Development of Novel DNA Gyrase Inhibitors Targeting *Mycobacterium tuberculosis*

THESIS

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by

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CERTIFICATE

This is to certify that the thesis entitled "**Development of Novel DNA Gyrase Inhibitors Targeting *Mycobacterium tuberculosis***" and submitted by **BOBESH K ANDREWS** ID No. **2012PHXF537H** for award of Ph.D. of the Institute embodies original work done by him under my supervision.

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Abstract

The search for new TB drugs that can overcome the increasing spread of multidrug resistant tuberculosis (MDR-TB) and emerging extremely drug resistant tuberculosis (XDR-TB) would be sucessful by the exploration of known and clinically validated targets for new chemical series or modification of existing drug classes. Over the past decade, type II topoisomerases have drawn much attention as selected targets for the discovery of potent antibacterial agents. Among the type II topoisomerases are DNA gyrase and topoisomerase IV.

In the present study we have chosen DNA Gyrase enzyme as the target for developing promising anti-tubercular compounds. This can be achieved by design, synthesis and anti-mycobacterial evaluation of compounds based on reported promising anti-tubercular agents acts via inhibiting DNA Gyrase enzyme.

In the present work, six classes of inhibitors (total 192 compounds) were designed and synthesized by simple and commercially feasible methods. Compound **BKA_35** (1-(4-fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea) emerged as the most promising inhibitor with an IC_{50} of 78 nM against *Mycobacterium tuberculosis* DNA Gyrase enzyme, with a good MTB MIC of 0.62 µM. **BKA_35** showed better anti mycobacterial potency than standard drug isoniazid. **PZ_11**, **BZ_10**, **BZ_38**, **QU_30**, **QU_08**, **BT_30** and **BB_27** evolved as promising inhibitors from different series.

The most active anti mycobacterial compounds were taken for nutrient starvation model to check the effect against dormant *MTB* , *in vivo* anti-bacterial screening using zebra fish model, zERG channel inhibition in a zebra fish model, safety profile of synthesized compounds were evaluated by checking their *in vitro* cytotoxicity against RAW 264.7 cell line (mouse leukemic monocyte macrophage) MTT assay.

We believe that the present class of inhibitors reported owing to the potency, selectivity and limited cytotoxicity emerged as valid leads for further optimization targeting DNA Gyrase enzyme.

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List of Abbreviations

µg	:	Microgram
µL	:	Microlitre
µM	:	Micromolar
^{13}C NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
3D	:	Three Dimensional
ADMET	:	Absorption, Distribution, Metabolism, Elimination and Toxicity
ADPNP	:	5'Adenylyl β,γ Imido Diphosphate
Anhy.	:	Anhydrous
ANOVA	:	Analysis of Variance
ATP	:	Adenosine Triphosphate
AZ	:	AstraZeneca
$(BOC)_2O$	:	Di-tert-butyl dicarbonate
BSA	:	Bovine serum albumin
$CDCl_3$	:	Chloroform deuterated
CoA	:	Coenzyme A
Cu	:	Copper
d	:	Doublet
DCM	:	Dichloromethane
dd	:	Doublet of doublet
DIPEA	:	Diisopropylethylamine
DMF	:	*N*,*N*-Dimethylformamide

DMSO	:	Dimethyl sulfoxide
DMSO-d_6	:	Dimethyl sulphoxide deuterated
DNA	:	Deoxyribonucleic acid
DOTS	:	Directly Observed Treatment, Short course
DTT	:	1, 4 Dithiothreitol
E	:	Ethambutol
EDTA	:	Ethylene diamine tetra acetic acid
EMA	:	European Medical Agency
ESI	:	Electron Spray Ionization
Et_3N	:	Triethylamine
EtOH	:	Ethanol
FQ	:	Fluoroquinolone
GLP	:	Good Lab Practices
GSK	:	Glaxo Smith Kline
H	:	Isoniazid
HEPES	:	4-(2-Hydroxyethyl)-1-Piperazineethanesulfonic acid
hERG	:	human Ether-a-go-go-Related Gene
HIV	:	Human Immuno Deficiency Virus
HPLC	:	High Pressure Liquid Chromatography
IC_{50}	:	Half Maximal Inhibitory Concentration
IPTG	:	Isopropyl-β-D-thiogalactopyranoside
J	:	Coupling constant
KCl	:	Potassium chloride
KOH	:	Potassium hydroxide
LB	:	Luria Broth

LCMS	:	Liquid chromatography–Mass Spectrometry
LHS	:	Left Hand Side
m	:	Multiplet
M.p	:	Melting point
MDR-TB	:	Multidrug-Resistant Tuberculosis
MeOH	:	Methanol
mg	:	Milligram
$MgCl_2$	:	Magnesium chloride
MHz	:	Mega hertz
MIC	:	Minimum Inhibitory Concentration
mL	:	Milliliter
Mmol	:	Millimole
Msm	:	*Mycobacterium smegmatis*
Mtb	:	*Mycobacterium tuberculosis*
MTT	:	(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	:	Microwave
NaCl	:	Sodium chloride
NADH	:	Nicotinamide Adenine Dinucleotide
NBTIs	:	Novel Bacterial Topoisomerase Inhibitors
NIH	:	National Institutes of Health
nM	:	Nanomolar
P	:	Para
PDB	:	Protein Data Bank
PMSF	:	Phenylmethylsulfonyl fluoride
Ppm	:	Parts per million

R	:	Rifampicin
RNA	:	Ribonucleic acid
Rpm	:	Rotations per minute
RPMI	:	Roswell Park Memorial Institute
Rt	:	Room temperature
S	:	Singlet
SAR	:	Structure Activity Relationship
SDS-PAGE	:	Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis
t	:	Triplet
TAE	:	Trisbase, Acetic acid, EDTA mixture
TB	:	Tuberculosis
TFA	:	Trifluoroacetic acid
THF	:	Tetrahydrofuran
TLC	:	Thin-layer chromatography
T_m	:	Melting temperature
TMS	:	Trimethylsilane
Toprim	:	Topoisomerase-primase
Tox	:	Toxicology
UV	:	Ultraviolet
WHO	:	World Health Organization
XDR-TB	:	Extensively Drug-Resistant Tuberculosis
XP	:	Extra Precision
Z	:	Pyrazinamide
zERG	:	Zebrafish Ether-a-go-go-Related Gene
δ	:	Chemical shift

Introduction

Chapter 1

"TB is like living with a bomb in your lungs. You just lie around very quietly hoping it won't go off"

Sylvia Path (1963)

Tuberculosis earned the sobriquet "The Captain of All These Men of Death" as it was greatest killer of mankind during 18 and 19th centuries. It is caused by rod shaped, aerobic, non-spore forming bacteria *Mycobacterium tuberculosis.* DNA analysis of Egyptian mummies revealed that tuberculosis was prevalent in Egypt more than 5000 years ago. Théophile Laennec Jean-Antoine Villemin (1865), Robert Koch (1882), Clemens von Pirquet (1907) made significant contributions in understanding pathogenesis of tuberculosis [Thomas M.D., *et al*., 2006].

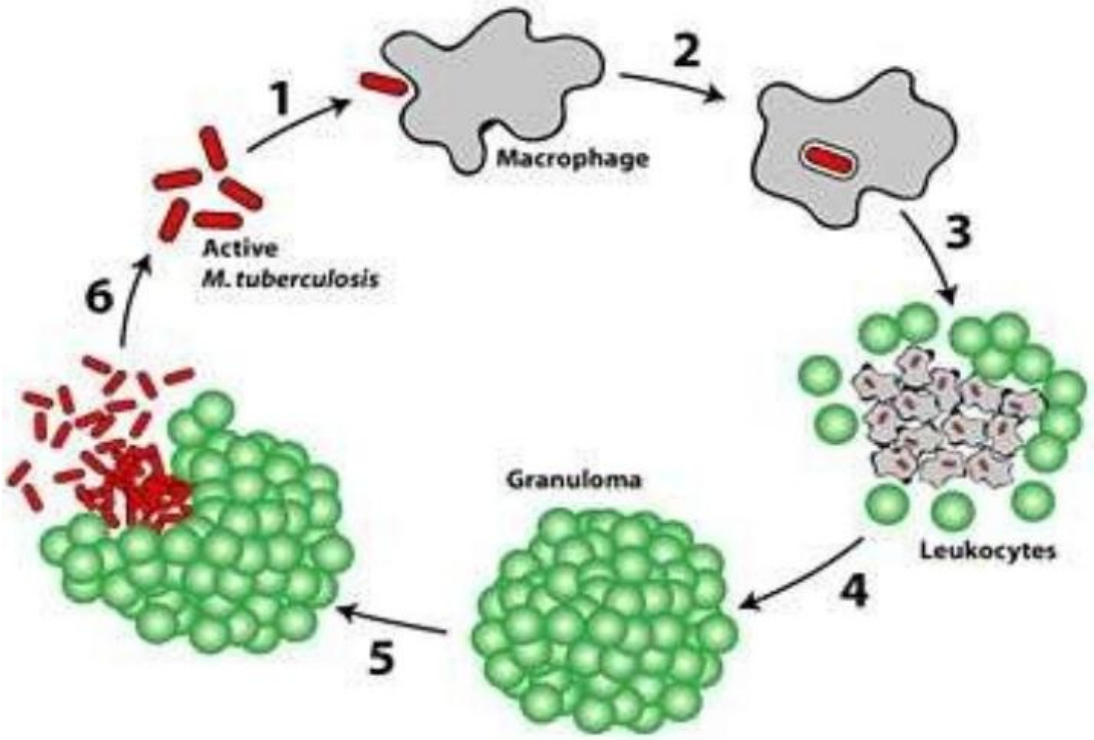

Fig 1.1: Pathogenesis of tuberculosis [Thomas M.D., *et al*., 2006].

It is an airborne infection spread by droplets produced during coughing or sneezing by infected individual [Daniel, T. M., et al., 2006]. To establish infection mycobacterium has to bypass host defense mechanisms like mucociliary clearance, phagocytosis mediated by complement protein in macrophages, cell mediated immunity by T cells. Depending on host immunity mycobacterium either establishes infection or enters into dormant non replicative phase called latent phase. In granuloma bacteria would be subjected to stress due to lack of availability of oxygen, nutrients, low pH, free radicals like ROS and RNS. When the immunity of host is compromised the mycobacterium will be actively replicating and causes signs of diseases. The symptoms of disease include coughing, fatigue, malaise, wasting, finger clubbing, low grade fever accompanied by chills and night sweats, hemoptysis, dyspnea or orthopnea (in severe stages), anemia and leukocytosis[Knechel NA, *et al*., 2009, Payne, D. J., *et al*., 2013].

1.1. Treatment of TB

The first ray of hope in treatment of tuberculosis was discovery of streptomycin in 1943. In 1950, several other antitubercular drugs are discovered namely- para-amino salicylic acid, isoniazid, pyrazinamide, cycloserine and kanamycin [Coxon, G. D., *et al*., 2012]. To combat resistance and to shorten duration of therapy combination of drugs were used [Balganesh, T. S., *et al*., 2004]. The treatment duration was further decreased to 9 months after discovery of rifampicin in 1960. Currently available drugs in market can be categorized into five groups based on their efficacy, potency, drug class [Zumla A., *et al*., 2013 and 2014].

FIRST LINE DRUGS	**GROUP 1** (oral)	Isoniazid, Rifampicin, Pyrazinamide, Ethambutol, Rifapentine or Rifabutin
SECOND LINE DRUGS	**GROUP 2** (injectable amino glycosides, poly peptides)	Streptomycin, Kanamycin, Amikacin, Capreomycin, Viomycin
	GROUP 3 (oral & injectable fluoro quinolones)	Ciprofloxacin, Levofloxacin, Moxifloxacin, Ofloxacin, Gatifloxacin
	GROUP 4 (oral)	p-amino salicylic acid, Cycloserine, Terizidone, Ethionamide,Prothionamide, Thioacetazone, Linezolid
THIRD LINE DRUGS	**GROUP 5**	Clofazimine, Linezolid, Clarithromycin, Amoxycillin + Clavulanate, Imipenem + Cilastatin

Table 1.1: Classification drugs.

1.1.1 Current TB therapy

WHO recommends standard regimen of antibiotics in DOTS therapy for newly infected individuals for period of 6 months which covers 2 phases [Ginsberg, A. M., *et al*., 2007]. The primary phase i.e., Intensive phase treatment involves use of Isoniazid, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin for a period of 2 months; the latter phase namely Continuation phase involves use of Isoniazid and Rifampicin for 4 months to completely eliminate bacteria and avoid development of resistance [Dover, L. G., *et al*., 2011]. WHO recommended doses of first line drugs for adults are as follows [WHO Guidelines for treatment of tuberculosis fourth edition-2010, Sharma, S. K., *et al*., 2013].

Drug	Recommended dose			
	Daily		3 times per week	
	Dose and range (mg/kg body weight)	Maximum (mg)	Dose and range (mg/kg body weight)	Daily maximum (mg)
Isoniazid	5 (4–6)	300	10 (8–12)	900
Rifampicin	10 (8–12)	600	10 (8–12)	600
Pyrazinamide	25 (20–30)	–	35 (30–40)	–
Ethambutol	15 (15–20)	–	30 (25–35)	–
Streptomycin[a]	15 (12–18)		15 (12–18)	1000

Table 1.2: Drug dosage

1.2. Drug resistance in TB

Even though many drugs are available in the market for treatment of tuberculosis; irrational use of antibiotics, non-compliance with standard regimen led to development of resistance [Gillespie, S. H., *et al*., 2002, Raoot, A., *et al*]. Tuberculosis is termed MDR TB (multi drug resistant) if it is resistant to isoniazid or rifampicin [Green, K., *et al*., 2013] Treatment of MDR strains is limited due to lesser options, expensive, less potency and more side effects. XDR TB [Haydel, S. E., *et a*l., 2010, Parida, S. K., *et al*., 2015] (extensive drug resistant) [Dheda, K., et al., 2010, O'Donnell, M. R., *et al*., 2013] is MDR TB having additional resistance to any of the fluoroquinolones (such as levofloxacin or moxifloxacin) and to at least one of three injectable second-line drugs (amikacin, capreomycin or kanamycin).

According to WHO global tuberculosis report 2015, 9.6 million new cases were reported in 2014. The majority of cases were reported from India, Indonesia and China. Globally 3.3% of new cases and 20% of previously treated cases have MDR TB. Half of the MDR TB patients were from India, China and Russia. 1,90,000 people died out of MDR TB in 2014. 9.7% cases of MDR TB have XDR TB [Matteelli, A., *et al*., 2014, Udwadia, Z. F., *et al*., 2012]. By 2015 105 countries had been reported to have XDR TB [WHO Global Tuberculosis report-2015, Jean B. Nachega]. (5,6)

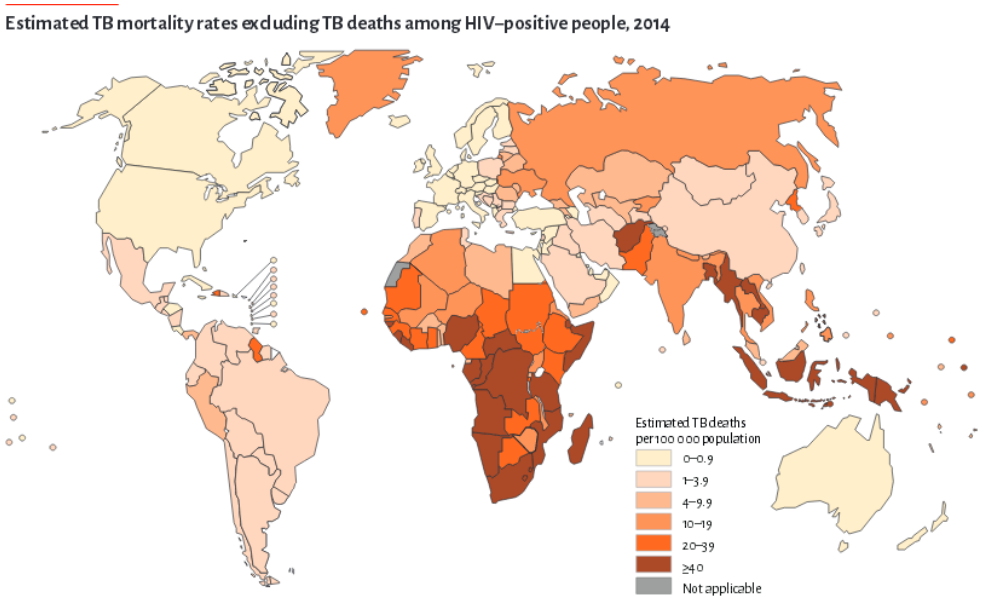

Fig 1.2: Estimated TB mortality rates excluding TB deaths among HIV-positive people, 2014

Newer diagnostic methods have been constantly reviewed by WHO to facilitate effective diagnosis of TB [Glaziou, P., et al., 2011]. Some of the diagnostic methods approved in 2015 are - LF LAM (Lateral Flow test for Lipoarabinomannam), LPA (Line Probe Assays), Xpert Ultra.

1.2.1. Treatment for drug resistant -TB

Drug resistant TB can be largely cured with the appropriate combination and rational use of available anti-tubercular drugs [Caminero J., *et al*., 2006, Pham, T., *et al*., 2013]. For the treatment of MDR-TB, WHO recommends the use of DOTS-Plus therapy, which includes drugs used in DOTS therapy plus second line TB drugs (**Figure 1.6**) [Ahuja S.D., *et al*., 2012].

Composition of MDR-TB drug regimen [Van Deun A., *et al*., 2010, Sullivan, T., *et al*., 2013]:

1. Choose, if possible:

a) Injectable second line drug (e.g. Amikacin)

b) Later generation fluoroquinolone (e.g. Levofloxacin)

c) Ethionamide or prothionamide

d) Cycloserine or terizidone

2. Choose at least 4 drugs (it is unclear whether all patients with MDR-TB/XDR-TB should be treated with pyrazinamide)

3. Choose group five drugs only if needed to sum up to at least four active drugs

4. Treatment for a total of 24 months with an intensive phase of 8 months

5. Prolongation of duration of therapy should be considered based on treatment success

1.3. Drug development pipeline

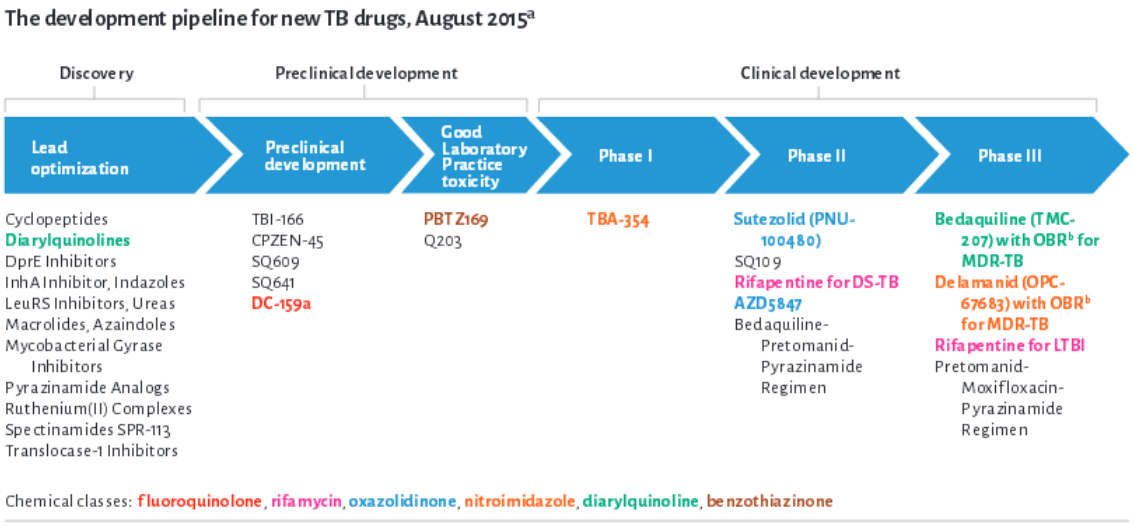

Fig 1.3: TB drugs pipeline

The figure depicts new and repurposed drugs for treatment of drug susceptible, MDR and latent forms of TB [Guzman, J. D., et al., 2013]. 8 drugs successfully entered clinical testing on humans; but 2 drugs AZD5847 and Sutezolid have not made much progress in last 2 years. TBA-354, a nitroimidazole derivative has successfully entered phase I trials. Bedaquiline was approved in Dec, 2012 by US FDA for treatment of MDR TB. Delamanid was approved in 2013 by European Medical Agency (EMA) for treating MDR TB [Verma, A. K., *et al*., 2012]. Global alliance for TB drug [Gupta, P., *et al*., 2004] development extensively works on development of new treatment a regimen that shortens treatment duration and effective against

resistant, latent forms of bacteria [Burki, T., et al 2014, Shehzad, A., et al., 2013]. Based on findings in NC-003 trail, combination of Bedaquiline, Pretomanid and Pyrazanamide are now taken further for phase IIB studies. END TB trail organized by Global TB alliance scheduled to start at end of this year evaluates efficacy of bedaquiline or delamanid, moxifloxacin or levofloxacin and pyrazinamide + linezolid or clofazimine against MDR TB. Other trail TB-PRACTECAL will evaluate safety and efficacy of 6 months regimen consisting of bedaquiline, pretomanid and linezolid with or without moxifloxacin againt MDR and XDR TB [Lienhardt, C., *et al*., 2010 and 2012, Villemagne, B., *et al*., 2012].

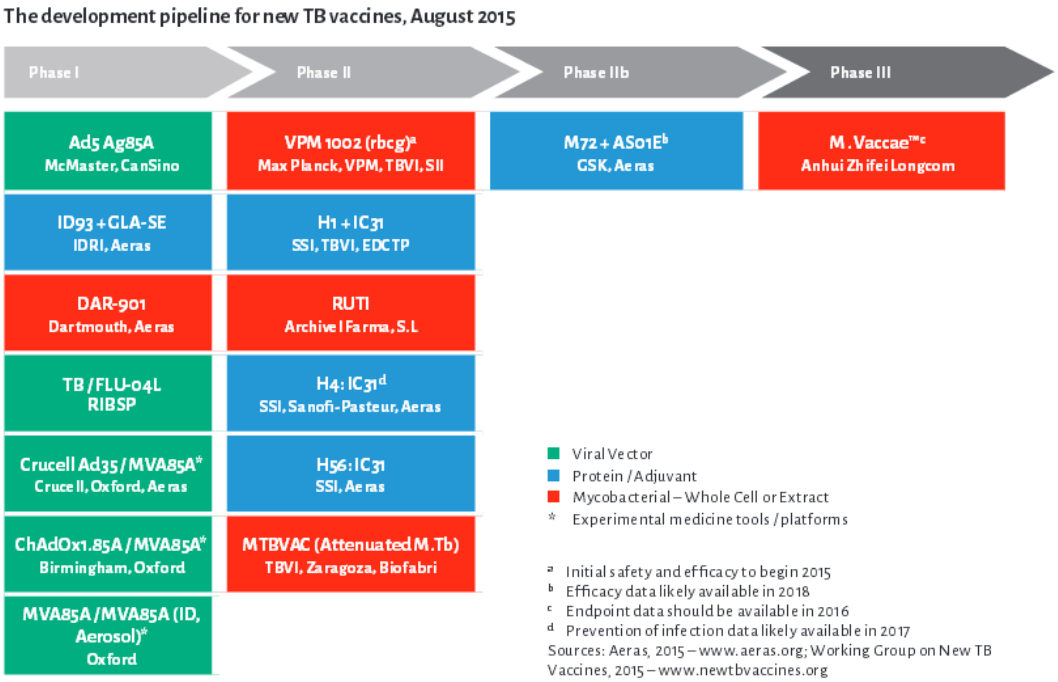

Fig 1.4: The development pipeline for TB vaccines

Vigorous efforts are made for development of vaccines to prevent infection or to prevent primary progression to disease or reactivation of latent TB [O'brien, R. J., *et al*., 2001]. The above figure illustrates different categories of vaccines and their development phases [WHO Global Tuberculosis report-2015].

Literature Review *Chapter* **2**

2.1. Classification of TB drugs based on mode of action

Targets employed for drug development against Mycobacterium tuberculosis-

Owing to development of resistant forms of Mtb quest for newer anti tubercular drugs never ends. The figure given below depicts existing and new targets for drug development against *TB* [Bocanegra-García, V., *et al*., 2011].

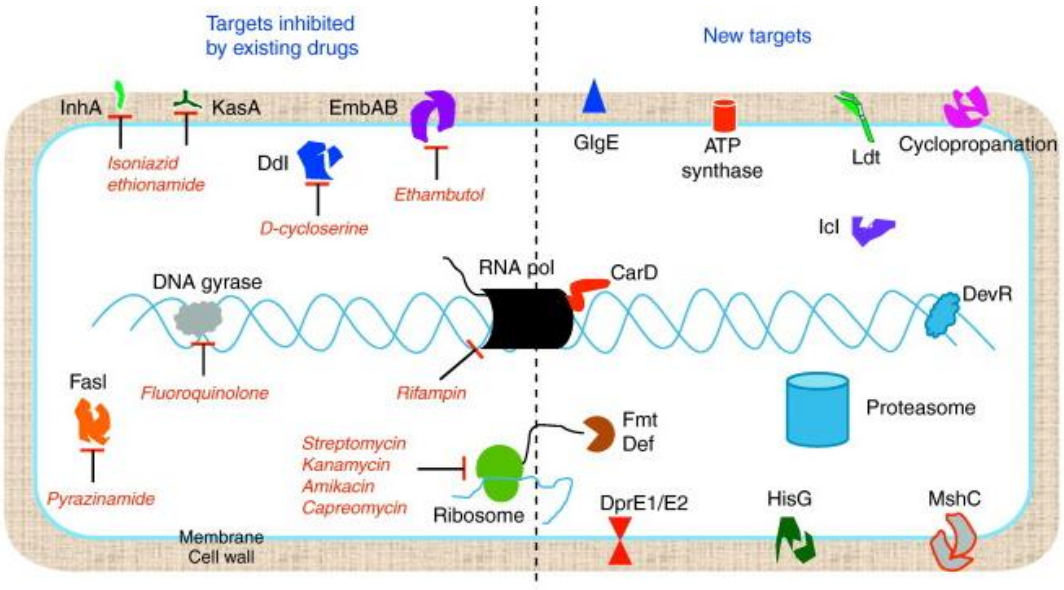

Fig 2.1: Targets employed for drug development againist *Mtb*

Drugs currently in clinical use or development can be categorized as-

a. Cell wall biosynthesis inhibitors (MEP pathway, Peptidoglycan biosynthesis, Arabinogalactan biosynthesis)
b. Mycolic acid biosynthesis (InhA, FAS-I,II pathway)
c. Energy production related pathways (Isocitrate lyase, ATP synthase)
d. Amino acid biosynthesis (Shikimate pathway, Lysine biosynthesis)[Williams, N. L., *et al*., 1999]
e. Cofactor related targets (Folic acid, NAD, Riboflavin biosynthesis, lumazine synthase)

f. DNA metabolism (Ribonucleotide reductase, Gyrase, Ligase) [Mdluli, K., *et al*., 2006, 2007 and 2014].
g. Menaquinone biosynthesis
h. Miscellaneous (Cytochrome inhibitors, Peptide deformylase, Siderophore biosynthesis, Signal transduction pathways).

2.2. Gyrase as druggable target in *Mtb*

Topoisomerases are crucial in maintaining topology of DNA essential for cellular activities. DNA gyrase is sole type II topoisomerase present in mtb. It's a heterotetramer consisting of 2 Gyr A and 2 Gyr B subunits and is responsible for inducing negative supercoils in DNA. Gyr A performs the function whereas Gyr B serves as ATPase domain providing energy required for the process. The sequence of events involved during action of gyrase on DNA is as shown in the figure-

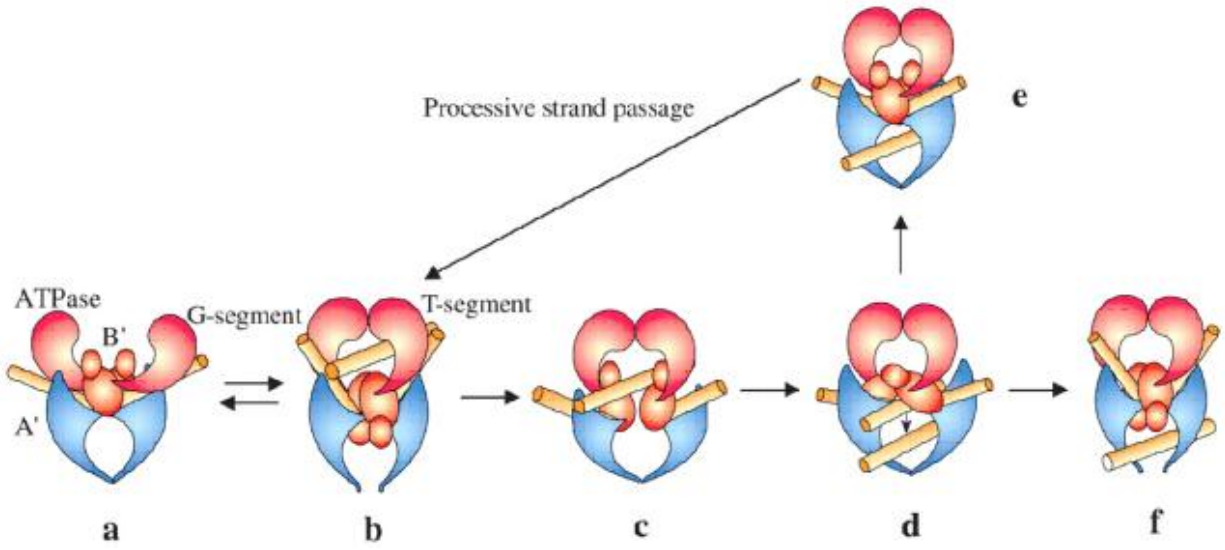

Fig 2.2: Mechanism of DNA Gyrase enzyme action

Initially a complex (a) is formed as a result of binding of Gyrase A and B subunits with G segment. The ATPase domains dimerize upon the binding of ATP [Tingey, A. P., et al., 1996], thereby capturing the T segment; the B′ dimer is opened, accompanying the distortion of the G segment, and finally converts to the cleavage–competent complex (b), where the G segment is cleaved by the active centre formed by the B′ and A′ domains [Laponogov, I., *et al*., 2009 and 2010]. A series of conformational changes occur and the cleaved G segment is separated (c),

including a conformational change shown in (d). Then an electrostatic potential gradient within the enzyme might be generated, thereby drawing the T segment through the break and towards the central hole (d). In (e) the enzyme recovers the conformation of the initial complex as shown in (a) and thus b–c–d–e–b forms a cycle of consecutive strand passage. The G segment is resealed and the T segment released from the exit gate [Champoux, J. J., *et al*., 2001, Wu, J., *et al*., 2011]

Quinolones, coumarins, cyclothialidines are well known gyrase inhibitors [Cheng, G., *et al*., 2013, Tanitame, A., *et al*., 2004 and 2005]. Quinolones form complex with gyrase A subunit thus blocking the DNA breakage reunion cycle resulting in death of bacteria [Boehm, H. J., *et al*., 2000, Mitton-Fry., et al., 2013]. They are mainly used as second line drugs for treatment of *TB*. Mtb has acquired resistance to available second line drugs by developing mutations at GyrA T80A, A90G and GyrB N510D. Owing to resistance for currently marketed fluoroquinolones attempts were made to develop more potent and efficacious quinolines as well as novel inhibitors targeting Gyr B subunit [East, S. P., et al., 2009]. Coumarins like novobiocin and clorobiocin have very good Gyr B inhibitory potential but the main disadvantage of this class of compounds is their eukaryotic toxicity[Alemparte-Gallardo, C., *et al* 2009 and 2010].

2.3. Quinolines scaffold as lead for development of anti-mycobacterial agents

Quninolines were one among the fascinating leads of natural origin identified among the libraries screened for antibacterial potency [Medapi, B., *et al*., 2015]. They are found widely spread in plants (Cinchona, Melicope etc.) and attempts to synthesize quinoline were also successful [Antonello, C., *et al*., 1993] To understand SAR many libraries were synthesized among them fluoroquinolines were found to more potent and used clinically [Anquetin, G., *et al*., 2006].

Initially quinolines were restricted for only urinary tract infections with advent of fluoroquinolines with wide spectrum of activity numerous substitutents were introduced in this class retaining 3-carboxy-6-fluoro-4-quinolone as core moiety for antibacterial effect [Migliori, G. B., *et al*., 2012, Xiao, Z. P., *et al*., 2014].

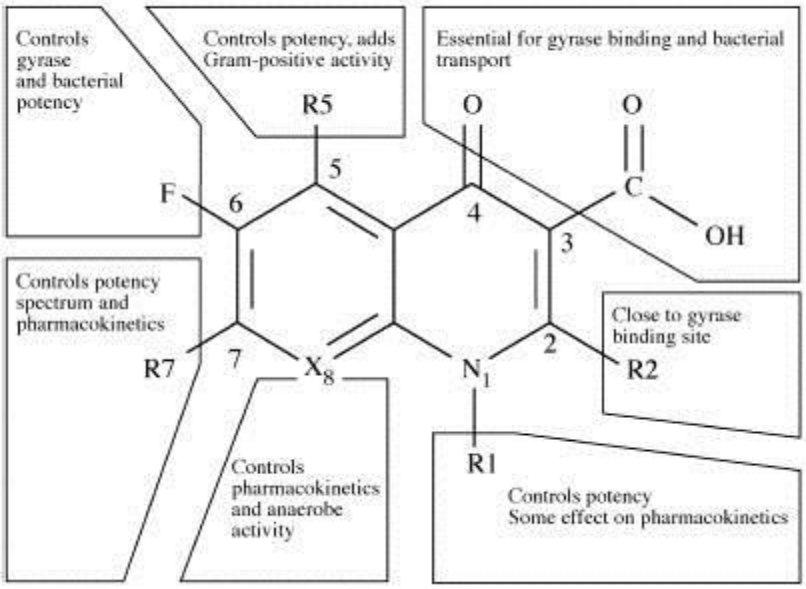

Fig 2.3: The effects of substituents at different positions of quinoline

2.3.1. Fluoroquinolones

The fluoroquinolones, moxifloxacin, gatifloxacin, ciprofloxacin and levofloxacin are most important bactericidal antibiotics and have broad spectrum activity [Sriram, D., *et al*., 2006]. They are active against both gram-positive and gram-negative bacteria [Alaa, A. M., *et al*., 2011, Plech, T., Kaproń, B., *et al*., 2015]. Gatifloxacin and moxifloxacin are under phase III clinical evaluation aiming at better TB treatment.

Fluoroquinoles inhibits both ATP dependent DNA gyrase (topoisomerase II) as well as ATP dependent topoisomerase IV [Takei M., *et al*., 2001; Kato J., *et al*., 1990]. Fluoroquinolones blocks the movement of replication works and transcription complexes [Drlica K., *et al*., 2003]. Resistance to fluoroquinolones in *M. tuberculosis* is due to mutations in the conserved quinolone resistant-determining region of gyrA and gyrB [Zhang, J., *et al*., 2015] involved in the interaction between the drug and DNA gyrase [Clairefond, P., *et al*., 1992, Pantel, A., *et al*., 2012].

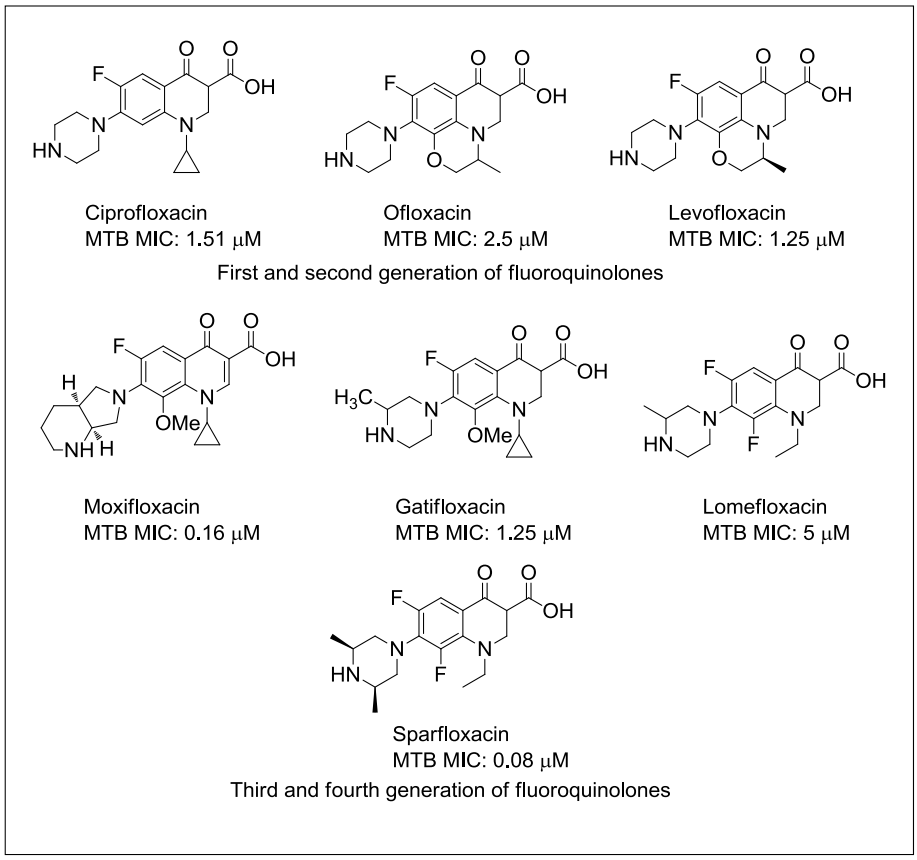

Fig 2.4: Different classes of fluoroquinolines used clinically

This class of compounds act mainly act by inhibiting Gyr A part of DNA gyrase thereby inhibiting DNA replication leading to bacterial death [Lübbers, T., et al., 2000]. Antibiotics with 7-piperazinyl-4-quinoline core showed good anti gram positive potency and were used against pencillin resistant bacteria. Quinoline antibiotics possess numerous advantages like high potency, broader spectrum ofactivity, better bioavailability, oral and intravenous formulations, high serum levels, a large volume of distribution indicating higher concentrations in tissues [Azéma, J., *et al*., 2011, Tran, T. P., *et al*., 2007]. The major drawback for this class of antibiotics is development of resistance. As the benefits outweigh disadvantages newer molecules are being developed in this class of antibiotics [Katritzky, A. R., *et al*., 2009]. The approval of Bedaquiline by FDA for treatment of MDR TB can be considered as milestone in drug development for resistance bacteria. Bedaquiline acts by inhibiting mycobacterial ATP synthetase and depletes cellular energy stores [Wohlkonig, A., *et al*., 2010]. With the success of

bedaquiline scientists focused on fusing quinolines with other scaffolds for newer molecules with potency against resistant TB.

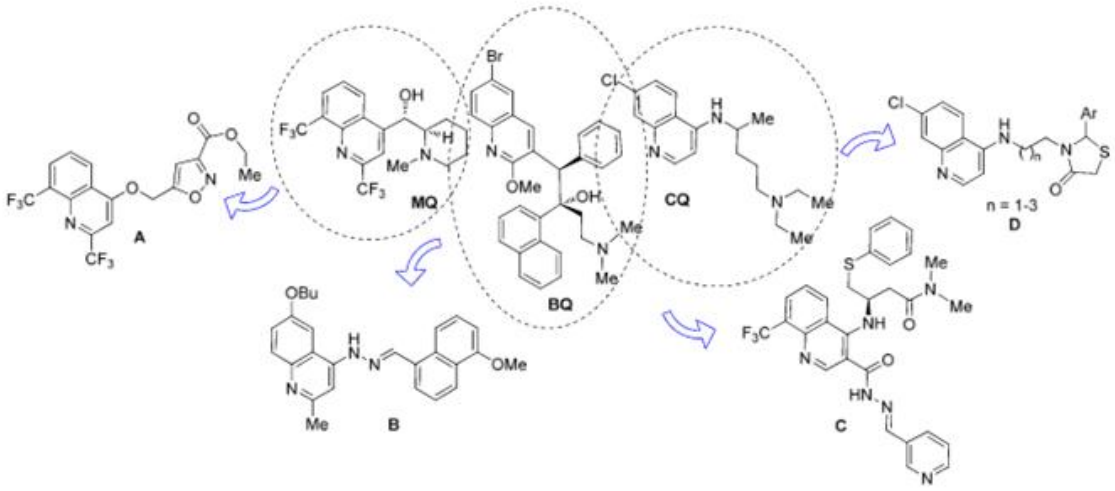

Fig 2.5: Modification of quinolone derivatives

Mefloquine-isoxazole derivatives (A) served as lead for development of -[(E)-2arylethenyl]-3-isoxazolecarboxylic acid alkyl ester derivatives which are active against replicating and non replicating bacteria and also has minimal toxicity. 4-Quinolinyl hydrazones (B), like INH-quinolone hybrid molecule (C) showed marked anti-TB activity possessing a good MIC (0.78 μg/mL), but poor selectivity for mycobacteria (SI = 6.67). Chloroquine- thiazolidinone hybrids (D) leads were less promising as antitubercular compounds [Senthilkumar, P., et al., 2009].

Chattopadhyaya and co-workers carried out structural, chemical and functional analysis of bedaquiline by dividing molecule into 4 hemispheres as shown in the figure. By using molecular modeling and docking studies modifications were done to enhance fitness in binding site and to improve physicochemical properties. The best molecule identified by this approach was 3-Benzyl-2-[4-fluoro-2-(1-imidazol-1-yl-ethyl)-phenoxy]-6-(4-phenyl-[1,2,3]triazol-1-yl)-quinoline having an MIC of 3.125 μg/mL and with clog P values (8.40) higher than that of bedaquiline.

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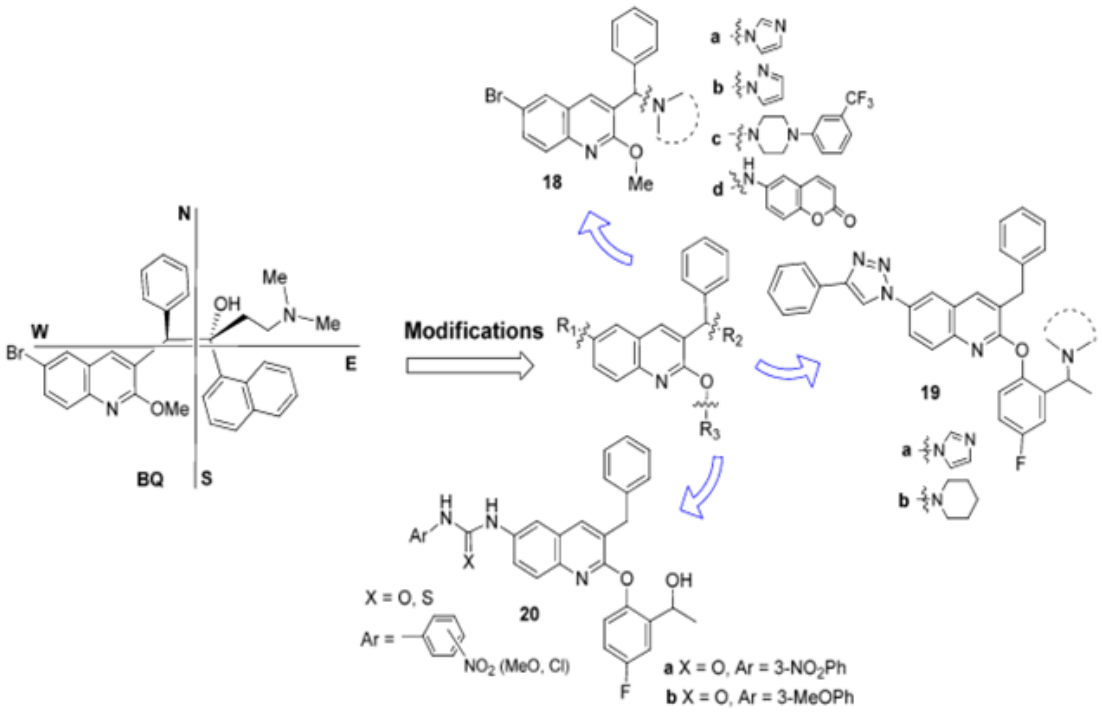


Fig 2.6: Modification of quinolone derivatives

2.4. Benzimidazoles as novel antimycobacterial agents

Most of the Bacterial Topoisomerase inhibitors possess quinoline or naphthyridine as left-hand side (LHS) ring, a mono- or bicyclic hydrophobic right-hand side (RHS) ring and a linker joining the RHS and LHS in a proper orientation [Charifson, P. S., *et al* 2008].

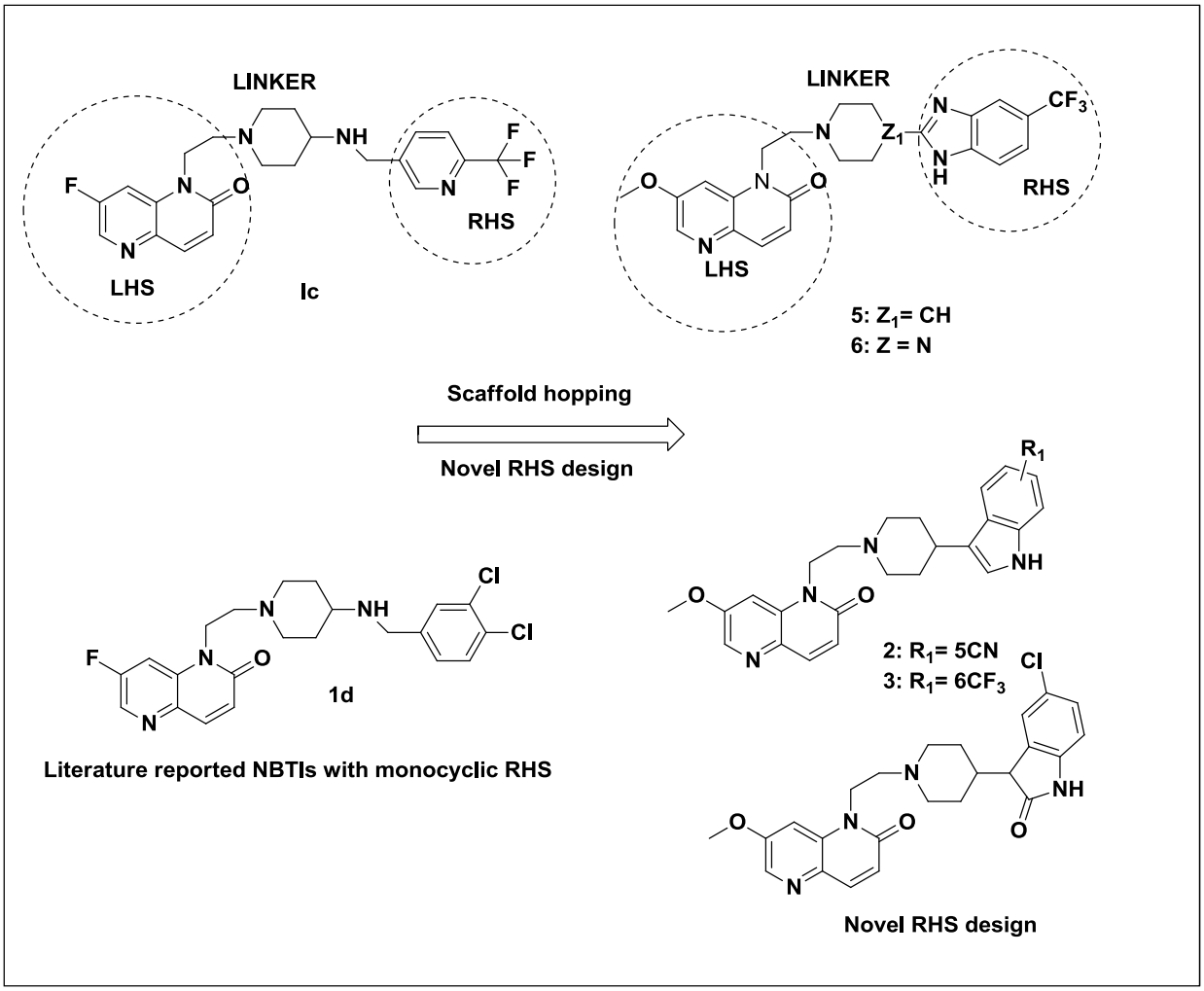
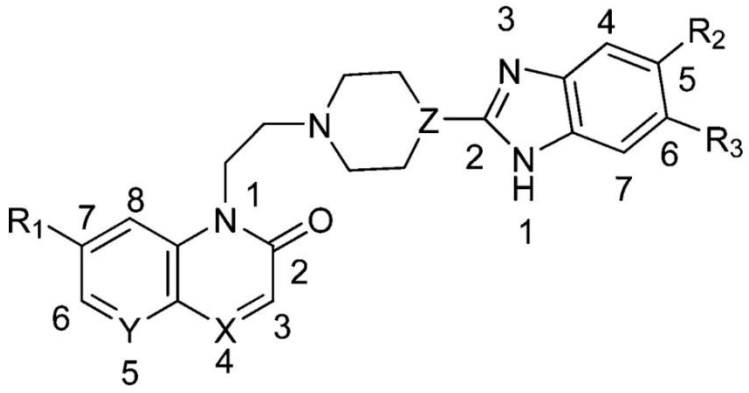


Fig 2.7: Design strategy employed for the development of benzimidazole moiety as novel anti-mycobacterial agents.

In 2014 Shahul hameed et.al., by using scaffold hopping approach developed novel gyrase inhibitors against fluoroquinoline resistant MTB. This class of inhibitors contains quinoline in the LHS and RHS was replaced with benzimidazole. To understand SAR modifications were attempted at R_1, R_2, R_3, X, Y, Z positions.



Based on MIC values SAR studies showed that piperidine linker is optimum linker when compared to piperazine. Bulky groups are favouring activity at R_1 and R_2 positions. Hydrophobic nature of benzimidazole exhibited synergism with hydrophobic electron withdrawing groups like CF_3 at C-5 position leading to most potent compound in the series. Introduction of other hydrophobic substituents such as methyl or fluorine enhanced the potency by 4-fold. The addition of a nitrogen at the 8 position of LHS ring as pyrido [2, 3-*b*] pyrazin-2(1*H*)-one or moving nitrogen to 4-position of LHS as 1,4-quinoxazolinone was broadly tolerated for potency. Initially, introduction of 3-pyridyloxy at R_1 instead of OCH_3 retained *Mtb* MIC but did not show any improvement in reducing hERG liability. Replacement of 3-pyridyloxy by 4-phenoxy sulphonamide at R_1 position, of LHS showed excellent improvement in *Mtb* MIC and hERG mitigation.

In 2013, Divya Awasthi et.al., identified 2 lead compounds belonging to 2,5,6-trisubstituted benzimidazoles class as Mtb-FtsZ inhibitors [Awasthi, D., et al., 2013]. Modifications were attempted at 5 and 6 positions for SAR purposes. This study has successfully led to the discovery of a highly potent advanced lead 5f (MIC = 0.06 μg/mL) and several other compounds with comparable potencies. In vitro experiments such as the FtsZ polymerization inhibitory assay and TEM analysis of Mtb-FtsZ treated with 5f and others indicate that Mtb-FtsZ is the molecular target for their antibacterial activity.

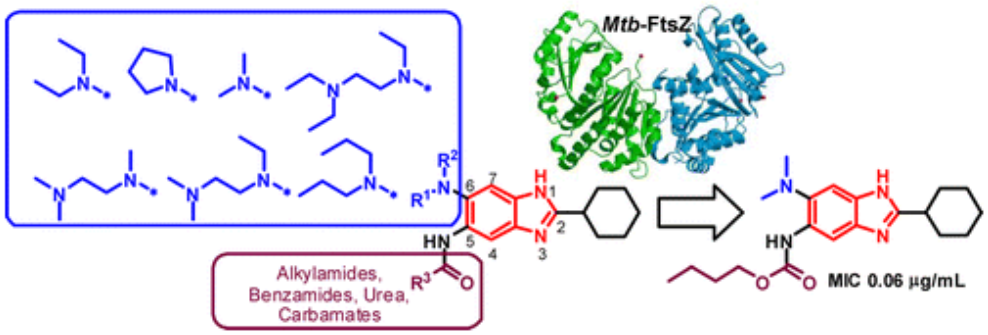


Fig 2.8: Design logic for the development of novel anti-mycobacterial agents

2.5. Benzothiazinone as new horizons in anti-mycobacterial class

The 1,3-benzothiazin-4-ones (BTZs) represent a new class of drugs, which have activity against M. tuberculosis in vitro, ex vivo, and in murine TB models [Makarov V., *et al*., 2009]. BTZs are activated in M. tuberculosis by reduction of an essential nitro group to a nitroso derivative as shown in figure below, which then specifically reacts with a cysteine residue in the active site of the enzyme decaprenylphosphoryl-D-ribose 2'-epimerase (DprE1) to form covalent semimercaptable adduct (Trefzer, RengifoGonzalez et al. 2010). Inhibition of this enzymatic activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor that is required for the synthesis of the cell-wall arabinans, thus causing bacterial lysis and death [Makarov V., *et al*., 2009]. Although spontaneous BTZ-resistant laboratory mutants were found to have a Ser or Gly substitution at codon Cys387 of dprE1, resistance to BTZs has not been reported in clinical *M. tuberculosis* isolates [Pasca M R., *et al*., 2010]. Recently, a novel resistance mechanism to BTZ was described in *M. smegmatis* involving the overexpression of the nitroreductase NfnB, which leads to the inactivation of the drug by reduction of a critical nitro-group to an amino-group [Manina G., *et al*., 2010]. However, *M. tuberculosis* seems to lack nitroreductases able to inactivate BTZs.

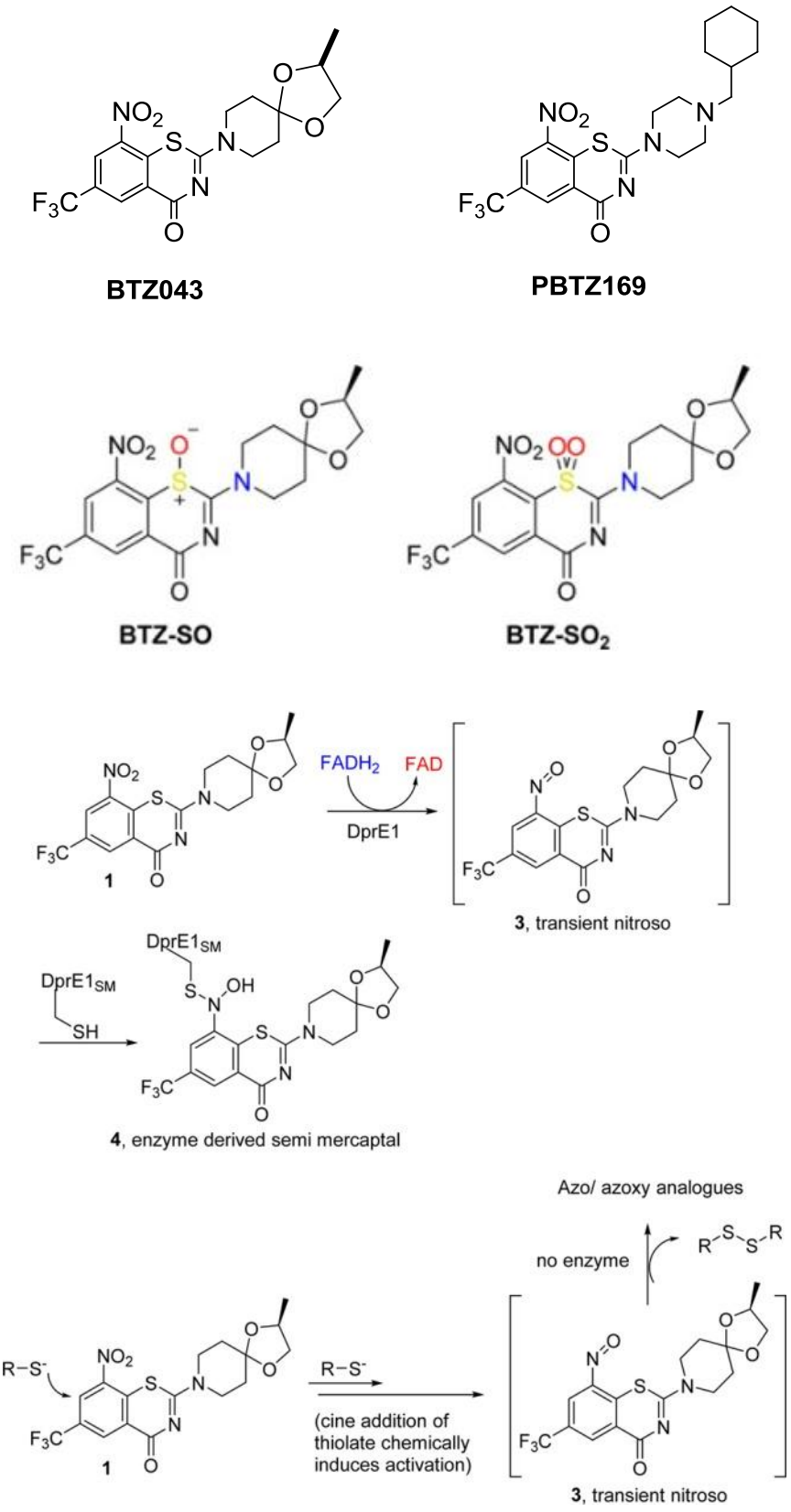


Fig 2.9: Mechanism of action of **BTZ043** [Makarov V., *et al*., 2009]

In 2014 Rohit tiwari et al., carried out synthesis, computational and NMR studies and anti-TB activity of oxidation products, 1,3-benzothiazinone sulfoxide (BTZ-SO) and 1,3-benzothiazinone sulfone (BTZ-SO_2) derived from BTZ043. BTZ-SO possesses potent activity against nonpathogenic and pathogenic mycobacterial strains, but BTZ-SO_2 is only weakly active [Tiwari, R., *et al*., 2014].

In 2015 Manoj Chandran *et.al*., has developed benzothiazinone based hybrids targeting MTB DNA gyrase. Identified inhibitor PBTZ169 possess benzothiazinone in LHS region and aryl thio urea in RHS.(fig below). Out of 36 synthesised compounds, compd 18 was identified as potent with supercoiling IC50 of 0.51 ± 0.16 µM and MIC of 4.41 µM [Manoj C., *et al* 2015]

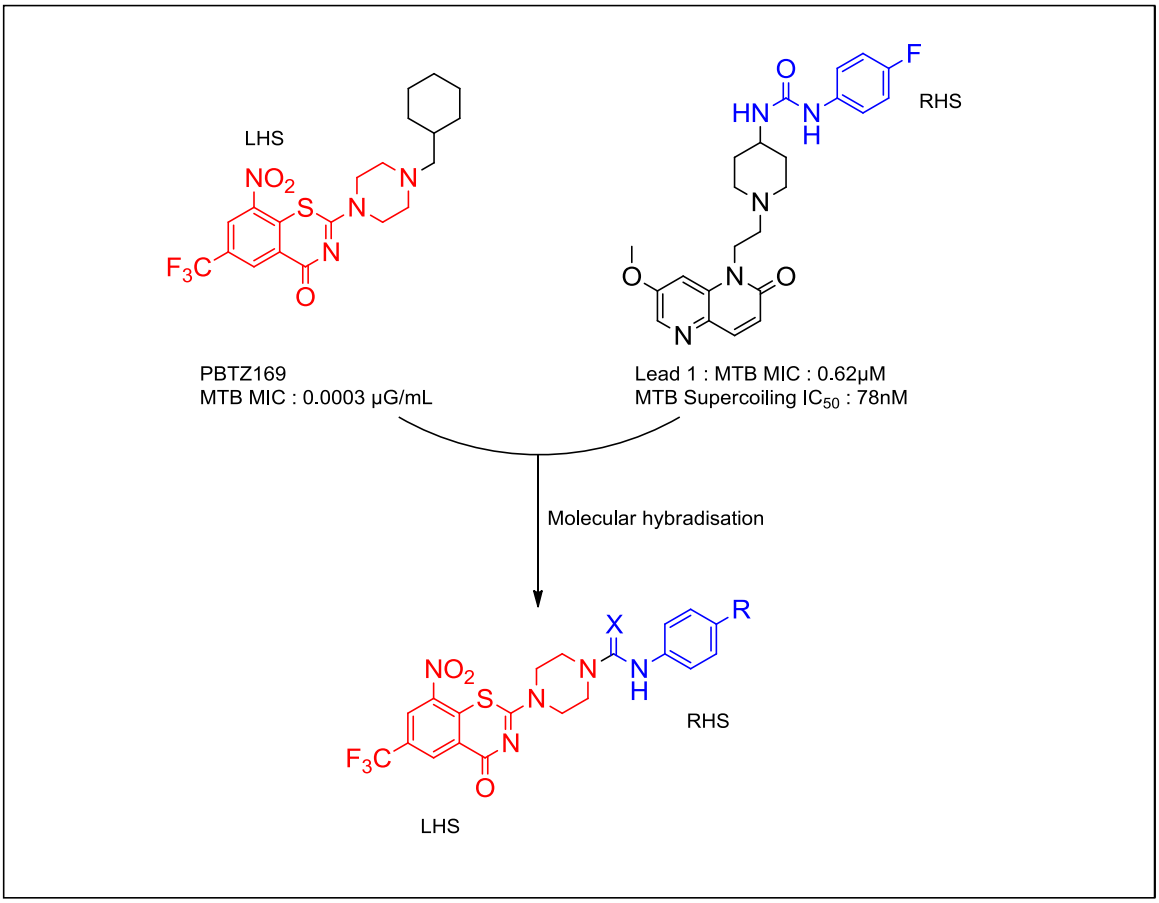
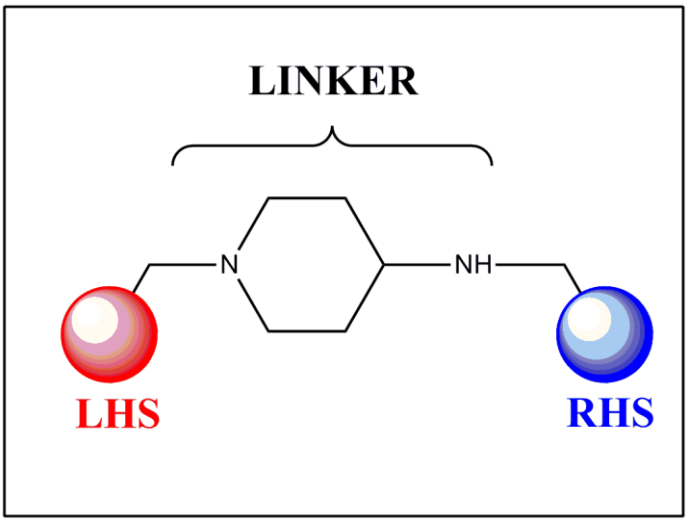


Fig 2.10: Design strategy for lead molecule [Manoj C., *et al* 2015]

2.6. Bacterial novel topoisomerase inhibitors (NBTIs)

The main challenge associated with antibacterial research is the cross resistance developed by bacteria against well known drugs. Quinolones are well validated class of antibacterial agents [Jayagobi, M., *et al*., 2011]. From the crystal structures of quinolone–BT binding mode and novel class of BT inhibitors revealed that novel bacterial topoisomerase inhibitors (NBTIs) and quinolones occupy different binding pockets, providing a structural basis for their lack of cross-resistance to quinolone-resistant strains[Hameed, S., *et al*., 2014]

The chemical structure of NBTIs consist of bicyclic LHS and bicyclic/monocyclic RHS connected through a linker mostly aminopiperidine [Jeankumar, V. U., *et al*., 2013].



The mechanism of inhibition and the binding mode of NBTIs for the antibacterial series has been reported [Bax, B.D., *et al.*, 2010]. From the crystal structure studies of methoxyquinoline-3-carbonitrile (**GSK299423**, **Figure 2.5**), one could infer that the LHS portion of the NBTIs bind to DNA substrate, whereas the RHS portion interact with the protein dimer interface of GyrA subunits. The binding site of NBTIs was distinct from the FQ binding and they were reported to be active against FQ-resistant strains of *S. aureus*.

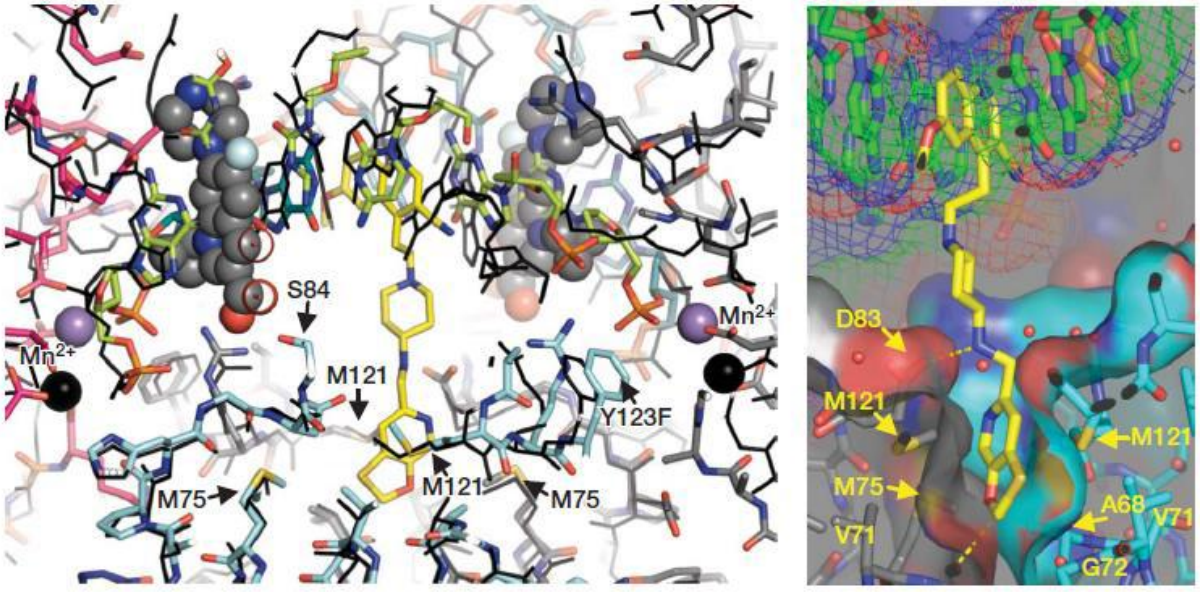


Figure 2.11: The 2.1A° GyrB27–A56 complex with GSK299423 and DNA (Bax, B.D. *et al.*, 2010). (a) The 2.1A ° GSK299423 complex is shown in stick representation with GSK299423 (yellow) half-way between the two Mn^{2+} ions (purple) at the active site. The 3.35A° ciprofloxacin structure (black lines) is shown superposed with ciprofloxacin in space filling (grey carbons) representation, and Mn^{2+} ions are shown in black. (b) The oxathiolopyridine ring of **GSK299423** sits in a largely hydrophobic pocket at the dimer interface (Bax, B.D. *et al.*, 2010).

Like fluoroquinolones, these compounds also interact with both the protein and DNA and inhibit supercoiling but unlike fluoroquinolone that uncouple ATP hydrolysis from supercoiling such that futile ATP hydrolysis occurs in the absence of supercoiling [Jeankumar, V. U., et al., 2016, Shirude, P. S., *et al*., 2012 and 2013]; NBTIs and related compounds, 22 on the other hand, do not uncouple ATP hydrolysis from supercoiling. Instead, a ternary gyrase-DNA-inhibitor complex forms that cannot pass through the catalytic cycle so that neither supercoiling nor ATP hydrolysis occurs [Jeankumar, V. U., et al., 2015].

The binding site of NBTIs in the DNA-bound gyrase complex of *Staphylococcus aureus* has been determined by crystallography. The binding site for the NBTI is close to, but does not overlap with, the two quinolone binding sites [Pissinate, K., *et al*., 2016]. The crystal structure shows that there is only one inhibitor molecule in the complex and indicates that the compound does not directly inhibit the DNA cleavage–religation reaction as it is not bound in the active

sites. The crystal structure reveals the left hand core of the inhibitors sitting in between the two central base pairs of the stretched DNA and the right hand core occupying the non-catalytic pocket that opens up between the two Gyr A subunits. The structure indicates that the compound does not directly inhibit the DNA cleavage–religation reaction as it is not bound in the active sites (allosteric inhibition), with some single-strand cleavage. The compound stabilizes a precleavage enzyme–DNA complex and inhibits strand separation. The novel binding mode provides a structural basis for why NBTIs are able to overcome target-mediated fluoroquinolone resistance.

Though obtaining the crystal structure of NBTIs with the mycobacterial gyrase domain has not been successful till date, their overall structural similarity with their antibacterial counterpart's points towards a similar mode of action for mycobacterial class as well.

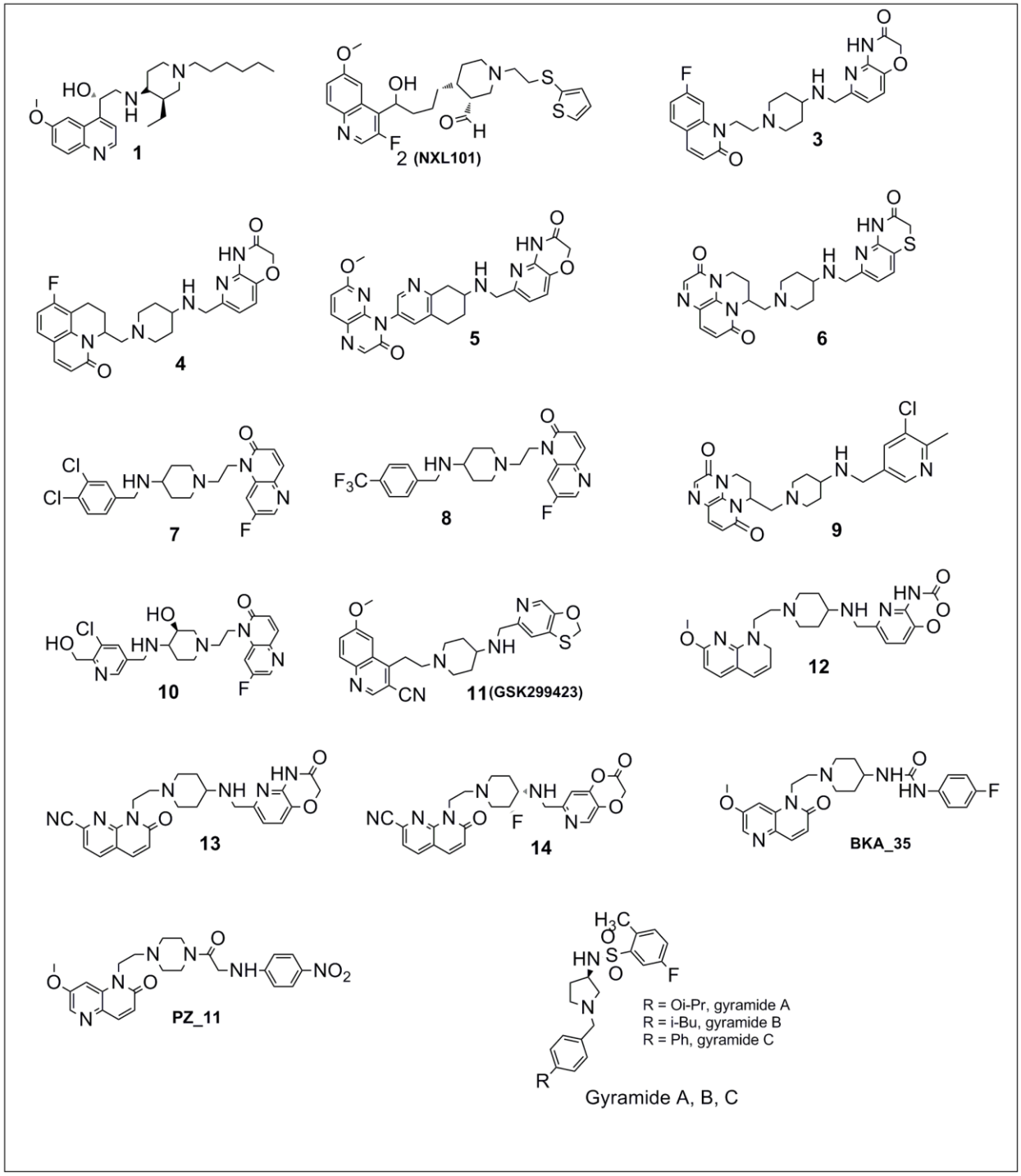


Fig 2.12: Known novel bacterial topoisomerase inhibitors

Compound (**1**) was reported by GSK in 1999 for antibacterial activity [W. J. Coates. *et al*., 1999], but no information regarding mechanism of action. This compound showed MIC of 4 μg/ml against *E. coli* strain. Compound 2 was developed by novexel (NXL101). The compound

2 acted through inhibition of type II topoisomerase (both Topo IV and gyrase) in *E. coli* and *S. pneumoniae*. Compound **2** also showed good activity against FQ-resistant strains of *S. aureus* with known mutations in the quinolone resistance-determining region (QRDR). This indicated that mechanism of inhibition of topoisomerases by compound **2** was very different from that of FQ mechanism [Black, M.T. *et al.*, 2008]. The compound **2** was advanced to Phase I studies but discontinued from the clinical trial due to QT prolongation signals in healthy volunteers (Press release, June 30, 2008; http://www.novexel.com/).

Compounds **3-6** from quinolone, quinoxazolinone and naphthyridone series with bicyclic right hand side (RHS) fragments showed *Mtb* MICs ranging from 0.3 – 2 μg/ml. However there were no data related to *Mtb* gyrase inhibition reported for this set of compounds. Although this was believed to be through *Mtb* gyrase inhibition as they were structurally very close to compound **11**, for which potent inhibition of DNA gyrase in *E. coli* and *S. aureus* with crystal structures reported [Bax, B.D. *et al*., 2010]. Further medicinal chemistry efforts aimed towards improving *Mtb* activity for monocyclic RHS subseries were reported. Compound 7 – 10 is example. There *Mtb* MIC ranges from 0.01-0.3 μg/ml against *Mtb* [Alemparte-Gallardo, C. *et al.*, 2009 and Alemparte-Gallardo, C. *et al*., 2010].

Scientists at AstraZeneca had reported novel N-linked aminopiperidines compound (compounds **12-14**) as potent inhibitors of the bacterial type II topoisomerases with potent anti-bacterial activity [Reck, F. *et al.*, 2011 and 2012]. It was shown that N-linked aminopiperidines with a bicyclic RHS and an optimised linker (compound **15**), showed lesser hERG liability [Reck, F. *et al.*, 2012].

Bobesh, KA. *et a*l., 2014 and 2015 reported N- linked aminopiperidines and piperazine compounds (BKA_35 and PZ_11) as novel bacterial type II topoisomerase inhibitors with potent anti-mycobacterial activity [Bobesh K A., 2014 and 2015]. These compounds are reported as DNA gyrase inhibitors.

2.7. Gyramides

Gyramides are another important class of antibacterial DNA Gyrase inhibitors with promising antibacterial potency, which are in early stage of discovery and believed to act through a mechanism of action similar to that of NBTIs.

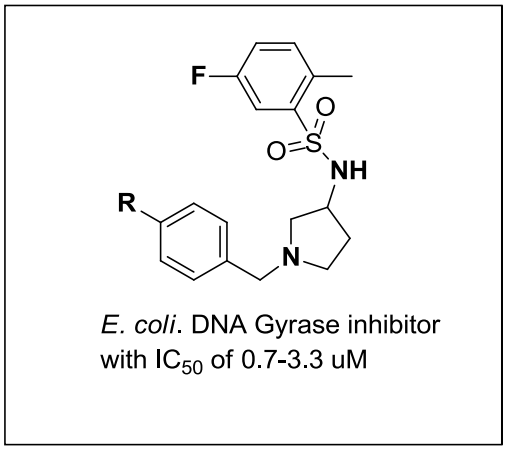


Fig 2.13: *E. coli*. DNA Gyrase inhibitor [Foss M., *et al*.,2009]

Foss M., *et a*l.,2009 reported gyramides as DNA gyrase inhibitors. The gyramides has a very good MIC againist *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, and *Streptococcus pneumonia* [Foss M., *et al*.,2009]. However, it remains to be seen whether they will also exhibit activity towards *Mycobacterium tuberculosis*.

Objectives and Plan of Work

Chapter **3**

3.1. Objective

Even though many drugs are available in the market for treatment of tuberculosis; irrational use of antibiotics, non-compliance with standard regimen led to development of resistance. This serious situation can only be addressed through the development of new anti-tubercular agents. Extensive literature review lead us to DNA Gyrase enzyme as an interesting drug target. We concluded that lot more work can be done in developing better anti-tubercular agents having superior qualities over the existing ones in terms of potency against drug resistant bacteria. The Gyr B portion of *Mtb* was less exploited thus present study focus on developing promising mycobacterial cellular potency achieving through *Mtb* DNA gyrase inhibitors.

The main objectives of the proposed work are:

1. To design novel *Mtb* DNA gyrase inhibitors based on reported anti-tubercular leads.
2. To synthesize and characterize the designed compounds using various synthetic protocols and analytical techniques.
3. To evaluate the inhibitory potency of the synthesized compounds by *in vitro Mtb* DNA Gyr B and supercoiling assay.
4. To undertake *in vitro* anti-mycobacterial screening of the synthesized compounds against *Mtb* by MABA assay.
5. *In vivo* evaluation of anti-mycobacterial potency of the synthesized compounds in *M.marinum* induced Zebra fish model.
6. To determine the efficacy of synthesized compounds against persistent phase of bacteria using dormant culture of *Mtb*
7. To perform the *in vitro* cytotoxicity studies of the synthesized compounds by MTT assay
8. To evaluate the cardiotoxicity zERG channel inhibition assay, and

9. Docking studies (comparision of existing leads and new compounds)

3.2. Plan of work

The plan of work was classified into following categories:

3.2.1. Design of anti-tubercular agents

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

1. *Molecular hybridisation strategy*

2. *Molecular derivatization strategy*

3.2.2. Synthesis and characterization of designed molecules

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

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

3.2.3. *In vitro* DNA gyrase enzyme inhibitory potency

The synthesized compounds were evaluated *in vitro* for their *Msm* DNA Gyr B inhibitory potency and *Mtb* DNA Gyrase supercoiling assay.

3.2.4. *In vitro Mtb* activity studies

All the synthesized compounds were further screened for their *in vitro* antimycobacterial activity against *Mtb* H_{37}Rv (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.

3.2.6. *In vitro* zERG channel inhibition screening

Since most of the previously reported aminopiperidine based DNA gyrase inhibitors showed hERG toxicity, the more potent Gyr B leads were also evaluated for hERG channel inhibition by assessing arrhythmogenic potential on Zebrafish ether-a-go-go-related gene (zERG) which is orthologous to the human ether-a-go-go-related gene (hERG).

3.2.7. Docking studies (Comparison docking study of known to synthesized inhibitors)

Synthesized compounds were subjected to docking studies to develop SAR (Structure activity studies) using commercially available computational software's. The synthesized compounds which were active in Gyr B as well as in supercoiling assay were docked onto the active pocket of the Gyr B ATPase domain of *Mycobacterium smegmatis* (*Msm*) retrieved from protein data bank.

3.2.8. *In vivo* anti-mycobacterial screening using adult zebra fish

The synthesized compounds are evaluated for its *In vivo* studies using adult zebra fish model, against *Mycobacterium marinum* strain (ATCC BAA-535) grown at 30 °C in Middlebrook 7H9 broth. The reduction in bacterial count was evaluated by MPN assay.

3.2.9. Nutrient starvation model

Synthesized compounds are further studied in dormant model of *Mtb* as reported by J. C. Betts et.al. i.e., Nutrient starvation model. The reduction in bacterial count was evaluated by MPN assay.

