Design and Synthesis of Piperazine/1,2,3-Triazole Tailored Heterocycles and Their Anti-tubercular and Photophysical Applications

THESIS

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CERTIFICATE

This is to certify that the thesis entitled "**Design and Synthesis of Piperazine/1,2,3-Triazole Tailored Heterocycles and Their Anti-tubercular and Photophysical Applications**" submitted by **NAGESH H N** ID No: **2011PHXF010H** for award of Ph.D. of the Institute embodies original work done by him under my supervision.

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Abstract

We herein focused on design, synthesis and antimycobacterial evaluation of synthesized compounds based on hybridization approach strategy. The synthesized compounds were evaluated for their antimycobacterial activity against $H_{37}Rv$ strain.

In **chapter 1**, introduction on Tuberculosis followed by cell envelope, emergence of drug resistance, current TB drugs and their mechanism of action are discussed. Also, strategies involved in developing suitable lead molecules have been briefed.

In **chapter 2**, objectives of the research work is described, **part-A** focusses on designing molecules emphasizing on molecular hybridization strategy followed by synthesis and evaluation of their *in vitro* antimycobacterial activity have been described. In **part-B** efforts were put forth to construct 1,2,3-triazole tethered heterocycles in one-pot employing click and activate strategy. Also, we envisaged to develop suitable azide source and simpler protecting group which readily undergoes deprotection under mild conditions.

In **chapter 3**, twenty two ciprofloxacin analogues were synthesized and evaluated for their antimycobacterial activity against MTB $H_{37}Rv$ strain. Among the synthesized compounds, **3c** and **3d** exhibited good activity with MIC **8** and **4 µg/mL** respectively.

In **chapter 4**, we designed two schemes based on phenanthridine skeleton. In **scheme 1** seventeen and in **scheme 2** nineteen phenanthridine analogues were synthesized and evaluated for their antimycobacterial activity against MTB $H_{37}Rv$ strain. Among the synthesized

compounds, **8e**, **8j**, **8k** and **11a** exhibited good antimycobacterial activity with MIC = 1.56 µg/mL. The selectivity index values were found to be >15, indicating compounds likeliness in drug development for tuberculosis.

In **chapter 5**, a series of twenty six aminothiazole analogues were synthesized over seven steps and evaluated for their anti-tubercular activity. Among the tested compounds, **18h** emerged as a prospective candidate by inhibiting the MTB H_{37}Rv strain at concentration 1.56 µg/mL. In addition, all the active compounds were subjected to cytotoxic studies against mouse macrophage (RAW264.7) cell lines and the selectivity index values for most of the compounds is >10 indicating suitability of compounds in an endeavour to attain lead molecule for further drug development.

In **chapter 7**, twenty one 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinolines were synthesized through a multi-component reaction. The domino/cascade reaction proceeds via click reaction, in which 1,2,3-triazole motif augment methylene group reactivity/N–C bond formation/Knoevenagel condensation in sequence. Overall one C–C bond and three C–N bonds were formed in a single step. In addition, photophysical properties of these new compounds were studied and compound **83u** emerged as good fluorogenic substrate with quantum yield ~0.21.

In **chapter 8**, fourteen 4-substituted-1*H*-1,2,3-triazoles were synthesized employing elemental sulfur to deprotect the methylene nitrile group. The operationally simpler strategy revealed good tolerance to various substrates affording desired products in moderate to good yields.

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List of Abbreviations

µg	:	Microgram
µM	:	Micromolar
^{13}C NMR	:	Carbon nuclear magnetic resonance
^{1}H NMR	:	Proton nuclear magnetic resonance
ACN	:	Acetonitrile
Ag_2CO_3	:	Silver(II) carbonate
ATP	:	Adenosine triphosphate
BCG	:	Bacillus Calmettee Guerin
t-BuOH	:	*tert*-butanol
t-BuOK	:	Potassium *tert*-butoxide
t-BuOLi	:	Lithium *tert*-Butoxide
CCDC	:	Cambridge Crystallographic Data Center
$CDCl_3$	:	Chloroform- deuterated
CO_2	:	Carbon dioxide
CP	:	Ciprofloxacin
Cs_2CO_3	:	Cesium carbonate
CuAAC		Copper-catalyzed azide-alkyne cycloaddition
CuBr	:	Copper(I) bromide
CuCl	:	Copper(I) chloride
$CuCl_2$	:	Copper(II) chloride
$CHCl_3$	:	Chloroform
$Cu(NO_3)_2.3H_2O$	:	Copper(II) nitrate trihydrate
$Cu(OAc)_2$	:	Copper(II) acetate
Cu_2O	:	Copper(I) oxide
CuI	:	Copper(I) iodide
$CuSO_4.5H_2O$	:	Copper sulphate pentahydrate
CuTC	:	Copper(I)-thiophene-2-carboxylate

d	:	Doublet
DBU	:	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	:	Dichloromethane
dd	:	Doublet of doublet
DOTS	:	Directly observed treatment short course
DIPEA	:	*N*,*N*-Diisopropylethylamine
DMEDA	:	*N*,*N*^{1}-Dimethylethylene diamine
DMF	:	*N*,*N*-Dimethylformamide
DMSO	:	Dimethyl sulfoxide
DMSO-d_6	:	Dimethyl sulfoxide deuterated
DNA	:	Deoxyribonucleic acid
EtOH	:	Ethanol
EMB	:	Ethambutol
EU	:	European Union
EtOAc	:	Ethyl acetate
FDA	:	Food and Drug Administration
FQ	:	Fluoroquinolone
G	:	Gram
H	:	Hour
HBr	:	Hydrobromic acid
H_2SO_4	:	Sulfuric acid
H_2O	:	Water
HIV	:	Human immunodeficiency virus
HRMS	:	High-resolution mass spectra
$NH_2OH.HCl$	:	Hydroxylamine hydrochloride
Hz	:	Hertz
INH	:	Isoniazid
IC_{50}	:	Inhibitory concentration
IR	:	Infrared Spectroscopy
J	:	Coupling constant
K_2CO_3	:	Potassium carbonate

KBr	:	Potassium bromide
KI	:	Potassium iodide
K_3PO_4	:	Potassium phosphate tribasic
LEV	:	Levofloxacin
m	:	Multiplet
m.p.	:	Melting point
MABA	:	Micro alamar blue assay
MCR	:	Multi-component reaction
MDR	:	Multi-drug resistant
MIC	:	Minimum inhibitory concentration
MOA	:	Mechanism of action
MeOH	:	Methanol
mg	:	Milligram
MHz	:	Megahertz
mmol	:	Millimolar
MS	:	Mass spectrometry
MTB	:	*Mycobacterium tuberculosis*
MH	:	Molecular hybridization
MTT assay	:	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay
MWI	:	Microwave irradiation
N_2	:	Nitrogen
nm	:	Nanometer
NAG-NAM	:	*N*-acetyl glucosamine – *N*-acetyl muramic acid
NaN_3	:	Sodium azide
NaOH	:	Sodium hydroxide
Na_2SO_4	:	Sodium sulfate
NTZ	:	Nitazoxanide
O_2	:	Oxygen
OLED	:	Organic light-emitting diode
PAS	:	Paraaminosalicylic acid
PEG	:	Polyethylene glycol

POA	:	Pyrazinoic acid
$POCl_3$	:	Phosphorus oxychloride
P_2O_5	:	Phosphorous pentoxide
ppm	:	Parts per million
PPA	:	Polyphosphoricacid
PZA	:	Pyrazinamide
RIF	:	Rifampicin
RNA	:	Ribonucleic acid
RT	:	Room temperature
s	:	Singlet
SI	:	Selectivity index
NaOAc	:	Sodium acetate
t	:	Triplet
TAACF	:	Tuberculosis antimicrobial acquisition and coordinating facility
TB	:	Tuberculosis
TBAB	:	Tetrabutyl ammonium bromide
TDR	:	Totally drug-resistant
TEA	:	Triethylamine
TFA	:	Trifluoroacetic acid
THF	:	Tetrahydrofuran
TIZ	:	Tizoxanide
TLC	:	Thin-layer chromatography
TMEDA	:	Tetramethylethylene diamine
$TMSN_3$	:	Trimethylsilyl azide
p-TsOH	:	*p*-Toluenesulfonic acid
Tt	:	Triplet of triplet
UV	:	Ultra violet
WHO	:	World Health Organization
XDR	:	Extensively-drug resistant

Part-A

Chapter I

Introduction

Introduction

Chapter 1

1. Tuberculosis

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB) ("white plague"), was identified by Robert Koch in 1882. MTB is one of the world's most successful and sophisticated pathogens, as it causes persistent infection leading to estimated deaths of over 2 billion people [1-3]. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB). TB is also a major public health problem in India. India accounts for one-fifth of the global TB incident cases. Each year nearly 2 million people in India develop TB, of which around 0.87 million are infectious cases. It is estimated that annually around 330,000 Indians die due to TB [4]. However, in recent times population of TB patients caused by MTB are increasing in an alarming rate, revealing ineptitude of currently available medicine in the market. Despite Bacillus Calmettee Guerin (BCG) vaccine and the combined chemotherapy with first-line (isoniazid, ethambutol, rifampicin, pyrazinamide) **figure 1.1** or second-line (ethionamide, ciprofloxacin, moxifloxacin) antibiotics, **figure 1.2**, MTB is the agent which causes more deaths from infection in the world [5]. Two factors mainly associated with the spread of TB are human immunodeficiency virus (HIV) infection and the emergence of MTB strains that are resistant to one or more drugs. Besides effective implementation of Directly Observed Treatment Short course (DOTS), perceptible transformation could be accounted to rapid spread of multi-drug resistant (MDR) and extensively-drug resistant (XDR) mycobacterium strains and the recently identified totally-drug resistant (TDR) TB strains [6]. The recent influx of immigrants from countries endemic for disease and the co-infection with

human immunodeficiency virus (HIV) compounded TB into a serious problem in developed/developing countries [7].

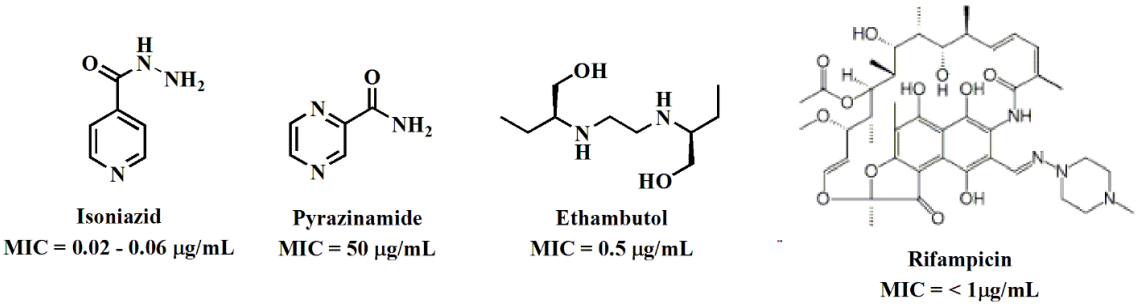

Figure 1.1 First line anti-TB drugs

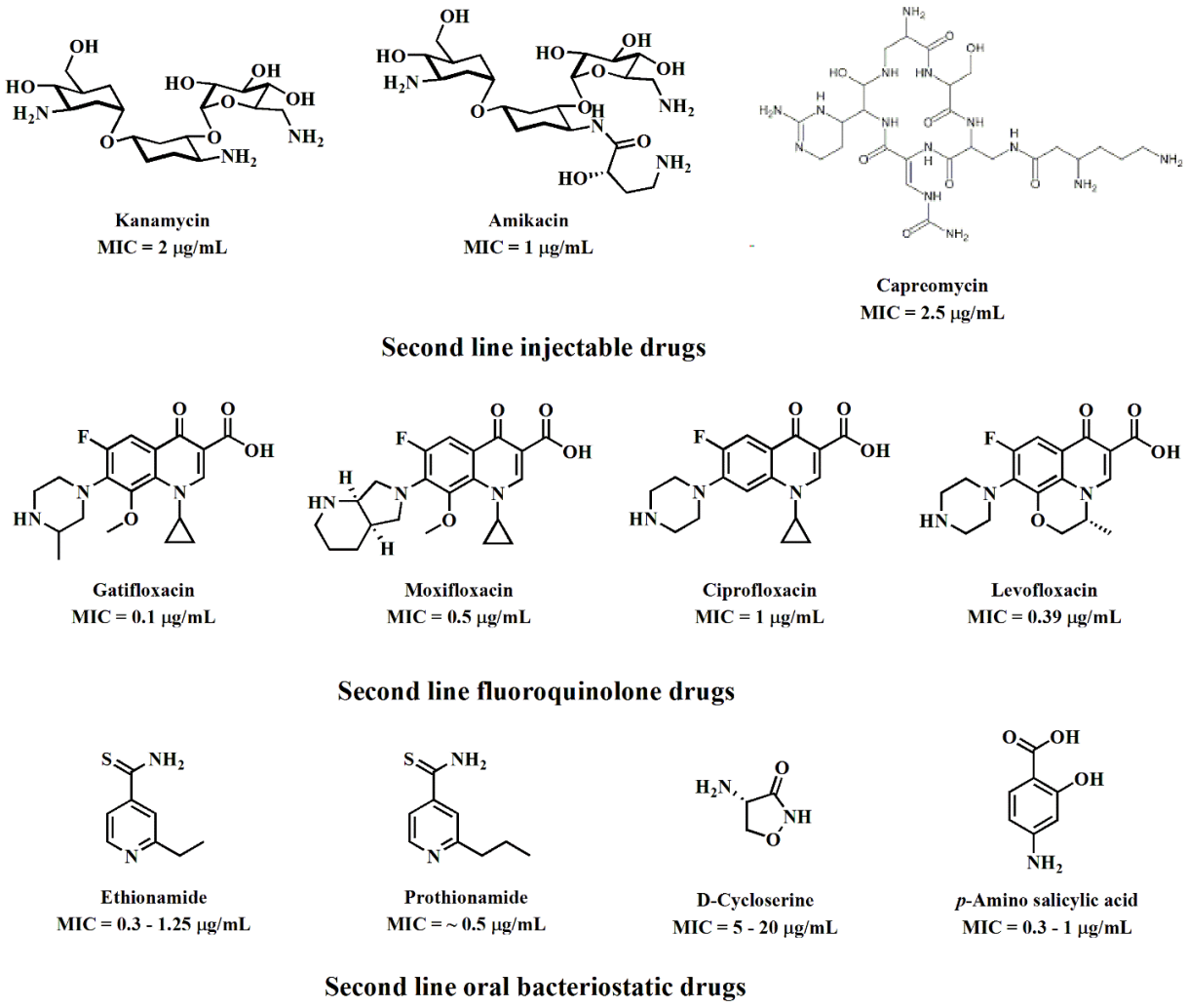

Figure 1.2 Second line anti-TB drugs

1.1 Cell envelope

The cell envelope of MTB, is composed of three distinct macromolecules: peptidoglycan, arabinogalactan and mycolic acids, which are surrounded by a non-covalently linked outer capsule of proteins and polysaccharides (**figure 1.3**) [8]. The cell wall is the most common target of antitubercular drugs, and many compounds that are in clinical use or under development target enzymes that synthesize distinct layers of the cell wall. The three polymers in the cell wall, arabinogalactan-mycolate [9], covalently linked with peptidoglycan and trehalose dimycolate, provide a thick layer that protects the tubercle bacillus from general antibiotics and the host's immune system [10].

The peptidoglycan layer surrounds the plasma membrane and comprises long polymers of the repeating disaccharide *N*-acetyl glucosamine–*N*-acetyl muramic acid (NAG–NAM) that are linked via peptide bridges. Compared with other model bacteria, such as *Escherichia coli* and *Bacillus subtilis*, mycobacterial peptidoglycan is heavily crosslinked. Up to 80% of the peptidoglycan contains non-traditional 3–3 peptide crosslinks instead of traditional 4–3 crosslinks [11].

A layer of arabinogalactan surrounds the peptidoglycan layer. Galactan comprises a repeating disaccharide unit of 6-D-Gal*f*β1–5-D-Gal*f*β and is synthesized by the galactofuranosyl transferases Glf, GlfT1 and GlfT2.

Most arabinan is ligated with long-carbon-chain mycolic acids [12] which form the characteristic thick waxy lipid coat of mycobacteria [13] and are major contributors to the impermeability of the cell wall [14] and to virulence [15].

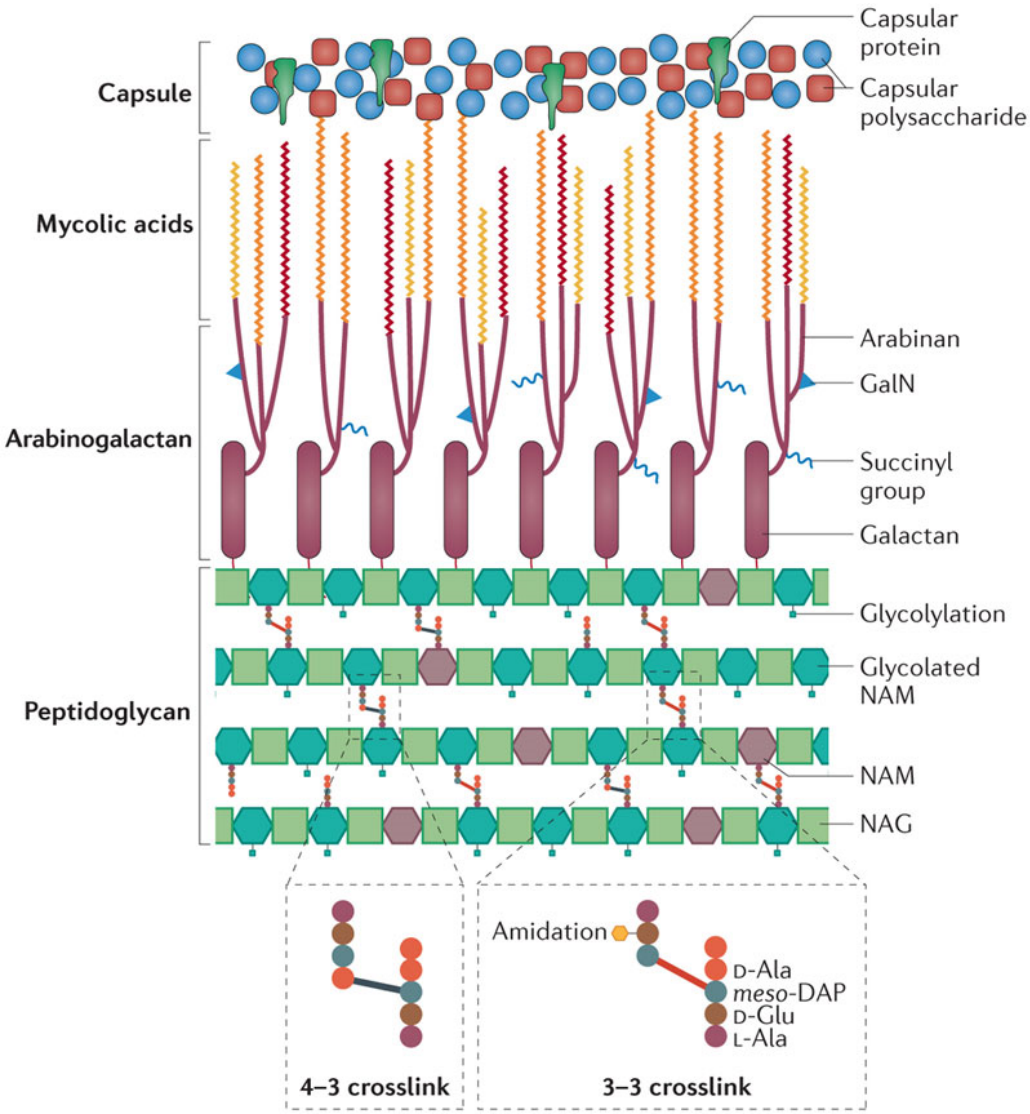

Figure 1.3 Schematic representation of cell envelope [8].

Mycolic acids are the hallmark of the cell envelope of MTB, which are long chain α-alkyl-β-hydroxy fatty acids, the major constituents of this protective layer, has been shown to be critical for the survival of MTB **figure 1.4** [16]. InhA, an enoyl-ACP reductase, involved in mycolic acid synthesis, is a well-known target for front-line anti-tubercular drugs [17] such as isoniazid [18], and ethionamide [19], much interest has been devoted to deciphering the chemistry and biosynthesis of mycolic acids in the alarming context of the emergence of multidrug-resistant (MDR), extremely drug-resistant (XDR), and totally drug-resistant (TDR) tuberculosis (TB). Mycolic acids are processed and matured by a cascade of enzymes [20], which results in three distinct meromycolate variants: α-meroacids, methoxy-meroacids and keto-meroacids [21]. All three variants are required for full virulence during infection and have varying levels of saturation, cyclopropanation and oxygenation [22].

