Design, Synthesis and Biological Activity of Novel Heterocyclic Compounds as Anti-tubercular Agents

THESIS

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By
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CERTIFICATE

This is to certify that the thesis entitled "**Design, Synthesis and Biological Activity of Novel Heterocyclic Compounds as Anti-tubercular Agents**" submitted by **MAHA LAKSHMI NAIDU KALAGA, ID.No: 2011PHXF409H**, for the award of Ph.D. degree of the Institute, embodies the original work done by him under my supervision.

Signature of the supervisor:

Name in capital letters: Prof. K.V.G. CHANDRA SEKHAR

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Date:

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ABSTRACT

Mycobacterium tuberculosis, the fatal agent of humans is estimated to claim two million deaths annually. Even though the existing drugs possess huge value in controlling disease to some extent, there are still several shortcomings. The drug discovery efforts are progressively becoming more rational, focusing at various enzymes and identification of appropriate targets become fundamental pre-requisite. In the present study we paid attention on achieving promising anti-tubercular agents by design, synthesis and anti-mycobacterial evaluation of compounds based on reported promising anti-tubercular agents. In the present work, four series of compounds (total 232 compounds) were designed and synthesized by conventional methods. Wherever possible, reactions were carried out using environmental benign techniques such as microwave assisted organic synthesis or under solvent free conditions with moderate to excellent yields. All synthesized novel compounds were characterized by various spectroscopic techniques (IR, NMR and MS), elemental analysis and structures of few compounds are confirmed by single crystal X-ray Diffraction (XRD). All synthesized compounds were screened for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain and furthermore, most active compounds were evaluated for their *in vitro* cytotoxicity against RAW 264.7 cell line (mouse leukemic monocyte macrophage) using MTT assay. Amongst, 232, twenty one compounds were found to be more active in inhibiting MTB H37Rv compared to standard anti-TB drugs viz. ethambutol and pyrazinamide, whereas one compound possessed better MTB MIC than ciprofloxacin. Over all the synthesized compounds, **BIP 53, BIP 58, BIP 63, BIP 65, QNP 17, QNP 19, QNP 22, QNP 32, QNP 45, QNP 48, QNP 53, PPA 18**, **PPA 24**, **PPA 28, IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57** (MTB MIC = 1.56 µg/mL SI ≥ 34) emerged as the most promising anti-tubercular drug candidates. Compound **QNP 27** emerged as the most potent compound with MTB MIC = 0.78 µg/mL and SI > 135. The present set up inhibitors will serve on potentioal leads for further chemical optimization to yield potentioal anti-tubercular agents.

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List of Abbreviations / Symbols

^{13}C NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
ACN	:	Acetonitrile
ADH	:	Alanine dehydrogenase
ATP	:	Adenosine Triphosphate
b	:	Broad
$CDCl_3$	:	Chloroform deuterated
CFU	:	Colony-forming unit
CI	:	Confidence intervals
d	:	Doublet
DCM	:	Dichloromethane
DIPEA	:	*N*,*N*-diisopropylethylamine
DMF	:	*N*,*N*-dimethylformamide
DMSO-d_6	:	Dimethyl sulphoxide deuterated
DNA	:	Deoxyribonucleic acid
DOTS	:	Directly Observed Treatment, Short course
DSF	:	Differential Scanning Fluorimeter
EBA	:	Early Bactericidal Activity
EDCI	:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EMA	:	European Medicines Agency
EMB	:	Ethambutol
EU	:	European Union
FAD	:	Flavin adenine dinucleotide
FDA	:	Food and Drug Administration
g		Gram
HIV	:	Human Immunodeficiency Virus
HOBt	:	Hydroxybenzotriazole
hr	:	Hour
HTS	:	High throughput screening
IBD	:	Iodobenzene diacetate
IC_{50}	:	Half Maximal Inhibitory Concentration

INH	:	Isoniazid
IR	:	Infra Red
J	:	Coupling constant
Kg	:	Kilogram
KM	:	Kanamycin
LAT	:	Lysine aminotransferase
LCMS	:	Liquid chromatography–Mass Spectrometry
LJ medium	:	Lowenstein–Jensen medium
m	:	Multiplet
M.P.	:	Melting point
MDR-TB	:	Multidrug-Resistant *Mycobacterium tuberculosis*
mg	:	Milligram
MIC	:	Minimum Inhibitory Concentration
mL	:	Milliliter
mmol	:	Millimole
MTT	:	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADH	:	Nicotinamide Adenine Dinucleotide
NIAID	:	National Institute of Allergy and Infectious Diseases
nM	:	Nanomolar
OADC	:	Oleic Albumin Dextrose Catalase
PDB	:	Protein Data Bank
ppm	:	Parts per million
PS	:	Pantothenate synthetase
PTSA	:	*p*-Toluenesulfonic acid
PZA	:	Pyrazinamide
RB flask	:	Round bottom flask
RMP	:	Rifampicin
RNA	:	Ribonucleic acid
rRNA	:	Ribosomal Ribonucleic acid
rt	:	Room temperature
s	:	Singlet
SAR	:	Structure Activity Relationship

SM	:	Streptomycin
t	:	Triplet
T_3P	:	Propylphosphonic anhydride
TAACF	:	Tuberculosis Antimicrobial Acquisition and Coordinating Facility
TB	:	Tuberculosis
TDR-TB	:	Totally Drug-Resistant *Mycobacterium tuberculosis*
TEA	:	Triethylamine
TFA	:	Trifluoroacetic acid
THF	:	Tetrahydrofuran
TLC	:	Thin-layer chromatography
T_m	:	Melting temperature
TMS	:	Tetramethylsilane
US	:	United States
VS	:	Virtual Screening
W	:	Watt
WHO	:	World Health Organisation
XDR-TB	:	Extensively Drug-Resistant *Mycobacterium tuberculosis*
XP	:	Extra Precision
Å	:	Angstrom (10^{-10} meter)
δ	:	Chemical shift
µg	:	Microgram
µM	:	Micromolar
µW	:	Microwave

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Chapter I
Introduction

Chapter 1

INTRODUCTION

1.1. Tuberculosis

Tuberculosis (TB) is an airborne contagious disease, caused by *Mycobacterium bacilli* or *Mycobacterium tuberculosis* (MTB) (**Figure 1.1**). It generally attacks the lungs and is known as pulmonary tuberculosis. It is transmitted from person to person through droplets from cough or sneeze. It can also attack any part of the human body such as kidney, spine and brain leading to extra pulmonary TB. TB develops from a recent infection caused by inhalation of aerosol containing TB bacilli and energising of dormant tubercle bacilli which are already present in the body for years or decades. The present scenario of two billion TB cases comprises both individuals with new exogenous disease and old reactivated endogenous disease. The risk of extra pulmonary TB is very high in patients with active HIV infection [Backer *et al*., 2006]. It is an ancient enemy of humanity, being considered as leading source of death from the start of last century [Khalid *et al*., 2012; Wong *et al*., 2013; Minch *et al*., 2015].

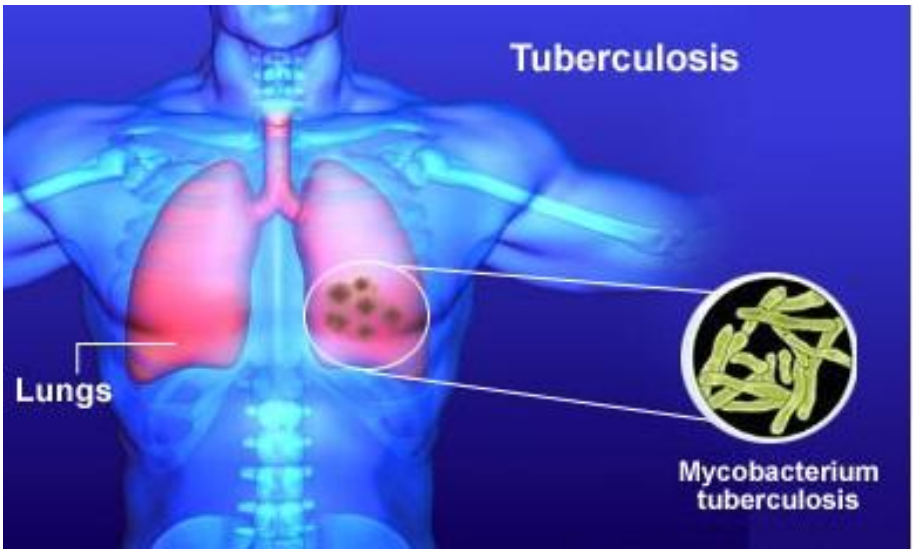


Figure 1.1: *Mycobacterium tuberculosis* scanning electron micrograph

Amongst, all the infectious diseases known to mankind, TB dangles on the lethal. This devastating disease has been wiping out humankind throughout known history from the period immemorial. Despite the fact that many other diseases like cholera, plague and smallpox have destructed the lives of lakhs of people, they demised in a short period of time,

but MTB has been ever present [Ducati *et al*., 2006; Sharma *et al*., 2013]. The era between 18th and 19th century, TB arrived epidemic in Europe and North America and consumed millions of lives by earning the sobriquet "Captain among these men of death [Daniel *et al*., 2006; Ducati *et al*., 2006]. TB as an infection of respiratory tract attack lungs primarily, and if not treated properly diffuses to other parts of the body except hair and nail [Pal *et al*., 2014]. In general, scrutiny revealed that older (≥ 60 years) TB patients have significantly more risk of hepatotoxicity when compared with middle aged patients. These studies suggest that a gentler regimen of treatment for older individuals could benefit health outcomes in these inhabitants of TB patients and reduce adventure to the public's health [Hosford *et al*., 2015].

According to World Health Organization (WHO), globally 2 billion people are infected with latent MTB, which is accountable for 8 to 10 million new cases of TB and 2 million deaths annually throughout the globe. In 2013, an estimated 9.0 million people developed new TB cases of which 1.1 million (13%) people were HIV positive. In this 1.5 million people died, including 0.36 million among individuals who have HIV-positive. Among the new TB cases 5% population belongs to age group of 0-14 years [Jeon *et al*., 2014]. Worldwide in 2013, estimations revealed that 0.48 million people developed MDR-TB and there were estimated 0.21 million deaths from MDR-TB. In 2013, 8.6 million people developed TB and 1.4 million people died of TB (including 0.43 million deaths from TB among HIV-positive people). Further, 0.5 million deaths occurred among women, making TB one of the top killers of women worldwide. The majority of TB cases in 2011 occurred in Asia (59%), Africa (26%) and more than 50% of all deaths occurred in Asia alone (**Figure 1.2**) [WHO Global TB report - 2014].

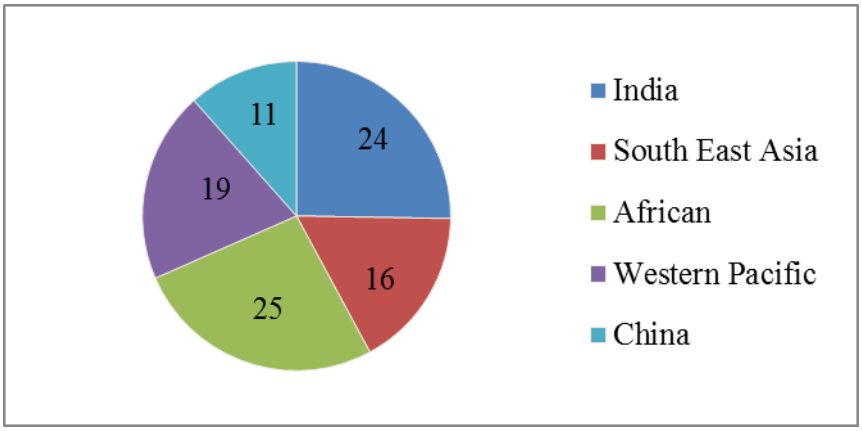


Figure 1.2: Worldwide majority of TB cases [WHO global TB report - 2014]

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

The burden of this fatal disease can be measured in terms of: Incidence (number of new and relapse cases of TB in particular time period), Prevalence (number of cases of TB at a given point of time) and Mortality (number of deaths caused by TB in a given time period). According to WHO estimated burden of TB in India, 2012 is depicted in **Table 1.1** [TB India 2014-Annual Status Report].

Table 1.1: Estimated burden of TB in India 2012 by WHO

TB burden	Number (Millions)	Rate Per 0.1 Million Persons
Incidence	2.2	176
Prevalence	2.8	230
Mortality	0.27	22
HIV among estimated incident TB patients	0.13	5.6[a]
MDR-TB among notified pulmonary TB patients	0.064	-
MDR-TB among notified new pulmonary TB patients	0.021	2.2[a]
MDR-TB among notified re-treatment pulmonary TB patients	0.043	15[a]

[a]in percentage out of 95 notified TB patients in respective disease

TB drug discovery of isoniazid (1952), pyrazinamide (1952), ethambutol (1961) and rifampin (1966) had flourished in the middle decades of last century (**Figure 1.3** and **Table 1.2**) and the initiation of combination chemotherapy led TB from incurable scourge to curable illness by drastically reducing mortality rate and number of incident cases [WHO Global TB report–2014; Diacon *et al*., 2009]. The blooming outcomes of combination treatment throve expectations of total eradication of TB. As expected TB incidence and mortality rates declined, but this was limited to developed countries. Whereas, in case of poor and

developing countries, it increased and prevailed owing to improper treatment options, poor surveillance and also TB co-infection with HIV. In the last two decades, development of drug resistance and HIV/AIDS co-infection has fuelled the TB, as a result the plentiful blazes of TB has fired millions of lives across the globe and has been continuing steadily [Tripathi *et al*., 2012].

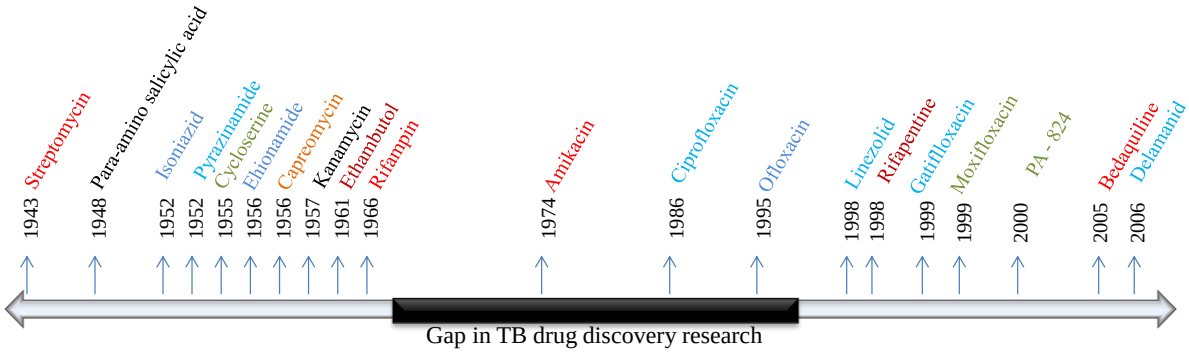


Figure 1.3: History of TB drug discovery [Wong E.B., *et al*., 2013]

1.2. The history to TB

TB is broad chronicle disease, and it has been recorded in history of Egyptian civilisation (3000-2400 BC), the earliest reference was found in ancient Indian Holy Scripture "The Vedas", where TB was referred as "Yakshma" (meaning of wasting disease) written between 1500 and 700 BC [Dhillon *et al*., 2001]. In standard Greece, TB was called as "Phthisis". Hippocrates wrote in his book of epidemics "Consumption was the most considerable of the diseases which then prevailed and only one which proved fatal to many persons". Franciscus identified between the various forms of TB viz. pulmonary and ganglion. TB was first conceived to be contagious by Fracastorius (1443-1553) and Thomas Willis first documented the clinical presentation in his treatise *Pthisilogica*. However the name TB has been ascribed to Laennec in the 1800's [Backer *et al*., 2006]. On 24th March 1882, Robert Koch announced the discovery of the tubercle bacillus as the causative agent of the disease at Berlin Physiological Society [Daniel *et al*., 2006]. Immortalising the centennial of this great discovery, WHO commenced celebrating 24th March as "World TB Day" globally [Herbert *et al*., 2014]. Various forms of TB regimens were developed from time to time. In the year 1921, a French bacteriologist Calmette together with Guerin created the Bacilli Calmette-Guerin (BCG) vaccine. Even though relatively ineffective, it is the only vaccine approved by WHO till date. Presently, BCG is only available vaccine for TB. TB was not epidemic until the second half of the 19th century; ensuing with migration caused by industrialization, migrations from high prevalence nations to developed ones; immigrants on their return to

home country could "bring back" tubercle bacilli and diffusing of the infection [Backer A.D., *et al*., 2006; Jeon *et al*., 2014; Losch *et al.*, 2015]. Since then TB prevalence has progressively increased till mid decades of last century.

TB and HIV pandemics are the leading causes of death in people with HIV/AIDS. Treatment of patients with MTB/HIV co-infection is complicated by drug interactions and toxicity that presents enormous disputes for clinical intervention. Discovery efforts to identify novel compounds with increased effectiveness and decreased drug-drug interactions against MTB/HIV is on progress. Drug resistance as a result of anthropic mistakes such as improper treatment, patient's non-compliance and poor surveillance led to resumption of consumption of TB in the last decade [Arcuri *et al*., 2011; Vijayakumar *et al*., 2013]. The resurged TB unfolded its paw during 1990s and TB was recognised by WHO as a global public health problem in 1990. The fatal conditions even dragged WHO to announce TB as a "Global Public Health Emergency" in 1993, and it was the only disease so far warranted by this designation [Ducati *et al.*, 2006; Akgun *et al*., 2012].

Table 1.2: Group name and mechanism of action of first and second line anti-TB agents

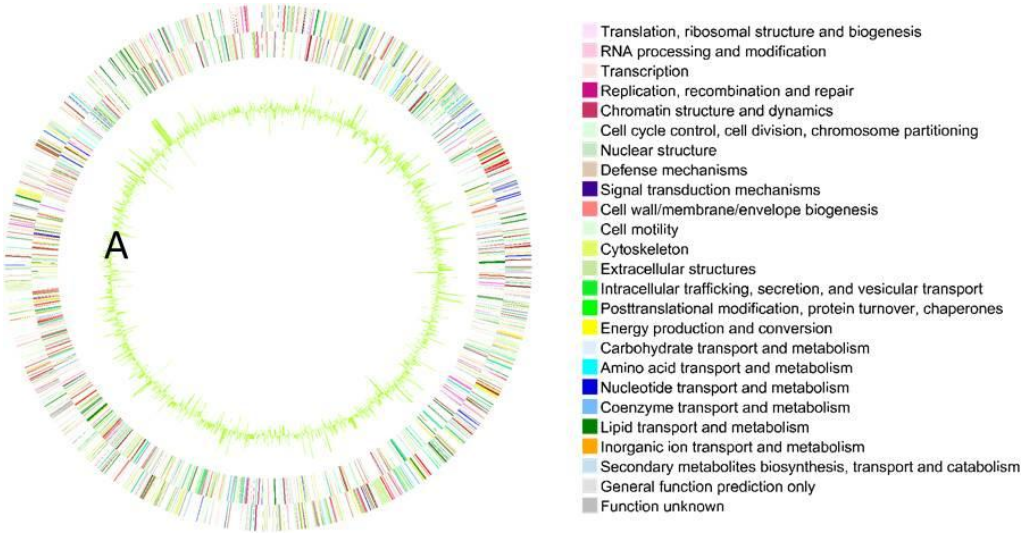
Name of the group	Drug*	Mechanism of action
First line anti-TB drugs	**Isoniazid**	Inhibition of mycolic acid biosynthesis
	Rifampin	Inhibition of RNA synthesis
	Pyrazinamide	Disruption of electron transport across the membrane
	Ethambutol	Arabinogalactone synthesis inhibitor
Second line Injectable anti-TB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

Name of the group	Drug*	Mechanism of action
		metabolism

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

1.3. *Mycobacterium tuberculosis* (MTB): An overview

Robert Koch discovered MTB in 1882. MTB has a strange, waxy coating on its cell surface [Daniel *et al.*, 2006]. The bacterium MTB is one of the cleverest bacteria. It has various unique properties compared to other microorganisms. MTB has rigid cell wall which prevents the penetration of many drugs [Brennan *et al.*, 2003; Dobrikov *et al.*, 2013; Cappoen *et al.*, 2014]. The genome sequence of MTB is one of the first complete genomes to be sequenced, and was decoded in 1998 by Cole and co-workers. It consists of high guanine-cytosine content, and comprises 44,11,529 base pairs, and contains around 4,000 genes (**Figure 1.4**). The H37Rv strain of MTB was isolated in 1905, since then it has been found extensively in many applications related to biomedical research [Cole *et al.*, 1998]. Several of the MTB genes discussed are attractive targets for healing intervention, either through drug development or through incorporation into vaccine strains. It is also previously obvious that a more complete understanding of the pathogenic strategies of this highly successful intracellular pathogen will elucidate novel features of macrophage defenses and the host immune response. In addition to this anticipated scientific dividend, the dividend of greatest immediate importance is the development of new drugs and vaccines against this deadly disease [Moody *et al.*, 2000].



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

Figure 1.4: Genome of *Mycobacterium tuberculosis* [Cole *et al.*, 1998]

1.4. Tuberculosis: Drug resistance

Drug-resistant TB is TB caused by MTB organisms that are resistant to at least one first-line anti-TB drug. Drug resistance in TB therapy is not an immediate past, as the strains of MTB that were resistant to streptomycin were observed soon after its introduction for TB treatment in 1944 [Zhang *et al*., 2009; Keshavjee *et al*., 2012]. These days resistance to all the available anti-TB drugs has been found in different constituents of the world. The most important factors causing drug resistance is incomplete and inadequate treatment procedures (**Figure 1.5**) and it emerges mostly where TB control programmes are feeble [Black *et al*., 2014]. There has been inprogress debate on whether the development of drug resistance in MTB reduces its relative fitness and its ability to cause disease. Demonstration of clinical efficacy in drug-sensitive TB is challenging, given high success rates for existing regimens, concerns about substituting an investigational agent for the most effective agents in a regimen and difficulties in determining the effect size of the components of a combination regimen [Sacks *et al*., 2008; Morcillo *et al*., 2014].

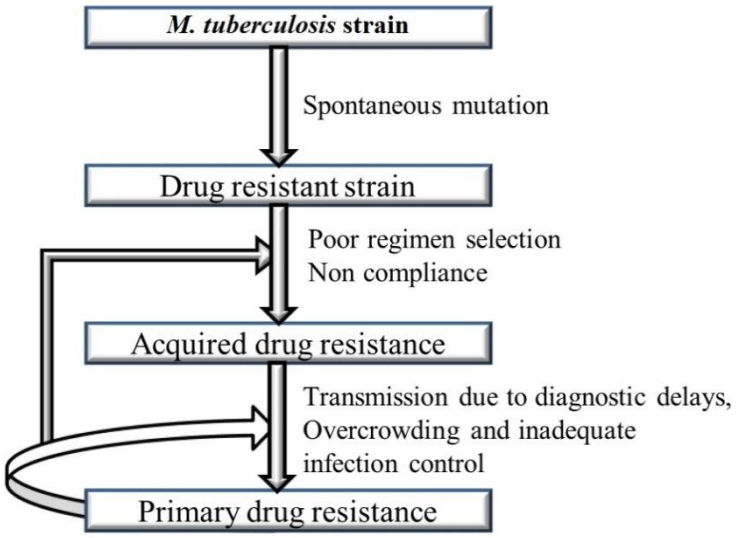


Figure 1.5: Conception in the exploitation of drug resistant TB [Zhang *et al*., 2009]

1.4.1. The intrinsic and acquired drug resistance

The intrinsic drug resistance pertains to the inborn ability of a bacterium to resist the functioning of a particular drug through its inherent structural properties and functions [Karakousis *et al*., 2008]. In case of MTB, intrinsic drug resistance has been ascribed to its unique cell wall structure. The cell wall of MTB built with mycolic acids which are high molecular weight α-alkyl, β-hydroxy fatty acids covalently bonded to arabinogalactan, and

form a rich hydrophobic barrier responsible for resistance to certain hydrophilic antibiotics [Karakoshis *et al*., 2008; Michalska *et al*., 2013]. Apart from hydrophobic cell wall, MTB also possesses β-lactamase enzyme which shows intrinsic resistance to β-lactam antibiotics [Kolyva *et al*., 2009; Dover *et al*., 2011]. These enzymes open the β-lactam ring of the antibiotic thereby altering the chemical structure of the drug, and this causes either failing to reach the target of action or no intended action at the target site. Bacterial efflux mechanism also plays an important role in intrinsic resistance [Webber *et al*., 2003; Pal *et al*., 2014]. Acquired drug resistance happens when MTB obtains the power to resist the activity of a particular antimicrobial agent to which it was previously sensitive [Ducati *et al*., 2006]. The spontaneous mutations in chromosomal gene of MTB, is the cause of acquired drug resistance [Zhang *et al*., 2009]. The rate of genetic mutations in MTB leading to drug resistance varies between ~10^{-5} to ~10^{-6} organisms for isoniazid and 10^{-7} to ~10^{-8} for rifampin.

1.4.2. Multidrug-resistant TB (MDR-TB)

MDR-TB is a form of TB which occurs when MTB strain turns resistant to the most effective anti-TB drugs i.e. isoniazid and rifampin [Moraski *et al*., 2012; Lechartier *et al*., 2014]. This circumstance is a consequence of incompatible chemotherapy which is defined as the use of monotherapy (single drug administration) [Petrini *et al*., 1999], use of inappropriate combination of TB drugs and short treatment periods resultant of patients non-compliance [Trauner *et al*., 2014]. In these situations the MTB pathogen would be exposed to sub-lethal anti-bacterial conditions, which favours the growth of resistant bacilli among an originally drug susceptible pathogenic population [Ducati *et al*., 2006].

Globally in 2012, an estimated 0.45 million people developed MDR-TB and 0.17 million died of this drug-resistant strain [WHO MDR-TB fact sheet 2014]. India, China and Russian federation contribute more than half of the world's cases of MDR-TB [Zignol *et al*., 2012]. The "Global Plan to Stop TB" estimates that one million MDR-TB patients need to be set on treatment in between 2011-2015 (**Figure 1.6**) [WHO MDR-TB fact sheet 2013]. An estimated 0.48 million cases of MDR-TB arise each year among both primary and acquired drug resistance cases [WHO Global TB report-2014].

In general, MTB resistant strains are in regions where there is less availability of drugs to fight disease, because non treated patients either die or become chronic bacilli disseminators, but their infecting bacteria usually do not develop any kind of drug resistance. However, among the nations with a greater and ready availability of anti-tubercular regimen (developed

countries), the drug resistance rates were observed to be higher although having less TB incident cases [Ducati *et al.*, 2006]. MDR-TB was much more difficult and pricey to treat than fully drug susceptible TB, and also the cure rate is found to be less (50-60%) compared to cure rate of (94-97%) patients with drug sensitive TB [Kwon *et al.*, 2014].

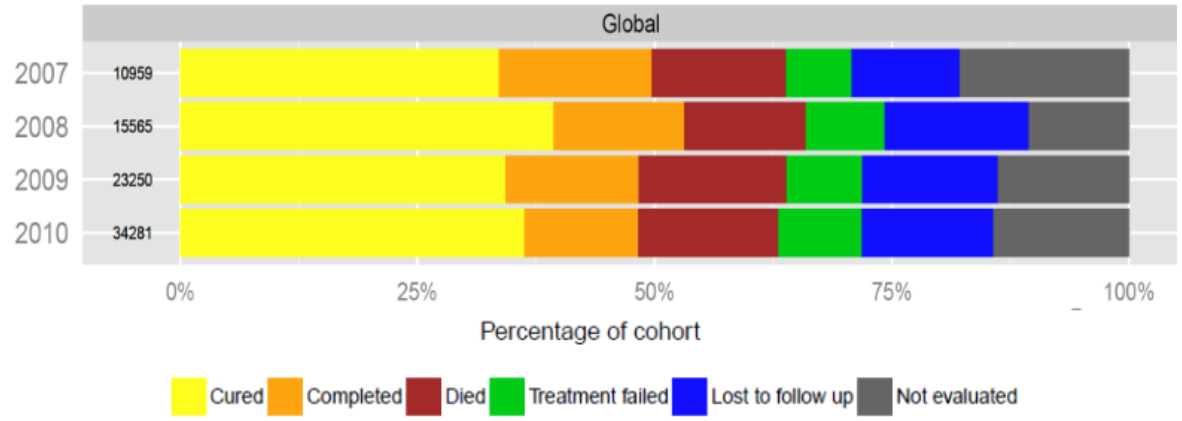


Figure 1.6: Worldwide treatment outcomes (2007-2010) for MDR-TB patients who started undergoing treatment [WHO MDR-TB 2013 update]

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

The XDR-TB is a form of MDR-TB which occurs when MTB strain is resistant to at least isoniazid and rifampin in addition to being resistant to one of the fluoroquinolones, as well as resistant to at least one of the second line injectable drugs i.e. amikacin, kanamycin or capreomycin. Inappropriate, poor and partial management of MDR-TB cases resulted in a new type of drug resistant TB, well known as extensively drug-resistant TB [Shah *et al.*, 2007; Migliori *et al.*, 2007; Fauci *et al.*, 2008]. A total of 100 countries have reported XDR-TB in 2013. On average, an estimated 9% of people with MDR-TB have XDR-TB. Globally, 54 countries and territories enrolled XDR-TB cases for healing in 2013, majority of which were notified from Ukraine (1006), South Africa (612), India (364) and Kazakhstan (305) [WHO Global Tuberculosis report-2014].

1.4.4. Totally drug resistant TB (TDR-TB) or extremely drug resistant TB (XXDR-TB)

The TDR-TB or XXDR-TB has not been clearly defined by WHO. It occurs when MTB strain becomes resistant to all first and second line anti-TB drugs, in addition to other drugs rifabutin, thiacetazone, clofazamine, dapsone and clarithromycin [Sharma *et al.*, 2013]. The emergence of HIV/AIDS fuelled the resurgence of TB globally [Ginsberg *et al.*, 2009]. Recently, some parts of the world have reported the cases of XXDR-TB and TDR-TB also

called as super XDR-TB [Migliori *et al.,* 2007; Loewenberg *et al.,* 2012]. The clinical isolates of TDR-TB were observed in Italy for the first time in 2003 [Migliori *et al.*, 2007], next in Iran [Velayati *et al.,* 2009] and now it has been reported from India that there were TB patients who did not respond to any of the anti-tubercular regimens [Udwadia *et al.*, 2012; Udwadia *et al.,* 2013]. Despite the fact that it is not yet prevalent, chances are bright to through a challenge of TDR-TB to researchers encircling the world.

1.4.5. Rifampicin-resistant TB (RR-TB)

The RR-TB is caused by MTB strains resistant to rifampicin, with or without resistance to other drugs. It includes any resistance to rifampicin, whether mono-resistance, multidrug resistance, polydrug resistance or extensive drug resistance. Both MDR-TB and XDR-TB are forms of RR-TB [WHO Global Tuberculosis report-2014].

1.5. Present therapy for TB

1.5.1. Treatment for drug susceptible-TB

Generally, TB treatment duration is 6-18 months, depending on drug susceptible or drug resistant form of TB. The standard therapy of TB is 6 months, the result of a sequence of intensive trials conducted over 20 years by the British Medical Research Council [Fox *et al*., 1999; Lienhardt *et al*., 2010]. The existing TB treatment consists of isoniazid, ethambutol, rifampicin and pyrazinamide for two months followed by isoniazid and rifampicin for four months (**Table 1.3**) [Yee *et al.,* 2003; Wong *et al.*, 2013]. This standard TB therapy is prolonged as patients have to take the drugs for six months and often leads to patient's non-adherence (**Figure 1.7**) [Ma *et al*., 2009; Yew *et al*., 2011; Trauner *et al.,* 2014;]. In these circumstances an incomplete treatment results in development of drug resistance. To confront this situation, WHO promoted a program known as "Directly Observed Treatment-Short course (DOTS)", DOTS is effective in many controlled trials, few studies have evaluated its effectiveness under programmatic conditions. DOTS based Revised National TB Control Programme (RNTCP) was initiated in June 2000. [Hegymegi-Barakonyi *et al*., 2008; Chan *et al*., 2013; Pieroni *et al*., 2014; Joseph *et al.,* 2015]. In this type of treatment there is a direct observation by trained personnel on patients undergoing treatment. The DOTS therapy has established to be one of the most cost effective health interventions available today around the world [Singhal *et al*., 2011; Villemagne *et al*., 2012; Elkomy *et al*., 2013; Getahun *et al*., 2013].

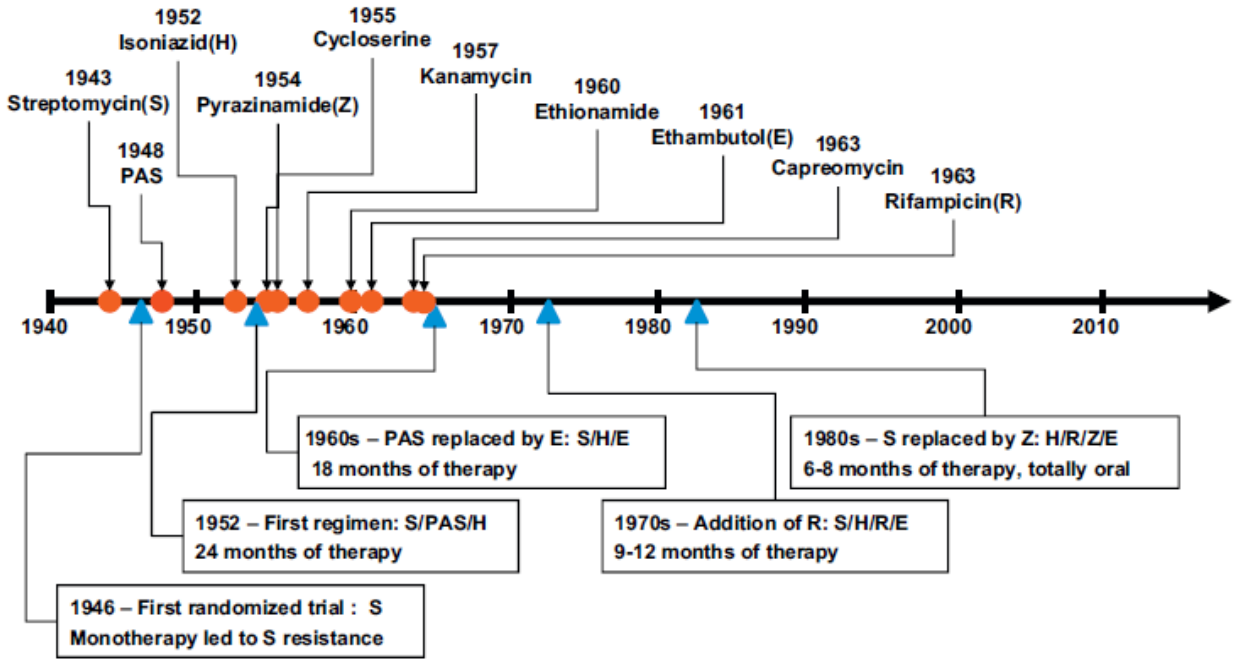


Figure 1.7: Treatment duration of TB drug discovery and regimen development [Ma *et al.*, 2009]

Table 1.3: WHO acceptable regimen for drug-susceptible TB [Wong *et al.*, 2013]

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

[a]Maximum daily dose 300 mg; [b]maximum daily dose 600 mg.

1.5.2. Therapy for drug resistant-TB

The drug resistant TB can be mostly cured with the appropriate combination and rational use of available anti-tubercular drugs. The poly drug-resistant TB therapy of contagions involving (INH + EMB, INH + PZA, EMB + PZA, or INH + EMB + PZA) MTB strains entails careful clinical evaluation but can be managed with extended treatment (~18 months) by regimens containing first-line drugs, fluoroquinolones, KAN/AMI/CAP and some second-line agents. Treatment of MDR-TB is lengthy, expensive, toxic, and associated with higher rates of clinical failure and disease relapse [Caminero *et al.*, 2006; Lew *et al.*, 2008; Ahmad and Mokaddas 2014]. The flourishing supervision of MDR-TB requires drug susceptibility testing (DST) for first- and second-line drugs and monitoring of patients for bacteriological

(sputum smear and culture) and radiological improvement and unfavourable drug reactions. In developing countries, treatment of MDR-TB is difficult due to delayed diagnosis, insufficient DST facilities, and the lack of adequate supplies of second-line drugs. Two basic healing approaches are used. Either standardized treatment regimens are designed based on representative drug-resistance surveillance data, or individualized regimens are adapted based on a previous chronicle of anti-TB treatment and DST results. 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 1.8** and **Figure 1.9**), composition of MDR-TB drug regimen [[Orenstein *et al*., 2009; Caminero *et al*., 2010; Van Deun *et al*., 2010; Ahuja *et al*., 2012; Lange *et al*., 2014].

1. Choose, if possible:

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

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

c) Ethionamide or prothionamide

d) Cycloserine or terizidone

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

3. Healing for a total of 24 months with an intensive phase of 8 months

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

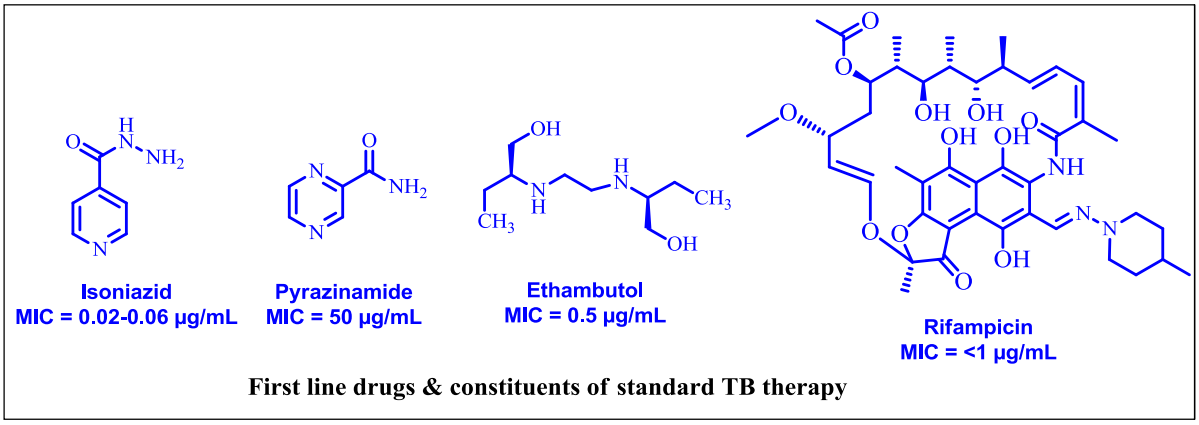


Figure 1.8: First line line anti-TB drug structures with MIC values.

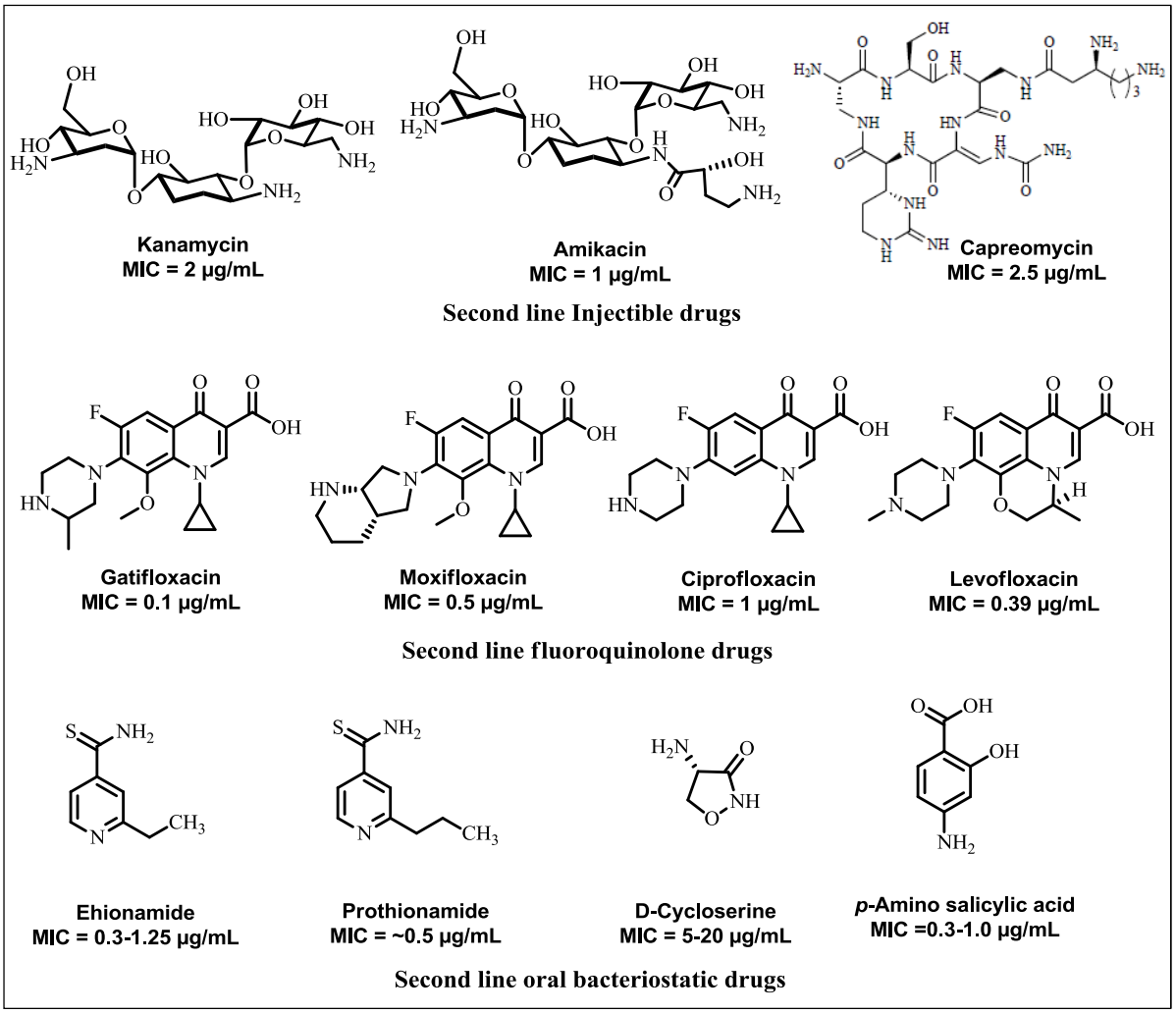


Figure 1.9: Second line anti-TB drug structures with MIC values.

Recently USFDA has approved bedaquiline (TMC-207) and delamanid (OPC-67683) for the treatment of MDR-TB in adults [Koul *et al*., 2007; Koul *et al*., 2008; Ashish et al., 2014; WHO Global TB report, 2014;].

Introduction of bedaquiline to standard therapy for MDR-TB importantly shortens the treatment time [Diacon *et al*., 2009]. WHO and the US centre for disease control and prevention (CDC) recently suggested that, bedaquiline may be used for treatment of MDR-TB in adults when MDR-TB patients in serious or life-threatening conditions an effective treatment regimen is not available. However there are safety issues with this drug, as it showed an increased risk of death and QT prolongation [Mase *et al*., 2013]. SIRTURO (bedaquiline fumarate) a bedaquiline drug for oral administration is available as 100 mg tablets. SIRTURO has received conditional approval from EMA in March 2014 to market in EU region for the treatment of MDR-TB. Each tablet contains 120.89 mg of bedaquiline

fumarate, which is equal to 100 mg of bedaquiline. SIRTURO should only be used in combination with at least three other drugs to which the patient's MDR-TB isolate is susceptible *in vitro*.

The OPC-67683 used for treatment of MDR-TB in adults, when MDR-TB patients in serious or life-threatening conditions an effective treatment regimen is not available. Curative rates (measured as sputum culture conversion) were significantly better in patients who additionally used Delamanid [Gler *et al*., 2012; Spreitzer *et al.*, 2013]. To appraise the cost-effectiveness of adding delamanid (Deltyba^{TM}) to a background regimen (BR) for treating MDR-TB. Deltyba^{TM} (Delamanid) marketed in Germany for pulmonary MDR-TB patients. MDR-TB patients if they use Deltyba^{TM}, duration of treatment period and cost also reduces (**Table 1.4**) [Diel *et al.*, 2015; Lessem *et al.*, 2015].

For healing XDR-TB or TDR-TB, drugs need to be selected stepwise on basis of safety and efficacy. New drugs (PA-824, OPC-67683 and TMC-207) and novel regimens (PA-824-Moxifloxacin-Pyrazinamide (PaMZ) and NC-003) for healing drug resistant TB are now available. These novel chemospheres are reducing the treatment period and cost of therapy. [Wong *et al.*, 2013].

Table 1.4: Delamanid results of MDR-TB treatment in Trial

Treatment outcome	MITT solid total N = 421	
	delamanid ≤ 2 months	delamanid ≥6 months
	Consent to trial 116 N = 229	Consent to trial 116 N = 192
Favourable treatment outcome	126 (55.0%)	143 (74.5%)
Unfavourable treatment outcome	103 (45.0%)	49 (25.5%)
Cured	111 (48.5%)	110 (57.3%)
Treatment completed	15 (6.6%)	33 (17.2%)
Failed	26 (11.4%)	32 (16.7%)
Defaulted	58 (15.3%)	15 (7.8%)
Died	19 (18.3%)	2 (1.0%)

MITT = modified intent-to-treat

1.5.3. Treatment for latent-TB infection

The Latent-TB infection (LTBI) is the presence of MTB organisms without symptoms or radiographic evidence of active disease [Menzies *et al*., 2011]. LTBI testing is very mandatory for: a) health care workers (HCWs), b) close contacts of infectious TB patients, and c) frequent travellers abroad. Because TB-infected patients ultimately present themselves to healthcare providers, HCWs are particularly susceptible to TB exposure and infection. The HCWs who have duties that involve face-to-face contact with patients with suspected or confirmed TB (including transport staff) should be included in a TB screening program [Demkow *et al*., 2008; Caglayan *et al*., 2011; Ringshausen *et al*., 2011; Lee *et al*., 2012; Adjoh *et al*., 2013; El-Helaly *et al*., 2014]. For treating LTBI there are few regimens used based on the results of drug susceptibility testing [Panickar *et al*., 2007].

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

b. Rifampicin four months therapy: Though this therapy is shorter than isoniazid therapy, it cannot be suggested for routine use until the reviewing of regular results of the efficacy trial. This therapy requires direct observation treatment.

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

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

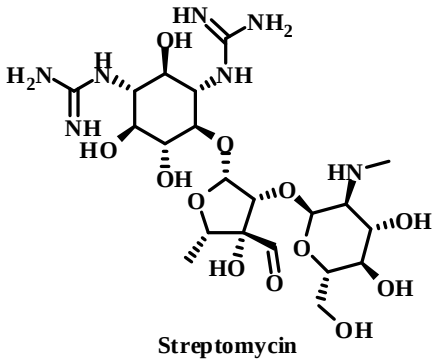
1.6. Classification of anti-TB drugs

TB still remains one of the deadliest communicable diseases in the globe. Present chemotherapy mostly owes to persistence of tubercle bacillus and inadequacy. The rising emergence of drug-resistant TB along with the HIV threatens infection control, to understand working of present drugs and necessitates development of more effective drugs. The

accessible anti-TB drugs can be classified based on their mechanism of action or inhibition. These are classified under following heads i) protein synthesis (amikacin, kanamycin & capreomycin), ii) cell wall synthesis (isoniazid, ethambutol, ethionamide and cycloserine), iii) nucleic acid synthesis (rifampin and quinolones), and iv) electron transport across the bacterial membrane (pyrazinamide) [Zhang 2005; Wong *et al.*, 2013]. Even though drug susceptible TB success rates are high as 95-98%, once the bacterium develops drug resistance (MDR-TB & XDR-TB) these infections are uncurable completely. The bacterial sub populations although drug-susceptible, can display phenotypic drug resistance in response to altered environmental signals. The strains of TB resistant to all the above drugs have been isolated from clinical isolates of different stages of TB-infected patients. Hence for novel drugs possessing different mechanisms of action to kill different bacterial sub populations is extremely necessary [Mak *et al.*, 2012; Wong *et al.*, 2013]. The current anti-tubercular regimen, their mechanism of action and healing confinement has been briefed below.

1.6.1. Protein synthesis Inhibitors

Streptomycin

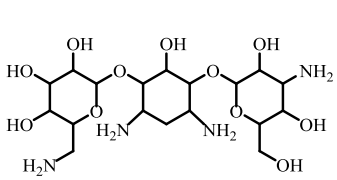


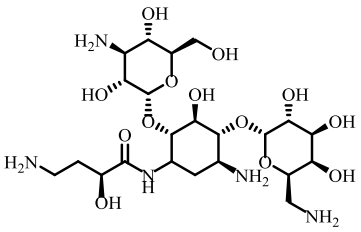
Streptomycin

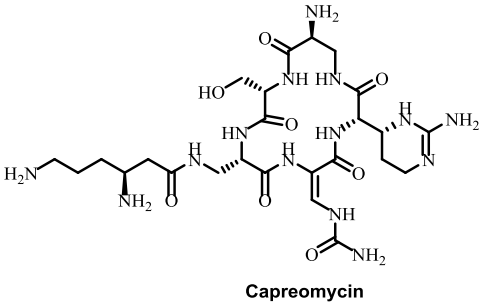
Streptomycin was first isolated in 1943. It is the first antibiotic drug used against TB and was derived from the actinobacterium *Streptomyces griseus*. Streptomycin has MIC 1 μg/mL with a half-life of 5-7 h. Because of its poor absorption through gastrointestinal tract, the action of administration is intramuscular and very occasionally by intrathecal route [Tripathi *et al.*, 2012]. Streptomycin acts as inhibitor of protein synthesis by binding to the S12 protein of the 30S subunit of the bacterial ribosome and interfering with the binding of formyl-methionyl-tRNA to the 30S subunit of the ribosome. These results in precarious ribosomal-mRNA complex, foremost to a frameshift mutation and flawed protein synthesis and further to cell death. Although resistance to streptomycin has become less common owing to its side effects it is not used currently in TB chemotherapy. Streptomycin exhibits toxic manifestations on

peripheral and central nervous system at higher doses and leads to hypersensitivity reactions [Sharma *et al.*, 2007].

Kanamycin, Amikacin and Capreomycin





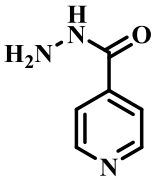


Kanamycin (KAN), Amikacin (AMK) and Capreomycin (CAP) are significant second-line drugs used to treat patients with MDR-TB. KAN and AMK belong to aminoglycoside family of drugs and these are protein synthesis inhibitors. These drugs target the 30S subunit of ribosome [Alangaden *et al.*, 1998; Suzuki *et al.*, 1998] in MTB strain. The A1401G mutation has been assorted with AMK resistance in MTB, and change in their *rrs* genes. CAP is a macrocyclic polypeptide, like streptomycin and KAN modifies the ribosomal structure at 16S RNA there by inhibits protein synthesis [Maus *et al.*, 2005; Johansen *et al.*, 2006]. MTB resistant to KAN and CAP has been associated with mutations in the *rrs* gene encoding 16S rRNA [Alangaden *et al.*, 1998; Maus *et al.*, 2005].

1.6.2. Cell wall synthesis inhibitors

Currently, cell wall synthesis inhibitors such as Isoniazid, Ethambutol, Ethionamide, Prothionamide and Cycloserine are used for TB treatment. Their brief mechanism of action and effects is outlined below:

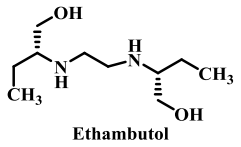
Isoniazid



Isonicotinyl hydrazide or Isonicotinic acid hydrazide or INH was introduced in 1951 for the treatment of TB and it is more potent drug than streptomycin and *p*-aminosalicylic acid. INH has in vitro activity against MTB MIC in the range of 0.01–0.2 μg/mL. It is the most widely used first line anti-tubercular orally active drug [Singh *et al.*, 1954; Vilcheze *et al.*, 2011]. It

is a prodrug activated by 'catalase peroxidase' enzyme (*KatG*) [Matsumoto *et al*., 2007; Murillo *et al*., 2007] and active against growing tubercle bacilli, but not active against non-replicating bacilli. For INH inhibition "enoyl acyl carrier protein reductase (InhA)" enzyme is primary target [Tonge *et al*., 2007]. InhA is involved in elongation of fatty acids in mycolic acid synthesis. *KatG* activates isoniazid to produce a range of highly reactive species, which attack multiple targets. Such reactive species isonicotinic-acyl anion or radical reacts with NAD(H) to form an isoniazid-NAD adduct, and then attacks InhA. Recent research shows that besides InhA it also attacks DfrA ('dihyrofolate reductase' involved in DNA synthesis) [Wilming and Johnsson, 1999; Zhang *et al*., 2009]. Since its wide range of usage, resistance to INH has been seen more repeatedly among clinical isolates of MTB infected patients. Resistance to INH occurs due to the mutations in *KatG* gene; as a result the ability of catalase peroxidase to activate INH prodrug reduces (**Table 2.1**). The Hepatitis, lupus-like syndrome, peripheral neuropathy and drug-drug interactions are major adverse reactions of INH [Rozwarski *et al.,* 1998; Hazbon *et al.,* 2006; Vilcheze *et al*., 2011].

Ethambutol

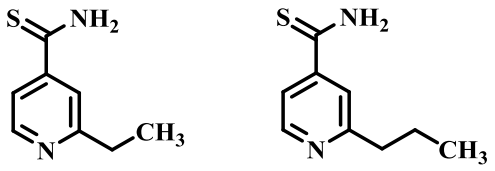


Ethambutol

Ethambutol (EMB) or chemically ethylene diamino-di-1-butanol, is discovered in 1961. EMB is a first line anti-tubercular drug. Jointly with isoniazid, rifampicin and pyrazinamide constitutes short course for the treatment of drug sensitive TB. It intervenes with the biosynthesis of cell wall of MTB, and it disrupts cell wall synthesis by particularly targeting the polymerization of arabinogalactans and lipoarabinomannan. Inhibition of synthesis of arabinogalactan (the chief constituent of bacterial cell wall) leads to increased permeability of the bacterial cell wall. (S, S)-isomer (dextro) form of EMB is 600 times more active than (R, R)-isomer [Lee *et al*., 1995; Shepherd *et al*., 1996; Yendapally *et al*., 2008; Tripathi *et al*., 2012]. The enzyme arabinoyl transferase encoded by the gene *embB* involved in the synthesis of arabinogalactan has been proposed as the target of EMB in MTB [Hasan *et al*., 2006]. Resistance to EMB is generally associated with mutations in the *embCAB* operon, in particular *embB* and infrequently *embC*. Some inconsistent reports revealed that one quarter of all EMB resistant MTB isolates do not harbour mutations in any of the above named

genes, investigations suggesting further canvases needed to explore possible mechanism of EMB resistance [Escuyer *et al*., 2001; Vilcheze *et al*., 2011].

Ethionamide and prothionamide



Ethionamide **Prothionamide**

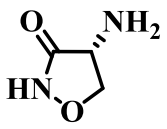
Thioamide drugs are Ethionamide (ETH) and Prothionamide (PTH), these are generally considered second-line drugs for treatment of TB, ETH, 2-ethylisothionicotinamide are structurally related to INH and are bactericidal against MTB. Like isoniazid, ETH and PTH are also a prodrug requiring activation by the monooxygenase EthA/EtaA [Vannelli *et al*., 2002; Debarber *et al*., 2000]. EtaA/EthA is a flavin adenosine dinucleotide (FAD) containing enzyme that oxidises ETH to the corresponding S-oxide. Similar to isoniazid, ETH inhibits mycolic acid synthesis by binding to the enzyme InhA. PTH is almost similar to ETH with regard to structure and activity [Wang *et al*., 2007; Yew *et al*., 2011]. Mutations of a gene chosen ethA were repeatedly found in the clinical isolates resistant to ETH [Baulard *et al*., 2000; Debarber *et al*., 2000; Morlock *et al*., 2003; Wang *et al*., 2007; Vilcheze *et al*., 2008]. The sublimated ETH-NAD and PTH-NAD adducts are showed nanomolar K_is against MTB [Wang *et al*., 2007]. Particularly, few drugs of the various genes involved in drug resistance are enlisted in **Table 1.5**.

Table 1.5: MTB genes and associated drug resistance [Wong *et al*., 2013]

Drug	**Effect on bacterial cell**	**MTB gene**	**Role of gene product**
		katG	Catalase/peroxidise
Isoniazid	Bactericidal	*inhA*	Enoyl reductase
		aphC	Alkyl hydroperoxide reductase
Rifampicin	Bactericidal	*rpoB*	β-subunit of RNA polymerase
Pyrazinamide	Bactericidal	*pncA*	Pyrazinamidase/nicotinamidase
Ethambutol	Bacteriostatic	*embB*	Arabinosyl transferase
Streptomycin	Bacteriostatic	*rpsL*	S12 ribosomal protein

Drug	Effect on bacterial cell	MTB gene	Role of gene product
		rrs	16S rRNA
		gidB	7-methylguanosine methyltransferase
Fluoroquinolones	Bactericidal	*gyrA/gyrB*	DNA gyrase
Kanamycin/ Amikacin	Bactericidal	*rrs*	16S rRNA
Ehionamide	Bacteriostatic	*inhA*	Enoyl reductase
p-aminosalicylic acid	Bacteriostatic	*thyA*	Thymidylate synthase A

Cycloserine

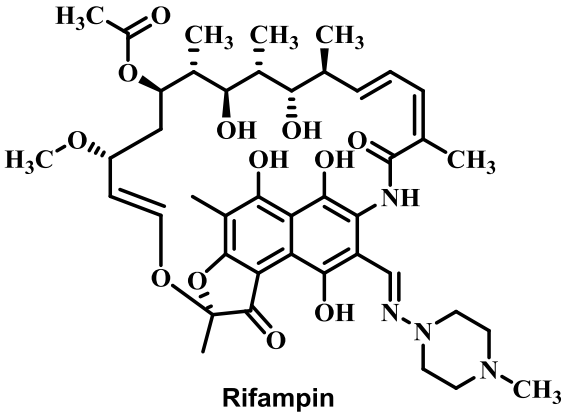


D-Cycloserine

Cycloserine (4-amino-3-isoxazolidinone or (R)-4-Amino-1,2-oxazolidin-3-one) is used for the treatment of TB, in particularly healing drug resistant TB (MDR-TB and XDR-TB). It is classified as a second-line drug. Cycloserine is a structural analog of the amino acid D-alanine, which inhibits the synthesis of cell wall mycobacteria by obstructing the mode of D-alanine racemase and D-alanine: D-alanine ligase [Lambert *et al*., 1972; Strych *et al*., 2001]. Cycloserine possesses activity against a wide range of bacteria [Otten *et al.*, 1998], and inhibits MTB at concentrations of 5-20 μg/mL [David *et al*., 1969]. Cycloserine produces side effects in the central nervous system that could also generate psychotic states with suicidal inclinations and epileptic paroxysm [Cacers *et al*., 1997]. Resistance to cycloserine is due to overexpression of AlrA and Dd1 [Feng *et al*., 2003].

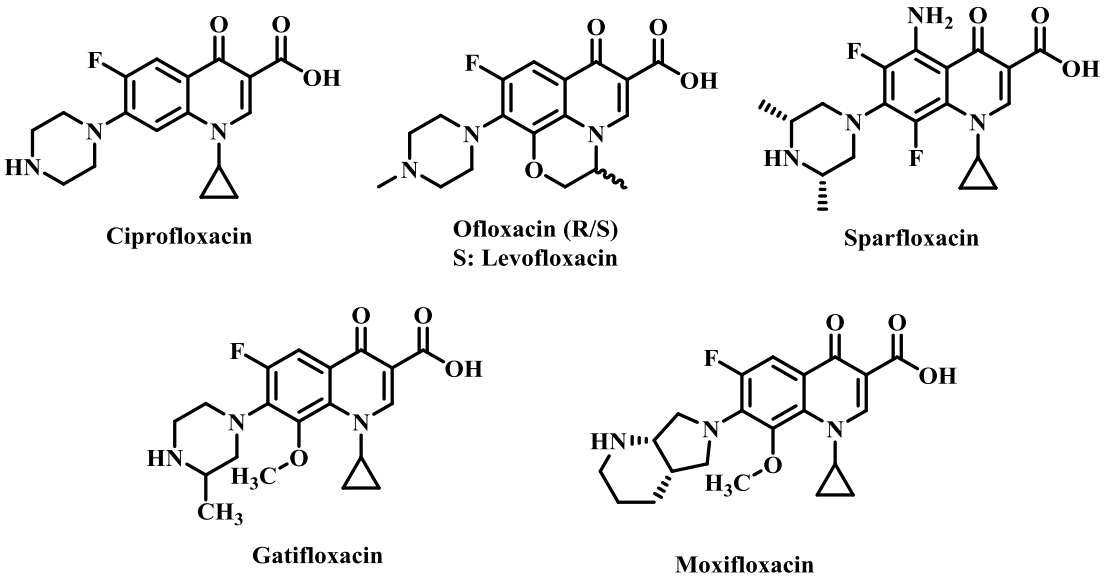
1.6.3. Nucleic acid synthesis inhibitors

Rifampin



Rifampin (RMP) or Rifampicin (RIF) introduction into the standard anti-TB regimen reduced the duration of treatment from 18 months to 9 months. Higher doses of newly emerged drug rifapentine, has the potential to further decrease the duration of TB treatment, although further studies are needed to evaluate maximum tolerated dose of this drug [Ginsberg *et al*., 2009]. The mode of action of RMP is inhibition of DNA-dependent RNA polymerase. Rifampin binds to β-subunit of the enzyme RNA polymerase, an enzyme necessary for RNA synthesis, thus preventing transcription to RNA and subsequent translation to proteins. Resistance to RMP is a result of mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase [Campbell *et al*., 2001; Feklistov *et al*., 2008].

Fluoroquinolones

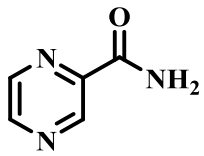


The fluoroquinolones viz. ciprofloxacin, moxifloxacin, gatifloxacin and levofloxacin are furthermost significant bactericidal antibiotics and have broad spectrum activity. These are

active against both gram-positive and gram-negative bacteria. Gatifloxacin and moxifloxacin are under phase III clinical evaluation aiming at better TB treatment. Fluoroquinoles inhibits both ATP dependent DNA gyrase (topoisomerase II) as well as ATP dependent topoisomerase IV [Kato *et al*., 1990; Takei *et al*., 2001]. Fluoroquinolones block the movement of replication works and transcription complexes [Drlica *et al*., 2003]. Resistance to fluoroquinolones in MTB is due to mutations in the conserved quinolone resistant-determining region of gyrA and gyrB involved in the interaction between the drug and DNA gyrase. The cytotoxicity of fluoroquinolones is probably a 2-step process involving, i) transformation of the topoisomerase quinolone-DNA complex to an irreversible form and ii) generation of a double-strand break by denaturation of the topoisomerase, which are conceived to be essential for the bacterial deadliness produced by fluoroquinolones [Cole *et al*., 1998; Khodursky *et al*., 1998; Fournier *et al*., 2000; Hooper 2001].

1.6.4. Electron transport across membrane inhibitors

Pyrazinamide



Pyrazinamide

Pyrazinamide (PZA) or pyrazine-2-carboxamide is an important front-line drug for the treatment of TB, it is used in combination with additional drugs viz. isoniazid and rifampicin for the treatment of MTB. It is still part of WHO suggested standard TB therapy. This can be accredited to its unique role in shortening TB treatment from preceding 9-12 months to 6 months. The PZA kills the semi-dormant populace of bacilli residing within an acidic environment. Like isoniazid, PZA is a prodrug and it necessitates activation to its active form pyrazinoic acid by the enzyme pyrazinamidase/nicotinamidase [Silva *et al*., 2011; Almeida *et al*., 2014]. The anti-tubercular activity of PZA has been attributed to distraction of electron transport transversely the membrane. However, mutations in this gene are merely associated with roughly 70% of observed PZA resistance. The PZA resistance in MTB is due to mutations in the *pncA*, furthermore to PZA resistance associated with *pncA*, *rpsA* or *panD* mutations, it has also been described it can change *pncA* expression, altered PZA update, or dysregulated pyrazinoic acid efflux, which creates fault in the functioning of pyrazinamidase. Hyperuricemia, gouty arthritis and rarely nephritis are major adverse reactions observed with

PZA [Cheng *et al*., 2000; Jureen *et al*., 2008; Alexander *et al*., 2012; Simons *et al*., 2012; Zimic *et al*., 2012; Zhang *et al*., 2013].

An overview of all these anti-TB drugs, a pictorial representation of various mechanism of action has been delineated in **Figure 1.10**.

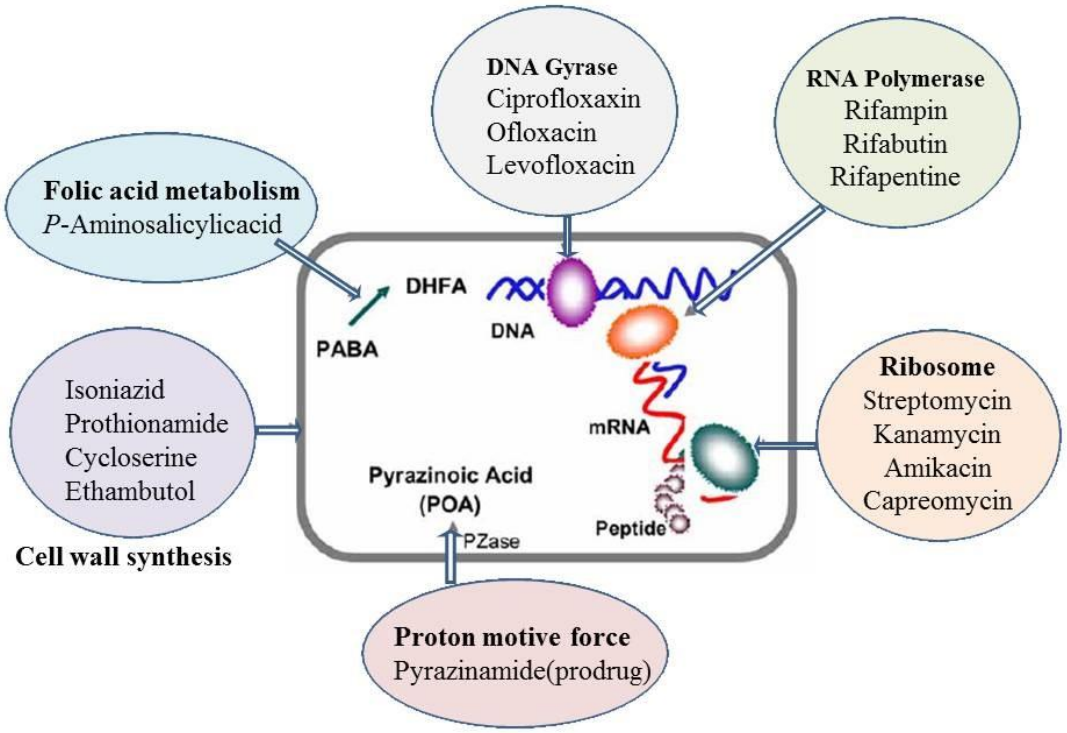


Figure 1.10: Present anti-TB drugs and their mechanism of action [Laurenzi *et al*., 2007]

1.7. Pipeline of Anti-TB drugs in drug discovery

An important hurdle to the victorious therapy of TB with current drugs is the time-span and complexity of the treatment protocols. The assortment of anti-TB agents currently in research and development is usually referred as TB drug-pipeline [Laurenzi *et al*., 2007; Ahmad *et al*., 2014]. The current decade blossoms with a promising anti-TB drug pipeline, with various potential drugs targeting diverse MTB terminating sites in several stages of drug development (**Figure 1.11**). Few drug candidates in various stages of drug progress are also in market (e.g., Deltyba, Situro) before finishing complete process of phase III trials. The novel drug combinations which propose to combat drug resistant TB and reduce the duration of therapy are in pipeline. The 21st century has seen coordinated efforts on TB drug development that has included the cooperation of non-profit organizations, Global Alliance (TB Alliance), international funders and researchers. The different phases of clinical trials in drug discovery

pipeline are presented in **Table 1.6** [Lienhardt *et al.*, 2010; Wong *et al.*, 2013; WHO Global Tuberculosis report-2014].

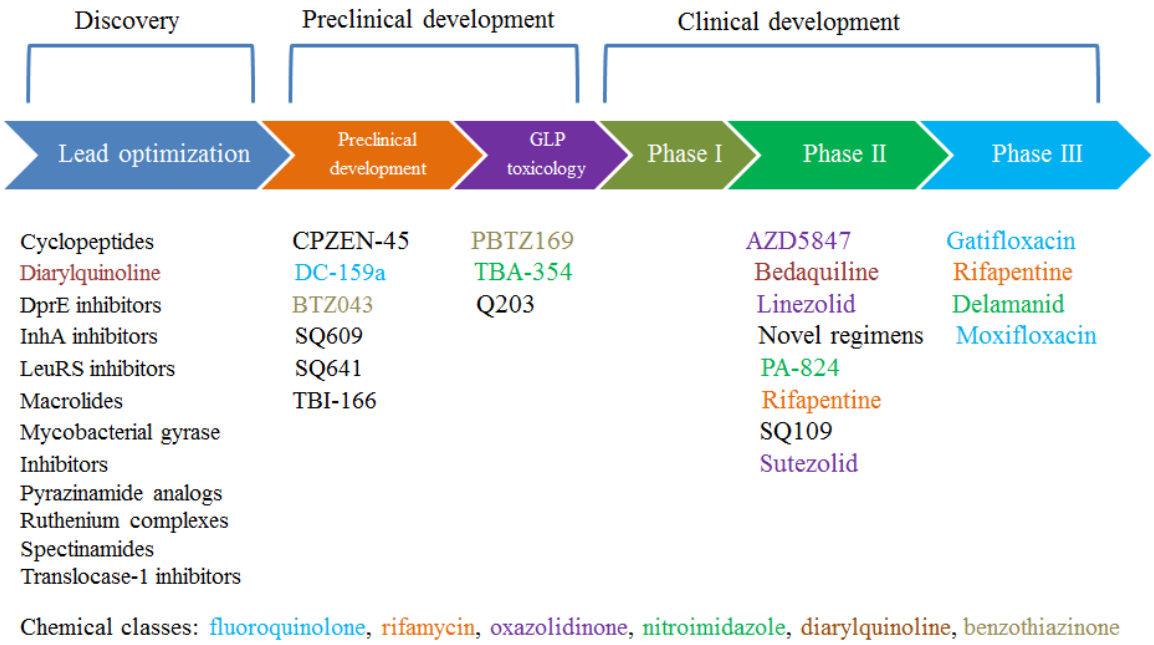


Figure 1.11: Various agents that are currently being investigated for TB therapy

Several individual efforts to develop new TB diagnostics, drugs, and vaccines have heightened throughout the past decade. However, significant progress and investment is still essential for the development of an accurate, easy-to-use, reasonable point-of-care assay for the hasty and early diagnosis of TB. According to WHO Global TB report, presently there are seven drugs and one novel regimen in phase II clinical trials, four drugs in phase III clinical studies and several new potent entity drug candidates and set of molecules that are in lead optimization and preclinical development [Lienhardt *et al.*, 2010; WHO Global TB report-2014].

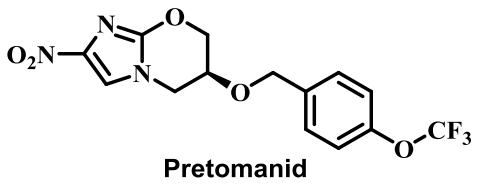
Table 1.6: Various phases of anti-TB agents in clinical trials

Phases	Description
Phase I trials	Preliminary studies to ascertain the metabolism and pharmacologic actions of drugs in humans, the side effects versus quantity of doses, and to gain early evidence of effectiveness; may include healthy participants or patients.
Phase II trials	Controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks. This

Phases	Description
	phase can also be used to establish dose ranges and dose-response relationships.
Phase IIa	Addresses dose and dose-response with limited numbers of participants (typically 30-50)
Phase IIb	Addresses risks and efficacy with large number of participants (typically 200-500)
Phase III trials	Expanded controlled and uncontrolled trials conducted after preliminary evidence suggesting effectiveness of the drug has been obtained, that are intended to gather additional information to evaluate the overall benefit-risk relationship of the drug and provide adequate basis for physician labelling based on established short and long-term safety and efficacy of the drug.
Phase IV trials	Post marketing studies to delineate additional information, including the drug's risks, benefits, and optimal use in populations.

1.7.1. Phase II clinical trials of TB therapy drugs

1.7.1.1. Pretomanid (PA-824)

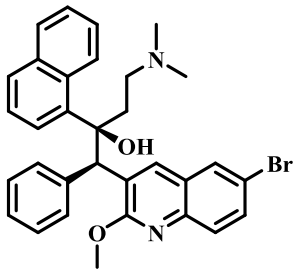


Pretomanid

Pretomanid (PA-824) is an investigational anti-TB drug, PA-824 is a bicyclic nitroimidazole-identical molecule with a very complex mechanism of action. New regimens based on nitroimidazole novel agents are sought in order to shorten or abridge the treatment of both drug-susceptible and drug-resistant forms of TB. PA-824 is a nitroimidazo-oxazine now in phase II trials and has shown significant early bactericidal activity alone and in combination with the newly approved agent bedaquiline or with pyrazinamide with or without moxifloxacin. PA-824 is a new potential drug in clinical development pipeline developed by TB-alliance (**Table 1.7**). It has entered phase-I studies in 2005 and after successfully completing phase-I trials it is now in phase-II clinical trials. PA-824 is capable of treating drug sensitive as well as MDR/XDR-TB [Laurenzi *et al*., 2007; Anderson *et al*., 2008; Kmentova *et al*., 2010; Palmer *et al*., 2010; Tasneen *et al*., 2015]. PA-824 is also a prodrug like isoniazid and requires the activation of aromatic nitro group by F420-dependent

mechanism [Yew *et al*., 2011]. **PA-824** inhibits both protein and lipid synthesis but does not affect nucleic acid synthesis. It can undergo nitro reduction producing highly reactive intermediates which then react with multiple targets inside the bacterial cell. **PA-824** *in vitro* studies indicate that it is active at MIC similar to that of isoniazid [Lenaerts *et al*., 2005; Tasneen *et al*., 2015]. Furthermore, *in vitro* studies with anaerobic culture models indicate that PA-824 has activity against non-replicating bacilli as well [Stover *et al*., 2000]. It also showed activity against strains with known resistance to standard TB treatment [Tyagi *et al*., 2005]. **PA-824** has been observed to kill bacteria in two distinct mechanisms: a) by interfering with the synthesis of ketomycolate which is an essential component of the mycobacterial cell wall, and b) by acting as a nitric oxide donor and causing respiratory poisoning [Singh *et al*., 2008]. A commoving role of **PA-824** has been identified in novel drug combinations which appear to enhance treatment in both the murine model and in a human (Early bactericidal activity) EBA trial. The novel combination PaMZ (PA-824 + moxifloxacin + pyrazinamide) has showed superior bactericidal and sterilizing activity in murine model [Wong *et al*., 2013].

1.7.1.2. Bedaquiline



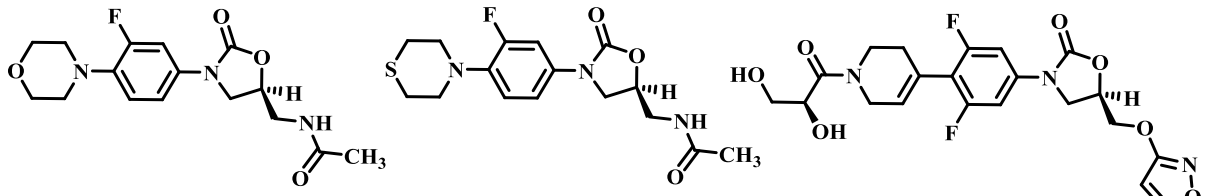
Bedaquiline

Bedaquiline (TMC-207) belongs to new class of diarylquinoline drug, the TMC-207 discovered by Janssen Pharmaceutica. TMC-207 was approved on 28th December 2012 by the FDA, and a drug of novel class to be approved over 40 years [Mahajan *et al*., 2013; Chan *et al*., 2013]. TMC-207 inhibits mycobacterial ATP synthase resulting in decreased ATP levels and pH imbalance in the organism. The bactericidal bedaquiline has an unusual long half-life of >24 hours in human [Andries *et al*., 2005; Goel *et al*., 2014]. Bedaquiline is one of the probables to fulfil an unmet therapeutic necessitate for the treatment of MDR-TB and will reduce the risk of development of resistance to other anti-TB drugs in the standard regimen [Deoghare *et al*., 2013].

Table 1.7: New anti-TB drugs, stage of development and their targets [Yew *et al*., 2011]

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

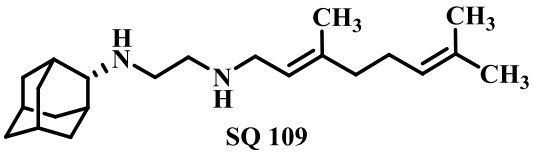
1.7.1.3. Oxazolidinones (Linezolid, sutezolid and Posizolid)



Oxazolidinones have a broad spectrum of antibiotic activity on anaerobic and gram positive aerobic bacteria as well as mycobacteria. Oxazolidinones inhibit the protein synthesis by binding to 23S RNA in the 50S ribosomal subunit of MTB [Zhang *et al*., 2005]. Linezolid was approved in the year 2000 for the treatment of drug resistant gram-positive bacterial infections (WHO Global Tuberculosis report-2013). It was initially developed for resistant Gram positive organisms and generally used to treat methicillin resistant *Staphylococcus aureus* [Wong *et al*., 2013]. It has good anti-mycobacterial activity *in vitro* and is used off-label in combination regimens for the treatment of MDR-TB, but its efficacy is unclear. Serious unpleasant effects such as peripheral and optic neuropathies, thrombocytopenia and anaemia have been reported with the use of linezolid. Therefore trials were conducted to evaluate the efficacy at lower doses (600 or 300 mg/day) compared with that of standard

doses (1200 mg/day). In recent trial patients with XDR-TB were given lower doses (300-600 mg/day), about 87% of all patients achieved negative conversion within 6 months [Lee *et al*., 2012]. Amongst four patients acquired linezolid resistance during TB treatment, 3 of them received only 300 mg/day of linezolid. Thus further canvas are needed to determine the potential efficient doses of this drug, while preventing the side effects [Chang *et al*., 2012; Bolhuis *et al*., 2013; Wong *et al*., 2013]. Sutezolid (PNU-100480) is an antibiotic, presently in progress as a treatment for extensively drug-resistant TB. New oxazolidinone developed by Pfizer is a close analogue of linezolid and demonstrated better activity *in vitro* than linezolid [Cynamon *et al*., 1999]. Its activity and pharmacokinetic data shows that sutezolid converts into sulfone and sulfoxide metabolites, the sulfoxide metabolite is more active and reaches four times higher in concentration than parent compound [Barbachyn *et al*., 1996]. Sutezolid has been tested in an EBA study at doses of either 600 mg twice a day or 1200 mg once a day. Results express that sutezolid lead to significant reduction in log colony forming units (CFU) with both dosage options. Mouse model studies showed that addition of sutezolid to current first line TB drugs improved the bactericidal activity. It also gave better results when used in combination with moxifloxacin and pyrazinamide. These outputs suggest that sutezolid has the potential to reduce the treatment duration in both drug susceptible and drug resistant TB. Sutezolid is currently in phase-II clinical trials [Balasubramanian *et al*., 2014; Wallis *et al*., 2014]. Posizolid (AZD 5847) is another class of oxazolidinone drug in pipeline, developed by AstraZeneca for the treatment of pulmonary TB [Wookey *et al*., 2004]. AZD 5847 mechanism of action is almost identical to Sutezolid, it binds to 50S ribosomal subunit and blocks initiation of protein synthesis [Kwon *et al*., 2014]. It is active against extracellular, intracellular and slowly and rapidly dividing mycobacteria in mouse models. AZD 5847 was well endured in both the studies and no major side effects were identified, in toxicological testing AZD 5847 has only minor haematological effects (decrease in RBCs and WBCs) but has no effect on bone marrow. Currently AZD 5847 is in phase II clinical studies [Wong *et al*., 2013].