Materials and Methods

Chapter 4

4.1. Design of the molecules

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

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

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

4.2. Chemistry and methodology

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

4.2.1. Synthesis of the designed molecules

Scheme – 1: Synthesis of 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives as potent Mycobacterium tuberculosis DNA Gyrase inhibitors

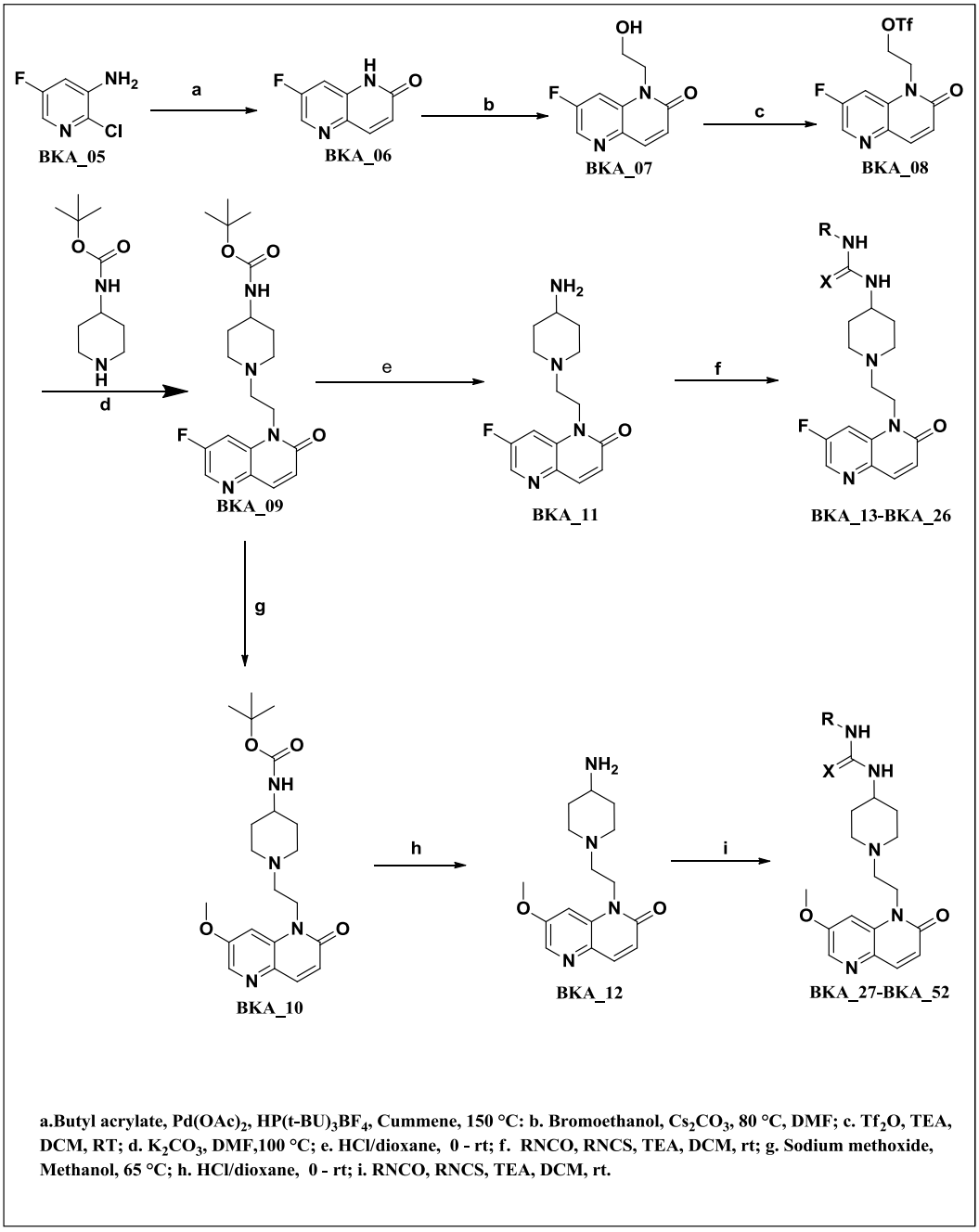


Figure 4.1: Synthetic protocol utilized for the synthesis of compounds **BKA_05 – BKA_52**

Scheme – 2: Synthesis of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives as potent Mycobacterium tuberculosis DNA Gyrase inhibitors

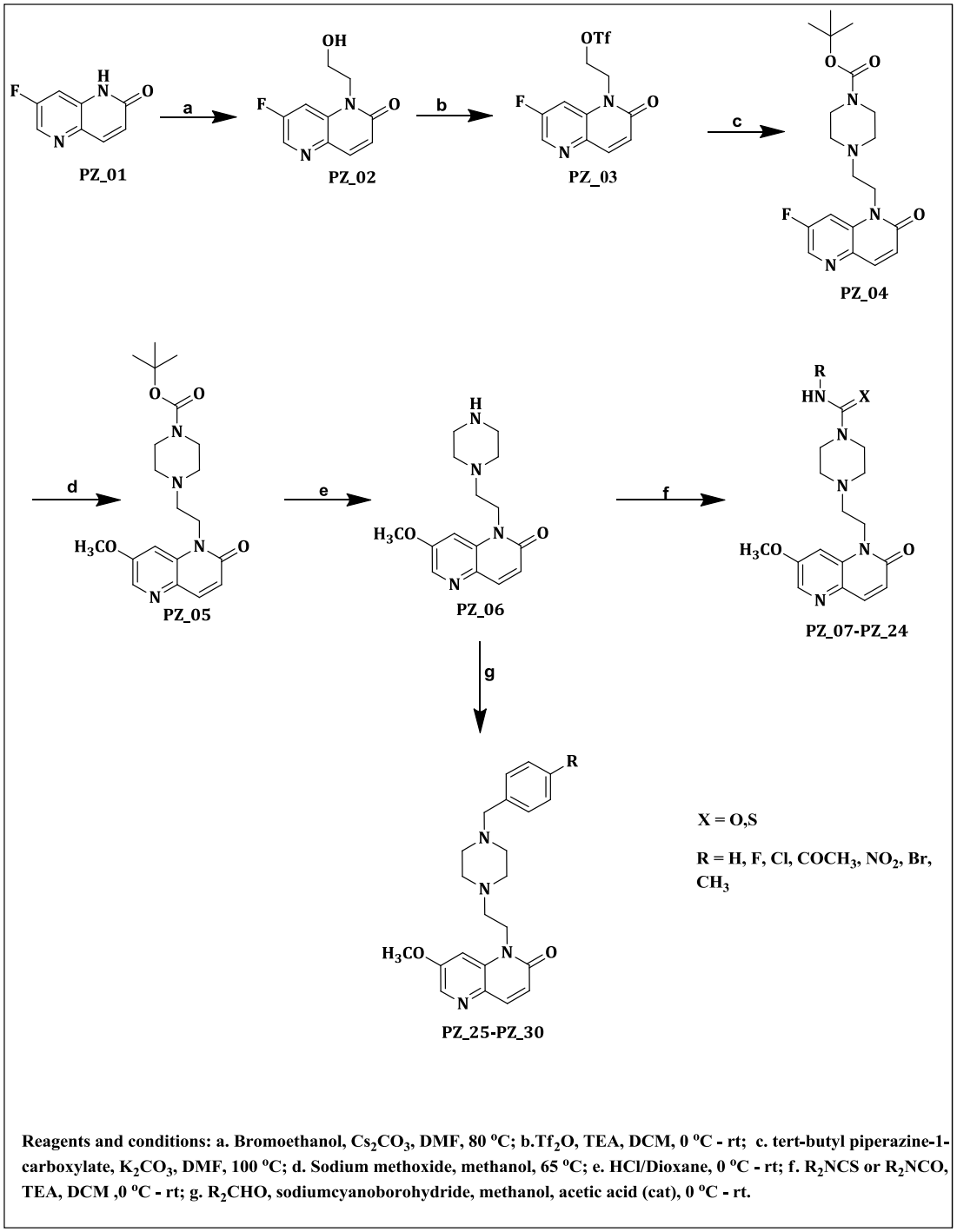


Figure 4.2: Synthetic protocol utilized for the synthesis of molecules **PZ_01 – PZ_30**

Scheme – 3: Synthesis of N-(4-(5-sub-1H-benzo[d]imidazol-2-yl) phenyl)-5-sub-1H-indole-2-carboxamide/(4-(5-sub-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-sub-1H-indol-2-yl)methanone derivatives as novel DNA Gyrase inhibitors

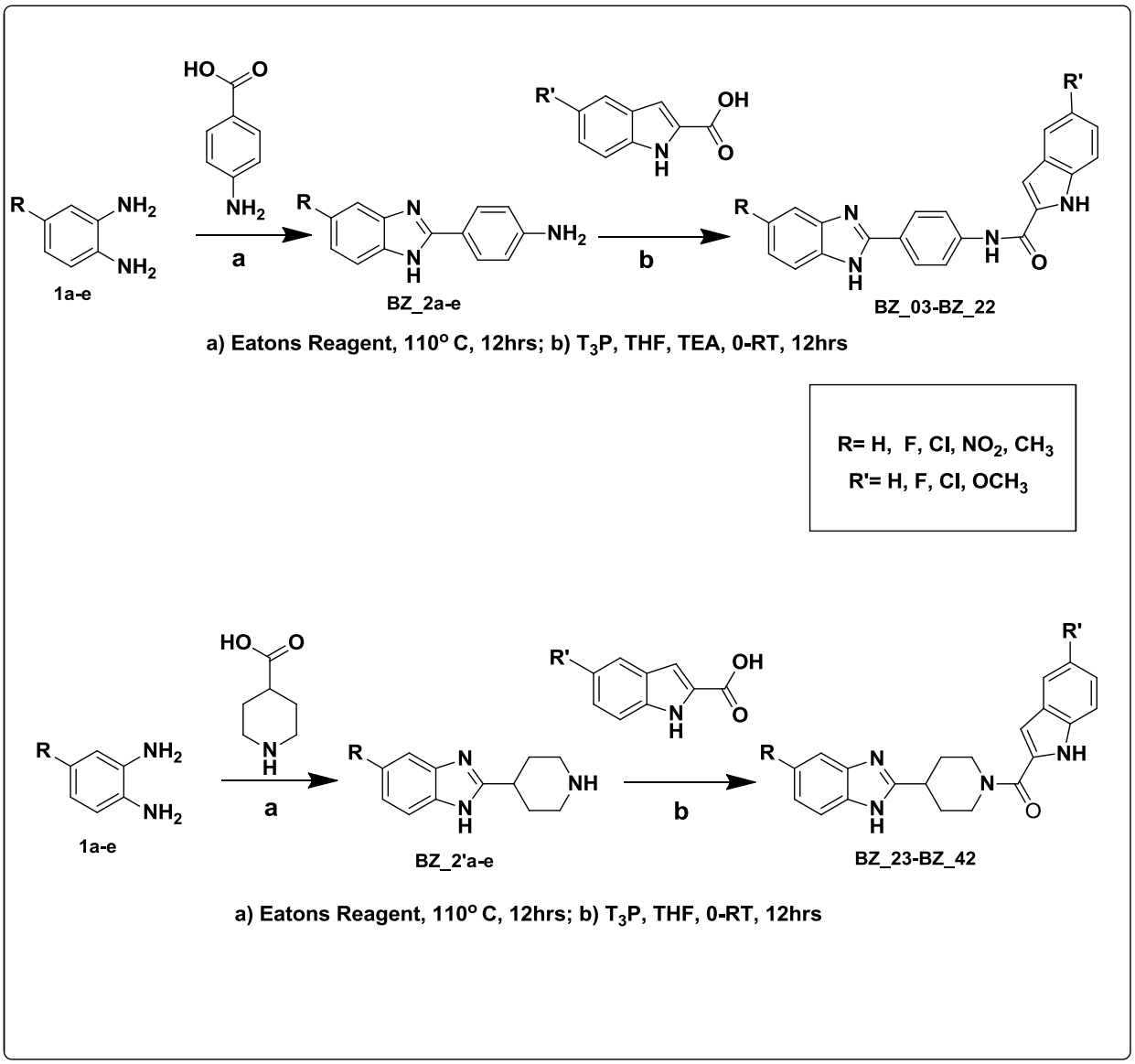


Figure 4.3: Synthetic protocol utilized for the synthesis of molecules **BZ_01a – BZ_42**

Scheme – 4: Synthesis of 1-(7-chloroquinolin-4-yl)piperidin-4-amine derivatives as novel DNA Gyrase inhibitors

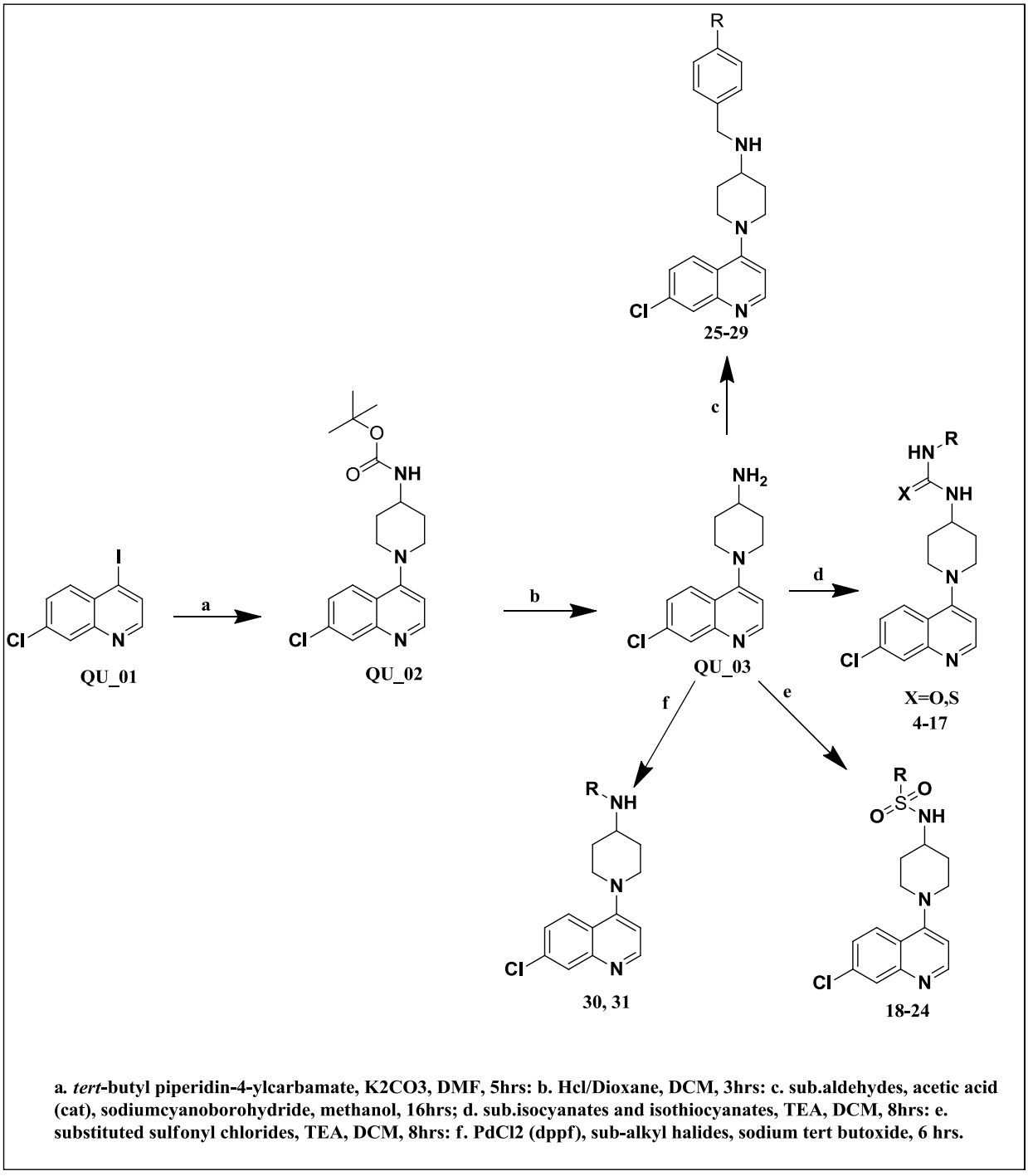


Figure 4.4: Synthetic protocol utilized for the synthesis of molecules **QU_01 – QU_31**

Scheme – 5: Synthesis of of 2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-phenylacetamide derivatives as potent Mycobacterium tuberculosis DNA Gyrase inhibitors

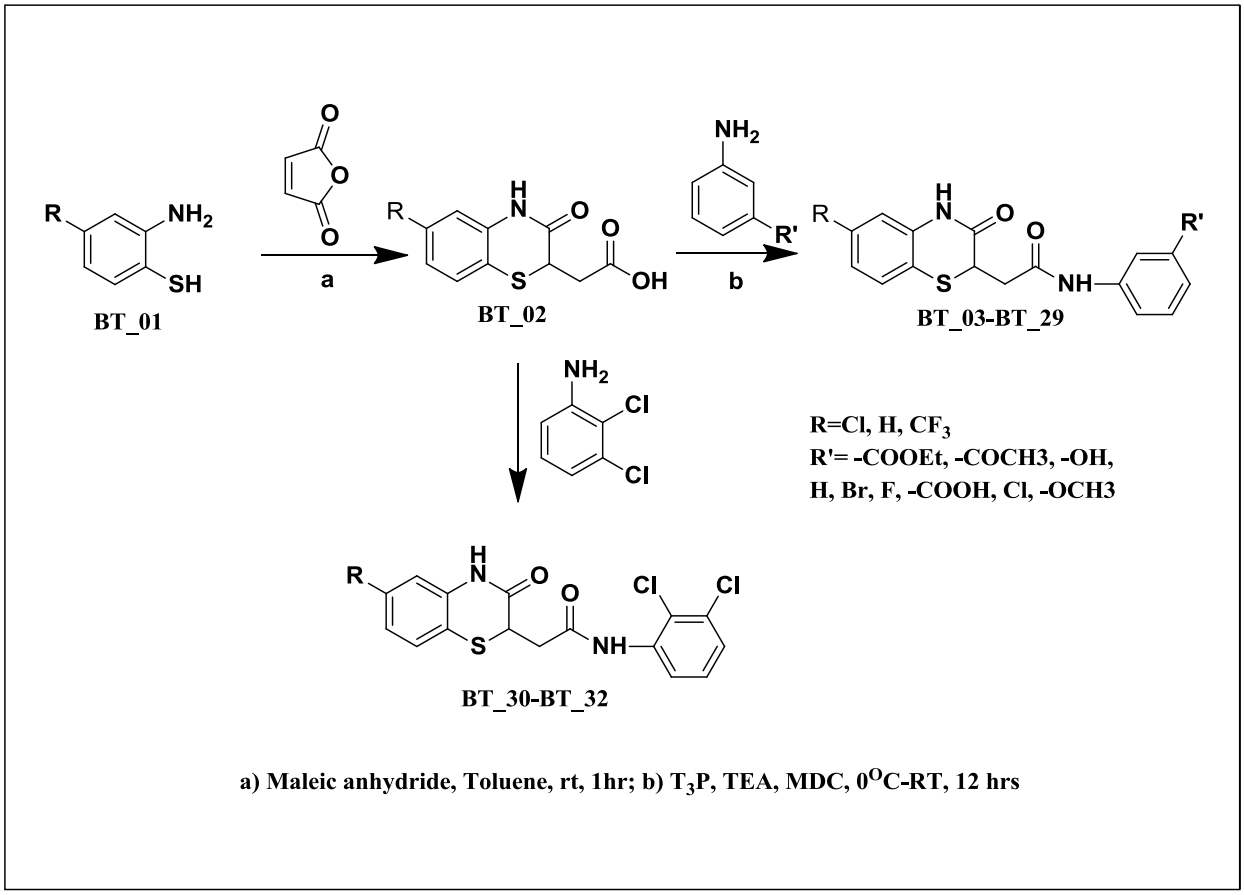
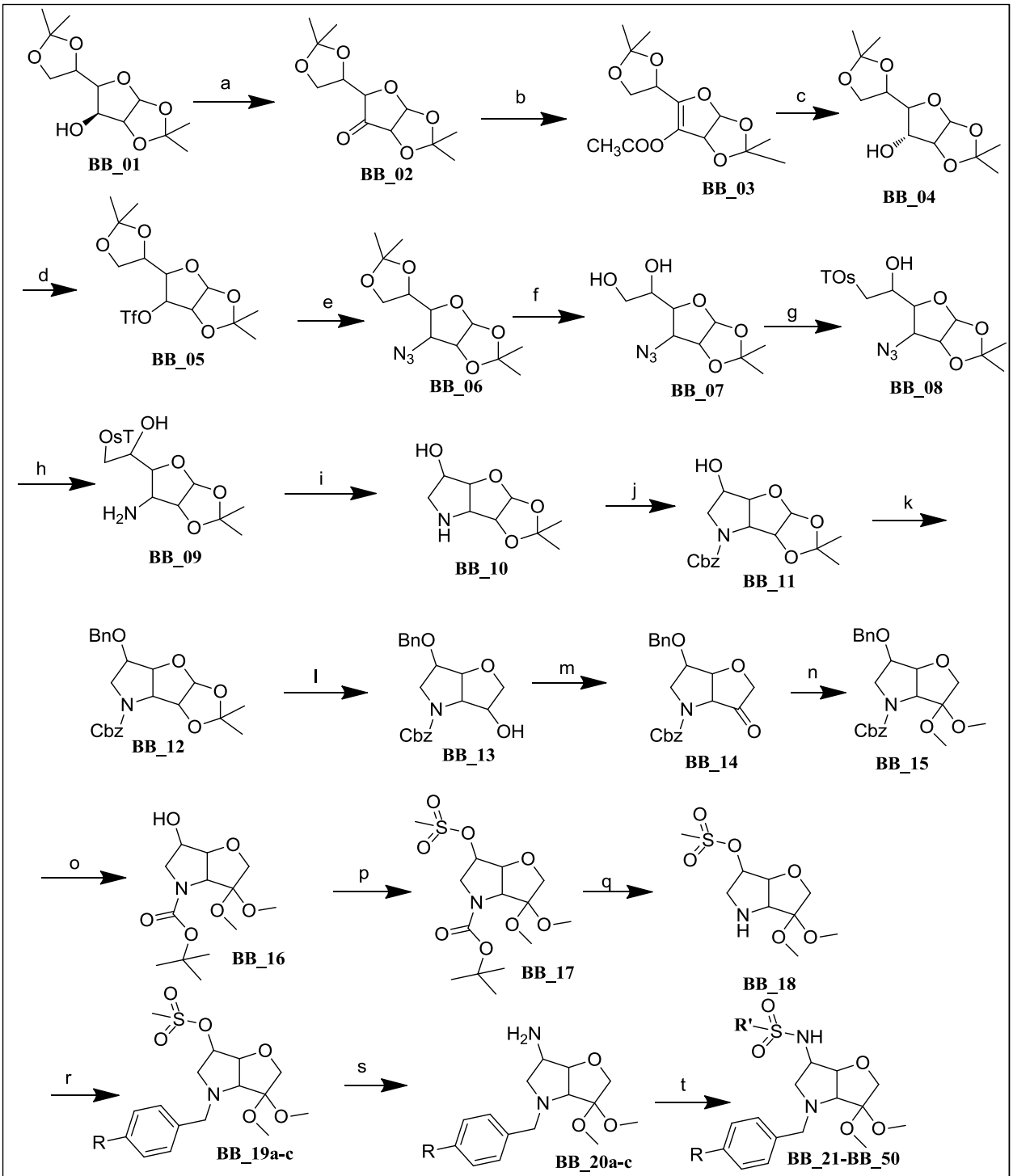


Figure 4.5: Synthetic protocol utilized for the synthesis of molecules **BT_01a – BT_32**

Scheme – 6: Synthesis of N-(4-sub-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-sub-sulfonamide derivatives as novel DNA Gyrase inhibitors



a. PCC, CH2Cl2, molecular sieve, 1hr; b. pyridine, Ac2O, 12hrs; c. Pd black, H2, EtOAc, 7hrs; then K2CO3 in MeOH; d. Tf2O,pyridine, DCM, 15mts; e. NaN3, DMF, 2hrs; f. aq. acetic acid, 2.5hrs; g. p-toluenesulfonyl chloride, pyridine, DCM, 4hrs; h. H2, Pd/C, methanol, 6hrs; i. TEA, MeOH, H2O, then Et3N, 50°C, 16hrs; j. benzylchloroformate, pyridine, DCM, 8hrs; k. benzyl bromide, sodium hydride, DMF, 1hr; l. BF3.Et2O, Et2SiH, DCM, 3days; m. Dess-Martin Periodinane, DCM, 2hrs; n. AcCl, MeOH, 16hrs; o. H2, Pd/C, 6hrs Boc20, TEA, 12hrs; p. MsCl, TEA, DCM, 14hrs; q. DCM, TFA, 3hrs; r. Sub.Benzaldehyd, NaCNBH3, acetic acid (cat), MeOH, 16hrs; s.NH3 solution, microwave, 12hrs; t. sub sulphonyl chlorides, TEA, DCM, 6hrs.

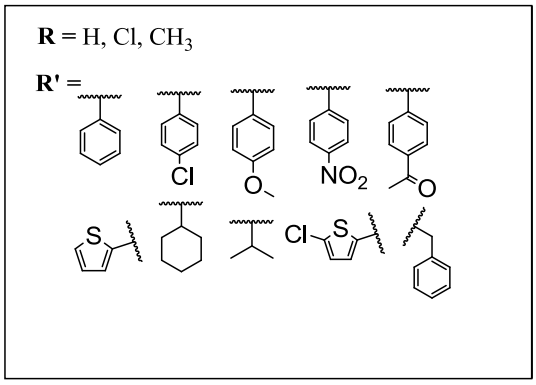


Figure 4.6: Synthetic protocol utilized for the synthesis of molecules **BB_01 – BB_50**

4.3. Biological screening of synthesized inhibitors

4.3.1. *Mycobacterium smegmatis* (*Msm*) DNA Gyr B 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 *Msm* DNA Gyr B was performed by amplifying the gene from mc2155 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), 500 mM NaCl, 2 mM KCl, 1.3 mM K_2HPO_4, 10 mM Na_2HPO_4, 5% Glycerol, 1 mM DTT. Protein was eluted with 25 mM Tris-HCl (pH 8), 140 mM NaCl, 5% Glycerol, 1 mM DTT, and 1 mM 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, 140 mM NaCl, 2 mM dithiothreitol, 15% glycerol, 1 mM 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 (1 mm, 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, 1 mM boric acid, 1 mM 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 Msm* Gyr B assay for the determination of IC_{50}

The *in vitro* ATPase assay was performed by *Msm* DNA Gyr B subunit. As the assay does not involve any substrate it is called as DNA independent assay. Gyr B 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 60 mM HEPES-KOH (pH ~ 7.7), 200 mM KCl, 250 mM potassium glutamate, 2 mM $MgCl_2$, 1 mM DTT, 2% Glycerol, 4% DMSO, 0.001% BriJ-35, 0.65 mM ATP, 40 nM Gyr B. 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 Mtb* DNA gyrase supercoiling assay

DNA supercoiling assay was performed using gyrase of *Mtb* 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 *Mtb* 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), 10 mM magnesium acetate, 10 mM DTT, 2 mM ATP, 500 mM 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, 100 mM Tris-HCl (pH ~ 7.5), 1 mM 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 (40 mM Tris acetate, 2 mM 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 densitometer and NIH image through Bio-Rad GelDoc image viewer.

4.3.4. *In vitro Mtb* MABA assay for MIC determination

MABA assay was performed to check the MIC of the *Mtb* 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 µL was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested [Franzblau, S. G., *et al*., 1998, 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 µL 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all 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, 50 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. Nutrient starvation model

Mycobacterium tuberculosis nutrient starved culture was prepared using nutrient starvation method [Betts, J. C., et al 2002]. In nutrient starvation model, culture of *Mycobacterium tuberculosis* H37Rv (NIRT, Chennai) grown in Middlebrook 7H9 medium supplemented with OADC(nutrient rich medium) was pelleted and washed twice with PBS(Phosphate Buffer Saline, obtained from HiMedia Laboratories). The pellet was resuspended in PBS in sealed bottles and was incubated at 37 °C for 6 weeks. These cultures were treated with standard drugs like isoniazid, rifampicin and moxifloxacin along with the synthesized compounds for 7 days at a concentration of 10 µg/ml.The treated cell suspensions were diluted 10-fold up to 10^{-6} using Middlebrook 7H9 medium supplemented with OADC and 100 µl of each dilutions were plated in 48 well plates in triplicates along with 450 µl of Middlebrook 7H9 medium (HiMedia Laboratories) supplemented with OADC (HiMedia Laboratories). The plates were incubated at 37 °C for 3-4 weeks the wells with visible bacterial growth were counted as positive. The bacterial count was down by using standard statistical methods using MPN assay.

4.3.6. Anti-mycobacterial screening using adult zebra fish

The most active compound was further evaluated for its in-vivo activity using adult zebrafish model established by us in a laboratory setup [Sridevi, J. P., *et al*., 2014]. We used

Mycobacterium marinum strain (ATCC BAA-535) grown at30 °C in Middlebrook 7H9 broth. Fish were initially weighed and monitored for its locomotor activities and were divided into control and treatment groups (n=6). All the fish were injected by intraperitonial injection with 20 µl of thawed bacterial stocks (around 0.75 million bacteria) [De Man, J. C., *et al*., 1975]. They were observed for lesions, reduction in swimming activities and squamous eruptions in the initial 7 day infection stage which was followed by treatment stage. The drug solutions were prepared based on the fish's body weight and oral dosing amount of 5 µL. Fish were then administered drug orally using micropipette, on each day during the treatment phase and were noted for their recovery symptoms. They were allowed to swim in 1.5mg/mL solution of kanamycin sulphate before proceeding for sacrifice at the end of study i.e., 14th day. Finally, all of them were sacrificed using homogenization technique and the tissue sample was prepared in Middlebrook 7H9 broth [Salina, E., *et al*., 2014]. The collected homogenate was serially diluted to 10^{-6} times and plated into 48-well plates, incubated at 30 °C for 24 h. The plates were checked for the bacterial counts using graph pad prism software.

4.3.7. Cell cytotoxicity studies by MTT assay

As the *Mtb* organism targets the macrophage cell lines, the toxicity studies were performed in the RAW cell lines [Ferrari, M., *et al*., 1990]. Cytotoxic safety profiling of all the test compounds was done on RAW 264.7 mouse leukemic monocyte macrophage cell line from ATCC (Gerlier, D., *et al*., 1986, 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-2H-tetrazolium bromide) into formazan crystals using Perkin Elmer Victor X3 Titre 96 plate reader at 590 and 620 nm. Ciprofloxacin (3% inhibition) and novobiocin (9.8% inhibition) were used as a standards in this assay. The percentage inhibition was calculated from the following formula:

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

4.3.8. Evaluation of zERG channel inhibition in a zebra fish model

The most potent compounds were evaluated for zERG toxicity inhibition by assessing arrhythmogenic potential on Zebrafish ether-a-go-go-related gene (zERG) which was orthologous to the human ether-a-go-go-related gene (hERG). Zebrafish acquired commercially were maintained as described earlier (Pushkar K., *et al*., 2014; Langheinrich, U., *et al*., 2003). In brief, zebrafish were maintained in the recirculatory system with 14 h light and 10 h dark cycles, with 28˚C as optimum temperature. Breeding, embryo collection and drug exposure was carried out as described previously [Mittelstadt, S. W., *et al*., 2008, Milan, D. J., *et al*., 2003]. Briefly, breeding was carried out using 2 females: 3 males in a separate breeding cage. Embryos were collected into petridishes containing E3 medium and incubated at 28˚C temperature. Three day post fertilized embryos were used for screening cardiotoxic potential of test drugs. The embryos were segregated and washed and distributed in 24-well plate i.e. six embryos with 250 µL of 0.1% DMSO per well. Stock solution of the drug was prepared in 100% DMSO and the 2X working concentrations were prepared accordingly by serial dilutions. Each well was then added with 250 µL of 2X working concentration and embryos were allowed to incubate at the optimum temperature for 4 h. After 4 h of incubation, the respective drug concentration exposed embryos were treated with tricaine and the heart rate was measured. The time taken for 30 atrial and ventricular beats was measured for each embryo, from which number of heart beats per minute was calculated by using the formula 1800/X = beats /minute (where X = time in seconds) (Ram Shankar U., *et al*., 2010). The most active compounds were exposed from 1 µM to 30 µM concentrations with 0.1% DMSO as vehicle. All the statistical analysis was done using GraphPad Prism software applying one way ANOVA and Dunnett's test.

4.4. Molecular docking studies

All computations were carried out on an Intel Core 2 Duo 63 E7400 2.80 GHz capacity processor with memory of 2 GB RAM 64 running with the RHEL 5.2 operating system. The synthesized compounds which were active in Gyr B as well as in supercoiling assay were docked onto the active pocket of the Gyr B ATPase domain of *M. smegmatis* retrieved from

protein data bank (PDB ID: 4B6C). The protein was initially processed using the Protein Preparation Wizard of Schrödinger Suite 2012. The optimization of the hydrogen-bonding network and the ligands to be docked were sketched in Maestro panel of Schrödinger and optimized with OPLS force field. The virtual screening options for HTVS (High Throughput Virtual Screening), SP (Standard Precision) and Glide XP (extra precision) docking were all checked to be executed. Glide XP (extra precision) module of Schrödinger 9.3 (Maestro version 9.3, Glide version 5.7, Schrödinger, LLC, New York, NY, 81 2012) was utilized for docking.

Results and Discussion *Chapter* 5

5.1. Design, synthesis and biological evaluation of 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives as potent *Mycobacterium tuberculosis* DNA Gyrase inhibitors

5.1.1. Design of the molecules

Bacterial DNA gyrase is a well-established and clinically validated target to develop novel antibacterials. Our effort was designated to search for synthetically better compounds with possibility of hit to lead development. With this as objective, a series of 1-(2-(4-aminopiperidin-1-yl) ethyl)-1, 5-naphthyridin-2(1H)-one derivatives were designed by molecular hybridization strategy. With target-based/phenotypic screening approaches offering few tangible successes in discovering novel antitubercular drugs, the concept of molecular hybridization could be of significant use to generate newer scaffold as potential antimycobacterial leads [Hameed P, S., *et al.*, 2014]. Molecular hybridization approach is an emerging structural modification tool involving adequate fusion of the two or more pharmacophoric units derived from previously reported leads/drugs in the design of new hybrid architecture that could maintain preselected characteristics of the original template [do Couto Maia, R., et al., 2010].We therefore envisaged that re-engineering the previously reported NBTIs could deliver a new scaffold/lead with better antimycobacterial activity *via* inhibition of the gyrase domain.

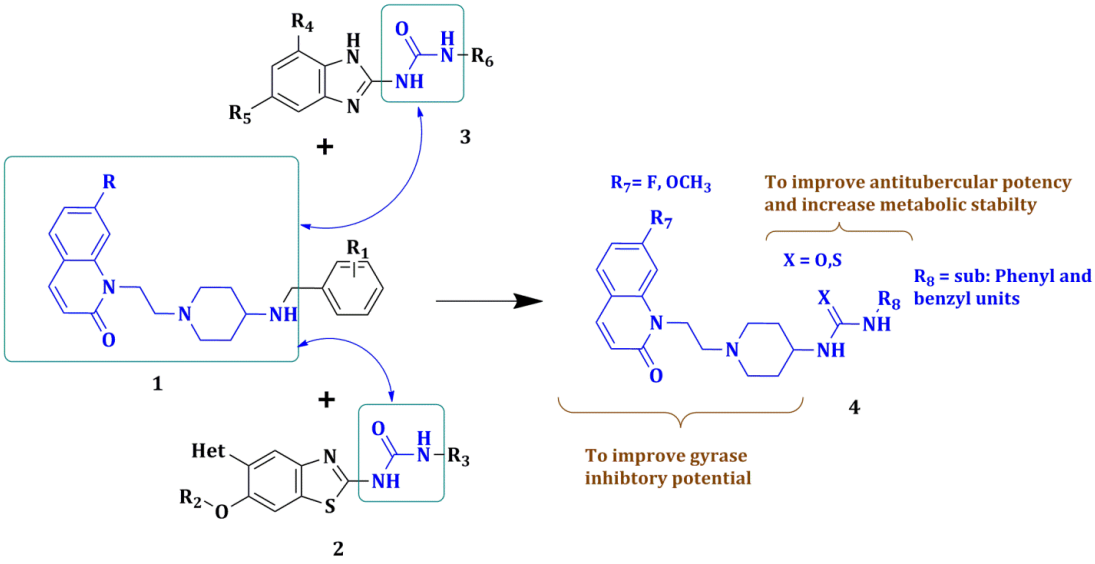


Figure 5.1: Strategy employed for designing the lead. Chemical structure of previously reported synthetic inhibitors of DNA gyrase bearing1,5-naphthyridin-2(1H)-one core (1), carbamide side chain (2-3) and the inhibitor designed through molecular hybridization (4).

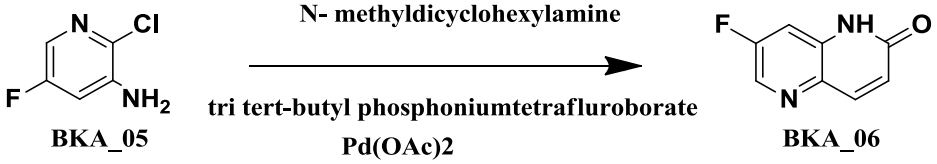
5.1.2. Experimental procedures utilized for the synthesis of BKA_05 – BKA_52

The design strategy utilized for developing the inhibitor has been sketched in **Fig 5.1**. It was decided to retain the 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one scaffold in our initial structure-activity exploration as it was understood to be an important requisite in retaining the gyrase inhibitory potential [Jeankumar, V. U., *et al*., 2014]. Since fluoro and methoxy groups at 7th position of the 1,5-naphthyridin-2(1H)-one core significantly improved the gyrase inhibition of the previously reported antibacterial NBTIs, these were also retained in our studies [Widdowson, K., *et al*., 2010]. Various carbamide/thiocarbamide derivatives were introduced as right hand core to increase stability and also to evaluate the steric and electronic effects on the anti-mycobacterial potency.

Synthesis of the compounds started with the construction of 7-fluoro1-5-naphthyridin-2(1H)-one via a Heck coupling reaction of 2-chloro-5-fluoropyridin-3-amine (**BKA_5**) with butyl acrylate. Though various literatures have explored a variety of conditions and reagents to afford the Heck product, the protocol reported by Voightet al was the most beneficial as it underwent an in-situ cyclisation of the so obtained Heck product butyl 3-(3-amino-5-fluoropyridin-2-

yl)acrylate to give the desired 7-fluoro1,5-naphthyridin-2(1H)-one (**BKA_6**) in good yield [Voight, E. A., et al., 2010]. The ethyl bridge that connected the naphthyridinone core to the aminopiperdine linker was introduced at N-1 position by alkylating the so obtained 7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_6**) with bromoethanol in presence of Cs_2CO_3.Small amounts of the O-alkylated product obtained were removed by column chromatography. Compound **BKA_7** on further treatment with trifluoromethanesulfonic anhydride and pyridine afforded the corresponding triflate (**BKA_8**) in good yield. This was further condensed with 4-N-Boc-aminopiperidine via a SnAr displacement to obtain the nitrogen-linked analog (**BKA_9**). Subsequent Boc deprotection afforded the scaffold **BKA_11** in good yields. Concordantly, the so obtained tert-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**BKA_9**) was also treated with sodium methoxide in methanol under reflux to introduce the methoxy substituent at the 7th position of 1,5-naphthyridin-2(1H)-one core via nucleophilic displacement of the fluoro group. This was subsequently subjected to Boc de protection in a similar fashion to the fluoro analogue to give the desired product (**BKA_12**). The final library was then assembled by treating the obtained scaffolds **BKA_11** and **BKA_12** with the desired isocyanates/isothiocyantes to afford compounds **BKA_13 – BKA_52** in excellent yields.

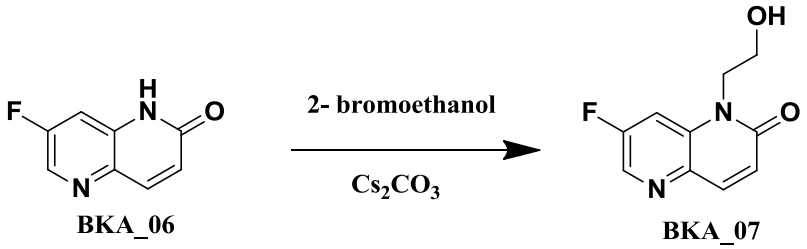
Preparation of 2-chloro-5-fluoropyridin-3-amine (BKA_06)



To a stirred mixture of substituted 2-chloro-5-fluoropyridin-3-amine **(BKA_05)** (2 g, 13.65 mmol) and butyl acrylate (2.09 g, 16.38 mmol) in cumene (10 mL) at room temperature, was added N- methyldicyclohexylamine (7.99 g, 40.94 mmol), followed by catalytic amounts of tri *tert*-butyl phosphoniumtetrafluroborate (0.158 g, 0.546 mmol). This reaction mixture was purged for 5 min with argon followed by the addition of $Pd(OAc)_2$ (0.061g, 0.273 mmol). The reaction mixture was heated at 150 °C for 4 hours (monitored by TLC & LCMS for completion), and the reaction mixture was filtered through celite bed and solvent was evaporated under reduced pressure. The reaction mixture was further extracted with ethyl

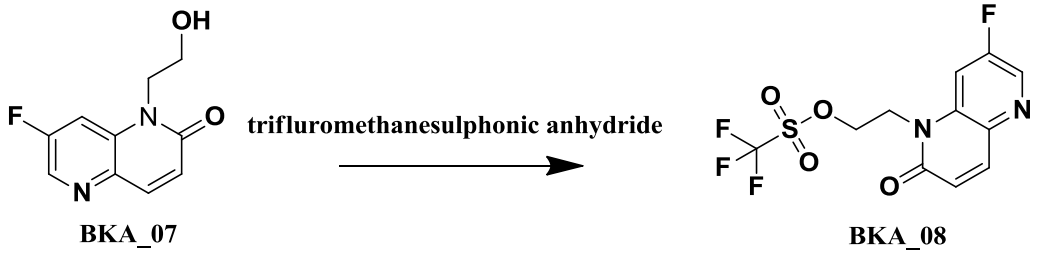
acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give 7-fluoro-1, 5-naphthyridin-2(1H)-one (**BKA_06**) (1.23 g, 53.6 %) as brown solid. M.p: 282-284°C. ^{1}H NMR [300 MHz, DMSO-d_6] δ_H 12.31 (s, 1H), 8.21 – 6.95 (m, 4H).^{13}C NMR [100 MHz, DMSO-d_6] δ_c: 163.5, 157.9, 142.8, 140.5, 132.3, 127.1, 123.2, 110.2 ESI-MS *m/z* 165 $(M+H)^+$. Anal Calcd for $C_8H_5FN_2O$: C, 58.54; H, 3.07; N, 17.07; Found: C, 58.58; H, 3.12; N, 17.03.

Preparation of 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (BKA_07)



To a solution corresponding 7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_06**)(1g , 6.09 mmol) in dry N,N' dimethyl formamide (10 mL) was added Cs_2CO_3 (4.95 g, 15.23 mmol) and followed by 2- bromoethanol (0.913 g, 7.31 mmol) and heated in sealed tube at 120 °C for 4 h (monitored by TLC & LCMS for completion). The reaction mixture was then filtered through celite and washed with dichloromethane. The filtrate was concentrated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (**BKA_07**) (0.953 g, 75.6 %) as pale yellow solid. M.p: 241-243 °C. ^{1}H NMR [300 MHz, DMSO-d_6] δ_H 8.14 – 6.52 (m, 4H), 3.74 – 3.47 (m, 5H). ^{13}C NMR [DMSO-d_6] δ_C: 162.3, 157.8, 147.5, 140.1, 132.3, 125.6, 123.2, 110.1, 64.5, 46.2. ESI-MS *m/z* 209 $(M+H)^+$. Anal Calcd for $C_{10}H_9FN_2O_2$: C, 57.69; H, 4.36; N, 13.46; Found: C, 57.72; H, 4.38; N, 13.45.

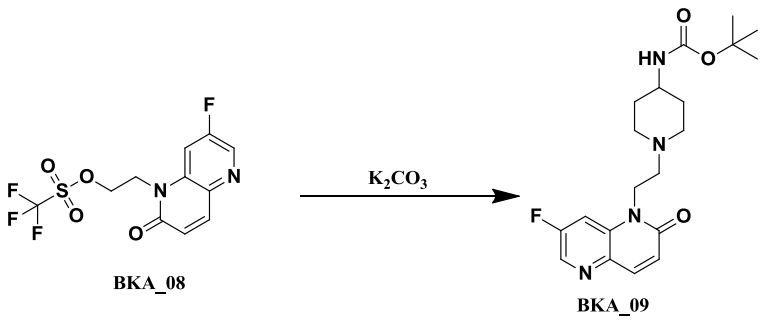
Preparation of 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl trifluoromethanesulfonate (BKA_08)



BKA_07 **BKA_08**

A solution of 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (**BKA_07**) (1 g, 4.80 mmol**)** in dichloromethane (10 mL) and pyridine (1.45 g, 14.41 mmol)was cooled to -20 ^{0}C. To this was added a solution of trifluromethanesulphonic anhydride (1.49. g, 5.28 mmol) in dichloromethane (10 mL) drop wise. The resultant reaction mixture was stirred at the same temperature for half an hour. Completion of the reaction was confirmed by TLC. Reaction mixture was then warmed to room temperature and given sodium bicarbonate (3 × 20 mL)**,** water (3 × 20 mL) and brine (3 × 20 mL) washes. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to get a brownish thick liquid**.** The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl) ethyltrifluoromethanesulfonate (**BKA_08**) (01.23 g, 73.6 %) as Yellow oil. ^{1}H NMR [DMSO-d_6]: δ_H 8.23 – 6.95 (m, 4H), 3.81–3.64 (m, 4H). ^{13}C NMR [DMSO-d_6] δ: 162.8, 157.8, 145.2, 140.2, 132.9, 125.6, 123.5, 119.2, 110.1, 61.2, 42.5. ESI-MS *m/z:* 341 $(M+H)^+$. Anal Calcd for $C_{11}H_8F_4N_2O_4S$: C, 38.83; H, 2.37; N, 8.23; Found: C, 38.85; H, 2.35; N, 8.27.

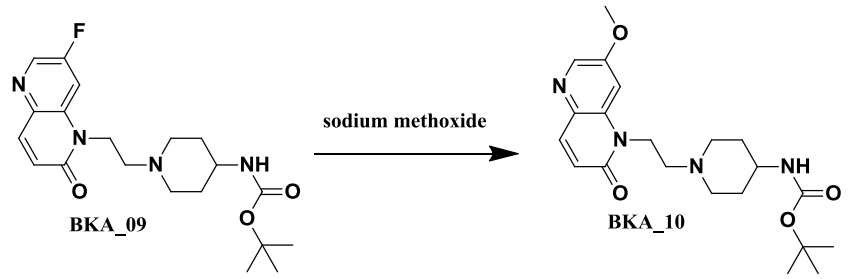
Preparation of *tert*-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (BKA_**09).**



BKA_08 **BKA_09**

To a solution of 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl trifluoromethane sulfonate (**BKA_08**) (1 g, 2.93 mmol) in dry N,N' dimethyl formamide (10 mL) was added K_2CO_3 (1.22 g, 8.82 mmol) and followed by *tert*-butyl piperidin-4-ylcarbamate (0.706 g, 3.52 mmol) and was heated in sealed tube at 100 ^{0}C for 4 h (monitored by TLC & LCMS for completion). The reaction mixture was then filtered through celite and washed with dichloromethane. The filtrate was concentrated under reduced pressure.The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried (MgSO4) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give *tert*-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**BKA_09**) (0.724 g, 63.2 %) as pale yellow solid. M.p: 210-212^{0}C. ^{1}H NMR [DMSO-d_6]: δ_H 10.32 (s, 1H), 8.21 – 6.73 (m, 4H), 4.63–1.39 (m, 13H), 1.38 (s, 9H). ^{13}C NMR [DMSO-d_6] δ: 162.3, 157.8, 156.2, 146.2, 140.1, 132.5, 125.6, 123.1, 110.2, 80.2, 55.2, 52.3 (2C), 50.2, 49.5, 30.2 (2C), 27.6 (3C).ESI-MS *m/z:* 391 $(M+H)^+$. Anal Calcd for $C_{20}H_{27}FN_4O_3$: C, 61.52; H, 6.97; N, 14.35; Found: C, 61.55; H, 6.93; N, 14.37.

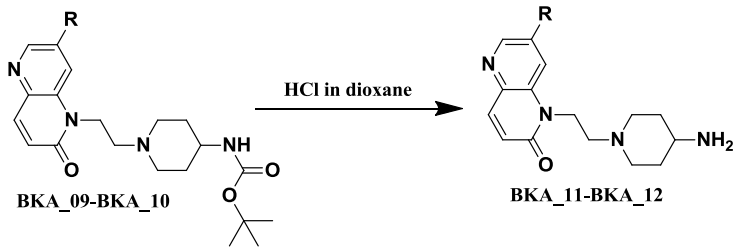
Preparation of *tert*-butyl(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (BKA_10)



A solution of corresponding *tert*-butyl (1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**BKA_09**) (2.0 g, 5.12 mmol) in methanol (10 mL) at room temperature was added sodium methoxide (0.553 g, 10.2 mmol). The reaction mixture was heated at 65 °C for 3h (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the

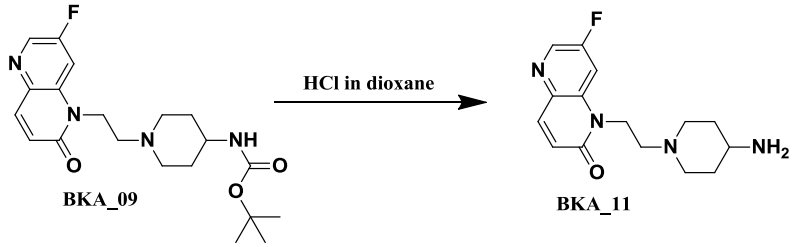
corresponding *tert*-butyl (1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**BKA_10**) (1.35 g, 65.5 %) as pale yellow solid. M.p: 232-234 °C. ^{1}H NMR [DMSO-d_6]: 10.32(s, 1H), 8.21 – 6.73 (m, 4H), 4.63–1.39 (m, 16H), 1.38 (s, 9H). ^{13}C NMR [DMSO-d_6] δ_c: 161.5, 156.7, 155.2, 146.2, 136.1, 132.5, 125.6, 119.1, 105.2, 80.3, 56.2, 55.2, 52.3 (2C), 50.2, 49.8, 30.2 (2C), 27.6 (3C). ESI-MS *m/z:* 403 $(M+H)^+$. Anal Calcd for $C_{21}H_{30}N_4O_4$: C, 62.67; H, 7.51; N, 13.92; Found: C, 62.61; H, 7.56; N, 13.90.

General procedure for synthesis of (BKA_11-BKA_12)



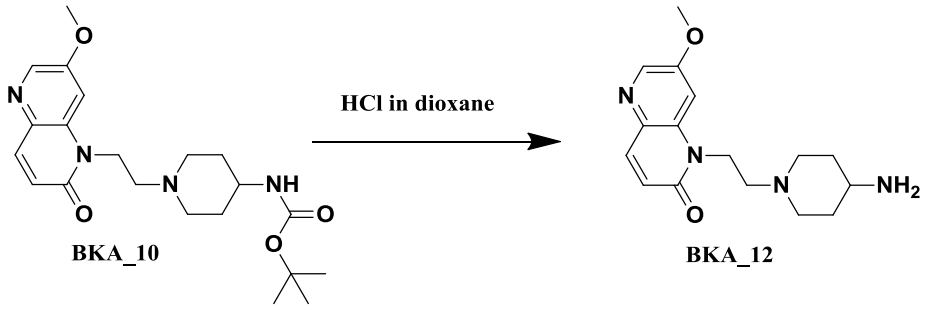
A solution of corresponding substituted tert-butyl (1-(2-(2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate(**BKA_9-BKA_10**) (1 eq) in dichloromethane (10 mL) was cooled to 0 ^{0}C followed by drop wise addition of HCl in dioxane (2 mL) and stirred for 1 hour. After completion of the reaction (monitored by TLC & LCMS), quenched with ice and concentrated under reduced pressure. The residue was partitioned between ethyl acetate (20 mL) and water (20 mL). Aqueous layer was basified with sodium carbonate solution and extracted with ethyl acetate (3 × 20 mL) and washed with water (3 × 10 mL) and brine (3 × 10 mL). Organic layer was dried over anhydrous sodium sulphate, filtered, and concentrated under reduced pressure to give the corresponding 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**BKA_11-BKA_12**) in good yield.

Preparation of 1-(2-(4-Aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (BKA_11).



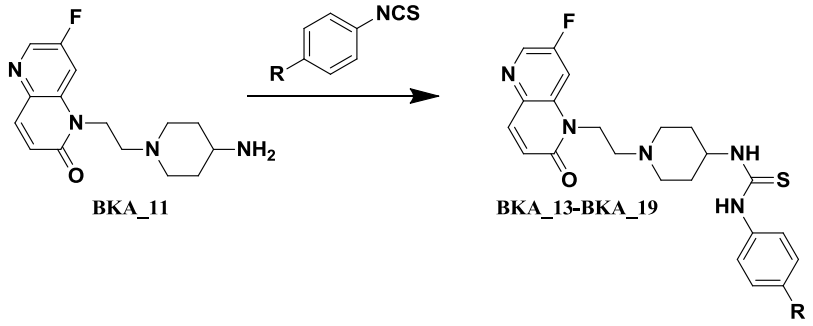
The compound was synthesized according to the general procedure described above by using tert-butyl(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**BKA_09**) (2.0 g, 5.12 mmol) and HCl in dioxane (10 mL) to afford **BKA_11** (1.25 g, 84.5 %) as pale yellow solid. M.p: 232-234 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.26 – 6.72 (m, 4H), 6.56 (s, 2H), 4.35–1.38 (m, 13H). ^{13}C NMR [DMSO-d_6] δ: 162.8, 155.9, 145.6, 140.2, 132.9, 125.1, 123.4, 109.7, 55.2, 52.6 (2C), 50.3, 45.2, 33.5 (2C). ESI-MS *m/z:* 291 $(M+H)^+$. Anal Calcd for $C_{15}H_{19}FN4O$: C, 62.05; H, 6.60; N, 19.30; Found: C, 62.01; H, 6.64; N, 19.31.

Preparation of 1-(2-(4-Aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (BKA_12).



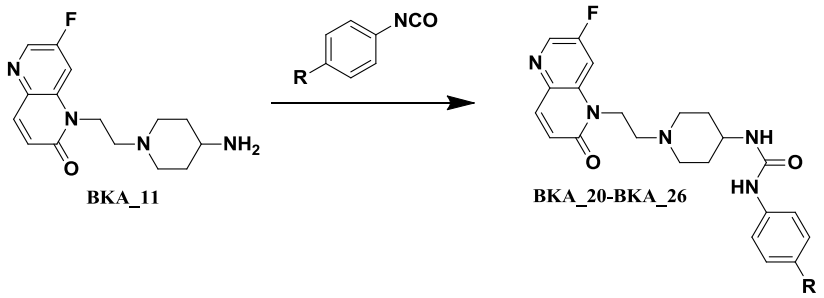
The compound was synthesized according to the general procedure by utilizing *tert*-butyl (1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)carbamate (**BKA_10**) (2.0 g, 4.97 mmol) and HCl in dioxane (10 mL) to afford **BKA_12** (1.35 g, 88 %) as Yellow solid. M.p: 225-227 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.11 – 6.63 (m, 4H), 6.42 (s, 2H), 4.48 (t, *J*=7.2 Hz, 2H), 3.92 (s, 3H), 3.65–1.36 (m, 11H) .^{13}C NMR [DMSO-d_6] δ: 162.3, 155.6, 145.7, 136.2, 132.8, 125.4, 119.2, 107.2, 56.2, 54.5, 52.3 (2C), 58.5, 46.2, 33.2 (2C) ESI-MS *m/z:* 303 $(M+H)^+$. Anal Calcd for $C_{16}H_{22}N4O_2$: C, 63.55; H, 7.33; N, 18.53; Found: C, 63.58; H, 7.31; N, 18.55.

General procedure for the synthesis of final molecules (BKA_13–BKA_19)



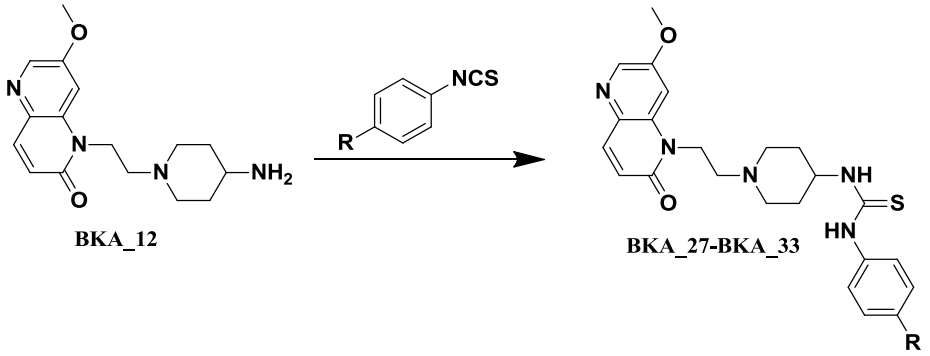
A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one **(BKA_11)** (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 °C followed by the addition of triethylamine (1.03 mmol). Corresponding substituted phenyl isothiocyanate was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was then washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted 1-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**BKA_13-BKA_19**) in good yields.

General procedure for the synthesis of final molecules (BKA_20–BKA_26)



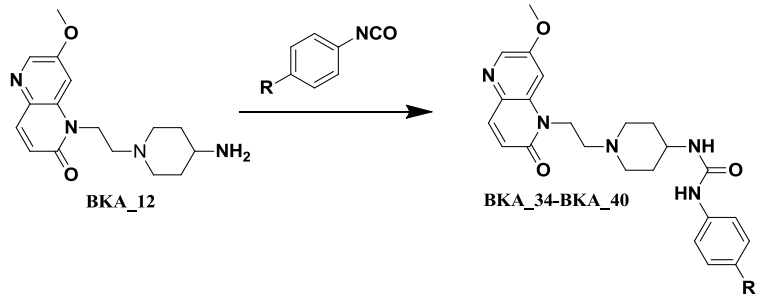
A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (1.03 mmol). Corresponding substituted phenyl isocyanate (0.413 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC &LCMS for completion). The reaction mixture was then washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted 1-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylurea (**BKA_20-BKA_26**) in good yield.

General procedure for the synthesis of final molecules (BKA_27–BKA_33)



A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.33 mmol) in dichloromethane (1 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (0.99 mmol). Corresponding substituted phenyl isothiocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted 1-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**BKA_27-BKA_33**) in good yields.

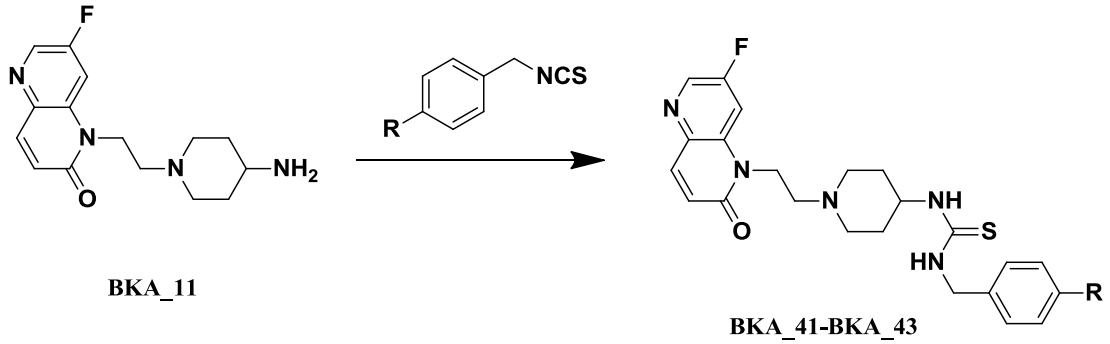
General procedure for the synthesis of final molecules (BKA_34–BKA_40)



A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.33 mmol) in dichloromethane (1 mL) was cooled to 0°C followed by the addition of triethylamine (0.99 mmol). Corresponding substituted phenyl isocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent

to give the corresponding substituted 1-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**BKA_34-BKA_40**) in good yield.

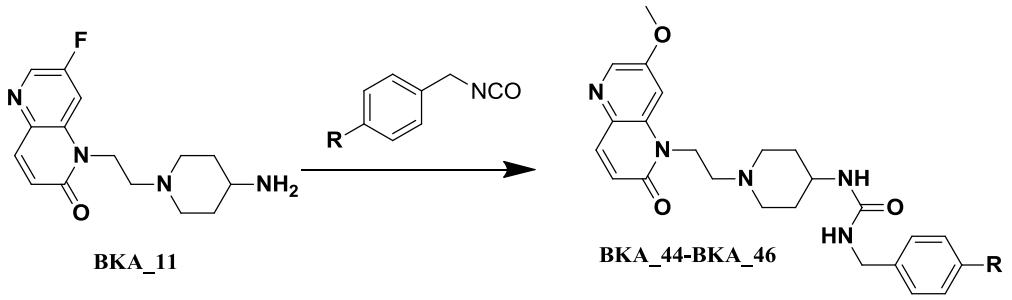
General procedure for the synthesis of final molecules (BKA_41–BKA_43)



BKA_11 **BKA_41-BKA_43**

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (1.02 mmol). Corresponding substituted benzyl isothiocyanate (0.413 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted 1-benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_41-BKA_43**) in good yields.

General procedure for the synthesis of final molecules (BKA_44–BKA_46)

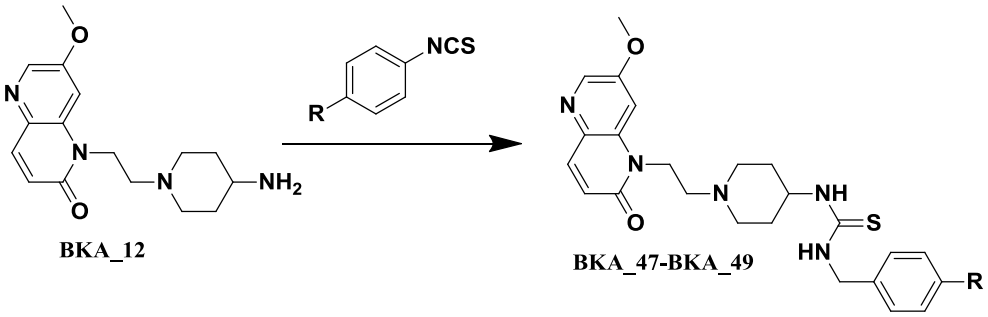


BKA_11 **BKA_44-BKA_46**

A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.344 mmol) in dichloromethane (3 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (1.03 mmol). Corresponding substituted benzyl isocyanate (0.413

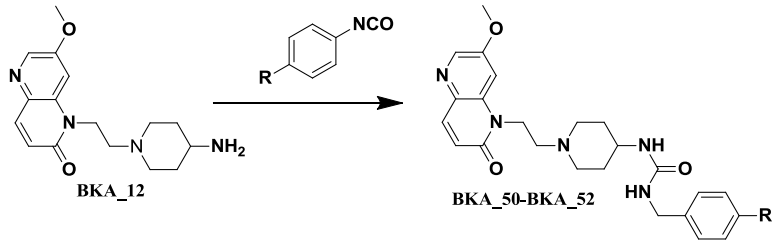
mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted 1-benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_44-BKA_46)** in good yield.

General procedure for the synthesis of final molecules (BKA_47–BKA_49)



A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.330 mmol) in dichloromethane (1 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (0.99 mmol). Corresponding substituted benzyl isothiocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted 1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_47-BKA_49**)in good yield.

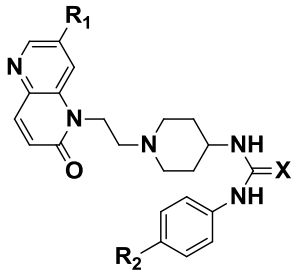
General procedure for the synthesis of final molecules (BKA_47–BKA_49)



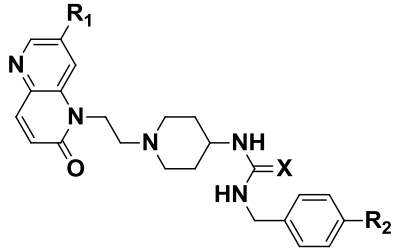
A solution of 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one**(BKA_12)** (0.33 mmol) in dichloromethane (1 mL) was cooled to 0 ^{O}C followed by the

addition of triethylamine (0.99 mmol). Corresponding substituted benzyl isocyanate (0.397 mmol) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding substituted1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_50-BKA_52**) in good yield.

Table 5.1: Physiochemical properties of the synthesized compounds **BKA_13–BKA_52**



BKA_13- BKA_40



BKA_41 - BKA_52

Cmpd.	R_1	X	R_2	Yield (%)	Molecular formula	Molecular weight	Melting point (°C)
BKA_13	F	S	H	58	$C_{22}H_{24}FN_5OS$	425.52	165-168
BKA_14	F	S	F	70	$C_{22}H_{23}F_2N_5OS$	443.51	184-186
BKA_15	F	S	Cl	65	$C_{22}H_{23}ClFN_5OS$	459.97	188-190
BKA_16	F	S	NO_2	86	$C_{22}H_{23}FN_6O_3S$	470.52	205-207
BKA_17	F	S	$COCH_3$	76	$C_{24}H_{26}FN_5O_2S$	467.56	188-190
BKA_18	F	S	OCH_3	81	$C_{23}H_{26}FN_5O_2S$	455.55	130-132
BKA_19	F	S	CH_3	78	$C_{23}H_{26}FN_5OS$	439.55	181-183
BKA_20	F	O	H	76	$C_{22}H_{24}FN_5O_2$	409.46	212-214
BKA_21	F	O	F	83	$C_{22}H_{23}F_2N_5O_2$	427.45	239-241
BKA_22	F	O	Cl	88	$C_{22}H_{23}ClFN_5O_2$	443.90	275-277
BKA_23	F	O	NO_2	63	$C_{22}H_{23}FN_6O_4$	454.45	261-263
BKA_24	F	O	$COCH_3$	67	$C_{24}H_{26}FN_5O_3$	451.49	224-226

BKA_25	F	O	OCH_3	78	$C_{23}H_{26}FN_5O_3$	439.48	196-198
BKA_26	F	O	CH_3	83	$C_{23}H_{26}FN_5O_2$	423.48	242-244
BKA_27	OCH_3	S	H	82	$C_{23}H_{27}N_5O_2S$	437.56	178-180
BKA_28	OCH_3	S	F	72	$C_{23}H_{26}FN_5O_2S$	455.55	165-167
BKA_29	OCH_3	S	Cl	78	$C_{23}H_{26}ClN_5O_2S$	472.00	167-169
BKA_30	OCH_3	S	NO_2	62	$C_{23}H_{26}N_6O_4S$	482.56	229-231
BKA_31	OCH_3	S	$COCH_3$	70	$C_{25}H_{29}N_5O_3S$	479.59	194-196
BKA_32	OCH_3	S	OCH_3	81	$C_{24}H_{29}N_5O_3S$	467.58	121-123
BKA_33	OCH_3	S	CH_3	77	$C_{24}H_{29}N_5O_2S$	451.58	134-136
BKA_34	OCH_3	O	H	73	$C_{23}H_{27}N_5O_3$	421.49	186-188
BKA_35	OCH_3	O	F	81	$C_{23}H_{26}FN_5O_3$	439.48	209-211
BKA_36	OCH_3	O	Cl	85	$C_{23}H_{26}ClN_5O_3$	455.94	206-208
BKA_37	OCH_3	O	NO_2	60	$C_{23}H_{26}N_6O_5$	466.49	221-223
BKA_38	OCH_3	O	$COCH_3$	79	$C_{25}H_{29}N_5O_4$	463.53	230-232
BKA_39	OCH_3	O	OCH_3	77	$C_{24}H_{29}N_5O_4$	451.52	217-219
BKA_40	OCH_3	O	CH_3	72	$C_{24}H_{29}N_5O_3$	435.52	225-227
BKA_41	F	S	H	72	$C_{23}H_{26}FN_5OS$	439.55	174-176
BKA_42	F	S	Cl	81	$C_{23}H_{25}ClFN_5OS$	473.99	163-165
BKA_43	F	S	OCH_3	80	$C_{24}H_{28}FN_5O_2S$	469.57	133-135
BKA_44	F	O	H	80	$C_{23}H_{26}FN_5O_2$	423.4	180-182
BKA_45	F	O	Cl	67	$C_{23}H_{25}ClFN_5O_2$	457.93	215-217
BKA_46	F	O	OCH_3	80	$C_{24}H_{28}FN_5O_3$	453.51	137-139
BKA_47	OCH_3	S	H	78	$C_{24}H_{29}N_5O_2S$	451.58	149-151
BKA_48	OCH_3	S	Cl	71	$C_{24}H_{28}ClN_5O_2S$	486.03	151-153
BKA_49	OCH_3	S	OCH_3	77	$C_{25}H_{31}N_5O_3S$	481.61	184-186
BKA_50	OCH_3	O	H	73	$C_{24}H_{29}N_5O_3$	435.52	184-186
BKA_51	OCH_3	O	Cl	84	$C_{24}H_{28}ClN_5O_3$	469.96	169-171
BKA_52	OCH_3	O	OCH_3	78	$C_{25}H_{31}N_5O_4$	465.54	185-187

5.1.3. Characterization of the synthesized molecules

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**BKA_13**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and phenyl isothiocyanate (0.055 g, 0.413 mmol) to afford **BKA_13** (0.085 g, 58 %) as white solid. M.p:165-168 ^{0}C. ^{1}H NMR [DMSO-d_6]: δ_H 10.33 (b, 1H), 7.98–6.69 (m, 9H), 4.33 (t, *J*=6.6Hz, 2H), 4.05 (b, 1H), 2.81–1.22 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 178.1, 160.2, 157.9, 146.2, 140.3, 139.5, 133.8, 130.3 (2C), 129.2, 127.7 (2C), 125.2, 124.8, 109.5, 56.6, 54.2, 52.8 (2C), 47.6, 30.3 (2C). ESI-MS *m/z:* 426 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}FN_5OS$: C, 62.10; H, 5.68; N, 16.46. Found: C, 62.11; H, 5.66; N, 16.45.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-fluorophenyl)thiourea (**BKA_14**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4 fluoro phenyl isothiocyanate (0.063 g, 0.413 mmol) to afford **BKA_14** (0.112 g, 70.4 %) as pale yellow solid. M.p: 184-186°C. ^{1}H NMR [DMSO-d_6]: δ_H 10.37 (b, 1H), 7.95–6.67 (m, 8H), 4.35 (t ,*J*= 6.9 Hz, 2H), 4.06 (b, 1H), 2.92–1.24 (m, 11H).^{13}C NMR [DMSO-d_6] δ: 178.9, 162.5, 161.7, 157.8, 145.3, 140.2, 135.6, 132.8, 130.8 (2C), 125.6, 123.8, 116.2 (2C), 109.5, 56.8, 54.5, 52.9 (2C), 47.5, 30.6 (2C). ESI-MS *m/z:*444 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}F_2N_5OS$: C, 59.58; H, 5.23; N, 15.79. Found: C, 59.60; H, 5.20; N, 15.80.

1-(4-Chlorophenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_15**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-chloro phenyl isothiocyanate (0.069 g, 0.413 mmol) to afford **BKA_15** (0.102 g, 63 %) as brown solid. M.p: 188-190 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.37 (b, 1H), 7.92 –6.55 (m, 8H),4.30 (t, *J*= 6.6 Hz, 2H), 4.12 (b, 1H), 2.88–1.29 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 178.1, 161.7, 157.8, 145.9, 140.2, 137.2, 134.5, 132.8, 131.9 (2C), 130.3 (2C), 125.6, 123.8, 109.6, 56.8, 54.5, 52.3 (2C), 47.5, 30.3 (2C). ESI-MS *m/z:*460 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}ClFN_5OS$: C, 57.45; H, 5.04; N, 15.23. Found: C, 57.47; H, 5.07; N, 15.29.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-nitrophenyl)thiourea (**BKA_16**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-nitro phenyl isothiocyanate (0.074 g, 0.413 mmol) to afford **BKA_16** (0.138 g, 86 %)as yellow solid. M.p: 205-207 °C. ^{1}H NMR [300 MHz, DMSO-d_6]: δ_H 9.97 (b, 1H), 8.57–6.82 (m, 8H), 4.34 (t, *J*= 6.3 Hz, 2H), 4.09 (b, 1H), 2.93–1.28 (m, 11H).^{13}C NMR [DMSO-d_6] δ: 178.9, 160.7, 157.3, 146.4, 142.3, 142.1, 141.6, 139.9, 132.8, 124.4 (2C), 123.9 (2C), 120.1, 109.3, 54.94, 53.16, 52.2 (2C), 50.6,3 0.7 (2C).ESI-MS *m/z*: 471 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}FN_6O_3S$: C, 56.16; H, 4.93; N, 17.86; Found: C, 56.18; H, 4.90; N, 17.87.

1-(4-Acetylphenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (BKA_17): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-acetyl phenyl isothiocyanate (0.073 g, 0.413 mmol) to afford **BKA_17** (0.122 g, 76 %) as white solid. M.p: 188-190 °C. ^{1}H NMR [DMSO-d_6]: δ_H10.49 (b, 1H), 8.38 – 6.75 (m, 8H), 4.62 (t, *J*=7.5 Hz, 2H), 4.15 (b,1H), 3.63 –3.02 (m, 3H), 2.52 (s, 3H), 2.43–1.18 (m, 8H).^{13}C NMR [DMSO-d_6] δ_c: 197.5, 178.9, 160.1, 157.8, 145.3, 143.4, 140.2, 138.3, 132.5, 130.2 (2C), 127.8 (2C), 125.3, 109.8, 55.8, 54.2, 53.5, 52.9 (2C), 47.8, 30.2 (2C), 25.8. ESI-MS *m/z*: 468 $(M+H)^+$. Anal Calcd for $C_{24}H_{26}FN_5O_2S$: C, 61.65; H, 5.60; N, 14.98; Found: C, 61.63; H, 5.57; N, 14.97.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxyphenyl)thiourea (**BKA_18**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4 methoxy phenyl isothiocyanate (0.068g, 0.413 mmol) to afford **BKA_18** (0.126 g, 81 %) as Pale yellow solid. M.p: 130-132 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.45 (b, 1H), 8.25 – 6.75 (m, 8H), 4.33 (t, *J*=6.0 Hz, 2H,), 4.05 (b, 1H), 3.85 (s, 3H), 2.84–1.31 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 178.2, 161.2, 160.5, 157.8, 145.7, 140.2, 132.3, 129.5, 128.2 (2C), 125.6, 123.5, 115.1 (2C), 110.1, 56.2, 55.9, 52.5, 51.9 (2C), 48.5, 30.3 (2C). ESI-MS *m/z:* 456 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_2S$: C, 60.64; H, 5.75; N, 15.37. Found: C, 60.69; H, 5.70; N, 15.35.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(p-tolyl)thiourea (**BKA_19**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-methyl phenyl isothiocyanate (0.062 g, 0.413 mmol) to afford **BKA_19** (0.118 g, 78 %) as White solid. M.p: 181-183 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.42 (b, 1H), 7.94–6.58 (m, 8H), 4.31 (t, *J*=6.9 Hz, 2H,), 4.15 (b, 1H), 2.92–1.31 (m, 14H).^{13}C NMR [DMSO-d_6] δ_c: 178.1, 160.2, 157.8, 145.8, 140.2, 138.5, 136.7, 132.8, 130.3 (2C), 126.7 (2C), 125.2, 123.8, 109.5, 56.8, 54.2, 52.8 (2C), 47.6, 30.3 (2C), 22.4. ESI-MS *m/z:* 440 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5OS$: C, 62.85; H, 5.96; N, 15.93. Found: C, 62.87; H, 5.95; N, 15.92.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylurea (**BKA_20**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and phenyl isocyanate (0.049 g, 0.413 mmol) to afford **BKA_20** (0.107 g, 76 %) as Pale yellow solid. M.p: 212-214 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.36 (b, 1H), 10.28 (b, 1H), 7.95 – 6.67 (m, 9H), 4.64 – 1.38 (m, 13H).^{13}C NMR [DMSO-d_6] δ_c: 160.7, 157.8, 155.6, 145.3, 140.2, 138.9, 132.9, 130.3 (2C), 129.2, 125.6, 123.4, 120.6 (2C), 109.8, 54.6, 52.8 (2C), 47.9, 46.8, 30.2 (2C). ESI-MS *m/z:*410 $(M+H)^+$. AnalCalcd for $C_{22}H_{24}FN_5O_2$: C, 64.53; H, 5.91; N, 17.10. Found: C, 64.55; H, 5.96; N, 17.07.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-fluorophenyl)urea (**BKA_21**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-fluoro phenyl isocyanate (0.056 g, 0.413 mmol) to afford **BKA_21** (0.122 g, 83 %) as pale yellow solid. M.p: 239-241 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.12 (b, 1H), 9.99 (b, 1H), 8.63 – 6.84 (m, 8H), 4.36 – 1.42 (m, 13H).^{13}C NMR [DMSO-d_6] δ_c: 161.8, 160.7, 155.8, 154.9, 145.9, 140.2, 136.7, 132.2, 125.5, 123.7, 120.2 (2C), 116.2 (2C), 109.8, 54.5, 52.3 (2C), 47.8, 46.7, 30.3 (2C). ESI-MS *m/z:*428 $(M+H)^+$. AnalCalcd for $C_{22}H_{23}F_2N_5O_2$: C, 61.82; H, 5.42; N, 16.38; Found: C, 61.83; H, 5.44; N, 16.43.

1-(4-Chlorophenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_22**): The compound was synthesized according to the general procedure using 1-

(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-chloro phenyl isocyanate (0.063 g, 0.413 mmol) to afford **BKA_22** (0.133 g, 88 %) as brown solid. M.p: 275-277 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.11(b,1H), 10.05 (b, 1H), 8.65 – 6.82 (m, 8H), 4.33 – 1.37 (m, 13H). ^{13}C NMR [DMSO-d_6] δ_c: 160.7, 157.8, 155.8, 145.9, 140.2, 138.6, 134.7, 132.3, 130.4 (2C), 125.6,123.7,120.5 (2C), 109.7, 54.2, 52.8 (2C), 47.9, 46.5, 30.4 (2C). ESI-MS *m/z* :444 $(M+H)^+$. AnalCalcd for $C_{22}H_{23}ClFN_5O_2$: C, 59.53; H, 5.22; N, 15.78; Found: C, 59.57; H, 5.20; N, 15.76.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-nitrophenyl)urea (**BKA_23**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-nitro phenyl isocyanate (0.067 g, 0.413 mmol) to afford **BKA_23** (0.097 g, 63 %) as pale yellow solid. M.p: 261-263 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.12 (b, 1H), 9.99 (b, 1H), 8.63 – 6.83 (m, 8H), 4.36 – 1.42 (m, 13H).^{13}C NMR [DMSO-d_6] δ_c: 160.9, 155.2, 154.8, 144.3, 143.3, 142.9, 139.7, 132.8, 125.2, 124.9 (2C), 123.8, 120.2 (2C), 109.5, 54.5, 52.8 (2C), 47.8, 46.9, 30.2 (2C). ESI-MS *m/z*: 455 $(M+H)^+$. AnalCalcd for $C_{22}H_{23}FN_6O_4$: C, 58.14; H, 5.10; N, 18.49; Found: C, 58.17; H, 5.13; N, 18.45.