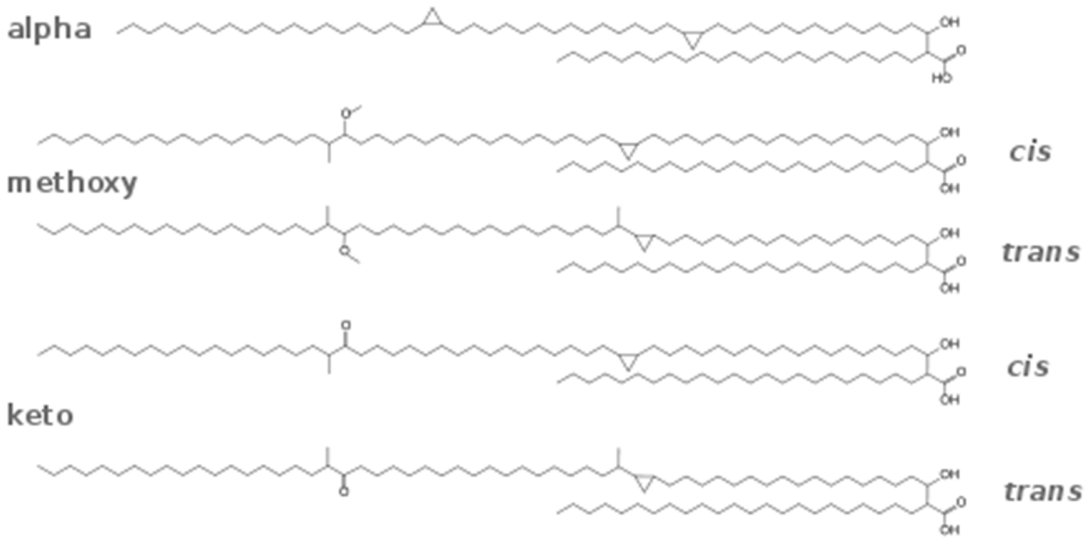

Figure 1.4 Schematic representation of mycolic acids in *Mycobacterium tuberculosis* [16].

1.2 Tuberculosis: Drug resistance

Situation of resistance to all the drugs used to treat TB have been reported. As a result of drug resistance the patient survival percentage has shrunk and worsened. This in turn led spreading of drug resistance TB and pose substantial threat to disease control [23]. The World Health Organization (WHO) classifies tuberculosis resistant to isoniazid and rifampicin as MDR-TB, when a switch to second line treatment is advised [24]. Resistance of MDR-TB strains to the fluoroquinolones and aminoglycosides are classed as XDR-TB. The term totally drug-resistant TDR-TB has been used to describe strains found resistant to all available drugs, but there is not yet an agreed definition of TDR-TB [23]. Treatment of drug resistant disease is prolonged and expensive, possesses heightened toxicity and adverse reactions are common and may be severe and irreversible [25]. Poor tolerance leads to reduced compliance, which in turn reduces cure rates and can result in amplification of resistance [26]. Studies have shown that one of the important causes for default of treatment is adverse drug reactions. Drugs used for MDR-TB treatment have a great potential to cause adverse effects. They range from mild gastrointestinal disturbances, arthralgia to major psychosis, suicidal tendencies and severe hypothyroidism [27].

1.2.1 Multidrug-resistant TB:

Multidrug resistance continues to emerge and spread because of mismanagement of TB treatment and person-to-person transmission. Most people with TB are cured by a strictly followed, six-month drug regimen that is provided to patients with support and supervision. Inappropriate or incorrect use of antimicrobial drugs, or use of ineffective formulations of drugs (e.g. use of single drugs, poor quality medicines or bad storage conditions), and premature treatment interruption can cause drug resistance. In some countries, it is becoming

increasingly difficult to treat MDR-TB. Treatment options are limited and expensive, recommended medicines are not always available, and patients experience many adverse effects from the drugs [28].

1.2.2 Extensively Drug-Resistant TB:

XDR-TB is a relatively rare type of MDR-TB. It is resistant to almost all drugs used to treat TB, including the two best first-line drugs: isoniazid and rifampin. XDR-TB is also resistant to the best second-line medications: fluoroquinolones and at least one of three injectable drugs (i.e., amikacin, kanamycin, or capreomycin). Because XDR-TB is resistant to the most powerful first-line and second-line drugs, patients are left with treatment options that are much less effective and often have worse treatment outcomes [29].

1.3 TB and AIDS

One of the key factors behind the resurgence of TB, apart from the emergence of drug-resistant strains, is the HIV pandemic. As the immune system is compromised due to HIV infection, the probability of developing TB increases by up to 30 times [30]. HIV infection also represents the major risk for the progression of a latent TB infection to active disease. Further, TB induces the onset of AIDS in HIV-positive patients by the production of stimulatory cytokines and a decrease in CD4 cell charge. Thus, the HIV-MTB co-infection is a serious concern both to infected patients and the global population [31]. It must also be noted that several of the problems associated with TB, such as resistance and HIV co-infection are highly coupled. For example, due to the high abandon rate of treatment by co-infected patients, there is a higher

emergence of drug resistant strains [32]. Such opportunistic infections have a disastrous effect on the mortality rate in infected patients.

1.4 Current treatment and control methods:

Tuberculosis is a curable disease if the standard TB drug treatment regimen is faithfully administered for six months [a combination of rifampicin (RIF), isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA) for two months, followed by a four-month continuation phase of RIF and INH] upon early and accurate diagnosis [33]. Non-compliance to the long treatment period has led to the emergence of MDR-TB. To halt the spread of MDR-TB, the WHO recommends pre-therapy drug susceptibility testing prior to initiating a 20-month treatment entailing appropriate second-line drugs, which are often associated with multiple (and sometimes serious) side effects and lower cure rates. With the same drugs prescribed for HIV-TB co-infections, their efficacy and tolerability has been affected by the interactions between anti-TB and antiretroviral therapies [34]. Recognising that a concerted effort is necessary to reduce the disease burden, the WHO developed and strongly encouraged the implementation of the DOTS, for countries to better grasp the disease situation with thorough disease surveillance, systematic diagnosis and treatment, and patient support. It has been proven to be one of the most efficient approaches to fight the global TB epidemics [33]. Effectiveness and tolerability relation of first- and second-line drugs used in TB treatment is depicted in **figure 1.5**.

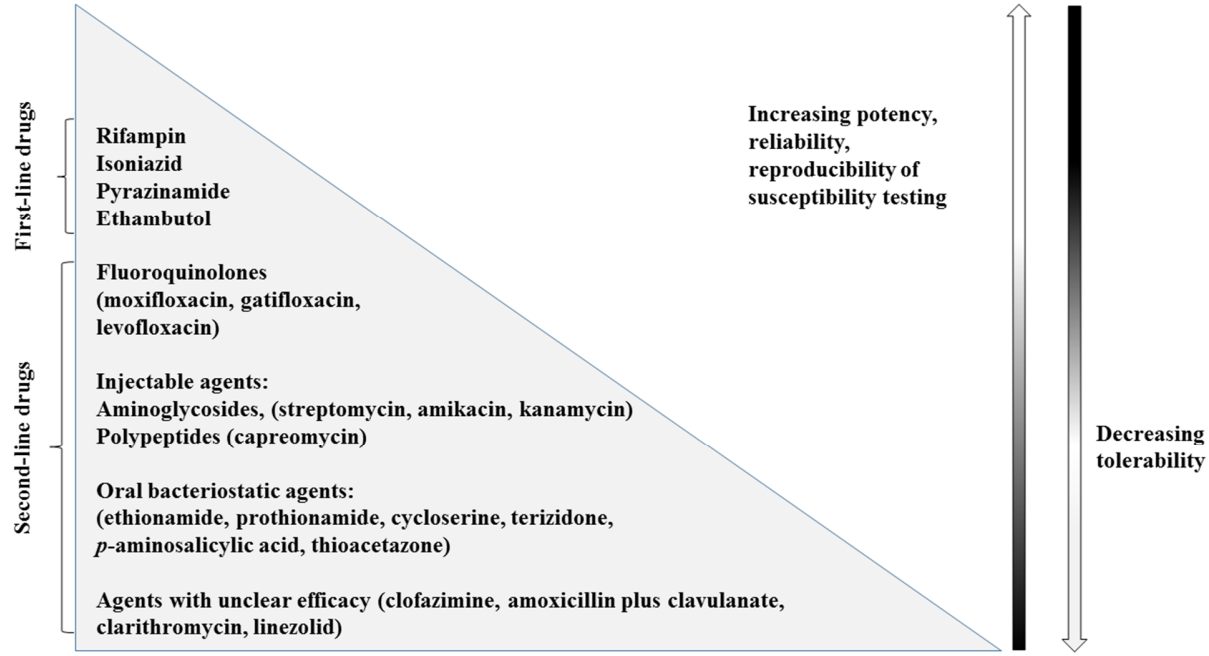

Figure 1.5 Effectiveness and tolerability relation of first- and second-line drugs used in TB treatment.

1.5 Molecular Modification:

Molecular modification is a chemical alteration to a lead compound or a drug, which enhances its pharmaceutical, pharmacokinetic or pharmacodynamic parameters. Among molecular modifications three approaches have generally been explored: prodrug approach, molecular hybridization and bioisosterism [35]. Among them, molecular hybridization is a leading one in developing suitable lead molecules for target disease.

1.5.1 Prodrug approach:

The first definition of prodrug was introduced by Albert in 1958 which define prodrug as “any compound that undergoes biotransformation prior to exhibit its pharmacological effects” [36]. In

order to improve this definition, Haper in 1959 proposed the term latentiation. Drug latentiation is understood as "the chemical modification of biologically active compound to form a new compound that, upon *in vivo* enzymatic attack, will liberate the parent compound". In general the prodrugs could be classified into two main classes: bioprecursors and carrier prodrugs. Bioprecursors is a molecular modification strategy that generates a new compound substrate for the metabolizing enzymes that after this biotransformation demonstrate biological activity. This approach generally does not use carriers. Several examples of drugs are available in the market used this strategy such as sulindac, acyclovir, losartan among others [37]. Carrier's prodrugs are designed using labile linkage between a carrier group and an active compound. This prodrug after chemical or biological biotransformation releases the parental drug responsible for the biological activity. The prodrug, per se, is usually inactive or less active than parental drug.

1.5.2 Molecular Hybridization (MH):

Molecular hybridization is a structural modification strategy useful in the design of new optimized ligands and prototypes with new molecular architectures composed of two or more known bioactive pharmacophoric fragments, through the adequate fusion into a single molecule. The advantage of using MH is to activate different targets by a single molecule, thereby increasing therapeutic efficacy as well as to improve the bioavailability profile [38]. Presently MH appears as an important tool for the design of new prototypes of innovating drugs because many such hybrid derivatives possessed improved efficiency and efficacy, when compared to the parent compounds [39].

Schematically, the **figure 1.6** shows the use of MH strategy. The drug A interacts only with the receptor A. The drug B interacts only with the receptor B. However the interaction between drug A and receptor B (and vice versa) is forbidden. But it is possible to design compounds that can interact with both receptors contributing synergistically for a desire effect [35].

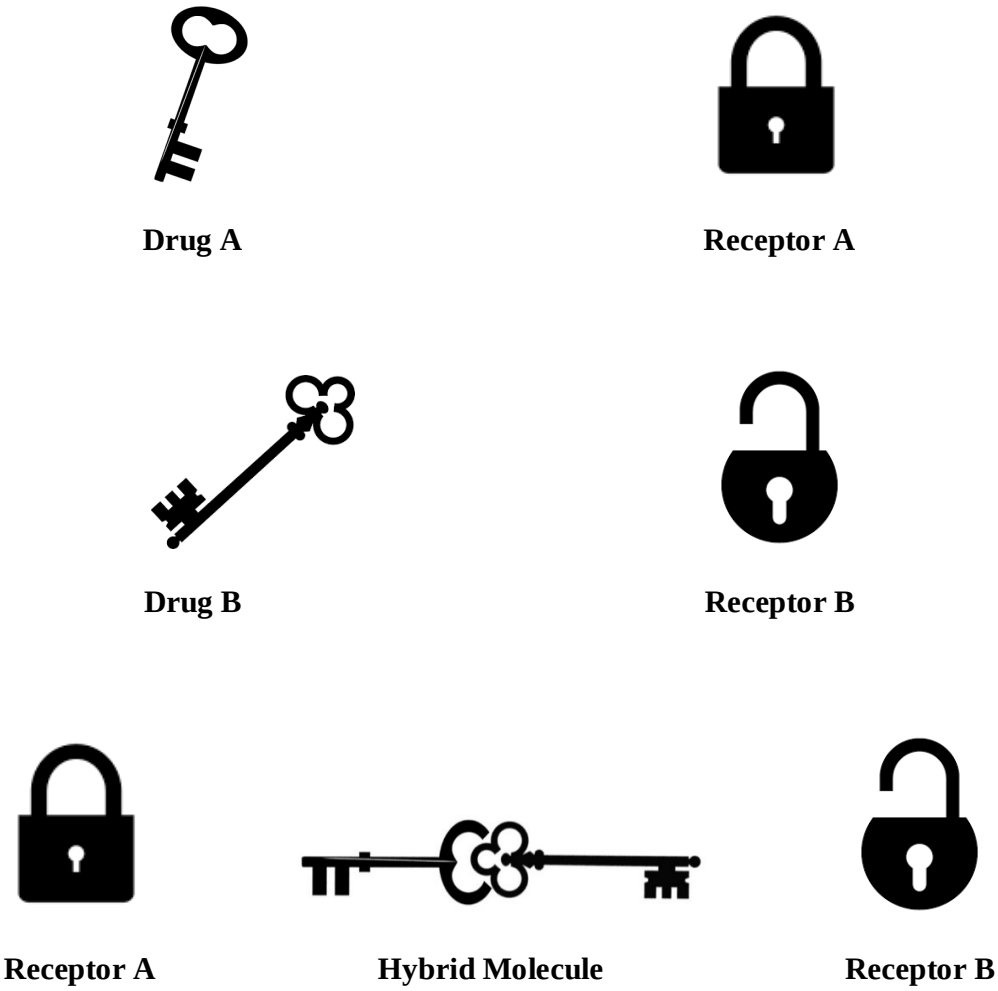

Figure 1.6 Molecular hybridization strategy

The combination of multiple actions in the same drug is an interesting strategy in TB treatment in order to contribute to therapy compliance, improve the activity and reduce resistance. One fruitful example using this strategy could be represented by **figure 1.7** which demonstrated MH of isoniazid and quinolone derivative to increase the antimycobacterial activity of the novel compounds. This compound was able to maintain high survival rate reducing *in vivo* colony-forming unit with few lung lesion and reduced splenomegaly [40].

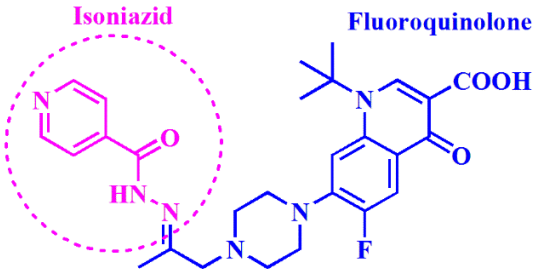

Figure 1.7 Molecular hybridization between fluoroquinolone and isoniazid

1.5.3 Bioisosterism

The term isosterism was first defined by Langmuir in 1919 as atoms or organic or inorganic molecules which possess the same number and/or arrangement of electrons examples such as C=O and N=N; CO_2 and NO_2 [41].

Currently bioisosteres are understood as groups or molecules which have a chemical and physical similarity producing broadly similar biological effects [42]. Burger classified and subdivided bioisosteres in two broad categories: classic and non-classic. The classical bioisosteres are subdivided in: a) monovalent atoms or groups; b) divalent atoms or groups; c) trivalent atoms or groups; tetravalent atoms and e) ring equivalents (**Table 1.1**).

Table 1.1 Classic bioisosteres atoms and groups.

Monovalent	**Divalent**	**Trivalent**	**Tetravalent**
-OH, -NH_2, -CH_3, -OR	-CH_2-	=CH-	=C=
-F, -Cl, -Br, -I, -SH, -PH_2	-O-	=N-	=Si=
-$SiCH_3$, -SR	-S-	=P-	$=N^+=$
	-Se-	=As-	$=As^+=$
	-Te-	=Sb-	$=Sb^+=$
			$=P^+=$

The non-classical bioisosteres do not present the steric and electronic definition of classical isosteres, furthermore they do not have the same number of atoms of the substituent or moiety replaced. Among non-classical bioisosteres we could cite: functional groups, noncyclic or cyclic and retroisosterism.

The bioisosterism approach is an important molecular modification tool that allows the discovery of several drugs in the market. The drugs discovered using this strategy that are in the markets usually known as “me too” [43]. This strategy has been used to discover new compounds to treat TB. One example is the class of fluoroquinolones. Fluoroquinolones demonstrated antitubercular activity besides Gram-negative and Gram- positive activity. This class of drugs is known to inhibit bacterial DNA replication and transcription by binding to DNA-gyrase-DNA complex. The use of fluoroquinolones occur mainly in patients with MDR-TB. The most active quinolones

for the treatment of TB are: ciprofloxacin, sparfloxacin, ofloxacin, moxifloxacin and levofloxacin [44]. Studies comparing the bactericidal activity of various fluoroquinolones against TB in the latent and exponential growth phases has demonstrated that most promising drugs are moxifloxacin and levofloxacin [45]. Most of the fluoroquinolone drugs were obtained using bioisosteric replacement (**figure 1.8**).

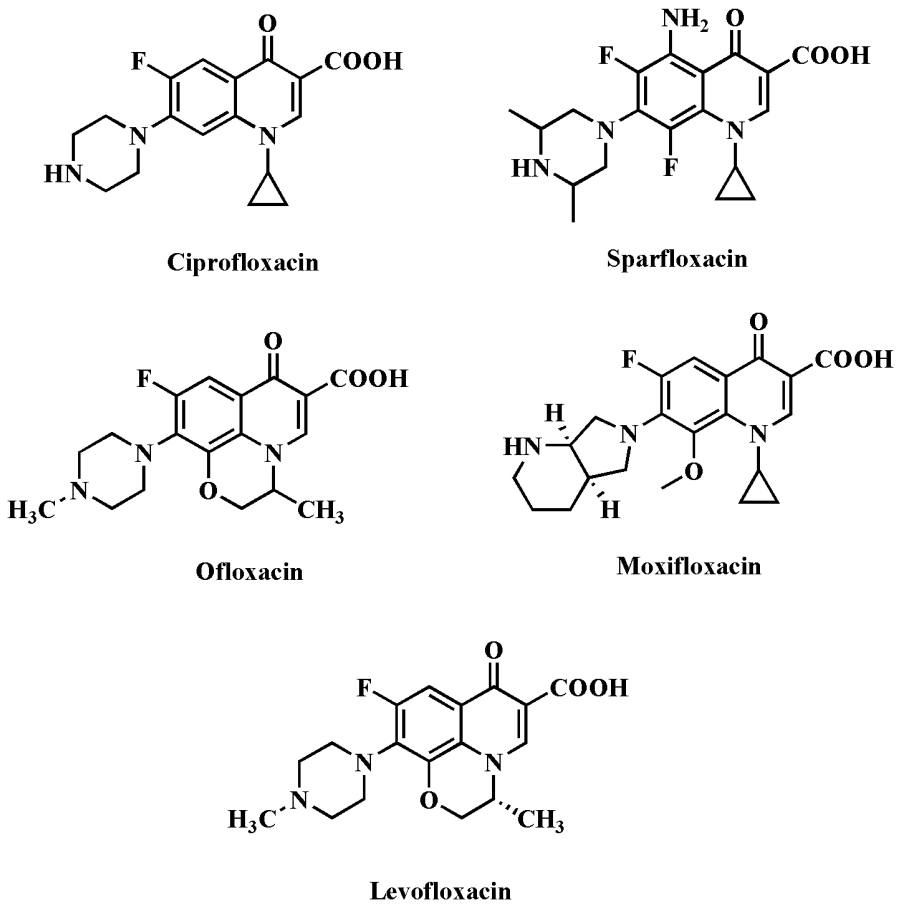

Figure 1.8 Fluoroquinolone drugs were obtained using bioisosteric replacement.

1.6 Current TB drugs and their mechanism of action

1.6.1 Isoniazid (INH)

INH has been the most commonly used antituberculosis drug since recognition of its clinical activity in 1952 [46]. INH consists of a pyridine ring and a hydrazide group, which is structurally related to the TB drugs ethionamide and pyrazinamide. Due to its potency of being a bactericidal agent, it emerged as a vital component of the first-line antituberculous regimens.

1.6.1.1 Mechanism of action

INH appears to penetrate into the host cells and able to diffuse smoothly across the MTB membrane [47]. It is a pro-drug, which undergoes oxidative activation by the MTB catalase-peroxidase enzyme KatG [48]. The crucial pathway for the antitubercular activity of the drug is inhibition of mycolic acid synthesis [49] along with the inhibition of synthesis of nucleic acids, phospholipids and NAD metabolism [50]. KatG links the isonicotinic acyl part to NADH resulting in an isonicotinic acyl-NADH complex. This complex binds efficiently to the InhA which is an enoyl-acyl carrier protein reductase, and blocks the natural enoyl-AcpM substrate and the action of fatty acid synthase. Consequently, the inhibition of synthesis of mycolic acids is terminated [51].

1.6.2 Rifampin and other rifamycins

The rifamycins were first isolated in 1957 from Amycolatopsis (formerly Streptomyces) mediterranei as part of an Italian antibiotic screening program [52]. Administering Rifampin in combination with standard anti-tuberculosis regimen allowed decrease in the treatment time from

18 to 9 months. Although the early bactericidal activity of the rifamycins is inferior to that of INH. These are well known for inhibiting drug-resistant tuberculosis in the 1960s [53].