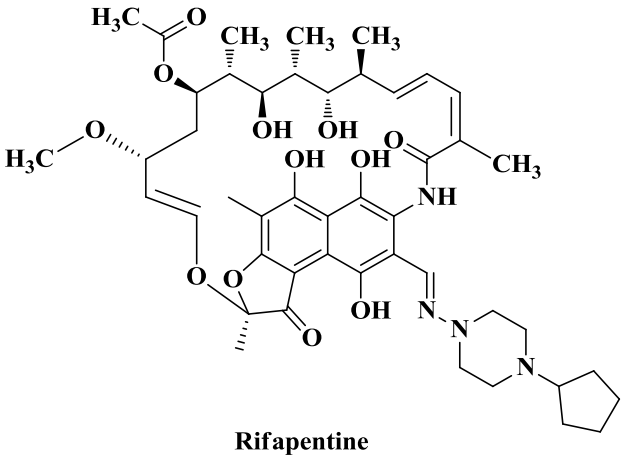
1.7.1.4. SQ 109



SQ 109

SQ 109 is a new orally active diamine antibiotic for the healing of TB, it is a derivative of ethambutol and has activity against both drug sensitive and drug resistant-TB. SQ 109 mechanism of action remains ill-defined, it is targeting MmpL3 a transporter of mycobacterial trehalose monomycolate (TMM) in MTB there by causing inhibition of protein synthesis [Meng *et al*., 2009; Sacksteder *et al*., 2012]. The replacement of ethambutol with SQ 109 in standard regimen increased the efficacy in mouse model [Nikonenko *et al*., 2007]. It has MIC of 0.16-0.64 µg/mL and no cross resistance with ethambutol, it has synergetic effects with TMC-207 and favourable interactions with sutezolid *in vitro* [Reddy *et al*., 2010; Reddy *et al*., 2012]. In phase-I studies SQ 109 was proved to be safe and well tolerated in single doses up to 300 mg and currently is in phase-II clinical studies [Sacksteder *et al*., 2012].

1.7.1.5. Rifapentine



Rifapentine

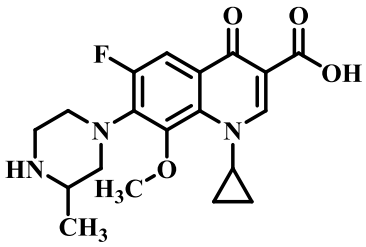
Rifapentine (RPT) is an antibiotic with excellent antimicrobial activity against MTB *in vitro* and *in vivo*, employed in the healing of TB. RPT is cyclopentyl rifampin and a lengthy acting analogue of rifamycin which inhibits DNA-dependent RNA polymerase. RPT exhibits bactericidal activity against both intracellular and extracellular MTB organisms and has a better pharmacokinetic profile in mice. Human arylacetamide deacetylase metabolizes RPT into its major metabolite 25-O-deacetylrifapentine and it retains activity. The half-life and protein binding (13-14 hours and 97%) of RPT is much longer than rifampin (2-3 hours and 85%). RPT is marketed as PRIFTIN for oral administration and it contains 150 mg of active ingredient per tablet [Acocella *et al*., 1971; Keung *et al*., 1999; Gao *et al*., 2009; Chan *et al*., 2014].

1.7.1.6. Phase II trials of Novel regimens

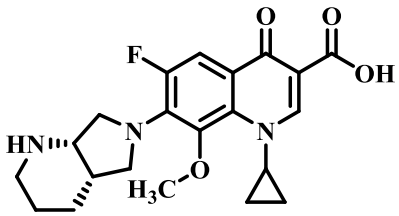
The deadly bacterium MTB has been recuperating its resistance to anti-TB drugs day by day, there should be combination of drugs essential to attack MTB rather than single potential agent. Besides the potential individual anti-TB agents and standard regimens, exploration for combinations has been continuing for years. Newly there has been a set of novel combination of drugs which are efficient in simplifying TB therapy by dropping the treatment complexities such as duration period, acquired drug resistance and increased relapse rates which are in phase II clinical trials. Present novel combinations of drugs in phase II clinical studies are: i) NC-001: Moxifloxacin, PZA and PA-824, ii) NC-002: Same regimen NC-001, testing in a two month trial, iii) NC-003: Bedaquiline, PA-824, clofazimine and PZA, iv) MAMS-TB-01: INH, RMP, PZA, EMB, moxifloxacin and SQ-109 [Zumla *et al*., 2014].

1.8. Phase III clinical trials of TB therapy drugs

1.8.1. Fluoroquinolones



Gatifloxacin



Moxifloxacin

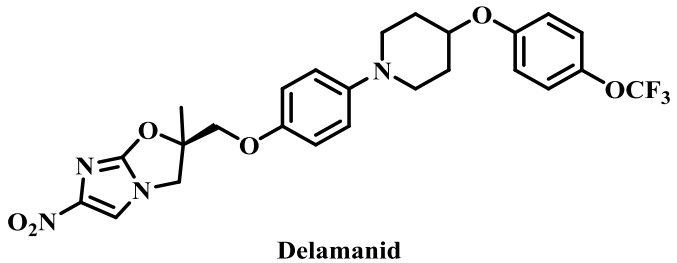
Currently, two fluoroquinolone class compounds are in phase III clinical trials. These are significant fluoroquinolone drug candidates viz. gatifloxacin and moxifloxacin. Fluoroquinolones are the keystone of treatment for MDR-TB and their potential has been tested in many studies [Falzon *et al*., 2013; Johnston *et al*., 2009]. These are also capable in reducing the treatment duration in drug susceptible TB [Rustomjee *et al*., 2008; Conde *et al*., 2009; Dorman *et al*., 2009]. These are bacterial enzymes DNA gyrase and topoisomerase IV inhibitors. Recent reports suggested that these drugs possess potent activity against MTB than the other members of this class including ofloxacin [Hu *et al*., 2003].

Gatifloxacin is the fourth-generation of fluoroquinolone. FDA approved in 1999, gatifloxacin for the treatment of patients with bronchitis, pneumonia and various infections including those of the urinary tract, kidneys and skin. Presently OFLOTUB consortium is conducting gatifloxacin clinical development programme. In Durban (South Africa) phase II studies were conducted on newly diagnosed patients by treating them with four drug regimen comprising

isoniazid, rifampin and pyrazinamide in combination with gatifloxacin for first two months (OFLOTUB phase II surrogate marker study). An outcome of the study shows that when substituted in place of ethambutol in standard TB therapy, both moxifloxacin and gatifloxacin killed MTB significantly faster than the control or ofloxacin based regimens. These results support that introduction of these fluoroquinolones in place of ethambutol in standard therapy may reduce the treatment duration from 9-6 to 6-3 months. The association is continuing the evaluation of gatifloxacin substituted regimen versus criterion 6 months treatment in phase III [Laurenzi *et al.*, 2007]. Moxifloxacin, a fourth generation synthetic fluoroquinolone developed by Bayer AG, was marketed worldwide under the brand names of Avelox and Avelon for oral treatment [www. Avelox.com], the moxifloxacin hydrochloride (Avelox) was approved by USFDA in 1999 for use in US. During 2005, in association with TB-alliance, Bayer started further exploration of moxifloxacin. The studies at John Hopkins University, used mice models where the infected mice were treated for one month with sparfloxacin, clinafloxacin, moxifloxacin or isoniazid [Ji *et al.*, 1998] and it was found that moxifloxacin had greatest bactericidal activity as compared to isoniazid. Another study suggested that moxifloxacin also had potent sterilizing activity [Cremades *et al.*, 2011; Ahmad *et al.*, 2013; Kwon *et al.*, 2014]. *In vitro* activities of gatifloxacin and moxifloxacin are better than the older fluoroquinolones ciprofloxacin and ofloxacin and their MICs against MTB H37Rv are as follows [Rodriguez *et al.*, 2001; Villemagne *et al.*, 2012]:

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

1.8.2. Delamanid



Delamanid

The Delamanid (OPC-67683) is a nitroimidazooxazole derivative. It has received approval by European Medicines Agency (EMA) for the treatment of MDR-TB in November 2013. Like isoniazid and pyrazinamide, delamanid is also a prodrug which is activated by the enzyme

deazaflavin dependent nitroreductase (Rv3547). OPC-67683 acts by inhibiting the synthesis of MTB cell wall components methoxy mycolic acid and ketomycolic acid [Gler *et al*., 2012; Skripconoka *et al*., 2013; Xavier *et al*., 2014]. Dissimilar, first-line anti-TB drugs which should be taken on empty stomach, resembling Delamanid is instructed to take along with food. After oral management the maximum concentration is observed at 4-5 h. The half-life is 38 h after drug discontinuation. During *in vitro* studies, it showed good anti-bacterial activity against drug sensitive and drug resistant strains of MTB with observed MIC of an extremely lower range of 0.006-0.024 µg/mL. In one study, OPC-67683 was tested on HIV negative MDR patients along with WHO standard therapy for 2 months. The results showed that higher sputum culture conversion rates were observed in the treatment group compared to patients on placebo and background regimen [Gler *et al*., 2012]. In clinical studies, OPC-67683 showed no significant interactions with other drugs such as lopinavir, tenofovir and efavirenz. This shows that OPC-67683 can be combined with other anti-TB drugs as there are no adverse drug-drug interactions. Presently it is marketed as Deltyba, 50 mg tablet used as a part of an appropriate combination for pulmonary MDR-TB in adults for whom current approved regimen fails [Xavier *et al*., 2014].

1.9. Drug optimisation and structure-activity relationship (SAR) studies

1.9.1. Rifamycin derivatives structure-activity relationship

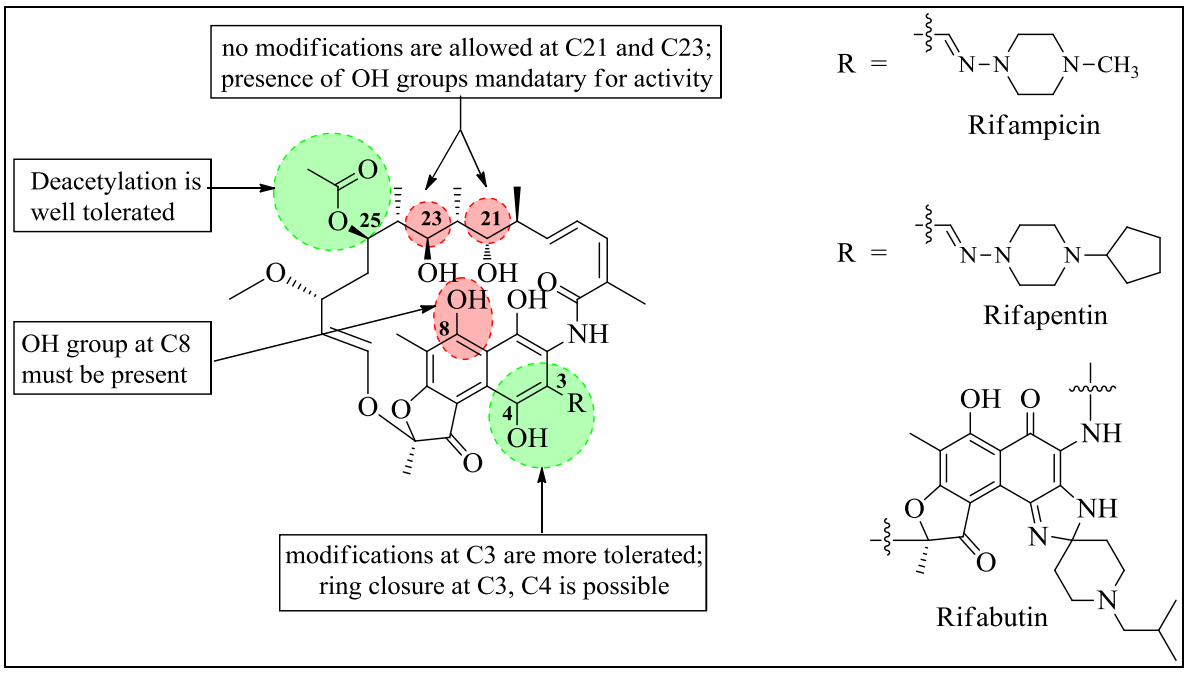


Figure 1.12: Rifamycins SAR

Rifampicin is the foremost drug from the family of rifamycins. Although it is one of the mainly employed first line anti-TB drug to treat drug sensitive TB, its usage was limited in case of drug resistant bacterial strains. If drug susceptible TB, rifampicin has to be taken for six months, such a long time leads to patient's non adherence which leads to as development of drug resistance. To overcome these difficulties rifampicin was structurally modified to get better agent which showed better activity for both drug susceptible and drug resistant TB strains and also to shorten present long treatment time. Rifabutine and rifapentine, the structural analogues of rifampicin are present generation rifamycins possessing high efficacy than rifampicin. Rifapentine is presently in phase III trials, the studies conducted in phase II shows that though it is more potent than rifampicin and active against MDR-TB, it possesses few side effects which was explained in previous section. There is a scope to develop even more potent compounds than rifapentine thorough research on SAR of rifamycins core structure. Based on the extensive research work on rifamycin motifs SAR analysis shows that (**Figure 1.12**) [Aristoff *et al*., 2010].

a) Suitable changings are allowed at C3, C4 and C25 positions.
b) A modification at naphthalene group is possible without altering C8-hydroxyl group.
c) The hydroxyl groups at C8, C21 and C23 must be present in order to retain its activity.
d) Ring closure between C3 and C4 positions are more abide.

1.9.2. Fluoroquinolones structure-activity relationship

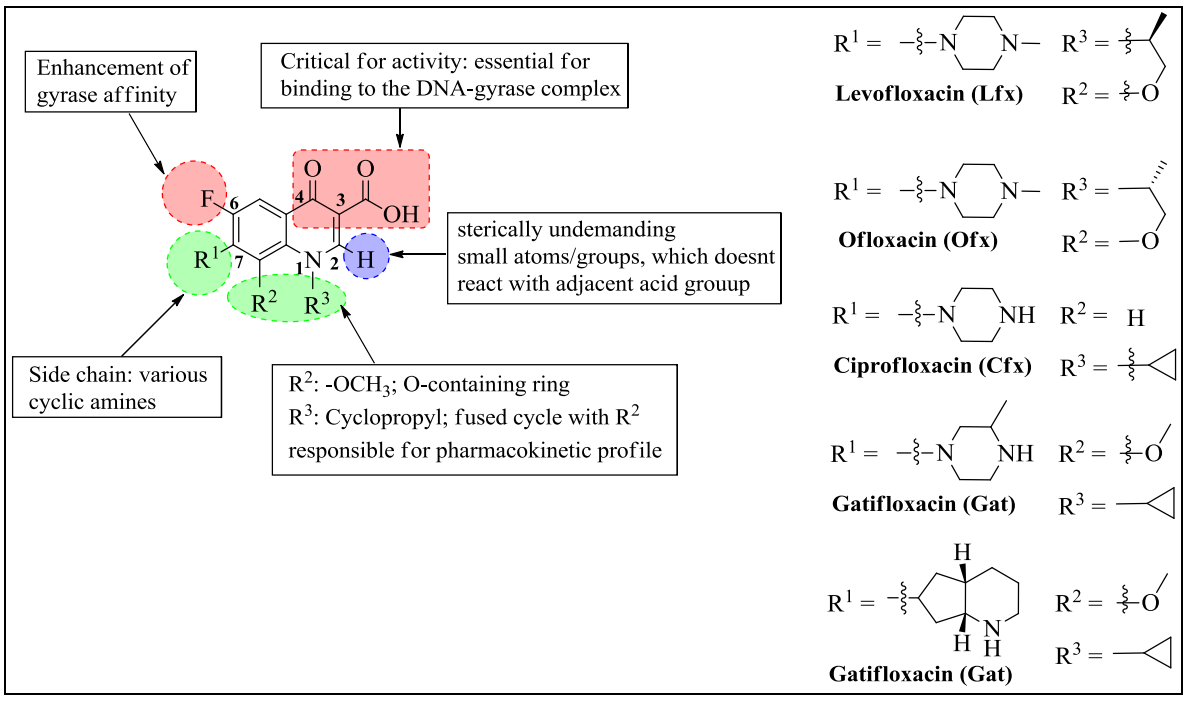


Figure 1.13: Fluoroquinolones SAR

Fluoroquinolones have broad range of anti-TB and anti-bacterial activities. These attack DNA gyrase enzyme. Their complete details is described in **Figure 1.13**, the SAR of fluoroquinolones could be abridged as follows:

a) Fluoroquinolones presence of carboxylic acid group at C3 and carbonyl group at C4 are accountable and essential for the binding with DNA gyrase, hence changes are not allowed at those positions.
b) The suitable groups at C2 are small atoms, H-atom is preferred. Bulky or large groups causes steric crowd which may interfere with adjacent carboxylic acid group.
c) The fluorine atom at C6 is essential for the activity, increases affinity for the targeted enzyme.
d) Modifications at C7 position are allowed, substituent at this position responsible for both pharmacokinetic profile and activity. Best groups are cyclic five or six membered rings containing nitrogen atoms.

1.9.3. Oxazolidinones structure-activity relationship

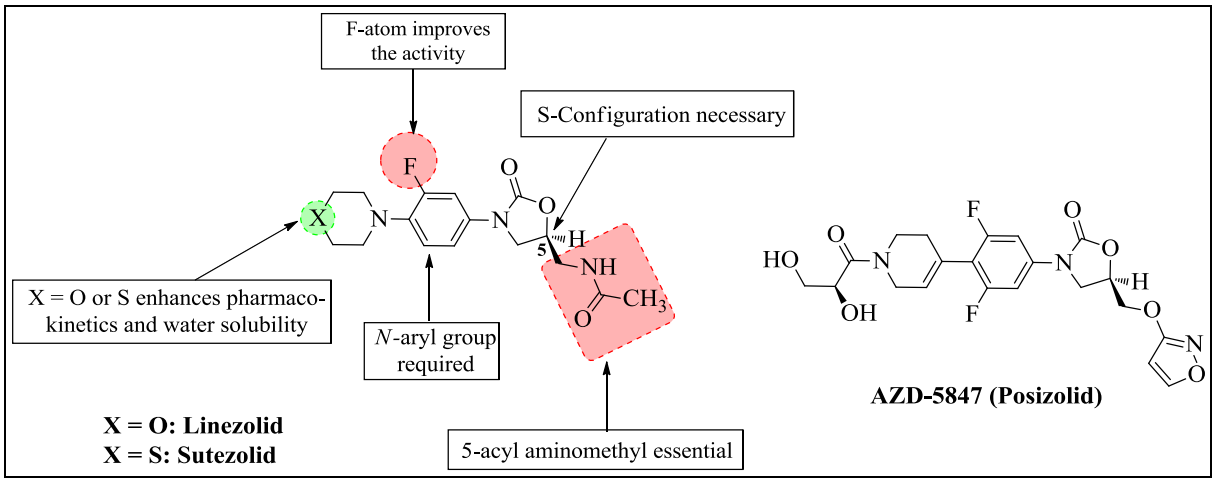


Figure 1.14: Oxazolidinones SAR

The oxazolidinone potential agents namely linezolid, sutezolid and AZD-5847 are presently in phase-II clinical trials. Linezolid was first drug in this class, which possessed activity (MIC<1µg/mL) against both drug sensitive and drug resistant forms of TB [Barbachyn *et al.*, 2003; Tokuyama *et al.*, 2001]. It is associated with few side effects due to which it cannot be used for long time therapy especially in patients infected with HIV. Sutezolid is obtained by replacing with sulphur atom in morpholine ring of linezolid. This modification enhanced sutezolid (MIC = 0.125 µg/mL) activity than parent linezolid (MIC<1µg/mL). Sutezolid was well tolerated than linezolid at a dosage of 1000 mg [Wallis *et al.*, 2011]. Another oxazolidinone drug AZD-5847 was obtained by few modifications on core structure: i)

replacement of morpholine ring with (*S*)-1-(5,6-dihydropyridin-1(2*H*)-yl)-2,3-dihydroxypropan-1-one, ii) di-substitution of fluorine atom on aromatic ring, and iii) replacement of acylamino group with isoxazol-3-yloxy (**Figure 1.14**). Latest reports showed that its *in vitro* efficacy was similar to that of linezolid (MIC against H37Rv = 1µg/mL) and it is currently in phase-IIa trials [Balasubramanian *et al.*, 2014]. SAR of oxazolidinones described below:

a) At 5th position of oxazolidinone *S*-configuration ring is required for activity
b) Existence of acylaminomethyl at 5th position is necessary (sutezolid has better MIC than AZD-5847 which doesn't have acylaminomethyl group);
c) On aromatic ring electron withdrawing substitutions are allowed.

1.9.4. Nitroimidazoles structure-activity relationship

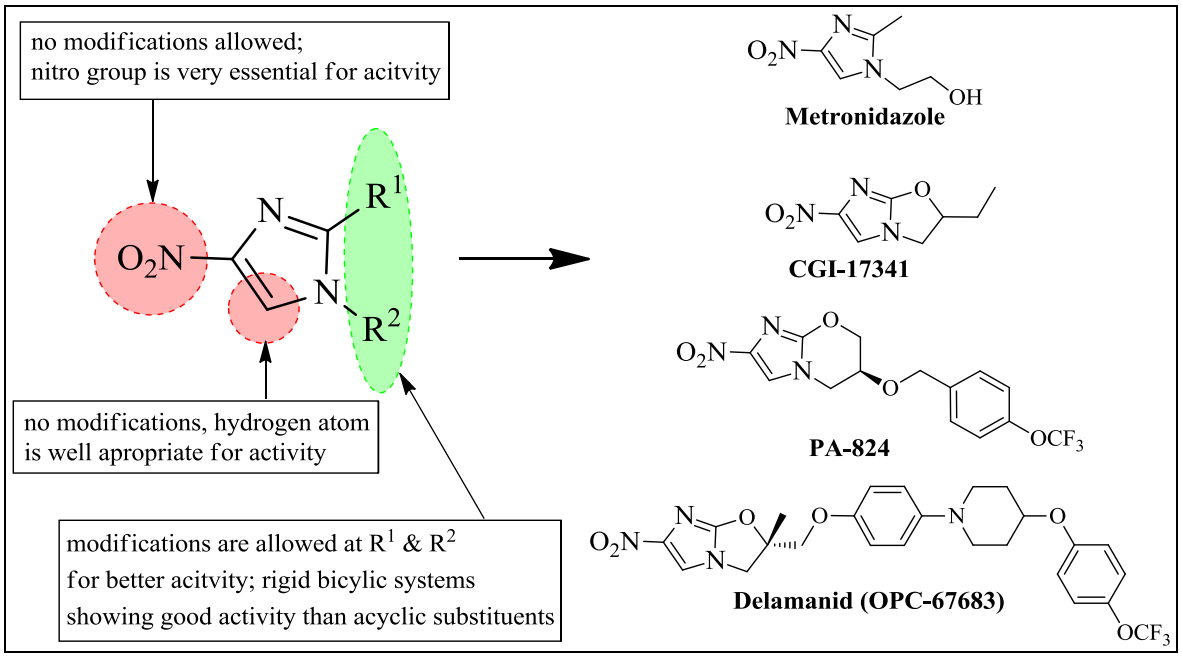


Figure 1.15: Nitroimidazoles SAR

Nitroimidazole derivatives have the ability to inhibit anaerobic bacteria; hence they can be used for treating latent TB infection. CGI-17341 is the most active compound in this class, but due to its mutagenicity it has not developed [Ashtekar *et al.*, 1993]. Additional evaluations showed that, this problem might be overcome by introduction of side chain at 2nd position of oxazole ring. Based on these SAR findings new series of nitroimidazoles were developed.

a) On imidazole nucleus, nitro group is essential for anti-bacterial activity.
b) Rigid bicyclic system is required, open ring compounds are not active.

Replacement of oxygen atom in bicyclic system with methylene group causes loss of activity, whereas sulphur and nitrogen atoms are well tolerated.

Chapter II
Literature Review

Chapter 2

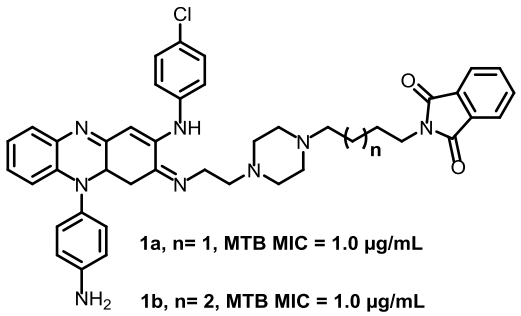
LITERATURE REVIEW

2.1. Literature review of promising anti-TB agents

Heterocyclic derivatives have broad range of biological importance and applications. This work includes heterocyclic motifs such as piperazines, hydrazones, oxindoles, benzisoxazoles, quinoxalines, phenanthradines, indoles, imidazoles, amides, sulphonamides, homopiperazines, and triazoles. This chapter describes previous reports available related to anti-TB activity based on these heterocyclic motifs.

2.2. Piperazines as anti-tubercular agents

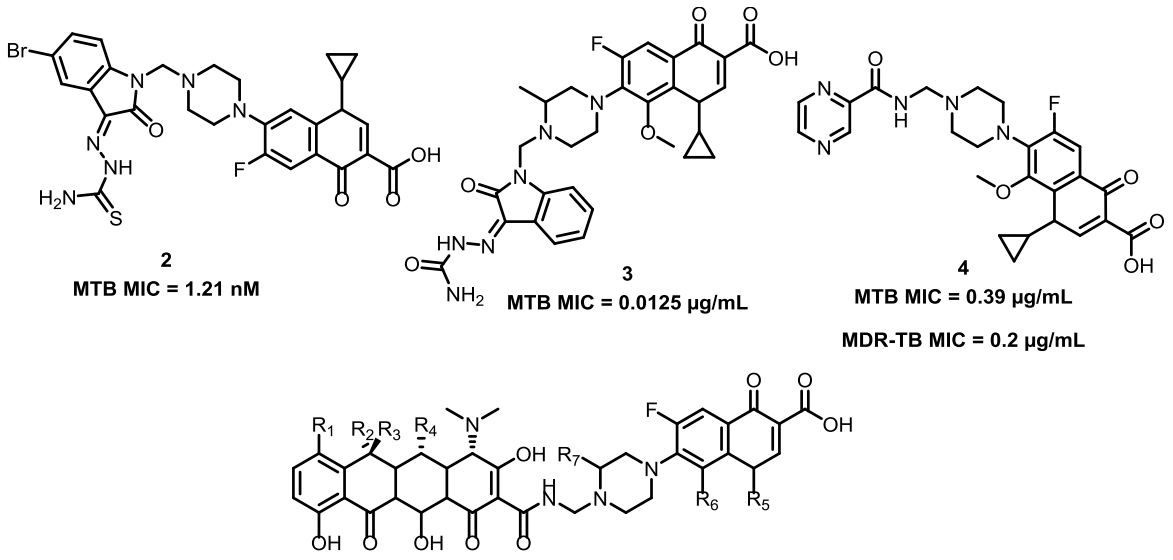
Emergence of drug resistance to a number of drugs is a new threat, and has covered for development of novel drugs [Upadhayaya *et al.*, 2010]. The significance of piperazine and lipophilicity of chemophores play vital role in the physiochemical properties and biological activities. Attaching piperazine analogues enriches the lipophilicity of compounds (Mortenson *et al*., 2011; Kakwani *et al*., 2011). Accordingly, piperazine derivatives are known to exhibit wide range of pharmacological activities and physiochemical properties. Indeed, this piperazine analogues display enhanced anti-TB activity. Various discovery groups have developed piperazine based anti-tubercular agents. Kamal *et al.*, prepared a series phthalimido and naphthalimido linked phenazines and screened them for anti-TB activity. All synthesized compounds screened against MTB H37Rv ATCC 27294, MTB clinical isolates (Sensitive and Resistant), MTB *avium* ATCC 49601 MTB *intracellulare* ATCC 13950. Among them, two compounds **1a** and **1b** were the most active compounds of the series against MTB H37Rv with MIC of 1.0 μg/mL, in MTB H37Rv, drug sensitive and resistant cultures of MTB [Kamal *et al.*, 2005].



1a, n= 1, MTB MIC = 1.0 μg/mL

1b, n= 2, MTB MIC = 1.0 μg/mL

7-substituted ciprofloxacin derivatives developed by Sriram *et al.,* were screened for antimycobacterial activity against MTB H37Rv strain, compound **2** was reported as the most active compound of the series with MIC of 1.21 nM, five times more active than ciprofloxacin *in vitro* [Sriram *et al.,*2005a]. Gatifloxacin piperazine analogues prepared by Sriram *et al.,* were evaluated for anti-TB activity against MTB H37Rv and MDR-TB. Amongst the synthesized compounds, compound **3** was found to be most active with MIC of 0.0125 µg/mL and ≤0.78 µg/mL against MTB and MDR-TB correspondingly [Sriram *et al.,* 2006a]. Pyrazinamide Mannich bases were developed by Sriram *et al,* and among them compound **4** was found to be most active *in vitro* with MIC of 0.39 and 0.2 µg/mL against MTB H37Rv and MDR-TB respectively [Sriram *et al.,* 2006b]. The same authors reported tetracycline derivatives as antimycobacterial agents. Among them three compound **5a, 5b**, and **5c** were found to be most active *in vitro* with MICs of each 0.2 µg/mL against MTB H37Rv [Sriram *et al.,* 2007].



2
MTB MIC = 1.21 nM

3
MTB MIC = 0.0125 µg/mL

4
MTB MIC = 0.39 µg/mL
MDR-TB MIC = 0.2 µg/mL

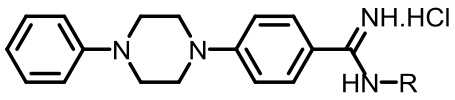
5a, R1 = H, R2 = CH3, R3 = OH, R4 = H, R5 = Cycloprpyl, R6 = OCH3, R7 = CH3; MTB MIC = 0.2 µg/mL

5b, R1 = NMe2, R2 = H, R3 = H, R4 = H, R5 = Et, R6 = F, R7 = CH3; MTB MIC = 0.2 µg/mL

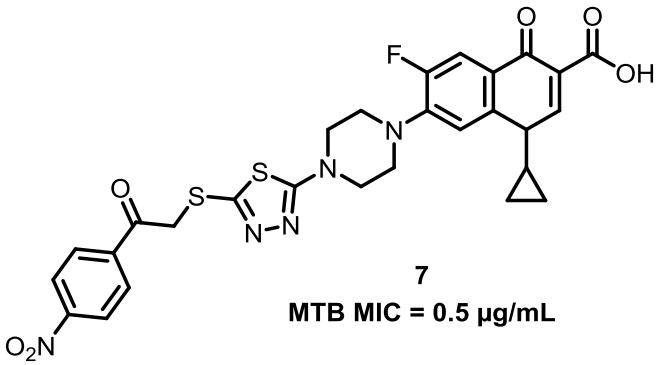
5c, R1 = NMe2, R2 = H, R3 = H, R4 = H, R5 = Cycloprpyl, R6 = OCH3, R7 = CH3; MTB MIC = 0.2 µg/mL

Forge *et al.,* reported 1,4-diarylpiperazines and analogs as anti-tubercular agents. Among these analogues **6a**, **6b** and **6c** compounds have excellent anti-TB activity with MIC 1.0 µM against MTB H37Rv [Forge *et al.,* 2012]. Amongst, four derivatives exhibited excellent anti-TB activity against MTB H37Rv with MICs ≤ 1.0 µg/mL. Among the compounds, 1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-{5-(2-oxo-2-pnitrophenylethylthio)-1,3,4-thiadiazol-

2-yl}piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**7**) displayed significant activity with MIC value 0.5 µg/mL [Agrawal and Talele 2013].



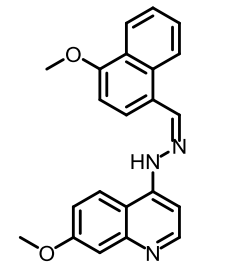
6a, R = C7H15; MTB MIC = 1.0 µM
6b, R = C8H17; MTB MIC = 1.0 µM
6c, R = C12H23; MTB MIC = 1.0 µM



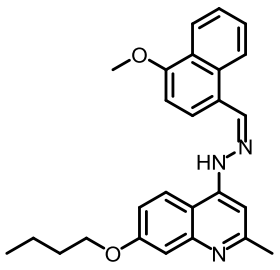
7
MTB MIC = 0.5 µg/mL

2.3. Hydrazones as anti-tubercular agents

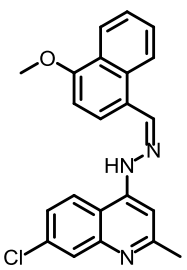
This class of derivatives have wide range of pharmacological and physiochemical properties. They play vital role in drug discovery. Synthesis and anti-tubercular evaluation of 4-quinolylhydrazones derivatives were published by Savini *et al.*, and tested against MTB H37Rv. Amongst, three (**8a, 8b** and **8c**) derivates have excellent anti-TB activity with MIC 0.78 µg/mL [Savini *et al.*, 2002]. Rando and his team reported hydrozones as potential tuberculostatic agents. In these, compound **9** exhibited better anti-TB activity with MIC 2.0 µg/mL against MTB H37Rv strain [Rando *et al.*, 2002]. Novel coupling products were synthesized and evaluated for their antimycobacterial activity against MTB H37Rv by Küçükgüzel *et al.*, in this compound **10** was found to be the most potent analogue with the MIC value of 6.25 µg/mL [Küçükgüzel and Rollas 2002].



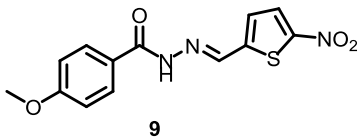
8a
MTB MIC = 0.78 µg/mL



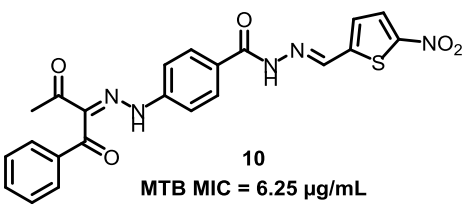
8b
MTB MIC = 0.78 µg/mL



8c
MTB MIC = 0.78 µg/mL

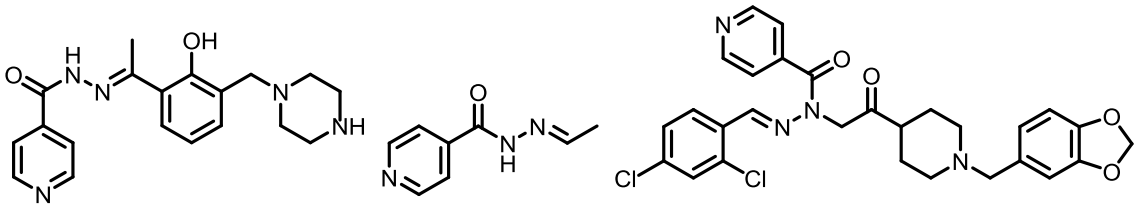


9
MTB MIC = 2.0 µg/mL



10
MTB MIC = 6.25 µg/mL

Sriram *et al.,* reported synthesis and *in vitro* and *in vivo* antimycobacterial activity of isonicotinoyl hydrazones. Of the series derivatives, *N′*-{1-[2-hydroxy-3-(piperazine-1-yl-ethyl)phenyl]ethylidene}isonicotinohydrazide (**11**) was found to be the most active with MIC of 0.56 μM against MTB H37Rv, and it was more potent than INH (MIC of 2.04 μM) [Sriram *et al.*,2005b]. *In vitro* advanced antimycobacterial screening of isoniazid-related hydrazones, hydrazides and cyanoboranes was published by Maccari *et al.* In this series compound **12** has excellent anti-TB activity against MTB H37Rv with MIC of 0.025 μg/mL and it was more potent than INH with MIC 0.78 μg/mL [Maccari *et al.,* 2005]. Sinha *et al.,* developed isonicotinic acid *N′*-arylidene-*N*-[2-oxo-2-(4-aryl-piperazin-1-yl)-ethyl]-hydrazides as anti-TB agents. Among the series of compounds, compound **13** was most active with MIC 1.0 μg/mL against MTB H37Rv [Sinha *et al.,* 2005].



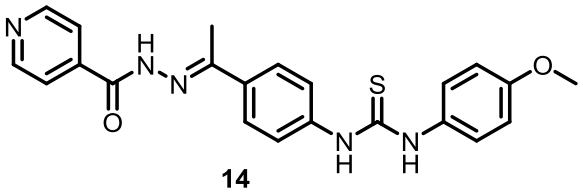
11
MTB MIC = 0.56 μM

12
MTB MIC = 0.025 μg/mL

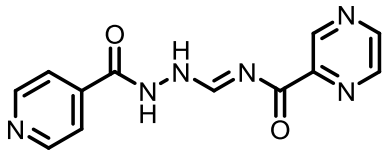
13
MTB MIC = 1.0 μg/mL

Synthesis and *in vitro* anti-tubercular activity of some 1-[(4-sub)phenyl]-3-(4-{1-[(pyridine-4-carbonyl)hydrazono]ethyl} phenyl)thiourea was published by Sriram *et al*. Of these derivatives, 1-(4-fluorophenyl)-3-(4-{1-[pyridine-4-carbonyl)hydrazono]ethyl}phenyl) thiourea (**14**) was found to be most potent compound, with MIC value of 0.47 μM against MTB H37Rv and INH-resistant MTB. It is more potent than standard anti-TB drugs viz. INH, PZA and EMB [Sriram *et al*., 2006c]. Nayyar *et al.,* reported synthesis, anti-TB activity, and 3D-QSAR study of ring-substituted-2/4-quinolinecarbaldehyde derivatives. *N*-(2-fluorophenyl)-*N′*-quinoline-2-yl-methylenehydrazine, *N*-(2-adamantan-1-yl)-*N′*-quinoline-4-yl-methylene)-*N′*-4-fluorophenyl) hydrazine and *N*-(2-cyclohexyl)-*N′*-quinoline-4-yl-methylene)-(2-fluorophenyl)hydrazine were found to be most active compounds, with MIC 3.125 μg/mL against drug-sensitive MTB H37Rv strain [Nayyar *et al.,* 2006]. Synthesis of new *S*-derivatives clubbed with triazolyl thiazole as anti-*Mycobacterium tuberculosis* agents was developed by Shiradkar *et al.* *N*-[3-({2-[(2*E*)-2-benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-(-({2[(benzoyl)amino]-1,3-thiazol-4-yl} methyl)-4H-1,2,4-triazol-4-yl]-3-nitrobenzamide (**15**) was the most active, with MIC 0.39 μg/mL against MTB H37Rv [Shiradkar *et al*., 2007]. Imramovský *et al.,* reported, a new modification of anti-tubercular

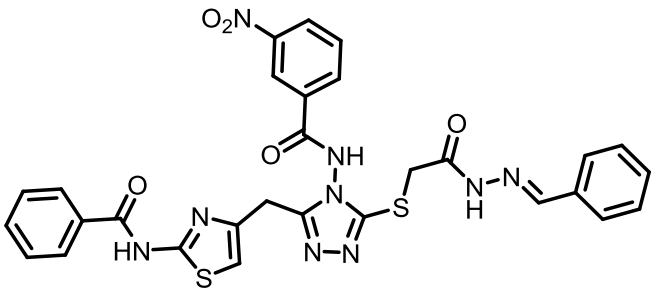
active molecules, amongst them 2-hydroxy-4-{[(isonicotinoylhydrazono)methyl]amino}benzoic acid (**16**) displayed promising inhibition of MTB H37Rv at 0.1 μg/mL [Imramovský *et al.*, 2007].



14
MTB MIC = 0.47 μM



16
MTB MIC = 0.1 μg/mL

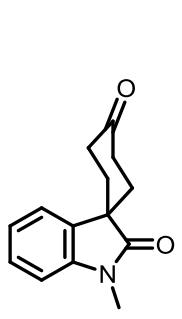


15
MTB MIC = 0.39 μg/mL

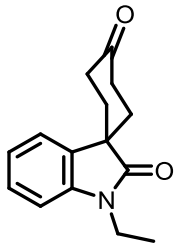
2.4. Oxindoles as anti-tubercular agents

Heterocyclic derivatives have broad spectrum of biological importances and applications. Chiefly, oxindole derivatives have known pharmacological activities, notably, few reports as anti-TB agents. Facile synthesis of active antitubercular, cytotoxic and antibacterial agents: a Michael addition approach was published by Chande *et al.* In these analogues, six compounds exhibited excellent anti-TB activity against MTB H37Rv with MIC ≤ 0.1 μg/mL, from this two viz. 1'-methylspiro[cyclohexane-1,3'-indoline]-2',4-dione (**17a**) and 1'-ethylspiro[cyclohexane-1,3'-indoline]-2',4-dione (**17b**) have splendid anti-TB activity with MIC 0.05 μg/mL [Chande *et al.*, 2005]. Sriram *et al.*, reported Gatifloxacin derivatives: synthesis, antimycobacterial activities, and inhibition of *Mycobacterium tuberculosis* DNA gyrase. Out of sixteen compounds, nine derivatives had excellent anti-TB activity against drug sensitive and drug resistant MTB strains with MIC ≤ 0.2 μg/mL, among them compound **3** has excellent activity with MIC 0.0125 μg/mL and 0.05 μg/mL respectively [Sriram *et al.*, 2006a]. *N*-cyclohexyl-2-(1-(morpholinomethyl)-5-nitro-2-oxoindolin-3-ylidene) hydrazinecarbothioamide compound with good activity against MTB H37Rv were developed by Karali *et al.* [Karali *et al.*, 2007]. Gözel *et al.*, reported synthesis and antituberculosis activity of 5-methyl/trifluoromethoxy-1H-indole-2,3-dione 3-

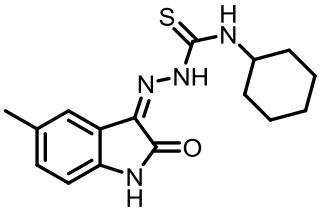
thiosemicarbazone derivatives. In this series, *N*-cyclohexyl-2-(5-methyl-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**18**) is the most active with MIC 0.79 µg/mL against MTB H37Rv (as IC_{90}) [Gözel *et al.,* 2008]. A facile synthesis and antimycobacterial evaluation of novel spiro-pyrido-pyrrolizines and pyrrolidines was developed by Ranjith Kumar *et al*. In these derivatives, 1-methyl-4-(2,4-dichlorophenyl)pyrrolo(spiro[2.3′′] oxindole)spiro[3.3′]-1′-methylpiperidin-4′-one (**19**) has excellent anti-TB activity against MTB with MIC 1.76 µM [Ranjith Kumar et al., 2009].



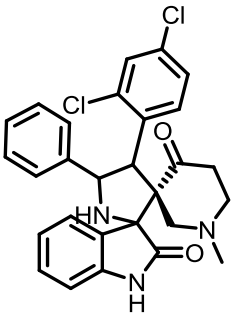
17a
MTB MIC = 0.05 µg/mL



17b
MTB MIC = 0.05 µg/mL



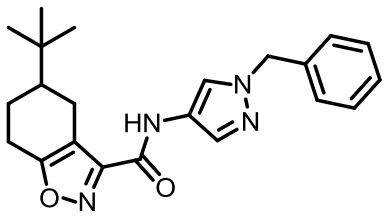
18
MTB MIC = 0.79 µg/mL



19
MTB MIC = 1.76 µM

2.5. Benzisoxazoles as anti-tubercular agents

Benzisoxazoles and its derivatives have broad spectrum of biological activities, in particular Subash *et al.,* reported 5-tert-butyl-Npyrazol-4-yl-4,5,6,7-tetrahydrobenzo[*d*]isoxazole-3-carboxamide derivatives with excellent anti-TB activity. Amongst, *N*-(1-benzyl-1H-pyrazol-4-yl)-5-tert-butyl-4,5,6,7-tetrahydrobenzo[*d*]isoxazole-3-carboxamide (**20**) was found to exhibit good anti-TB activity by MABA [Subash *et al.,* 2008].

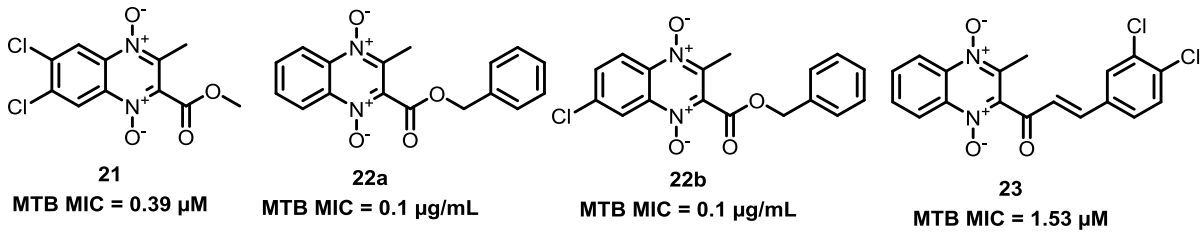


20
84% inhibition at 128µM

2.6. Quinoxalines as anti-tubercular agents

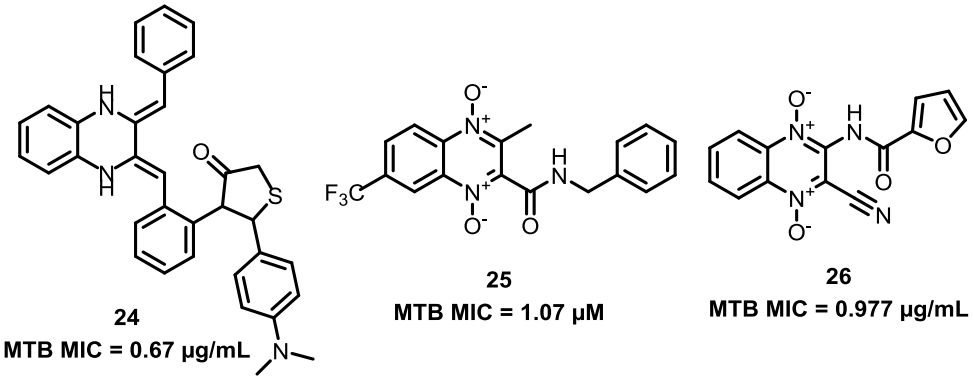
Hetero atoms viz., nitrogen containing molecules have wide range of biological and physiochemical properties in drug discovery development and material science discovery. Particularly, quinoxaline derivatives are significant core motifs found in a variety of natural

products and alkaloids and other synthetic vital molecules with a wide range of biological activities and physiochemical properties. Jaso and his group reported, twenty eight quinoxaline derivatives in 2003. Amongst these, one compound 2-acetyl-6,7-dichloro-3-methylquinoxaline 1,4-di-*N*-oxide (**21**) has excellent anti-TB activity with MIC 0.39 μM [Jaso *et al.,* 2003]. Same group published, synthesis of new quinoxaline-2-carboxylate 1,4-dioxide derivatives as anti-*Mycobacterium tuberculosis* agents. Out of these ten derivatives have admirable anti-TB activity against MTB H37Rv with MIC ≤ 0.78 μg/mL; amongst these two compounds, benzyl 3-methylquinoxaline-2-carboxylate-1,4-dioxide (**22a**) and benzyl 7-chloro-3-methylquinoxaline-2-carboxylate-1,4-dioxide (**22b**) exhibited excellent activity with MIC 0.10 μg/mL, when compared with standard TB regimens viz. INH, PZA, PAS and EMB [Jaso *et al.,* 2005]. Das *et al*., developed *E*-2-[3-(3,4-Dichlorophenyl)-1-oxo-2-propenyl]-3-methylquinoxaline-1,4-dioxide: A lead anti-tubercular agent which alters mitochondrial respiration in rat liver. From these, compound *E*-2-[3-(3,4-Dichlorophenyl)-1-oxo-2-propenyl]-3-methylquinoxaline-1,4-dioxide (**23**) has excellent anti-TB activity against MTB H37Rv with MIC value 1.53 μM [Das *et al*., 2010].



Puratchikody *et al*., reported synthesis, *in vitro* anti-tubercular activity and 3D-QSAR of novel quinoxaline derivatives. In these derivatives, five compounds had excellent anti-TB activity against MTB H37Rv with MIC values < 1.0 μg/mL. Out of these one compound 2-(4-dimethylaminophenyl)-3-(2-((*Z*)-((*E*)-3-(phenylimino)-3,4-dihydroquinoxalin-2-(1H)-ylidene)amino)phenyl)thiazolidin-4-one (**24**) has excellent activity with MIC 0.67 μg/mL [Puratchikody *et al*., 2011]. New 1,4-di-*N*-oxide-quinoxaline-2-ylmethylene isonicotinic acid hydrazide derivatives as anti-*Mycobacterium tuberculosis* agents was published by Torres *et al*. Amongst them four analogues have excellent activity against MTB H37Rv with MIC values ≤ 1.32 μM. Among the compounds one analogue (**25**) possesses excellent anti-TB activity with MIC 1.07 μM [Torres *et al*., 2011]. $AlCl_3$ induced (hetero) arylation of 2,3-dichloroquinoxaline: A one-pot synthesis of mono/disubstituted quinoxalines as potential antitubercular agents was developed by Shiva Kumar *et al.* In these derivatives 2-chloro-3-(5,6-difluoro-1H-indol-3-yl)quinoxaline has moderate inhibition against chorismate mutase

[Shiva Kumar *et al.,* 2012]. Hadda *et al.,* reported, *Mycobacterium tuberculosis* inhibition of heterocyclic-2-carboxylic acid (3-cyano-1,4-dinoxidequinoxalin-2-yl)amide derivatives. Out of 40, four derivatives have excellent anti-TB activity against MTB H37Rv with MIC ≤ 1.70 µg/mL. Amongst them one compound (**26**) has excellent anti-TB activity with MIC 0.977 µg/mL [Hadda *et al.,* 2014].



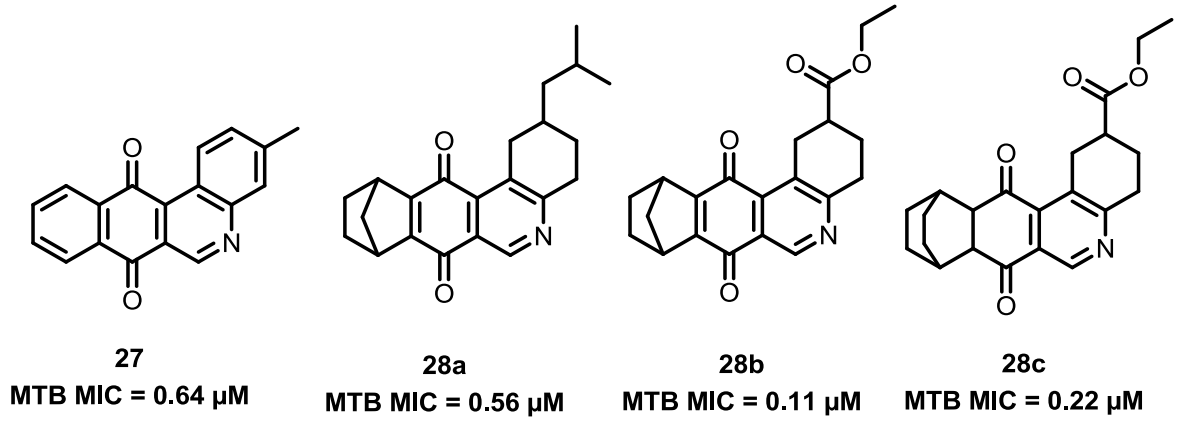
2.7. Phenanthradines as anti-tubercular agents

Phenanthridine derivatives are significant core moieties found in a variety of natural products, important class of alkaloids and other synthetic vital molecules with a wide range of biological activities and applications, including as antitubercular agents. Straightforward palladium-mediated synthesis and biological evaluation of benzo[*j*]phenanthridine-7,12-diones as anti-TB agents was reported by Cappoen *et al.* Amongst, the reported compounds 3-methylbenzo[*j*]phenanthridine-7,12-dione (**27**) has excellent anti-TB activity against MTB H37Rv with MIC < 0.64 µM [Cappoen *et al.,* 2012]. Recently, Cappoen *et al.,* reported 1,2,3,4,8,9,10,11-octahydrobenzo[*j*]phenanthridine-7,12-diones as new leads against *Mycobacterium tuberculosis.* All derivatives were screened against MTB H37Rv strain, amongst them three compounds (**28a**, **28b** and **28c**) had excellent anti-TB activity with ≤ 0.59 µM. Furthermore, these three compounds were evaluated against five more strains such as MTB H37Rv, MTB Bejing 17919l, *Mycobacterium bovis* (*M. Bovis*) AN5, *M. avium subsp. Paratuberculosis* ATCC19698 and *M. avium subsp. avium* ATCC15769, their MIC summarised in **Table 2.1** and INH was used as control [Cappoen *et al.,* 2014].

Table 2.1: *In vitro* MIC (µM) of Compounds **28a**, **28b** and **28c** against various *Mycobacterials*

Name of strain	***In vitro* MIC (µM)**

	28a	**28b**	**28c**	**INH**
MTB H37Rv	0.56	0.11	0.22	0.13
MTB Bejing 17919l	0.33	0.14	0.16	0.09
M. Bovis AN5	0.78	0.09	0.57	0.08
M. avium subsp. Paratuberculosis ATCC19698	0.69	0.44	0.21	0.99
M. avium subsp. avium ATCC15769	0.8	1.22	0.37	0.76



27
MTB MIC = 0.64 µM

28a
MTB MIC = 0.56 µM

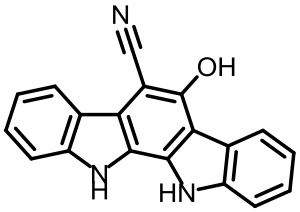
28b
MTB MIC = 0.11 µM

28c
MTB MIC = 0.22 µM

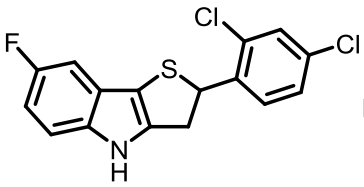
2.8. Indole as anti-tubercular agents

Heterocyclic compounds play an important role in an untiring effort aimed at developing new anti-tubercular agents. Chiefly, the indole moiety is probably the most widely spread nitrogen heterocycle in nature. It as an essential part of the amino acid tryptophan and the neurotransmitter serotonin, and the indole scaffold is also found in a manifold of naturally occurring plant based alkaloids. The biological importance of indole heterocylces and, directly associated with this, their pharmacological and medinal potential, has made indoles extremely attractive and rewarding research targets and have motivated countless researchers to study their synthesis, pharmacological and physiochemical properties. Guo and his team reported, natural product leads for drug discovery: Isolation, synthesis and biological evaluation of 6-cyano-5-methoxyindolo[2,3-*a*]carbazole based ligands as antibacterial agents. Among them, 6-hydroxy-11,12-dihydroindolo[2,3-*a*]carbazole-5-carbonitrile (**29**) was found to be moderately active in MTB H37Rv with MIC 15 µM [Guo *et al.,* 2009]. Karthikeyan *et al.,* reported microwave-assisted facile regioselective Fischer indole synthesis and antitubercular evaluation of novel 2-aryl-3,4-dihydro-2H-thieno[3,2-*b*]indoles. Among them, four compounds possessed excellent anti-TB activity against both MTB H37Rv and MDR-TB with MIC ≤ 0.78 µg/mL. Out of them 2-(2,4-dichlorophenyl)-7-fluoro-3,4-dihydro-2H-

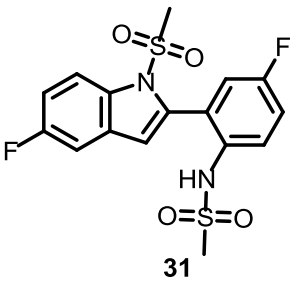
thieno[3,2-*b*]indole (**30**) has admirable anti-TB activity against MTB H37Rv and MDR-TB with MICs 0.4 µg/mL [Karthikeyan *et al.,* 2009]. Nakhi *et al.,* reported indoles via in situ desilylation–Sonogashira strategy: and their potential anti-TB activity. Amongst, *N*-(4-fluoro-2-(5-fluoro-1-(methylsulfonyl)-1H-indol-2-yl)phenyl)methanesulfonamide (**31**) displayed moderate anti-TB activity with MIC 50 µM [Nakhi *et al.,* 2011]. Yamuna *et al.,* reported synthesis, antimicrobial, antimycobacterial and structure-activity relationship of substituted pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimido cyclohepta[*b*]indoles. Three compounds (**32a**, **32b** and **32c**) were found to exhibit good anti-TB activity against MTB H37Rv with MICs 3.12 µg/mL [Yamuna *et al.,* 2012].



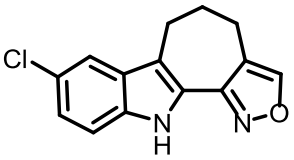
29
MTB MIC = 15 µM



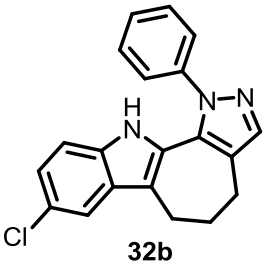
30
MTB MIC = 0.4 µg/mL



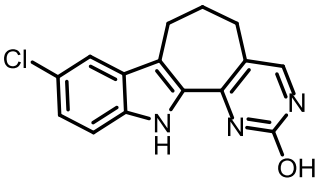
31
MTB MIC = 50 µM



32a
MTB MIC = 3.12 µg/mL

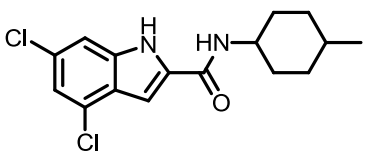


32b
MTB MIC = 3.12 µg/mL

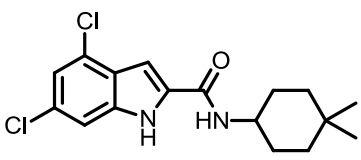


32c
MTB MIC = 3.12 µg/mL

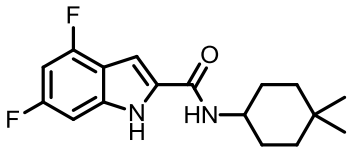
Kondreddi *et al.,* reported design, synthesis, and biological evaluation of fourty indole-2-carboxamide derivatives as anti-TB agents. Among them twenty five compounds were found to have admirable activity in MTB H37Rv with MIC < 1 µM. Three compounds (**33a**, **33b** and **33c**) have outstanding anti-TB activity with MIC < 0.1 µM. The SAR on indole ring functionalities suggested that indole ring is important for MTB activity [Kondreddi *et al.,* 2013].



33a
MTB MIC = 0.053 µM

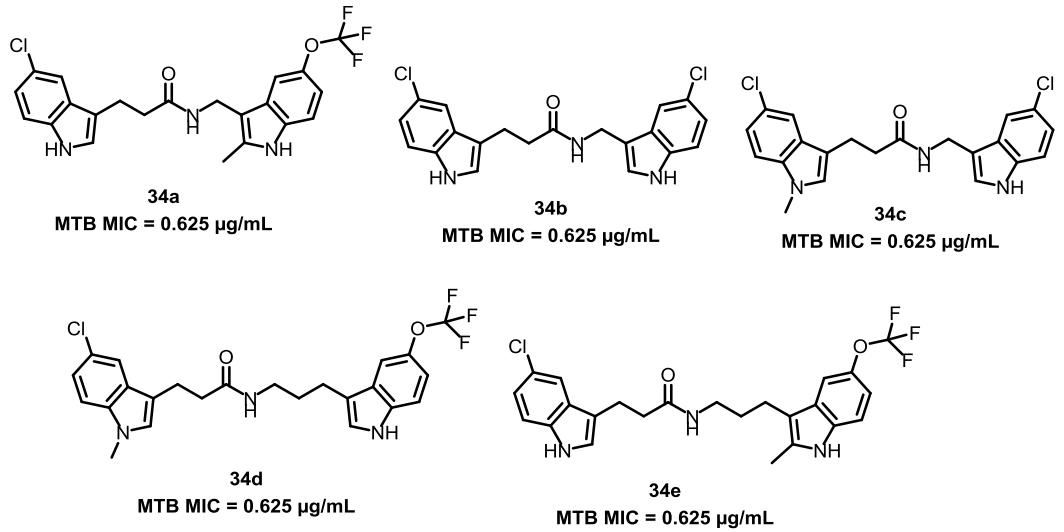


33b
MTB MIC = 0.015 µM

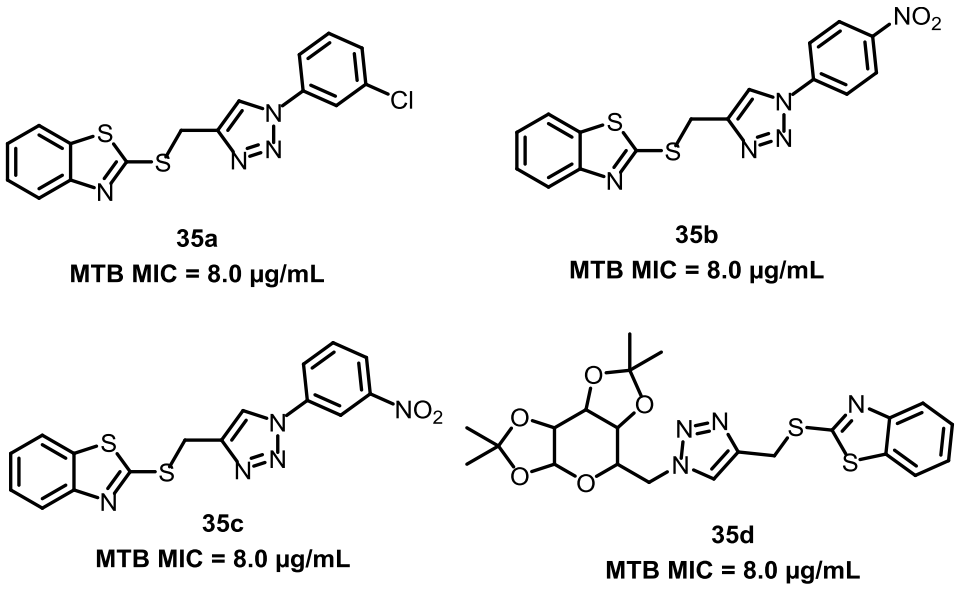


33c
MTB MIC = 0.023 µM

Synthesis, characterization, and SAR studies of new (1H-indol-3-yl)alkyl-3-(1H-indol-3-yl)propanamide derivatives as possible antimicrobial and anti-tubercular agents were developed by Ranjith and his team. All derivatives were screened against four strains like MTB H37Rv (ATCC 27294), *Mycobacterium smegmatis* (ATCC 19420), *Mycobacterium fortuitum* (ATCC 19542) and drug resistant strain (MDR-TB). Five compounds (**34a**, **34b, 34c, 34d** and **34e**) were found to possess excellent anti-TB activity against MTB H37Rv with MIC values 0.625 µg/mL [Ranjith *et al.,* 2013].

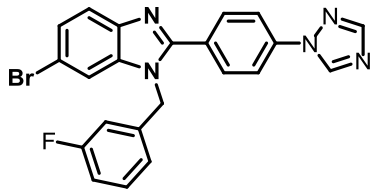


Mir *et al.,* reported sulphur rich 2-mercaptobenzothiazole and 1,2,3-triazole conjugates as novel anti-tubercular agents. Among the derivatives, four compounds (**35a**, **35b, 35c** and **35d**) were found to exhibit moderate activity with MIC 8 µg/mL [Mir *et al.,* 2014].

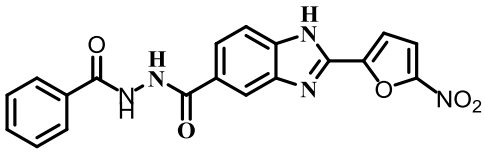


2.9. Imidazoles as anti-tubercular agents

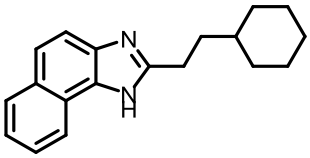
Imidazole derivatives are found in a variety of natural products and alkaloids and other synthetic vital molecules with a wide range of biological activities and physiochemical properties. Jadhav *et al.,* reported hybrids of [1,2,4]-triazolyl with fluoro benzimidazoles as antimicrobial and anti-TB agents. Among them 2-(4-(1H-1,2,4-triazol-1-yl)phenyl)-6-bromo-1-(3-fluorobenzyl)-1H-benzo[*d*]imidazole (**36**) exhibited admirable anti-TB activity in MTB H37Rv with MIC 0.36 µg/mL [Jadhav *et al.,* 2009]. Synthesis and biological evaluation of benzimidazole-5-carbohydrazide derivatives as anti-malarial, cytotoxic and anti-tubercular agents was published by Camacho *et al*. Among the derivatives, *N*′-Benzoyl-2-(5-nitrofuran-2-yl)-3H-benzo[*d*]imidazole-5-carbohydrazide (**37**) has moderate activity in MTB H37Rv and drug-resistant strain with MIC 12.5 µg/mL and 6.25 µg/mL respectively [Camacho *et al*., 2011]. Synthesis of novel 3-cyclohexylpropanoic acid-derived nitrogen heterocyclic compounds and their evaluation for tuberculostatic activity was developed by Gobis *et al.* Among them, 2-(2-cyclohexylethyl)-1H-naphto[2,3-*d*]imidazole (**38**) has admirable anti-TB activity against MTB H37Rv, Spec. 192 and Spec. 210 with MIC 1.5 µg/mL [Gobis *et al.*, 2012].



36
MTB MIC = 0.36 µg/mL

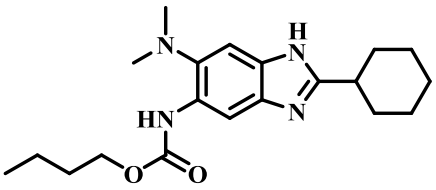


37
MTB MIC = 12.5 µg/mL

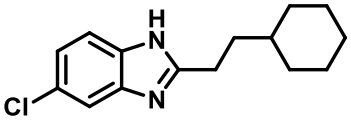


38
MTB MIC = 1.5 µg/mL

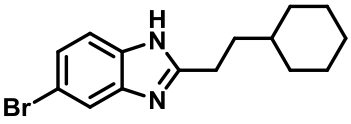
Aswathi and Ojima, reported synthesis and MTB H37Rv screening results of 63 new trisubstituted benzimidazoles. The synthesized analogues were studied, based on the screening data of 587 compounds against H37Rv. Out of these 587 compounds, 81 hit compounds have been found to possess MIC less than 5.0 µg/mL. Further screening at lower concentrations revealed 11 hit compounds possessing MIC of 0.39-6.1 µg/mL. Based on top most four molecules, SAR was built and further they synthesized 63 molecules. The compounds that emerged as the most active have MIC $\leq$ 0.16 µg/mL. Among them, one compound (**39**) possesses an outstanding anti-TB activity in MTB H37Rv with 0.06 µg/mL [Aswathi *et al.,* 2013]. Gobis *et al.*, reported synthesis and *in vitro* antimycobacterial activity of novel 1*H*-benzo[*d*]imidazole derivatives. Among them, four derivatives (**40a, 40b, 40c** and **40d**) have excellent anti-TB activity against MTB H37Rv, Spec. 192, Spec. 210 and MTB *bovis* with MIC values 0.75 µg/mL [Gobis *et al.*, 2015].



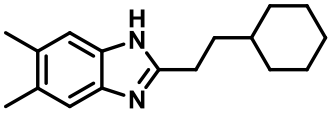
39
MTB MIC = 0.06 µg/mL



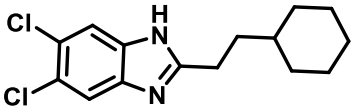
40a
MTB MIC = 0.75 µg/mL



40b
MTB MIC = 0.75 µg/mL



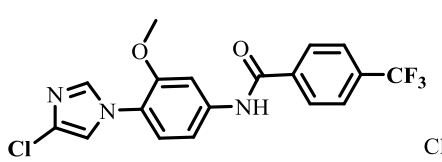
40c
MTB MIC = 0.75 µg/mL



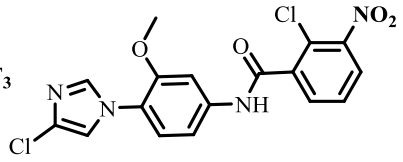
40d
MTB MIC = 0.75 µg/mL

2.10. Amides and Sulphonamide as anti-tubercular agents

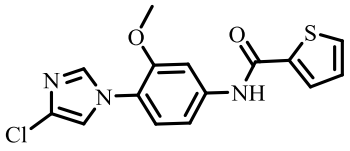
Amides and Sulphonamide derivatives play a part of important drug discovery groups. Attaching an alkyl/aryl amide and sulphonamides enhances the lipophilicity of compounds [Mortenson *et al.,* 2011; Ranjith *et al.,* 2014]. Consequently, alkyl/aryl amide and alkyl/aryl sulphonamide derivatives are known to exhibit wide range of pharmacological activities and physiochemical properties. Indeed, these alkyl/aryl amide and alkyl/aryl sulphonamide analogues display enhanced antibacterial and anti-TB activity [Tangallapally *et al.,* 2005; Punkvang *et al.,* 2010; Thomas *et al.,* 2011; Ranjith *et al.,* 2014; Patel and Telvekar 2014]. Ranjith and co-workers reported synthesis and anti-tubercular activity of *N*-(4-(4-chloro-1*H*-imidazol-1-yl)-3-methoxyphenyl) amide/sulphonamide derivatives. Among the amides, three molecules **41a, 41b** and **41c** were found to be active against MTB H37Rv with MIC 1 µg/mL and among the sulphonamides four derivatives **42a, 42b, 42c** and **42d** were found to have excellent activity with MIC 1 µg/mL [Ranjith *et al.,* 2014].



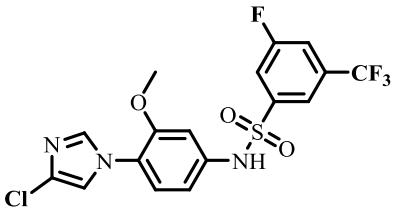
41a
MTB MIC = 1.0 µg/mL



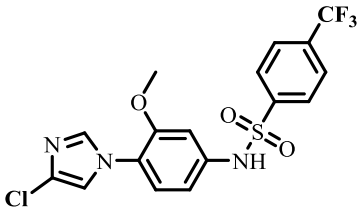
41b
MTB MIC = 1.0 µg/mL



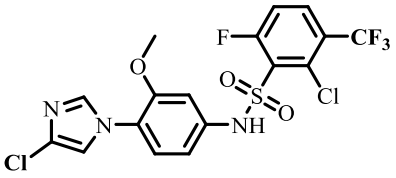
41c
MTB MIC = 1.0 µg/mL



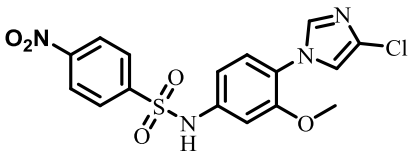
42a
MTB MIC = 1.0 µg/mL



42b
MTB MIC = 1.0 µg/mL



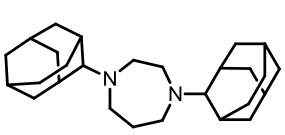
42c
MTB MIC = 1.0 µg/mL



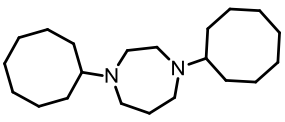
42d
MTB MIC = 1.0 µg/mL

2.11. Homopiperazine as anti-tubercular agents

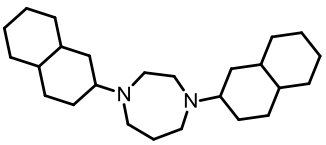
Homopiperazine is a class of diamine compound, which received extensive interest from medicinal chemists across the globe due to its significant role in the drug discovery. Bogatcheva *et al.*, reported identification of new diamine scaffolds with activity against *mycobacterium tuberculosis*. A library of 5000-compounds were synthesized from commercially available diamines and screened for activity against MTB *in vitro*, revealing 143 hits with MIC ≤ 12.5 µM. Amongst them, four homopiperazine derivatives **43a, 43b, 43c** and **43d** were found to have excellent anti-TB activity with MIC 1.56 µM [Bogatcheva *et al.*, 2006].



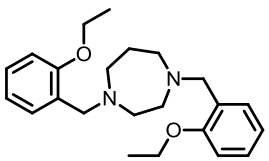
43a
MTB MIC = 1.56 µM



43b
MTB MIC = 1.56 µM



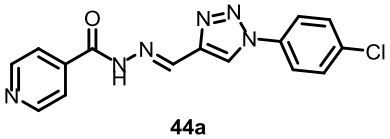
43c
MTB MIC = 1.56 µM



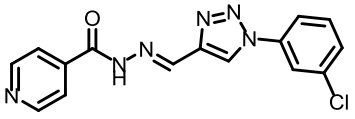
43d
MTB MIC = 1.56 µM

2.12. Triazoles as anti-tubercular agents

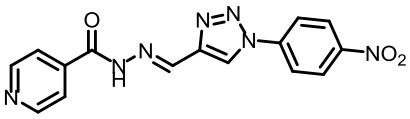
Heterocyclic motifs have been much exploited in drug discovery development owing to their ample biological spectrum. Triazole core moieties found in a variety of natural products, important class of alkaloids and other synthetic vital molecules with a wide range of biological activities and applications, mainly, 1,2,3-triazoles have been postulated to generate a nonclassic bioisostere of amide bond, which is necessary feature to increase binding affinity towards receptor. Greatly, attention has been waged to click chemistry arena due to easily accessible novel complex and diversified heterocycles using environmentally benign and relatively inexpensive reagents [Galli *et al.,* 2008; Valverde *et al.,* 2013]. Boechat *et al.,* reported novel 1,2,3-triazole derivatives for use against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) strain. Among them, four derivatives **44a, 44b, 44c** and **44d** possess admirable activity against MTB H37Rv with MIC 0.62 µg/mL [Boechat *et al.,* 2011]. Triazole-fused spirochromone conjugates were synthesized and screened for their *in vitro* antimycobacterial activity against MTB H37Rv. Most of the compounds depicted a significant *in vitro* activity against MTB with a MIC value in the range, 0.78–6.25 µg/mL. Among them, one compound (**45**) was more active with MIC 0.78 µg/mL, SAR reveals that compounds possessing cyclohexyl group at position 2 of the chromone ring favour better activity than piperidinyl moiety; and aromatic substitution at position 4 of the triazole is favorable than alkyl substitution [Muthukrishnan *et al.,* 2011].



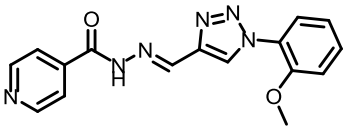
44a
MTB MIC = 0.62 µg/mL



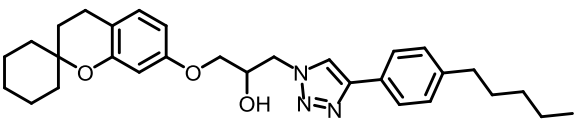
44b
MTB MIC = 0.62 µg/mL



44c
MTB MIC = 0.62 µg/mL



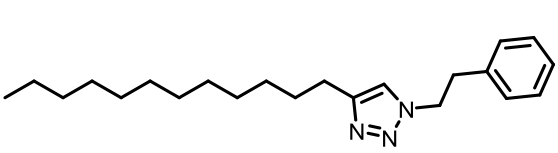
44d
MTB MIC = 0.62 µg/mL



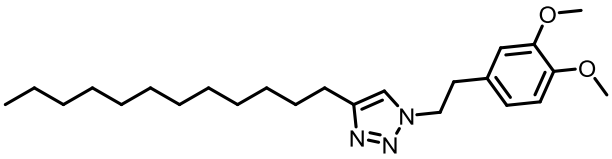
45
MTB MIC = 0.78 µg/mL

Menendez and his team reported synthesis of 1,2,3-triazoles bearing alkyl and aryl/alkyl chain using click chemistry and examined their anti-TB activity against MTB H37Rv strain. Among them, two derivatives **46a** and **46b** evinced promising inhibition effects with MIC values 0.50 and 0.25 µg/mL, respectively. The length of the alkyl chain is important, which in turn affects the anti-TB activity, the 12-carbon chain derivatives were found to be eight to ten fold more active than 9 and 10 carbon chain [Menendez *et al.,* 2012]. Patpi and co-workers,

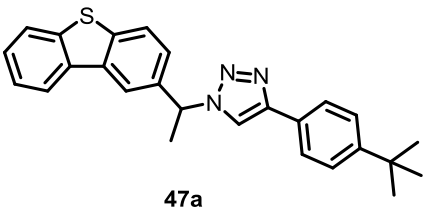
reported a series of novel dibenzo[*b,d*]furan, dibenzo[b,d]thiophene, and *N*-methylcarbazole clubbed 1,2,3-triazoles as potent inhibitors of *Mycobacterium tuberculosis.* Among them nine derivatives were excellent with MICs ≤ 1.56 µg/mL against MTB H37Rv. From these, 4-(4-*tert*-butylphenyl)-1-(1-(dibenzo[*b,d*]thiophen-2-yl)ethyl)-1H-1,2,3-triazole (**47a**) and 4-(2-chloro-4-fluorophenyl)-1-(1-(dibenzo[*b,d*]thiophen-2-yl)-ethyl)-1H-1,2,3-triazole (**47b**) have admirable anti-TB activity with MIC value 0.78 µg/mL [Patpi *et al.*, 2012].



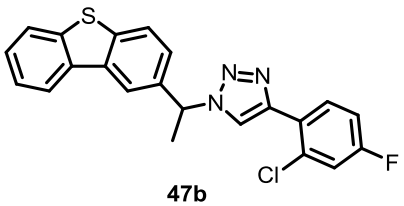
46a
MTB MIC = 0.5 µg/mL



46b
MTB MIC = 0.25 µg/mL

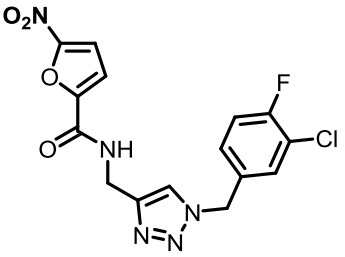


47a
MTB MIC = 0.78 µg/mL

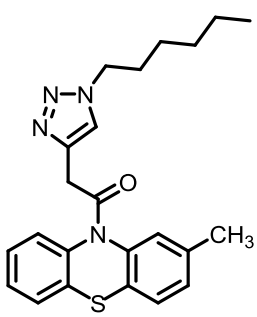


47b
MTB MIC = 0.78 µg/mL

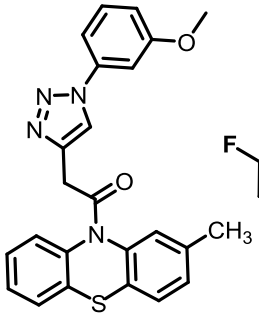
Kamal and coworkers synthesized nitrofuran-triazole conjugates and evaluated their anti-TB activity against MTB H37Rv. Amongst them, compound **48** exhibited excellent anti-TB activity with MIC value 0.25 µg/mL [Kamal *et al.*, 2013]. Addla and his co-workers reported 2-(trifluoromethyl) phenothiazine-[1,2,3]triazole hybrids from 2-(trifluoromethyl)-1′H-phenothiazine in three steps and evaluated for their anti-tubercular activity. Among the 18 molecules, **49a, 49b** and **49c** derivatives emerged as moderately active compounds by inhibiting MTB H37Rv with MICs 6.25 µg/mL [Addla *et al*., 2014]. Kumar and coworkers reported the synthesis of Isoniazid-triazole conjugates and their *in vitro* evaluation as possible anti-TB agents against MTB H37Rv. The compounds exhibited potent activity against MTB strain with MIC values ranging from 0.195 to 1.56 µM. Among them **50** was the most active derivative with MIC 0.195µM [Kumar *et al.*, 2014].