1-(4-Acetylphenyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_24**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-acetyl phenyl isocyanate (0.066 g, 0.413 mmol) to afford **BKA_24** (0.104 g, 67 %) as white solid. M.p: 224-226 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.56 (b, 1H), 10.44 (b, 1H), 8.39 – 6.72 (m, 8H), 4.68 (t, *J*=7.8 Hz, 2H), 3.78 – 3.09 (m, 3H), 2.53 (s,3H), 2.49–1.17 (m, 8H).^{13}C NMR [DMSO-d_6] δ_c: 196.1, 161.2, 156.8, 154.1, 145.1, 143.2, 140.1, 136.7, 131.3, 129.1 (2C), 124.5, 123.8, 120.2 (2C), 109.8,5 4.5, 51.4 (2C), 47.6, 45.5, 29.6 (2C), 26.2. ESI-MS *m/z*: 452 $(M+H)^+$. Anal Calcd for $C_{24}H_{26}FN_5O_3$: C, 63.85; H, 5.80; N, 15.51; Found: C, 63.86; H, 5.88; N, 15.49.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxyphenyl)urea (**BKA_25**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-methoxy phenyl isocyanate (0.062 g, 0.413 mmol) to

afford **BKA_25** (0.117 g, 78 %) as white solid. M.p: 196-198 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.16 (b, 1H), 10.03 (b,1H), 8.57 – 6.73 (m, 8H), 4.37 (t, *J*=6.6 Hz, 2H), 3.81(s, 3H), 4.32–1.38 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 160.7, 159.7, 157.8, 155.3, 145.2, 140.1, 131.7, 130.8, 125.6, 123.8, 120.2 (2C), 115.6 (2C), 109.5, 56.2, 54.2, 52.3 (2C), 47.8, 46.7, 30.3 (2C). ESI-MS *m/z:*440 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_3$: C, 62.86; H, 5.96; N, 15.94; Found: C, 62.88; H, 5.95; N, 15.99.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(p-tolyl)urea (**BKA_26**).**:** The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-methyl phenyl isocyanate (0.054 g, 0.413 mmol) to afford **BKA_26** (0.120 g, 83 %) as brown solid. M.p: 242-244 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.39 (b,1H), 10.26 (b,1H), 7.88 – 6.65 (m, 8H), 4.40 (t, *J*=7.2 Hz, 2H,), 3.05–1.36 (m, 14H).^{13}C NMR [DMSO-d_6] δ_c: 160.7, 157.2, 155.4, 145.8, 140.2, 137.9, 137.2, 132.7, 130.2 (2C), 125.6, 123.8, 120.8 (2C), 109.8, 54.6, 52.5 (2C), 47.8, 46.5, 30.2 (2C), 20.8. ESI-MS *m/z* 424 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_2$: C, 65.23; H, 6.19; N, 16.54. Found: C, 65.25; H, 6.18; N, 16.55.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylthiourea (**BKA_27**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and phenyl isothiocyanate (0.055 g, 0.397 mmol) to afford **BKA_27** (0.142 g, 82 %) as white solid. M.p: 178-180 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.33 (b, 1H), 7.92 – 6.65 (m, 9H), 4.34 (t, *J*=6.6 Hz, 2H), 4.09 (b, 1H), 3.82 (s, 3H), 2.86 – 1.23 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 178.2, 160.5, 157.8, 145.2, 141.3, 138.5, 134.8, 130.3 (2C), 129.7, 126.7 (2C), 125.2, 124.8, 110.5, 56.6, 55.3, 53.2, 52.8 (2C), 47.6, 30.3 (2C). ESI-MS *m/z;* 438 $(M+H)^+$. Anal Calcd for $C_{23}H_{27}N_5O_2S$: C, 63.13; H, 6.22; N, 16.01. Found: C, 63.11; H, 6.25; N, 16.09.

1-(4-Fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_28**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-fluoro phenyl isothiocyanate (0.060g, 0.397 mmol) to afford **BKA_28** (0.108 g, 72 %) as yellow solid. M.p: 165-167°C. ^{1}H NMR [DMSO-d_6]: δ_H 10.35 (b,

1H), 7.98 – 6.65 (m, 8H), 4.33 (t, *J*=7.2 Hz, 2H), 4.05 (b, 1H), 3.84 (s, 3H), 2.97–1.26 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 178.9, 162.5, 161.7, 157.8, 145.3, 140.2, 135.6, 132.8, 130.8 (2C), 125.6, 123.8, 116.2 (2C), 109.5, 56.8, 55.7, 54.5, 52.9 (2C), 47.5, 30.6 (2C). ESI-MS *m/z:* 456 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_2S$: C, 60.64; H, 5.75; N, 15.37. Found: C, 60.68; H, 5.72; N, 15.39.

1-(4-Chlorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_29**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-chloro phenyl isothiocyanate (0.067g, 0.397 mmol) to afford **BKA_29** (0.121 g, 78 %) as brown solid. M.p: 167-169°C. ^{1}H NMR [DMSO-d_6]: δ_H 10.46 (b, 1H), 8.01 – 6.71 (m, 8H), 4.30 (t, *J*=6.3Hz, 2H), 4.15 (b, 1H), 3.91 (s, 3H), 2.91–1.35 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 178.1, 161.7, 157.8, 145.9, 140.2, 137.2, 134.5, 132.8, 131.9 (2C), 130.3 (2C), 125.6, 123.8, 109.6, 56.8, 55.4, 54.5, 52.3 (2C), 47.5, 30.3 (2C). ESI-MS *m/z:*473 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}ClN_5O_2S$: C, 58.53; H, 5.55; N, 14.84. Found: C, 58.57; H, 5.50; N, 14.89.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-nitrophenyl)thiourea (BKA_30): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-nitro phenyl isothiocyanate (0.071 g, 0.397 mmol) to afford **BKA_30** (0.099 g, 62 %) as yellow solid. M.p: 229-231 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.03 (b, 1H), 8.62–6.87 (m, 8H), 4.41 (t, *J*=6.3 Hz, 2H), 4.18 (b,1H), 3.95 (s, 3H), 3.05–1.42 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 178.9, 160.7, 157.3, 146.4, 142.3, 142.1, 141.6, 139.9, 132.8, 124.4 (2C), 123.9 (2C), 120.1, 109.3, 55.9, 54.9, 53.1, 52.2 (2C), 50.6, 30.7 (2C). ESI-MS *m/z*: 483$(M+H)^+$. Anal Calcd for $C_{23}H_{26}N_6O_4S$: C, 57.25; H, 5.43; N, 17.42. Found: C, 57.21; H, 5.42; N, 17.38.

1-(4-Acetylphenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (BKA_**31**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-acetyl phenyl isothiocyanate (0.070g, 0.397 mmol) to afford

BKA_31 (0.11 g, 70 %) as white solid. M.p: 194-196 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.35 (b, 1H), 8.25–6.62 (m, 8H), 4.41 (t, *J*=7.8 Hz, 2H), 4.18 (b,1H), 3.85 (s, 3H), 3.54–2.99 (m, 3H), 2.56 (s, 3H), 2.44–1.2 3(m, 8H). ^{13}C NMR [DMSO-d_6] δ_c: 197.8, 177.5, 162.3, 155.3, 143.9, 143.5, 138.5, 136.2, 132.8, 130.2 (2C), 125.2 (2C), 124.9, 119.2, 105.8, 56.5, 54.2, 53.5, 51.5 (2C), 47.5, 30.2 (2C), 25.6.ESI-MS *m/z*: 480$(M+H)^+$. Anal Calcd for $C_{25}H_{29}N_5O_3S$: C, 62.61; H, 6.09; N, 14.60; Found: C, 62.66; H, 6.11; N, 14.56.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxyphenyl)thiourea (**BKA_32**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-methoxy phenyl isothiocyanate (0.065g, 0.397 mmol) to afford **BKA_32** (0.125 g, 81 %) as brown solid. M.p: 121-123 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.32 (b, 1H), 7.83–6.58 (m, 8H), 4.49 (t, *J*=6.3 Hz, 2H,), 4.12 (b, 1H), 3.85 (s, 3H), 3.75 (s, 3H), 2.93–1.41 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 178.3, 163.5,160.2, 155.8,143.3, 136.8, 132.3, 131.6, 128.1 (2C), 125.6, 119.2, 115.6 (2C), 105.2, 55.8(2C), 55.2, 54.3, 52.8 (2C), 50.2, 30.5 (2C). ESI-MS *m/z:* 468 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_3S$: C, 61.65; H, 6.25; N, 14.98. Found: C, 61.63; H, 6.27; N, 14.97.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(p-tolyl)thiourea (BKA_**33**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-methyl phenyl isothiocyanate (0.059 g, 0.397 mmol) to afford **BKA_33** (0.115 g, 77 %) as white solid. M.p: 134-136 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.37 (b, 1H), 7.89–6.52 (m, 8H), 4.29 (t, *J*=6.9 Hz, 2H), 4.11 (b, 1H), 3.75 (s, 3H), 2.92–1.31 (m, 14H).^{13}C NMR [DMSO-d_6] δ_c: 180.2, 163.3, 155.6, 142.9, 136.2, 135.3, 134.9, 132.1, 130.3 (2C), 126.8 (2C), 124.4, 119.2, 104.3, 56.4, 55.8, 53.8, 53.1 (2C), 50.1, 30.8 (2C), 22.6. ESI-MS *m/z* 452 $(M+H)^+$. Anal.Calcd.for:$C_{24}H_{29}N_5O_2S$: C, 63.83; H, 6.47; N, 15.51. Found: C, 63.82; H, 6.45; N, 15.55.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-phenylurea (BKA_**34**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330

mmol) and phenyl isocyanate (0.047 g, 0.397 mmol) to afford **BKA_34** (0.102 g, 73 %) as yellow solid. M.p: 186-188 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.46 (b, 1H), 10.38 (b, 1H), 7.92–6.65 (m, 9H), 4.67 (t, *J*= 6.9*Hz*, 2H), 3.85 (s, 3H), 3.01–1.42 (m, 11H). ^{13}C NMR [DMSO-d_6] δ_c: 161.7, 156.8, 155.6, 145.3, 140.2, 136.9, 132.9, 130.3 (2C), 129.2, 125.6, 123.4 (2C), 120.6, 106.8, 56.2, 53.6, 52.8 (2C), 47.9, 46.8, 30.2 (2C). ESI-MS *m/z:*422 $(M+H)^+$. Anal.Calcd.for $C_{23}H_{27}N_5O_3$: C, 65.54; H, 6.46; N, 16.62. Found: C, 65.55; H, 6.40; N, 16.67.

1-(4-Fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_35**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-fluoro phenyl isocyanate (0.059 g, 0.397 mmol) to afford **BKA_35** (0.117 g, 81 %) as pale yellow solid. M.p: 209-211°C. ^{1}H NMR [DMSO-d_6]: δ_H10.42 (bs,1H), 10.35 (bs,1H), 7.98–6.65 (m, 8H), 4.52 (t, *J*=6.6 Hz, 2H), 3.82 (s, 3H), 3.48–1.38 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c:163.1, 160.7, 155.8, 154.9, 145.9, 136.7, 134.9, 132.2, 125.5, 120.2 (2C), 119.1, 116.2 (2C), 106.8, 56.5, 54.5, 52.3 (2C), 49.5, 46.7, 30.3 (2C). ESI-MS *m/z:* 440 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_3$: C, 62.86; H, 5.96; N, 15.94. Found: C, 62.82; H, 5.99; N, 15.91.

1-(4-Chlorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_36**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-chloro phenyl isocyanate (0.061g, 0.397 mmol) to afford **BKA_36** (0.128 g, 85 %) as pale brown solid. M.p: 206-208 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.49(b, 1H), 10.41 (b, 1H), 7.96–6.68 (m, 8H), 4.49 (t, *J* =6.3 Hz, 2H), 3.85 (s, 3H), 3.25–1.39 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 161.7, 156.8, 155.8, 145.9, 138.6, 135.7, 132.9, 132.3, 130.4 (2C), 125.6, 120.5 (2C), 119.5, 106.7, 56.2, 54.2, 52.8 (2C), 47.9, 46.5, 30.4 (2C). ESI-MS *m/z:* 456 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}ClN_5O_3$: C, 60.59; H, 5.75; N, 15.36. Found: C, 60.57; H, 5.78; N, 15.38.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-nitrophenyl)urea (BKA_37): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-nitro phenyl isocyanate (0.065 g, 0.397 mmol) to afford **BKA_37** (0.092 g, 60 %) as yellow solid. M.p: 221-223 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.52(b,

1H), 10.43 (b, 1H), 8.15–6.68 (m, 8H), 4.52 (t, *J*=6.9 Hz, 2H), 3.86 (s, 3H), 3.36–1.48 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 162.9, 155.2, 154.8, 144.3, 143.3, 142.9, 135.7, 132.8, 125.2, 124.9 (2C), 120.2 (2C), 119.2, 106.5, 56.2, 54.1, 52.8 (2C), 47.8, 46.9, 30.2 (2C) .ESI-MS *m/z*: 467 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}N_6O_5$: C, 59.22; H, 5.62; N, 18.02. Found: C, 59.25; H, 5.58; N, 18.08.

1-(4-Acetylphenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_38**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-acetyl phenyl isocyanate (0.063 g, 0.397 mmol) to afford **BKA_38** (0.12 g, 79 %)as white solid. M.p: 230-232 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.59 (b, 1H). 10.42 (b, 1H), 8.34–6.70 (m, 8H), 4.68 (t, *J*=6.6 Hz, 2H), 4.05 (s,3H), 3.73–3.05 (m, 3H), 2.51 (s, 3H), 2.45–1.15 (m, 8H).^{13}C NMR [DMSO-d_6] δ_c: 196.1, 161.0, 156.8, 154.2, 145.0, 140.8, 136.7, 135.1, 131.3, 129.6 (2C), 125.4, 120.9 (2C), 116.5, 104.9, 57.0, 52.0,51.48 (2C), 51.0, 47.6, 29.2 (2C),26.2. ESI-MS *m/z*: 464 $(M+H)^+$. Anal Calcd for $C_{25}H_{29}N_5O_4$: C, 64.78; H, 6.31; N, 15.11; Found: C, 64.77; H, 6.29; N, 15.14.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxyphenyl)urea (**BKA_39**):The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-methoxy phenyl isothiocyanate (0.059 g, 0.397 mmol) to afford **BKA_39** (0.114 g, 77 %) as yellow solid. M.p: 217-219 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.42 (b, 1H), 10.53 (b, 1H), 7.88–6.63 (m, 8H), 4.51 (t, *J*=6.9 Hz, 2H,), 3.85 (s, 3H), 3.81 (s,3H), 3.27–1.39 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 162.7, 159.7, 157.8, 155.3, 145.2, 136.1, 131.7, 130.8, 125.6, 120.2 (2C), 119.5, 115.6 (2C), 106.5, 56.2 (2C), 54.2, 52.3 (2C), 47.8, 46.7, 30.3 (2C). ESI-MS *m/z:* 452 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_4$: C, 63.84; H, 6.47; N, 15.51. Found: C, 63.88; H, 6.43; N, 15.55.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(p-tolyl)urea (**BKA_40**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-methyl phenyl isocyanate (0.053 g, 0.397 mmol) to afford **BKA_40** (0.103 g,

72.0 %) as pale brown solid. M.p: 225-227 °C. ^{1}H NMR [DMSO-d_6]: δ_H10.42 (b,1H), 10.36 (b,1H), 7.83–6.66 (m, 8H), 4.42 (t, *J*=7.5 Hz, 2H,), 3.87 (s, 3H) 3.12–1.39 (m, 14H).^{13}C NMR [DMSO-d_6] δ_c: 161.7, 157.2, 155.4, 145.8, 137.9, 137.2, 135.3, 132.7, 130.2 (2C), 125.6, 120.8 (2C), 119.2, 106.8, 56.2, 54.6, 52.5 (2C), 47.8, 46.5, 30.2 (2C), 20.8. ESI-MS *m/z:* 436 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_3$: C, 66.19; H, 6.71; N, 16.08. Found: C, 66.22; H, 6.78; N, 16.12%.

1-Benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_41**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and benzyl isothiocyanate (0.062 g, 0.413 mmol) to afford **BKA_41** (0.108 g, 72 %) as pale yellow solid. M.p: 174-176 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.68–6.82 (m, 9H), 5.52 (b, 1H), 5.26 (b, 1H), 4.36–4.12 (m, 4H), 2.89–1.25 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 179.8, 160.7, 157.3, 145.3, 139.8, 137.2, 132.7, 128.3 (2C), 127.8 (2C), 127.5, 124.6, 123.8, 109.8, 54.2, 53.4, 52.8 (2C), 47.3, 43.5, 30.2 (2C). ESI-MS *m/z:* 440 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5OS$: C, 62.85; H, 5.96; N, 15.93; Found: C, 62.82; H, 5.95; N, 15.95.

1-(4-Chlorobenzyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_42**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**11**) (0.1 g, 0.344 mmol) and 4-chloro benzyl isothiocyanate (0.075 g, 0.413 mmol) to afford **BKA_42** (0.132 g, 81 %) as yellow solid. M.p: 163-165°C. ^{1}H NMR [DMSO-d_6]: δ_H 8.65 –6.66 (m, 8H), 5.51(b, 1H), 5.32(b, 1H), 4.27– 4.07 (m, 4H), 2.76–1.19 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 179.8, 160.7, 157.8, 145.2, 140.3, 137.8, 135.2 (2C), 132.9, 132.5, 129.5 (2C), 125.6, 123.5, 109.8, 55.4, 53.5, 51.5 (2C), 47.8, 46.5, 30.2 (2C). ESI-MS *m/z:* 474 $(M+H)^+$. Anal Calcd for $C_{23}H_{25}ClFN_5OS$: C, 58.28; H, 5.32; N, 14.78; Found: C, 58.30; H, 5.30; N, 14.75.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxybenzyl)thiourea (**BKA_43**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-methoxy benzyl isothiocyanate (0.074 g, 0.413 mmol) to afford **BKA_43** (0.128 g, 80 %)as brown solid. M.p: 133-135°C. ^{1}H NMR [DMSO-d_6]: δ_H 8.52–6.73 (m, 8H), 5.34 (b, 1H), 5.21 (b, 1H), 4.29–4.05 (m, 4H), 3.69 (s, 3H), 2.81–1.12 (m,

11H).^{13}C NMR [DMSO-d_6] δ_c: 179.8, 160.6, 158.0, 157.3, 145.6, 139.9, 132.7, 129.8 (2C), 129.6, 125.6, 123.9, 113.6 (2C), 109.3, 55.7, 54.9, 53.6, 52.8 (2C), 47.8, 45.2, 30.3 (2C). ESI-MS *m/z:* 470 $(M+H)^+$. Anal Calcd for $C_{24}H_{28}FN_5O_2S$: C, 61.39; H, 6.01; N, 14.91; Found: C, 61.35; H, 6.05; N, 14.93.

1-Benzyl-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_44**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and benzyl isocyanate (0.055 g, 0.413 mmol) to afford **BKA_44** (0.116 g, 80 %) as yellow solid. M.p: 180-182 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.71–6.84 (m, 9H), 6.12 (b,1H), 5.84 (b, 1H), 4.36–4.12 (m, 4H), 2.89–1.25 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 161.6, 158.1, 157.3, 144.2, 139.9, 137.2, 132.7, 128.3 (2C), 127.8 (2C), 127.2, 124.6, 123.9, 109.4, 53.1, 52.4 (2C), 51.2, 48.1, 45.5, 31.3 (2C). ESI-MS *m/z:*424 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}FN_5O_2$: C, 65.23; H, 6.19; N, 16.54; Found: C, 65.21; H, 6.17; N, 16.53.

1-(4-Chlorobenzyl)-3-(1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_45**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-chloro benzyl isocyanate (0.069 g, 0.413 mmol) to afford **BKA_45** (0.105 g, 67 %) as yellow solid. M.p: 215-217 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.77–6.86 (m, 8H), 6.11(b, 1H), 5.89 (b, 1H), 4.32– 4.11 (m, 4H), 2.88–1.21 (m,11H).^{13}C NMR [DMSO-d_6] δ_c: 160.7, 158.6, 157.4, 144.5, 139.9, 138.7, 137.5 (2C),132.9, 132.7, 128.3 (2C), 124.8, 123.5, 109.8, 53.3, 52.8 (2C), 52.5, 46.5, 42.6, 31.3 (2C). ESI-MS *m/z:*458 $(M+H)^+$. Anal Calcd for $C_{23}H_{25}ClFN_5O_2$: C, 60.33; H, 5.50; N, 15.29; Found: C, 60.35; H, 5.53; N, 15.27.

1-(1-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxybenzyl)urea (**BKA_46**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (**BKA_11**) (0.1 g, 0.344 mmol) and 4-methoxy benzyl isocyanate (0.067 g, 0.413 mmol) to afford **BKA_46** (0.125 g, 80 %)as brown solid. M.p: 137-139 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.67–6.81 (m,8H), 6.84 (b, 1H), 5.81 (b, 1H), 4.34–4.08 (m, 4H), 3.71 (s, 3H), 2.83–1.14 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 160.6, 160.4, 158.0, 157.3, 139.9, 134.4, 133.1, 132.8 (2C), 132.7, 128.3, 123.9, 113.6 (2C), 109.3, 55.0, 53.1, 52.4 (2C), 52.2, 46.0, 42.2, 32.3 (2C). ESI-

MS *m/z:* 454 $(M+H)^+$. Anal Calcd for $C_{24}H_{28}FN_5O_3$: C, 63.56; H, 6.22; N, 15.44; Found: C, 63.59; H, 6.25; N, 15.41.

1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_47**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and benzyl isothiocyanate (0.059 g, 0.397 mmol) to afford **BKA_47** (0.108 g, 78 %) as pale yellow solid. M.p: 149-151 °C. ^{1}H NMR [DMSO-d_6]: δ_H 7.79–7.61 (m, 2H), 7.37 (b, 1H), 7.31 (b, 1H), 7.23–6.65 (m, 7H), 4.68 (s, 2H), 4.42 (t, *J*=6.6 Hz, 2H), 3.91 (s, 3H), 3.08–1.25 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 180.91, 161.9, 155.6, 146.2, 138.2, 136.2, 132.4, 129.1(2C), 126.2(2C), 125.9, 124.5, 119.1, 105.2, 56.2, 54.9, 54.1, 52.5 (2C), 51.2, 48.5, 30.2 (2C). ESI-MS *m/z:* 452 $(M+H)^+$.Anal Calcd for $C_{24}H_{29}N_5O_2S$: C, 63.83; H, 6.47; N, 15.51. Found: C, 63.86; H, 6.44; N, 15.55.

1-(4-Chlorobenzyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)thiourea (**BKA_48**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-chloro benzyl isothiocyanate (0.072 g, 0.397 mmol) to afford **BKA_48** (0.114 g, 71 %) as white solid. M.p: 151-153 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.28-.7.85 (m, 2H), 7.70 (b, 1H), 7.47 (b, 1H), 7.41–6.64 (m, 6H), 4.64 (s, 2H), 4.38 (t, *J*=7.2Hz, 2H), 3.98 (s, 3H), 2.91–1.33 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 180.2, 161.5, 155.6, 146.2, 137.1, 136.5, 134.7 (2C), 132.5, 132.1, 129.2 (2C), 125.1, 119.2, 105.8, 56.2, 55.7, 54.5, 52.5 (2C), 51.2, 50.1, 30.2 (2C). ESI-MS *m/z* 487 $(M+H)^+$. Anal Calcd for $C_{24}H_{28}ClN_5O_2S$: C, 59.31; H, 5.81; N, 14.41; Found: C, 59.41; H, 5.79; N, 14.45.

1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxybenzyl)thiourea (**BKA_49**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-methoxy benzyl isothiocyanate (0.071 g, 0.397 mmol) to afford **BKA_49** (0.122 g, 77 %) as brown solid. M.p: 184-186 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.2–7.87 (m, 2H), 7.58 (b, 1H), 7.43 (b, 1H), 7.45–6.61 (m, 6H), 4.57 (s, 2H), 4.35 (t, *J*=6.6 Hz, 2H), 3.98 (s, 3H), 395 (s, 3H), 2.87–1.35 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 180.5, 161.5, 159.5, 155.1, 146.2, 135.3, 132.5, 130.2 (2C), 129.5, 125.3, 119.1, 115.2 (2C), 104.1, 56.2 (2C),

55.8, 54.5, 52.5 (2C), 51.2, 50.3, 30.2 (2C). ESI-MS *m/z:* 482 $(M+H)^+$. Anal Calcd for $C_{25}H_{31}N_5O_3S$: C, 62.35; H, 6.49; N, 14.54; Found: C, 62.38; H, 6.42; N, 14.56.

1-benzyl-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_50**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and benzyl isocyanate (0.052 g, 0.397 mmol) to afford **BKA_50** (0.105 g, 73 %) as pale yellow solid. M.p: 184-186 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.75 (b, 1H), 10.52 (s, 1H),7.92–6.67 (m, 9H), 4.56 (s, 2H), 4.41 (t, *J*=7.2 Hz, 2H), 3.87 (s, 3H), 3.28–1.36 (m, 11H).^{13}C NMR [DMSO-d_6]δ_c: 163.1, 157.8, 155.3, 145.7, 138.1, 136.1, 132.3, 129.1 (2C), 127.1 (2C), 126.8, 125.1, 119.2, 104.2, 56.2, 54.5, 52.3 (2C), 51.3, 48.5, 45.6, 30.1 (2C). ESI-MS *m/z:*436 $(M+H)^+$.Anal Calcd for $C_{24}H_{29}N_5O_3$: C, 66.19; H, 6.71; N, 16.08. Found: C, 66.22; H, 6.75; N, 16.05.

1-(4-Chlorobenzyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea (**BKA_51**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-chloro benzyl isocyanate (0.066 g, 0.397 mmol) to afford **BKA_51** (0.129 g, 84 %) as pale brown solid. M.p: 169-171 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.65 (b, 1H), 10.32 (s, 1H), 8.03–6.62 (m, 8H), 4.51 (s, 2H), 4.43 (t, *J*=6.3Hz, 2H), 3.86 (s, 3H), 3.31–1.42 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 161.2, 158.2, 155.6, 145.6, 137.2, 135.9, 135.1 (2C), 132.5, 131.9, 129.5 (2C), 125.2, 119.1, 105.1, 56.2, 54.5, 52.5 (2C), 51.5, 48.5, 45.6, 30.2 (2C). ESI-MS *m/z:* 470 $(M+H)^+$. Anal Calcd for $C_{24}H_{28}ClN_5O_3$: C, 61.34; H, 6.01; N, 14.90; Found: C, 61.39; H, 6.01; N, 14.97.

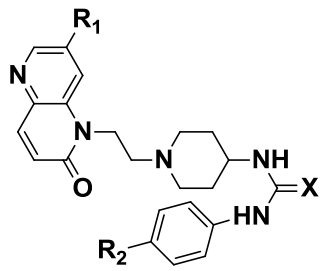
1-(1-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)-3-(4-methoxybenzyl)urea (**BKA_52**): The compound was synthesized according to the general procedure using 1-(2-(4-aminopiperidin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (**BKA_12**) (0.1 g, 0.330 mmol) and 4-methoxy benzyl isocyanate (0.064 g, 0.397 mmol) to afford **BKA_52** (0.119 g, 78 %) as brown solid. M.p: 185-187 °C. ^{1}H NMR [DMSO-d_6]: δ_H 10.44 (b, 1H), 10.29 (s, 1H), 8.12–6.71 (m, 8H), 4.65 (s, 2H), 4.48 (t, *J*=6.6Hz, 2H), 3.92 (s, 3H), 3.87 (s, 3H), 3.35–1.43 (m, 11H).^{13}C NMR [DMSO-d_6] δ_c: 162.1, 159.7, 158.1, 155.6, 146.2, 136.6, 132.8, 130.7 (2C), 129.1, 124.8, 119.2, 115.2 (2C), 105.1, 56.2 (2C), 54.6, 52.5

(2C), 51.2, 48.6, 45.6, 30.2 (2C). ESI-MS *m/z:*466 $(M+H)^+$. Anal Calcd for $C_{25}H_{31}N_5O_4$: C, 64.50; H, 6.71; N, 15.04; Found: C, 64.55; H, 6.69; N, 15.09.

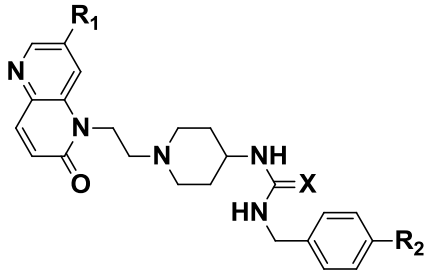
5.1.4. *In vitro* supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were evaluated for their *in vitro* supercoiling assay for the derivation of SAR and lead optimization. The compounds were further subjected to a whole cell screening against *Mtb* $H_{37}Rv$ strain to understand their bactericidal potency using the agar dilution method and later the safety profile of these molecules were evaluated by checking the *in vitro* cytotoxicity against RAW 264.7 cell line (mouse macrophage) at 50 µM concentration by MTT assay, and the results are shown in **Table 5.2.**

Table 5.2: *In vitro* biological evaluation of the synthesized compounds **BKA_13** – **BKA_52**



BKA_13- BKA_40



BKA_41 - BKA_52

Cmpd.	R_1	X	R_2	MTB Supercoiling assay (IC_{50}) (µM)	MTB MIC (µM)	RAW264.7 Cytotoxicity at 50 µM (% inhib.)
BKA_13	F	S	H	0.5±0.22	1.83	31.68
BKA_14	F	S	F	7.1±0.35	6.80	29.78
BKA_15	F	S	Cl	3.72±0.25	8.52	28.02
BKA_16	F	S	NO_2	0.28±0.14	0.91	24.09
BKA_17	F	S	$COCH_3$	11.3±0.67	21.22	30.02
BKA_18	F	S	OCH_3	1.8±0.34	1.71	26.09
BKA_19	F	S	CH_3	3.6±0.72	3.54	32.54
BKA_20	F	O	H	0.44±0.1	1.90	34.47
BKA_21	F	O	F	3.12±0.66	3.64	27.11
BKA_22	F	O	Cl	0.3±0.05	1.29	21.91
BKA_23	F	O	NO_2	0.25±0.09	3.77	32.34
BKA_24	F	O	$COCH_3$	0.2±0.15	2.91	31.75

BKA_25	F	O	OCH_3	2.2±0.55	3.23	17.75
BKA_26	F	O	CH_3	0.67±0.12	1.84	25.39
BKA_27	OCH_3	S	H	5.2±0.89	7.14	31.37
BKA_28	OCH_3	S	F	4.1±0.43	3.42	31.21
BKA_29	OCH_3	S	Cl	2.3±0.69	1.65	24.64
BKA_30	OCH_3	S	NO_2	3.9±0.25	6.46	23.84
BKA_31	OCH_3	S	$COCH_3$	3.1±0.42	6.89	32.97
BKA_32	OCH_3	S	OCH_3	8.6±0.24	13.19	28.18
BKA_33	OCH_3	S	CH_3	3.8±0.17	3.45	31.19
BKA_34	OCH_3	O	H	10.4±0.44	14.82	35.46
BKA_35	OCH_3	O	F	0.078±0.02	0.62	11.94
BKA_36	OCH_3	O	Cl	0.092±0.05	1.85	21.15
BKA_37	OCH_3	O	NO_2	1.91±0.3	3.94	30.05
BKA_38	OCH_3	O	$COCH_3$	11.6±0.78	6.60	30.16
BKA_39	OCH_3	O	OCH_3	0.2±0.06	3.78	21.28
BKA_40	OCH_3	O	CH_3	10.9±0.72	30.30	31.89
BKA_41	F	S	H	1.56±0.31	7.10	35.49
BKA_42	F	S	Cl	1.9±0.77	1.64	35.56
BKA_43	F	S	OCH_3	0.4±0.43	1.66	38.57
BKA_44	F	O	H	1.5±0.31	7.37	19.64
BKA_45	F	O	Cl	2.9±0.23	3.70	38.36
BKA_46	F	O	OCH_3	0.41±0.2	1.89	23.64
BKA_47	OCH_3	S	H	1.87±0.54	7.50	28.21
BKA_48	OCH_3	S	Cl	1.53±0.22	1.60	25.12
BKA_49	OCH_3	S	OCH_3	3.2±0.81	12.97	13.75
BKA_50	OCH_3	O	H	1.87±0.26	13.42	23.86
BKA_51	OCH_3	O	Cl	0.45±0.04	1.65	36.52
BKA_52	OCH_3	O	OCH_3	2.76±0.35	3.35	13.66
Moxifloxacin				11.2±0.23	2.4	ND
Novobiocin				46±28nM	>200	19.3
Ethambutol				NT	9.84	ND

IC_{50}, 50% inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; NT, not tested; nM, nanomolar

Mtb DNA gyrase supercoiling enzyme inhibition activity

In vitro activity against *Mtb* $H_{37}Rv$

Cytotoxicity against RAW 264.7 cells (mouse macrophage cell line)

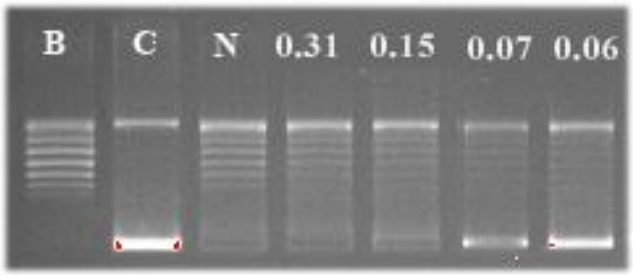


Fig.5.2: Depicting the supercoiling assay picture of compound **BKA_35** at four different concentrations of 0.31, 0.15, 0.07, 0.06 µM and novobiocin as standard where R-Relaxed DNA substrate +DMSO; C- Relaxed DNA substrate

5.1.5. SAR and discussion

All the forty compounds synthesized were screened for their enzyme inhibition studies using MTB DNA gyrase kit (Inspiralis, Norwich). Preliminary screening was performed at concentrations of 500, 125, 31.3, 7.8, and 1.95 µM and those which were active were further tested at 250, 62.5, 15.6, 3.9 and 0.97 µM concentrations. Finally molecules that exhibited more than 60% inhibitory activity at 0.97 µM were further screened at lower concentrations of 0.48, 0.24, 0.12, and 0.06 µM to ensure the activity profile. Among the entire series of forty compounds, thirteen compounds showed IC_{50}s less than 0.97 µM. While the most active compound **BKA_35** showed an IC_{50} of 78 nM which possessed electronegative group (fluorine) and the chlorine compound **BKA_36** exhibited an IC_{50} of 92 nM which indicated that the substitution of electronegative groups at the para position of the phenyl ring and the most electropositive methoxy group at 7^{th} position of 1,5-naphthyridin-2(1H)-one could ensure excellent inhibitory property. Compounds **BKA_34** and **BKA_40** with hydrogen and methyl group at para position of phenyl group exhibited IC_{50} of about 10 µMand lost their inhibitory potential by a factor of hundred, thus marking the importance of the electronegative groups at this position. Eleven other compounds (**BKA_13, BKA_16, BKA_20, BKA_22-BKA_24, BKA_26, BKA_39, BKA_43, BKA_46 and BKA_51**) showed IC_{50}s in between 0.2 and 0.6µM which signified the importance of amino-piperidine moiety in the inhibition of DNA gyrase enzyme. Novobiocin and moxifloxacin were considered as positive controls as they have been shown to be potent inhibitors of DNA supercoiling of mycobacterial DNA gyrase. All the compounds in this study showed better enzyme supercoiling inhibitions compared to standard

moxifloxacin drug whose IC_{50} was 11.2 µM and has been considered as one of the potent DNA gyrase inhibitor till date. Similarly the other broad spectrum standard drug novobiocin showed an IC_{50} of 46 nM. Dose-dependent inhibition profile of the most active compound **BKA_35** at different inhibitor concentrations along with standard novobiocin is illustrated in **Fig 5.2.**

The compounds were further screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strain by microplate Alamar blue assay method. As these compounds were mostly hydrophilic, the *in vitro* antimycobacterial activities were in commendable range of 0.49-30.3 µM. Ethambutol (MIC: 15.31 µM), Isoniazid (MIC: 0.66 mM), Moxifloxacin (MIC: 1.2 µM) and Novobiocin (MIC: >200 µM) were considered as standard drugs for comparison in this assay. Compared to the first-line antitubercular drugs like Ethambutol and Isoniazid, the most active compound **BKA_35** showed a better MIC of 0.62 µM. Twelve compounds (**BKA_13, BKA_16, BKA_20, BKA_22, BKA_26, BKA_29, BKA_36, BKA_42-BKA_43, BKA_46, BKA_48** and **BKA_51**) exhibited good antimycobacterial inhibitory profiles with MIC less than 2 µM. These compounds could be considered as promising antitubercular leads with best *in vitro* DNA gyrase enzyme inhibitory profile.

The eukaryotic cell safety profile of all the compounds was observed by testing there *in vitro* cytotoxicity against RAW 264.7 cell (Mouse leukemic monocyte macrophage cell line) at 50 µM concentration by using 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide (MTT) assay. All 40 molecules showed lesser cytotoxicity within a range of 11-38 % as shown in **Table 5.2.** The most promising anti-TB compound **BKA_35** showed only 11.9 % cytotoxicity which was within the safety profile limit. The R and R' dimethoxy substituted groups comparatively showed lesser toxicity when compared to other compounds in the series. Novobiocin was used as standard that exhibited 19.36 % inhibition.

5.1.6. Highlights of the study

By combining molecular hybridization strategy with biological assays we could successfully re-engineer the previously reported antibacterial leads that exhibited promising attributes of synthetic accessibility, excellent *in vitro* enzyme inhibition profiles, and antitubercular activity. A series of 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives were designed by molecular hybridization strategy and synthesized nine step reaction combining

various ureas and thioureas to yield activity in low nanomolar range and commendable antibacterial activities. Compound **BKA_35** (1-(4-fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea) emerged as the most promising inhibitor with an IC_{50} of 78 nM against *Mycobacterium tuberculosis* DNA gyrase enzyme, with a good MTB MIC of 0.62 µM, and not cytotoxic at a higher concentration of 50 µM in eukaryotic cell line.

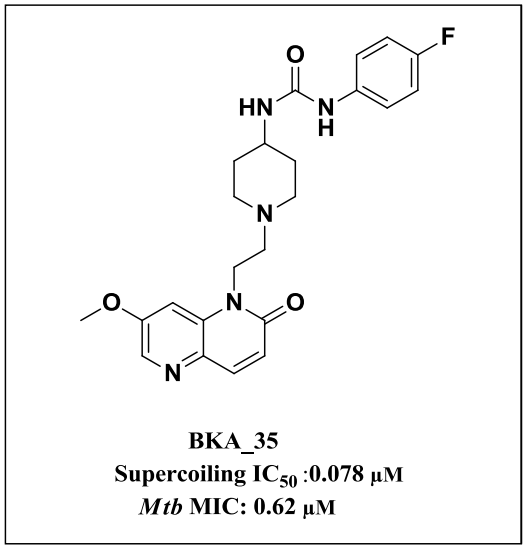


Fig 5.3: Chemical structure and biological activity of the most active compound **BKA_35**

5.2. Design, synthesis and biological evaluation of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives as potent *Mycobacterium tuberculosis* DNA Gyrase inhibitors

5.2.1. Design of the molecules

The chemical structures of NBTIs comprise a bicyclic heteroaromatic left-hand side (LHS) ring, a mono- or bicyclic hydrophobic right-hand side (RHS) ring and a middle aminopiperidine linker part (**figure 1**). As per literature, it is reported that aminopiperidines having a bicyclic aromatic moiety, generally show potent broad-spectrum antibacterial activity, but usually suffer from potent hERG inhibition leading to hERG toxicity [Reck, F., *et al*., 2011 and 2012]. In order to overcome this toxicity issues, we have made an attempt to synthesize simple piperazine linker replacing the aminopiperidine as shown in **figure 5.4** resulting in no/reduced hERG channel inhibition

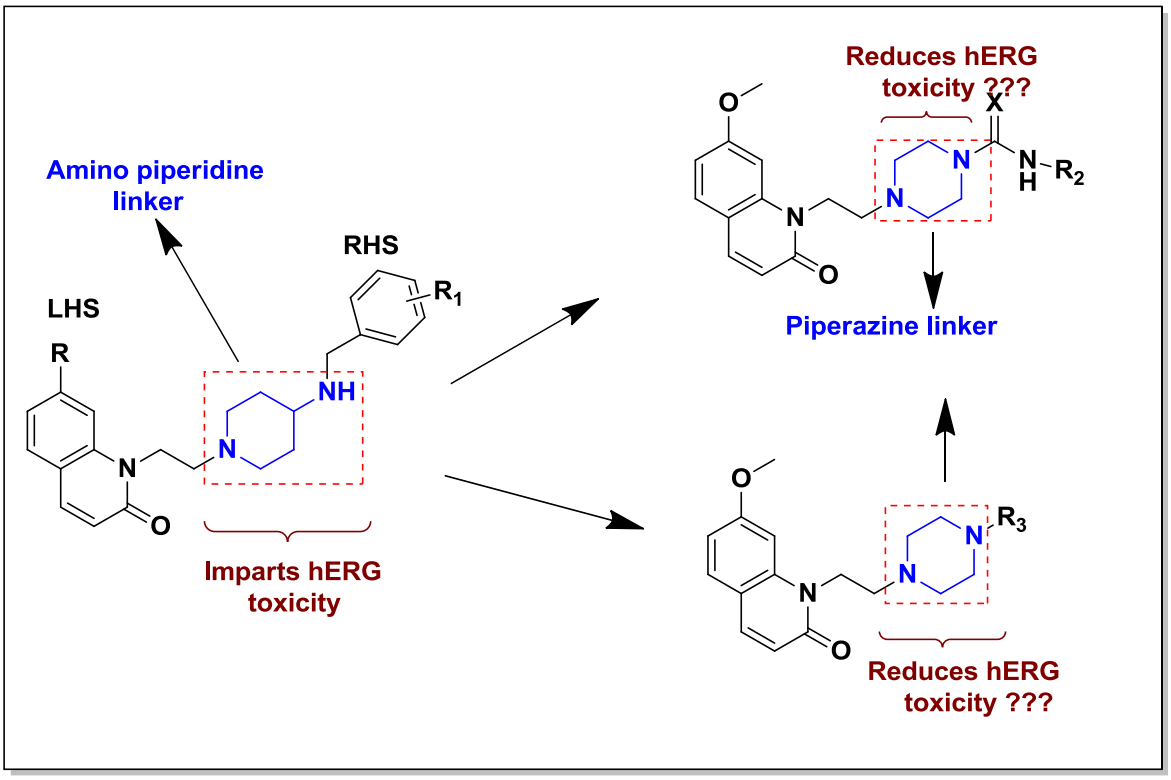


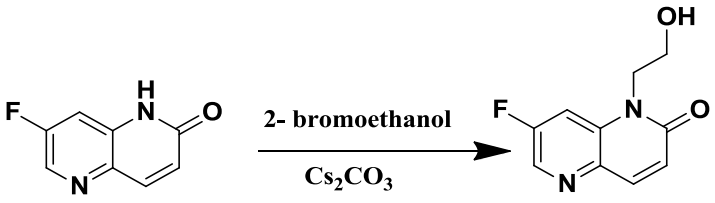
Fig.5.4: Strategy employed for designing the piperazine based linker from the previously reported aminopiperidines [Bobesh, K. A., *et al*., 2014, Hameed P, S., *et al*., 2014 and 2015].

5.2.2. Experimental procedures utilized for the synthesis of PZ_02 – PZ_30

To synthesize the designed compounds we followed the multistep synthetic protocol started with the preparation of 7-fluoro1,5-naphthyridin-2(1H)-one (**PZ_1**). The formation of naphthopyridinone core (**PZ_1**) was achieved via Heck coupling, which was reported in our earlier paper [Bobesh, K. A., *et al.*, 2014]. 7-fluoro1,5-naphthyridin-2(1H)-one (**PZ_1**) alkylated at the N-1 position with bromoethanol using Cs_2CO_3 as base resulted in the formation of 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (**PZ_2**). Conversion of hydroxyl group in compound (**PZ_2**) to easily leaving group triflate was achieved using trifluoromethane sulphonic anhydride and pyridine. Introduction of linker piperazine to compound (**PZ_3**) was achieved by condensation with 1-boc piperazine to yield compound (**PZ_4**). As methoxy group at 7^{th} position showed good activity in our earlier work, nucleophilic displacement of fluoro to methoxy group was achieved by sodium methoxide and methanol at reflux temperature, and resulted in compound (**PZ_5**). Boc deprotection of compound (**PZ_5**) using dioxane/HCl to

afford scaffold 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_6**) [Bobesh, K. A., *et al*., 2014]. The final library of 24 compounds were synthesized by treating the scaffold (**PZ_6**) with corresponding isocyanates or isothiocyanates to get the urea and thio urea derivatives (**PZ_7–PZ_24**) and with aldehydes to get reductive amination product (**PZ_25–PZ_30**) [Dangerfield, E. M., *et al*., 2010] in good yield. We have selected particular isocyanate, isothiocyanate and aldehyde based on the activity from our earlier paper with aminopiperidines.

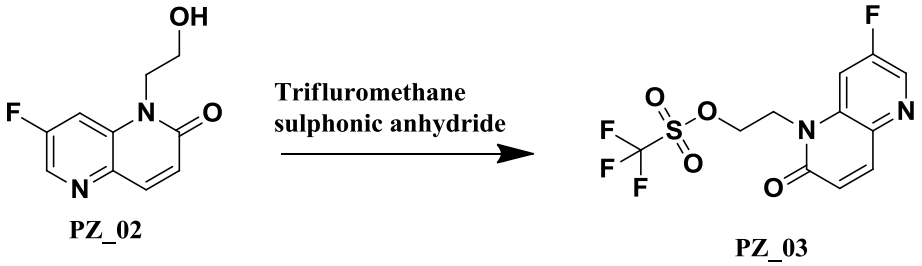
Preparation of 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (PZ_02)



PZ_01 **PZ_02**

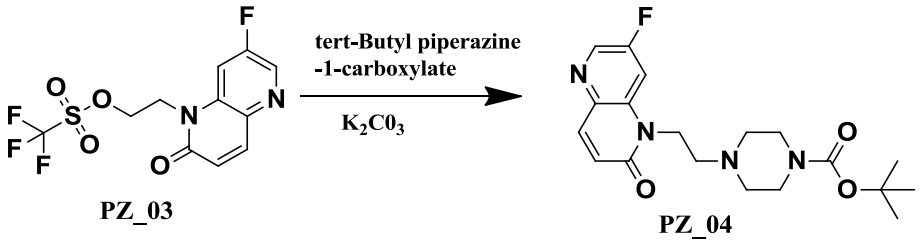
To a solution of corresponding 7-fluoro-1,5-naphthyridin-2(1H)-one (**PZ_01**) (1g , 6.09 mmol) (1 eq) in dry N,N' dimethyl formamide (10 mL) was added Cs_2CO_3 (4.95 g, 15.23 mmol) (2.5 eq) and followed by 2- bromoethanol (0.913 g, 7.31 mmol) (1.2 eq) and heated in sealed tube at 120 ^{0}C for 4 h (monitored by TLC & LCMS for completion). The reaction mixture was then filtered through celite and washed with dichloromethane. The filtrate was concentrated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (40 %): ethyl acetate (60 %) as eluent to give **PZ_02** (0.953 g, 75.6 %) as pale yellow solid. ^{1}H NMR [300 MHz, DMSO-d_6] δ_H: 8.37 (d, J = 2.6 Hz, 1H), 8.13 (s, 1H), 7.86 (d, J = 9.6 Hz, 1H), 7.51 (s, 1H), 6.67 (d, J = 9.6 Hz, 1H), 4.28 (t, J=6 Hz, 2H), 3.68 (t, J = 6 Hz, 2H). ^{13}C NMR [DMSO-d_6] δ_c: 162.3, 157.8, 147.5, 140.1, 132.3, 125.6, 123.2, 110.1, 64.5, 46.2. ESI-MS *m/z*: 209.24 $(M+H)^+$. Anal Calcd for $C_{10}H_9FN_2O_2$: C, 57.69; H, 4.36; N, 13.46; Found: C, 57.72; H, 4.38; N, 13.45.

Preparation of 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl Trifluoromethanesulfonate (PZ_03)



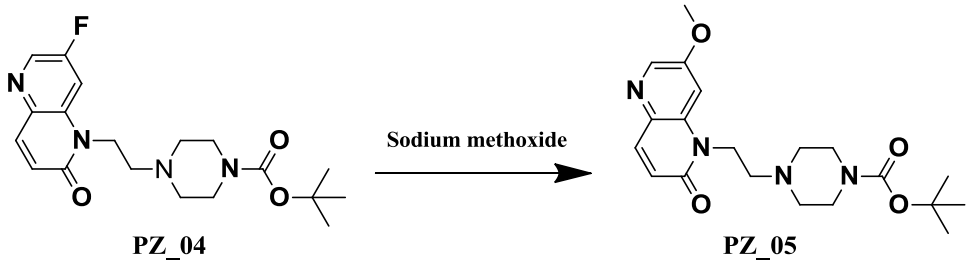
A solution of 7-fluoro-1-(2-hydroxyethyl)-1,5-naphthyridin-2(1H)-one (**PZ_02**) (1 g, 4.80 mmol) (1 eq) in dichloromethane (10 mL) and pyridine (1.45 g, 14.41 mmol) (3 eq) was cooled to -20 ^{0}C. and stirred for 10 mts. To this was added a solution of trifluoromethanesulphonic anhydride (1.49. g, 5.28 mmol) (1.1 eq) in dichloromethane (10 mL) drop wise. The resultant reaction mixture was stirred at the same temperature for half an hour. Completion of the reaction was confirmed by TLC. Reaction mixture was then warmed to room temperature and given sodium bicarbonate (3 × 20 mL**)**, water (3 × 20 mL) and brine (3 × 20 mL) washes. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to get a brownish thick liquid. The crude product was purified by silica gel column chromatography using hexane (80 %) : ethyl acetate (20 %) as eluent to give **PZ_03** (1.23 g, 73.6 %) as Yellow oil. ^{1}H NMR [DMSO-d_6]: δ_H: 8.36 (d, J = 2.6 Hz, 1H), 7.89 (d, J = 9.6 Hz, 1H), 7.49 (s, 1H), 6.68 (d, J =9.6 Hz, 1H), 4.26 (t, J = 6 Hz, 2H), 3.71 (t, J = 6 Hz, 2H). ^{13}C NMR [DMSO-d_6] δ_c: 162.8, 157.8, 145.2, 140.2, 132.9, 125.6, 123.5, 119.2, 110.1, 61.2, 42.5. ESI-MS *m/z*: 341.14 $(M+H)^+$. Anal Calcd for $C_{11}H_8F_4N_2O_4S$: C, 38.83; H, 2.37; N, 8.23; Found: C, 38.89; H, 2.35; N, 8.25.

Preparation of *tert*-butyl 4-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxylate (PZ_04)



To a solution of corresponding 2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl trifluoromethanesulfonate (**PZ_03**) (1 g, 2.93 mmol) (1 eq) in dry N,N' dimethyl formamide (10 mL) was added K_2CO_3 (1.22 g, 8.82 mmol) (3 eq) and followed by *tert*-Butyl piperazine-1-carboxylate (0.656 g, 3.52 mmol) (1.2eq) and was heated in sealed tube at 100 ^{0}C for 4 h (monitored by TLC & LCMS for completion). The reaction mixture was then filtered through celite and washed with dichloromethane. The filtrate was concentrated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (60) : ethyl acetate (40) as eluent to **PZ_04** (0.67 g, 60 %) as yellow solid. ^{1}H NMR [DMSO-d_6]: δ_H: 8.39 (d, J = 2.6 Hz, 1H), 7.87 (d, J = 9.6 Hz, 1H), 7.48(s, 1H), 6.69 (d, J = 9.6 Hz, 1H), 4.41 (t, J = 6.8 Hz, 2H), 3.34(t, J = 4 Hz, 4H), 2.59–2.48 (m, 6H), 1.41 (s, 9H). ^{13}C NMR [DMSO-d_6] δ_c: 162.3, 155.8, 155.2, 143.2, 140.1, 131.5, 124.6, 123.1, 110.2, 80.2, 58.2 (2C), 51.5, 50.2, 45.5 (2C), 28.2 (3C).ESI-MS *m/z* : 377.27 $(M+H)^+$. Anal Calcd for $C_{19}H_{25}FN_4O_3$: C, 60.62; H, 6.69; N, 14.88; Found: C, 60.70; H, 6.70; N, 14.90.

Preparation of *tert*-butyl 4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxylate (PZ_05).

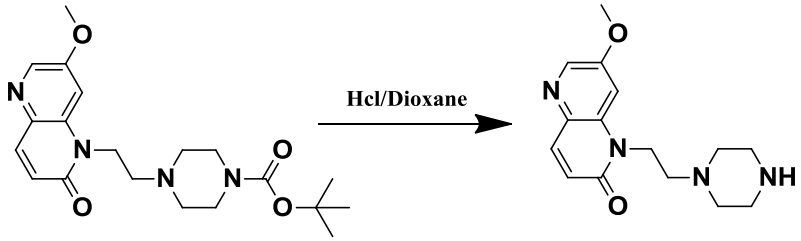


PZ_04 PZ_05

A solution of corresponding *tert*-butyl 4-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxylate (**PZ_04**) (2.0 g, 5.12 mmol) (1 eq) in methanol (10 mL) at room temperature was added sodium methoxide (0.556 g, 10.2 mmol) (2 eq). The reaction mixture was heated at 65 ^{0}C for 3 h (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The reaction mixture was further extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (2 × 40 mL) and water (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude residue

was purified by silica gel column chromatography using hexane (50 %) : ethylacetate (50 %) as eluent to give **PZ_05** (1.22 g, 61 %) as pale yellow solid. ^{1}H NMR [DMSO-d_6]: δ_H 8.23 (d, J = 2 Hz, 1H), 7.85 (d, J = 9.6 Hz, 1H), 7.45 (s, 1H), 6.69 (d, J = 9.6 Hz, 1H), 4.42 (t, J = 6.8 Hz, 2H), 4.01 (s, 3H), 3.32 (t, J = 4 Hz, 4H), 2.58-2.47 (m, 6H), 1.41 (s, 9H). ^{13}C NMR [DMSO-d_6] δ_c: 162.5, 155.7, 154.2, 143.2, 136.1, 131.5, 125.6, 117.1, 105.2, 80.3, 58.2 (2C), 56.2, 52.5, 50.2, 45.8 (2C), 28.6 (3C). ESI-MS *m/z:* 389.51 $(M+H)^+$. Anal Calcd for $C_{20}H_{28}N_4O_4$: C, 61.84; H, 7.27; N, 14.42; Found: C, 61.72; H, 7.26; N, 14.40.

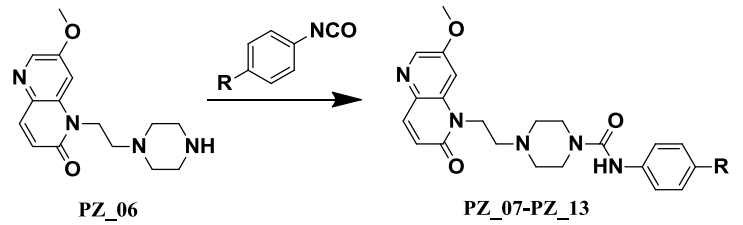
Preparation of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06)**.



PZ_05 **PZ_06**

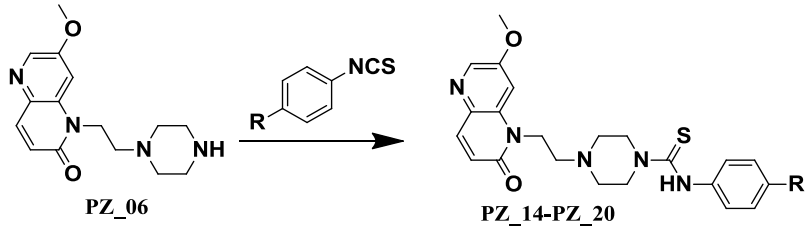
A solution of corresponding substituted *tert*-butyl 4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxylate (**PZ_05**) (2.0g, 5.15 mmol) (1 eq) in dichloromethane (10 mL) was cooled to 0 ^{0}C followed by drop wise addition of HCl in dioxane (10 mL) and stirred for 1 hour. After completion of the reaction (monitored by TLC & LCMS), quenched with ice and concentrated under reduced pressure. The residue was partitioned between ethyl acetate (20 mL) and water (20 mL). Aqueous layer was basified with sodium carbonate solution and extracted with ethyl acetate (3 × 20 mL) and washed with water (3 × 10 mL) and brine (3 × 10 mL). Organic layer was dried over anhydrous sodium sulphate, filtered, and concentrated under reduced pressure to give **PZ_06** as yellow solid. ^{1}H NMR [DMSO-d_6]: δ_H: 8.21 (d, J = 2 Hz, 1H), 7.86 (d, J = 9.6 Hz, 1H), 7.44 (s, 1H), 6.68(d, J = 9.6 Hz, 1H), 4.41 (t, J = 6.8 Hz, 2H), 4.00 (s, 3H), 2.86 (t, J = 4 Hz, 4H), 2.56-2.35 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 162.8, 155.6, 143.6, 136.2, 132.9, 123.4, 119.2, 105.7, 56.2 (2C), 55.6, 51.3, 50.5, 45.2, (2C). ESI-MS *m/z:* 289.33 $(M+H)^+$. Anal Calcd for $C_{15}H_{20}N_4O_2$: C, 62.48; H, 6.99; N, 19.43; Found: C, 62.57; H, 6.98; N, 19.41.

General procedure for the preparation of final compounds PZ_07–PZ_13



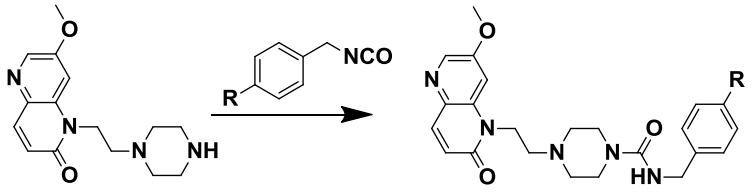
A solution of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one **(PZ_06)** (0.344 mmol) (1 eq) in dichloromethane (3 mL) was cooled to 0 ^{0}C followed by the addition of triethylamine (1.03 mmol) (3 eq). Corresponding substituted phenyl isocyanate (0.416 mmol) (1.2 eq) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was then washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (20 %) : ethyl acetate (80 %) as eluent to give **PZ_07–PZ_013** in good yields.

General procedure for the preparation of final compounds PZ_14–PZ_20



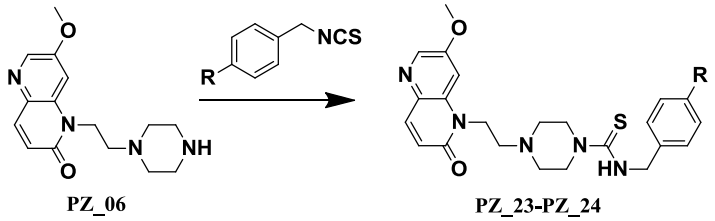
A solution of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.346 mmol) (1 eq) in dichloromethane (3 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (1.03 mmol) (3 eq). Corresponding substituted phenyl isothiocyanate (0.416 mmol) (1.2 eq) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC &LCMS for completion). The reaction mixture was then washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (20 %): ethyl acetate (80 %) as eluent to give (**PZ_14–PZ_20**) in good yield.

General procedure for the preparation of final compounds PZ_21-PZ_22



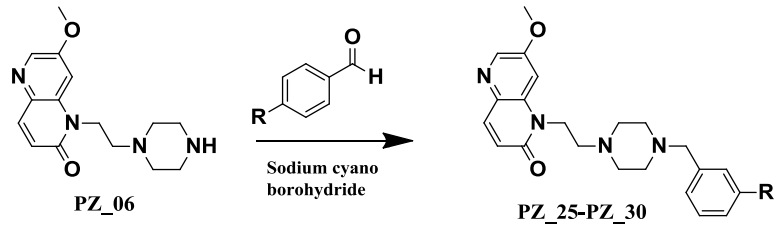
A solution of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) in dichloromethane (1 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (0.99 mmol) (3 eq). Corresponding substituted benzyl isocyanate (0.416 mmol) (1.2 eq) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (30 %) : ethyl acetate (70 %) as eluent to give **PZ_21-PZ_22** in good yield.

General procedure for the preparation of final compounds PZ_23-PZ_24



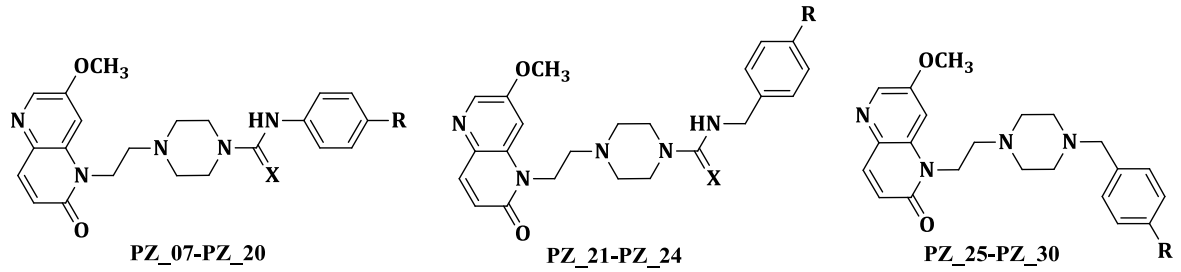
A solution of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.346 mmol) (1 eq) in dichloromethane (3 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (1.02 mmol) (3 eq). Corresponding substituted benzyl isothiocyanate (0.416 mmol) (1.2 eq) was added to the reaction mixture and was stirred in room temperature for 12 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (30 %) : ethyl acetate (70 %) as eluent to give (**PZ_23-PZ_24**) in good yields.

General procedure for the preparation of final compounds PZ_25–PZ_30



A solution of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.346) (1 eq) in methanol (3 mL) was cooled to 0 ^{O}C followed by the addition of triethylamine (1.02 mmol) (3 eq) and allowed the reaction mixture to stir at room temperature for 10 mts, inorder to neutralize the HCl salt. Corresponding substituted benzaldehyde (0.416 mmol) (1.2) was added to the reaction mixture and was stirred in room temperature for 12 h followed by the addition of sodium cyanoborohydide (0.519 mmol) (1.5 eq) at 0 ^{0}C and allowed the reaction mixture to stand room temperature for 4 h (monitored by TLC & LCMS for completion). The solvent methanol was distilled and the reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (40 %): ethyl acetate (60 %) as eluent to give (**PZ_25–PZ_30**) in good yields.

Table 5.3: Physiochemical properties of the synthesized compounds **PZ_07 – PZ_3**



Compd.	X	R	Yield (%)	Molecular Formula	Molecular Weight	Melting Point (°C
PZ_07	O	H	63	$C_{22}H_{25}N_5O_3$	407.47	185-187

PZ_08	O	F	65	$C_{22}H_{24}FN_5O_3$	425.46	201-203
PZ_09	O	Cl	63	$C_{22}H_{24}ClN_5O_3$	441.91	197-199
PZ_10	O	$COCH_3$	66	$C_{24}H_{27}N_5O_4$	449.50	191-193
PZ_11	O	NO_2	57	$C_{22}H_{24}N_6O_5$	452.46	201-203
PZ_12	O	Br	64	$C_{22}H_{24}BrN_5O_3$	486.36	175-177
PZ_13	O	CH_3	63	$C_{23}H_{27}N_5O_3$	421.49	185-187
PZ_14	S	H	75	$C_{22}H_{25}N_5O_2S$	423.53	192-194
PZ_15	S	F	73	$C_{22}H_{24}FN_5O_2S$	441.52	176-178
PZ_16	S	Cl	63	$C_{22}H_{24}ClN_5O_2S$	457.98	187-189
PZ_17	S	$COCH_3$	70	$C_{24}H_{27}N_5O_3S$	465.57	193-195
PZ_18	S	NO_2	65	$C_{22}H_{24}N_6O_4S$	468.53	199-201
PZ_19	S	Br	72	$C_{22}H_{24}BrN_5O_2S$	502.43	211-213
PZ_20	S	CH_3	65	$C_{23}H_{27}N_5O_2S$	437.56	184-186
PZ_21	O	OCH_3	71	$C_{24}H_{29}N_5O$	451.52	162-164
PZ_22	O	Cl	60	$C_{23}H_{26}ClN_5O_3$	455.94	169-171
PZ_23	S	OCH_3	75	$C_{24}H_{29}N_5O_3S$	467.58	153-155
PZ_24	S	Cl	60	$C_{23}H_{26}ClN_5O_2S$	472.00	166-168
PZ_25	-	H	62	$C_{22}H_{26}N_4O_2$	378.47	221-223
PZ_26	-	Cl	54	$C_{22}H_{25}ClN_4O_2$	412.91	234-236
PZ_27	-	F	60	$C_{22}H_{25}FN_4O_2$	396.46	228-230
PZ_28	-	OCH_3	73	$C_{23}H_{28}N_4O_3$	408.49	214-216
PZ_29	-	NO2	60	$C_{22}H_{25}N_5O_4$	424.43	218-220
PZ_30	-	Br	458.33	$C_{22}H_{25}BrN_4O_2$	457.36	238-240

5.2.3. Characterization of the synthesized molecules

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-phenylpiperazine-1-carboxamide (PZ_07): The compound was synthesized according to the general procedure using 4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-phenylpiperazine-1-carboxamide (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and phenyl isocyanate (0.049 g, 0.416 mmol) (1.2 eq) to afford **PZ_07** (0.088 g, 63 %) as white solid. M.p: 185-187 ^{0}C. ^{1}H NMR [DMSO-

d_6]: δ_H: 8.51 (b, 1H), 8.29 (d, *J* = 2 Hz, 1H), 7.89 (d, *J* = 9.6 Hz, 1H), 7.47 (s, IH), 7.46 (d, *J* = 8.4 Hz, 2H), 7.23 (t, *J* = 7.6 Hz, 2H), 6.94 (t, *J* = 7.2 Hz, 1H), 6.69 (d, *J* = 9.6 Hz, 1H),4.43 (t, *J* = 6.8 Hz, 2H), 4.00 (s, 3H), 3.41 (t, *J* = 4 Hz, 4H), 2.50 – 2.63 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 160.7, 156.3, 154.9, 140.4, 140.1, 137.0, 134.4, 131.1, 128.2 (2C), 121.6, 121.2, 119.5 (2C), 117.8, 104.8, 56.07 (2C), 54.76, 52.82 (2C), 43.72. ESI-MS *m/z:* 408.55 $(M+H)^+$. Anal Calcd for $C_{22}H_{25}N_5O_3$: C, 64.85; H, 6.18; N, 17.19. Found: C, 64.71; H, 6.19; N, 17.16.

N-(4-Fluorophenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxamide (PZ_08): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-fluoro phenyl isocyanate (0.057 g, 0.416 mmol) (1.2 eq) to afford **PZ_08** (0.96 g, 65 %) as white solid. M.p: 201-203 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.64 (b,1H), 8.31 (d, *J* = 2 Hz, 1H), 7.88 (d, *J* = 9.6 Hz, 1H), 7.47 (dd, *J* = 9 Hz, *J* = 2.4 Hz, 2H), 7.43 (s, 1H), 7.28 (dd, *J* = 9 Hz, *J* = 9.6 Hz, 2H), 6.68 (d, *J* = 9.6 Hz, 1H), 4.41 (t, *J* = 6.8 Hz , 2H), 4.02 (s, 3H), 3.42 (t, *J* = 4 Hz, 4H), 2.50 –2.67 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 163.5, 161.6, 157.8, 156.9, 143.9, 136.7, 135.8, 130.2, 125.5, 120.2 (2C), 119.2, 116.8 (2C), 105.8, 55.3 (2C), 54.6, 52.5, 51.5 (2C), 48.8. ESI-MS *m/z:* 426.44 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}FN_5O_3$: C, 62.11; H, 5.69; N, 16.46; Found: C, 62.18; H, 5.68; N, 16.49.

N-(4-Chlorophenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxamide (PZ_09): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-chloro phenyl isocyanate (0.063 g, 0.416 mmol) (1.2 eq) to afford **PZ_09** (0.097 g, 63 %) as brown solid. M.p: 197-199 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.66 (b, 1H), 8.30 (d, *J* = 2 Hz, 1H), 7.89 (d, *J* = 9.6 Hz, 1H), 7.50 (d, *J* = 9.2 Hz, 2H), 7.44 (s, 1H), 7.30 (t, *J* = 9.2 Hz, 2H), 6.69 (d, *J* = 9.6 Hz, 1H), 4.42 (t, *J* = 6.8 Hz , 2H), 4.00 (s, 3H), 3.41 (t, *J* = 4 Hz, 4H), 2. 49–2.65 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 160.8, 156.3, 154.6, 140.3, 139.4, 136.9, 134.4, 131.2 (2C), 128.1, 125.2, 121.2 (2C), 120.9, 104.9, 56.1 (2C), 54.5, 53.5, 52.6 (2C), 43.5. ESI-MS *m/z:* 442.24 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}ClN_5O_3$: C, 59.79; H, 5.47; N, 15.85; Found: C, 59.68; H, 5.45; N, 15.82.

N-(4-Acetylphenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxamide (PZ_10): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-acetyl phenyl isocyanate (0.066 g, 0.416 mmol) (1.2 eq) to afford **PZ_10** (0.105 g, 66 %)as white solid. M.p: 191-193 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.57 (bs, 1H), 8.28 (d, *J* = 2 Hz, 1H), 7.93 (d, *J* = 9.6 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.45 (s, 1H), 7.26 (t, *J* = 8.2 Hz, 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 4.41 (t, *J* = 6.8 Hz, 2H), 4.01 (s, 3H), 3.41 (t, *J* = 4 Hz, 4H), 2.47 – 2.61 (m, 6H), 2.45 (s, 3H). ^{13}C NMR [DMSO-d_6] δ_c: 196.1, 161.2, 155.8, 154.2, 144.1, 143.2, 135.9, 134.7, 132.3, 130.1 (2C), 124.5, 120.2 (2C), 119.2, 105.8, 55.6 (2C), 54.8, 51.9, 51.6 (2C) 50.4, 26.3. ESI-MS *m/z*: 450.25 $(M+H)^+$. Anal Calcd for $C_{24}H_{27}N_5O_4$: C, 64.13; H, 6.05; N, 15.58; Found: C, 64.05; H, 6.06; N, 15.60.

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(4-nitrophenyl)piperazine-1-carboxamide (**PZ_11**): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1eq) and 4-nitro phenyl isocyanate (0.068 g, 0.416 mmol) (1.2 eq) to afford **PZ_11** (0.090 g, 57 %) as yellow solid. M.p: 201-203 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.69 (b, 1H), 8.33 (d, *J* = 2 Hz, 1H), 7.96 (d, *J* = 9.6 Hz, 1H), 7.63 (d, *J* = 9.4 Hz, 2H), 7.44 (s, 1H), 7.33 (t, J = 9.4 Hz, 2H), 6.73 (d, *J* = 9.6 Hz, 1H), 4.44 (t, *J* = 6.8 Hz , 2H), 4.02 (s, 3H), 3.42 (t, *J* = 4 Hz, 4H), 2.51 – 2.65 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 161.5, 155.6, 154.8, 146.3, 143.3, 142.9, 136.7, 132.1, 124.2, 123.9 (2C), 120.2 (2C), 117.6, 105.5, 55.5 (2C), 54.6, 52.1, 51.8 (2C), 50.1. ESI-MS *m/z*: 453. 43 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}N_6O_5$: C, 58.40; H, 5.35; N, 18.57; Found: C, 58.52; H, 5.37; N, 18.55.

N-(4-Bromophenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxamide (PZ_12): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-bromo phenyl isocyanate (0.082 g, 0.416 mmol) (1.2 eq) to afford **PZ_12** (0.13 g, 64 %)as brown solid. M.p: 175-177 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.61 (b, 1H), 8.30 (d, *J* = 2 Hz, 1H), 7.88 (d, *J* = 9.6 Hz, 1H), 7.48 (d, *J* = 9 Hz , 2H), 7.45 (s, 1H), 7.29 (t, *J* = 9 Hz, 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 4.41 (t, *J* = 6.8 Hz , 2H), 4.01 (s, 3H), 3.40 (t, *J* = 4 Hz, 4H), 2.49 – 2.64 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 162.5, 155.6, 154.9, 143.5, 139.6, 136.2, 132.3 (2C),

131.9, 125.6, 121.5, 120.2 (2C), 119.6, 105.8, 56.5 (2C), 55.8, 53.6, 51.9 (2C), 50.6. ESI-MS *m/z:* 486.31 $(M)^+$. Anal Calcd for $C_{22}H_{24}BrN_5O_3$: C, 54.33; H, 4.97; N, 14.40; Found: C, 54.42; H, 4.96; N, 14.42.

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(p-tolyl)piperazine-1-carboxamide (PZ_13): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-methyl phenyl isocyanate (0.055 g, 0.416 mmol) (1.2 eq) to afford **PZ_13** (0.092 g, 63 %) as brown solid. M.p: 185-187 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.48 (b, 1H), 8.27 (d, *J* = 2 Hz, 1H), 7.85 (d, *J* = 9.6 Hz, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.44 (s, 1H), 7.22 (t, *J* = 7.8 Hz, 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 4.41 (t, *J* = 6.8 Hz, 2H), 4.00 (s, 3H), 3.40 (t, *J* = 4 Hz, 4H), 2.47 – 2.62 (m, 6H), 2.39 (s, 3H). ^{13}C NMR [DMSO-d_6] δ_c: 162.5, 156.1, 153.4, 142.3, 135.9, 135.2, 134.9, 132.1, 130.2 (2C), 125.6, 120.5 (2C), 119.2, 105.8, 55.6 (2C), 54.6, 53.5, 51.9 (2C) 50.2, 20.9. ESI-MS *m/z:* 422.37 $(M+H)^+$. Anal Calcd for $C_{23}H_{27}N_5O_3$: C, 65.54; H, 6.46; N, 16.62. Found: C, 65.42; H, 6.47; N, 16.60.

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-phenylpiperazine-1-carbothioamide (PZ_14): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and phenyl isothiocyanate (0.056g, 0.416 mmol) (1.2 eq) to afford **PZ_14** (0.11 g, 75 %) as white solid. M.p: 192-194 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.26 (d, *J* = 2 Hz, 1H), 7.77 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.21 (t, *J* = 7.4 Hz , 2H), 6.88 (t, *J* = 7 Hz, 1H), 6.66 (d, *J* = 9.6 Hz, 1H), 6.53 (b, 1H),4.42 (t, *J* = 6.8 Hz , 2H), 4.01 (s, 3H), 3.42 (t, *J* = 4 Hz, 4H), 2.48 – 2.61 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 179.9, 162.5, 155.8, 143.2, 139.6, 136.5, 132.8, 130.3 (2C), 129.7, 125.7 (2C), 124.8, 119.5, 105.2, 56.6 (2C), 55.3 (2C), 54.2, 52.8 50.5. ESI-MS *m/z*: 424.49 $(M+H)^+$. Anal Calcd for $C_{22}H_{25}N_5O_2S$: C, 62.39; H, 5.95; N, 16.54. Found: C, 62.48; H, 5.96; N, 16.56.

N-(4-Fluorophenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carbothioamide (PZ_15): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-fluoro phenyl isothiocyanate (0.063 g, 0.316 mmol) (1.2 eq) to afford **PZ_15** (0.112 g, 73 %) as pale brown solid. M.p: 176-178 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.30

(d, *J* = 2 Hz, 1H), 7.89 (d, *J* = 9.6 Hz, 1H), 7.45 (s, 1H), 7.33 (dd, *J* = 9 Hz, *J* = 9.4 Hz, 2H), 7.18 (dd, *J* = 9 Hz, *J* = 2.4 Hz, 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 6.53 (b, 1H), 4.40 (t, *J* = 6.8 Hz, 2H), 4.01 (s, 3H), 3.41 (t, *J* = 4 Hz, 4H), 2. 51–2.68 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 179.9, 164.5, 162.7, 155.8, 143.3, 136.2, 134.6, 132.5, 130.8 (2C), 125.6, 119.8, 116.2 (2C), 105.5, 56.8 (2C), 55.7 (2C), 54.5, 51.9, 50.5. ESI-MS *m/z:* 442.57 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}FN_5O_2S$: C, 59.85; H, 5.48; N, 15.86. Found: C, 59.72; H, 5.49; N, 15.85.

N-(4-Chlorophenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carbothioamide (**PZ_16**): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-chloro phenyl isothiocyanate (0.07 g, 0.416 mmol) (1.2 eq) to afford **PZ_16** (0.098 g, 63 %) as white solid. M.p: 187-189 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.26 (d, *J* = 2 Hz, 1H), 7.75 (d, *J* = 9.6 Hz, 1H), 7.45 (s, 1H), 7.38 (d, *J* = 9 Hz, 2H), 7.21 (t, *J* = 9 Hz , 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 6.53 (b, 1H), 4.41 (t, *J* = 6.8 Hz , 2H), 4.01 (s, 3H), 3.40 (t, *J* = 4 Hz, 4H), 2.49 – 2.64 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 179.9, 162.3, 155.6, 142.8, 135.6, 134.2, 133.2, 130.9, 129.5 (2C), 128.5 (2C), 125.6, 119.2, 105.6, 56.5, (2C), 55.2 (2C), 54.5, 53.2, 50.8. ESI-MS *m/z:* 458.31 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}ClN_5O_2S$: C, 57.70; H, 5.28; N, 15.29. Found: C, 57.79; H, 5.27; N, 15.27.

N-(4-Acetylphenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carbothioamide (PZ_17): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-acetyl phenyl isothiocyanate (0.073g, 0.416 mmol) (1.2 eq) to afford **PZ_17** (0.113 g, 70 %) as white solid. M.p: 193-195 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.25 (d, *J* = 2 Hz, 1H), 7.88 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.21 (t, *J* = 8 Hz , 2H), 6.66 (d, *J* = 9.6 Hz, 1H), 6.55 (b, 1H), 4.40 (t, *J* = 6.8 Hz , 2H), 4.00 (s, 3H), 3.51 (t, *J* = 4 Hz, 4H), 2.48 –2.59 (m, 6H), 2.46 (s, 3H). ^{13}C NMR [DMSO-d_6] δ_c: 196.8, 179.5, 162.3, 155.3, 143.9, 142.5, 136.5, 134.2, 132.8, 130.2 (2C), 125.2 (2C), 123.9, 119.2, 105.8, 56.5 (2C), 55.8 (2C), 54.2, 51.5, 50.5, 25.3. ESI-MS *m/z*: 466.52 $(M+H)^+$. Anal Calcd for $C_{24}H_{27}N_5O_3S$: C, 61.92; H, 5.85; N, 15.04; Found: C, 61.85; H, 5.83; N, 15.03

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(4-nitrophenyl)piperazine-1-carbothioamide (PZ_18): The compound was synthesized according to the general procedure

using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-nitro phenyl isothiocyanate (0.074 g, 0.416 mmol) (1.2 eq) to afford **PZ_18** (0.105 g, 65 %) as yellow solid. M.p: 199-201 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.31 (d, *J* = 2 Hz, 1H), 7.89 (d, *J* = 9.6 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.44 (s, 1H), 7.31 (t, *J* = 9.2 Hz , 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 6.59 (b, 1H), 4.43 (t, *J* = 6.8 Hz , 2H), 4.01 (s, 3H), 3.49 (t, *J* = 4 Hz, 4H), 2.50 –2.64 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 179.5, 162.3, 155.3, 146.4, 145.3, 143.1, 136.6, 132.9, 125.4 (2C), 125.1, 124.8 (2C), 119.1, 105.3, 56.5 (2C), 55.8 (2C) 54.5, 51.8, 50.5. ESI-MS *m/z*: 469.25 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}N_6O_4S$: C, 56.40; H, 5.16; N, 17.94. Found: C, 56.51; H, 5.14; N, 17.93.

N-(4-Bromophenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carbothioamide (**PZ_19**): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-bromo phenyl isothiocyanate (0.89g, 0.416 mmol) (1.2 eq) to afford **PZ_19** (0.126 g, 72 %) as white solid. M.p: 211-213 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.27 (d, *J* = 2 Hz, 1H), 7.86 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.38 (d, *J* = 9 Hz, 2H), 7.22 (t, *J* = 8.8 Hz , 2H), 6.66 (d, *J* = 9.6 Hz, 1H), 6.54 (b, 1H), 4.40 (t, *J* = 6.8 Hz , 2H), 4.0 (s, 3H), 3.50 (t, *J* = 4 Hz, 4H), 2.47 – 2.62 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 179.3, 162.4, 155.6, 142.3, 138.2, 136.5, 132.9 (2C), 132.2, 131.5 (2C), 125.6, 122.6, 119.1, 105.6, 56.8 (2C), 55.4 (2C), 54.8, 52.7, 50.3. ESI-MS *m/z:* 502.19 $(M)^+$. Anal Calcd for $C_{22}H_{24}BrN_5O_2S$: C, 52.59; H, 4.81; N, 13.94. Found: C, 52.65; H, 4.80; N, 13.96.