1.6.2.1 Mechanism of action

Rifamycins contain an aromatic nucleus linked on both sides by an aliphatic bridge so as to easily diffuse across the *MTB* cell membrane due to their lipophilic profile. These have ability to inhibit bacterial DNA-dependent RNA synthesis, due to high bind affinity with RNA polymerase [54].

1.6.3 Pyrazinamide (PZA)

PZA was discovered in 1952 [55], it shortens the treatment time from 9–12 months to the current 6 months [56]. The treatment-shortening potential of PZA has been attributed to the drug's ability to target semi-dormant populations of bacilli residing within an acidic environment [57]. Thus PZA is inferior in bactericidal activity to that of INH and rifampin [58].

1.6.3.1 Mechanism of action

PZA is an amide derivative of pyrazine-2-carboxylic acid and nicotinamide analog, whose mechanism of action of PZA remains poorly understood. PZA is a pro-drug, requiring activation to its active form, pyrazinoic acid (POA), by the enzyme pyrazinamidase (PZase) [59]. As PZA is administered for initial 2 months of the therapy, it has been hypothesized that the PZA might act against bacilli residing in acidified compartments of the lung that are present during the early inflammatory stages of infection [60]. PZA disrupts the proton motive force required for essential membrane transport functions by POA at acidic pH [61].

1.6.4 Ethambutol (EMB)

EMB - dextro-2,2.-(ethylenediimino)-di-1-butanol was discovered as anti-tubercular agent in 1961. It has become one of the key ingredients in modern-day short-course treatment of drug-susceptible TB. EMB was reported to inhibit actively multiplying bacilli and has very poor sterilizing activity.

1.6.4.1 Mechanism of action

EMB inhibits the cell wall of arabinan polymerization, which in turn affects the arabinogalactan biosynthesis [62]. EMB also has been known to inhibit several other cellular pathways, including RNA metabolism [63], transfer of mycolic acids into the cell wall [64], phospholipid synthesis [65], and spermidine biosynthesis [66].

1.6.5 Aminoglycosides

Streptomycin was first discovered in early 1940s, which led to the breakthrough in TB chemotherapy. Due to the poor oral absorption of streptomycin, as well as the toxicity profile of the aminoglycosides, streptomycin is substituted for EMB and favored the use of EMB in first-line anti-tuberculosis therapy. Other aminoglycosides with significant antimycobacterial activity include kanamycin and amikacin. Aminoglycosides are used currently as second-line drugs primarily in the treatment of MDR-TB.

1.6.5.1 Mechanism of action

Aminoglycosides bind to the 30S ribosomal subunit, which affects polypeptide synthesis, thereby resulting in inhibition of translation.

1.6.6 Fluoroquinolones

The fluoroquinolones (moxifloxacin, gatifloxacin, sparfloxacin, levofloxacin, ofloxacin, and ciprofloxacin), possess excellent activity against MTB and are used as second-line drugs in TB treatment. New-generation fluoroquinolones [moxifloxacin, gatifloxacin] are under clinical evaluation as first-line antibiotics focusing on shortening the duration of TB treatment.

1.6.6.1 Mechanism of action

Fluoroquinolones are known for their binding ability to gyrase and topoisomerase IV on DNA. Thus blocking the movement of replication forks and transcription complexes [67].

1.6.7 Ethionamide

Ethionamide, is a pro-drug and structurally related to INH, which gets activated by the monooxygenase EthA [68].

1.6.7.1 Mechanism of action

Ethionamide has ability to inhibit mycolic acid synthesis by binding to the ACP reductase InhA.

1.6.8 Capreomycin

Capreomycin is a macrocyclic polypeptide antibiotic isolated from *Streptomyces capreolus* [69]. Capreomycin, like streptomycin and kanamycin, inhibits protein synthesis through modification of ribosomal structures at the 16S rRNA [54]. Recent studies using site-directed mutagenesis have identified the binding site of capreomycin on 16S rRNA helix 44 [70].

1.6.9 Cycloserine

Cycloserine is a d-alanine analogue, which interrupts peptidoglycan synthesis by inhibiting the enzymes d-alanine racemase (AlrA) and d-alanine:d-alanine ligase (Ddl) [71].

1.6.10 Paraaminosalicylic acid (PAS)

PAS is thought to inhibit folic acid biosynthesis and uptake of iron [54]. Mutations in the thyA gene encoding the enzyme thimidylate synthesis of the folate biosynthesis pathway have been identified in PAS-resistant MTB clinical isolates, suggesting that PAS may act as a folate antagonist [72]. However, only slightly more than a third of the evaluated PAS-resistant strains had mutations in thyA, suggesting the existence of additional mechanisms of PAS resistance. Thr202Ala has been reported as the most common mutation associated with PAS resistance, although this mutation has also been identified in several PAS-susceptible isolates [73].

1.7 TB drugs in pipeline

Table 1.2 TB drugs in pipeline

Preclinical Development	Phase I	Phase II	Phase III
TBI-166 Riminophenazines antibiotic	**Q203** - Novel anti TB agent Imidazopyridine	**Sutezolid** (PNU - 100480) Oxazolidinone	**Bedaquiline** (TMC207)
CPZEN-45 Caprazene nucleoside		**SQ109** Ethylenediamine	**Delamanid** (OPC-67683)
SQ641 Capuramycin		**Bedaquiline-Pretomanid-Pyrazinamide**	**Rifapentine**
Spectinamide 1599 Spectinomycin analogs		**Levofloxacin**	**Pretomanid-oxifloxacin-Pyrazinamide**
PBTZ 169 Benzothiazinone			
BTZ 043 Benzothiazinone			

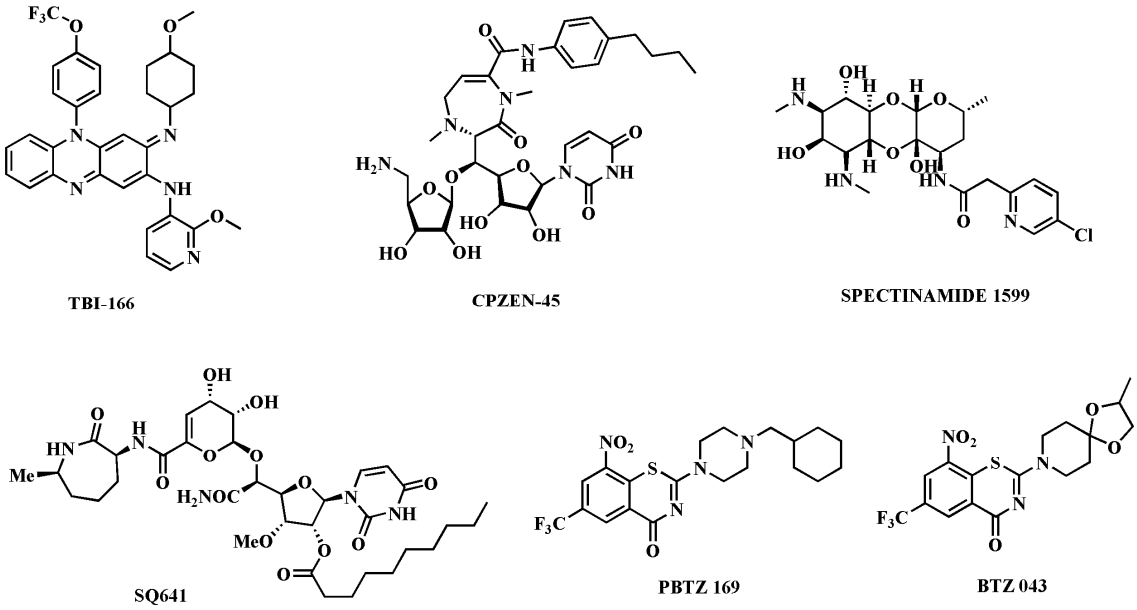

Figure 1.9 Structure of anti-TB agents under preclinical development.

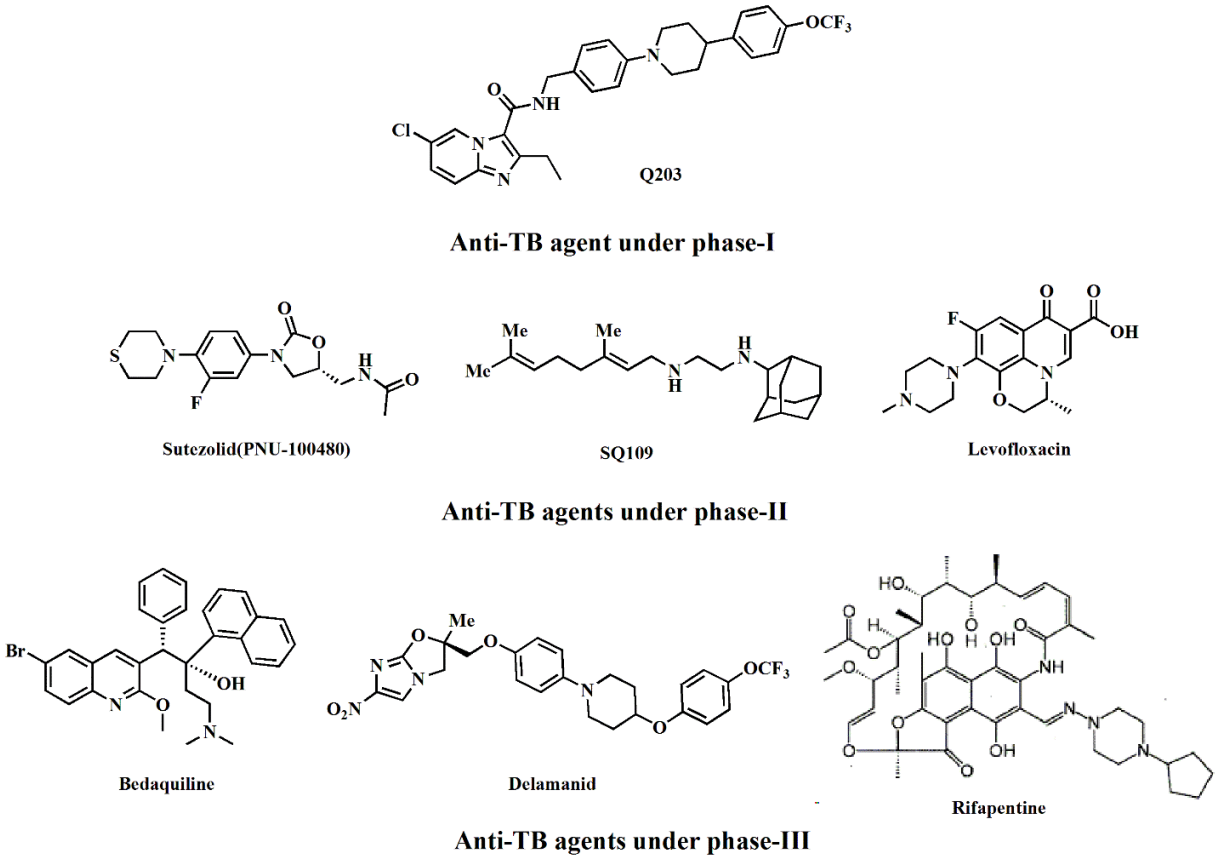

Figure 1.10 Structure of anti-TB agents under various phase of clinical trials.

1.7.1 Q203

Q203 is under phase I clinical trials capable of rapidly inhibiting ATP synthesis which indicates the inhibition of cytochrome *bc*1 activity, which is a bacterial enzyme complex needed for respiration. It is found that Q203, being a new chemical entity is able to inhibit MDR-TB and XDR-TB [74].

1.7.2 Sutezolid

Sutezolid has better antimycobacterial activity and an improved safety profile than linezolid [75-77]. Sutezolid exhibits time dependent inhibition in an *in vitro* whole blood culture test [78]. In addition, it has activity against both drug susceptible and drug resistant TB [79].

1.7.3 SQ109

SQ109 is structurally analogues to EMB with amplified lipophilic framework. Unlike EMB, SQ109 has different mechanism of action and belongs to the classes of cell wall inhibitors. SQ109 demonstrated synergistic interactions with INH and RIF, and additive effects when combined with EMB or streptomycin during *in vitro* study. It also revealed no adverse drug-drug interactions, good activity against drug susceptible and drug resistant TB. These results promise that SQ109 could be added to MDR-TB regimen [80].

1.7.4 Levofloxacin (LEV)

LEV is a new-generation fluoroquinolone antibiotic. The *in vitro* and *in vivo* activities of LEV against MTB are two to three-fold greater than for ofloxacin [81]. LEV has a MIC of 1 μg/L against MTB, while the MIC of ofloxacin and ciprofloxacin are 2 and 4 μg/L [82,83]

respectively. LEV has become a commonly used fluoroquinolone in North America because of its superior *in vivo* activity, as well as its more-convenient dosing schedule (LEV is administered once daily compared with the twice-daily schedule for ciprofloxacin/ofloxacin) [84]. In August 2001, the TB Control Service at the British Columbia Centre for Disease Control switched from ciprofloxacin to levofloxacin for the treatment of patients with drug-resistant TB infection and for patients who had TB and were intolerant to first-line medications [81].

1.7.5 Bedaquiline

The US Food and Drug Administration (FDA) in 2012 granted accelerated approval to Johnson and Johnson's drug bedaquiline to treat resistant TB, more prevalent in India, China and Eastern Europe [85]. The MOA of Bedaquiline is inhibition of the proton pump of mycobacterial ATP synthase. ATP synthase is a critical enzyme in the ATP synthesis of MTB. Binding of bedaquiline to the oligomeric and proteolipic subunit-c of mycobacterial ATP synthase leads to inhibition of ATP synthesis, which subsequently results in bacterial death [86].

Bedaquiline can affect the heart's electrical activity causing prolongation of the QT interval, which could lead to an abnormal and potentially fatal heart rhythm. Accordingly, the FDA has approved bedaquiline as part of combination therapy to treat adults with MDR pulmonary TB when other alternatives are not available. The FDA also granted fast-track designation, priority review and orphan-product designation to bedaquiline [87].

1.7.6 Delamanid

Delamanid (Deltyba), a non-mutagenic nitroimidazooxazole (or nitroimidazopyran) [88] with early bactericidal activity [89] has been approved in the EU and Japan for the treatment of MDR-TB, when administered in combination with an optimized background regimen.

Delamanid acts as a mycolic acid biosynthesis inhibitor, thereby disrupting metabolism of the cell wall and facilitating better drug penetration into mycobacteria. It is a pro-drug that requires bioreduction of its nitro group by MTB to produce a reactive species. This activation is dependent on the actions of the reduced deazaflavin cofactor F_{420}, its reductively activating enzyme F_{420}-dependent glucose-6-phosphate dehydrogenase and the nitroreductase gene product of *Rv3547*. Delamanid interferes with mycolic acid production by inhibiting the synthesis of ketomycolic and methoxymycolic, but not alphamycolic, acids [90].

1.7.7 Rifapentine

Rifapentine is a cyclopentyl-substituted semisynthetic rifamycin that was first synthesized in 1965 by the Italian company that developed rifampin. Like other rifamycins, rifapentine inhibits bacterial DNA-dependent RNA polymerase. Rifamycins are unique among drugs that work by this mechanism, because the inhibition of RNA polymerase will occur even when enzyme exposure to the drug is very brief in otherwise metabolically dormant organisms; this has implications for the use of these drugs for treatment of latent tuberculosis infection. Rifapentine should be given with isoniazid during the continuation phase of the treatment of drug-susceptible pulmonary tuberculosis after an intensive phase that consists of at least rifampin (or rifabutin), isoniazid, pyrazinamide, and ethambutol administered for two months [91].

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Chapter II

Objectives

Objectives

Chapter 2

PART-A

Tuberculosis (TB) is a deadly disease caused due to the victorious pathogen *Mycobacterium tuberculosis* (MTB). TB is a major public health problem in India. India accounts for one-fifth of the global TB incident cases. Ascribable to complexity and toxicity of the current TB drug regimens and emergence of various forms of drug resistant TB warranted the scientific community to focus on exploring novel chemotherapeutical agents. As TB is an inevitable disease among HIV patients, it is identified that current TB drugs (Rifabutin, Rifampicin and Rifapentine) interact with the antiretroviral drugs taken by HIV positive people; hence it is essential to scrutinize cost effective, less toxic chemical entities preferably with new biochemical pathways to shorten treatment time, and to interrupt drug-drug interaction.

The main objectives of the proposed research are as follows:

1. Design molecules emphasizing on molecular hybridization strategy.
2. Synthesize and evaluation of their *in vitro* antimycobacterial activity.
3. Investigate mechanism of action and identify the target site.

PART-B

One-pot reactions turned out to be a potentially more efficient synthetic strategy. Owing to instinctive drawbacks involved in a multistep synthesis, one-pot synthesis has recently the choice of interest.

1. To construct 1,2,3-triazole tethered heterocycles in one-pot employing click and activate strategy.
2. Develop suitable azide source and simpler protecting group which readily undergoes deprotection under mild conditions.

Chapter III

Synthesis of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-{2-(4-substitutedpiperazin-1-yl)acetyl}piperazine-1-yl]quinoline-3-carboxylic acid derivatives as anti-tubercular agents

Chapter 3

Synthesis of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-{2-(4-substitutedpiperazin-1-yl)acetyl}piperazine-1-yl]quinoline-3-carboxylic acid derivatives as anti-tubercular agents

3.1. Introduction

Quinolones are a group of synthetic antibacterials that effectively inhibit DNA replication and are commonly used as treatments for many infectious diseases [1]. The addition of a fluorine atom to the quinolone antibiotics at position 7, yielded a new class of drugs, known as fluoroquinolones (FQ), which have a broader activity spectrum and improved pharmacokinetic properties [2].

Fluoroquinolones are used for the clinical control of MDR-TB, that is, resistant to both isoniazid and rifampin due to its bacilli. Several of the quinolone antibacterials, such as gatifloxacin, moxifloxacin and sitafloxacin, (**figure 3.1**) have been examined as inhibitors of MTB [6]. Quinolones inhibit bacterial type II topoisomerase, DNA gyrase, and topoisomerase IV [7], which are essential enzymes that maintain the supercoils in DNA.

The reasons for its wide applicability include: multiresistant pathogens are susceptible to Ciprofloxacin (CP) alone; the pharmacokinetic profile of CP, which demonstrates equivalent or greater bioavailability; higher plasma concentrations; and increased tissue penetration, as reflected in the greater volume of distribution. The problem of drug-resistant bacteria has been

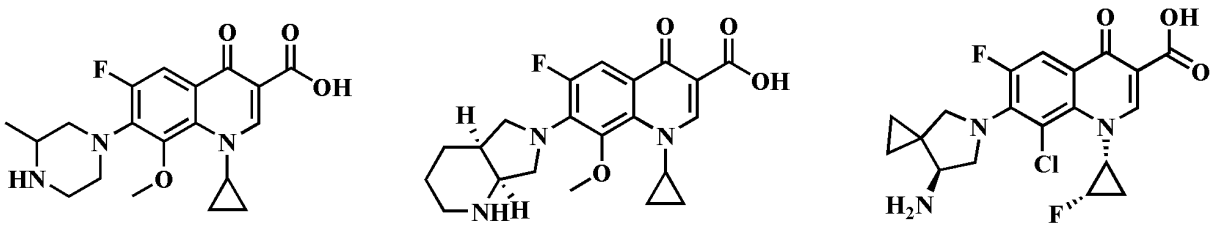


Figure 3.1 Quinolone antibacterials examined as inhibitors of MTB

the driving force for the development of CP derivatives. One major factor relevant to the design of new antitubercular agents is the transport of compounds through the cell wall of mycobacteria. This is difficult since it is well known that mycolic acids and surface-associated lipids of these organisms form a transport barrier when compared with the cell wall of other eubacteria [8]. According to structure activity relationship (SAR) of FQ antibacterial agents, substituents at the C-7 position are crucial and attribute to the physicochemical properties of FQs exhibiting antibacterial activity, bioavailability and safety [9-11]. Carboxylic acid group at C-3 position and keto group at C-4 position are essential for hydrogen bonding interactions with DNA bases [12]. Hence assimilation of the right substituent at the 4^{th} position of piperazine FQ creates interest in exploring molecular diversity to synthesize therapeutically important framework thereby anticipating greater lipophilicity.

3.2 Literature review

Considering all these facts, two separate groups led by Tzeng [13] and Sriram [14], tested various 7-substituted CP derivatives **(A-D; figure 3.2)** against MTB. Preliminary results indicated that most of the compounds demonstrated better *in vitro* antimycobacterial activity than

CP. These studies have revealed that increasing the lipophilic side chain at C-7 improved the *in vitro* antimycobacterial activity and showed no cytotoxicity at the active concentration. Talath *et al.* evaluated a series of 7-[4-(5-amino-1,3,4-thiadiazole-2-sulfonyl)]-1-piperazinyl fluoroquinolone derivatives **(E; figure 3.2)** [15]. The compounds were evaluated for their *in vitro* antitubercular activity against MTB $H_{37}Rv$ strain by the broth dilution assay method. The antitubercular data of the tested CP derivatives indicated that the synthesized compounds showed moderate activity against the MTB compared with the reference drug CP.