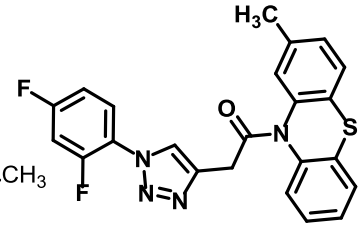
48
MTB MIC = 0.25 µg/mL



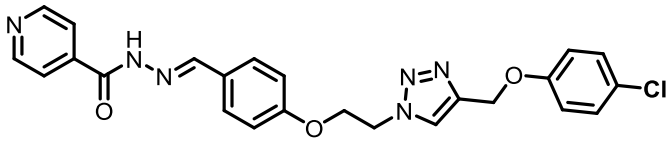
49a
MTB MIC = 6.25 µg/mL



49b
MTB MIC = 6.25 µg/mL

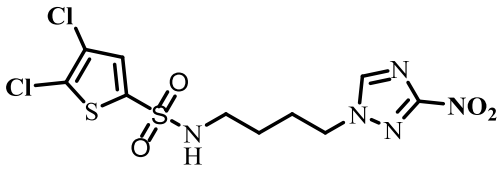


49c
MTB MIC = 6.25 µg/mL

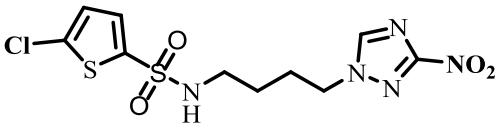


50
MTB MIC = 0.195 µM

Recently, Papadopoulou *et al*., reported anti-mycobacterial screening results of nitrotriazole and imidazole based amides and sulphonamides. Among the series of molecules, 4,5-dichloro-*N*-(4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl)thiophene-2-sulfonamide (**51a**) and 5-chloro-*N*-(4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl)thiophene-2-sulfonamide (**51b**) demonstrated MIC values of 1.56 µM and 3.13 µM respectively. These molecules could act as potential leads for further development [Papadopoulou *et al*., 2014].



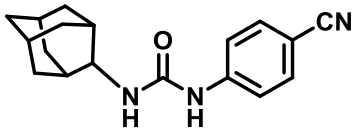
51a
MTB MIC = 1.56 µM



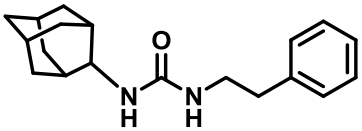
51b
MTB MIC = 3.13 µM

2.13. Miscellaneous hetero derivatives as promising anti-tubercular agents

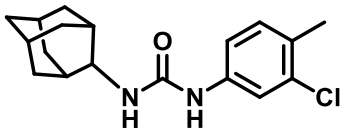
Brown and co-workers, reported synthesis of adamantyl urea derivatives and evaluated for *in vitro* anti-mycobacterial activity against MTB. Among the synthesized compounds **52a, 52b, 52c** and **52d** showed MICs < 1 µg/mL [Brown *et al*., 2011].



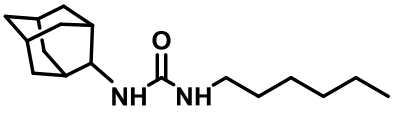
52a
MTB MIC = 0.4 µg/mL



52b
MTB MIC = 0.01 µg/mL

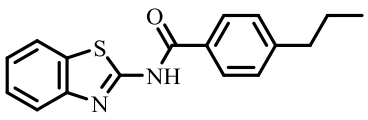


52c
MTB MIC = 0.02 µg/mL

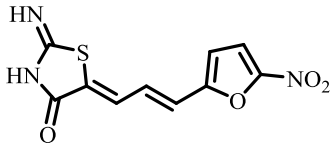


52d
MTB MIC = 0.01 µg/mL

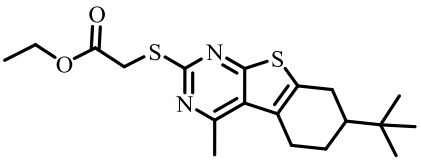
Vilcheze *et al*., screened 300 compounds against MTB H37Rv. Out of these compounds, 11 compounds were found to be active by inhibiting the growth of MTB. Out of which, four compounds (**53a, 53b, 53c** and **53d**) showed MICs below 10 µM [Vilcheze *et al*., 2011].



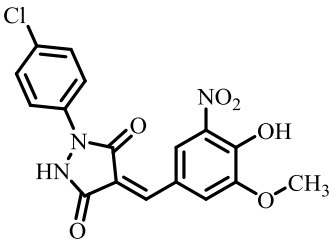
53a
MIC H37Rv = 8.5 µM



53b
MIC H37Rv = 9.0 µM

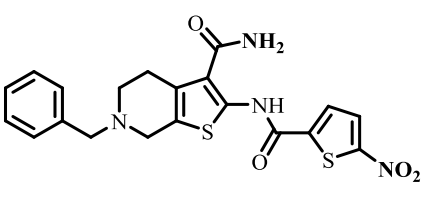


53c
MIC H37Rv = 1.0 µM

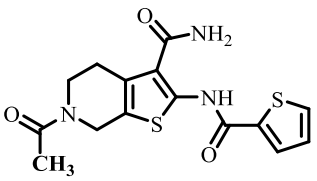


53d
MIC H37Rv = 1.5 µM

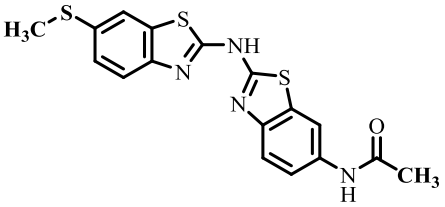
Reynolds and co-workers reported highthroughput screening results of a total of 25,671 compounds against MTB in a single dose assay at a concentration of 10 µg/mL. Out of these, 1,329 compounds were found to be active based on their ability to inhibit growth of organism by ≥ 85%. These active compounds were further evaluated in a dose-response format against MTB where 584 compounds possessed MTB IC_{90} values of < 10 µg/mL. The structures of most potent and non-cytotoxic compounds (**54a, 54b, 54c, 54d, 54e** and **54f**) are shown below [Reynolds *et al.,* 2012].



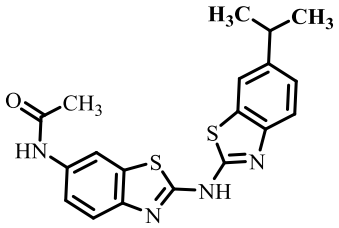
54a
MTB MIC_{90} = 0.5 µg/mL



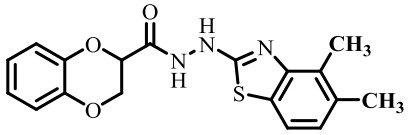
54b
MTB MIC_{90} = 3.2 µg/mL



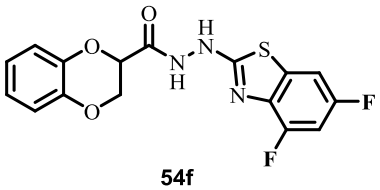
54c
MTB MIC_{90} = <0.2 µg/mL



54d
MTB MIC_{90} = 2.0 µg/mL

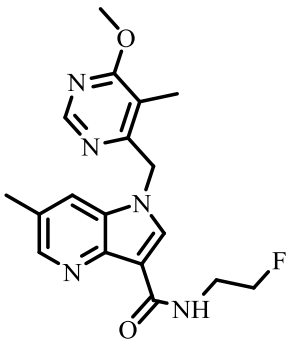


54e
MTB MIC_{90} = 0.9 **µg/mL**

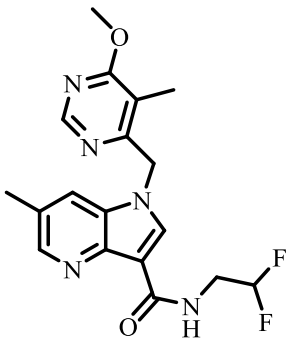


54f
MTB MIC_{90} = 3.3 **µg/mL**

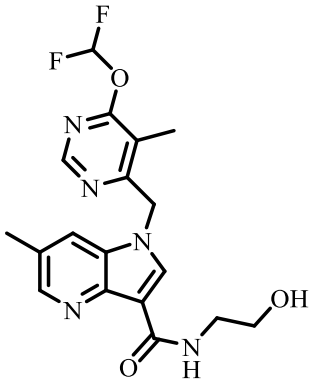
Shirude and team have recently reported synthesis of 1,4-azaindoles and evaluated for *in vitro* anti-mycobacterial activity against MTB H37Rv. Among a total of 37 derivatives, three compounds (**55a, 55b** and **55c**) exhibited excellent anti-TB activity with MICs 0.39 µM [Shirude *et al.*, 2012].



55a
MTB MIC = 0.39 µM

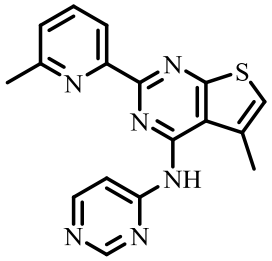


55b
MTB MIC = 0.39 µM

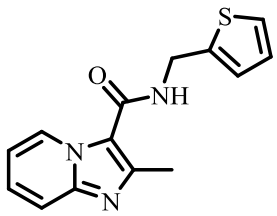


55c
MTB MIC = 0.39 µM

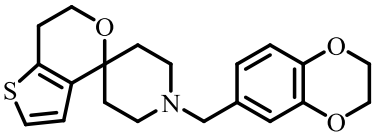
To invade the growing TB infection, researchers at GlaxoSmithKline and others have reported most promising families, from high throughput screening of their corporate compound libraries (>2 × 10^6 chemical entities) against mycobacteria. The most effective compounds with their MICs against H37Rv and code numbers are presented [Ballell *et al.*, 2013].



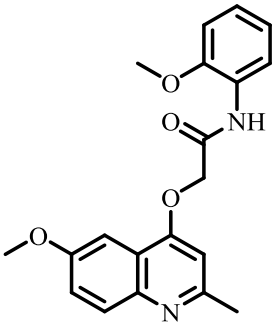
GSK163574A
MIC H37Rv: 0.76 µM



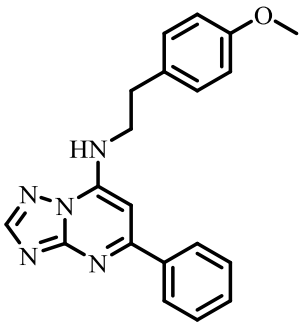
GSK1829820A
MIC H37Rv: 0.19 µM



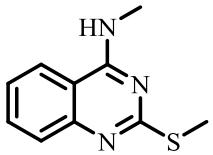
GSK2200150A
MIC H37Rv: 0.38 µM



GSK358607A
MIC H37Rv: 0.70 µM

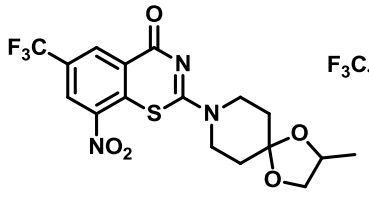


GSK888636A
MIC H37Rv: 0.94 µM

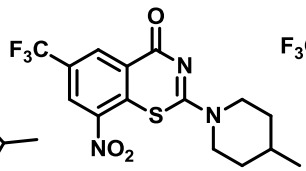


GSK353069A
MIC H37Rv: 0.13 µM

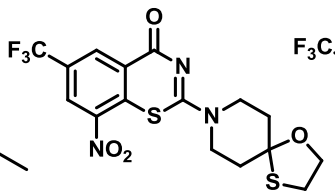
Gao and co-workers studied the anti-mycobacterial activity of *N*-alkyl substituted 1,3-benzothiazin-4-one derivatives. Out of them sixteen derivatives possessed excellent anti-TB activity with MICs < 1 µM. From these analogues four compounds (**56a, 56b, 56c** and **56d**) displayed admirable anti-TB, with MIC ≤ 0.02 µM. Amongst them, compound **56d**, which contained an azaspirodithiolane group, showed MIC of 0.0001 µM against MTB H37Rv and it was also non-toxic to Vero cells. SAR analysis evinced that extended or branched alkyl chain could enhance the potency of *N*-alkyl substituted 1,3-benzothiazin-4-ones [Gao *et al.*, 2013].



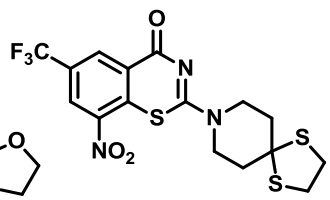
56a
MTB MIC = 0.002 µM



56b
MTB MIC = 0.08 µM

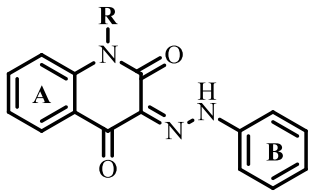


56c
MTB MIC = 0.002 µM



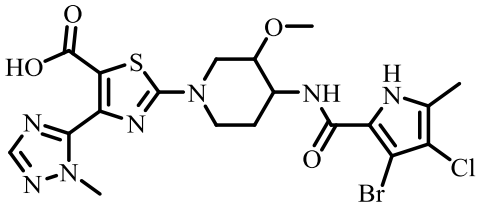
56d
MTB MIC = 0.0001 µM

Manvar and co-workers synthesized 79 new quinolylhydrazides and evaluated them for anti-tubercular activity. Out of seventy nine, forty compounds (**57**$_{40}$) exhibited MICs of < 6.25 µg/mL against MTB H37Rv strains [Manvar A., *et al.*, 2013].



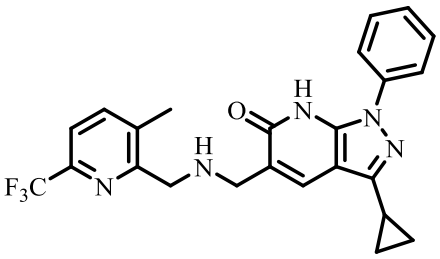
57_{40} MIC <6.25 µg/mL,
R = H and CH_3;
ring A, B = various *o,m,p*- and mono, di-substituted benzenes

Ehmann and Sushmita reported the compound **58** as potential inhibitor for bacterial DNA topoisomerase with MIC 0.25 µM [Ehmann *et al*., 2014].

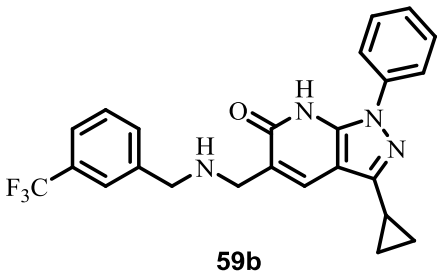


58
MTB MIC = 0.25 µM

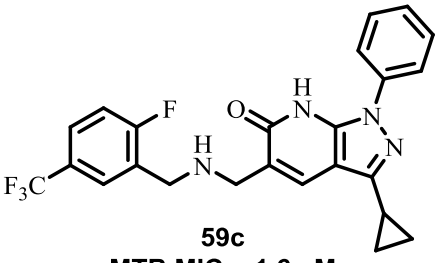
Panda *et al*., from AstraZeneca-India, published the synthesis and anti-mycobacterial activity of pyrazolopyridones. Four derivatives (**59a, 59b, 59c** and **59d**) were found to be very active from their study, against MTB with MIC values ≤ 3.1 µM. Among the compounds, **59a** possess excellent anti-TB activity with MIC 1.5 µM [Panda M., *et al.*, 2014].



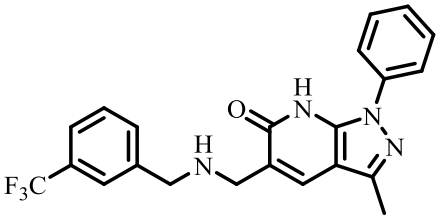
59a
MTB MIC = 1.5 µM



59b
MTB MIC = 1.6 µM

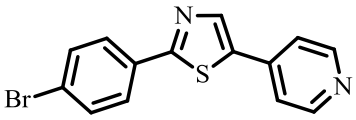


59c
MTB MIC = 1.6 µM



59d
MTB MIC = 3.1 µM

Parameshwar and Tharanikkarasu, have published synthesis of 2-aminothiazoles starting with substituted acetophenones and evaluated for their anti-mycobacterial activity against MTB H37Rv. Among the reported 34 molecules, compound **60** emerged as the most active compound with MIC 6.25 µM. Compound **60** also possessed, three H-acceptors, one H-donor, molecular weight of 332.2, logP of 4.34 and three freely rotatable bonds making zero violations to Lipinski rule [Parameshwar and Tharanikkarasu, 2014].



60
MTB MIC = 6.25 µM

Chapter III
Objectives & Plan of Work

Chapter 3

OBJECTIVES & PLAN OF WORK

3.1. Objectives

Based on the broad range of literature review of accessible and new promising anti-tubercular agents, we summarise that more work can be done in developing enhanced anti-tubercular agents which reduce the cost and side effects minimizing the treatment period.

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

1. To design novel anti-tubercular agents based on reported anti-tubercular agent leads by molecular hybridisation strategy and rational drug derivatization based medicinal chemistry approach.

2. To synthesize the designed molecules by conventional methods, an environmental benign technique, microwave assisted organic synthesis and solvent free organic synthesis wherever practicable.

3. To carry out *in vitro* anti-mycobacterial screening of the synthesized compounds against *Mycobaterium tuberculosis.*

4. To evaluate *in vitro* cytotoxicity of the most active compounds and determine the selectivity profile.

3.2. Plan of work

The plan of work is classified into following categories:

3.2.1. Design of novel anti-tubercular agents

We designed the new anti-tubercular agents by adopting two approaches: i) Molecular hybridisation strategy from lead motifs. ii) Rational drug derivatization medicinal chemistry approach.

3.2.2. Design strategy of novel scheme 1 (benzisoxazole) derivatives

Based on broad range of literature review (chapter 2) about heterocyclic motifs such as benzisoxazole, oxindole, indole, triazole, piperazine, homopiperazine and sulphonamide derivatives possessing anti-TB activity, we stitched some of these heterocycles and designed novel compounds as depicted in **Figure 3.1** [Chande *et al.*, 2005; Bogatcheva *et al.*, 2006; Sriram *et al.*, 2006a; Gözel *et al.*, 2008; Subash *et al.*, 2008; Karthikeyan *et al.*, 2009; Ranjith Kumar *et al.*, 2009; Kamal *et al.*, 2013; Kondreddi *et al.*, 2013; Ranjith *et al.*, 2014].

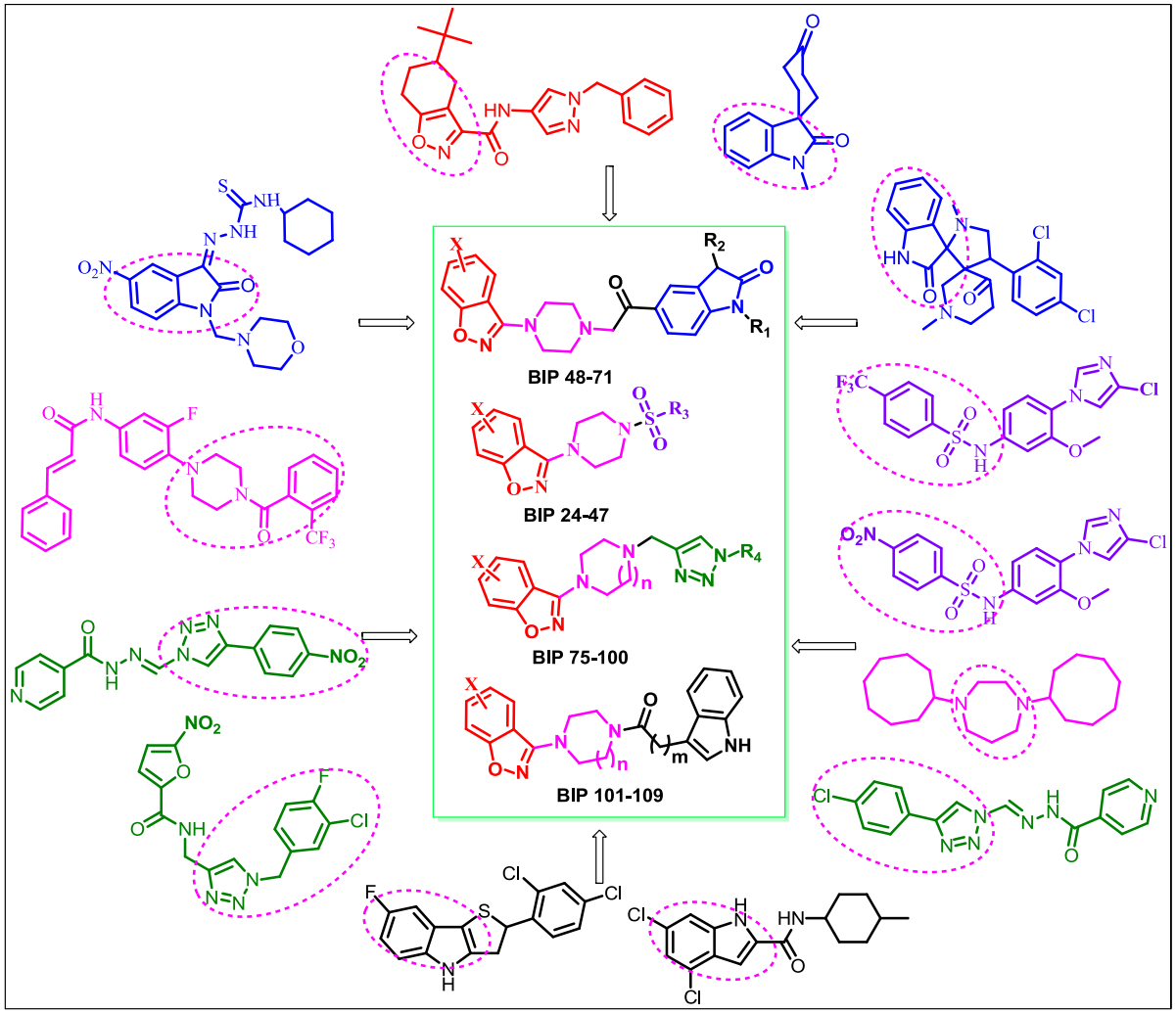


Figure 3.1: Design strategy to achieve the **scheme 1** compounds

3.2.3. Design strategy of novel scheme 2 (quinoxaline) derivatives

Based on quinoxaline, triazoles, piperazine and hydrazine derivatives possessing anti-TB activity, we crafted scheme 2 compounds by hybridisation approach [Savini *et al.*, 2002; Jaso *et al.*, 2005; Sriram *et al.*, 2005; Imramovský *et al.*, 2007; Boechat *et al.*, 2011; Puratchikody *et al.*, 2011; Torres *et al.*, 2011; Menendez *et al.*, 2012; Kamal et al., 2013; Shiva Kumar *et*

al., 2013; Asif *et al.*, 2014; Hadda *et al.*, 2014]. Sketched new derivatives are portrayed in **Figure 3.2.**

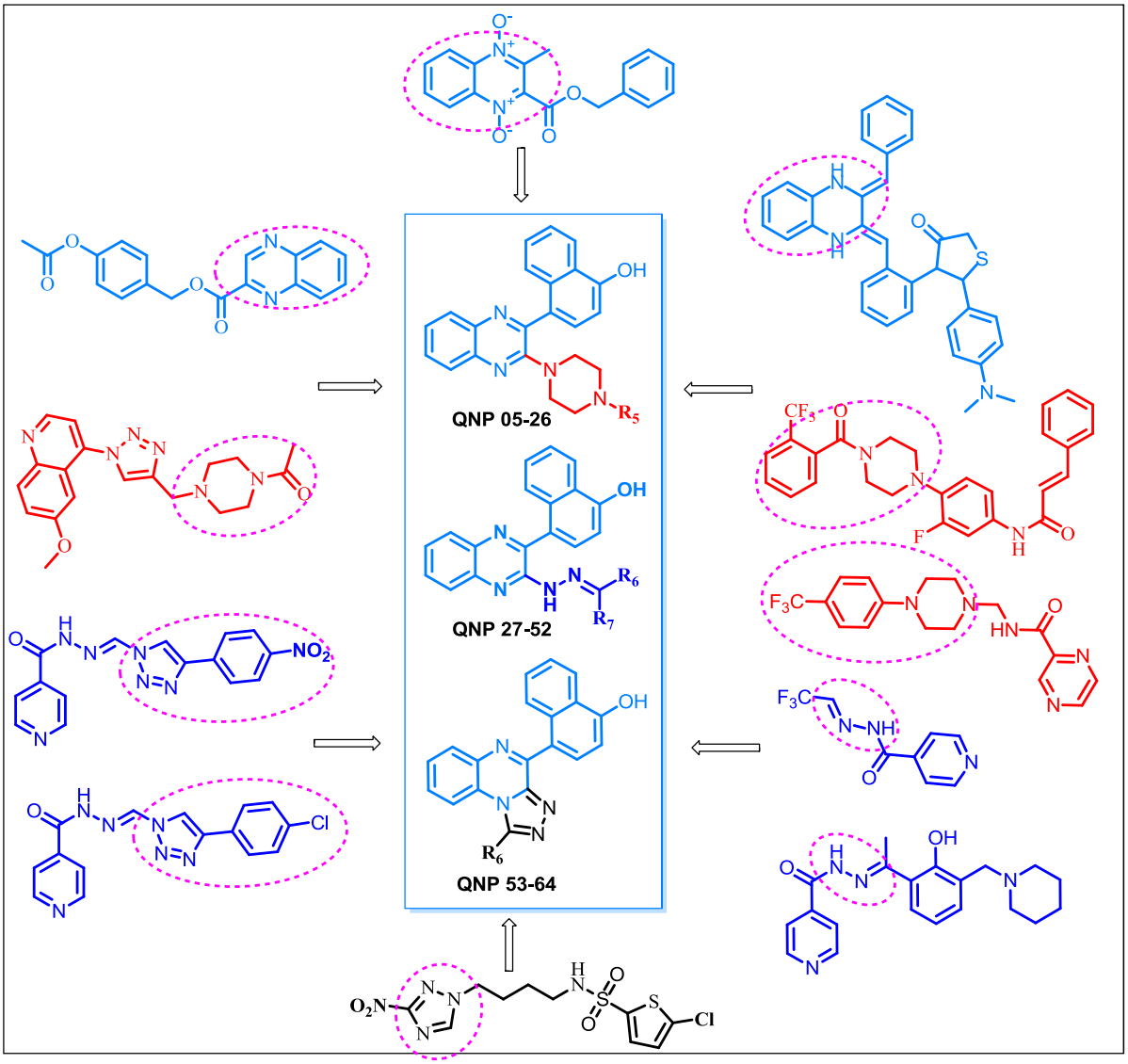


Figure 3.2: Design strategy to achieve the **scheme 2** derivatives

3.2.4. Design strategy of novel scheme 3 (phenanthridine) derivatives

Based on literature survey (chapter 2), phenanthridine, amide and sulphonamide derivatives possesing anti-TB activity, we derivatized some of these heterocycles and designed novel compounds as depicted in **Figure 3.3** [Tangallapally *et al.*, 2005; Punkvang et al., 2010; Thomas *et al.*, 2011; Cappoen *et al.*, 2012; Cappoen *et al.*, 2014].

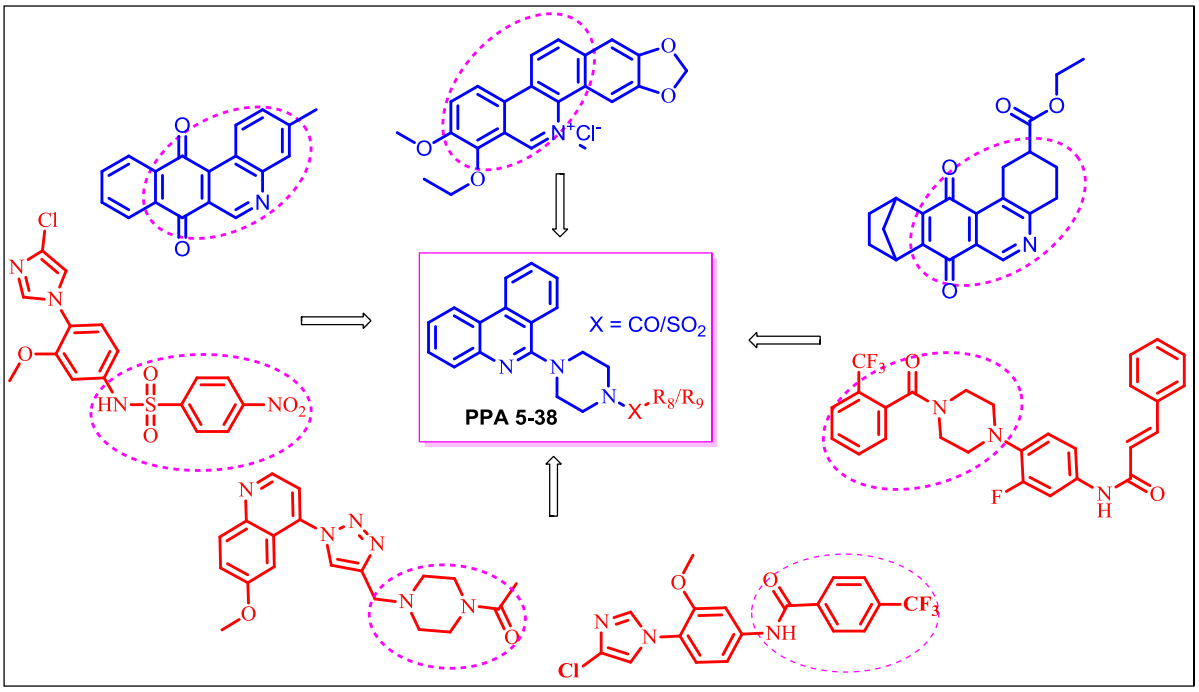


Figure 3.3: Design strategy to achieve the **scheme 3** analogues

3.2.5. Design strategy of novel scheme 4 (indole) derivatives

Based on broad range of literature study (chapter 2), heterocyclic molecules such as imidazole, indole, oxindole and triazole derivatives possessing anti-TB activity, we draw scheme 3 compounds by hybridisation and rational approach depicted in **Figure 3.4** [Chande *et al.*, 2005; Sriram *et al.*, 2006; Bogatcheva *et al.*, 2006; Gözel *et al.*, 2008; Subash *et al.*, 2008; Jadhav *et al.*, 2009; Karthikeyan *et al.*, 2009; Ranjith Kumar et al., 2009; Gobis *et al.*, 2012; Aswathi *et al.*, 2013; Kamal *et al.*, 2013; Kondreddi *et al.*, 2013; Mir *et al.*, 2014; Ranjith *et al.*, 2014; Gobis *et al.*, 2015; Yoon *et al.*, 2015].

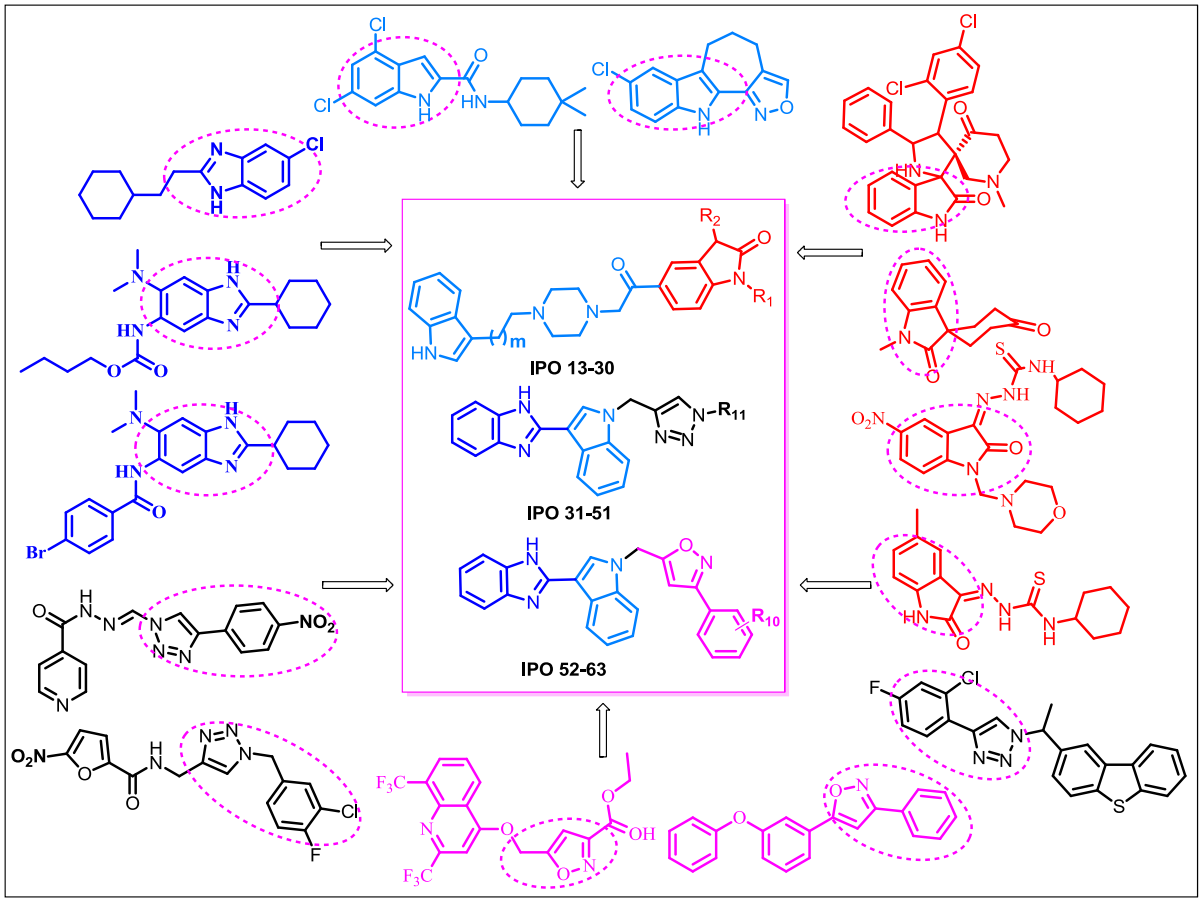


Figure 3.4: Design strategy to achieve the **scheme 4** derivatives

3.2.6. Synthesis and characterization of designed molecules

Synthesis: The designed molecules 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. Synthetic strategies for other compounds was developed in house. 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 or biotage chromatography.

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

3.2.7. *In vitro* anti-mycobacterial activity against MTB H37Rv

The *in vitro* anti-mycobacterial screening of the synthesized compounds was carried out against MTB H37Rv strain. This investigation was performed by micro plate alamar blue assay (MABA) method.

3.2.8. *In vitro* cytotoxicity screening

The most active compounds were evaluated for *in vitro* cytotoxic activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay method and further selectivity index was determined.

Chapter IV
Materials and Methods

Chapter 4

MATERIALS AND METHODS

4.1. Materials and Methods

Thin-layer chromatography (TLC) was carried out on aluminium-supported silica gel plates (Merck 60 F254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 100-200 and 230-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on 300 MHz using a Bruker AV 300 spectrometer or 400 MHz using a Bruker AV 400 spectrometer (Bruker CO., Switzerland) in $CDCl_3$ or DMSO-d_6 solution with tetramethylsilane as the internal standard, and chemical shift values (δ) were given in ppm. Microwave reactions are performed in closed vessel using Biotage Initiator microwave synthesizer (Uppsala, Sweden). IR spectra were recorded on a FT-IR spectrometer (Schimadzu) and peaks are reported in cm^{-1}. Melting points were determined on an electro thermal melting point apparatus (Stuart-SMP30) in open capillary tubes and are uncorrected. Elemental analyses were analysed by Elementar Analysensysteme GmbH vario MICRO cube CHNS/O Analyzer. Mass spectra (ESI-MS) were recorded on Schimadzu MS/ESI mass spectrometer. Chemical Crystallography was analysed by Single crystal X-ray diffraction (XRD), Bruker. Unless otherwise indicated, all the reagents were purchased from commercial suppliers and were used without further purification.

4.2. Chemistry experimental work

Present thesis work is split into four schemes under the following heads.

4.2.1. 3-(piperazin-1-yl/homopiperazine)benzo[*d*]isoxazole (Scheme 1) derivatives

Scheme 1 methodology involved following stages, key intermediate synthesis of substituted-3-(piperazin-1-yl)benzo[*d*] isoxazole derivatives (**BIP 17-20**), cardinal synthesis of 3-(1,4-diazepan-1-yl)benzo[*d*]isoxazole (**BIP 23**) and chief intermediate synthesis of 5-(2-chloroacetyl)-substituted indolin-2-one derivatives (**OIP 16-21**). Synthesis of 3-(4-(substituted sulfonyl)piperazin-1-yl)benzo[*d*]isoxazole analogues (**BIP 24-47**), 5-(2-(4-(benzo[*d*]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one derivatives (**BIP 48-71**), 3-(4-((substituted-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[*d*]isoxazole derivatives

(**BIP 75-100**) and 1-(4-(substituted benzo[*d*]isoxazol-3-yl) piperazin-1-yl)-2-(1H-indol-3-yl) substituted-1-one analogues (**BIP 101-109**) out lined in **Figure 4.1.**

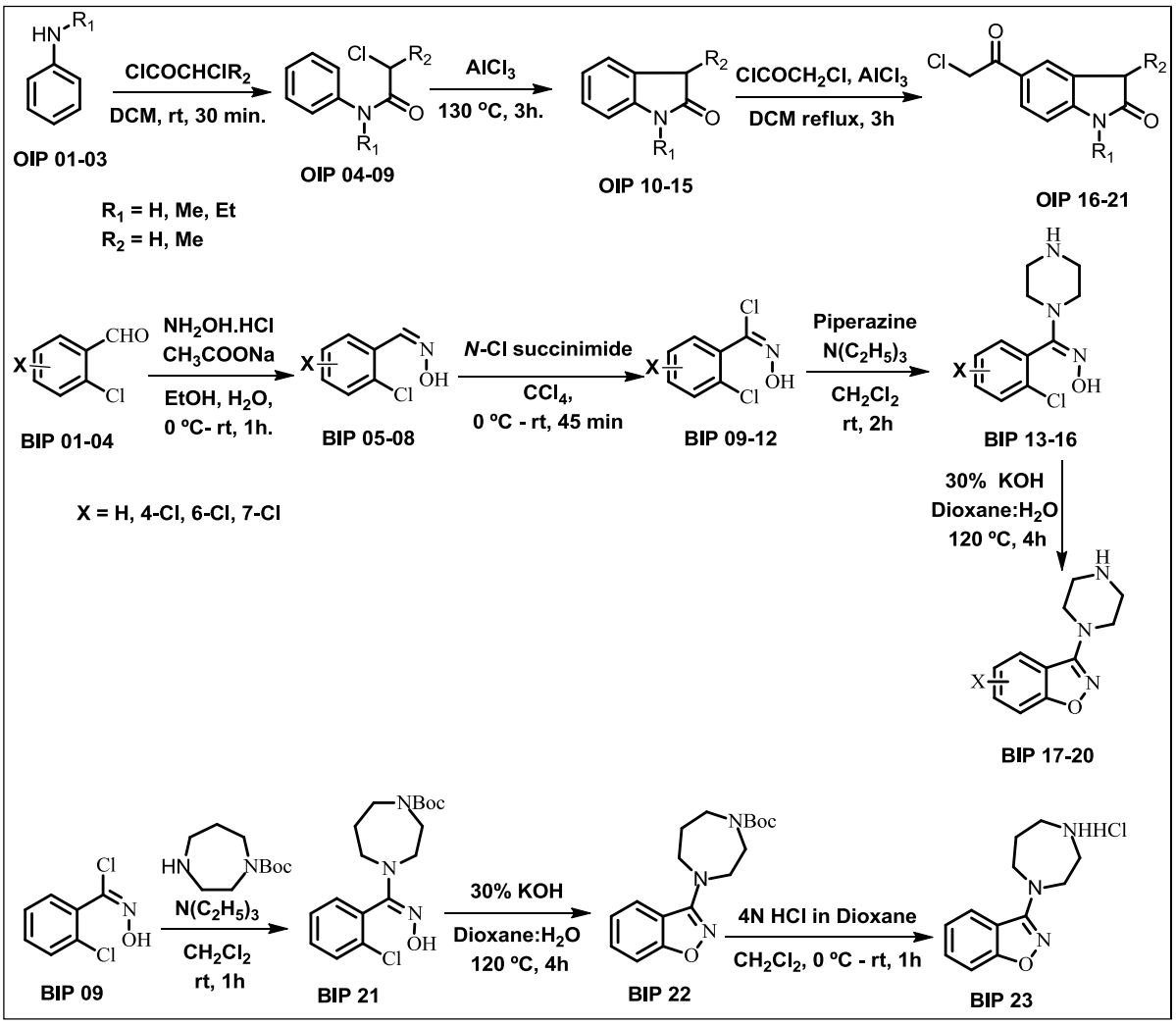


Figure 4.1: Synthetic protocol employed for the synthesis of key intermediates for scheme 1

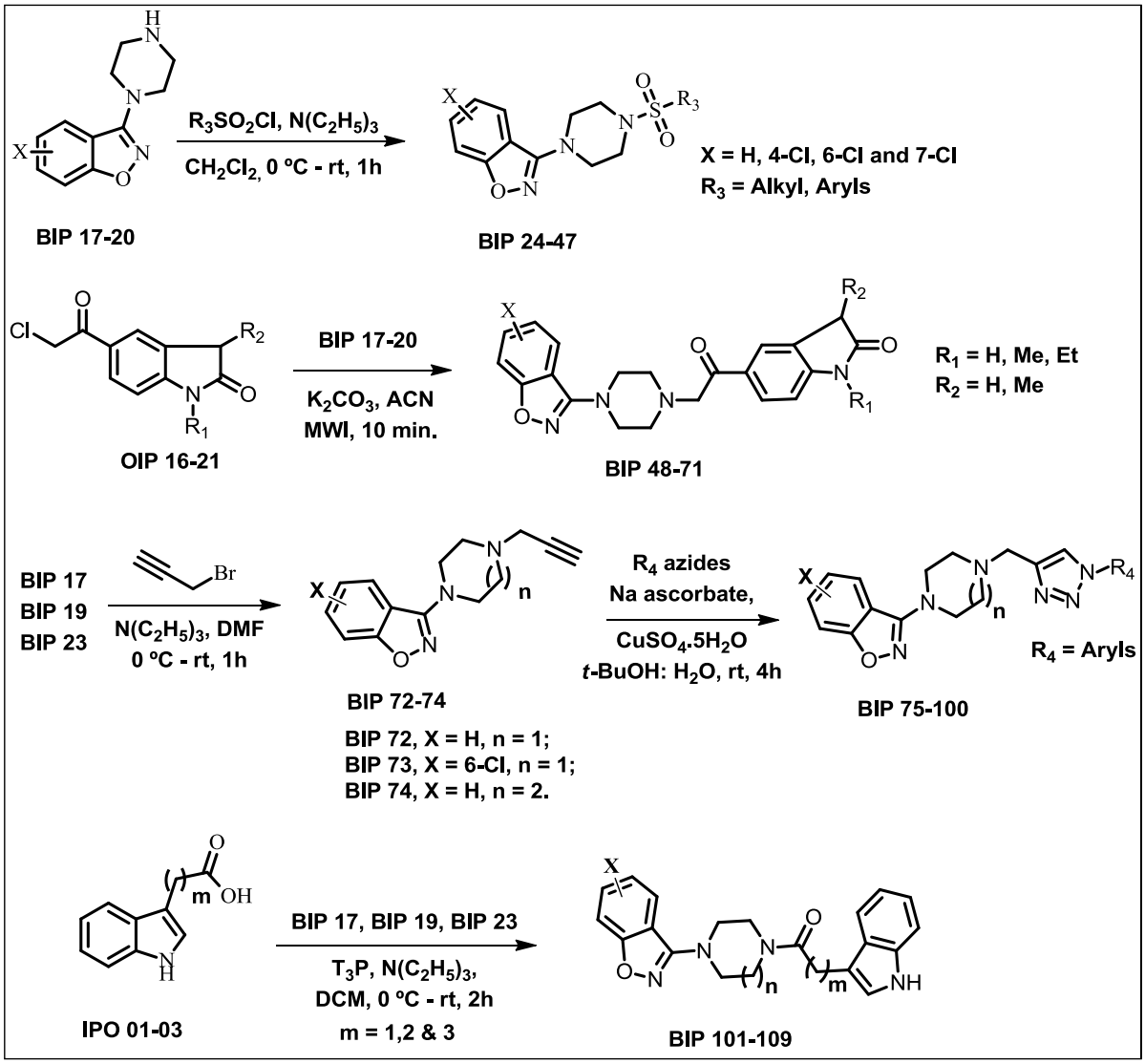
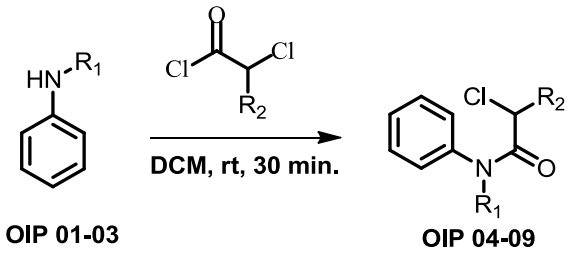


Figure 4.2: Synthetic protocol employed for the synthesis of compounds **BIP 24-109** of scheme 1

General experimental procedure utilized for the synthesis of 5-(2-chloroacetyl)indolin-2-one derivatives (OIP 16-21) and characterization

The *N*-substituted aniline **(OIP 01-03)** on treatment with 2-chloroacetyl chloride / 2-chloropropanoyl chloride and $N(C_2H_5)_3$ in CH_2Cl_2 at 0 °C - rt for 1h yielded 2-chloro-*N*-substituted-*N*-phenyl amide derivatives **(OIP 04-09)**. Further cyclisation with $AlCl_3$ under solvent free conditions at 130 °C for 3h afforded substituted indolin-2-one derivatives **(OIP 10-15)**. Substituted 5-(2-chloroacetyl)indolin-2-ones (**OIP 16-21**) were synthesized by reacting 2-chloroacetyl chloride with anhydrous $AlCl_3$ in CH_2Cl_2 as Friedel craft acylation at rt for 3h with moderate to good yield [Sumpter *et al.*, 1945; Howard *et al.*, 1996] (**Figure 4.1)**.

Preparation of 2-chloro-*N*-phenylacetamide derivatives (OIP 04-09)



The *N*-substituted anilines **(OIP 01-03)** (5.00 g, 1.0 equiv.) in CH_2Cl_2 were cooled to 0 °C. After 4 min, 2-chloroacetyl chloride / 2-chloropropanoyl chloride (1.20 equiv.) were added to above mixture and warmed to rt for 30 min. After completion of the reaction, as indicated by TLC, the reaction were quenched with water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (05 - 20 %)] to get **OIP 04-09** in good yield ranging from 78-95%.

Characterization of the synthesized OIP 04-09 compounds

***2-chloro-N-phenylacetamide* (OIP 04)**

Appearance: white solid; Yield: 85% (7.74g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (b, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.42–7.30 (m, 3H), 4.38 (s, 2H); ESI-MS: (m/z) calcd. for C_8H_8ClNO: 169.02, found: 170.11 $[M+H]^+$.

***2-chloro-N-phenylpropanamide* (OIP 05)**

Appearance: white solid; Yield: 78% (6.78g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (b, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.43–7.30 (m, 3H), 4.71 (q, 1H), 1.84 (d, J = 4.0 Hz, 3H); ESI-MS: (m/z) calcd. for $C_9H_{10}ClNO$: 183.04, found: 184.13 $[M+H]^+$.

***2-chloro-N-methyl-N-phenylacetamide* (OIP 06)**

Appearance: white solid; Yield: 95% (9.36g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 8.4 Hz, 2H), 7.40–7.28 (m, 3H), 3.92 (s, 2H), 3.35 (s, 3H); ESI-MS: (m/z) calcd. for $C_9H_{10}ClNO$: 183.04, found: 184.11 $[M+H]^+$.

***2-chloro-N-methyl-N-phenylpropanamide* (OIP 07)**

Appearance: white solid; Yield: 92% (9.76g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.4 Hz, 2H), 7.39–7.27 (m, 3H), 4.32 (q, 1H), 3.31 (s, 3H), 1.58 (d, J = 1.2 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{10}H_{12}ClNO$: 197.06, found: 198.15 $[M+H]^+$.

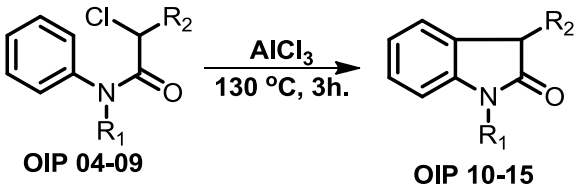
***2-chloro-N-ethyl-N-phenylacetamide* (OIP 08)**

Appearance: brown oil; Yield: 88% (9.33g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 8.0 Hz, 2H), 7.33–7.21 (m, 3H), 4.48 (q, 2H), 3.78 (s, 2H), 1.20 (t, J = 1.2 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{10}H_{12}ClNO$: 197.06, found: 198.14 $[M+H]^+$.

2-chloro-N-ethyl-N-phenylpropanamide **(OIP 09)**

Appearance: brown oil; Yield: 84% (8.91g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 8.0 Hz, 2H), 7.31–7.23 (m, 3H), 4.45 (q, 1H), 3.89 (q, 2H), 1.76 (d, J = 1.2 Hz, 3H), 1.6 (t, J = 1.6 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{11}H_{14}ClNO$: 211.07, found: 212.15 $[M+H]^+$.

Preparation of indolin-2-one derivatives (OIP 10-15)



OIP 04-09 **OIP 10-15**

The 2-chloro-*N*-phenylacetamide derivatives **(OIP 04-09)** (5.00 g, 1.0 equiv.) on cyclisation with $AlCl_3$ (3.0 equiv.) under solvent free conditions at 130 °C for 3h afforded substituted indolin-2-one derivatives **(OIP 10-15)**. After completion of the reaction, as indicated by TLC, the reaction was quenched with 4N HCl and water was slowly added and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (10 - 30 %)] to get the **OIP 10-15** in moderate to good yields (60-75%).

Characterization of the synthesized OIP 10-15 compounds

Indolin-2-one **(OIP 10)**

Appearance: white solid; Yield: 61% (2.03g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (b, 1H), 7.36 (m, 1H), 7.32 (d, J = 8.0 Hz, 2H), 7.05 (m, 1H), 3.48 (s, 2H); ESI-MS: (m/z) calcd. for C_8H_7NO: 133.05, found: 134.11 $[M+H]^+$.

3-methylindolin-2-one **(OIP 11)**

Appearance: white solid; Yield: 60% (2.40g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (b, 1H), 7.35 (m, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 6.89–6.78 (m, 1H), 3.48 (q, 1H), 1.51 (d, J = 1.2 Hz, 3H); ESI-MS: (m/z) calcd. for C_9H_9NO: 147.06, found: 148.13 $[M+H]^+$.

1-methylindolin-2-one **(OIP 12)**

Appearance: white solid; Yield: 78% (3.12g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 8.0 Hz, 1H), 7.22–6.99 (m, 2H), 6.91 (d, J = 8.0 Hz, 1H), 3.51 (s, 2H), 3.34 (s, 3H); ESI-MS: (m/z) calcd. for C_9H_9NO: 147.06, found: 148.11 $[M+H]^+$.

1,3-dimethylindolin-2-one **(OIP 13)**

Appearance: white solid; Yield: 73% (2.97g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.97 (m, 1H), 3.50 (q, 1H), 3.38 (s, 3H), 1.49 (d, J = 1.6 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{10}H_{11}NO$: 161.08, found: 162.15 $[M+H]^+$.

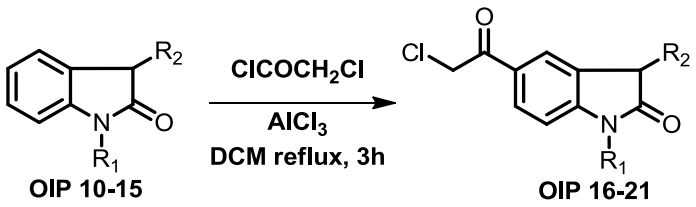
1-ethylindolin-2-one **(OIP 14)**

Appearance: brown oil; Yield: 72% (2.93g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 8.0 Hz, 1H), 7.24–7.02 (m, 2H), 6.99 (d, J = 8.0 Hz, 1H), 3.90 (q, 2H), 3.32 (s, 2H), 1.56 (t, J = 1.6 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{10}H_{11}NO$: 161.08, found: 162.19 $[M+H]^+$.

1-ethyl-3-methylindolin-2-on **(OIP 15)**

Appearance: brown oil; Yield: 70% (2.89g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.97 (m, 1H), 3.97 (q, 1H), 3.65 (q, 2H), 1.58 (t, J = 1.6 Hz, 3H), 1.16 (d, J = 2.0 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{11}H_{13}NO$: 175.09, found: 176.21 $[M+H]^+$.

Preparation of 5-(2-chloroacetyl)indolin-2-one derivatives (OIP 16-21)



OIP 10-15 OIP 16-21

Substituted 5-(2-chloroacetyl)indolin-2-ones (**OIP 16-21**) were synthesized from **OIP 10-15** (4.00 g, 1.0 equiv.) by reacting with 2-chloroacetyl chloride (1.30 equiv.) anhydrous $AlCl_3$ (3.00 equiv.) in CH_2Cl_2 at rt for 3h. After completion of the reaction, as indicated by TLC, the reaction was quenched with 4N HCl and water was slowly added and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (30 - 50 %)] to get the **OIP 10-15** in moderate to good yields (60-80%).

Characterization of the synthesized OIP 16-21 compounds

5-(2-chloroacetyl) indolin-2-one **(OIP 16)**

Appearance: off white solid; Yield: 65% (4.09g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (b, 1H), 8.12 (s, 1H), 8.09 (d, J = 8.8 Hz, 1H), 7.36 (d, J = 9.6 Hz, 1H), 4.66–3.49 (s, 4H); ESI-MS: (m/z) calcd. for $C_{10}H_8ClNO_2$: 209.20, found: 210.11 $[M+H]^+$.

***5-(2-chloroacetyl)-3-methylindolin-2-one* (OIP 17)**

Appearance: off white solid; Yield: 62% (4.09g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (b, 1H), 8.10 (s, 1H), 8.08 (d, J = 8.8 Hz, 1H), 7.37 (d, J = 9.6 Hz, 1H), 4.67 (s, 2H), 3.54 (q, 1H), 1.69 (d, J = 1.2 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{11}H_{10}ClNO_2$: 223.04, found: 224.15 $[M+H]^+$.

***5-(2-chloroacetyl)-1-methylindolin-2-one* (OIP 18)**

Appearance: off white solid; Yield: 78% (4.73g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.35 (d, J = 9.6 Hz, 1H), 4.68–3.34 (s, 7H); ESI-MS: (m/z) calcd. for $C_{11}H_{10}ClNO_2$: 223.04, found: 224.11 $[M+H]^+$.

***5-(2-chloroacetyl)-1,3-dimethylindolin-2-one* (OIP 20)**

Appearance: off white solid; Yield: 80% (4.71g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (s, 1H), 8.00 (d, J = 9.2 Hz, 1H), 7.36 (d, J = 9.6 Hz, 1H), 4.68 (s, 2H), 3.53 (q, 1H), 3.38 (s, 3H), 1.68 (d, 3H); ESI-MS: (m/z) calcd. for $C_{12}H_{12}ClNO_2$: 237.05, found: 238.13 $[M+H]^+$.

***5-(2-chloroacetyl)-1-ethylindolin-2-one* (OIP 21)**

Appearance: brown oil; Yield: 75% (4.41g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 10.0 Hz, 1H), 4.67 (s, 2H), 3.86 (q, 2H), 3.77 (s, 2H), 1.65 (t, J = 1.6 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{12}H_{12}ClNO_2$: 237.05, found: 238.14 $[M+H]^+$.

***5-(2-chloroacetyl)-1-ethyl-3-methylindolin-2-one* (OIP 22)**

Appearance: brown oil; Yield: 74% (4.24g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (s, 1H), 7.96 (d, J = 9.6 Hz, 1H), 7.35 (d, J = 10.0 Hz, 1H), 4.67 (s, 2H), 3.85 (q, 2H), 3.49 (q, 1H), 1.64 (t, J = 1.6 Hz, 3H), 1.32 (d, J = 2.0 Hz, 3H); ESI-MS: (m/z) calcd. for $C_{13}H_{14}ClNO_2$: 251.07, found: 252.15 $[M+H]^+$.

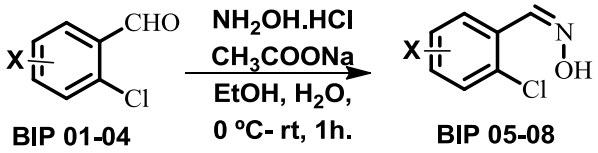
General experimental procedures utilized for the synthesis of 3-(piperazin-1-yl)benzo[*d*]isoxazole (BIP 17-20) derivatives and characterization

The **BIP 24-47,** 3-(4-substituted sulfonylpiperazin-1-yl)benz[*d*]isoxazole derivatives are synthesized as per synthetic protocol given in **Figure 4.1.** Substituted 2-chlorobenzaldehydes **(BIP 01-04)** on treatment with $NH_2OH.HCl$ and CH_3COONa in EtOH and H_2O at 0 °C - rt for 1h yielded substituted 2-chlorobenzaldehyde oxime derivatives **(BIP 05-08)** [Soni *et al*.,

2013]. Further chlorination with *N*-chlorosuccinimide in CCl_4 stirred at 0 °C–rt for 45 min afforded substituted 2-chloro-N-hydroxybenzimidoyl chloride derivatives **(BIP 09-12)** [Yan *et al*., 2011]. Substituted (2-chlorophenyl) (piperazin-1-yl)methanone oxime (**BIP 13-16**) [Daniel *et al*., 2012] was synthesized by reacting **BIP 09-12** with anhydrous piperazine and $N(C_2H_5)_3$ as base in CH_2Cl_2 at rt for 2h. Cyclization of **BIP 13-16** to afford **BIP 17-20** by recting with KOH in dioxane: water, is depicted in **Figure 4.1** [Iranpoor *et al*., 2006; Chen *et al*., 2011; Hou *et al*., 2013].

BIP 17-20 derivatives were synthesized in good yield. Further, title compounds **BIP 24-47** were synthesized by reacting substituted-3-(piperazin-1-yl)benzo[*d*]isoxazole (**BIP 17-20**) with various sulfonyl chlorides, using $N(C_2H_5)_3$ as base and CH_2Cl_2 as solvent at 0 °C to rt. Yield of **BIP 24-47** compounds were good to excellent.

Preparation of 2-chlorobenzaldehyde oxime derivatives (BIP 05-08)



2-chlorobenzaldehyde derivatives **(BIP 01-04)** (5.00 g, 1.0 equiv.) and $NH_2OH.HCl$ (1.2 equiv.) were taken in ethanol (30 mL) and coold to 0 °C, after 5 min CH_3COONa (1.2 equiv.) in water was added to above mixture and warmed to rt for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water and filtered by buchner funnel and dried *in vacuo*. The resultant products are used for next step without purification. Compound yields ranged from 85-96%.

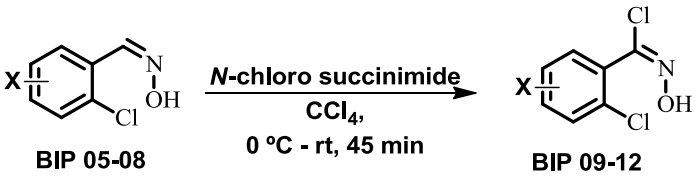
***2-chlorobenzaldehyde oxime* (BIP 05):** yield: 89% (4.92g), exact mass: 155.01, ESI-MS showed 156.12 $[M+H]^+$

2,3-dichlorobenzaldehyde oxime (**BIP 06**): yield: 96% (5.21g), exact mass: 188.97, ESI-MS showed 190.10 $[M+H]^+$

2,4-dichlorobenzaldehyde oxime (**BIP 06**): yield: 92% (4.99g), exact mass: 188.97, ESI-MS showed 190.10 $[M+H]^+$

2,6-dichlorobenzaldehyde oxime (**BIP 06**): yield: 86% (4.66g), exact mass: 188.97, ESI-MS showed 190.14 $[M+H]^+$

Preparation of 2-chloro-*N*-hydroxybenzimidoyl chloride derivatives (BIP 09-12)



2-chlorobenzaldehyde oxime derivatives **(BIP 05-08)** (4.50 g, 1.0 equiv.) were in carbon tetra chloride (50 mL) and cooled to 0 °C, after 5 min and *N*-chloro succinimide (1.2 equiv.) is added to above mixture and warm to rt for 45 min. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant products are used for next step without purification. Compound yields ranged from 85-94%.

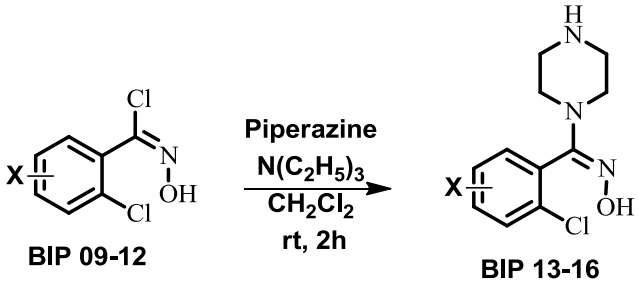
2-chloro-N-hydroxybenzimidoyl chloride (**BIP 09**): yield: 90% (4.94g), exact mass: 188.97, ESI-MS showed 190.10 $[M+H]^+$

2,3-dichloro-N-hydroxybenzimidoyl chloride (**BIP 10**): yield: 86% (4.57g), exact mass: 222.94, ESI-MS showed 224.05 $[M+H]^+$

2,4-dichloro-N-hydroxybenzimidoyl chloride (**BIP 11**): yield: 94% (4.99g), exact mass: 222.94, ESI-MS showed 224.10 $[M+H]^+$

2,6-dichloro-N-hydroxybenzimidoyl chloride (**BIP 12**): yield: 90% (4.78g), exact mass: 222.94, ESI-MS showed 224.07 $[M+H]^+$

Preparation of (2-chlorophenyl)(piperazin-1-yl)methanone oxime derivatives (BIP 13-16)



2-chloro-*N*-hydroxybenzimidoyl chloride derivatives **(BIP 09-12)** (5.00 g, 1.0 equiv.) were added in DCM (50 mL) and cooled to 0 °C, after four min, piperazine (8.0 equiv.) is added to above mixture and warmed to rt for 1-2 h. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4

and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (50 - 70 %) to get the **BIP 13-16** in yields ranging from 80-92%.

Characterization of the synthesized BIP 13-16 compounds

(2-chlorophenyl)(piperazin-1-yl)methanone oxime (**BIP 13**)

Appearance: white solid; Yield: 85% (5.35g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.52–7.48 (m, 2H), 3.48 (b, 1H), 3.19–2.88 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{14}ClN_3O$: 239.08, found: 240.09 $[M+H]^+$.

(2,3-dichlorophenyl)(piperazin-1-yl)methanone oxime (**BIP 14**)

Appearance: white solid; Yield: 80% (4.88g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.44 (m, 1H), 3.42 (b, 1H), 3.13–2.84 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{13}Cl_2N_3O$: 273.04, found: 274.11 $[M+H]^+$.

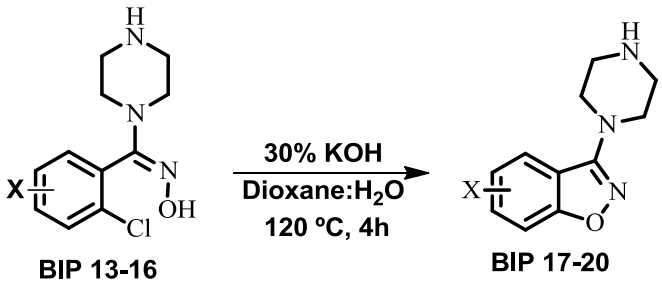
(2,4-dichlorophenyl)(piperazin-1-yl)methanone oxime (**BIP 15**)

Appearance: white solid; Yield: 92% (5.62g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.82 (s, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 3.40 (b, 1H), 3.16–2.78 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{13}Cl_2N_3O$: 273.04, found: 274.13 $[M+H]^+$.

(2,6-dichlorophenyl)(piperazin-1-yl)methanone oxime (**BIP 16**)

Appearance: white solid; Yield: 88% (5.37g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.82 (s, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 3.40 (b, 1H), 3.16–2.78 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{13}Cl_2N_3O$: 273.04, found: 274.13 $[M+H]^+$.

Preparation of 3-(piperazin-1-yl)benzo[*d*]isoxazole derivatives (BIP 17-20)



(2-chlorophenyl)(piperazin-1-yl)methanone oxime derivatives **(BIP 13-16)** (4.00 g, 1.0 equiv.) were added in Dioxane:H_2O (3:1) (40 mL) and cooled to 0 °C, after 7 min KOH (3.0 equiv.) is added to above mixture and warmed to 120 °C for 4 h in sealed tube. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold

water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (70 - 80 %) to get the key intermediates **BIP 17-20** in moderate to good yields.

Characterization of the synthesized BIP 17-20 compounds

3-(piperazin-1-yl)benzo[d]isoxazole (**BIP 17**)

Appearance: white solid; Yield: 85% (2.88g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, J = 8.8 Hz, 1H), 8.15–7.82 (m, 2H), 7.48 (d, J = 8.4 Hz, 1H), 4.92 (b, 1H), 3.38–2.98 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{13}N_3O$: 203.11, found: 204.19 $[M+H]^+$.

4-chloro-3-(piperazin-1-yl)benzo[d]isoxazole (**BIP 18**)

Appearance: white solid; Yield: 70% (2.42g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.51 (m, 1H), 4.90 (b, 1H), 3.34–2.99 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{12}ClN_3O$: 237.07, found: 238.15 $[M+H]^+$.

6-chloro-3-(piperazin-1-yl)benzo[d]isoxazole (**BIP 19**)

Appearance: white solid; Yield: 82% (2.84g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.37 (s, 1H), 4.92 (b, 1H), 3.32–2.95 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{12}ClN_3O$: 237.07, found: 238.17 $[M+H]^+$.

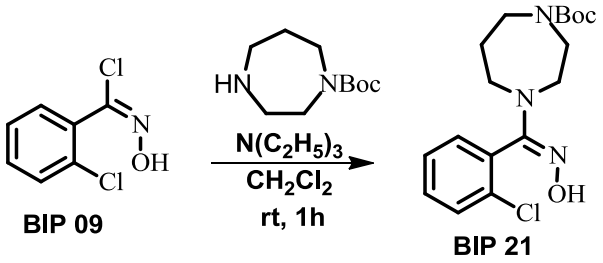
7-chloro-3-(piperazin-1-yl)benzo[d]isoxazole (**BIP 20**)

Appearance: white solid; Yield: 73% (2.53g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.48 (m, 1H), 4.90 (b, 1H), 3.35–2.92 (t, J = 4.0 Hz, 8H); ESI-MS: (m/z) calcd. for $C_{11}H_{12}ClN_3O$: 237.07, found: 238.13 $[M+H]^+$.

General experimental procedures utilized for the synthesis of 3-(1,4-diazepan-1-yl)benzo[*d*]isoxazole hydrochloride (BIP 23) and characterization

2-chloro-*N*-hydroxybenzimidoyl chloride **(BIP 09)** is reacted with 1-Boc homopiperazine in the presence of $N(C_2H_5)_3$ and CH_2Cl_2 to yield *tert*-butyl 4-((2-chlorophenyl)(hydroxyimino)methyl)-1,4-diazepane-1-carboxylate (**BIP 21**) with good yield. Cyclization of **BIP 21** with KOH at 120 °C afforded *tert*-butyl 4-(benzo[*d*]isoxazol-3-yl)-1,4-diazepane-1-carboxylate (**BIP 22**). Simple de Boc reaction in the presence of 4N HCl gave the key intermediate 3-(1,4-diazepan-1-yl)benzo[*d*]isoxazole (**BIP 23**) with excellent yield [Iranpoor *et al*., 2006; Chen *et al*., 2011; Hou *et al*., 2013]. Synthetic protocol employed is portrayed in **Figure 4.1**.

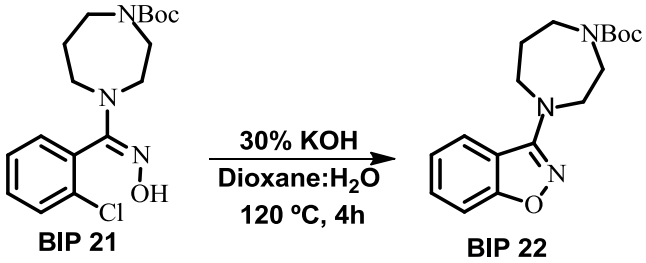
Preparation of *tert*-butyl 4-((2-chlorophenyl)(hydroxyimino)methyl)-1,4-diazepane-1-carboxylate (BIP-21)



2-chloro-*N*-hydroxybenzimidoyl chloride derivatives **(BIP 09)** (5.00 g, 1.0 equiv.) are added in DCM (50 mL) and cooled to 0 °C; After four min, 1-Boc homopiperazine (1.0 equiv.) is added to above mixture and warmed to rt for 1 h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product is purified by column chromatography in ethyl acetate / hexane (30 - 40 %) to get the **BIP 21** with good yield 8.03g (86%).

Tert-butyl4-((2-chlorophenyl)(hydroxyimino)methyl)-1,4-diazepane-1-carboxylate (**BIP 21**) Appearance: white solid; Yield: 86% (8.03g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.01 (s, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 80 Hz, 1H), 7.60–7.48 (m, 2H), 3.28–2.88 (t, J = 4.0 Hz, 8H), 1.74–1.64 (m, 2H), 1.42 (s, 9H); ESI-MS: (m/z) calcd. for $C_{17}H_{24}ClN_3O_3$: 353.15, found: 354.23 $[M+H]^+$.

Preparation of *tert*-butyl 4-(benzo[*d*]isoxazol-3-yl)-1,4-diazepane-1-carboxylate (BIP-22)



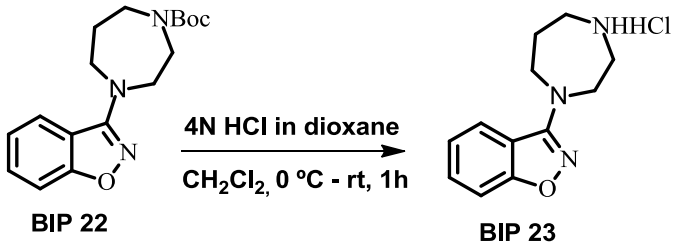
Tert-butyl 4-((2-chlorophenyl)(hydroxyimino)methyl)-1,4-diazepane-1-carboxylate (**BIP 21**) (8.00 g, 1.0 equiv.) was added in Dioxane:H_2O (3:1) (80 mL) and cooled to 0 °C; after 7 min KOH (3.0 equiv.) is added to above mixture and warmed to 120 °C for 4 h in sealed tube. After completion of the reaction, as indicated by TLC, the reaction was quenched with water

and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product is purified by column chromatography [ethyl acetate / hexane (30 - 40%)] to get the **BIP 22** in good yield 5.77g (80%).

***tert-butyl 4-(benzo[d]isoxazol-3-yl)-1,4-diazepane-1-carboxylate* (BIP-22)**

Appearance: white solid; Yield: 80% (5.77g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 8.8 Hz, 1H), 8.18–7.83 (m, 2H), 7.47 (d, J = 8.4 Hz, 1H), 3.72–3.28 (t, J = 4.0 Hz, 8H), 1.86–1.82 (m, 2H), 1.44 (s, 9H); ESI-MS: (m/z) calcd. for $C_{17}H_{23}N_3O_3$: 317.17, found: 318.25 $[M+H]^+$.

Preparation of 3-(1,4-diazepan-1-yl)benzo[*d*]isoxazole hydrochloride (BIP-23)



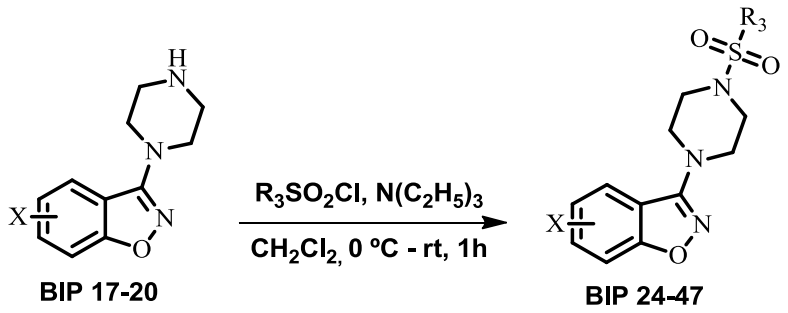
BIP 22 BIP 23

Tert-butyl 4-(benzo[*d*]isoxazol-3-yl)-1,4-diazepane-1-carboxylate (**BIP 22**) (6.00 g, 1.0 equiv.) was added in CH_2Cl_2, (60 mL) and cooled to 0 °C; after 5 min, 4N HCl in dioxane (2ml) is added to above mixture and warmed to rt for 1h. After completion of the reaction, as indicated by TLC, the reaction was concentrated *in vacuo* and washed with *n*-pentane. **BIP 23** in excellent yield 4.38g (95%) was obtained.

***3-(1,4-diazepan-1-yl)benzo[d]isoxazole hydrochloride* (BIP-23)**

Appearance: white solid; Yield: 95% (4.38g); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.86 (s, 1H), 8.65 (d, J = 8.8 Hz, 1H), 8.19–7.85 (m, 2H), 7.50 (d, J = 8.4 Hz, 1H), 3.73–3.31 (t, J = 4.0 Hz, 8H), 1.89–1.85 (m, 2H); ESI-MS: (m/z) calcd. for $C_{12}H_{15}N_3O$+HCl: 217.12+35.98, found: 218.23 $[M+H]^+$.

4.2.1.1. Synthesis of 3-(4-(substituted)piperazin-1-yl)benzo[*d*]isoxazole (BIP 24-47) derivatives and its characterization



To a stirred solution of 3-(piperazin-1-yl)benzo[*d*]isoxazole derivatives **(BIP 17-20)** (1.0 equiv.), triethylamine (2.0 equiv.) was added in dichloromethane and cooled to 0 °C; to this mixture substituted sulfonylchlorides (1.2 equiv.) were added and allowed to rt, stirred for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (45 - 80 %)] to get the title compounds in yields ranging from 80-98%.

Characterization of the synthesized title compounds BIP 24-47

***3-(4-(methylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 24)**

To a stirred solution of 3-(piperazin-1-yl)benzo[*d*]isoxazole **(BIP 17)** 0.3g (1.0 equiv.), triethylamine (2.0 equiv.) was added in dichloromethane and cooled to 0 °C; to this mixture substituted methylsulfonylchloride (1.2 equiv.) was added and allowed to rt, stirred for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (45 - 50 %)] to achieve 3-(4-(methylsulfonyl)piperazin-1-yl)benzo[*d*]isoxazole (**BIP 24**).

Appearance: white solid; Yield: 94% (0.39g); IR (KBr) ν_{max}: 3104, 3030, 1651, 1522, 1500, 1446, 1210 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.0 Hz, 1H), 7.53–7.46 (m, 2H), 7.27 (d, *J* = 12.0 Hz, 1H), 3.70 (t, *J* = 4.0 Hz, 4H), 3.44 (t, *J* = 4.0 Hz, 4H), 2.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.06, 160.66, 129.90, 122.71, 121.72, 115.74, 110.62, 48.00, 44.93, 34.65; ESI-MS (m/z) calculated for $C_{12}H_{15}N_3O_3S$: 281.09, found: 282.15 $(M+H)^+$. Anal. Calcd for $C_{12}H_{15}N_3O_3S$: (%) C 51.23, H 5.37, N 14.94. Found: C 51.39, H 5.44, N 15.11.

***3-(4-(phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 25)**

Appearance: white solid; Yield: 88% (0.44g); IR (KBr) ν_{max}: 3110, 3025, 1650, 1534, 1505, 1455, 1210 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 7.2 Hz, 2H), 7.65–7.48 (m, 4H), 7.45 (d, J = 7.6 Hz, 2H), 7.19 (m, 1H), 3.68 (t, J = 4.8 Hz, 4H), 3.24 (t, J = 4.8 Hz 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.99, 160.59, 135.44, 133.18, 129.81, 129.26, 127.76, 122.61, 121.65, 115.68, 110.57, 47.75, 45.27; ESI-MS (m/z) calculated for $C_{17}H_{17}N_3O_3S$: 343.10, found: 344.17 $(M+H)^+$. Anal. Calcd for $C_{17}H_{17}N_3O_3S$: (%) C 59.46, H 4.99, N 12.24. Found: C 59.61, H 5.11, N 12.37.

***3-(4-tosylpiperazin-1-yl)benzo[d]isoxazole* (BIP 26)**

Appearance: pale yellow solid; Yield: 85% (0.44g); IR (KBr) ν_{max}: 3108, 3050, 1660, 1530, 1450, 1210 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 7.2 Hz, 1H), 7.81 (d, J = 9.2 Hz, 1H), 7.68–7.64 (m, 1H), 7.52 (d, J = 7.2 Hz, 4H), 7.34 (d, J = 7.4 Hz, 1H), 3.52 (t, J = 4.8 Hz, 4H), 3.25 (t, J = 4.8 Hz, 4H), 2.96 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.48, 160.12, 135.14, 133.28,129.81, 129.26, 127.76, 123.42, 122.61, 121.65, 110.64, 47.75, 45.27, 28.45. ESI-MS (m/z) calculated for $C_{18}H_{19}N_3O_3S$: 357.12, found: 358.13 $(M+H)^+$. Anal. Calcd for $C_{17}H_{19}N_3O_3S$: (%) C 60.49, H 5.36, N 11.76. Found: C 60.64, H 5.18, N 11.93.

***3-(4-(4-(trifluoromethoxy)phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 27)**

Appearance: white solid; Yield: 80% (0.50g); IR (KBr) ν_{max}: 3100, 3045, 1650, 1533, 1500, 1446, 1210, 850 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 7.4 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.52 (d, J = 7.2 Hz, 2H), 7.34 (d, J = 7.4 Hz, 1H), 7.08 (d, J = 7.6 Hz, 2H), 3.68 (t, J = 5.2 Hz, 4H), 3.24 (t, J = 5.2 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.29, 160.23, 154.62, 135.14, 132.28, 129.32, 128.72, 127.76,123.42, 122.61, 116.76, 110.64, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{18}H_{16}F_3N_3O_4S$: 427.08, found: 428.11 $(M+H)^+$. Anal. Calcd for $C_{18}H_{16}F_3N_3O_4S$: (%) C 50.58, H 3.77, N 9.83. Found: C 50.68, H 3.54, N 9.97.

***3-(4-(4-nitrophenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 28)**

Appearance: pale yellow solid; Yield: 82 (0.42g)%; IR (KBr) ν_{max}: 3090, 3040, 1650, 1530, 1500, 1446, 1330, 1210, 850 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 8.4 Hz, 1H), 8.24 (d, J = 8.0 Hz, 2H), 8.14 (d, J = 7.6 Hz, 2H), 8.04 (d, J = 7.2 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 7.4 Hz, 1H), 3.56 (t, J = 4.8 Hz, 4H), 3.26 (t, J = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.32, 160.41, 159.62, 134.64, 133.28, 129.54, 129.22, 127.76, 123.42, 122.61, 110.64, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{17}H_{16}N_4O_5S$: 388.09, found: 389.13 $(M+H)^+$. Anal. Calcd for $C_{17}H_{16}N_4O_5S$: (%) C 52.57, H 4.15, N 14.43. Found: C

52.73, H 4.32, N 14.57.

***3-(4-(4-bromophenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 29)**

Appearance: white solid; Yield: 85% (0.52g); IR (KBr) ν_{max}: 3110, 3040, 1658, 1530, 1505, 1452, 1215, 900, 710 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 7.6 Hz, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 7.6 Hz, 2H), 7.86 (d, J = 7.2 Hz, 2H), 7.54 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 3.68 (t, J = 4.8 Hz, 4H), 3.24 (t, J = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.89, 161.08, 139.12, 135.14, 133.28, 129.81, 129.42, 127.76,123.42, 122.61, 110.64, 47.64, 45.25. ESI-MS (m/z) calculated for $C_{17}H_{16}BrN_3O_3S$: 421.01, found: 422.07 $(M+H)^+$. Anal. Calcd for $C_{17}H_{16}BrN_3O_3S$: (%) C 48.35, H 3.82, N 9.95. Found: C 48.47, H 4.03, N 10.13.