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(p-tolyl)piperazine-1-carbothioamide (**PZ_20**): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-methyl phenyl isothiocyanate (0.062g, 0.416 mmol) (1.2 eq) to afford **PZ_20** (0.099 g, 65 %) as white solid. M.p: 184-186 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.24 (d, *J* = 2 Hz, 1H), 7.84 (d, *J* = 9.6 Hz, 1H), 7.45 (s, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.17 (t, *J* = 8.4 Hz , 2H), 6.65 (d, *J* = 9.6 Hz, 1H), 6.52 (b, 1H), 4.40 (t, *J* = 6.8 Hz , 2H), 4.01 (s, 3H), 3.41 (t, *J* = 4 Hz, 4H), 2.44 – 2.61 (m, 6H), 2.40 (s, 3H). ^{13}C NMR [DMSO-d_6] δ_c: 179.2, 162.3, 155.6, 142.3, 138.2, 136.3, 135.9, 132.1, 130.3 (2C), 125.8 (2C), 123.4, 119.2, 105.3, 56.4 (2C), 55.8 (2C),

54.8, 51.9, 50.1, 20.8. ESI-MS *m/z:* 438.46 $(M+H)^+$. Anal Calcd for $C_{23}H_{27}N_5O_2S$: C, 63.13; H, 6.22; N, 16.01. Found: C, 63.02; H, 6.20; N, 16.03.

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(4-methoxybenzyl)piperazine-1-carboxamide (PZ_21): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-methoxy benzyl isocyanate (0.067 g, 0.416 mmol) (1.2 eq) to afford **PZ_21** (0.110 g, 71 %) as brown solid. M.p: 162-164 °C. ^{1}H NMR [DMSO-d_6]: δ_H 8.46 (b, 1H), 8.25 (d, *J* = 2 Hz, 1H), 7.79 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.41 (d, *J* = 8 Hz, 2H), 7.19 (t, *J* = 7.8 Hz , 2H), 6.68 (d, *J* = 9.6 Hz, 1H), 4.40 (t, *J* = 6.8 Hz , 2H), 4.24 (s, 2H), 4.01 (s, 6H), 3.40 (t, *J* = 4 Hz, 4H), 2.45 – 2.62 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 162.3, 159.7, 156.1, 155.6, 142.2, 136.6, 132.8, 130.7 (2C), 129.1, 125.8, 119.2, 115.2 (2C), 105.1, 55.2 (2C), 54.6 (2C), 53.5, 51.8 (2C), 50.5, 45.6. ESI-MS *m/z:* 452.44 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_4$: C, 63.84; H, 6.47; N, 15.51; Found: C, 63.95; H, 6.46; N, 15.53.

N-(4-Chlorobenzyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxamide (PZ_22): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-chloro benzyl isocyanate (0.069 g, 0.416 mmol) (1.2 eq) to afford **PZ_22** (0.115 g, 60 %) as pale brown solid. M.p: 169-171 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.61 (b, 1H), 8.25 (d, *J* = 2 Hz, 1H), 7.74 (d, *J* = 9.6 Hz, 1H), 7.45 (s, 1H), 7.43 (d, *J* = 9.2 Hz, 2H), 7.25 (t, *J* = 9 Hz , 2H), 6.66 (d, *J* = 9.6 Hz, 1H), 4.41 (t, *J* = 6.8 Hz , 2H), 4.00 (s, 3H), 4.28 (s, 2H), 3.40 (t, *J* = 4 Hz, 4H), 2.48 – 2.63 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 162.2, 158.2, 155.6, 142.6, 136.2, 134.9, 133.1 (2C), 132.5, 131.9, 129.5 (2C), 124.2, 119.1, 105.1, 55.2 (2C), 54.5, 52.5, 51.8 (2C), 50.5, 48.2. ESI-MS *m/z:* 456.21 $(M+H)^+$. Anal Calcd for $C_{23}H_{26}ClN_5O_3$: C, 60.59; H, 5.75; N, 15.36; Found: C, 60.68; H, 5.74; N, 15.35.

4-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(4-methoxybenzyl)piperazine-1-carbothioamide (PZ_23): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-methoxy benzyl isothiocyanate (0.077 g, 0.416 mmol) (1 eq) to afford PZ_23 (0.12 g, 75 %)as brown solid. M.p: 153-155 °C. ^{1}H NMR [DMSO-d6]: δH: 8.44 (b, 1H), 8.24 (d, *J* = 2 Hz, 1H), 7.76 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.39 (d, *J* = 8 Hz,

2H), 7.16 (t, *J* = 7.6 Hz , 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 4.79 (s, 2H), 4.40 (t, *J* = 6.8 Hz , 2H), 4.01 (s, 6H), 3.41 (t, *J* = 4 Hz, 4H), 2.45 – 2.62 (m, 6H). ^{13}C NMR [DMSO-d6] δc: 179.8, 162.6, 158.6, 155.3, 142.6, 136.9, 132.7, 130.8 (2C), 129.6, 124.6, 119.2, 116.6 (2C), 105.3, 56.7 (2C), 55.9 (2C), 54.6 (2C), 53.8, 52.5, 50.6. ESI-MS m/z: 468.67 $(M+H)^+$. Anal Calcd for $C_{24}H_{29}N_5O_3S$: C, 61.65; H, 6.25; N, 14.98; Found: C, 61.73; H, 6.23; N, 14.95.

N-(4-Chlorobenzyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carbothioamide (PZ_24): The compound was synthesized according to the general procedure using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4-chloro benzyl isothiocyanate (0.078 g, 0.416 mmol) (1.2 eq) to afford PZ_24 (0.098 g, 60 %) as white solid. M.p: 166-168 °C. ^{1}H NMR [DMSO-d6]: δH: 8.60 (b, 1H), 8.23 (d, *J* = 2 Hz, 1H), 7.73 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.41 (d, *J* = 9 Hz, 2H), 7.24 (t, *J* = 8.8 Hz , 2H), 6.66 (d, *J* = 9.6 Hz, 1H), 4.85 (s, 2H), 4.40 (t, *J* = 6.8 Hz , 2H), 4.00 (s, 3H), 3.41 (t, *J* = 4 Hz, 4H), 2.47 – 2.64 (m, 6H). ^{13}C NMR [DMSO-d6] δc: 179.2, 162.3, 155.8, 142.2, 135.3, 134.3, 133.9 (2C), 132.9, 131.5, 129.5 (2C), 124.6, 119.5, 105.8, 56.4 (2C), 55.3 (2C), 54.5,53.5, 51.5, 50.5 ESI-MS m/z: 473.18 (M+H)+. Anal Calcd for $C_{23}H_{26}ClN_5O_2S$: C, 58.53; H, 5.55; N, 14.84; Found: C, 58.59; H, 5.56; N, 14.82.

1-(2-(4-Benzylpiperazin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (PZ_25): The compound was synthesized according to the general procedure using using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and benzaldehyde (0.044 g, 0.416 mmol) (1.2 eq) and sodium cyanoborohydride (0.032 g, 0.519 mmol) (1.5 eq) to afford **PZ_25** (0.082 g, 62 %) as white solid. M.p: 221-223 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.27 (d, *J* = 2 Hz, 1H), 7.76 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.18 (t, *J* = 7.4 Hz, 2H), 7.05 (t, *J* = 7 Hz, 1H), 6.66 (d, *J* = 9.6 Hz, 1H), 4.42 (t, *J* = 6.8 Hz , 2H), 4.00 (s, 3H), 3.59 (s, 2H), 2.61 (t, *J* = 4 Hz, 4H), 2.38 – 2.55 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 162.5, 155.6, 142.3, 139.5, 136.5, 132.3, 129.6 (2C), 128.5 (2C), 126.5, 123.9, 119.2, 105.6, 66.2, 56.7 (2C), 54.9, 54.2 (2C), 53.1, 50.5. ESI-MS *m/z:* 379.55 $(M+H)^+$. Anal Calcd for $C_{22}H_{26}N_4O_2$: C, 69.82; H, 6.92; N, 14.80; Found: C, 69.96; H, 6.93; N, 14.77.

1-(2-(4-(4-Chlorobenzyl)piperazin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (PZ_26): The compound was synthesized according to the general procedure using using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol)

(1 eq) and 4 chloro benzaldehyde (0.058g, 0.416 mmol) (1.2 eq) and sodium cyanoborohydride (0.032 g, 0.519 mmol) (1.5 eq) to afford (0.078 g, 54 %) as white solid M.p: 234-236 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.26 (d, *J* = 2 Hz, 1H), 7.74 (d, *J* = 9.6 Hz, 1H), 7.45 (s, 1H), 7.44 (d, *J* = 9 Hz, 2H), 7.26 (t, *J* = 9Hz , 2H), 6.66 (d, *J* = 9.6 Hz, 1H), 4.42 (t, *J* = 6.8 Hz , 2H), 4.00 (s, 3H), 3.58 (s, 2H), 2.65 (t, *J* = 4 Hz, 4H), 2.37 – 2.53 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 162.3, 155.6, 142.3, 135.2, 134.8, 132.8, 130.9, 130.2 (2C), 129.5 (2C), 125.6, 119.2, 105.6, 66.8, 56.5 (2C), 54.8, 53.9 (2C), 52.8, 50.6. ESI-MS *m/z*: 413.55 $(M+H)^+$. Anal Calcd for $C_{22}H_{25}ClN_4O_2$: C, 63.99; H, 6.10; N, 13.57. Found: C, 63.88; H, 6.09; N, 13.59.

1-(2-(4-(4-Fluorobenzyl)piperazin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (PZ_27): The compound was synthesized according to the general procedure using using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4 fluoro benzaldehyde (0.051g, 0.416 mmol) (1.2 eq) and sodium cyanoborohydride (0.032 g, 0.519 mmol) (1.5 eq) to afford **PZ_27** (0.083 g, 60 %) as white solid M.p: 228-230 °C. ^{1}H NMR [DMSO-d_6]: $\delta_{H:}$ 8.27 (d, *J* = 2 Hz, 1H), 7.75 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 2H), 7.41 (dd, *J* = 8.8 Hz, J = 2.4 Hz, 2H), 7.23 (dd, *J* = 8.8 Hz , *J* = 9.2 Hz, 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 4.41 (t, *J* = 6.8 Hz , 2H), 4.02 (s, 3H), 3.57 (s, 2H), 2.66 (t, *J* = 4 Hz, 4H), 2.35 – 2.52 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 162.3, 160.8, 155.6, 142.3, 136.4, 15.6, 132.2, 130.5 (2C), 125.2, 119.2, 116.2 (2C), 105.8, 66.3, 56.5 (2C), 54.5, 53.8 (2C), 52.6, 49.9. ESI-MS *m/z*: 397.33 $(M+H)^+$. Anal Calcd for $C_{22}H_{25}FN_4O_2$: C, 66.65; H, 6.36; N, 14.13. Found: C, 66.51; H, 6.37; N, 14.11.

7-Methoxy-1-(2-(4-(4-methoxybenzyl)piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (PZ_28): The compound was synthesized according to the general procedure using using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4 methoxy benzaldehyde (0.056 g, 0.416 mmol) (1.2 eq) and sodium cyanoborohydride (0.032 g, 0.519 mmol) (1.5 eq) to afford **PZ_28** (0.103 g, 73 %) as brown solid M.p: 214-216 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.23 (d, *J* = 2 Hz, 1H), 7.77 (d, *J* = 9.6 Hz, 1H), 7.44 (s, 1H), 7.39 (d, *J* = 8 Hz, 2H), 7.18 (t, *J* = 7.8 Hz , 2H), 6.66 (d, *J* = 9.6 Hz, 1H), 4.41 (t, *J* = 6.8 Hz , 2H), 4.00 (s, 6H), 3.56 (s, 2H), 2.60 (t, *J* = 4 Hz, 4H,), 2.36 – 2.53 (m, 6H).^{13}C NMR [DMSO-d_6] δ_c: 162.3, 160.1, 155.6, 142.4, 136.2, 131.8 (2C), 130.9, 129.6, 125.6, 119.2, 116.2 (2C), 105.3, 66.7, 56.7 (2C), 54.8 (2C), 53.9 (2C), 52.3, 49.8. ESI-MS *m/z*: 409.24

$(M+H)^+$. Anal Calcd for $C_{23}H_{28}N_4O_3$: C, 67.63; H, 6.91; N, 13.72. Found: C, 67.77; H, 6.93; N, 13.70.

7-Methoxy-1-(2-(4-(4-nitrobenzyl)piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (PZ_29): The compound was synthesized according to the general procedure using using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4 nitro benzaldehyde (0.062 g, 0.416 mmol) (1.2 eq) and sodium cyanoborohydride (0.032 g, 0.519 mmol) (1.5 eq) to afford **PZ_29** (0.088 g, 60 %) as brown solid M.p: 218-220 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.31 (d, *J* = 2 Hz, 1H), 7.94 (d, *J* = 9.6 Hz, 1H), 7.56 (d, *J* = 9.4 Hz, 2H), 7.44 (s, 1H), 7.32 (t, *J* = 9.4 Hz , 2H), 6.69 (d, *J* = 9.6 Hz, 1H), 4.42 (t, *J* = 6.8 Hz , 2H), 4.02 (s, 3H), 3.54 (s, 2H), 2.65 (t, *J* = 4 Hz, 4H), 2.39 – 2.56 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 162.3, 155.6, 147.5, 145.6, 142.3, 135.8, 132.2, 130.2 (2C), 125.6, 122.5 (2C), 119.6, 105.6, 66.2, 56.5 (2C), 54.5, 53.8 (2C), 51.9, 50.1. ESI-MS *m/z*: 424.43 $(M+H)^+$. Anal Calcd for $C_{22}H_{25}N_5O_4$: C, 62.40; H, 5.95; N, 16.54. Found: C, 62.55; H, 5.93; N, 16.55.

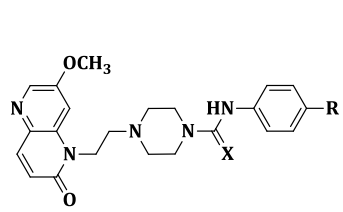
1-(2-(4-(4-Bromobenzyl)piperazin-1-yl)ethyl)-7-methoxy-1,5-naphthyridin-2(1H)-one (PZ_30): The compound was synthesized according to the general procedure using using 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one (**PZ_06**) (0.1 g, 0.346 mmol) (1 eq) and 4 bromo benzaldehyde (0.076 g, 0.416 mmol) (1.2 eq) and sodium cyanoborohydride (0.032 g, 0.519 mmol) (1.5 eq) to afford **PZ_30** (0.09 g, 56 %) as brown solid M.p: 238-240 °C. ^{1}H NMR [DMSO-d_6]: δ_H: 8.31 (d, *J* = 2 Hz, 1H), 7.85 (d, *J* = 9.6 Hz, 1H), 7.50 (d, *J* = 9 Hz, 2H), 7.45 (s, 1H), 7.29 (t, *J* = 8.8 Hz , 2H), 6.67 (d, *J* = 9.6 Hz, 1H), 4.42 (t, *J* = 6.8 Hz , 2H), 4.01 (s, 3H), 3.57 (s, 2H,), 2.65 (t, *J* = 4 Hz, 4H), 2.38 – 2.55 (m, 6H). ^{13}C NMR [DMSO-d_6] δ_c: 162.3, 155.8, 142.5, 138.1, 136.2, 132.2, 130.5 (2C), 129.9 (2C), 125.6, 122.5, 119.2, 105.8, 66.2, 56.5 (2C), 54.5, 53.8 (2C), 51.8, 50.5. ESI-MS *m/z*: 458.33 $(M+H)^+$. Anal Calcd for $C_{22}H_{25}BrN_4O_2$: C, 57.77; H, 5.51; N, 12.25. Found: C, 57.66; H, 5.52; N, 12.24.

5.2.4. In *vitro* supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

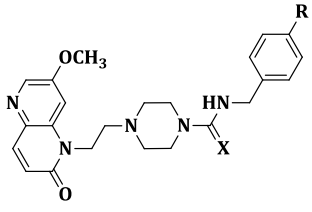
All the synthesized derivatives were evaluated for their *in vitro* supercoiling assay for the derivation of SAR and lead optimization. The compounds were further subjected to a whole cell screening against *Mtb* H_{37}Rv strain to understand their bactericidal potency using the agar

dilution method and later the safety profile of these molecules were evaluated by checking the *in vitro* cytotoxicity against RAW 264.7 cell line (mouse macrophage) at 50 µM concentration by MTT assay, and the results are shown in **Table 5.4**.

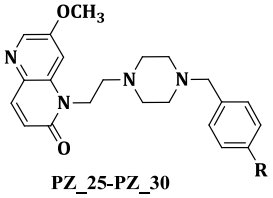
Table 5.4: *In vitro* biological evaluation of synthesized compounds **PZ_07 – PZ_30**



PZ_07-PZ_20



PZ_21-PZ_24



PZ_25-PZ_30

Compd.	X	R	Supercoiling assay (IC_{50}) (µM)	MIC (µM)	Cytotoxicity at 50 µM (% inhib:)
PZ_07	O	H	13.52±0.23	30.69	9.54
PZ_08	O	F	11.61±0.45	14.69	25.22
PZ_09	O	Cl	0.32±0.15	7.06	26.96
PZ_10	O	$COCH_3$	19.4±0.51	26.90	18.56
PZ_11	O	NO_2	0.29±0.22	3.45	5.08
PZ_12	O	Br	0.75±0.53	3.21	24.35
PZ_13	O	CH_3	2.68±0.19	7.41	32.31
PZ_14	S	H	15.41±0.57	29.51	32.59
PZ_15	S	F	14.66±0.68	14.16	21.55
PZ_16	S	Cl	12.84±0.64	13.64	6.59
PZ_17	S	$COCH_3$	16.41±0.58	10.65	3.16
PZ_18	S	NO_2	18.52±0.46	26.68	17.33
PZ_19	S	Br	0.49±0.22	3.8	1.06
PZ_20	S	CH_3	11.46±0.39	10.65	26.80
PZ_21	O	OCH_3	14.58±0.59	27.68	37.58
PZ_22	O	Cl	0.92±0.19	6.6	24.62
PZ_23	S	OCH_3	15.53±0.81	26.73	17.13

PZ_24	S	Cl	0.95±0.19	4.31	24.95
PZ_25	-	H	0.94±0.15	4.12	15.01
PZ_26	-	Cl	0.81±0.22	10.09	26.48
PZ_27	-	F	0.91±0.16	15.01	26.43
PZ_28	-	OCH_3	1.34±0.11	3.82	15.95
PZ_29	-	NO_2	0.98±0.26	7.38	37.57
PZ_30	-	Br	6.44±0.35	13.67	2.15
Moxifloxacin			11.2±0.23	2.4	ND
Novobiocin			46±28nM	>200	19.3
Ethambutol			NT	9.84	ND

IC_{50}, 50% inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; NT, not tested; nM, nanomolar

Mtb DNA gyrase supercoiling enzyme inhibition activity

In vitro activity against *Mtb* $H_{37}Rv$

Cytotoxicity against RAW 264.7 cells (mouse macrophage cell line)

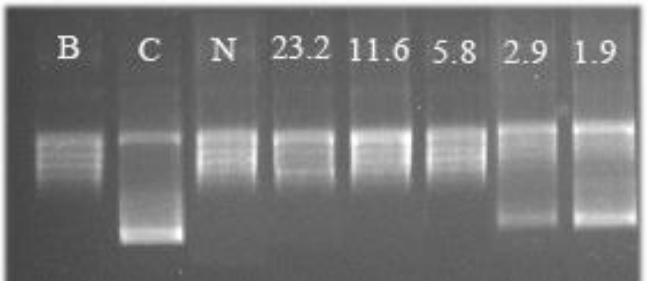


Fig.5.5: Illustrating the supercoiling assay picture of compound **PZ_11** at five different concentrations of 23.2, 11.6, 5.8, 2.9 and 1.9 µM and novobiocin as standard; B-Relaxed DNA substrate + DMSO; C- Relaxed DNA substrate + DNA Gyrase + DMSO; N-Novobiocin.

5.2.5. Evaluation of zERG channel inhibition in a zebra fish model

We evaluated the effect of compounds **PZ_09** and **PZ_11** on heart rate and atrio-ventricular block of zebrafish embryos and the assessment of arrhythmogenic potential of test compounds. Arrhythmia is a condition with diverse group of symptoms in which there is lengthening of

cardiac repolarization, leading to QT interval prolongation and torsades de pointes.The Proarrhythmic effect is mainly due to inhibition of human ether-a-go-go-related gene (hERG) channel which play a key role in repolarization. Milan *et al*., reported that current *in-vitro* models of hERG inhibition are lack of evidence for main mechanism of action. More-over electrophysiological studies are quite expensive and time consuming. Zebrafish (*Danio rerio*) is an attractive preliminary *in-vivo* model for screening compounds with potential Pro- pro-arrhythmic , hERG blockade & QT prolongation. Langheinrich U *et al*., reported Zebrafish ether-a-go-go-related gene (zERG) has been identified and an 80% homology to human hERG channel. zERG is highly similar to hERG mainly in the pore and drug-binding regions which explain bradycardia in wild-type embryos. Mittelstadt *et al*., have reported the Primary advantage of this i*n –vivo* assay is it permits to assess the drug effects on multiple ion currents whereas the *in-vitro* hERG models allow only single ion current investigation. In this model we used Terfenadine (20 µM) was used as a positive control. Compound **PZ_09** treated groups showed mild toxicity at 30 µM. Embryos treated with Compound **PZ_11** showed mild toxicity at 10 µM and moderate toxicity at 30 µM **Figure 5.5 and 5.6**.

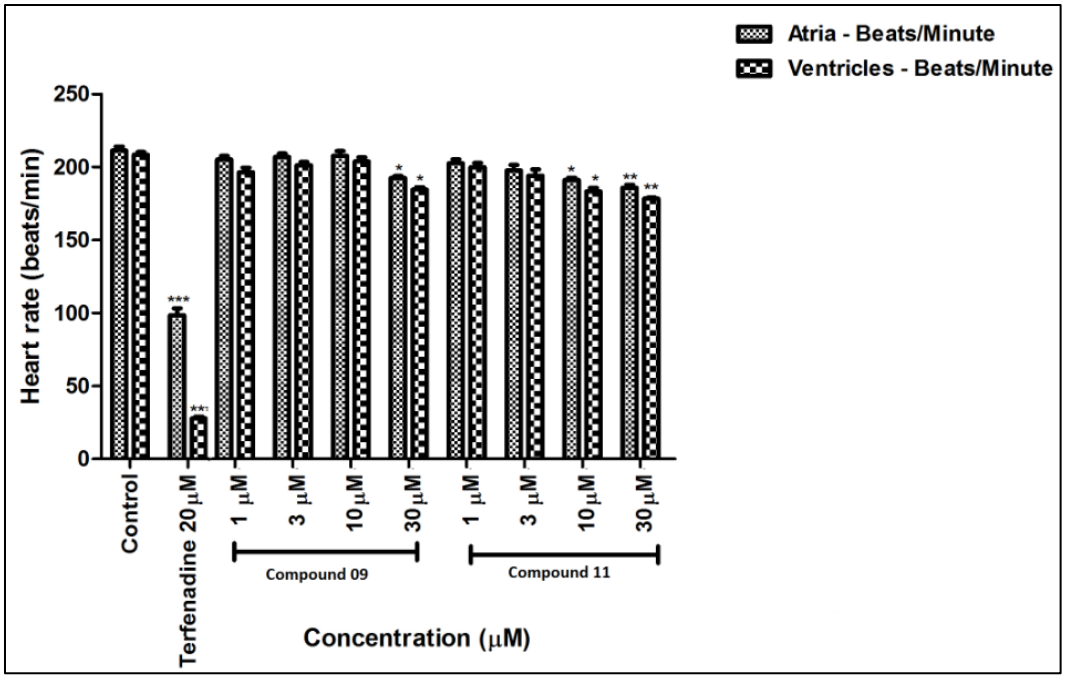


Fig 5.6: Depicts the results of the heart rates of atria and ventricles. Mean (±S.E.M.) of the heart rates of atria and ventricles of treatment groups. (*p<0.05, **p<0.01 and ***p<0.001). Statistical significance was analyzed with respect to the control group.

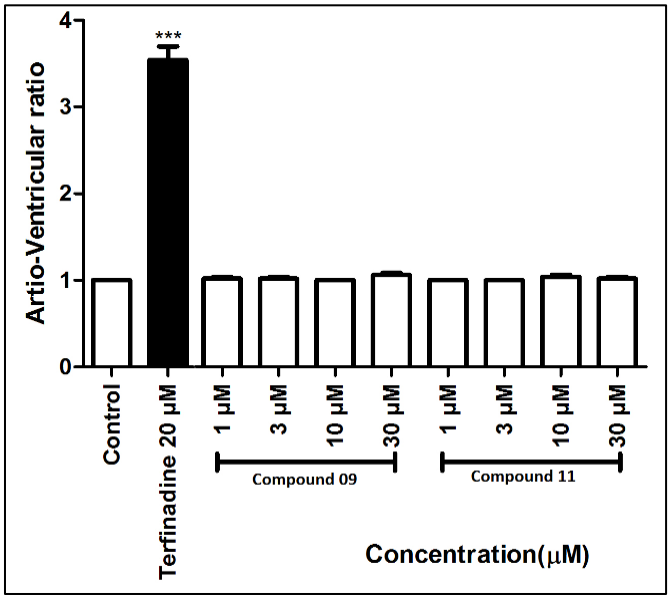


Fig 5.7: Depicts the atrio-venticular beat ratio. Mean (± S.E.M.) score of atrio ventricular ratio of treatment groups. (*p<0.05, **p<0.01 and ***p<0.001). Statistical significance was analyzed comparing control group Vs all groups

5.2.6. SAR and Discussion

In the initial screening all the twenty four synthesized compounds were subjected to screening for their biological inhibition studies using MTB DNA gyrase supercoiling assay kit (Inspiralis, Norwich, UK). The compounds were preliminarily screened at a concentrations of 500, 125, 31.3, 7.8, 1.95 µM respectively, those which were active were further tested at 250, 62.5, 15.6, 3.9 and 0.97 µM concentrations, molecules showing greater than 50% inhibitory profile at 0.97 µM were further screened at lower concentrations of 0.48, 0.24, 0.12 µM to ensure the activity profile of the best inhibitory compounds. Among the twenty four compounds ten showed good activity with IC_{50} lower than 1 µM. Compound 4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(4-nitrophenyl)piperazine-1-carboxamide (**PZ_11**) was found to be the most active compound with IC_{50} of 0.29±0.22 µM. Dose dependent inhibitory profile of the most active compound **PZ_11** along with standard drug novobiocin is illustrated in **Figure.5.5.** When compared to standard MTB DNA gyrase inhibitor Moxifloxacin (IC_{50} of 11.2±0.23 µM) fourteen compounds were more active. None of the compounds were more potent than another potent standard DNA gyrase inhibitor Novobiocin (IC_{50} of 46±28 µM), at the same time Novobiocin is inactive against MTB (MIC of >200 µM). With respect to structure activity relationship (SAR) among the phenyl urea derivatives (**PZ_07–PZ_13**); phenyl group with chloro, bromo and nitro substituents at 4^{th} position showed nano molar activity. Substitution with methyl, acetyl and fluoro at 4^{th} position or unsubstituted showed moderate activity. In the case of phenyl thiourea derivatives (**PZ_14–PZ_20**); only bromo derivatives showed good activity whereas all other substituents reduces the activity drastically. In general phenyl urea derivatives were more potent than phenyl thiourea derivatives. In the case of benzyl urea/thiourea derivatives chloro derivatives showed good activity than methoxy derivatives. In the case benzyl piperazines prepared by reductive amination (**PZ_25–PZ_30**) except bromo derivative all the other compounds showed good inhibitory effect. In most cases activity was not correlated with the structure and in general we were not able to conclude any proper SAR from this study. Further few of the synthesized compounds were screened for the GyrB inhibitory potency against *Mycobacterium smegmatis* GyrB protein using the malachite green assay and none of the molecules showed any inhibition on GyrB indicates that these molecules targets GyrA subunit of DNA gyrase.

Further to check the activity of the synthesized compounds in actively replicated MTB we evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strain by a standard protocol of microplate Alamar blue assay method. We have used Moxifloxacin and Ethambutol as the positive controls for comparison (**Table 1**). All the compounds inhibited MTB with MIC ranging from 3.21-30.69 µM. Nine compounds inhibited MTB with MIC < 10 µM and compounds **PZ_11** and **PZ_12** (N-(4-bromophenyl)-4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperazine-1-carboxamide) were found to be more potent with MTB MIC of ~ 3.5 µM. When compared Moxifloxacin (MIC of 2.4 µM) none of the molecules showed better activity and ten compounds were found to be more potent than standard first line anti-TB drug Ethambutol (MIC of 9.84 µM). In the case of most potent DNA gyrase inhibitor compound **PZ_11** showed supercoiling inhibitory IC_{50} of 0.29±0.22 µM showed MTB MIC of 3.45 µM. This difference in activity might be due to problem in MTB cell wall penetration of the compound or efflux pump present in the bacteria might be pumped out the molecule effectively.

Subsequently, the eukaryotic cell safety profile of all the twenty four compounds was observed by testing their *in vitro* cytotoxicity against the RAW 264.7 cell line (Mouse leukemic monocyte macrophage cell line) at 50 µM concentration by (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Since the mycobacteria resides in the macrophages during the infection stages, this cell line was selected. At 50 µM tested compounds showed cytotoxicity range of 1.06-37.58 % as shown in **Table 5.4**. The most promising anti-TB compound among the synthesized set of compounds was **11** with only 5.08 % cytotoxicity which is within the safety profile limits. Novobiocin was used as standard with 19.36 % inhibition in the above cell line.

Finally we evaluated the effect of compounds **PZ_09** and **PZ_11** on heart rate and atrio-ventricular block of zebrafish embryos & the assessment of arrhythmogenic potential of test compounds. Compound **PZ_09** treated groups showed mild toxicity at 30 µM. Embryos treated with Compound **PZ_11** showed mild toxicity at 10 µM and moderate toxicity at 30 µM **Figure 5.5 and 5.6**.

5.2.7. Highlights of the study

Synthetically, by replacement of aminopiperidine linker with piperazine moiety, we identified new derivatives of mycobacterial DNA gyrase inhibitors with reduced hERG toxicity, excellent *in vitro* enzyme inhibition profiles, and antitubercular activity. We developed a series of twenty four compounds with a piperazine linker 1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one. Among them compound 4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(4-nitrophenyl)piperazine-1-carboxamide (**PZ_11**) was the most promising inhibitor with *Mycobacterium tuberculosis* (MTB) DNA Gyrase enzyme supercoiling IC_{50} of 0.29±0.22 µM, with a good MTB MIC of 3.45 µM. These kind of compounds retains good potency and showed reduced cardiotoxicity compared to aminopiperidines.

As there is an urgent requirement of new anti-TB agents, we strongly believe that the present class of DNA gyrase inhibitors reported in this work provides an interesting potential for further optimization of the leads to yield novel drugs that are aimed to combat ever-increasing bacterial infections.

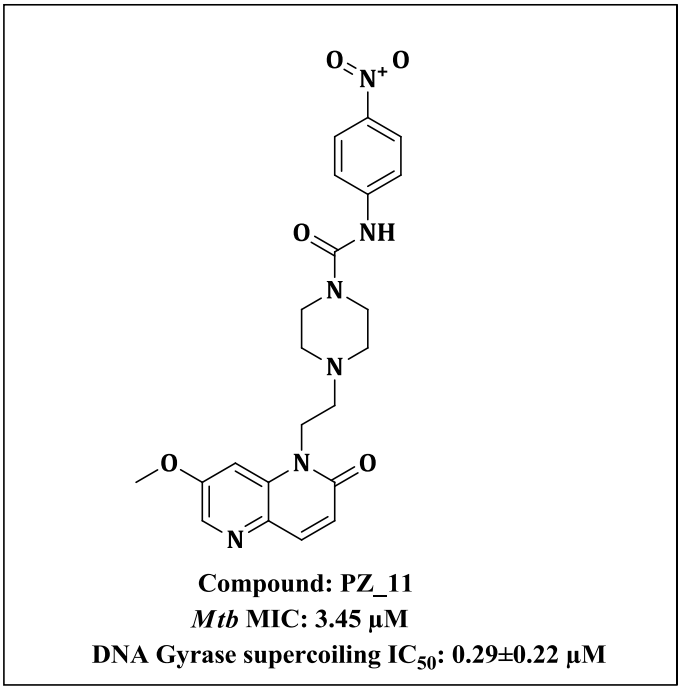


Fig 5.8: Chemical structure and biological activity of the most active compound **PZ_11**

5.3. Design, synthesis and biological evaluation of N-(4-(5-sub-1H-benzo[d]imidazol-2-yl) phenyl)-5-sub-1H-indole-2-carboxamide/(4-(5-sub-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-sub-1H-indol-2-yl)methanone derivatives as novel DNA Gyrase inhibitors

5.3.1. Design of the molecules

Extensive research about the binding mode of novel bacterial topoisomerase inhibitors (NBTIs) led to the identification of a novel class of scaffold, benzimidazoles as DNA gyrase inhibitors with potent anti-TB activity [Hameed P, S., *et al*., 2014 and 2015, Zhang, L., *et al*., 2016]. To confirm the above statement, docking of benzimidazoles to a NBTI bound crystal structure was carried out. The result suggested that benzimidazoles made key contacts in the enzyme active site similar to the reported NBTIs. More proof to the above observation was done using measurement of DNA gyrase inhibition, mutated Mtb strains resistant to aminopiperidines based NBTIs and to moxifloxacin.

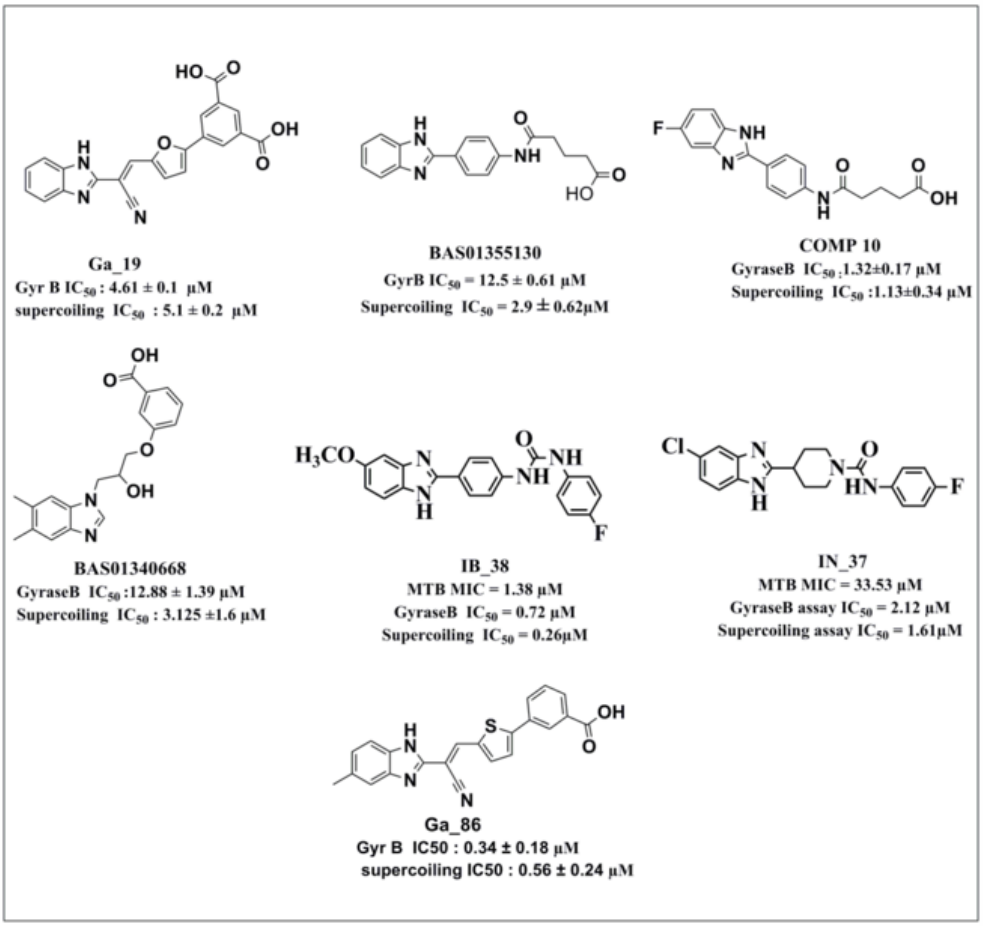


Figure 5.9: Structures of reported benzimidazole based DNA gyrase inhibitors from our lab as well as Asinex database.

In 2014 Shahul hameed. *et al*.,[Hameed P, S., *et a*l., 2014] by using scaffold hopping approach developed a novel gyrase inhibitors against fluoroquinoline resistant MTB. This class of inhibitors contains quinoline in the LHS and RHS was replaced with benzimidazole

In order to obtain a novel anti tubercular molecule with improved DNA gyrase activity, based on earlier literatures and compounds synthesized from our own lab we designed 2 sets of compounds using molecular hybridization technique, one with phenyl and other with amino piperidine linker. For getting a clear structure activity relation we made substitution on both benzimidazole moiety and indole moiety and proposed a library of 40 molecules.

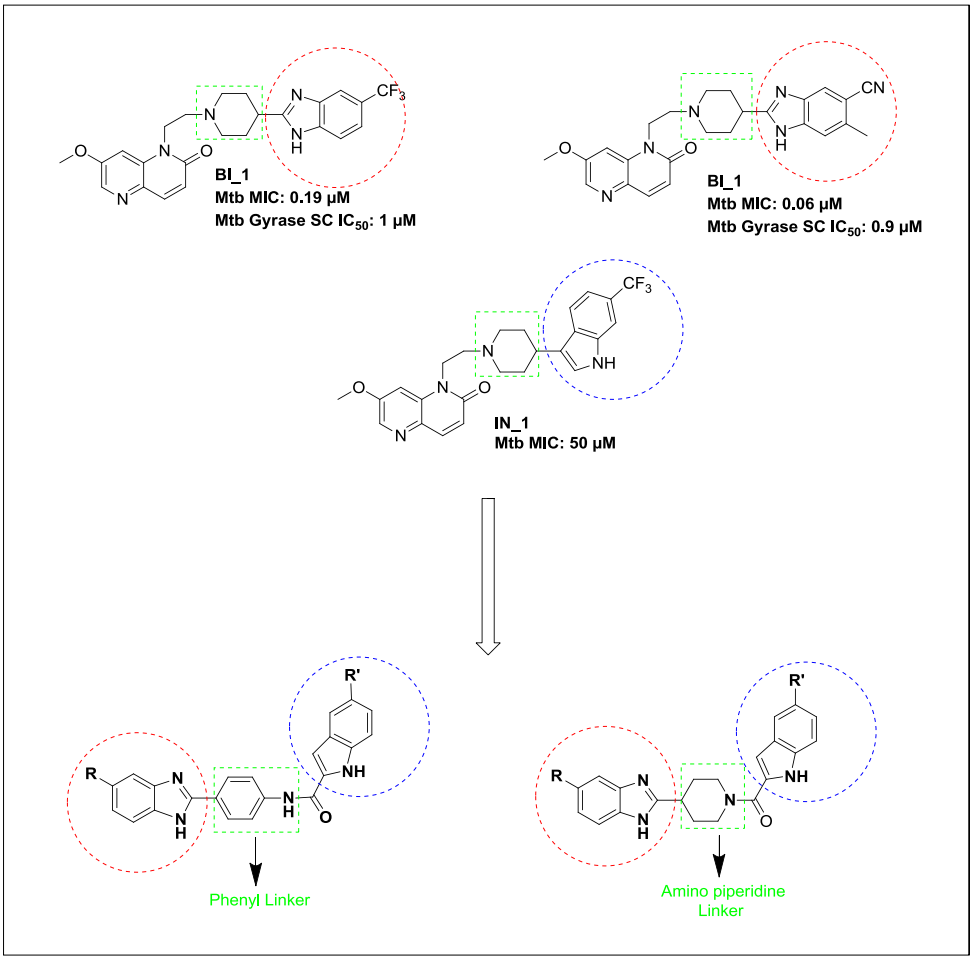
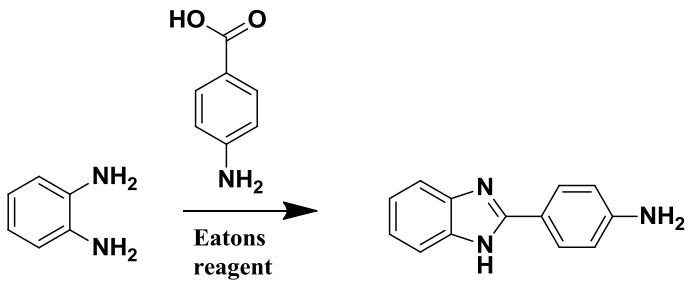


Fig 5.10: Design strategy employed for the synthesis of final compounds **BZ_03 – BZ_42**.

5.3.2. Experimental procedures utilized for the synthesis of BZ_01a – BZ_42

The synthetic pathway used to achieve lead modifications is delineated in **Fig 4.3**. The synthesis of target molecules began with the condensation of commercially available substituted 1, 2 phenylene diamine (compounds **BZ_1a-e**) with 4-aminobenzoic acid in the presence of Eaton's reagent to yield the corresponding 4-(sub:-1H-benzo[d]imidazol-2-yl) aniline (compounds **BZ_2a-e**) in good yields [Bahrami, K., et al., 2007]. Same way condensation of substituted 1,2 phenylenediamine (compounds **BZ_1a-e**) with iso nipecotic acid using Eaton's reagent resulted in the formation of corresponding substituted 2-(piperidin-4-yl)-1H-benzo[d]imidazole (**2'a-e**) [Axford, L. C., et al., 2013]. This method utilizing Eaton's reagent was highly beneficial in improving the yields of the so obtained condensation product, when compared to other reagents/protocols available in literatures. Functionalization of 4-aminophenyl linker and piperadine was then brought about by treatment with 5 substituted indole acids to yield compounds **BZ_3 - BZ_42**. This was achieved by using coupling reagent, propyl phosphonic anhydride [Dunetz, J. R., *et al*., 2011]. A library of forty derivatives were synthesized (**BZ_3** – **BZ_42**, as shown in Table 5.5), characterized and evaluated for their ability to inhibit the Gyrase B activity as an effort towards the derivatization of structure-activity relationships and lead optimization.

Preparation of 4-(1H-benzo[d]imidazol-2-yl)aniline (BZ_02a)

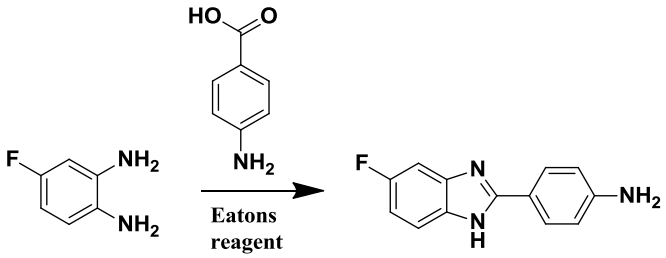


1a BZ_02a

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of 1,2-phenylenediamine (1a) (1 g, 9.25 mmol) and 4-amino benzoic acid (1.27 g, 9.25 mmol) at 0^{o}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 to afford **BZ_02a** (1.42 g, 74% yield) as pale brown solid. ^{1}H NMR (DMSO-d_6): δ_H 6.82-7.98 (m, 8H), 6.42 (s, 2H) . ^{13}C NMR (DMSO-d_6): δ_c 153.1, 145.4, 141.5, 128.7, 123.5, 116.7, 115.5. EI-MS *m/z*: 210.45 $(M+H)^+$. 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.

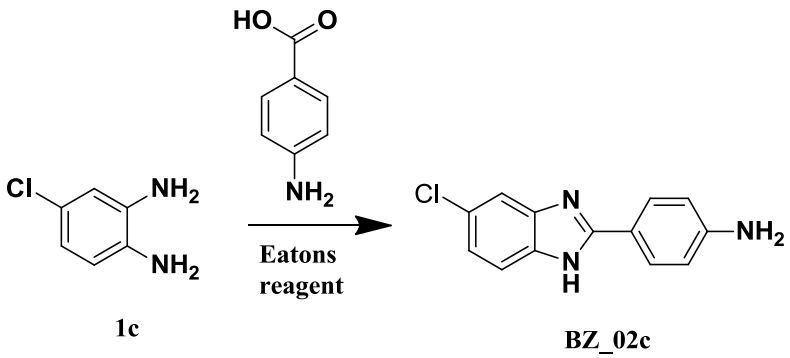
Preparation of 4-(5-Fluoro-1H-Benzo[d]imidazol-2-yl)aniline (BZ_02b)



1b **BZ_02b**

Eaton's reagent (10 mL)) was added drop wise to a well pulverised mixture of using 4-fluoro-1,2-phenylenediamine (1b) (1 g, 7.93 mmol) and 4-amino benzoic acid (1.09 g, 7.93 mmol) 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 to afford **BZ_02b** (1.02 g, 56 % yield) as brown solid. ^{1}H NMR (DMSO-d6): δ_H 7.00 - 8.09 (m, 7H), 6.33 (s, 2H). ^{13}C NMR (DMSO-d6): δc 156.6, 153.3, 145.6, 139.1, 137.8, 128.2, 116.8, 115.6, 110.3, 101.9. EI-MS m/z: 228.26 $(M+H)^+$. 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.

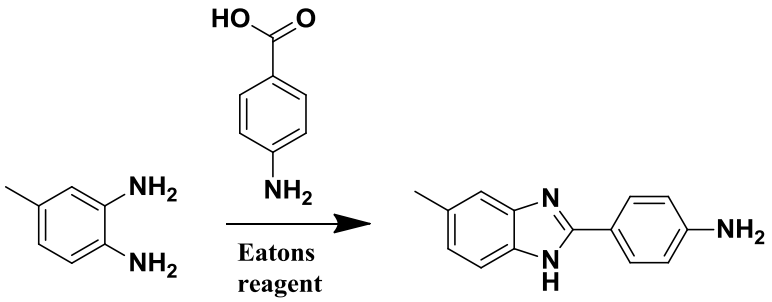
Preparation of 4-(5-Chloro-1H-Benzo[d]imidazol-2-yl) aniline (BZ_02c)



1c **BZ_02c**

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of 4-chloro-1,2-phenylenediamine (1c) (1 g, 7.02 mmol) and 4-amino benzoic acid (0.96 g, 7.02 mmol) 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 to afford **BZ_02c** (1.1 g, 64 % yield) as buff coloured solid. ^{1}H NMR (DMSO-d_6): δ_H 6.45 (s, 2H), 7.08 - 8.39 (m, 7H). ^{13}C NMR (DMSO-d_6): δ_c 153.1, 145.7, 133.2, 131.2, 129.6, 128.4, 124.3, 116.7, 116.3, 115.9, 115.2. EI-MS *m/z*: 244.42$(M+H)^+$. 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.

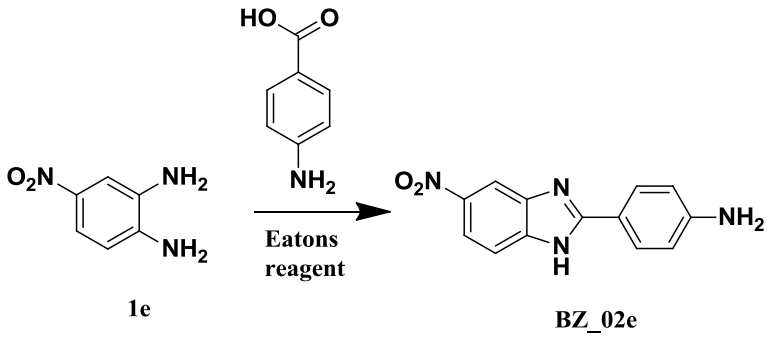
Preparation of 4-(5-methyl-1H-benzo[d]imidazol-2-yl)aniline (BZ_02d)



1d BZ_02d

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of 4-methyl-1,2-phenylenediamine (1d) (1 g, 8.19 mmol) and 4-amino benzoic acid (1.12 g, 8.19 mmol) 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 to afford **BZ_02d** (1.05 g, 57 %) as reddish brown solid. ^{1}H NMR (DMSO-d6): δ_H 7.04 - 8.18 (m, 7H), 6.51 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (DMSO-d6): δc 153.5, 146.2, 134.2, 133.5, 130.8, 128.5 (2c), 117.2, 115.4, 115.3, 115.1 (2C), 22.5. EI-MS m/z: 224 $(M+H)^+$. Anal Calcd for $C_{14}H_{13}N_3$: C, 75.31; H, 5.87; N, 18.82; Found: C, 75.25; H, 5.88; N, 18.85.

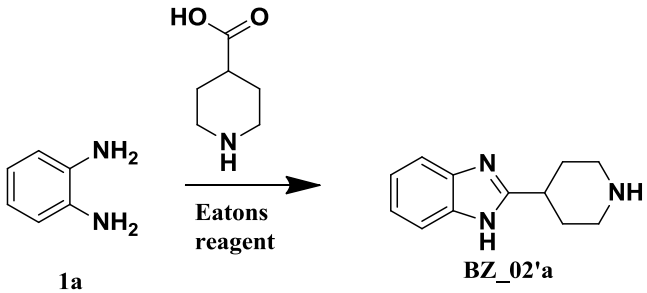
Preparation of 4-(5-Nitro-1H-Benzo[d]imidazol-2-yl)aniline (BZ_02e)



1e BZ_02e

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of 4-nitro-1,2-phenylenediamine (1e) (1 g, 6.53 mmol) and 4-amino benzoic acid (0.9 g, 6.53 mmol) 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 to afford **BZ_02e** (1.2 g, 72 % yield) as yellowish brown solid. ^{1}H NMR (DMSO-d6): δ_H 7.21 - 8.49 (m, 7H), 6.53 (s, 2H). ^{13}C NMR (DMSO-d6): δc 153.3, 148.6, 145.7, 144.6, 140.2, 128.3, 118.9, 116.3, 116.1, 115.3, 113.2. EI-MS m/z: 255.44 $(M+H)^+$. Anal Calcd for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96 N, 22.04; Found C, 61.44; H, 3.94 N, 22.06.

Preparation of 2-(piperidin-4-yl)-1H-benzo[d]imidazole (BZ_02'a)

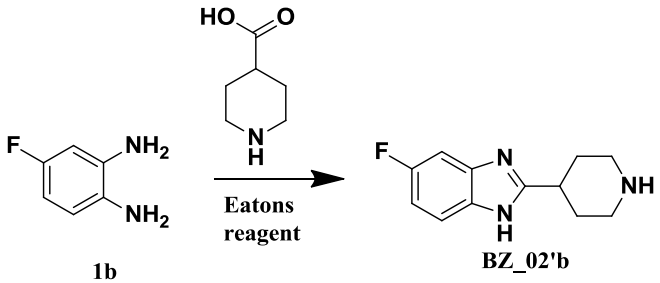


1a BZ_02'a

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of the corresponding 1,2-phenylenediamine (1a) (1 g, 9.25 mmol) and piperidine-4-carboxylic acid (1.19 g, 9.25 mmol) at 0 °C. The reaction mixture was then heated at 130 ^{o}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 **BZ_02'a** (1.55 g, 83 % yield) as pale brown solid. ^{1}H NMR (DMSO-d6): δ_H 10.56 (s, 1H), 7.65 – 7.18 (m, 4H), 6.81 (s, 1H), 3.81 (d, *J* = 10.8 Hz, 2H), 3.16 (m, 3H), 2.18 (t, *J* = 10.6 Hz, 2H), 1.92 (t, *J* = 10.6 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 139.8, 138.5 (2C), 121.5 (2C), 116.4 (2C), 40.5 (2C), 36.4, 32.5 (2C). EI-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, 71.58; H, 7.50; N, 20.90.

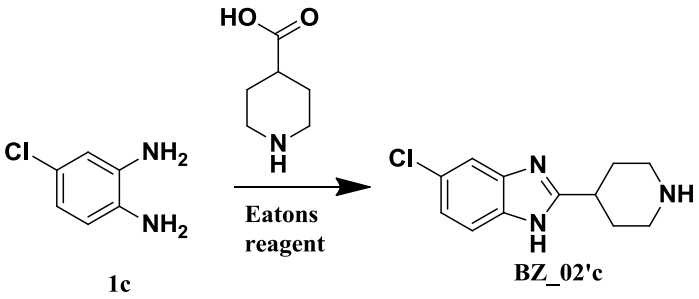
Preparation of 5-fluoro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (BZ_02'b)



1b BZ_02'b

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of using 4-fluoro-1,2-phenylenediamine (1b) (1 g, 7.93 mmol) and piperidine-4-carboxylic acid (1.02 g, 7.93 mmol) 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 to afford **BZ_02'b** (1.3202 g, 76 % yield) as brown solid. ^{1}H NMR (DMSO-d6): δ_H 10.48 (s, 1H), 7.64 – 6.91 (m, 3H), 6.79 (s, 1H), 3.78 (d, *J* = 10.6 Hz, 2H), 3.19 (m, 3H), 2.14 (t, *J* = 10.8 Hz, 2H), 1.90 (t, *J* = 10.4 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 155.8, 142.1, 139.8, 135.6, 115.9, 110.2, 102.5, 40.5 (2C), 36.2, 32.5 (2C). EI-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, 65.82; H, 6.43; N, 19.18.

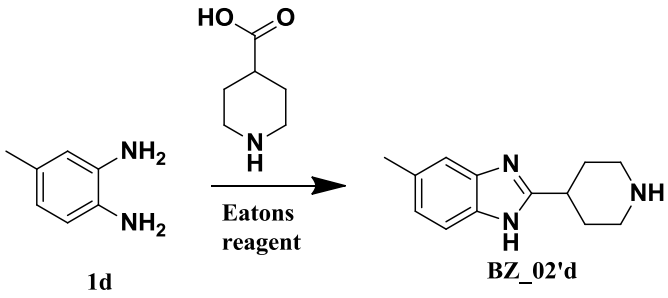
Preparation of 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (BZ_02'c)



1c BZ_02'c

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of 4-chloro-1,2-phenylenediamine (1c) (1 g, 7.02 mmol) and piperidine-4-carboxylic acid (0.91 g, 7.04 mmol) 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 to afford **BZ_02'c** (1.21 g, 73 % yield) as buff coloured solid .^{1}H NMR (DMSO-d6): δ_H 10.46 (s, 1H), 8.24 – 7.18 (m, 3H), 6.84 (s, 1H), 3.75 (d, *J* = 10.7 Hz, 2H), 3.22 (m, 3H), 2.16 (t, *J* = 10.8 Hz, 2H), 1.86 (t, *J* = 10.4 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 142.1, 139.5, 136.8, 130.5, 125.2, 116.8, 116.1, 40.5 (2C), 36.4, 32.5 (2C). EI-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, 61.23; H, 6.00; N, 17.81.

Preparation of 5-methyl-2-(piperidin-4-yl)-1H-benzo[d]imidazole (BZ_02'd)

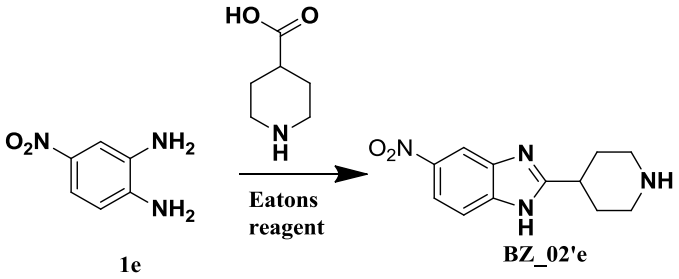


1d BZ_02'd

Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of 4-methyl-1,2-phenylenediamine (1d) (1 g, 8.19 mmol) and piperidine-4-carboxylic acid (1.05 g, 8.19 mmol) 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 to afford **BZ_02'd** (1.15 g, 65 %) as reddish brown solid. ^{1}H NMR (DMSO-d6): δ_H 10.61 (s, 1H), 7.61 – 7.09 (m, 3H), 6.81 (s, 1H), 3.76 (d, *J* = 10.8 Hz, 2H), 3.19 (m, 3H), 2.35 (s, 3H), 2.18 (t, *J* = 10.6 Hz, 2H), 1.85 (t, *J* = 10.4 Hz, 2H).^{13}C NMR (DMSO-d6): δc 142.1, 139.7, 136.2, 133.5, 126.3, 115.8, 115.6, 40.5 (2C), 36.3, 32.5 (2C), 22.5. EI-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, 72.58; H, 7.95; N, 19.54.

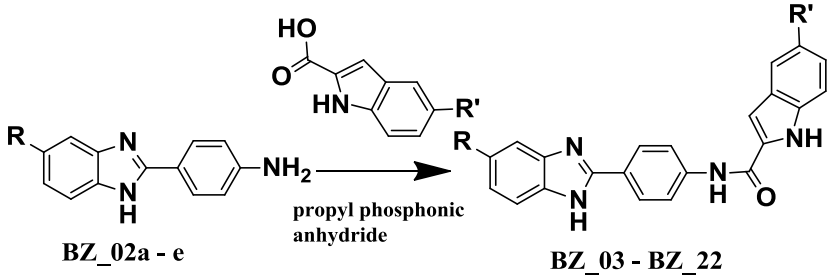
Preparation of 5-nitro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (BZ_02'e)



1e BZ_02'e

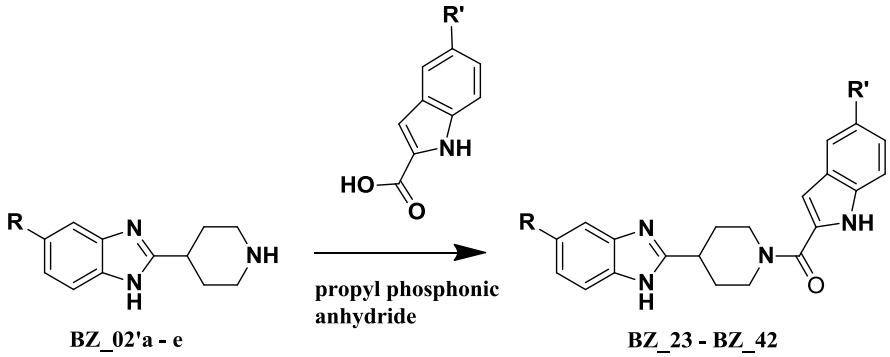
Eaton's reagent (10 mL) was added drop wise to a well pulverised mixture of 4-nitro-1,2-phenylenediamine (1e) (1 g, 6.53 mmol) and piperidine-4-carboxylic acid (0.84 g, 6.53 mmol) at 0 °C. The reaction mixture was then heated at 130 °C for 5 - 6 h (monitored by TLC and LCMS for completion). The reaction 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 to afford **BZ_02'e** (1.1 g, 69 % yield) as yellowish brown solid. ^{1}H NMR (DMSO-d6): δ_H 10.65 (s, 1H), 8.35 – 7.52 (m, 3H), 6.87 (s, 1H), 3.74 (d, *J* = 10.5 Hz, 2H), 3.20 (m, 3H), 2.21 (t, *J* = 10.4 Hz, 2H), 1.86 (t, *J* = 10.8 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 146.1, 145.2, 143.2, 142.3, 119.2, 116.5, 113.2, 40.5 (2C), 36.2, 32.5 (2C). EI-MS m/z: 247 $(M+H)^+$. Anal Calcd for $C_{12}H_{14}N_4O_2$: C, 58.53; H, 5.73 N, 22.75; Found C, 58.45; H, 5.72, N, 22.77.

General procedure for the synthesis of final compounds BZ_03 – BZ_22



The synthesis followed the literature procedure. To a well stirred solution of the corresponding 4-(sub:-1H-benzo[d]imidazol-2-yl)aniline (**BZ_2a-e**) (1 equiv) in anhydrous tetrahydrofuran was added triethylamine (3 equiv) followed by the addition of corresponding 5-sub- 2-indole carboxylic acid (1.2 equiv) shown in Table 5.5 at room temperature. To the above mixture was added propyl phosphonic anhydride (T_3P) (2 equiv). The reaction mixture was then stirred at rt for 12 h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water and the aqueous layer was extracted with dichloromethane; the combined organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using dichloromethane: methanol as to afford the desired product in good yield and purity as described below.

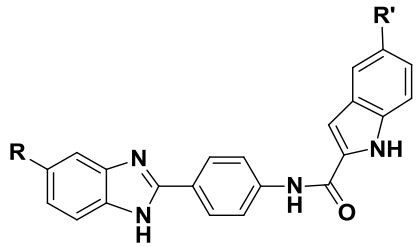
General procedure for the synthesis of final compounds BZ_23 – BZ_42



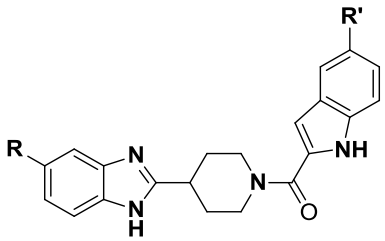
The synthesis followed the literature procedure. To a well stirred solution of the corresponding 2-(piperidin-4-yl)-1H-benzo[d]imidazole (**2'a-e**) (1 equiv) in anhydrous tetrahydrofuran was

added, triethylamine (3 equiv) followed by the addition of corresponding 5-sub- 2-indole carboxylic acid (1.2 equiv) shown in Table 5.5 at room temperature. To the above mixture was added propyl phosphonic anhydride (T_3P) (2 equiv). The reaction mixture was then stirred at rt for 12 h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water and the aqueous layer was extracted with dichloromethane; the combined organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using dichloromethane: methanol as to afford the desired product in good yield and purity as described below.

Table 5.5: Physiochemical properties of the synthesized compounds **BZ__03 – BZ_42**



BZ_03-BZ_22



BZ_23-BZ_42

Comp	R	R'	Yield (%)	M.P (°C)	Molecular Formula	Molecular Weight
BZ_03	H	Cl	24	-	$C_{22}H_{15}ClN_4O$	386.83
BZ_04	H	OCH_3	22	197-199	$C_{23}H_{18}N_4O_2$	382.41
BZ_05	H	H	21	-	$C_{22}H_{16}N_4O$	352.39
BZ_06	H	F	28	-	$C_{22}H_{15}FN_4O$	370.38
BZ_07	F	Cl	26	-	$C_{22}H_{14}ClN_4O$	404.82
BZ_08	F	OCH_3	25	181-183	$C_{23}H_{17}FN_4O_2$	400.41
BZ_09	F	H	30	173-175	$C_{22}H_{15}FN_4O$	370.38
BZ_10	F	F	31	226-228	$C_{22}H_{14}F_2N_4O$	388.37
BZ_11	Cl	Cl	22	-	$C_{22}H_{14}Cl_2N_4O$	421.28
BZ_12	Cl	OCH_3	25	213-215	$C_{23}H_{17}ClN_4O_2$	416.86

BZ_13	Cl	H	27	-	$C_{22}H_{15}ClN_4O$	386.83
BZ_14	Cl	F	22	-	$C_{22}H_{14}ClFN_4O$	404.82
BZ_15	CH_3	Cl	26	231-233	$C_{23}H_{17}ClN_4O$	400.86
BZ_16	CH_3	OCH_3	28	171-173	$C_{24}H_{20}N_4O_2$	396.44
BZ_17	CH_3	H	26	197-199	$C_{23}H_{18}N_4O$	366.42
BZ_18	CH_3	F	32	206-208	$C_{23}H_{17}FN_4O$	384.41
BZ_19	NO_2	Cl	21	-	$C_{22}H_{14}ClN_5O_3$	431.83
BZ_20	NO_2	OCH_3	23	-	$C_{23}H_{17}N_5O_4$	427.41
BZ_21	NO_2	H	24	-	$C_{22}H_{15}N_5O_3$	397.39
BZ_22	NO_2	F	20	-	$C_{22}H_{14}FN_5O_3$	415.38
BZ_23	H	Cl	31	250-252	$C_{21}H_{19}ClN_4O$	378.85
BZ_24	H	OCH_3	26	232-234	$C_{22}H_{22}N_4O_2$	374.44
BZ_25	H	H	36	230-232	$C_{21}H_{20}N_4O$	344.41
BZ_26	H	F	24	242-244	$C_{21}H_{19}FN_4O$	362.40
BZ_27	F	Cl	29	146-148	$C_{21}H_{18}ClFN_4O$	396.85
BZ_28	F	OCH_3	25	202-204	$C_{22}H_{21}FN_4O_2$	392.43
BZ_29	F	H	31	231-233	$C_{21}H_{19}FN_4O$	362.40
BZ_30	F	F	25	144-146	$C_{21}H_{18}F_2N_4O$	380.39
BZ_31	Cl	Cl	33	188-190	$C_{21}H_{18}Cl_2N_4O$	413.30
BZ_32	Cl	OCH_3	22	143-145	$C_{22}H_{21}ClN_4O_2$	408.88
BZ_33	Cl	H	33	225-227	$C_{21}H_{19}ClN_4O$	378.85
BZ_34	Cl	F	31	215-217	$C_{21}H_{18}ClFN_4O$	396.85
BZ_35	CH_3	Cl	21	243-245	$C_{22}H_{21}ClN_4O$	392.88
BZ_36	CH_3	OCH_3	23	133-135	$C_{23}H_{24}N_4O_2$	388.46
BZ_37	CH_3	H	23	251-252	$C_{12}H_{22}N_4O$	358.44
BZ_38	CH_3	F	27	226-228	$C_{22}H_{21}FN_4O$	376.43
BZ_39	NO_2	Cl	32	150-152	$C_{21}H_{18}ClN_5O_3$	423.85
BZ_40	NO_2	OCH_3	28	124-126	$C_{22}H_{21}N_5O_4$	419.43
BZ_41	NO_2	H	31	120-122	$C_{21}H_{19}N_5O_3$	389.41
BZ_42	NO_2	F	30	142-144	$C_{21}H_{18}FN_5O_3$	407.40

5.3.3. Characterization of the synthesized molecules

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-5-chloro-1H-indole-2-carboxamide (**BZ_03**): The compound was synthesized according to the general procedure by utilizing 4-(1H-Benzo[d]imidazol-2-yl)aniline (**2a**) (0.1 g, 0.48 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.11 g, 0.573 mmol) and triethyl amine (0.2 ml, 1.43 mmol) to afford **BZ_03** (0.095 g, 51 %) as brown gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.29 (s, 1H), 11.82 (s, 1H), 9.54 (s, 1H), 7.97 (m, 2H), 7.75 (m, 2H), 7.65 (d, *J* = 2 Hz, 1H), 7.51 (s, 1H), 7.42 (d, *J* = 8.6 Hz, 2H), 7.25 – 7.16 (m, 3H), 6.83 (d, *J* = 1.6 Hz, 1H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 139.8 (2C), 137.5, 131.8 (2C), 133.2, 128.2 (2C), 126.2, 122.5 (2C), 121.8, 121.6, 120.9, 120.5 (2C), 116.2 (2C), 115.8, 114.9. ESI-MS *m/z:* 387 $(M+H)^+$. Anal Calcd for $C_{22}H_{15}ClN_4O$: C, 68.31; H, 3.91; N, 14.48; Found: C, 68.39; H, 3.90; N, 14.46.

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-5-methoxy-1H-indole-2-carboxamide (**BZ_04**): The compound was synthesized according to the general procedure by utilizing 4-(1H-Benzo[d]imidazol-2-yl)aniline (**2a**) (0.1 g, 0.48 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.109 g, 0.573 mmol) and triethyl amine (0.2 ml, 1.43 mmol) to afford **BZ_04** (0.102 g, 56 %) as yellow solid. M.p: 197-199 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.28 (s, 1H), 11.85 (s, 1H), 9.49 (s, 1H), 7.91 (m, 2H), 7.72 (m, 2H), 7.66 (d, *J* = 2 Hz, 1H), 7.54 (s, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 7.21 – 7.14 (m, 3H), 6.85 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 155.1, 153.1, 139.7 (2C), 138.7, 138.2, 133.4, 133.1, 128.2 (2C), 122.5 (2C), 121.8, 120.1 (2C), 116.1, 115.8, 112.5, 112.2, 111.9, 55.6. ESI-MS *m/z:* 383 $(M+H)^+$. Anal Calcd for $C_{23}H_{18}N_4O_2$: C, 72.24; H, 4.74; N, 14.65; Found: C, 72.15; H, 4.75; N, 14.64.

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_05**): The compound was synthesized according to the general procedure by utilizing 4-(1H-Benzo[d]imidazol-2-yl)aniline (**2a**) (0.1 g, 0.48 mmol), 1H-indole-2-carboxylic acid (0.092 g, 0.573 mmol) and triethyl amine (0.2 ml, 1.43 mmol) to afford **BZ_05** (0.088 g, 52 %) as pale yellow gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.28 (s, 1H), 11.82 (s, 1H), 9.51 (s, 1H), 7.96 (m, 2H), 7.70 (m, 2H), 7.60 – 7.44 (m, 4H), 7.38 (s, 1H), 7.20 – 6.89 (m, 4H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.2, 139.8 (2C), 138.2, 137.9, 137.5, 130.9, 128.1 (2C), 122.5 (2C), 121.5, 121.2, 120.9, 118.9 (2C), 118.5, 116.1 (2C), 115.5, 112.1 ESI-MS *m/z:* 353 $(M+H)^+$. Anal Calcd for $C_{22}H_{16}N_4O$: C, 74.98; H, 4.58; N, 15.90; Found: C, 74.87; H, 4.57; N, 15.92.

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-5-fluoro-1H-indole-2-carboxamide (**BZ_06**): The compound was synthesized according to the general procedure by utilizing 4-(1H-Benzo[d]imidazol-2-yl)aniline (**2a**) (0.1 g, 0.48 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.102 g, 0.573 mmol) and triethyl amine (0.2 ml, 1.43 mmol) to afford **BZ_06** (0.098 g, 55 %) as yellow gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.29 (s, 1H), 11.82 (s, 1H), 9.48 (s, 1H), 7.92 (m, 2H), 7.73 (m, 2H), 7.48 (m, 1H), 7.40 (s, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.26 – 7.17 (m, 3H), 6.98 (m, 1H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 149.9, 139.7 (2C), 138.7, 138.2, 133.5, 128.2 (2C), 122.5 (2C), 121.8, 120.5 (2C), 115.6 (2C), 115.1, 110.5, 110.1. ESI-MS *m/z:* 371 $(M+H)^+$. Anal Calcd for $C_{22}H_{15}FN_4O$: C, 71.34; H, 4.08; N, 15.13; Found: C, 71.42; H, 4.07; N, 15.11.

5-chloro-N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_07**): The compound was synthesized according to the general procedure by utilizing 4-(5-fluoro-1H-benzo[d]imidazol-2-yl)aniline (**2b**) (0.1 g, 0.44 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.1 g, 0.528 mmol) and triethyl amine (0.18 ml, 1.32 mmol) to afford **BZ_07** (0.085 g, 44 %) as brown gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.29 (s, 1H), 11.85 (s, 1H), 9.52 (s, 1H), 7.95 (m, 2H), 7.76 (m, 2H), 7.67 (d, *J* = 2 Hz, 1H), 7.44 (d, J = 8.8 Hz, 1H), 7.43 (m, 1H), 7.28 (s, 1H), 7.17 – 6.95 (m, 2H), 6.82 (d, *J* = 1.6 Hz, 1H).^{13}C NMR [DMSO-d_6] δ: 161.5, 155.8, 153.5, 139.8, 138.5, 138.1 (2C), 137.9, 133.1, 128.2 (2C), 126.2, 123.1, 122.5, 120.5 (2C), 116.5, 115.2, 114.5, 110.2, 101.3. ESI-MS *m/z:* 405 $(M+H)^+$. Anal Calcd for $C_{22}H_{14}ClN_4O$: C, 65.27; H, 3.49; N, 13.84; Found: C, 65.35; H, 3.48; N, 13.86.

N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-5-methoxy-1H-indole-2-carboxamide (**BZ_08**): The compound was synthesized according to the general procedure by utilizing 4-(5-fluoro-1H-benzo[d]imidazol-2-yl)aniline (2b) (0.1 g, 0.44 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.103 g, 0.528 mmol) and triethyl amine (0.18 ml, 1.32 mmol) to afford **BZ_08** (0.1 g, 52 %) as yellow solid. M.p:181-183 ºC. ^{1}H NMR [DMSO-d_6]: δ_H 12.25 (s, 1H), 11.81 (s, 1H), 9.51 (s, 1H), 7.91 (m, 2H), 7.75 (m, 2H), 7.57 (d, *J* = 2.0 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.35 (m, 1H), 7.24 (s, 1H), 7.15 – 6.95 (m, 2H), 6.83 (d, *J* = 1.4 Hz, 1H), 3.88 (m, 3H).^{13}C NMR [DMSO-d_6] δ: 161.5, 155.5, 154.2, 153.1, 138.5, 138.2, 137.5, 137.1, 133.5, 133.1, 128.5 (2C), 122.3, 120.5 (2C), 116.5, 115.2, 113.1, 112.9, 112.1, 110.2, 102.5, 55.6. ESI-MS *m/z:* 401 $(M+H)^+$. Anal Calcd for $C_{23}H_{17}FN_4O_2$: C, 68.99; H, 4.28; N, 13.99; Found: C, 68.91; H, 4.29; N, 13.97.

N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_09**): The compound was synthesized according to the general procedure by utilizing 4-(5-fluoro-1H-benzo[d]imidazol-2-yl)aniline (2b) (0.1 g, 0.44 mmol), 1H-indole-2-carboxylic acid (0.07 g, 0.528 mmol) and triethyl amine (0.18 ml, 1.32 mmol) to afford **BZ_09** (0.088 g, 54 %) as dark brown solid. M.p: 173-175 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.26 (s, 1H), 11.86 (s, 1H), 9.55 (s, 1H), 7.90 (m, 2H), 7.78 (m, 2H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.40 (m, 1H), 7.24 (s, 1H), 7.18 – 6.85 (m, 4H).^{13}C NMR [DMSO-d_6] δ: 161.5, 155.6, 153.1, 139.1, 138.9, 138.3, 137.9, 137.5, 132.5, 128.5 (2C), 122.5, 122.3, 120.9, 120.5, 120.1 (2C), 117.2, 115.2, 110.9, 102.5. ESI-MS *m/z:* 371 $(M+H)^+$. Anal Calcd for $C_{22}H_{15}FN_4O$: C, 71.34; H, 4.08; N, 15.13; Found: C, 71.42; H, 4.09; N, 15.11.

5-fluoro-N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_10**): The compound was synthesized according to the general procedure by utilizing 4-(5-fluoro-1H-benzo[d]imidazol-2-yl)aniline (2b) (0.1 g, 0.44 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.094 g, 0.528 mmol) and triethyl amine (0.18 ml, 1.32 mmol) to afford **BZ_10** (0.075 g, 44 %) as yellow solid. M.p: 226-228 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.29 (s, 1H), 11.82 (s, 1H), 9.51 (s, 1H), 7.92 (m, 2H), 7.76 (m, 2H), 7.64 (d, *J* = 2.4 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.42 (m, 1H), 7.28 (s, 1H), 7.15 – 6.82 (m, 3H).^{13}C NMR [DMSO-d_6] δ: 161.5, 155.6, 153.2, 149.8, 139.9, 139.2, 138.8, 138.2, 136.2, 133.5, 128.2 (2C), 122.5, 120.5 (2C), 115.9, 115.2, 114.8, 110.5, 110.2 (2C), 101.5. ESI-MS *m/z:* 389 $(M+H)^+$. Anal Calcd for $C_{22}H_{14}F_2N_4O$: C, 68.04; H, 3.63; N, 14.43; Found: C, 68.13; H, 3.62; N, 14.45.

5-chloro-N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_11**): The compound was synthesized according to the general procedure by utilizing 4-(5-chloro-1H-benzo[d]imidazol-2-yl)aniline (**2c**) (0.1 g, 0.41 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.096 g, 0.492 mmol) and triethyl amine (0.17 ml, 1.23 mmol) to afford **BZ_11** (0.092 g, 53 %) as yellow gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.24 (s, 1H), 11.82 (s, 1H), 9.52 (s, 1H), 7.95 (m, 2H), 7.78 (m, 2H), 7.74 (d, *J* = 2 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.46 – 7.25 (m, 3H), 7.02 (t, *J* = 1.4 Hz, 1H,), 6.85 (d, *J* = 1.6 Hz, 1H,).^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 138.6, 138.2 (2C), 133.4, 133.1, 130.1, 129.5, 128.2 (2C), 125.7, 125.2, 123.4, 122.6, 122.4, 120.5 (2C), 115.8, 115.5, 114.8, 114.5. ESI-MS *m/z:* 422 $(M+H)^+$. Anal Calcd for $C_{22}H_{14}Cl_2N_4O$: C, 62.72; H, 3.35; N, 13.30; Found: C, 62.65; H, 3.36; N, 13.31.

N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)-5-methoxy-1H-indole-2-carboxamide (**BZ_12**): The compound was synthesized according to the general procedure by utilizing 4-(5-chloro-1H-benzo[d]imidazol-2-yl)aniline (2c) (0.1 g, 0.41 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.093 g, 0.492 mmol) and triethyl amine (0.17 ml, 1.23 mmol) to afford **BZ_12** (0.07 g, 41 %) as yellow solid. M.p: 213-215 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.28 (s, 1H), 11.84 (s, 1H), 9.50 (s, 1H), 7.90 (m, 2H), 7.80 (m, 2H), 7.76 (d, *J* = 2 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.48 – 7.23 (m, 3H), 7.01 (t, *J* = 1.6 Hz, 1H), 6.82 (d, *J* = 1.6 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 155.1, 153.5, 139.2, 138.5, 133.5, 133.1, 132.9, 129.8, 129.7, 128.5 (2C), 124.3, 122.1, 120.5 (2C), 116.8, 116.2, 115.5, 113.1, 112.8, 112.1, 55.6. ESI-MS *m/z:* 417 $(M+H)^+$. Anal Calcd for $C_{23}H_{17}ClN_4O_2$: C, 66.27; H, 4.11; N, 13.44; Found: C, 66.21; H, 4.12; N, 13.42.

N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_13**): The compound was synthesized according to the general procedure by utilizing 4-(5-chloro-1H-benzo[d]imidazol-2-yl)aniline (2c) (0.1 g, 0.41 mmol), 1H-indole-2-carboxylic acid (0.079 g, 0.492 mmol) and triethyl amine (0.17 ml, 1.23 mmol) to afford **BZ_13** (0.068 g, 43 %) as pale yellow gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.27 (s, 1H), 11.82 (s, 1H), 9.49 (s, 1H), 7.94 (m, 2H), 7.82 (m, 2H), 7.75 (d, *J* = 2 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.46 – 7.22 (m, 3H), 7.04 – 6.85 (m, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 140.2, 139.5, 138.2, 133.5, 132.1, 130.5, 130.2, 128.2 (2C), 125.6, 122.3, 122.1, 120.9, 120.6, 120.3 (2C), 117.1, 116.5, 115.5, 112.5. ESI-MS *m/z:* 387 $(M+H)^+$. Anal Calcd for $C_{22}H_{15}ClN_4O$: C, 68.31; H, 3.91; N, 14.48; Found: C, 68.24; H, 3.90; N, 14.47.

N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)-5-fluoro-1H-indole-2-carboxamide (**BZ_14**): The compound was synthesized according to the general procedure by utilizing 4-(5-chloro-1H-benzo[d]imidazol-2-yl)aniline (2c) (0.1 g, 0.41 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.088 g, 0.492 mmol) and triethyl amine (0.17 ml, 1.23 mmol) to afford **BZ_14** (0.079 g, 47 %) as brown gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.28 (s, 1H), 11.84 (s, 1H), 9.54 (s, 1H), 7.97 (m, 2H), 7.80 (m, 2H), 7.78 (d, *J* = 2 Hz, 1H), 7.49 (d, *J* = 8.8 Hz, 1H), 7.44 – 7.21 (m, 3H), 6.97 – 6.81 (m, 2H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 150.5, 139.7, 138.5, 136.5, 133.5, 133.2, 131.5, 130.2, 128.2 (2C), 125.1, 122.3, 120.5 (2C), 115.9, 115.5, 115.2, 114.8, 110.5, 110.2. ESI-MS *m/z:* 405 $(M+H)^+$. Anal Calcd for $C_{22}H_{14}ClFN_4O$: C, 65.27; H, 3.49; N, 13.84; Found: C, 65.35; H, 3.50; N, 13.82.