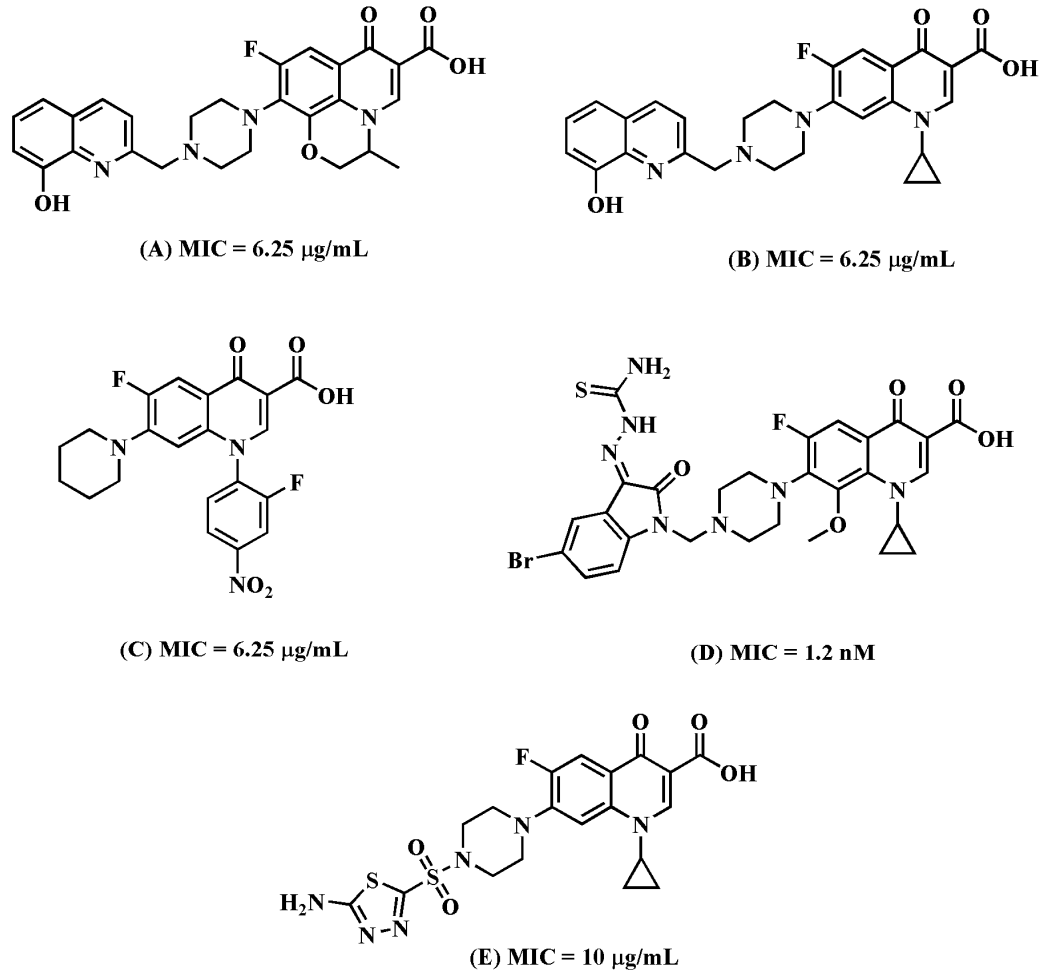


Figure 3.2 Active FQ derivatives against MTB

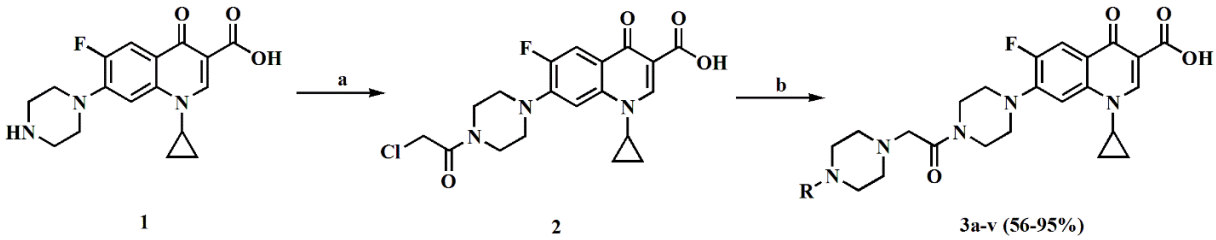
Based on these beneficial information we were prompted to explore the substituted piperazine derivatives of CP anticipating enhanced activity against this infectious bacterial disease.

3.3 Results and Discussion

3.3.1 Chemistry

Twenty two new substituted-7-(piperazin-1-yl) derivatives of CP were synthesized. Initially, 7-(4-(2-chloroacetyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2**) was synthesized by coupling CP with chloroacetyl chloride following reported procedure [16] and then various substituted piperazines were reacted with **2** to increase the lipophilicity.

Acylation of CP**, 1** with chloroacetyl chloride and triethylamine (TEA) in dichloromethane (DCM) at 0 °C to room temperature (RT) afforded compound **2** in 70% after purification. The series of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substitutedpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid derivatives of CP were prepared by coupling commercially available substituted piperazines with **2** using TEA in *N,N*-dimethylformamide (DMF) to obtain the final compounds **3a-v** in yields ranging from 55- 95%. (**Scheme 1**, **Table 3.1**)

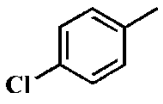
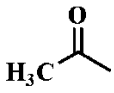


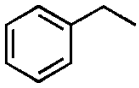
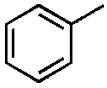
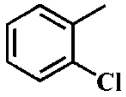
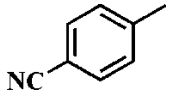
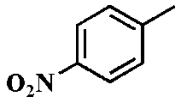
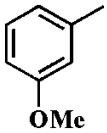
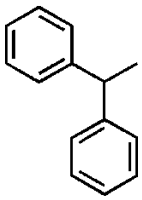
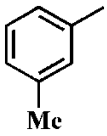
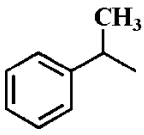
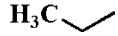
Scheme 1 General route for the synthesis of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-substitutedpiperazin-1-yl)acetyl)piperazin-1-yl)quinoline-3-carboxylic acid derivatives (**3a-v**)

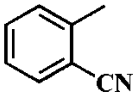
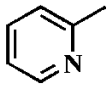
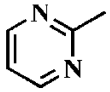
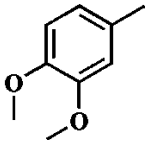
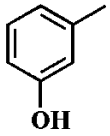
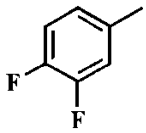
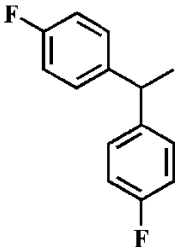
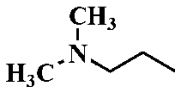
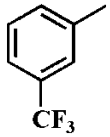
Reagents and conditions: (a) TEA (2 eq), $ClCH_2COCl$ (1.2 eq), DCM, 0 °C-RT, 1h (b) TEA (2 eq), potassium iodide (0.1 eq), aromatic or aliphatic substituted piperazines (1 eq), DMF, 125 °C, 8-12h.

In general ^1H NMR of all the title compounds displayed two triplets in the range 1.10-1.37 ppm and a multiplet in the range 3.75–3.90 ppm corresponding to the protons of cyclopropyl ring. Two multiplets of piperazine protons resonated in the range 3.30-3.70 ppm and two sharp doublets resonated in the range 7.20-7.90 ppm due to C-5 and C-8 protons of the FQ moiety. The C-2 protons of FQ resonated in the range 8.63-8.71 ppm with a sharp singlet and a broad peak due to the proton of carboxylic acid functional group resonated in the range 15.15-15.25 ppm. The acetyl link protons displayed multiplet in the range 3.29-3.39 ppm and second piperazine protons resonated in the range 2.20-2.60 ppm.

Table 3.1 Synthesized compounds: structure, yield, and lipophilicity **(3a-v)**.

Entry	R	Yield (%)	clogP[a]	MIC(μg/ml) against MTB $H_{37}R_V$
3a		57	-1.20	64
3b		68	2.54	64
3c		88	-0.81	8

3d		89	1.95	4
3e		88	1.66	>64
3f		95	2.54	16
3g		68	1.49	>64
3h		92	1.77	>64
3i		87	1.61	>64
3j		82	3.30	16
3k		65	2.09	16
3l		94	2.19	16
3m		94	-0.67	16

3n		63	1.49	>64
3o		81	0.71	>64
3p		91	-0.05	32
3q		69	1.27	>64
3r		79	0.02	>64
3s		72	2.10	>64
3t		66	3.58	>64
3u		75	-0.82	64
3v		56	2.84	>64
Ciprofloxacin			-0.72	ND
Rifampicin			ND	0.12

[a] clogP was calculated by software (Chem Draw Ultra 10.0)

3.3.2 Antimycobacterial activity

The compounds, **3a-v**, were tested for anti-tubercular activity against MTB $H_{37}Rv$ strain by Microplate Alamar Blue Assay (MABA). The active compounds exhibited minimum inhibition concentration (MIC) in the range 4-64 µg/mL. Compounds **3c, 3d** and **3f** were the most active compounds with MIC 8, 4 and 16 µg/mL respectively (**Table 3.1**). The SAR study revealed that when 'R' is Phenyl (**3e**) the compound was found to be inactive. Introducing electron withdrawing and releasing groups at various positions on phenyl ring exhibited moderate anti-tubercular activity. Introduction of nitrogen atom in the phenyl ring (**3o** and **3p**) didn't alter the activity spectrum. Replacing 'R' by aliphatic chain with/without nitrogen atom (**3a, 3m,** and **3u**) was found to be critical. Interestingly, immediate branching at α-position of the aliphatic chain (**3c**, MIC 8 µg/mL) enhanced the activity by two fold as compared to **3m**. The enhanced activity of **3c** might be attributed to the presence of carbonyl functional group. Also, immediate branching at α-position of aromatic ring (**3j, 3l,** and **3t**) showed moderate activity except **3d**, which exhibited excellent activity amongst the series with MIC 4 µg/mL. These encouraging results further pave the way to explore different substituents on the benzyl group.

3.3.3 Cytotoxicity assay

Compounds with MIC ≤ **32 µg/mL** were further examined for their toxicity in mouse macrophage cell lines (RAW 264.7) at 50 µM concentration. The approximate IC_{50} values [17] and selectivity index (SI) are tabulated in **Table 3.2**. Among the eight compounds tested, two compounds **3c** and **3d** were found to have SI values **9.58** and **22.0** respectively. This indicates their effectiveness towards drug development for TB. These encouraging results unraveled the

interest to consider lipophilicity as a vital platform to fetch CP derivatives as potential therapeutic agent.

Table 3.2 IC_{50} (µg/mL) and selectivity index (SI) values of active compounds (**3c-d, 3f, 3j-m** and **3p**) against mouse macrophage cell lines (RAW264.7)

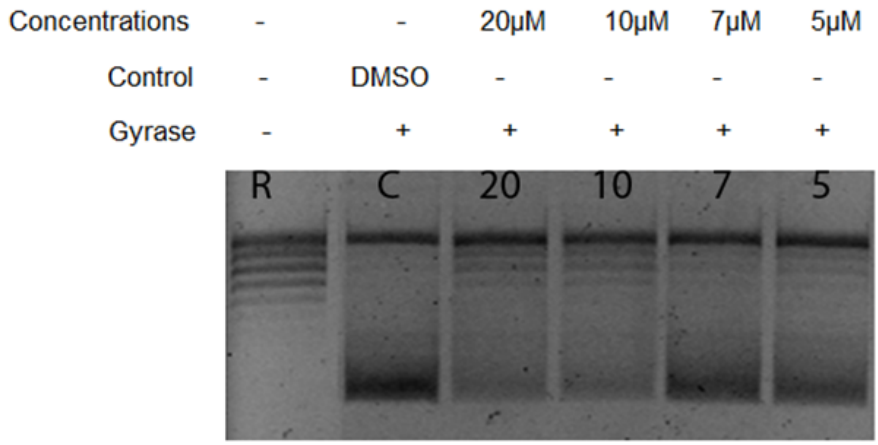
Entry	Compound	[a]MIC (µg/mL) in MTB $H_{37}Rv$	% Cell Inhibition at 50 µg/mL	[b]IC_{50} (µg/mL) approximation	[c]SI value
1	**3c**	8	**32.6**	**76.68**	9.58
2	**3d**	4	**28.4**	**88.02**	22.00
3	**3f**	16	**26.4**	**94.69**	5.91
4	**3j**	16	**41.6**	**60.09**	3.75
5	**3k**	16	**29.7**	**84.17**	5.26
6	**3l**	16	**30.2**	**82.78**	5.17
7	**3m**	16	**27.5**	**90.90**	5.68
8	**3p**	32	**20.6**	**121.35**	3.79

[a]Minimum inhibitory concentration against $H_{37}Rv$ strain of MTB (µg/mL).
[b]Measurement of cytotoxicity in RAW264.7 cells: 50% inhibitory concentrations µg/mL).
[c]Selectivity index (in vitro): IC_{50} in RAW264.7 cells/MIC against MTB.

3.3.4 DNA gyrase supercoiling assay

DNA gyrase is a bacterial type II DNA topoisomerase which controls the topological state of DNA [18]. The relaxed form of DNA is crucial for its replication and transcription. In order to catalyze the negative super coiling of double-stranded circular DNA, the free energy of ATP hydrolysis is utilized [19]. Therefore, suppressing the role of DNA gyrase hampers the relaxation process of super coiled DNA, which ultimately causes bacterial cell death. The supercoiling

activity studies were carried out at 20, 10, 7, and 5µM concentrations of compound **3d** using inspiralis kit and the results are illustrated in **figure 3.3**. The activity results indicated the IC_{50} of compound **3d** to be **7µM**.



Lane 1 : Relaxed circular DNA (R)

Lane 2 : Supercoiling reaction (Control- C) in presence of 4% DMSO

Lane 3 to 6 : Reaction in the presence of 20, 10, 7, and 5µM of compound **3d** respectively.

Figure 3.3 Illustration of the supercoiling activity of **3d** compound with an IC_{50} of **7µM**

3.4 Conclusion

In conclusion, this work has revealed the synthesis and *in vitro* antimycobacterial studies of new CP derivatives. Amongst, the synthesized compounds, **3p** exhibited 99% inhibition of MTB H_{37}Rv strain with MIC **32 µg/mL**. Compounds **3f** and **3j-m** were significantly active against MTB with MIC **16 µg/mL.** Compound **3c** and **3d** exhibited good activity with MIC **8** and **4 µg/mL** respectively. The anti-tubercular SAR profile suggests that tailoring benzyl and acetyl

group by means of appropriate substituents or functional groups might provide an insight to obtain the lead compound.

3.5 Experimental section

3.5.1 Materials and methods

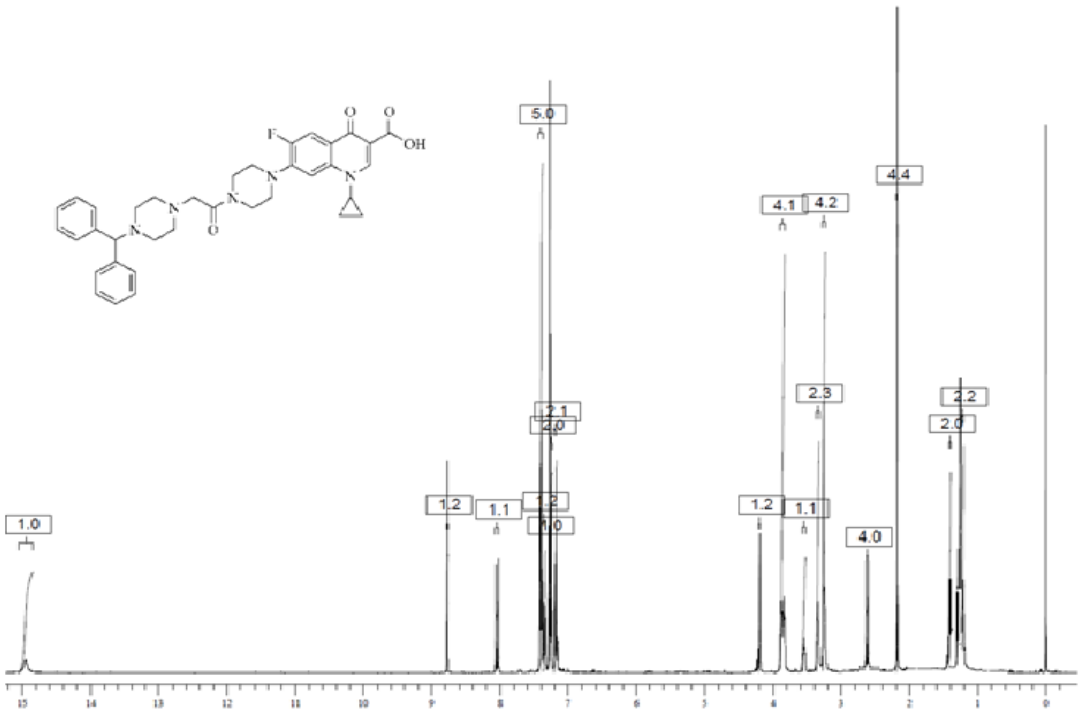
All reagents were purchased from commercial sources and used without further purification. CP was purchased from Sigma Aldrich (>98%). All reactions were monitored by analytical Thin Layer Chromatography (TLC) preformed on E-Merck 0.25 mm pre-coated silica gel aluminium plates (60 F254). Visualization of the spots on TLC plates was achieved by exposure to UV light (254nm). Column chromatography was performed using silica gel (Acme, 100-200 mesh). Melting points were obtained using Stuart SMP30 system and are uncorrected. Infrared (IR) spectra were recorded in KBr pellets on Schimadzu IR Prestige-21 FT-IR spectrophotometer (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on Avance 300 (300.132 MHz for ^{1}H, 75 MHz for ^{13}C), in $CDCl_3$ or DMSO-d_6. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane ($\delta = 0.0$) as an internal standard and coupling constants (J) in Hertz. Low-resolution mass spectra (ESI-MS) were recorded on Schimadzu.

Synthesis of 7-[4-(2-Chloroacetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***(2)***

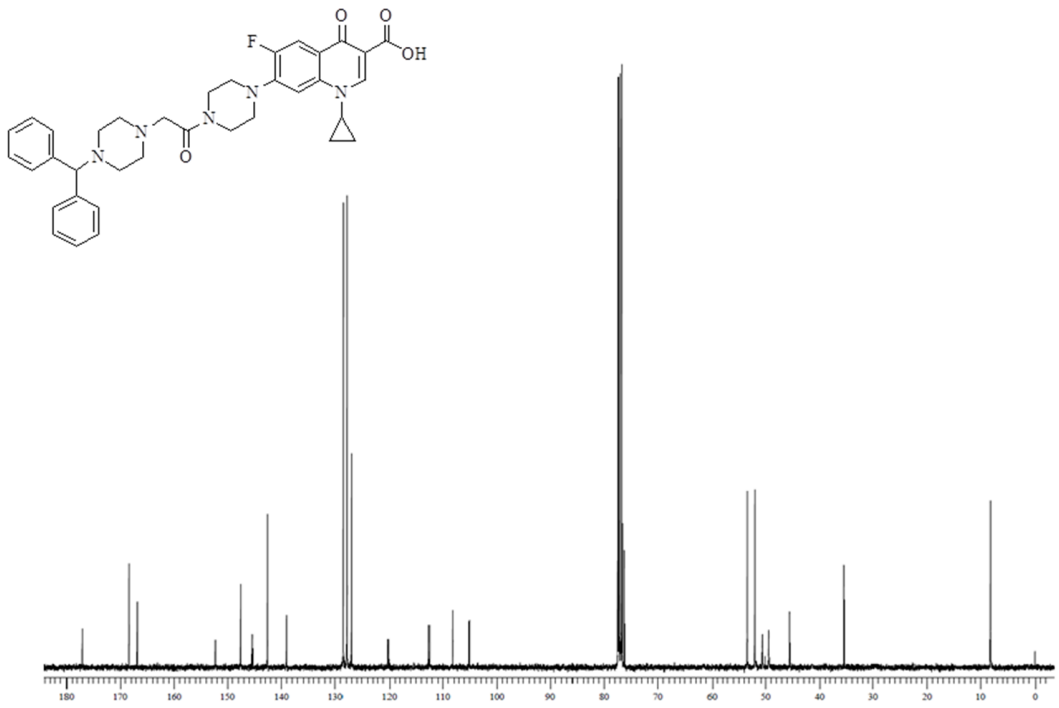
The compound **2** was synthesized according to the literature protocol [16]. mp: 262-263 °C (Lit. mp: > 260 °C [16]).

General procedure for the preparation of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substituted piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic acid derivatives of CP ***(3a-v)***

To a solution of substituted piperazines (0.9819 mmol) in dry DMF (4 mL), TEA (0.27 mL, 1.9638 mmol) and potassium iodide (16.29 mg, 0.0981 mmol) were added at RT under N_2 atmosphere. Compound **2** (0.4 g, 0.9819 mmol) was added to the above reaction mixture and resultant mixture was heated at 125 °C. After the reaction was complete, as indicated by TLC, DMF was evaporated in vacuo. The obtained residue was diluted with 20 mL of water. The compound was extracted with DCM (3 × 5mL). The organic layers were collected, washed with saturated brine solution, dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated in vacuo. The resultant crude was purified by column chromatography [DCM/MeOH (1-10%)] to get the title compounds.