***4-chloro-3-(4-(methylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 30)**

Appearance: white solid; Yield: 95% (0.37g); IR (KBr) ν_{max}: 3125, 3045, 1650, 1520, 1510, 1452, 1210, 910, 840 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 7.6 Hz, 1H), 7.58 (m, 1H), 7.21 (d, J = 8.0 Hz, 1H), 3.58 (t, J = 4.8 Hz, 4H), 3.25 (t, J = 4.8 Hz, 4H), 2.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.54, 160.59, 133.18, 123.11, 122.61, 121.65, 115.65, 47.75, 45.27, 38.18. ESI-MS (m/z) calculated for $C_{12}H_{14}ClN_3O_3S$: 315.05, found: 316.11 $(M+H)^+$. Anal. Calcd for $C_{12}H_{14}ClN_3O_3S$: (%) C 45.64, H 4.47, N 13.31. Found: C 45.47, H 4.63, N 13.55.

***4-chloro-3-(4-(phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 31)**

Appearance: white solid; Yield: 84% (0.40g); IR (KBr) ν_{max}: 3115, 3045, 1650, 1530, 1500, 1450, 1220, 910, 850 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 7.2 Hz, 1H), 7.71–7.64 (m, 3H), 7.52 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 8.2 Hz, 1H), 3.68 (t, J = 4.8 Hz, 4H), 3.24 (t, J = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.99, 160.59, 135.44, 133.18, 129.81, 129.26, 127.76, 122.61, 121.65, 115.68, 110.57, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{17}H_{16}ClN_3O_3S$: 377.06, found: 378.13 $(M+H)^+$. Anal. Calcd for $C_{17}H_{16}ClN_3O_3S$: (%) C 54.04, H 4.27, N 11.12. Found: C 54.27, H 4.45, N 11.37.

***4-chloro-3-(4-tosylpiperazin-1-yl)benzo[d]isoxazole* (BIP 32)**

Appearance: white solid; Yield: 95% (0.46g); IR (KBr) ν_{max}: 3112, 3038, 1650, 1520, 1505, 1450, 1210, 915, 855 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.4 Hz, 1H), 7.39 (d, J = 8.0 Hz, 2H), 7.37 (m, 1H), 7.23 (d, J = 8.4 Hz, 1H), 3.53 (t, J = 4.8 Hz, 4H), 3.25 (t, J = 4.8 Hz, 4H), 2.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.98, 160.42, 138.14, 133.28, 129.22, 129.04, 123.76, 123.42, 122.61, 121.65, 110.64, 47.75, 45.27, 28.14. ESI-MS (m/z) calculated for $C_{18}H_{18}ClN_3O_3S$: 391.08, found: 392.13 $(M+H)^+$.

Anal. Calcd for $C_{18}H_{18}ClN_3O_3S$: (%) C 55.17, H 4.63, N 10.72 Found: C 55.34, H 4.84, N 10.91.

***4-chloro-3-(4-(4-(trifluoromethoxy)phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 33)**

Appearance: white solid; Yield: 82% (0.47g); IR (KBr) ν_{max}: 3110, 3045, 1650, 1520, 1510, 1450, 1215, 915, 810 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 8.8 Hz, 1H), 7.45 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 5.2 Hz, 1H), 7.39–7.35 (m, 1H), 7.25 (d, J = 8.4 Hz, 2H), 3.53 (t, J = 4.4 Hz, 4H), 3.25 (t, J = 4.4 Hz, 4H), 2.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.45, 161.12, 138.22, 133.28, 129.81, 129.26, 127.76, 123.42, 122.61, 121.23, 121.02, 110.64, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{18}H_{15}ClF_3N_3O_4S$: 461.05, found: 462.11 $(M+H)^+$. Anal. Calcd for $C_{18}H_{15}ClF_3N_3O_4S$: (%) C 46.81, H 3.27, N 9.10. Found: C 47.02, H 3.38, N 9.31.

***4-chloro-3-(4-(4-nitrophenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 34)**

Appearance: pale yellow solid; Yield: 85% (0.45g); IR (KBr) ν_{max}: 3120, 3050, 1652, 1520, 1500, 1450, 1220, 910, 860 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 7.6 Hz, 2H), 8.14 (d, J = 8.4 Hz, 2H), 7.88 (d, J = 7.6 Hz, 1H), 7.54 (d, J = 7.2 Hz, 1H), 7.38–7.34 (m, 1H), 3.56 (t, J = 4.4 Hz, 4H), 3.26 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.92, 160.41, 156.62, 138.64, 133.28, 129.80, 125.22, 124.76, 122.42, 121.61, 110.64, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{17}H_{15}ClN_4O_5S$: 422.05, found: 423.10 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}ClN_4O_5S$: (%) C 48.29, H 3.58, N 13.25. Found: C 48.47, H 3.72, N 13.48.

***3-(4-(4-bromophenylsulfonyl)piperazin-1-yl)-4-chlorobenzo[d]isoxazole* (BIP 35)**

Appearance: white solid; Yield: 85% (0.48g); IR (KBr) ν_{max}: 3112, 3045, 1650, 1520, 1510, 1450, 1215, 910, 850, 710 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 7.6 Hz, 1H), 8.08 (dd, J = 9.2 Hz, 1H), 7.94 (d, J = 7.6 Hz, 2H), 7.86 (d, J = 7.2 Hz, 2H), 7.54 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 3.68 (t, J = 4.4 Hz, 4H), 3.24 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.89, 161.08, 139.12, 135.14, 133.28, 129.81, 129.24, 124.76, 123.42, 121.61, 110.64, 47.64, 45.25. ESI-MS (m/z) calculated for $C_{17}H_{15}BrClN_3O_3S$: 454.97, found: 456.07 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}BrClN_3O_3S$: (%) C 44.70, H 3.31, N 9.20. Found: C 44.89, H 3.54, N 9.63.

***6-chloro-3-(4-(methylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 36)**

Appearance: white solid; Yield: 92% (0.36g); IR (KBr) ν_{max}: 3120, 3040, 1650, 1560, 1500, 1450, 1210, 910 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 7.6 Hz, 1H), 7.52 (d, J =

8.0 Hz, 1H), 7.36 (s, 1H), 3.58 (t, *J* = 4.4 Hz, 4H), 3.25 (t, *J* = 4.4 Hz, 4H), 2.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.12, 152.32, 133.18, 123.11, 122.61, 121.65, 115.65, 47.75, 45.27, 38.18. ESI-MS (m/z) calculated for $C_{12}H_{14}ClN_3O_3S$ calculated: 315.05, found: 316.11 $(M+H)^+$. Anal. Calcd for $C_{12}H_{14}ClN_3O_3S$: (%) C 45.64, H 4.47, N 13.31. Found: C 45.82, H 4.61, N 13.59.

***6-chloro-3-(4-(phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 37)**

Appearance: white solid; Yield: 98% (0.46g); IR (KBr) ν_{max}: 3110, 3045, 1650, 1560, 1512, 1454, 1212, 910, 818 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.4 Hz, 2H), 7.78–7.67 (m, 3H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.36 (s, 1H), 3.68 (t, *J* = 4.4 Hz, 4H), 3.24 (t, *J* = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.57, 156.64, 136.44, 134.18, 129.81, 128.24, 127.76, 122.61, 121.65, 115.68, 110.57, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{17}H_{16}ClN_3O_3S$: 377.06, found: 378.10 $(M+H)^+$. Anal. Calcd for $C_{17}H_{16}ClN_3O_3S$: (%) C 54.04, H 4.27, N 11.12. Found: C 54.24, H 4.41, N 11.39.

***6-chloro-3-(4-tosylpiperazin-1-yl)benzo[d]isoxazole* (BIP 38)**

Appearance: white solid; Yield: 85% (0.42g); IR (KBr) ν_{max}: 3122, 3040, 1646, 1544, 1513, 1450, 1218, 923, 831 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.34 (s, 1H), 3.56 (t, *J* = 4.4 Hz, 4H), 3.29 (t, *J* = 4.4 Hz, 4H), 2.76 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.98, 152.42, 137.14, 133.28, 129.81, 128.24, 127.76, 123.42, 122.61, 121.65, 110.64, 47.75, 45.27, 28.14. ESI-MS (m/z) calculated for $C_{18}H_{18}ClN_3O_3S$: 391.08, found: 392.13 $(M+H)^+$. Anal. Calcd for $C_{18}H_{18}ClN_3O_3S$: (%) C 55.17, H 4.63, N 10.72 Found: C 55.38, H 4.88, N 10.97.

***6-chloro-3-(4-(4-(trifluoromethoxy)phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 39)**

Appearance: white solid; Yield: 80% (0.46g); IR (KBr) ν_{max}: 3110, 3045, 1650, 1510, 1450, 1320, 1214, 910, 848 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.46 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 1H), 3.68 (t, *J* = 4.0 Hz,, 4H), 3.27 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.32, 160.19, 152.55, 136.46, 133.94, 129.90, 123.63, 121.15, 121.05, 118.91, 114.46, 110.98, 47.72, 45.12. ESI-MS (m/z) calculated for $C_{18}H_{15}ClF_3N_3O_4S$: 461.05, found: 462.11 $(M+H)^+$. Anal. Calcd for $C_{18}H_{15}ClF_3N_3O_4S$: (%) C 46.81, H 3.27, N 9.10. Found: C 46.98, H 3.42, N 9.35.

***6-chloro-3-(4-(4-nitrophenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 40)**

Appearance: yellow solid; Yield: 85% (0.45g); IR (KBr) ν_{max}: 3122, 3040, 1650, 1580, 1510,

1450, 1210, 915, 850 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 8.0 Hz, 2H), 8.14 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.38 (s, 1H), 3.56 (t, J = 4.4 Hz,, 4H), 3.29 (t, J = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.96, 154.41, 152.62, 138.64, 133.28, 129.81, 125.26, 124.76, 122.42, 121.61, 110.64, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{17}H_{15}ClN_4O_5S$: 422.05, found: 423.10 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}ClN_4O_5S$: (%) C 48.29, H 3.58, N 13.25. Found: C 48.44, H 3.69, N 13.07.

***3-(4-(4-bromophenylsulfonyl)piperazin-1-yl)-6-chlorobenzo[d]isoxazole* (BIP 41)**

Appearance: white solid; Yield: 94% (0.54g); IR (KBr) ν_{max}: 3110, 3045, 1650, 1520, 1510, 1450, 1215, 915, 710 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 7.6 Hz, 2H), 8.08 (d, J = 7.4 Hz, 2H), 7.56 (d, J = 7.6 Hz, 1H), 7.42 (d, J = 7.2 Hz, 1H), 7.32 (s, 1H), 3.58 (t, J = 4.4 Hz, 4H), 3.29 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.88, 152.08, 139.12, 135.14, 133.28, 129.81, 129.04, 124.76,123.42, 122.61, 110.64, 47.64, 45.25. ESI-MS (m/z) calculated for $C_{17}H_{15}BrClN_3O_3S$: 454.97, found: 456.11 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}BrClN_3O_3S$: (%) C 44.70, H 3.31, N 9.20. Found: C 44.91, H 3.50, N 9.57.

***7-chloro-3-(4-(methylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 42)**

Appearance: white solid; Yield: 95% (0.37g); IR (KBr) ν_{max}: 3110, 3045, 1650, 1590, 1515, 1450, 1215, 920, 854 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.0 Hz, 1H), 7.54–7.51 (m, 1H), 7.46 (d, J = 8.0 Hz, 1H), 3.56 (t, J = 4.8 Hz,, 4H), 3.28 (t, J = 4.8 Hz, 4H), 2.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.14, 152.54, 132.18, 124.26, 122.61, 121.65, 115.65, 47.75, 45.27, 38.18. ESI-MS (m/z) calculated for $C_{12}H_{14}ClN_3O_3S$: 315.05, found: 316.12 $(M+H)^+$. Anal. Calcd for $C_{12}H_{14}ClN_3O_3S$: (%) C 45.64, H 4.47, N 13.31. Found: C 45.78, H 4.62, N 13.51.

***7-chloro-3-(4-(phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 43)**

Appearance: white solid; Yield: 93% (0.44g); IR (KBr) ν_{max}: 3120, 3045, 1650, 1595, 1510, 1450, 1215, 910 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 2H), 7.74–7.66 (m, 3H), 7.52 (d, J = 7.2 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.38–7.34 (m, 1H), 3.68 (t, J = 4.4 Hz,, 4H), 3.29 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.44, 154.12, 136.44, 133.18, 129.81, 129.26, 127.76, 122.61, 121.65, 116.68, 110.57, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{17}H_{16}ClN_3O_3S$: 377.06, found: 378.11 $(M+H)^+$. Anal. Calcd for $C_{17}H_{16}ClN_3O_3S$: (%) C 54.04, H 4.27, N 11.12. Found: C 54.28, H 4.42, N 11.33.

***7-chloro-3-(4-tosylpiperazin-1-yl)benzo[d]isoxazole* (BIP 44)**

Appearance: white solid; Yield: 85% (0.42g); IR (KBr) ν_{max}: 3124, 3040, 1650, 1595, 1508, 1450, 1215, 900 cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.82 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.0

Hz, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.38–7.33 (m, 1H), 3.54 (t, *J* = 4.4 Hz, 4H), 3.29 (t, *J* = 4.4 Hz, 4H), 2.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.18, 159.42, 136.22, 132.34, 129.81, 128.14, 124.76, 123.42, 122.61, 121.65, 110.64, 47.75, 45.27, 28.14. ESI-MS (m/z) calculated for $C_{18}H_{18}ClN_3O_3S$: 391.08, found: 392.13 $(M+H)^+$. Anal. Calcd for $C_{18}H_{18}ClN_3O_3S$: (%) C 55.17, H 4.63, N 10.72 Found: C 55.41, H 4.82, N 10.93.

***7-chloro-3-(4-(4-(trifluoromethoxy)phenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 45)**

Appearance: white solid; Yield: 84% (0.48g); IR (KBr) ν_{max}: 3120, 3045, 1650, 1590, 1510, 1450, 1330, 1210, 910, 810 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.38 (m, 1H), 7.18 (d, *J* = 7.2 Hz, 2H), 3.56 (t, *J* = 4.4 Hz, 4H), 3.27 (t, *J* = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.14, 160.12, 148.22, 136.28, 132.81, 129.26, 124.76, 123.42, 122.61, 120.23, 114.14, 110.64, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{18}H_{15}ClF_3N_3O_4S$ calculated: 461.05, found: 462.11 $(M+H)^+$. Anal. Calcd for $C_{18}H_{15}ClF_3N_3O_4S$: (%) C 46.81, H 3.27, N 9.10. Found: C 47.04, H 3.46, N 9.29.

***7-chloro-3-(4-(4-nitrophenylsulfonyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 46)**

Appearance: yellow solid; Yield: 85% (0.45g); IR (KBr) ν_{max}: 3120, 3042, 1650, 1590, 1510, 1450, 1340, 1210, 900 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 7.6 Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.41 (m, 1H), 3.56 (t, *J* = 4.8 Hz, 4H), 3.29 (t, *J* = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.92, 160.41, 156.62, 137.64, 132.28, 128.82, 125.26, 122.76, 122.42, 120.24, 116.64, 47.75, 45.27. ESI-MS (m/z) calculated for $C_{17}H_{15}ClN_4O_5S$: 422.05, found: 423.13 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}ClN_4O_5S$: (%) C 48.29, H 3.58, N 13.25. Found: C 48.49, H 3.71, N 13.44.

***3-(4-(4-bromophenylsulfonyl)piperazin-1-yl)-7-chlorobenzo[d]isoxazole* (BIP 47)**

Appearance: white solid; Yield: 85% (0.48g); IR (KBr) ν_{max}: 3128, 3044, 1640, 1592, 1510, 1450, 1215, 910, 715 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.0 Hz, 2H), 8.02 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 7.2 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.36–7.32 (m, 1H), 3.58 (t, *J* = 4.8 Hz,, 4H), 3.26 (t, *J* = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.74, 160.08, 139.12, 133.14, 132.28, 129.81, 124.84, 123.76, 122.42, 121.61, 111.12, 47.64, 45.25. ESI-MS (m/z) calculated for $C_{17}H_{15}BrClN_3O_3S$ calculated: 454.97, found: 456.13 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}BrClN_3O_3S$: (%) C 44.70, H 3.31, N 9.20. Found: C 44.94, H 3.58, N 9.59.

4.2.1.2. Crystal growth and X-ray crystallographic study of compound BIP 25

3-(4-(phenylsulfonyl)piperazin-1-yl)benzo[*d*]isoxazole (**BIP 25**) crystals were grown from the slow evaporation of a 1:2:7 ratio of methanol/dichloromethane/hexane solvent mixture at rt, to get a white flake crystal. A suitable crystal (Crystal size/mm^3 0.39 × 0.34 × 0.22), was selected and mounted on an Xcalibur, Eos, Gemini diffractometer. The crystal was kept at 293.15 K during data collection. Using Olex2 [Dolomanov *et al*., 2009] the structure was solved with the Unknown structure solution program using Unknown and refined with the olex2.refine [Bourhis *et al*., 2015] refinement package using Gauss-Newton minimization. The basic crystallographic data and structure refinement are shown in **Table 4.1** and molecular structure is given as an ORTEP diagram in **Figure 4.3**. Crystallographic data of the compound **BIP 25** was deposited with the Cambridge Crystallographic Data Centre and the deposition number is **CCDC 968913**.

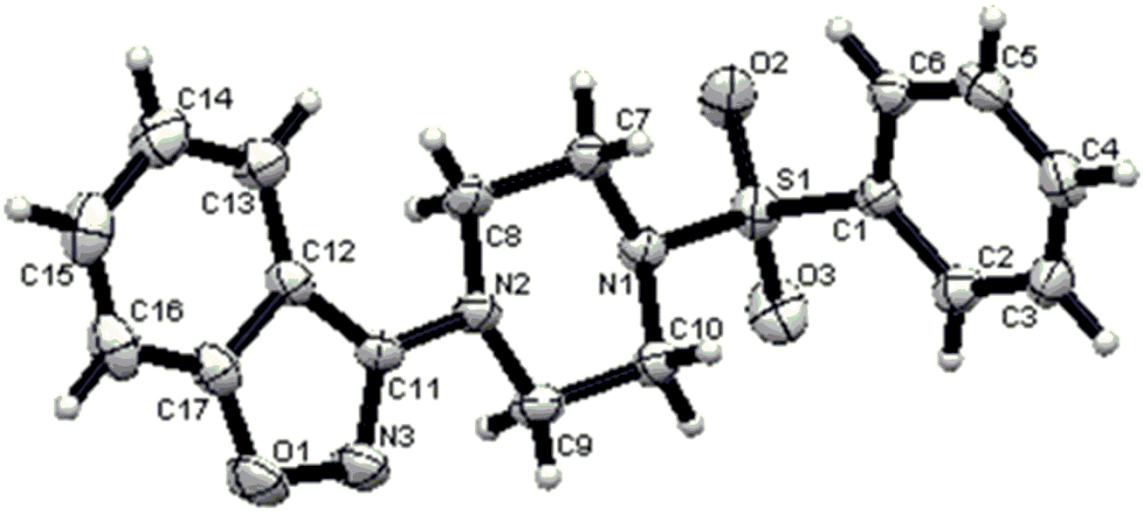


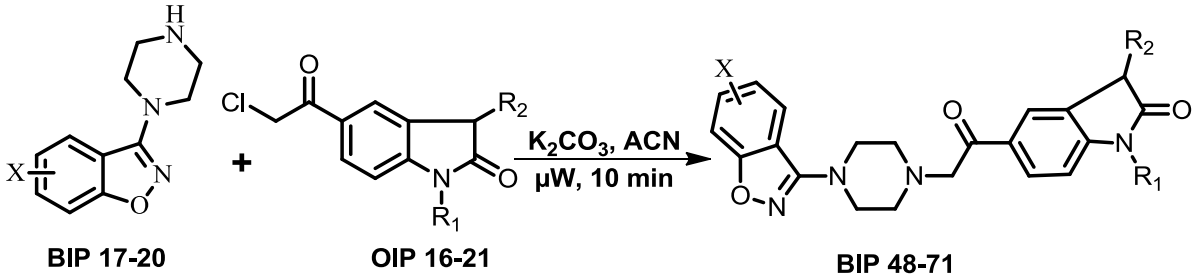
Figure 4.3: ORTEP plot for the compound **BIP 25**. All the non-hydrogen atoms are presented by their 30% probability thermal ellipsoids.

Table 4.1: Crystal data and structure refinement for **BIP 25**

Compound **BIP 25**	
Formula	$C_{17}H_{17}N_3O_3S$
Formula weight	343.39
Temperature/K	293.15
Wavelength (Å)	0.7107

Crystal system	monoclinic
Space group	$P2_1/c$
a (Å)	8.2112(6)
b (Å)	19.5119(9)
c (Å)	10.7941(8)
α (°)	90
β (°)	109.787(7)
γ (°)	90
V ($Å^3$)	1627.3(2)
Z	4
F(000)	720.0
D_{calc} (g/mm^3)	1.402
μ (mm^{-1})	0.220
2Θ (°)	5.67 to 52.744°
R_{int}	0.0284
Crystal size/mm^3	0.39 × 0.34 × 0.22
Goodness-of-fit on F^2	1.039
R_1 indices [I>2sigma(I)]	0.0501
wR_2 (all data)	0.1148
Largest diff. peak/hole (e $Å^{-3}$)	0.18/-0. 0.27

4.2.1.3. Synthesis of 5-(2-(4-(benzo[*d*]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one (BIP 48-71) analogues and characterization



To a mixture of 3-(piperazin-1-yl)benzo[*d*]isoxazole derivatives **(BIP 17-20)** (1.0 equiv.), 5-(2-chloroacetyl)indolin-2-one derivatives **(OIP 16-21)** (1.1 equiv.), anhydrous potassium carbonate (1.2 equiv.) was added in acetonitrile and refluxed in microwave oven for 10 min at 500W. Once the reaction is complete, as indicated by TLC, the reaction mixture was

allowed to cool to rt and quenched with water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography (1-5 % methanol in CH_2Cl_2) to achieved the **BIP 48-71** final compounds in yields ranging from 60–90%.

Characterization of the synthesized 5-(2-(4-(benzo[*d*]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one (BIP 48-71) analogues

5-(2-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one (**BIP 48**)

A mixture of 3-(piperazin-1-yl)benzo[*d*]isoxazole **(BIP 17)** 0.3g (1.0 equiv.), 5-(2-chloroacetyl)indolin-2-one derivatives **(OIP 16)** (1.1 equiv.) and anhydrous potassium carbonate (1.2 equiv.) were added in acetonitrile and refluxed in microwave oven for 10 min at 500W. Once the reaction is complete, as indicated by TLC, the reaction mixture was allowed to cool to rt and quenched with water and extracted with CH_2Cl_2. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography (2-5 % methanol in CH_2Cl_2) to achieved 0.33g of 5-(2-(4-(benzo[*d*]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one (**BIP 48)** compound in yield 60%.

Appearance: pale orange solid; Yield: 60% (0.33g); IR (KBr) ν_{max}: 3366, 3174, 3037, 1726, 1681, 1532, 1500, 1446, 908 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.92 (b, 1H), 8.08 (d, J = 9.6 Hz, 1H), 7.96 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.48–7.44 (m, 2H), 7.24 (d, J = 8.0 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 3.88 (s, 2H), 3.69 (t, J = 4.4 Hz, 4H), 3.57 (s, 2H), 2.80 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 190.81, 178.78, 163.31, 160.08, 148.56, 131.22, 130.45, 129.94, 127.89, 123.41, 122.97, 122.75, 115.12, 110.28, 108.44, 63.23, 52.98, 44.34, 34.12. ESI-MS: (m/z) calculated for $C_{21}H_{20}N_4O_3$, calculated: 376.15, found: 377.24 $(M+H)^+$. Anal. Calcd for $C_{21}H_{20}N_4O_3$: (%) C 67.01, H 5.36, N 14.88. Found: C 67.12, H 5.41, N 14.94.

5-(2-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-3-methylindolin-2-one **(BIP 49)**

Appearance: pale yellow semi solid; Yield: 62% (0.35g); IR (KBr) ν_{max}: 3385, 3156, 3061, 1721, 1699, 1550, 1502, 1451, 920 cm^{-1}. ^1H NMR (400 MHz, DMSO- d_6) δ 8.81 (b, 1H), 8.04 (d, J = 9.6 Hz, 1H), 7.91 (s, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.52–7.46 (m, 2H), 7.25 (d, J = 8.0 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 3.87 (s, 2H), 3.65 (t, J = 4.4 Hz, 4H), 3.48 (q, 1H), 2.92 (t, J = 4.4 Hz, 4H), 1.51 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 191.02,

178.94, 162.75, 161.12, 149.03, 132.11, 130.29, 129.88, 127.45, 124.12, 123.94, 122.67, 116.23, 111.35, 107.98, 64.01, 53.11, 43.89, 41.56, 15.78. ESI-MS: (m/z) calculated for $C_{22}H_{22}N_4O_3$, calculated: 390.16, found: 391.24 $(M+H)^+$. Anal. Calcd for $C_{22}H_{22}N_4O_3$: (%) C 67.68, H 5.68, N 14.35. Found: C 67.76, H 5.74, N 14.42.

5-(2-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-methylindolin-2-one **(BIP 50)** Appearance: pale yellow solid; Yield: 84% (0.48g); IR (KBr) ν_{max}: 3098, 3042, 1718, 1700, 1548, 1499, 1438, 918 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 9.6 Hz, 1H), 7.95 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.51–7.47 (m, 2H), 7.24 (d, J = 8.0 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 3.86 (s, 2H), 3.67 (t, J = 4.4 Hz, 4H), 3.58 (s, 2H), 3.26 (s, 3H), 2.83 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.01, 179.27, 162.68, 161.11, 149.84, 130.86, 130.09, 129.66, 128.22, 124.57, 123.34, 122.79, 117.14, 116.68, 110.24, 63.55, 52.61, 43.94, 36.33, 34.28. ESI-MS: (m/z) calculated for $C_{22}H_{22}N_4O_3$, calculated: 390.16, found: 391.26 $(M+H)^+$. Anal. Calcd for $C_{22}H_{22}N_4O_3$: (%) C 67.68, H 5.68, N 14.35. Found: C 67.78, H 5.72, N 14.46.

5-(2-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1,3-dimethylindolin-2-one **(BIP 51)** Appearance: brown solid; Yield: 79% (0.47g); IR (KBr) ν_{max}: 3148, 3068, 1722, 1698, 1544, 1502, 1446, 915 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 9.6 Hz, 1H), 7.94 (s, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.49–7.46 (m, 2H), 7.23 (dd, J = 8.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 3.92 (s, 2H), 3.68 (t, J = 4.4 Hz, 4H), 3.52 (q, 1H), 3.27 (s, 3H), 2.88 (t, 4H), 1.48 (d, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.48, 178.74, 163.08, 160.89, 149.57, 131.80, 130.22, 129.01, 128.31, 124.38, 123.12, 122.04, 118.22, 117.21, 110.99, 63.65, 53.01, 44.23, 41.28, 37.64, 16.25. ESI-MS: (m/z) calculated for $C_{23}H_{24}N_4O_3$, calculated: 404.18, found: 405.23 $(M+H)^+$. Anal. Calcd for $C_{23}H_{24}N_4O_3$: (%) C 68.30, H 5.98, N 13.85. Found: C 68.41, H 5.92, N 13.94.

5-(2-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethylindolin-2-one **(BIP 52)** Appearance: pale yellow solid; Yield: 90% (0.53g); IR (KBr) ν_{max}: 3154, 3068, 1720, 1688, 1549, 1500, 1451, 921 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.6 Hz, 1H), 7.92 (s, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.50–7.48 (m, 2H), 7.24 (d, J = 8.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 3.90 (s, 2H), 3.66 (t, J = 4.4 Hz, 4H), 3.54 (s, 2H), 3.31 (q, 2H), 2.92 (t, J = 4.4 Hz, 4H), 1.31 (t, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.98, 178.11, 163.75, 161.02, 148.24, 131.77, 130.45, 130.11, 128.88, 124.65, 123.13, 122.40, 117.02, 116.27, 109.44, 61.02, 52.13, 44.52, 40.56, 36.04, 13.18. ESI-MS: (m/z) calculated for $C_{23}H_{24}N_4O_3$, calculated: 404.18, found: 405.26 $(M+H)^+$. Anal. Calcd for $C_{23}H_{24}N_4O_3$: (%) C 68.30, H 5.98, N 13.85. Found: C 68.43, H 5.94, N 13.91.

5-(2-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethyl-3-methylindolin-2-one **(BIP 53)**

Appearance: orange solid; Yield: 83% (0.51g); IR (KBr) ν_{max}: 3160, 3075, 1714, 1692, 1547, 1508, 1450, 920 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.4 Hz, 1H), 7.94 (s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.51–7.47 (m, 2H), 7.24 (d, J = 8.0 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 3.87 (s, 2H), 3.81 (q, 2H), 3.68 (t, J = 4.4 Hz, 4H), 3.50 (q, 1H), 2.84 (t, J = 4.4 Hz, 4H), 1.52 (d, J = 7.6 Hz, 3H), 1.30 (t, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.83, 177.84, 163.30, 160.09, 148.54, 131.12, 130.32, 129.91, 127.89, 123.40, 122.97, 122.80, 115.11, 110.18, 108.30, 59.75, 51.09, 44.34, 40.12, 34.19, 14.78, 12.55. ESI-MS: (m/z) calculated for $C_{24}H_{26}N_4O_3$, calculated: 418.20, found: 419.28 $(M+H)^+$. Anal. Calcd for $C_{24}H_{26}N_4O_3$: (%) C 68.88, H 6.26, N 13.39. Found: C 68.94, H 6.12, N 13.42.

5-(2-(4-(4-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one **(BIP 54)**

Appearance: Pale yellow semi solid; Yield: 61% (0.31g); IR (KBr) ν_{max}: 3381, 3165, 3038, 1721, 1688, 1539, 1501, 1446, 900, 780 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (b, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.90 (s, 1H), 7.72 (d, J = 1.2 Hz, 1H), 7.68–6.66 (m, 1H), 7.49 (d, J = 0.8 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 3.91 (s, 2H), 3.72 (t, J = 4.4 Hz, 4H), 3.48 (s, 2H), 2.81 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.76, 179.08, 163.45, 161.11, 149.03, 132.33, 131.48, 130.92, 129.89, 129.47, 123.97, 122.75, 118.12, 111.35, 109.87, 63.11, 52.91, 44.13, 34.83. ESI-MS: (m/z) calculated for $C_{21}H_{19}ClN_4O_3$, calculated: 410.11, found: 411.18 $(M+H)^+$. Anal. Calcd for $C_{21}H_{19}ClN_4O_3$: (%) C 61.39, H 4.66, N 13.64. Found: C 61.47, H 4.72, N 13.72.

5-(2-(4-(4-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-3-methylindolin-2-one **(BIP 55)**

Appearance: pale yellow solid; Yield: 62% (0.33g); IR (KBr) ν_{max}: 3368, 3164, 3030, 1718, 1679, 1518, 1508, 1451, 902, 758 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (b, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.74 (d, J = 1.2 Hz, 1H), 7.68–7.65 (m, 1H), 7.47 (d, J = 0.8 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 3.93 (s, 2H), 3.67 (t, J = 4.4 Hz, 4H), 3.60 (q, 1H), 2.62 (t, J = 4.4 Hz, 4H), 1.53 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.08, 179.12, 162.23, 160.92, 149.45, 132.02, 131.27, 130.66, 129.47, 129.11, 124.14, 123.23, 120.04, 112.35, 111.05, 64.01, 52.44, 43.84, 40.88, 35.12, 16.13. ESI-MS: (m/z) calculated for $C_{22}H_{21}ClN_4O_3$, calculated: 424.13, found: 425.22 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: (%) C 62.19, H 4.98, N 13.19. Found: C 62.27, H 5.03, N 13.26.

5-(2-(4-(4-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-methylindolin-2-one (**BIP**

56)

Appearance: pale yellow solid; Yield: 81% (0.43g); IR (KBr) ν_{max}: 3179, 3048, 1717, 1670, 1512, 1508, 1450, 895, 792 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.4 Hz, 1H), 7.90 (s, 1H), 7.71 (d, J = 1.6 Hz, 1H), 7.58–7.54 (m, 1H), 7.46 (d, J = 1.2 Hz, 1H), 7.28 (d, J = 7.6 Hz, 1H), 3.88 (s, 2H), 3.69 (t, J = 4.4 Hz, 4H), 3.63 (s, 2H), 3.34 (s, 3H), 2.81 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.71, 177.84, 163.03, 160.57, 150.12, 132.24, 131.11, 130.26, 129.86, 129.01, 124.38, 123.09, 119.84, 116.91, 110.12, 63.87, 52.22, 43.07, 35.19, 33.82. ESI-MS: (m/z) calculated for $C_{22}H_{21}ClN_4O_3$, calculated: 424.13, found: 425.25 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: (%) C 62.19, H 4.98, N 13.19. Found: C 62.23, H 5.05, N 13.24.

***5-(2-(4-(4-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1,3-dimethylindolin-2-one* (BIP 57)**

Appearance: pale yellow solid; Yield: 90% (0.49g); IR (KBr) ν_{max}: 3182, 3045, 1723, 1663, 1534, 1500, 1458, 898, 799 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.8 Hz, 1H), 7.91 (s, 1H), 7.71 (d, J = 1.2 Hz, 1H), 7.65–7.63 (m, 1H), 7.46 (d, J = 1.6 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 3.92 (s, 2H), 3.68 (t, J = 4.4 Hz, 4H), 3.51 (q, 1H), 3.29 (s, 3H), 2.92 (t, J = 4.4 Hz, 4H), 1.54 (d, J = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.23, 178.18, 163.24, 159.11, 150.20, 132.21, 131.01, 130.67, 129.45, 129.23, 124.18, 123.31, 119.89, 116.78, 110.54, 63.18, 52.12, 43.11, 41.19, 35.24, 15.69. ESI-MS: (m/z) calculated for $C_{23}H_{23}ClN_4O_3$, calculated: 438.14, found: 439.28 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}ClN_4O_3$: (%) C 62.94, H 5.28, N 12.77. Found: C 63.02, H 5.33, N 12.84.

***5-(2-(4-(4-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethylindolin-2-one* (BIP 58)**

Appearance: pale brown solid; Yield: 88% (0.48g); IR (KBr) ν_{max}: 3180, 3048, 1720, 1651, 1534, 1498, 1461, 899, 801 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.4 Hz, 1H), 7.90 (s, 1H), 7.72 (d, J = 1.2 Hz, 1H), 7.68–7.66 (m, 1H), 7.48 (d, J = 1.2 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 3.91 (s, 2H), 3.80 (q, 2H), 3.69 (s, 2H), 3.46 (t, J = 4.4 Hz, 4H), 2.51 (t, J = 4.4 Hz, 4H), 1.18 (t, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.74, 178.79, 163.46, 160.23, 149.53, 132.04, 131.66, 130.23, 129.12, 129.00, 124.21, 123.22, 118.17, 115.78, 110.28, 63.89, 52.56, 43.09, 40.78, 36.00, 16.21. ESI-MS: (m/z) calculated for $C_{23}H_{23}ClN_4O_3$, calculated: 438.14, found: 439.22 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}ClN_4O_3$: (%) C 62.94, H 5.28, N 12.77. Found: C 63.00, H 5.35, N 12.82.

***5-(2-(4-(4-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethyl-3-methylindolin-2-one* (BIP 59)**

Appearance: pale yellow solid; Yield: 82% (0.46g); IR (KBr) ν_{max}: 3179, 3049, 1720, 1656, 1555, 1503, 1450, 896, 800 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.74 (d, J = 1.6 Hz, 1H), 7.70–7.67 (m, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 3.98 (s, 2H), 3.82 (q, 2H), 3.76 (t, J = 4.4 Hz, 4H), 3.61 (q, 1H), 2.94 (t, J = 4.4 Hz, 4H), 1.52 (d, J = 7.2 Hz, 3H), 1.24 (t, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.08, 178.65, 163.63, 160.37, 149.77, 132.11, 131.28, 130.45, 129.11, 129.47, 124.04, 123.25, 118.28, 116.09, 110.24, 63.17, 52.62, 43.45, 41.18, 39.79, 16.24, 14.92. ESI-MS: (m/z) calculated for $C_{24}H_{25}ClN_4O_3$, calculated: 452.16, found: 453.22 $(M+H)^+$. Anal. Calcd for $C_{24}H_{25}ClN_4O_3$: (%) C 63.64, H 5.56, N 12.37. Found: C 63.71, H 5.59, N 12.42.

5-(2-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one **(BIP 60)**

Appearance: pale yellow semi solid; Yield: 65% (0.33g); IR (KBr) ν_{max}: 3402, 3188, 3062, 1718, 1650, 1552, 1508, 1451, 897, 810 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (b, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.67 (s, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.34 (s, 1H), 3.90 (s, 2H), 3.76 (t, J = 4.4 Hz, 4H), 3.41 (s, 2H), 2.84 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.81, 179.84, 163.12, 160.25, 149.76, 135.08, 131.75, 130.24, 129.39, 124.14, 123.88, 122.72, 118.02, 110.95, 109.27, 63.23, 52.57, 43.28, 33.89. ESI-MS: (m/z) calculated for $C_{21}H_{19}ClN_4O_3$, calculated: 410.11, found: 411.22 $(M+H)^+$. Anal. Calcd for $C_{21}H_{19}ClN_4O_3$: (%) C 61.39, H 4.66, N 13.64. Found: C 61.45, H 4.75, N 13.69.

5-(2-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-3-methylindolin-2-one **(BIP 61)**

Appearance: pale yellow solid; Yield: 60% (0.37g); IR (KBr) ν_{max}: 3398, 3182, 3053, 1716, 1650, 1551, 1502, 1459, 912, 804 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (b, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.69 (s, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.32 (s, 1H), 3.86 (s, 2H), 3.68 (t, J = 4.4 Hz, 4H), 3.54 (q, 1H), 2.68 (t, J = 4.4 Hz, 4H), 1.44 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.94, 179.82, 163.59, 160.01, 149.76, 136.11, 131.45, 130.07, 129.21, 124.02, 123.48, 122.63, 118.58, 111.23, 108.98, 63.44, 53.02, 43.74, 39.98, 14.12. ESI-MS: (m/z) calculated for $C_{22}H_{21}ClN_4O_3$, calculated: 424.13, found: 425.20 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: (%) C 62.19, H 4.98, N 13.19. Found: C 62.26, H 5.01, N 13.24.

5-(2-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-methylindolin-2-one **(BIP 62)**

Appearance: pale yellow solid; Yield: 94% (0.50g); IR (KBr) ν_{max}: 3168, 3044, 1718, 1668,

1550, 1501, 1454, 906, 810 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.31 (s, 1H), 7.29 (s, 1H), 3.84 (s, 2H), 3.68 (t, J = 4.4 Hz, 4H), 3.60 (s, 2H), 3.37 (s, 3H), 2.72 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.35, 177.79, 163.24, 160.22, 149.88, 135.72, 130.91, 130.11, 129.23, 124.00, 123.57, 122.52, 119.40, 115.24, 112.64, 63.23, 53.11, 44.24, 34.92, 34.08. ESI-MS: (m/z) calculated for $C_{22}H_{21}ClN_4O_3$, calculated: 424.13, found: 425.25 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: (%) C 62.19, H 4.98, N 13.19. Found: C 62.23, H 5.05, N 13.24.

***5-(2-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1,3-dimethylindolin-2-one* (BIP 63)**

Appearance: pale brown solid; Yield: 89% (0.49g); IR (KBr) ν_{max}: 3167, 3048, 1721, 1650, 1548, 1500, 1452, 902, 805 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.8 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.43 (s, 1H), 7.23 (s, 1H), 3.87 (s, 2H), 3.69 (t, J = 4.4 Hz, 4H), 3.54 (q, 1H), 3.28 (s, 3H), 2.82 (t, J = 4.4 Hz, 4H), 1.48 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.08, 178.48, 163.82, 160.43, 149.89, 134.85, 130.76, 130.22, 129.34, 124.38, 123.21, 122.78, 118.35, 116.14, 111.83, 63.86, 53.24, 43.65, 40.68, 38.54, 16.18. ESI-MS: (m/z) calculated for $C_{23}H_{23}ClN_4O_3$, calculated: 438.14, found: 439.24 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}ClN_4O_3$: (%) C 62.94, H 5.28, N 12.77. Found: C 63.07, H 5.31, N 12.86.

***5-(2-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethylindolin-2-one* (BIP 64)**

Appearance: pale brown solid; Yield: 81% (0.44g); IR (KBr) ν_{max}: 3172, 3054, 1720, 1649, 1547, 1498, 1450, 900, 815 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 8.8 Hz, 1H), 8.00 (d, J = 9.6 Hz, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.43 (s, 1H), 7.28 (s, 1H), 4.11 (s, 2H), 3.78 (q, 2H), 3.69 (s, 2H), 3.38 (t, J = 4.4 Hz, 4H), 2.51 (t, J = 4.4 Hz, 4H), 1.18 (t, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.14, 178.23, 163.67, 160.52, 149.95, 135.12, 131.47, 130.04, 129.28, 124.21, 123.63, 122.09, 118.85, 116.67, 111.69, 63.45, 53.32, 43.88, 40.11, 35.23, 15.02. ESI-MS: (m/z) calculated for $C_{23}H_{23}ClN_4O_3$, calculated: 438.14, found: 439.26 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}ClN_4O_3$: (%) C 62.94, H 5.28, N 12.77. Found: C 63.03, H 5.34, N 12.84.

***5-(2-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethyl-3-methylindolin-2-one* (BIP 65)**

Appearance: pale brown solid; Yield: 80% (0.45g); IR (KBr) ν_{max}: 3170, 3060, 1724, 1651, 1555, 1499, 1452, 909, 812 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.4 Hz, 1H),

7.94 (d, *J* = 9.2 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.40 (s, 1H), 7.31 (s, 1H), 3.92 (s, 2H), 3.81 (q, 2H), 3.79 (t, *J* = 4.4 Hz, 4H), 3.57 (q, 1H), 2.85 (t, *J* = 4.4 Hz, 4H), 1.25 (d, *J* = 6.0 Hz, 3H), 1.24 (t, *J* = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.74, 178.65, 163.23, 160.11, 149.84, 135.31, 131.68, 130.35, 129.69, 124.11, 123.64, 122.54, 118.63, 116.98, 111.84, 63.40, 53.12, 44.08, 40.74, 39.82, 16.45, 14.87. ESI-MS: (m/z) calculated for $C_{24}H_{25}ClN_4O_3$, calculated: 452.16, found: 453.24 $(M+H)^+$. Anal. Calcd for $C_{24}H_{25}ClN_4O_3$: (%) C 63.64, H 5.56, N 12.37. Found: C 63.70, H 5.62, N 12.46.

5-(2-(4-(7-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one **(BIP 66)**

Appearance: pale yellow semi solid; Yield: 67% (0.34g); IR (KBr) ν_{max}: 3398, 3168, 3058, 1721, 1652, 1550, 1498, 1455, 902, 810 cm-1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (b, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.77 (s, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.30–7.28 (m, 1H), 3.88 (s, 2H), 3.76 (t, *J* = 4.4 Hz, 4H), 3.50 (s, 2H), 2.65 (t, *J* = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.97, 179.82, 161.12, 159.45, 148.92, 131.75, 130.46, 129.34, 127.88, 123.45, 122.29, 122.46, 119.92, 116.28, 109.48, 63.21, 52.98, 44.34, 34.36. ESI-MS: (m/z) calculated for $C_{21}H_{19}ClN_4O_3$, calculated: 410.11, found: 411.22 $(M+H)^+$. Anal. Calcd for $C_{21}H_{19}ClN_4O_3$: (%) C 61.39, H 4.66, N 13.64. Found: C 61.45, H 4.71, N 13.74.

5-(2-(4-(7-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-3-methylindolin-2-one **(BIP 67)**

Appearance: pale yellow solid; Yield: 63% (0.33g); IR (KBr) ν_{max}: 3405, 3172, 3063, 1720, 1658, 1557, 1500, 1464, 908, 821 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (b, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.68 (s, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.32–7.29 (m, 1H), 3.91 (s, 2H), 3.70 (t, *J* = 4.4 Hz, 4H), 3.68 (q, 1H), 2.65 (t, *J* = 4.4 Hz, 4H), 1.25 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.82, 179.85, 161.31, 159.87, 148.90, 131.98, 130.33, 129.28, 127.58, 124.42, 123.39, 122.17, 120.31, 117.20, 109.76, 63.34, 52.79, 44.02, 40.81, 15.74. ESI-MS: (m/z) calculated for $C_{22}H_{21}ClN_4O_3$, calculated: 424.13, found: 425.21 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: (%) C 62.19, H 4.98, N 13.19. Found: C 62.26, H 5.05, N 13.24.

5-(2-(4-(7-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-methylindolin-2-one **(BIP 68)**

Appearance: creamy off white solid; Yield: 92% (0.49g); IR (KBr) ν_{max}: 3170, 3068, 1722, 1654, 1555, 1504, 1468, 902, 820 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.8 Hz, 1H), 7.83 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.41–7.38 (m, 1H), 7.30

(d, *J* = 8.0 Hz, 1H), 3.89 (s, 2H), 3.72 (t, *J* = 4.4 Hz, 4H), 3.60 (s, 2H), 3.36 (s, 3H), 2.57 (t, *J* = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.99, 178.24, 161.64, 159.22, 148.45, 131.75, 130.92, 129.36, 127.72, 124.81, 123.44, 122.66, 120.57, 116.96, 116.18, 63.14, 52.64, 43.80, 35.36, 34.78. ESI-MS: (m/z) calculated for $C_{22}H_{21}ClN_4O_3$, calculated: 424.13, found: 425.24 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: (%) C 62.19, H 4.98, N 13.19. Found: C 62.27, H 5.02, N 13.22.

***5-(2-(4-(7-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1,3-dimethylindolin-2-one* (BIP 69)**

Appearance: pale yellow solid; Yield: 86% (0.47g); IR (KBr) ν_{max}: 3180, 3072, 1717, 1664, 1560, 1511, 1465, 900, 820 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 1H), 7.90 (s, 1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.41–7.38 (m, 1H), 7.33 (s, 1H), 3.92 (s, 2H), 3.69 (t, *J* = 4.4 Hz, 4H), 3.52 (q, 1H), 3.34 (s, 3H), 2.65 (t, *J* = 4.4 Hz, 4H), 1.24 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.42, 179.08, 162.22, 159.35, 149.81, 132.22, 130.07, 129.71, 127.88, 124.65, 123.23, 122.31, 120.68, 116.98, 115.97, 63.63, 52.89, 43.87, 40.86, 34.25, 15.78. ESI-MS: (m/z) calculated for $C_{23}H_{23}ClN_4O_3$, calculated: 438.14, found: 439.25 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}ClN_4O_3$: (%) C 62.94, H 5.28, N 12.77. Found: C 63.06, H 5.31, N 12.82.

***5-(2-(4-(7-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethylindolin-2-one* (BIP 70)**

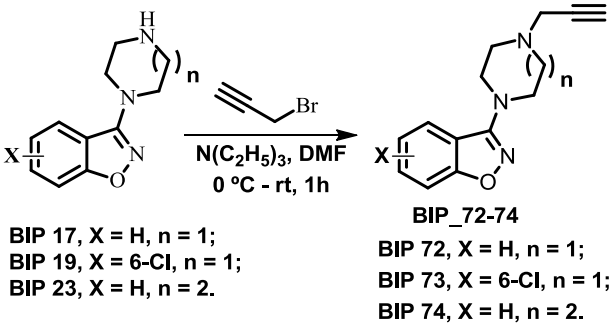
Appearance: yellow solid; Yield: 84% (0.46g); IR (KBr) ν_{max}: 3181, 3070, 1718, 1662, 1567, 1512, 1468, 904, 819 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.85 (s, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.40–7.37 (m, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 3.88 (s, 2H), 3.80 (q, 2H), 3.64 (s, 2H), 3.63 (t, *J* = 4.4 Hz, 4H), 2.52 (t, *J* = 4.4 Hz, 4H), 1.22 (t, *J* = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.88, 179.73, 162.09, 159.21, 149.72, 132.31, 130.64, 129.45, 127.36, 124.38, 123.59, 122.44, 120.02, 116.77, 115.84, 63.48, 52.99, 43.82, 41.15, 36.13, 16.23. ESI-MS: (m/z) calculated for $C_{23}H_{23}ClN_4O_3$, calculated: 438.14, found: 439.28 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}ClN_4O_3$: (%) C 62.94, H 5.28, N 12.77. Found: C 63.02, H 5.36, N 12.84.

***5-(2-(4-(7-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)acetyl)-1-ethyl-3-methylindolin-2-one* (BIP 71)**

Appearance: pale yellow solid; Yield: 90% (0.51g); IR (KBr) ν_{max}: 3178, 3068, 1715, 1660, 1562, 1508, 1462, 908, 816 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.0 Hz, 1H), 7.92 (s, 1H), 7.74 (d, *J* =8.4 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.40–7.37 (m, 1H), 7.36 (s, 1H), 3.92 (s, 2H), 3.79 (q, 2H), 3.70 (t, *J* = 4.4 Hz, 4H), 3.62 (q, 1H), 2.67 (t, *J* = 4.4 Hz, 4H),

1.51 (d, *J* = 6.8 Hz, 3H), 1.32 (t, *J* = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.23, 179.68, 162.31, 159.45, 149.63, 132.84, 130.79, 129.23, 127.48, 124.21, 123.68, 122.24, 120.11, 116.89, 115.72, 63.79, 53.18, 43.78, 40.89, 39.88, 16.21, 15.45. ESI-MS: (m/z) calculated for $C_{24}H_{25}ClN_4O_3$, calculated: 452.16, found: 453.28 $(M+H)^+$. Anal. Calcd for $C_{24}H_{25}ClN_4O_3$: (%) C 63.64, H 5.56, N 12.37. Found: C 63.72, H 5.62, N 12.44.

Preparation of 3-(4-(prop-2-ynyl)piperazin-1-yl/homopiperazine-1-yl))benzo[*d*]isoxazole (BIP 72-74) and characterization



BIP 17, X = H, n = 1;
BIP 19, X = 6-Cl, n = 1;
BIP 23, X = H, n = 2.

BIP_72-74
BIP 72, X = H, n = 1;
BIP 73, X = 6-Cl, n = 1;
BIP 74, X = H, n = 2.

A solution of 3-(piperazin-1-yl/homo piperazine-1-yl)benzo[*d*]isoxazole (**BIP 17, BIP 19** and **BIP 23**) derivatives (5.00 g, 1.0 equiv.) in DMF was cooled to 0 °C and triethylamine (2.0 equiv.) and propargyl bromide (1.1 equiv.) were added and allowed to reach rt, stirred for 1h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with diethyl ether. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (60 - 80 %)] to get the key intermediates **BIP 72-74** in yields ranging from 80-94%.

Characterization of the synthesized BIP 72-74 molecules

***3-(4-(prop-2-ynyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 72)**

Appearance: white solid; Yield: 92% (5.45g); IR (KBr) ν_{max}: 3150, 3062, 2184, 1645, 1550, 1508, 1462, 910 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.8 Hz, 1H), 7.65–7.51 (m, 2H), 7.30 (d, *J* = 8.4 Hz, 1H), 3.54 (t, *J* = 4.4 Hz, 4H), 3.38 (s, 2H), 3.19 (s, 1H), 2.65 (t, *J* = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.29, 154.59, 132.44, 125.76, 123.61, 122.65, 112.68, 81.57, 75.12, 52.16, 47.85, 45.47. ESI-MS: (m/z) calculated for $C_{14}H_{15}N_3O$: 241.12, found: 242.19 $(M+H)^+$. Anal. Calcd for $C_{14}H_{15}N_3O$: (%) C 69.96, H 6.27, N 17.41. Found: C 69.99, H 6.33, N 17.47.

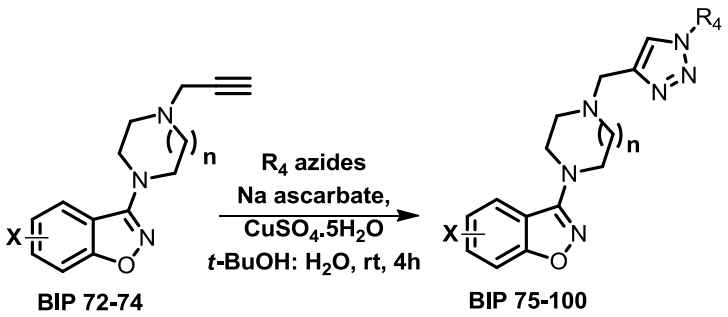
***6-chloro-3-(4-(prop-2-ynyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 73)**

Appearance: white solid; Yield: 94% (5.45g); IR (KBr) ν_{max}: 3156, 3060, 2188, 1650, 1560, 1515, 1468, 915 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.42 (s, 1H), 3.52 (t, J = 4.4 Hz, 4H), 3.37 (s, 2H), 3.28 (s, 1H), 2.70 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.04, 146.12, 137.40, 124.79, 124.35, 121.47, 113.84, 81.43, 77.91, 52.44, 47.92, 45.44. ESI-MS: (m/z) calculated for $C_{14}H_{14}ClN_3O$: 275.08, found: 276.16 $(M+H)^+$. Anal. Calcd for $C_{14}H_{14}ClN_3O$: (%) C 60.98, H 5.12, N 15.24. Found: C 61.06, H 5.15, N 15.27.

***3-(4-(prop-2-ynyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 74)**

Appearance: white solid; Yield: 90% (4.52g); IR (KBr) ν_{max}: 3170, 3064, 2184, 1640, 1554, 1510, 1458, 914 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 8.8 Hz, 1H), 8.07–7.74 (m, 2H), 7.44 (s, 1H), 3.94 (s, 2H), 3.82 (t, J = 4.4 Hz, 4H), 3.14 (s, 1H), 3.03 (t, J = 5.2 Hz, 2H), 2.88 (t, J = 4.4 Hz, 2H), 1.98–1.94 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.04, 146.12, 137.40, 124.79, 124.35, 121.47, 113.84, 81.43, 77.91, 52.44, 47.92, 45.44. ESI-MS: (m/z) calculated for $C_{15}H_{17}N_3O$: 255.14, found: 256.21 $(M+H)^+$. Anal. Calcd for $C_{15}H_{17}N_3O$: (%) C 70.56, H 6.71, N 16.46. Found: C 70.61, H 6.75, N 16.53.

4.2.1.4. Synthesis of 3-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl/homopiperazin-1-yl)benzo[*d*]isoxazole (BIP 72-100) derivatives and characterization



A solution of 3-(4-(prop-2-ynyl)piperazin-1-yl/homopiperazine-1-yl) benzo[*d*]isoxazole (**BIP 72-74**) derivatives (0.20 g, 1.0 equiv.) is reacted with varius aryl azides (1.2 equiv.) in the presence of Na ascorbate (0.01 equiv.), $CuSO_4.5H_2O$ (0.02 equiv.) and *t*-BuOH: H_2O (2:1), at rt for 4h [Himo *et al*., 2005]. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with ethyl acetate. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography

[ethyl acetate / hexane (70 -100 %)] to get the **BIP 75-100** title compounds in yields ranging from 65-95%.

Characterization of the synthesized BIP 75-100 compounds

***3-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 75)**

A solution of 3-(4-(prop-2-ynyl)piperazin-1-yl) benzo[*d*]isoxazole (**BIP 72**) 0.20g (1.0 equiv.) is reacted with 1-azido-4-ethylbenzene (1.2 equiv.) in the presence of Na ascorbate (0.01 equiv.), $CuSO_4.5H_2O$ (0.02 equiv.) and *t*-BuOH: H_2O (2:1), at rt for 4h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with ethyl acetate. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (70 -80 %)] to achieve 0.27g of 3-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[*d*]isoxazole (**BIP 75**) in yield 85%.

Appearance: white solid; Yield: 85% (0.27g); IR (KBr) ν_{max}: 3110, 3058, 1650, 1600, 1545, 1504, 1452, 1255, 908 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.49–7.30 (m, 2H), 7.16 (d, J = 8.8 Hz, 1H), 3.76 (s, 2H), 3.51 (t, J = 4.0 Hz, 4H), 2.74 (t, J = 4.0 Hz, 4H), 2.68 (q, 2H), 1.24 (t, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.36, 148.11, 145.24, 136.08, 134.74, 133.58, 131.12, 127.88, 124.04, 123.35, 122.44, 120.28, 114.88, 67.02, 54.19, 48.98, 30.12, 16.78. ESI-MS: (m/z) calculated for $C_{22}H_{24}N_6O$: 388.20, found: 389.26 $(M+H)^+$. Anal. Calcd for $C_{22}H_{24}N_6O$: (%) C 68.02, H 6.23, N 21.63. Found: C 68.13, H 6.27, N 21.70.

***3-(4-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 76)**

Appearance: white solid; Yield: 80% (0.25g); IR (KBr) ν_{max}: 3118, 3055, 1650, 1590, 1546, 1510, 1453, 1259, 912, 730 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 1H), 7.99 (d, J = 8.8 Hz, 1H), 7.98–7.92 (m, 2H), 7.60 (d, J = 8.4 Hz, 2H), 7.48–7.41 (m, 2H), 7.30–7.28 (m, 1H), 3.78 (s, 2H), 3.50 (t, J = 4.0 Hz, 4H), 2.70 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.36, 163.08, 147.24, 134.11, 131.24, 127.05, 123.12, 122.88, 122.36, 121.69, 120.44, 117.04, 113.37, 66.11, 54.23, 48.45. ESI-MS: (m/z) calculated for $C_{20}H_{19}FN_6O$: 378.16, found: 379.24 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}FN_6O$: (%) C 63.48, H 5.06, N 22.21. Found: C 63.53, H 5.11, N 22.28.

***3-(4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 77)**

Appearance: white solid; Yield: 95% (0.31g); IR (KBr) ν_{max}: 3115, 3050, 1648, 1603, 1544, 1495, 1450, 1255, 908, 852 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.01 (d, J = 8.8 Hz, 3H), 7.70 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.31–7.28 (m, 1H), 3.77 (s, 2H), 3.54 (t, J = 4.0 Hz, 4H), 2.70 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.36, 163.08, 147.24, 134.11, 131.24, 127.05, 123.12, 122.88, 122.36, 121.69, 120.44, 117.04, 113.37, 66.11, 54.23, 48.45. ESI-MS: (m/z) calculated for $C_{20}H_{19}ClN_6O$: 394.13, found: 395.25 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}ClN_6O$: (%) C 60.84, H 4.85, N 21.28. Found: C 60.90, H 4.88, N 21.34.

***3-(4-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 78)**

Appearance: white solid; Yield: 92% (0.33g); IR (KBr) ν_{max}: 3120, 3048, 1645, 1590, 1545, 1504, 1452, 1255, 908, 728 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.00 (d, J = 8.8 Hz, 3H), 7.59 (d, J = 8.4 Hz, 2H), 7.49–7.41 (m, 2H), 7.29–7.27 (m, 1H), 3.78 (s, 2H), 3.52 (t, J = 4.0 Hz, 4H), 2.69 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.21, 147.08, 136.11, 134.27, 133.06, 131.12, 128.12, 124.04, 123.81, 122.75, 121.60, 120.44, 113.78, 66.04, 54.36, 48.09. ESI-MS: (m/z) calculated for $C_{20}H_{19}BrN_6O$: 438.08, found: 439.15 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}BrN_6O$: (%) C 54.68, H 4.36, N 19.13. Found: C 54.76, H 4.39, N 19.18.

***3-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 79)**

Appearance: yellow solid; Yield: 90% (0.30g); IR (KBr) ν_{max}: 3115, 3046, 1650, 1605, 1545, 1505, 1452, 1345, 1255, 908 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.24 (d, J = 8.8 Hz, 2H), 7.99 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.8 Hz, 2H), 7.51–7.42 (m, 2H), 7.32 (d, J = 8.8 Hz, 1H), 3.79 (s, 2H), 3.53 (t, J = 4.0 Hz, 4H), 2.71 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.04, 148.12, 147.45, 144.22, 133.77, 131.04, 125.03, 124.00, 123.22, 122.08, 121.45, 119.87, 113.46, 65.57, 54.24, 48.00. ESI-MS: (m/z) calculated for $C_{20}H_{19}N_7O_3$: 405.15, found: 406.23 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}N_7O_3$: (%) C 59.25, H 4.72, N 24.18. Found: C 59.31, H 4.77, N 24.25.

***3-(4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 80)**

Appearance: pale solid; Yield: 90% (0.29g); IR (KBr) ν_{max}: 3118, 3055, 1640, 1590, 1545,

1504, 1452, 1272, 1255, 910 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.32–7.29 (m, 1H), 7.14 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H), 3.72 (s, 2H), 3.52 (t, *J* = 4.0 Hz, 4H), 2.70 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.10, 161.25, 147.22, 134.20, 131.08, 130.14, 124.42, 123.22, 122.11, 121.77, 120.33, 120.10, 112.89, 65.47, 56.02, 54.22, 48.46. ESI-MS: (m/z) calculated for $C_{21}H_{22}N_6O_2$: 390.18, found: 391.24 $(M+H)^+$. Anal. Calcd for $C_{21}H_{22}N_6O_2$: (%) C 64.60, H 5.68, N 21.52. Found: C 64.71, H 5.73, N 21.57.

***3-(4-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 81)**

Appearance: white solid; Yield: 92% (0.30g); IR (KBr) ν_{max}: 3116, 3052, 1648, 1600, 1544, 1495, 1450, 1258, 908, 854 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.92–7.88 (m, 1H), 7.88 (s, 1H), 7.68–7.64 (m, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.30–7.27 (m, 1H), 3.76 (s, 2H), 3.52 (t, *J* = 4.0 Hz, 4H), 2.70 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.48, 148.02, 137.24, 134.45, 133.20, 132.11, 131.12, 129.04, 128.33, 127.02, 125.23, 122.15, 121.84, 120.11, 112.58, 66.24, 55.51, 48.22. ESI-MS: (m/z) calculated for $C_{20}H_{19}ClN_6O$: 394.13, found: 395.25 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}ClN_6O$: (%) C 60.84, H 4.85, N 21.28. Found: C 60.89, H 4.89, N 21.35.

***3-(4-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 82)**

Appearance: pale solid; Yield: 81% (0.26g); IR (KBr) ν_{max}: 3122, 3053, 1640, 1598, 1544, 1505, 1452, 1270, 1256, 910 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.80–7.69 (m, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.39–7.35 (m, 1H), 7.12 (s, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 3.84 (s, 3H), 3.71 (s, 2H), 3.53 (t, *J* = 4.0 Hz, 4H), 2.71 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.22, 163.08, 148.14, 144.12, 134.06, 131.14, 130.44, 123.78, 122.45, 121.18, 120.12, 116.44, 115.80, 114.18, 65.40, 57.08, 54.72, 48.25. ESI-MS: (m/z) calculated for $C_{21}H_{22}N_6O_2$: 390.18, found: 391.26 $(M+H)^+$. Anal. Calcd for $C_{21}H_{22}N_6O_2$: (%) C 64.60, H 5.68, N 21.52. Found: C 64.68, H 5.72, N 21.87.

***3-(4-((1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 83)**

Appearance: pale solid; Yield: 80% (0.28g); IR (KBr) ν_{max}: 3115, 3050, 1650, 1590, 1540, 1510, 1453, 1255, 915, 784 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.94 (s, 1H), 7.88–7.72 (m, 2H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 8.8 Hz,

1H), 7.41 (d, *J* = 9.2 Hz, 1H), 7.30–7.27 (m, 1H), 3.72 (s, 2H), 3.68 (t, *J* = 4.0 Hz, 4H), 2.72 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.04, 148.11, 134.08, 133.45, 131.12, 130.44, 130.02, 125.48, 124.23, 123.11, 122.84, 122.07, 121.41, 121.48, 120.08, 112.55, 65.12, 57.24, 48.01. ESI-MS: (m/z) calculated for $C_{21}H_{19}F_3N_6O$: 428.15, found: 429.23 $(M+H)^+$. Anal. Calcd for $C_{21}H_{19}F_3N_6O$: (%) C 58.87, H 4.47, N 19.62. Found: C 58.95, H 4.52, N 19.68.

***3-(4-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 84)**

Appearance: pale solid; Yield: 81% (0.28g); IR (KBr) ν_{max}: 3118, 3055, 1650, 1590, 1545, 1510, 1454, 1259, 910, 710 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.94–7.90 (m, 1H), 7.90 (s, 1H), 7.81–7.77 (m, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 3.70 (s, 2H), 3.69 (t, *J* = 4.0 Hz, 4H), 2.02 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.88, 148.06, 134.00, 132.11, 131.25, 130.78, 130.12, 128.04, 123.19, 123.04, 122.42, 122.02, 121.75, 120.85, 120.04, 112.82, 65.48, 56.72, 48.14. ESI-MS: (m/z) calculated for $C_{21}H_{18}BrF_3N_6O$: 428.15, found: 429.23 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}BrF_3N_6O$: (%) C 49.72, H 3.58, N 16.57. Found: C 49.79, H 3.63, N 16.64.

***6-chloro-3-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d] isoxazole* (BIP 85)**

Appearance: white solid; Yield: 65% (0.19g); IR (KBr) ν_{max}: 3110, 3048, 1650, 1592, 1545, 1510, 1454, 1250, 911, 830 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 1H), 7.38 (s, 1H), 3.74 (s, 2H), 3.52 (t, *J* = 4.0 Hz, 4H), 2.73 (t, *J* = 4.0 Hz, 4H), 2.69 (q, 2H), 1.20 (t, *J* = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.58, 148.08, 145.64, 137.01, 135.44, 133.16, 128.42, 124.56, 123.80, 123.32, 121.22, 120.04, 114.24, 67.11, 54.18, 48.54, 30.27, 16.64. ESI-MS: (m/z) calculated for $C_{22}H_{23}ClN_6O$: 422.16, found: 423.24 $(M+H)^+$. Anal. Calcd for $C_{22}H_{23}ClN_6O$: (%) C 62.48, H 5.48, N 19.87. Found: C 62.54, H 5.53, N 19.92.

***6-chloro-3-(4-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d] isoxazole* (BIP 86)**

Appearance: white solid; Yield: 84% (0.25g); IR (KBr) ν_{max}: 3115, 3045, 1650, 1590, 1544, 1510, 1454, 1250, 915, 848, 710 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.36 (s, 1H), 7.30–7.27 (m, 2H), 3.77 (s, 2H), 3.53 (t, *J* = 4.0 Hz, 4H), 2.71 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz,

DMSO-d_6) δ 167.18, 163.74, 147.88, 136.82, 133.24, 132.18, 124.74, 124.52, 122.42, 121.60, 120.14, 117.12, 112.24, 66.08, 54.47, 48.34. ESI-MS: (m/z) calculated for $C_{20}H_{18}ClFN_6O$: 412.12, found: 413.24 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}ClFN_6O$: (%) C 58.19, H 4.39, N 20.36. Found: C 58.25, H 4.43, N 20.41.

***6-chloro-3-(4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d] isoxazole* (BIP 87)**

Appearance: white solid; Yield: 90% (0.28g); IR (KBr) ν_{max}: 3115, 3051, 1650, 1596, 1545, 1510, 1454, 1255, 918, 850 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.4 Hz, 3H), 7.30 (s, 1H), 3.78 (s, 2H), 3.53 (t, J = 4.0 Hz, 4H), 2.71 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.02, 147.11, 137.28, 136.54, 133.24, 133.02, 124.08, 123.12, 122.88, 122.36, 121.69, 120.44, 112.14, 66.10, 54.27, 48.41. ESI-MS: (m/z) calculated for $C_{20}H_{18}Cl_2N_6O$: 428.09, found: 429.18 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}Cl_2N_6O$: (%) C 55.96, H 4.23, N 19.58. Found: C 56.03, H 4.27, N 19.64.

***3-(4-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-chlorobenzo[d] isoxazole* (BIP 88)**

Appearance: brown solid; Yield: 88% (0.30g); IR (KBr) ν_{max}: 3111, 3045, 1650, 1595, 1545, 1510, 1454, 1250, 914, 862, 712 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 1H), 7.88 (d, J = 80 Hz, 2H), 7.67 (d, J = 8.4 Hz, 3H), 7.56 (d, J = 8.0 Hz, 1H), 7.32 (s, 1H), 3.77 (s, 2H), 3.53 (t, J = 4.0 Hz, 4H), 2.71 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.84, 147.92, 136.54, 136.12, 133.24, 132.34, 128.60, 124.04, 123.95, 122.44, 121.60, 120.49, 112.88, 66.23, 54.74, 48.14. ESI-MS: (m/z) calculated for $C_{20}H_{18}BrClN_6O$: 472.04, found: 473.12 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}BrClN_6O$: (%) C 50.70, H 3.83, N 17.74. Found: C 50.76, H 3.87, N 17.79.

***6-chloro-3-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d] isoxazole* (BIP 89)**

Appearance: yellow solid; Yield: 92% (0.29g); IR (KBr) ν_{max}: 3110, 3048, 1650, 1592, 1545, 1510, 1454, 1330, 1255, 918, 860, 705 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.35 (d, J = 8.8 Hz, 2H), 8.12 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.34 (s, 1H),3.74 (s, 2H), 3.53 (t, J = 4.0 Hz, 4H), 2.72 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.11, 148.65, 147.22, 144.38, 136.42, 133.08, 123.82, 123.46, 122.48, 122.12, 121.08, 120.28, 114.59, 65.08, 54.36, 48.22. ESI-MS: (m/z) calculated for $C_{20}H_{18}ClN_7O_3$: 439.11, found: 440.19 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}ClN_7O_3$: (%) C 54.61,

H 4.12, N 22.29. Found: C 54.66, H 4.15, N 22.35.

***6-chloro-3-(4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d] isoxazole* (BIP 90)**

Appearance: pale solid; Yield: 80% (0.24g); IR (KBr) ν_{max}: 3115, 3046, 1650, 1590, 1545, 1510, 1454, 1250, 910, 850 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 7.86 (d, J = 8.8 Hz, 3H), 7.77 (d, J = 8.4 Hz, 1H), 7.38 (s, 1H), 7.15 (d, J = 8.4 Hz, 2H), 3.83 (s, 3H), 3.74 (s, 2H), 3.58 (t, J = 4.0 Hz, 4H), 2.71 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.08, 161.42, 147.90, 136.74, 133.68, 130.11, 124.02, 123.81, 122.78, 121.45, 121.01, 118.12, 113.78, 65.40, 56.11, 54.48, 48.07. ESI-MS: (m/z) calculated for $C_{21}H_{21}ClN_6O_2$: 424.14, found: 425.18 $(M+H)^+$. Anal. Calcd for $C_{21}H_{21}ClN_6O_2$: (%) C 59.36, H 4.98, N 19.78. Found: C 59.36, H 4.98, N 19.78.

***3-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 91)**

Appearance: white solid; Yield: 68% (0.21g); IR (KBr) ν_{max}: 3120, 3018, 1650, 1592, 1545, 1510, 1451, 13322, 1255, 918 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.69 (d, J = 8.0 Hz, 3H), 7.60–7.55 (m, 2H), 7.45 (d, J = 8.0 Hz, 2H), 7.18 (s, 1H), 3.94 (s, 2H), 3.86–2.88 (t, J = 4.0 Hz, 8H), 2.72 (q, 2H), 2.08-2.02 (m, 2H), 1.42 (t, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.31, 148.04, 145.12, 136.46, 134.88, 133.50, 131.49, 127.98, 124.02, 123.38, 122.42, 120.58, 114.06, 64.02, 58.34, 57.18, 55.12, 51.22, 30.45, 29.08, 16.74. ESI-MS: (m/z) calculated for $C_{23}H_{26}N_6O$: 402.21, found: 403.28 $(M+H)^+$. Anal. Calcd for $C_{23}H_{26}N_6O$: (%) C 68.63, H 6.51, N 20.88. Found: C 68.69, H 6.55, N 20.94.

***3-(4-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 92)**

Appearance: white solid; Yield: 83% (0.25g); IR (KBr) ν_{max}: 31106, 3040, 1652, 1595, 1550, 1504, 1452, 1335, 1250, 916, 862 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H), 8.10 (d, J = 8.8 Hz, 1H), 7.98–7.84 (m, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.50–7.34 (m, 2H), 7.30 (d, J = 8.4 Hz, 1H), 3.92 (s, 2H), 3.86–2.88 (t, J = 4.0 Hz, 8H), 2.03–1.94 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 166.48, 163.22, 147.68, 134.25, 133.14, 131.62, 123.68, 123.52, 122.97, 122.58, 122.14, 120.04, 114.34, 63.24, 58.11, 57.45, 55.08, 51.46, 29.84. ESI-MS: (m/z) calculated for $C_{21}H_{21}FN_6O$: 392.17, found: 393.24 $(M+H)^+$. Anal. Calcd for $C_{21}H_{21}FN_6O$: (%) C 64.27, H 5.39, N 21.42. Found C 64.32, H 5.43, N 21.47.

***3-(4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 93)**

Appearance: white solid; Yield: 92% (0.29g); IR (KBr) ν_{max}: 3110, 3049, 1653, 1598, 1542, 1510, 1453, 1325, 1250, 930, 863 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.04 (d, J = 8.8 Hz, 2H), 7.82–7.79 (m, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.8 Hz, 1H), 3.95 (s, 2H), 3.87–2.89 (t, J = 4.0 Hz, 8H), 2.04–1.90 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.86, 147.65, 137.01, 135.10, 133.28, 132.15, 130.02, 123.85, 122.88, 122.36, 121.96, 120.44, 113.37, 63.24, 58.14, 57.48, 55.11, 51.40, 29.88. ESI-MS: (m/z) calculated for $C_{21}H_{21}ClN_6O$: 408.14, found: 409.22 $(M+H)^+$. Anal. Calcd for $C_{21}H_{21}ClN_6O$: (%) C 61.69, H 5.18, N 20.55. Found: C 61.75, H 5.21, N 20.59.

***3-(4-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 94)**

Appearance: pale brown solid; Yield: 90% (0.31g); IR (KBr) ν_{max}: 3110, 3030, 1650, 1593, 1548, 1510, 1450, 1328, 1245, 918, 860, 706 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (s, 1H), 7.70 (d, J = 8.8 Hz, 3H), 7.68 (d, J = 8.4 Hz, 2H), 7.49–7.40 (m, 2H), 7.18 (d, J = 8.8 Hz, 1H), 3.94 (s, 2H), 3.88–2.88 (t, J = 4.0 Hz, 8H), 2.09–2.04 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.82, 147.56, 136.08, 133.77, 133.22, 131.35, 129.52, 124.01, 123.67, 122.72, 122.69, 120.41, 114.88, 63.55, 58.18, 57.48, 55.08, 51.87, 29.64. ESI-MS: (m/z) calculated for $C_{21}H_{21}BrN_6O$: 452.09, found: 453.16 $(M+H)^+$. Anal. Calcd for $C_{21}H_{21}BrN_6O$: (%) C 55.64, H 4.67, N 18.54. Found: C 55.69, H 4.72, N 18.58.

***3-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 95)**

Appearance: yellow solid; Yield: 86% (0.28g); IR (KBr) ν_{max}: 3112, 3044, 1647, 1598, 1550, 1510, 1454, 1330, 1250, 915, 860 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H), 8.23 (d, J = 8.8 Hz, 2H), 8.11 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.8 Hz, 2H), 7.70–7.64 (m, 2H), 7.43 (d, J = 8.8 Hz, 1H), 3.94 (s, 2H), 3.89–2.86 (t, J = 4.0 Hz, 8H), 2.10–2.06 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.54, 148.78, 147.40, 144.27, 133.71, 131.32, 125.18, 124.01, 123.12, 122.11, 121.40, 120.89, 115.08, 63.63, 58.18, 57.54, 54.11, 52.04, 29.85. ESI-MS: (m/z) calculated for $C_{21}H_{21}N_7O_3$: 419.17, found: 420.23 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}N_7O_3$: (%) C 60.13, H 5.05, N 23.38. Found: C 60.19, H 5.07, N 23.45.

***3-(4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)benzo[d]isoxazole* (BIP 96)**

Appearance: pale solid; Yield: 75% (0.23g); IR (KBr) ν_{max}: 3112, 3045, 1651, 1594, 1550, 1505, 1451, 1332, 1251, 922, 860 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 8.04 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 8.0 Hz, 2H), 7.62–7.56 (m, 2H), 7.44 (d, J = 8.4 Hz, 1H),

7.18 (d, *J* = 8.8 Hz, 2H), 3.94 (s, 2H), 3.90 (s, 3H), 3.84–2.82 (t, *J* = 4.0 Hz, 8H), 2.06–2.02 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.18, 161.44, 147.52, 134.12, 131.80, 130.19, 124.34, 123.25, 122.78, 122.42, 121.30, 120.12, 114.88, 65.55, 63.62, 58.22, 57.44, 54.08, 52.14, 29.85. ESI-MS: (m/z) calculated for $C_{22}H_{24}N_6O_2$: 404.19, found: 405.24 $(M+H)^+$. Anal. Calcd for $C_{22}H_{24}N_6O_2$: (%) C 65.33, H 5.98, N 20.78. Found: C 65.38, H 6.02, N 20.83.

***3-(4-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 97)**

Appearance: white solid; Yield: 90% (0.28g); IR (KBr) ν_{max}: 3112, 3035, 1652, 1599, 1543, 1510, 1450, 1330, 1255, 923, 859 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.94–7.90 (m, 1H), 7.86 (s, 1H), 7.68–7.63 (m, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.32–7.29 (m, 1H), 3.92 (s, 2H), 3.87–2.85 (t, *J* = 4.0 Hz, 8H), 2.02–1.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.44, 148.15, 138.56, 134.48, 133.85, 132.10, 131.43, 129.64, 128.79, 126.12, 123.48, 122.90, 122.42, 120.63, 114.98, 63.58, 61.24, 58.44, 56.10, 50.11, 29.82. ESI-MS: (m/z) calculated for $C_{21}H_{21}ClN_6O$: 408.14, found: 409.20 $(M+H)^+$. Anal. Calcd for $C_{21}H_{21}ClN_6O$: (%) C 61.69, H 5.18, N 20.55. Found: C 61.74, H 5.22, N 20.58.

***3-(4-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d] isoxazole* (BIP 98)**

Appearance: pale solid; Yield: 82% (0.25g); IR (KBr) ν_{max}: 3117, 3045, 1652, 1596, 1545, 1510, 1454, 1330, 1255, 918, 860 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.82–7.68 (m, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.40–7.37 (m, 1H), 7.18 (s, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 3.92 (s, 2H), 3.89 (s, 3H), 3.85–2.82 (t, *J* = 4.0 Hz, 8H), 2.04–2.00 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.38, 163.51, 148.40, 144.10, 134.22, 131.88, 130.72, 123.58, 122.64, 122.18, 121.02, 116.84, 113.66, 114.24, 65.84, 63.63, 58.27, 56.44, 54.14, 52.20, 29.74. ESI-MS: (m/z) calculated for $C_{22}H_{24}N_6O_2$: 404.19, found: 405.26 $(M+H)^+$. Anal. Calcd for $C_{22}H_{24}N_6O_2$: (%) C 65.33, H 5.98, N 20.78. Found: C 65.39, H 6.03, N 20.82.

***3-(4-((1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 99)**

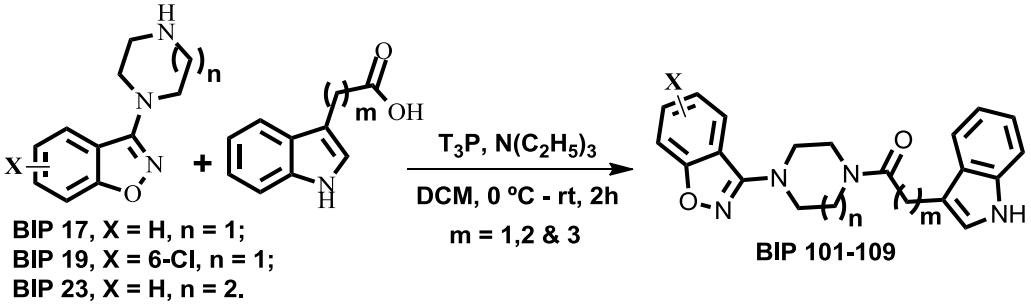
Appearance: pale solid; Yield: 80% (0.27g); IR (KBr) ν_{max}: 3111, 3049, 1650, 1590, 1555, 1510, 1451, 1330, 1245, 924, 848 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.95 (s, 1H), 7.88–7.70 (m, 2H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.42 (d, *J* = 9.2 Hz, 1H), 7.33–7.30 (m, 1H), 3.93 (s, 2H), 3.88–2.84 (t, *J* = 4.0 Hz,

8H), 2.02–1.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.15, 148.34, 134.08, 133.88, 131.10, 130.72, 129.82, 125.65, 124.98, 123.27, 122.80, 122.11, 121.40, 120.90, 120.22, 114.52, 63.81, 60.24, 58.44, 56.10, 52.24, 29.80. ESI-MS: (m/z) calculated for $C_{22}H_{21}F_3N_6O$: 442.17, found: 443.23 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}F_3N_6O$: (%) C 59.72, H 4.78, N 18.99. Found: C 59.78, H 4.81, N 19.03.