5-chloro-N-(4-(5-methyl-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_15**): The compound was synthesized according to the general procedure by utilizing 4-(5-methyl-1H-benzo[d]imidazol-2-yl)aniline (**2d**) (0.1 g, 0.45 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.105 g, 0.537 mmol) and triethyl amine (0.19 ml, 1.35 mmol) to afford **BZ_15** (0.104 g, 57 %) as yellow solid. M.p: 231-233 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.28 (s, 1H), 11.88 (s, 1H), 9.43 (s, 1H), 7.93 (m, 2H), 7.76 (m, 2H), 7.69 (d, *J* = 2 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.28 (s, 1H), 7.18 (m, 1H), 6.92 (t, 1H, *J* = 1.2 Hz), 6.83 (d, 1H, *J* = 1.6 Hz), 2.38 (s, 3H).^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 138.5, 138.2 (2C), 136.1, 133.6 (2C), 132.5, 128.2 (2C), 126.2, 125.5, 123.4, 122.6, 122.1, 120.5 (2C), 116.1, 115.9, 115.5, 114.9, 22.5. ESI-MS *m/z:* 401 $(M+H)^+$. Anal Calcd for $C_{23}H_{17}ClN_4O$: C, 68.69; H, 4.27; N, 13.98; Found: C, 68.72; H, 4.26; N, 14.00.

5-methoxy-N-(4-(5-methyl-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_16**): The compound was synthesized according to the general procedure by utilizing 4-(5-methyl-1H-benzo[d]imidazol-2-yl)aniline (2d) (0.1 g, 0.45 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.102 g, 0.537 mmol) and triethyl amine (0.19 ml, 1.35 mmol) to afford **BZ_16** (0.096 g, 54 %) as yellow solid. M.p: 171-173 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.26 (s, 1H), 11.85 (s, 1H), 9.46 (s, 1H), 7.91 (m, 2H), 7.78 (m, 2H), 7.66 (d, *J* = 2 Hz, 1H), 7.42 (d, *J* = 9.2 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 1H), 7.34 (s, 1H), 7.23 (m, 1H), 7.04 (t, *J* = 1.6 Hz, 1H), 6.83 (d, *J* = 1.6 Hz, 1H), 3.87 (s, 3H), 2.39 (s, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.8, 152.5, 139.2, 138.3, 136.2, 133.5, 132.8, 132.5, 132.1, 128.5 (2C), 126.2, 122.3, 120.5 (2C), 116.2, 115.9, 115.2, 112.5, 112.3, 111.8, 55.6, 22.5. ESI-MS *m/z:* 397 $(M+H)^+$. Anal Calcdfor$C_{24}H_{20}N_4O_2$: C, 72.71; H, 5.08; N, 14.13; Found: C, 72.65; H, 5.09; N, 14.12.

N-(4-(5-methyl-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_17**): The compound was synthesized according to the general procedure by utilizing 4-(5-methyl-1H-benzo[d]imidazol-2-yl)aniline (2d) (0.1 g, 0.45 mmol), 1H-indole-2-carboxylic acid (0.086 g, 0.537 mmol) and triethyl amine (0.19 ml, 1.35 mmol) to afford **BZ_17** (0.091 g, 55%) as pale yellow solid. M.p: 197-199 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.29 (s, 1H), 11.86 (s, 1H), 9.57 (s, 1H), 7.94 (m, 2H), 7.81 (m, 2H), 7.61 (m, 1H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.35 (s, 1H), 7.17 (m, 1H), 7.04 – 6.79 (m, 3H), 2.38 (s, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 140.1, 138.6, 138.2, 136.2, 133.5, 132.5, 131.9, 128.5 (2C), 126.2, 122.3, 122.1,

121.5, 120.8, 120.5 (2C), 116.3, 116.1, 115.8, 111.8, 22.5. ESI-MS *m/z:* 367 $(M+H)^+$. Anal Calcd for $C_{23}H_{18}N_4O$: C, 75.39; H, 4.95; N, 15.29; Found: C, 75.48; H, 4.94; N, 15.30.

5-fluoro-N-(4-(5-methyl-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_18**): The compound was synthesized according to the general procedure by utilizing 4-(5-methyl-1H-benzo[d]imidazol-2-yl)aniline (2d) (0.1 g, 0.45 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.096 g, 0.537 mmol) and triethyl amine (0.19 ml, 1.35 mmol) to afford **BZ_18** (0.071 g, 41 %) as orange solid. M.p: 206-208 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.25 (s, 1H), 11.85 (s, 1H), 9.54 (s, 1H), 7.92 (m, 2H), 7.78 (m, 2H), 7.54 (m, 1H), 7.42 (d, *J* = 9.2 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.29 (s, 1H), 7.23 (m, 1H), 7.04 – 6.83 (m, 2H), 2.40 (s, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 150.6, 139.1, 136.9, 135.8, 135.6, 133.8, 133.5, 132.9, 132.3, 128.5 (2C), 126.5, 122.5, 120.5 (2C), 116.3, 116.2, 115.9, 110.6, 110.2, 22.5. ESI-MS *m/z:* 385 $(M+H)^+$. Anal Calcd for $C_{23}H_{17}FN_4O$: C, 71.86; H, 4.46; N, 14.57; Found: C, 71.97; H, 4.45; N, 14.55.

5-chloro-N-(4-(5-nitro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_19**): The compound was synthesized according to the general procedure by utilizing 4-(5-nitro-1H-benzo[d]imidazol-2-yl)aniline (**2e**) (0.1 g, 0.39 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.092 g, 0.472 mmol) and triethyl amine (0.16 ml, 1.17 mmol) to afford **BZ_19** (0.077 g, 48 %) as pale yellow gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.29 (s, 1H), 11.85 (s, 1H), 9.54 (s, 1H), 7.92 (m, 2H), 7.85 (d, *J* = 2 Hz, 1H), 7.78 (m, 2H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.54 – 7.28 (m, 3H), 7.12 (t, *J* = 1.4 Hz, 1H), 6.93 (d, *J* = 1.6 Hz, 1H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 148.6, 145.6, 140.2, 138.8, 137.5 (2C), 133.5, 128.5 (2C), 126.2, 123.2, 122.9, 122.7, 120.5 (2C), 119.4, 116.6, 115.3, 114.8, 113.6. ESI-MS *m/z:* 432 $(M+H)^+$. Anal Calcd for $C_{22}H_{14}ClN_5O_3$: C, 61.19; H, 3.27; N, 16.22; Found: C, 61.12; H, 3.28; N, 16.24.

5-methoxy-N-(4-(5-nitro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_20**): The compound was synthesized according to the general procedure by utilizing 4-(5-nitro-1H-benzo[d]imidazol-2-yl)aniline (2e) (0.1 g, 0.39 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.90 g, 0.472 mmol) and triethyl amine (0.16 ml, 1.17 mmol) to afford **BZ_20** (0.073 g, 44 %) as brown gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.27 (s, 1H), 11.80 (s, 1H), 9.54 (s, 1H), 7.94 (m, 2H), 7.81 (m, 2H), 7.78 (d, *J* = 2 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.51 – 7.22 (m, 3H), 7.02 (t, *J* = 1.6 Hz, 1H), 6.87 (d, *J* = 1.6 Hz, 1H), 3.88 (s, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 155.1, 153.6, 146.9, 145.2, 140.2, 139.6, 138.6, 133.7, 133.5, 128.5 (2C), 122.6, 120.5

(2C), 119.2, 116.6, 115.4, 113.4, 112.8, 115.5, 111.9, 55.6 ESI-MS *m/z:* 428 $(M+H)^+$. Anal Calcd for $C_{23}H_{17}N_5O_4$: C, 64.63; H, 4.01; N, 16.39; Found: C, 64.68; H, 4.00; N, 16.40.

N-(4-(5-nitro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_21**): The compound was synthesized according to the general procedure by utilizing 4-(5-nitro-1H-benzo[d]imidazol-2-yl)aniline (2e) (0.1 g, 0.39 mmol), 1H-indole-2-carboxylic acid (0.076 g, 0.472 mmol) and triethyl amine (0.16 ml, 1.17 mmol) to afford **BZ_21** (0.081 g, 53 %) as yellow gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.26 (s, 1H), 11.83 (s, 1H), 9.49 (s, 1H), 7.97 (m, 2H), 7.79 (m, 2H), 7.70 (d, *J* = 2.2 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.49 – 7.21 (m, 3H), 7.01 – 6.89 (m, 3H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 148.5, 145.6, 140.2 (2C), 139.3, 138.6, 132.3, 128.5 (2C), 122.5, 122.3, 121.6, 120.8, 120.5 (2C), 119.7, 116.6, 115.2, 113.5, 112.2. ESI-MS *m/z:* 398 $(M+H)^+$. Anal Calcd for $C_{22}H_{15}N_5O_3$: C, 66.49; H, 3.80; N, 17.62; Found: C, 66.55; H, 3.79; N, 17.64.

5-fluoro-N-(4-(5-nitro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide (**BZ_22**): The compound was synthesized according to the general procedure by utilizing 4-(5-nitro-1H-benzo[d]imidazol-2-yl)aniline (2e) (0.1 g, 0.39 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.080 g, 0472 mmol) and triethyl amine (0.16 ml, 1.17 mmol) to afford **BZ_22** (0.088 g, 55 %) as pale yellow gum. ^{1}H NMR [DMSO-d_6]: δ_H 12.29 (s, 1H), 11.82 (s, 1H), 9.53 (s, 1H), 7.95 (m, 2H), 7.79 (m, 2H), 7.72 (d, *J* = 2 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.55 – 7.24 (m, 3H), 7.04 – 6.88 (m, 2H). ^{13}C NMR [DMSO-d_6] δ: 161.5, 153.5, 150.6, 148.5, 145.6, 140.2, 139.6, 138.8, 136.2, 133.5, 128.5 (2C), 122.6, 120.5 (2C), 119.5, 116.7, 115.6, 115.1, 113.3, 110.5, 110.2 ESI-MS *m/z:* 416 $(M+H)^+$. Anal Calcd for $C_{22}H_{14}FN_5O_3$: C, 63.61; H, 3.40; N, 16.86; Found: C, 63.68; H, 3.39; N, 16.85.

(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-chloro-1H-indol-2-yl)methanone (**BZ_23**): The compound was synthesized according to the general procedure by utilizing 2-(piperidin-4-yl)-1H-benzo[d]imidazole (**2'a**) (0.1 g, 0.496 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.116 g, 0.596 mmol) and triethyl amine (0.2 ml, 1.48 mmol) to afford **BZ_23** (0.11 g, 59 %) as pale yellow solid. M.p: 250-252 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.27 (s, 1H), 11.83 (s, 1H), 7.68 (d, *J* = 2 Hz, 1H), 7.49 (s, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.21 – 7.12 (m, 3H), 6.81 (d, *J* = 1.6 Hz, 1H), 4.51 (d, *J* = 11.6 Hz, 2H), 3.23 (m, 3H), 2.15 (t, *J* = 10.4 Hz, 2H), 1.88 (t, *J* = 10.4 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 142.3, 140.2, 139.2 (2C), 138.6, 133.5, 126.2, 122.5 (2C), 121.8, 121.5, 115.7 (2C), 115.1, 114.8, 45.6 (2C), 36.3, 30.2 (2C). ESI-MS *m/z:* 379

$(M+H)^+$. Anal Calcd for $C_{21}H_{19}ClN_4O$: C, 66.58; H, 5.05; N, 14.79; Found: C, 66.42; H, 5.04; N, 14.80.

(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-methoxy-1H-indol-2-yl)methanone (**BZ_24**): The compound was synthesized according to the general procedure by utilizing 2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'a) (0.1 g, 0.496 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.113 g, 0.596 mmol) and triethyl amine (0.2 ml, 1.48 mmol) to afford **BZ_24** (0.115 g, 62 %) as yellow solid.M.p: 232-234 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.31 (s, 1H), 11.81 (s, 1H), 7.62 (d, *J* = 2 Hz, 1H), 7.52 (s, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.18 – 7.09 (m, 3H), 6.82 (d, *J* = 2 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 2H), 3.85 (s, 3H), 3.26 (m, 3H), 2.18 (t, *J* = 10.6, 2H), 1.85 (t, *J* = 10.5 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 155.6, 142.1, 140.2 (2C), 139.2, 132.8, 132.6, 122.5 (2C), 115.8 (2C), 115.2, 113.6, 112.7, 111.4, 56.5, 45.5 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 375 $(M+H)^+$. Anal Calcd for $C_{22}H_{22}N_4O_2$: C, 70.57; H, 5.92; N, 14.96; Found: C, 70.50; H, 5.91; N, 14.94.

(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(1H-indol-2-yl)methanone (**BZ_25**): The compound was synthesized according to the general procedure by utilizing 2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'a) (0.1 g, 0.496 mmol), 1H-indole-2-carboxylic acid (0.096 g, 0.596 mmol) and triethyl amine (0.2 ml, 1.48 mmol) to afford **BZ_25** (0.101 g, 60 %) as white solid. M.p: 230-232 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.30 (s, 1H), 11.84 (s, 1H), 7.58 – 7.42(m, 4H), 7.40 (s, 1H), 7.18 – 6.95 (m, 4H), 4.54 (d, *J* = 11.3 Hz, 2H), 3.23 (m, 3H), 2.14 (t, *J* = 10.8, 2H), 1.83 (t, *J* = 10.5 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 142.3, 140.2, 139.5 (2C), 138.8, 130.5, 122.5 (2C), 122.1, 121.5, 120.5, 115.8 (2C), 115.3, 112.5, 45.5 (2C), 36.2, 30.2 (2C). ESI-MS m/z: 345 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27; Found: C, 73.31; H, 5.84; N, 16.25.

(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-fluoro-1H-indol-2-yl)methanone (**BZ_26**): The compound was synthesized according to the general procedure by utilizing 2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'a) (0.1 g, 0.496 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.106 g, 0596 mmol) and triethyl amine (0.2 ml, 1.48 mmol) to afford **BZ_26** (0.098 g, 55 %) as pale yellow solid. M.p: 242-244 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.34 (s, 1H), 11.86 (s, 1H), 7.45 (m, 1H), 7.41 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.25 – 7.14 (m, 3H), 7.08 (m, 1H), 4.51 (d, *J* = 11.2 Hz, 2H), 3.22 (m, 3H), 2.16 (t, *J* = 10.6, 2H), 1.87 (t, *J* = 10.6 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 150.4, 142.1, 140.2 (2C), 139.6, 136.5, 133.34, 122.5 (2C), 115.6 (2C),

115.4, 115.1, 110.5, 110.2, 45.5 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 363 $(M+H)^+$. Anal Calcd for $C_{21}H_{19}FN_4O$: C, 69.60; H, 5.28; N, 5.46; Found: C, 69.68; H, 5.27; N, 5.45.

(5-chloro-1H-indol-2-yl)(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_27**): The compound was synthesized according to the general procedure by utilizing 5-fluoro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (**2'b**) (0.1 g, 0.456 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.107 g, 0.547 mmol) and triethyl amine (0.19 ml, 1.37 mmol) to afford **BZ_27** (0.108 g, 59 %) as yellow solid. M.p: 146-148 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.25 (s, 1H), 11.82 (s, 1H), 7.65 (d, *J* = 2 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.41 (m, 1H), 7.25 (s, 1H), 7.14 – 7.01 (m, 2H), 6.82 (d, *J* = 1.8 Hz, 1H), 4.52 (d, *J* = 11.0 Hz, 2H), 3.35 (m, 3H), 2.14 (t, *J* = 11.6, 2H), 1.95 (t, *J* = 11.4 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 156.2, 142.1, 139.8, 139.2, 138.5, 135.2, 133.5, 126.2, 123.2, 122.2, 117.2, 115.7, 115.1, 110.2, 101.8, 45.2 (2C), 36.3, 30.2 (2C). ESI-MS *m/z:* 397 $(M+H)^+$. Anal Calcd for $C_{21}H_{18}ClFN_4O$: C, 63.56; H, 4.57; N, 14.52; Found: C, 63.61; H, 4.56; N, 14.11.

(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-methoxy-1H-indol-2-yl)methanone (**BZ_28**): The compound was synthesized according to the general procedure by utilizing 5-fluoro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'b) (0.1 g, 0.456 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.104 g, 0.547 mmol) and triethyl amine (0.19 ml, 1.37 mmol) to afford **BZ_28** (0.105 g, 58 %) as white solid. M.p: 202-204 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.26 (s, 1H), 11.85 (s, 1H), 7.61 (d, *J* = 2.2 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.39 (m, 1H), 7.27 (s, 1H), 7.16 – 6.98 (m, 2H), 6.85 (d, *J* = 1.8 Hz, 1H), 4.50 (d, *J* = 11.2 Hz, 2H), 3.88 (m, 3H), 3.31 (m, 3H), 2.11 (t, *J* = 11.2, 2H), 1.91 (t, *J* = 11.6 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 156.3, 155.2, 142.2, 139.5, 138.3, 135.6, 133.5, 133.2, 117.2, 115.5, 112.4, 111.9, 110.5, 101.5, 556.6, 45.2 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 393 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}FN_4O_2$: C, 67.33; H, 5.39; N, 14.28; Found: C, 67.25; H, 5.40; N, 14.27.

(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(1H-indol-2-yl)methanone (**BZ_29**): The compound was synthesized according to the general procedure by utilizing 5-fluoro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'b) (0.1 g, 0.456 mmol), 1H-indole-2-carboxylic acid (0.088 g, 0.547 mmol) and triethyl amine (0.19 ml, 1.37 mmol) to afford **BZ_29** (0.104 g, 62 %) as white solid. M.p: 231-233 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.24 (s, 1H), 11.82 (s, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.42 (m, 1H), 7.29 (s, 1H), 7.16 – 6.83 (m, 4H), 4.52 (d, *J* = 11.0 Hz, 2H), 3.34 (m, 3H), 2.14 (t, *J* = 11.2, 2H), 1.94 (t, *J* = 11.8 Hz, 2H).^{13}C

NMR [DMSO-d_6] δ: 172.5, 155.6, 142.2, 139.5, 138.9, 138.4, 135.6, 132.1, 122.8, 121.3, 120.5, 117.2, 115.6, 112.3, 110.2, 101.5, 45.2 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 363 $(M+H)^+$. Anal Calcd for $C_{21}H_{19}FN_4O$: C, 69.60; H, 5.28; N, 15.46; Found: C, 69.71; H, 5.29; N, 15.45.

(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-fluoro-1H-indol-2-yl)methanone (**BZ_30**): The compound was synthesized according to the general procedure by utilizing 5-fluoro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'b) (0.1 g, 0.456 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.098 g, 0.547 mmol) and triethyl amine (0.19 ml, 1.37 mmol) to afford **BZ_30** (0.111 g, 64 %) as pale brown solid. M.p: 144-146 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.27 (s, 1H), 11.84 (s, 1H), 7.69 (d, *J* = 2.2 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.40 (m, 1H), 7.31 (s, 1H), 7.18 – 6.85 (m, 3H), 4.47 (d, *J* = 11.4 Hz, 2H), 3.36 (m, 3H), 2.15 (t, *J* = 11.6, 2H), 1.92 (t, *J* = 11.6 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 155.7, 150.8, 141.2, 139.5, 138.8, 136.2, 135.6, 133.4, 117.2, 115.8, 115.5, 110.8, 110.5, 110.4, 101.5, 45.2 (2C), 36.3, 30.2 (2C). ESI-MS *m/z:* 381 $(M+H)^+$. Anal Calcd for $C_{21}H_{18}F_2N_4O$: C, 66.31; H, 4.77; N, 14.73; Found: C, 66.39; H, 4.76; N, 14.75.

(4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-chloro-1H-indol-2-yl)methanone (**BZ_31**): The compound was synthesized according to the general procedure by utilizing 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (**2'c**) (0.1 g, 0.424 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.099 g, 0.509 mmol) and triethyl amine (0.17 ml, 1.27 mmol) to afford **BZ_31** (0.106 g, 61 %) as pale brown solid. M.p: 188-190 °C ^{1}H NMR [DMSO-d_6]: δ_H 12.26 (s, 1H), 11.85 (s, 1H), 7.75 (d, *J* = 2 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.40 – 7.21 (m, 3H), 7.06 (t, *J* = 1.4 Hz, 1H,), 6.82 (d, *J* = 1.6 Hz, 1H,), 4.53 (d, *J* = 11.8 Hz, 2H), 3.32 (m, 3H), 2.16 (t, *J* = 11.0, 2H), 1.92 (t, *J* = 11.2 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 142.1, 139.5, 138.9, 138.2, 137.5, 133.5, 130.2, 125.7, 125.2, 123.5, 122.7, 117.1, 116.5, 115.2, 114.9, 45.2 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 414 $(M+H)^+$. Anal Calcd for $C_{21}H_{18}Cl_2N_4O$: C, 61.03; H, 4.39; N, 13.56; Found: C, 61.13; H, 4.40; N, 13.55.

(4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-methoxy-1H-indol-2-yl)methanone (**BZ_32**): The compound was synthesized according to the general procedure by utilizing 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'c) (0.1 g, 0.424 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.97 g, 0.509 mmol) and triethyl amine (0.17 ml, 1.27 mmol) to afford **BZ_32** (0.1 g, 58 %) as brown solid. M.p: 143-145^{0}C. ^{1}H NMR [DMSO-d_6]: δ_H 12.27 (s, 1H), 11.82 (s, 1H), 7.78 (d, *J* = 2 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.46 – 7.25 (m,

3H), 7.04 (t, *J* = 1.4 Hz, 1H), 6.85 (d, *J* = 1.4 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 2H), 3.85 (s, 3H), 3.35 (m, 3H), 2.18 (t, *J* = 11.6, 2H), 1.94 (t, *J* = 11.4 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 154.8, 142.1, 139.5, 138.8, 138.1, 133.4, 133.2, 130.2, 124.8, 117.1, 116.5, 115.2, 113.5, 113.9, 112.4, 55.6, 45.5 (2C), 36.3, 30.2 (2C). ESI-MS *m/z:* 409 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}ClN_4O_2$: C, 64.62; H, 5.18; N, 13.70; Found: 64.55; H, 5.19; N, 13.69.

(4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(1H-indol-2-yl)methanone (**BZ_33**): The compound was synthesized according to the general procedure by utilizing 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'c) (0.1 g, 0.424 mmol), 1H-indole-2-carboxylic acid (0.082 g, 0.509 mmol) and triethyl amine (0.17 ml, 1.27 mmol) to afford **BZ_33** (0.099 g, 62 %) as white solid. M.p: 225-227 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.24 (s, 1H), 11.81 (s, 1H), 7.79 (d, *J* = 2 Hz, 1H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.44 – 7.26 (m, 3H), 7.00 – 6.85 (m, 3H), 4.53 (d, *J* = 11.6 Hz, 2H), 3.36 (m, 3H), 2.15 (t, *J* = 11.8, 2H), 1.92 (t, *J* = 11.6 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 142.1, 139.5, 138.9, 138.2, 137.5, 132.5, 130.2, 124.5, 122.3, 121.7, 120.5, 117.3, 116.1, 115.5, 112.2, 45.2 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 379 $(M+H)^+$. Anal Calcd for $C_{21}H_{19}ClN_4O$: C, 65.58; H, 5.05; N, 14.79; Found: C, 65.51; H, 5.04; N, 14.81.

(4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-fluoro-1H-indol-2-yl)methanone (**BZ_34**): The compound was synthesized according to the general procedure by utilizing 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'c) (0.1 g, 0.424 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.091 g, 0.509 mmol) and triethyl amine (0.17 ml, 1.27 mmol) to afford **BZ_34** (0.106 g, 64 %) as brown solid. M.p: 215-217 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.22 (s, 1H), 11.80 (s, 1H), 7.81 (d, *J* = 2 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.45 – 7.26 (m, 3H), 7.03 – 6.85 (m, 2H), 4.55 (d, *J* = 11.4 Hz, 2H), 3.32 (m, 3H), 2.14 (t, *J* = 11.4, 2H), 1.93 (t, *J* = 11.8 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 150.5, 142.1, 139.5, 138.8, 138.1, 136.1, 133.5, 130.2, 125.6, 117.1, 116.1, 115.8, 115.2, 110.5, 110.2, 45.2 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 397 $(M+H)^+$. Anal Calcd for $C_{21}H_{18}ClFN_4O$: C, 63.56; H, 4.57; N, 14.12; Found: C, 63.49; H, 4.58; N, 14.11.

(5-chloro-1H-indol-2-yl)(4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_35**): The compound was synthesized according to the general procedure by utilizing 5-methyl-2-(piperidin-4-yl)-1H-benzo[d]imidazole (**2'd**) (0.1 g, 0.464 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.109 g, 0.557 mmol) and triethyl amine (0.19 ml, 1.39 mmol) to afford **BZ_35** (0.116 g, 64 %) as yellow solid. M.p: 243-245 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.25

(s, 1H), 11.86 (s, 1H), 7.67 (d, *J* = 2 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.27 (s, 1H), 7.20 (m, 1H), 6.96 (t, *J* = 1.2 Hz, 1H), 6.81 (d, *J* = 1.6 Hz, 1H), 4.49 (d, *J* = 12.8 Hz, 2H), 3.34 (m, 3H), 2.39 (s, 3H), 2.13 (t, *J* = 10.8, 2H), 1.89 (t, *J* = 11.2 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 142.1, 139.1, 138.8, 138.4, 138.1, 136.2, 132.8 (2C), 126.2, 125.8, 123.1, 122.5, 115.8, 115.4, 45.2 (2C), 36.2, 30.2 (2C), 22.5. ESI-MS *m/z:* 393 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}ClN_4O$: C, 67.26; H, 5.39; N, 14.26; Found: C, 67.31; H, 5.40; N, 14.25.

(5-methoxy-1H-indol-2-yl)(4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_36**): The compound was synthesized according to the general procedure by utilizing 5-methyl-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'd) (0.1 g, 0.464 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.106 g, 0.557 mmol) and triethyl amine (0.19 ml, 1.39 mmol) to afford **BZ_36** (0.109 g, 61 %) as dark brown solid. M.p: 133-135 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.23 (s, 1H), 11.83 (s, 1H), 7.65 (d, *J* = 2 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 7.39 (d, *J* = 8.6 Hz, 1H), 7.31 (s, 1H), 7.21 (m, 1H), 7.02 (t, *J* = 1.6 Hz, 1H), 6.85 (d, *J* = 1.4 Hz, 1H), 4.51 (d, *J* = 12.2 Hz, 2H), 3.85 (s, 3H), 3.37 (m, 3H), 2.37 (s, 3H), 2.14 (t, *J* = 10.6, 2H), 1.85 (t, *J* = 11.4 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 134.3, 142.5, 139.4, 138.8, 136.3, 133.5, 133.1, 132.8, 126.2, 116.1, 115.8, 113.5, 113.1, 112.5, 55.6, 45.2 (2C), 36.2, 30.2 (2C), 22.5. ESI-MS *m/z:* 389 $(M+H)^+$. Anal Calcd for $C_{23}H_{24}N_4O_2$: C, 71.11; H, 6.23; N, 14.42; Found: C, 71.20; H, 6.22; N, 14.44.

(1H-indol-2-yl)(4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_37**): The compound was synthesized according to the general procedure by 5-methyl-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'd) (0.1 g, 0.464 mmol), 1H-indole-2-carboxylic acid (0.089 g, 0.557 mmol) and triethyl amine (0.19 ml, 1.39 mmol) to afford **BZ_37** (0.102 g, 62 %) as white solid. M.p: 251-252 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.27 (s, 1H), 11.84 (s, 1H), 7.59 (m, 1H), 7.44 (d, *J* = 9.0 Hz, 1H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.31 (s, 1H), 7.18 (m, 1H), 7.00 – 6.76 (m, 3H), 4.47 (d, *J* = 12.4 Hz, 2H), 3.33 (m, 3H), 2.38 (s, 3H), 2.13 (t, *J* = 10.6, 2H), 1.88 (t, *J* = 10.8 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 142.1, 140.2, 139.6, 138.9, 136.2, 133.5, 132.2, 126.5, 122.5, 121.6, 120.5, 115.8, 115.5, 114.5, 112.3, 45.2 (2C), 36.2, 30.2 (2C), 22.5. ESI-MS *m/z:* 359 $(M+H)^+$. Anal Calcd for $C_{12}H_{22}N_4O$: C, 73.72; H, 7.33; N, 15.63; Found: C, 73.81; H, 6.18; N, 15.65.

(5-fluoro-1H-indol-2-yl)(4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_38**):The compound was synthesized according to the general procedure by utilizing 5-methyl-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'd) (0.1 g, 0.464 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.091 g, 0.557 mmol) and triethyl amine (0.19 ml, 1.39 mmol) to afford **BZ_38** (0.096 g, 56 %) as pale yellow solid. M.p: 226-228 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.24 (s, 1H), 11.86 (s, 1H), 7.56 (m, 1H), 7.45 (d, *J* = 9.2 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 1H), 7.32 (s, 1H), 7.25 (m, 1H), 7.05 – 6.85 (m, 2H), 4.51 (d, *J* = 11.8 Hz, 2H), 3.36 (m, 3H), 2.40 (s, 3H), 2.15 (t, *J* = 10.8, 2H), 1.90 (t, *J* = 11.0 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 150.8, 142.1, 139.5, 138.9, 136.2, 135.8, 133.5, 133.1, 126.5, 115.7, 115.3 (2C), 114.5, 110.5, 110.2, 45.2 (2C), 36.2, 30.2 (2C), 22.5. ESI-MS *m/z:* 377 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}FN_4O$: C, 70.20; H, 5.62; N, 14.88; Found: C, 70.29; H, 5.63; N, 14.90.

(5-chloro-1H-indol-2-yl)(4-(5-nitro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_39**): The compound was synthesized according to the general procedure by utilizing 5-nitro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (**2'e**) (0.1 g, 0.406 mmol), 5-chloro-1H-indole-2-carboxylic acid (0.095 g, 0.487 mmol) and triethyl amine (0.2 ml, 1.46 mmol) to afford **BZ_39** (0.114 g, 66 %) as brown solid. M.p: 150-152 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.27 (s, 1H), 11.83 (s, 1H), 7.85 (d, *J* = 2 Hz, 1H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.52 – 7.25 (m, 3H), 7.10 (t, *J* = 1.6 Hz, 1H), 6.89 (d, *J* = 1.4 Hz, 1H), 4.52 (d, *J* = 11.4 Hz, 2H), 3.30 (m, 3H), 2.17 (t, *J* = 11.8, 2H), 1.93 (t, *J* = 11.4 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 146.2, 144.9, 143.1, 142.1, 139.4, 138.2, 133.5, 125.8, 122.8, 122.2, 119.5, 116.6, 115.2, 114.8, 114.2, 45.2 (2C), 36.3, 30.2 (2C) ESI-MS *m/z:* 424 $(M+H)^+$. Anal Calcd for $C_{21}H_{18}ClN_5O_3$: C, 59.51; H, 4.28; N, 16.52; Found: C, 59.42; H, 4.27; N, 16.53.

(5-methoxy-1H-indol-2-yl)(4-(5-nitro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_40**): The compound was synthesized according to the general procedure by utilizing 5-nitro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'e) (0.1 g, 0.406 mmol), 5-methoxy-1H-indole-2-carboxylic acid (0.093 g, 0.487 mmol) and triethyl amine (0.2 ml, 1.46 mmol) to afford **BZ_40** (0.097 g, 56 %) as brown solid. M.p: 124-126 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.24 (s, 1H), 11.81 (s, 1H), 7.79 (d, *J* = 2 Hz, 1H), 7.62 (d, *J* = 8.6 Hz, 1H), 7.50 – 7.23 (m, 3H), 7.08 (t, *J* = 1.8 Hz, 1H), 6.86 (d, *J* = 1.6 Hz, 1H), 4.55 (d, *J* = 11.4 Hz, 2H), 3.87 (s, 3H), 3.32 (m, 3H), 2.19 (t, *J* = 11.6, 2H), 1.91 (t, *J* = 11.2 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 154.6, 146.2, 144.9, 143.2, 142.1, 139.3, 133.4, 133.1, 119.5, 116.6, 115.2, 113.5, 113.1, 112.7, 112.3,

55.6, 45.2 (2C), 36.3, 30.2 (2C). ESI-MS *m/z:* 420 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}N_5O_4$: C, 63.00; H, 5.05; N, 16.70; Found: C, 63.15; H, 5.04; N, 16.68.

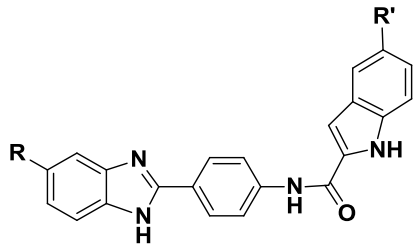
(1H-indol-2-yl)(4-(5-nitro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_41**):
The compound was synthesized according to the general procedure by 5-nitro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'e) (0.1 g, 0.406 mmol), 1H-indole-2-carboxylic acid (0.078 g, 0.487mmol) and triethyl amine (0.2 ml, 1.46 mmol) to afford **BZ_41** (0.102 g, 64 %) as yellow solid. M.p: 120-122 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.21 (s, 1H), 11.82 (s, 1H), 7.71 (d, *J* = 2 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.46 – 7.22 (m, 3H), 7.04 – 6.92 (m, 3H), 4.52 (d, *J* = 11.6 Hz, 2H), 3.34 (m, 3H), 2.13 (t, *J* = 11.4, 2H), 1.94 (t, *J* = 11.8 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 146.2, 144.9, 143.1, 142.1, 140.2, 139.4, 131.8, 122.5, 121.3, 120.5, 119.4, 116.3, 115.3, 113.5, 112.6, 45.2 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 390 $(M+H)^+$. Anal Calcd for $C_{21}H_{19}N_5O_3$: C, 64.77; H, 4.92; N, 17.98; Found: C, 64.70; H, 4.91; N, 17.97.

(5-Fluoro-1H-indol-2-yl)(4-(5-nitro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)methanone (**BZ_42**):The compound was synthesized according to the general procedure by utilizing 5-nitro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (2'e) (0.1 g, 0.406 mmol), 5-fluoro-1H-indole-2-carboxylic acid (0.087 g, 0.487 mmol) and triethyl amine (0.2 ml, 1.46 mmol) to afford **BZ_42** (0.09g, 55 %) as yellow solid. M.p: 142-144 °C. ^{1}H NMR [DMSO-d_6]: δ_H 12.26 (s, 1H), 11.84 (s, 1H), 7.73 (d, *J* = 2 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.54 – 7.21 (m, 3H), 7.02 – 6.85 (m, 2H), 4.49 (d, *J* = 11.2 Hz, 2H), 3.35 (m, 3H), 2.15 (t, *J* = 11.6, 2H), 1.92 (t, *J* = 11.4 Hz, 2H).^{13}C NMR [DMSO-d_6] δ: 172.5, 150.5, 146.3, 144.9, 143.2, 142.1, 139.6, 136.2, 135.5, 119.5, 116.4, 115.6, 115.3, 113.6, 110.9, 110.5, 45.2 (2C), 36.2, 30.2 (2C). ESI-MS *m/z:* 408 $(M+H)^+$. Anal Calcd for $C_{21}H_{18}FN_5O_3$: C, 61.91; H, 4.45; N, 17.19; Found: C, 61.83; H, 4.46; N, 17.20.

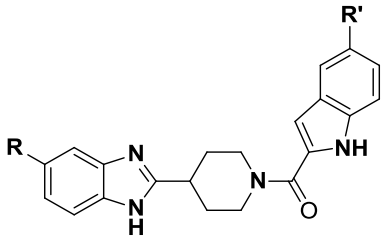
5.3.4. *In vitro* supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were evaluated for their *in vitro* supercoiling assay for the derivation of SAR and lead optimization. The compounds were further subjected to a whole cell screening against *Mtb* H_{37}Rv strain to understand their bactericidal potency using the agar dilution method and later the safety profile of these molecules were evaluated by checking the *in vitro* cytotoxicity against RAW 264.7 cell line (mouse macrophage) at 50 μM concentration by MTT assay, and the results are shown in **Table 5.6**.

Table 5.6: *In vitro* biological evaluation of the synthesized compounds **BZ_03** – **BZ_42**



BZ_03-BZ_22



BZ_23-BZ_42

Comp no	R	R'	Super coiling assay IC_{50} (µM)	MTB MIC (µM)	RAW264.7 Cytotoxicity at 50 µM (% inhib.)
BZ_03	H	Cl	4.50±0.28	32.31	20.70
BZ_04	H	OCH_3	25.41±0.8	8.17	13.73
BZ_05	H	H	11.45±0.34	70.94	16.24
BZ_06	H	F	5.40±0.5	67.50	12.10
BZ_07	F	Cl	13.24±0.15	61.76	27.18
BZ_08	F	OCH_3	11.65±0.8	31.22	15.32
BZ_09	F	H	11.88±0.47	67.50	10.41
BZ_10	F	F	4.10±0.21	16.09	18.84
BZ_11	Cl	Cl	24.38±0.48	14.84	23.28
BZ_12	Cl	OCH_3	13.21±0.62	7.50	18.51
BZ_13	Cl	H	31.60±0.11	32.31	15.12
BZ_14	Cl	F	9.84±0.31	30.88	19.74
BZ_15	CH_3	Cl	28.29±0.5	15.59	21.44
BZ_16	CH_3	OCH_3	26.41±0.17	63.06	18.97
BZ_17	CH_3	H	35.65±0.33	68.23	24.33
BZ_18	CH_3	F	29.30±0.52	32.32	20.30
BZ_19	NO_2	Cl	15.33±0.14	7.24	17.05
BZ_20	NO_2	OCH_3	9.85±0.44	58.49	24.30
BZ_21	NO_2	H	19.94±0.62	62.91	13.96

BZ_22	NO_2	F	21.26±0.7	30.09	25.60
BZ_23	H	Cl	23.91±0.24	32.99	20.83
BZ_24	H	OCH_3	4.28±0.52	16.69	23.56
BZ_25	H	H	24.40±0.26	9.07	15.70
BZ_26	H	F	4.80±0.9	17.25	18.72
BZ_27	F	Cl	35.16±0.6	7.87	12.69
BZ_28	F	OCH_3	46.11±.012	63.71	35.86
BZ_29	F	H	23.65±0.42	34.49	19.28
BZ_30	F	F	25.42±0.63	65.72	20.68
BZ_31	Cl	Cl	16.34±0.5	60.49	17.80
BZ_32	Cl	OCH_3	24.32±0.54	61.14	10.36
BZ_33	Cl	H	24.20±0.4	65.99	25.28
BZ_34	Cl	F	12.88±0.32	63.00	14.45
BZ_35	CH_3	Cl	16.51±0.18	31.82	11.52
BZ_36	CH_3	OCH_3	18.72±0.5	8.04	19.18
BZ_37	CH_3	H	17.39±0.17	17.44	15.87
BZ_38	CH_3	F	5.75±0.26	4.15	14.87
BZ_39	NO_2	Cl	38.28±0.31	58.98	12.88
BZ_40	NO_2	OCH_3	25.80±0.61	59.60	14.72
BZ_41	NO_2	H	29.27±0.49	32.10	15.08
BZ_42	NO_2	F	36.14±0.4	30.68	17.88
Moxifloxacin			11.2±0.23	2.4	ND
Novobiocin			46±28	>200	19.3
Ethambutol			NT	9.84	NT

IC_{50}, 50% inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; NT, not tested; nM, nanomolar

Mtb DNA gyrase supercoiling enzyme inhibition activity

In vitro activity against *Mtb* $H_{37}Rv$

Cytotoxicity against RAW 264.7 cells (mouse macrophage cell line)

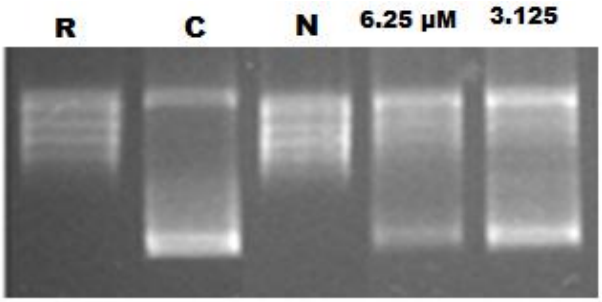


Fig 5.11: Supercoiling assay of most active compound **BZ_10** at two different concentrations 6.25 and 3.125 µM; N-Novobiocin; C-Control (Relaxed DNA substrate + DNA Gyrase + DMSO); R-Relaxed DNA (substrate + DMSO).

5.3.5. Nutrient Starvation Model

Compound **BZ_38** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e., Nutrient starvation model. **BZ_38** showed 2.4 log reductions in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold).

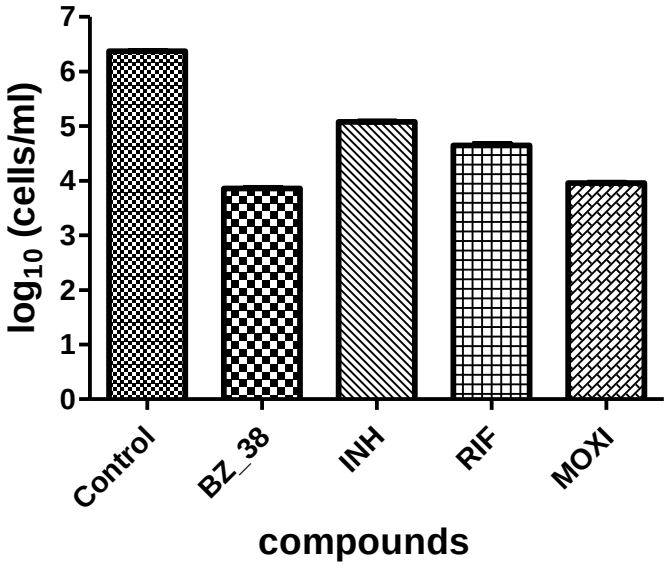


Fig 5.12: Nutrient starvation graph of **BZ_38**

Bacterial count estimation (Mean ± S.D., n = 3) for control and treated groups conducted by using the MPN (most probable number) assay. The dormant cell suspension was treated with the compounds at a concentration of 10 µM. Most of the compounds gave significant inhibition of growth of *M. tuberculosis* in this model as compared to the control ($p < 0.0001$, two way ANOVA usingGraphPad Prism Software).

5.3.6. Anti-mycobacterial screening for most active compound using adult zebrafish (Zebrafish Model)

To evaluate preliminary pharmacokinetic of **BZ_38** compound it was also tested in *Mycobacterium marinum* induced adult zebra fish model. The compound was administered orally at a dose of 10 mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. **BZ_38** exhibited good pharmacokinetic profile and also log reduction of 2.1 fold in bacteria when compared to first line drugs- isoniazid (2.9 fold),and moxifloxacin (2.8 fold).

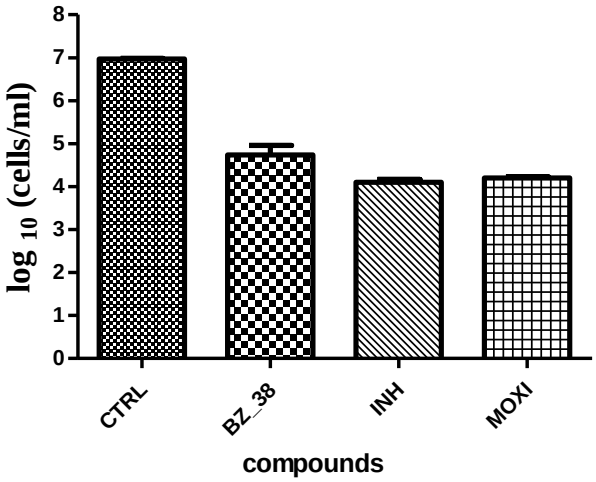


Fig 5.13: Zebra fish model graph if **BZ_38**

Bacterial count estimation (Mean ± S.E.M., n = 6) for control and treated groups conducted by using MPN (most probable number) assay. The statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p<0.0001$) with respect to infected control group has been analyzed by Two-way ANOVA using GraphPad Prism Software

5.3.7 SAR and discussion

All the forty compounds synthesized were screened for their enzyme inhibition studies using MTB DNA gyrasekit (Inspiralis, Norwich). Preliminary screening was performed at concentrations of 500, 125, 31.3, 7.8, and 1.95 µM and those which were active were further tested at 250, 62.5, 15.6, and 3.9 µM concentrations. Among the entire series of forty compounds, four compounds showed IC_{50}s less than 5 µM. While the most active compound **BZ_10** showed an IC_{50} of 4.1 µM which has an electronegative group (fluorine) at both (5F of benzimidazole and Fluorine at 5^{th} position of indole) positions are connected through a phenyl group as linker. Compound **BZ_24** exhibited an supercoiling IC_{50} of 4.2 µM possess electropositive groups –H at 5^{th} position of benzimidazole group and methoxy group at indole both joined by aminopiperidine linker. Compound **BZ_3** and **BZ_26** shows IC_{50} 4.5 µM and 4.8 µM respectively. Both compounds **BZ_3** and **BZ_26** have electronegative group's chlorine and fluorine on indole group. Substitution of electronegative groups at the 5^{th} position of the indole ensured excellent inhibitory property. Compound **BZ_6, BZ_14, BZ_20,** and **BZ_38** shows IC_{50} less than 10 µM. Twelve compounds (**BZ_5, BZ_7 – BZ_9, BZ_12, BZ_19, BZ_21, BZ_31, BZ_34 – BZ_37**) shows potency less than 20 µM. Novobiocin and moxifloxacin were considered as positive controls as they have been shown to be potent inhibitors of DNA supercoiling of mycobacterial DNA gyrase. Eight compounds in this study showed better enzyme supercoiling inhibitions compared to standard moxifloxacin drug whose IC_{50} was 11.2 µM and has been considered as one of the potent DNA gyrase inhibitor till date. Similarly the other broad spectrum standard drug novobiocin showed an IC_{50} of 46 nM. Further few of the synthesized compounds were screened for the GyrB inhibitory potency against *Mycobacterium smegmatis* GyrB protein using the malachite green assay and none of the molecules showed any inhibition on GyrB indicates that these molecules targets GyrA subunit of DNA gyrase.

The compounds were further screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strain by microplateAlamar blue assay method. Ethambutol (MIC: 15.31 µM), Isoniazid (MIC: 0.66 mM), Moxifloxacin (MIC: 1.2 µM) and Novobiocin (MIC: >200 µM) were considered as standard drugs for comparison in this assay. All the compounds inhibited MTB with MIC ranging from 4.15-70.94 µM. Seven compounds inhibited MTB with MIC < 10 µM (**BZ_4, BZ_12, BZ_19, BZ_25, BZ_27, BZ_36, BZ_38**). Seven compounds were found to be more potent than standard first line anti-TB drug Ethambutol. When compared

to Moxifloxacin (MIC of 2.4 µM) none of the molecules showed better activity. In the case of most potent DNA gyrase inhibitor compound **BZ_10** showed supercoiling inhibitory IC_{50} of 4.1±0.21µM showed MTB MIC of 16.09 µM. This difference in activity might be due to problem in MTB cell wall penetration of the compound or efflux pump present in the bacteria might have pumped out the molecule.

Compound **BZ_38** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e.,Nutrient starvation model. **BZ_38** showed 2.4 log reduction in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold). These results suggest that compound **BZ_38** is not only effective against replicative stage of mtb but also against persistent stages of bacteria.

To evaluate invivo activity of compound **BZ_38,** it was also tested in *Mycobacterium marinum* induced adult zebra fish model. The compound was administered orally at a dose of 10mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. Compound **BZ_38** exhibited good anti-mycobacterial potency and also log reduction of 2.1 fold in bacteria when compared to first line drugs- isoniazid (2.9 fold), and moxifloxacin (2.8 fold). It is an indicative that compound **BZ_38** is best candidate for further drug development studies.

Subsequently, the eukaryotic cell safety profile of all the forty compounds were observed by testing there *in vitro* cytotoxicity against the RAW 264.7 cell line (Mouse leukemic monocyte macrophage cell line) at 50 µM concentration by (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Since the mycobacteria resides in the macrophages during the infection stages, this cell line was selected. At 50 µM tested compounds showed cytotoxicity range of 14.37-49.13 % as shown in **Table 5.6**.The most promising anti-TB compound among the synthesized set of compounds was **BZ_38** with only 14.87 % cytotoxicity which is within the safety profile limits. Novobiocin was used as standard with 19.36 % inhibition in the above cell line.

5.3.8. Highlights of the study

In summary, we identified and synthesized a novel class of benzimidazole derivatives from earlier reported antitubercular compounds from different research groups. Many of the compounds showed potent DNA gyrase supercoiling inhibition and *M. tuberculosis* MIC. Compound **BZ_10** (5-fluoro-N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide) was found to be the most active DNA gyrase inhibitor with supercoiling IC_{50} of 4.10±0.21µM and inhibited drug sensitive *M. tuberculosis* with MIC of 16.09 µM. Also we identified **BZ_38** as most active anti-mycobacterial compound with an MIC of 4.15 µM and with supercoiling IC_{50} of 5.75±0.26 µM. The most active anti-mycobacterial compound **BZ_38** in, Nutrient starved model showed 2.4 log reduction in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold). Also compound **BZ_38;** was tested in *Mycobacterium marinum* induced adult zebra fish model. Compound **BZ_38** exhibited good pharmacokinetic profile and also log reduction of 2.1 fold in bacteria when compared to first line drugs isoniazid (2.9 fold),and moxifloxacin (2.8 fold). It is an indicative that compound **BZ_38** is best candidate for further drug development studies.

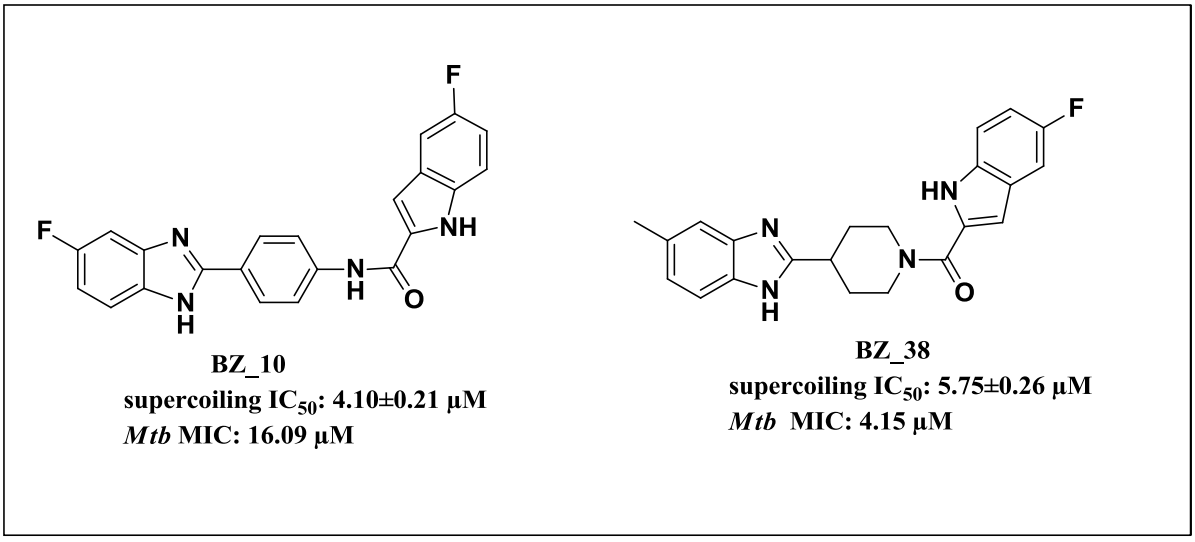


Figure 5.14: Chemical structure and biological activity of the most active compounds **BZ_10** and **BZ_38.**

5.4. Design, synthesis and biological evaluation of 1-(7-chloroquinolin-4-yl)piperidin-4-amine derivatives as novel DNA Gyrase inhibitors

5.4.1. Design of the molecule

BITS database lead, Compound **Gb_1** [**2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide,** Gyr B inhibitory IC_{50} = 12.2 ± 0.09 μM**]** exhibited a docking score of -6.26 kcal/mol^{-1} and was found to be in the vicinity of the amino acid Ala53, Asp79, Val125, Val49, Ile171, Val77, Val98,Val99, Pro85, Asn52, Glu56 and Gly83 amino acid residues (which is also characterized to be the active site pocket). Also compound reported by Jean *et al*., **Gb_29** and **Gb_36** exhibited Gyr B inhibitory IC_{50} of 2.5 ± 0.1 μM and 3.1 ± 0.2 μM respectively [Jeankumar, V. U., *et al*., 2016].

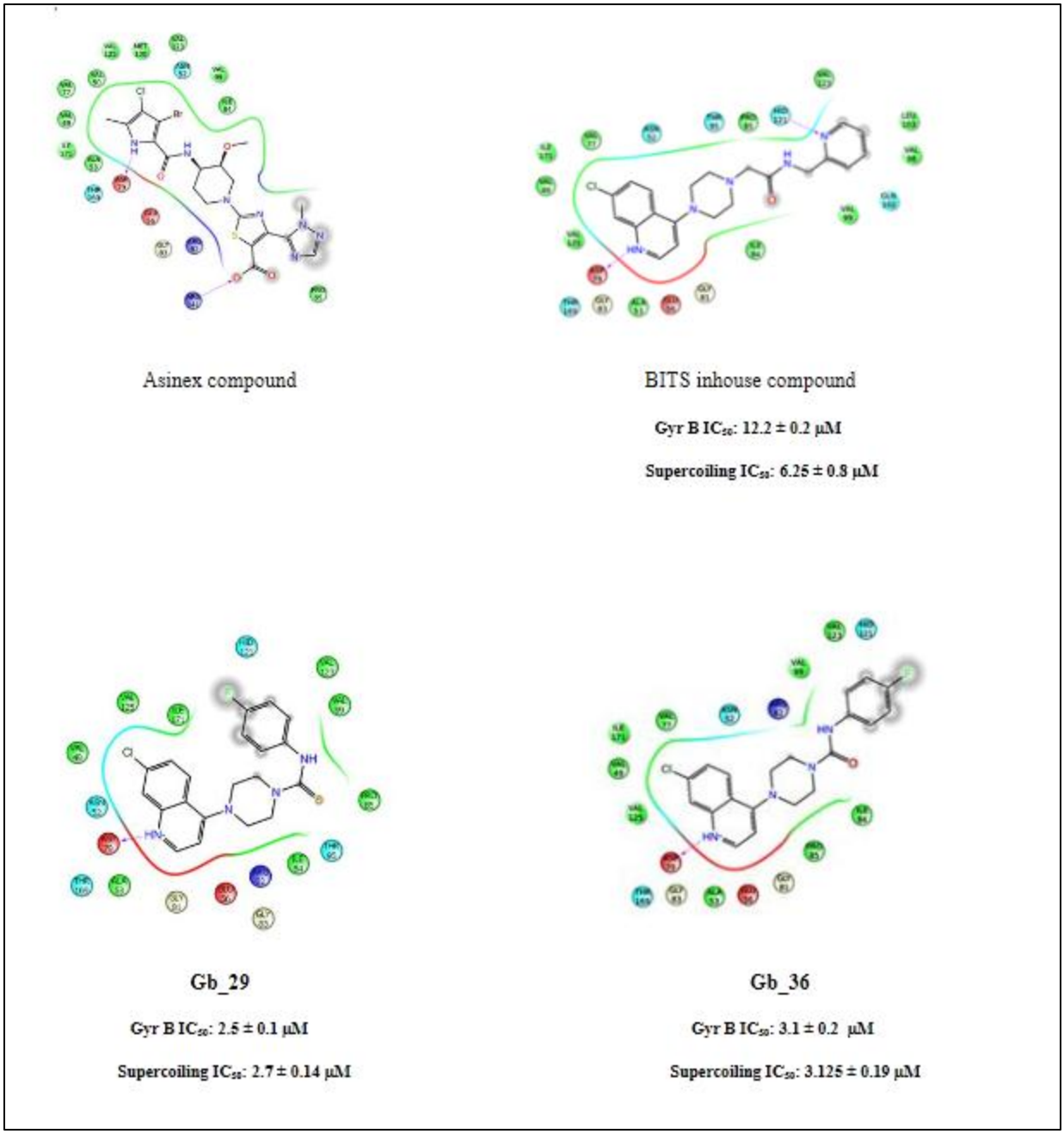


Fig 5.15: Interaction profile picture of most active compounds used for design

We have taken Gb_29 reported by Jean *et al* [Jean kumar., *et al*., 2016]as the lead molecule for designing new target

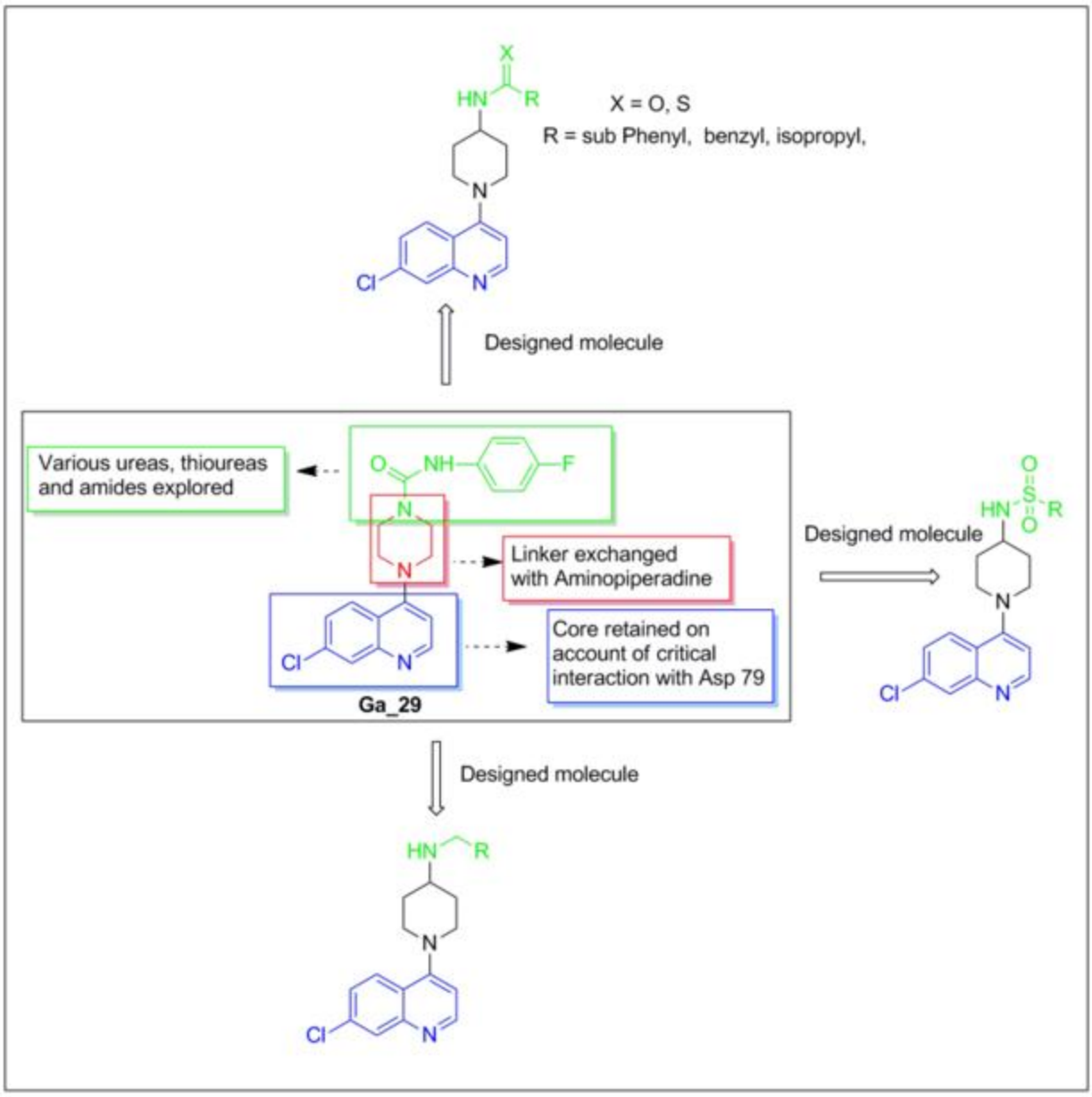


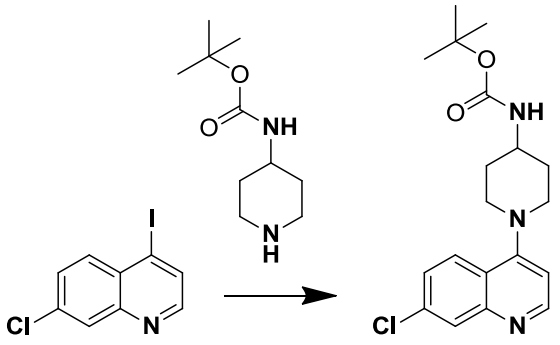
Fig 5.16: Design logic employed for generating final molecules **QU_04** – **QU_31**

5.4.2. Experimental procedures utilized for the synthesis of QU_01 – BZ_31

A closer look at the interaction profile diagram of the lead molecule **Gb_29** showed the quinoline nitrogen (N-4) to be involved in a prominent hydrogen bonding interaction with Asp 79, analogues to the one observed in the crystal ligand. This interaction is believed to be critical in retaining the activity. Based on this observation synthesise starts with 7-chloro-4-iodoquinoline. The synthetic strategy utilized for the synthesis of final compounds **QU_04** –

QU_31 was depicted **in Fig 4.4**. In the first step easy leaving group iodo was replaced with tert-butyl piperidin-4-ylcarbamate using K_2CO_3 as base to obtain **QU_02**. The boc protected **QU_02** was treated with HCl in dioxane to get scaffold **QU_03** as amine [Jeankumar, V. U., *et al*., 2016].Treatment of **QU_03** with isocyanates and isothiocyanates leads to the formation of ureas and thioureas as final compounds **QU_04** – **QU_17** [Suh, Y., *et al*., 2001]**.** Compounds **QU_18** – **QU_24** was obtained by treating **QU_03** with various substituted sulfonyl chlorides to get sulphonamides [Marshall, D. R., et al., 2007]. Reductive amination of **QU_03** with substituted aldehydes using sodium cyanoborohydride resulted in final compounds **QU_25 – QU_29** [Dangerfield, E. M., *et al*., 2010]. Buchwald-Hartiwig Cross coupling of bromo benzene and isopropyl bromide with **QU_03** using $PdCl_2$ (dppf) as catalyst and sodium tertiary butoxide as a base resulted in QU_**30** and **QU_31** [Fors, B. P., *et al*., 2010].

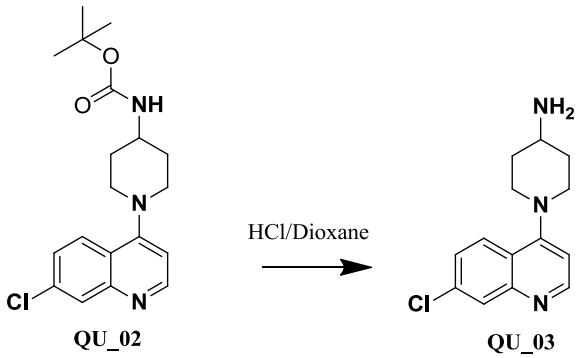
Preparation of tert-butyl (1-(7-chloroquinolin-4-yl)piperidin-4-yl)carbamate (QU_02)



Tert-butyl piperidin-4-ylcarbamate (0.83 g, 4.14 mmol) was dissolved in dry DMF (5 ml) and stirred at room temperature under nitrogen atmosphere. To the above solution anhydrous potassium carbonate (1.19 g, 8.63 mmol) was added and stirred for half an hour at room temperature. 7-chloro-4-iodoquinoline (**QU_01**) (1 g, 3.45 mmol) was dissolved in dry DMF (5 ml) and added to the reaction mixture at 0 °C dropwise. The reaction mixture was allowed to come to room temperature slowly and stir it for 5 hr (monitored by TLC and LCMS for completion). The reaction mixture was diluted with ice cold water and the aqueous layer was extracted with ethyl acetate, the combined organic layer was then washed with saturated brine solution to remove excess of DMF. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified

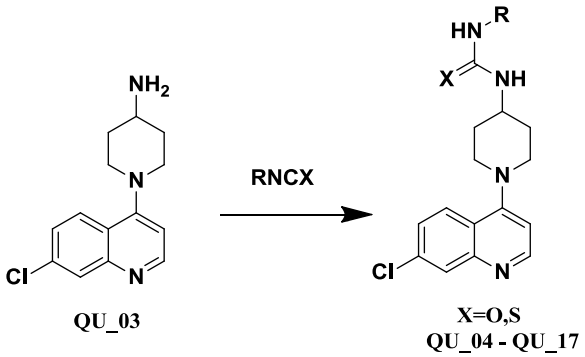
using column chromatography using pet ether and ethyl acetate as eluent to afford **QU_02** (0.77 g, 62 % yield) as brown solid.^{1}H NMR (DMSO-d_6): δ_H 8.69 (d, *J* = 5.6 Hz, 1H), 8.01 (dd, *J* = 12.6, 3.2 Hz, 2H), 7.36 – 7.18 (m, 2H), 6.82 (t, *J* = 5.6 Hz, 1H), 3.70 (m, 1H), 3.51 (d, *J* = 11.8 Hz, 2H), 3.03 (t, *J* = 11.6 Hz, 2H), 2.07 (d, *J* = 12.6 Hz, 2H), 1.72 (t, *J* = 10.6 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (DMSO-d_6): δ_c 160.1, 154.3, 152.4, 151.5, 135.8, 129.5, 129.1, 127.2, 121.5, 120.1, 80.2, 52.5 (2C), 50.2, 30.2 (2C), 27.5 (2C). EI-MS *m/z*: 361 $(M)^+$. Anal Calcd for $C_{19}H_{24}ClN_3O_2$: C, 63.06; H, 6.68; N, 11.61. Found: C, 63.15; H, 6.67; N, 11.60.

Preparation of 1-(7-chloroquinolin-4-yl)piperidin-4-amine (QU_03)



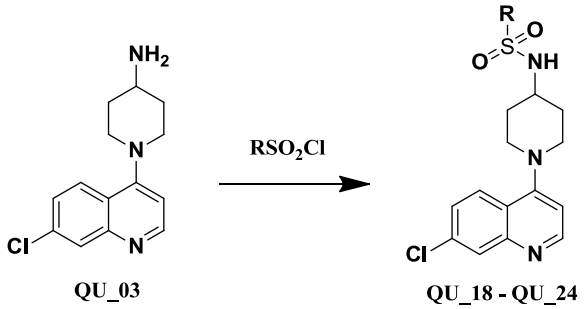
Tert-butyl (1-(7-chloroquinolin-4-yl)piperidin-4-yl)carbamate (**QU_02**) (0.5g, 1.38 mmol) was dissolved in dry DCM (5 ml) under inert atmosphere. The reaction mixture was cooled to 0 ° C. To the cooled reaction mixture HCl/Dioxane (1 ml) was added drop wise and allows the reaction to stir at room temperature for 3 h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water and basified using sodim carbonate. The aqueous layer was extracted with ethyl acetate, the combined organic layer was dried over sodium sulphate and concentrated under reduced pressure to afford **QU_03** (0.3 g, 83 % yield) as pale brown oil and carried out to final reactions without further purification. EI-MS *m/z*: 261 $(M)^+$.

Preparation of final compounds QU_04 – QU_17



1-(7-chloroquinolin-4-yl)piperidin-4-amine (**QU_03**) (1 equiv) was dissolved in dichloromethane under nitrogen atmosphere. The reaction mixture was cooled to 0 °C, followed by the drop wise addition of triethyl amine (3 equiv). To this ice cold mixture corresponding substituted isocyanate / isothiocyanate (1.1 equiv) was added and allow the reaction to stand room temperature for 8 h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water, the aqueous layer was extracted with ethyl acetate, the combined organic layer was dried over sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using pet ether and ethyl acetate as eluent to afford to desired product in good yield.

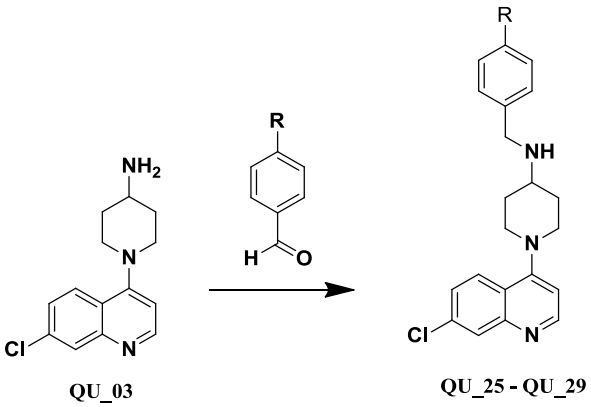
Preparation of final compounds QU_18 – QU_24



1-(7-chloroquinolin-4-yl) piperidin-4-amine (**QU_03**) (1 equiv) was dissolved in dichloromethane under nitrogen atmosphere. The reaction mixture was cooled to 0 °C, followed by the drop wise addition of triethyl amine (3 equiv). To this ice cold mixture corresponding sulphonyl chloride (1.1 equiv) was added and allows the reaction to stand room temperature for 8 h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum,

diluted with water, the aqueous layer was extracted with ethyl acetate, the combined organic layer was dried over sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using pet ether and ethyl acetate as eluent to afford to desired product in good yield.

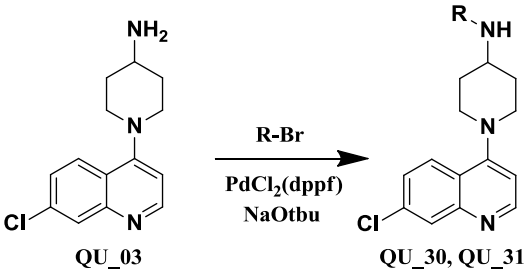
Preparation of final compounds QU_25 – QU_29



QU_03 QU_25 - QU_29

1-(7-chloroquinolin-4-yl)piperidin-4-amine (**QU_03**) (1 equiv) was dissolved in methanol under nitrogen atmosphere. To this added substituted aromatic aldehyde (1.1 equiv) and catalytic amount of acetic acid, stirred at room temperature for 12 h. Then the reaction mixture was cooled to 0 °C, sodium cyano borohydride (1.5 equiv) was added portion wise. Reaction mixture was allowed to stand at room temperature for 4 h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water, the aqueous layer was extracted with ethyl acetate, the combined organic layer was dried over sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using pet ether and ethyl acetate as eluent to afford to desired product in good yield.

Preparation of final compounds QU_30



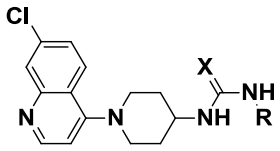
QU_03 QU_30, QU_31

A solution of 1-(7-chloroquinolin-4-yl) piperidin-4-amine (**QU_03**) (1 equiv) in dioxane was taken in a sealed tube. To this dry sodium tert butoxide (2 equiv) was added and stirred for 15 mts, followed by the addition of corresponding alkyl halides (1.2 equiv). To the above mixture catalytic amount of $PdCl_2$ (dppf) (5 mol %) and stirred at 100 °C for 6 h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water, the aqueous layer was extracted with ethyl acetate, and the combined organic layer was dried over sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using pet ether and ethyl acetate as eluent to afford desired product in good yield.

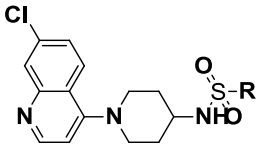
Preparation of final compounds QU_31

1-(7-chloroquinolin-4-yl)piperidin-4-amine (**QU_03**) (1 equiv) was dissolved in dry DMF (5 vol) and stirred at room temperature under nitrogen atmosphere. To the above solution anhydrous potassium carbonate (2 equiv) was added and stirred for half an hour at room temperature. 2-bromopropane (1.2 equiv) was dissolved in dry DMF (5 vol) and added to the reaction mixture at 0 °C dropwise. The reaction mixture was allowed to come to room temperature slowly and stir it for 5 hr (monitored by TLC and LCMS for completion). The reaction mixture was diluted with ice cold water and the aqueous layer was extracted with ethyl acetate, the combined organic layer was then washed with saturated brine solution to remove excess of DMF. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using pet ether and ethyl acetate as eluent to afford desired product in good yield.

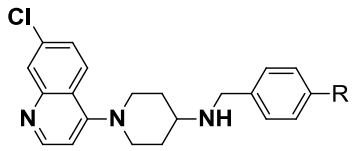
Table 5.7: Physiochemical properties of the synthesized compounds **QU_04 – QU_31**



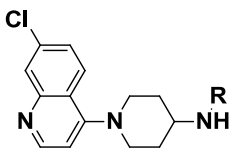
X=O,S
QU_04-QU_17



QU_18-QU_24



QU_25-QU_29



QU_30, QU_31

Comp	X	R	Yield (%)	MP (°C)	Molecular Formula	Molecular Weight
QU_04	S	benzyl	60	135 - 137	$C_{22}H_{23}ClN_4S$	410.96
QU_05	S	phenyl	57	109 -111	$C_{21}H_{21}ClN_4S$	396.94
QU_06	S	4-chloro phenyl	65	116 – 118	$C_{21}H_{20}Cl_2N_4S$	431.38
QU_07	S	4-fluoro phenyl	57	172 – 174	$C_{21}H_{20}ClFN_4S$	414.93
QU_08	S	4-nitro phenyl	58	133 – 135	$C_{21}H_{20}ClN_5S$	441.93
QU_09	S	4-methoxy phenyl	64	97 – 99	$C_{22}H_{23}ClN_4OS$	426.96
QU_10	S	isopropyl	63	155 – 157	$C_{18}H_{23}ClN_4S$	362.92
QU_11	O	benzyl	68	198 - 200	$C_{22}H_{23}ClN_4O$	394.90
QU_12	O	phenyl	60	208 -210	$C_{21}H_{21}ClN_4O$	380.87
QU_13	O	4-chloro phenyl	57	171 – 173	$C_{21}H_{20}Cl_2N_4O$	415.32
QU_14	O	4-fluoro phenyl	71	205 – 207	$C_{21}H_{20}ClFN_4O$	398.86
QU_15	O	4-nitro phenyl	61	222 – 224	$C_{21}H_{20}ClN_4O$	425.87
QU_16	O	4-methoxy phenyl	61	201 – 203	$C_{22}H_{23}ClN_4O_2$	410.90
QU_17	O	isopropyl	68	-	$C_{18}H_{23}ClN_4O$	346.85
QU_18	-	benzyl	71	137 - 139	$C_{21}H_{22}ClN_3O_2S$	415.94
QU_19	-	phenyl	64	143 -145	$C_{20}H_{20}ClN_2S$	401.91
QU_20	-	4-chloro phenyl	63	155 – 157	$C_{20}H_{19}Cl_2N_3O_2S$	436.35
QU_21	-	4-fluoro phenyl	62	174 – 176	$C_{20}H_{19}ClFN_3O_2S$:	419.90
QU_22	-	4-nitro phenyl	67	198 – 200	$C_{20}H_{19}ClN_4O_4S$	446.91
QU_23	-	4-methoxy phenyl	60	137 – 139	$C_{21}H_{22}ClN_3O_3S$	431.94
QU_24	-	isopropyl	64	-	$C_{17}H_{22}ClN_3O_2S$	367.89
QU_25	-	H	57	-	$C_{21}H_{22}ClN_3$	351.87
QU_26	-	4-chloro	55	-	$C_{21}H_{21}Cl_2N_3$	386.32

QU_27	-	4-fluoro	68	-	$C_{21}H_{21}ClFN_3$	369.86
QU_28	-	4-nitro	66	125 -127	$C_{21}H_{21}ClN_4O_2$	396.87
QU_29	-	4-methoxy	65	74 -76	$C_{22}H_{24}ClN_3O$	381.90
QU_30	-	phenyl	42	-	$C_{20}H_{20}ClN_3$	337.85
QU_31	-	isopropyl	38	-	$C_{17}H_{22}ClN_3$	303.83

5.4.3. Characterization of Synthesized compounds

1-benzyl-3-(1-(7-chloroquinolin-4-yl) piperidin-4-yl) thiourea (QU_04): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), benzyl isothiocyanate (0.062 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_04** (0.095 g, 60 % yield) as brown solid. M.P: 135 - 137 ^{o}C.^{1}H NMR (DMSO-d_6): δ_H 10.12 (s, 1H), 8.71 (d, *J* = 7 Hz, 1H), 8.03 – 7.62 (m, 3H), 7.50(d, *J* = 11.2 Hz, 2H), 7.21 (t, *J* = 10.5 Hz, 2H), 7.02 (d, *J* = 6.6 Hz, 1H), 6.88 (t, *J* = 11.2 Hz, 1H), 6.33 (d, *J* = 10.2 Hz, 1H), 4.79 (s, 2H), 4.31 (m, 1H), 3.57 (d, *J* = 9.6 Hz, 2H), 3.08 (t, *J* = 11.2 Hz, 2H), 2.21 (d, *J* = 10.6 Hz, 2H), 1.88 (t, *J* = 10.2 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ_c 179.5, 160.3, 155.2, 149.5, 138.2, 136.3, 129.5, 129.2, 127.9 (2C), 127.1 (2C), 125.8, 125.0, 123.1, 117.9, 56.2, 53.5 (2C), 51.2, 29.8 (2C). EI-MS *m/z*: 411 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}ClN_4S$: C, 64.30; H, 5.64; N, 13.63; Found C, 64.18; H, 5.65; N, 13.61.

1-(1-(7-chloroquinolin-4-yl) piperidin-4-yl)-3-phenylthiourea (QU_05): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), phenyl isothiocyanate (0.056 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_05** (0.088 g, 57 % yield) as reddish brown solid. MP: 109 -111 ^{o}C. ^{1}H NMR (DMSO-d_6): δ_H 10.18 (s, 1H), 8.70 (d, *J* = 6.6 Hz, 1H), 8.03 –7.64 (m, 3H), 7.55 (d, *J* = 11.5 Hz, 2H), 7.26 (t, *J* = 11Hz, 2H), 7.04 (d, *J* = 6.8 Hz, 1H), 6.92 (t, *J* = 11.6 Hz, 1H), 6.52 (d, *J* = 10.5 Hz, 1H), 4.30 (m, 1H), 3.59 (d, *J* = 9.8 Hz, 2H), 3.06 (t, *J* = 11.4 Hz, 2H), 2.20 (d, *J* = 10.2 Hz, 2H), 1.86 (t, *J* = 10.2 Hz, 2H).^{13}C NMR (DMSO-d_6): δ_c 178.2, 160.1, 154.9, 149.6, 139.1, 137.3, 129.8, 129.5 (2C), 128.9, 128.5, 127.2 (2C), 126.5, 123.4, 117.6, 56.1, 53.5 (2C), 29.8 (2C). EI-MS *m/z*: 397 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4S$: C, 63.54; H, 5.33; N, 14.11; Found: C, 63.68; H, 5.32; N, 14.10.

1-(4-chlorophenyl)-3-(1-(7-chloroquinolin-4-yl) piperidin-4-yl) thiourea (QU_06): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-chloro phenyl isothiocyanate (0.070 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_06** (0.11 g, 65 % yield) as brown solid. MP: 116 – 118 ^{o}C.^{1}H NMR (DMSO-d_6): δ_H 10.14 (s, 1H), 8.69 (d, *J* = 5.6 Hz, 1H), 8.38 (s, 1H), 8.22 – 8.04 (m, 4H), 7.85 (d, *J* = 9.4 Hz, 2H), 7.58 (m, 1H), 7.03 (d, *J* = 5.8 Hz, 1H), 4.29 (m, 1H), 3.57 (d, *J* = 8.9 Hz, 2H), 3.04 (t, *J* = 10.4 Hz, 2H), 2.21 (d, *J* = 10.6 Hz, 2H), 1.88 (t, *J* = 10.2 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ_c 178.5, 160.2, 154.8, 149.6, 137.8, 136.2, 134.8, 132.5 (2C), 130.8, 130.2 (2C), 129.8, 126.5, 123.4, 117.7, 56.2, 53.4 (2C), 29.8 (2C). EI-MS *m/z*: 432 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}Cl_2N_4S$: C, 58.47; H, 4.67; N, 12.99; Found C, 58.39; H, 4.66; N, 13.00.