^{1}H NMR spectrum of compound **3j**



^{13}C NMR spectrum of compound **3j**

1-cyclopropyl-6-fluoro-7-[4-(2-{4-methylpiperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3a)

Colorless solid (57%); mp 248-250 °C; IR (KBr, cm^{-1}) 3250, 3025, 1725, 1690, 1670, 1250, 1045. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.52 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 3.81 (tt, J = 7.2 Hz, J = 6.9 Hz, 1H), 3.78-3.76 (m, 4H), 3.30-3.28 (m, 2H), 3.19-3.16 (m, 4H), 3.02 (s, 3H), 2.92-2.89 (m, 4H), 2.69-2.67 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, $J_{C\text{-}F}$ = 2.2 Hz), 167.56, 166.43, 153.21 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 138.95, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 53.67, 51.12,

50.89, 49.16, 45.91, 43.78, 41.39, 35.58, 8.12. ESI-MS (m/z): calcd for $C_{24}H_{30}FN_5O_4$ 471.23, found 472.39 $[M+H]^+$.

7-[4-(2-{4-(4-chlorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3b)

Pale yellow solid (68%); mp 249-250 °C; IR (KBr, cm^{-1}) 3265, 3018, 1719, 1686, 1675, 1245, 1050, 800, 600. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.1 (d, J = 5.8 Hz, 2H), 6.52 (d, J = 8.5 Hz, 2H), 3.79 (tt, J = 7.2, 6.9 Hz, 1H), 3.69-3.67 (m, 4H), 3.35-3.32 (m, 2H), 3.29-3.26 (m, 4H), 3.01-2.98 (m, 4H), 2.69-2.67 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.84, 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.57, 125.51, 119.91 (d, J_{C-F} = 8.1 Hz), 116.38, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.89, 51.12, 50.46, 49.12, 45.84, 35.82, 8.43. ESI-MS (m/z): calcd for $C_{29}H_{31}ClFN_5O_4$ 567.20, found 568.29 $[M+H]^+$.

7-[4-(2-{4-acetylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c)

Colorless solid (88%); mp 230-231 °C; IR (KBr, cm^{-1}) 3234, 3027, 1722, 1695, 1668, 1253, 1042. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 3.82 (tt, J = 7.2, 6.9 Hz, 1H), 3.79-3.76 (m, 4H), 3.34-3.31 (m, 2H), 3.23-3.21 (m, 4H), 2.88-2.86 (m, 4H), 2.54-2.52 (m, 4H), 2.15 (s, 3H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 168.39, 167.56, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz),

111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 62.45, 52.83, 51.15, 48.86, 35.81, 25.14, 8.17. ESI-MS (m/z): calcd for $C_{25}H_{30}FN_5O_5$ 499.22, found 500.42 $[M+H]^+$.

7-[4-(2-{4-benzylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3d)

Colorless solid (89%) m.p. 104-106 °C; IR (KBr, cm^{-1}) 3267, 3036, 1730, 1696, 1675, 1251, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.52 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 7.32-7.30 (m, 5H), 3.71 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.65-3.62 (m, 4H), 3.48-3.46 (m, 2H), 3.34-3.32 (m, 4H), 3.15 (s, 2H), 2.96-2.93 (m, 4H), 2.74-2.72 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, $J_{C\text{-}F}$ = 2.2 Hz), 168.33, 153.6 (d, $J_{C\text{-}F}$ = 249.3 Hz), 146.21, 145.49 (d, $J_{C\text{-}F}$ = 10.3 Hz), 139.01, 127.12, 126.75, 126.13, 120.15 (d, $J_{C\text{-}F}$ = 8.1 Hz), 112.51 (d, $J_{C\text{-}F}$ = 24.14 Hz), 108.16, 105.08 (d, $J_{C\text{-}F}$ = 3.7 Hz), 61.41, 54.31, 51.17, 51.48, 49.11, 45.07, 35.12, 8.65. ESI-MS (m/z): calcd for $C_{30}H_{34}FN_5O_4$ 547.26, found 548.62 $[M+H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-phenylpiperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (3e)

Colorless solid (88%); mp 212-214 °C; IR (KBr, cm^{-1}) 3247, 3031, 1732, 1694, 1672, 1252, 1038. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H). 8.63 (s, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.52 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 7.26-7.23 (m, 5H), 3.81 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.71-3.68 (m, 4H), 3.46-3.44 (m, 2H), 3.30-3.28 (m, 4H), 2.93-2.91 (m, 4H), 2.64-2.62 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, $J_{C\text{-}F}$ = 2.2 Hz), 168.33, 166.82, 153.6 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.53, 145.49 (d, $J_{C\text{-}F}$ = 10.3 Hz), 144.21, 139.01, 130.09,

128.41, 127.74, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 54.65, 52.42, 51.17, 49.33, 45.40, 34.86, 8.18. ESI-MS (m/z): calcd for $C_{29}H_{32}FN_5O_4$ 533.24, found 534.31 $[M+H]^+$.

7-[4-(2-{4-(2-chlorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3f)

Colorless solid (95%); mp 138-139 °C; IR (KBr, cm^{-1}) 3238, 3021, 1718, 1689, 1672, 1251, 1087, 728. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.58-7.55 (m, 2H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.13 (d, *J* = 7.7 Hz, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 3.76 (tt, *J* = 7.2 Hz, *J* = 6.9 Hz, 1H), 3.74-3.72 (m, 4H), 3.39-3.37 (m, 2H), 3.20-3.18 (m, 4H), 2.84-2.82 (m, 4H), 2.58-2.56 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 143.84, 139.01, 130.95, 129.13, 128.64, 124.2, 122.26, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 53.11, 51.75, 50.83, 48.96, 44.72, 35.42, 7.87. ESI-MS (m/z): calcd for $C_{29}H_{31}ClFN_5O_4$ 567.20, found 568.35 $[M+H]^+$.

7-[4-(2-{4-(4-cyanophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3g)

Colorless solid (68%); mp 278-279 °C; IR (KBr, cm^{-1}) 3282, 3018, 2245, 1741, 1682, 1668, 1245, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.3 (d, *J* = 5.8 Hz, 2H), 6.65 (d, *J* = 8.5 Hz, 2H), 3.71 (tt, *J* = 7.2 Hz, *J* = 6.9 Hz, 1H), 3.70-3.67 (m, 4H), 3.34-3.32 (m, 2H), 3.23-3.21 (m, 4H), 2.81-2.79

(m, 4H), 2.48-2.45 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.84, 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 131.86, 126.93, 119.91 (d, J_{C-F} = 8.1 Hz), 117.12, 116.91, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.43, 51.75, 50.22, 49.72, 45.89, 35.23, 7.13. ESI-MS (m/z): calcd for $C_{30}H_{31}FN_6O_4$ 558.24, found 559.38 $[M+H]^+$.

1-cyclopropyl-6-fluoro-7-[4-(2-{4-(4-nitrophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3h)

Yellow solid (92%); mp 242-244 °C; IR (KBr, cm^{-1}) 3265, 3035, 1732, 1675, 1655, 1512, 1375, 1253, 1044. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.9 (d, J = 5.8 Hz, 2H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 6.7 (d, J = 8.5 Hz, 2H), 3.79 (tt, J = 7.2 Hz, J = 6.9 Hz, 1H), 3.71-3.69 (m, 4H), 3.41-3.38 (m, 2H), 3.25-3.23 (m, 4H), 2.85-2.83 (m, 4H), 2.56-2.54 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 148.42, 146.93, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.45, 128.41, 119.91 (d, J_{C-F} = 8.1 Hz), 117.12, 116.91, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.39, 51.52, 50.79, 49.23, 35.85, 8.34. ESI-MS (m/z): calcd for $C_{29}H_{31}FN_6O_6$ 578.23, found 579.32 $[M+H]^+$.

1-cyclopropyl-6-fluoro-7-[4-(2-{4-(3-methoxyphenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3i)

Pale brown solid (87%); mp 144-145 °C; IR (KBr, cm^{-1}) 3243, 3024, 1718, 1686, 1671, 1252, 1145, 1052. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.15 (t, J = 7.4 Hz, 1H), 6.57 (d, J = 7.3 Hz, 1H), 6.5 (s,

1H), 6.42 (d, *J* = 7.3 Hz, 1H), 3.79 (tt, 1H, *J* = 7.2 Hz, *J* = 6.9 Hz), 3.76 (s, 3H), 3.71-3.69 (m, 4H), 3.48-3.46 (m, 2H), 3.17-3.14 (m, 4H), 2.75-2.72 (m, 4H), 2.43-2.41 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 146.27, 145.49 (d, J_{C-F} = 10.3 Hz), 144.56, 139.01, 131.29, 129.72, 129.13, 123.18, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 110.3, 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 53.85, 52.11, 51.73, 48.17, 46.14, 44.72, 35.42, 8.18. ESI-MS (m/z): calcd for $C_{30}H_{34}FN_5O_5$ 563.25, found 564.42 $[M+H]^+$.

7-[4-(2-{4-benzhydrylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3j)

Colorless solid (82%); mp 199-200 °C, IR (KBr, cm^{-1}) 3265, 3018, 1723, 1695, 1668, 1246, 1038. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.27-7.25 (m, 10H), 4.12 (s, 1H), 3.83 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.72-3.69 (m, 4H), 3.48-3.46 (m, 2H), 3.21-3.18 (m, 4H), 2.96 (s, 3H), 2.70-2.68 (m, 4H), 2.45-2.43 (m, 4H), 1.32 (t, *J* = 6.9 Hz, 2H), 1.20 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 142.60, 139.01, 128.52, 127.86, 127.00, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 76.27, 53.35, 51.92, 50.65, 49.45, 45.48, 35.33, 8.26. ESI-MS (m/z): calcd for $C_{36}H_{38}FN_5O_4$ 623.29, found 624.41 $[M+H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-m-tolylpiperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (3k)

Colorless solid (65%); mp 158-160 °C; IR (KBr, cm^{-1}) 3268, 3054, 1719, 1697, 1668, 1251, 1082. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.73 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.42 (d, J_{H-F} = 7.5 Hz, 1H), 7.04 (t, *J* = 7.6 Hz, 1H), 6.71 (s, 1H), 6.68 (d, *J* = 7.5 Hz, 1H), 6.55 (d, *J* = 7.6 Hz, 1H), 3.76 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.74-3.72 (m, 4H), 3.43-3.41 (m, 2H), 3.40-3.38 (m, 4H), 2.68-2.66 (m, 4H), 2.40-2.38 (m, 4H), 2.19 (s, 3H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 146.03, 145.49 (d, J_{C-F} = 10.3 Hz), 141.48, 139.01, 129.5, 127.59, 127.11, 126.58, 122.34, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 53.87, 51.12, 50.86, 49.43, 45.12, 35.39, 22.29, 8.18. ESI-MS (m/z): calcd for $C_{30}H_{34}FN_5O_4$ 547.26, found 548.39 $[M+H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(1-phenylethyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (3l)

Colorless solid (94%); mp 212-214 °C; IR (KBr, cm^{-1}) 3239, 3026, 1728, 1696, 1672, 1254, 1076. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.30-7.28 (m, 5H), 3.78 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.64-3.62 (m, 4H), 3.38-3.36 (m, 2H), 3.33-3.31 (m, 4H), 3.06 (q, *J* = 6.9 Hz, 1H), 2.54-2.52 (m, 4H), 2.41-2.39 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.20 (d, *J* = 5.9 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 142.60, 139.01, 128.52, 127.86, 127.00, 120.15 (d, J_{C-F} = 8.1 Hz),

112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 72.21, 54.11, 52.19, 51.07, 48.69, 46.18, 34.04, 22.67, 8.13. ESI-MS (m/z): calcd for $C_{31}H_{36}FN_5O_4$ 561.28, found 562.41 $[M+H]^+$.

1-cyclopropyl-7-[4-(2-{4-ethylpiperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3m)

Colorless solid (94%); mp 239-240 °C; IR (KBr, cm^{-1}) 3287, 3032, 1734, 1698, 1672, 1253, 1048. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H),7.55 (d, J_{H-F} = 7.5 Hz, 1H), 3.79 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.69-3.67 (m, 4H), 3.54-3.52 (m, 2H), 3.35-3.32 (m, 4H), 2.9 (q, *J* = 6.1 Hz, 2H), 2.51-2.48 (m, 4H), 2.38-2.36 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H), 1.08 (t, *J* = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 54.56, 52.23, 51.67, 49.71, 48.87, 44.76, 34.74, 14.87, 8.45. ESI-MS (m/z): calcd for $C_{25}H_{32}FN_5O_4$ 485.24, found 486.37 $[M+H]^+$.

7-[4-(2-{4-(2-cyanophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3n)

Colorless solid (63%) mp 222-223 °C; IR (KBr, cm^{-1}) 3245, 3022, 2238, 1729, 1692, 1671, 1255, 1043. ^{1}H NMR (300 MHz, DMSO- d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.92 (d, J_{H-F} = 13.2 Hz, 1H), 7.66 (d, J_{H-F} = 7.5 Hz, 1H), 7.57-7.55 (m, 2H), 7.13 (d, *J* = 6.9 Hz, 1H), 7.05 (t, *J* = 7.1 Hz, 1H), 3.82 (tt, 1H, *J* = 7.2, 6.9 Hz), 3.79-3.77 (m, 4H), 3.68-3.66 (m, 2H), 3.36-3.34 (m, 4H), 3.16-3.14 (m, 4H), 2.65-2.62 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz),

147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 142.43, 138.95, 128.2, 127.43, 126.32, 122.78, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 108.23, 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 81.42, 53.59, 51.12, 50.58, 49.33, 45.06, 35.99, 8.54. ESI-MS (m/z): calcd for $C_{30}H_{31}FN_6O_4$ 558.24, found 559.36 $[M+H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2- {4-(pyridine-2-yl)piperazin-1-yl}acetyl) piperazin-1-yl]- 1,4-dihydroquinoline-3-carboxylic acid (3o)

Colorless solid (81%); mp 204-205 °C; IR (KBr, cm^{-1}) 3268, 3028, 1729, 1695, 1678, 1253, 1054. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.92 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.66 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 7.65-7.62 (m, 2H), 7.21 (d, J = 6.9 Hz, 1H), 7.16 (t, J = 6.8 Hz, 1H), 3.77 (tt, J = 7.2, 6.9 Hz, 1H), 3.63-3.61 (m, 4H), 3.58-3.56 (m, 2H), 3.33-3.31 (m, 4H), 3.26-3.23 (m, 4H), 2.59-2.57 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C\text{-}F}$ = 2.2 Hz), 167.56, 166.43, 154.34, 153.21 (d, $J_{C\text{-}F}$ = 249.3 Hz), 150.78, 148.43, 147.23, 146.07, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 138.95, 121.56, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 53.78, 53.12, 50.96, 48.76, 45.78, 37.23, 8.45. ESI-MS (m/z): calcd for $C_{28}H_{31}FN_6O_4$ 534.24, found 535.36 $[M+H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(pyrimidine-2-yl)piperazin-1-l}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (3p)

Pale yellow solid (91%); m.p. 189-190 °C; IR (KBr, cm^{-1}) 3278, 3018, 1724, 1698, 1669, 1234, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 8.31 (d, J = 4.6 Hz, 2H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.55 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 7.01(t, J = 6.8 Hz, 1H), 3.77 (tt, J = 7.2, 6.9 Hz, 1H), 3.69-3.67 (m, 4H), 3.36-3.34 (m, 2H), 3.07-3.05 (m, 4H), 2.60-2.58 (m, 4H), 2.48-

2.46 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C\text{-}F}$ = 2.2 Hz), 167.56, 166.43, 160.67, 154.89, 153.21 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 138.95, 122.45, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 54.12, 52.23, 51.08, 49.67, 44.97, 36.24, 8.14. ESI-MS (m/z): calcd for $C_{27}H_{30}FN_7O_4$ 535.23, found 536.39 $[M+H]^+$.

1-cyclopropyl-7-[4-(2-{4-(3,4-dimethoxyphenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3q)

Colorless solid (69%); mp 189-190 °C; IR (KBr, cm^{-1}) 3250, 3025, 1725, 1690, 1670, 1250, 1187, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.55 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 6.63 (d, J = 6.8 Hz, 1H), 6.56 (d, J = 6.9 Hz, 1H), 6.25 (s, 1H), 3.79 (tt, J = 7.2, 6.9 Hz, 1H), 3.73 (s, 6H), 3.73-3.71 (m, 4H), 3.41-3.38 (m, 2H), 3.27-3.25 (m, 4H), 2.45-2.42 (m, 4H), 2.37-2.35 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, $J_{C\text{-}F}$ = 2.2 Hz), 167.56, 166.43, 153.21 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 143.24, 140.67, 138.08, 138.95, 121.78, 116.02, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 117.32, 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 61.45, 60.95, 59.34, 53.35, 51.92, 50.65, 49.45, 35.33, 8.26. ESI-MS (m/z): calcd for $C_{31}H_{36}FN_5O_6$ 593.26, found 594.39 $[M+H]^+$.

1-cyclopropyl-6-fluoro-7-[4-(2-{4-(3-hydroxyphenyl)piperazin-1-yl}acetylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3r)

Colorless solid (79%); mp 220-222 °C; IR (KBr, cm^{-1}) 3450, 3245, 3026, 1732, 1692, 1669, 1236, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2

Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.05 (t, J = 6.9 Hz, 1H), 6.52 (d, J = 6.6 Hz, 1H), 6.45 (s, 1H), 6.39 (d, J = 6.7 Hz, 1H), 5.23 (s, 1H), 3.81 (tt, J = 7.2, 6.9 Hz, 1H), 3.67-3.65 (m, 4H), 3.48-3.46 (m, 2H), 3.36-3.34 (m, 4H), 2.45-2.43 (m, 4H), 2.35-2.32 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 146.58, 145.12 (d, J_{C-F} = 10.3 Hz), 142.03, 138.95, 134.61, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.28, 107.12, 106.07, 104.89 (d, J_{C-F} = 3.7 Hz), 101.72, 54.15, 50.91, 51.27, 49.81, 44.73, 35.99, 8.12. ESI-MS (m/z): calcd for $C_{29}H_{32}FN_5O_5$ 549.24, found 550.45 $[M+H]^+$.

1-cyclopropyl-7-[4-(2-{4-(3,4-difluorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3s)

Pale yellow solid (72%); mp 198-200 °C; IR (KBr, cm^{-1}) 3256, 3023, 1721, 1696, 1675, 1252, 1044. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.63 (d, J_{H-F} = 7.5 Hz, 1H), 7.25 (dd, J_{H-F} = 12.11, 7.01 Hz, 1H), 7.15 (dt, J_{H-F} = 13.2, 6.8 Hz, 1H), 6.98 (dd, J_{H-F} = 7.1, 3.4 Hz, 1H), 3.85 (tt, J = 7.2, 6.9 Hz, 1H), 3.81-3.79 (m, 4H), 3.41-3.39 (m, 2H), 3.38-3.35 (m, 4H), 2.58-2.56 (m, 4H), 2.35-2.32 (m, 4H), 1.37 (t, J = 6.9 Hz, 2H), 1.24 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 162.58 (dd, J_{C-F} = 252.67 Hz, 22.68 Hz),153.21 (d, J_{C-F} = 249.3 Hz), 151.52 (dd, J_{C-F} = 247.28 Hz, 21.39 Hz),147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 141.49 (d, J_{C-F} = 2.8 Hz), 138.95, 125.23 (d, J_{C-F} = 3.07 Hz), 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 110.87 (dd, J_{C-F} = 23.81 Hz, 3.7 Hz), 109.42 (dd, J_{C-F} = 20.65 Hz, 2.95 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 55.32, 51.49, 51.11, 49.72, 45.29, 35.37, 8.42. ESI-MS (m/z): calcd for $C_{29}H_{30}F_3N_5O_4$ 569.22, found 570.49 $[M+H]^+$.

7-[4-(2-{4-(bis(4-fluoroophenyl)methyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclo propyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3t)

Colorless solid (66%); mp 195-196 °C; IR (KBr, cm^{-1}) 3265, 3045, 1724, 1686, 1672, 1251, 1044. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.61 (d, J_{H-F} = 7.5 Hz, 1H), 7.51 (dd, J_{H-F} = 5.8, 7.5 Hz, 4H), 7.21 (dd, J_{H-F} = 8.5 Hz, 4H), 4.2 (s, 1H), 3.86 (tt, J = 7.2, 6.9 Hz, 1H), 3.76-3.74 (m, 4H), 3.35-3.32 (m, 2H), 3.27-3.25 (m, 4H), 2.56-2.54 (m, 4H), 2.39-2.37 (m, 4H), 1.32 (t, J = 6.9 Hz, 2H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 176.97 (d, J_{C-F} = 2.2 Hz), 168.23, 166.76, 161.83 (d, J_{C-F} = 246.49 Hz), 153.57 (d, J_{C-F} = 251.52 Hz), 147.50, 145.45 (d, = 10.3 Hz), 139.01, 138.07 (d, J_{C-F} = 2.9 Hz), 129.21 (d, J_{C-F} = 7.3 Hz), 120.08 (d, d, J_{C-F} = 8.1 Hz), 115.43 (d, J_{C-F}= 21.27 Hz), 112.46 (d, J_{C-F} = 23.48 Hz), 108.11, 105.05 (d, J_{C-F} = 3.7 Hz), 74.43, 53.31, 51.70, 50.61, 49.39, 45.45, 35.32, 8.25. ESI-MS (m/z): calcd for $C_{36}H_{36}F_3N_5O_4$ 659.27, found 660.62 $[M+H]^+$.