***3-(4-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4-diazepan-1-yl)benzo[d]isoxazole* (BIP 100)**

Appearance: pale solid; Yield: 81% (0.26g); IR (KBr) ν_{max}: 3110, 3048, 1650, 1592, 1545, 1510, 1454, 1330, 1255, 918, 860, 709 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.95–7.91 (m, 1H), 7.86 (s, 1H), 7.81–7.78 (m, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.41 (d, J = 9.2 Hz, 1H), 3.92 (s, 2H), 3.86–2.88 (t, J = 4.0 Hz, 8H), 2.00–1.96 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.28, 148.32, 134.04, 132.45, 131.20, 130.98, 130.12, 128.72, 123.58, 123.12, 122.79, 122.23, 122.08, 121.35, 120.12, 115.05, 63.80, 60.27, 58.28, 56.42, 52.12, 29.84. ESI-MS: (m/z) calculated for $C_{22}H_{20}BrF_3N_6O$: 520.08, found: 521.15 $(M+H)^+$. Anal. Calcd for $C_{22}H_{20}BrF_3N_6O$: (%) C 50.68, H 3.87, N 16.12. Found: C 50.73, H 3.91, N 16.16.

4.2.1.5. Synthesis of 1-(4-(benzo[*d*]isoxazol-3-yl)piperazin-1-yl/ homopiperazin-1-yl)-2-(1H-indol-3-yl)alkylone (BIP 101-109) derivatives and characterization



A stirred solution of 3-(piperazin-1-yl/homo piperazine-1-yl)benzo[*d*]isoxazole (**BIP 17, BIP 19** and **BIP 23**) derivatives (0.30 g, 1.0 equiv.) in CH_2Cl_2 was cooled to 0 °C and 2-(1H-indol-3-yl)acetic acid analogues (1.05 equiv.), Propylphosphonic anhydride (T_3P) (1.2 equiv.) and triethyl amine (2.0 equiv.) were added to above mixture, & stirred for 2h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude

products were purified by column chromatography [methanol/ CH_2Cl_2 (2-6 %)] to get the **BIP 101-109** title compounds in yields ranging from 75-90%.

Characterization of the synthesized 1-(4-(benzo[*d*]isoxazol-3-yl)piperazin-1-yl/ homopiperazin-1-yl)-2-(1H-indol-3-yl)alkyl-one (BIP 101-109) derivatives

1-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)-2-(1H-indol-3-yl)ethan-1-one **(BIP 101)**

A stirred solution of 3-(piperazin-1-yl)benzo[*d*]isoxazole (**BIP 17**) 0.30g (1.0 equiv.) in CH_2Cl_2 was added and cooled to 0 °C and 2-(1H-indol-3-yl)acetic acid (1.05 equiv.), T_3P (1.2 equiv.) and triethyl amine (2.0 equiv.) were added to above mixture and stirred for 2h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [methanol/ CH_2Cl_2 (3-6 %)] to achived 0.36g of 1-(4-(benzo[*d*]isoxazol-3-yl)piperazin-1-yl)-2-(1H-indol-3-yl)ethanone (**BIP 101**).

Appearance: white solid; Yield: 82% (0.36g); IR (KBr) ν_{max}: 3280, 3109, 3044, 1765, 1650, 1598, 1545, 1510, 1451, 1320, 1255, 925 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.63 (b, 1H), 8.18 (d, J = 8.8 Hz, 1H), 7.94–7.72 (m, 2H), 7.65 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.52 (d, J = 9.2 Hz, 1H), 7.38 (s, 1H), 7.32–7.18 (m, 2H), 3.83 (s, 2H), 3.80–3.65 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.58, 161.47, 148.33, 138.25, 131.82, 129.12, 123.90, 123.11, 122.70, 122.54, 121.69, 121.18, 120.24, 119.78, 118.73, 114.42, 58.22, 54.08, 35.46. ESI-MS: (m/z) calculated for $C_{21}H_{20}N_4O_2$: 360.15, found: 361.22 $(M+H)^+$. Anal. Calcd for $C_{21}H_{20}N_4O_2$: (%) C 69.98, H 5.59, N 15.55. Found: C 69.98, H 5.59, N 15.55.

1-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)-3-(1H-indol-3-yl)propan-1-one **(BIP 102)**

Appearance: white solid; Yield: 78% (0.36g); IR (KBr) ν_{max}: 3275, 3105, 3042, 1760, 1649, 1595, 1544, 1508, 1450, 1325, 1245, 926 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.68 (b, 1H), 8.14 (d, J = 8.8 Hz, 1H), 7.96–7.74 (m, 2H), 7.70 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 7.40 (s, 1H), 7.32–7.20 (m, 2H), 3.82–2.64 (t, J = 4.4 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.28, 166.44, 148.12, 138.20, 131.81, 129.08, 123.94, 123.18, 122.74, 122.54, 121.65, 121.18, 120.21, 119.78, 118.55, 114.68, 57.01, 54.42, 36.80, 25.04. ESI-MS: (m/z) calculated for $C_{22}H_{22}N_4O_2$: 374.17, found: 375.24 $(M+H)^+$. Anal. Calcd for $C_{22}H_{22}N_4O_2$: (%) C 70.57, H 5.92, N 14.96. Found: C 70.63, H 5.95, N 15.02.

1-(4-(benzo[d]isoxazol-3-yl)piperazin-1-yl)-4-(1H-indol-3-yl)butan-1-one **(BIP 103)**

Appearance: white solid; Yield: 75% (0.36g); IR (KBr) ν_{max}: 3268, 3110, 3051, 1772, 1650, 1600, 1550, 1508, 1450, 1320, 1252, 927 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (b, 1H), 8.10 (d, J = 8.8 Hz, 1H), 7.94–7.72 (m, 2H), 7.70 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 8.8 Hz, 1H), 7.50 (d, J = 9.2 Hz, 1H), 7.40 (s, 1H), 7.33–7.21 (m, 2H), 3.83–2.61 (t, J = 4.4 Hz, 12H), 1.75–1.71 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.09, 166.67, 148.23, 138.11, 131.34, 129.42, 123.90, 123.45, 122.23, 122.09, 121.68, 121.22, 120.48, 119.66, 118.82, 114.89, 57.25, 54.01, 32.38, 30.12., 29.26. ESI-MS: (m/z) calculated for $C_{23}H_{24}N_4O_2$: 388.18, found: 389.26 $(M+H)^+$. Anal. Calcd for $C_{23}H_{24}N_4O_2$: (%) C 71.11, H 6.23, N 14.42. Found: C 71.16, H 6.27, N 14.45.

1-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)-2-(1H-indol-3-yl)ethan-1-one **(BIP 104)**

Appearance: white solid; Yield: 90% (0.38g); IR (KBr) ν_{max}: 3280, 3109, 30445, 1764, 1650, 1599, 1545, 1510, 1451, 1320, 1252, 925, 866 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (b, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.42–7.34 (s, 2H), 7.30–7.19 (m, 2H), 3.84 (s, 2H), 3.80–3.64 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.48, 161.25, 148.08, 138.22, 136.88, 124.52, 124.14, 123.72, 123.23, 122.50, 121.14, 120.20, 119.88, 118.75, 116.42, 115.11, 56.46, 54.02, 35.80. ESI-MS: (m/z) calculated for $C_{21}H_{19}ClN_4O_2$: 394.11, found: 395.18 $(M+H)^+$. Anal. Calcd for $C_{21}H_{19}ClN_4O_2$: (%) C 63.88, H 4.85, N 14.19. Found: C 63.93, H 4.89, N 14.24.

1-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)-3-(1H-indol-3-yl)propan-1-one **(BIP 105)**

Appearance: white solid; Yield: 82% (0.36g); IR (KBr) ν_{max}: 3280, 3109, 3044, 1765, 1650, 1598, 1545, 1510, 1451, 1320, 1253, 922, 864 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (b, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 8.8 Hz, 2H), 7.42–7.38 (s, 2H), 7.31–7.20 (m, 2H), 3.82–2.65 (t, J = 4.4 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.87, 166.64, 148.35, 137.21, 136.43, 127.79, 123.90, 123.35, 122.89, 122.24, 121.44, 121.18, 120.04, 119.78, 117.55, 114.68, 57.10, 54.22, 37.18, 24.89. ESI-MS: (m/z) calculated for $C_{22}H_{21}ClN_4O_2$: 408.13, found: 409.18 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}ClN_4O_2$: (%) C 64.62, H 5.18, N 13.70. Found: C 64.68, H 5.21, N 13.74.

1-(4-(6-chlorobenzo[d]isoxazol-3-yl)piperazin-1-yl)-4-(1H-indol-3-yl)butan-1-one **(BIP 106)**

Appearance: white solid; Yield: 78% (0.35g); IR (KBr) ν_{max}: 3266, 3100, 3049, 17656, 1648,

1598, 1545, 1510, 1451, 1323, 1250, 925, 865 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.66 (b, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 8.8 Hz, 2H), 7.40–7.36 (s, 2H), 7.32–7.18 (m, 2H), 3.83–2.52 (t, J = 4.4 Hz, 12H), 1.74–1.71 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.14, 166.62, 148.09, 138.02, 136.57, 128.46, 123.77, 123.35, 122.23, 122.09, 121.68, 121.22, 120.48, 119.66, 118.82, 114.89, 56.25, 54.24, 37.33, 30.87, 28.75. ESI-MS: (m/z) calculated for $C_{23}H_{23}ClN_4O_2$: 422.15, found: 423.22 $(M+H)^+$. Anal. Calcd for $C_{23}H_{23}ClN_4O_2$: (%) C 65.32, H 5.48, N 13.25. Found: C 65.36, H 5.52, N 13.31.

***1-(4-(benzo[d]isoxazol-3-yl)-1,4-diazepan-1-yl)-2-(1H-indol-3-yl)ethan-1-one* (BIP 107)**

Appearance: white solid; Yield: 85% (0.37g); IR (KBr) ν_{max}: 3282, 3108, 3048, 1768, 1652, 1598, 1549, 1510, 1451, 1328, 1259, 927 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.68 (b, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.93–7.70 (m, 2H), 7.62 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.50 (d, J = 9.2 Hz, 1H), 7.40 (s, 1H), 7.33–7.21 (m, 2H), 3.84 (s, 2H), 3.79–3.65 (t, J = 4.4 Hz, 8H), 2.04–1.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.55, 161.88, 148.42, 138.24, 131.80, 129.02, 123.90, 123.24, 122.23, 122.04, 121.60, 121.01, 120.24, 119.88, 118.73, 116.42, 56.12, , 54.40, 52.46, 52.10, 37.75, 29.22. ESI-MS: (m/z) calculated for $C_{22}H_{22}N_4O_2$: 374.17, found: 375.26 $(M+H)^+$. Anal. Calcd for $C_{22}H_{22}N_4O_2$: (%) C 70.57, H 5.92, N 14.96. Found: C 70.65, H 5.96, N 15.02.

***1-(4-(benzo[d]isoxazol-3-yl)-1,4-diazepan-1-yl)-3-(1H-indol-3-yl)propan-1-one* (BIP 108)**

Appearance: white solid; Yield: 83% (0.37g); IR (KBr) ν_{max}: 3282, 3108, 3045, 1766, 1652, 1599, 1544, 1512, 1454, 1325, 1254, 922 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.65 (b, 1H), 8.10 (d, J = 8.8 Hz, 1H), 7.94–7.75 (m, 2H), 7.71 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.50 (d, J = 9.2 Hz, 1H), 7.42 (s, 1H), 7.33–7.21 (m, 2H), 3.84–2.66 (t, J = 4.4 Hz, 12H), 1.98–1.94 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.97, 166.54, 148.10, 138.25, 131.64, 129.02, 123.91, 123.25, 122.68, 122.33, 121.60, 121.12, 120.21, 119.18, 116.74, 115.08, 60.22, 54.12, 52.30, 52.05, 36.48, 29.24, 25.15. ESI-MS: (m/z) calculated for $C_{23}H_{24}N_4O_2$: 388.18, found: 389.25 $(M+H)^+$. Anal. Calcd for $C_{23}H_{24}N_4O_2$: (%) C 71.11, H 6.23, N 14.42. Found: C 71.17, H 6.27, N 14.46.

***1-(4-(benzo[d]isoxazol-3-yl)-1,4-diazepan-1-yl)-4-(1H-indol-3-yl)butan-1-one* (BIP 109)**

Appearance: white solid; Yield: 79% (0.37g); IR (KBr) ν_{max}: 3272, 3108, 3044, 1765, 1650, 1598, 1545, 1510, 1451, 1320, 1251, 923 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.70 (b, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.95–7.74 (m, 2H), 7.70 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 8.8 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 7.42 (s, 1H), 7.36–7.24 (m, 2H), 3.84–2.48 (t, J = 4.4 Hz, 12H), 2.02–1.74 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.12, 166.60, 148.34, 138.20,

131.78, 129.06, 123.94, 123.33, 122.48, 122.02, 121.54, 121.11, 120.77, 119.24, 118.39, 115.75, 60.12, 54.08, 52.22, 51.78, 32.38, 30.12., 29.26, 27.84. ESI-MS: (m/z) calculated for $C_{24}H_{26}N_4O_2$: 402.20, found: 403.26 $(M+H)^+$. Anal. Calcd for $C_{24}H_{26}N_4O_2$: (%) C 71.62, H 6.51, N 13.92. Found: C 71.68, H 6.55, N 13.96.

4.2.2. 4-(quinoxalin-2-yl)naphthalen-1-ol (Scheme 2) derivatives

Scheme 2 methodology involved following stages, cardinal synthesis of 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol (**QNP 4**). 4-(3-(substituted piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol derivatives (**QNP 5-26**), 4-(3-(2-substituted hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol analogoues (**QNP 28-52**) and 4-(substituted-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)naphthalen-1-ol derivatives (**QNP 53-64**) (**Figure 4.4)**.

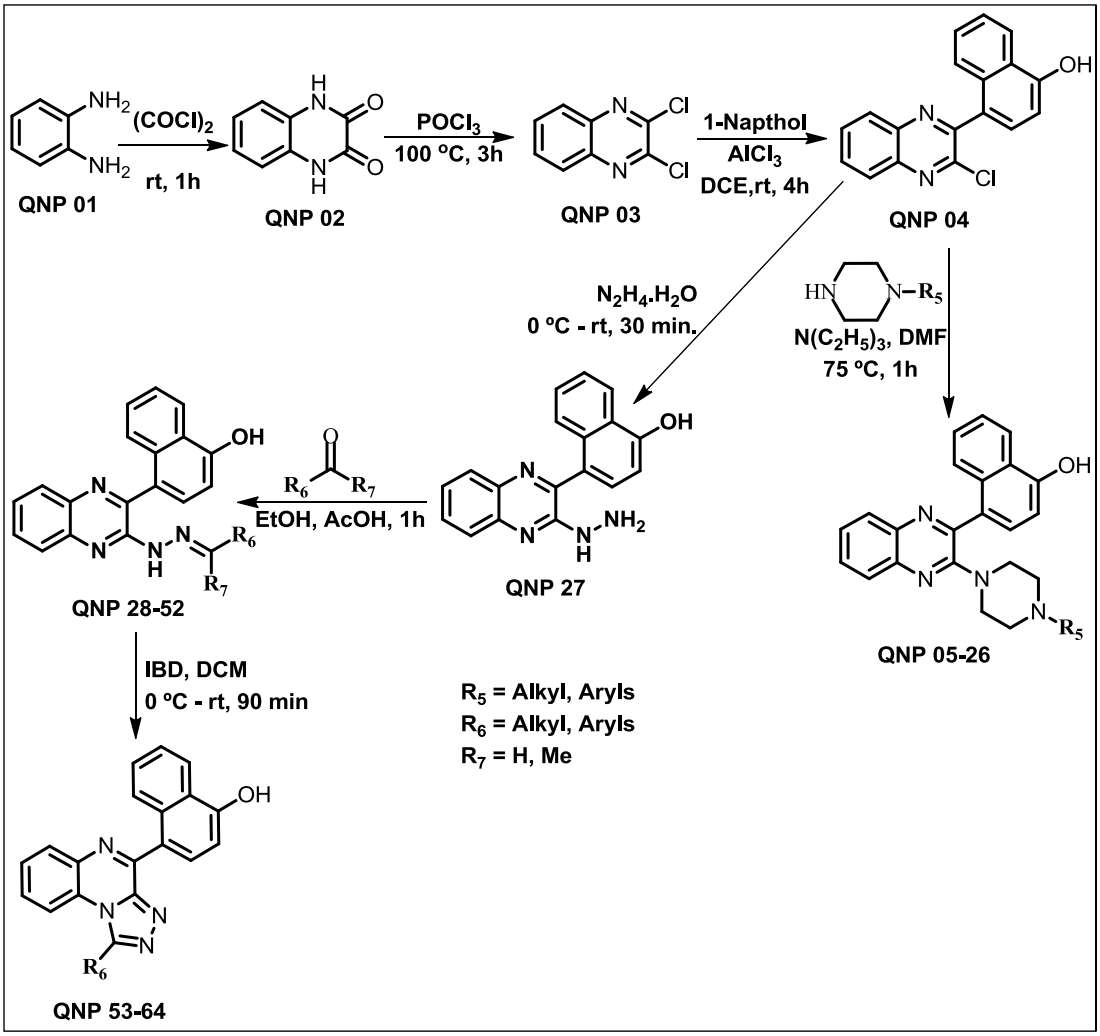
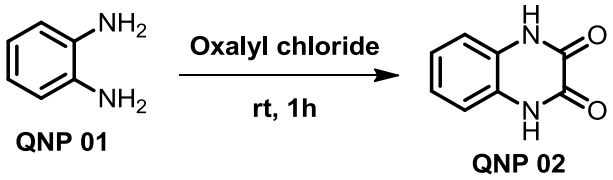


Figure 4.4: Synthetic protocol employed for the synthesis of compounds **QNP 05-64** of scheme 2

4.2.2.1. General experimental procedures utilized for the synthesis of 4-(3-(4-phenylpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol (QNP 5-26)

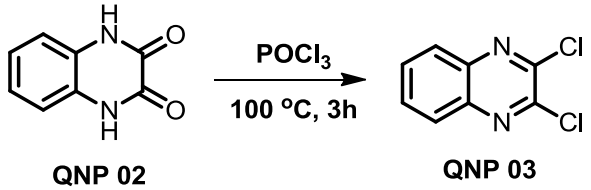
The target molecules were synthesized by following a four step synthetic protocol (**Figure 4.4**), wherein the first step was reaction of benzene-1,2-diamine (**QNP 01**) with oxalyl chloride to yield the quinoxaline-2,3(1H,4H)-dione (**QNP 02**) in good yield. In the next step, reaction of amide on chlorination with $POCl_3$ afforded 2,3-dichloroquinoxaline (**QNP 03**) in good yield. Followed by friedel craft protocol, **QNP 03** reaction with 1-napthol in the presence of $AlCl_3$ and DCE yielded 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol (**QNP 04**) [Kumar *et al*., 2012]. Synthesis of 4-(3-(substitutedpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol derivatives (**QNP 5-26**), *N*-alkylation of **QNP 04** reaction with various piperazine analogues yielded **QNP 5-26** final compounds [Kumar *et al*., 2012] with moderate to good yields (**Figure 4.4).**

Preparation of quinoxaline-2,3(1H,4H)-dione (QNP 02)



To a solution of benzene-1,2-diamine (**QNP 01**) (5.00 g, 1.0 equiv.) oxalyl chloride (10 ml) was added & stirred for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water and observed, white semi solid is filterd through buchner funnel under vaccum to yield the quinoxaline-2,3(1H,4H)-dione (**QNP 02**) with excellent yield (90%). Appearance: white solid; Yield: 90% (6.74g); ESI-MS: (m/z) calcd. for $C_8H_6N_2O_2$: 162.04, found: 163.10 $[M+H]^+$.

Preparation of 2,3-dichloroquinoxaline (QNP 03)

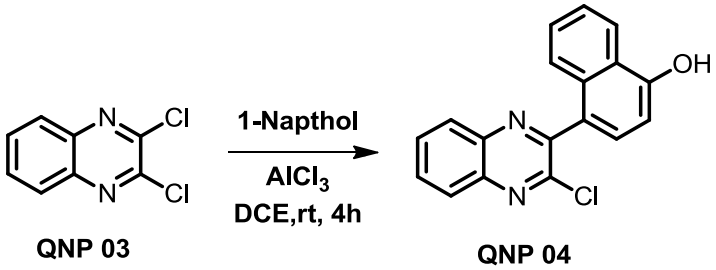


To a stirred solution of quinoxaline-2,3(1H,4H)-dione (**QNP 02**) (5.00 g, 1.0 equiv.), $POCl_3$

was added (20 ml) and refluxed at 100 °C for 3h. After completion of the reaction, as indicated by TLC, the reaction mass was distilled under vaccum and quenched with ice cold water. Off white semi solid was formed and is filtered through buchner funnel under vaccum to yield the 2,3-dichloroquinoxaline (**QNP 03**) in excellent yield.

Appearance: off white solid; Yield: 92% (5.64g); ESI-MS: (m/z) calcd. for $C_8H_4Cl_2N_2$: 197.98, found: 199.10 $[M+H]^+$.

Preparation of 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol (QNP 04)

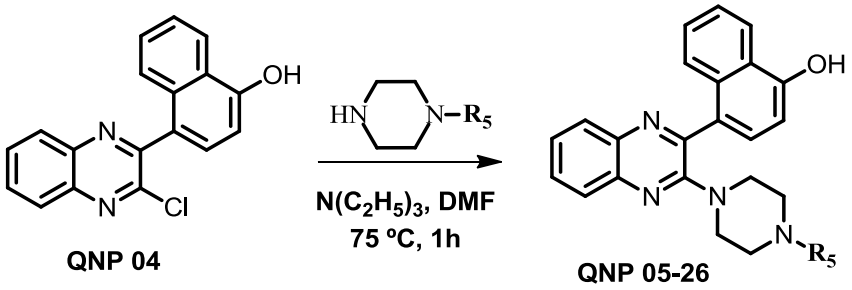


QNP 03 QNP 04

A solution of 2,3-dichloroquinoxaline (**QNP 03**) (5.00 g, 1.0 equiv.) in 1,2-Dichloroethene (DCE) was cooled to 0 °C and $AlCl_3$ (3.0 equiv.) was added to above solution for 20 min 1-napthol (1.20 equiv.) was added to above mixture, stirred at rt for 4h. After completion of the reaction, as indicated by TLC, the reaction mass was quenched with 4N HCl and ice cold water was added & extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography (in ethyl acetate / hexane) to get the 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol in good yield (80%).

Appearance: plae brown solid; Yield: 80% (6.16g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (b, 1H), 8.28 (d, J = 8.8 Hz, 1H), 8.16 (d, J = 8.4 Hz, 2H), 8.02–7.52 (m, 4H), 7.42 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.28, 147.84, 146.30, 145.58, 141.64, 141.23, 134.69, 134.02, 133.08, 132.80, 131.14, 130.77, 129.60, 128.18, 127.78, 127.55, 126.43, 116.44. ESI-MS: (m/z) calculated for $C_{18}H_{11}ClN_2O$: 306.05, found: 307.21 $(M+H)^+$. Anal. Calcd for $C_{18}H_{11}ClN_2O$: (%) C 70.48, H 3.61, N 9.13. Found: C 70.52, H 3.64, N 9.17.

4.2.2.2. Synthesis of 4-(3-(substitutedpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol (QNP 05-26) derivatives and characterization



A stirred solution of 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol (**QNP 04**) (0.30 g, 1.0 equiv.) in DMF was cooled to 0 °C and various piperazine analogues (1.00 equiv. Note: for **QNP 05**; piperazine (3.00 equiv.)), triethyl amine were added to above mixture, stirred at 75 °C for 1h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (70 -95 %) to get the **QNP 05-26** title compounds in yields ranging from 64-90%.

Characterization of the synthesized QNP 05-26 compounds

***4-(3-(piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 05)**

A stirred solution of 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol (**QNP 04**) (0.30 g, 1.0 equiv.) in DMF was cooled to 0 °C and piperazine (3.00 equiv), triethyl amine were added to above mixture, stirred at 75 °C for 1h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (80 - 90 %) to achieve 0.23g of 4-(3-(piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol (**QNP 05**).

Appearance: off white solid; Yield: 68% (0.23g); IR (KBr) ν_{max}: 3585, 3105, 3042, 1650, 1598, 1544, 1508, 1451, 1320, 1245, 920 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.62 (b, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.8 Hz, 3H), 8.77–7.54 (m, 4H), 7.04 (d, J = 8.8 Hz, 1H), 4.16 (b, 1H) 3.64–2.88 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.75, 155.45, 142.12, 135.63, 135.22, 134.09, 133.41, 130.38, 130.02, 129.98, 129.40, 128.92, 128.24, 127.76, 127.10, 126.57, 124.78, 115.85, 55.42, 47.12. ESI-MS: (m/z) calculated for $C_{22}H_{20}N_4O$: 356.16, found: 357.22 $(M+H)^+$. Anal. Calcd for $C_{22}H_{20}N_4O$: (%) C 74.14, H 5.66, N 15.72. Found: C 74.21, H 5.69, N 15.76.

***4-(3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 06)**

Appearance: off white solid; Yield: 81% (0.29g); IR (KBr) ν_{max}: 3582, 3104, 3045, 1652, 1599, 1545, 1509, 1452, 1324, 1245, 926 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.59 (b, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 8.74–7.55 (m, 4H), 7.02 (d, J = 8.8 Hz, 1H), 3.65–2.84 (t, J = 4.0 Hz, 8H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.44, 155.32, 141.74, 136.81, 136.12, 134.65, 134.34, 130.72, 130.11, 129.67, 129.25, 127.78, 127.24, 126.81, 126.23, 125.44, 124.62, 116.47, 56.48, 52.04, 48.08. ESI-MS: (m/z) calculated for $C_{23}H_{22}N_4O$: 370.17, found: 371.25 $(M+H)^+$. Anal. Calcd for $C_{23}H_{22}N_4O$: (%) C 74.57, H 5.99, N 15.12. Found: C 74.61, H 6.03, N 15.18.

***4-(3-(4-ethylpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 07)**

Appearance: off white solid; Yield: 84% (0.31g); IR (KBr) ν_{max}: 3580, 3100, 3041, 1650, 1598, 1544, 1508, 1451, 1320, 1242, 922 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (b, 1H), 8.28 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 8.83–7.59 (m, 4H), 7.02 (d, J = 8.8 Hz, 1H), 3.66–3.35 (t, J = 4.0 Hz, 8H), 2.24 (q, 2H), 1.27 (t, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.44, 155.32, 141.74, 136.81, 136.12, 134.65, 134.34, 130.72, 130.11, 129.67, 129.25, 127.78, 127.24, 126.81, 126.23, 125.44, 124.62, 116.47, 56.62, 52.47, 51.48, 18.08. ESI-MS: (m/z) calculated for $C_{24}H_{24}N_4O$: 384.19, found: 385.24 $(M+H)^+$. Anal. Calcd for $C_{24}H_{24}N_4O$: (%) C 74.97, H 6.29, N 14.57. Found: C 75.04, H 6.33, N 14.62.

***4-(3-(4-benzylpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 08)**

Appearance: pale brown solid; Yield: 75% (0.32g); IR (KBr) ν_{max}: 3586, 3108, 3044, 1652, 1598, 1544, 1508, 1451, 1321, 1233, 922 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (b, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.8 Hz, 1H), 7.86 (d, J = 8.0 Hz, 2H), 8.80–7.36 (m, 7H), 7.32 (d, J = 8.4 Hz, 2H), 7.04 (d, J = 8.8 Hz, 1H), 3.72 (s, 2H), 3.65–3.33 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.12, 155.36, 141.72, 140.20, 136.84, 136.10, 134.64, 134.11, 130.70, 130.10,129.94, 129.64, 129.22, 128.92, 128.24, 127.88, 127.29, 126.88, 126.21, 125.44, 124.62, 116.23, 66.40, 56.44, 52.11. ESI-MS: (m/z) calculated for $C_{29}H_{26}N_4O$: 446.21, found: 447.28 $(M+H)^+$. Anal. Calcd for $C_{29}H_{26}N_4O$: (%) C 78.00, H 5.87, N 12.55. Found: C 78.08, H 5.91, N 12.59.

***4-(3-(4-benzhydrylpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 09)**

Appearance: off whit solid; Yield: 72% (0.36g); IR (KBr) ν_{max}: 3579, 3103, 3038, 1650, 1599, 1545, 1507, 1454, 1324, 1244, 925 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.89 (d, J =

8.0 Hz, 2H), 8.80–7.62 (m, 4H), 7.59 (d, *J* = 8.4 Hz, 4H), 7.36–7.32 (m, 6H), 7.08 (d, *J* = 8.8 Hz, 1H), 4.64 (s, 1H), 3.63–2.86 (t, *J* = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.45, 154.87, 144.35, 141.28, 136.84, 136.04, 134.64, 134.11, 131.12, 130.70, 130.10, 129.94, 129.11, 128.90, 128.20, 127.88, 126.84, 126.29, 125.56, 124.64, 116.78, 86.12, 56.25, 52.01. ESI-MS: (m/z) calculated for $C_{35}H_{30}N_4O$: 522.24, found: 523.32 $(M+H)^+$. Anal. Calcd for $C_{35}H_{30}N_4O$: (%) C 80.43, H 5.79, N 10.72. Found: C 80.49, H 5.83, N 10.78.

***4-(3-(4-phenylpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 10)**

Appearance: off whit solid; Yield: 80% (0.33g); IR (KBr) ν_{max}: 3572, 3099, 3046, 1649, 1598, 1550, 1505, 1449, 1324, 1248, 944 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (b, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 8.79–7.34 (m, 7H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.8 Hz, 1H), 3.74–3.45 (t, *J* = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.11, 155.53, 150.30, 141.12, 136.82, 136.14, 134.42, 134.18, 131.45, 130.48, 130.01, 129.98, 129.00, 128.92, 128.45, 127.23, 126.67, 126.28, 125.56, 120.12, 116.04, 54.22, 53.68. ESI-MS: (m/z) calculated for $C_{28}H_{24}N_4O$: 432.19, found: 433.26 $(M+H)^+$. Anal. Calcd for $C_{28}H_{24}N_4O$: (%) C 77.75, H 5.59, N 12.95. Found: C 77.82, H 5.63, N 12.99.

***4-(3-(4-(p-tolyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 11)**

Appearance: brown solid; Yield: 78% (0.34g); IR (KBr) ν_{max}: 3578, 3102, 3040, 1654, 1599, 1545, 1505, 1451, 1320, 1245, 920 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (b, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 8.82–7.58 (m, 4H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 8.8 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 3.65–3.42 (t, *J* = 4.0 Hz, 8H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.23, 155.22, 148.72, 141.08, 136.89, 136.21, 134.80, 134.08, 132.49, 131.92, 131.22, 130.77, ,129.84, 129.21, 128.87, 128.40, 127.22, 126.67, 126.22, 125.60, 120.11, 116.32, 54.58, 53.71. ESI-MS: (m/z) calculated for $C_{29}H_{26}N_4O$: 446.21, found: 447.32 $(M+H)^+$. Anal. Calcd for $C_{29}H_{26}N_4O$: (%) C 78.00, H 5.87, N 12.55. Found: C 78.07, H 5.91, N 12.59.

***4-(3-(4-(4-fluorophenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 12)**

Appearance: pale brown solid; Yield: 80% (0.35g); IR (KBr) ν_{max}: 3583, 3102, 3045, 1649, 1598, 1548, 1504, 1455, 1318, 1245, 923 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (b, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 8.82–7.38 (m, 6H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.8 Hz, 1H), 3.62–3.45 (t, *J* = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.87, 155.01, 148.79, 141.12, 136.56, 136.20, 134.82, 134.22, 132.34, 131.87, 131.33, 130.65, 129.73, 129.24, 128.80,

128.42, 127.14, 126.60, 126.29, 120.78, 118.11, 116.65, 54.34, 53.86. ESI-MS: (m/z) calculated for $C_{28}H_{23}FN_4O$: 450.18, found: 451.24 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}FN_4O$: (%) C 74.65, H 5.15, N 12.44. Found: C 74.72, H 5.19, N 12.51.

***4-(3-(4-(4-chlorophenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 13)**

Appearance: off white solid; Yield: 84% (0.38g); IR (KBr) ν_{max}: 3573, 3099, 3048, 1650, 1598, 1544, 1508, 1448, 1320, 1248, 928, 862 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.58 (b, 1H), 8.29 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.8 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 8.83–7.56 (m, 4H), 7.44 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.8 Hz, 1H), 3.64–3.44 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.25, 155.22, 148.88, 141.30, 136.62, 136.22, 134.65, 134.12, 132.38, 131.99, 131.24, 130.60, 130.35, 129.70, 129.24, 128.80, 128.42, 127.14, 126.60, 126.29, 120.04, 116.72, 54.22, 53.64. ESI-MS: (m/z) calculated for $C_{28}H_{23}ClN_4O$: 466.15, found: 467.22 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}ClN_4O$: (%) C 72.02, H 4.96, N 12.00. Found: C 72.11, H 4.99, N 12.04.

***4-(3-(4-(4-bromophenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 14)**

Appearance: pale brown solid; Yield: 80% (0.39g); IR (KBr) ν_{max}: 3574, 3101, 3048, 1650, 1599, 1544, 1510, 1455, 1320, 1245, 925, 705 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.60 (b, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.0 Hz, 2H), 8.84–7.57 (m, 4H), 7.53 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 8.8 Hz, 2H), 3.62–3.48 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.20, 155.16, 148.64, 141.22, 136.54, 136.61, 134.53, 134.33, 133.82, 132.25, 131.95, 131.24, 130.87, 130.22, 129.62, 129.23, 128.76, 128.44, 127.14, 126.60, 120.11, 117.01, 54.13, 53.32. ESI-MS: (m/z) calculated for $C_{28}H_{23}BrN_4O$: 510.10, found: 511.12 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}BrN_4O$: (%) C 65.76, H 4.53, N 10.96. Found: C 65.81, H 4.57, N 11.02.

***4-(3-(4-(4-nitrophenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 15)**

Appearance: pale yellow solid; Yield: 85% (0.39g); IR (KBr) ν_{max}: 3585, 3105, 3042, 1649, 1599, 1552, 1501, 1451, 1320, 1248, 928 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.64 (b, 1H), 8.48 (d, J = 8.8 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.0 Hz, 2H), 8.84–7.57 (m, 4H), 7.53 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 8.8 Hz, 2H), 3.62–3.48 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.20, 155.16, 148.64, 141.22, 136.54, 136.61, 134.53, 134.33, 133.82,132.25, 131.95, 131.24, 130.87, 130.22, 129.62, 129.23, 128.76, 128.44, 127.14, 126.60, 120.11, 117.01, 54.13, 53.32. ESI-MS: (m/z) calculated for $C_{28}H_{23}N_5O_3$: 477.18, found: 477.26 $(M+H)^+$. Anal.Calcd for $C_{28}H_{23}N_5O_3$: (%) C 70.43, H 4.85, N 14.67. Found: C 70.49, H 4.88, N 14.72.

***4-(3-(4-(4-hydroxyphenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 16)**

Appearance: pale brown solid; Yield: 70% (0.30g); IR (KBr) ν_{max}: 3581, 3104, 3045, 1650, 1599, 1545, 1507, 1452, 1325, 1245, 931 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84–10.62 (b, 2H), 8.34 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 8.85–7.56 (m, 4H), 7.40 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.8 Hz, 1H), 3.68–3.46 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.22, 155.87, 149.68, 148.91, 141.34, 137.66, 134.50, 134.22, 133.80, 131.43, 130.92, 129.54, 128.76, 127.82, 127.11, 126.75, 126.24, 125.82, 124.12, 119.45, 118.78, 114.22, 54.65, 54.08. ESI-MS: (m/z) calculated for $C_{28}H_{24}N_4O_2$: 448.18, found: 449.24 $(M+H)^+$. Anal. Calcd for $C_{28}H_{24}N_4O_2$: (%) C 74.98, H 5.39, N 12.49. Found: C 75.24, H 5.43, N 12.55.

***4-(3-(4-(4-methoxyphenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 17)**

Appearance: pale brown solid; Yield: 75% (0.33g); IR (KBr) ν_{max}: 3580, 3104, 3046, 1647, 1598, 1544, 1502, 1451, 13204, 1245, 928 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (b, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 2H), 8.80–7.60 (m, 4H), 7.40 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 1H), 3.67–3.44 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.48, 155.87, 149.52, 148.88, 141.28, 137.44, 134.34, 134.70, 133.72, 131.25, 130.90, 129.55, 128.70, 127.76, 127.04, 126.68, 126.50, 125.80, 124.11, 119.42, 119.13, 114.08, 54.68, 54.24. ESI-MS: (m/z) calculated for $C_{29}H_{26}N_4O_2$: 462.20, found: 463.28 $(M+H)^+$. Anal. Calcd for $C_{29}H_{26}N_4O_2$: (%) C 75.30, H 5.67, N 12.11. Found: C 75.37, H 5.71, N 12.15.

***4-(3-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 18)**

Appearance: brown solid; Yield: 81% (0.39g); IR (KBr) ν_{max}: 3568, 3106, 3050, 1650, 1602, 1540, 1504, 1451, 1320, 1245, 928 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (b, 1H) 8.34 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 2H), 8.78–7.59 (m, 4H), 7.54 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 8.8 Hz, 1H), 3.68–3.46 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.55, 155.66, 152.28, 149.44, 142.22, 137.50, 134.36, 134.64, 133.40, 131.11, 130.88, 129.18, 128.45, 127.42, 127.22, 127.02, 126.61, 126.23, 125.89, 125.58, 124.15, 117.44, 114.43, 54.65, 54.22. ESI-MS: (m/z) calculated for $C_{29}H_{23}F_3N_4O$: 500.18, found: 501.26 $(M+H)^+$. Anal. Calcd for $C_{29}H_{23}F_3N_4O$: (%) C 69.59, H 4.63, N 12.11. Found: C 69.65, H 4.67, N 12.18.

***4-(3-(4-(2-fluorophenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 19)**

Appearance: pale brown solid; Yield: 78% (0.34g); IR (KBr) ν_{max}: 3578, 3100, 3042, 1650, 1598, 1544, 1508, 1451, 1320, 1244, 918 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (b, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.0 Hz, 2H), 8.81–7.59 (m, 4H), 8.33–7.26 (m, 3H), 7.22 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 3.61–3.46 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.88, 155.22, 148.40, 141.11, 136.44, 136.32, 134.74, 134.09, 132.67, 131.88, 131.14, 130.64, 129.78, 129.22, 128.84, 128.42, 127.14, 126.60, 126.29, 120.78, 119.12, 118.60, 114.08, 54.34, 53.80. ESI-MS: (m/z) calculated for $C_{28}H_{23}FN_4O$: 450.18, found: 451.24 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}FN_4O$: (%) C 74.65, H 5.15, N 12.44. Found: C 74.71, H 5.18, N 12.50.

***4-(3-(4-(2-chluorophenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 20)**

Appearance: off white solid; Yield: 82% (0.37g); IR (KBr) ν_{max}: 3577, 3105, 3042, 1650, 1598, 1544, 1508, 1451, 1320, 1245, 926 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (b, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.0 Hz, 2H), 8.85–7.58 (m, 4H), 7.46 (d, J = 8.4 Hz, 1H), 7.40–7.34 (m, 2H), 7.24 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 3.62–3.48 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.40, 155.18, 148.75, 141.34, 136.60, 136.28, 134.63, 134.18, 132.38, 131.79, 131.24, 130.44, 130.36, 129.44, 129.36, 128.81, 128.42, 127.14, 126.82, 126.34, 120.04, 115.94, 54.14, 53.88. ESI-MS: (m/z) calculated for $C_{28}H_{23}ClN_4O$: 466.15, found: 467.24 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}ClN_4O$: (%) C 72.02, H 4.96, N 12.00. Found: C 72.09, H 5.02, N 12.06.

***4-(3-(4-(3-chlorophenyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 21)**

Appearance: pale brown white solid; Yield: 80% (0.36g); IR (KBr) ν_{max}: 3582, 3105, 3042, 1650, 1599, 1544, 1508, 1451, 1333, 1248, 928 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (b, 1H), 8.26 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 8.0 Hz, 2H), 8.86–7.45 (m, 5H), 7.34 (s, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 3.63–3.50 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.38, 155.25, 148.72, 142.08, 136.32, 135.41, 134.63, 134.22, 133.04, 132.01, 131.45, 131.24, 130.44, 130.36, 129.44, 127.32, 127.02, 126.40, 125.88, 118.11, 117.12, 115.94, 54.08, 53.91. ESI-MS: (m/z) calculated for $C_{28}H_{23}ClN_4O$: 466.15, found: 467.25 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}ClN_4O$: (%) C 72.02, H 4.96, N 12.00. Found: C 72.08, H 5.01, N 12.04.

***4-(3-(4-(pyridin-4-yl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-olol* (QNP 22)**

Appearance: pale yellow solid; Yield: 75% (0.31g); IR (KBr) ν_{max}: 3581, 3104, 3045, 1650,

1595, 1544, 1501, 1451, 1320, 1254, 918 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (b, 1H), 8.48 (d, J = 8.8 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.0 Hz, 2H), 7.92 (d, J = 8.0 Hz, 2H), 7.84–7.63 (m, 4H), 7.23 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.4 Hz, 1H), 3.62–3.58 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.33, 155.07, 153.25, 152.34, 141.20, 136.48, 134.72, 134.13, 133.38, 131.70, 130.84, 130.40, 129.44, 128.46, 128.01, 127.42, 127.14, 126.82, 126.34, 116.04, 115.94, 54.10, 53.89. ESI-MS: (m/z) calculated for $C_{27}H_{23}N_5O$: 433.19, found: 434.24 $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_5O$: (%) C 74.81, H 5.35, N 16.16. Found: C 74.87, H 5.39, N 16.21.

***4-(3-(4-(pyridin-2-yl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 23)**

Appearance: pale yellow solid; Yield: 82% (0.34g); IR (KBr) ν_{max}: 3567, 3095, 3048, 1650, 1595, 1548, 1504, 1452, 1330, 1245, 923 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (b, 1H), 8.49 (d, J = 8.8 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.85–7.38 (m, 6H), 7.21 (d, J = 8.0 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 3.62–3.58 (t, J = 4.0 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.28, 158.11, 154.82, 150.33, 141.28, 136.35, 134.87, 134.24, 133.65, 131.62, 130.75, 130.41, 129.42, 129.22, 128.48, 128.42, 127.78, 127.14, 125.54, 123.34, 120.33, 116.04, 115.94, 54.10, 53.89. ESI-MS: (m/z) calculated for $C_{27}H_{23}N_5O$: 433.19, found: 434.26 $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_5O$: (%) C 74.81, H 5.35, N 16.16. Found: C 74.86, H 5.39, N 16.22.

***4-(3-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 24)**

Appearance: off white solid; Yield: 84% (0.41g); IR (KBr) ν_{max}: 3579, 3225, 3101, 3048, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 920 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88–10.62 (b, 2H), 8.42 (d, J = 8.8 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.84–7.63 (m, 4H), 7.60 (d, J = 8.0 Hz, 1H), 7.56 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.38–7.28 (m, 2H), 7.14 (d, J = 8.4 Hz, 1H), 3.74–2.65 (t, J = 4.0 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.25, 155.16, 142.64, 141.22, 136.54, 136.22, 134.53, 134.33, 133.82, 132.25, 131.95, 131.24, 130.87, 130.22, 129.62, 129.23, 128.76, 128.44, 127.14, 124.36, 123.44, 121.60, 120.84, 120.11, 117.01, 114.52, 66.24, 54.13, 53.32, 24.88. ESI-MS: (m/z) calculated for $C_{32}H_{29}N_5O$: 499.23, found: 500.15 $(M+H)^+$. Anal. Calcd for $C_{32}H_{29}N_5O$: (%) C 76.93, H 5.85, N 14.02. Found: C 76.99, H 5.89, N 14.08.

***4-(3-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 25)**

Appearance: off white solid; Yield: 85% (0.42g); IR (KBr) ν_{max}: 3572, 3230, 3103, 3049, 1650, 1602, 15445, 1502, 1452, 1330, 1245, 928 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90–10.61 (b, 2H), 8.40 (d, J = 8.8 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 7.6 Hz,

1H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.87–7.65 (m, 4H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.50 (s, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.36–7.29 (m, 2H), 7.15 (d, *J* = 8.4 Hz, 1H), 3.74–2.55 (t, *J* = 4.0 Hz, 12H), 2.01–1.97 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.33, 155.08, 142.45, 141.11, 136.70, 136.12, 134.78, 134.32, 133.77, 132.01, 131.94, 131.20, 130.88, 130.25, 129.44, 129.26, 128.76, 127.44, 126.22, 124.36, 123.44, 121.44, 120.65, 120.14, 117.12, 114.54, 58.23, 54.24, 53.09, 25.11, 24.84. ESI-MS: (m/z) calculated for $C_{33}H_{31}N_5O$: 513.25, found: 514.20 $(M+H)^+$. Anal. Calcd for $C_{33}H_{31}N_5O$: (%) C 77.17, H 6.08, N 13.63. Found: C 77.22, H 6.14, N 13.69.

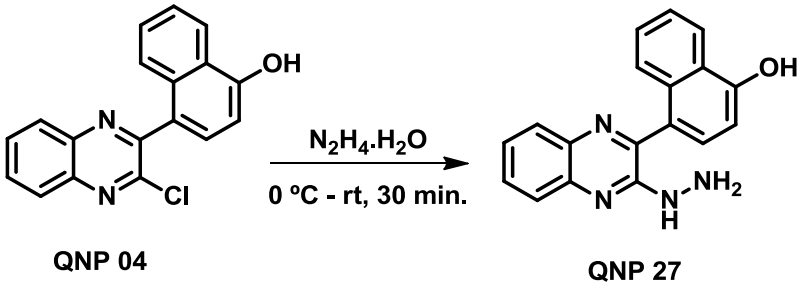
***4-(3-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 26)**

Appearance: off white solid; Yield: 78% (0.40g); IR (KBr) ν_{max}: 3568, 3224, 3103, 3044, 1650, 1601, 1544, 1505, 1452, 1335, 1245, 932 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90–10.62 (b, 2H), 8.40 (d, *J* = 8.8 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.86–7.66 (m, 4H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.50 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.36–7.30 (m, 2H), 7.16 (d, *J* = 8.4 Hz, 1H), 3.76–2.60 (t, *J* = 4.0 Hz, 12H), 1.82–1.68 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.44, 145.82, 142.22, 141.08, 136.72, 136.11, 134.46, 134.11, 133.64, 132.02, 131.87, 131.20, 130.64, 130.22, 129.44, 129.12, 128.75, 127.44, 126.20, 124.36, 123.44, 121.23, 120.55, 120.04, 117.28, 114.12, 58.54, 54.67, 53.44, 45.22, 32.10, 25.52. ESI-MS: (m/z) calculated for $C_{34}H_{33}N_5O$: 527.26, found: 528.30 $(M+H)^+$. Anal. Calcd for $C_{34}H_{33}N_5O$: (%) C 77.39, H 6.30, N 13.27. Found: C 77.45, H 6.34, N 13.31.

4.2.2.3. General experimental procedures utilized for the synthesis of 4-(3-hydrazinylquinoxalin-2-yl)naphthalen-1-ol (QNP 27-64) derivatives

The title compounds were synthesized by following a five step synthetic protocol for **QNP 28-52** (**Figure 4.4)** and six step synthetic protocols for **QNP 53-64** (**Figure 4.4**), wherein the first 3 steps of the reactions are already described. 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol (**QNP 04**) on reaction with hydrazine monohydrate yielded **QNP 27** [Dubey *et al*., 2005] in good yield and it is further reacted with various carbonyl compounds to yield **QNP 28-52** in good to excellent yields (**Figure 4.4).** 12 compounds are obtained through cyclization in the presence of IBD and DCM, **QNP 53-64** final compounds were obtained in moderate to good yields [Viktor 2008; Viktor 2011; Tang *et al*., 2013].

Preparation of 4-(3-hydrazinylquinoxalin-2-yl)naphthalen-1-ol (QNP 27)

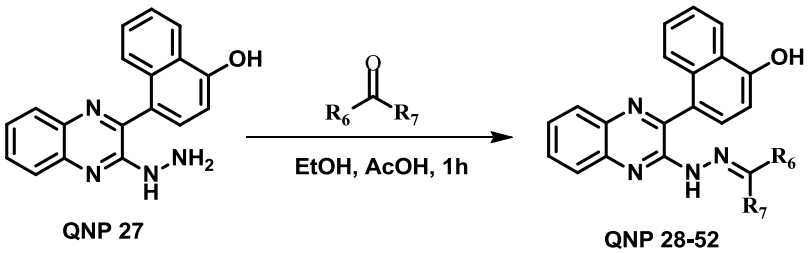


QNP 04 **QNP 27**

To a stirred solution of 4-(3-chloroquinoxalin-2-yl)naphthalen-1-ol (**QNP 04**) (5.0 g, 1.0 equiv.), $N_2H_4.H_2O$ was (10ml) added and stirred at 0 °C for 30 min. Once the reaction is complete, as indicated by TLC, the mixture was quenched with cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was washed with 10% ethyl acetate in hexane two times, to get the 4-(3-hydrazinylquinoxalin-2-yl)naphthalen-1-ol **(QNP 27**) in good yield.

Appearance: plae yellow solid; Yield: 85% (4.18g); IR (KBr) ν_{max}: 3579, 3101, 3045, 1650, 1602, 1545, 1502, 1452, 1333, 1245, 924, 784 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.78–9.54 (b, 2H), 8.34 (d, J = 8.8 Hz, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 8.4 Hz, 3H), 8.01–7.58 (m, 4H), 7.36 (d, J = 8.8 Hz, 1H), 5.68 (b, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.18, 155.88, 141.30, 140.58, 139.64, 138.22, 136.69, 135.02, 134.88, 134.10, 131.14, 130.72, 129.65, 128.18, 126.78, 126.12, 125.40, 116.23. ESI-MS: (m/z) calculated for $C_{18}H_{14}N_4O$: 302.11, found: 303.20 $(M+H)^+$. Anal. Calcd for $C_{18}H_{14}N_4O$: (%) C 71.51, H 4.67, N 18.53. Found: C 71.56, H 4.71, N 18.59.

4.2.2.4. Synthesis of 4-(3-(2-(substituted)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol (QNP 28-52) derivatives and characterization



QNP 27 **QNP 28-52**

A solution of 4-(3-hydrazinylquinoxalin-2-yl)naphthalen-1-ol **(QNP 27**) (0.30 g, 1.0 equiv.) in Ethanol (EtOH) was cooled to 0 °C and then various carbonyl compounds (1.05 equiv.) and catalytic amount of acetic acid were added, stirred at rt for 1h. Once the reaction is

complete, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (60 -100 %) to get the **QNP 28-52** title compounds in yields ranging from 75-95%.

Characterization of the synthesized QNP 28-52 compounds

***4-(3-(2-ethylidenehydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 28)**

A solution of 4-(3-hydrazinylquinoxalin-2-yl)naphthalen-1-ol **(QNP 27**) (0.30 g, 1.0 equiv.) in EtOH was cooled to 0 °C and then acetaldehyde (1.05 equiv.) and catalytic amount of acetic acid were added, stirred at rt for 1h. Once the reaction is complete, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (60-70%)] to achive 0.26g of 4-(3-(2-ethylidenehydrazinyl)quinoxalin-2-yl)naphthalen-1-ol (**QNP 28).**

Appearance: pale yellow solid; Yield: 81% (0.26g); IR (KBr) ν_{max}: 3568, 3220, 3102, 3051, 1650, 1600, 1544, 1502, 1452, 1330, 1245, 928 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.81–10.60 (b, 2H), 8.38 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.08 (m, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.84–7.61 (m, 4H), 7.08 (d, J = 8.4 Hz, 1H), 1.24 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.12, 154.33, 150.98, 140.30, 136.20, 135.71, 135.00, 134.64, 134.01, 133.89, 130.00, 129.64, 128.10, 127.11, 126.62, 126.08, 125.23, 124.75, 116.23, 25.46. ESI-MS: (m/z) calculated for $C_{20}H_{16}N_4O$: 328.13, found: 329.10 $(M+H)^+$. Anal. Calcd for $C_{20}H_{16}N_4O$: (%) C 73.15, H 4.91, N 17.06. Found: C 73.21, H 4.95, N 17.10.

***4-(3-(2-(3-methylbutylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 29)**

Appearance: pale yellow solid; Yield: 75% (0.27g); IR (KBr) ν_{max}: 3565, 3230, 3099, 3052, 1650, 1600, 1545, 1504, 1451, 1330, 1245, 933 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.82–10.60 (b, 2H), 8.40 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 7.6 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.83–7.62 (m, 4H), 7.20 (t, J = 7.2 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 2.52 (d, J = 9.2 Hz, 2H), 2.04–1.99 (m, 1H), 1.27 (d, J = 5.2 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.34, 154.48, 150.88, 140.11, 136.47, 135.44, 135.01, 134.82, 134.12, 133.24, 130.66, 129.45, 128.10, 127.11, 126.62, 126.00, 125.44, 124.48, 115.88,

37.40, 28.12, 24.78. ESI-MS: (m/z) calculated for $C_{23}H_{22}N_4O$: 370.17, found: 370.22 $(M+H)^+$. Anal. Calcd for $C_{23}H_{22}N_4O$: (%) C 74.57, H 5.99, N 15.12. Found: C 74.61, H 6.05, N 15.16.

***4-(3-(2-benzylidenehydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 30)**

Appearance: off white solid; Yield: 80% (0.30g); IR (KBr) ν_{max}: 3568, 3218, 3101, 3048, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 920 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.83–10.61 (b, 2H), 8.33 (d, J = 8.4 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.12 (s, 1H), 8.08 (d, J = 8.0 Hz, 2H), 7.93 (d, J = 8.0 Hz, 2H), 7.83–7.68 (m, 7H), 7.07 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.64, 151.26, 144.74, 140.55, 137.20, 136.67, 135.11, 134.46, 134.10, 133.80, 131.25, 130.70, 129.68, 128.10, 127.68, 127.00, 126.80, 126.44, 126.10, 125.69, 125.08, 124.89, 115.87. ESI-MS: (m/z) calculated for $C_{25}H_{18}N_4O$: 390.14, found: 391.18 $(M+H)^+$. Anal. Calcd for $C_{25}H_{18}N_4O$: (%) C 76.91, H 4.65, N 14.35. Found: C 76.97, H 4.69, N 14.39.

***4-(3-(2-(4-fluorobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 31)**

Appearance: pale yellow solid; Yield: 83% (0.33g); IR (KBr) ν_{max}: 3572, 3222, 3099, 3048, 1650, 1604, 1545, 1504, 1452, 1330, 1245, 933 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.92–10.64 (b, 2H), 8.39 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 7.6 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.10 (s, 1H), 8.02 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.0 Hz, 2H), 7.83–7.39 (m, 6H), 7.08 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.38, 154.22, 144.89, 138.42, 137.11, 136.45, 135.10, 134.62, 134.07, 132.64, 131.33, 130.58, 129.66, 128.22, 127.44, 127.01, 126.80, 126.33, 126.01, 125.25, 124.00, 119.22, 115.90. ESI-MS: (m/z) calculated for $C_{25}H_{17}FN_4O$: 408.13, found: 409.18 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}FN_4O$: (%) C 73.52, H 4.20, N 13.72. Found: C 73.56, H 4.25, N 13.77.

***4-(3-(2-(4-chlorobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 32)**

Appearance: pale yellow solid; Yield: 84% (0.35g); IR (KBr) ν_{max}: 3578, 3225, 3101, 3048, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 922 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.93–10.65 (b, 2H), 8.41 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.16 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.82–7.65 (m, 4H), 7.61 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.85, 150.90, 144.76, 138.40, 137.68, 136.93, 135.82, 134.70, 134.22, 131.88, 130.14, 129.58, 129.04, 128.22, 127.70, 127.11, 126.45, 126.12, 126.00, 125.68, 124.08, 114.63. ESI-MS: (m/z) calculated for $C_{25}H_{17}ClN_4O$: 424.10, found: 425.18 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}ClN_4O$: (%) C 70.67, H 4.03, N 13.19. Found: C 70.73, H 4.09, N 13.23.

***4-(3-(2-(4-bromobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 33)**

Appearance: pale yellow solid; Yield: 80% (0.37g); IR (KBr) ν_{max}: 3580, 3220, 3100, 3048, 1650, 1601, 1544, 1505, 1453, 1330, 1245, 918 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94–10.66 (b, 2H), 8.42 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.14 (s, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.82–7.79 (m, 3H), 7.74 (d, J = 7.6 Hz, 2H), 7.69–7.64 (m, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.23, 150.83, 144.65, 138.48, 137.90, 136.94, 135.80, 134.69, 134.23, 131.20, 130.33, 129.67, 129.11, 128.20, 127.45, 127.10, 126.48, 126.10, 126.23, 125.60, 124.41, 114.89. ESI-MS: (m/z) calculated for $C_{25}H_{17}BrN_4O$: 468.05, found: 469.16 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}BrN_4O$: (%) C 63.98, H 3.65, N 11.94. Found: C 64.04, H 3.69, N 11.99.

***4-(3-(2-(4-nitrobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 34)**

Appearance: yellow solid; Yield: 82% (0.35g); IR (KBr) ν_{max}: 3581, 3234, 3111, 3049, 1651, 1598, 1544, 1505, 1452, 1330, 1248, 930 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78–10.54 (b, 2H), 8.36 (d, J = 8.4 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 8.0 Hz, 2H), 8.11 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.81–7.62 (m, 4H), 7.04 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.88, 152.04, 150.46, 145.30, 140.58, 139.64, 137.22, 136.68, 135.02, 134.88, 134.10, 133.82, 131.14, 130.72, 129.65, 128.18, 126.78,126.08, 126.12, 125.40, 125.12, 124.88, 116.23. ESI-MS: (m/z) calculated for $C_{25}H_{17}N_5O_3$: 435.13, found: 436.18 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}N_5O_3$: (%) C 68.96, H 3.94, N 16.08. Found: C 69.02, H 3.99, N 16.13.

***4-(3-(2-(4-hydroxybenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 35)**

Appearance: pale brown solid; Yield: 78% (0.31g); IR (KBr) ν_{max}: 3572, 3230, 3141, 3045, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 920 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12–10.68 (b, 3H), 8.36 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 7.6 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.13 (s, 1H), 8.06 (d, J = 7.6 Hz, 2H), 7.94 (d, J = 8.0 Hz, 2H), 7.82–7.68 (m, 6H), 7.18 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.36, 154.78, 150.21, 144.68, 138.23, 137.54, 136.21, 135.78, 134.52, 134.11, 131.25, 130.08, 129.68, 129.00, 128.40, 127.48, 127.12, 126.77, 126.35, 126.07, 124.68, 119.24, 114.76. ESI-MS: (m/z) calculated for $C_{25}H_{18}N_4O_2$: 406.14, found: 407.18 $(M+H)^+$. Anal. Calcd for $C_{25}H_{18}N_4O_2$: (%) C 73.88, H 4.46, N 13.78. Found: C 73.93, H 4.50, N 13.82.

***4-(3-(2-(4-methoxybenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-oll* (QNP 36)**

Appearance: pale brown solid; Yield: 80% (0.33g); IR (KBr) ν_{max}: 3581, 3225, 3101, 3048, 1650, 1599, 1545, 1508, 1454, 1328, 1244, 928 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ

11.89–10.67 (b, 2H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 2H), 7.81–7.63 (m, 6H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.12, 154.65, 150.08, 144.63, 138.44, 137.23, 136.22, 135.80, 134.55, 134.10, 131.68, 130.35, 129.22, 129.01, 128.56, 127.77, 127.09, 126.89, 126.22, 126.00, 124.58, 118.11, 115.11, 59.68. ESI-MS: (m/z) calculated for $C_{26}H_{20}N_4O_2$: 420.15, found: 421.18 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_4O_2$: (%) C 74.27, H 4.79, N 13.33. Found: C 74.33, H 4.84, N 13.39.

***4-(3-(2-(4-(dimethylamino)benzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 37)**

Appearance: off white solid; Yield: 75% (0.32g); IR (KBr) ν_{max}: 3588, 3233, 3103, 3049, 1648, 1602, 1544, 1507, 1452, 1325, 1240, 922 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92–10.68 (b, 2H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 8.12 (s, 1H), 7.94 (d, *J* = 8.0 Hz, 3H), 7.86–7.66 (m, 4H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 1H), 3.18 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.90, 154.68, 153.99, 144.60, 138.12, 137.44, 136.89, 135.88, 134.71, 134.22, 131.74, 131.02, 129.34, 129.00, 128.87, 127.65, 127.12, 126.88, 126.34, 126.01, 124.67, 118.00, 114.98, 36.70. ESI-MS: (m/z) calculated for $C_{27}H_{23}N_5O$: 433.19, found: 434.24 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_4O_2$: (%) C 74.81, H 5.35, N 16.16. Found: C 74.87, H 5.39, N 16.22.

***4-(3-(2-(2-fluorobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 38)**

Appearance: pale yellow solid; Yield: 79% (0.32g); IR (KBr) ν_{max}: 3579, 3225, 3101, 3048, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 934 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94–10.68 (b, 2H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 8.17 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 4H), 7.81–7.36 (m, 7H), 7.14 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.82, 161.64, 154.11, 144.62, 138.40, 137.08, 136.41, 135.22, 134.78, 134.00, 132.33, 131.45, 130.54, 129.60, 128.28, 127.41, 127.22, 126.83, 126.32, 126.22, 125.40, 124.33, 123.88, 119.76, 115.68. ESI-MS: (m/z) calculated for $C_{25}H_{17}FN_4O$: 408.13, found: 409.20 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}FN_4O$: (%) C 73.52, H 4.20, N 13.72. Found: C 73.58, H 4.26, N 13.78.

***4-(3-(2-(3-fluorobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 39)**

Appearance: off white solid; Yield: 78% (0.31g); IR (KBr) ν_{max}: 3581, 3235, 3104, 3052, 1651, 1601, 1544, 1504, 1453, 1335, 1245, 933 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93–10.66 (b, 2H), 8.36 (d, *J* = 8.0 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.18 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 4H), 7.83–7.44 (m, 7H), 7.19 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-

d_6) δ 164.64, 162.59, 154.20, 144.60, 138.23, 137.20, 136.80, 135.11, 134.65, 134.12, 132.09, 131.48, 130.68, 129.22, 128.44, 127.39, 127.31, 126.80, 126.62, 126.31, 125.66, 124.06, 123.67, 119.70, 115.54. ESI-MS: (m/z) calculated for $C_{25}H_{17}FN_4O$: 408.13, found: 409.19 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}FN_4O$: (%) C 73.52, H 4.20, N 13.72. Found: C 73.57, H 4.25, N 13.77.

***4-(3-(2-(3-nitrobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 40)**

Appearance: pale yellow solid; Yield: 89% (0.38g); IR (KBr) ν_{max}: 3580, 3240, 3101, 3045, 1648, 1595, 1551, 1502, 1450, 1330, 1245, 925 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.81–10.60 (b, 2H), 8.38 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 2H), 8.12 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.83–7.62 (m, 5H), 7.12 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.24, 151.94, 150.52, 144.86, 140.23, 139.11, 137.29, 136.07, 135.72, 135.12, 134.88, 134.02, 133.82, 131.25, 130.18, 129.61, 129.05, 128.18, 126.78, 126.44, 126.12, 125.40, 125.12, 124.62, 116.17. ESI-MS: (m/z) calculated for $C_{25}H_{17}N_5O_3$: 435.13, found: 436.10 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}N_5O_3$: (%) C 68.96, H 3.94, N 16.08. Found: C 69.01, H 3.98, N 16.14.

***4-(3-(2-(3-(trifluoromethyl)benzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 41)**

Appearance: pale yellow solid; Yield: 85% (0.38g); IR (KBr) ν_{max}: 3584, 3228, 3105, 3048, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 930 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.75–10.59 (b, 2H), 8.34 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.08–8.02 (s, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.4 Hz, 1H), 7.80–7.63 (m, 6H), 7.10 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.68, 151.45, 150.72, 143.99, 140.11, 138.02, 136.55, 136.12, 135.33, 135.11, 134.58, 134.12, 133.66, 131.22, 130.56, 129.25, 129.00, 128.47, 127.22, 126.78, 126.08, 125.40, 125.22, 124.38, 114.04. ESI-MS: (m/z) calculated for $C_{26}H_{17}F_3N_4O$: 458.13, found: 456.20 $(M+H)^+$. Anal. Calcd for $C_{26}H_{17}F_3N_4O$: (%) C 68.12, H 3.74, N 12.22. Found: C 68.19, H 3.78, N 12.27.

***4-(3-(2-(2,3-dichlorobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 42)**

Appearance: off white solid; Yield: 90% (0.41g); IR (KBr) ν_{max}: 3565, 3225, 3101, 3048, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 922, 864 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.84–10.65 (b, 2H), 8.42 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.15 (s, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.86–7.52 (m, 7H), 7.12 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.88, 151.22, 144.90, 143.72, 138.42, 137.62, 136.54, 135.88, 135.12, 134.66, 134.35, 133.08, 131.80, 130.23, 129.58, 129.22, 127.58, 127.14,

126.48, 126.24, 126.10, 125.88, 125.28, 124.14, 116.64. ESI-MS: (m/z) calculated for $C_{25}H_{16}Cl_2N_4O$: 458.07, found: 459.14 $(M+H)^+$. Anal. Calcd for $C_{25}H_{16}Cl_2N_4O$: (%) C 65.37, H 3.51, N 12.20. Found: C 65.42, H 3.55, N 12.26.

***4-(3-(2-(2,4-dichlorobenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 43)**

Appearance: off white solid; Yield: 95% (0.43g); IR (KBr) ν_{max}: 3560, 3235, 3101, 3048, 1650, 1599, 1544, 1500, 1452, 1333, 1245, 925 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.86–10.64 (b, 2H), 8.45 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 7.6 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 7.6 Hz, 2H), 7.92 (s, 1H), 7.88–7.64 (m, 4H), 7.58 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.91, 150.78, 144.44, 138.53, 137.38, 136.68, 135.82, 135.22, 134.70, 134.22, 133.12, 132.08, 131.54, 130.24, 129.66, 128.11, 127.59, 127.22, 126.64, 126.16, 126.05, 125.90, 125.30, 124.07, 117.01. ESI-MS: (m/z) calculated for $C_{25}H_{16}Cl_2N_4O$: 458.07, found: 459.16 $(M+H)^+$. Anal. Calcd for $C_{25}H_{16}Cl_2N_4O$: (%) C 65.37, H 3.51, N 12.20. Found: C 65.41, H 3.56, N 12.27.

***4-(3-(2-(3,4-dimethoxybenzylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 44)**

Appearance: pale brown solid; Yield: 81% (0.36g); IR (KBr) ν_{max}: 3574, 3234, 3100, 3048, 1650, 1601, 1544, 1452, 1332, 1245, 925 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.88–10.66 (b, 2H), 8.42 (d, J = 8.0 Hz, 1H), 8.24 (d, J = 7.2 Hz, 1H), 8.17 (d, J = 7.6 Hz, 1H), 8.09 (s, 1H), 7.94 (d, J = 7.6 Hz, 3H), 7.86–7.65 (m, 5H), 7.61 (d, J = 7.6 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 3.63 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.24, 154.56, 151.08, 150.36, 144.50, 138.11, 137.25, 136.62, 135.39, 135.11, 134.33, 134.07, 133.64, 132.02, 130.33, 129.27, 128.30, 127.61, 127.34, 126.28, 126.08, 125.63, 125.11, 124.28, 116.25, 64. 45. ESI-MS: (m/z) calculated for $C_{27}H_{22}N_4O_3$: 450.16, found: 451.22 $(M+H)^+$. Anal. Calcd for $C_{27}H_{22}N_4O_3$: (%) C 71.99, H 4.92, N 12.44. Found: C 72.06, H 4.96, N 12.49.

***4-(3-(2-(pyridin-2-ylmethylene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 45)**

Appearance: pale yellow solid; Yield: 78% (0.30g); IR (KBr) ν_{max}: 3582, 3225, 3102, 3055, 1650, 1601, 1544, 1505, 1452, 1333, 1245, 925 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.80–10.25 (b, 2H), 8.62 (d, J = 8.8 Hz, 1H), 8.40 (d, J = 8.0 Hz, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 8.0 Hz, 4H), 7.93–7.68 (m, 6H), 7.33 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.26, 154.08, 152.64, 151.25, 144.22, 138.36, 137.89, 136.26, 135.12, 134.65, 134.23, 132.12, 131.53, 130.08, 129.38, 128.39, 127.72, 127.38, 126.54, 126.11, 125.56, 124.65, 123.82, 121.76, 115.65. ESI-MS: (m/z) calculated for

$C_{24}H_{17}N_5O$: 391.14, found: 392.21 $(M+H)^+$. Anal. Calcd for $C_{24}H_{17}N_5O$: (%) C 73.64, H 4.38, N 17.89. Found: C 73.69, H 4.44, N 17.95.

***4-(3-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 46)**

Appearance: pale yellow solid; Yield: 79% (0.31g); IR (KBr) ν_{max}: 3582, 3225, 3103, 3045, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 924 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.81–10.22 (b, 2H), 8.72 (d, J = 8.8 Hz, 2H), 8.41 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H), 8.04 (d, J = 8.0 Hz, 3H), 7.96 (d, J = 8.0 Hz, 2H),7.94–7.68 (m, 4H), 7.19 (d, J = 8.4 Hz, 1H), 3.12 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.27, 155.36, 154.22, 150.78, 139.79, 137.44, 136.11, 135.05, 134.75, 134.29, 132.12, 131.53, 130.08, 129.38, 128.39, 127.90, 127.38, 126.54, 125.48, 124.77, 124.40, 114.94, 25.88. ESI-MS: (m/z) calculated for $C_{25}H_{19}N_5O$: 405.15, found: 406.27 $(M+H)^+$. Anal. Calcd for $C_{25}H_{19}N_5O$: (%) C 74.06, H 4.72, N 17.27. Found: C 74.12, H 4.76, N 17.33.

***4-(3-(2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 47)**

Appearance: pale yellow solid; Yield: 77% (0.30g); IR (KBr) ν_{max}: 3582, 3224, 3101, 3048, 1650, 1604, 1545, 1504, 1452, 1332, 1245, 934 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.83–10.20 (b, 2H), 8.64 (d, J = 8.8 Hz, 1H), 8.41 (d, J = 8.0 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 7.6 Hz, 3H), 7.94–7.68 (m, 6H), 7.16 (d, J = 8.4 Hz, 1H), 3.16 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.88, 156.25, 155.37, 151.71, 146.22, 138.39, 137.88, 136.22, 135.10, 134.33, 134.06, 132.77, 131.14, 130.71, 129.45, 128.54, 127.44, 127.38, 126.11, 125.56, 124.65, 123.80, 121.64, 115.60, 24.08. ESI-MS: (m/z) calculated for $C_{25}H_{19}N_5O$: 405.15, found: 406.29 $(M+H)^+$. Anal. Calcd for $C_{25}H_{19}N_5O$: (%) C 74.06, H 4.72, N 17.27. Found: C 74.12, H 4.75, N 17.34.

***4-(3-(2-((1H-pyrrol-2-yl)methylene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 48)**

Appearance: off white solid; Yield: 82% (0.30g); IR (KBr) ν_{max}: 3582, 3233, 3108, 3054, 1650, 1605, 1545, 1505, 1452, 1330, 1245, 928 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.62–10.21 (b, 3H), 8.38 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 8.0 Hz, 3H), 7.92–7.64 (m, 4H), 7.32 (s, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.02, (d, J = 8.0 Hz, 1H), 6.91 (d, J = 7.6 Hz, 1H), 6.84–6.79 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.12, 144.30, 138.62, 137.88, 136.22, 135.34, 134.62, 134.09, 132.45, 131.28, 130.18, 129.23, 128.00, 127.70, 127.36, 126.76, 126.21, 125.55, 124.68, 123.88, 122.76, 120.42, 114.69. ESI-MS: (m/z) calculated for $C_{23}H_{17}N_5O$: 379.14, found: 380.21 $(M+H)^+$. Anal. Calcd for $C_{23}H_{17}N_5O$: (%) C 72.81, H 4.52, N 18.46. Found: C 72.85, H 4.56, N 18.52.

***4-(3-(2-(thiophen-2-ylmethylene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 49)**

Appearance: pale brown solid; Yield: 85% (0.33g); IR (KBr) ν_{max}: 3581, 3224, 3101, 3050, 1648, 1604, 1545, 1508, 1450, 1338, 1240, 934 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78–10.34 (b, 2H), 8.39 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.15 (s, 1H), 8.00 (d, J = 7.6 Hz, 2H), 7.88–7.38 (m, 7H), 7.18 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.10, 155.62, 144.24, 138.33, 137.45, 136.18, 135.57, 134.34, 134.12, 132.33, 131.12, 130.24, 129.78, 129.14, 128.02, 127.45, 127.08, 126.88, 126.05, 125.63, 124.71, 124.90, 114.70. ESI-MS: (m/z) calculated for $C_{23}H_{16}N_4OS$: 396.10, found: 397.18 $(M+H)^+$. Anal. Calcd for $C_{23}H_{16}N_4OS$: (%) C 69.68, H 4.07, N 14.13. Found: C 69.73, H 4.12, N 14.19.

***4-(3-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 50)**

Appearance: pale yellow solid; Yield: 90% (0.37g); IR (KBr) ν_{max}: 3585, 3225, 3101, 3048, 1650, 1598, 1544, 1504, 1452, 1333, 1245, 930 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81–10.60 (b, 2H), 8.63 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 7.6 Hz, 2H), 8.12 (s, 1H), 7.92 (d, J = 7.6 Hz, 2H), 7.82–7.65 (m, 4H), 7.32 (d, J = 8.4 Hz, 1H). 7.12 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.36, 155.97, 152.32, 151.24, 138.12, 137.40, 136.19, 135.36, 134.78, 134.25, 132.62, 131.04, 130.27, 129.23, 128.56, 127.71, 127.00, 126.15, 126.00, 125.01, 124.72, 119.25, 114.23. ESI-MS: (m/z) calculated for $C_{23}H_{15}N_5O_3S$: 425.11, found: 426.19 $(M+H)^+$. Anal. Calcd for $C_{23}H_{15}N_5O_3S$: (%) C 64.94, H 3.55, N 16.46. Found: C 64.99, H 3.59, N 16.52.

***4-(3-(2-(9H-fluoren-9-ylidene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 51)**

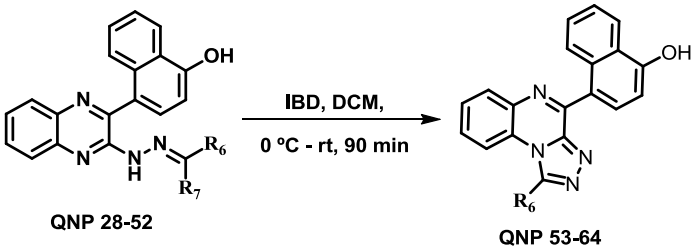
Appearance: off white solid; Yield: 78% (0.35g); IR (KBr) ν_{max}: 3590, 3234, 3105, 3048, 1645, 1604, 1544, 1498, 1452, 1330, 1245, 932 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96–10.72 (b, 2H), 8.67 (d, J = 8.4 Hz, 1H), 8.42 (d, J = 7.6 Hz, 1H), 8.38 (d, J = 7.6 Hz, 1H), 8.29 (d, J = 7.6 Hz, 1H), 8.14 (d, J = 7.6 Hz, 1H), 8.08 (d, J = 8.0 Hz, 2H), 7.95 (d, J = 7.6 Hz, 2H), 7.90–7.64 (m, 8H), 7.10 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.98, 155.72, 145.33, 138.45, 137.47, 136.10, 135.01, 134.25, 134.12, 132.35, 131.47, 130.25, 129.24, 128.87, 128.44, 127.77, 127.12, 126.72, 126.13, 125.45, 124.72, 124.12, 122.09, 116.11. ESI-MS: (m/z) calculated for $C_{31}H_{20}N_4O$: 464.16, found: 465.24 $(M+H)^+$. Anal. Calcd for $C_{31}H_{20}N_4O$: (%) C 80.15, H 4.34, N 12.06. Found: C 80.22, H 4.39, N 12.09.

***4-(3-(2-((1H-indol-3-yl)methylene)hydrazinyl)quinoxalin-2-yl)naphthalen-1-ol* (QNP 52)**

Appearance: off white solid; Yield: 75% (0.31g); IR (KBr) ν_{max}: 3580, 3235, 3102, 3055, 1650, 1601, 1544, 1505, 1452, 1330, 1245, 925 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ

10.91–10.70 (b, 2H), 8.59 (d, *J* = 8.4 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.15 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.88–7.62 (m, 5H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.22–7.18 (m, 2H), 7.12 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.98, 155.72, 145.33, 138.45, 137.47, 136.10, 135.01, 134.25, 134.12, 132.35, 131.47, 130.25, 129.24, 128.87, 128.44, 127.77, 127.12, 126.72, 126.13, 125.45, 124.72, 124.12, 122.09, 116.11. ESI-MS: (m/z) calculated for $C_{27}H_{19}N_5O$: 429.15, found: 430.22 $(M+H)^+$. Anal. Calcd for $C_{27}H_{19}N_5O$: (%) C 75.51, H 4.46, N 16.31. Found: C 75.57, H 4.50, N 16.38.

4.2.2.5. Synthesis of 4-(1-(substituted)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)naphthalen-1-ol (QNP 53-64) derivatives and characterization



QNP 28-52 QNP 53-64

A solution of **QNP 28-52** (amongst only 12 compounds used for cyclization) (0.20 g, 1.0 equiv.) in DCM was cooled to 0 °C and then iodobenzene diacetate (IBD) was added at inert atmosphere to above mixture, stirred at rt for 90 min. Once the reaction is complete, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (70 -90 %) to get the **QNP 53-64** title compounds in yields ranging from 60-85%.

Characterization of the synthesized QNP 53-64 title compounds

***4-(1-isobutyl-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 53)**

A solution of 4-(-3-(3-methylbutylidene)hydrazono)-3,4-dihydroquinoxalin-2-yl)naphthalen-1-ol (**QNP 28**) 0.20 g (1.0 equiv.) in DCM was cooled to 0 °C and then iodobenzene diacetate IBD was added at inert atmosphere to above mixture, stirred at rt for 90 min. Once the reaction is complete, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layer was collected, washed with saturated brine solution,

dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (70 -80 %) to achieve 0.14g of 4-(1-isobutyl-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl)naphthalen-1-ol (**QNP 53**).

Appearance: pale brown solid; Yield: 73% (0.14g); IR (KBr) ν_{max}: 3582, 3101, 3048, 1650, 1600, 1543, 1504, 1451, 1333, 1248, 935 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.88 (b, 1H), 8.42 (d, J = 8.0 Hz, 1H), 8.24 (d, J = 7.6 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.86–7.63 (m, 5H), 7.12 (d, J = 8.4 Hz, 1H), 2.68 (d, J = 9.2 Hz, 2H), 2.10–2.06 (m, 1H), 1.21 (d, J = 2.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.34, 154.33, 152.90, 145.47, 142.62, 141.44, 135.22, 134.28, 134.05, 133.45, 130.41, 129.48, 128.18, 127.52, 126.60, 126.12, 125.27, 118.11, 115.08, 39.78, 32.40, 26.01. ESI-MS: (m/z) calculated for $C_{23}H_{20}N_4O$: 368.16, found: 369.24 $(M+H)^+$. Anal. Calcd for $C_{23}H_{20}N_4O$: (%) C 74.98, H 5.47, N 15.21. Found: C 75.04, H 5.53, N 15.25.