1-(1-(7-chloroquinolin-4-yl) piperidin-4-yl)-3-(4-fluorophenyl) thiourea (**QU_07**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-fluoro phenyl isothiocyanate (0.064 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_07** (0.091 g, 57 % yield) as brown solid. MP: 172 – 174 ^{o}C.^{1}H NMR (DMSO-d_6): δ_H 10.12 (s, 1H), 8.70 (d, *J* = 6 Hz, 1H), 8.36 (s, 1H), 8.23 – 7.96 (m, 4H), 7.76 (d, *J* = 9.6 Hz, 2H), 7.55 (m, 1H), 7.04 (d, *J* = 6 Hz, 1H), 4.32 (m, 1H), 3.59 (d, *J* = 9 Hz, 2H), 3.05 (t, *J* = 10.6 Hz, 2H), 2.18 (d, *J* = 10.8 Hz, 2H), 1.89 (t, *J* = 10.4 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ_c 178.1, 162.1, 160.2, 154.8, 149.8, 136.2, 135.2, 130.8 (2C), 130.5, 129.5, 125.8, 123.5, 117.5, 116.2 (2C), 56.2, 53.5 (2C), 29.8 (2C). EI-MS *m/z*: 415 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}ClFN_4S$: C, 60.79; H, 4.86; N, 13.50; Found C, 60.90; H, 4.87; N, 13.51.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-(4-nitrophenyl)thiourea (**QU_08**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-nitro phenyl isothiocyanate (0.075 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_08** (0.098 g, 58 % yield) as yellow solid. MP: 133 – 135 °C. ^{1}H NMR (DMSO-d6): δ_H 10.10 (s, 1H), 8.71 (d, *J* = 5.2 Hz, 1H), 8.41 (s, 1H), 8.20 – 8.03 (m, 4H), 7.87 (d, *J* = 9.2 Hz, 2H), 7.60 (m, 1H), 7.05 (d, *J* = 5.2 Hz, 1H), 4.28 (m, 1H), 3.55 (d, *J* = 9.2 Hz, 2H), 3.06 (t, *J* = 10.8 Hz, 2H), 2.19 (d, *J* = 10.8 Hz, 2H), 1.86 (t, *J* = 10.4 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 178.2, 160.3, 154.2, 149.8, 145.6,

144.8, 136.2, 130.5, 130.1, 125.2, 124.9 (2C), 124.1 (2C), 123.4, 117.6, 56.1, 53.5 (2C), 29.8 (2C). EI-MS m/z: 442 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}ClN_5S$: C, 57.07; H, 4.56; N, 15.85; Found C, 57.17; H, 4.55; N, 15.84.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-(4-methoxyphenyl)thiourea (**QU_09**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-methoxy phenyl isothiocyanate (0.069 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_09** (0.105 g, 64 % yield) as pale brown solid. MP: 97 – 99 °C. ^{1}H NMR (DMSO-d6): δ_H 10.14 (s, 1H), 8.71 (d, *J* = 5 Hz, 1H), 8.38 (s, 1H), 8.05 – 7.61 (m, 3H), 7.28 – 6.92 m, 4H), 6.42 (d, *J* = 7.8 Hz, 1H), 4.31 (m, 1H), 3.75 (s, 3H), 3.49 (d, *J* = 13.1 Hz, 2H), 3.05 (t, *J* = 11 Hz, 2H), 2.06 (d, *J* = 10.2 Hz, 2H), 1.81 (t, *J* = 10.6 Hz, 2H).^{13}C NMR (DMSO-d6): δc 178.5, 160.3, 159.8, 154.2, 149.8, 136.2, 131.5, 130.8, 129.8, 128.2 (2C), 126.2, 123.6, 117.4, 115.1 (2C), 56.5, 56.1, 53.5 (2C), 29.8 (2C). EI-MS m/z: 427 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}ClN_4OS$: C, 61.89; H, 5.43; N, 13.12; Found C, 61.96; H, 5.44; N, 13.14.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-isopropylthiourea (**QU_10**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), isopropyl isothiocyanate (0.042 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_09** (0.075 g, 63 % yield) as yellowish brown solid. MP: 155 – 157 °C.^{1}H NMR (DMSO-d6): δ_H 8.71 (d, *J* = 5.2 Hz, 1H), 7.99 – 7.31 (m, 5H), 7.04 (t, *J* = 5.2 Hz, 1H), 4.27 (m, 1H), 4.14 (m, 1H), 3.52 (d, *J* = 12 Hz, 2H), 3.02 (t, *J* = 11.2 Hz, 2H), 2.09 (d, *J* = 12.4 Hz, 2H), 1.76 (t, *J* = 10.4 Hz, 2H), 1.12 (s, 6H). ^{13}C NMR (DMSO-d6): δc 180.5, 160.1, 154.8, 149.5, 136.2, 129.9, 129.1, 126.5, 123.4, 117.7, 56.2, 53.5 (2C), 52.5, 29.8 (2C), 23.9 (2C). EI-MS m/z: 363 $(M+H)^+$. Anal Calcd for $C_{18}H_{23}ClN_4S$: C, 59.57; H, 6.39; N, 15.44; Found C, 59.45; H, 6.40; N, 15.43

1-benzyl-3-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)urea (**QU_11**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), benzyl isocyanate (0.055 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_11** (0.103 g, 68 % yield) as pale yellow solid. M.P: 198 - 200 °C.^{1}H NMR (DMSO-d6): δ_H 8.69 (d, J = 6.8 Hz, 1H), 8.21 (s, 1H), 8.04 – 7.59 (m, 3H), 7.48 (d, *J* = 11.6 Hz, 2H), 7.19 (t, *J* = 10.6 Hz, 2H), 7.00 (d, *J* = 6.8 Hz, 1H), 6.85 (t, *J* = 11.4 Hz, 1H), 6.35 (d, *J* = 10.4 Hz, 1H), 4.18 (s, 2H), 3.72 (m, 1H), 3.61 (d, *J* = 10 Hz, 2H), 3.10 (t, *J* = 11.4 Hz,

2H), 2.02 (d, *J* = 10.8 Hz, 2H), 1.89 (d, *J* = 10.6 Hz, 2H).^{13}C NMR (DMSO-d6): δc 160.2, 158.5, 153.6, 149.8, 138.2, 136.2, 129.8, 129.2, 128.8 (2C), 127.2 (2C), 127.0, 126.3, 123.5, 117.7, 52.8 (2C), 49.8, 45.6, 28.9 (2C). EI-MS m/z: 395 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}ClN_4O$: C, 66.91; H, 5.87; N, 14.19; Found C, 66.99; H, 5.86; N, 14.20.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-phenylurea (**QU_12**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine QU_03 (0.1 g, 0.38 mmol), phenyl isocyanate (0.050 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_12** (0.87 g, 60 % yield) as yellow solid. MP: 208 -210 °C. ^{1}H NMR (DMSO-d6): δ_H 8.70 (d, *J* = 6.4 Hz, 1H), 8.38 (s, 1H), 8.03 – 7.59 (m, 3H), 7.40 (d, *J* = 10.4 Hz, 2H), 7.25 (t, *J* = 10.4 Hz, 2H), 7.04 (d, *J* = 6.8 Hz, 1H), 6.91 (t, *J* = 10 Hz, 1H), 6.30 (d, *J* = 10 Hz, 1H), 3.76 (m, 1H), 3.48 (d, *J* = 16.4 Hz, 2H), 3.04 (t, *J* = 14.4 Hz, 2H), 2.06 (d, *J* = 14.8 Hz, 2H), 1.77 (d, *J* = 13.2 Hz, 2H).^{13}C NMR (DMSO-d6): δc 160.4, 155.6, 154.2, 149.5, 140.2, 136.2, 129.8, 129.2 (2C), 129.0, 128.5, 126.8, 123.6, 120.8 (2C), 117.5, 52.5 (2C), 49.7, 28.9 (2C). EI-MS m/z: 381 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4O$: C, 66.22; H, 5.56; N, 14.71; Found: C, 66.36; H, 5.57; N, 14.69.

1-(4-chlorophenyl)-3-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)urea (**QU_13**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-chloro phenyl isocyanate (0.065 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_13** (0.092 g, 57 % yield) as pale yellow solid. MP: 171 – 173 °C. ^{1}H NMR (DMSO-d6): δ_H 8.68 (d, *J* = 5.8 Hz, 1H), 8.41 (s, 1H), 8.23 (dd, *J* = 10, 6 Hz, 2H), 8.05 (dd, *J* = 22.4, 9.8 Hz, 2H), 7.79 (d, *J* = 9.6 Hz, 2H) 7.61 (dd, *J* = 11.4, 6.7 Hz, 1H), 6.81 (d, *J* = 6.2 Hz, 1H), 3.68 (m, 1H), 3.61 (d, *J* = 10.4 Hz, 2H), 3.05 (t, *J* = 10.6 Hz, 2H), 2.08 (d, *J* = 10 Hz, 2H), 1.85 (d, *J* = 11 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.3, 155.7, 154.2, 149.6, 138.2, 136.2, 132.5, 129.8, 129.2 (2C), 128.9, 126.3, 123.4, 120.5 (2C), 117.5, 52.5 (2C), 49.8, 28.9 (2C). EI-MS m/z: 432 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}Cl_2N_4O$: C, 60.73; H, 4.85 N, 13.49; Found C, 60.64; H, 4.86 N, 13.48.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-(4-fluorophenyl)urea (**QU_14**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-fluoro phenyl isocyanate (0.057 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_14** (0.109 g, 71 % yield) as pale yellow solid. MP: 205 – 207 °C. ^{1}H NMR (DMSO-d6): δ_H 8.68 (d, *J* = 5.2 Hz, 1H), 8.36 (s, 1H), 8.20 – 8.03 (m,

4H), 7.71 (d, *J* = 9.6 Hz, 2H), 7.53 – 7.26 (m, 2H), 6.72 (d, *J* = 7.6 Hz, 1H), 3.71 (m, 1H), 3.61 (d, *J* = 9 Hz, 2H), 3.02 (t, *J* = 10.6 Hz, 2H), 2.08 (d, *J* = 10.8 Hz, 2H), 1.92 (t, *J* = 10.6 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 163.5, 160.2, 155.6, 154.2, 149.8, 136.2, 134.8, 129.8, 129.3, 126.5, 123.4, 119.8 (2C), 117.5, 116.2 (2C), 52.5 (2C), 49.6, 28.9 (2C). EI-MS m/z: 399 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}ClFN_4O$: C, 63.24; H, 5.05; N, 14.05; Found C, 63.31; H, 5.04; N, 14.03.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-(4-nitrophenyl)urea (**QU_15**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-nitro phenyl isocyanate (0.068 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_15** (0.099 g, 61 % yield) as pale yellow solid. MP: 222 – 224 °C. ^{1}H NMR (DMSO-d6): δ_H 9.21 (s, 1H), 8.71 (d, *J* = 6.8 Hz, 1H), 8.16 (d, *J* = 12 Hz, 2H), 8.03 – 7.65 (m, 5H)), 7.04 (d, *J* = 6.8 Hz, 1H), 6.64 (d, *J* = 10 Hz, 1H), 3.78 (m, 1H), 3.50 (d, *J* = 16.4 Hz, 2H), 3.06 (t, *J* = 14.4 Hz, 2H), 2.08 (d, *J* = 14.4 Hz, 2H), 1.81 (t, *J* = 13.2 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 155.6, 154.2, 149.8, 146.2, 144.2, 136.2, 129.8, 129.2, 127.6, 125.2 (2C), 123.5, 119.7 (2C), 117.6, 52.5 (2C), 49.6, 28.9 (2C). EI-MS m/z: 426 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}ClN_4O$: C, 59.23; H, 4.73; N, 16.44; Found C, 59.09; H, 4.74; N, 16.42.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-(4-methoxyphenyl)urea (**QU_16**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-methoxy phenyl isocyanate (0.062 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_16** (0.095 g, 61 % yield) as white solid. MP: 201 – 203 °C. ^{1}H NMR (DMSO-d6): δ_H 8.70 (d, *J* = 4.8 Hz, 1H), 8.18 (s, 1H), 8.02 – 7.58 (m, 3H), 7.30 (m, 2H) 7.05 (d, *J* = 5.2 Hz, 1H), 6.83 (m, 2H), 6.18 (d, *J* = 7.6 Hz, 1H) 3.72 (m, 1H), 3.69 (s, 3H), 3.48 (d, *J* = 12.4 Hz, 2H), 3.03 (t, *J* = 10.8 Hz, 2H), 2.05 (d, *J* = 10 Hz, 2H), 1.75 (t, *J* = 10.4 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 159.7, 155.4, 154.5, 149.8, 136.2, 132.5, 129.8, 129.3, 127.2, 123.5, 119.6 (2C), 117.5, 115.6 (2C), 56.2, 52.5 (2C), 49.6, 28.9 (2C). EI-MS m/z: 411 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}ClN_4O_2$: C, 64.31; H, 5.64; N, 13.64; Found C, 64.45; H, 5.65; N, 13.66.

1-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-3-isopropylurea (**QU_17**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), isopropyl isocyanate (0.035 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_17** (0.091 g, 68 % yield) as colourless gum. ^{1}H NMR (DMSO-

d6): δ_H 8.69 (d, *J* = 5.6 Hz, 1H), 8.01 (m, 2H), 7.36 – 7.18 (m, 3H), 6.82 (t, *J* = 5.6 Hz, 1H), 4.16 (m, 1H), 3.70 (m, 1H), 3.51 (d, *J* = 11.8 Hz, 2H), 3.03 (t, *J* = 11.6 Hz, 2H), 2.07 (d, *J* = 12.6 Hz, 2H), 1.72 (t, *J* = 10.6 Hz, 2H), 1.13 (s, 6H). ^{13}C NMR (DMSO-d6): δc 160.2, 158.2, 154.2, 149.7, 136.2, 129.8, 129.2, 126.5, 123.5, 117.5, 52.5 (2C), 49.6, 46.4, 28.9 (2C), 23.2 (2C). EI-MS m/z: 347 $(M+H)^+$. Anal Calcd for $C_{18}H_{23}ClN_4O$: C, 62.33; H, 6.68; N, 16.15; Found C, 62.49; H, 6.69; N, 16.16.

N-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-1-phenylmethanesulfonamide (**QU_18**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine QU**_03** (0.1 g, 0.38 mmol), phenylmethanesulfonylchloride (0.08 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_18** (0.113 g, 71% yield) as white solid. M.P: 137 - 139 °C. ^{1}H NMR (DMSO-d6): δ_H 8.68 (d, *J* = 6.5 Hz, 1H), 8.02 – 7.65 (m, 3H), 7.48 (d, *J* = 12 Hz, 2H), 7.28 (t, *J* = 11 Hz, 2H), 7.22 (d, *J* = 6.8 Hz, 1H), 7.14 (t, *J* = 11 Hz, 1H), 7.05 (d, *J* = 10.6 Hz, 1H), 4.26 (s, 2H), 4.32 (m, 1H), 3.61 (d, *J* = 9.8 Hz, 2H), 3.11 (t, *J* = 11.6 Hz, 2H), 2.23 (d, *J* = 10.6 Hz, 2H), 1.91 (t, *J* = 10.6 Hz, 2H).^{13}C NMR (DMSO-d6): δc 160.2, 154.3, 149.5, 136.2, 133.6, 130.5 (2C), 129.8, 129.2, 128.8 (2C), 126.5, 126.1, 123.5, 117.7, 65.2, 52.5 (2C), 46.2, 28.5 (2C). EI-MS m/z: 416 $(M+H)^+$. Anal Calcd for $C_{21}H_{22}ClN_3O_2S$: C, 60.64; H, 5.33; N, 10.10; Found C, 60.55; H, 5.34; N, 10.11.

N-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)benzenesulfonamide (**QU_19**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine QU**_03** (0.1 g, 0.38 mmol), benzenesulfonyl chloride (0.074 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_19** (0.098 g, 64 % yield) as reddish brown solid. MP: 143 -145 °C. ^{1}H NMR (DMSO-d6): δ_H 8.67 (d, *J* = 6.8 Hz, 1H), 8.01 – 7.68 (m, 3H), 7.52 (d, *J* = 11.5 Hz, 2H), 7.32 (t, *J* = 11.5 Hz, 2H), 7.18 (d, *J* = 6.8 Hz, 1H), 7.12 (t, *J* = 12.1 Hz, 1H), 6.98 (d, *J* = 10.8 Hz, 1H), 4.29 (m, 1H), 3.60 (d, *J* = 10 Hz, 2H), 3.08 (t, *J* = 11.6 Hz, 2H), 2.19 (d, *J* = 10 Hz, 2H), 1.87 (t, *J* = 10.2 Hz, 2H).^{13}C NMR (DMSO-d6): δc 160.2, 154.3, 149.5, 141.2, 136.2, 132.5, 130.5 (2C), 129.8, 129.2, 128.8 (2C), 126.5, 123.5, 117.5, 52.5 (2C), 46.2, 28.5 (2C). EI-MS m/z: 402 $(M+H)^+$. Anal Calcd for $C_{20}H_{20}ClN_2S$: C, 59.77; H, 5.02; N, 10.46; Found: C, 59.85; H, 5.03; N, 10.48.

4-chloro-N-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)benzenesulfonamide (**QU_20**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-chlorobenzenesulfonyl chloride (0.088 g, 0.42

mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_20** (0.106 g, 63 % yield) as yellowish brown solid. MP: 155 – 157 ° C. ^{1}H NMR (DMSO-d6): δ_H 8.72 (d, *J* = 5.8 Hz, 1H), 8.29 – 8.02 (m, 4H), 7.82 (d, *J* = 9.2 Hz, 2H), 7.63 (m, 1H), 7.48 (s, 1H), 7.18 (d, *J* = 5.6 Hz, 1H), 4.31 (m, 1H), 3.62 (d, *J* = 9 Hz, 2H), 3.01 (t, *J* = 10.6 Hz, 2H), 2.24 (d, *J* = 10.9 Hz, 2H), 1.92 (t, *J* = 10.6 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 154.2, 149.6, 138.2, 136.2, 129.8, 129.5 (2C), 129.2 (2C), 129.0, 126.5, 123.5, 117.5, 52.5 (2C), 46.2, 28.5 (2C). EI-MS m/z: 437 $(M+H)^+$. Anal Calcd for $C_{20}H_{19}Cl_2N_3O_2S$: C, 55.05; H, 4.39; N, 9.63; Found C, 54.96; H, 4.40; N, 9.62.

N-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-4-fluorobenzenesulfonamide (**QU_21**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-fluoro phenyl isothiocyanate (0.081 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_21** (0.1 g, 62 % yield) as brown solid. MP: 174 – 176 ° C. ^{1}H NMR (DMSO-d6): δ_H 8.71 (d, *J* = 6.2 Hz, 1H), 8.24 – 8.02 (m, 4H), 7.71 (d, *J* = 9.8 Hz, 2H), 7.61 (m, 1H), 7.52 (s, 1H), 7.15 (d, *J* = 6.4 Hz, 1H), 4.28 (m, 1H), 3.61 (d, *J* = 9.2 Hz, 2H), 3.01 (t, *J* = 10.8 Hz, 2H), 2.20 (d, *J* = 11 Hz, 2H), 1.92 (t, *J* = 10.6 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 165.8, 160.2, 154.2, 149.6, 139.8, 136.2, 130.2 (2C), 129.8, 129.2, 126.5, 123.5, 117.5, 114.8 (2C), 52.5 (2C), 46.2, 28.5 (2C). EI-MS m/z: 420 $(M+H)^+$. Anal Calcd for $C_{20}H_{19}ClFN_3O_2S$: C, 57.21; H, 4.56; N, 10.01; Found C, 57.09; H, 4.57; N, 10.03.

N-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-4-nitrobenzenesulfonamide (**QU_22**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-nitrobenzenesulfonyl chloride (0.093 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_22** (0.116 g, 67 % yield) as yellow solid. MP: 198 – 200 ° C. ^{1}H NMR (DMSO-d6): δ_H 8.73 (d, *J* = 5.8 Hz, 1H), 8.24 – 8.00 (m, 4H), 7.85 (d, *J* = 9.6 Hz, 2H), 7.66 (m, 1H), 7.51 (s, 1H), 7.21 (d, *J* = 5.8 Hz, 1H), 4.29 (m, 1H), 3.62 (d, *J* = 9.8 Hz, 2H), 3.05 (t, *J* = 10.4 Hz, 2H), 2.21 (d, *J* = 10.9 Hz, 2H), 1.88 (t, *J* = 10.6 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 154.5, 150.8, 150.2, 149.6, 136.2, 129.8, 129.2, 128.5 (2C), 126.6, 125.2 (2C), 123.5, 117.7, 52.5 (2C), 46.2, 28.5 (2C). EI-MS m/z: 447 $(M+H)^+$. Anal Calcd for $C_{20}H_{19}ClN_4O_4S$: C, 53.75; H, 4.29; N, 12.54; Found C, 53.69; H, 4.30; N, 12.56.

N-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)-4-methoxybenzenesulfonamide (**QU_23**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-methoxybenzenesulfonyl chloride (0.086 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_23** (0.099 g, 60 % yield) as dark brown solid. MP: 137 – 139 ° C. ^{1}H NMR (DMSO-d6): δ_H 8.73 (d, *J* = 5.2 Hz, 1H), 8.03 – 7.67 (m, 3H), 7.55 (s, 1H), 7.42 – 7.21 (m, 4H), 7.14 (d, *J* = 8.0 Hz, 1H), 4.30 (m, 1H), 3.76 (s, 3H), 3.51 (d, *J* = 12.8 Hz, 2H), 3.04 (t, *J* = 11.6 Hz, 2H), 2.05 (d, *J* = 10.4 Hz, 2H), 1.82 (t, *J* = 11.2 Hz, 2H).^{13}C NMR (DMSO-d6): δc 164.5, 160.2, 154.2, 149.5, 136.9, 136.2, 129.8, 129.2, 126.8 (2C), 126.4, 123.5, 117.5, 114.9 (2C), 56.2, 52.5 (2C), 46.2, 28.5 (2C). EI-MS m/z: 432 $(M+H)^+$. Anal Calcd for $C_{21}H_{22}ClN_3O_3S$: C, 58.39; H, 5.13; N, 9.73; Found C, 58.49; H, 5.14; N, 9.72.

N-(1-(7-chloroquinolin-4-yl)piperidin-4-yl)propane-2-sulfonamide (**QU_24**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), isopropylbenzenesulfonyl chloride (0.059 g, 0.42 mmol) and triethyl amine (0.16 ml, 1.15 mmol) to afford **QU_24** (0.094 g, 64 % yield) as brown gum. ^{1}H NMR (DMSO-d6): δ_H 8.69 (d, *J* = 5 Hz, 1H), 8.02 – 7.46 (m, 4H), 7.20 (t, *J* = 5.6 Hz, 1H), 4.29 (m, 1H), 4.16 (m, 1H), 3.55 (d, *J* = 12.2 Hz, 2H), 3.05 (t, *J* = 11.6 Hz, 2H), 2.12 (d, *J* = 12.6 Hz, 2H), 1.81 (t, *J* = 10.6 Hz, 2H), 1.14 (s, 6H).^{13}C NMR (DMSO-d6): δc 160.2, 154.3, 149.6, 136.2, 129.8, 129.3, 126.5, 123.5, 117.5, 63.5, 52.5 (2C), 47.1, 28.5 (2C), 12.8 (2C). EI-MS m/z: 368 $(M+H)^+$. Anal Calcd for $C_{17}H_{22}ClN_3O_2S$: C, 55.50; H, 6.03; N, 11.42; Found C, 55.63; H, 6.02; N, 11.44.

N-benzyl-1-(7-chloroquinolin-4-yl) piperidin-4-amine (**QU_25**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), benzaldehyde (0.044 g, 0.42 mmol) and sodium cyanoborohydride (0.035 g, 0.57 mmol) to afford **QU_25** (0.077 g, 57 % yield) as yellow gum.^{1}H NMR (DMSO-d6): δ_H 8.72 (d, *J* = 7.6 Hz, 1H), 8.04 – 7.58 (m, 3H), 7.46 (d, *J* = 11.4 Hz, 2H), 7.18 (t, *J* = 10.8 Hz, 2H), 7.04 (d, *J* = 6.8 Hz, 1H), 6.91 (t, *J* = 11.6 Hz, 1H), 6.42 (d, *J* = 10.5 Hz, 1H), 3.81 (s, 2H), 3.68 (m, 1H), 3.60 (d, *J* = 9.8 Hz, 2H), 3.09 (t, *J* = 11.5 Hz, 2H), 2.20 (d, *J* = 10.8 Hz, 2H), 1.91 (d, *J* = 10.5 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 154.5, 149.5, 141.2, 136.2, 132.3, 130.2 (2C), 129.8, 129.5 (2C), 128.9, 125.3, 123.5, 117.5, 52.5 (2C), 46.2, 28.5 (2C). EI-

MS m/z: 352 $(M+H)^+$. Anal Calcd for $C_{21}H_{22}ClN_3$: C, 71.68; H, 6.30; N, 11.94; Found: C, 71.80; H, 6.31; N, 11.93.

N-(4-chlorobenzyl)-1-(7-chloroquinolin-4-yl)piperidin-4-amine (**QU_26**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-chloro benzaldehyde (0.059 g, 0.42 mmol) and sodium cyanoborohydride (0.035 g, 0.57 mmol) to afford **QU_26** (0.082 g, 55 % yield) as brown gum. ^{1}H NMR (DMSO-d6): δ_H 8.70 (d, *J* = 5.8 Hz, 1H), 8.21 – 8.01 (m, 4H), 7.81 (d, *J* = 9.6 Hz, 2H) 7.51 (m, 1H), 7.41 (s, 1H), 6.96 (d, *J* = 6 Hz, 1H), 3.79 (s, 2H), 3.67 (m, 1H), 3.60 (d, *J* = 9.6 Hz, 2H), 3.12 (t, *J* = 12 Hz, 2H), 2.18 (d, *J* = 11 Hz, 2H), 1.90 (d, *J* = 10.8 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 154.2, 149.5, 139.2, 136.2, 133.2, 130.2 (2C), 129.8, 129.2, 128.8 (2C), 126.4, 123.4, 117.5, 60.2, 52.9 (2C), 52.5, 29.9 (2C). EI-MS m/z: 387 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}Cl_2N_3$: C, 65.29; H, 5.48; N, 10.88; Found: C, 65.17; H, 5.49; N, 10.87.

1-(7-chloroquinolin-4-yl)-N-(4-fluorobenzyl)piperidin-4-amine (**QU_27**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-chloro benzaldehyde (0.052 g, 0.42 mmol) and sodium cyanoborohydride (0.035 g, 0.57 mmol) to afford **QU_27** (0.096 g, 68 % yield) as brown gum. ^{1}H NMR (DMSO-d6): δ_H 8.68 (d, J = 6.2 Hz, 1H), 8.18 – 7.94 (m, 4H), 7.81 (d, *J* = 9.4 Hz, 2H), 7.48 (m, 1H), 7.45 (s, 1H), 7.02 (d, *J* = 5.8 Hz, 1H), 3.79 (s, 2H), 3.68 (m, 1H), 3.62 (d, *J* = 9.8 Hz, 2H), 3.02 (t, *J* = 10.8 Hz, 2H), 2.18 (d, *J* = 11 Hz, 2H), 1.92 (t, *J* = 10.6 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 161.5, 160.2, 154.5, 149.5, 136.2, 136.1, 129.8, 129.4 (2C), 129.0, 126.3, 123.5, 117.5, 115.1 (2C), 60.2, 52.9 (2C), 52.7, 29.9 (2C). EI-MS m/z: 370 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClFN_3$: C, 68.19; H, 5.72; N, 11.36; Found: C, 68.28; H, 5.71; N, 11.38.

1-(7-chloroquinolin-4-yl)-N-(4-nitrobenzyl)piperidin-4-amine (**QU_28**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-nitro benzaldehyde (0.063 g, 0.42 mmol) and sodium cyanoborohydride (0.035 g, 0.57 mmol) to afford **QU_28** (0.101 g, 66 % yield) as yellow solid. MP: 125 -127 °C. ^{1}H NMR (DMSO-d6): δ_H 8.73 (d, *J* = 5.6 Hz, 1H), 8.19 – 7.94 (m, 4H), 7.85 (d, *J* = 9.6 Hz, 2H), 7.54 (m, 1H), 7.42 (s, 1H), 7.01 (d, *J* = 5.6 Hz, 1H), 3.82 (s, 2H), 3.68 (m, 1H), 3.58 (d, *J* = 9 Hz, 2H), 2.98 (t, *J* = 11.4 Hz, 2H), 2.16 (d, *J* = 12.4 Hz, 2H), 1.92 (t, *J* = 13.6 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.1, 154.5, 149.8, 145.8, 145.6, 136.2, 129.8, 129.2, 129.0 (2C), 126.4, 124.2 (2C), 123.5, 117.6, 60.2, 52.8 (2C), 52.6, 29.8 (2C). EI-MS m/z: 397

$(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4O_2$: C, 63.55; H, 5.33; N, 14.12; Found: C, 63.44; H, 5.32; N, 14.10.

1-(7-chloroquinolin-4-yl)-N-(4-methoxybenzyl)piperidin-4-amine (**QU_29**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 4-methoxy benzaldehyde (0.057 g, 0.42 mmol) and sodium cyanoborohydride (0.035 g, 0.57 mmol) to afford **QU_29** (0.095 g, 65 % yield) as brown solid. MP: 74 -76 °C. ^{1}H NMR (DMSO-d6): δ_H 8.68 (d, *J* = 5.6 Hz, 1H), 8.06 – 7.56 (m, 3H), 7.48 (s, 1H), 7.35 – 6.95 (m, 4H), 6.38 (d, *J* = 7.8 Hz, 1H), 3.81 (s, 2H), 3.78 (s, 3H), 3.68 (m, 1H), 3.52 (d, *J* = 13.4 Hz, 2H), 3.08 (t, *J* = 11.5 Hz, 2H), 2.04 (d, *J* = 10 Hz, 2H), 1.85 (t, *J* = 10.2 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 159.8, 154.5, 149.8, 136.2, 133.6, 130.8 (2C), 129.8, 129.2, 126.4, 123.4, 117.4, 114.6 (2C), 60.2, 56.5, 52.8 (2C), 52.7, 30.3 (2C). EI-MS m/z: 382 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}ClN_3O$: C, 69.19; H, 6.33; N, 11.00; Found: C, 69.31; H, 6.32; N, 11.01.

1-(7-chloroquinolin-4-yl)-N-phenylpiperidin-4-amine (**QU_30**): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), bromo benzene (070 g, 0.45 mmol), sodium tertiary butoxide (0.74 g, 0.76 mmol), and $PdCl_2$ (dppf) (0.013 g, 0.019 mol) to afford **QU_30** (0.054 g, 42 % yield) as brown gum. ^{1}H NMR (DMSO-d6): δ_H 8.69 (d, *J* = 6.8 Hz, 1H), 7.99 – 7.61 (m, 3H), 7.52 (d, *J* = 11.8 Hz, 2H), 7.48 (s, 1H), 7.31 (t, *J* = 12 Hz, 2H), 6.98 (t, *J* = 11 Hz, 1H), 6.54 (d, *J* = 10.2 Hz, 1H), 3.68 (m, 1H), 3.61 (d, *J* = 10 Hz, 2H), 3.01 (t, *J* = 11.2 Hz, 2H), 2.14 (d, *J* = 10.5 Hz, 2H), 1.88 (t, *J* = 10.3 Hz, 2H). ^{13}C NMR (DMSO-d6): δc 160.2, 154.5, 149.6, 148.2, 136.2, 129.8, 129.3 (2C), 129.0, 126.6, 123.4, 119.8, 117.6, 114.5 (2C), 57.2, 52.7 (2C), 29.5 (2C). EI-MS m/z: 338 $(M+H)^+$. Anal Calcd for $C_{20}H_{20}ClN_3$: C, 71.10; H, 5.97; N, 12.44; Found: C, 71.20; H, 5.96; N, 12.46.

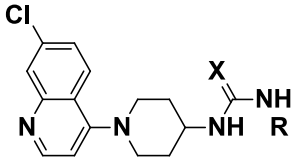
1-(7-chloroquinolin-4-yl)-N-isopropylpiperidin-4-amine (QU_31): The compound was prepared according to the general procedure using 1-(7-chloroquinolin-4-yl) piperidin-4-amine **QU_03** (0.1 g, 0.38 mmol), 2-bromopropane (055 g, 0.45 mmol), potassium carbonate (0.104 g, 0.76 mmol) to afford **QU_31** (0.045 g, 38 % yield) as pale yellow gum. ^{1}H NMR (DMSO-d6): δ_H 8.66 (d, *J* = 5.6 Hz, 1H), 8.01 – 7.33 (m, 4H), 7.05 (t, *J* = 5.6 Hz, 1H), 4.16 (m, 1H), 3.67 (m, 1H), 3.55 (d, *J* = 12.5 Hz, 2H), 3.03 (t, *J* = 11.8Hz, 2H), 2.09 (d, *J* = 12.1 Hz, 2H), 1.82 (t, *J* = 10.8 Hz, 2H), 1.15 (s, 6H). ^{13}C NMR (DMSO-d6): δc 160.2, 154.4, 149.5, 136.2, 129.8,

129.2, 126.4, 123.4, 117.5, 57.2, 52.9 (2C), 50.8, 30.2 (2C), 23.8 (2C). EI-MS m/z: 304 (M+H)+. Anal Calcd for $C_{17}H_{22}ClN_3$: C, 67.20; H, 7.30; N, 13.83; Found: C, 67.12; H, 7.31; N, 13.81.

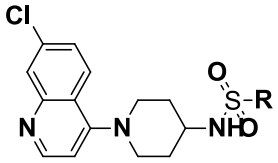
5.4.4. *In vitro Msm* Gyr B assay, supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were evaluated for their *in vitro Msm* Gyr B assay and supercoiling assay for the derivation of SAR and lead optimization. The compounds were further subjected to a whole cell screening against *Mtb* $H_{37}Rv$ strain to understand their bactericidal potency using the agar dilution method and later the safety profile of these molecules were evaluated by checking the *in vitro* cytotoxicity against RAW 264.7 cell line (mouse macrophage) at 50 μM concentration by MTT assay, and the results are shown in **Table 5.8**:

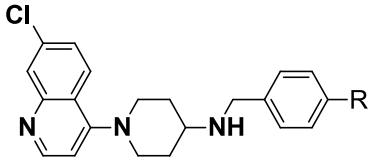
Table 5.8: *In vitro* biological evaluation of the synthesized compounds **QU_04** – **QU_31**



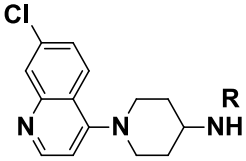
X=O,S
QU_4-QU_17



QU_18-QU_24



QU_25-QU_29



QU_30, QU_31

Comp	X	R	*Msm* Gyr B Assay (IC_{50}) (μM)	*Mtb* SUPER COILING IC_{50} (μM)	*Mtb* MIC (μM)	RAW264.7 Cytotoxicity at 50 μM (% inhib.)
QU_04	S	benzyl	27.18±0.06	50.12±0.6	30.42	28.39
QU_05	S	phenyl	24.14±0.21	55.42±0.55	15.75	29.39
QU_06	S	4-chloro phenyl	21.20±0.15	23.5±0.32	28.98	34.89
QU_07	S	4-fluoro phenyl	35.61±0.08	48.25±0.42	30.13	38.83
QU_08	S	4-nitro phenyl	25.22±0.43	25.24±0.14	1.72	24.69
QU_09	S	4-methoxy phenyl	32.48±0.32	62.18±0.52	58.55	8.20
QU_10	S	isopropyl	28.32±0.05	40.26±0.59	68.89	37.91
QU_11	O	benzyl	26.15±0.42	39.85±0.44	63.31	39.03

QU_12	O	phenyl	34.23±0.33	50.10±0.12	65.64	36.02
QU_13	O	4-chloro phenyl	27.81±0.55	39.40±0.66	30.10	14.59
QU_14	O	4-fluoro phenyl	36.15±0.28	50.52±0.11	62.68	28.42
QU_15	O	4-nitro phenyl	33.24±0.11	50.10±0.71	7.34	45.94
QU_16	O	4-methoxy phenyl	25.63±0.52	51.88±0.65	15.21	9.85
QU_17	O	isopropyl	23.16±0.04	46.21±0.35	72.08	37.49
QU_18	-	benzyl	22.01±0.62	56.13±0.42	60.10	36.40
QU_19	-	phenyl	23.15±0.27	33.72±0.28	62.20	39.70
QU_20	-	4-chloro phenyl	29.86±0.16	52.20±0.22	28.65	35.17
QU_21	-	4-fluoro phenyl	23.18±0.34	48.20±0.47	59.54	39.67
QU_22	-	4-nitro phenyl	28.15±0.52	51.20±0.68	55.94	24.63
QU_23	-	4-methoxy phenyl	39.14±0.06	53.52±0.74	57.88	34.87
QU_24	-	isopropyl	29.33±0.42	50.20±0.81	67.96	15.50
QU_25	-	H	18.23±0.29	20.52±0.26	18.50	25.35
QU_26	-	4-chloro	18.65±0.65	21.22±0.18	8.09	29.68
QU_27	-	4-fluoro	22.34±0.72	38.14±0.59	67.59	39.07
QU_28	-	4-nitro	20.29±0.88	29.65±0.9	31.50	13.38
QU_29	-	4-methoxy	25.31±0.04	38.32±0.41	32.73	39.73
QU_30	-	phenyl	14.98±0.17	18.25±0.32	8.88	25.89
QU_31	-	isopropyl	25.84±0.13	50.81±0.29	82.28	4.98
Moxifloxacin			>50	11.2±0.23	2.4	NT
Novobiocin			180±3.9nM	46±28nM	>200	19.3
Ethambutol			NT	NT	9.84	NT

IC_{50}, 50% inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; NT, not tested; nM, nanomolar

Mtb DNA gyrase supercoiling enzyme inhibition activity

In vitro activity against *Mtb* $H_{37}Rv$

Cytotoxicity against RAW 264.7 cells (mouse macrophage cell line)

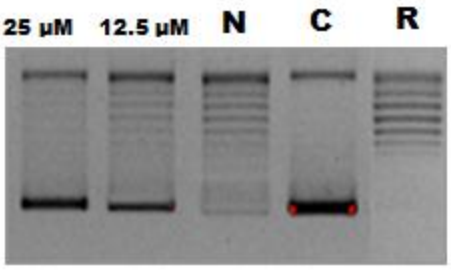


Fig 5.17: Supercoiling assay of most active compound **QU_30** at two different concentrations 25 and 12.5 µM; N-Novobiocin; C-Control (Relaxed DNA substrate + DNA Gyrase + DMSO); R-Relaxed DNA (substrate + DMSO).

5.4.5. Docking studies

In order to fully explore the structure–activity relationship associated with the *Msm* Gyr B inhibitors, compounds were docked to the Gyr B ATPase domain of *Msm* retrieved from protein data bank (PDB ID: 4B6C) using extra precision mode (XP) of Glide module. BITS database lead, Compound **Gb_1** [**2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide,** Gyr B inhibitory IC_{50} = 12.2 ± 0.09 µM**]** exhibited a docking score of -6.26 kcal/mol^{-1} and was found to be in the vicinity of the amino acid Ala53, Asp79, Val125, Val49, Ile171, Val77, Val98,Val99, Pro85, Asn52, Glu56 and Gly83 amino acid residues (which is also characterized to be the active site pocket). Also compound reported by Jean *et al*., **Gb_29** and **Gb_36** exhibited Gyr B inhibitory IC_{50} of 2.5 ± 0.1 µM and 3.1 ± 0.2 µM respectively. A closer look at the interaction profile diagram of these molecules showed the quinoline nitrogen (N-4) to be involved in a prominent hydrogen bonding interaction with Asp 79, analogues to the one observed in the crystal ligand. This interaction is believed to be critical in retaining the activity.

It has been observed that the most active Gyr B compound shown the same interaction, quinoline nitrogen (N-4) to be involved in a prominent hydrogen bonding interaction with Asp 79, analogues to the one observed in the crystal ligand.

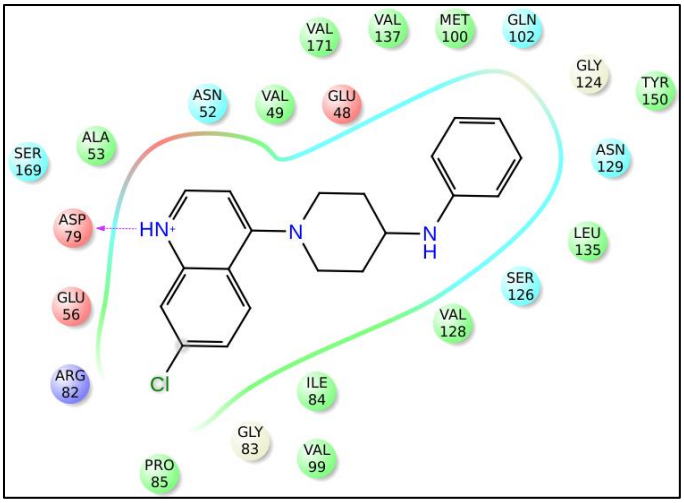


Fig 5.18: Interaction profile picture of most active Gyr B compound **QU_30**

5.4.6. Nutrient Starvation Model

The most active anti-mycobacterial compound **QU_08** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e., Nutrient starvation model. **QU_08** showed 2.7 log reductions in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold).

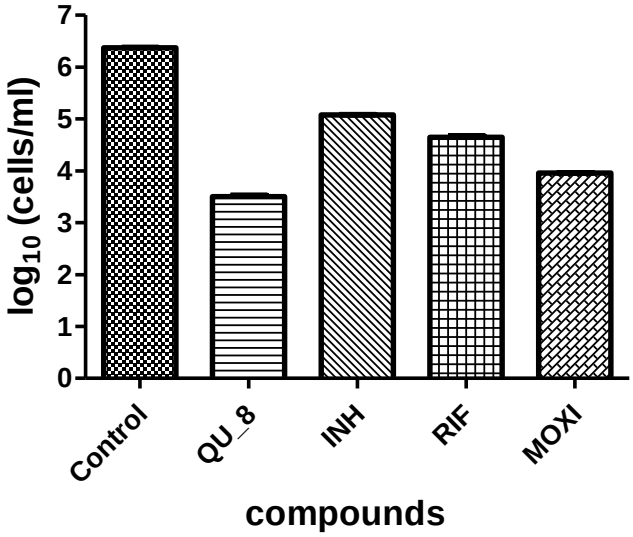


Fig 5.19: Nutrient starvation model graph of **QU_08**

Bacterial count estimation (Mean ± S.D., n = 3) for control and treated groups conducted by using the MPN (most probable number) assay. The dormant cell suspension was treated with the compounds at a concentration of 10 µM. most of the compounds gave significant inhibition of growth of *M. tuberculosis* in this model as compared to the control ($p < 0.0001$, two way ANOVA using GraphPad Prism Software).

5.4.7. Anti-mycobacterial screening for most active compound using adult zebrafish (Zebrafish Model)

To evaluate anti-mycobacterial potency of compound **QU_08** *In vivo* it was also tested in *Mycobacterium marinum* induced adult zebra fish model. The compound was administered orally at a dose of 10 mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. **QU_08** not exhibited significant log reduction in bacteria when compared to first line drugs- Isoniazid (2.9 fold), and Moxifloxacin (2.8 fold).

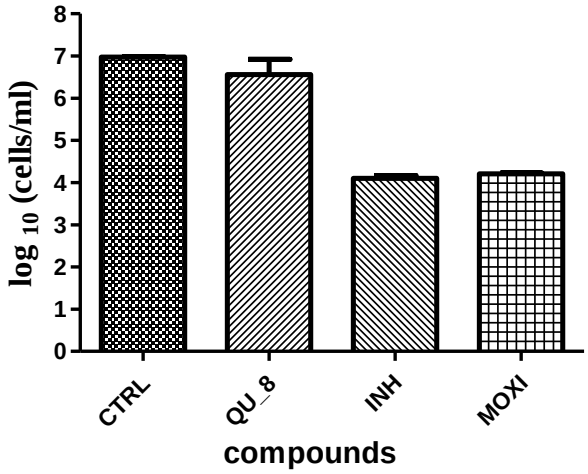


Fig 5.20: Zebra fish model graph of **QU_08**

Bacterial count estimation (Mean ± S.E.M., n = 6) for control and treated groups conducted by using MPN (most probable number) assay. The statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p<0.0001$) with respect to infected control group has been analyzed by Two-way ANOVA using GraphPad Prism Software

5.4.8. SAR and Discussion

BITS database lead, Compound **Gb_1** [**2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide,** Gyr B inhibitory IC_{50} = 12.2 ± 0.09 µM**]** exhibited a docking

score of -6.26 kcal/mol^{-1} and was found to be in the vicinity of the amino acid Ala53, Asp79, Val125, Val49, Ile171, Val77, Val98, Val99, Pro85, Asn52, Glu56 and Gly83 amino acid residues (which is also characterized to be the active site pocket). Also compound reported by Jean *et al*., **Gb_29** and **Gb_36** exhibited Gyr B inhibitory IC_{50} of 2.5 ± 0.1 µM and 3.1 ± 0.2 µM respectively. A closer look at the interaction profile diagram of these molecules showed the quinoline nitrogen (N-4) to be involved in a prominent hydrogen bonding interaction with Asp 79, analogues to the one observed in the crystal ligand. This interaction is believed to be critical in retaining the activity. An additional hydrogen bonding interaction was also observed between the pyridyl nitrogen on the right hand core and -NH_2 sidechain of His12. Further the compound was also found to be stabilized by the hydrophobic interaction with Val125, Val49, Ile171, Val77, Ala53, Ile84, Val98, Val99, and Val123 amino acid residues

Based on the informations from the earlier reported molecules, modifications where made on linker and substitutions on RHS with various amides, isocyanates, thiocyanates and reductive amination products. A library of 28 derivatives were prepared as described in **Fig 4.4**.

The synthesized derivatives were then evaluated for the Gyr B inhibitory potency using the malachite green assay as described in the materials and methods section. Among the 28 derivatives evaluated for their Gyr B inhibitory potency. Twelve compounds showed an inhibitory IC_{50} of less than 25 µM, out of which one compound exhibited inhibition of Gyr B activity with IC_{50} less than 15 µM. **QU_30** emerged as the most promising Gyr B lead with IC_{50} 14.98±0.17 µM. Two compounds exhibited Gyr B activity IC_{50} less than 20 µM. **QU_25**, **QU_26** exhibited Gyr B IC_{50} of 18.23±0.29 µM and 18.65±0.65 µM.

The interaction profile diagram of these molecules showed that the quinoline nitrogen (N-4) to be involved in a prominent hydrogen bonding interaction with Asp 79. This interaction is critical for the activity of these compounds.

Structure activity relationship of compounds **QU_25** – **QU_31,** the most active Gyr B inhibitor **QU_30** is having phenyl group at RHS without any substitution. Compounds **QU_25** is having benzyl group attached to RHS without any substitution. In case of **QU_26,** a phenyl group with chloro attached to RHS showing reduced Gyr B activity compared to unsubstituted phenyl ring. The presence of electron donating and electron withdrawing substituents on RHS aromatic ring reduces the activity when compared to unsubstituted aromatic rings (**QU_27**, **QU_28** and

QU_29). Also the presence of aliphatic groups on RHS instead of aromatic rings resulted in loss of activity (**QU_31**).

SAR of compounds **QU_18** – **QU_24,** sulfonamides derivatives are not shown much significant activity related to Gyr B inspite of various substitutions on RHS.

In case of compounds **QU_4** – **QU_17**, urea and thiourea derivatives not showed any significant Gyr B IC_{50}.

All the synthesized compounds were further evaluated for their supercoiling inhibition studies. Out of 28 molecules, four molecules shown IC_{50} less than 25 µM. The most active Gyr B compound **QU_30** showed supercoiling activity of 18.25±0.32 µM; **QU_25** showed activity of 20.52±0.26 µM and QU_26 showed activity of 21.22±0.18 µM.

The antimycobacterial potency of synthesized molecules was evaluated by *invitro* MABA assay as defined in in materials and methods section. Out of 28 molecules 13 molecules showed MIC less than 50 µM. Out of which 7 molecules showed MIC less than 20 µM, 4 molecules shown MIC less than 10 µM. **QU_08** emerged as the most antimycobacterial compound with MIC of 1.72 µM. The most active compound **QU_30** also showed significant antimycobacterial potency with MIC of 8.88 µM. **QU_25** and **QU_26** showed significant MIC of 18.50 µM and 8.09 µM respectively.

The most potent antimycobacterial compound in the series **QU_08** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e., Nutrient starvation model detailed procedure was described in materials and methods section. **QU_08** showed significant 2.7 log reduction in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold). These results suggest that compound **QU_08** is not only effective against replicative stage of mtb but also against persistent stages of bacteria.

To evaluate *In vivo* activity of most potent antimycobacterial compound **QU_08** was also tested in *Mycobacterium marinum* induced adult zebra fish model, detailed procedure was explained in materials and methods. The compound was administered orally at a dose of 10 mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. Compound **QU_08** not exhibited significant log reduction in bacteria when compared to first line drugs- Isoniazid, Rifmapicin and Moxifloxacin.

Finally the eukaryotic cell safety profile of all the twenty eight compounds were observed by testing there *in vitro* cytotoxicity against the RAW 264.7 cell line (Mouse leukemic monocyte macrophage cell line) at 50 µM concentration by (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Since the mycobacteria resides in the macrophages during the infection stages, this cell line was selected. At 50 µM tested compounds showed cytotoxicity range of 8.20-45.94 % as shown in **Table 5.8**. The most of the synthesized compounds showed cytotoxicity within the acceptable range.

5.4.9. Highlights of the study

In summary, we identified and synthesized a novel class of DNA Gyrase inhibitors from earlier reported lead molecules from our own research group. Compound **QU_30** (1-(7-chloroquinolin-4-yl)-N-phenylpiperidin-4-amine) was found to be the most active DNA gyrase B inhibitor with Gyr B IC_{50} of 14.98±0.17 µM, supercoiling IC_{50} of 18.25±0.32 µM and inhibited drug sensitive *M. tuberculosis* with MIC of 8.88 µM. Also we identified **QU_08** as most active antimycobacterial compound with an MIC of 1.72 µM. Nutrient starvation model, **QU_08** showed 2.7 log reductions in bacterial count when compared to standard drugs namely isoniazid (1.5 log fold), rifampicin (1.7 log fold) and moxifloxacin (2.0 log fold). These results suggest that compound **QU_08** is not only effective against replicative stage of mtb but also against persistent stages of bacteria.

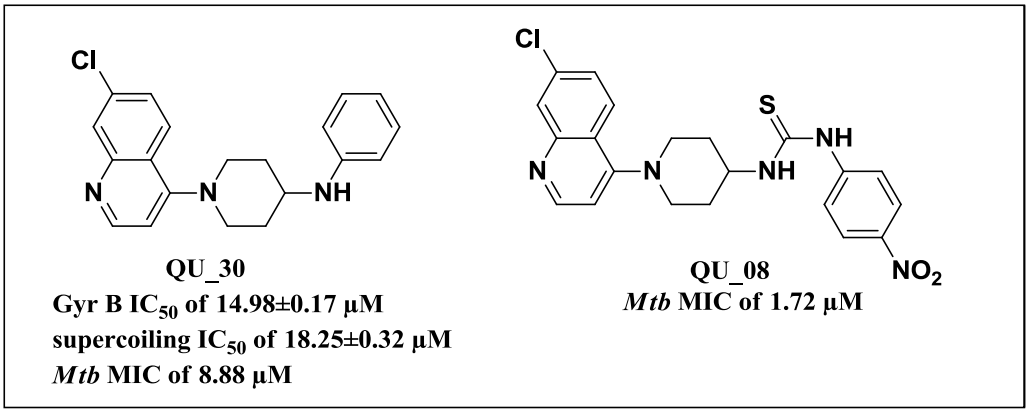


Figure 5.21: Chemical structure and biological activity of the most active compounds **QU_30** and **QU_08**.

5.5. Design, synthesis and biological evaluation of 2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-phenylacetamide derivatives as potent *Mycobacterium tuberculosis* DNA Gyrase inhibitors

5.5.1. Design of the molecules

1,3-benzothiazin-4-ones (BTZs) discovered by Makarov *et al* in 2009 [Markarov., et al., 2009] lead to new anti TB drug with an MIC of 0.001 µG/mL.). BTZ043 a nitroaromatic compound, was reported to be active against MDR-clinical isolates of *Mycobacterium tuberculosis tuberculosis* [Pasca., *et al.*, 2010; Makarov M., *et al.*, 2009]. Manoj Chandran *et al* reported Benzothiazinone – piperazine derivatives as DNA gyrase inhibitors [Manoj Chandran., *et al*, 2015].

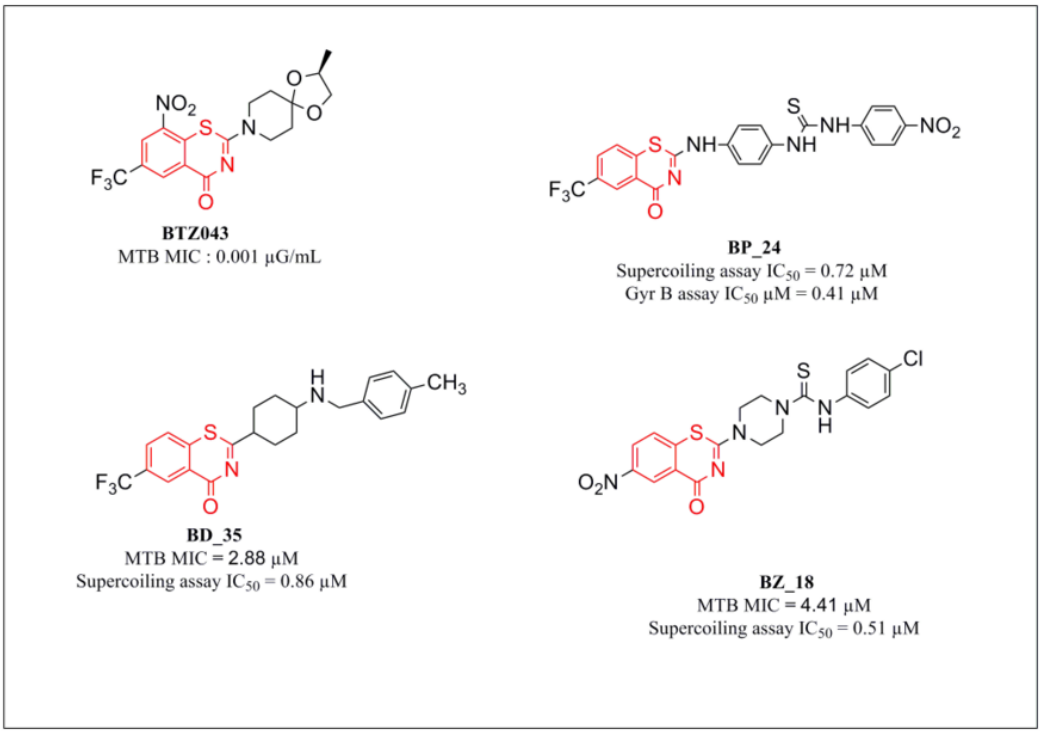


Fig 5.22: Inhibitors reported as DNA gyrase inhibitors and lead molecule BTZ043 [Manoj Chandran, *et al.*, 2015, Makarov M., *et al.*, 2009].

Based on the above reported inhibitors we have taken benzo thiazine core as the major building block to synthesize novel DNA gyrase inhibitors with improved activity. We have synthesized substituted 2-(3-Oxo-3, 4-dihydro-2H-1,4-benzothiazin-2-yl)acetic acid as scaffold and coupled

with different substituted anilines to obtain amides as the final compounds [Jangir, R., *et al*., 2015].

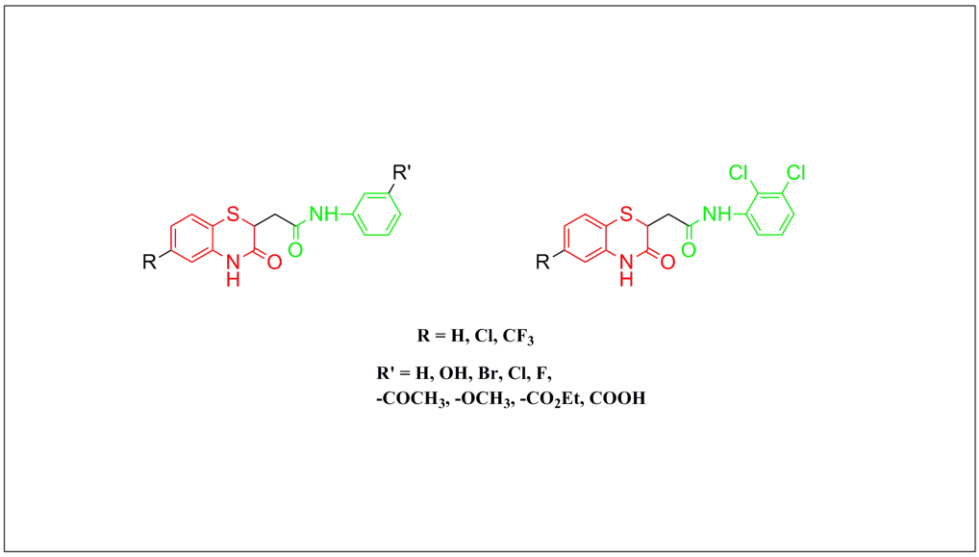


Fig 5.23: Designed two set of molecules **BT_03 – BT_32**

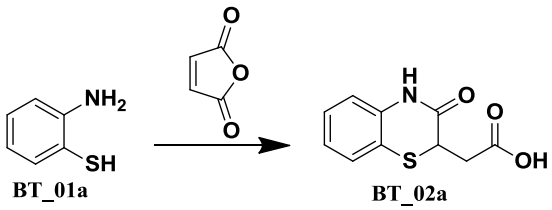
We have designed two set of molecules with different substitution and synthesized a library of 30 derivatives.

5.5.2. Experimental procedures utilized for the synthesis of BT_01a – BT_32

From the earlier reported we find out that benzothiazine scaffold have antibacterial potency and can be functioned as potent DNA gyrase inhibitors too. Construction of benzothiazine core was achieved by following well known procedure reported by R. Jangir *et al*., [Jangir, R., *et al*., 2015]. The reaction of substituted o-aminothiophenol (**BT_01a - BT_01c**) with maleic anhydride in diethyl ether at room temperature gives the desired 1,4-benzothiazinylacetic acid (**BT_02a – BT_02c**) in good yield by a literature procedure [Gupta, R. R., et al., 1987]. This reaction plausibly takes place via the Michael-type addition of the thiol unit of O-aminothiophenol to the C=C bond in maleic anhydride, followed by regioselective in situ intramolecular aminolysis. Thus obtained substituted benzothiazinylacetic acid was coupled with 3-substituted anilines to get corresponding amides (**BT_03 – BT_29**). Amide coupling was executed by using propyl phosphonic anhydride reagent. Compounds **BT_30 – BT_32** was

obtained by treating benzothiazinylacetic acid with 2,3 dichloroaniline using propyl phosphonic anhydride.

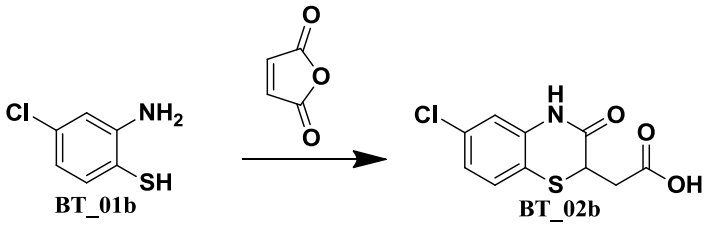
Preparation of 2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetic acid (BT_02a)



BT_01a BT_02a

To a stirred solution of maleic anhydride (2.45 g, 25 mmol) in Et_2O (40 mL) was added O-aminothiophenol (3.13 g, 25 mmol) in a dropwise fashion at 25 °C under argon; the mixture was stirred for 1 h. The separated precipitate was filtered on a Buchner funnel and washed with Et2O (25 mL). The obtained product was dried under vacuum to afford **BT_02a** (5.50 g, 98% yield) as white solid. ^{1}H NMR (DMSO-d_6): δ_H 12.56 (br s, 1 H), 10.69 (s, 1H), 7.34 (d, J = 8 Hz, 1 H, 7.20 (t, J = 8 Hz, 1 H), 6.73 (t, J = 8 Hz, 1 H), 6.66 (d, J = 8 Hz, 1 H), 3.77 (dd, J = 8, 8 Hz, 1 H), 2.84 (dd, J = 16, 8 Hz, 1 H), 2.42 (dd, J = 16, 8 Hz, 1 H). ^{13}C NMR (DMSO-d_6): δ_c 171.1, 165.7, 136.9, 127.6, 127.3, 123.1, 118.2, 117.1, 37.5, 33.7. EI-MS *m/z*: 224 $(M+H)^+$. Anal Calcd for $C_{10}H_9NO_3S$: C, 53.80; H, 4.06; N, 6.27. Found: C, 53.88; H, 4.05; N, 6.26.

Preparation of 2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetic acid (BT_02b)

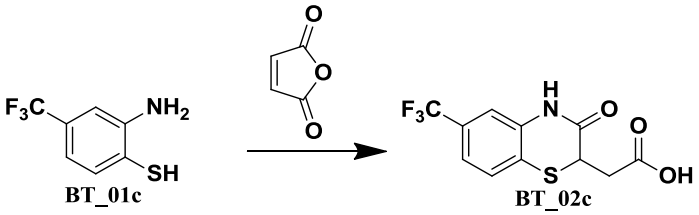


BT_01b BT_02b

To a stirred solution of maleic anhydride (1.84 g, 18 mmol) in Et_2O (40 mL) was added 2-amino-4-chlorobenzenethiol (3 g, 18 mmol) in a dropwise fashion at 25 °C under argon; the mixture was stirred for 1 h. The separated precipitate was filtered on a Buchner funnel and washed with Et_2O (25 mL). The obtained product was dried under vacuum to afford **BT_02b** (3.92 g, 80 % yield) as pale yellow solid. ^{1}H NMR (DMSO-d_6): δ_H 12.48 (br s, 1 H), 10.58 (s, 1H), 7.81(s, 1H), 7.28 (d, J = 8.2 Hz, 1 H), 7.18 (d, J = 8 Hz, 1 H), 3.82 (dd, J = 8, 8 Hz, 1 H),

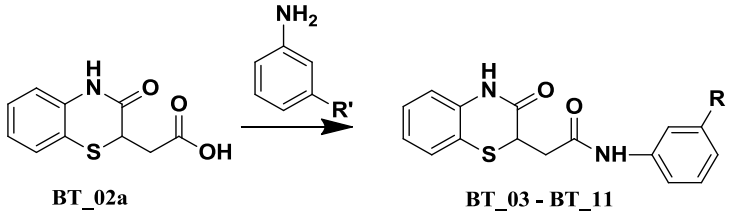
2.81 (dd, *J* = 16, 8 Hz, 1 H), 2.41 (dd, *J* = 16, 8 Hz, 1 H). ^{13}C NMR (DMSO-d_6): δ_c 171.2, 166.2, 141.5, 131.2, 127.5, 125.6, 122.5, 115.9, 37.6, 33.6. EI-MS *m/z*: 257 $(M)^+$. Anal Calcd for $C_{10}H_8ClNO_3S$: C, 46.61; H, 3.13; N, 5.44. Found: C, 46.69; H, 3.12; N, 5.45.

Preparation of 2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetic acid (BT_02c)



To a stirred solution of maleic anhydride (1.84 g, 15 mmol) in Et_2O (40 mL) was added 2-amino-4-(trifluoromethyl)benzenethiol (3 g, 15 mmol) in a dropwise fashion at 25 °C under argon; the mixture was stirred for 1 h. The separated precipitate was filtered on a Buchner funnel and washed with Et_2O (25 mL). The obtained product was dried under vacuum to afford **BT_02C** (4.12 g, 91 % yield) as white solid. ^{1}H NMR (DMSO-d_6): δ_H 12.51 (br s, 1 H), 10.62 (s, 1H), 7.96 (s, 1H), 7.28 (d, *J* = 8 Hz, 1 H), 7.12 (d, *J* = 7.8 Hz, 1 H), 3.86 (dd, *J* = 8, 8 Hz, 1 H), 2.88 (dd, *J* = 16, 8 Hz, 1 H), 2.46 (dd, *J* = 16, 8 Hz, 1 H). ^{13}C NMR (DMSO-d_6): δ_c 171.3, 166.2, 140.8, 128.5, 128.1, 125.6, 122.5, 120.8, 116.8, 37.8, 33.5. EI-MS *m/z*: 292 $(M+H)^+$. Anal Calcd for $C_{11}H_8F_3NO_3S$: C, 45.36; H, 2.77; N, 4.81. Found: C, 45.30; H, 2.78; N, 4.80.

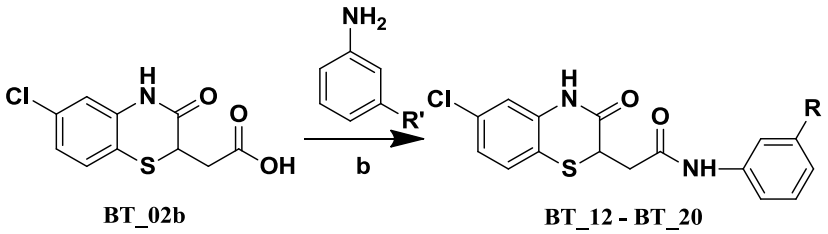
Preparation of final compounds BT_03 – BT_11



To a well stirred solution of 2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetic acid **(BT_02a)** (1 equiv) in anhydrous dichloromethane was added triethylamine (3 equiv) followed by the addition of corresponding 3- sub aniline (1.2 equiv) at room temperature. To the above

mixture was added propyl phosphonic anhydride ≥50 wt. % in ethyl acetate (T_3P) (2 equiv). The reaction mixture was then stirred at rt for 12h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water and the aqueous layer was extracted with dichloromethane, the combined organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using hexane : ethylacetate as to afford the desired product in good yield.

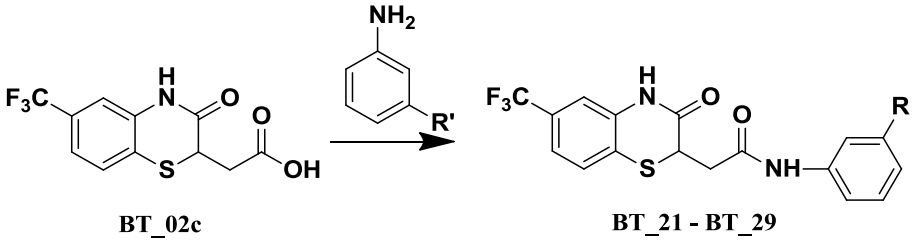
Preparation of final compounds BT_12 – BT_20



BT_02b **BT_12 - BT_20**

To a well stirred solution of 2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetic acid (**BT_02b**) (1 equiv) in anhydrous dichloromethane was added triethylamine (3 equiv) followed by the addition of corresponding 3- sub aniline (1.2 equiv) at room temperature. To the above mixture was added propyl phosphonic anhydride ≥50 wt. % in ethyl acetate (T_3P) (2 equiv). The reaction mixture was then stirred at rt for 12h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water and the aqueous layer was extracted with dichloromethane, the combined organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using hexane : ethylacetate as to afford the desired product in good yield.

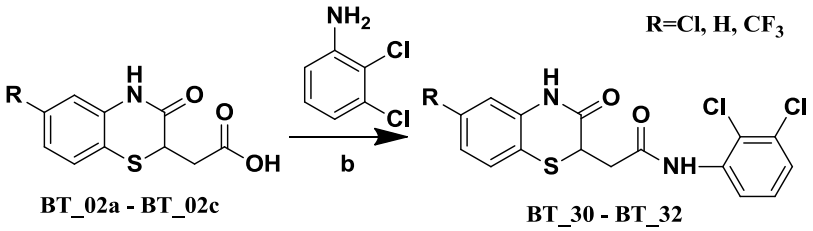
Preparation of final compounds BT_21 – BT_29



BT_02c **BT_21 - BT_29**

To a well stirred solution of 2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetic acid (**BT_02c**) (1 equiv) in anhydrous dichloromethane was added triethylamine (3 equiv) followed by the addition of corresponding 3- sub aniline (1.2 equiv) at room temperature. To the above mixture was added propyl phosphonic anhydride ≥50 wt. % in ethyl acetate (T_3P) (2 equiv). The reaction mixture was then stirred at rt for 12h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water and the aqueous layer was extracted with dichloromethane, the combined organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using hexane : ethylacetate as to afford the desired product in good yield.

Preparation of final compounds BT_31 – BT_33

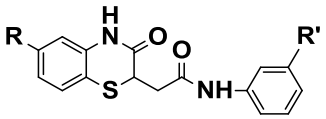


BT_02a - BT_02c **BT_30 - BT_32**

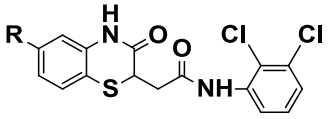
To a well stirred solution of the corresponding 2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetic acid **(BT_02a – BT_02c)** (1 equiv) in anhydrous dichloromethane was added triethylamine (3 equiv) followed by the addition of 2,3-dichloroaniline (1.2 equiv) at room

temperature. To the above mixture was added propyl phosphonic anhydride ≥50 wt. % in ethyl acetate (T_3P) (2 equiv). The reaction mixture was then stirred at rt for 12h (monitored by TLC and LCMS for completion). The solvent was removed under vacuum, diluted with water and the aqueous layer was extracted with dichloromethane, the combined organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was further purified using column chromatography using hexane : ethylacetate as to afford the desired product in good yield.

Table 5.9: Physiochemical properties of the synthesized compounds **BT_03 – BT_32**



BT_ 03 - BT_29



BT_30 - BT_32

COMP	R	R'	Yield (%)	MP (°C)	Molecular Formula	Molecular Weight
BT_03	H	-COOEt	67	198 – 200	$C_{19}H_{18}N2O_4S$	370.42
BT_04	H	-$COCH_3$	67	204 – 206	$C_{18}H_{16}N_2O_3S$	340.40
BT_05	H	-OH	65	219 – 221	$C_{16}H_{14}N_2O_3S$	314.36
BT_06	H	H	80	228 – 230	$C_{16}H_{14}N_2O_2S$	298.36
BT_07	H	Br	73	208 – 210	$C_{16}H_{13}BrN_2O_2S$	377.26
BT_08	H	F	77	225 – 227	$C_{16}H_{13}FN_2O_2S$	316.35
BT_09	H	-COOH	66	185 – 187	$C_{17}H_{14}N_2O_4S$	342.37
BT_10	H	Cl	79	206 – 208	$C_{16}H_{13}ClN_2O_2S$	332.80
BT_11	H	-OCH_3	74	203 – 205	$C_{17}H_{16}N_2O_3S$	328.39
BT_12	Cl	-COOEt	63	-	$C_{19}H_{17}ClN_2O_4S$	404.87
BT_13	Cl	-$COCH_3$	78	-	$C_{18}H_{15}ClN_2O_3S$	374.84
BT_14	Cl	-OH	61	-	$C_{16}H_{13}ClN_2O_3S$	348.80
BT_15	Cl	H	69	-	$C_{16}H_{13}ClN_2O_2S$	332.80
BT_16	Cl	Br	75	222 – 224	$C_{16}H_{12}BrClN_2O_2S$	411.70
BT_17	Cl	F	76	-	$C_{16}H_{12}ClFN_2O_2S$	350.80

BT_18	Cl	-COOH	72	-	$C_{17}H_{13}ClN_2O_4S$	376.81
BT_19	Cl	Cl	55	-	$C_{16}H_{12}Cl_2N_2O_2S$	367.25
BT_20	Cl	$-OCH_3$	54	229 – 231	$C_{17}H_{15}ClN_2O_2S$	362.83
BT_21	CF_3	-COOEt	71	251 – 253	$C_{20}H_{17}F_3N_2O_4S$	438.42
BT_22	CF_3	$-COCH_3$	68	204 – 206	$C_{19}H_{15}F_3N_2O_3S$	408.39
BT_23	CF_3	-OH	65	214 – 216	$C_{17}H_{13}F_3N_2O_3S$	382.36
BT_24	CF_3	H	63	255 – 257	$C_{17}H_{13}F_3N_2O_2S$	366.36
BT_25	CF_3	Br	69	237 – 239	$C_{17}H_{12}BrF_3N_2O_2S$	445.25
BT_26	CF_3	F	74	250 – 252	$C_{17}H_{12}F_4N_2O_2S$	384.35
BT_27	CF_3	-COOH	63	198 – 200	$C_{18}H_{13}F_3N_2O_4S$	410.37
BT_28	CF_3	Cl	72	230 – 232	$C_{17}H_{12}ClF_3N_2O_2S$	400.80
BT_29	CF_3	$-OCH_3$	68	228 – 230	$C_{18}H_{15}F_3N_2O_3S$	396.38
BT_30	H	-	67	221 – 223	$C_{16}H_{12}Cl_2N_2O_2S$	367.25
BT_31	Cl	-	63	-	$C_{16}H_{11}C_{l3}N_2O_2S$	401.69
BT_32	CF3	-	71	224 – 226	$C_{17}H_{11}Cl_2F_3N_2O_2S$	435.25

5.5.3. Characterization of the synthesized molecules BT_03 – BT_32

Ethyl 3-(2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamido)benzoate (BT_03): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), ethyl 3-aminobenzoate (0.088 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_03** (0.11 g, 67 % yield) as white solid. M.P - 198 – 200 ^oC.^1H NMR (DMSO-d_6): δ_H 10.71 (s, 1H), 10.12 (s, 1H), 8.16 (s, 1H), 7.81 – 7.61 (m, , 3H), 7.35 – 6.65 (m, 4H), 4.12 (m, 2H), 3.84 (dd, J = 14.4, 2.8 Hz, 1H), 2.88 (dd, J = 19.5, 8.5 Hz, 1H), 2.51 (dd, J = 21.5, 10 Hz, 1H), 1.35 (t, J = 7.8 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ_c 172.6, 165.2, 164.1, 138.2, 135.8, 129.8, 129.7, 129.1, 126.2, 125.8, 125.2, 123.2, 121.1, 119.1, 117.2, 58.2, 40.6, 32.1, 13.8. EI-MS *m/z*: 371 $(M+H)^+$. Anal Calcd for $C_{19}H_{18}N2O_4S$: C, 61.61; H, 4.90; N, 7.56. Found: C, 61.69; H, 4.91; N, 7.55.

N-(3-acetylphenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_04): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), 3 amino acetophenone (0.072 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_04** (0.102 g, 67 % yield) as white solid. M.P - 204 – 206 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.65 (s, 1H), 10.15 (s, 1H), 8.09 (s, 1H), 7.76 – 7.58 (m, 3 H), 7.35 – 6.63 (m, 4H), 3.92 (dd, *J* = 14.4, 3 Hz, 1H), 2.85 (dd, *J* = 20.5, 10.5 Hz, 1H), 2.49 (dd, *J* = 22, 8.8 Hz, 1H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_c 195.2, 172.5, 165.6, 140.8, 135.5, 135.1, 132.2, 129.8, 125.2, 124.8, 124.1, 123.1, 119.1, 118.8, 117.2, 40.8, 32.6, 25.8. EI-MS *m/z*: 341 $(M+H)^+$. Anal Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.58; H, 4.75; N, 8.22.

N-(3-hydroxyphenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_05): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), 3 amino phenol (0.058 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_05** (0.091 g, 65 % yield) as pale yellow solid. M.P - 219 – 221 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.67 (s, 1H), 10.02 (s, 1H), 7.34 – 7.20 (m, 4 H), 7.14 – 6.72 (m, 4H), 6.02 (s, 1H), 3.89 (dd, *J* = 15.4, 4 Hz, 1H), 2.83 (dd, *J* = 20.4, 10 Hz, 1H), 2.43 (dd, *J* = 21.2, 9 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.8, 160.2, 140.2, 137.2, 129.8, 129.5, 125.2, 123.2, 119.1, 116.3, 114.9, 109.8, 104.2, 40.6, 32.5. EI-MS *m/z*: 315 $(M+H)^+$. Anal Calcd for $C_{16}H_{14}N_2O_3S$: C, 31.13; H, 4.49; N, 8.91. Found: C, 31.17; H, 4.50; N, 8.90.

2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-phenylacetamide (BT_06): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), aniline (0.05 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_06** (0.108 g, 80 % yield) as pale brown solid. M.P – 228 – 230 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.71 (s, 1H), 10.12 (s, 1H), 7.73 – 7.51 (m, 4H), 7.33 – 6.73 (m, 5H), 3.92 (dd, *J* = 15.1, 4 Hz, 1H), 2.82 (dd, *J* = 18.5, 10.5 Hz, 1H), 2.42 (dd, *J* = 20.5, 9.2 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.8, 165.6, 140.2, 137.2, 129.8, 129.5 (2C), 128.9, 125.2, 123.2, 120.8 (2C), 118.9, 117.2, 40.2, 32.6. EI-MS *m/z*: 299 $(M+H)^+$. Anal Calcd for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73; N, 9.39. Found: C, 64.49; H, 4.72; N, 9.40.

N-(3-bromophenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_07): The compound was prepared according to the general procedure using **BT_02a** (0.1

g, 0.44 mmol), 3-bromoaniline (0.092 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_07** (0.123 g, 73 % yield) as pale yellow solid. M.P - 208 – 210 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.68 (s, 1H), 10.08 (s, 1H), 7.93 (s, 1H), 7.28 – 7.20 (m, 3H), 7.14 – 6.68 (m, 4H), 3.91 (dd, *J* = 15.2, 3 Hz, 1H), 2.86 (dd, *J* = 19.2, 10.5 Hz, 1H), 2.45 (dd, *J* = 20.2, 9 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.1, 165.6, 137.6, 135.8, 129.8, 129.6, 128.5, 124.7, 123.2, 123.0, 121.8, 121.4, 118.9, 117.2, 40.5, 32.9. EI-MS m/z: 379 $(M+2H)^+$. Anal Calcd for $C_{16}H_{13}BrN_2O_2S$: C, 50.94; H, 3.47; N, 7.43. Found: C, 50.89; H, 3.48; N, 7.42.

N-(3-fluorophenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_08): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), 3-fluoroaniline (0.059 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_08** (0.112 g, 77 % yield) as white solid. M.P - 225 – 227 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.69 (s, 1H), 10.14 (s, 1H), 7.81 – 7.36 (m, 3H) 7.33 – 6.67 (m, 5H), 3.87 (dd, *J* = 15.2, 4 Hz, 1H), 2.86 (dd, *J* = 20.5, 10.8 Hz, 1H), 2.46 (dd, *J* = 22.6, 10 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.5, 165.4, 164.6, 139.8, 137.2, 131.2, 130.8, 124.6, 123.2, 118.8, 117.4, 116.9, 116.4, 115.8, 40.6, 32.7. EI-MS *m/z*: 317 $(M+H)^+$. Anal Calcd for $C_{16}H_{13}FN_2O_2S$: C, 60.75; H, 4.14; N, 8.86. Found: C, 60.70; H, 4.15; N, 8.87.

3-(2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamido)benzoic acid (BT_09): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), 3-aminobenzoic acid (0.073 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_09** (0.101 g, 66 % yield) as brown solid. M.P - 185 – 187 ^oC. ^1H NMR (DMSO-d_6): δ_H 12.60 (br s, 1 H), 10.62 (s, 1H), 10.11 (s, 1H), 8.21 (s, 1H), 8.04 –7.71 (m, 3H) 7.33 – 6.69 (m, 4H), 3.94 (dd, *J* = 16.6, 4 Hz, 1H), 2.88 (dd, *J* = 21, 10.5 Hz, 1H), 2.47 (dd, *J* = 22.5, 9.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.6, 164.2, 140.8, 137.2, 129.8, 128.8, 128.5, 127.1, 126.4, 124.6, 123.2, 121.5, 118.9, 117.2, 40.5, 32.1. EI-MS *m/z*: 341 $(M-H)^+$. Anal Calcd for $C_{17}H_{14}N_2O_4S$: C, 59.64; H, 4.12; N, 8.18. Found: C, 59.68; H, 4.11; N, 8.19.

N-(3-chlorophenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_10): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), 3-chloroaniline (0.069 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_10** (0.118 g, 79 % yield) as yellow solid. M.P - 206 – 208 ^oC.^1H NMR

(DMSO-d_6): δ_H 10.68 (s, 1H), 10.11 (s, 1H), 8.02 (s, 1H), 7.54 – 7.33 (m, 3H), 7.26 – 6.71 (m, 4H), 3.85 (dd, *J* = 15.2, 3 Hz, 1H), 2.81 (dd, *J* = 20.8, 11 Hz, 1H), 2.44 (dd, *J* = 22.6, 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.5, 165.4, 140.2, 137.2, 133.8, 129.8, 129.6, 128.2, 125.6, 123.2, 121.2, 120.2, 118.9, 117.2, 40.2, 32.1. EI-MS *m/z*: 332 $(M)^+$. Anal Calcd for $C_{16}H_{13}ClN_2O_2S$: C, 57.74; H, 3.94; N, 8.42. Found: C, 57.81; H, 3.95; N, 8.41.