1-cyclopropyl-7-[4-(2-{4-(2-(dimethylamino)ethyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3u)

Colorless solid (75%), mp 138-140 °C; IR (KBr, cm^{-1}) 3248, 3025, 1728, 1696, 1672, 1250, 1045. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.63 (d, J_{H-F} = 7.5 Hz, 1H), 3.89 (tt, J = 7.2, 6.9 Hz, 1H), 3.78-3.76 (m, 4H), 3.41-3.39 (m, 2H), 3.38-3.35 (m, 4H), 2.83 (s, 6H), 2.70 (t, J = 7.2 Hz, 4H), 2.59-2.57 (m, 4H), 2.44-2.42 (m, 4H), 1.37 (t, J = 6.9 Hz, 2H), 1.24 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.95,

53.53, 51.67, 50.22, 49.20, 48.63, 47.97, 41.11, 35.12, 8.09. ESI-MS (m/z): calcd for $C_{27}H_{37}FN_6O_4$ 528.29, found 530.41 $[M+H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(3-(trifluoromethyl)phenyl)piperazin-1-yl}acetyl) piperazin -1-yl]-1,4-dihydroquinoline-3-carboxylic acid (3v)

Colorless solid (56%); mp 190-192 °C; IR (KBr, cm^{-1}) 3278, 3028, 1732, 1698, 1674, 1252, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.63 (d, J_{H-F} = 7.5 Hz, 1H), 7.48 (t, *J* = 8.03 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.26 (s, 1H), 7.14 (d, *J* = 7.78 Hz, 1H), 3.89 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.78-3.76 (m, 4H), 3.42-3.39 (m, 2H), 3.37-3.35 (m, 4H), 2.61-2.59 (m, 4H), 2.44-2.42 (m, 4H), 1.37 (t, *J* = 6.9 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 2H). ^{13}CNMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 148.38 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.94, 126.39, 124.62, 119.91 (d, J_{C-F} = 8.1 Hz), 119.19, 116.47, 112.72, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 54.11, 50.89, 50.16, 49.92, 45.14, 35.49, 8.08. ESI-MS (m/z): calcd for $C_{30}H_{31}F_4N_5O_4$ 601.23, found 602.56 $[M+H]^+$.

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Chapter IV

Synthesis of 6-[4-substitutedpiperazin-1-yl]phenanthridine analogues as antimycobacterial agents

Chapter 4

Synthesis of 6-[4-substitutedpiperazin-1-yl]phenanthridine analogues as antimycobacterial agents

4.1 Introduction

Quinoline frameworks constitute important class of alkaloids displaying wide biological activities such as antimicrobial, antimalarial, antibacterial, antifungal and anti-tubercular agents [1-5]. Ease of practical manipulation to fetch structural complexity and biological diversity crafts quinoline as an exceptional versatile biological synthon. The diaryl quinoline derivative, bedaquiline was approved for use in TB treatment, in cases of MDR and XDR-TB [6]. Some of the reported quinoline skeletals which exhibit anti-tubercular activity are shown in **figure 4.1** [7-11].

The presence of variety of aryl and aryl piperazine groups at the 2nd position on quinoline skeleton has strikingly improved the potency of the molecule [7,11]. On the basis of above beneficial information and as part of our ongoing research on TB program [12], we earmarked to design new 6-(4-substitutedpiperazin-1-yl)phenanthridine derivatives anticipating enhanced biological activity to combat this lethal disease.

Heterocyclic motifs have been much exploited in drug discovery development owing to their ample biological spectrum. Mainly, 1,2,3-triazoles have been postulated to generate a nonclassic bioisostere of amide bond [13] which is an essential feature to increase binding affinity towards

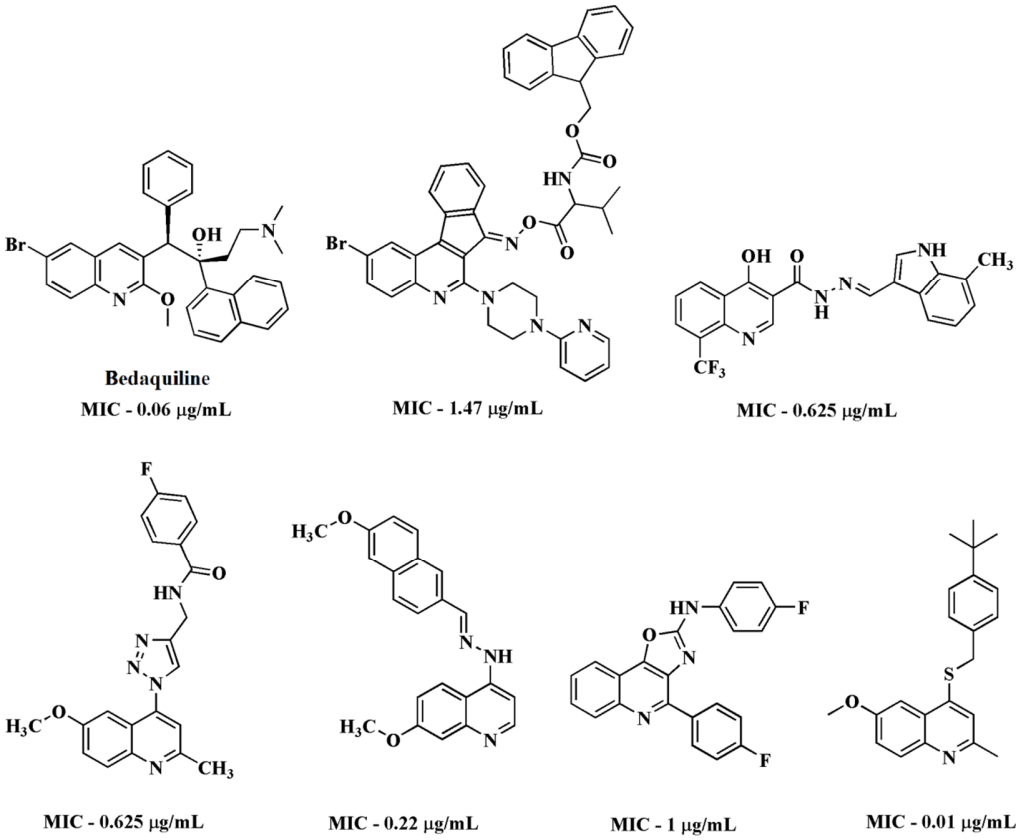


Figure 4.1 Some of the quinoline based anti-tubercular agents.

receptor. Much attention has been paid to click chemistry arena due to easily accessible novel complex and diversified heterocycles using environmentally benign and relatively inexpensive catalyst and starting materials. 1,2,3-Triazoles are identified as antifungal, anticancer, antiprotozoal, antiphotoaging, HIV type-1 protease inhibitor, β-lactum antibiotic, histone deacetylase inhibitor etc., [14-18]. Some of the drugs based on 1,2,3-triazoles which are currently in use are depicted in **figure 4.2**. Hence, we synthesized 6-(4-((substituted-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine derivatives as sketched in **scheme 1**.

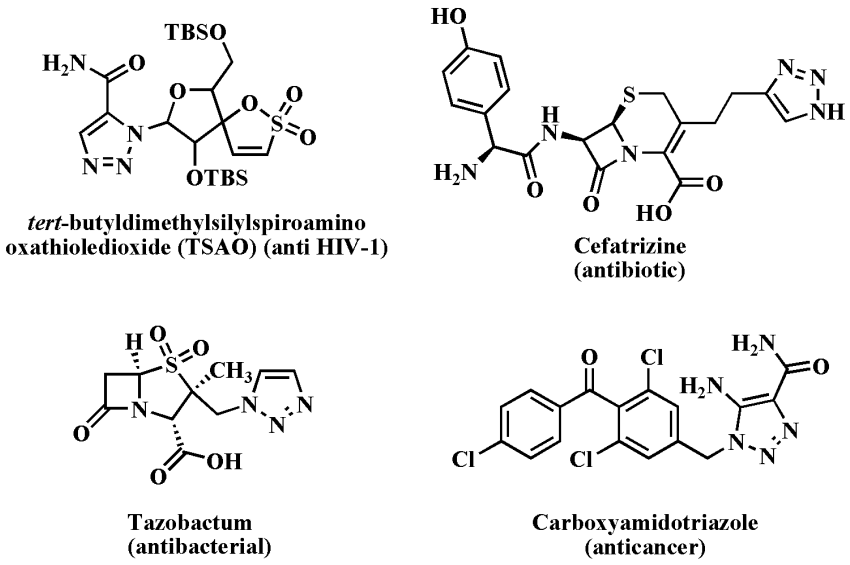
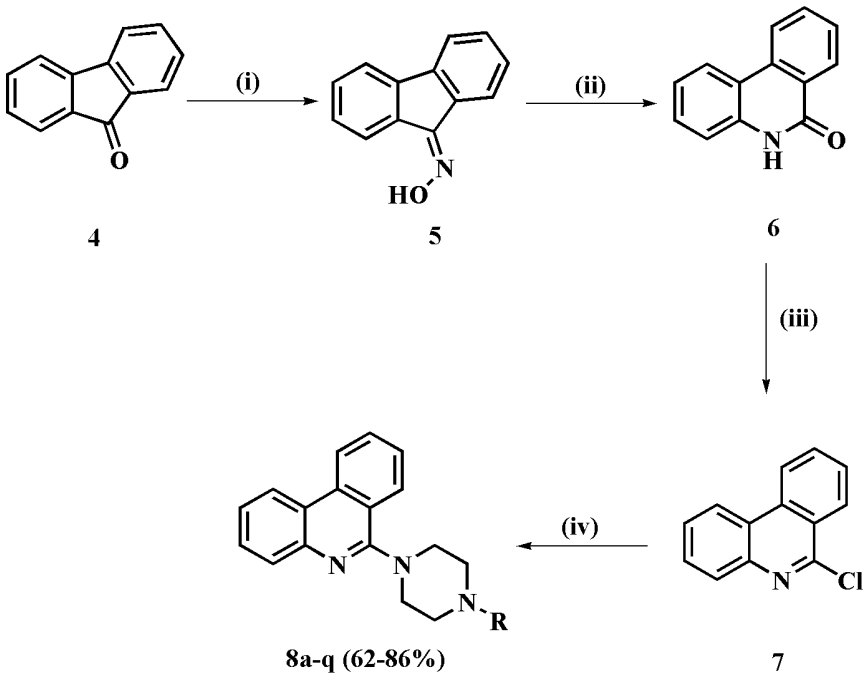


Figure 4.2 Pharmaceutically used 1,2,3-triazoles based molecules.

4.2 Results and Discussion

4.2.1 Chemistry

We sketched the synthesis of 6-(4-substitutedpiperazin-1-yl)phenanthridine derivatives starting from 9-fluorenone as outlined in **scheme 1**. We synthesized up to compound **7** according to the literature protocol with slight modification [19,20]. 9-Fluorenone upon treatment with hydroxylamine hydrochloride ($NH_2OH.HCl$) and sodium acetate (NaOAc) under reflux condition afforded *N*-hydroxy-9*H*-fluoren-9-imine (**5**). Further heating **5** with polyphosphoric acid (PPA) and phosphorus pentoxide (P_2O_5) gave phenanthridin-6(5*H*)-one (**6**). 6-chlorophenanthridine was synthesized by refluxing **6** with phosphorus oxychloride ($POCl_3$) and *N*,*N*-dimethylaniline.



Scheme 1 Synthetic protocol utilized for the synthesis of molecules **8a-q**

Reagents and conditions: (i) $NH_2OH.HCl$ (2 eq, NaOAc (2 eq), EtOH:H_2O (3:1), reflux 1.5 h (ii) PPA (10 eq), P_2O_5 (0.5 eq), heating at 150 °C, 0.5 h (iii) $POCl_3$ (10 eq), *N*,*N*-dimethylaniline (0.5 eq), reflux 3h (iv) substituted piperazines (1.2 eq), TEA (1.5 eq), DMF, MWI, 455 Watt, 20 min.

Initially, we set off our investigation for the synthesis of **8b** under conventional thermal conditions. As a model reaction, **7** was treated with 1-(3-methylphenyl)piperazine in presence of TEA using dry DMF as solvent on an oil bath at 150 °C for 4 h to yield **8b**. Unfortunately, the desired compound wasn't obtained. With this set back we tried to monitor reaction conditions with variety of bases and solvents as summarized in **Table 4.1**.

Table 4.1 Optimization of reaction conditions of **8b**[a]

Entry	Solvent	Base	Temperature (°C)	Heating source	Time	Yield (%)[b]
1	DMF	TEA	150	conventional	4 h	-
2	DMF	TEA	150	conventional	12 h	47
3	DMF	DIPEA	150	conventional	12 h	41
4	DMF	K_2CO_3	150	conventional	12 h	42
5	DMSO	TEA	150	conventional	12 h	35
6	THF	K_2CO_3	90	conventional	12 h	15
7	DMSO	TEA	150	microwave	10 min	40
8	DMF	K_2CO_3	150	microwave	10 min	45
9	DMF	TEA	150	microwave	10 min	56
10	DMF	TEA	150	microwave	15 min	68
11	**DMF**	**TEA**	**150**	**microwave**	**20 min**	**84**
12	DMF	TEA	150	microwave	25 min	81

[a]All the reactions were carried out with **7** (2.34 mmol), 1-(3-methylphenyl)piperazine (2.57 mmol) and base (3.51 mmol) in solvent (5 mL), [b]Isolated yield after column chromatography.

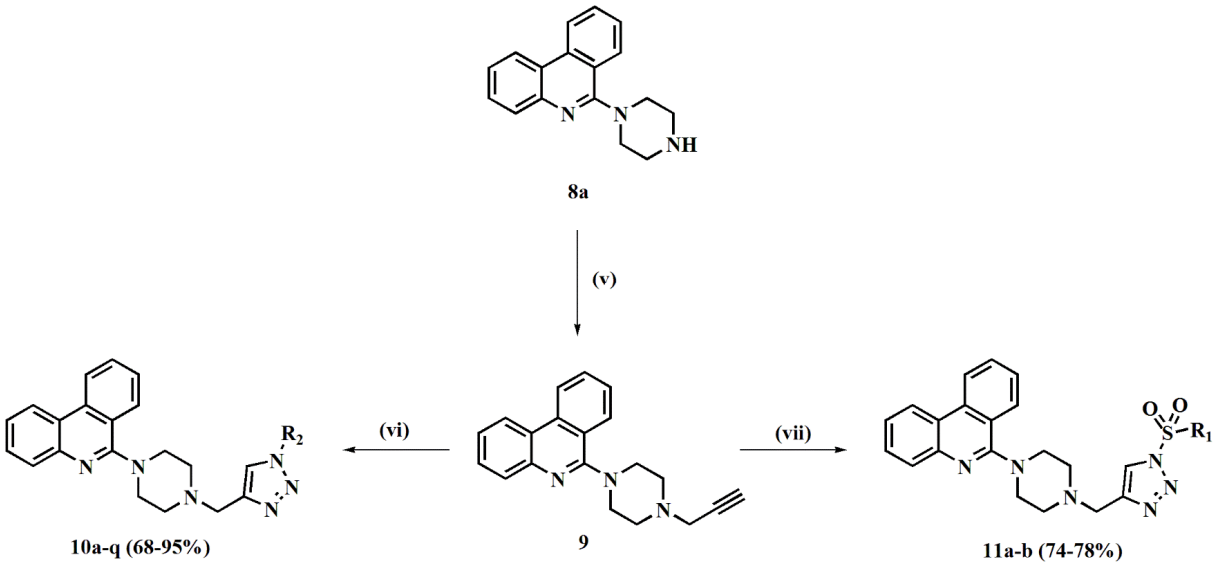
To improve the yield of target compound **8b**, reaction was carried out under reflux condition using TEA and DMF for 12 h to get the desired product in about 47%. There was drop in the yield (**entry 3**) when we employed *N*,*N*-diisopropylethylamine (DIPEA) as base under same reaction conditions. Next, hopping to an inorganic base potassium carbonate (K_2CO_3) under

reflux condition for 12 h didn't have much impact on yield. The reaction employing dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) with base TEA and K_2CO_3 respectively, found to be critical (**entry 5-6**). Alternatively emphasizing on microwave assisted organic synthesis, reaction was carried out at 150 °C in Biotage initiator with a pre-stirring of 30 s and stirring rate at 600 rpm (**entry 7-12**). Initially, the reaction was carried out by irradiating microwaves for 10 min under various bases and solvents (**entry 7-9**) to give the desired compound in moderate yield. The reaction was further optimized with TEA and DMF at various intervals of time (**entry 10-12**), to give **8b** in good yield. These findings markedly enhanced the yield and diminished the reaction time to 20 min in contrast to the conventional method. Having the optimized reaction condition handy, a library of **17** compounds **8a-q** was synthesized in good yield, thus validating the scope of the present protocol. Both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and HRMS) of all the synthesized compounds were confirmed prior to their use in the evaluation of their antimycobacterial activity. In general, nucleophilic aromatic substitution at 6-chlorophenanthridine was confirmed by FTIR spectrum of final compounds **8a-q** which showed the disappearance of IR band at 756 cm^{-1} due to aromatic C-Cl stretching frequencies. Furthermore, ^{1}H NMR spectrum displayed 8 aromatic protons of phenanthridine in the range 7.40-8.55 ppm and 8 aliphatic piperazine (-CH_2-) protons in the range δ 3.60-3.90 ppm. The spectral data of final compounds are provided in experimental section.

Scheme 2

Compound **9** was obtained by heating **8a** with propargyl bromide (80% in toluene) in the presence of TEA using DMF as solvent. The final compounds were synthesized from **9** by means of click chemistry employing catalytic amount of copper sulfate pentahydrate ($CuSO_4.5H_2O$) and

sodium ascorbate in 1:2 ratio of water and *tert*-butanol (*t*-BuOH) to get desired compound **10a–q**. While catalytic amount of copper(I)-thiophene-2-carboxylate (CuTC) and toluene as solvent was used to synthesize the compounds **11a–b**. All the final compounds displayed multiplet in the range 2.75–2.95 ppm and 3.45–3.65 ppm corresponding to piperazine (–CH_2–) protons, singlet in the range 3.85–4.00 ppm corresponding to methylene proton, and proton of 1,2,3-triazole ring resonated in the range 7.8–8.2 ppm. Both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and HRMS) of all the synthesized compounds were confirmed and employed further for their antimycobacterial evaluation.



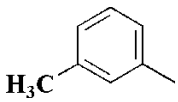
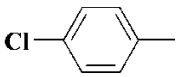
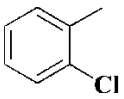
Scheme 2 Synthetic protocol utilized for the synthesis of molecules **10a-q** and **11a-b**

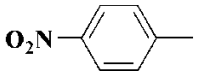
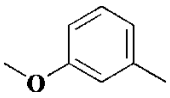
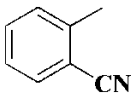
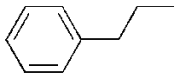
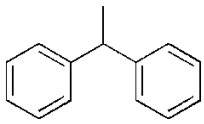
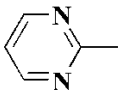
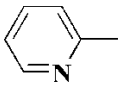
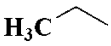
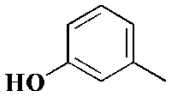
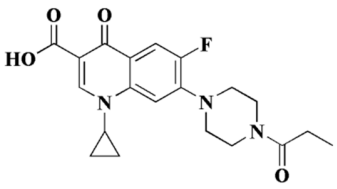
Reagents and conditions: (v) propargyl bromide (80% in toluene) (1.2 eq), TEA (1.5 eq), DMF, heating at 70 °C 1.5 h; (vi) substituted azides, $CuSO_4.5H_2O$ (0.1 eq), sodium ascorbate (0.1 eq), H_2O:*t*-BuOH (1:2), RT 3 h; (vii) substituted sulfonyl azides (1 eq), CuTC (0.1 eq), toluene, RT, 1 h.

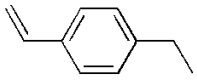
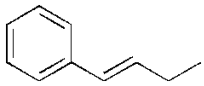
4.2.2. Antimycobacterial activity

In **scheme 1** all the synthesized compounds were tested for their ability to inhibit the growth of MTB $H_{37}Rv$ using Microplate Alamar Blue Assay (MABA) [21,22] with compound concentration ranging from 50 to 0.78 µg/mL. Isoniazid and rifampicin were used as the positive drug standard. The *in vitro* test results for final compounds are tabulated in **Table 4.2** as MIC and the activity ranged from 1.56 to 50 µg/mL. Compounds with MIC 1.56 µg/mL were further subjected to cytotoxicity studies. From the MTB activity profile, three compounds **8e**, **8j** and **8k** were found to be active and inhibit 99% growth of MTB $H_{37}Rv$ at the concentration 1.56 µg/mL. Based on the MTB activity summary structure-activity relationships were established.