***4-(1-(4-fluorophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 54)**

Appearance: pale brown solid; Yield: 80% (0.15g); IR (KBr) ν_{max}: 3582, 3101, 3049, 1650, 1604, 1555, 1505, 1454, 1330, 1254, 932,780 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.90 (b, 1H), 8.48 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 7.6 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.82–7.36 (m, 7H), 7.11 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.55, 154.74, 153.24, 151.78, 144.88, 143.33, 135.10, 134.48, 133.22, 132.62, 132.07, 131.63, 130.33, 130.08, 129.66, 128.22, 127.44, 126.84, 126.23, 124.04, 118.22, 115.42. ESI-MS: (m/z) calculated for $C_{25}H_{15}FN_4O$: 406.12, found: 407.20 $(M+H)^+$. Anal. Calcd for $C_{25}H_{15}FN_4O$: (%) C 73.88, H 3.72, N 13.79. Found: C 73.94, H 3.76, N 13.85.

***4-(1-(4-chlorophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 55)**

Appearance: off white solid; Yield: 85% (0.16g); IR (KBr) ν_{max}: 3582, 3101, 3048, 1650, 1600, 1545, 1504, 1451, 1330, 1248, 935, 845 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.93 (b, 1H), 8.48 (d, J = 8.0 Hz, 1H), 8.30 (d, J = 7.6 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 7.6 Hz, 2H), 7.94 (d, J = 8.0 Hz, 1H), 7.84–7.63 (m, 7H), 7.14 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.98, 153.44, 151.90, 144.72, 143.40, 135.68, 134.93, 134.34, 133.70, 133.22, 131.47, 130.14, 129.66, 129.12, 128.20, 127.78, 126.45, 126.12, 124.68, 120.08, 114.63. ESI-MS: (m/z) calculated for $C_{25}H_{15}ClN_4O$: 422.09, found: 423.17 $(M+H)^+$. Anal. Calcd for $C_{25}H_{15}ClN_4O$: (%) C 71.01, H 3.58, N 13.25. Found: Elemental Analysis: C 71.08, H 3.61, N 13.29.

***4-(1-(4-bromophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 56)**

Appearance: pale brown solid; Yield: 82% (0.16g); IR (KBr) ν_{max}: 3580, 3100, 3048, 1648, 1604, 1543, 1504, 1451, 1333, 1248, 932, 708 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (b, 1H), 8.48 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 7.81–7.58 (m, 5H), 7.14 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.88, 153.25, 151.22, 144.64, 142.20, 135.66, 134.61, 134.62, 131.09, 130.52, 129.55, 129.02, 128.63, 127.48, 127.10, 126.48, 126.10, 125.60, 125.11, 124.41, 118.52, 114.63. ESI-MS: (m/z) calculated for $C_{25}H_{15}BrN_4O$: 466.04, found: 467.15 $(M+H)^+$. Anal. Calcd for $C_{25}H_{15}BrN_4O$: (%) C 64.25, H 3.24, N 11.99. Found: C 64.29, H 3.31, N 12.06.

***4-(1-(4-nitrophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 57)**

Appearance: yellow solid; Yield: 82% (0.16g); IR (KBr) ν_{max}: 3580, 3099, 3045, 1650, 1600, 1543, 1508, 1452, 1332, 1248, 933 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (b, 1H), 8.54 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.0 Hz, 2H), 8.10 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.0 Hz, 1H), 7.87–7.63 (m, 5H), 7.12 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.62, 153.23, 151.46, 149.10, 145.58, 143.64, 141.28, 135.22, 134.80, 134.15, 133.80, 131.23, 130.80, 129.60, 128.22, 126.80,126.12, 126.00, 125.44, 124.12, 118.72, 116.24. ESI-MS: (m/z) calculated for $C_{25}H_{15}N_5O_3$: 433.11, found: 434.16 $(M+H)^+$. Anal. Calcd for $C_{25}H_{15}N_5O_3$: (%) C 69.28, H 3.49, N 16.16. Found: C 69.33, H 3.53, N 16.22.

***4-(1-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 58)**

Appearance: pale brown solid; Yield: 70% (0.13g); IR (KBr) ν_{max}: 3585, 3104, 3054, 1650, 1605, 1545, 1505, 1450, 1330, 1248, 930 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (b, 1H), 8.43 (d, J = 8.0 Hz, 1H), 8.28 (d, J = 7.6 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.0 Hz, 1H), 7.81–7.63 (m, 5H), 7.29 (d, J = 7.2 Hz, 2H), 7.14 (d, J = 8.4 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.12, 154.90, 143.33, 152.08, 144.56, 143.11, 134.23, 133.22, 131.62, 130.33, 129.20, 128.92, 128.56, 127.66, 127.12, 126.89, 125.22, 124.14, 120.58, 118.08, 115.40, 59.23. ESI-MS: (m/z) calculated for $C_{26}H_{18}N_4O_2$: 418.14, found: 419.22 $(M+H)^+$. Anal. Calcd for $C_{26}H_{18}N_4O_2$: (%) C 74.63, H 4.34, N 13.39. Found C 74.69, H 4.84, N 13.44.

***4-(1-(3-fluorophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 59)**

Appearance: pale brown solid; Yield: 75% (0.14g); IR (KBr) ν_{max}: 3582, 3104, 3048, 1650, 1600, 1543, 1504, 1451, 1328, 1248, 933 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (b, 1H), 8.41 (d, J = 8.0 Hz, 1H), 8.29 (d, J = 7.6 Hz, 1H), 8.20 (d, J = 7.6 Hz, 1H), 8.13 (d, J =

8.0 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.84–7.39 (m, 8H), 7.16 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.68, 154.29, 153.22, 151.07, 144.60, 143.23, 134.89, 133.56, 133.11, 132.67, 131.80, 131.09, 130.48, 130.10, 129.22, 128.44, 127.39, 126.80, 126.22, 125.63, 124.06, 123.67, 119.70, 115.54. ESI-MS: (m/z) calculated for $C_{25}H_{15}FN_4O$: 406.12, found: 407.22 $(M+H)^+$. Anal. Calcd for $C_{25}H_{15}FN_4O$: (%) C 73.92, H 3.77, N 13.84. Found: C 73.96, H 3.82, N 13.89.

***4-(1-(3-nitrophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 60)**

Appearance: pale yellow solid; Yield: 85% (0.16g); IR (KBr) ν_{max}: 3578, 3106, 3048, 1650, 1604, 1545, 1500, 1455, 1330, 1246, 928 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (b, 1H), 8.52 (d, *J* = 8.4 Hz, 1H), 8.34 (d, *J* = 7.6 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.89–7.63 (m, 6H), 7.13 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.68, 152.94, 151.44, 149.88, 143.23, 141.22, 135.59, 135.07, 134.72, 134.12, 133.88, 133.02, 132.82, 131.33, 130.44, 129.21, 129.00, 128.41, 126.44, 126.40, 125.11, 124.65, 119.78, 115.28. ESI-MS: (m/z) calculated for $C_{25}H_{15}N_5O_3$: 433.11, found: 434.18 $(M+H)^+$. Anal. Calcd for $C_{25}H_{15}N_5O_3$: (%) C 69.28, H 3.49, N 16.16. Found: C 69.33, H 3.53, N 16.22.

***4-(1-(2,3-dichlorophenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 61)**

Appearance: off white solid; Yield: 78% (0.15g); IR (KBr) ν_{max}: 3580, 3103, 3048, 1650, 1600, 1543, 1504, 1454, 1330, 1258, 932, 835 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (b, 1H), 8.63 (d, *J* = 8.0 Hz, 1H), 8.36 (d, *J* = 7.6 Hz, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.84–7.52 (m, 7H), 7.12 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.94, 153.22, 151.90, 145.72, 143.42, 141.69, 135.54, 133.88, 133.12, 132.66, 132.10, 131.28, 131.04, 130.43, 129.81, 129.12, 127.48, 127.11, 126.42, 126.21, 125.14, 124.88, 119.14, 116.23. ESI-MS: (m/z) calculated for $C_{25}H_{14}Cl_2N_4O$: 456.05, found: 457.12 $(M+H)^+$. Anal. Calcd for $C_{25}H_{14}Cl_2N_4O$: (%) C 65.42, H 3.55, N 12.26. Found: C 65.49, H 3.59, N 12.31.

***4-(1-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 62)**

Appearance: pale brown solid; Yield: 79% (0.15g); IR (KBr) ν_{max}: 3580, 3101, 3048, 1650, 1600, 1543, 1504, 1451, 1330, 1248, 932 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (b, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.33 (d, *J* = 7.6 Hz, 1H), 8.21 (d, *J* = 7.6 Hz, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.82–7.64 (m, 5H), 7.32 (s, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 3.76 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.86,

153.56, 151.22, 1501.07, 145.63, 143.29, 134.43, 132.28, 131.64, 131.02, 130.62, 129.27, 128.34, 127.62, 127.22, 126.64, 126.12, 125.64, 125.10, 124.27, 119.48, 117.22, 116.88, 115.25, 63.38. ESI-MS: (m/z) calculated for $C_{27}H_{20}N_4O_3$: 448.15, found: 449.18 $(M+H)^+$. Anal. Calcd for $C_{27}H_{20}N_4O_3$: (%) C 72.31, H 4.50, N 12.49. Found: Elemental Analysis: C 72.37, H 4.55, N 12.55.

***4-(1-(1H-pyrrol-2-yl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 63)**

Appearance: pale brown solid; Yield: 75% (0.14g); IR (KBr) ν_{max}: 3580, 3105, 3058, 1650, 1600, 1544, 1501, 1450, 1330, 1258, 923 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.63–10.82 (b, 2H), 8.44 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 8.0 Hz, 3H), 7.93 (d, J = 7.6 Hz, 3H), 7.88–7.59 (m, 5H), 7.14 (d, J = 8.4 Hz, 1H), 7.02, (d, J = 8.0 Hz, 1H), 6.88 (d, J = 7.6 Hz, 1H), 6.78–6.74 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.38, 151.65, 144.78, 143.62, 142.89, 134.69, 134.11, 132.36, 131.45, 130.22, 129.45, 128.28, 127.05, 126.76, 125.55, 124.68, 123.27, 122.87, 121.42, 118.12, 115.69, 113.41. ESI-MS: (m/z) calculated for $C_{23}H_{15}N_5O$: 377.12, found: 378.18 $(M+H)^+$. Anal. Calcd for $C_{23}H_{15}N_5O$: (%) C 73.20, H 4.01, N 18.56. Found: C 73.20, H 4.01, N 18.56.

***4-(1-(thiophen-2-yl)-[1,2,4]triazolo[4,3-a]quinoxalin-4-yl)naphthalen-1-ol* (QNP 64)**

Appearance: pale brown solid; Yield: 77% (0.15g); IR (KBr) ν_{max}: 3582, 3104, 3045, 1655, 1602, 1543, 1504, 1451, 1328, 1255, 934 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.86 (b, 2H), 8.48 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 7.2 Hz, 1H), 7.87–7.36 (m, 6H), 7.16 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.46, 152.89, 151.42, 144.04, 143.33, 142.87.45, 134.67, 134.11, 132.48, 131.04, 130.27, 129.62, 129.11, 128.31, 127.35, 127.14, 126.88, 126.11, 125.54, 124.24, 119.64, 116.12. ESI-MS: (m/z) calculated for $C_{23}H_{14}N_4OS$: 394.04, found: 395.14 $(M+H)^+$. Anal. Calcd for $C_{23}H_{14}N_4OS$: (%) C 70.03, H 3.58, N 14.20. Found: C 70.09, H 3.52, N 14.26.

4.2.3. 6-(piperazin-1-yl)phenanthridine (Scheme 3) derivatives

Scheme 3 methodology involves following stages, cardinal synthesis of 6-(piperazin-1-yl)phenanthridine (**PPA 05**). (4-(phenanthridin-6-yl)piperazin-1-yl) methanone derivatives (**PPA 06-24**) and 6-(4-(substitutedsulfonyl)piperazin-1-yl)phenanthridine analogoues (**PPA 25-38**). Details of scheme is presented in **Figure 4.5.**

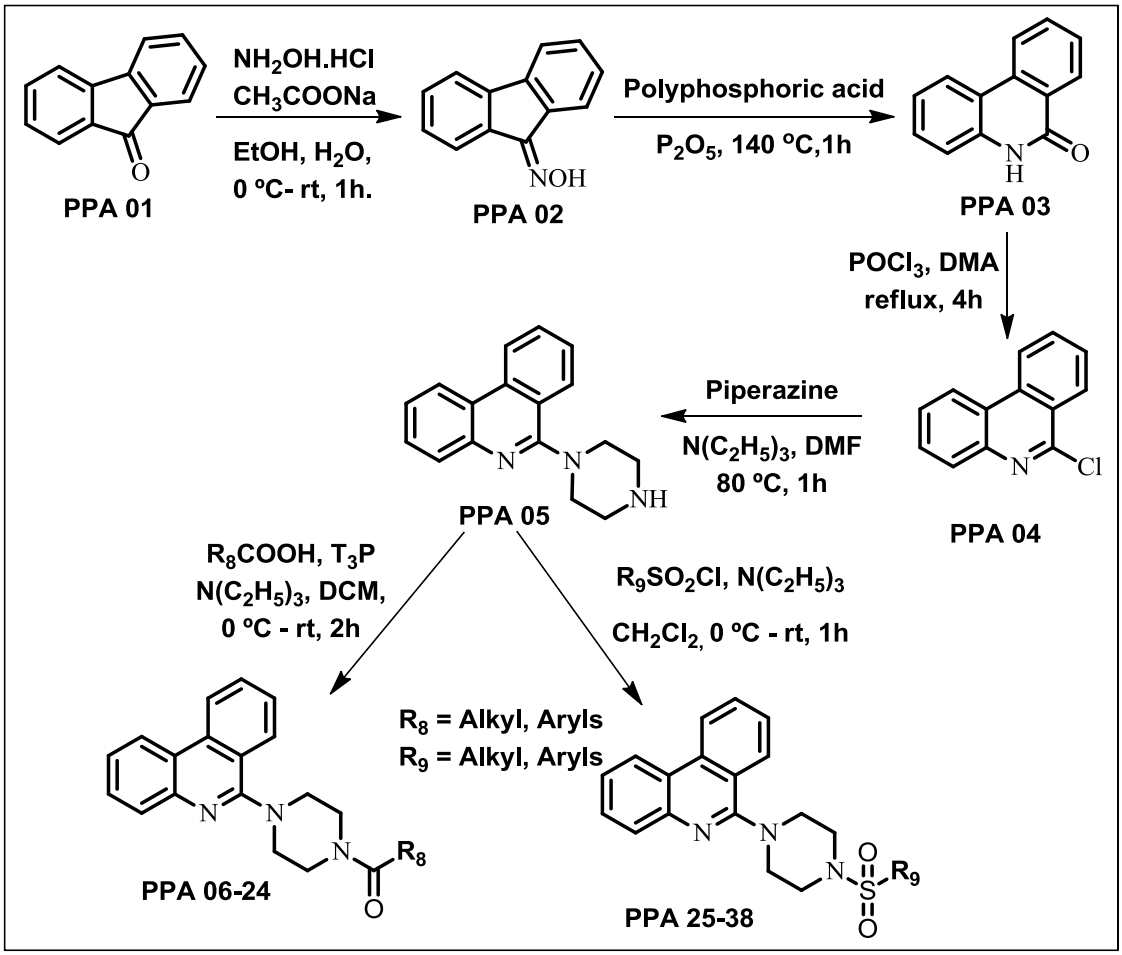


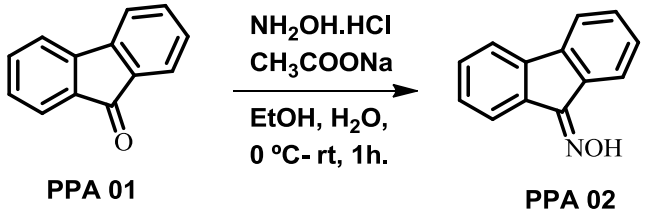
Figure 4.5: Synthetic protocol employed for the synthesis of compounds **PPA 05-38** of scheme 3

4.2.3.1. General experimental procedure utilized for the synthesis of 6-(piperazin-1-yl)phenanthridine derivatives (PPA 05-38)

The target molecules were synthesized by following a five steps synthetic protocol. Starting from 9-fluorenone (**PPA 01**) as outlined in **Figure 4.5**, we synthesized compound 6-(piperazin-1-yl)phenanthridine (**PPA 05**) according to reported protocol with slight modification [Meseroll *et al*., 2011; Badger *et al*., 1951]. 9-fluorenone upon treatment with hydroxylamine hydrochloride and sodium acetate afforded *N*-hydroxy-9*H*-fluoren-9-imine **(PPA 02)**. Further heating **PPA 02** with polyphosphoric acid and phosphorus pentoxide afforded Beckmann rearrangement product phenanthridin-6(5*H*)-one (**PPA 03**). 6-chlorophenanthridine (**PPA 04**) was synthesized by refluxing **PPA 03** with phosphorus oxychloride and *N*,*N*-dimethylaniline. Cardinal synthon 6-(piperazin-1-yl) phenanthridine (**PPA 05**) was prepared from a mixture of 6-chlorophenanthridine, anhydrous piperazine,

triethylamine and DMF at 80 °C, (**Figure 4.5).** The optimised reaction conditions handy, a series of **19** compounds **PPA 06-24** were synthesized in good yield, thus validating the scope of the present protocol sketched in **Figure 4.5**. Further, compound **PPA 05** was reacted with various sulfonyl chlorides, using TEA as base and DCM as solvent at 0 ºC to rt for 1-2 h to yield **PPA 25-38** in good to excellent yields (**Figure 4.5)**. All the title compounds were purified by column chromatography.

Preperation of 9H-fluoren-9-one oxime (PPA 02)

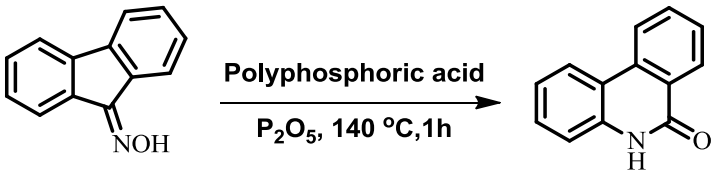


PPA 01 **PPA 02**

A solution of 9-fluorenone (**PPA 01**) (5.00 g, 1.0 equiv.) and $NH_2OH.HCl$ (1.2 equiv.) were taken in ethanol (30 mL) and cooled to 0 °C, After 5 min CH_3COONa (1.2 equiv.) in water was added to above mixture and warmed to rt for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water and formed as white semi solid. It is filtered by buchner funnel and dried over *in vacuo*. The resultant product *N*-hydroxy-9*H*-fluoren-9-imine **(PPA 02)** is used for next step without purification.

Appearance: white solid; Yield: 95% (5.14g); ESI-MS: (m/z) calculated for $C_{13}H_9NO$: 195.06, found: 196.11 $(M+H)^+$.

Preparation of phenanthridin-6(5H)-one (PPA 03)

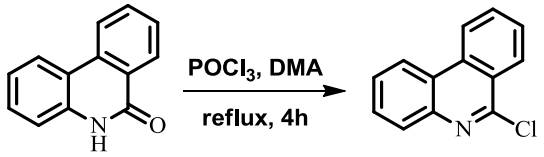


PPA 02 **PPA 03**

We adopted *N*-hydroxy-9*H*-fluoren-9-imine **(PPA 02)** (5.00 g, 1.0 equiv.), polyphosphoric acid (6.0 equiv.) and phosphorus pentoxide (catalytic) were added and stirred at 140 °C for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with 4N HCl and water was added & stirred for 2h at rt. The mixture was extracted with ethyl acetate. The organic layers were collected, washed with saturated brine solution, dried over

anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (70 -100 %) to get the compound **PPA 03.**
Appearance: off white solid; Yield: 80% (4.00g); ESI-MS: (m/z) calculated for $C_{13}H_9NO$: 195.06, found: 196.13 $(M+H)^+$.

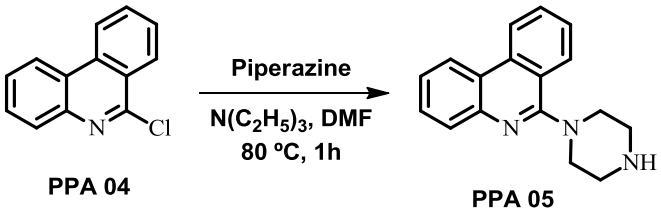
Preperation of 6-chlorophenanthridine (PPA 04)



PPA 03 **PPA 04**

A solution of phenanthridin-6(5H)-one **(PPA 03)** (4.00 g, 1.0 equiv.), $POCl_3$ (20 ml) and DMA were (catalytic) added and refluxed for 4h. After completion of the reaction, as indicated by TLC, the reaction was distilled under reduced vaccum and quenched with ice cold water and stirred for 1h, the product is filtered by buchner funnel and dried over *in vacuo*. The resultant product 6-chlorophenanthridine **(PPA 04)** is used for next step without purification.
Appearance: off white solid; Yield: 85% (3.75g); ESI-MS: (m/z) calculated for $C_{13}H_8ClN$: 213.03, found: 214.10 $(M+H)^+$.

Preparation of 6-(piperazin-1-yl)phenanthridine (PPA 05)



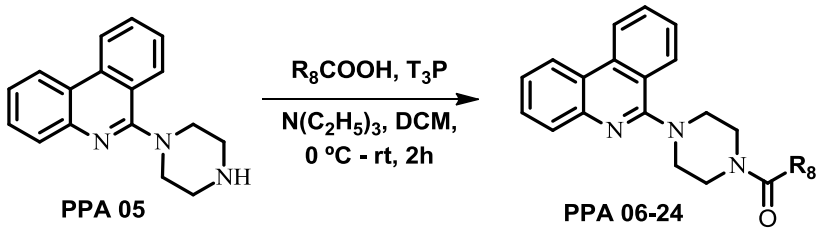
PPA 04 **PPA 05**

A stirred solution of piperazine (3.0 equiv.), TEA (2.0 equiv.) in DMF was added and the resultant solution was cooled to 0 °C. 6-chlorophenanthridine **(PPA 04)** (5.00 g, 1.0 equiv.) was further added to above mixture and it was kepted at 80 °C for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with ethyl acetate. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (60 -80 %) to achieved 4.62g of 6-(piperazin-

1-yl)phenanthridine **(PPA 05)** with good yield.

Appearance: off white solid; Yield: 75% (4.62g); IR (KBr) ν_{max}: 3268, 3105, 3044, 1648, 1600, 1552, 1504, 1450, 1330, 1248, 933 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 8.4 Hz, 1H), 8.42 (d, J = 7.2 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.82–7.48 (m, 4H), 3.82–3.54 (t, J = 4.0 Hz, 8H), 3.46 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.14, 161.68, 142.40, 136.22, 134.45, 131.41, 130.37, 128.83, 127.19, 125.14, 124.17, 122.13, 121.58, 120.26, 119.85, 51.60, 47.12, 31.28. ESI-MS: (m/z) calculated for $C_{17}H_{17}N_3$: 263.14, found: 264.18 $(M+H)^+$. Anal. Calcd for $C_{17}H_{17}N_3$; (%) C 72.71, H 5.49, N 16.96; found: C 72.82, H 5.54, N 17.04.

4.2.3.2. Synthesis of (4-(phenanthridin-6-yl)piperazin-1-yl)(phenyl)methanone (PPA 06-24) derivatives and characterization



Initially, we tried monitoring the reaction conditions with various bases and solvents as summarised in **Table 4.2**. We set off our investigation for the synthesis of **PPA 07,** under various reaction conditions. As a model reaction, compound (**PPA 05**) was treated with various acids and amide coupling reagents [Valeur *et al*., 2009; Joshua *et al*., 2011; El-Faham *et al*., 2011; Pattabiraman *et al*., 2011; Goodreid *et al*., 2014] were employed to get desired compound (entry 1-11). Initially, we employed TEA as base, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC.HCl) and 1-hydroxybenzotriazole (HOBt) as amide coupling reagents in the presence of dichloromethane (DCM) as solvent at 0 ^oC to rt. Resultant mixture was stirred for 8h to yield **PPA 07** in about 58% (entry 1). Changing the solvent to DMF under similar reaction conditions yielded **PPA 07** in about 52%. Alternatively, changed amide coupling reagents such as 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[*4,5-b*]pyridinium 3-oxid hexafluoro phosphate (HATU), (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and solvent as *N,N*-diisopropylethylamine (DIPEA) under similar condition yields are 38-42% (entry 3–5). Next we hope to improve the yields, 1-Propanephosphonic anhydride (T_3P) was employed to

alleviate the yield (entry 6-11). Initially, the reaction was carried out with various bases and solvents (entry 6-7) to give the desired compound in moderate yield. The reaction was further optimised with TEA and DCM at various intervals of time (entry 8-11), to give **PPA 07** in good yield. Found entries 9-10 potently improved the yield and actuate reaction period 6-8 h and reputable technique. Having the optimised reaction conditions handy, a series of **19** compounds **PPA 06-24** was synthesized in good yield, thus validating the scope of the present protocol sketched **Figure 4.5**.

Table 4.2: Optimisation of reaction conditions of **PPA 07**[a]

Entry	Solvent	Base	Reagent	Temperature (°C)	Time	Yield (%)[b]
1	DCM	TEA	EDC.HCl, HOBt	rt	8 h	58
2	DMF	TEA	EDC.HCl, HOBt	rt	8 h	52
3	DMF	DIPEA	HATU	rt	8 h	41
4	DMF	DIPEA	BOP	rt	8 h	42
5	DMF	DIPEA	PyBOP	rt	8 h	38
6	DCM	Pyridine	T_3P	rt	4 h	54
7	DCM	TEA	T_3P	rt	2 h	56
8	DCM	TEA	T_3P	rt	4 h	68
9	DCM	TEA	T_3P	rt	6 h	84
10	DCM	TEA	T_3P	rt	8 h	85
11	DCM	TEA	T_3P	rt	12 h	84

[a]All the reactions were carried out with **PPA 07** (1.0 equiv.), base (2.0 equiv.) and reagent (1.0-1.6 equiv.) in solvent, [b]Isolated yield after column chromatography.

Characterization of the synthesized PPA 06-24 final compounds

***3-oxo-3-(4-(phenanthridin-6-yl)piperazin-1-yl)propanenitrile* (PPA 06)**

Appearance: off white solid; yield = 78% (0.29g); IR (KBr) ν_{max}: 3104, 3045, 2238, 1649, 1603, 1550, 1507, 1450, 1329, 1248, 930 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 8.4 Hz, 1H), 8.42 (d, J = 7.2 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.82–7.48 (m, 4H), 3.82–3.54 (t, J = 4.4 Hz, 8H), 3.46 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.14, 161.68, 142.40, 136.22, 134.45, 131.41, 130.37, 128.83, 127.19, 125.14, 124.17, 122.13, 121.58, 120.26, 119.85, 51.60, 47.12, 31.28. FT-IR: ν 2248 (CN); ESI-MS: (m/z)

calcd. for $C_{20}H_{18}N_4O$, 330.14, found: 331.22 $(M + H)^+$. Anal. Calcd for $C_{20}H_{18}N_4O$; (%) C 72.71, H 5.49, N 16.96; found: C 72.82, H 5.54, N 17.04.

***(4-(phenanthridin-6-yl)piperazin-1-yl)(phenyl)methanone* (PPA 07)**

A stirred solution of 6-(piperazin-1-yl)phenanthridine **(PPA 05)** 0.30g (1.0 equiv.), benzoic acid (1.05 equiv), T_3P (1.2 equiv), TEA (2.0 equiv.) in DCM were added at rt and stirred for 6h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with DCM. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (60 -70 %) to achieved 0.35g of (4-(phenanthridin-6-yl)piperazin-1-yl)(phenyl)methanone **(PPA 07)** with good yield.

Appearance: white solid; yield = 85% (0.35g); IR (KBr) ν_{max}: 3106, 3044, 1770, 1646, 1604, 1548, 1507, 1450, 1328, 1248, 931 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 8.4 Hz, 1H), 8.46 (d, J = 7.2 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 7.78–7.74 (m, 2H), 7.66–7.45 (m, 6H), 4.12–3.51 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.74, 159.62, 143.50, 135.74, 135.02, 133.41, 130.37, 130.12, 129.83, 128.83, 128.59, 127.15, 126.95, 126.14, 125.17, 122.83, 121.91, 121.26, 51.63, 47.82. ESI-MS: (m/z) calcd. for $C_{24}H_{21}N_3O$, 367.16, found: 368.23 $(M + H)^+$. Anal. Calcd for $C_{24}H_{21}N_3O$; (%) C 78.45, H 5.76, N 11.44; found: C 78.61, H 5.81, N 11.53.

***(4-chlorophenyl)(4-(phenanthridin-6-yl)piperazin-1-yl)methanone* (PPA 08)**

Appearance: off white solid; yield = 81% (0.37g); IR (KBr) ν_{max}: 3095, 3050, 1782, 1648, 1602, 1550, 1498, 1450, 1335, 1254, 930, 847 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 8.4 Hz, 1H), 8.46 (d, J = 8.8 Hz, 2H), 8.25 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 10.8 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.81–7.48 (m, 4H), 3.67–3.44 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.52, 160.14, 144.22, 136.18, 135.81, 132.87, 131.08, 130.64, 129.54, 128.63, 128.32, 127.11, 126.84, 126.33, 125.46, 122.57, 121.87, 121.18, 52.28, 47.54. ESI-MS: (m/z) calcd. for $C_{24}H_{20}ClN_3O$, 401.12, found: 402.25 $(M + H)^+$. Anal. Calcd for $C_{24}H_{20}ClN_3O$; (%) C 71.73, H 5.02, N 10.46; found: C 71.81, H 5.09, N 10.53.

***(4-nitrophenyl)(4-(phenanthridin-6-yl)piperazin-1-yl)methanone* (PPA 09)**

Appearance: yellow solid; yield = 68% (0.31g); IR (KBr) ν_{max}: 3108, 3044, 1778, 1648, 1602, 1548, 1508, 1450, 1332, 1248, 933 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, J = 8.4 Hz, 1H), 8.48 (d, J = 8.0, 1H), 8.25 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 9.6 Hz, 2H), 7.97 (d, J =

7.6 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.82–7.54 (m, 4H), 3.72–3.68 (t, *J* = 5.2 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.12, 164.51, 146.86, 142.02, 139.53, 137.84, 132.07, 130.84, 129.74, 128.44, 128.01, 127.69, 126.80, 126.11, 125.47, 123.78, 122.35, 120.88, 52.23, 48.59. ESI-MS: (m/z) calcd. for $C_{24}H_{20}N_4O_3$, 412.15, found: 413.10 $(M + H)^+$. Anal. Calcd for $C_{24}H_{20}N_4O_3$; (%) C 69.89, H 4.89, N 13.58; found: C 69.98, H 4.95, N 13.70.

***(3-nitrophenyl)(4-(phenanthridin-6-yl)piperazin-1-yl)methanone* (PPA 10)**

Appearance: pale yellow solid; yield = 63% (0.29g); IR (KBr) ν_{max}: 3108, 3045, 1783, 1649, 1605, 1548, 1505, 1454, 1334, 1248, 935 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.63 (d, *J* = 8.4 Hz, 1H), 8.42 (d, *J* = 8.0, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.21 (d, *J* = 9.2 Hz, 1H), 7.97 (d, *J* = 7.6 Hz, 1H), 7.93 (m, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.82–7.54 (m, 4H), 3.69–3.57 (t, *J* = 5.2 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.08, 162.31, 147.36, 143.47, 141.11, 139.62, 135.24, 132.04, 131.84, 130.02, 129.21, 128.68, 128.32, 127.34, 126.01, 125.82, 125.21, 123.52, 121.76, 120.88, 53.62, 47.84. ESI-MS: (m/z) calcd. for $C_{24}H_{20}N_4O_3$, 412.15, found: 413.20 $(M + H)^+$. Anal. Calcd for $C_{24}H_{20}N_4O_3$; (%) C 69.89, H 4.89, N 13.58; found: C 69.96, H 4.96, N 13.66.

***(3,5-dinitrophenyl)(4-(phenanthridin-6-yl)piperazin-1-yl)methanone* (PPA 11)**

Appearance: yellow solid; yield = 64% (0.33g); IR (KBr) ν_{max}: 3105, 3048, 1774, 1645, 1605, 1549, 1507, 1450, 1324, 1244, 938 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08–8.83 (s, 3H), 8.46 (d, *J* = 8.0, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.85–7.48 (m, 4H), 3.71–3.62 (t, *J* = 5.2 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.32, 159.22, 154.08, 144.75, 142.47, 139.81, 138.02, 133.83, 132.38, 130.39, 129.21, 128.52, 127.01, 125.44, 124.23, 122.89, 121.22, 119.65, 52.63, 47.82. ESI-MS: (m/z) calcd. for $C_{24}H_{19}N_5O_5$, 457.13, found: 458.21 $(M + H)^+$. Anal. Calcd for $C_{24}H_{19}N_5O_5$; (%) C 63.02, H 4.19, N 15.31; found: C 63.11, H 4.24, N 15.39.

***(2-aminophenyl)(4-(phenanthridin-6-yl)piperazin-1-yl)methanone* (PPA 12)**

Appearance: white solid; yield = 60% (0.26g); IR (KBr) ν_{max}: 3268, 3104, 3045, 1780, 1649, 1605, 1550, 1506, 1450, 1330, 1248, 932 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.80–7.18 (m, 6H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.02 (br, 2H), 3.68–3.52 (t, *J* = 5.2 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.78, 158.13, 146.87, 142.42, 141.44, 138.23, 137.54, 131.12, 130.66, 130.08, 129.23, 128.84, 126.45, 124.87, 124.12, 123.88, 121.86, 120.22, 119.82, 118.52, 52.42, 46.57. ESI-MS: (m/z) calcd. for $C_{24}H_{22}N_4O$, 382.17, found: 383.26 $(M + H)^+$. Anal. Calcd for $C_{24}H_{22}N_4O$; (%) C 75.37, H 5.80, N 14.65;

found: C 75.51, H 5.85, N 14.54.

***(4-aminophenyl)(4-(phenanthridin-6-yl)piperazin-1-yl)methanone* (PPA 13)**

Appearance: off white semi solid; yield = 62% (0.27g); IR (KBr) ν_{max}: 3268, 3102, 3055, 1783, 1648, 1604, 1548, 1500, 1450, 1328, 1240, 930 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (d, J = 8.4 Hz, 1H), 8.43 (d, J = 8.0, 1H), 8.22 (d, J = 7.8 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.84 (m, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.71–7.52 (m, 3H), 7.18 (d, J = 8.2 Hz, 2H), 5.84 (br, 2H), 3.78–3.54 (t, J = 4.8 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.84, 163.58, 147.18, 146.09, 140.25, 134.28, 131.12, 130.82, 129.98, 128.22, 127.84, 127.02, 126.45, 125.34, 124.72, 123.21, 122.87, 119.24, 52.64, 47.82. ESI-MS: (m/z) calcd. for $C_{24}H_{22}N_4O$, 382.17, found: 383.22 $(M + H)^+$. Anal. Calcd for $C_{24}H_{22}N_4O$; (%) C 75.37, H 5.80, N 14.65; found: C 75.54, H 5.86, N 14.52.

***2-(naphthalen-1-yl)-1-(4-(phenanthridin-6-yl)piperazin-1-yl)ethanone* (PPA 14)**

Appearance: pale yellow solid; yield = 75% (0.36g); IR (KBr) ν_{max}: 3095, 3040, 1778, 1650, 1602, 1550, 1506, 1450, 1329, 1248, 932 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, J = 8.4 Hz, 1H), 8.65 (d, J = 8.0 Hz, 1H), 8.28 (d, J = 8.8 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.78–7.42 (m, 7H), 7.31 (d, J = 8.0 Hz, 1H), 4.28 (s, 2H), 3.93–3.40 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.09, 163.24, 150.86, 148.13, 141.18, 140.45, 138.16, 134.22, 133.72, 132.07, 131.28, 130.88, 129.12, 128.33, 126.57, 125.78, 124.02, 123.31, 123.04, 122.89, 122.23, 121.64, 121.02, 120.58, 50.82, 48.54. ESI-MS: (m/z) calcd. for $C_{29}H_{25}N_3O$, 431.19, found: 432.33 $(M + H)^+$. Anal. Calcd for $C_{29}H_{25}N_3O$; (%) C 80.72, H 5.84, N 9.74; found: C 80.79, H 5.87, N 9.81.

***(E)-1-(4-(phenanthridin-6-yl)piperazin-1-yl)-3-phenylprop-2-en-1-one* (PPA 15)**

Appearance: off white greenish solid; yield = 72% (0.32g); IR (KBr) ν_{max}: 3098, 3052, 1784, 1650, 1604, 1550, 1508, 1450, 1329, 1248, 930 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 8.4 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.0 Hz, 2H), 7.75–7.48 (m, 7H), 7.38 (d, J = 15.2 Hz, 1H), 7.29 (d, J = 15.8 Hz, 1H), 3.68–3.54 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.08, 161.22, 154.16, 148.12, 140.82, 138.45, 133.16, 132.84, 131.90, 128.37, 127.78, 125.23, 124.88, 123.65, 122.44, 122.21, 121.84, 121.22, 120.36, 118.41, 51.59, 46.38. ESI-MS: (m/z) calcd. for $C_{26}H_{23}N_3O$, 393.18, found: 394.27 $(M + H)^+$. Anal. Calcd for $C_{26}H_{23}N_3O$; (%) C 79.36, H 5.89, N 10.68; found: C 79.48, H 5.93, N 10.75.

***(4-(phenanthridin-6-yl)piperazin-1-yl)(pyridin-2-yl)methanone* (PPA 16)**

Appearance: off white semi solid; yield = 78% (0.32g); IR (KBr) ν_{max}: 3095, 3040, 1785, 1648, 1602, 1550, 1506, 1452, 1328, 1245, 928 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 8.8 Hz, 1H), 8.46 (d, J = 8.4 Hz, 1H), 8.25 (d, J = 8.8 Hz, 1H), 8.14 (m, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.78–7.48 (m, 5H), 3.92–3.52 (t, J = 4.8 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.23, 160.89, 154.04, 151.12, 149.34, 142.81, 140.86, 133.17, 132.34, 130.14, 129.13, 128.47, 126.05, 124.60, 123.37, 122.88, 122.14, 121.22, 120.80, 51.74, 46.58. ESI-MS: (m/z) calcd. for $C_{23}H_{20}N_4O$, 368.16, found: 369.24 $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}N_4O$; (%) C 74.98, H 5.47, N 15.21; found: C 75.07, H 5.41, N 15.14.

***(4-(phenanthridin-6-yl)piperazin-1-yl)(pyridin-3-yl)methanone* (PPA 17)**

Appearance: off white solid; yield = 74% (0.31g); IR (KBr) ν_{max}: 3098, 3052, 1782, 1655, 1602, 1550, 1504, 1450, 1328, 1248, 925 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.72 (d, J = 8.4 Hz, 2H), 8.64 (d, J = 8.0 Hz, 1H), 8.28 (d, J = 8.8 Hz, 1H), 7.94 (m, 2H), 7.83 (d, J = 8.0 Hz, 1H), 7.78–7.51 (m, 4H), 3.98–3.44 (t, J = 4.8 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.84, 162.45, 153.76, 153.23, 151.88, 141.54, 139.72, 132.65, 132.11, 130.81, 129.33, 128.28, 126.32, 124.86, 123.42, 122.46, 122.02, 121.85, 120.43, 52.68, 47.54. ESI-MS: (m/z) calcd. for $C_{23}H_{20}N_4O$, 368.16, found: 369.28 $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}N_4O$; (%) C 74.98, H 5.47, N 15.21; found: C 75.05, H 5.45, N 15.12.

***(4-(phenanthridin-6-yl)piperazin-1-yl)(pyridin-4-yl)methanone* (PPA 18)**

Appearance: off white semi solid; yield = 81% (0.34g); IR (KBr) ν_{max}: 3105, 3040, 1774, 1650, 1602, 1550, 1450, 1325, 1248, 938 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, J = 8.8 Hz, 2H), 8.45 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 7.6 Hz, 1H), 7.75–7.44 (m, 4H), 3.88–3.54 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.06, 159.15, 152.74, 150.89, 147.21, 144.39, 139.82, 133.45, 130.23, 128.06, 127.14, 126.23, 124.18, 123.42, 122.01, 121.79, 120.12, 52.36, 47.52. ESI-MS: (m/z) calcd. for $C_{23}H_{20}N_4O$, 368.16, found: 369.23 $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}N_4O$; (%) C 74.98, H 5.47, N 15.21; found: C 75.12, H 5.40, N 15.16.

***6-(4-(phenanthridin-6-yl)piperazine-1-carbonyl)picolinic acid* (PPA 19)**

Appearance: off white semi solid; yield = 65% (0.30g); IR (KBr) ν_{max}: 3275, 3098, 3052, 1774, 1653, 1600, 1558, 1506, 1450, 1320, 1245, 930 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1H), 8.88 (d, J = 8.4 Hz, 1H), 8.82 (d, J = 9.2 Hz, 1H), 8.61 (m, 1H), 8.57 (d, J = 8.4 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H), 7.79–7.46 (m, 4H), 3.82–3.52 (t, J = 4.8 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.36,

171.81, 160.12, 152.45, 150.86, 148.32, 143.75, 140.48, 135.08, 134.53, 131.24, 129.04, 128.14, 126.87, 124.12, 123.44, 122.81, 122.63, 121.07, 120.24, 52.72, 47.50. ESI-MS: (m/z) calcd. for $C_{24}H_{20}N_4O_3$, 412.15, found: 413.28 $(M + H)^+$. Anal. Calcd for $C_{24}H_{20}N_4O_3$; (%) C 69.89, H 4.89, N 13.58; found: C 69.98, H 4.95, N 13.64.

***furan-2-yl(4-(phenanthridin-6-yl)piperazin-1-yl)methanone* (PPA 20)**

Appearance: off white solid; yield = 76% (0.30g); IR (KBr) ν_{max}: 3098, 3048, 1782, 1654, 1605, 1551, 1506, 1450, 1330, 1248, 930 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 8.8 Hz, 1H), 8.44 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.86–7.54 (m, 4H), 7.43 (d, J = 7.8 Hz, 1H), 7.22 (m, 1H), 4.94–3.68 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.74, 157.68, 148.15, 142.44, 135.22, 133.45, 130.87, 130.12, 129.18, 128.84, 128.04, 127.21, 126.90, 126.12, 125.18, 122.18, 121.98, 118.24, 51.68, 48.84. ESI-MS: (m/z) calcd. for $C_{22}H_{19}N_3O_2$, 357.14, found: 358.25 $(M + H)^+$. Anal. Calcd for $C_{22}H_{19}N_3O_2$; (%) C 73.93, H 5.36, N 11.76; found: C 73.99, H 5.41, N 11.83.

***(4-(phenanthridin-6-yl)piperazin-1-yl)(thiophen-2-yl)methanone* (PPA 21)**

Appearance: white solid; yield = 81% (0.34g); IR (KBr) ν_{max}: 3102, 3047, 2015, 1774, 1650, 1602, 1550, 1450, 1328, 1245, 933 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, J = 8.8 Hz, 1H), 8.58 (d, J = 8.0 Hz, 1H), 8.42 (d, J = 8.0 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.88–7.37 (m, 5H), 4.89–3.53 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.02, 158.88, 149.22, 141.89, 136.72, 131.93, 130.12, 129.94, 129.34, 128.84, 128.11, 127.26, 126.95, 125.87, 125.03, 122.48, 121.68, 120.87, 52.46, 47.52. ESI-MS: (m/z) calcd. for $C_{22}H_{19}N_3OS$, 373.12, found: 374.27 $(M + H)^+$. Anal. Calcd for $C_{22}H_{19}N_3OS$; (%) C 70.75, H 5.13, N 11.25; found: C 70.83, H 5.19, N 11.15.

***2-(1H-indol-3-yl)-1-(4-(phenanthridin-6-yl)piperazin-1-yl)ethanone* (PPA 22)**

Appearance: off white solid; yield = 80% (0.38g); IR (KBr) ν_{max}: 3288, 3098, 3048, 1774, 1649, 1606, 1550, 1450, 1333, 1248, 930 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 7.6 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.84 (m, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.58–7.48 (m, 3H), 7.42 (d, J = 8.0 Hz, 1H), 7.34 (s, 1H), 7.20–7.16 (m, 2H), 6.08 (br, 1H), 3.88 (t, J = 4.4 Hz, 4H), 3.80 (s, 2H), 3.52 (t, J = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.54, 159.62, 144.83, 143.23, 140.23, 138.64, 135.49, 135.05, 133.63, 132.84, 129.47, 128.43, 127.19, 126.82, 126.11, 124.46, 123.23, 121.52, 119.88, 119.04, 117.74, 108.04, 52.64, 48.48, 40.68. ESI-MS: (m/z) calcd. for $C_{27}H_{24}N_4O$ 420.19, found: 421.15 $(M + H)^+$. Anal. Calcd for $C_{27}H_{24}N_4O$: (%) C 77.12, H 5.75, N 13.32; Found: C 77.24, H 5.88, N 13.41.

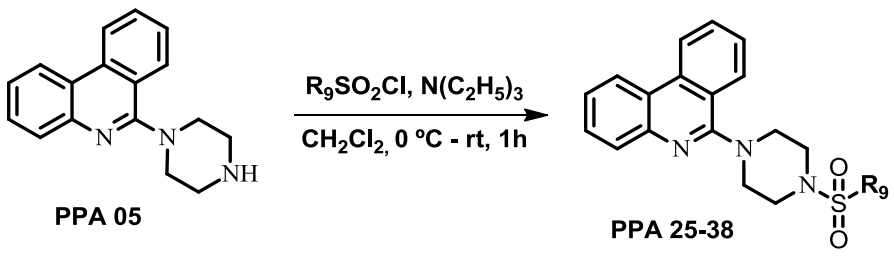
3-(1H-indol-3-yl)-1-(4-(phenanthridin-6-yl)piperazin-1-yl)propan-1-one **(PPA 23)**

Appearance: off white solid; yield = 83% (0.41g); IR (KBr) ν_{max}: 3248, 3095, 3049, 1788, 1650, 1602, 1548, 1506, 1450, 1329, 1256, 930 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.86 (d, J = 7.2 Hz, 1H), 7.80 (m, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.61–7.44 (m, 3H), 7.41 (d, J = 8.0 Hz, 1H), 7.38 (s, 1H), 7.22–7.19 (m, 2H), 6.84 (br, 1H), 3.92–2.70 (t, J = 4.4 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.22, 162.48, 145.16, 144.06, 141.18, 139.21, 136.42, 135.84, 133.82, 133.08, 128.92, 128.11, 127.64, 126.74, 126.32, 124.22, 123.68, 120.94, 119.24, 118.48, 117.38, 110.28 52.12, 47.39, 39.94, 32.98. ESI-MS: (m/z) calcd. for $C_{28}H_{26}N_4O$ 434.21, found: 435.28 $(M + H)^+$. Anal. Calcd for $C_{28}H_{26}N_4O$: (%) C 77.39, H 6.03, N 12.89; Found: C 77.46, H 6.12, N 12.94.

4-(1H-indol-3-yl)-1-(4-(phenanthridin-6-yl)piperazin-1-yl)butan-1-one **(PPA 24)**

Appearance: off white solid; yield = 75% (0.38g); IR (KBr) ν_{max}: 3280, 3095, 3047, 1778, 1650, 1602, 1558, 1505, 1450, 1335, 1248, 938 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 7.6 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.4 Hz, 1H), 7.84 (m, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.62–7.39 (m, 3H), 7.35 (d, J = 8.0 Hz, 1H), 7.30 (s, 1H), 7.18–7.14 (m, 2H), 6.42 (br, 1H), 3.89–2.60 (t, J = 4.4 Hz, 12H), 1.68–1.63 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.84, 163.04, 144.87, 143.21, 141.85, 139.42, 135.08, 134.91, 133.09, 132.44, 129.48, 128.74, 127.04, 126.23, 123.11, 122.41, 122.26, 121.39, 120.12, 119.94, 118.87, 111.46, 52.88, 46.24, 40.37, 30.42, 39.87. ESI-MS: (m/z) calcd. for $C_{29}H_{28}N_4O$ 448.22, found: 449.24 $(M + H)^+$. Anal. Calcd for $C_{29}H_{28}N_4O$: (%) C 77.65, H 6.29, N 12.49; Found: C 77.72, H 6.38, N 12.38.

4.2.3.3. Synthesis of 6-(4-(substitutedsulfonyl)piperazin-1-yl)phenanthridine (PPA 25-38) analogues and characterization



To a stirred solution of 6-(piperazin-1-yl)phenanthridine (**PPA 05**) (1.0 equiv.), triethylamine (1.2 equiv.) was added in CH_2Cl_2 and cooled to 0 °C; to this mixture substituted

sulfonylchlorides (1.2 equiv.) were added and allowed to reach rt, stirred for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (40 - 80 %)] to get the title compounds in yields ranging from 74–95%.

Characterization of the synthesized PPA 25-38 title compounds

***6-(4-(methylsulfonyl)piperazin-1-yl)Phenanthridine* (PPA 25)**

To a stirred solution of 6-(piperazin-1-yl)phenanthridine (**PPA 05**) 0.2g (1.0 equiv.), TEA (1.2 equiv.) was added in CH_2Cl_2 and cooled to 0 °C; to this mixture substituted methyl sulfonylchloride (1.1 equiv.) were added and allowed to rt, stirred for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with CH_2Cl_2. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (40 - 50 %)] to achive 0.20g of 6-(4-(methylsulfonyl)piperazin-1-yl)phenanthridine (**PPA 25**).

Appearance: off white semi solid; yield = 78% (0.20g); IR (KBr) ν_{max}: 3108, 3052, 1654, 1602, 1550, 1508, 1450, 1333, 1248, 938 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 7.4 Hz, 1H), 7.74–7.41 (m, 4H), 3.87–3.53 (t, J = 4.4 Hz, 8H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.24, 148.76, 140.08, 133.13, 131.67, 130.43, 129.88, 123.24, 122.41, 121.82, 120.34, 119.92, 114.34, 53.65, 48.21, 38.98. ESI-MS: (m/z) calcd. for $C_{18}H_{19}N_3O_2S$ 341.12, found: 342.14 $(M + H)^+$. Anal. Calcd for $C_{18}H_{19}N_3O_2S$: (%) C 63.32, H 5.61, N 12.31; found: C 63.64, H 5.74, N 12.28.

***6-(4-(phenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 26)**

Appearance: off white solid; yield = 88% (0.26g); IR (KBr) ν_{max}: 3105, 3050, 1653, 1604, 1558, 1507, 1450, 1330, 1247, 932 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, J = 8.0 Hz, 1H), 8.41 (d, J = 7.6 Hz, 1H), 8.02 (d, J = 7.8 Hz, 2H), 7.90 (dd, J = 9.0 Hz, J = 0.8 Hz, 1H), 7.82 (d, J = 7.4 Hz, 1H), 7.77–7.39 (m, 7H), 3.68–3.44 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.86, 152.08, 141.12, 138.47, 132.24, 131.82, 130.12, 129.64, 124.28, 123.81, 122.89, 121.24, 121.08, 120.42, 119.92, 118.42, 112.47, 50.48, 48.07. ESI-MS: (m/z) calcd. for $C_{23}H_{21}N_3O_2S$ 403.13, found: 403.22 $(M + H)^+$. Anal. Calcd for $C_{23}H_{21}N_3O_2S$: (%)

C 68.46, H 5.25, N 10.41; found: C 68.55, H 5.32, N 10.60.

***6-(4-tosylpiperazin-1-yl)phenanthridine* (PPA 27)**

Appearance: pale yellow solid; yield = 95% (0.30g); IR (KBr) ν_{max}: 3114, 3050, 1652, 1604, 1550, 1508, 1450, 1330, 1254, 932 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.91 (dd, J = 8.4 Hz, J = 0.8 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.80 (d, J = 7.8 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.74–7.46 (m, 4H), 3.64–3.38 (t, J = 4.4 Hz, 8H), 2.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.42, 153.14, 144.46, 139.08, 138.81, 132.11, 131.81, 129.64, 128.43, 124.82, 123.08, 122.28, 121.63, 120.42, 119.92, 118.42, 113.22, 52.18, 47.11, 24.08. ESI-MS: (m/z) calcd. for $C_{24}H_{23}N_3O_2S$ 417.15, found: 417.24 $(M + H)^+$. Anal. Calcd for $C_{24}H_{23}N_3O_2S$: (%) C 69.04, H 5.55, N 10.06; found: C 69.11, H 5.64, N 10.12.

6-(4-(4-tert-butylphenylsulfonyl)piperazin-1-yl)phenanthridine **(PPA 28)**

Appearance: white solid; yield = 92% (0.32g); IR (KBr) ν_{max}: 3109, 3050, 1651, 1608, 1555, 1504, 1450, 1327, 1250, 932 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 8.0 Hz, 1H), 8.45 (d, J = 8.2 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 6.4 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.76–7.72 (m, 1H), 7.74 (d, J = 7.2 Hz, 1H), 7.64–7.55 (m, 4H), 7.51 (d, J = 8.0 Hz, 1H), 3.60–3.35 (t, J = 4.4 Hz, 8H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.24, 156.71, 143.45, 134.98, 132.83, 130.30, 128.82, 128.62, 127.79, 126.81, 126.17, 125.95, 125.14, 122.85, 122.65, 121.86, 121.03, 50.48, 46.03, 35.23, 31.13. ESI-MS: (m/z) calcd. for $C_{27}H_{29}N_3O_2S$ 459.19, found: 460.25 $(M + H)^+$. Anal. Calcd for $C_{27}H_{29}N_3O_2S$: (%) C 70.56, H 6.36, N 9.14; found: C 70.64, H 6.28, N 9.24.

***6-(4-(mesitylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 29)**

Appearance: white solid; yield = 81% (0.27g); IR (KBr) ν_{max}: 3107, 3055, 1652, 1608, 1554, 1500, 1455, 1328, 1252, 934 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, J = 8.0 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 7.2 Hz, 1H), 7.72–7.58 (m, 4H), 7.24 (s, 2H), 3.62–3.42 (t, J = 4.4 Hz, 8H), 2.88–2.62 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.38, 157.24, 148.12, 145.31, 139.45, 138.08, 131.78, 131.41, 130.21, 127.09, 125.22, 124.62, 123.26, 122.08, 121.96, 121.14, 120.42, 51.04, 47.21, 31.08, 30.88. ESI-MS: (m/z) calcd. for $C_{26}H_{27}N_3O_2S$, 445.18, found: 446.23 $(M + H)^+$. Anal. Calcd for $C_{26}H_{27}N_3O_2S$; (%) C 70.08, H 6.11, N 9.43; found: C 70.14, H 6.23, N 9.59.

***6-(4-(4-chloro-2,5-dimethylphenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 30)**

Appearance: white solid; yield = 87% (0.30g); IR (KBr) ν_{max}: 3105, 3050, 1650, 1594, 1550, 1504, 1452, 1332, 1254, 933, 844 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 8.4 Hz,

1H), 8.46 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.85 (s, 1H), 7.78–7.53 (m, 4H), 7.34 (s, 1H), 3.56–3.46 (t, *J* = 4.4 Hz, 8H), 2.62–2.42 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.21, 158.73, 149.08, 142.74, 141.03, 139.22, 137.63, 137.24, 134.09, 131.11, 130.87, 129.32, 128.08, 126.43, 123.28, 122.92, 121.03, 120.25, 119.46, 51.82, 47.04, 36.42, 30.12. ESI-MS: (m/z) calcd. for $C_{25}H_{24}ClN_3O_2S$, 465.12, found: 466.15 $(M + H)^+$. Anal. Calcd for $C_{25}H_{24}ClN_3O_2S$; (%) C 64.44, H 5.19, N 9.02; found: C 64.58, H 5.30, N 9.14.

***6-(4-(4-fluorophenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 31)**

Appearance: pale yellow solid; yield = 74% (0.23g); IR (KBr) ν_{max}: 3105, 3050, 1658, 1601, 1550, 1509, 1450, 1328, 1252, 928 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.2 Hz, 1H), 8.42 (d, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.48–7.29 (m, 8H), 3.64–3.32 (t, *J* = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.86, 160.08, 151.11, 149.23, 138.14, 137.67, 135.62, 132.16, 131.23, 128.32, 126.45, 124.82, 123.88, 122.44, 121.21, 120.08, 114.05, 52.24, 46.42. ESI-MS: (m/z) calcd. for $C_{23}H_{20}FN_3O_2S$, 421.12, found: 422.25 $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}FN_3O_2S$; (%) C 65.54, H 4.78, N 9.97; found: C 65.62, H 4.88, N 10.08.

***6-(4-(4-chlorophenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 32)**

Appearance: white solid; yield = 82% (0.27g); IR (KBr) ν_{max}: 3110, 3050, 1652, 1612, 1551, 1510, 1450, 1323, 1249, 930, 842 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 10.8 Hz, 1H), 8.46 (d, *J* = 10.8 Hz, 1H), 8.25 (d, *J* = 11.2 Hz, 1H), 7.96 (d, *J* = 10.8 Hz, 1H), 7.82–7.48 (m, 4H), 7.28 (d, *J* = 6.8 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 1H), 3.67–3.44 (t, *J* = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.14, 159.28, 150.88, 142.23, 139.86, 134.07, 132.47, 131.52, 129.71, 128.91, 127.20, 124.88, 124.25, 123.62, 122.89, 121.03, 120.21, 51.72, 46.05. ESI-MS: (m/z) calcd. for $C_{23}H_{20}ClN_3O_2S$; 437.09, found: 438.15 $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_2S$; (%) C 63.08, H4.60, N 9.59; found: 63.19, H4.64, N 9.65.

***6-(4-(4-bromophenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 33)**

Appearance: pale yellow solid; yield = 78% (0.28g); IR (KBr) ν_{max}: 3105, 3050, 1654, 1602, 1550, 1508, 1450, 1330, 1238, 928, 715 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 8.4 Hz, 1H), 8.61 (d, *J* = 7.6 Hz, 1H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.88 (m, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 7.82–7.51 (m, 5H), 7.22 (d, *J* = 7.2 Hz, 2H), 3.51–3.20 (t, *J* = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.80, 159.12, 148.46, 144.02, 139.14, 138.07, 134.61, 133.72, 131.98, 131.34, 127.45, 126.82, 123.74, 122.46, 121.84, 121.03, 120.42, 51.68, 47.04. ESI-MS: (m/z) calcd. for $C_{23}H_{20}BrN_3O_2S$, 481.04, found: 482.13 $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}BrN_3O_2S$;

(%) C 57.27, H 4.18, N 8.71; found: C 57.34, H 4.23, N 8.85.

***6-(4-(4-(trifluoromethyl)phenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 34)**

Appearance: pale yellow solid; yield = 75% (0.26g); IR (KBr) ν_{max}: 3105, 3051, 1654, 1608, 1550, 1508, 1450, 1334, 1248, 932 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, J = 8.2 Hz, 1H), 8.44 (d, J = 7.6 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.98–7.49 (m, 9H), 3.63–3.38 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.24, 156.68, 149.12, 142.81, 138.37, 134.12, 131.28, 130.98, 130.12, 129.35, 128.84, 128.02, 126.87, 124.07, 123.89, 121.25, 120.81, 119.48, 52.38, 48.65. ESI-MS: (m/z) calcd. for $C_{24}H_{20}F_3N_3O_2S$; 471.12, found: 472.18 $(M + H)^+$. Anal. Calcd for $C_{24}H_{20}F_3N_3O_2S$; (%) C 61.14, H 4.28, N 8.91; found: C 61.18, H 4.31, N 8.86.

***6-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 35)**

Appearance: pale yellow solid; yield = 78% (0.25g); IR (KBr) ν_{max}: 3112, 3051, 1655, 1605, 1551, 1508, 1450, 1327 1248, 931 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, J = 8.4 Hz, 1H), 8.63 (d, J = 7.6 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.88 (m, 1H), 7.82 (dd, J = 8.4 Hz, J = 1.2 Hz, 1H), 7.78–7.74 (m, 2H), 7.69 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.55–7.51 (m, 1H), 7.22 (d, J = 7.2 Hz, 2H), 3.88 (s, 3H), 3.47–3.32 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.08, 160.28, 151.42, 140.23, 133.86, 132.46, 131.27, 130.52, 128.74, 127.91, 126.24, 124.88, 124.21, 123.63, 122.87, 120.03, 118.77, 51.78, 46.53. ESI-MS: (m/z) calcd. for $C_{24}H_{23}N_3O_3S$; 433.14, found: 434.23 $(M + H)^+$. Anal. Calcd for $C_{24}H_{23}N_3O_3S$; (%) C 66.49, H 5.35, N 9.69; found: C 66.54, H 5.42, N 9.77.

***6-(4-(4-nitrophenylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 36)**

Appearance: pale yellow solid; yield = 85% (0.28g); IR (KBr) ν_{max}: 3108, 3052, 1654, 1602, 1550, 1508, 1450, 1330, 1248, 944 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 7.8 Hz, 3H), 8.06 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 8.0 Hz, 1H), 7.79–7.48 (m, 4H), 3.64–3.38 (t, J = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.25, 154.71, 146.36, 137.11, 132.87, 132.23, 131.08, 128.52, 127.79, 126.82, 126.18, 125.94, 125.14, 123.65, 121.86, 121.02, 120.48, 50.79, 47.04. ESI-MS: (m/z) calcd. for $C_{23}H_{20}N_4O_4S$; 448.12, found: 449.22 $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}N_4O_4S$; (%) C 61.59, H 4.49, N 12.49; found: C 61.64, H 4.61, N 12.54.

***1-(4-(4-(phenanthridin-6-yl)piperazin-1-ylsulfonyl)phenyl)ethanone* (PPA 37)**

Appearance: off white solid; yield = 76% (0.25g); IR (KBr) ν_{max}: 3098, 3048, 1652, 1601, 1550, 1508, 1450, 1330, 1254, 955 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, J = 8.8 Hz, 1H), 8.46 (d, J = 8.0 Hz, 2H), 8.40 (d, J = 8.2 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.04 (d, J =

8.0 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.81–7.44 (m, 4H), 3.64–3.38 (t, *J* = 4.4 Hz, 8H), 2.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.47, 160.68, 152.23, 145.22, 138.22, 132.46, 132.67, 130.97, 129.42, 128.84, 127.46, 126.17, 124.24, 124.08, 123.68, 121.22, 120.88, 120.56, 50.64, 46.03, 29.44. ESI-MS: (m/z) calcd. for $C_{25}H_{23}N_3O_3S$; 445.14, found: 446.25 $(M + H)^+$. Anal. Calcd for $C_{25}H_{23}N_3O_3S$; (%) C 67.40, H 5.20, N 9.43; found: C 67.49, H 5.24, N 9.47.

***6-(4-(5-bromothiophen-2-ylsulfonyl)piperazin-1-yl)phenanthridine* (PPA 38)**

Appearance: pale yellow solid; yield = 80% (0.29g); IR (KBr) ν_{max}: 3112, 3052, 1654, 1602, 1550, 1508, 1450, 1330, 1248, 935 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 8.4 Hz, 1H), 8.45 (d, *J* = 7.8 Hz, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 8.93 (d, *J* = 8.0 Hz, 1H), 7.81–7.50–7.45 (m, 4H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.16 (d, *J* = 7.4 Hz, 1H), 3.66–3.40 (t, *J* = 4.4 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.24, 151.47, 144.78, 139.41, 134.89, 133.01, 131.22, 128.41, 126.84, 126.12, 125.08, 124.86, 123.25, 123.11, 122.47, 120.78, 119.23, 50.48, 46.14. ESI-MS: (m/z) calcd. For $C_{21}H_{18}BrN_3O_2S_2$; 487.00, found: 488.13 $(M + H)^+$. Anal. Calcd for $C_{21}H_{18}BrN_3O_2S_2$; (%) C 51.64, H 3.71, N 8.60; found: C 51.78, H 3.75, N 8.69.

4.2.3.4. Crystal growth and X-ray crystallographic study of PPA 07 and PPA 28

The X-ray crystallographic analysis of the compounds **PPA 07** and **PPA 28** was carried out: Crystals were grown from the slow evaporation of a 1:3:6 ratio of methanol/dichloromethane/ethyl acetate solvent mixture at rt, to get white flake crystals. A desirable crystal under triclinic system with space groups $P\bar{1}$ of **PPA 07**, crystal size/mm^3 0.6 × 0.5 × 0.4 and crystal under monoclinic system with space groups $P2_1/n$ of **PPA 28**, crystal size/mm^3 0.6 × 0.5 × 0.4 respectively. These crystals were kept at 293 K during data collection. Using Computer programs SHELXL97 (Sheldrick, 2008) the structure was solved with the unknown structure solution program using unknown and refined with the SHELXS-97 (Sheldrick, 2008) [Sheldrick 1997]. The crystal data, data collection and structure refinement details are summarized in **Table 4.3** and molecular structures are given as an ORTEP diagram pictured in **Figure 4.6** and **Figure 4.7**. Crystallographic data of the compounds **PPA 07** and **PPA 28** were deposited with the Cambridge Crystallographic Data Centre (CCDC). These compounds have been assigned the following deposition numbers **CCDC 1012376** and **CCDC 1012377** respectively. The X-ray structure parameters and complete details of compound **PPA 07** and **PPA 28** are provided in **Table 4.3**.

Table 4.3: Crystal data and structure refinement details for **PPA 07** and **PPA 28**

Compound	**PPA 07**	**PPA 28**
Formula	$C_{24}H_{21}N_3O$	$C_{27}H_{29}N_3O_2S$
Sum formula weight	367.44	459.60
Temperature/K	293	293
Crystal system	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P2_1/n$
a (Å)	9.4717 (13)	12.5058(13)
b (Å)	9.6936 (14)	14.6774 (17)
c (Å)	11.8432 (16)	13.3524 (14)
Angle α, β, γ (°)	73.767 (12), 74.207 (12), 65.560 (14)	90, 89.451 (9), 90
Volume (Å^3)	935.1 (2)	2450.8(5)
Z	2	4
F(000)	388.0	976.0
D_{calc} (g/mm^3)	1.305	1.246
μ (mm^{-1})	0.081	0.16
Absorption correction:	Multi-scan	Multi-scan
Radiation wavelength (Å)	0.71073	0.71073
Radiation type	MoK_α	MoK_α
Radiation monochromator	Graphite	Graphite
$(h, k, l)_{max}$, $(h, k, l)_{min}$	(12, 13, 14), (−12, −13, −14)	(17, 20, 15), (−9, −16, −18)
2Θ range for data collection	6.6 − 58.2°	6.4 − 58.4°
*T*min, *T*max	0.246, 1.000	0.922, 1.000
No. of measured, independent and observed [I > 2σ(I)] reflections	6305, 5019, 2213	7784, 6663, 3305
R_{int}	0.038	0.018
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.684	0.687
Crystal size/mm^3	0.6 × 0.5 × 0.4	0.6 × 0.5 × 0.4
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.0757 (2213), 0.2206 (4075), 0.95	0.050 (3305), 0.121 (5149), 1.02
No. of parameters	253	298
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.27, −0.28	0.14, −0.25
Structure refinement	SHELXL97 (Sheldrick, 2008)	

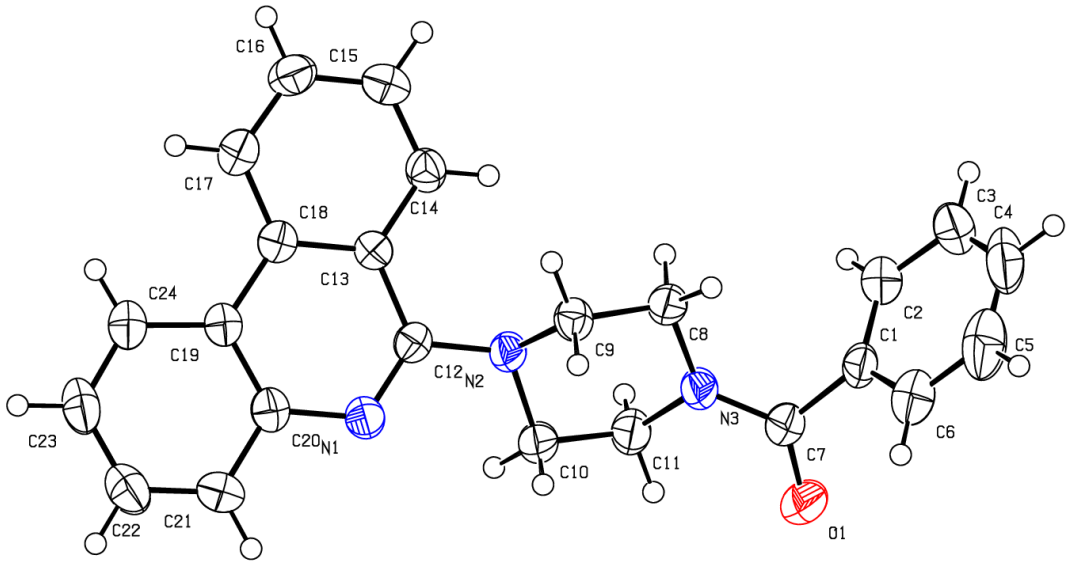


Figure 4.6: ORTEP plot for the compound **PPA 07**. All the non-hydrogen atoms are presented by their 50% probability thermal ellipsoids.

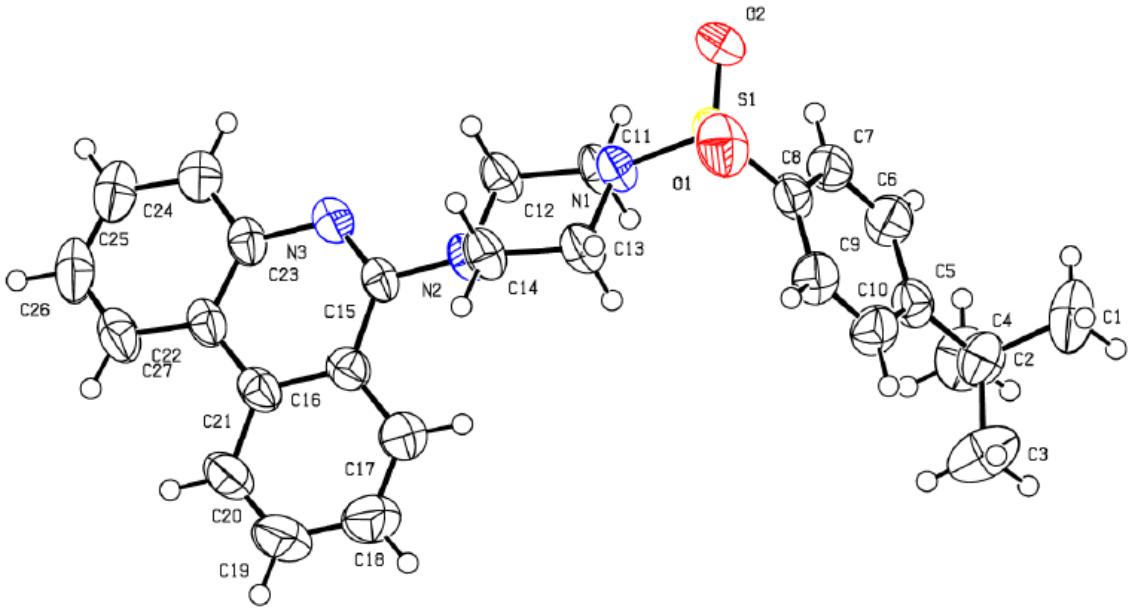


Figure 4.7: ORTEP plot for the compound **PPA 28**. All the non-hydrogen atoms are presented by their 50% probability thermal ellipsoids.

4.2.4. 5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-substitutedindolin-2-one (IPO 13-30), 2-(1-((substituted-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[*d*] imidazole (IPO 31-51) and 5-((3-(1H-benzo[*d*]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-substituted isoxazole (IPO 52-63) derivatives (Scheme 4):

Scheme 4 methodology involves following stages, key intermediate synthesis of 3-(2-(piperazin-1-yl)substituted)-1H-indole (**IPO 07-09**), cardinal synthesis of 2-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) and chief intermediate synthesis of *N*-hydroxybenzimidoyl chloride (**IIS 25-36**) derivatives. Details are sketched in **Figure 4.8**.

5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-substitutedindolin-2-one (**IPO 13-30**), 2-(1-((substituted-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[*d*] imidazole (**IPO 31-51**) and 5-((3-(1H-benzo[*d]*imidazol-2-yl)-1H-indol-1-yl)methyl)-3-substituted isoxazole (**IPO 52-63**) derivatives are sketched in **Figure 4.9.**

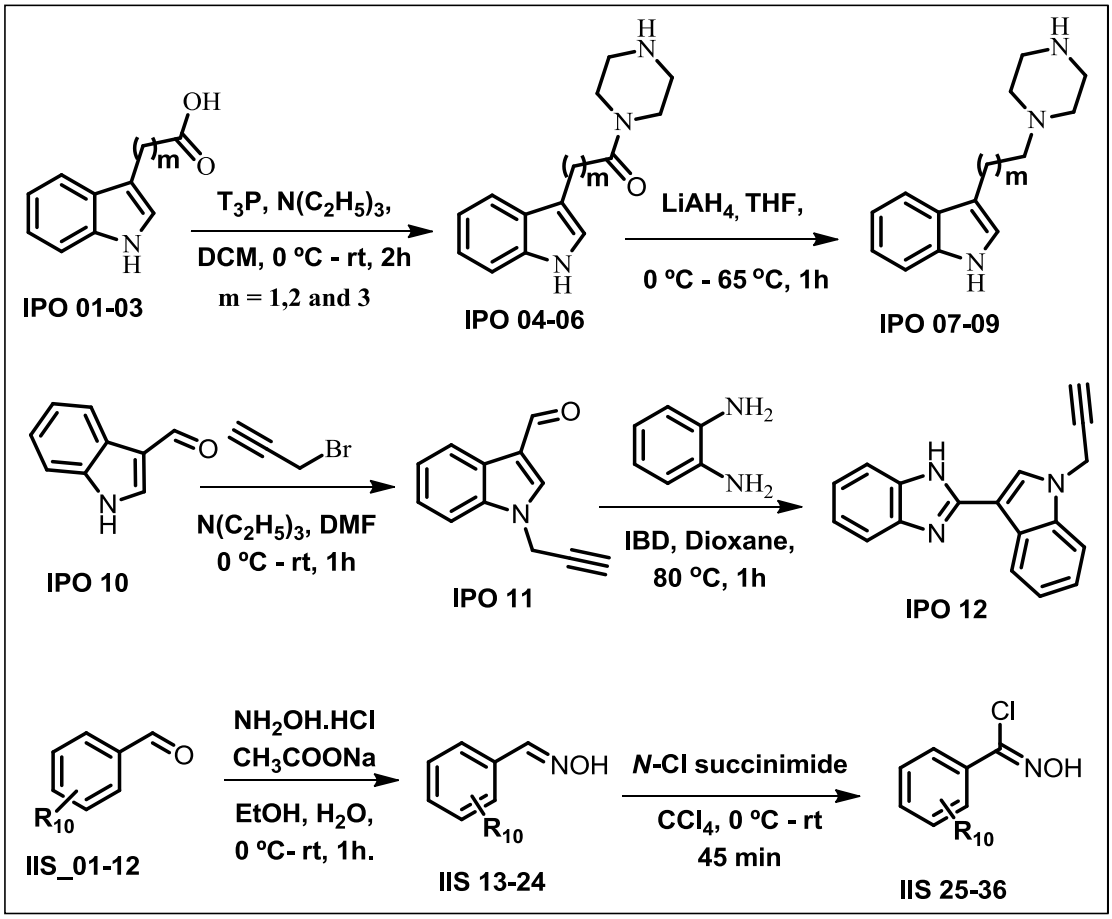


Figure 4.8: Synthetic protocol employed for the synthesis of cardinal synthons for scheme 4

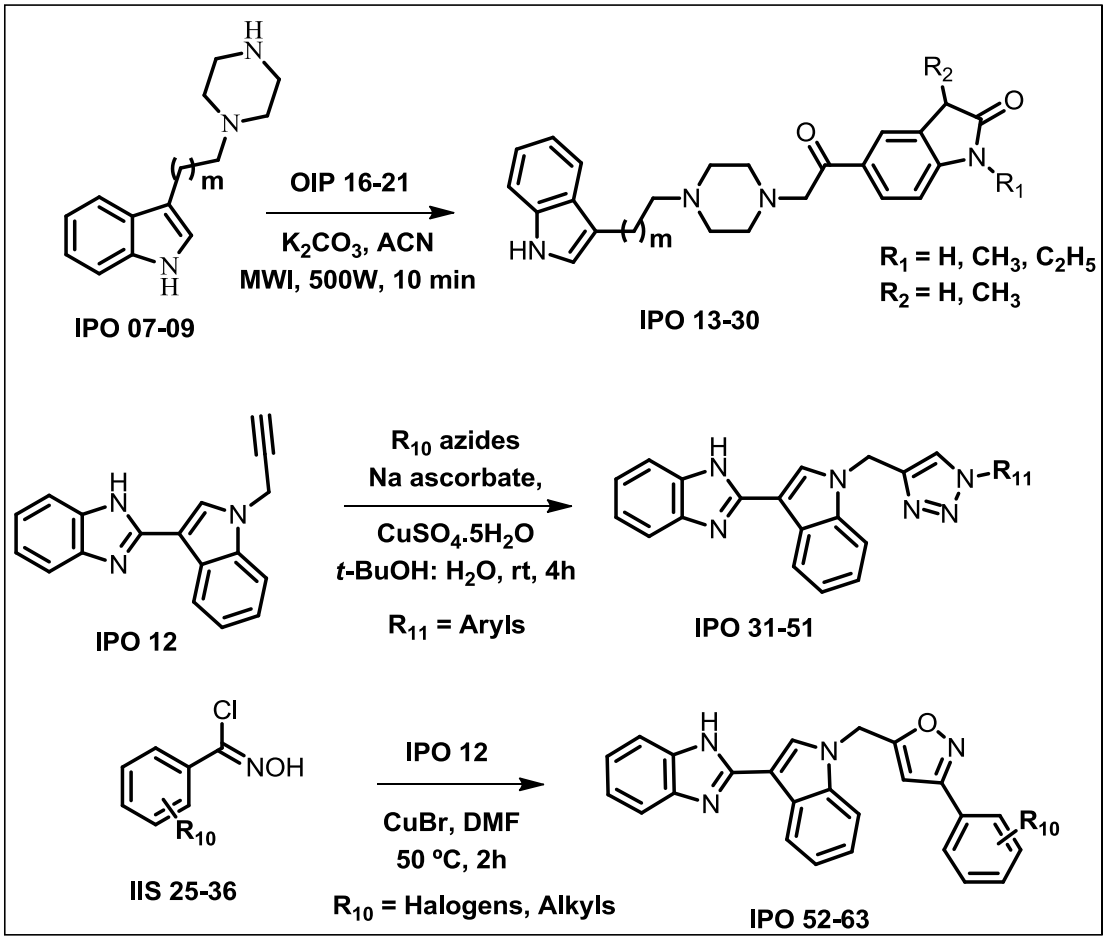


Figure 4.9: Synthetic protocol employed for the synthesis of compounds **IPO 13-63** of scheme 4

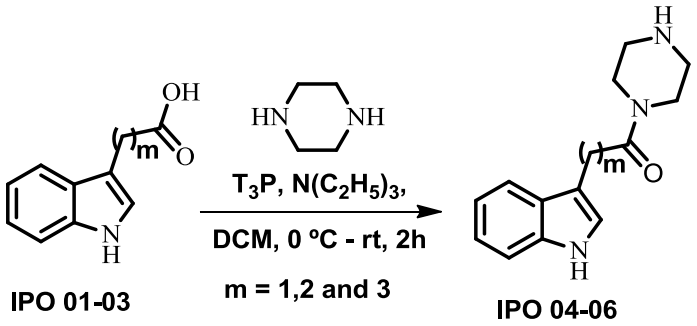
4.2.4.1. General experimental procedure utilized for the synthesis of Indole derivatives (IPO 13-63)

The target molecules were synthesized by following a three to six steps synthetic protocol. We synthesized **IPO 07-09** according to reported protocol with slight modification. Starting from 2-(1H-indol-3-yl) substituted acid (**IPO 01-03**) analogues as outlined in **Figure 4.8**. 2-(1H-indol-3-yl) substituted acid (**IPO 01-03**) on treatment with piperazine, T_3P, TEA and DCM afforded 2-(1H-indol-3-yl)-1-(piperazin-1-yl) substituted-1-one (**IPO 04-06**) [Joshua *et al*., 2011]. Further, **IPO 04-06** compounds are reduced in the presence of $LiAlH_4$ and THF to yield key intermediates 3-(2-(piperazin-1-yl) substituted)-1H-indole (**IPO 07-09**) in good yields [Assimomytis *et al*., 2009]. An essential intermediate 2-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) was synthezed from 1H-indole-3-carbaldehyde (**IPO 10**) on treatment with propargyl bromide in the presence of TEA and DMF to yield 1-(prop-

2-yn-1-yl)-1H-indole-3-carbaldehyde (**IPO 11**) in excellent yield. Further, **IPO 11** on reacting with benzene-1,2-diamine and iodobenzene diacetate (IBD) in the presence of dioxane afforded 2-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) in good yield (**Figure 4.8)** [Du *et al*., 2007]. Another cardinal synthon substituted *N*-hydroxybenzimidoyl chloride (**IIS 25-36**) is prepared from substituted benzaldehydes (**IIS 01-12**) on treatment with $NH_2OH.HCl$ and CH_3COONa in EtOH and H_2O to yield substituted benzaldehyde oxime derivatives (**IIS 13-24**) [Ajay *et al*., 2013]. Further chlorination of **IIS 13-24** with *N*-chlorosuccinimide in CCl_4 afforded substituted *N*-hydroxybenzimidoyl chloride derivatives (**IIS 25-36**) in good to excellent yields (**Figure 4.8)** [Qiao *et al*., 2011].