N-(3-methoxyphenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_11): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), 3-methoxyaniline (0.066 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_11** (0.109 g, 74 % yield) as white solid. M.P - 203 – 205 ^oC. ^{1}H NMR (DMSO-d_6): δ_H 10.69 (s, 1H), 10.04 (s, 1H), 7.35 – 7.23 (m, 4H), 7.19 – 6.61 (m, 4H), 3.94 (dd, *J* = 14.4, 2.8 Hz, 1H), 3.71 (s, 3H), 2.98 (dd, *J* = 21.2, 10 Hz, 1H), 2.59 (dd, *J* = 24, 6.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.5, 165.6, 159.8, 140.2, 137.5, 129.9, 129.6, 125.2, 123.2, 118.9, 117.2, 116.6, 111.3, 109.8, 56.5, 40.3, 32.8. EI-MS *m/z*: 329 $(M+H)^+$. Anal Calcd for $C_{17}H_{16}N_2O_3S$: C, 62.18; H, 4.91; N, 8.53. Found: C, 62.25; H, 40; N, 8.54.

Ethyl 3-(2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamido)benzoate (BT_12): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), ethyl 3-aminobenzoate (0.077 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_12** (0.099 g, 63 % yield) as brown liquid. ^{1}H NMR (DMSO-d_6): δ_H 10.69 (s, 1H), 10.09 (s, 1H), 8.13 (s, 1H), 7.95 (s, 1H), 7.85 – 7.58 (m, 3H), 7.35 – 7.02 (m, 2H), 4.08 (m, 2H), 3.87 (dd, *J* = 18.5, 3.2 Hz, 1H), 2.88 (dd, *J* = 21.5, 10.8 Hz, 1H), 2.51 (dd, *J* = 23.4, 7.5 Hz, 1H), 1.35 (t, *J* = 7.8 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ_c 172.8, 165.6, 164.8, 141.6, 139.6, 131.5, 130.8, 129.6, 129.2, 126.4, 126.0, 125.3, 122.5, 121.1, 117.1, 58.5, 40.8, 32.3, 13.9. EI-MS *m/z*: 405 $(M+H)^+$. Anal Calcd for $C_{19}H_{17}ClN_2O_4S$: C, 56.36; H, 4.23; N, 6.92. Found: C, 56.42; H, 4.22; N, 6.91.

N-(3-acetylphenyl)-2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_13): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 3 amino acetophenone (0.063 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_13** (0.113 g, 78 % yield) as brown liquid. ^{1}H NMR (DMSO-d_6): δ_H 10.66 (s, 1H), 10.16 (s, 1H), 8.06 (s, 1H), 7.96 (s, 1H), 7.74 – 7.61 (m, 3H), 7.32 – 7.05 (m, 2H), 3.90 (dd, *J* = 16.6, 3.8 Hz, 1H), 2.85 (dd, *J* = 20.5, 10.5 Hz, 1H),

2.49 (dd, *J* = 21.6, 8 Hz, 1H), 2.51 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_c 195.3, 172.6, 165.6, 141.2, 140.6, 135.8, 132.1, 131.6, 129.5, 126.6, 124.8, 124.5, 121.9, 118.9, 117.2, 40.6, 32.4, 25.7. EI-MS *m/z*: 375 $(M+H)^+$. Anal Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.01; N, 7.47. Found: C, 57.75; H, 4.02; N, 7.48.

2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-(3-hydroxyphenyl)acetamide (BT_14): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 3 amino phenol (0.051 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_14** (0.082 g, 61 % yield) as yellow gum.^{1}H NMR (DMSO-d_6): δ_H 10.68 (s, 1H), 10.08 (s, 1H), 7.92 (s, 1H), 7.35 (s, 1H), 7.29 (d, *J* = 8 Hz, 1H), 7.21 (t, *J* = 8 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 7.05 (d, *J* = 7.8Hz, 1H), 6.71 (d, *J* = 8 Hz, 1H), 6.03 (s, 1H), 3.87 (dd, *J* = 19.2, 4 Hz, 1H), 2.86 (dd, *J* = 21.5, 10.5 Hz, 1H), 2.45 (dd, *J* = 22.4, 9.3 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.8, 165.8, 168.2, 141.2, 140.5, 131.3, 130.6, 129.5, 125.2, 121.8, 117.1, 115.2, 109.8, 104.6, 40.5, 32.4. EI-MS *m/z*: 349 $(M+H)^+$. Anal Calcd for $C_{16}H_{13}ClN_2O_3S$: C, 55.09; H, 3.76; N, 8.03. Found: C, 55.02; H, 3.75; N, 8.04.

2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-phenylacetamide (BT_15): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), aniline (0.043 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_15** (0.09 g, 69 % yield) as yellow gum. ^{1}H NMR (DMSO-d_6): δ_H 10.73 (s, 1H), 10.15 (s, 1H), 7.91 (s, 1H), 7.65 – 7.48 (m, 4H) 7.34 – 7.07 (m, 3H), 3.89 (dd, *J* = 18.2, 3 Hz, 1H), 2.86 (dd, *J* = 22.5, 10.6 Hz, 1H), 2.44 (dd, *J* = 24.1, 8.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.1, 165.4, 141.6, 139.2, 131.5, 129.5 (2C), 129.1, 128.8, 125.6, 122.5, 121.4 (2C), 117.1, 40.2, 32.6. EI-MS *m/z*: 333 $(M+H)^+$. Anal Calcd for $C_{16}H_{13}ClN_2O_2S$: C, 57.74; H, 3.94; N, 8.42. Found: C, 57.69; H, 3.95; N, 8.41.

N-(3-bromophenyl)-2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_16): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 3-bromoaniline (0.08 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_16** (0.121 g, 75 % yield) as brown solid. M.P - 222 – 224 ^{o}C. ^{1}H NMR (DMSO-d_6): δ_H 10.66 (s, 1H), 10.14 (s, 1H), 7.92 (s, 1H), 7.85 (s, 1H), 7.37– 7.33 (m, 2H), 7.19 – 6.96 (m, 3H), 3.88 (dd, *J* = 17.8, 3.2 Hz, 1H), 2.88 (dd, *J* = 25.3, 10.6 Hz, 1H), 2.46

(dd, J = 26.5, 9.2 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.1, 165.4, 141.2, 139.6, 131.2, 129.8, 129.1, 128.5, 125.2, 124.4, 123.1, 121.0, 120.8, 117.1, 40.2, 32.6. EI-MS *m/z*: 411 $(M)^+$. Anal Calcd for $C_{16}H_{12}BrClN_2O_2S$: C, 46.68; H, 2.94; N, 6.80. Found: C, 46.73; H, 2.93; N, 6.81.

2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-(3-fluorophenyl)acetamide (BT_17): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 3-fluoroaniline (0.052 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_17** (0.103 g, 76 % yield) as yellow gum.^{1}H NMR (DMSO-d_6): δ_H 10.72 (s, 1H), 10.15 (s, 1H), 7.94 (s, 1H), 7.81 – 7.43 (m, 3H), 7.33 – 6.89 (m, 3H), 385 (dd, J = 17.2, 3.8 Hz, 1H), 2.82 (dd, J = 22.3, 10.5 Hz, 1H), 2.51 (dd, J = 23.4, 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.4, 162.1, 142.1, 139.8, 131.2, 130.8, 129.2, 125.4, 121.9, 117.6, 117.2, 116.9, 116.5, 40.2, 32.5. EI-MS *m/z*: 351 $(M+H)^+$. Anal Calcd for $C_{16}H_{12}ClFN_2O_2S$: C, 54.78; H, 3.45; N, 7.99. Found: C, 54.85; H, 3.46; N, 8.00.

3-(2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamido)benzoic acid (BT_18): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 3-aminobenzoic acid (0.064 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_18** (0.105 g, 72 % yield) as brown gum. ^{1}H NMR (DMSO-d_6): δ_H 12.65 (br s, 1 H), 10.66 (s, 1H), 10.09 (s, 1H), 8.24 (s, 1H), 8.06 – 7.68 (m, 3H), 7.33 – 7.04 (m, 3H), 3.92 (dd, J = 18.5, 2.9 Hz, 1H), 2.82 (dd, J = 20.5, 10.8 Hz, 1H), 2.43 (dd, J = 22.8, 9.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.5, 165.6, 162.4, 143.8, 131.2, 129.5, 128.9, 128.6, 128.4, 127.2, 126.2, 125.4, 122.4, 121.8, 117.2, 40.6, 32.4. EI-MS *m/z*: 375 $(M-H)^+$. Anal Calcd for $C_{17}H_{13}ClN_2O_4S$: C, 54.19; H, 3.48; N, 7.43. Found: C, 54.25; H, 3.49; N, 7.42.

2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-(3-chlorophenyl)acetamide (BT_19): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 3-chloroaniline (0.059 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_19** (0.078 g, 55 % yield) as yellow gum.^{1}H NMR (DMSO-d_6): δ_H 10.69 (s, 1H), 10.14 (s, 1H), 8.04 (s, 1H), 7.96 (s, 1H), 7.55 – 7.33 (m, 3H), 7.18 – 7.02 (m, 2H), 3.88 (dd, J = 17.9, 4 Hz, 1H), 2.83 (dd, J = 24.2,10.8 Hz, 1H), 2.49 (dd, J = 26,8.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.4, 142.5, 140.8, 133.2, 131.2, 130.6, 129.2, 128.1, 125.2, 122.4, 122.1, 120.5, 117.1, 40.8, 32.1. EI-MS *m/z*: 367 $(M)^+$. Anal Calcd for $C_{16}H_{12}Cl_2N_2O_2S$: C, 52.33; H, 3.29; N, 7.63. Found: C, 52.38; H, 3.30; N, 7.62.

2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-(3-methoxyphenyl)acetamide (BT_20): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 3-methoxyaniline (0.057 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_20** (0.075 g, 54 % yield) as brown solid. M.P - 229 – 231 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.66 (s, 1H), 10.08 (s, 1H), 7.85 (s, 1H), 7.62 (s, 1H), 7.35 – 7.33 (m, 2H), 7.11 – 6.62 (m, 3H) 3.91 (dd, *J* = 16.1, 2.8 Hz, 1H), 3.73 (s, 3H), 2.95 (dd, *J* = 22.2, 10 Hz, 1H), 2.5 (dd, *J* = 24, 7.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.5, 165.6, 161.2, 142.5, 140.2, 131.2, 130.5, 129.1, 125.6, 122.4, 117.2, 116.8, 110.5, 109.8, 56.5, 40.6, 32.5. EI-MS *m/z*: 363 $(M+H)^+$. Anal Calcd for $C_{17}H_{15}ClN_2O_2S$: C, 56.27; H, 4.17; N, 7.72. Found: C, 56.32; H, 4.18; N, 7.73

Ethyl-3-(2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamido)benzoate (BT_21): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), ethyl 3-aminobenzoate (0.068 g, 0.41 mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_21** (0.107 g, 71 % yield) as white solid. M.P - 251 – 253 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.67 (s, 1H), 10.08 (s, 1H), 8.18 (s, 1H), 8.06 (s, 1H), 7.77 – 7.61 (m, 3H) 7.33 – 6.89 (m, 2H), 4.10 (m, 2H), 3.84 (dd, *J* = 16.1, 4 Hz, 1H), 2.84 (dd, *J* = 22.5, 10.6 Hz, 1H), 2.55 (dd, *J* = 24.4, 8 Hz, 1H), 1.37 (t, *J* = 7.8 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ_c 172.8, 165.6, 161.6, 142.5, 140.8, 130.8, 129.2, 127.6, 127.1, 126.2, 125.3, 122.5, 122.1, 121.2, 120.8, 118.2, 58.2, 40.7, 32.5, 13.6. EI-MS *m/z*: 439 $(M+H)^+$. Anal Calcd for $C_{20}H_{17}F_3N_2O_4S$: C, 54.79; H, 3.91; N, 6.39. Found: C, 54.72; H, 3.90; N, 6.40.

N-(3-acetylphenyl)-2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_22): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), 3 amino acetophenone (0.055 g, 0.41 mmol) triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_22** (0.097 g, 68 % yield) as pale yellow solid. M.P - 204 – 206 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.65 (s, 1H), 10.11 (s, 1H), 8.08 (s, 1H), 7.97 (s, 1H), 7.61 – 7.48 (m, 3H), 7.32 – 6.88 (m, 2H), 3.41 (dd, *J* = 16.8, 3.2 Hz, 1H), 2.85 (dd, *J* = 23.3, 10.8 Hz, 1H), 2.52 (dd, *J* = 25.6, 9.5 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_c 195.2, 172.1, 165.4, 142.1, 140.8, 137.2, 132.1, 126.8, 126.2, 125.9, 125.2, 124.8, 122.3,

121.5, 118.8, 118.1, 40.4, 32.2, 25.8. EI-MS *m/z*: 409 $(M+H)^+$. Anal Calcd for $C_{19}H_{15}F_3N_2O_3S$: C, 55.80; H, 3.70; N, 6.86. Found: C, 55.88; H, 3.69; N, 6.87.

N-(3-hydroxyphenyl)-2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_23): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), 3 amino phenol (0.045 g, 0.41 mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_23** (0.086 g, 65 % yield) as pale brown solid. M.P - 214 – 216 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.62 (s, 1H), 10.05 (s, 1H), 7.98 (s, 1H), 7.36 (s, 1H), 7.33 – 7.12 (m, 3H), 6.92 – 6.71 (m, 2H), 6.08 (s, 1H), 3.84 (dd, *J* = 17.6, 3.6 Hz, 1H), 2.85 (dd, *J* = 20.8, 11 Hz, 1H), 2.48 (dd, *J* = 22.8, 9.7 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.1, 165.4, 159.6, 142.5, 140.2, 129.8, 128.5, 128.2, 123.9, 122.5, 121.5, 118.1, 115.1, 109.8, 104.8, 40.8, 32.6. EI-MS *m/z*: 383 $(M+H)^+$. Anal Calcd for $C_{17}H_{13}F_3N_2O_3S$: C, 53.40; H, 3.43; N, 7.33. Found: C, 53.48; H, 3.42; N, 7.32.

2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-phenylacetamide (BT_24): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), aniline (0.032 g, 0.41 mmol) triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_24** (0.079 g, 63 % yield) as white solid. M.P - 255 – 257 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.74 (s, 1H), 10.14 (s, 1H), 8.04 (s, 1H), 7.72 – 7.52 (m, 4H), 7.33 – 6.94 (m, 3H), 3.89 (dd, *J* = 16.8, 3 Hz, 1H), 2.81 (dd, *J* = 23.5, 10.2 Hz, 1H), 2.52 (dd, *J* = 25.1, 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.4, 142.8, 139.2, 129.2 (2C), 128.9, 128.5, 128.1, 124.8, 121.6, 120.8 (2C), 120.1, 118.1, 40.3, 32.8. EI-MS *m/z*: 367 $(M+H)^+$. Anal Calcd for $C_{17}H_{13}F_3N_2O_2S$: C, 55.73; H, 3.58; N, 7.65. Found: C, 55.80; H, 3.59; N, 7.64.

N-(3-bromophenyl)-2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_25): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), 3-bromoaniline (0.071 g, 0.41 mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_25** (0.105 g, 69 % yield) as pale yellow solid. M.P - 237 – 239 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.61(s, 1H), 10.12 (s, 1H), 7.96 (s, 1H), 7.82 (s, 1H), 7.37 – 7.09 (m, 3H), 6.98 – 6.85 (m, 2H), 3.86 (dd, *J* = 16, 3.6 Hz, 1H), 2.81 (dd, *J* = 23.3, 10.6 Hz, 1H), 2.49 (dd, *J* = 25.5, 9.4 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.8, 165.4, 142.1, 139.6, 129.8, 128.5, 128.6, 127.9, 125.2, 123.8, 122.5, 121.8, 121.4, 121.1, 118.2, 40.2,

32.6. EI-MS *m/z*: 447 $(M+2H)^+$. Anal Calcd for $C_{17}H_{12}BrF_3N2O_2S$: C, 45.86; H, 2.72; N, 6.29. Found: C, 45.91; H, 2.73; N, 6.30.

N-(3-fluorophenyl)-2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_26): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), 3-fluoroaniline (0.045 g, 0.41 mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_26** (0.098 g, 74 % yield) as white solid. M.P - 250 – 252 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.67 (s, 1H), 10.11 (s, 1H), 7.96 (s, 1H), 7.78 – 7.36 (m, 3H), 7.33 – 6.75 (m, 3H), 3.81 (dd, *J* = 17.7, 3Hz, 1H), 2.84 (dd, *J* = 23.3, 11Hz, 1H), 2.49 (dd, *J* = 25.4, 9.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.5, 165.6, 161.6, 142.1, 138.9, 129.8, 128.1, 127.8, 125.2, 122.5, 121.4, 118.2, 117.5, 116.8, 115.8, 40.8, 32.7. EI-MS *m/z*: 385 $(M+H)^+$. Anal Calcd for $C_{17}H_{12}F_4N_2O_2S$: C, 53.12; H, 3.15; N, 7.29. Found: C, 53.18; H, 3.16; N, 7.30.

3-(2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamido)benzoic acid (BT_27): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), 3-aminobenzoic acid (0.057 g, 0.41mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_27** (0.088 g, 63 % yield) as yellow solid. M.P - 198 – 200 ^oC. ^1H NMR (DMSO-d_6): δ_H 12.71 (br s, 1 H), 10.61 (s, 1H), 10.10 (s, 1H), 8.26 (s, 1H), 8.04 (s, 1H), 7.98 –7.72 (m, 3H) 7.33 – 6.87 (m, 2H), 3.88 (dd, *J* = 17, 3 Hz, 1H), 2.85 (dd, *J* = 22.5, 10.4 Hz, 1H), 2.46 (dd, *J* = 24.6, 8.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.4, 162.5, 141.2, 139.5, 129.2, 128.5, 128.2, 127.9, 127.5, 126.2, 124.6, 122.4, 121.9, 121.5, 118.1, 40.2, 32.4. EI-MS *m/z*: 409 $(M-H)^+$. Anal Calcd for $C_{18}H_{13}F_3N_2O_4S$: C, 52.68; H, 3.19; N, 6.83. Found: C, 52.64; H, 3.20; N, 6.84.

N-(3-chlorophenyl)-2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_28): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), 3-chloroaniline (0.053 g, 0.41 mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_28** (0.1 g, 72 % yield) as brown solid. M.P - 230 – 232 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.62 (s, 1H), 10.08 (s, 1H), 8.06 (s, 1H), 8.03 (s, 1H), 7.56 – 7.35 (m, 3H), 7.17 – 6.92 (m, 2H), 3.84 (dd, *J* = 16.2, 3 Hz, 1H), 2.85 (dd, *J* = 22.2, 10.5 Hz, 1H), 2.46 (dd, *J* = 24.2,7.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.2, 165.4, 141.2, 138.6, 133.2, 129.5, 128.5, 128.1, 127.8, 125.4, 122.5, 122.2, 121.6, 120.5, 118.1, 40.8, 32.6. EI-MS *m/z*: 400

$(M)^+$. Anal Calcd for $C_{17}H_{12}ClF_3N_2O_2S$: C, 50.94; H, 3.02; N, 6.99. Found: C, 50.99; H, 3.03; N, 7.00.

N-(3-methoxyphenyl)-2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_29): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), 3-methoxyaniline (0.051 g, 0.47 mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_29** (0.093 g, 68 % yield) as white solid. M.P - 228 – 230 ^oC.^1H NMR (DMSO-d_6): δ_H 10.59 (s, 1H), 10.07 (s, 1H), 8.04 (s, 1H), 7.82 (s, 1H), 7.34 – 7.12 (m, 3H), 6.89 – 6.65 (m, 2H), 3.94 (dd, *J* = 16.5, 3.8 Hz, 1H), 3.75 (s, 3H), 2.94 (dd, *J* = 21.2, 10.6 Hz, 1H), 2.53 (dd, *J* = 23 Hz, 6.9 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.6, 158.2, 141.5, 140.2, 130.2, 128.6, 128.2, 125.6, 122.4, 121.1, 118.1, 116.8, 110.8, 109.8, 109.8, 56.6, 40.2, 32.8. EI-MS *m/z*: 397 $(M+H)^+$. Anal Calcd for $C_{18}H_{15}F_3N_2O_3S$: C, 54.54; H, 3.81; N, 7.07. Found: C, 54.60; H, 3.80; N, 7.08.

N-(2,3-dichlorophenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_30): The compound was prepared according to the general procedure using **BT_02a** (0.1 g, 0.44 mmol), 2,3-dichloroaniline (0.085 g, 0.53 mmol), triethyl amine (0.18 ml, 1.34 mmol), T_3P (0.5 ml, 0.89 mmol) to afford **BT_30** (0.11 g, 67 % yield) as white solid. M.P - 221 – 223 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.68 (s, 1H), 10.12 (s, 1H), 7.96 –7.34 (m, 3H), 7.29 – 6.94 (m, 4H), 3.91 (dd, *J* = 16, 3.2 Hz, 1H), 2.91 (dd, *J* = 22.2, 10.3Hz, 1H), 2.51 (dd, *J* = 24.2, 8.4 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.5, 165.4, 140.2, 135.4, 131.5, 130.8, 129.5 (2C), 128.9, 128.2, 125.4, 122.8, 119.2, 117.2, 40.3, 32.6. EI-MS *m/z*: 367 $(M)^+$. Anal Calcd for $C_{16}H_{12}Cl_2N_2O_2S$: C, 52.33; H, 3.29; N, 7.63. Found: C, 52.28; H, 3.30; N, 7.65.

2-(6-chloro-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)-N-(2,3-dichlorophenyl)acetamide (BT_31): The compound was prepared according to the general procedure using **BT_02b** (0.1 g, 0.39 mmol), 2,3-dichloroaniline (0.076 g, 0.47 mmol), triethyl amine (0.16 ml, 1.16 mmol), T_3P (0.5 ml, 0.77 mmol) to afford **BT_31** (0.099 g, 63 % yield) as brown gum.^1H NMR (DMSO-d_6): δ_H 10.71 (s, 1H), 10.15 (s, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.82 (s, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8 Hz, 1H), 7.21 (t, *J* = 8 Hz, 1H) 7.04 (d, *J* = 7.8 Hz, 1H), 3.88 (dd, *J* = 17.8, 4 Hz, 1H), 2.84 (dd, *J* = 22.2, 10.8 Hz, 1H), 2.51 (dd, *J* = 24.5, 7.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.8, 165.6, 142.1, 140.1, 131.8, 130.4, 130.0, 129.6, 129.2

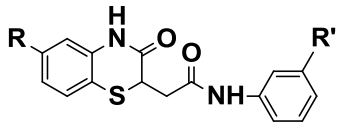
(2C), 128.5, 128.2, 122.5, 117.1, 40.9, 32.6. EI-MS *m/z*: 401 $(M)^+$. Anal Calcd for $C_{16}H_{11}Cl_3N_2O_2S$: C, 47.84; H, 2.76; N, 6.97. Found: C, 47.90; H, 2.77; N, 6.98.

N-(2,3-dichlorophenyl)-2-(3-oxo-6-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide (BT_32): The compound was prepared according to the general procedure using **BT_02c** (0.1 g, 0.34 mmol), ethyl 3-aminobenzoate (0.066 g, 0.41 mmol), triethyl amine (0.14 ml, 1.03 mmol), T_3P (0.5 ml, 0.68 mmol) to afford **BT_32** (0.107 g, 71 % yield) as pale yellow solid. M.P - 224 – 226 ^oC. ^1H NMR (DMSO-d_6): δ_H 10.64 (s, 1H), 10.11 (s, 1H), 8.06 (s, 1H), 7.91 – 7.34 (m, 3H), 7.20 – 6.87 (m, 2H), 3.81 (dd, *J* = 16.8, 4 Hz, 1H), 2.86 (dd, *J* = 23.2, 10.8 Hz, 1H), 2.46 (dd, *J* = 25.5, 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_c 172.4, 165.6, 141.8, 137.5, 131.8, 130.8, 130.1, 129.8, 129.2, 128.6, 128.3, 125.6, 122.4, 121.1, 117.9, 40.6, 32.4. EI-MS *m/z*: 435 $(M)^+$. Anal Calcd for $C_{17}H_{11}Cl_2F_3N_2O_2S$: C, 46.91; H, 2.55; N, 6.44. Found: C, 46.97; H, 2.54; N, 6.43.

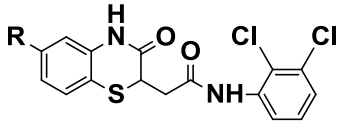
5.5.4. *In vitro Msm* Gyr B assay, supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were evaluated for their *in vitro Msm* Gyr B assay and supercoiling assay for the derivation of SAR and lead optimization. The compounds were further subjected to a whole cell screening against *Mtb* $H_{37}Rv$ strain to understand their bactericidal potency using the agar dilution method and later the safety profile of these molecules were evaluated by checking the *in vitro* cytotoxicity against RAW 264.7 cell line (mouse macrophage) at 50 μM concentration by MTT assay, and the results are shown in **Table 5.10**.

Table 5.10: *In vitro* biological evaluation of the synthesized compounds **BT_03** – **BT_32**



BT_ 03 - BT_29



BT_30 - BT_32

COMP	R	R'	Mtb Supercoiling assay (% of Inhibition at 25 µM)	Mtb MIC (µM)	RAW264.7 Cytotoxicity at 50 µM (% inhib.)
BT_03	H	-COOEt	10	4.21	37.32
BT_04	H	$-COCH_3$	<5	73.44	33.01
BT_05	H	-OH	15	79.53	23.13
BT_06	H	H	20	20.95	21.63
BT_07	H	Br	10	16.57	22.58
BT_08	H	F	30	9.88	37.24
BT_19	H	-COOH	<5	18.26	43.20
BT_10	H	Cl	<5	37.56	34.41
BT_11	H	$-OCH_3$	25	76.13	29.85
BT_12	Cl	-COOEt	20	3.85	23.14
BT_13	Cl	$-COCH_3$	40	4.16	19.74
BT_14	Cl	-OH	50	2.24	23.47
BT_15	Cl	H	50	4.69	25.34
BT_**16**	Cl	Br	5	60.72	14.05
BT_17	Cl	F	25	8.91	22.65
BT_18	Cl	-COOH	50	4.14	32.04
BT_19	Cl	Cl	10	68.07	49.68
BT_20	Cl	$-OCH_3$	15	68.90	34.32
BT_21	CF_3	-COOEt	20	28.51	30.70
BT_22	CF_3	$-COCH_3$	5	61.22	44.96
BT_23	CF_3	-OH	15	8.17	32.33
BT_24	CF_3	H	10	68.24	23.13
BT_25	CF_3	Br	30	3.50	23.55
BT_26	CF_3	F	<5	65.04	29.99
BT_27	CF_3	-COOH	20	15.23	22.78
BT_28	CF_3	Cl	25	62.38	38.75

BT_29	CF_3	$-OCH_3$	35	3.94	20.72
BT_30	H	-	60	2.12	19.68
BT_31	Cl	-	10	31.12	31.44
BT_32	CF_3	-	5	14.36	39.96
Moxifloxacin			11.2±0.23	2.4	NT
Novobi0cin			46±28nM	>200	19.3
Ethambutol			NT	9.84	NT

IC_{50}, 50% inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; NT, not tested; nM, nanomolar

Mtb DNA gyrase supercoiling enzyme inhibition activity

In vitro activity against *Mtb* $H_{37}Rv$

Cytotoxicity against RAW 264.7 cells (mouse macrophage cell line)

5.5.5. Nutrient Starvation Model

The most active anti-mycobacterial compound **BT_30** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e., Nutrient starvation model. **BT_30** not showed significant reductions in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold).

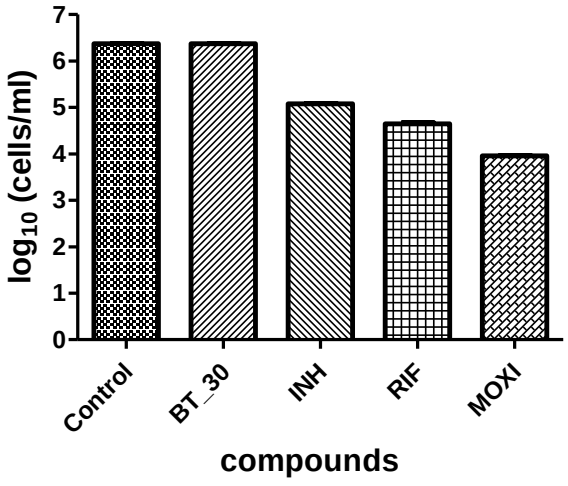


Fig 5.24: Nutrient starvation model graph of **BT_30**

Bacterial count estimation (Mean ± S.D., n = 3) for control and treated groups conducted by using the MPN (most probable number) assay. The dormant cell suspension was treated with the compounds at a concentration of 10 µM. most of the compounds gave significant inhibition of growth of *M. tuberculosis* in this model as compared to the control ($p < 0.0001$, two way ANOVA using GraphPad Prism Software).

5.5.6. Anti-mycobacterial screening for most active compound using adult zebrafish (Zebrafish Model)

To evaluate anti-mycobacterial potency of compound **BT_30** *In vivo,* it was also tested in *Mycobacterium marinum* induced adult zebra fish model. The compound was administered orally at a dose of 10 mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. **BT_30** exhibited good pharmacokinetic profile and also log reduction of 1.9 fold in bacteria when compared to first line drugs- Isoniazid (2.9 fold), and Moxifloxacin (2.8 fold).

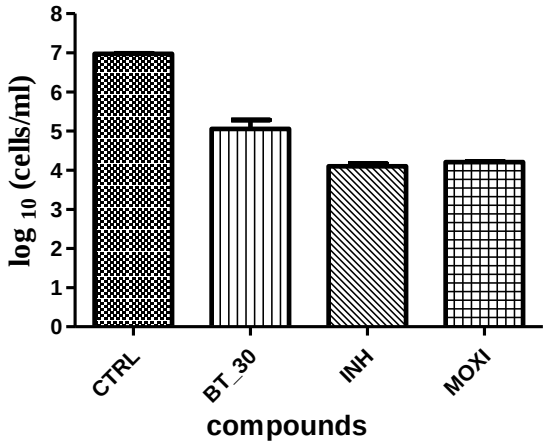


Fig 5.25: Zebra fish model graph of **BT_30**

Bacterial count estimation (Mean ± S.E.M., n = 6) for control and treated groups conducted by using MPN (most probable number) assay. The statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p<0.0001$) with respect to infected control group has been analyzed by Two-way ANOVA using GraphPad Prism Software

5.5.7. SAR and discussion

Based on earlier literature [Makarov M., *et al.,* 2009] we have identified benzothiazine is a potent scaffold for developing novel anti tubercular drug. Manoj chandran *et al.,* reported

benzothiazine as potent inhibitors of DNA gyrase. We have synthesized a library of 30 derivatives based on benzothiazine scaffold.

All the synthesized compounds were screened for their enzyme inhibition studies using MTB DNA gyrase kit (Inspiralis, Norwich). Preliminary screening was performed at 25 µM concentration. Four compounds (**BT_14**, **BT_15**, **BT_18**, and **BT_30**) showed percentage inhibition of 50 – 60%. BT_31 showed better percentage of inhibition of 50% AT 25 µM. Eleven compounds showed percentage inhibition of 20 – 40%. Fifteen compounds are having percentage inhibition less than 20%.

All the synthesized compounds were screened for their in-vitro antimycobacterial activity against *Mtb* H_{37}Rv by microplate alamar blue assay method. Ethambutol (MIC: 15.31 µM), Isoniazid (MIC: 0.66 mM), Moxifloxacin (MIC: 1.2 µM) and Novobiocin (MIC: >200 µM) were considered as standard drugs for comparison in this assay. The MIC values varied from 2.12 – 79.53 µM. Nine compounds inhibited *Mtb* with MIC less than 5 µM. **BT_30** with an MIC of 2.12 µM turned out as the most anti-mycobacterial compound. **BT_14** with an MIC of 2.24 µM becomes second most active anti-mycobacterial compound. The other seven compounds shown MIC values less than 5 µM, are in the order **BT_25** < **BT_12** < **BT_29** < **BT_18** < **BT_13** < **BT_03** < **BT_15**. Three compounds inhibited *Mtb* with an MIC between 5 – 10 µM (**BT_23**, **BT_17** and **BT_08**). Three compounds exhibited MIC values less than 20 µM (**BT_07**, **BT_09**, and **BT_32**). Four compounds (**BT_06, BT_10, BT_21** and **BT_31**) showed MIC less than 40 µM. All the other compounds showed MIC more than 50 µM.

From structure activity relation point of view the most active antimycobacterial compound **BT_30** possess electron donating hydrogen at the 7^{th} position of benzothiazine moiety. Also it possesses a 2,3 dichloro phenyl group attached to the benzothiazine moiety through amide bonding. The second most potent antimycobacterial compound **BT_14** also possesses a electron withdrawing chloro group at the 7 th position of 1,4 benzothiazine group.**BT_14** had a 3 hydroxy phenyl group attached through a amide bond. It has been observed that in case of second set of compounds (**BT_30 – BT_32**) with 2,3 dichloro phenyl group attached to benzothiazine group without any substitution at 7^{th} position shown better activity. But in case of compounds with chloro and CF_3 at 7^{th} position of benzothiazine group bearing 2,3 dichloro phenyl group activity got reduced drastically (**BT_31** and **BT_32**).

In the first set of compounds (**BT_03 – BT_29**) possess H, Cl, CF_3 at 7^{th} position of benzothiazine group and 3 substituted phenyl group attached through amide bond. When the 7^{th} position is substituted with H and CF_3 there is no significant improvement in MIC irrespective of substitution on amide bonded phenyl group (**BT_03** – **BT_11** and **BT_21** – **BT_29**). In the case of compounds with chloro substitution at 7^{th} position of benzothiazine group shown significant MIC. Chloro substituted compounds with electron withdrawing groups at third position of amide bonded phenyl group shows excellent activity compared to electron dontating substituents (**BT_12**, **BT_13**, and **BT_18**). **BT_14** and **BT_15** are exceptions. Whereas in general electron donating substituents in 3^{rd} position of amide bonded phenyl group reduces the activity.

When compared to Moxifloxacin (MIC of 2.4 µM) two molecules (**BT_30** and **BT_14**) showed better activity. Compared to standard first line anti-TB drug Ethambutol (MIC of 9.84 µM), eleven molecules showed better activity. When compared to isoniazid (0.66 µM), none of the molecules showed better activity.

The most active anti-mycobacterial compound **BT_30** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e., Nutrient starvation model. **BT_30** not showed reduction in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold). These results suggest that compound **BT_30** is only effective against replicative stage of mtb not against persistent stages of bacteria.

To evaluate *In vivo* activity of **BT_30,** it was also tested in *Mycobacterium marinum* induced adult zebra fish model. The compound was administered orally at a dose of 10mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. Compound **BT_30** exhibited good anti-mycobacterial potency and also log reduction of 1.9 fold in bacteria when compared to first line drugs- Isoniazid (2.9 fold),and Moxifloxacin (2.8 fold). It is an indicative that compound **BT_30** is best candidate for further drug development studies.

Subsequently, the eukaryotic cell safety profile of all the forty compounds were observed by testing there *in vitro* cytotoxicity against the RAW 264.7 cell line (Mouse leukemic monocyte macrophage cell line) at 50 µM concentration by (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Since the mycobacteria resides in the macrophages during the infection stages, this cell line was selected. At 50 µM tested compounds showed

cytotoxicity range of 14.05-49.68 % as shown in **Table 5.10.** The most promising anti-TB compound among the synthesized set of compounds was **BT_30** with only 19.68 % cytotoxicity which is within the safety profile limits. Novobiocin was used as standard with 19.36 % inhibition in the above cell line.

5.5.8. Highlights of the study

Compound **BT_30** (N-(2,3-dichlorophenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide) was found to be the most active antimycobacterial compound with an MIC of 2.12 µM. BT_30 showed 60% inhibition of DNA Gyrase enzyme via supercoiling assay. BT**_30** was also tested in *Mycobacterium marinum* induced adult zebra fish model. Compound **BT_30** exhibited good anti-mycobacterial potency and also log reduction of 1.9 fold in bacteria when compared to first line drugs- Isoniazid (2.9 fold),and Moxifloxacin (2.8 fold). It is an indicative that compound **BT_30** is best candidate for further drug development studies.

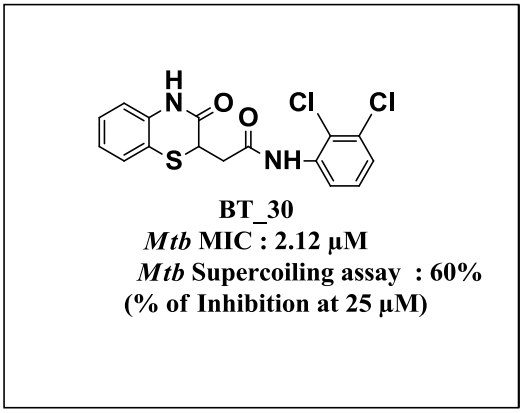


Fig 5.26: Chemical structure and biological activity of the most active compounds **BT_30**

5.6. Design, synthesis and biological evaluation of N-(4-sub-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-sub-sulfonamide derivatives as novel DNA Gyrase inhibitors

5.6.1. Design of the molecules

Foss M H in 2011 reported E. coli DNA Gyrase inhibitor with an IC_{50} of 0.7-3.33 µM [Foss, M. H., *et a*l., 2011]. We re designed the reported molecule with central bicyclic pyrrolidine ring to Bicyclic central hexahydro-2H-furo[3,2-b]pyrrolyl core ring as possible bacterial DNA gyrase inhibitors. Also we made modifications on R' position with aromatic, aliphatic and cyclic groups.We have designed a libray of 30 derivatives via different substitutions at R and R' positions.

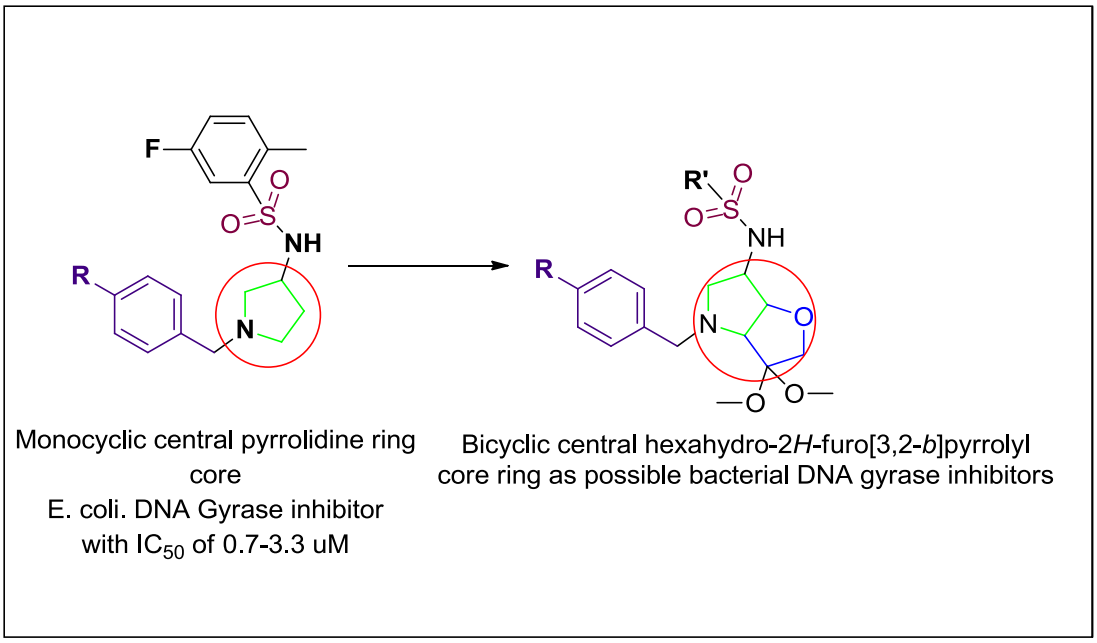


Fig 5.27: Design strategy employed in synthesis of final compounds

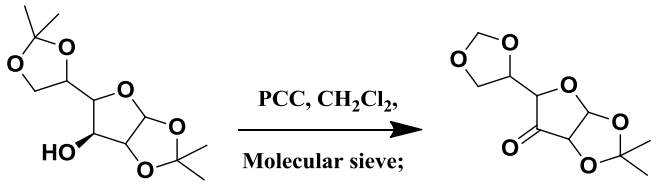
5.6.2. Experimental procedures utilized for the synthesis of BB_01-BB_50

Synthetic protocol employed was shown in Fig 4.6. Synthesis started with oxidation of diacetone glucose **BB_01** with PCC in dichloromethane in the presence of molecular sieves gave the ketone **BB_02.** Resulted ketone on treatment with acetic anhydride in pyridine afforded the enol acetate **BB_03.** Hydrogenation of the enol acetate **BB_03** in the presence of

palladium black in ethyl acetate, followed by ester exchange, gave the *gulo*-alcohol 4, epimeric with **BB_01** at both C-3 and C-4 [Watterson, M. P., et al., 1999, Legler, G., *et al*., 1986]. Triflation of the free hydroxyl group in **BB_04** resulted in the formation of **BB_05**. Displacement of easy leaving group pseudo halide triflate leads to azide **BB_06** in good yield. **BB_06** one treatment with a mild acid, such as diluted acetic acid or similar, which can selectively hydrolyze the 5,6-acetal of compound **BB_06**, to obtain a diol **BB_07**. The primary alcohol present in **BB_07** can be selectively reacted with an alkyl- or aryl sulfonyl chloride like p- toluenesulfonyl chloride to give compound **BB_08**. The azide group of derivative **BB_08** is reduced for example by catalytic hydrogenation using palladium on charcoal in a suitable solvent such as an alcohol, like ethanol or methanol into the free amine **BB_09**. The obtained nucleophilic nitrogen reacts spontaneously, or optionally in the presence of a suitable base like such as triethyl amine or sodium acetate, with the C-6 position forming a 5,5-bicycle **BB_10.** Thus obtained **BB_10** with pyrrole -NH was N-protected with a suitable protecting group such as benzyl chloroformate (Cbz) to give compound **BB_11**. Thus obtained **BB_11** was then protected with a suitable acid stable protecting group such as substituted in particular benzyl ether. This can be achieved by treatment of the mono hydroxyl group of **BB_11** with a base such as sodium hydride or sodium hydroxide in an aprotic solvent N,N- dimethylformamide (DMF) in the presence of the desired alkylating agent such as the benzyl halide, in particular benzyl bromide to obtain **BB_12**. The obtained **BB_12** bearing Di-oxo group can then be reduced to hydroxyl group according to methods described by G. J. Ewing *et al.*, to obtain **BB_13 [**Ewing, G. J., *et al*., 1999]. Preferably the reduction is performed with excess boron trifluoride etherate in the presence of a reducing agent such as trialkylsilane, in particular with excess triethylsilane in a suitable non-protic solvent such as dichloromethane. Treatment of **BB_13** with Dess-Martin Periodinane resulted in oxidation of alcohol to corresponding ketone **BB_14**. Conversion of keto in **BB_14** to dimethoxy was achieved by employing treatment of **BB_14** with acetyl chloride resulted in **BB_15**. Hydrogenation of compound **BB_15** using palladium-on-charcoal in a suitable solvent or solvent mixture such as ethyl acetate-ethanol in a hydrogen atmosphere, in the presence of di-tert-butyl dicarbonate and pyridine gives intermediate **BB_16 [**Nilsson M., *et al*., 2013]. By repeated catalytic hydrogenation, as described above, the mono-ol **BB_16** was obtained. **BB_16** on treatment with methane sulphonyl chloride in presence of triethyl amine resulted in the formation of mesylated product

BB_17. In next stage boc anhydride was cleaved using trifluoro acetic acid to get **BB_18**. Reductive amination of **BB_18** using substituted aldehydes and sodium cyano borohydride leads to the formation of N alkylated product (**BB_19a – BB_19C)** [Dangerfield, E. M., *et al*., 2010]. In the next step the nucleophilic displacement of easy leaving mesyl group take place in presence of excess ammonia under microwave condition to give free amine (**BB_20a – BB_20C)**. So obtained amine served as the basic building blocks for synthesizing a library of 30 derivatives. The scaffold was treated with various substituted sulphonyl chlorides using organic base triethyl amine to synthesize final compounds **BB_21 – BB_50** as sulphonamides [Marshall, D. R., et al., 2007].

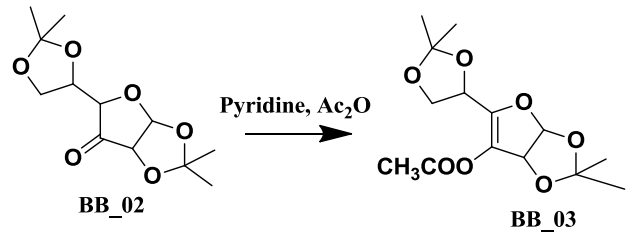
Preparation of 5-(1,3-dioxolan-4-yl)-2,2-dimethyldihydrofuro[2,3-d][1,3]dioxol-6(3aH)-one (BB_02)



BB_01 BB_02

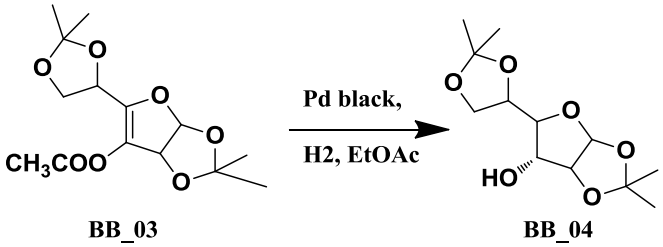
A solution of **BB_01** (3 g, 9.9mmol), pyridinium dichromate (2.3 g, 5.9 mmol), and acetic anhydride (3 mL) in dichloromethane (30 mL) was heated under reflux for 1 h, and then concentrated to about half its volume. The chromium salts were precipitated by the addition of 2 vol. of ethyl acetate and removed, and the dark filtrate was passed through a column (4 x 20 cm) of silica gel equilibrated and eluted with ethyl acetate. The eluate was concentrated, and toluene was evaporated from the residue to remove traces of pyridine and acetic acid to obtain **BB_02**, colourless, viscous oil (2.5 g, 94%).^{1}H NMR (DMSO-d_6): δ_H 5.65 (m, 1H), 5.12 (m, 1H), 4.21 (m, 1H), 4.05 (m, 1H), 4.01 – 3.62 (m, 2H), 1.34 (s, 12H). ^{13}C NMR (DMSO-d_6): δ_c 201.2, 120.5, 117.6, 108.2, 79.8, 76.9, 69.5, 65.6, 25.8 (4C). EI-MS *m/z*: 259 $(M+H)^+$. Anal Calcd for $C_{12}H_{18}O_6$: C, 55.81; H, 7.02. Found: C, 55.89; H, 7.01.

Preparation of 5-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-3a,6a-dihydrofuro[2,3-d][1,3]dioxol-6-yl acetate(BB_03)



Acetic anhydride (6.5 mL) was added to a solution of **BB_02** (5 g, 17.4 mmol), in pyridine (50 mL) and the reaction mixture was heated at 60°C for overnight. Reaction mixture was then concentrated to obtain a residue. Ethyl acetate and water were added to the residue. Organic layer was separated, washed with dilute hydrochloric acid, water and brine. The solvent was evaporated under reduced pressure and the residue thus obtained was purified by silica gel column chromatography using 8% ethyl acetate in hexane as eluent to furnish the title compound **BB_03**, yield 2 g. ^{1}H NMR (DMSO-d_6): δ_H 6.02 (m, 1H), 4.96 (m, 1H), 4.34 (m, 1H), 4.12 – 3.72 (m, 2H), 2.18 (s, 3H), 1.34 (s, 12H). ^{13}C NMR (DMSO-d_6): δ_c 169.2, 145.6, 119.8, 118.2, 108.4, 96.2, 89.5, 76.2, 65.6, 25.9 (4C), 20.8. EI-MS *m/z*: 301 $(M+H)^+$. Anal Calcd for $C_{14}H_{20}O_7$: C, 55.99; H, 6.71. Found: C, C, 56.08; H, 6.70.

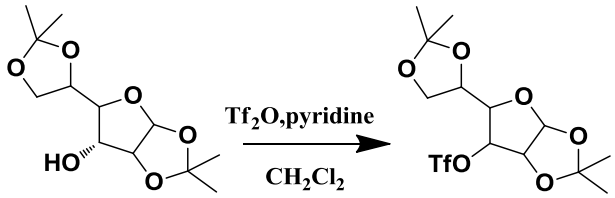
Preparation of (6R)-5-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-ol (BB_04)



To a solution of **BB_03** (3 g, 106 mmol) in methanol (75 mL) was added 20% Pd(OH), on charcoal (-1 g, Aldrich), and the mixture was cooled to -25 °C and rapidly stirred under hydrogen. The temperature was then slowly raised to -15 to -10 °C and stirred for 7h (monitored by TLC and LCMS for completion). The catalyst was removed and the filtrate concentrated to viscous oil (3 g, 91%) which was de acetylated by methanolysis without further purification to

get **BB_04**. ^{1}H NMR (DMSO-d_6): δ_H 5.38 (m, 1H), 4.82 (m, 1H), 4.31 – 4.17 (m, 2H), 4.12 (m, 1H), 4.00 – 3.69 (m, 2H), 3.62 (s, 1H), 1.35 (s, 12H). ^{13}C NMR (DMSO-d_6): δ_c 122.3, 120.5, 110.5, 85.6, 79.8, 76.2, 75.8, 68.2, 25.8 (4C). EI-MS *m/z*: 261 $(M+H)^+$. Anal Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.42; H, 7.75.

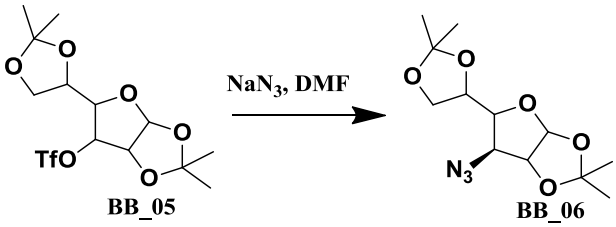
Preparation of 5-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-yl trifluoromethanesulfonate (**BB_05**)



BB_04 **BB_05**

To a solution of **BB_04** (1.03 g, 3.96 mmol), in 19: 1 CH_2Cl_2-pyridine (40 mL) at 0 °C was added dropwise triflic anhydride (2.83 mL, 16.8 mmol) in CH_2Cl_2 (2 mL). After stirring for 15 min, TLC showed that the starting material was replaced by a new spot. The mixture was then extracted with ice-cold 5% HCl and water, dried with sodium sulphate and evaporated to an orange liquid **BB_05**. This was taken for further step without purification.

Preparation of 6-azido-5-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxole (**BB_06**)

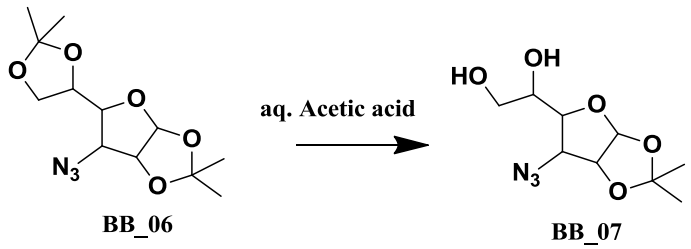


BB_05 **BB_06**

BB_05 was directly dissolved in dry DMF (100 mL) and cooled to 0 °C and sodium azide (1.29 g, 19.8 mmol) was added. After stirring for 2 h and warming to room temperature the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine. The residue left after evaporation was chromatographed (6: 1 hexane: EtOAc) to give **BB_06** (985 mg, 87%) as a colorless oil. ^{1}H NMR (DMSO-d_6): δ_H 5.34 (m, 1H), 4.27 – 4.19 (m, 2H), 4.14 (m, 1H), 4.02 – 3.71 (m, 2H), 3.51(t, J = 5.5 Hz, 1H), 1.37 (s, 12H). ^{13}C NMR (DMSO-d_6): δ_c 122.5, 120.5,

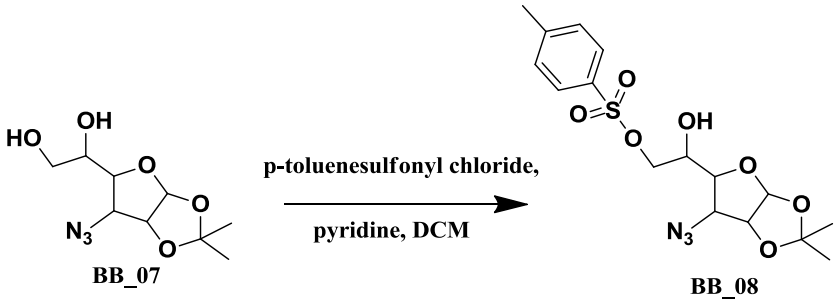
109.8, 84.2, 82.3, 77.8, 65.8, 55.3, 25.8 (4C). EI-MS *m/z*: 261 $(M+H)^+$. Anal Calcd for $C_{12}H_{19}N_3O_5$: C, 50.52; H, 6.71; N, 14.73. Found: C, 50.63; H, 6.70; N, 14.75.

Preparation of 1-(6-azido-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)ethane-1,2-diol (**BB_07**)



A solution of **BB_06** (10 g, 36.3 mmol) in acetic acid-water (7:3, 100 ml) was heated to 50 °C for 2.5 h. The solvent was then removed under vacuum, using toluene to co-evaporate traces of acid, to afford the title compound **BB_07** (8 gm, 93 %), which was used without further purification in the next step. ^{1}H NMR (DMSO-d_6): δ_H 5.41 (d, J = 4.5 Hz, 1H), 4.36 (m, 1H), 3.91 – 3.68 (m, 3H), 3.61 (m, 1H), 3. 55 (s, 1H), 3.50 (t, J = 5.5 Hz, 1H), 3.44 (s, 1H), 1.37 (s, 6H). ^{13}C NMR (DMSO-d_6): δ_c 122.8, 109.5, 82.7, 81.5, 70.6, 65.6, 52.3, 25.8 (2C). EI-MS *m/z*: 246 $(M+H)^+$. Anal Calcd for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.13. Found: C, 44.15; H, 6.18; N, 17.11.

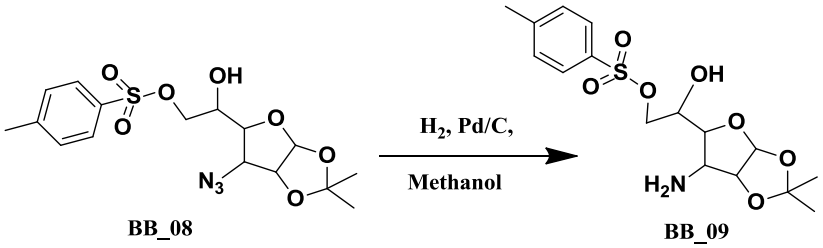
Preparation of 2-(6-azido-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2-hydroxyethyl 4-methylbenzenesulfonate (**BB_07**)



Compound **BB_07** (10.35 g, 36.3 mmol) was dissolved in dichloromethane (100 ml) and treated with pyridine (8.8 ml, 108.9 mmol) followed by tosyl chloride (6.92 g, 36.3 mmol). The reaction mixture was stirred at 48 °C for 4 h (monitored by TLC and LCMS for completion). The mixture was then washed with 1M HCl and saturated $NaHCO_3$, dried and concentrated. The

residue was purified by column chromatography to yield the title compound **BB_08** (13.5 g, 93.2 %).[1]H NMR (DMSO-d6): δ_H 7.83 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* =7.8 Hz, 2H), 5.52 (d, *J* = 4.6 Hz, 1H), 4.29 – 4.01 (m, 3H), 3.94 – 3.71 (m, 2H), 3.53 (t, *J* = 5.5 Hz, 1H), 3.46 (s, 1H), 2.38 (s, 3H), 1.37 (s, 6H). ^{13}C NMR (DMSO-d6): δ_c 145.6, 139.2, 129.8 (2c), 128.5 (2C), 122.5, 109.2, 84.9, 84.2, 72.3, 68.2, 52.5, 25.8 (2c), 22.4. EI-MS m/z: 400 $(M+H)^+$. Anal Calcd for $C_{16}H_{21}N_3O_7S$: C, 48.11; H, 5.30; N, 10.52. Found: C, 48.19; H, 5.29; N, 10.53.

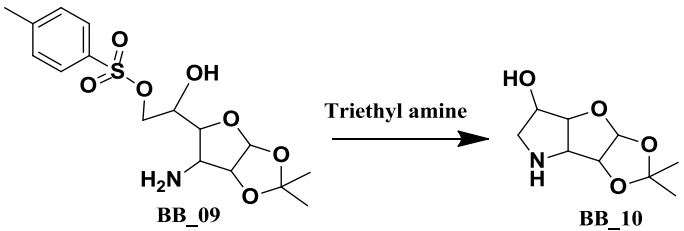
Preparation of 2-(6-amino-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2-hydroxyethyl 4-methylbenzenesulfonate (**BB_09**)



BB_08 BB_09

BB_08 (10 g, 25 mmol) was dissolved in methanol (100 ml) . Pd/C was added portion wise to the reaction mixture and stirred at room temperature under pressure for 6 h. After completion of reaction (monitored by TLC and LCMS), reaction mixture was passed through celite bed to remove Pd/C. The filtrate was concentrate to get the product **BB_09** (8.5 g, 86 %). [1]H NMR (DMSO-d6): δ_H 7.81 (d, *J* = 7.6 Hz, 2H), 7.65 (d, *J* =7.8 Hz, 2H), 5.57 (d, *J* = 4.6 Hz, 1H), 5.25 (b, 2H),4.62 (t, *J* = 5.2 Hz, 1H), 4.34 – 3.91 (m, 3H), 3.71 (m, 1H), 3.64 (t, *J* = 5.5 Hz, 1H), 3.41 (s, 1H), 2.38 (s, 3H), 1.37 (s, 6H). ^{13}C NMR (DMSO-d6): δ_c 145.6, 139.2, 129.5 (2C), 128.2 (2C), 122.5, 109.5, 88.2, 84.5, 72.6, 68.2, 60.2, 25.8 (2C), 22.5. EI-MS m/z: 374 $(M+H)^+$. Anal Calcd for $C_{16}H_{23}NO_7S$: C, 51.46; H, 6.21; N, 3.75. Found: C, 51.52; H, 6.22; N, 3.74.

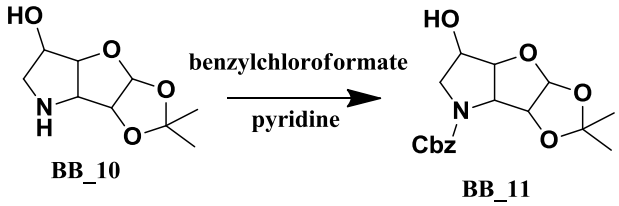
Preparation of 2,2-dimethylhexahydro-3aH-[1,3]dioxolo[4',5':4,5]furo[3,2-b]pyrrol-5-ol (**BB_10**)



BB_09 BB_10

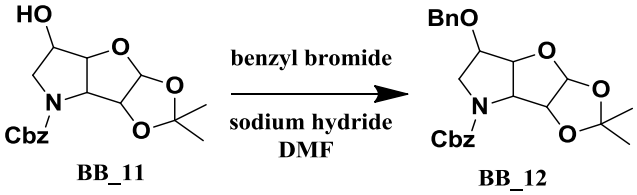
Triethyl amine (0.58 ml, 4.17 mmol) was added to a solution of compound **BB_09** (1.04 g, 2.78 mmol) dissolved in MeOH (50 mL) and H_2O (5 mL). The reaction was stirred at room temperature overnight. LC-MS showed presence of starting material. The reaction mixture was heated to 50 ° C. After 4 h LC-MS showed reaction completion. The solvent was evaporated and the crude product purified by flash chromatography (heptane:ethyl acetate (3:2)) to give desired product **BB_10** (0.49 g, 54 %). ^{1}H NMR (DMSO-d6): δ_H 5.48 (d, *J* = 4.6 Hz, 1H), 4.59 (t, *J* = 5.2 Hz, 1H), 3.98 (t, *J* = 5.4 Hz, 1H), 3.71 (m, 1H), 3.46 (t, *J* = 4.6 Hz, 1H), 3.38 (s, 1H), 3.11 – 2.81 (m, 2H), 1.37 (s, 6H). ^{13}C NMR (DMSO-d6): δ*c* 122.5, 109.5, 94.5, 86.2, 77.8, 60.2, 54.2, 25.8 (2C). EI-MS m/z: 202$(M+H)^+$. Anal Calcd for $C_9H_{15}NO_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.81; H, 7.52; N, 6.97.

Preparation of benzyl 5-hydroxy-2,2-dimethyltetrahydro-3a*H*-[1,3]dioxolo[4',5':4,5]furo[3,2-b]pyrrole-7(4aH)-carboxylate (**BB_11**)



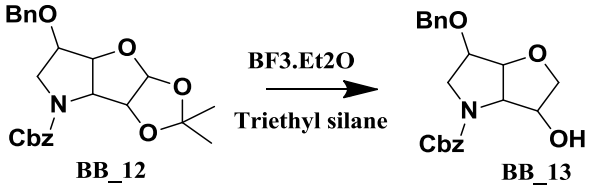
The compound **BB_10** (2 gm, 49 mmol) was dissolved in dry dichloromethane. Then the reaction mixture was cooled to 0 °C. Pyridine (12 ml, 149 mmol) was added to the reaction mixture at cooled condition and allowed the reaction mixture to stir for 10 mt. Benzylchloroformate (10.1 ml, 74 mmol) was added dropwise to the reaction mixture at 0 °C and allowed the reaction mixture to come to rt and stand for 8 hr. After completion of reaction (monitored by TLC and LCMS), reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layer was washed with sodium bicarbonate and dried over dry magnesium sulphate followed by concentration under reduced pressure to get crude, which inturn purified by column chromatography to get desired product **BB_11** (1.2 g, 68 %). ^{1}H NMR (DMSO-d6): δ_H 7.62 – 7.26 (m, 5H), 5.52 (d, *J* = 4.6 Hz, 1H), 5.13 (s, 2H), 5.01 – 4.76 (m, 2H), 4.53 (t, *J* = 5.2 Hz, 1H), 3.75 – 3.56 (m, 3H), 3.39 (s, 1H), 1.37 (s, 6H). ^{13}C NMR (DMSO-d6): δ_c 158.2, 137.5, 130.1 (2C), 128.2, 127.9 (2C), 122.5, 109.5, 91.5, 84.2, 81.2, 74.5, 68.2, 54.5, 25.9 (2C). EI-MS m/z: 336 $(M+H)^+$. Anal Calcd for $C_{17}H_{21}NO_6$: C, 60.89; H, 6.31; N, 4.18. Found: C, 60.78; H, 6.30; N, 4.19.

Preparation of benzyl 5-(benzyloxy)-2,2-dimethyltetrahydro-3a*H*-[1,3]dioxolo[4',5':4,5]furo[3,2-b]pyrrole-7(4aH)-carboxylate (**BB_12**)



To a stirred suspension of sodium hydride (60% in mineral oil, Aldrich, 0.34 g, 8.4 mmol) and compound **BB_11** (2.17 g, 6.47 mmol) in dimethylformamide (30 ml) was added benzyl bromide (0.81 mmol, 6.8 mmol) during 5 minutes. After stirring 1 h (TLC: ethyl acetate in petroleum ether 2:3), methanol (approx 2 ml) was added to destroy excess reagent, then immediately partitioned between ethyl acetate (180 ml) and water (150 ml). The organic layer was washed with water (3 x 100 ml), then dried (sodium sulfate), filtered and concentrated onto silica. Flash chromatography of the residue using ethyl acetate in petroleum ether gave a colorless syrup **BB_12** (2.7 g, 98 %). ^{1}H NMR (DMSO-d6): δ_H 7.52 – 7.27 (m, 10H), 5.46 (d, *J* = 4.6 Hz, 1H), 5.12 (s, 2H), 4.93 – 4.71 (m, 3H), 4.72 (s, 2H), 3.72 – 3.41 (m, 2H), 3.35 (m, 1H), 1.36 (s, 6H). ^{13}C NMR (DMSO-d6): δ_c 158.4, 136.9, 135.8, 129.2 (2C), 128.5 (2C), 127.8, 127.6, 127.2 (2C), 126.8 (2C), 122.5, 109.5, 89.6, 84.1, 82.3, 76.2, 73.8, 68.2, 52.5, 25.9 (2C). EI-MS m/z: 426 $(M+H)^+$. Anal Calcd for $C_{24}H_{27}NO_6$: C, 67.75; H, 6.41; N, 3.29. Found: C, 67.69; H, 6.40; N, 3.30.

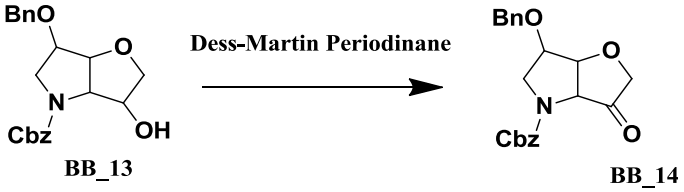
Preparation of benzyl 6-(benzyloxy)-3-hydroxytetrahydro-2H-furo[3,2-b]pyrrole-4(5H)-carboxylate (**BB_13**)



Triethyl silane (18 ml, 116 mmol) was added to compound **BB_12** (5.70 g, 11.6 mmol) dissolved in dry DCM (40 mL).The round bottomed flask was placed under an inert atmosphere (N_2) in an ice bath, and allowed to cool, before slow addition of $BF_3.Et_2O$ (14.5 ml, 116 mmol). The reaction proceeded slowly and was stirred for 3 days. After this time, starting material was

still present. Slow addition of $NaHCO_3$ (70 mL) was followed by portion wise addition of solid NaHCO3 until the gas evolution ceased. The aqueous phase was extracted with DCM (150 mL) and washed with$NaHCO_3$ (70 mL) and subsequently NH_4Cl (70 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (Hexane:Ethyl acetate (2: 1) to give desired product **BB_13** (2 gm, 46%). ^{1}H NMR (DMSO-d6): δ_H 7.53 – 7.31 (m, 10H), 5.10 (s, 2H), 4.72 – 4.65 (m, 2H), 4.42(s, 2H), 4.15 – 3.82 (m, 3H), 3.73 (s, 1H), 3.59 – 3.28 (m, 3H). ^{13}C NMR (DMSO-d6): δ_c 158.6, 139.2, 137.8, 130.1 (2C), 129.5 (2C), 128.5 (2C), 127.6 (2C), 126.2 (2C), 95.6, 90.2, 74.3, 73.9, 73.2, 69.8, 68.2, 52.3. EI-MS m/z: 370 $(M+H)^+$. Anal Calcd for $C_{21}H_{23}NO_5$: C, 68.28; H, 6.28; N, 3.79. Found: C, 68.38; H, 6.29; N, 3.78.

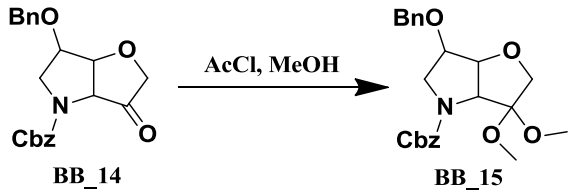
Preparation of benzyl 6-(benzyloxy)-3-oxotetrahydro-2H-furo[3,2-b]pyrrole-4(5H)-carboxylate (BB_14)



BB_13 BB_14

Dess-Martin periodinane (0.60 g, 1.48 mmol) was added to a solution of **BB_13** (0.5 g, 1.35 mmol) dissolved in dry DCM. The reaction was stirred under N_2 for 2 hrs when the reaction was deemed to have reached completed by TLC. The solution was washed 3 times (3*20 mL) with a 1:1 mixture of 10% $Na_2S_2O_3$ and $NaHCO_3$.The organic phase was dried with Na2SO4, filtered and concentrated in vacuo The crude product was purified by flash chromatography (Hexane:Ethyl acetate (3:1) to give desired product **BB_14** (0.32 g, 64 %).^{1}H NMR (DMSO-d6): δ_H 7.52 – 7.30 (m, 10H), 5.11 (s, 2H), 4.62 – 4.49 (m, 3H), 4.41 (s, 2H), 3.72 – 3.29 (m, 4H). ^{13}C NMR (DMSO-d6): δ_c 208.2, 155.6, 138.9, 136.9, 130.2 (2C), 129.6 (2C), 128.8 (2C), 127.7 (2C), 127.4 (2C), 78.2, 75.9, 73.5, 70.2, 68.2, 67.5, 51.2. EI-MS m/z: 368 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.59; H, 5.77; N, 3.80.

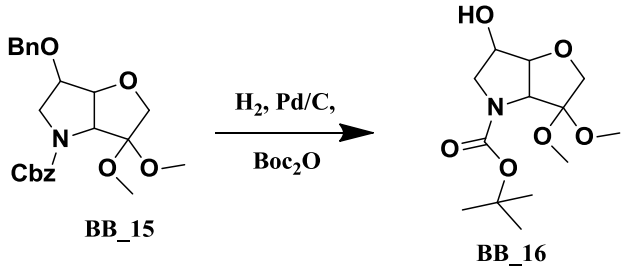
Preparation of benzyl 6-(benzyloxy)-3,3-dimethoxytetrahydro-2H-furo[3,2-b]pyrrole-4(5H)-carboxylate (**BB_15**)



BB_14 **BB_15**

A pre-mixed solution of AcCl (0.058 ml, 0.816 mmol) and MeOH (5 mL) was added to a solution of compound **BB_14** (0.5 g, 1.36 mmol). After stirring for 2 hrs, additional AcCl (0.13 mL, 1.90 mmol) was added and again after stirring for 16 hrs, additional AcCl (1.3 mL, 19 mmol) was added. The reaction was completed shortly thereafter concentrated in vacuo and subsequently any residual solvent was removed by high vacuum to give crude.The crude product was purified by flash chromatography (Hexane:Ethyl acetate (3:1) to give desired product **BB_15** (0.31 g, 55 %). ^{1}H NMR (DMSO-d6): δ_H 7.54 – 7.28 (m, 10H), 5.28 (d, J = 4.8 Hz, 1H), 5.16 (s, 2H), 4.77 (t, 1H, J = 5.2 Hz), 4.61 (s, 2H), 4.02 – 3.83 (m, 2H), 3.79 (s, 6H), 3.72 – 3.21 (m, 3H). ^{13}C NMR (DMSO-d6): δ_c 159.4, 138.2, 137.5, 130.2 (2C), 129.5 (2C), 128.5 (2C), 128.2 (2C), 127.5 (2C), 121.6, 90.2, 76.2, 72.8, 71.5, 70.2, 68.2, 52.3, 50.2 (2C). EI-MS m/z: 414 $(M+H)^+$. Anal Calcd for $C_{23}H_{27}NO_6$: C, 66.81; H, 6.58; N, 3.39. Found: C, 66.95; H, 6.59; N, 3.40.

Preparation of tert-butyl 6-hydroxy-3,3-dimethoxytetrahydro-2H-furo[3,2-b]pyrrole-4(5H)-carboxylate (**BB_16**)

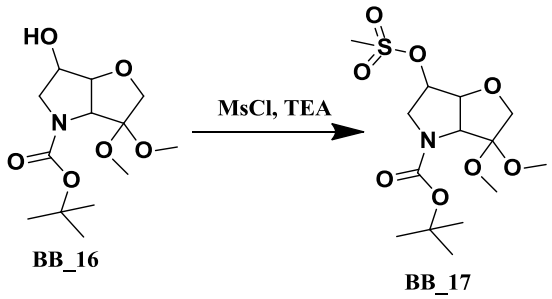


BB_15 **BB_16**

Compound **BB_15** (1 g, 2.4 mmol) was dissolved in methanol (10 ml) . Pd/C was added portion wise to the reaction mixture and stirred at room temperature under pressure for 6 h. After completion of reaction (monitored by TLC and LCMS), reaction mixture was passed through celite bed to remove Pd/C. The filtrate was directly taken to next step for boc protection. Tri ethyl amine (1.0 ml, 7.28 mmol), was added to the filtrate after filtration. To this Boc anhydride (0.63 ml, 2.90 ml) was added dropwise. The reaction was stirred overnight and thereafter the

solvent was removed by concentration in vacuo. The crude product was purified by flash column chromatography (heptane:ethyl acetate) to give the product **BB_16** in good yield (0.52 g, 75 %). ^{1}H NMR (DMSO-d6): δ_H 5.27 (d, *J* = 4.4 Hz, 1H), 4.57 (t, *J* = 5.2 Hz, 1H), 3.98 – 3.85 (m, 2H), 3.76 (s, 6H), 3.72 –3.61 (m, 3H), 3.42 (s, 1H), 1,39 (s, 9H). ^{13}C NMR (DMSO-d6): δ_c 156.2, 121.5, 92.3, 80.2, 74.5, 73.5, 70.2, 54.5, 50.2 (2C), 28.9 (3C). EI-MS m/z: 290 $(M+H)^+$. Anal Calcd for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84. Found: C, 54.06; H, 8.00; N, 4.85.

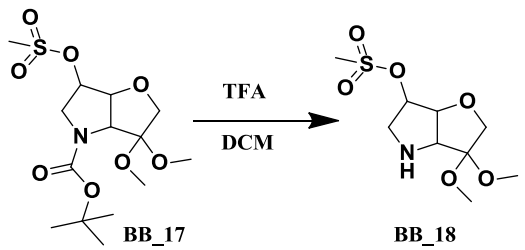
Preparation of tert-butyl 3,3-dimethoxy-6-((methylsulfonyl)oxy)tetrahydro-2H-furo[3,2-b]pyrrole-4(5H)-carboxylate (**BB_17**)



BB_16 **BB_17**

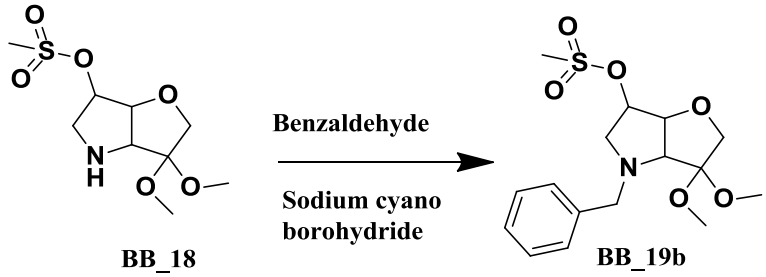
Compound **BB_16** (0.5 g, 1.73 mmol) was dissolved in dry dichloromethane (5 ml) and the solution cooled to 0 ° C. Tri ethyl amine (0.72 ml, 5.19 mmol) was added to the reaction mixture. Mesyl chloride (0.2 ml, 2.59 mmol) was slowly added to the solution drop wise and allowed to warm up to room temperature. The reaction was stirred overnight and after 14 hrs (Reaction completion was monitored by TLC and LCMS). The solution was washed three times with 2 M H_2SO_4 (aq) (3*100 mL) and two times with $NaHCO_3$ sat.(aq) (2*100 mL) and thereafter the organic phase was dried with Na_2SO_4, filtered and concentrated in vacuo to get crude. The crude was purified by flash chromatography using (Hexane ; ethylacetate) as eluent to yield the desired product **BB_17**as white solid (0.48 g, 75 %). ^{1}H NMR (DMSO-d6): δ_H 5.38 (m, 1H), 5.25 (d, *J* = 4.5 Hz, 1H), 4.61 (t, *J* = 5.2 Hz, 1H), 4.01 – 3.89 (m, 2H), 3.81 (s, 6H), 3.71 –3.52 (m, 2H), 3.21 (s, 3H), 1,39 (s, 9H). ^{13}C NMR (DMSO-d6): δ_c 155.2, 121.2, 88.2, 80.2, 73.5, 70.2, 68.5, 51.2, 50.3 (2C), 40.2, 28.9 (3C). EI-MS m/z: 368 $(M+H)^+$. Anal Calcd for $C_{14}H_{25}NO_8S$: C, 45.77; H, 6.86; N, 3.81. Found: C, 45.68; H, 6.87; N, 3.80.

Preparation of 3, 3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl methanesulfonate (**BB_18**)



Compound **BB_17** (1 g, 2.74 mmol) was dissolved in dry dichloromethane (10 ml). The reaction mixture was cooled to 0 °C, followed by the drop wise addition of HCl/Dioxane (5 ml). After the addition reaction mixture was allowed to stand at room temperature for 3 h (reaction completion was monitored by TLC and LCMS). Reaction mixture was washed with sodium carbonate, and dried over sodium sulphate and concentrated to get the desired product **BB_18** as yellow oil (0.65 g, 90 %). ^{1}H NMR (DMSO-d6): δ_H 5.35 (m, 1H), 4.25 (t, *J* = 5.2 Hz, 1H), 4.16 (b, 1H), 4.02 – 3.86 (m, 2H), 3.79 (s, 6H), 3.52 (d, *J* = 4.8 Hz, 1H), 3.21 (s, 3H), 3.16 – 2.81 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 124.6, 91.2, 73.5, 70.2, 68.2, 50.8, 50.2 (2C), 40.1. EI-MS m/z: 268 $(M+H)^+$. Anal Calcd for $C_9H_{17}NO_6S$: C, 40.44; H, 6.41; N, 5.24. Found: C, 40.49; H, 6.40; N, 5.25.

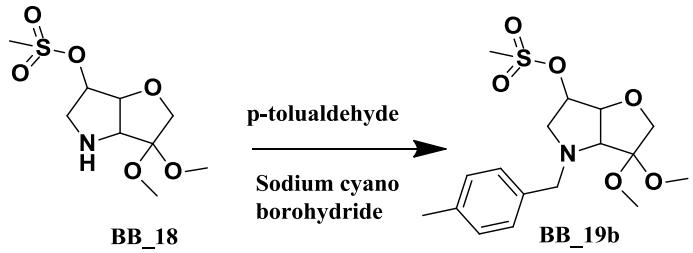
Preparation of 4-benzyl-3, 3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl methanesulfonate (**BB_19a**)



A solution of compound **BB_18** (1 g, 3.74 mmol) in methanol (10 volumes) was stirred under argon atmosphere. To the above solution benzaldehyde (0.45 ml, 4.49 mmol), acetic acid (cat) was added to the reaction mixture and was stirred in room temperature for 12 h followed by the addition of sodium cyanoborohydide (0.35 g, 5.61 mmol) at 0 ^{0}C and allowed the reaction mixture to stand room temperature for 4 h (monitored by TLC & LCMS for completion). The solvent methanol was distilled and the reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (40%): ethyl acetate (60 %) as eluent to afford **BB_19a** (0.82 g, 62 %). ^{1}H NMR (DMSO-d6):

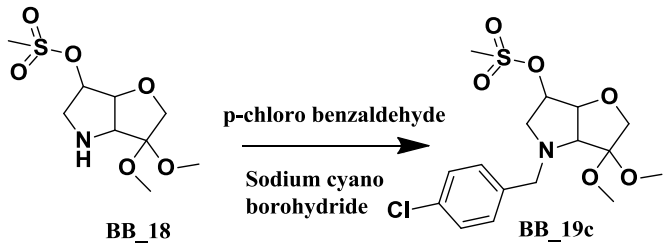
δ_H 7.41 – 7.18 (m, 5H), 5.38 (m, 1H), 4.23 (t, *J* = 5.2 Hz, 1H), 3.99 – 3.85 (m, 2H), 3.81 (s, 2H), 3.75 (s, 6H), 3.21 (s, 3H), 3.15 (d, *J* = 4.6 Hz, 1H), 2.65 – 2.31 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 139.4, 129.5 (2C), 129.1 (2C), 128.1, 122.5, 89.5, 74.5, 73.2, 68.5, 62.5, 59.1, 50.2 (2C), 40.2. EI-MS m/z: 358 $(M+H)^+$. Anal Calcd for $C_{16}H_{23}NO_6S$: C, 53.77.; H, 6.49; N, 3.92. Found: C, 53.69.; H, 6.50; N, 3.91.