Table 4.2 Antimycobacterial activity of compounds (**8a-q**) against MTB $H_{37}Rv$

Compound No.	R	MIC(µg/mL) against MTB $H_{37}Rv$	clogp[a]
8a	H	25	2.69
8b		6.25	5.75
8c		50	6.13
8d		6.25	6.13

8e		1.56	5.36
8f		12.5	5.27
8g		50	5.08
8h		6.25	5.06
8i		3.125	6.27
8j		1.56	3.54
8k		1.56	4.30
8l		6.25	3.66
8m		3.125	3.68
8n		12.5	1.66

8o	H_3C—	6.25	3.13
8p		25	5.64
8q		25	5.33
Isoniazid		0.36	-0.668
Rifampicin		0.02	ND

[a] Calculated using chemdraw Ultra 8.0

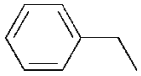
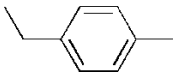
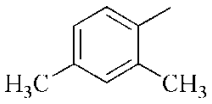
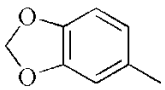
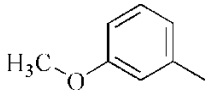
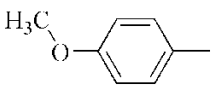
ND = not determined

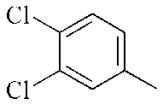
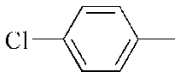
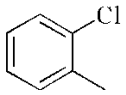
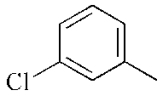
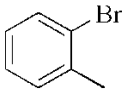
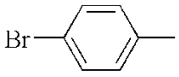
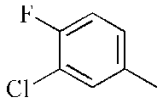
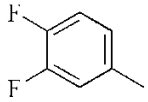
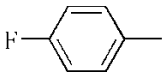
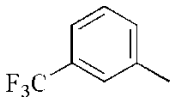
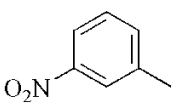
Among the series, compounds bearing alkyl or alkyl-aryl groups at the 4th position of piperazine are found to exhibit moderate anti-tubercular activity with MIC 6.25 µg/mL (**8h**, **8l**, **8o-q**). While immediate branching at the α-position of alkyl-aryl group (**8i**) and hydroxyl group at the *meta* position of phenyl ring (**8m**) enhanced the activity by 2 folds with MIC 3.125 µg/mL. Methyl or Chloro group at the *meta* and *ortho* position of phenyl ring respectively displayed moderate activity with MIC 6.25 µg/mL (**8b**, **8d**). Notably, nitro group at *para* position of phenyl ring (**8e**) and introducing heteroaryl groups viz., pyridine, pyrimidine ring at 4th position of piperazine (**8j**, **8k**) greatly enhanced the activity. This projected **8e**, **8j** and **8k** to be promising ligand of interest with MIC 1.56 µg/mL.

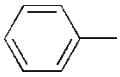
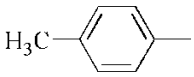
In **scheme 2**, the synthesized compounds were tested for their ability to inhibit the growth of MTB $H_{37}R_V$ strain by MABA. Isoniazid and Rifampicin were used as the positive drug standard.

The *in vitro* antimycobacterial test results of final compounds are tabulated in **Table 4.3** as MIC and the activity ranged from 1.56-50 µg/mL. Compounds with MIC <3.125 µg/mL were further subjected to cytotoxicity studies. Amongst the series, compounds **10f** and **10j** (with *p*-methoxy and *m*-chloro substituent respectively), inhibit 99% growth of MTB $H_{37}Rv$ strain at a concentration 3.125 µg/mL. Nevertheless, compound **11a** with sulfonyl functional group sandwiched between five-membered 1,2,3-triazole and phenyl group, emerged as a promising candidate by inhibiting 99% growth of MTB $H_{37}Rv$ strain at a concentration 1.56 µg/mL.

Table 4.3 Antimycobacterial activities of compound **10a-q** and **11a-b** against MTB $H_{37}Rv$

Compound No.	R	MIC (µg/mL) against MTB $H_{37}Rv$	clogp[a]
10a		50	4.02
10b		50	5.50
10c		12.5	5.47
10d		50	4.68
10e		50	4.70
10f		3.125	4.70

10g		50	6.00
10h		25	5.35
10i		12.5	5.35
10j		3.125	5.35
10k		50	5.50
10l		12.5	5.50
10m		50	5.55
10n		25	4.91
10o		25	4.78
10p		12.5	5.65
10q		6.25	4.58

11a		1.56	2.93
11b		12.5	3.43
Isoniazid		0.36	-0.668
Rifampicin		0.02	ND

[a] Calculated using chemdraw Ultra 8.0

ND=Not determined

Among the synthesized compounds, electron releasing groups like ethyl and methyl exhibited moderate anti-tubercular activity whereas 1,3-benzodioxole and methoxy at *meta* position had no effect on the activity spectrum. Notably, the introduction of methoxy (**10f**) at *para* position improved the activity by 15 folds (MIC = 3.125 µg/mL). Presence of chloro group (**10j**) at *meta* position (MIC = 3.125 µg/mL) exhibited increase in activity by eight and four folds compared to *para* and *ortho* positions (**10h** and **10i**) respectively. Replacing with bromo substituent (**10k** and **10l**) had less effect on the activity spectrum. When electron withdrawing fluoro substituent (**10m**, **10n** and **10o**) was introduced not much change in the activity was observed. Trifluoromethyl group at *meta* position exhibited moderate activity (**10p**, MIC = 12.5 µg/mL). Hopping to nitro group improved the activity by two folds (**10q**, MIC = 6.25 µg/mL). Eventually, we intended to sandwich a sulfonyl group between triazole and the aromatic unit to fetch compounds **11a** and **11b**. This trajectory conferred **11a** as promising ligand of interest with MIC 1.56 µg/mL. Antimycobacterial activity profile suggests that, functional group which has ability to act as hydrogen bond acceptor preferably through its lone pair is essential which might attribute to the enhanced binding interactions.

4.2.3 Cytotoxicity assay

RAW 264.7 cells are macrophage-like cell line derived from BALB/c mice and they maintain many of the properties of macrophages including nitric oxide production, phagocytosis. MTB generally reside and multiply inside the macrophages, so to carry out the cytotoxicity against RAW 264.7 cells is imperative for the selectivity of compounds against MTB rather than host macrophage [23].

From the **scheme 1** most active compounds **8e**, **8j** and **8k** were subjected to Promega Cell Titer 96 non-radioactive cell proliferation assay to evaluate the *in vitro* cytotoxicity against mouse macrophage (RAW264.7) cell lines. The approximate IC_{50} values [24] and selectivity index (SI) are tabulated in **Table 4.4**. These findings indicate that all three new compounds target and kill MTB to a greater extent compared to macrophage cell lines thereby without disordering immune system and justified to consider for further development of phenanthridinyl piperazine scaffold as lead to attenuate the treatment of this devastating disease.

Table 4.4 IC_{50} (µM) and selectivity index (SI) value of active compounds (**8e, 8j** and **8k**) against mouse macrophage cell lines (RAW264.7)

Entry	Compound	MIC (µg/mL) in MTB $H_{37}Rv$	% cell inhibition at 50 µM	IC_{50} approximation	SI value IC_{50}/MIC
1	**8e**	1.56	21.4	**>40**	>25
2	**8j**	1.56	18.2	**>40**	>25
3	**8k**	1.56	16.4	**>40**	>25

From the **scheme 2** most active compounds, **10f**, **10j** and **11a** were subjected to *in vitro* cytotoxicity studies against mouse macrophage (RAW264.7) cell lines. The IC_{50} and selectivity index (SI) values are tabulated in **Table 4.5** and the results imply the suitability of the compounds in drug development for tuberculosis.

Table 4.5 IC_{50} (µg/mL) and selectivity index (SI) values of active compounds **10f, 10j** and **11a**

Entry	Compound	MIC (µg/mL) in MTB $H_{37}Rv$	% cell inhibition at 50µg/mL	IC_{50} approximation	SI value IC_{50}/MIC
1	**10f**	3.125	19.16	**>125**	>15
2	**10j**	3.125	12.62	**>150**	>20
3	**11a**	1.56	5.82	**>425**	>125

4.2.4 X-ray crystallographic study of compound 8k

The X-ray crystallographic analysis of the target molecule **8k** was carried out on a pale yellow crystal, with dimensions 0.42 mm x 0.36 mm x 0.32 mm, grown from the slow evaporation of a dichloromethane and ethanol (1:1) solvent mixture at room temperature. A suitable crystal was selected and mounted on an Xcalibur, Eos, Gemini diffractometer. The crystal was kept at 293.15 K during data collection. Using Olex2 [25], the structure was solved with the unknown structure solution program using unknown and refined with the olex2.refine [26] refinement package using Gausse Newton minimization. The basic crystallographic data and structure refinement are shown in **Table 4.6** and molecular structure is given as an ORTEP diagram in **figure 4.3**. Crystallographic data for the structure **8k** has been deposited with the Cambridge Crystallographic Data Center and the deposition number is CCDC 948870.

Table 4.6 Crystal data and structure refinement for compound **8k**

Crystal data	
Empirical formula	$C_{22}H_{20}N_4$
Formula weight	340.1688
Crystal system	Monoclinic
Crystal size/mm^3	0.42 mm × 0.36 mm × 0.32 mm
Space group	$P2_1/c$
a/Å	9.6106(9)
b/Å	14.7485(19)
c/Å	15.525(2)
Volume/Å^3	1733.0(4)
Angle α/°, β/°, γ/°	90 , 128.043(9), 90
Z	4
Crystal density mg/mm^3	1.3047
F(000)	720.2
μ/mm^{-1}	0.079
Radiation wavelength	0.71073
Radiation type	MoK_α
Radiation monochromator	Graphite
2Θ range for data collection	6.06 to 52.74°
Index ranges	$-12 \leq h \leq 9$, $-16 \leq k \leq 18$, $-17 \leq l \leq 19$

Reflections collected	5060
Independent reflections	3040[R(int) = 0.0198]
Data/restraints/parameters	3040/0/235
Goodness-of-fit on F^2	1.045
Final R indexes [I>=2σ (I)]	R_1 = 0.0492
Final R indexes [all data]	R_1 = 0.0772, wR_2 = 0.1353
Largest diff. peak/hole / e $Å^{-3}$	0.21/-0.23
Structure refinement	OLEX 2

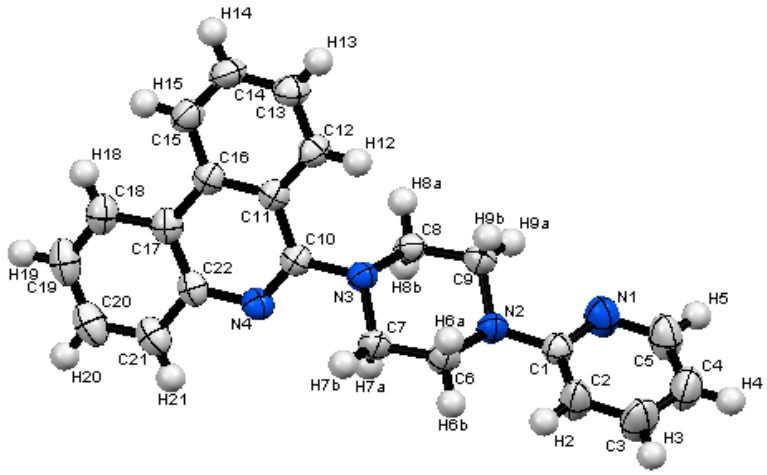


Figure 4.3 ORTEP diagram showing the X-ray crystal structure of compound **8k** with 50% probability thermal ellipsoids.

4.3 Conclusion

From the **scheme 1** preliminary *in vitro* anti-tubercular screening results warrant 6-(4-substitutedpiperazin-1-yl)phenanthridine derivatives as highly potent anti-tubercular agents. In

light of these findings, MTB activity profile point out that further activity can be enhanced by opting appropriate substituent on the phenyl ring at the 4th position of 6-(piperazin-1-yl)phenanthridine. Incorporating pyridine (**8j**, MIC = 1.56 µg/mL) and pyrimidine (**8k**, MIC = 1.56 µg/mL) ring drew a significant attention to employ other heterocycles as well. Nitro group at the *para* position on the phenyl ring has greatly increased the activity of the compound (**8e**, MIC = 1.56 µg/mL).

From the **scheme 2** preliminary anti-tubercular screening results drive us to engineer the chemical structure of phenanthridine derivative to generate essential pharmacophoric features that could lead to the synthesis of a promising candidate to develop anti-tubercular agent. We discovered that incorporating sulfonyl group in the moiety (**11a**) plays a pivotal role in the activity profile. These findings unfold the possibility of employing various functional groups on this derivative.

4.4 Experimental section

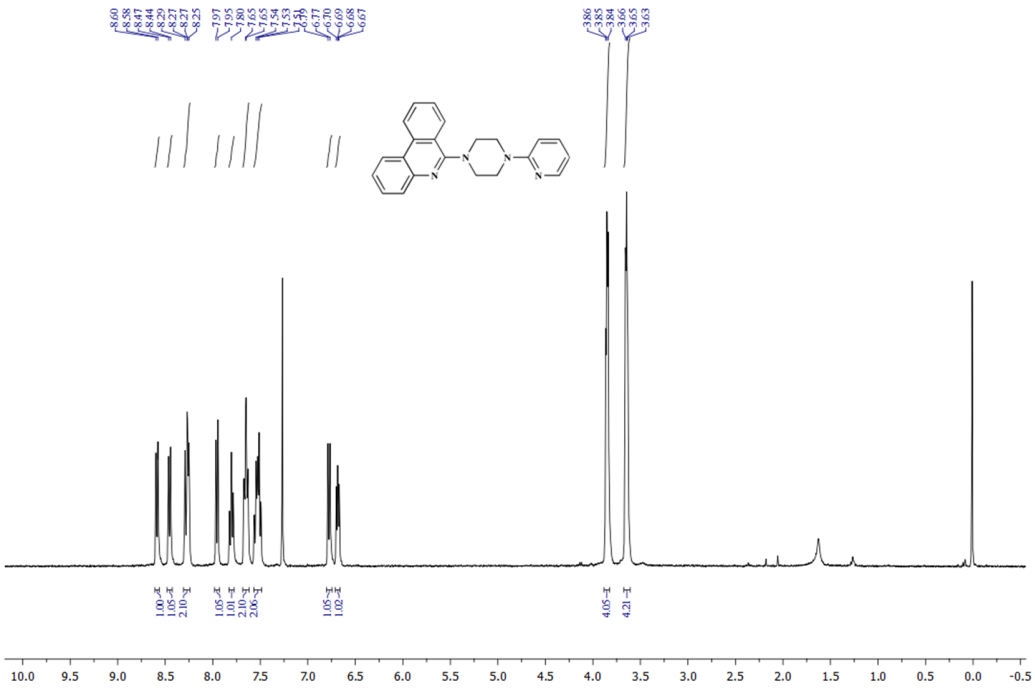
4.4.1 Materials and methods

Chemicals and solvents were procured from commercial sources and are analytically pure. 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 230-400 mesh). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded at 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) are given in ppm. Microwave reactions

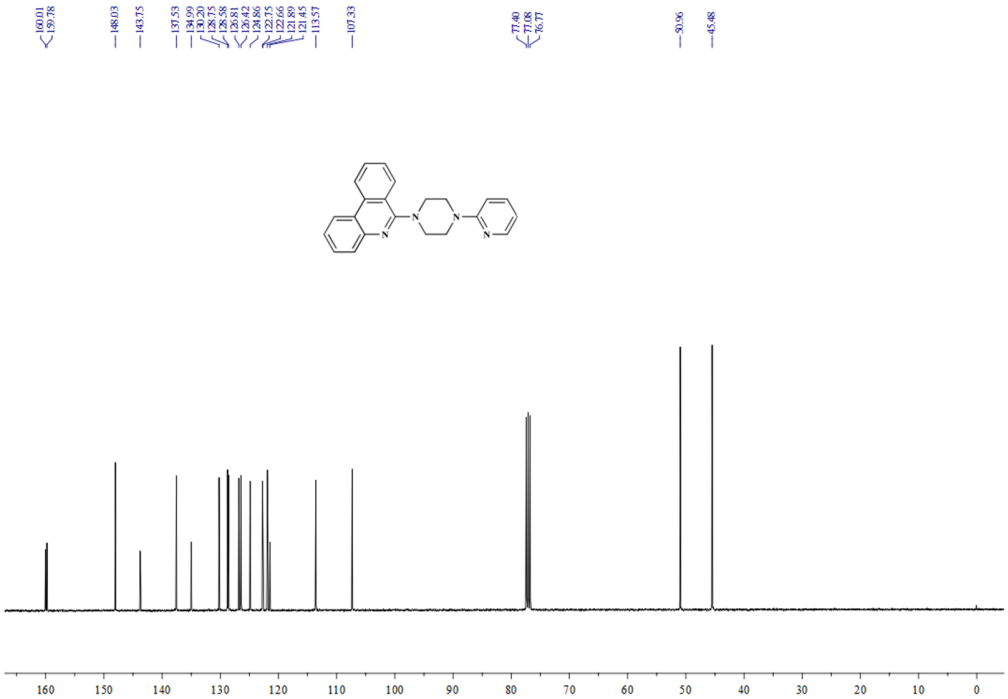
were performed in closed vessel using Biotage Initiator microwave synthesizer (Uppsala, Sweden). IR spectra were recorded on a FT-IR spectrometer (Schimadzu) and peaks are reported in cm^{-1}. Melting points were determined on an electro thermal melting point apparatus (Stuart-SMP30) in open capillary tubes and are uncorrected. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer.

Synthesis of title compounds (8a-q)

6-chlorophenanthridine (2.34 mmol) was dissolved in DMF (5 mL) in an oven dry microwave vial. Then TEA (3.51 mmol) followed by anhydrous piperazine (4.68 mmol)/substituted piperazine (2.57 mmol) were added. Microwave vial was sealed with aluminium cap and the resultant mixture was subjected to microwave irradiation at 150 °C for 20 min. Completion of the reaction was monitored by TLC using 10% MeOH in DCM as mobile phase. After the reaction was complete, reaction mixture was transferred into round bottom flask and DMF was evaporated in vacuo. Resultant residue was extracted using EtOAc (3 x 5 mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous Na_2SO_4 and evaporated in vacuo. Column chromatography of the obtained residue using gradient 2-10% MeOH in DCM as mobile phase gave title compounds **8a-q**.



^{1}H NMR spectrum of compound **8k**



^{13}C NMR spectrum of compound **8k**

6-(piperazin-1-yl)phenanthridine (8a)

Beige solid (62%); mp 118-119 °C; IR (KBr, cm^{-1}) 3316, 3052, 2863, 1687, 1568, 1368, 779. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.2 Hz, 1H), 8.45 (d, *J* = 7.9 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 1H), 4.81 (s, br 1H). 3.82 – 3.25 (m, 8H).^{13}C NMR (100.61 MHz, $CDCl_3$) δ 171.87, 147.68, 136.79, 134.12, 129.97, 128.32, 126.11, 125.56, 124.13, 122.86, 121.64, 120.76, 113.78, 51.23, 46.69. HRMS: (ESI, m/z) for $C_{17}H_{18}N_3$ calcd: 264.3449, found: 264.3453 $(M+H)^+$.

6-[4-(3-methylphenyl)piperazin-1-yl]phenanthridine (8b)

Colorless solid (78%); mp 102-104 °C; IR (KBr, cm^{-1}) 3069, 2876, 1675, 1579, 1377, 815, 768. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 8.2 Hz, 1H), 8.45 (dd, *J* = 8.1, 1.1 Hz, 1H), 8.26 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.96 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.82 – 7.76 (m, 1H), 7.64 (tt, *J* = 8.3, 1.4 Hz, 2H), 7.50 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 7.6 Hz, 2H), 6.73 (d, *J* = 7.5 Hz, 1H), 3.69 – 3.64 (m, 4H), 3.52 – 3.46 (m, 4H), 2.36 (s, 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.12, 151.76, 148.45, 142.58, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 114.85, 109.34, 107.36, 50.74, 45.78, 26.23. HRMS: (ESI, m/z) for $C_{24}H_{24}N_3$ calcd: 354.1965, found: 354.1982 $(M+H)^+$.

6-[4-(4-chlorophenyl)piperazin-1-yl]phenanthridine (8c)

Pale yellow solid (71%); mp 173-175 °C; IR (KBr, cm^{-1}) 3043, 2871, 1669, 1531, 1357, 819, 763. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 8.2 Hz, 1H), 8.45 (dd, *J* = 8.1, 1.2 Hz, 1H), 8.24 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.95 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.83 – 7.76 (m, 1H), 7.64 (tdd, *J* = 8.2, 2.2, 1.4 Hz, 2H), 7.51 (ddd, *J* = 8.2, 7.1, 1.3 Hz, 1H), 7.28 (d, *J* = 3.3 Hz, 1H), 7.25 – 7.23 (m,

1H), 6.98 – 6.95 (m, 1H), 6.95 – 6.92 (m, 1H), 3.69 – 3.64 (m, 4H), 3.49 – 3.43 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.38, 151.17, 148.72, 143.56, 137.98, 135.82, 130.39, 129.58, 128.42, 127.74, 126.60, 125.73, 124.86, 123.65, 122.58, 122.55, 121.93, 114.32, 108.11, 52.71, 47.68. HRMS: (ESI, m/z) for $C_{23}H_{21}ClN_3$ calcd: 374.1419, found: 374.1431 $(M+H)^+$.