The target compounds **IPO 13-30** were prepared from 3-(2-(piperazin-1-yl) substituted)-1H-indole (**IPO 07-09**) on reacting with **OIP 16-21** in the presence of K_2CO_3 and acetonitrile to yield 5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)indolin-2-one (**IPO 13-30**) in moderate to good yields (depicted in **Figure 4.9)**. The final compounds **IPO 31-51** are prepared from 2-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) on treatment with R_{11} azides in the presence of Na ascorbate, $CuSO_4.5H_2O$ t-BuOH and H_2O to afford 2-(1-((1-substituted-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 31-51**) derivatives in good to excellent yields. The title compounds **IPO 52-63** are prepared from 2-(1-(prop-2-yn-1-yl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) on treatment with *N*-hydroxybenzimidoyl chloride derivatives (**IIS 25-36**) in the presence of CuBr and DMF to yield 5-((3-(1H-benzo[*d*]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(substituted)isoxazole analogues (**IPO 52-63**) in moderate to good yields [Himo *et al*., 2005; Hansen *et al*., 2005; Waldo and Larock 2005; Kaiser *et al*., 2013; Chen *et al*., 2014].

Preparation of 2-(1H-indol-3-yl)-1-(piperazin-1-yl) substituted-1-one (IPO 04-06)



IPO 01-03 **IPO 04-06**

A solution of 2-(1H-indol-3-yl) substituted acid (**IPO 01-03**) analogues (5.00 g, 1.0 equiv.) in DCM (30 mL) was cooled to 0 °C, After 5 min T_3P (1.1 equiv.) and TEA were added to above mixture and warmed to rt for 2h. After completion of the reaction, as indicated by TLC, the reaction was quenched with water and extracted with DCM. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [DCM / methanol (1 - 6 %)] to afford 2-(1H-indol-3-yl)-1-(piperazin-1-yl) substituted-1-one (**IPO 04-06**) in yields ranging from 80-85%.

2-(1H-indol-3-yl)-1-(piperazin-1-yl)ethan-1-one (**IPO 04)**

Appearance: white solid; Yield: 85% (5.90g); ESI-MS: (m/z) calculated for $C_{14}H_{17}N_3O$: 243.13, found: 244.19 $(M+H)^+$.

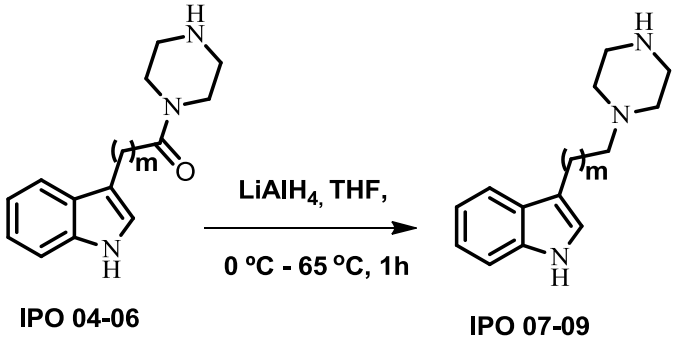
3-(1H-indol-3-yl)-1-(piperazin-1-yl)propan-1-one (**IPO 05)**

Appearance: white solid; Yield: 80% (5.44g); ESI-MS: (m/z) calculated for $C_{15}H_{19}N_3O$: 257.15, found: 258.21 $(M+H)^+$.

4-(1H-indol-3-yl)-1-(piperazin-1-yl)butan-1-one (**IPO 06)**

Appearance: white solid; Yield: 84% (5.61g); ESI-MS: (m/z) calculated for $C_{16}H_{21}N_3O$: 271.16, found: 272.20 $(M+H)^+$.

Preperation of 3-(2-(piperazin-1-yl) substituted)-1H-indole (IPO 07-09)



IPO 04-06 **IPO 07-09**

A solution of 2-(1H-indol-3-yl)-1-(piperazin-1-yl) substituted-1-one (**IPO 04-06**) analogues (5.00 g, 1.0 equiv.) in THF (30 mL) was cooled to -10 °C, after 10 min $LiAlH_4$ (1.0 equiv.) was added slowly to above mixture and warmed to 65 °C for 1h. After completion of the reaction, as indicated by TLC, the reaction was diluted with ethyl acetate and quenched with 5% NaOH and extracted with ethyl acetate. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [DCM / methanol (1 - 5

%)] to afforded key intermediates 3-(2-(piperazin-1-yl) substituted)-1H-indole (**IPO 07-09**) in yields ranging from 75-80%.

3-(2-(piperazin-1-yl)ethyl)-1H-indole (**IPO 07)**

Appearance: off white solid; Yield: 78% (3.67g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (b, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.53 (s, 1H), 7.35–7.30 (m, 2H), 5.12 (b, 1H), 2.78–2.46 (t, 12H). ESI-MS: (m/z) calculated for $C_{14}H_{19}N_3$: 229.15, found: 230.21 $(M+H)^+$.

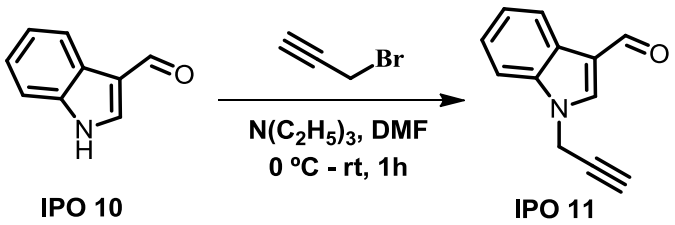
3-(3-(piperazin-1-yl)propyl)-1H-indole (**IPO 08)**

Appearance: white solid; Yield: 75% (3.54g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (b, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.45 (s, 1H), 7.36–7.30 (m, 2H), 5.18 (b, 1H), 3.08–2.46 (t, 12H), 2.03 (m, 2H). ESI-MS: (m/z) calculated for $C_{15}H_{21}N_3$: 243.17, found: 244.23 $(M+H)^+$.

3-(4-(piperazin-1-yl)butyl)-1H-indole (**IPO 09)**

Appearance: white solid; Yield: 80% (3.79g); MP: 142-144 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (b, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.45 (s, 1H), 7.34–7.29 (m, 2H), 5.21 (b, 1H), 3.22–2.45 (t, 12H), 1.68–1.53 (m, 4H). ESI-MS: (m/z) calculated for $C_{16}H_{23}N_3$: 257.18, found: 258.24 $(M+H)^+$.

Preparation of 1-(prop-2-ynyl)-1H-indole-3-carbaldehyde (IPO 11)

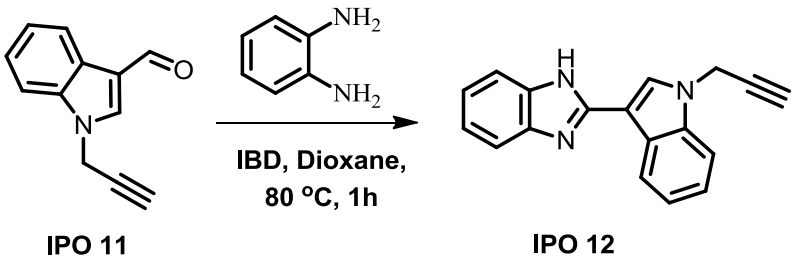


IPO 10 IPO 11

A solution of 1H-indole-3-carbaldehyde (**IPO 10**) (5.00 g, 1.0 equiv.) in DMF was added and cooled to 0 ºC. To this mixture triethylamine (2.0 equiv.) and propargyl bromide (1.1 equiv.) was added and allowed to rt, and stirred for 1h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water, and filtered by buchner funnel and dried over *in vacuo*. The resultant product 1-(prop-2-ynyl)-1H-indole-3-carbaldehyde (**IPO 11**) is used for next step with out purification.

Appearance: white solid; yield: 92% (5.80g); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (b, 1H), 8.39 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 8.07 (s, 1H), 7.68–7.63 (m, 2H), 4.73–3.11 (s, 3H). ESI-MS: (m/z) calculated for $C_{12}H_9NO$: 183.06, found: 184.11 $(M+H)^+$.

Preparation of 2-(1-(prop-2-ynyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (IPO 12)

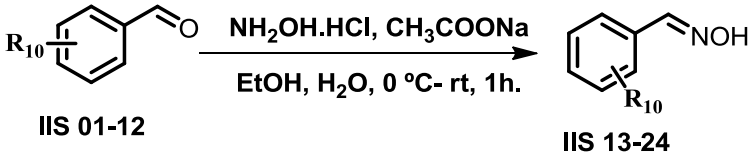


IPO 11 **IPO 12**

To a solution of 1-(prop-2-ynyl)-1H-indole-3-carbaldehyde (**IPO 11**) (5.00 g, 1.0 equiv.), benzene-1,2-diamine (1.0 equiv.) is added in dioxane and cooled to 0 ºC, After 10 min IBD (0.5 equiv.) was added and warmed to 80 ºC, stirred for 1h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with hypo solution and water, extracted with diethyl ether. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (40 - 80 %)] to get the cardinal synthon 2-(1-(prop-2-ynyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) in good yield (80%).

Appearance: brown solid; Yield: 80% (5.92g); IR (KBr) ν_{max}: 3277, 3089, 3048, 2230, 1652, 1601, 1550, 1508, 1450, 1254, 950 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.67 (b, 1H), 8.32 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 1H), 7.58–7.36 (m, 4H), 7.30 (s, 1H), 4.80–3.10 (s, 3H). ESI-MS: (m/z) calculated for $C_{18}H_{13}N_3$: 271.11, found: 272.14 $(M+H)^+$.

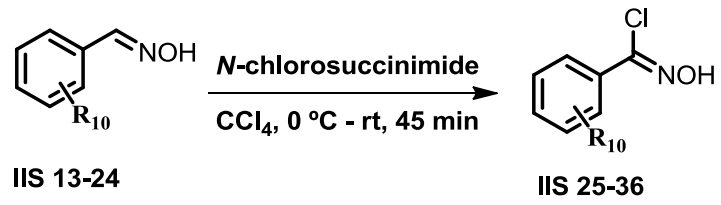
Preparation of benzaldehyde oxime derivatives (IIS 13-24)



IIS 01-12 **IIS 13-24**

A solution of benzaldehyde derivatives **(IIS 01-12)** (2.00 g, 1.0 equiv.) and $NH_2OH.HCl$ (1.2 equiv.) were taken in ethanol (30 mL) and cooled to 0 °C. After 5 min CH_3COONa (1.2 equiv.) in water was added to above mixture and warmed to rt for 1h. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water and filtered by buchner funnel and dried over *in vacuo*. The resultant products are used for next step without purification. The compound yields ranged from 85-96%. All these analaogoues were confirmed by mass spectrometry.

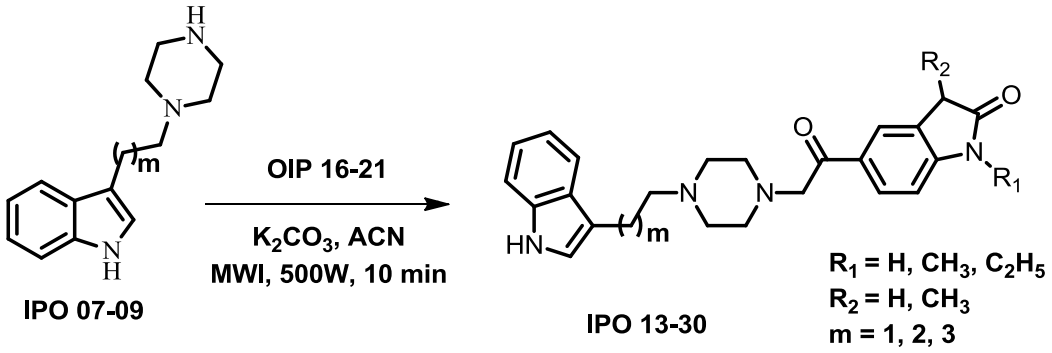
Preparation of *N*-hydroxybenzimidoyl derivatives (IIS 25-36)



IIS 13-24 **IIS 25-36**

A solution of benzaldehyde oxime derivatives **(IIS 13-24)** (1.00 g, 1.0 equiv.) in carbon tetra chloride (10 mL) was cooled to 0 °C. After 5 min *N*-chloro succinimide (1.2 equiv.) is added to above mixture and warmed to rt for 45 min. After completion of the reaction, as indicated by TLC, the reaction was quenched with ice cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. *N*-hydroxybenzimidoyl (**IIS 25-36**) derivatives were obtained in good to excellent yields (85-94%). The resultant products are used for next step with out purification. All these analaogoues were confirmed by mass spectrometry.

4.2.4.2. Synthesis of 5-(2-(4-(2-(1H-indol-3-yl) substituted) piperazin-1-yl) acetyl)-substituted indolin-2-one (IPO 13-30) derivatives and characterization



IPO 07-09 **IPO 13-30**

A solution of 3-(2-(piperazin-1-yl) substituted)-1H-indole (**IPO 07-09**) derivatives (0.30 g, 1.0 equiv.) and K_2CO_3 (1.2 equiv.) were added in ACN (4 ml) and irradiatied on μW at 500W for 10 min. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [methanol / DCM (2 –6 %) to get the title compounds **IPO 13-30** in yields ranging from 60-90%.

Characterization of the synthesized IPO 13-30 title compounds

***5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)indolin-2-one* (IPO 13)**

A solution of 3-(2-(piperazin-1-yl) substituted)-1H-indole (**IPO 07**) 0.30g (1.0 equiv.) and K_2CO_3 (1.2 equiv.) were added in ACN (4 ml) and irradiatied on μW at 500W for 10 min. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with CH_2Cl_2. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [methanol / DCM (4 - 8 %) to achieve 0.34g of 5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)indolin-2-one (**IPO 13**).

Appearance: off white solid; yield = 65% (0.34g); IR (KBr) ν_{max}: 3308, 3098, 3048, 1692, 1601, 1550, 1508, 1450, 1330, 1254, 952 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.95–10.78 (b, 2H), 8.27 (s, 1H), 8.14 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.58 (s, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.22–7.14 (m, 2H), 3.92–3.75 (s, 4H), 2.78–2.58 (t, J = 4.0 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.84, 175.44, 150.72, 136.22, 133.65, 130.11, 129.33, 126.28, 125.32, 124.86, 123.42, 121.46, 120.12, 116.85, 115.43, 114.19, 72.88, 63.47, 63.42, 62.22, 39.68, 27.54. ESI-MS: (m/z) calcd. for $C_{24}H_{26}N_4O_2$, 402.20, found: 403.28 $(M + H)^+$. Anal. Calcd for $C_{24}H_{26}N_4O_2$; (%) C 71.62, H 6.51, N 13.92. Found: C 71.68, H 6.55, N 13.96.

***5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-3-methylindolin-2-one* (IPO 14)**

Appearance: off white solid; yield = 60% (0.32g); IR (KBr) ν_{max}: 3305, 3100, 3052, 1702, 1602, 1548, 1508, 1450, 1324, 1250, 936 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.94–10.79 (b, 2H), 8.29 (s, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.58 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.23–7.16 (m, 2H), 3.98 (q, 1H), 3.88 (s, 2H), 2.83–2.52 (t, J = 4.0 Hz, 12H), 1.61 (d, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.68, 175.47, 150.66, 136.46, 133.28, 130.14, 129.48, 126.08, 124.42, 124.11, 123.33, 121.58, 120.08, 116.88, 116.12, 114.42, 72.74, 63.57, 63.55, 62.39, 45.68, 28.23, 18.27. ESI-MS: (m/z) calcd. for $C_{25}H_{28}N_4O_2$, 416.22, found: 417.16 $(M + H)^+$. Anal. Calcd for $C_{25}H_{28}N_4O_2$; (%) C 72.09, H 6.78, N 13.45. Found: C 72.15, H 6.84, N 13.49.

***5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-1-methylindolin-2-one* (IPO 15)**

Appearance: white solid; yield = 80% (0.43g); IR (KBr) ν_{max}: 3295, 3094, 3048, 1698, 1601, 1550, 1508, 1450, 1254, 959 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.91 (b, 1H), 8.18 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 7.6 Hz, 1H), 7.22–7.14 (m, 2H), 3.90–3.66 (s, 7H), 2.82–2.53 (t, J = 4.0 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.23, 175.40, 150.54, 136.20, 133.61, 130.28,

129.24, 126.31, 125.11, 124.82, 123.36, 121.08, 120.38, 116.81, 115.11, 114.74, 73.08, 63.22, 63.07, 62.78, 42.12, 39.42, 28.12. ESI-MS: (m/z) calcd. for $C_{25}H_{28}N_4O_2$, 416.22, found: 417.24 $(M + H)^+$. Anal. Calcd for $C_{25}H_{28}N_4O_2$; (%) C 72.09, H 6.78, N 13.45. Found: C 72.14, H 6.83, N 13.51.

5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-1,3-dimethylindolin-2-one **(IPO 16)**

Appearance: white solid; yield = 85% (0.47g); IR (KBr) ν_{max}: 3308, 3098, 3048, 1692, 1601, 1550, 1508, 1450, 1330, 1254, 952 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.92 (b, 1H), 8.17 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.24–7.16 (m, 2H), 3.94 (q, 1H), 3.88–3.64 (s, 5H), 2.85–2.52 (t, J = 4.0 Hz, 12H), 1.48 (d, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.48, 175.33, 150.62, 136.45, 133.54, 130.33, 129.22, 126.58, 125.12, 124.46, 123.45, 121.22, 120.08, 116.74, 115.23, 114.88, 73.45, 63.64, 63.12, 62.56, 42.22, 39.78, 28.34, 21.08. ESI-MS: (m/z) calcd. for $C_{26}H_{30}N_4O_2$, 430.23, found: 431.28 $(M + H)^+$. Anal. Calcd for $C_{26}H_{30}N_4O_2$; (%) C 72.53, H 7.02, N 13.01. Found: C 72.59, H 7.06, N 13.08.

5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-1-ethylindolin-2-one **(IPO 17)**

Appearance: off white solid; yield = 79% (0.44g); IR (KBr) ν_{max}: 3301, 3090, 3044, 1707, 1600, 1548, 1502, 1450, 1330, 1251, 951 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.88 (b, 1H), 8.18 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.24–7.15 (m, 2H), 4.22 (q, 2H), 3.92–3.88 (s, 4H), 2.82–2.54 (t, J = 4.0 Hz, 12H), 1.63 (t, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.88, 175.42, 150.73, 136.63, 133.44, 130.08, 129.46, 126.23, 125.34, 124.65, 123.46, 121.71, 120.11, 116.55, 115.22, 114.66, 73.63, 63.42, 63.31, 62.75, 48.29, 39.69, 27.34, 18.22. ESI-MS: (m/z) calcd. for $C_{26}H_{30}N_4O_2$, 430.23, found: 431.26 $(M + H)^+$. Anal. Calcd for $C_{26}H_{30}N_4O_2$; (%) C 72.53, H 7.02, N 13.01. Found: C 72.57, H 7.07, N 13.06.

5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-1-ethyl-3-methylindolin-2-one **(IPO 17)**

Appearance: off white solid; yield = 75% (0.43g); IR (KBr) ν_{max}: 3292, 3102, 3048, 1699, 1601, 1547, 1450, 1329, 1254, 948 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.91 (b, 1H), 8.20 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.23–7.16 (m, 2H), 3.94 (q, 1H), 3.90 (s, 2H), 3.61 (q, 2H), 2.81–2.55 (t, J = 4.0 Hz, 12H), 1.64 (t, J = 2.0 Hz, 3H), 1.51 (d, J = 1.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.43, 175.36, 150.65, 136.52, 133.32, 130.21, 129.55, 126.41, 125.11, 124.81, 123.23, 121.72, 120.24, 116.58, 115.08, 114.58, 73.44, 63.38, 63.11, 62.43,

48.33, 41.58, 27.54, 18.68, 17.22. ESI-MS: (m/z) calcd. for $C_{27}H_{32}N_4O_2$, 444.25, found: 445.28 $(M + H)^+$. Anal. Calcd for $C_{27}H_{32}N_4O_2$; (%) C 72.94, H 7.26, N 12.60. Found: C 72.99, H 7.29, N 12.67.

***5-(2-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)acetyl)indolin-2-one* (IPO 18)**

Appearance: off white solid; yield = 64% (0.32g); IR (KBr) ν_{max}: 3288, 3102, 3048, 1699, 1605, 1550, 1504, 1452, 1325, 1250, 964 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.96–10.81 (b, 2H), 8.29 (s, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.24–7.17 (m, 2H), 3.93–3.88 (s, 4H), 2.83–2.52 (t, J = 4.0 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.55, 175.23, 150.64, 136.28, 133.12, 130.59, 129.11, 126.45, 124.37, 124.23, 123.47, 121.58, 120.12, 116.84, 116.36, 114.41, 72.77, 63.68, 63.12, 62.40, 45.09, 32.11, 27.45. ESI-MS: (m/z) calcd. for $C_{25}H_{28}N_4O_2$, 416.22, found: 417.30 $(M + H)^+$. Anal. Calcd for $C_{25}H_{28}N_4O_2$; (%) C 72.09, H 6.78, N 13.45. Found: C 72.14, H 6.82, N 13.50.

***5-(2-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)acetyl)-3-methylindolin-2-one* (IPO 20)**

Appearance: white solid; yield = 68% (0.36g); IR (KBr) ν_{max}: 3302, 3096, 3049, 1698, 1601, 1550, 1508, 1450, 1330, 1250, 950 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.92–10.88 (b, 2H), 8.18 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.24–7.16 (m, 2H), 3.94 (q, 1H), 3.88 (s, 2H), 2.74–2.50 (t, J = 4.0 Hz, 12H), 2.10–2.06 (m, 2H), 1.49 (d, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.45, 175.28, 150.66, 136.37, 133.54, 130.31, 129.20, 126.58, 125.08, 124.46, 123.45, 121.31, 120.12, 116.74, 115.41, 114.75, 73.52, 63.89, 63.75, 62.22, 42.63, 31.78, 26.34, 18.78. ESI-MS: (m/z) calcd. for $C_{26}H_{30}N_4O_2$, 430.23, found: 431.27 $(M + H)^+$. Anal. Calcd for $C_{26}H_{30}N_4O_2$; (%) C 72.53, H 7.02, N 13.01. Found: C 72.58, H 7.07, N 13.07.

***5-(2-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)acetyl)-1-methylindolin-2-one* (IPO 21)**

Appearance: white solid; yield = 80% (0.42g); IR (KBr) ν_{max}: 3292, 3090, 3059, 1695, 1601, 1550, 1508, 1450, 1330, 1248, 948 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.91 (b, 1H), 8.18 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.23–7.16 (m, 2H), 3.93–3.61 (s, 7H), 2.84–2.57 (t, J = 4.0 Hz, 12H), 2.12–2.07 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.45, 175.69, 150.94, 136.72, 133.40, 130.25, 129.31, 126.22, 125.34, 124.64, 123.46, 121.71, 120.28, 116.63, 115.15, 114.34, 73.68, 63.69, 63.22, 62.01, 40.45, 39.82, 32.34, 26.41. ESI-MS: (m/z) calcd. for $C_{26}H_{30}N_4O_2$, 430.23, found: 431.26 $(M + H)^+$. Anal. Calcd for $C_{26}H_{30}N_4O_2$; (%) C 72.53, H 7.02, N 13.01. Found: C 72.57, H 7.08, N 13.06.

5-(2-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)acetyl)-1,3-dimethylindolin-2-one **(IPO 22)**

Appearance: white solid; yield = 75% (0.41g); IR (KBr) ν_{max}: 3308, 3101, 3048, 1702, 1601, 1550, 1505, 1450, 1328, 1250, 950 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (b, 1H), 8.20 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.42 (d, J = 7.6 Hz, 1H), 7.25–7.16 (m, 2H), 3.96 (q, 1H), 3.90–3.58 (s, 5H), 2.82–2.51 (t, J = 4.0 Hz, 12H), 2.12–2.08 (m, 2H), 1.58 (d, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.56, 175.33, 150.78, 135.91, 133.11, 130.40, 129.34, 126.11, 125.20, 124.42, 123.46, 121.72, 120.24, 116.40, 115.12, 114.27, 73.21, 63.72, 63.20, 62.55, 40.58, 32.11, 30.54, 26.68, 18.43. ESI-MS: (m/z) calcd. for $C_{27}H_{32}N_4O_2$, 444.25, found: 445.32 $(M + H)^+$. Anal. Calcd for $C_{27}H_{32}N_4O_2$; (%) C 72.94, H 7.26, N 12.60. Found: C 72.98, H 7.32, N 12.66.

5-(2-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)acetyl)-1-ethylindolin-2-one **(IPO 23)**

Appearance: white solid; yield = 75% (0.41g); IR (KBr) ν_{max}: 3294, 3099, 3047, 1699, 1601, 1550, 1450, 1333, 1255, 958 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (b, 1H), 8.19 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.57 (s, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.24–7.13 (m, 2H), 3.95 (q, 2H), 3.92–3.86 (s, 4H), 2.83–2.53 (t, J = 4.0 Hz, 12H), 2.11–2.06 (m, 2H), 1.61 (t, J = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.32, 175.64, 150.12, 135.76, 133.40, 130.39, 129.18, 126.24, 125.22, 124.33, 123.91, 121.72, 120.23, 116.40, 115.08, 114.46, 73.65, 61.77, 61.20, 60.55, 42.97, 38.34, 31.50, 26.02, 18.38. ESI-MS: (m/z) calcd. for $C_{27}H_{32}N_4O_2$, 444.25, found: 445.31 $(M + H)^+$. Anal. Calcd for $C_{27}H_{32}N_4O_2$; (%) C 72.94, H 7.26, N 12.60. Found: C 72.99, H 7.31, N 12.64.

5-(2-(4-(3-(1H-indol-3-yl)propyl)piperazin-1-yl)acetyl)-1-ethyl-3-methylindolin-2-one **(IPO 24)**

Appearance: off white solid; yield = 80% (0.45g); IR (KBr) ν_{max}: 3308, 3098, 3048, 1692, 1601, 1550, 1508, 1450, 1327, 1254, 950 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (b, 1H), 8.20 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.55 (s, 1H), 7.49 (d, J = 7.6 Hz, 1H), 7.41 (d, J = 7.2 Hz, 1H), 7.25–7.16 (m, 2H), 4.32 (q, 2H), 3.89 (s, 2H), 2.82–2.56 (t, J = 4.0 Hz, 12H), 2.51 (q, 1H), 2.12–2.07 (m, 2H), 1.63 (t, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.36, 175.55, 150.14, 135.81, 133.34, 130.45, 129.60, 126.38, 125.20, 124.35, 123.85, 121.66, 120.33, 116.42, 115.12, 114.46, 73.73, 61.82, 61.30, 60.64, 43.12, 40.64, 31.64, 26.33, 20.08, 18.38. ESI-MS: (m/z) calcd. for $C_{28}H_{34}N_4O_2$, 458.26, found: 459.30 $(M + H)^+$. Anal. Calcd for $C_{28}H_{34}N_4O_2$; (%) C 73.33, H 7.47, N 12.22. Found: C 73.39, H 7.49, N 12.26.

5-(2-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)acetyl)indolin-2-one **(IPO 25)**

Appearance: off white solid; yield = 62% (0.31g); IR (KBr) ν_{max}: 3284, 3094, 3048, 1702, 1608, 1550, 1508, 1450, 1327, 1248, 963 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92–10.86 (b, 2H), 8.01 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.87 (s, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.42 (d, J = 7.2 Hz, 1H), 7.34 (s, 1H), 7.18–7.14 (m, 2H), 3.91–3.84 (s, 4H), 3.16–2.62 (t, J = 4.0 Hz, 12H), 2.12–1.81 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.88, 176.71, 150.14, 136.33, 133.20, 130.11, 129.63, 126.56, 125.62, 124.33, 123.23, 121.90, 120.38, 116.65, 115.70, 114.55, 73.53, 61.78, 61.69, 60.80, 46.12, 40.23, 33.34, 27.42. ESI-MS: (m/z) calcd. for $C_{26}H_{30}N_4O_2$, 430.23, found: 431.28 $(M + H)^+$. Anal. Calcd for $C_{26}H_{30}N_4O_2$; (%) C 72.53, H 7.02, N 13.01. Found: C 72.59, H 7.07, N 13.04.

***5-(2-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)acetyl)-3-methylindolin-2-one* (IPO 26)**

Appearance: off white solid; yield = 70% (0.36g); IR (KBr) ν_{max}: 3303, 3105, 3033, 1699, 1601, 1549, 1508, 1450, 1334, 1254, 955 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92–10.84 (b, 2H), 8.03 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.88 (s, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 7.2 Hz, 1H), 7.34 (s, 1H), 7.20–7.16 (m, 2H), 3.94 (q, 1H), 3.85 (s, 2H), 3.15–2.61 (t, J = 4.0 Hz, 12H), 2.10–1.82 (m, 4H), 1.46 (d, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.84, 176.67, 150.10, 136.28, 133.22, 130.14, 129.65, 126.55, 125.60, 124.29, 123.24, 121.92, 120.38, 116.65, 115.64, 114.52, 73.48, 61.79, 61.66, 60.74, 46.22, 41.98, 33.47, 27.40, 18.37. ESI-MS: (m/z) calcd. for $C_{27}H_{32}N_4O_2$, 444.25, found: 445.31 $(M + H)^+$. Anal. Calcd for $C_{27}H_{32}N_4O_2$; (%) C 72.94, H 7.26, N 12.60. Found: C 72.99, H 7.31, N 12.64.

***5-(2-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)acetyl)-1-methylindolin-2-one* (IPO 27)**

Appearance: white solid; yield = 81% (0.41g); IR (KBr) ν_{max}: 3305, 3098, 3048, 1697, 1601, 1550, 1508, 1450, 1331, 1250, 954 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (b, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.88 (s, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.33 (s, 1H), 7.19–7.16 (m, 2H), 3.92–3.65 (s, 7H), 3.14–2.62 (t, J = 4.0 Hz, 12H), 2.12–1.80 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.69, 176.65, 150.10, 136.38, 133.24, 130.22, 129.64, 126.56, 125.62, 124.38, 123.21, 121.87, 120.36, 116.34, 115.62, 114.53, 73.50, 61.76, 61.67, 60.74, 46.08, 41.27, 40.11, 33.48, 27.40. ESI-MS: (m/z) calcd. for $C_{27}H_{32}N_4O_2$, 444.25, found: 445.31 $(M + H)^+$. Anal. Calcd for $C_{27}H_{32}N_4O_2$; (%) C 72.94, H 7.26, N 12.60. Found: C 72.99, H 7.32, N 12.65.

***5-(2-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)acetyl)-1,3-dimethylindolin-2-one* (IPO 28)**

Appearance: off white solid; yield = 83% (0.44g); IR (KBr) ν_{max}: 3308, 3098, 3048, 1698, 1604, 1556, 1508, 1454, 1333, 1249, 961 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (b,

1H), 8.16 (s, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.32 (s, 1H), 7.23–7.17 (m, 2H), 3.98 (q, 1H), 3.93–3.58 (s, 5H), 3.17–2.63 (t, *J* = 4.0 Hz, 12H), 2.11–1.79 (m, 4H), 1.67 (d, *J* = 2.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.44, 175.71, 150.10, 135.72, 133.32, 130.56, 129.62, 126.33, 125.22, 124.34, 123.74, 121.68, 120.32, 116.45, 115.22, 114.48, 73.78, 61.90, 61.42, 60.64, 43.17, 40.64, 33.64, 29.33, 26.08, 18.72. ESI-MS: (m/z) calcd. for $C_{28}H_{34}N_4O_2$, 458.26, found: 459.33 $(M + H)^+$. Anal. Calcd for $C_{28}H_{34}N_4O_2$; (%) C 73.33, H 7.47, N 12.22. Found: C 73.38, H 7.49, N 12.28.

5-(2-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)acetyl)-1-ethylindolin-2-one **(IPO 29)**

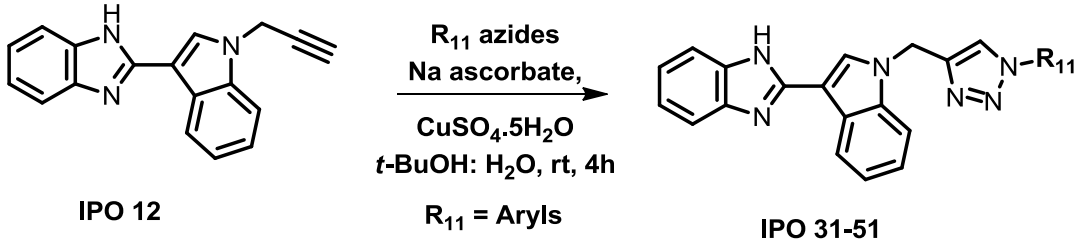
Appearance: off white solid; yield = 79% (0.42g); IR (KBr) ν_{max}: 3297, 3088, 3054, 1698, 1594, 1550, 1505, 1450, 1333, 1259, 958 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (b, 1H), 8.17 (s, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.82(d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.33 (s, 1H), 7.23–7.17 (m, 2H), 4.38 (q, 2H), 3.93–3.64 (s, 4H), 3.18–2.61 (t, *J* = 4.0 Hz, 12H), 2.10–1.81 (m, 4H), 1.66 (t, *J* = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.58, 176.43, 150.12, 136.38, 133.24, 130.22, 129.64, 126.56, 125.62, 124.38, 123.21, 121.87, 120.36, 116.33, 115.62, 114.12, 73.48, 61.88, 61.24, 60.89, 47.11, 45.33, 38.18, 33.48, 28.27, 18.81. ESI-MS: (m/z) calcd. for $C_{28}H_{34}N_4O_2$, 458.26, found: 459.32 $(M + H)^+$. Anal. Calcd for $C_{28}H_{34}N_4O_2$; (%) C 73.33, H 7.47, N 12.22. Found: C 73.37, H 7.52, N 12.29.

5-(2-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)acetyl)-1-ethyl-3-methylindolin-2-one **(IPO 30)**

Appearance: white solid; yield = 81% (0.44g); IR (KBr) ν_{max}: 3295, 3089, 3037, 1704, 1608, 1550, 1508, 1450, 1332, 1254, 959 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (b, 1H), 8.19 (s, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.33 (s, 1H), 7.25–7.19 (m, 2H), 4.08 (q, 1H), 3.89 (s, 2H), 3.78 (q, 2H), 3.18–2.61 (t, *J* = 4.0 Hz, 12H), 2.10–1.78 (m, 4H), 1.69 (t, *J* = 2.0 Hz, 3H), 1.62 (d, *J* = 2.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.63, 175.52, 150.11, 135.63, 133.25, 130.57, 129.64, 126.27, 125.20, 124.34, 123.72, 121.68, 120.32, 116.45, 115.22, 114.48, 73.74, 61.91, 61.43, 60.63, 45.23, 43.18, 40.62, 33.68, 28.30, 19.28, 18.04. ESI-MS: (m/z) calcd. for $C_{29}H_{36}N_4O_2$, 472.28, found: 473.34 $(M + H)^+$. Anal. Calcd for $C_{29}H_{36}N_4O_2$; (%) C 73.70, H 7.68, N 11.85. Found: C 73.76, H 7.82, N 11.89.

4.2.4.3. Synthesis of 2-(1-((1-substituted-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-

1H-benzo[*d*]imidazole (IPO 31-51) derivatives and characterization



A solution of 2-(1-(prop-2-ynyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) (0.30 g, 1.0 equiv.) is reacted with varius aryl azides (1.2 equiv.) in the presence of $CuSO_4.5H_2O$ (0.02 equiv.), Na ascorbate (0.01 equiv.) and *t*-BuOH: H_2O (2:1), at rt for 4h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with ethyl acetate. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (70 - 90 %)] to get the **IPO 31-51** compounds in yields ranging from 70-92%.

Characterization of the synthesized IPO 31-51 title compounds

2-(1-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole **(IPO 31)**

A solution of 2-(1-(prop-2-ynyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) 0.30g (1.0 equiv.) is reacted with azidobenzene (1.2 equiv.) in the presence of $CuSO_4.5H_2O$ (0.02 equiv.), Na ascorbate (0.02 equiv.) and *t*-BuOH: H_2O (2:1), at rt for 4h. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with ethyl acetate. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (70 - 80 %)] to achieved 0.31g of 2-(1-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 31**).

Appearance: off white solid; yield = 74% (0.31g); IR (KBr) ν_{max}: 3292, 3090, 3045, 1654, 1600, 1555, 1508, 1450, 1330, 1258, 962 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.62 (b, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.21 (s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.83 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 7.2 Hz, 1H), 7.69–7.40 (m, 7H), 7.23 (s, 1H), 5.51 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.74, 146.48, 138.24, 137.81, 134.58, 131.20, 131.61, 130.85, 125.24, 123.58,

123.11, 122.57, 121.74, 120.90, 119.62, 114.53, 112.86, 58.76. ESI-MS: (m/z) calcd. for $C_{24}H_{18}N_6$, 390.15, found: 391.24 $(M + H)^+$. Anal. Calcd for $C_{24}H_{18}N_6$; (%) C 73.83, H 4.65, N 21.52. Found: C 73.87, H 4.69, N 21.56.

***2-(1-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 32)**

Appearance: off white solid; yield = 70% (0.31g); IR (KBr) ν_{max}: 3295, 3092, 3048, 1650, 1602, 1550, 1508, 1450, 1333, 1258, 956 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.60 (b, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.23 (s, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 7.6 Hz, 2H), 7.68–7.41 (m, 6H), 7.21 (s, 1H), 5.60 (s, 2H), 2.64 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.81, 146.42, 140.20, 139.44, 136.90, 135.63, 133.68, 131.82, 130.68, 125.22, 123.54, 123.12, 121.81, 120.64, 118.31, 114.97, 111.55, 58.72, 26.18. ESI-MS: (m/z) calcd. for $C_{25}H_{20}N_6$, 404.17, found: 405.26 $(M + H)^+$. Anal. Calcd for $C_{25}H_{20}N_6$; (%) C 74.24, H 4.98, N 20.78. Found: C 74.29, H 5.02, N 20.82.

***2-(1-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 33)**

Appearance: pale brown solid; yield = 80% (0.37g); IR (KBr) ν_{max}: 3298, 3099, 3055, 1654, 1600, 1552, 1504, 1450, 1335, 1258, 960 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.61 (b, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.22 (s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 7.6 Hz, 1H), 7.68–7.40 (m, 6H), 7.19 (s, 1H), 5.61 (s, 2H) 2.91 (q, 2H), 1.48 (t, J = 2.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.90, 146.11, 143.23, 139.04, 136.87, 135.71, 133.59, 131.63, 130.71, 129.34, 124.88, 123.32, 122.79, 121.82, 120.69, 118.22, 114.50, 112.08, 58.70, 33.20, 18.12. ESI-MS: (m/z) calcd. for $C_{26}H_{22}N_6$, 418.19, found: 419.22 $(M + H)^+$. Anal. Calcd for $C_{26}H_{22}N_6$; (%) C 74.62, H 5.30, N 20.08. Found: C 74.66, H 5.35, N 20.12.

***2-(1-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 34)**

Appearance: off white solid; yield = 75% (0.33g); IR (KBr) ν_{max}: 3288, 3097, 3045, 1651, 1600, 1555, 1508, 1450, 1327, 1258, 955, 782 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.59 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.18 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 3H), 7.68–7.32 (m, 6H), 7.23 (s, 1H), 5.62 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.71, 155.64, 144.31, 139.36, 134.94, 131.10, 125.25, 124.71, 123.62, 122.81, 121.64, 120.48, 119.94, 119.24, 114.52, 112.07, 58.70. ESI-MS: (m/z) calcd. for $C_{24}H_{17}FN_6$, 408.14, found: 409.21 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}FN_6$; (%) C 70.58, H 4.20, N 20.58. Found: C

70.62, H 4.24, N 20.63.

***2-(1-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 35)**

Appearance: white solid; yield = 92% (0.43g); IR (KBr) ν_{max}: 3290, 3093, 3045, 1654, 1590, 1551, 1508, 1450, 1333, 1258, 960, 828 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.63 (b, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.19 (s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 7.6 Hz, 2H), 7.67–7.42 (m, 6H), 7.20 (s, 1H), 5.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.11, 144.36, 139.26, 136.68, 135.94, 131.68, 130.88, 125.13, 124.50, 123.62, 121.10, 120.73, 119.31, 114.98, 112.51, 58.68. ESI-MS: (m/z) calcd. for $C_{24}H_{17}ClN_6$, 424.12, found: 425.21 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}ClN_6$; (%) C 67.84, H 4.03, N 19.78. Found: C 67.88, H 4.07, N 19.82.

***2-(1-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 36)**

Appearance: white solid; yield = 86% (0.40g); IR (KBr) ν_{max}: 3290, 3088, 3005, 1655, 1608, 1550, 1509, 1450, 1336, 1246, 955, 704 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.63 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.20 (s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 7.6 Hz, 2H), 7.71 (d, J = 78.0 Hz, 2H), 7.65–7.40 (m, 4H), 7.21 (s, 1H), 5.68 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.23, 144.33, 139.11, 136.97, 135.25, 133.72, 130.84, 130.02, 124.88, 124.50, 123.60, 122.23, 121.84, 120.78, 118.76, 114.12, 112.04, 58.73. ESI-MS: (m/z) calcd. for $C_{24}H_{17}BrN_6$, 424.12, found: 425.21 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}BrN_6$; (%) C 61.42, H 3.65, N 17.91. Found: C 61.48, H 3.69, N 17.94.

***2-(1-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 37)**

Appearance: white solid; yield = 88% (0.42g); IR (KBr) ν_{max}: 3287, 3089, 3052, 1654, 1605, 1555, 1508, 1450, 1333, 1247, 955 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.63 (b, 1H), 8.47 (d, J = 8.4 Hz, 2H), 8.31 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 7.6 Hz, 2H), 8.18 (s, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 1H), 7.66–7.42 (m, 4H), 7.23 (s, 1H), 5.73 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.64, 149.27, 144.80, 143.46, 139.02, 134.75, 131.72, 130.84, 126.19, 125.21, 123.68, 122.47, 122.07, 121.78, 120.76, 118.07, 114.73, 111.40, 58.72. ESI-MS: (m/z) calcd. for $C_{24}H_{17}BrN_6$, 435.14, found: 436.22 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}BrN_6$; (%) C 66.20, H 3.94, N 22.52. Found: C 66.26, H 3.99, N 22.56.

***2-(1-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 38)**

Appearance: pale brown solid; yield = 78% (0.36g); IR (KBr) ν_{max}: 3290, 3095, 3022, 1651, 1604, 1558, 1500, 1450, 1331, 1253, 972, 848 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.62 (b, 1H), 8.38 (d, J = 8.4 Hz, 1H), 8.19 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 7.6 Hz, 1H), 7.63–7.40 (m, 4H), 7.23 (s, 1H), 5.70–4.12 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.27, 155.30, 144.10, 139.72, 134.91, 131.28, 130.88, 130.10, 124.82, 124.12, 123.58, 122.62, 121.72, 120.16, 118.91, 118.10, 114.81, 112.31, 59.11, 58.88. ESI-MS: (m/z) calcd. for $C_{25}H_{20}N_6O$, 420.16, found: 421.12 $(M + H)^+$. Anal. Calcd for $C_{25}H_{20}N_6O$; (%) C 71.41, H 4.79, N 19.99. Found: C 71.45, H 4.83, N 20.03.

***2-(1-((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 39)**

Appearance: off white solid; yield = 75% (0.38g); IR (KBr) ν_{max}: 3295, 3087, 3049, 1653, 1600, 1555, 1508, 1450, 1330, 1258, 955, 788 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.59 (b, 1H), 8.38 (d, J = 8.4 Hz, 1H), 8.21 (s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.80 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 7.6 Hz, 2H), 7.69–7.40 (m, 6H), 7.21 (s, 1H), 5.70 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.72, 144.12, 141.08, 139.63, 137.22, 134.90, 132.68, 130.88, 129.08, 125.78, 124.68, 124.11, 122.10, 121.73, 121.23, 120.45, 118.10, 114.92, 111.98, 58.63. ESI-MS: (m/z) calcd. for $C_{25}H_{17}F_3N_6$, 458.14, found: 459.22 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}F_3N_6$; (%) C 65.50, H 3.74, N 18.33. Found: C 65.54, H 3.79, N 18.37.

***2-(1-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 40)**

Appearance: off white solid; yield = 87% (0.40g); IR (KBr) ν_{max}: 3292, 3090, 3045, 1654, 1600, 1555, 1508, 1450, 1330, 1258, 962, 862 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.62 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.18 (s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.66–7.42 (m, 7H), 7.21 (s, 1H), 5.72 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.23, 144.71, 139.10, 134.88, 134.25, 133.92, 133.12, 131.76, 131.08, 130.83, 130.28, 124.92, 123.62, 122.78, 121.22, 120.65, 118.47, 114.75, 112.63, 58.69. ESI-MS: (m/z) calcd. for $C_{24}H_{17}ClN_6$, 424.12, found: 425.21 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}ClN_6$; (%) C 67.84, H 4.03, N 19.78. Found: C 67.89, H 4.08, N 19.83.

***2-(1-((1-(2-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 41)**

Appearance: off white solid; yield = 82% (0.38g); IR (KBr) ν_{max}: 3288, 3092, 3005, 1655, 1606, 1550, 1505, 1450, 1333, 1246, 955, 712 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.67 (b, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.22 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.80 (d, J = 7.6 Hz,

1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.71–7.43 (m, 7H), 7.20 (s, 1H), 5.69 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.60, 144.21, 138.89, 136.25, 135.68, 134.73, 132.84, 131.58, 130.23, 128.22, 124.87, 124.24, 123.70, 122.45, 121.89, 120.46, 118.72, 114.68, 112.63, 58.78. ESI-MS: (m/z) calcd. for $C_{24}H_{17}BrN_6$, 424.12, found: 425.21 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}BrN_6$; (%) C 61.42, H 3.65, N 17.91. Found: C 61.47, H 3.68, N 17.96.

2-(1-((1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole **(IPO 42)**

Appearance: off white solid; yield = 84% (0.40g); IR (KBr) ν_{max}: 3290, 3085, 3005, 1650, 1604, 1550, 1509, 1450, 1330, 1249, 959, 834 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (b, 1H), 8.48 (d, *J* = 8.4 Hz, 2H), 8.21 (s, 1H), 8.26 (m, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.79–7.44 (m, 5H), 7.23 (s, 1H), 5.75 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.87, 148.12, 144.39, 139.08, 136.24, 134.70, 131.87, 130.76, 129.65, 129.10, 126.62, 124.49, 123.88, 122.19, 121.64, 120.54, 118.42, 114.35, 112.00, 58.69. ESI-MS: (m/z) calcd. for $C_{24}H_{17}BrN_6$, 435.14, found: 436.22 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}BrN_6$; (%) C 66.20, H 3.94, N 22.52. Found: C 66.25, H 3.99, N 22.58.

2-(1-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole **(IPO 43)**

Appearance: pale brown solid; yield = 79% (0.36g); IR (KBr) ν_{max}: 3288, 3097, 3010, 1652, 1609, 1550, 1512, 1450, 1331, 1245, 953, 854 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (b, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.18 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.64–7.42 (m, 6H), 7.23 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.71–4.12 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.89, 155.22, 144.68, 143.74, 189.72, 134.83, 131.76, 130.84, 130.12, 124.79, 124.10, 123.63, 122.64, 121.55, 120.80, 118.88, 118.13, 114.88, 113.24, 112.07, 59.12, 58.83. ESI-MS: (m/z) calcd. for $C_{25}H_{20}N_6O$, 420.16, found: 421.24 $(M + H)^+$. Anal. Calcd for $C_{25}H_{20}N_6O$; (%) C 71.41, H 4.79, N 19.99. Found: C 71.46, H 4.82, N 20.05.

2-(1-((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole **(IPO 44)**

Appearance: off white solid; yield = 80% (0.38g); IR (KBr) ν_{max}: 3289, 3098, 3012, 1650, 1605, 1550, 1500, 1450, 1332, 1249, 950, 848 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (b, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.33 (d, *J* = 7.6 Hz, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 8.21–8.08 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.74–7.43 (m, 5H), 7.22 (s, 1H), 5.76 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.65, 144.71, 139.89, 137.74, 137.22, 134.87, 131.76, 130.85, 130.31, 125.62, 124.21, 123.87, 122.63, 121.77, 120.65, 118.40,

114.28, 113.50, 112.09, 58.72. ESI-MS: (m/z) calcd. for $C_{24}H_{17}BrN_6$, 435.14, found: 436.24 $(M + H)^+$. Anal. Calcd for $C_{24}H_{17}BrN_6$; (%) C 66.20, H 3.94, N 22.52. Found: C 66.24, H 3.98, N 22.57.

***2-(1-((1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 45)**

Appearance: off white solid; yield = 75% (0.38g); IR (KBr) ν_{max}: 3292, 3075, 3011, 1652, 1608, 1550, 1509, 1450, 1335, 1248, 950, 784 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.61 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.22–8.14 (s, 2H), 7.93 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.67–7.42 (m, 5H), 7.23 (s, 1H), 5.71 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.66, 144.54, 139.11, 137.22, 134.84, 134.32, 132.88, 130.84, 130.22, 130.58, 126.78, 125.64, 124.23, 123.34, 122.17, 121.44, 121.45, 120.55, 118.07, 114.78, 112.32, 58.68. ESI-MS: (m/z) calcd. for $C_{25}H_{17}F_3N_6$, 458.14, found: 459.18 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}F_3N_6$; (%) C 65.50, H 3.74, N 18.33. Found: C 65.55, H 3.77, N 18.39.

***2-(1-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 46)**

Appearance: off white solid; yield = 78% (0.36g); IR (KBr) ν_{max}: 3282, 3090, 3012, 1652, 1608, 1552, 1505, 1450, 1328, 1255, 955, 822 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.66 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.21 (s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.71 (d, J = 7.6 Hz, 1H), 7.64–7.45 (m, 4H), 7.29 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 5.60 (s, 2H), 2.69 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.69, 143.82, 139.48, 138.74, 134.93, 133.76, 130.82, 130.28, 126.46, 125.54, 124.82, 123.61, 122.64, 121.38, 120.48, 118.11, 114.87, 112.44, 58.77, 25.94. ESI-MS: (m/z) calcd. for $C_{25}H_{20}N_6$, 418.19, found: 419.20 $(M + H)^+$. Anal. Calcd for $C_{25}H_{20}N_6$; (%) C 74.62, H 5.30, N 20.08. Found: C 74.66, H 5.35, N 20.12.

***2-(1-((1-(3,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 47)**

Appearance: white solid; yield = 74% (0.34g); IR (KBr) ν_{max}: 3290, 3095, 3005, 1655, 1608, 1550, 1509, 1450, 1336, 1246, 955, 768 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.61 (b, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.19 (s, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.74 (d, J = 8.0 Hz, 1H), 7.69–7.32 (m, 7H), 7.21 (s, 1H), 5.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.87, 151.49, 143.96, 138.42, 134.28, 131.02, 130.74, 127.33, 126.69, 123.60, 122.83, 122.64, 121.73, 120.44, 119.90, 119.11, 118.68, 114.63, 112.18, 58.75. ESI-MS: (m/z) calcd. for

$C_{24}H_{16}F_2N_6$, 426.14, found: 427.22 $(M + H)^+$. Anal. Calcd for $C_{24}H_{16}F_2N_6$; (%) C 67.60, H 3.78, N 19.71. Found: C 67.64, H 3.82, N 19.75.

***2-(1-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 48)**

Appearance: white solid; yield = 85% (0.43); IR (KBr) ν_{max}: 3292, 3054, 3012, 1655, 1608, 1550, 1509, 1450, 1332, 1246, 955, 860 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.61 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.17 (s, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 7.6 Hz, 1H), 7.64–7.41 (m, 6H), 7.21 (s, 1H), 5.76 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.79, 144.65, 139.29, 136.23, 134.92, 134.34, 133.46, 131.08, 130.68, 124.76, 123.55, 123.78, 122.63, 121.87, 120.72, 120.08, 118.69, 114.58, 112.44, 58.72. ESI-MS: (m/z) calcd. for $C_{24}H_{16}Cl_2N_6$, 458.08, found: 459.10 $(M + H)^+$. Anal. Calcd for $C_{24}H_{16}Cl_2N_6$; (%) C 62.76, H 3.51, N 18.30. Found: C 62.81, H 3.55, N 18.34.

***2-(1-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 49)**

Appearance: white solid; yield = 78% (0.38g); IR (KBr) ν_{max}: 3291, 3084, 3012, 1650, 1610, 1550, 1509, 1450, 1336, 1246, 958, 868, 733 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.64 (b, 1H), 8.38 (d, J = 8.4 Hz, 1H), 8.23–8.03 (s, 2H), 7.92 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.70–7.32 (m, 6H), 7.21 (s, 1H), 5.67 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.28, 155.36, 143.91, 138.44, 134.63, 131.00, 130.79, 128.33, 124.78, 124.24, 123.88, 122.92, 121.23, 120.80, 120.11, 119.12, 118.90, 118.33, 114.56, 112.22, 58.74. ESI-MS: (m/z) calcd. for $C_{24}H_{16}ClFN_6$, 442.11, found: 443.20 $(M + H)^+$. Anal. Calcd for $C_{24}H_{16}ClFN_6$; (%) C 65.09, H 3.64, N 18.98. Found: C 65.13, H 3.69, N 19.02.

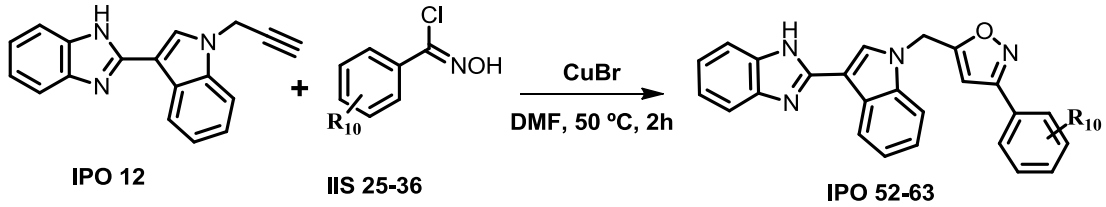
***2-(1-((1-(4-chloro-2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole* (IPO 50)**

Appearance: white solid; yield = 81% (0.42g); IR (KBr) ν_{max}: 3287, 3063, 3011, 1652, 1607, 1550, 1504, 1450, 1333, 1248, 955, 862 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.69 (b, 1H), 8.53 (s, 1H), 8.38 (d, J = 8.4 Hz, 1H), 8.21 (s, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 8.8 Hz, 2H), 7.76 (d, J = 7.6 Hz, 1H), 7.68–7.43 (m, 3H), 7.24 (s, 1H), 5.76 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.88, 147.10, 144.64, 138.78, 136.90, 135.63, 134.72, 131.12, 130.78, 129.65, 127.60, 125.42, 124.88, 123.19, 122.64, 121.54, 120.68, 118.54, 114.68, 112.22, 58.71. ESI-MS: (m/z) calcd. for $C_{24}H_{16}ClN_7O_2$, 469.10, found: 470.18 $(M + H)^+$. Anal. Calcd for $C_{24}H_{16}ClN_7O_2$; (%) C 61.35, H 3.43, N 20.87. Found: C 61.39, H 3.47, N 20.91.

2-(1-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[d]imidazole **(IPO 51)**

Appearance: off white solid; yield = 74% (0.35g); IR (KBr) ν_{max}: 3292, 3063, 3003, 1654, 1610, 1550, 1506, 1450, 1329, 1246, 955, 712 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (b, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.18–8.01 (s, 2H), 7.93 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.63–7.42 (m, 4H), 7.23 (s, 1H), 5.74 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.88, 144.63, 138.29, 134.47, 133.92, 132.34, 131.46, 131.11, 130.78, 129.76, 124.95, 124.42, 123.97, 123.11, 122.72, 122.28, 121.24, 121.02, 120.63, 118.48, 114.72, 112.47, 58.74. ESI-MS: (m/z) calcd. for $C_{25}H_{16}BrF_3N_6$, 536.05, found: 537.14 $(M + H)^+$. Anal. Calcd for C $C_{25}H_{16}BrF_3N_6$; (%) C 55.88, H 3.00, N 15.64. Found: C 55.92, H 3.04, N 15.69.

4.2.4.4. Synthesis of 5-((3-(1H-benzo[*d*]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-phenylisoxazole (IPO 52-63) derivatives and characterization



A solution of 2-(1-(prop-2-ynyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) (0.30 g, 1.0 equiv.) is reacted with varius *N*-hydroxybenzimidoyl (**IIS 25-36**) derivatives (1.2 equiv.) in the presence of CuBr (0.5 equiv.) in DMF and warmed to 50 °C for 2h. It is Sonaghasira coupling followed by cyclization. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with ethyl acetate. The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude products were purified by column chromatography [ethyl acetate / hexane (60 - 80 %)] to get the **IPO 52-63** compounds in yields ranging from 65-85%.

Characterization of the synthesized IPO 52-63 compounds

5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-phenylisoxazole **(IPO 52)**

A solution of 2-(1-(prop-2-ynyl)-1H-indol-3-yl)-1H-benzo[*d*]imidazole (**IPO 12**) 0.30g (1.0 equiv.) is reacted with *N*-hydroxybenzimidoyl chloride (**IIS 25**) (1.2 equiv.) in the presence of CuBr (0.5 equiv.) in DMF and warmed to 50 °C for 2h. It is sonaghasira coupling followed

by cyclization. Once completion of the reaction, as indicated by TLC, the reaction was quenched with cold water and extracted with ethyl acetate. The organic layer was collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude product was purified by column chromatography [ethyl acetate / hexane (60 – 70 %)] to achieve 0.31g of 5-((3-(1H-benzo[*d*]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-phenylisoxazole (**IPO 52**).

Appearance: pale brown solid; yield = 72% (0.31g); IR (KBr) ν_{max}: 3285, 3068, 3012, 1652, 1600, 1548, 1508, 1450, 1333, 1251, 950 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.26 (b, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 7.6 Hz, 2H), 7.89 (d, J = 8.0 Hz, 3H), 7.77–7.72 (s, 2H), 7.64–7.39 (m, 7H), 5.27 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.34, 161.22, 154.08, 149.67, 138.24, 131.64, 130.88, 130.27, 128.91, 128.35, 125.06, 124.58, 124.11, 121.37, 116.62, 114.53, 108.86, 58.76. ESI-MS: (m/z) calcd. for $C_{25}H_{18}N_4O$, 390.14, found: 391.22 $(M + H)^+$. Anal. Calcd for $C_{25}H_{18}N_4O$; (%) C 76.91, H 4.65, N 14.35. Found: C 76.95, H 4.69, N 14.38.

***5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-p-tolylisoxazole* (IPO 53)**

Appearance: pale brown solid; yield = 77% (0.34g); IR (KBr) ν_{max}: 3292, 3065, 3002, 1650, 1605, 1551, 1508, 1450, 1332, 1251, 953 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.29 (b, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 7.6 Hz, 2H), 7.77 (d, J = 8.0 Hz, 3H), 7.72–7.68 (s, 2H), 7.63 (m, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.38 (m, 2H), 5.25–2.67 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.68, 162.06, 154.34, 149.60, 138.39, 132.12, 131.22, 130.75, 130.04, 128.92, 128.31, 127.34, 124.67, 123.18, 121.66, 117.52, 115.53, 109.22, 58.24, 27.11. ESI-MS: (m/z) calcd. for $C_{26}H_{20}N_4O$, 404.14, found: 404.20 $(M + H)^+$. Anal. Calcd for $C_{26}H_{20}N_4O$; (%) C 77.21, H 4.98, N 13.85. Found: C 77.27, H 5.03, N 13.88.

***5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(4-fluorophenyl)isoxazole* (IPO 54)**

Appearance: off whit solid; yield = 78% (0.35g); IR (KBr) ν_{max}: 3292, 3080, 3002, 1652, 1607, 1548, 1500, 1450, 1331, 1251, 950, 756 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.46 (b, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 7.6 Hz, 2H), 7.92 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 8.0 Hz, 1H), 7.65–7.48 (m, 6H), 7.44–7.38 (s, 2H), 5.36 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.36, 164.17, 161.12, 155.62, 144.24, 138.32, 133.17, 130.95, 128.91, 125.74, 124.63, 124.23, 124.01, 123.65, 121.35, 118.44, 114.21, 109.81, 58.54. ESI-MS: (m/z) calcd. for $C_{25}H_{17}ClN_4O$, 408.13, found: 409.18 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}ClN_4O$; (%) C 73.52, H 4.20, N 13.72. Found: C 73.58, H 4.24, N 13.77.

5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(4-chlorophenyl)isoxazole **(IPO 55)**

Appearance: off white solid; yield = 80% (0.37g); IR (KBr) ν_{max}: 3285, 3063, 3010, 1653, 1605, 1548, 1508, 1450, 1333, 1251, 950, 848 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.30 (b, 1H), 8.35 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 7.6 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.69 (d, J = 8.0 Hz, 1H), 7.63–7.41 (m, 4H), 7.23–7.20 (s, 2H), 5.69 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.50, 162.12, 153.77, 144.64, 138.44, 135.12, 131.32, 130.86, 130.44, 127.88, 126.11, 124.68, 124.20, 122.61, 120.22, 116.55, 109.67, 58.24. ESI-MS: (m/z) calcd. for $C_{25}H_{17}ClN_4O$, 424.10, found: 425.18 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}ClN_4O$; (%) C 70.67, H 4.03, N 13.19. Found: C 70.72, H 4.08, N 13.23.

5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(4-bromophenyl)isoxazole **(IPO 56)**

Appearance: pale brown solid; yield = 72% (0.37g); IR (KBr) ν_{max}: 3292, 3055, 3011, 1650, 1604, 1548, 1508, 1450, 1333, 1251, 950, 715 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.30 (b, 1H), 8.39 (d, J = 8.4 Hz, 1H), 8.08 (d, J = 7.6 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.71 (d, J = 8.0 Hz, 1H), 7.64–7.40 (m, 4H), 7.24–7.20 (s, 2H), 5.71 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.52, 162.07, 153.80, 144.60, 138.42, 135.15, 131.28, 130.84, 130.49, 127.90, 126.10, 124.70, 124.22, 122.34, 120.21, 116.48, 109.15, 58.20. ESI-MS: (m/z) calcd. for $C_{25}H_{17}BrN_4O$, 468.05, found: 469.12 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}BrN_4O$; (%) C 63.98, H 3.65, N 11.94. Found: C 64.04, H 3.68, N 11.99.

5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(4-nitrophenyl)isoxazole **(IPO 57)**

Appearance: pale brown solid; yield = 80% (0.38g); IR (KBr) ν_{max}: 3292, 3055, 3011, 1650, 1607, 1555, 1506, 1450, 1331, 1251, 958, 825 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.29 (b, 1H), 8.58 (d, J = 8.0 Hz, 2H), 8.33 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 7.6 Hz, 2H), 7.91 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.0 Hz, 1H), 7.65–7.41 (m, 4H), 7.23–7.21 (s, 2H), 5.68 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.12, 162.33, 153.80, 150.28, 143.42, 137.24, 133.31, 132.84, 130.33, 127.91, 126.22, 125.70, 124.31, 123.27, 121.11, 120.38, 116.46, 111.20, 58.32. ESI-MS: (m/z) calcd. for $C_{25}H_{17}N_5O_3$, 435.13, found: 436.18 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}N_5O_3$; (%) C 68.96, H 3.94, N 16.08. Found: C 68.99, H 3.98, N 16.12.

4-(5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)isoxazol-3-yl)phenol **(IPO 58)**

Appearance: brown solid; yield = 65% (0.29g); IR (KBr) ν_{max}: 3518, 3294, 3072, 3010, 1652, 1605, 1548, 1508, 1450, 1331, 1251, 950, 855 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.36–

10.91 (b, 2H), 8.31 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 2H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.64 (m, 1H), 7.59 (d, *J* = 7.6 Hz, 2H), 7.55–7.41 (m, 3H), 7.24–7.20 (s, 2H), 5.69 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.22, 162.13, 161.76, 154.07, 142.38, 137.59, 132.90, 132.25, 130.12, 128.44, 126.22, 125.12, 124.82, 123.65, 121.80, 120.64, 116.97, 111.34, 58.11. ESI-MS: (m/z) calcd. for $C_{25}H_{18}N_4O_2$, 406.14, found: 407.18 $(M + H)^+$. Anal. Calcd for $C_{25}H_{18}N_4O_2$; (%) C 73.88, H 4.46, N 13.78. Found: C 73.92, H 4.49, N 13.83.

***5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(2-fluorophenyl)isoxazole* (IPO 59)**

Appearance: pale brown solid; yield = 73% (0.32g); IR (KBr) ν_{max}: 3277, 3065, 3008, 1652, 1600, 1548, 1508, 1450, 1330, 1249, 955, 768 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (b, 1H), 8.32 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 7.6 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.67–7.45 (m, 7H), 7.25–7.36 (s, 2H), 5.45 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.44, 161.38, 161.11, 142.26, 138.32, 132.34, 131.99, 128.91, 125.74, 124.61, 124.22, 124.02, 123.65, 122.08, 121.35, 119.4, 114.21, 109.84, 58.57. ESI-MS: (m/z) calcd. for $C_{25}H_{17}ClN_4O$, 408.13, found: 409.21 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}ClN_4O$; (%) C 73.52, H 4.20, N 13.72. Found: C 73.58, H 4.25, N 13.76.

***5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(2-chlorophenyl)isoxazole* (IPO 60)**

Appearance: off white solid; yield = 80% (0.37g); IR (KBr) ν_{max}: 3284, 3066, 3011, 1652, 1600, 1548, 1508, 1450, 1329, 1251, 958, 872 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (b, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.61–7.40 (m, 6H), 7.22–7.18 (s, 2H), 5.79 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.53, 161.82, 153.89, 144.23, 138.07, 134.12, 132.30, 131.86, 130.11, 127.88, 126.11, 124.68, 124.20, 122.61, 121.03, 120.22, 116.11, 109.14, 58.72. ESI-MS: (m/z) calcd. for $C_{25}H_{17}ClN_4O$, 424.10, found: 425.20 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}ClN_4O$; (%) C 70.67, H 4.03, N 13.19. Found: C 70.71, H 4.09, N 13.22.

***5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(2-bromophenyl)isoxazole* (IPO 61)**

Appearance: off white solid; yield = 75% (0.38g); IR (KBr) ν_{max}: 3288, 3067, 3009, 1650, 1610, 1553, 1508, 1450, 1332, 1249, 964, 718 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (b, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.65–7.42 (m, 6H), 7.25–7.18 (s, 2H), 5.63 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.48, 162.12, 154.89, 144.63, 141.11, 138.01, 133.49,

131.23, 130.52, 129.11, 127.72, 126.13, 124.72, 124.45, 122.12, 120.78, 116.32, 109.10, 58.23. ESI-MS: (m/z) calcd. for $C_{25}H_{17}BrN_4O$, 468.05, found: 469.18 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}BrN_4O$; (%) C 63.98, H 3.65, N 11.94. Found: C 64.03, H 3.69, N 11.98.

***5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(2-nitrophenyl)isoxazole* (IPO 62)**

Appearance: pale brown solid; yield = 74% (0.35g); IR (KBr) ν_{max}: 3292, 3064, 3010, 1658, 1600, 1548, 1508, 1450, 1329, 1251, 953, 812 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.41 (b, 1H), 8.41 (d, J = 8.0 Hz, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.19 (d, J = 7.6 Hz, 1H), 7.98–7.94 (m, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.0 Hz, 1H), 7.66–7.42 (m, 4H), 7.22–7.19 (s, 2H), 5.69 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.65, 162.08, 154.92, 150.20, 143.72, 138.08, 136.33, 132.85, 130.53, 130.11, 127.91, 126.22, 125.70, 124.31, 123.88, 122.38, 121.11, 120.09, 118.25, 117.46, 110.07, 58.62. ESI-MS: (m/z) calcd. for $C_{25}H_{17}N_5O_3$, 435.13, found: 436.21 $(M + H)^+$. Anal. Calcd for $C_{25}H_{17}N_5O_3$; (%) C 68.96, H 3.94, N 16.08. Found: C 68.98, H 3.99, N 16.15.

***5-((3-(1H-benzo[d]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-(2,4-dichlorophenyl)isoxazole* (IPO 63)**

Appearance: white solid; yield = 78% (0.39g); IR (KBr) ν_{max}: 3288, 3067, 3010, 1655, 1606, 1548, 1508, 1450, 1330, 1251, 968, 854 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.38 (b, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.0 Hz, 1H), 7.68 (s, 1H), 7.60–7.42 (m, 5H), 7.23–7.18 (s, 2H), 5.68 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.88, 161.76, 154.37, 144.65, 138.28, 136.11, 134.72, 132.10, 131.02, 129.88, 128.68, 127.68, 124.22, 122.68, 121.88, 120.20, 118.02, 116.14, 112.20, 58.78. ESI-MS: (m/z) calcd. for $C_{25}H_{16}Cl_2N_4O$, 458.07, found: 459.14 $(M + H)^+$. Anal. Calcd for $C_{25}H_{16}Cl_2N_4O$; (%) C 65.37, H 3.51, N 12.20. Found: C 65.41, H 3.55, N 12.26.

4.3. Biological evauations

4.3.1. *In vitro* anti-mycobacterial screening by MABA assay

All the synthesized novel compounds were evaluated for anti-mycobacterial screening for their ability to inhibit the growth of MTB H37Rv (ATCC 27294) strain by Microplate Alamar Blue Assay (MABA) as per earlier reported procedure [Collins *et al.*, 1997; Franzblau *et al.*, 1998]. Briefly, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic

acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No.1, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37 °C in normal atmosphere. After 7 days of incubation, 30 µL of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidised state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour. Each reaction was carried out in triplicates.

4.3.2. Cytotoxicity assay

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

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

Chapter V
Results & Discussion

Chapter 5

RESULTS AND DISCUSSION

5.1. Physical and biological results and discussion of the title compounds

All four schemes of synthezed novel compounds physical properties and biological results are given in this chapter. To enhance the development of potent anti-TB drug discovery process, recently many non-profit organisations/institutes and commercial organisations are working together. They are providing preliminary screening results of thousands opounds, to allow the researchers worldwide to utilize the high quality screening data for developing new leads and potential anti-TB agents.

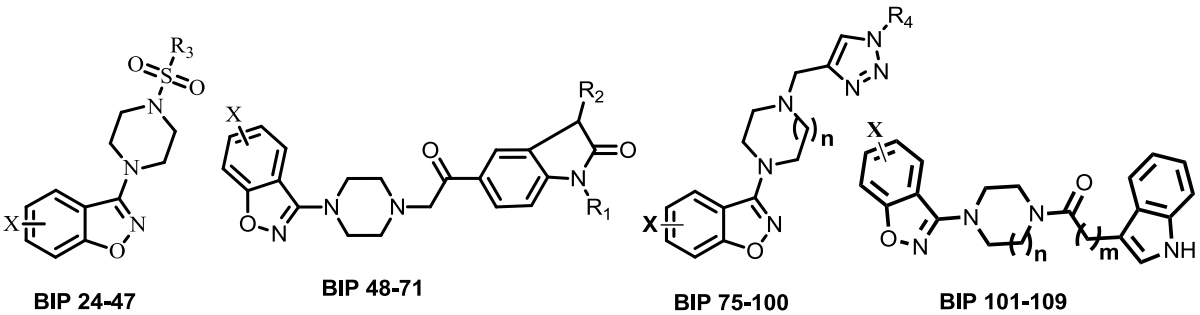
5.2. Physical properties and biological results of 3-(piperazin-1-yl/homopiperazine)benzo[*d*]isoxazole (scheme 1) derivatives

5.2.1. General spectral data and physical properties of scheme1 derivatives

IR, Solid compounds were analysed by KBr disc method and semi-solid compounds were recorded as neat samples. ν_{max} is expressed in cm^{-1}: 3610–3450 (O-H stretch), 3455–3250 (N-H stretch), 3062–3032 (aromatic C-H stretch); 2918–2744 (aliphatic C-H stretch); 2268–2140 (C≡C stretch); 1800–1646 (C=O stretch); 1650–1487 (aromatic C=C stretch); 1356–1150 (aliphatic C-O, C-N stretch); 1100–750 (C-Halogen stretch).

In NMR spectra, the NH and OH protons are seen as broad peaks in the range δ 12.40–9.50, aromatic protons in the range δ 9.90–6.52 and alphatic protons in the range δ 5.60–1.24. ^{13}C NMR, carbonyl group is obtained at δ 180.50–160.00 aromatic carbons in the range δ 160.00–105.00 and alphatic carbons in the range δ 80.00–12.00. Physiochemical properties of the synthesized compounds **BIP 24**–**109** are given in **Table 5.1**.

Table 5.1: Physiochemical properties of the synthesized compounds **BIP 24**–**109**



Compound	X	R_1 /n	$R_2/R_3/R_4$/m	Molecular formula	Molecular weight	M.P. (°C)
BIP 24	H	-	Me	$C_{12}H_{15}N_3O_3S$	281.33	168-169
BIP 25	H	-	Ph	$C_{17}H_{17}N_3O_3S$	343.40	174-176
BIP 26	H	-	4-Me Ph	$C_{17}H_{19}N_3O_3S$	345.41	162-163
BIP 27	H	-	4-OCF_3 Ph	$C_{18}H_{16}F_3N_3O_4S$	427.39	181-182
BIP 28	H	-	4-NO_2 Ph	$C_{17}H_{16}N_4O_5S$	388.39	230-232
BIP 29	H	-	4-Br Ph	$C_{17}H_{16}BrN_3O_3S$	422.30	202-204
BIP 30	4-Cl	-	Me	$C_{12}H_{14}ClN_3O_3S$	315.78	169-171
BIP 31	4-Cl	-	Ph	$C_{17}H_{16}ClN_3O_3S$	377.85	164-165
BIP 32	4-Cl	-	4-Me Ph	$C_{18}H_{18}ClN_3O_3S$	391.87	187-188
BIP 33	4-Cl	-	4-OCF_3 Ph	$C_{18}H_{15}ClF_3N_3O_4S$	461.84	116-118
BIP 34	4-Cl	-	4-NO_2 Ph	$C_{17}H_{15}ClN_4O_5S$	422.84	216-218
BIP 35	4-Cl	-	4-Br Ph	$C_{17}H_{15}BrClN_3O_3S$	456.74	195-196
BIP 36	6-Cl	-	Me	$C_{12}H_{14}ClN_3O_3S$	315.78	194-196
BIP 37	6-Cl	-	Ph	$C_{17}H_{16}ClN_3O_3S$	377.85	202-204
BIP 38	6-Cl	-	4-Me Ph	$C_{18}H_{18}ClN_3O_3S$	391.87	185-186
BIP 39	6-Cl	-	4-OCF_3 Ph	$C_{18}H_{15}ClF_3N_3O_4S$	461.84	176-177
BIP 40	6-Cl	-	4-NO_2 Ph	$C_{17}H_{15}ClN_4O_5S$	422.84	232-234
BIP 41	6-Cl	-	4-Br Ph	$C_{17}H_{15}BrClN_3O_3S$	456.74	212-213
BIP 42	7-Cl	-	Me	$C_{12}H_{14}ClN_3O_3S$	315.78	172-173
BIP 43	7-Cl	-	Ph	$C_{17}H_{16}ClN_3O_3S$	377.85	175-176
BIP 44	7-Cl	-	4-Me Ph	$C_{18}H_{18}ClN_3O_3S$	391.87	159-161
BIP 45	7-Cl	-	4-OCF_3 Ph	$C_{18}H_{15}ClF_3N_3O_4S$	461.84	170-172
BIP 46	7-Cl	-	4-NO_2 Ph	$C_{17}H_{15}ClN_4O_5S$	422.84	242-244
BIP 47	7-Cl	-	4-Br Ph	$C_{17}H_{15}BrClN_3O_3S$	456.74	187-188
BIP 48	H	H	H	$C_{21}H_{20}N_4O_3$	376.41	164-165
BIP 49	H	H	CH_3	$C_{22}H_{22}N_4O_3$	390.44	Oil
BIP 50	H	CH_3	H	$C_{22}H_{22}N_4O_3$	390.44	178-179

Compound	X	R_1 /n	$R_2/R_3/R_4$/m	Molecular formula	Molecular weight	M.P. (°C)
BIP 51	H	CH_3	CH_3	$C_{23}H_{24}N_4O_3$	404.47	114-115
BIP 52	H	C_2H_5	H	$C_{23}H_{24}N_4O_3$	404.47	174-175
BIP 53	H	C_2H_5	CH_3	$C_{24}H_{26}N_4O_3$	418.49	120-121
BIP 54	4-Cl	H	H	$C_{21}H_{19}ClN_4O_3$	410.85	Semi solid
BIP 55	4-Cl	H	CH_3	$C_{22}H_{21}ClN_4O_3$	424.88	149-150
BIP 56	4-Cl	CH_3	H	$C_{22}H_{21}ClN_4O_3$	424.88	191-192
BIP 57	4-Cl	CH_3	CH_3	$C_{23}H_{23}ClN_4O_3$	438.91	209-210
BIP 58	4-Cl	C_2H_5	H	$C_{23}H_{23}ClN_4O_3$	438.91	188-189
BIP 59	4-Cl	C_2H_5	CH_3	$C_{24}H_{25}ClN_4O_3$	452.93	158-159
BIP 60	6-Cl	H	H	$C_{21}H_{19}ClN_4O_3$	410.85	Oil
BIP 61	6-Cl	H	CH_3	$C_{22}H_{21}ClN_4O_3$	424.88	129-131
BIP 62	6-Cl	CH_3	H	$C_{22}H_{21}ClN_4O_3$	424.88	177-179
BIP 63	6-Cl	CH_3	CH_3	$C_{23}H_{23}ClN_4O_3$	438.91	99-101
BIP 64	6-Cl	C_2H_5	H	$C_{23}H_{23}ClN_4O_3$	438.91	169-171
BIP 65	6-Cl	C_2H_5	CH_3	$C_{24}H_{25}ClN_4O_3$	452.93	122-123
BIP 66	7-Cl	H	H	$C_{21}H_{19}ClN_4O_3$	410.85	Oil
BIP 67	7-Cl	H	CH_3	$C_{22}H_{21}ClN_4O_3$	424.88	168-170
BIP 68	7-Cl	CH_3	H	$C_{22}H_{21}ClN_4O_3$	424.88	203-204
BIP 69	7-Cl	CH_3	CH_3	$C_{23}H_{23}ClN_4O_3$	438.91	208-209
BIP 70	7-Cl	C_2H_5	H	$C_{23}H_{23}ClN_4O_3$	438.91	163-164
BIP 71	7-Cl	C_2H_5	CH_3	$C_{24}H_{25}ClN_4O_3$	452.93	149-151
BIP 75	H	1	4-Et Ph	$C_{22}H_{24}N_6O$	388.47	224-226
BIP 76	H	1	4-F Ph	$C_{20}H_{19}FN_6O$	378.41	146-147
BIP 77	H	1	4-Cl Ph	$C_{20}H_{19}ClN_6O$	394.86	159-160
BIP 78	H	1	4-Br Ph	$C_{20}H_{19}BrN_6O$	439.31	157-159
BIP 79	H	1	4-NO_2 Ph	$C_{20}H_{19}N_7O_3$	405.41	204-205
BIP 80	H	1	4-OMe Ph	$C_{21}H_{22}N_6O_2$	390.44	141-142
BIP 81	H	1	3-Cl Ph	$C_{20}H_{19}ClN_6O$	394.86	121-122

Compound	X	R_1 /n	$R_2/R_3/R_4$/m	Molecular formula	Molecular weight	M.P. (°C)
BIP 82	H	1	3-OMe Ph	$C_{21}H_{22}N_6O_2$	390.44	130-132
BIP 83	H	1	3-CF_3 Ph	$C_{21}H_{19}F_3N_6O$	428.41	108-109
BIP 84	H	1	4-Br-3-CF_3 Ph	$C_{21}H_{18}BrF_3N_6O$	507.31	Oil
BIP 85	6-Cl	1	4-Et Ph	$C_{22}H_{23}ClN_6O$	422.91	157-158
BIP 86	6-Cl	1	4-F Ph	$C_{20}H_{18}ClFN_6O$	412.85	Semi solid
BIP 87	6-Cl	1	4-Cl Ph	$C_{20}H_{18}Cl_2N_6O$	429.30	168-170
BIP 88	6-Cl	1	4-Br Ph	$C_{20}H_{18}BrClN_6O$	473.75	192-194
BIP 89	6-Cl	1	4-NO_2 Ph	$C_{20}H_{18}ClN_7O_3$	439.86	199-201
BIP 90	6-Cl	1	4-OMe Ph	$C_{21}H_{21}ClN_6O_2$	424.88	171-173
BIP 91	H	2	4-Et Ph	$C_{23}H_{26}N_6O$	402.50	102-103
BIP 92	H	2	4-F Ph	$C_{21}H_{21}FN_6O$	392.43	138-139
BIP 93	H	2	4-Cl Ph	$C_{21}H_{21}ClN_6O$	408.89	153-154
BIP 94	H	2	4-Br Ph	$C_{21}H_{21}BrN_6O$	453.34	146-147
BIP 95	H	2	4-NO_2 Ph	$C_{21}H_{21}N_7O_3$	419.44	176-177
BIP 96	H	2	4-OMe Ph	$C_{22}H_{24}N_6O_2$	404.47	108-109
BIP 97	H	2	3-Cl Ph	$C_{21}H_{21}ClN_6O$	408.89	174-176
BIP 98	H	2	3-OMe Ph	$C_{22}H_{24}N_6O_2$	404.47	Oil
BIP 99	H	2	3-CF_3 Ph	$C_{22}H_{21}F_3N_6O$	442.44	136-137
BIP 100	H	2	4-Br-3-CF_3 Ph	$C_{22}H_{20}BrF_3N_6O$	521.34	Oil
BIP 101	H	1	1	$C_{21}H_{20}N_4O_2$	360.41	153-154
BIP 102	H	1	2	$C_{22}H_{22}N_4O_2$	374.44	Oil
BIP 103	H	1	3	$C_{23}H_{24}N_4O_2$	388.41	162-164
BIP 104	6-Cl	1	1	$C_{21}H_{19}ClN_4O_2$	394.85	196-198
BIP 105	6-Cl	1	2	$C_{22}H_{21}ClN_4O_2$	408.88	164-166
BIP 106	6-Cl	1	3	$C_{23}H_{23}ClN_4O_2$	422.91	Oil
BIP 107	H	2	1	$C_{22}H_{22}N_4O_2$	374.44	147-149
BIP 108	H	2	2	$C_{23}H_{24}N_4O_2$	388.41	205-207
BIP 109	H	2	3	$C_{24}H_{26}N_4O_2$	402.49	107-108

5.2.2. *In vitro* MTB screening, cytotoxicity studies and selectivity index of the synthesized molecules

All the synthesized compounds were first screened for their *in vitro* anti-tubercular activity against MTB H37Rv (ATCC 27294) strain by Microplate Alamar Blue Assay (MABA) with compound concentration ranging from 50 to 0.78 µg/mL. Isoniazid, ethambutol and pyrazinamide were used as reference compounds for comparison. The *in vitro* test results for title compounds tabulated in **Table 5.2** indicate that MIC ranges from 1.56 to ≥ 50 µg/mL. Amongst, most active compounds were also tested for *in vitro* cytotoxicity against RAW 264.7cells at 50 µg/mL concentration using MTT assay. All the results are presented in **Table 5.2**.