Preparation of 3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl methanesulfonate (**BB_19b**)



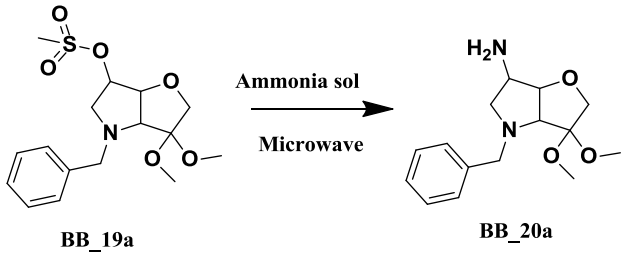
A solution of compound **BB_18** (1 g, 3.74 mmol) in methanol (10 volumes) was stirred under argon atmosphere. To the above solution p-tolualdehyde (0.53 ml, 4.49 mmol), acetic acid (cat) was added to the reaction mixture and was stirred in room temperature for 12 h followed by the addition of sodium cyanoborohydide (0.35 g, 5.61 mmol) at 0 ^{0}C and allowed the reaction mixture to stand room temperature for 4 h (monitored by TLC & LCMS for completion). The solvent methanol was distilled and the reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (40%): ethyl acetate (60 %) as eluent to afford **BB_19b** (0.78 g, 56 %). ^{1}H NMR (DMSO-d6): δ_H 7.18 – 7.09 (m, 4H), 5.36 (m, 1H), 4.25 (t, *J* = 5.2 Hz, 1H), 4.04 – 3.91 (m, 2H), 3.84 (s, 2H), 3.76 (s, 6H), 3.19 (s, 3H), 3.16 (d, *J* = 4.6 Hz, 1H), 2.62 – 2.35 (m, 2H), 2.32 (s, 3H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 136.2, 129.5 (2C), 128.5 (2C), 122.5, 89.5, 74.6, 73.5, 68.2, 62.5, 59.5, 50.2 (2C), 40.3, 22.5. EI-MS m/z: 372 $(M+H)^+$. Anal Calcd for $C_{17}H_{25}NO_6S$: C, 54.97.; H, 6.78; N, 3.77. Found: C, 55.08.; H, 6.79; N, 3.76.

Preparation of 4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl methanesulfonate (**BB_19c**)



A solution of compound **BB_18** (1 g, 3.74 mmol) in methanol (10 volumes) was stirred under argon atmosphere. To the above solution p-chloro benzaldehyde (0.52 g, 4.49 mmol), acetic acid (cat) was added to the reaction mixture and was stirred in room temperature for 12 h followed by the addition of sodium cyanoborohydide (0.35 g, 5.61 mmol) at 0 ^{0}C and allowed the reaction mixture to stand room temperature for 4 h (monitored by TLC & LCMS for completion). The solvent methanol was distilled and the reaction mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). Organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexane (40 %): ethyl acetate (60 %) as eluent to afford **BB_19c** (0.95 g, 65 %). ^{1}H NMR (DMSO-d6): δ_H 7.42 – 7.29 (m, 4H), 5.38 (m, 1H), 4.22 (t, *J* = 5.2 Hz, 1H), 4.02 – 3.90 (m, 2H), 3.82 (s, 2H), 3.77 (s, 6H), 3.22 (s, 3H), 3.14 (d, *J* = 4.6 Hz, 1H), 2.65 – 2.31 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 137.4, 133.5, 132.2 (2C), 129.8 (2C), 122.5, 89.5, 74.6, 73.5, 68.2, 62.5, 59.1, 50.3 (2C), 40.2. EI-MS m/z: 392 $(M+H)^+$. Anal Calcd for $C_{16}H_{22}ClNO_6S$: C, 49.04.; H, 5.66; N, 3.57. Found: C, 49.13.; H, 5.67; N, 3.58.

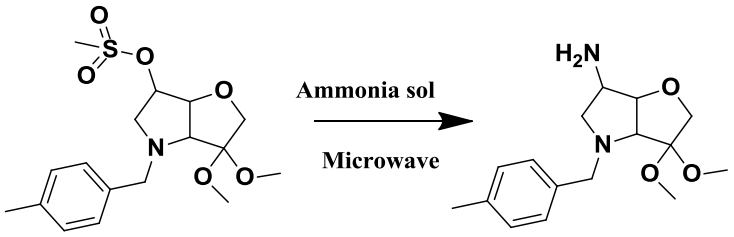
Preparation of 4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-amine (**BB_20a**)



BB_19a (1g, 2.80 mmol) was dissolved in methanol (5 mL) in microwave vial. To the above reaction mixture ammonia solution (2 M ammonia in methanol) (10 volume) was added and sealed the vial. The reaction mixture was irradiated under microwave condition at 100 °C using Biotage microwave initiator for 12 h (Reaction completion was monitored by TLC and LCMS). Reaction mixture was concentrated under vacuo to get the crude, which was purified using flash chromatography with neutral alumina using hexane:ethylacetate as eluent to afford **BB_20a**

(0.55 g, 65 %) as yellow liquid. ^{1}H NMR (DMSO-d6): δ_H 7.36 – 7.18 (m, 5H), 6.52 (b, 2H), 4.28 (t, *J* = 5.2 Hz, 1H), 4.01 – 3.91 (m, 2H), 3.85 (s, 2H), 3.74 (s, 6H), 3.14 (d, *J* = 4.6 Hz, 1H), 3.01 (m, 1H), 2.69 – 2.32 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 139.5, 129.5 (2C), 129.1 (2C), 128.2, 121.5, 82.6, 76.2, 73.5, 62.6, 60.5, 50.2 (2C), 50.1. EI-MS m/z: 279 $(M+H)^+$. Anal Calcd for $C_{15}H_{22}N_2O_3$: C, 64.73.; H, 7.97; N, 10.06. Found: C, 64.79.; H, 7.96; N, 10.05.

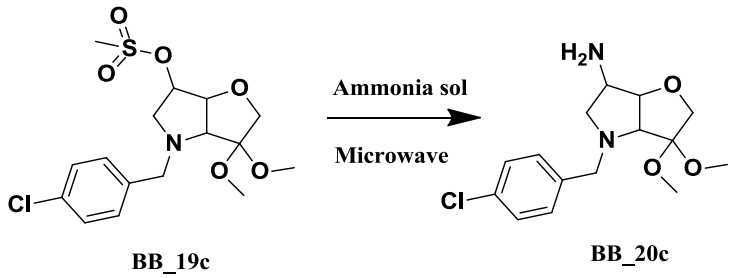
Preparation of 3, 3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-amine (BB_20b)



BB_19b BB_20b

BB_19b (1g, 3.42 mmol) was dissolved in methanol (5 mL) in microwave vial. To the above reaction mixture ammonia solution (2 M ammonia in methanol) (10 volume) was added and sealed the vial. The reaction mixture was irradiated under microwave condition at 100 °C using Biotage microwave initiator for 12h (Reaction completion was monitored by TLC and LCMS). Reaction mixture was concentrated under vacuo to get the crude, which was purified using flash chromatography with neutral alumina using hexane:ethylacetate as eluent to afford **BB_20b** (0.81 g, 63 %) as pale yellow liquid. ^{1}H NMR (DMSO-d6): δ_H 7.21 – 7.16 (m, 4H), 6.65 (b, 2H), 4.26 (t, *J* = 5.4 Hz, 1H), 3.98 – 3.86 (m, 2H), 3.81 (s, 2H), 3.76 (s, 6H), 3.16 (d, *J* = 4.5 Hz, 1H), 3.05 (m, 1H), 2.65 – 2.29 (m, 2H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d6): δ_c 138.2, 136.5, 129.9 (2C), 128.8 (2C), 121.2, 82.6, 76.2, 73.4, 62.5, 60.2, 50.2 (2C), 49.6, 22.5. EI-MS m/z: 293 (M+H)+. Anal Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73.; H, 8.27; N, 9.58. Found: C, 65.80.; H, 8.26; N, 9.57

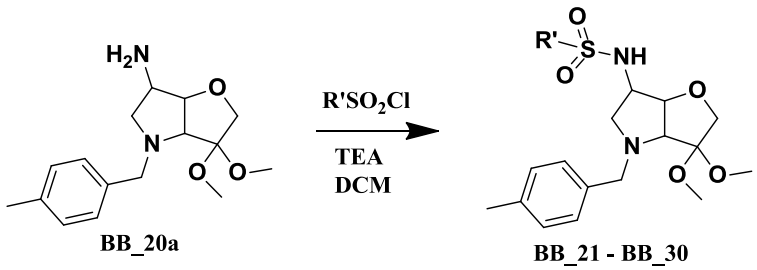
Preparation of 4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-amine (BB_20c)



BB_19c BB_20c

BB_19c (1g, 3.20 mmol) was dissolved in methanol (5 mL) in microwave vial. To the above reaction mixture ammonia solution (2 M ammonia in methanol) (10 volume) was added and sealed the vial. The reaction mixture was irradiated under microwave condition at 100 °C using Biotage microwave initiator for 12 h (Reaction completion was monitored by TLC and LCMS). Reaction mixture was concentrated under vacuo to get the crude, which was purified using flash chromatography with neutral alumina using hexane:ethylacetate as eluent to afford **BB_20c** (0.77 g, 61 %) as pale yellow liquid. ^{1}H NMR (DMSO-d6): δ_H 7.42 – 7.29 (m, 4H), 6.71 (b, 2H), 4.23 (t, *J* = 5.6 Hz, 1H), 4.01 – 3.96 (m, 2H), 3.85 (s, 2H), 3.72 (s, 6H), 3.15 (d, *J* = 4.8 Hz, 1H), 3.02 (m, 1H), 2.58 – 2.34 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 133.5, 132.5 (2C), 129.6 (2C), 121.5, 82.5, 76.2, 73.8, 62.5, 60.2, 50.2 (2C), 49.6. EI-MS m/z: 313 $(M+H)^+$. Anal Calcd for $C_{15}H_{21}ClN_2O_3$: C, 57.60.; H, 6.77; N, 8.96. Found: C, 57.72.; H, 6.78; N, 8.95.

Preparation of final compounds (BB_21 – BB_30)

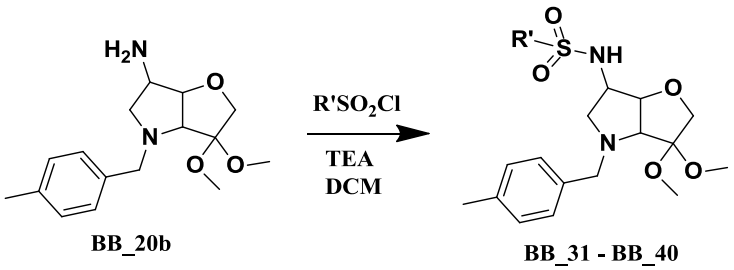


BB_20a BB_21 - BB_30

BB_20a (1 eq) was dissolved in dry dichloromethane and cooled to 0 °C, to the above solution triethylamine (3 eq) was added and stirred at room temperature for 5 mts. To the above mixture substituted sulfonyl chlorides (1.1 eq) are added and allowed to come to room temperature. The reaction mixture was stirred for 6 h (Reaction completion was checked by TLC and LCMS). Reaction mixture was diluted with water and extracted with dichloromethane. Organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo to get the crude product.

The crude was purified by flash chromatography using hexane;ethylacetate as eluent to yield final compounds (**BB_21-BB_30**) in good yield.

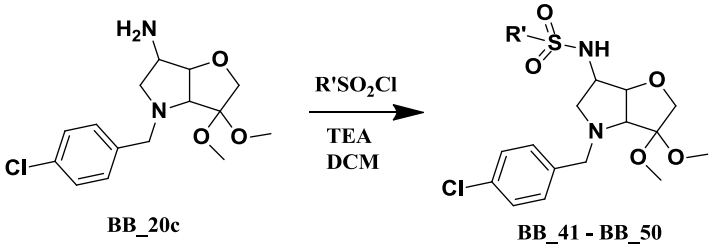
Preparation of final compounds (BB_31 – BB_40)



BB_20b BB_31 - BB_40

BB_20b (1 eq) was dissolved in dry dichloromethane and cooled to 0 °C, to the above solution triethylamine (3 eq) was added and stirred at room temperature for 5 mts. To the above mixture substituted sulfonyl chlorides (1.1 eq) are added and allowed to come to room temperature. The reaction mixture was stirred for 6 h (Reaction completion was checked by TLC and LCMS). Reaction mixture was diluted with water and extracted with dichloromethane. Organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo to get the crude product. The crude was purified by flash chromatography using hexane;ethylacetate as eluent to yield final compounds (**31-40**) in good yield.

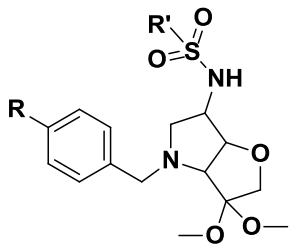
Preparation of (BB_41 – BB_50)



BB_20c BB_41 - BB_50

BB_20c (1 eq) was dissolved in dry dichloromethane and cooled to 0 °C, to the above solution triethylamine (3 eq) was added and stirred at room temperature for 5 mts. To the above mixture substituted sulfonyl chlorides (1.1 eq) are added and allowed to come to room temperature. The reaction mixture was stirred for 6 h (Reaction completion was checked by TLC and LCMS). Reaction mixture was diluted with water and extracted with dichloromethane. Organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo to get the crude product. The crude was purified by flash chromatography using hexane;ethylacetate as eluent to yield final compounds (**41-50**) in good yield.

Table 5.11: Physiochemical properties of the synthesized compounds **BB_21 – BB_50**



BB_21 - BB_50

Comp ID	R	R'	Yield (%)	MP (°C)	Molecular Formula	Molecular Weight
BB_21	H	Phenyl	55	80 – 82	$C_{21}H_{26}N_2O_5S$	418.51
BB_22	H	4 Fluoro Phenyl	56	69 – 71	$C_{21}H_{25}FN_2O_5S$	436.50
BB_23	H	4 Methoxy Phenyl	59	68 – 70	$C_{22}H_{28}N_2O_6S$	448.53
BB_24	H	4 Nitro Phenyl	55	-	$C_{21}H_{25}N_3O_7S$	463.50
BB_25	H	4 Acetyl phenyl	59	74 – 76	$C_{23}H_{28}N_2O_6S$	460.54
BB_26	H	2 Thiophene	57	83 – 85	$C_{19}H_{24}N_2O_5S_2$	424.53
BB_27	H	Cyclohexyl	63	-	$C_{21}H_{32}N_2O_5S$	424.55
BB_28	H	Isopropyl	56	-	$C_{18}H_{28}N_2O_5S$	384.49
BB_29	H	5 Chloro- 2-Thiophene	52	-	$C_{19}H_{23}ClN_2O_5S_2$	458.98
BB_30	H	Benzyl	50	-	$C_{22}H_{28}N_2O_5S$	432.53
BB_31	CH_3	Phenyl	57	125 – 127	$C_{22}H_{28}N_2O_5S$	432.53
BB_32	CH_3	4 Fluoro Phenyl	59	55 – 57	$C_{22}H_{27}FN_2O_5S$	450.52
BB_33	CH_3	4 Methoxy Phenyl	58	109 – 111	$C_{23}H_{30}N_2O_6S$	462.56
BB_34	CH_3	4 Nitro Phenyl	52	106 – 108	$C_{22}H_{27}N_3O_7S$	477.53
BB_35	CH_3	4 Acetyl phenyl	65	57 – 59	$C_{24}H_{30}N_2O_6S$	474.57
BB_36	CH_3	2 Thiophene	59	73 – 75	$C_{20}H_{26}N_2O_5S_2$	438.56
BB_37	CH_3	Cyclohexyl	62	111 – 113	$C_{22}H_{34}N_2O_5S$	438.58
BB_38	CH_3	Isopropyl	59	113 – 115	$C_{19}H_{30}N_2O_5S$	398.52
BB_39	CH_3	5 Chloro-2-Thiophene	65	-	$C_{20}H_{25}ClN_2O_5S_2$	473.01
BB_40	CH_3	Benzyl	55	-	$C_{23}H_{30}N_2O_5S$	446.56
BB_41	Cl	Phenyl	53	92 – 94	$C_{21}H_{25}ClN_2O_5S$	452.95

BB_42	Cl	4 Fluoro Phenyl	59	66 – 68	$C_{21}H_{24}ClFN_2O_5S$	470.94
BB_43	Cl	4 Methoxy Phenyl	51	-	$C_{22}H_{27}ClN_2O_6S$	482.98
BB_44	Cl	4 Nitro Phenyl	68	61 – 63	$C_{21}H_{24}ClN_3O_7S$	497.95
BB_45	Cl	4 Acetyl phenyl	58	-	$C_{23}H_{27}ClN_2O_6S$	494.99
BB_46	Cl	2 Thiophene	64	113 – 115	$C_{19}H_{23}ClN_2O_5S_2$	458.98
BB_47	Cl	Cyclohexyl	55	-	$C_{21}H_{31}ClN_2O_5S$	459.00
BB_48	Cl	Isopropyl	58	61 – 63	$C_{18}H_{27}ClN_2O_5S$	418.94
BB_49	Cl	5 Chloro-2-Thiophene	63	-	$C_{19}H_{22}Cl_2N_2O_5S_2$	493.42
BB_50	Cl	Benzyl	66	-	$C_{22}H_{27}ClN_2O_5S$	466.98

5.6.3. Characterization of the synthesized molecules BB_21 – BB_30

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)benzenesulfonamide (BB_21): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), benzenesulfonyl chloride (0.069 mg, 0.395 mmol) to afford **BB_21** (0.082 g, 55 %) as pale yellow solid. MP: 80 – 82 °C. ^{1}H NMR (DMSO-d6): δ_H 7.91 (d, *J* = 8.8 Hz, 2H), 7.75 – 7.59 (m, 3H), 7.38 (s, 1H), 7.33 – 7.18 (m, 5H), 4.28 (t, *J* = 5.2 Hz, 1H), 3.87 – 3.75 (m, 2H), 3.69 – 3.50 (m, 2H), 3.35 (s, 2H), 3.20 (s, 6H), 2.41 – 2.26 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 138.5, 137.8, 133.6, 130.8 (2C), 129.5 (2C), 128.6 (2C), 127.6 (2C), 126.2, 109.8, 78.5, 76.2, 69.9, 59.8, 54.6, 50.8 (2C), 48.2. EI-MS m/z: 419 $(M+H)^+$. Anal Calcd for $C_{21}H_{26}N_2O_5S$: C, 60.27; H, 6.26; N, 6.69. Found: C, 60.35.; H, 6.25; N, 6.68.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-fluorobenzenesulfonamide (BB_22): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), 4- fluoro benzenesulfonyl chloride (0.076 mg, 0.395 mmol) to afford **BB_22** (0.08 g, 56 %) as brown solid. MP: 69 – 71 °C. ^{1}H NMR (DMSO-d6): δ_H 8.02 (d, *J* = 8.6 Hz, 2H), 7.52 (m, 2H), 7.38 (s, 1H), 7.38 – 7.21 (m, 5H), 4.26 (t, *J* = 4.8 Hz, 1H), 3.90 – 3.77 (m, 2H), 3.72 – 3.48 (m, 2H), 3.32 (s, 2H), 3.24 (s, 6H), 2.46 – 2.24 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 165.2, 139.2, 137.9, 130.9 (2C), 128.5 (2C), 128.1 (2C), 126.5, 114.6 (2C), 109.3, 78.2, 76.5, 69.1,

60.5, 54.2, 50.6 (2C), 48.3. EI-MS m/z: 437 $(M+H)^+$. Anal Calcd for $C_{21}H_{25}FN_2O_5S$: C, 57.78; H, 5.77; N, 6.42. Found: C, 57.88; H, 5.78; N, 6.41.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-methoxybenzenesulfonamide (BB_23): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), 4- methoxy benzenesulfonyl chloride (0.081 g, 0.395 mmol) to afford **BB_23** (0.096 g, 59 %) as pale brown solid. MP: 68 – 70 °C. ^{1}H NMR (DMSO-d6): δ_H 7.73 (d, *J* = 8.8 Hz, 2H), 7.39 (s, 1H), 7.30 – 7.20 (m, 5H), 7.09 – 7.01 (m, 2H), 4.24 (t, *J* = 5.2 Hz, 1H), 3.84 – 3.77 (m, 5H), 3.55 – 3.46 (m, 2H), 3.34 (s, 2H), 3.21 (s, 6H), 2.44 – 2.21 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 164.2, 139.5, 137.4, 129.5 (2C), 128.8 (2C), 128.1, 127.2 (2C), 112.5 (2C), 109.8, 78.5, 76.4, 69.5, 60.2, 54.5, 54.3, 50.2 (2C), 48.3. EI-MS m/z: 449 $(M+H)^+$. Anal Calcd for $C_{22}H_{28}N_2O_6S$: C, 58.91; H, 6.29; N, 6.25. Found: C, 59.03; H, 6.30; N, 6.24.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-nitrobenzenesulfonamide (BB_24): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), 4- nitro benzenesulfonyl chloride (0.087 g, 0.395 mmol) to afford **BB_24** (0.090 g, 55 %) as pale yellow gum. ^{1}H NMR (DMSO-d6): δ_H 8.37 (d, *J* = 8.0 Hz, 2H), 8.05 (t, *J* = 8.4 Hz, 2H) 7.35 (s, 1H), 7.28 – 7.17 (m, 5H), 4.28 (t, *J* = 5.2 Hz, 1H), 3.87 – 3.75 (m, 2H), 3.69 – 3.50 (m, 2H), 3.35 (s, 2H), 3.20 (s, 6H), 2.41 – 2.25 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 149.3, 147.0, 139.6, 128.9 (2C), 128.0 (2C), 128.0 (2C), 126.7, 124.3, 109.8, 82.0, 69.7, 69.5, 59.2, 55.0, 54.5, 50.8 (2C), 48.5. EI-MS m/z: 464 $(M+H)^+$. Anal Calcd for $C_{21}H_{25}N_3O_7S$: C, 54.42; H, 5.44; N, 9.07. Found: C, 54.32; H, 5.45; N, 9.08.

4-acetyl-N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)benzenesulfonamide (BB_25): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), 4-acetylbenzene-1-sulfonyl chloride (0.086 g, 0.395 mmol) to afford **BB_25** (0.098 g, 59 %) as pale brown solid. MP: 74 – 76 °C. ^{1}H NMR (DMSO-d6): δ_H 8.24 (d, *J* = 8.2 Hz, 2H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.40 (s, 1H), 7.36 – 7.21 (m, 5H), 4.27 (t, *J* = 5.4 Hz, 1H), 3.86 – 3.72 (m, 2H), 3.64 – 3.51 (m, 2H), 3.37 (s, 2H), 3.22 (s, 6H), 2.52 (s, 3H), 2.42 – 2.21 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 198.2, 150.2, 140.2, 139.6, 130.2 (2C), 129.6 (2C), 128.9 (2C), 128.5

(2C), 128.1, 109.8, 82.6, 76.8, 69.5, 59.2, 54.0, 50.2 (2C), 48.3, 25.2. EI-MS m/z: 461 $(M+H)^+$. Anal Calcd for $C_{23}H_{28}N_2O_6S$: C, 59.98; H, 6.13; N, 6.08. Found: C, 60.10; H, 6.12; N, 6.09.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)thiophene-2-sulfonamide (BB_26): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), 2- thiophene-sulfonyl chloride (0.072 g, 0.395 mmol) to afford **BB_26** (0.088 g, 57 %) as brown solid. MP: 83 – 85 °C. ^{1}H NMR (DMSO-d6): δ_H 7.88 (d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.55 (t, *J* = 8.6 Hz, 1H), 7.41 (s, 1H), 7.14 – 7.11 (m, 5H), 4.28 (t, *J* = 5.6 Hz, 1H), 3.84 – 3.70 (m, 2H), 3.62 – 3.48 (m, 2H), 3.34 (s, 2H), 3.21 (s, 6H), 2.44 – 2.21 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 140.1, 129.2 (2C), 128.6 (2C), 127.6 (2C), 127.1, 126.2, 125.9, 109.8, 78.5, 76.2, 69.8, 60.2, 54.2, 50.2 (2C), 48.5. EI-MS m/z: 425 $(M+H)^+$. Anal Calcd for $C_{19}H_{24}N_2O_5S_2$: C, 53.75; H, 5.70; N, 6.60. Found: C, 53.83; H, 5.69; N, 6.61.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)cyclohexanesulfonamide (BB_27): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), cyclohexyl-sulfonyl chloride (0.057 ml, 0.395 mmol) to afford **BB_27** (0.101 g, 63 %) as pale yellow gum. ^{1}H NMR (DMSO-d6): δ_H 7.42 (s, 1H), 7.38 – 7.22 (m, 5H), 4.28 (t, *J* = 5.6 Hz, 1H), 3.88 – 3.74 (m, 2H), 3.66 – 3.50 (m, 2H), 3.36 (s, 2H), 3.21 (s, 6H), 3.05 (m, 1H), 2.47 – 2.21 (m, 2H), 2.13 – 1.92 (m, 4H), 1.72 – 1.58 (m, 4H), 1.51 – 1.39 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 140.1, 129.2 (2C), 128.6 (2C), 128.1, 109.1, 78.6, 76.2, 69.8, 64.8, 60.1, 54.3, 50.2 (2C), 48.4, 26.2, 25.4 (2C), 25.2 (2C). EI-MS m/z: 425 $(M+H)^+$. Anal Calcd for $C_{21}H_{32}N_2O_5S$: C, 59.41; H, 7.60; N, 6.60. Found: C, 59.52; H, 7.61; N, 6.59.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)propane-2-sulfonamide (BB_28): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), isopropyl-sulfonyl chloride (0.044 ml, 0.395 mmol) to afford **BB_28** (0.077 g, 56 %) as brown gum. ^{1}H NMR (DMSO-d6): δ_H 7.38 (s, 1H), 7.36 – 7.24 (m, 5H), 4.27 (t, *J* = 5.4 Hz, 1H), 3.85 – 3.70 (m, 2H), 3.67 – 3.54 (m, 2H), 3.38 (s, 2H), 3.28 (m, 1H), 3.22 (s, 6H), 2.46 – 2.20 (m, 2H), 1.36 (m, 6H). ^{13}C NMR (DMSO-d6): δ_c 140.2, 129.2 (2C), 128.6 (2C), 127.9, 109.8, 78.2, 76.5, 69.5, 63.4, 59.8, 54.2, 50.2 (2C), 48.4, 13.2 (2C). EI-MS m/z: 385 $(M+H)^+$. Anal Calcd for $C_{18}H_{28}N_2O_5S$: C, 56.23.; H, 7.34; N, 7.29. Found: C, 56.11.; H, 7.33; N, 7.30.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-5-chlorothiophene-2-sulfonamide (BB_29): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), 5-chloro thiophene-2-sulfonyl chloride (0.053 ml, 0.395 mmol) to afford **BB_29** (0.086 g, 52 %) as dark brown gum. ^{1}H NMR (DMSO-d6): δ_H 7.84 (d, *J* = 8.2 Hz, 1H), 7.39 (s, 1H), 7.35 – 7.21 (m, 5H), 7.18 (d, *J* = 7.8 Hz, 1H),4.29 (t, *J* = 5.4 Hz, 1H), 3.87–3.73 (m, 2H), 3.68 – 3.54 (m, 2H), 3.36 (s, 2H), 3.22 (s, 6H), 2.46 – 2.22 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 140.1, 129.5 (2C), 129.1 (2C), 128.2, 127.8, 127.5, 126.8, 126.2, 109.1, 78.2, 76.2, 69.5, 60.1, 54.8, 50.2 (2C), 48.5. EI-MS m/z: 458 $(M)^+$. Anal Calcd for $C_{19}H_{23}ClN_2O_5S_2$: C, 49.72; H, 5.05; N, 6.10. Found: C, 49.64; H, 5.04; N, 6.11.

N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-1-phenylmethane sulfonamide (BB_30): The compound was synthesized according to the general procedure by utilizing compound **BB_20a** (0.1 g, 0.359 mmol), triethyl amine (0.15 ml, 1.07 mmol), Phenylmethanesulfonyl chloride (0.075 g, 0.395 mmol) to afford **BB_30** (0.078 g, 50 %) as pale brown gum. ^{1}H NMR (DMSO-d6): δ_H 7.43 (s, 1H), 7.35 – 7.18 (m, 10H), 4.65 (s, 2H), 4.28 (t, *J* = 5.6 Hz, 1H), 3.81– 3.69 (m, 2H), 3.61 – 3.49 (m, 2H), 3.36 (s, 2H), 3.21 (s, 6H), 2.49 – 2.23 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 140.2, 132.1, 130.8 (2C), 130.1 (2C), 129.6 (2C), 128.8 (2C), 128.2, 126.5, 109.8, 78.2, 76.5, 69.5, 65.8, 59.6, 54.2, 50.2 (2C), 48.2. EI-MS m/z: 433 $(M+H)^+$. Anal Calcd for $C_{22}H_{28}N_2O_5S$: C, 61.09; H, 6.52; N, 6.48. Found: C, 60.96; H, 6.51; N, 6.47.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)benzenesulfonamide (BB_31): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), benzenesulfonyl chloride (0.069 g, 0.376 mmol) to afford **BB_31** (0.088 g, 57 %) as pale yellow solid. MP: 125 – 127 °C. ^{1}H NMR (DMSO-d6): δ_H 7.94 (d, *J* = 8.4 Hz, 2H), 7.74 – 7.53 (m, 3H), 7.38 (s, 1H), 7.22 – 7.12 (m, 4H), 4.28 (t, *J* = 5.6 Hz, 1H), 3.85 – 3.72 (m, 2H), 3.66 – 3.51 (m, 2H), 3.37 (s, 2H), 3.21 (s, 6H), 2.47 – 2.24 (m, 5H). ^{13}C NMR (DMSO-d6): δ_c 141.5, 137.2, 134.8, 132.3, 129.9 (2C), 129.7 (2C), 128.9 (2C), 128.6 (2C), 109.8, 78.3, 76.3, 69.5, 59.8, 54.6, 50.2 (2C), 48.6, 20.6. EI-MS m/z: 433 $(M+H)^+$. Anal Calcd for $C_{22}H_{28}N_2O_5S$: C, 61.09; H, 6.52; N, 6.48. Found: C, 61.20; H, 6.51; N, 6.49.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-fluorobenzenesulfonamide (BB_32): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), 4- fluoro benzenesulfonyl chloride (0.074 g, 0.376 mmol) to afford **BB_32** (0.092 g, 59 %) as pale brown solid. MP: 55 – 57 °C. ^{1}H NMR (DMSO-d6): δ_H 8.01 (d, *J* = 8.2 Hz, 2H), 7.62 (m, 2H), 7.35 (s, 1H), 7.25 – 7.11 (m, 4H), 4.29 (t, *J* = 5.6 Hz, 1H), 3.84 – 3.69 (m, 2H), 3.62 – 3.49 (m, 2H), 3.35 (s, 2H), 3.20 (s, 6H), 2.46 – 2.24 (m, 5H). ^{13}C NMR (DMSO-d6): δ_c 165.2, 138.2, 137.2, 136.5, 129.8 (2C), 129.6 (2C), 129.1 (2C), 114.8 (2C), 109.8, 78.1, 76.2, 69.5, 59.6, 54.3, 50.2 (2C), 48.1, 20.6. EI-MS m/z: 451 (M+H)+. Anal Calcd for $C_{22}H_{27}FN_2O_5S$: C, 58.65; H, 6.04; N, 6.22. Found: C, 58.76; H, 6.03; N, 6.21.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-methoxybenzenesulfonamide (BB_33): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 l mmol), 4- methoxy benzenesulfonyl chloride (0.077 g, 0.376 mmol) to afford **BB_33** (0.093 g, 58 %) as brown solid. MP: 109 – 111 °C. ^{1}H NMR (DMSO-d6): δ_H 7.69 (d, *J* = 8.6 Hz, 2H), 7.41 (s, 1H), 7.26 – 7.14 (m, 4H), 7.06 – 6.94 (m, 2H), 4.27 (t, *J* = 5.6 Hz, 1H), 3.87 – 3.74 (m, 5H), 3.54 – 3.42 (m, 2H), 3.36 (s, 2H), 3.22 (s, 6H), 2.46 – 2.20 (m, 5H). ^{13}C NMR (DMSO-d6): δ_c 164.2, 138.9, 137.2, 136.2, 130.1 (2C), 129.2 (2C), 127.2 (2C), 112.6 (2C), 109.8, 78.3, 76.2, 69.6, 59.2, 54.5, 54.2, 50.2 (2C), 48.2, 20.5. EI-MS m/z: 463 $(M+H)^+$. Anal Calcd for $C_{23}H_{30}N_2O_6S$: C, 59.72; H, 6.54; N, 6.06. Found: C, 59.65; H, 6.55; N, 6.05.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-nitrobenzenesulfonamide (BB_34): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), 4- nitro benzenesulfonyl chloride (0.083 g, 0.376 mmol) to afford **BB_34** (0.085 g, 52 %) as yellow solid. MP: 106 – 108 °C. ^{1}H NMR (DMSO-d6): δ_H 8.38 (d, *J* = 8.8 Hz, 2H), 8.07 (t, *J* = 8.4 Hz, 2H), 7.40 (s, 1H), 7.24 – 7.18 (m, 4H), 4.26 (t, *J* = 5.6 Hz, 1H), 3.86 – 3.71 (m, 2H), 356 – 3.41 (m, 2H), 3.38 (s, 2H), 3.23 (s, 6H), 2.47 – 2.19 (m, 5H). ^{13}C NMR (DMSO-d6): δ_c 152.3, 151.5, 137.2, 136.1, 129.8 (2C), 128.5 (2C), 127.8 (2C), 125.6 (2C), 109.8, 78.6, 76.6, 69.6, 59.8, 54.5, 50.2 (2C), 48.7, 20.5. EI-MS m/z: 478 $(M+H)^{+\cdot}$ Anal Calcd for $C_{22}H_{27}N_3O_7S$: C, 55.33; H, 5.70; N, 8.80. Found: C, 55.41; H, 5.69; N, 8.81.

4-acetyl-N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)benzenesulfonamide (BB_35): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), 4-acetylbenzene-1-sulfonyl chloride (0.082 g, 0.376 mmol) to afford **BB_35** (0.105 g, 65 %) as pale brown solid. MP: 57 – 59 °C. ^{1}H NMR (DMSO-d6): δ_H 8.28 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.38 (s, 1H), 7.25 – 7.16 (m, 4H), 4.29 (t, *J* = 5.4 Hz, 1H), 3.87 – 3.74 (m, 2H), 3.66 – 3.52 (m, 2H), 3.39 (s, 2H), 3.21 (s, 6H), 2.54 (s, 3H), 2.46 – 2.24 (m, 5H). ^{13}C NMR (DMSO-d6): δ_c 198.5, 149.6, 140.2, 137.2, 136.2, 130.2 (2C), 129.5 (2C), 128.6 (2C), 127.9 (2C), 109.8, 78.2, 76.2, 69.8, 60.1, 54.5, 50.2 (2C), 48.5, 25.2, 20.6. EI-MS m/z: 475 $(M+H)^+$. Anal Calcd for $C_{24}H_{30}N_2O_6S$: C, 60.74; H, 6.37; N, 5.90. Found: C, 60.81; H, 6.38; N, 5.89.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)thiophene-2-sulfonamide (BB_36): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol mmol), 2- thiophene-sulfonyl chloride (0.068 mg, 0.376 mmol) to afford **BB_36** (0.088 g, 59%) as pale brown solid. MP: 73 – 75 °C. ^{1}H NMR (DMSO-d6): δ_H 7.88 (d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 10.4 Hz, 1H), 7.55 (t, *J* = 8.2Hz, 1H), 7.42 (s, 1H), 7.14 – 7.12 (m, 4H), 4.27 (t, *J* = 5.6 Hz, 1H), 3.86 – 3.69 (m, 2H), 3.61 – 3.50 (m, 2H), 3.36 (s, 2H), 3.23 (s, 6H), 2.45 – 2.23 (m, 5H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 135.9, 130.5 (2C), 129.9 (2C), 128.5 (2C), 127.2, 126.8, 109.8, 78.2, 76.5, 69.5, 59.8, 54.5, 50.2 (2C), 48.5, 20.5. EI-MS m/z: 439 $(M+H)^+$. Anal Calcd for $C_{20}H_{26}N_2O_5S_2$: C, 54.77; H, 5.98; N, 6.39. Found: C, 54.89; H, 5.99; N, 6.38.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)cyclohexanesulfonamide (BB_37): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), cyclohexyl-sulfonyl chloride (0.054 ml, 0.376 mmol) to afford **BB_37** (0.92 g, 62 %) as pale yellow solid. MP: 111 – 113 °C. ^{1}H NMR (DMSO-d6): δ_H 7.38 (s, 1H), 7.25 – 7.17 (m, 4H), 4.27 (t, *J* = 5.2 Hz, 1H), 3.85 – 3.71 (m, 2H), 3.65 – 3.50 (m, 2H), 3.38 (s, 2H), 3.22 (s, 6H), 3.06 (m, 1H), 2.49 – 2.25 (m, 5H), 2.11 – 1.89 (m, 4H), 1.75 – 1.56 (m, 4H), 1.50 – 1.38 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 136.8, 130.3 (2C), 129.5 (2C), 109.8, 78.5, 76.2, 69.5, 67.2, 59.5, 54.5, 50.2 (2C), 48.2, 26.2, 25.4 (2C), 25.1 (2C), 20.5. EI-MS m/z: 439

(M+H)+. Anal Calcd for $C_{22}H_{34}N_2O_5S$: C, 60.25; H, 7.81; N, 6.39. Found: C, 60.32; H, 7.80; N, 6.40.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)propane-2-sulfonamide (BB_38): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), isopropyl-sulfonyl chloride (0.042 ml, 0.376 mmol) to afford **BB_38** (0.077 g, 59 %) as pale yellow solid. MP: 113 – 115 °C. ^{1}H NMR (DMSO-d6): δ_H 7.40 (s, 1H), 7.24 – 7.12 (m, 4H), 4.29 (t, *J* = 5.2 Hz, 1H), 3.84 – 3.68 (m, 2H), 3.65 – 3.52 (m, 2H), 3.36 (s, 2H), 3.29 (m, 1H), 3.23 (s, 6H), 2.47 – 2.18 (m, 5H), 1.37 (m, 6H). ^{13}C NMR (DMSO-d6): δ_c 137.5, 136.2, 130.5 (2C), 129.2 (2C), 109.1, 78.5, 76.2, 69.5, 63.5, 59.6, 54.5, 50.2 (2C), 48.6, 20.5, 13.2 (2C). EI-MS m/z: 399 $(M+H)^+$. Anal Calcd for $C_{19}H_{30}N_2O_5S$: C, 57.26; H, 7.59; N, 7.03. Found: C, 57.18; H, 7.60; N, 7.02.

5-chloro-N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)thiophene-2-sulfonamide (BB_39): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), 5-chloro thiophene-2-sulfonyl chloride (0.050 ml, 0.376 mmol) to afford **BB_39** (0.106 g, 65 %) as pale yellow oil. ^{1}H NMR (DMSO-d6): δ_H 7.99 (d, *J* = 8.9 Hz, 1H), 7.43 (s, 1H), 7.20 (d, *J* = 9.0 Hz, 1H), 7.16 – 7.10 (m, 4H), 4.33 (t, *J* = 4.8 Hz, 1H), 3.84– 3.79 (m, 2H), 3.69 – 3.50 (m, 2H), 3.39 (s, 2H), 3.22 (s, 6H), 2.40 – 2.29 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (DMSO-d6): δ_c 140.6, 136.5, 135.8, 134.15, 131.28, 128.7 (2C), 128.3 (2C), 127.8, 109.8, 82.0, 69.8, 69.5, 59.1, 54.6, 50.8 (2C), 48.58, 20.68. EI-MS m/z: 474 $(M)^+$. Anal Calcd for $C_{20}H_{25}ClN_2O_5S_2$: C, 50.78; H, 5.33; N, 5.92. Found: C, 50.89; H, 5.32; N, 5.93.

N-(3,3-dimethoxy-4-(4-methylbenzyl)hexahydro-2H-furo[3,2-b]pyrrol-6-yl)-1-phenylmethanesulfonamide (BB_40): The compound was synthesized according to the general procedure by utilizing compound **BB_20b** (0.1 g, 0.342 mmol), triethyl amine (0.14 ml, 1.02 mmol), Phenylmethanesulfonyl chloride (0.065 g, 0.376 mmol) to afford **BB_40** (0.083 g, 55 %) as brown liquid. ^{1}H NMR (DMSO-d6): δ_H 7.39 (s, 1H), 7.42 – 7.28 (m, 5H), 7.26 – 7.11 (m, 4H), 4.68 (s, 2H), 4.29 (t, *J* = 5.8 Hz, 1H), 3.80– 3.71 (m, 2H), 3.60 – 3.51 (m, 2H), 3.37 (s, 2H), 3.22 (s, 6H), 2.50 – 2.22 (m, 5H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 136.2, 134.5, 129.8 (2C), 129.0 (2C), 128.4 (2C), 128.0 (2C), 124.2, 109.1, 78.5, 76.2, 69.2, 65.2, 59.5,

54.5, 50.2 (2C), 48.5, 20.5. EI-MS m/z: 447 $(M+H)^+$. Anal Calcd for $C_{23}H_{30}N_2O_5S$: C, 61.86; H, 6.77; N, 6.27. Found: C, 61.94; H, 6.76; N, 6.26.

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)benzenesulfonamide (BB_41): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), benzenesulfonyl chloride (0.062 g, 0.352 mmol) to afford **BB_41** (0.077 g, 53 %) as yellow solid. MP: 92 – 94 °C. ^{1}H NMR (DMSO-d6): δ_H 8.46 (d, *J* = 8.8 Hz, 2H), 8.12 – 7.95 (m, 3H), 7.42(s, 1H), 7.33 – 7.25 (m, 4H), 4.28 (t, *J* = 5.41 Hz, 1H), 3.88 – 3.76 (m, 2H), 3.68 – 3.51 (m, 2H), 3.36 (s, 2H), 3.22 (s, 6H), 2.40 – 2.28 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 141.5, 137.2, 133.5, 132.5, 130.5 (2C), 129.6, 128.9 (2C), 128.4 (2C), 109.8, 78.2, 76.2, 69.8, 59.5, 54.2, 50.2 (2C), 48.5. EI-MS m/z: 452 $(M)^+$. Anal Calcd for $C_{21}H_{25}ClN_2O_5S$: C, 55.68; H, 5.56; N, 6.18. Found: C, 55.77; H, 5.57; N, 6.19.

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-fluorobenzenesulfonamide (BB_42): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), 4- fluoro benzenesulfonyl chloride (0.068 g, 0.352 mmol) to afford **BB_42** (0.089 g, 59 %) as pale brown solid. MP: 66 – 68 °C ^{1}H NMR (DMSO-d6): δ_H 8.05 (d, *J* = 8.8Hz, 2H), 7.56 (m, 2H), 7.40 (s, 1H), 7.36 – 7.28 (m, 4H), 4.28(t, *J* = 5.6 Hz, 1H), 3.91 – 3.76 (m, 2H), 3.75 – 3.51 (m, 2H), 3.34 (s, 2H), 3.22 (s, 6H), 2.47 – 2.25 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 165.2, 141.2, 137.8, 133.5, 131.6 (2C), 130.2 (2C), 129.4 (2C), 114.8 (2C), 109.8, 78.2, 76.5, 69.8, 60.1, 54.6, 50.2 (2C), 48.3. EI-MS m/z: 471 $(M+H)^+$. Anal Calcd for $C_{21}H_{24}ClFN_2O_5S$: C, 53.56; H, 5.14; N, 5.95. Found: C, 53.49; H, 5.15; N, 5.96.

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-methoxybenzenesulfonamide (BB_43): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), 4- methoxy benzenesulfonyl chloride (0.073 g, 0.352 mmol) to afford **BB_43** (0.079 g, 51 %) as brown gum. ^{1}H NMR (DMSO-d6): δ_H 7.75 (d, *J* = 8.6 Hz, 2H), 7.42 (s, 1H), 7.32 – 7.25 (m, 4H), 7.13 – 7.06 (m, 2H), 4.26 (t, *J* = 5.6 Hz, 1H), 3.86 – 3.75 (m, 5H), 3.57 – 3.42 (m, 2H), 3.36 (s, 2H), 3.20 (s, 6H), 2.46 – 2.23 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 164.5, 137.2, 136.9, 133.5, 130.8 (2C), 130.8 (2C), 129.5 (2C), 125.2 (2C), 112.5 (2C), 109.8, 78.4,

76.5, 69.5, 60.2, 55.3, 54.6, 50.2 (2C), 48.2. EI-MS m/z: 482 $(M)^+$. Anal Calcd for $C_{22}H_{27}ClN_2O_6S$: C, 54.71; H, 5.63; N, 5.80. Found: C, 54.78; H, 5.64; N, 5.79.

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-4-nitrobenzenesulfonamide (BB_44): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), 4- nitro benzenesulfonyl chloride (0.078 g, 0.352 mmol) to afford **BB_44** (0.108 g, 68 %) as brown solid. MP: 61 – 63 °C. ^{1}H NMR (DMSO-d6): δ_H 8.43 (d, *J* = 8.8 Hz, 2H), 8.08 (t, *J* = 8.0 Hz, 2H) 7.36 (s, 1H), 7.35 – 7.26 (m, 4H), 4.26 (t, *J* = 5.8 Hz, 1H), 3.85 – 3.72 (m, 2H), 3.65 – 3.48 (m, 2H), 3.34 (s, 2H), 3.22 (s, 6H), 2.39 – 2.20 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 152.2, 151.1, 137.8, 133.5, 131.5 (2C), 129.8 (2C), 128.9 (2C), 125.6 (2C), 109.8, 78.2, 76.2, 69.8, 60.2, 54.5, 50.2 (2C), 48.5. EI-MS m/z: 498 $(M+H)^+$. Anal Calcd for $C_{21}H_{24}ClN_3O_7S$: C, 50.65; H, 4.86; N, 8.44. Found: C, 50.71; H, 4.87; N, 8.45.

4-Acetyl-N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)benzenesulfonamide (BB_45): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), 4-acetylbenzene-1-sulfonyl chloride (0.077 g, 0.352 mmol) to afford **BB_45** (0.088 g, 58 %) as pale brown gum. ^{1}H NMR (DMSO-d6): δ_H 8.26 (d, *J* = 8.4 Hz, 2H), 7.2 (d, *J* = 8.0 Hz, 2H), 7.38 (s, 1H), 7.34 – 7.22 (m, 4H), 4.28 (t, *J* = 5.6 Hz, 1H), 3.87 – 3.71 (m, 2H), 3.65 – 349 (m, 2H), 3.36 (s, 2H), 3.21 (s, 6H), 2.54 (s, 3H), 2.46 – 2.19 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 199.5, 150.2, 140.2, 137.8, 133.5, 130.5 (2C), 130.2 (2C), 129.5 (2C), 128.3 (2C), 109.8, 78.2, 76.5, 69.5, 59.8, 54.5, 50.2 (2C), 48.2, 25.8. EI-MS m/z: 495 $(M+H)^+$. Anal Calcd for $C_{23}H_{27}ClN_2O_6S$: C, 55.81; H, 5.50; N, 5.66. Found: C, 55.74; H, 5.51; N, 5.65.

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)thiophene-2-sulfonamide (BB_46): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), 2-thiophene-sulfonyl chloride (0.064 g, 0.352 mmol) to afford **BB_46** (0.094 g, 64 %) as pale yellow solid. MP: 113 – 115 °C. ^{1}H NMR (DMSO-d6): δ_H 7.85 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.54 (t, *J* = 8.6 Hz, 1H), 7.38 (s, 1H), 7.34 – 7.19 (m, 4H), 4.26 (t, *J* = 5.6 Hz, 1H), 3.88 – 3.74 (m, 2H), 3.66 – 3.52 (m, 2H), 3.35 (s, 2H), 3.22 (s, 6H), 2.45 – 2.18 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 133.5, 130.2 (2C), 129.8 (2C), 128.1 (2C), 126.5 (2C), 126.2,

109.8, 78.5, 76.2, 69.5, 60.2, 54.6, 50.2 (2C), 48.2. EI-MS m/z: 459 $(M+H)^+$. Anal Calcd for $C_{19}H_{23}ClN_2O_5S_2$: C, 49.72; H, 5.05; N, 6.10. Found: C, 49.78; H, 5.04; N, 6.11.

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)cyclohexanesulfonamide (BB_47): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), cyclohexyl-sulfonyl chloride (0.051 ml, 0.352 mmol) to afford **BB_47** (0.81 g, 55 %) as yellow gum. ^{1}H NMR (DMSO-d6): δ_H 7.38 (s, 1H), 7.35 – 7.22 (m, 4H), 4.27 (t, *J* = 5.2 Hz, 1H), 3.89 – 3.70 (m, 2H), 3.5 – 3.54 (m, 2H), 3.37 (s, 2H), 3.20 (s, 6H), 3.08 (m, 1H), 2.49 – 2.20 (m, 2H), 2.11 – 1.95 (m, 4H), 1.74 – 1.62 (m, 4H), 1.55 – 1.37 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 133.5, 130.5 (2C), 129.5 (2C), 109.8, 78.5, 76.2, 69.5, 67.2, 59.8, 54.5, 50.2 (2C), 48.5, 26.2, 25.4 (2C), 25.1 (2C). EI-MS m/z: 459 $(M)^+$. Anal Calcd for $C_{21}H_{31}ClN_2O_5S$: C, 54.95; H, 6.81; N, 6.10. Found: C, 55.02; H, 6.80; N, 6.09

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)propane-2-sulfonamide (BB_48): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), isopropyl-sulfonyl chloride (0.040 ml, 0.352 mmol) to afford **BB_48** (0.079 g, 58 %) as brown gum. MP: 61 – 63 °C. ^{1}H NMR (DMSO-d6): δ_H 7.41 (s, 1H), 7.38 – 7.21 (m, 4H), 4.29 (t, *J* = 5.2 Hz, 1H), 3.90 – 3.68 (m, 2H), 3.65 – 3.50 (m, 2H), 3.39 (s, 2H), 3.27 (m, 1H), 3.21 (s, 6H), 2.45 – 2.18 (m, 2H), 1.37 (m, 6H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 133.5, 132.5 (2C), 130.2 (2C), 109.8, 78.2, 76.2, 69.5, 63.6, 60.2, 54.2, 50.2 (2C), 48.5, 13.2 (2C). EI-MS m/z: 419 $(M+H)^+$. Anal Calcd for $C_{18}H_{27}ClN_2O_5S$: C, 51.61; H, 6.50; N, 6.69. Found: C, 51.69; H, 6.49; N, 6.70.

5-chloro-N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)thiophene-2-sulfonamide (BB_49): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), 5-chloro thiophene-2-sulfonyl chloride (0.047 ml, 0.352 mmol) to afford **BB_49** (0.101 g, 63 %) as brown gum. ^{1}H NMR (DMSO-d6): δ_H 7.88 (d, *J* = 8.2 Hz, 1H), 7.38 (s, 1H), 7.36 – 7.20 (m, 5H), 7.16 (d, *J* = 7.8 Hz, 1H), 4.27 (t, *J* = 5.4 Hz, 1H), 3.86– 3.71 (m, 2H), 3.70 – 3.52 (m, 2H), 3.38 (s, 2H), 3.21 (s, 6H), 2.48 – 2.21 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 135.8, 133.5, 130.2 (2C), 129.8 (2C), 128.2, 127.6, 126.8, 125.2, 109.8, 78.2, 76.2, 69.5,

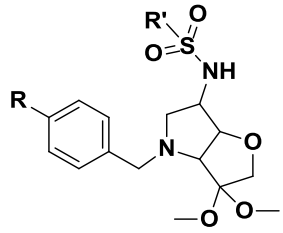
60.2, 54.5, 50.2 (2C), 48.5. EI-MS m/z: 493 $(M)^+$. Anal Calcd for $C_{19}H_{22}Cl_2N_2O_5S_2$: C, 46.25; H, 4.49; N, 5.68. Found: C, 46.32; H, 4.50; N, 5.67.

N-(4-(4-chlorobenzyl)-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)-1-phenylmethanesulfonamide (BB_50): The compound was synthesized according to the general procedure by utilizing compound **BB_20c** (0.1 g, 0.319 mmol), triethyl amine (0.13 ml, 0.961 mmol), Phenylmethanesulfonyl chloride (0.062 g, 0.352 mmol) to afford **BB_50** (0.098 g, 66 %) as pale yellow solid. MP: 74 – 76 °C. ^{1}H NMR (DMSO-d6): δ_H 7.41 (s, 1H), 7.37 – 7.21 (m, 9H), 4.63 (s, 2H), 4.26 (t, *J* = 5.4 Hz, 1H), 3.80– 3.71 (m, 2H), 3.63 – 3.52 (m, 2H), 3.35 (s, 2H), 3.22 (s, 6H), 2.50 – 2.21 (m, 2H). ^{13}C NMR (DMSO-d6): δ_c 137.2, 132.4, 131.6, 130.5 (2C), 129.6 (2C), 128.8 (2C), 127.9 (2C), 126.2, 109.8, 78.2, 76.5, 69.5, 65.2, 60.2, 54.5, 50.2 (2C), 48.5. EI-MS m/z: 467 $(M+H)^+$. Anal Calcd for $C_{22}H_{27}ClN_2O_5S$: C, 56.58; H, 5.83; N, 6.80. Found: C, 56.65; H, 5.84; N, 6.81.

5.6.4. *In vitro Msm* Gyr B assay, supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were evaluated for their *in vitro Msm* Gyr B assay and supercoiling assay for the derivation of SAR and lead optimization. The compounds were further subjected to a whole cell screening against *Mtb* H_{37}Rv strain to understand their bactericidal potency using the agar dilution method and later the safety profile of these molecules were evaluated by checking the *in vitro* cytotoxicity against RAW 264.7 cell line (mouse macrophage) at 50 μM concentration by MTT assay, and the results are shown in **Table 5.12**.

Table 5.12: *In vitro* biological evaluation of the synthesized derivatives **BB_21**– **BB_50**



BB_21 - BB_50

Comp ID	R	R’	Supercoiling (% inhibition at 25 µM)	*MTB* MIC (µM)	RAW264.7 Cytotoxicity at 50 µM (% inhib.)
BB_21	H	Phenyl	20	59.74	28.66
BB_22	H	4 Fluro Phenyl	25	57.27	21.20
BB_23	H	4 Methoxy Phenyl	-	27.87	38.22
BB_24	H	4 Nitro Phenyl	15	53.94	33.01
BB_25	H	4 Acetyl phenyl	60	54.28	25.22
BB_26	H	2 Thiophene	-	29.44	35.43
BB_27	H	Cyclohexyl	25	1.61	20.52
BB_28	H	Isopropyl	10	32.51	23.34
BB_29	H	5 Chloro- 2-Thiophene	-	54.47	45.67
BB_30	H	Benzyl	20	57.80	14.37
BB_31	CH_3	Phenyl	-	57.80	27.84
BB_32	CH_3	4 Fluro Phenyl	50	55.49	16.91
BB_33	CH_3	4 Methoxy Phenyl	20	54.05	34.03
BB_34	CH_3	4 Nitro Phenyl	-	52.35	20.23
BB_35	CH_3	4 Acetyl phenyl	-	26.34	24.93
BB_36	CH_3	2 Thiophene	15	57.00	21.07
BB_37	CH_3	Cyclohexyl	20	57.00	46.89
BB_38	CH_3	Isopropyl	35	31.37	27.94
BB_39	CH_3	5 Chloro-2-Thiophene	25	1.79	49.13
BB_40	CH_3	Benzyl	20	55.98	28.46

BB_41	Cl	Phenyl	35	13.80	37.87
BB_42	Cl	4 Fluro Phenyl	10	53.09	19.43
BB_43	Cl	4 Methoxy Phenyl	30	51.76	24.07
BB_44	Cl	4 Nitro Phenyl	10	50.21	28.54
BB_45	Cl	4 Acetyl phenyl	-	50.51	16.07
BB_46	Cl	2 Thiophene	10	54.47	18.36
BB_47	Cl	Cyclohexyl	10	54.47	19.56
BB_48	Cl	Isopropyl	50	14.92	24.10
BB_49	Cl	5 Chloro-2-Thiophene	20	50.67	29.70
BB_50	Cl	Benzyl	50	53.54	28.75
Moxifloxacin			11.2±0.23	2.4	NT
Novobi0cin			46±28nM	>200	19.3
Ethambutol			NT	9.84	NT

IC_{50}, 50% inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; NT, not tested; nM, nanomolar

Mtb DNA gyrase supercoiling enzyme inhibition activity

In vitro activity against *Mtb* $H_{37}Rv$

Cytotoxicity against RAW 264.7 cells (mouse macrophage cell line)

5.6.5. Nutrient Starvation Model

The most active anti-mycobacterial compound **BB_27** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e., Nutrient starvation model. **BB_27** showed 1.2 log reductions in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold).

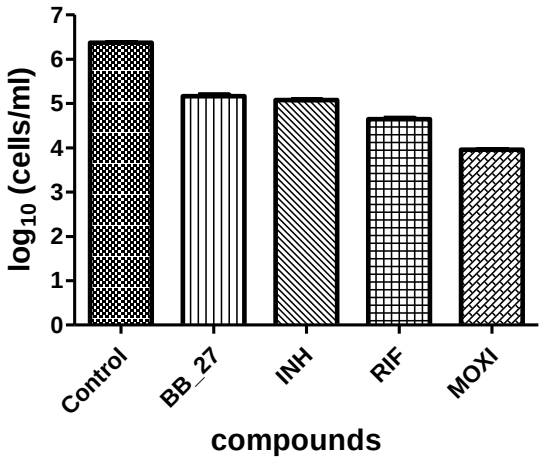


Fig 5.28: Nutrient starvation model graph of **BB_27**

Bacterial count estimation (Mean ± S.D., n = 3) for control and treated groups conducted by using the MPN (most probable number) assay. The dormant cell suspension was treated with the compounds at a concentration of 10 µM. most of the compounds gave significant inhibition of growth of *M. tuberculosis* in this model as compared to the control ($p < 0.0001$, two way ANOVA using GraphPad Prism Software).

5.6.6. Anti-mycobacterial screening for most active compound using adult zebrafish (Zebrafish Model)

To evaluate anti-mycobacterial potency of compound **BB_27** *In vivo,* it was also tested in *Mycobacterium marinum* induced adult zebra fish model. The compound was administered orally at a dose of 10 mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. **BB_27** exhibited good anti-mycobacterial potency and also log reduction of 2.7 fold in bacteria when compared to first line drugs- Isoniazid (2.9 fold), and Moxifloxacin (2.8 fold).

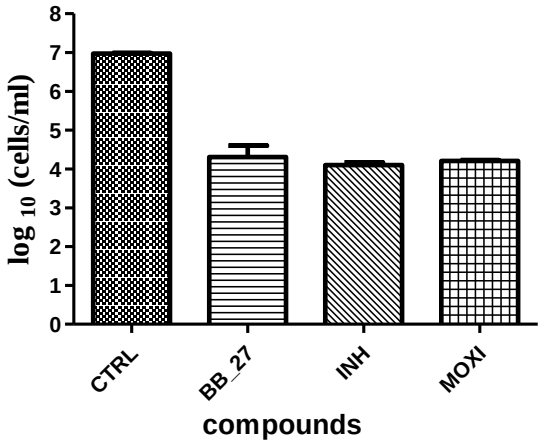


Fig 5.29: Zebra fish model graph of **BB_27**

Bacterial count estimation (Mean ± S.E.M., n = 6) for control and treated groups conducted by using MPN (most probable number) assay. The statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001 and ****p<0.0001) with respect to infected control group has been analyzed by Two-way ANOVA using GraphPad Prism Software

5.6.7. SAR and Discussion

Foss M., et al., 2009 has reported gyramides as DNA Gyrase inhibitors. The gyramides has a very good MIC againist *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, and *Streptococcus pneumonia*. In our studies we attempted to modify the above reported molecule using molecular hybridization and derivatized a library of 30 molecules to obtain novel *Mtb* DNA gyrase inhibitors.

All the synthesized compounds were screened for their enzyme inhibition studies using MTB DNA gyrase kit (Inspiralis, Norwich). Preliminary screening was performed at 25 µM concentration. Four compounds (**BB_25**, **BB_32**, **BB_48**, **and BB_50**) showed percentage inhibition of 50 – 60 %. Twelve compounds showed percentage inhibition of 20 – 35 %. Fourteen compounds are having percentage inhibition less than 20 %.

All the synthesized compounds were screened for their in-vitro antimycobacterial activity against *Mtb* H_{37}Rv by microplate alamar blue assay method. Ethambutol (MIC: 15.31 µM), Isoniazid (MIC: 0.66 mM),Moxifloxacin (MIC: 1.2 µM) and Novobiocin (MIC: >200 µM) were considered as standard drugs for comparison in this assay. The MIC values varied from 1.61 – 59.74 µM. Two compounds inhibited *Mtb* with MIC less than 5 µM. **BB_27** with an MIC

of 1.61 µM and **BB_39** with an MIC of 1.79 µM. Two compounds inhibited *Mtb* with MIC less than 15 µM. **BB_41** with an MIC of 13.80 µM and **BB_48** with an MIC of 14.92 µM. Four compounds (**BB_23**, **BB_28**, **BB_35** and **BB_38**) showed MIC less than 35 µM. All the other compounds showed MIC more than 50 µM. From structure activity relation point of view the most active antimycobacterial compound **BB_27** possess electron donating hydrogen at the para position of benzyl group attached to nitrogen of pyrrole. Also it possesses a cyclohexyl ring at the sulphonamide position. The second most potent antimycobacterial compound **BB_39** also possesses a electron donating methyl group at the 4^{th} position of benzyl group attached to nitrogen of pyrrole. **BB_39** had a thiophene group at the sulphonamide position. It is observed that when electon withdrawing substituent like chloro group attached to benzyl group connected to pyrrole nitrogen, there is drastic decrease in activity.

When compared to Moxifloxacin (MIC of 2.4 µM) and standard first line anti-TB drug Ethambutol (MIC of 9.84 µM), two molecules (**BB_27** and **BB_39**) showed better activity. When compared to isoniazid (0.66 µM), none of the molecules showed better activity.

The most active anti-mycobacterial compound **BB_27** was taken for further studies in dormant model of mtb as reported by J. C. Betts et.al. i.e.,Nutrient starvation model. **BB_27** showed 1.2 log reduction in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold). These results suggest that compound **BB_27** is not only effective against replicative stage of mtb but also against persistent stages of bacteria.

To evaluate invivo activity of **BB_27,** it was also tested in *Mycobacterium marinum* induced adult zebra fish model. The compound was administered orally at a dose of 10mg/kg body weight for a period of 7 days. The reduction in bacterial count was evaluated by MPN assay. Compound **BB_27** exhibited good anti-mycobacterial potency and also log reduction of 2.7 fold in bacteria when compared to first line drugs- isoniazid (2.9 fold),and moxifloxacin (2.8 fold). It is an indicative that compound **BB_27** is best candidate for further drug development studies.

Subsequently, the eukaryotic cell safety profile of all the forty compounds were observed by testing there *in vitro* cytotoxicity against the RAW 264.7 cell line (Mouse leukemic monocyte macrophage cell line) at 50 µM concentration by (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Since the mycobacteria resides in the macrophages

during the infection stages, this cell line was selected. At 50 µM tested compounds showed cytotoxicity range of 10.41-35.86 % as shown in **Table 5.12**.The most promising anti-TB compound among the synthesized set of compounds was **BB_27** with only 20.52 % cytotoxicity which is within the safety profile limits. Novobiocin was used as standard with 19.36 % inhibition in the above cell line.

5.6.8. Highlights of the study

Compound **BB_27** (N-(4-benzyl-3,3-dimethoxy hexahydro-2H-furo [3,2-b] pyrrol-6-yl) cyclohexane sulfonamide) was found to be the most active antimycobacterial compound with an MIC of 1.61µM. **BB_27** showed 25 % inhibition of DNA Gyrase enzyme via supercoiling assay. **BB_27** was also tested in *Mycobacterium marinum* induced adult zebra fish model. It exhibited good anti-mycobacterial potency and also log reduction of 2.7 fold in bacteria when compared to first line drugs- isoniazid (2.9 fold), and moxifloxacin (2.8 fold). It is an indicative that compound **BB_27** is best candidate for further drug development studies. Nutrient starvation model, **BB_27** showed 1.2 log reduction in bacterial count when compared to standard drugs namely Isoniazid (1.5 log fold), Rifampicin (1.7 log fold) and Moxifloxacin (2.0 log fold). These results suggest that compound **BB_27** is not only effective against replicative stage of mtb but also against persistent stages of bacteria.

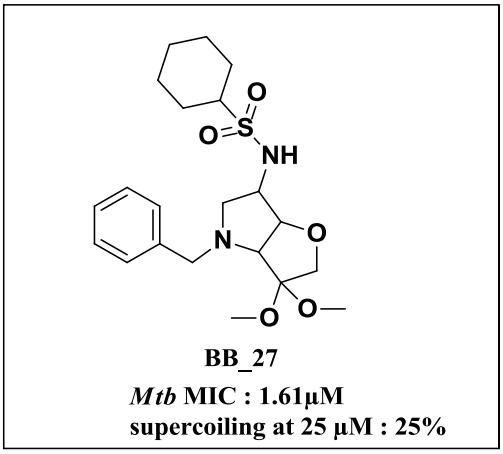


Fig 5.30: Chemical structure and biological activity of the most active compound **BB_27**

Summary and Conclusion *Chapter 6*

Tuberculosis continues to cause significant morbidity and mortality worldwide. Increasing rates of drug-resistant tuberculosis are a significant concern and pose serious implications for current and future treatment of the disease. The present decade has seen a reawakening of tuberculosis (TB) drug research and development (R&D), spurred on by an urgent need to stem the tide of the disease globally and develop new, more effective treatments against drug sensitive and resistant strains.

Treatment of tuberculosis was an obstacle for ages. In spite of good drugs, the disease is not under control. Thus, the need for more drugs existed. Searching for a new drug demands much investment on the time, money and human resources. There are many drugs with antitubercular action that are discovered and explored in the last century. The most common drug, Isoniazid, is now a frontrunner anti-tubercular drug. Like the same anti tubercular effect of Trifluoperazine which is known since 1990s, but due to the lack of research its anti-tubercular potential is not yet explored completely. Form literature search we had chosen reported DNA gyrase inhibitors having good MIC's as lead molecules and re-engineered to get more drugs like properties by maintaining its core structure for the activity. These leads were taken up for hit expansion by chemical synthesis and a total of 192 molecules from six different series were synthesized and biologically evaluated in our laboratory.

From the first set of molecules, 1-(2-(4-aminopiperidin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives, compound **BKA_35** (1-(4-fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea evolved as the most promising inhibitor with an IC_{50} of 78 nM against *Mycobacterium tuberculosis* DNA Gyrase enzyme, with a good MTB MIC of 0.62 µM, and not cytotoxic at a higher concentration of 50 µM in eukaryotic cell line.

Among the molecules of 7-methoxy-1-(2-(piperazin-1-yl)ethyl)-1,5-naphthyridin-2(1H)-one derivatives, compound **PZ_11** (4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)-N-(4-nitrophenyl)piperazine-1-carboxamide) was the most promising inhibitor with *Mycobacterium tuberculosis* (MTB) DNA Gyrase enzyme supercoiling IC_{50} of 0.29±0.22 µM, with a good

MTB MIC of 3.45 µM. These kind of compounds retains good potency and showed reduced cardio toxicity compared to aminopiperidines.

Among the third set of molecules of N-(4-(5-sub-1H-benzo[d]imidazol-2-yl) phenyl)-5-sub-1H-indole-2-carboxamide/(4-(5-sub-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)(5-sub-1H-indol-2-yl)methanone derivatives, Compound **BZ_10** (5-fluoro-N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-1H-indole-2-carboxamide) was found to be the most active DNA Gyrase inhibitor with supercoiling IC_{50} of 4.10±0.21 µM and inhibited drug sensitive *M. tuberculosis* with MIC of 16.09 µM. Also we identified **BZ_38** as most active anti-mycobacterial compound with an MIC of 4.15 µM and with supercoiling IC_{50} of 5.75±0.26 µM. The most active anti-mycobacterial compound **BZ_38** in, Nutrient starved model showed 2.4 log reduction in bacterial count when compared to standard drugs namely isoniazid (1.5 log fold), rifampicin (1.7 log fold) and moxifloxacin (2.0 log fold). Also compound **BZ_38;** was tested in *Mycobacterium marinum* induced adult zebra fish model. Compound **BZ_38** exhibited good ant-mycobacterial potency and also log reduction of 2.1 fold in bacteria when compared to first line drugs isoniazid (2.9 fold), and moxifloxacin (2.8 fold). It is an indicative that compound **BZ_38** is best candidate for further drug development studies.

In 1-(7-chloroquinolin-4-yl)piperidin-4-amine derivatives, Compound **QU_30** (1-(7-chloroquinolin-4-yl)-N-phenylpiperidin-4-amine) was found to be the most active DNA Gyrase B inhibitor with Gyr B IC_{50} of 14.98±0.17 µM, supercoiling IC_{50} of 18.25±0.32 µM and inhibited drug sensitive *M. tuberculosis* with MIC of 8.88 µM. **QU_08** emerged as the most active anti-mycobacterial compound with an MIC of 1.72 µM. Nutrient starvation model, **QU_08** showed 2.7 log reduction in bacterial count when compared to standard drugs namely isoniazid (1.5 log fold), rifampicin (1.7 log fold) and moxifloxacin (2.0 log fold). These results suggest that compound **QU_08** is not only effective against replicative stage of *Mtb* but also against persistent stages of bacteria.

Among the molecules of 2-(3-oxo-3,4- dihydro -2H – benzo [b] [1,4] thiazin-2-yl) -N-phenyl acetamide derivatives,compound **BT_30** (N-(2,3-dichlorophenyl)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetamide) was found to be the most active anti-mycobacterial compound with an MIC of 2.12 µM. **BT_30** showed 60% inhibition of DNA Gyrase enzyme via supercoiling assay. **BT_30** was also tested in *Mycobacterium marinum* induced adult zebra

fish model. Compound **BT_30** exhibited good anti-mycobacterial potency and also log reduction of 1.9 fold in bacteria when compared to first line drugs- isoniazid (2.9 fold),and moxifloxacin (2.8 fold). It is an indicative that compound **BT_30** is best candidate for further drug development studies.

In N-(4-sub-benzyl-3,3-dimethoxy hexahydro-2H-furo [3,2-b] pyrrol-6-yl)-sub-sulfonamide derivatives, Compound **BB_27** (N-(4-benzyl-3,3-dimethoxyhexahydro-2H-furo[3,2-b]pyrrol-6-yl)cyclohexanesulfonamide) was found to be the most active anti-mycobacterial compound with an MIC of 1.61 µM. **BB_27** showed 25 % inhibition of DNA Gyrase enzyme via supercoiling assay. **BB_27** was also tested in *Mycobacterium marinum* induced adult zebra fish model. It exhibited good anti-mycobacterial potency and also log reduction of 2.7 fold in bacteria when compared to first line drugs- isoniazid (2.9 fold), and moxifloxacin (2.8 fold). It is an indicative that compound **BB_27** is best candidate for further drug development studies. Nutrient starvation model, **BB_27** showed 1.2 log reductions in bacterial count when compared to standard drugs namely isoniazid (1.5 log fold), rifampicin (1.7 log fold) and moxifloxacin (2.0 log fold). These results suggest that compound **BB_27** is not only effective against replicative stage of mtb but also against persistent stages of bacteria.

Overall, sixty one compounds were found to have supercoiling IC_{50} better than standard drug Moxifloxacin. Sixty seven compounds were found to be more active in inhibiting *M. tuberculosis* compared to standard first line anti-TB drug ethambutol. Amongst all the synthesized compounds, Compound **BKA_35** (1-(4-fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea) emerged as the most promising inhibitor with an IC_{50} of 78 nM against *Mycobacterium tuberculosis* DNA Gyrase enzyme, with a good MTB MIC of 0.62 µM. **BKA_35** showed better anti-mycobacterial potency than standard drug isoniazid.

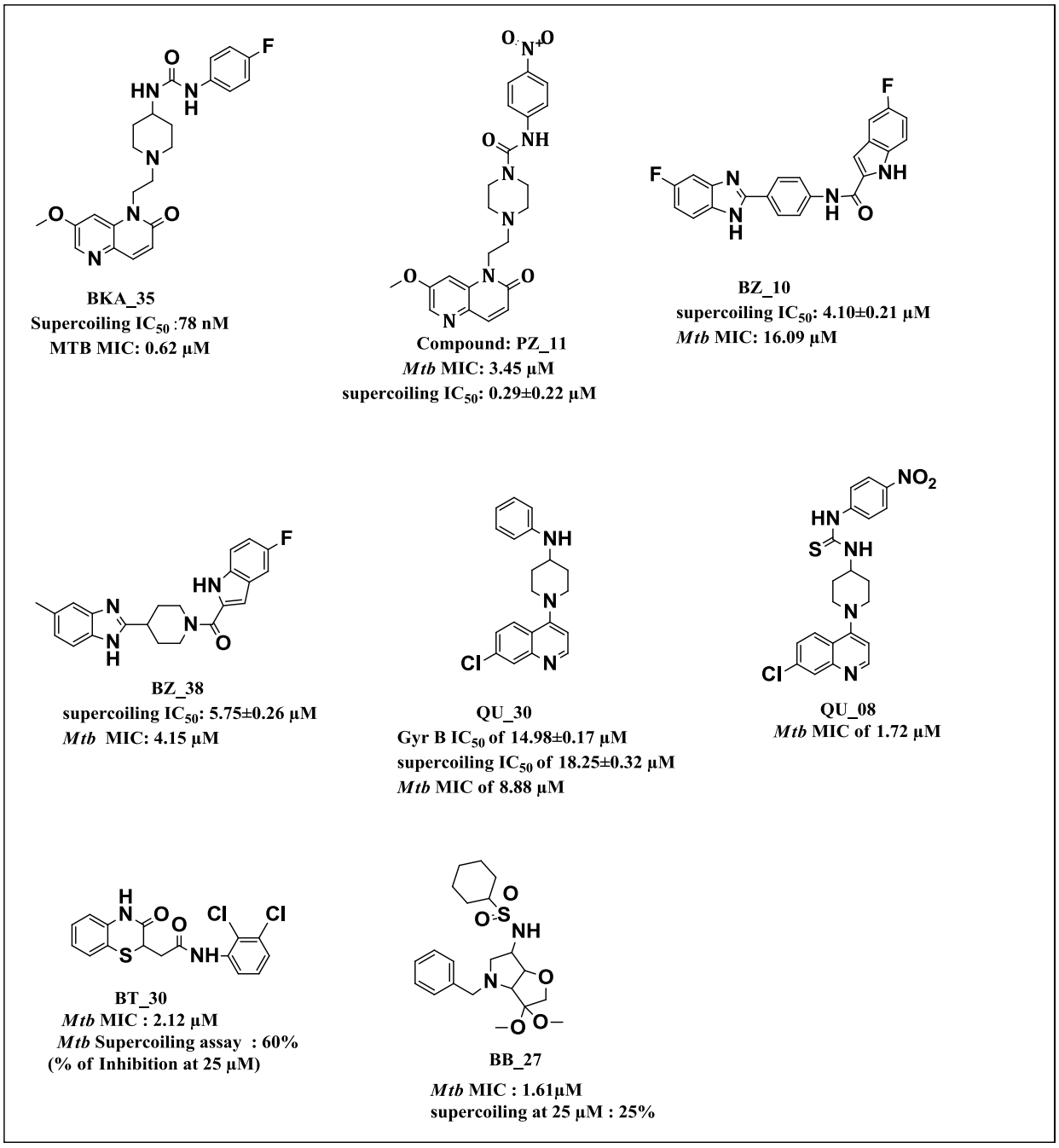


Fig 6.1: Structures of most potent compounds from each series

Our studies opens a new window to a set of NCE's which can be further developed into a novel drug candidate.

Future perspectives

The present thesis described the development of six chemically diverse series of molecules as potential DNA Gyrase inhibitors with promising *Mtb* MIC. The type II topoisomerase, DNA Gyrase are absent in humans but essential in microbial pathogens, suggesting that it provides potential targets for the development of novel antibacterial compounds.

The molecules reported here displayed excellent DNA Gyrase enzyme inhibition and considerable *in vitro* enzyme inhibition and potency against *M. tuberculosis* H37Rv strain. Although these results are encouraging, lead optimization is still needed.

Some of the active compounds reported in this studies shown significant reductions in bacterial count of dormant culture of *Mtb*, Nutrient starvation model. These results suggest that these compounds can be developed to target not only replicative stage of *Mtb* but also against persistent stages of bacteria.

Also some of the active anti-mycobacterial compounds reported in this thesis are screened for there *in vivo* activity using adult zebra fish. Extensive pharmacodynamics and pharmacokinetic studies of the safer compounds have to be undertaken in higher animal models.

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

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.

Cost effective and minimizing the number of steps involved in the synthesis of compounds reported in this thesis should be optimized.

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Appendix

List of Publications

- **Bobesh, K. A**., Renuka, J., Srilakshmi, R. R., Yellanki, S., Kulkarni, P., Yogeeswari, P., & Sriram, D. (2016). Replacement of cardiotoxic aminopiperidine linker with piperazine moiety reduces cardiotoxicity? Mycobacterium tuberculosis novel bacterial topoisomerase inhibitors.*Bioorganic & Medicinal Chemistry*, *24*(1), 42-52.

- **Bobesh, K. A**., Renuka, J., Jeankumar, V. U., Shruti, S. K., Sridevi, J. P., Yogeeswari, P., & Sriram, D. (2014). Extending the N-linked aminopiperidine class to the mycobacterial gyrase domain: Pharmacophore mapping from known antibacterial leads. *European Journal of Medicinal Chemistry*, *85*, 593-604.

- Janupally, R., Jeankumar, V. U., **Bobesh, K. A**., Soni, V., Devi, P. B., Pulla, V. K., ... & Sriram, D. (2014). Structure-guided design and development of novel benzimidazole class of compounds targeting DNA gyraseB enzyme of Staphylococcus aureus. *Bioorganic & Medicinal Chemistry*, *22*(21), 5970-5987.

- Reshma, R. S., Saxena, S., **Bobesh, K. A**., Jeankumar, V. U., Gunda, S., Yogeeswari, P., & Sriram, D. (2016). Design and development of new class of Mycobacterium tuberculosisL-alanine dehydrogenase inhibitors.*Bioorganic & Medicinal Chemistry*.

Papers presented at Conferences

International

- 6th International Symposium on "Current Trends in Drug Discovery & Research", CSIR-CDRI, Lucknow, Feb. 25th – 28th, 2016
- 13th Eurasia Conference on Chemical Sciences, IISC, Bangalore, India, Dec. 14th – 18th, 2014.
- 2nd UK-India Medchem Congress at IICT-Hyderabad, Hyderabad, Mar. 22nd - 23rd, **2013**.

National

- Drug Discovery & Development – Global scenario – Indian Perspective at NIPER-Hyderabad, Nov. 20th - 21th , **2015**.
- National conference on Drug Discovery and Development in Chemistry- Applications in Pharma Industry (DDDC-2015) at S.V. University, Tirupathi, Sep. 14th - 15th, **2015**.
- National Symposium on Human Diseases at BITS-Pilani, Hyderabad Campus, Hyderabad, Mar. 15th - 16th, **2014.**

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 16 years. He has 327 peer-reviewed research publications, 5 patents and 1 book 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. Prof. D Sriram has been selected for the prestigious Tata Innovation Fellowship of the Department of Biotechnology, Government of India for the year 2015-16 in recognition of his outstanding research contributions.He has guided 18 Ph.D. students and 9 students are pursuing Ph.D. currently. His research is funded by agencies like the UGC, CSIR, ICMR, DBT and DST.

BIOGRAPHY OF BOBESH K ANDREWS

Mr. Bobesh K Andrews completed his Bachelor of Science and Master of Science from St.Berchman's college, Changanasherry (M G University, Kottayam). He has about 7 years of industrial experience. He has worked at Syngene intl - Bangalore as Senior Research Associate. He has been appointed as DBT-ICMR Senior Research Fellow from Jan 2013 – April 2016 at Birla Institute of Technology & Science, Pilani, and Hyderabad campus under the supervision of Prof. D. Sriram. He has published four scientific papers in well-renowned international journals and also presented papers at national and international conferences.