Table 5.2: *In vitro* biological evaluation of synthesized compounds **BIP 24–109**

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
BIP 24	12.5	28.43	67.86	10.85
BIP 25	3.12	5.82	429.55	137.45
BIP 26	50	NT	NT	NT
BIP 27	>50	NT	NT	NT
BIP 28	>50	NT	NT	NT
BIP 29	50	NT	NT	NT
BIP 30	>50	NT	NT	NT
BIP 31	25	NT	NT	NT
BIP 32	6.25	21.60	115.74	18.51
BIP 33	12.5	42.16	59.29	4.74
BIP 34	50	NT	NT	NT
BIP 35	50	NT	NT	NT
BIP 36	>50	NT	NT	NT
BIP 37	25	NT	NT	NT
BIP 38	>50	NT	NT	NT
BIP 39	12.5	18.10	138.12	11.04

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
BIP 40	25	NT	NT	NT
BIP 41	>50	NT	NT	NT
BIP 42	25	NT	NT	NT
BIP 43	25	NT	NT	NT
BIP 44	>50	NT	NT	NT
BIP 45	>50	NT	NT	NT
BIP 46	50	NT	NT	NT
BIP 47	25	NT	NT	NT
BIP 48	50	NT	NT	NT
BIP 49	25	NT	NT	NT
BIP 50	6.25	35.62	70.18	11.22
BIP 51	6.25	22.80	109.64	17.54
BIP 52	3.12	38.42	65.07	20.85
BIP 53	1.56	36.46	68.56	43.95
BIP 54	25	NT	NT	NT
BIP 55	25	NT	NT	NT
BIP 56	12.5	NT	NT	NT
BIP 57	6.25	38.10	65.61	10.49
BIP 58	1.56	35.62	70.18	44.99
BIP 59	3.12	40.40	61.88	19.83
BIP 60	>50	NT	NT	NT
BIP 61	12.5	NT	NT	NT
BIP 62	6.25	42.80	58.41	9.34
BIP 63	1.56	46.43	53.84	34.51
BIP 64	3.12	31.76	78.71	25.22
BIP 65	1.56	44.60	56.05	35.93
BIP 66	>50	NT	NT	NT

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
BIP 67	50	NT	NT	NT
BIP 68	12.5	NT	NT	NT
BIP 69	6.25	28.92	86.44	13.83
BIP 70	3.12	30.16	82.89	26.56
BIP 71	3.12	22.16	112.81	36.15
BIP 75	>50	NT	NT	NT
BIP 76	50	NT	NT	NT
BIP 77	>50	NT	NT	NT
BIP 78	>50	NT	NT	NT
BIP 79	6.25	42.08	59.41	9.50
BIP 80	50	NT	NT	NT
BIP 81	12.5	31.76	78.71	6.29
BIP 82	50	NT	NT	NT
BIP 83	6.25	24.25	103.05	16.48
BIP 84	25	NT	NT	NT
BIP 85	>50	NT	NT	NT
BIP 86	3.12	19.16	130.48	41.82
BIP 87	25	NT	NT	NT
BIP 88	>50	NT	NT	NT
BIP 89	50	NT	NT	NT
BIP 90	6.25	22.12	113.01	18.08
BIP 91	>50	NT	NT	NT
BIP 92	12.5	26.52	94.26	7.54
BIP 93	50	NT	NT	NT
BIP 94	>50	NT	NT	NT
BIP 95	25	NT	NT	NT
BIP 96	50	NT	NT	NT

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
BIP 97	6.25	30.76	81.24	13.00
BIP 98	50	NT	NT	NT
BIP 99	12.5	42.16	59.29	4.74
BIP 100	25	NT	NT	NT
BIP 101	50	NT	NT	NT
BIP 102	>50	NT	NT	NT
BIP 103	25	NT	NT	NT
BIP 104	50	NT	NT	NT
BIP 105	12.5	20.45	122.24	9.77
BIP 106	25	NT	NT	NT
BIP 107	50	NT	NT	NT
BIP 108	6.25	18.55	134.77	21.56
BIP 109	25	NT	NT	NT
INH	0.1	-	-	-
TAP	0.33	-	-	-
EMB	1.56	-	-	-
PZA	6.25	-	-	-

[a]Minimum inhibitory concentration *in vitro* activity against MTB H37Rv strain; [b]Cytotoxicity against RAW 264.7 cells; [c]IC_{50}, 50% inhibitory concentration; [d]SI: selectivity index; NT: not tested; INH: Isoniazid; TAP: GSK888636A; EMB: Ethambutol; PZA: Pyrazinamide.

5.2.3. SAR and discussion

All the synthesized compounds showed activity against MTB H37Rv strain with MIC ranging from 1.56 to ≥50 µg/mL. Amongst twenty two compounds (**BIP 25**, **BIP 32, BIP 50-53, BIP 57-59, BIP 62-65, BIP 69-71, BIP 79, BIP 83, BIP 86, BIP 90, BIP 97** and **BIP 108**) inhibited MTB with MIC of ≤ 6.25 µg/mL, out of these eleven compounds (**BIP 25**, **BIP 52-53, BIP 58-59, BIP 63-65, BIP 70-71** and **BIP 86**) exhibited good anti-TB activity with MIC ≤ 3.12. Four compounds (**BIP 53, BIP 58, BIP 63** and **BIP 65**) were found to be better than ethambutol and pyrazinamide. Compounds **BIP 53, BIP 58, BIP 63** and **BIP 65** were found to be the most active compounds with *in vitro* MICs of ≤ 1.56 µg/mL and these

are more potent than pyrazinamide (MIC 6.25 µg/mL). When compared to ethambutol and pyrazinamide these four compounds are lead analogues showing better anti-TB activity in **scheme 1** derivatives.

Further, with respect to SAR, the compounds containing benzo[*d*]isoxazole with oxindole analogues (**BIP 48-71**) showed better activities than compounds with benzo[*d*]isoxazole with sulphonamides, triazoles and indole derivatives. The order of activity was benzo[*d*]isoxazole with oxindoles enhanced the activity 32-fold from ≥ 50 to 1.56 µg/mL. When benzo[*d*]isoxazole with sulphonamides, triazoles and indoles decreased the activity by 8-folds. Amongst, oxindole ring containing compounds substituted at 1 and 3rd position of analogues have enhanced activity by 32-fold.

Furthermore, most active anti-TB compounds exhibited good IC_{50} values and selectivity index are tabulated in **Table 5.2**. These compounds (**BIP 53, BIP 58, BIP 63** and **BIP 65**) showed SI ≥ 34, indicating that they are not toxic and might be considered for further structural modification, appropriation of the chemophores in further drug development.

5.2.4. Highlights of the scheme 1

Based on the activity results of previous reports, we anticipated that replacing benzo[*d*]isoxazole with oxindole, heterocyclic amines would lead to increase in activity. The most active compound (**BIP 53, BIP 58, BIP 63** and **BIP 65**) from the **scheme 1** were taken as lead molecules for further extension of library. We decided to synthesis molecules starting with Oxindole heterocyclic derivatives. As expected, we found four molecules exhibiting greater than or equal to MTB MIC than standard anti-TB drugs viz. ethambutol and pyrazinamide. The most active compounds **BIP 53, BIP 58, BIP 63** and **BIP 65** (**Figure 5.1**) showed MTB MIC 1.56 µg/mL.

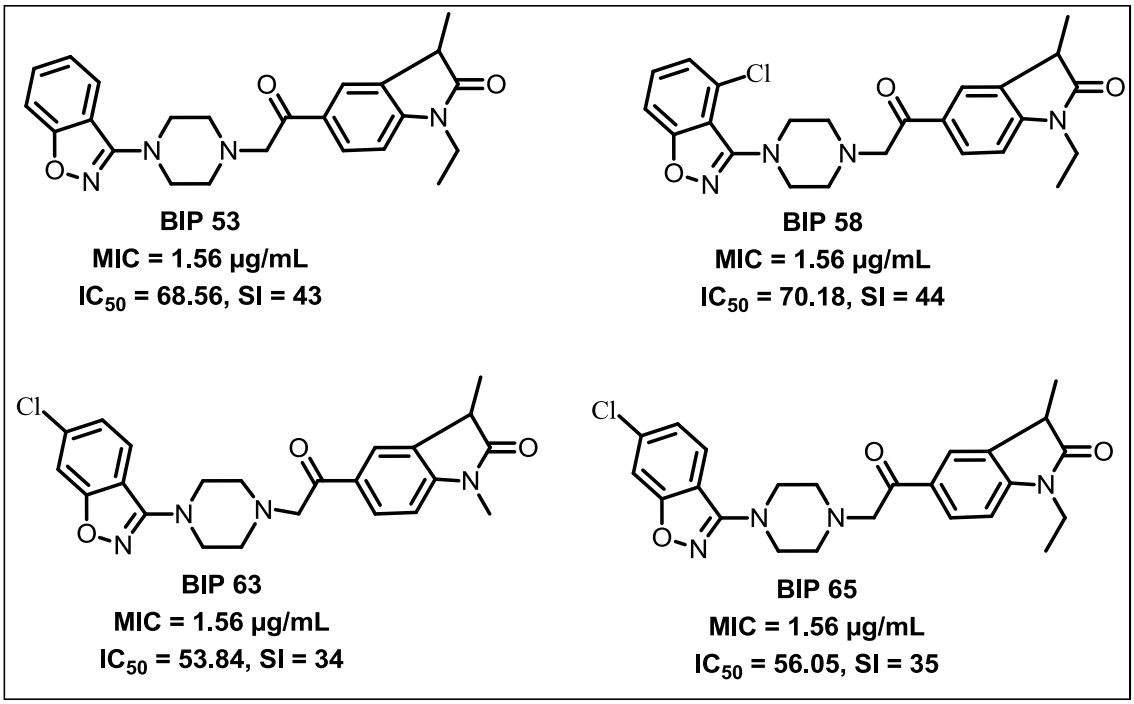


Figure 5.1: Chemical structures and anti-TB activity of the most active compounds (**BIP 53, BIP 58, BIP 63** and **BIP 65**)

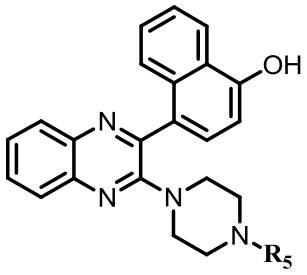
5.3. Physical properties and biological results of 4-(quinoxalin-2-yl)naphthalen-1-ol (scheme 2) derivatives

5.3.1. General spectral data and physical properties of scheme 2 derivatives

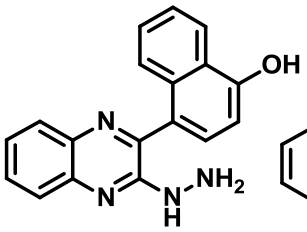
In IR, solid compounds were analysed by KBr disc method and semi-solid compounds were recorded as neat samples. ν_{max} is expressed in cm^{-1}: 3615–3460 (O-H stretch), 3450–3200 (N-H stretch), 3065–3030 (aromatic C-H stretch); 2920–2760 (aliphatic C-H stretch); 2260–2150 (C≡C stretch); 1800–1650 (C=O stretch); 1650–1480 (aromatic C=C stretch); 1350–1150 (aliphatic C-O, C-N stretch); 1100–750 (C-Halogen stretch).

In NMR spectra, the ^{1}H NMR NH and OH protons are seen as broad peaks in the range δ 12.50–9.50, aromatic protons in the range δ 9.90–6.50 and alphatic protons in the range δ 5.65–1.12. ^{13}C NMR, aromatic carbons in the range δ 160.50–105.80 and alphatic carbons in the range δ 80.50–12.50. Physiochemical properties of the synthesized compounds **QNP 05–64** are given in **Table 5.3**.

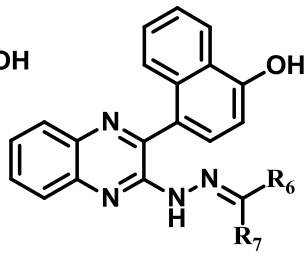
Table 5.3: Physiochemical properties of the synthesized compounds **QNP 05–64**



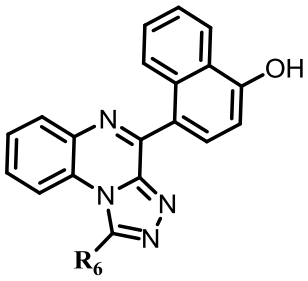
QNP 05-26



QNP 27



QNP 28-52



QNP 53-64

Compound	R_5 / R_6	R_7	Molecular formula	Molecular weight	M.P. (°C)
QNP 05	H	-	$C_{22}H_{20}N_4O$	356.42	Semi solid
QNP 06	Me	-	$C_{23}H_{22}N_4O$	370.45	149-150
QNP 07	Et	-	$C_{24}H_{24}N_4O$	384.48	136-137
QNP 08	$PhCH_2$	-	$C_{29}H_{26}N_4O$	446.55	118-119
QNP 09	Ph_2CH	-	$C_{35}H_{30}N_4O$	522.65	103-104
QNP 10	Ph	-	$C_{28}H_{24}N_4O$	432.52	188-189
QNP 11	4-Me Ph	-	$C_{29}H_{26}N_4O$	446.55	81-82
QNP 12	4-F Ph	-	$C_{28}H_{23}FN_4O$	450.51	Oil
QNP 13	4-Cl Ph	-	$C_{28}H_{23}ClN_4O$	466.96	162-163
QNP 14	4-Br Ph	-	$C_{28}H_{23}BrN_4O$	511.42	182-184
QNP 15	4-NO_2 Ph	-	$C_{28}H_{23}N_5O_3$	477.52	224-226
QNP 16	4-OH Ph	-	$C_{28}H_{24}N_4O_2$	448.52	132-133
QNP 17	4-OMe Ph	-	$C_{29}H_{26}N_4O_2$	462.55	Oil
QNP 18	4-CF_3 Ph	-	$C_{29}H_{23}F_3N_4O$	500.52	85-86
QNP 19	2-F Ph	-	$C_{28}H_{23}FN_4O$	450.51	Oil
QNP 20	2-Cl Ph	-	$C_{28}H_{23}ClN_4O$	466.96	172-173
QNP 21	3-Cl Ph	-	$C_{28}H_{23}ClN_4O$	466.96	154-156
QNP 22	Pyridin-4-yl	-	$C_{27}H_{23}N_5O$	433.51	Oil
QNP 23	Pyridin-2-yl	-	$C_{27}H_{23}N_5O$	433.51	124-125
QNP 24	(1H-indol-3-yl) Et	-	$C_{32}H_{29}N_5O$	499.61	Oil
QNP 25	(1H-indol-3-yl)	-	$C_{33}H_{31}N_5O$	513.64	188-190

Compound	R_5 / R_6	R_7	Molecular formula	Molecular weight	M.P. (°C)
	n-Pr				
QNP 26	(1H-indol-3-yl) n-Bu	-	$C_{34}H_{33}N_5O$	527.67	Oil
QNP 27	-	-	$C_{18}H_{14}N_4O$	302.33	245-247
QNP 28	Me	H	$C_{20}H_{16}N4O$	328.37	188-190
QNP 29	*t*-Bu	H	$C_{23}H_{22}N_4O$	370.45	171-172
QNP 30	Ph	H	$C_{25}H_{18}N_4O$	390.46	Oil
QNP 31	4-F Ph	H	$C_{25}H_{17}FN_4O$	408.43	174-175
QNP 32	4-Cl Ph	H	$C_{25}H_{17}ClN_4O$	424.88	184-186
QNP 33	4-Br Ph	H	$C_{25}H_{17}BrN_4O$	469.34	203-205
QNP 34	4-NO_2 Ph	H	$C_{25}H_{17}N_5O_3$	435.44	224-226
QNP 35	4-OH Ph	H	$C_{25}H_{18}N_4O_2$	406.44	229-230
QNP 36	4-OMe Ph	H	$C_{26}H_{20}N_4O_2$	420.47	143-145
QNP 37	4-NMe_2 Ph	H	$C_{27}H_{23}N_5O$	433.51	259-261
QNP 38	2-F Ph	H	$C_{25}H_{17}FN_4O$	408.43	199-200
QNP 39	3-F Ph	H	$C_{25}H_{17}FN_4O$	408.43	Semi solid
QNP 40	3-NO_2 Ph	H	$C_{25}H_{17}N_5O_3$	435.44	239-241
QNP 41	3-CF_3 Ph	H	$C_{26}H_{17}F_3N_4O$	458.44	230-231
QNP 42	2,3-diCl Ph	H	$C_{25}H_{16}Cl_2N_4O$	459.33	184-185
QNP 43	2,4-diCl Ph	H	$C_{25}H_{16}Cl_2N_4O$	459.33	289-291
QNP 44	3,4-diOMe Ph	H	$C_{27}H_{22}N_4O_3$	450.49	Oil
QNP 45	Pyridin-2-yl	H	$C_{24}H_{17}N_5O$	391.43	206-207
QNP 46	Pyridin-4-yl	Me	$C_{25}H_{19}N_5O$	405.46	139-141
QNP 47	Pyridin-2-yl	Me	$C_{25}H_{19}N_5O$	405.46	235-285
QNP 48	1H-pyrrol-2-yl	H	$C_{23}H_{17}N_5O$	379.42	201-203
QNP 49	Thiophen-2-yl	H	$C_{23}H_{16}N_4OS$	396.46	162-164
QNP 50	5-NO_2-furan-2-yl	H	$C_{23}H_{15}N_5O_3S$	441.46	197-198
QNP 51	9H-fluoren-9-yl	H	$C_{31}H_{20}N_4O$	464.52	Oil
QNP 52	1H-indol-3-yl	H	$C_{27}H_{19}N_5O$	429.48	198-200

Compound	R_5 / R_6	R_7	Molecular formula	Molecular weight	M.P. (°C)
QNP 53	*t*-Bu	-	$C_{23}H_{20}N_4O$	368.43	121-122
QNP 54	4-F Ph	-	$C_{25}H_{15}FN_4O$	406.41	Semi solid
QNP 55	4-Cl Ph	-	$C_{25}H_{15}ClN_4O$	422.86	181-182
QNP 56	4-Br Ph	-	$C_{25}H_{15}BrN_4O$	467.31	203-205
QNP 57	4-NO_2 Ph	-	$C_{25}H_{15}N_5O_3$	433.41	231-233
QNP 58	4-OMe Ph	-	$C_{26}H_{18}N_4O_2$	418.44	Oil
QNP 59	3-F Ph	-	$C_{25}H_{15}FN_4O$	406.41	239-240
QNP 60	3-NO_2 Ph	-	$C_{25}H_{15}N_5O_3$	433.41	228-229
QNP 61	2,3-diCl Ph	-	$C_{25}H_{14}Cl_2N_4O$	457.31	236-238
QNP 62	3,4-diOMe Ph	-	$C_{27}H_{20}N_4O_3$	448.47	121-122
QNP 63	1H-pyrrol-2-yl	-	$C_{23}H_{15}N_5O$	377.39	161-163
QNP 64	Thiophen-2-yl	-	$C_{23}H_{14}N_4OS$	394.44	Oil

5.3.2. *In vitro* MTB screening, cytotoxicity studies and selectivity index of the synthesized molecules

All the synthesized compounds were first screened for their *in vitro* anti-tubercular activity against MTB H37Rv (ATCC 27294) strain by MABA with compound concentration ranging from 50 to 0.78 µg/mL. Isoniazid, ethambutol and pyrazinamide were used as reference compounds for comparison. The *in vitro* test results for title compounds tabulated in **Table 5.4** indicate that MIC ranges from 1.56 to ≥ 50 µg/mL. Most active compounds were also tested for *in vitro* cytotoxicity against RAW 264.7cells at 50 µg/mL concentration using MTT assay. All the results are presented in **Table 5.4**.

Table 5.4: *In vitro* biological evaluation of synthesized compounds **QNP 05–64**

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
QNP 05	6.25	36.42	68.64	10.98
QNP 06	6.25	34.33	72.82	11.65
QNP 07	3.12	40.32	62.00	19.84

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
QNP 08	3.12	34.25	72.99	23.35
QNP 09	12.5	NT	NT	NT
QNP 10	6.25	44.62	52.02	8.96
QNP 11	3.12	33.65	74.29	23.77
QNP 12	25	NT	NT	NT
QNP 13	12.5	NT	NT	NT
QNP 14	25	NT	NT	NT
QNP 15	3.12	33.77	74.03	23.68
QNP 16	6.25	43.88	56.97	9.11
QNP 17	1.56	23.50	106.38	68.19
QNP 18	3.12	33.76	74.05	23.69
QNP 19	1.56	24.22	103.22	66.16
QNP 20	12.5	NT	NT	NT
QNP 21	25	NT	NT	NT
QNP 22	1.56	22.29	112.15	71.89
QNP 23	6.25	44.51	56.16	8.98
QNP 24	6.25	34.37	72.73	11.63
QNP 25	12.5	NT	NT	NT
QNP 26	12.5	NT	NT	NT
QNP 27	0.78	23.72	105.39	135.12
QNP 28	50	NT	NT	NT
QNP 29	12.5	NT	NT	NT
QNP 30	3.12	51.77	48.29	15.47
QNP 31	3.12	45.88	54.48	17.46
QNP 32	1.56	23.76	105.21	67.44
QNP 33	6.25	43.76	57.12	9.14
QNP 34	3.12	34.22	73.05	23.41

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
QNP 35	6.25	33.72	74.13	11.86
QNP 36	25	NT	NT	NT
QNP 37	50	NT	NT	NT
QNP 38	6.25	53.81	46.45	7.43
QNP 39	50	NT	NT	NT
QNP 40	12.5	NT	NT	NT
QNP 41	6.25	33.73	74.11	11.85
QNP 42	12.5	NT	NT	NT
QNP 43	25	NT	NT	NT
QNP 44	6.25	42.36	59.01	9.44
QNP 45	1.56	24.08	103.82	66.55
QNP 46	3.12	33.62	74.36	23.83
QNP 47	6.25	35.12	71.18	11.38
QNP 48	1.56	33.29	75.09	48.13
QNP 49	25	NT	NT	NT
QNP 50	12.5	NT	NT	NT
QNP 51	3.12	23.02	108.60	34.80
QNP 52	12.5	NT	NT	NT
QNP 53	1.56	29.14	85.79	54.99
QNP 54	12.5	NT	NT	NT
QNP 55	3.12	32.76	76.31	24.45
QNP 56	≥50	NT	NT	NT
QNP 57	6.25	42.77	58.45	9.35
QNP 58	25	NT	NT	NT
QNP 59	25	NT	NT	NT
QNP 60	3.12	33.45	74.73	23.95
QNP 61	12.5	NT	NT	NT

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
QNP 62	50	NT	NT	NT
QNP 63	6.25	21.88	114.25	18.28
QNP 64	25	NT	NT	NT
INH	0.1	-	-	-
EMB	1.56	-	-	-
PZA	6.25	-	-	-

[a]Minimum inhibitory concentration *in vitro* activity against MTB H37Rv strain; [b]Cytotoxicity against RAW 264.7 cells; [c]IC_{50}, 50% inhibitory concentration; [d]SI: selectivity index; NT: not tested; INH: Isoniazid; EMB: Ethambutol; PZA: Pyrazinamide.

5.3.3. SAR and discussion

In this series, all the synthesized compounds showed activity against MTB H37Rv strain with MIC ranging from 0.78 to ≥50 µg/mL. Amongst, thirty five compounds 99% inhibited the growth with MTB MIC ≤ 6.25 µg/mL, Out of these twenty compounds (**QNP 07-8, QNP 11, QNP 15, QNP 17-19, QNP 22, QNP 27, QNP 30-32, QNP 34, QNP 45-46, QNP 48, QNP 51, QNP 53, QNP 55** and **QNP 60**) exhibited good anti-TB activity with MIC ≤ 3.12. Eight compounds (**QNP 17, QNP 19, QNP 22, QNP 27, QNP 32, QNP 45, QNP 48** and **QNP 53**) were found to be better than or equivalent to standard drugs viz. ethambutol and pyrazinamide. One compound **QNP 27** was found to be outstanding anti-TB active compound *in vitro* with MIC≤ 0.78 µg/mL and this is more potent than ethambutol (MIC 1.56 µg/mL) and pyrazinamide (MIC 6.25 µg/mL). When compared to ethambutol and pyrazinamide these 8 compounds are showing better anti-TB activity. Furthermore, most active anti-TB compounds exhibited good IC_{50} values, low cytotoxicity and selectivity index (**Table 5.4**). The most active compounds (**QNP 17, QNP 19, QNP 22, QNP 27, QNP 32, QNP 45, QNP 48** and **QNP 53**) showed SI ≥ 48, indicating the nontoxic nature of the compounds and might be considered for further structural modification, leading to drug development.

5.3.4. Highlights of the scheme 2 derivatives

The quinoxalin naphthalen-1-ol derivatives were synthesized in moderate to excellent yields. The preliminary *in vitro* anti-TB screening results, toxicity studies and SI profile of

compounds **QNP 07-8, QNP 11, QNP 15, QNP 18, QNP 30-31, QNP 34, QNP 46, QNP 51, QNP 55** and **QNP 60** displayed good anti-TB activity (MIC 3.13 µg/mL) and SI profile >19. Compounds **QNP 17, QNP 19, QNP 22, QNP 32, QNP 45, QNP 48** and **QNP 53** exhibited excellent anti-TB activity (MIC 1.56 µg/mL) and SI profile >48. Lead compounds **QNP 27** exhibited admirable anti-TB activity (MIC 0.78 µg/mL) and SI profile >135. Quinoxalin naphthalen-1-ol hydrozones derivatives were more potent than quinoxalin naphthalen-1-ol piperazine and quinoxalin naphthalen-1-ol triazole motifs. The quinoxalin naphthalen-1-ol skeleton is established to be an important motif for further drug development of anti-tubercular agents. The most active compound **QNP 27** is depicted in **Figure 5.2**.

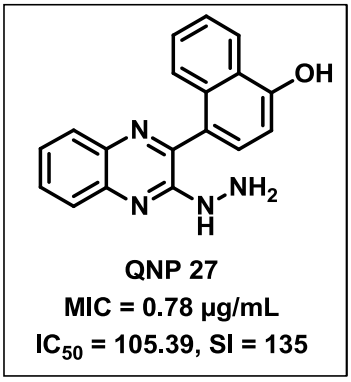


Figure 5.2: Chemical structure and anti-TB activity of the most active compound (**QNP 27**)

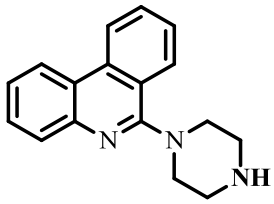
5.4. Physical properties and biological results of 6-(piperazin-1-yl)phenanthridine (Scheme 3) derivatives

5.4.1. General spectral data and physical properties of scheme 3 derivatives

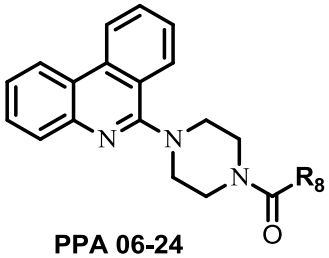
In IR, solid compounds were analysed by KBr disc method and semi-solid compounds were recorded as neat samples. ν_{max} is expressed in cm^{-1}: 3455–3250 (N-H stretch), 3060–3050 (aromatic C-H stretch); 2915–2740 (aliphatic C-H stretch); 1800–1646 (C=O stretch); 1650–1480 (aromatic C=C stretch); 1356–1150 (aliphatic C-O, C-N stretch); 1100–750 (C-Halogen stretch).

In NMR spectra, the NH protons are seen as broad peaks in the range δ 11.00–9.50, aromatic protons in the range δ 9.90–6.50 and alphatic protons in the range δ 5.50–1.14. ^{13}C NMR, carbonyl group present at δ 175.00–160.00 aromatic carbons in the range δ 160.00–105.00 and alphatic carbons in the range δ 80.00–12.00. Physiochemical properties of the synthesized compounds **PPA 05–38** are given in **Table 5.5**.

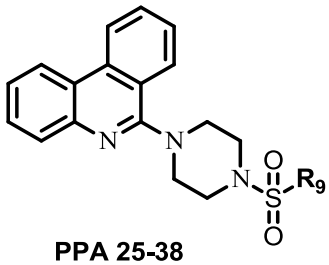
Table 5.5: Physiochemical properties of the synthesized compounds **PPA 05–38**



PPA 05



PPA 06-24



PPA 25-38

Compound	R_8 / R_9	Molecular formula	Molecular weight	M.P. (°C)
PPA 05	-	$C_{17}H_{17}N_3$	236.34	118-120
PPA 06	$CNCH_2$	$C_{20}H_{18}N_4O$	330.39	139-140
PPA 07	Ph	$C_{24}H_{21}N_3O$	367.45	108-109
PPA 08	4-Cl Ph	$C_{24}H_{20}ClN_3O$	401.89	132-133
PPA 09	4-NO_2 Ph	$C_{24}H_{20}N_4O_3$	412.44	148-149
PPA 10	3-NO_2 Ph	$C_{24}H_{20}N_4O_3$	412.44	230-231
PPA 11	3,5-NO_2 Ph	$C_{24}H_{19}N_5O_5$	457.44	196-197
PPA 12	2-NH_2 Ph	$C_{24}H_{22}N_4O$	382.46	164-165
PPA 13	4-NH_2 Ph	$C_{24}H_{22}N_4O$	382.46	Oil
PPA 14	Napth-1-CH_2	$C_{29}H_{25}N_3O$	431.53	181-182
PPA 15	Ph CHCH	$C_{26}H_{23}N_3O$	393.49	154-156
PPA 16	Pyridin-2-yl	$C_{23}H_{20}N_4O$	368.44	Oil
PPA 17	Pyridin-3-yl	$C_{23}H_{20}N_4O$	368.44	153-154
PPA 18	Pyridin-4-yl	$C_{23}H_{20}N_4O$	368.44	Oil
PPA 19	Picolinicacid-6	$C_{24}H_{20}N_4O_3$	412.44	Oil
PPA 20	Furan-2-yl	$C_{22}H_{19}N_3O_2$	357.41	118-119
PPA 21	Thiophen-2-yl	$C_{22}H_{19}N_3OS$	373.47	143-145
PPA 22	(1H-indol-3-yl)-CH_2	$C_{27}H_{24}N_4O$	420.51	162-163
PPA 23	(1H-indol-3-yl)-CH_2CH_2	$C_{28}H_{26}N_4O$	434.54	120-121
PPA 24	(1H-indol-3-yl)-n-Pr	$C_{29}H_{28}N_4O$	448.57	148-149
PPA 25	Me	$C_{18}H_{19}N_3O_2S$	341.42	Oil

Compound	R_8 / R_9	Molecular formula	Molecular weight	M.P. (°C)
PPA 26	Ph	$C_{23}H_{21}N_3O_2S$	403.50	238-239
PPA 27	4-Me Ph	$C_{24}H_{23}N_3O_2S$	417.52	188-189
PPA 28	4-*t*-Bu Ph	$C_{27}H_{29}N_3O_2S$	459.60	154-155
PPA 29	Mesityl	$C_{26}H_{27}N_3O_2S$	445.58	118-119
PPA 30	4-Cl-2,5-di Me Ph	$C_{25}H_{24}ClN_3O_2S$	465.99	163-164
PPA 31	4-F Ph	$C_{23}H_{20}FN_3O_2S$	421.49	188-189
PPA 32	4-Cl Ph	$C_{23}H_{20}ClN_3O_2S$	437.94	196-197
PPA 33	4-Br Ph	$C_{23}H_{20}BrN_3O_2S$	482.39	224-225
PPA 34	4-CF_3 Ph	$C_{24}H_{20}F_3N_3O_2S$	471.49	163-164
PPA 35	4-OMe Ph	$C_{24}H_{23}N_3O_3S$	433.52	208-209
PPA 36	4-NO_2 Ph	$C_{23}H_{20}N_4O_4S$	448.49	198-200
PPA 37	4-MeCO Ph	$C_{23}H_{20}N_4O_4S$	448.49	204-205
PPA 38	5-Br thiophen-2-yl	$C_{21}H_{18}BrN_3O_2S_2$	488.41	162-163

5.4.2. *In vitro* MTB screening, cytotoxicity studies and selectivity index of the synthesized molecules

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against MTB H37Rv (ATCC 27294) strain by MABA with compound concentration ranging from 50 to 0.78 µg/mL. Isoniazid, ethambutol and pyrazinamide were used as reference compounds for comparison. The *in vitro* test results for title compounds tabulated in **Table 5.6** indicate that MIC ranges from 1.56 to ≥ 50 µg/mL. Amongst, most active compounds were also tested for *in vitro* cytotoxicity against RAW 264.7cells at 50 µg/mL concentration using MTT assay, all the results are presented in **Table 5.6**.

Table 5.6: *In vitro* biological evaluation of synthesized compounds **PPA 05–38**

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
PPA 05	25	NT	NT	NT
PPA 06	25	NT	NT	NT

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
PPA 07	3.13	22.16	112.81	36.15
PPA 08	12.5	26.50	94.33	7.54
PPA 09	50	NT	NT	NT
PPA 10	3.13	34.64	72.17	23.13
PPA 11	25	NT	NT	NT
PPA 12	>50	NT	NT	NT
PPA 13	50	NT	NT	NT
PPA 14	>50	NT	NT	NT
PPA 15	12.5	22.12	113.01	9.04
PPA 16	3.13	42.16	59.29	19.00
PPA 17	6.25	19.16	130.48	20.87
PPA 18	1.56	27.90	89.60	57.43
PPA 19	3.13	26.52	94.26	30.21
PPA 20	25	NT	NT	NT
PPA 21	50	NT	NT	NT
PPA 22	25	NT	NT	NT
PPA 23	6.25	34.64	72.17	11.54
PPA 24	1.56	34.12	73.27	46.96
PPA 25	25	NT	NT	NT
PPA 26	6.25	30.72	81.38	13.02
PPA 27	>50	NT	NT	NT
PPA 28	1.56	28.92	86.44	55.41
PPA 29	12.5	26.52	94.26	7.54
PPA 30	6.25	34.64	72.17	11.54
PPA 31	6.25	32.76	76.31	12.21
PPA 32	3.13	20.92	119.50	38.30
PPA 33	3.13	28.16	88.77	28.45

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
PPA 34	25	NT	NT	NT
PPA 35	6.25	26.52	94.26	15.08
PPA 36	50	NT	NT	NT
PPA 37	25	NT	NT	NT
PPA 38	3.13	42.16	59.29	38.01
INH	0.1	-	-	-
EMB	1.56	-	-	-
PZA	6.25	-	-	-

[a]Minimum inhibitory concentration *in vitro* activity against MTB H37Rv strain; [b]Cytotoxicity against RAW 264.7 cells; [c]IC_{50}, 50% inhibitory concentration; [d]SI: selectivity index; NT: not tested; INH: Isoniazid; EMB: Ethambutol; PZA: Pyrazinamide.

5.4.3. SAR and discussion

All the synthesized compounds showed activity against MTB H37Rv strain with MIC ranging from 1.56 to ≥50 µg/mL. Among the synthesized compounds, electronic effects of substituent play an important role in displaying anti-TB activity. 6-(piperazin-1-yl)phenanthridine (**PPA 05**) was inhibiting 99% growth of MTB H37Rv strain at 25 µg/mL. Amongst, phenanthridine amides (**PPA 06-24)** and phenanthridine sulphonamide **(PPA 25-38)** derivatives MTB activity summary, SAR studies are described based on activity of compound **PPA 05**. Introduction of cyanoacetyl group (**PPA 06**) at the 4th position of compound **PPA 05** retained the anti-TB activity with MIC 25 µg/mL. Presence of amine an electron donating group (EDG) at either ortho (**PPA 12**) or para (**PPA 13**) position on phenyl ring decreased the activity by ≥ 2 fold. Introduction of nitro group, an electron withdrawing group (EWG), at meta (**6e**) position on phenyl ring greatly enhanced anti-TB activity by 8 fold with MIC 3.12 µg/mL. On the other hand nitro group at di-meta (**PPA 11**) and para (**PPA 09**) position on phenyl ring, decreased activity by 1-2 folds. Presence of chloro an EWG at para (**6c**) position on phenyl ring improved anti-TB activity by 2 fold with MIC 12.5 µg/mL. Benzoyl group (**PPA 07**) at 4th position of compound **PPA 05** significantly increased anti-TB activity by 8 folds with MIC 3.12 µg/mL; cinnamoyl group (**PPA 15**) at 4th position of **PPA 05** increased activity by 2 folds with MIC 12.5 µg/mL and 2-(naphthalen-1-yl)acetyl group (**PPA 14**) at 4th position of compound **PPA 05** critically decreased the anti-TB activity

by >2 folds with MIC >50 µg/mL. To establish extensive SAR we also varied heterocyclic compounds such as five membered rings (furan and thiophene), six membered ring (pyridyls) and indole analogues. Furan-2-carbonyl group (**PPA 20**) and thiophene-2-carbonyl group (**PPA 21**) at 4th position of 6-(piperazin-1-yl)phenanthridine (**PPA 05**) retained and decreased anti-TB activity by 1-2 folds with MIC 25 µg/mL and 50 µg/mL respectively. Introduction of six member heterocyclic rings such as nicotinoyl (**PPA 17**), picolinoyl (**PPA 16**), 6-formicacid picolinoyl (**PPA 19**) and isonicotinoyl groups (**PPA 18**) at 4th position of **PPA 05** greatly enhanced the anti-TB activity by 4–16 folds with MIC 6.25–1.56 µg/mL. While, 2-(1H-indol-3-yl)acetyl (**PPA 22**), 3-(1H-indol-3-yl)propanoyl (**PPA 23**) and 4-(1H-indol-3-yl)butanoyl groups (**PPA 24**) at 4th position of **PPA 05** witnessed either retention (**PPA 22**) or greatly enhanced (**PPA 23** and **PPA 24**) the anti-TB activity by 1–16 folds. In conclusion, **PPA 06-24** presence of EDGs on phenyl ring and five membered heterocyclic rings decreased anti-TB activity while EWGs, six membered and benzannulated heterocyclic rings increased the activity (exception **PPA 09**).

SAR profile of the fourteen phenanthridine sulphonamide **(PPA 25-38)** analogues is as follows. In the presence of simple methanesulfonyl group (**PPA 25**) at the 4th position of compound **5** the anti-TB activity is retained with MIC 25 µg/mL. Benzenesulfonyl group (**PPA 26**) at the 4th position of compound **PPA 05** increased the anti-TB activity by 4 fold with MIC 6.25 µg/mL. Presence of EDGs in the phenyl ring increased the activity in general exception being (**PPA 27**). 2,4,6-triethyl groups (**PPA 29**) on phenyl ring improved anti-TB activity by 2 fold with MIC 12.5 µg/mL. *Tert*-butyl group (**PPA 28**) at para position on phenyl ring remarkably enriched the anti-TB activity by 16 folds with MIC 1.56 µg/mL. Methoxy group at para (**PPA 35**) position on phenyl ring increased the anti-TB activity by 4 folds with MIC 6.25 µg/mL. Presence of electron withdrawing halogens (**PPA 31**), Cl (**PPA 32**) and Br (**PPA 33**)) at para position on phenyl ring impressively enhanced the anti-TB activity by 4–8 folds with MIC 6.25–3.12 µg/mL, including compound **PPA 30.** Electron withdrawing nitro (**PPA 36**) and acetyl (**PPA 37**) groups at para positions on phenyl ring either retained or decreased activity by 1–2 folds. 5-bromothiophene-2-sulfonyl group at 4th position of 6-(piperazin-1-yl)phenanthridine (**PPA 05**) prominently augmented the anti-TB activity by 8 folds with MIC 3.12 µg/mL. In general, EWGs on phenyl ring enhanced or retained the anti-TB activity exception being **PPA 36**. Wrapping up the results, we observe that in the series synthesized, sulphonamide analogues were more active than the amides.

Amongst, sixteen compounds (**PPA 7, PPA 10, PPA 16-19, PPA 23-24, PPA 26, PPA 28, PPA 30-33, PPA 35** and **PPA 38**) 99% inhibited with MTB MIC of ≤ 6.25 µg/mL, out of these ten compounds (**PPA 7, PPA 10, PPA 16, PPA 18-19, PPA 24, PPA 28, PPA 32-33** and **PPA 38**) good anti-TB activity with MIC ≤ 3.12. Three compounds (**PPA 18, PPA 24** and **PPA 28**) were found to be better than or equivalent to ethambutol and pyrazinamide. Compounds **PPA 18, PPA 24** and **PPA 28** were found to be the most active compound *in vitro* MICs of ≤ 1.56 µg/mL and these are more potent than pyrazinamide (MIC 6.25 µg/mL). When compared to ethambutol and pyrazinamide these 3 compounds are lead analogues were showing better anti-TB activities of scheme 3 analogues.

Furthermore, most active anti-TB compounds exhibited good IC_{50} values and selectivity index (**Table 5.6**). The most active compounds (**PPA 18, PPA 24** and **PPA 28**) showed SI ≥ 46, indicating the nontoxic nature of compounds and might be considered for further structural modification, leading to drug development.

5.4.4. Highlights of the scheme 3 derivatives

The phenanthridine derivatives were synthesized in moderate to excellent yields. The preliminary *in vitro* anti-TB screening results, toxicity studies and SI profile results of compounds **PPA 07**, **PPA 10, PPA 16, PPA 19, PPA 32-33** and **PPA 38** displayed good anti-TB activity (MIC 3.13 µg/mL) and SI profile >19. Compounds **PPA 18**, **PPA 24**, and **PPA 28** exhibited excellent anti-TB activity (MIC 1.56 µg/mL) and SI profile > 46. The 6-(4-substitutedpiperazin-1-yl)phenanthridine skeleton is established to be an important motif for further development of anti-tubercular agents. The most active compounds **PPA 18**, **PPA 24**, and **PPA 28** with MTB MIC 1.56 µg/mL are depicted in **Figure 5.3**.

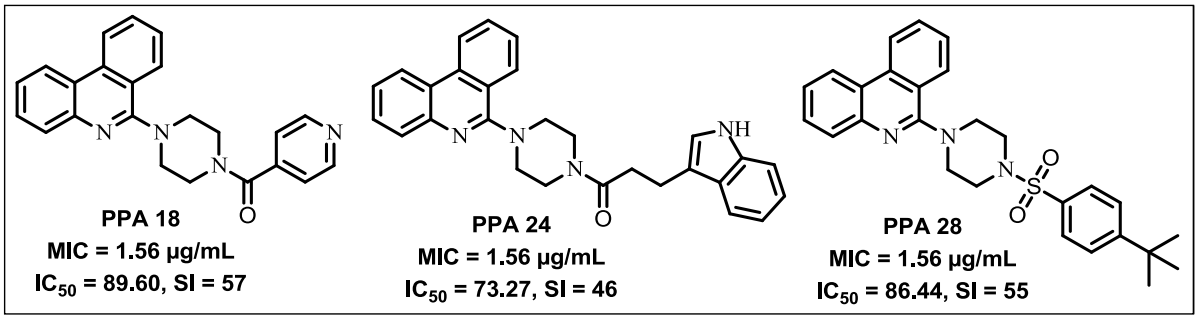


Figure 5.3: Chemical structures and anti-TB activity of the most active compounds (**PPA 18, PPA 24** and **PPA 28**)

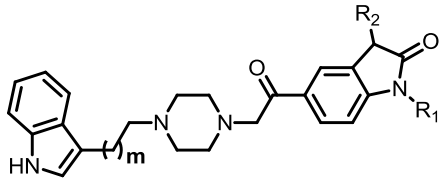
5.5. Physical properties and biological results of 5-(2-(4-(2-(1H-indol-3-yl)ethyl)piperazin-1-yl)acetyl)-substitutedindolin-2-one (IPO 13-30), 2-(1-((substituted-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-1H-benzo[*d*] imidazole (IPO 31-51) and 5-((3-(1H-benzo[*d*]imidazol-2-yl)-1H-indol-1-yl)methyl)-3-substituted isoxazole (IPO 52-63): scheme 4 derivatives

5.5.1. General spectral data and physical properties of scheme 4 derivatives

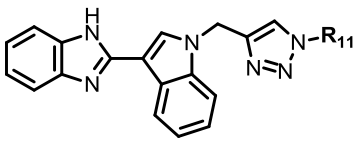
In IR, solid compounds were analysed by KBr disc method and semi-solid compounds were recorded as neat samples. ν_{max} is expressed in cm^{-1}: 3455–3250 (N-H stretch), 3060–3050 (aromatic C-H stretch); 2915–2740 (aliphatic C-H stretch);); 2260–2155 (C≡C stretch); 1800–1646 (C=O stretch); 1650–1480 (aromatic C=C stretch); 1356–1150 (aliphatic C-O, C-N stretch); 1100–750 (C-Halogen stretch).

In NMR spectra, the NH protons are seen as broad peaks in the range δ 11.00–9.50, aromatic protons in the range δ 9.90–6.50 and alphatic protons in the range δ 5.50–1.14. ^{13}C NMR, carbonyl group was present at δ 175.00–160.00; aromatic carbons in the range δ 160.00–105.00 and alphatic carbons in the range δ 80.00–12.00. Physiochemical properties of the synthesized compounds **IPO 13–63** are given in **Table 5.7**.

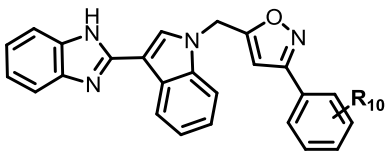
Table 5.7: Physiochemical properties of the synthesized compounds **IPO 13–63**



IPO 13-30



IPO 31-51



IPO 52-63

Compound	R_1	R_2, m/R_{10}/R_{11}	Molecular formula	Molecular weight	M.P. (°C)
IPO 13	H	H, 1	$C_{24}H_{26}N_4O_2$	402.48	Oil
IPO 14	H	Me, 1	$C_{25}H_{28}N_4O_2$	416.51	Oil
IPO 15	Me	H, 1	$C_{25}H_{28}N_4O_2$	416.51	197-199
IPO 16	Me	Me, 1	$C_{26}H_{30}N_4O_2$	430.54	172-173
IPO 17	Et	H, 1	$C_{26}H_{30}N_4O_2$	430.54	218-220
IPO 18	Et	Me, 1	$C_{27}H_{32}N_4O_2$	444.56	209-210
IPO 19	H	H, 2	$C_{25}H_{28}N_4O_2$	416.51	Semi solid
IPO 20	H	Me, 2	$C_{26}H_{30}N_4O_2$	430.54	Oil

Compound	R_1	R_2, m/R_{10}/R_{11}	Molecular formula	Molecular weight	M.P. (°C)
IPO 21	Me	H, 2	$C_{26}H_{30}N_4O_2$	430.54	Semi solid
IPO 22	Me	Me, 2	$C_{27}H_{32}N_4O_2$	444.56	194-196
IPO 23	Et	H, 2	$C_{27}H_{32}N_4O_2$	444.56	180-182
IPO 24	Et	Me, 2	$C_{28}H_{34}N_4O_2$	458.59	199-200
IPO 25	H	H, 3	$C_{26}H_{30}N_4O_2$	430.54	Oil
IPO 26	H	Me, 3	$C_{27}H_{32}N_4O_2$	444.56	Semi solid
IPO 27	Me	H, 3	$C_{27}H_{32}N_4O_2$	444.56	162-164
IPO 28	Me	Me, 3	$C_{28}H_{34}N_4O_2$	458.59	168-170
IPO 29	Et	H, 3	$C_{28}H_{34}N_4O_2$	458.59	172-173
IPO 30	Et	Me, 3	$C_{29}H_{36}N_4O_2$	472.62	158-160
IPO 31	-	Ph	$C_{24}H_{18}N_6$	390.43	184-185
IPO 32	-	4-Me Ph	$C_{25}H_{20}N_6$	404.46	136-137
IPO 33	-	4-Et Ph	$C_{26}H_{22}N_6$	418.49	163-164
IPO 34	-	4-F Ph	$C_{24}H_{17}FN_6$	408.43	140-141
IPO 35	-	4-Cl Ph	$C_{24}H_{17}ClN_6$	424.88	164-166
IPO 36	-	4-Br Ph	$C_{24}H_{17}BrN_6$	469.33	226-227
IPO 37	-	4-NO_2 Ph	$C_{24}H_{17}N_7O_2$	435.43	170-172
IPO 38	-	4-OMe Ph	$C_{25}H_{20}N_6O$	420.46	248-250
IPO 39	-	4-CF_3 Ph	$C_{25}H_{17}F_3N_6$	458.43	234-236
IPO 40	-	2-Cl Ph	$C_{24}H_{17}ClN_6$	424.88	234-235
IPO 41	-	2-Br Ph	$C_{24}H_{17}BrN_6$	469.33	239-240
IPO 42	-	2-NO_2 Ph	$C_{24}H_{17}N_7O_2$	435.43	164-165
IPO 43	-	3-OMe Ph	$C_{25}H_{20}N_6O$	420.46	218-220
IPO 44	-	3-NO_2 Ph	$C_{24}H_{17}N_7O_2$	435.43	132-133
IPO 45	-	3-CF_3 Ph	$C_{25}H_{17}F_3N_6$	458.43	137-138
IPO 46	-	3,4-Me Ph	$C_{26}H_{22}N_6$	418.49	Oil
IPO 47	-	3,4-F Ph	$C_{24}H_{16}F_2N_6$	426.42	118-119
IPO 48	-	3,4-Cl Ph	$C_{24}H_{16}Cl_2N_6$	459.33	204-206
IPO 49	-	3-Cl-4-F Ph	$C_{24}H_{16}ClFN_6$	442.87	Oil

Compound	R_1	R_2, m/R_{10}/R_{11}	Molecular formula	Molecular weight	M.P. (°C)
IPO 50	-	4-Cl-2-NO_2 Ph	$C_{24}H_{16}ClN_7O_2$	469.88	134-135
IPO 51	-	4-Br-3-CF_3 Ph	$C_{25}H_{16}BrF_3N_6$	537.33	134-135
IPO 52	-	H	$C_{25}H_{18}N_4O$	390.43	258-260
IPO 53	-	4-Me	$C_{26}H_{20}N_4O$	404.63	241-243
IPO 54	-	4-F	$C_{25}H_{17}FN_4O$	408.42	180-181
IPO 55	-	4-Cl	$C_{25}H_{17}ClN_4O$	424.88	143-145
IPO 56	-	4-Br	$C_{25}H_{17}BrN_4O$	469.33	244-246
IPO 57	-	4-NO_2	$C_{25}H_{17}N_5O_3$	435.43	140-142
IPO 58	-	4-OH	$C_{25}H_{18}N_4O_2$	406.43	130-132
IPO 59	-	2-F	$C_{25}H_{17}FN_4O$	408.42	180-181
IPO 60	-	2-Cl	$C_{25}H_{17}ClN_4O$	424.88	130-131
IPO 61	-	2-Br	$C_{25}H_{17}BrN_4O$	469.33	153-154
IPO 62	-	2-NO_2	$C_{25}H_{17}N_5O_3$	435.43	148-150
IPO 63	-	2,4-Cl	$C_{25}H_{16}Cl_2N_4O$	459.33	138-139

5.5.2. *In vitro* MTB screening, cytotoxicity studies and selectivity index of the synthesized molecules

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against MTB H37Rv (ATCC 27294) strain by MABA with compound concentration ranging from 50 to 0.78 µg/mL. Isoniazid, ethambutol and pyrazinamide were used as reference compounds for comparison. The *in vitro* test results for title compounds tabulated in **Table 5.8** indicate that MIC ranges from 1.56 to ≥ 50 µg/mL. Amongst, most active compounds were also tested for *in vitro* cytotoxicity against RAW 264.7 cells at 50 µg/mL concentration using MTT assay. All the results are presented in **Table 5.8**.

Table 5.8: *In vitro* biological evaluation of synthesized compounds **IPO 13–63**

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
IPO 13	25	NT	NT	NT

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
IPO 14	50	NT	NT	NT
IPO 15	25	NT	NT	NT
IPO 16	25	NT	NT	NT
IPO 17	1.56	19.16	130.48	83.64
IPO 18	3.12	22.45	111.35	35.69
IPO 19	6.25	42.03	59.48	9.51
IPO 20	25	NT	NT	NT
IPO 21	1.56	26.52	94.26	60.42
IPO 22	12.5	NT	NT	NT
IPO 23	3.12	31.76	78.71	25.22
IPO 24	12.5	NT	NT	NT
IPO 25	1.56	30.68	81.48	52.23
IPO 26	50	NT	NT	NT
IPO 27	25	NT	NT	NT
IPO 28	12.5	NT	NT	NT
IPO 29	1.56	34.15	73.20	46.92
IPO 30	6.25	24.28	102.96	16.47
IPO 31	≥ 50	NT	NT	NT
IPO 32	50	NT	NT	NT
IPO 33	≥ 50	NT	NT	NT
IPO 34	25	NT	NT	NT
IPO 35	50	NT	NT	NT
IPO 36	50	NT	NT	NT
IPO 37	1.56	26.72	93.56	59.97
IPO 38	25	NT	NT	NT
IPO 39	12.5	NT	NT	NT
IPO 40	25	NT	NT	NT

Compound	[a]MIC (µg/mL) against MTB H37Rv	[b]Cytotoxicity % cell inhibition at 50 µg/mL	[c]IC_{50} approximation	[d]SI
IPO 41	25	NT	NT	NT
IPO 42	6.25	33.58	74.44	11.91
IPO 43	25	NT	NT	NT
IPO 44	12.5	NT	NT	NT
IPO 45	6.25	40.11	62.32	9.97
IPO 46	50	NT	NT	NT
IPO 47	25	NT	NT	NT
IPO 48	6.25	38.07	65.66	10.50
IPO 49	25	NT	NT	NT
IPO 50	12.5	NT	NT	NT
IPO 51	6.25	22.29	112.15	17.94
IPO 52	≥ 50	NT	NT	NT
IPO 53	50	NT	NT	NT
IPO 54	12.5	NT	NT	NT
IPO 55	25	NT	NT	NT
IPO 56	25	NT	NT	NT
IPO 57	1.56	21.78	114.78	73.57
IPO 58	25	NT	NT	NT
IPO 59	3.12	30.72	81.38	26.03
IPO 60	25	NT	NT	NT
IPO 61	50	NT	NT	NT
IPO 62	12.5	NT	NT	NT
IPO 63	50	NT	NT	NT
INH	0.1	-	-	-
EMB	1.56	-	-	-
PZA	6.25	-	-	-

[a]Minimum inhibitory concentration *in vitro* activity against MTB H37Rv strain; [b]Cytotoxicity against RAW 264.7 cells; [c]IC_{50}, 50% inhibitory concentration; [d]SI: selectivity index; NT: not tested; INH: Isoniazid; EMB: Ethambutol; PZA: Pyrazinamide.

5.5.3. SAR and discussion

Herein, all the synthesized compounds showed activity against MTB H37Rv strain with MIC ranging from 0.78 to ≥ 50 µg/mL. Amongst, fifteen compounds (**IPO 17-19, IPO 21, IPO 23, IPO 25, IPO 29-30, IPO 37, IPO 42, IPO 45, IPO 48, IPO 51, IPO 57** and **IPO 59**) inhibited with MTB MIC of ≤ 6.25 µg/mL. Out of these nine compounds (**IPO 17-18, IPO 21, IPO 23, IPO 25, IPO 29, IPO 37, IPO 57** and **IPO 59**) exhibited good anti-TB activity with MIC ≤ 3.12 µg/mL. Six compounds (**IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57**) were found to be superior than or equivalent to standard drugs viz. ethambutol and pyrazinamide, and these are more potent than pyrazinamide (MIC 6.25 µg/mL). When compared to ethambutol and pyrazinamide these 6 compounds are lead analogues showing better anti-TB activities of scheme 4 analogues.

Furthermore, most active anti-TB compounds exhibited good IC_{50} values and selectivity index (**Table 5.8)**. The most active compounds (**IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57**) displayed SI ≥ 46, indicating the non toxic nature of the compounds and might be considered for further lead optimization to drug development.

5.5.4. Highlights of the scheme 4 derivatives

The indole derivatives were synthesized with moderate to excellent yields. The preliminary *in vitro* anti-TB screening results, toxicity studies and SI profile of compounds (**IPO 17-19, IPO 21, IPO 23, IPO 25, IPO 29-30, IPO 37, IPO 42, IPO 45, IPO 48, IPO 51, IPO 57** and **IPO 59**) displayed moderate anti-TB activity with MIC 6.25 µg/mL and SI profile > 9. Compounds **IPO 17-18, IPO 21, IPO 23, IPO 25, IPO 29, IPO 37, IPO 57** and **IPO 59** exhibited good anti-TB activity with MIC 3.12 µg/mL and SI profile >25. Lead compounds **IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57** exhibited admirable anti-TB activity (MIC 1.56 µg/mL) and SI profile >46. Amongst, the compounds indole piperazine oxindole derivatives were more potent than indole piperazine imidazole motifs. The indole piperazine oxindole skeleton is established to be an important motif for further drug development of anti-tubercular agents. The most active compounds **IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57** are depicted in **Figure 5.4**.

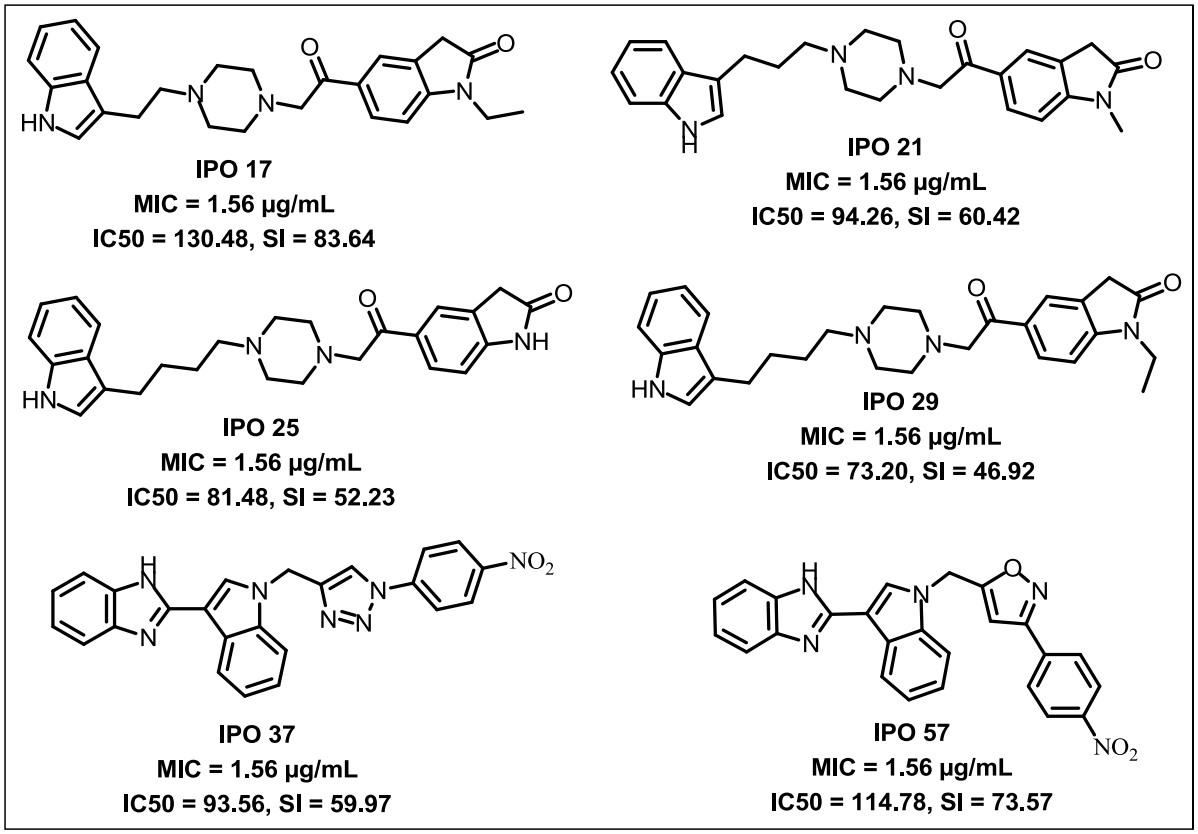


Figure 5.4: Chemical structure and anti-TB activity of the most active compounds (**IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57)**

Chapter VI
Summary & Conclusions

Chapter 6

SUMMARY AND CONCLUSIONS

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

John Bunyan (1680)

The whole time in known human history, the lethal TB transformed its names viz. phthisis, white plague, king's evil, wasting disease, pott's disease other than, it never changed its action of consumption. The Bacilli certainly not respected humankind, it has treated the king or soldier, the rich and the poor, the American and the African comparable with equal scorn.

During the broad range of literature search, we found that there are several good chemical motifs which are inhibiting MTB with MIC < 1 µM, but they were not turning into potent drug candidates owing to many other side reactions. We have selected reported anti-TB compounds with good MIC's as lead molecules and redesigned new chemical entities by retaining the core structure expecting improved activity. These leads were adopted for hit expansion by chemical synthesis and a total of 232 molecules from four different series were synthesized by conventional methods and wherever possible, reactions were carried out using benign techniques such as microwave irradiation organic synthesis or under solvent free conditions in moderate to excellent yields. All synthesized novel compounds were characterized by spectral data (IR, NMR and MS), elemental analysis and few compounds confirmed by single crystal XRD. All compounds were evaluated for their antimycobacterial activity and selected compounds were evaluated for cytotoxicity studies.

In **scheme 1**, eighty six benzo[*d*]isoxazole derivatives were synthesized in moderate to excellent yields (60-95%). The benzo[*d*]isoxazole oxindole, heterocyclic amines would lead to increase in activity. Out of 86 synthesized motifs four compounds were most active (**BIP 53, BIP 58, BIP 63** and **BIP 65**) with MIC 1.56 µg/mL and SI ≥ 34, indicating that these are non toxic and might be considered as lead molecules. We found four molecules exhibiting greater than or equal to MTB MIC than standard anti-TB drugs viz. ethambutol and pyrazinamide.

The quinoxalin naphthalen-1-ol (**Scheme 2**) derivatives were synthesized in moderate to excellent yields (60-95%). The preliminary *in vitro* anti-TB screening results, toxicity studies and SI profile of **QNP 17, QNP 19, QNP 22, QNP 32, QNP 45, QNP 48** and **QNP 53** exhibited excellent anti-TB activity (MIC 1.56 µg/mL) and SI profile > 48. Lead compound **QNP 27** exhibited admirable anti-TB activity (MIC 0.78 µg/mL) and SI profile >135. Out of sixty synthesized analogues, the most active eight compounds were non toxic and might be considered as lead molecules. We found 8 molecules exhibiting greater than or equal to MTB MIC than standard anti-TB drugs viz. ethambutol and pyrazinamide. Therefore, the quinoxalin naphthalen-1-ol skeleton is established to be an important motif for further drug development of anti-TB agents.

In **Scheme 3**, the phenanthridine derivatives were synthesized in moderate to excellent yields (60-98%). The preliminary *in vitro* anti-TB screening results, toxicity studies and SI profile results of compounds **PPA 18**, **PPA 24**, and **PPA 28** exhibited excellent anti-TB activity (MIC 1.56 µg/mL) and SI profile > 46. Out of 34 synthesized analogues, the active three compounds are formed to be non toxic and might be considered as lead molecules. We found 3 molecules exhibiting greater than or equal to MTB MIC than standard anti-TB drugs viz. ethambutol and pyrazinamide. Therefore, the 6-(4-substitutedpiperazin-1-yl)phenanthridine skeleton is established to be an important motif for further drug discovery of anti-tubercular agents.

The Indole derivatives (**Scheme 4**) were synthesized in moderate to excellent yields 60-95%). The preliminary *in vitro* anti-TB screening results, toxicity studies and SI profile of compounds **IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57** exhibited admirable anti-TB activity (MIC 1.56 µg/mL) and SI profile > 46. Amongst, indole piperazine oxindole derivatives were formed to be more potent than indole piperazine imidazole motifs. Out of 51 synthesized analogues, the most active six compounds were non toxic and might be considered as lead molecules. We found 6 molecules exhibiting greater than or equal to MTB MIC than standard anti-TB drugs viz. ethambutol and pyrazinamide. Hence, the indole piperazine oxindole skeleton is established to be an important motif for further drug development of anti-TB agents.

Amongst all the 232 compounds, twenty one compounds were found to be more active in inhibiting MTB H37Rv compared to standard first line anti-TB drug ethambutol and pyrazinamide, whereas one compound possessed better MTB MIC than ciprofloxacin. Over

all the synthesized compounds, **BIP 53, BIP 58, BIP 63, BIP 65, QNP 17, QNP 19, QNP 22, QNP 32, QNP 45, QNP 48, QNP 53, PPA 18**, **PPA 24**, **PPA 28, IPO 17, IPO 21, IPO 25, IPO 29, IPO 37** and **IPO 57** (MTB MIC = 1.56 µg/mL SI ≥ 34) emerged as the most promising anti-TB candidates. Compound **QNP 27** is the most potent compound with MTB MIC = 0.78 µg/mL and SI > 135. The exact mechanism of action for these agents is yet to be established. Structures of active compounds are depicted in **Figure 6.1**

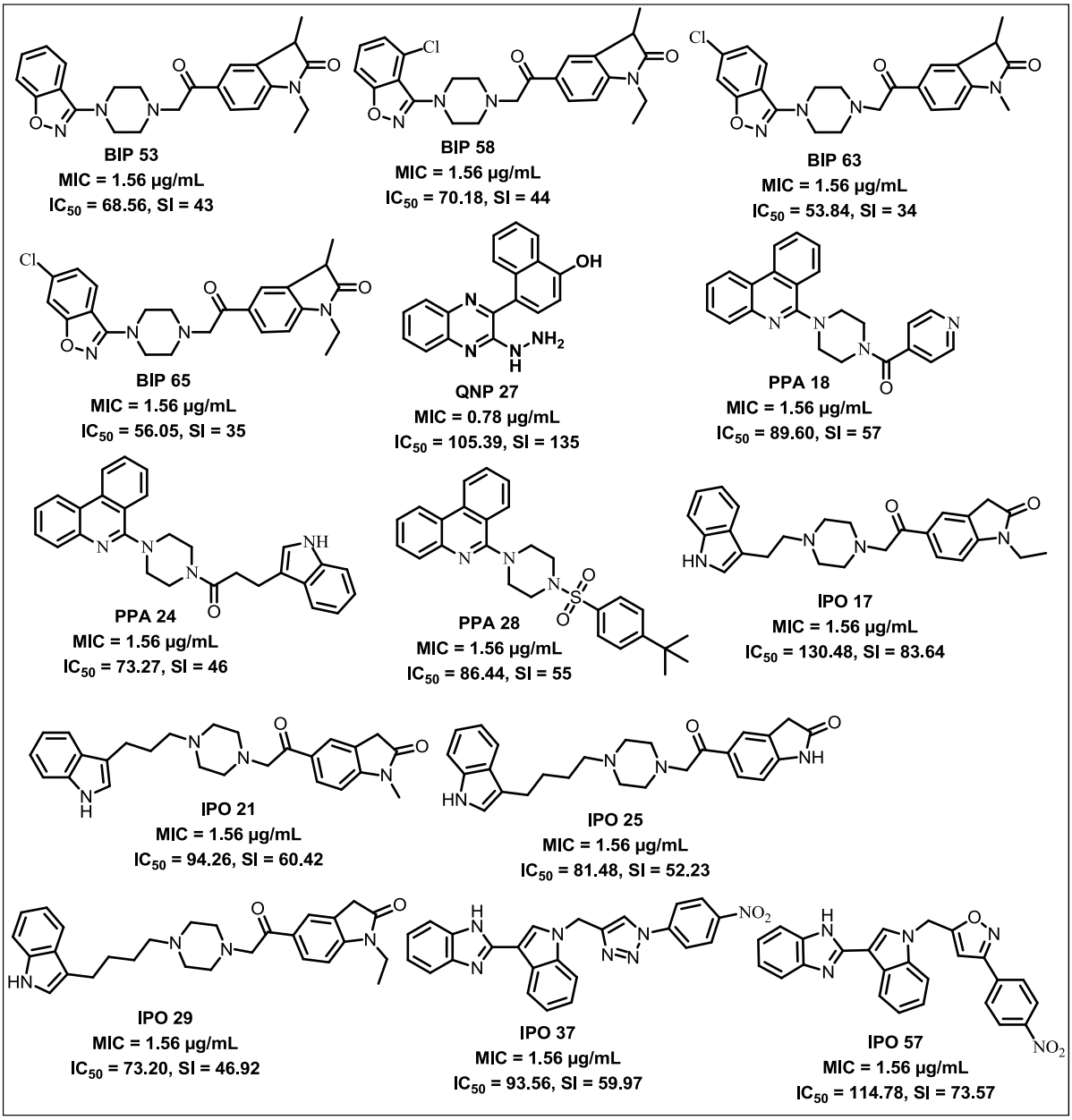


Figure 6.1: Structures of most potent anti-TB active compounds with MIC values

In conclusion, the potent anti-TB compounds depicted in **Figure 6.1** besets a collection of promising leads for further drug optimization and discovery to yield best novel drugs aimed

to combat ubiquitous and everywhere-present mycobacterial infections. This thesis work also provides the origin for further chemical optimization of these potent inhibitors as potential anti-TB agents.

FUTURE PERSPECTIVES

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

- Extensive side effect profile of all the synthesized compounds may be carried out. Sub-acute and acute toxicological screening of novel chemical entities needs to be done.
- Extensive pharmacodynamic and pharmacokinetic studies of the safer compounds have to be undertaken in higher animal models.
- Based on the pharmacophore model proposed, various substituents which lead to activity proposed could be incorporated into the compounds synthesized and studied further in various animal models.

Further, the viability, reduction in therapy period, cost effectiveness and reproducibility of synthesizing these compounds in big scale have to be attempted.

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Appendix

List of Publications

From Thesis Work

1. **K.M. Naidu,** G.N. Rudresh, K.V.G. Chandra Sekhar Design, synthesis and biological evaluation of 5-(2-(4-(substitutedbenzo[*d*]isoxazol-3-yl)piperazin-1-yl)acetyl)indolin-2-one and 5-(2-(4-substituted piperazin-1-yl)acetyl)indolin-2-one analogues as novel anti-tubercular agents. ***Arab. J. Chem.*** **2015**. DOI:10.1016/j.arabjc.2015.02.025.
2. **K.M. Naidu,** A. Suresh, J. Subbalakshmi, D. Sriram, P. Yogeeswari, P. Raghavaiah, K.V.G. Chandra Sekhar, Design, synthesis and antimycobacterial activity of various 3-(4-substituted sulfonylpiperazin-1-yl)benzo[*d*]isoxazole derivatives. ***Eur. J. Med. Chem***. **2014,** 87, 71-78.
3. **K.M. Naidu,** H.N. Nagesh, M. Singh, D. Sriram, P. Yogeeswari, K.V.G. Chandra Sekhar, Novel amide and sulphonamide derivatives of 6-(piperazin-1-yl)phenanthridine as potent *Mycobacterium tuberculosis* H37Rv inhibitors. ***Eur. J. Med. Chem***. **2015**, 92, 415-426.
4. **K.M. Naidu,** D. Sriram, P. Yogeeswari, K.V.G. Chandra Sekhar, Quest for potent antitubercular inhibitors: Design, synthesis and biological evaluation of 4-(3-(4-substitutedpiperazin-1-yl)quinoxalin-2-yl)naphthalen-1-ol analogues. Under review in ***Eur. J. Med. Chem.***
5. **K.M. Naidu,** N. Agnieszka, A. Ewa, K.V.G. Chandra Sekhar, Quest for potent anti-tubercular agents: Design, synthesis and anti-tubercular activity of various ((triazoles/indole)-piperazin-1-yl/1,4-diazepan-1-yl)benzo[*d*]isoxazole derivatives. Under review in ***Chem. Biol. & Drug Design.***

Other Publications

6. H.N. Nagesh, **K.M. Naidu,** D.H. Rao, J.P. Sridevi, D. Sriram, P. Yogeeswari, K.V.G. Chandra Sekhar, Design, synthesis and evaluation of 6-(4-((substituted-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl) phenanthridine analogues as antimycobacterial agents, ***Bioorg. Med. Chem. Lett.*** **2013**, 23, 6805-6810.
7. K.V.G. Chandra Sekhar, T.V.N.V. Tara Sasank, H.N. Nagesh, N. Suresh, **K.M. Naidu,** A. Suresh, Synthesis of 3,5-diarylisoxazoles under solvent-free conditions using

iodobenzene diacetate. ***Chin. Chem. Lett.*** **2013**, 24, 1045-1048.

8. H.N. Nagesh, N. Suresh, **K.M. Naidu,** B. Arun, J.P. Sridevi, D. Sriram, P. Yogeeswari, K.V.G. Chandra Sekhar, Synthesis and evaluation of anti-tubercular activity of 6-(4-substituted piperazin-1-yl)phenanthridine analogues. ***Eur. J. Med. Chem***. **2014**, 74, 333-339.

Papers in Conference / Symposium

9. 13th Eurasia Conference on Chemical Sciences at Indian Institute of Science, Bangalore, India, Dec. 14-18th **2014**.
10. National Symposium on Human Diseases at BITS-Pilani, Hyderabad Campus, Hyderabad, Mar. 15-16th, **2014**.
11. 5th National seminar on Bioinformatics, Sri Venkateshwara Institute of Medical Sciences, Tirupati, February 14-15th, **2014**. **(Won the best paper award in poster section).**
12. 16th CRSI Symposium in Chemistry, IIT Bombay, February 7 -9th, **2014**.
13. 5th International Conference on Drug Development and Orphan/Neglected Diseases, CDRI Lucknow, February 26-28th, **2013**.
14. 14th CRSI National Symposium on Chemistry, CSIR-NIIST, Thiruvananthapuram, February 3-5th, **2012**.

Biography of Prof. K.V.G. Chandra Sekhar

Prof. K.V.G. Chandra Sekhar completed his B. Pharm (Hons.) in 1999 from BITS, Pilani and later on worked as a faculty in Gurukul Vidyapeeth Junior College, Hyderabad for two years. He re-joined BITS, Pilani in 2001 as Teaching Assistant and completed his M. Pharm in 2003. He then worked as Assistant Lecturer for 1 year, and then as Lecturer up to 2008. He was awarded Ph.D in Synthetic medicinal chemistry in 2008. From 2008 to 2014 he worked as Assistant Professor and currently he is working as Associate Professor since 2015. His areas of interest include Synthetic medicinal chemistry and drug design. As investigator, he successfully completed major research projects funded by UGC, DST and DBT New Delhi. He has published over 25 research articles in well renowned international journals and presented around 35 papers in various conferences/symposia and workshops. He is a life member of Association of Pharmacy Teachers of India, CRSI, Indian pharmacological society, Indian council of chemists, Indian association of chemistry teachers etc.

Biography of Mr. K. Mahalakshmi Naidu

Mr. K. Mahalakshmi Naidu completed his B.Sc. (Maths, Chemistry and Physics) in 2005 from Andhra University, Visakhapatnam and M.Sc. (Organic Chemistry) in 2007 with distinction from Andhra University. He started his career as trianee chemist in Neuland Laboratories Limited (2007-09), Hyderabad, India & worked there for about two years. He later joined as Research Associate in Jubilant Chemsys Limited (2009-11), Noida, New delhi India for two years. To pursue higher degree studies, he resigned from the Senior Research Associate position and joined BITS-Pilani Hyderabad campus, Hyderabad, India since 2011 for his PhD. He has been appointed as DST project fellow from October 2011–July 2013 and BITS-Pilani research fellow since August 2013 at Birla Institute of Technology & Science, Pilani, Hyderabad campus under the supervision of Prof. K.V.G. Chandra Sekhar. He has published six scientific papers and five papers are under review in well-renowned international journals and also presented six papers at national and international conferences.