6-[4-(2-chlorophenyl)piperazin-1-yl]phenanthridine (8d)

Colorless solid (76%); mp 134-136 °C; IR (KBr, cm^{-1}) 3049, 2857, 1691, 1559, 1373, 798, 785. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 8.1 Hz, 1H), 8.43 (d, *J* = 8.2 Hz, 1H), 8.29 – 8.24 (m, 1H), 8.22 (s, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.66 – 7.61 (m, 2H), 7.54 (dd, *J* = 10.7, 3.8 Hz, 1H), 7.51 (t, *J* = 5.4 Hz, 1H), 6.69 (d, *J* = 8.6 Hz, 1H), 6.62 (dd, *J* = 6.7, 5.3 Hz, 1H), 3.85 – 3.77 (m, 4H), 3.66 – 3.61 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.78, 151.34, 148.87, 143.12, 136.92, 135.85, 130.93, 129.43, 128.78, 127.81, 127.11, 124.86, 122.71, 122.06, 121.83, 121.25, 115.47, 113.91, 107.33, 51.96, 42.48. HRMS: (ESI, m/z) for $C_{23}H_{21}ClN_3$ calcd: 374.1419, found: 374.1439 $(M+H)^+$.

6-[4-(4-nitrophenyl)piperazin-1-yl]phenanthridine (8e)

Yellow solid (69%); mp 272-274 °C; IR (KBr, cm^{-1}) 3072, 2881, 1688, 1586, 1567, 1366, 1322, 819, 775. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 8.2 Hz, 1H), 8.47 (d, *J* = 7.9 Hz, 1H), 8.25 (d, *J* = 8.3 Hz, 1H), 8.19 (d, *J* = 9.3 Hz, 2H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.67 (t, *J* = 7.6 Hz, 2H), 7.54 (t, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 9.4 Hz, 2H), 3.75 – 3.67 (m, 8H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.83, 155.26, 152.12, 148.96, 143.63, 138.12, 136.57, 131.12, 129.66, 128.89, 127.54, 126.18, 125.37, 124.14, 121.64, 120.89, 119.26, 114.75, 108.22, 52.2, 45.76. HRMS: (ESI, m/z) for $C_{23}H_{21}N_4O_2$ calcd: 385.1659, found: 385.1671 $(M+H)^+$.

6-[4-(3-methoxyphenyl)piperazin-1-yl]phenanthridine (8f)

Brown solid (79%); mp 113-115 °C; IR (KBr, cm^{-1}) 3077, 2861, 1688, 1578, 1365, 1056, 783, 719. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 8.2 Hz, 1H), 8.39 (dd, *J* = 8.1, 1.1 Hz, 1H), 8.22 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.93 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.79 – 7.72 (m, 1H), 7.61 (tt, *J* = 8.3, 1.4 Hz, 2H), 7.45 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.19 (t, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 7.6 Hz, 2H), 6.71 (d, *J* = 7.5 Hz, 1H), 3.96 (s, 3H), 3.69 – 3.64 (m, 4H), 3.52 – 3.46 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 159.87, 150.28, 148.24, 144.45, 137.23, 135.72, 130.35, 129.32, 128.46, 126.17, 125.34, 124.13, 123.56, 122.64, 121.85, 120.56, 115.34, 114.88, 106.42, 58.67, 52.63, 46.61. HRMS: (ESI, m/z) for $C_{24}H_{24}N_3O$ calcd: 370.1914, found: 370.1923 $(M+H)^+$.

2-[4-(phenanthridin-6-yl)piperazin-1-yl]benzonitrile (8g)

Beige solid (81%); mp 171-172 °C; IR (KBr, cm^{-1}) 3086, 2882, 2258, 1696, 1577, 1371, 791, 772. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.1 Hz, 1H), 8.47 (d, *J* = 8.2 Hz, 1H), 8.35 – 8.29 (m, 1H), 8.28 (s, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 7.68 – 7.63 (m, 2H), 7.58 (dd, *J* = 10.7, 3.8 Hz, 1H), 7.52 (t, *J* = 5.4 Hz, 1H), 6.63 (d, *J* = 8.6 Hz, 1H), 6.57 (dd, *J* = 6.7, 5.3 Hz, 1H), 3.78 – 3.65 (m, 4H), 3.61 – 3.58 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 161.17, 152.26, 149.87, 141.31, 138.39, 135.51, 131.22, 129.68, 127.78, 126.69, 126.13, 125.78, 124.56, 122.64, 121.64, 120.53, 118.45, 115.49, 116.23, 108.78, 51.70, 45.33. HRMS: (ESI, m/z) for $C_{24}H_{21}N_4$ calcd: 365.1761, found: 365.1779 $(M+H)^+$.

6-(4-phenethylpiperazin-1-yl)phenanthridine (8h)

Colorless solid (86%); mp 123-125 °C; IR (KBr, cm^{-1}) 3089, 2855, 1581, 1679, 1356, 783. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 8.2 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.15 (d, *J* = 8.3 Hz, 1H), 8.11 (d, *J* = 9.3 Hz, 2H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.77 (t, *J* = 7.6 Hz, 1H), 7.58 (m, 5H), 7.52 (t, *J* = 7.6 Hz, 1H), 3.72 – 3.65 (m, 8H), 3.46 (t, *J* = 7.12 Hz, 2H), 2.73 (t, *J* = 6.9 Hz , 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 159.21, 151.06, 148.19, 143.66, 140.61 138.84, 136.11, 131.75, 129.44, 128.92, 127.14, 126.51, 125.60, 123.17, 121.88, 120.47, 119.27, 115.85, 108.31, 50.74, 49.28, 45.78, 32.55. HRMS: (ESI, m/z) for $C_{25}H_{26}N_3$ calcd: 368.2121, found: 368.2137 $(M+H)^+$.

6-(4-benzhydrylpiperazin-1-yl)phenanthridine (8i)

Pale yellow solid (72%); mp 175-177 °C; IR (KBr, cm^{-1}) 3087, 2873, 1691, 1571, 1359, 768. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 8.2 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.23 (d, *J* = 8.3 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.82 (t, *J* = 7.6 Hz, 1H), 7.72 (m, 5H), 7.67 (t, *J* = 7.6 Hz, 2H), 7.5 (m, 5H), 7.41 (t, *J* = 7.6 Hz, 1H), 3.79 – 3.64 (m, 4H), 3.54 – 3.48 (m, 4H), 3.39 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.94, 155.65, 151.27, 149.08, 145.43, 141.35, 138.45, 135.22, 131.37, 129.11, 128.06, 127.57, 125.18, 124.77, 123.79, 121.31, 120.96, 120.02, 114.17, 70.86, 52.42, 43.26. HRMS: (ESI, m/z) for $C_{30}H_{28}N_3$ calcd: 430.2278, found: 430.2288 $(M+H)^+$.

6-(4-(pyrimidin-2-yl)piperazin-1-yl)phenanthridine (8j)

Beige solid (73%); mp 154-155 °C; IR (KBr, cm^{-1}) 3073, 2863, 1690, 1556, 1361, 778. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 8.2 Hz, 1H), 8.47 (d, *J* = 7.9 Hz, 1H), 8.25 (d, *J* = 8.3 Hz, 1H), 8.19 (d, *J* = 9.3 Hz, 2H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.67 (t, *J* = 7.6 Hz,

2H), 6.95 (d, *J* = 9.4 Hz, 2H), 3.75 – 3.67 (m, 8H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 161.83, 159.38, 157.69, 155.39, 150.21, 147.62, 144.74, 138.08, 136.73, 130.23, 128.98, 127.12, 126.78, 125.25, 123.43, 120.03, 113.78, 54.11, 44.64. HRMS: (ESI, m/z) for $C_{21}H_{20}N_5$ calcd: 342.1713, found: 342.1722 $(M+H)^+$.

6-[4-(pyridin-2-yl)piperazin-1-yl]phenanthridine (8k)

Colorless solid (75%); mp 147-149 °C ; IR (KBr, cm^{-1}) 3066, 2869, 1667, 1577, 1381, 788. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 8.1 Hz, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 8.32 – 8.27 (m, 1H), 8.25 (s, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.80 (t, *J* = 7.6 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.55 (dd, *J* = 10.7, 3.8 Hz, 1H), 7.51 (t, *J* = 5.4 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 6.68 (dd, *J* = 6.7, 5.3 Hz, 1H), 3.90 – 3.79 (m, 4H), 3.68 – 3.59 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.01, 150.78, 148.03, 143.75, 137.53, 134.99, 130.20, 128.75, 128.58, 126.81, 126.42, 124.86, 122.75, 122.66, 121.89, 121.45, 113.57, 107.33, 50.96, 45.48. HRMS: (ESI, m/z) for $C_{22}H_{21}N_4$ calcd: 341.1761, found: 341.1776 $(M+H)^+$.

6-(4-ethylpiperazin-1-yl)phenanthridine (8l)

Beige solid (80%); mp 78-80 °C; IR (KBr, cm^{-1}) 3054, 2891, 1669, 1575, 1387, 768. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 8.2 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.31 (d, *J* = 8.3 Hz, 1H), 7.83 (d, *J* = 7.5 Hz, 1H), 7.72 (t, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 2H), 7.45 (t, *J* = 7.6 Hz, 1H), 3.78 – 3.72 (m, 4H), 3.62 – 3.57 (m, 4H), 3.42 (q, 2H), 1.36 (t, 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.85, 151.53, 147.47, 142.80, 137.32, 135.27, 129.13, 128.46, 127.37, 123.33, 121.96, 119.25, 114.39, 51.14, 48.63, 42.49, 16.40. HRMS: (ESI, m/z) for $C_{19}H_{22}N_3$ calcd: 292.1808, found: 292.1821 $(M+H)^+$.

3-[4-(phenanthridin-6-yl)piperazin-1-yl]phenol (8m)

Brown solid (67%); mp 195-197 °C; IR (KBr, cm^{-1}) 3488, 3086, 2889, 1683, 1564, 1375, 775, 733. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 8.2 Hz, 1H), 8.46 (dd, *J* = 8.1, 1.1 Hz, 1H), 8.29 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.93 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.79 (m, 1H), 7.68 (tt, *J* = 8.3, 1.4 Hz, 2H), 7.58 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 2H), 6.73 (d, *J* = 7.5 Hz, 1H), 4.88 (s, 1H), 3.65 – 3.58 (m, 4H), 3.51 – 3.45 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.45, 151.67, 149.35, 144.68, 138.25, 135.15, 131.06, 128.75, 127.13, 126.29, 125.47, 124.28, 122.41, 121.23, 120.55, 119.34, 114.56, 109.23, 108.74, 51.33, 44.26. HRMS: (ESI, m/z) for $C_{23}H_{22}N_3O$ calcd: 356.1757, found: 356.1769 $(M+H)^+$.

1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-(phenanthridin-6-yl)piperazin-1-yl)acetyl)piperazin-1-yl)quinoline-3-carboxylic acid (8n)

Brown solid (77%); mp 270-271 °C; IR (KBr, cm^{-1}) 3267, 3087, 3018, 2877, 1733, 1695, 1667, 1375, 1244, 1038, 763. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 8.57 (d, *J* = 8.2 Hz, 1H), 8.45 (d, *J* = 7.9 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.83 (d, *J* = 7.5 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.52 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 3.81 (tt, *J* = 7.2 Hz, *J* = 6.9 Hz, 1H), 3.79 – 3.56 (m, 16H), 3.28 (m, 2H), 1.26 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82, 167.56, 166.43, 159.87, 150.28, 147.67, 146.23, 143.67, 138.95, 136.79, 134.12, 129.97, 128.32, 127.89, 126.11, 125.56, 124.13, 122.86, 121.64, 121.16, 120.76, 113.21, 111.97, 107.12, 106.33, 61.21, 53.67, 51.87, 51.12, 50.89, 49.16, 46.78, 45.91, 41.39, 35.58, 8.12. HRMS: (ESI, m/z) for $C_{36}H_{36}FN_6O_4$ calcd: 635.2777, found: 635.2796 $(M+H)^+$.

6-[4-methylpiperazin-1-yl]phenanthridine (8o)

Colorless solid (76%); mp 97-98 °C; IR (KBr, cm^{-1}) 3089, 2876, 1655, 1577, 1385, 791. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 8.2 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.27 (d, *J* = 8.3 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.86 (t, *J* = 7.6 Hz, 1H), 7.76 (t, *J* = 7.6 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 1H), 3.65 – 3.59 (m, 8H), (3.43 s, 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 161.12, 153.87, 151.89, 144.80, 144.16, 138.97, 128.68, 118.92, 118.12, 110.9, 110.71, 106.23, 106.11, 49.55, 44.46, 35.89. HRMS: (ESI, m/z) for $C_{18}H_{20}N_3$ calcd: 278.1652, found: 278.1668 $(M+H)^+$.

6-(4-(4-vinylbenzyl)piperazin-1-yl)phenanthridine (8p)

Beige solid (66%); mp 114-115 °C; IR (KBr, cm^{-1}) 3075, 2872, 1692, 1559, 1376, 987, 783, 721. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 8.1 Hz, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 8.32 – 8.27 (m, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.80 (t, *J* = 7.6 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.55 (dd, *J* = 10.7, 3.8 Hz, 1H), 7.51 (d, *J* = 5.4 Hz, 2H), 6.78 (d, *J* = 8.6 Hz, 2H), 6.56 (s, 1H), 5.56 (d, 2H), 3.90 – 3.79 (m, 4H), 3.68 – 3.59 (m, 6H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 160.12, 151.33, 147.42, 143.75, 138.24, 134.76, 130.15, 129.17, 128.56, 126.86, 125.85, 124.79, 122.39, 121.47, 120.42, 119.12, 118.32, 114.21, 108.12, 107.24, 106.77, 61.34, 51.23, 46.69. HRMS: (ESI, m/z) for $C_{26}H_{26}N_3$ calcd: 380.2121, found: 380.2134 $(M+H)^+$.

6-(4-cinnamylpiperazin-1-yl)phenanthridine (8q)

Pale yellow oil (70%); IR (KBr, cm^{-1}) 3091, 2873, 1692, 1559, 1383, 971, 787. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 8.1 Hz, 1H), 8.41 (d, *J* = 8.2 Hz, 1H), 8.28 – 8.25 (m, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.63 – 7.55 (m, 2H), 7.51 (dd, *J* = 10.7, 3.8 Hz, 1H), 7.32-7.12 (m, 5H), 6.78 (d, *J* = 8.6 Hz, 1H), 5.86 (s, 1H), 3.90 – 3.79 (m, 4H), 3.68 – 3.59 (m,

6H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 159.25, 151.41, 148.83, 144.23, 140.23, 137.64, 134.49, 132.45, 131.63, 128.47, 127.43, 126.12, 125.42, 124.31, 122.98, 121.63, 120.52, 119.82, 113.57, 112.74, 107.33, 58.43, 52.46, 43.68. HRMS: (ESI, m/z) for $C_{26}H_{26}N_3$ calcd: 380.2121, found: 380.2142 $(M+H)^+$.

Synthesis of 6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine (9)

6-(piperazin-1-yl)phenanthridine (0.0187 mol) was dissolved in DMF (50 mL), then TEA (0.0280 mol) followed by propargyl bromide (80% in toluene) (0.0280 mol) were added. Resultant mixture was heated at 70 °C for 1.5 h. Completion of the reaction was monitored by TLC using 5% MeOH in DCM as mobile phase. After the reaction was complete, DMF was evaporated in vacuo and added 50 mL of water. Compound was extracted using EtOAc (3 x 15 mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous Na_2SO_4 and evaporated in vacuo. Column chromatography of the residue using 1–2% MeOH in DCM gave 6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine.

Pale yellow solid (92%); mp 125-126 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 8.0 Hz, 1H), 8.36 (d, J = 7.6, Hz, 1H), 8.21 (d, J = 7.2 Hz, 1H), 7.92 (d, J = 9.6 Hz, 1H), 7.76–7.39 (m, 4H),3.94 (s, 2H), 3.58 (m, 4H), 2.92 (m, 4H), 2.42 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.27, 138.62, 134.53, 131.65, 129.44, 128.33, 127.45, 125.34, 124.56, 123.65,122.64, 121.16, 117.76, 78.64, 76.89, 58.72, 56.21 50.63. HRMS: (ESI m/z) for $C_{20}H_{20}N_3$ calcd: 302.3929, found: 302.3933 $(M+H)^+$.

Synthesis of 10a-q

6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine **(9)** (0.6571 mmol) was dissolved in 1:2 ratio of water and *t*-BuOH (3 mL). Then $CuSO_4.5H_2O$ (0.1314 mmol), sodium ascorbate (0.1314 mmol) and various substitued azide (0.7228 mmol) was added. Resultant mixture was stirred at RT for 3 h. Completion of the reaction was monitored by TLC using 2% MeOH in DCM as mobile phase. After the reaction was complete, volatile was evaporated in vacuo and the compound was extracted using EtOAc (3 x 5 mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous sodium sulphate and evaporated in vacuo. Column chromatography of the residue using 1–2% MeOH in DCM gave title compounds **10a-q**.

6-(4-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10a)

Colorless solid (91%); mp 132-133 °C; IR (KBr, cm^{-1}) 3086, 2843, 1667, 1556, 1349, 767. ^1H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.2, Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.88 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.32 (m, 9H), 4.98 (s, 2H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 172.46, 148.76, 141.28, 139.84, 138.32, 135.51, 134.42, 130.80, 129.47, 128.13, 127.69, 126.61, 124.60, 123.11, 122.24, 121.64, 120.43, 119.92, 116.85, 60.16, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{27}H_{27}N_6$ calculated: 435.2297, found: 435.2289 $(M+H)^+$.

6-(4-((1-(4-ethylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10b)

Colorless solid (89%); mp 122-123 °C; IR (KBr, cm^{-1}) 3053, 2852, 1673, 1548, 1357, 836, 773. ^1H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.46 (m, 8H), 3.92 (s, 2H), 3.55 (m, 4H),

2.90 (m, 4H), 2.76 (m, 2H), 1.36 (t, 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 151.76, 148.45, 142.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 114.85, 112.36, 58.18, 50.74, 45.27, 29.23, 17.25. HRMS: (ESI, m/z) for $C_{28}H_{29}N_6$ calculated: 449.2453, found: 449.2446 $(M+H)^+$.

6-(4-((1-(2,4-dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10c)

Colorless solid (94%); mp 114-115 °C; IR (KBr, cm^{-1}) 3061, 2851, 1660, 1547, 1354, 772. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.68 (d, *J* = 7.4 Hz, 1H), 7.58 (s, 1H), 7.56 – 7.37 (m, 4H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H), 2.22–2.56 (s, 6H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 151.76, 148.45, 142.58, 140.18, 139.84, 137.84, 136.14 135.51, 134.80, 132.26, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 114.88, 113.16, 58.18, 50.74, 45.27, 24.18, 21.58. HRMS: (ESI, m/z) for $C_{28}H_{29}N_6$ calculated: 449.2453, found: 449.2448 $(M+H)^+$.

6-(4-((1-(benzo[*d*][1,3]dioxol-5-yl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10d)

Colorless solid (88%); mp 124-125 °C; IR (KBr, cm^{-1}) 3095, 2856, 1685, 1576, 1366, 1138, 778. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 7.2 Hz, 1H), 7.02 (s, 1H), 7.68 – 7.42 (m, 4H), 5.94 (s, 2H), 3.91 (s, 2H), 3.59 (m, 4H), 2.91 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 173.42, 150.76, 148.81, 148.45, 144.58, 142.18, 139.84, 137.84,

136.14 135.51, 134.80, 132.26, 128.13, 127.69, 126.61, 125.60, 124.21, 122.64, 120.43, 119.92, 114.88, 104.16, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{27}H_{25}N_6O_2$ calculated: 465.2039, found: 465.2032 $(M+H)^+$.

6-(4-((1-(3-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10e)

Pale yellow semi solid (68%); mp N.D.; IR (KBr, cm^{-1}) 3093, 2867, 1683, 1576, 1363, 1132, 792, 697. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.46 (d, *J* = 8.0 Hz, 1H), 8.36 (d, *J* = 7.6, Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.00 (s, 1H),7.92 (d, *J* = 9.6 Hz, 1H), 7.90 (d, *J* = 7.2 Hz, 1H), 7.76 – 7.39 (m, 5H),6.96 (d, *J* = 7.6 Hz, 1H), 6.92 (s, 1H), 3.99 (s, 3H), 3.94 (s, 2H), 3.58 (m, 4H), 2.92 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.27, 150.45, 142.23, 141.28, 138.62, 136.45, 134.53, 131.65, 129.44, 128.33, 127.45, 126.89, 125.34, 124.56, 123.65, 122.64 121.32, 121.16, 119.42, 118.12, 117.76, 58.72, 56.21 50.63, 45.38. HRMS: (ESI, m/z) for $C_{27}H_{27}N_6O$ calculated: 451.2246, found: 451.2252 $(M+H)^+$.

6-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10f)

Pale green solid (78%); mp 128-129 °C; IR (KBr, cm^{-1}) 3062, 2854, 1648, 1565, 1357, 1211, 829, 784. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.56 (d, *J* = 8.4 Hz, 1H), 8.42 (d, *J* = 7.2, Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.68 – 7.37 (m, 4H), 6.92 (d, *J* = 7.2 Hz, 2H), 3.98 (s, 3H). 3.92 (s, 2H), 3.58 (m, 4H), 2.93 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.10, 162.42, 149.72, 140.58, 139.84, 138.84, 135.51, 134.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 121.12, 119.62, 118.45, 115.46, 62.34,

58.98, 51.74, 46.27. HRMS: (ESI, m/z) for $C_{27}H_{27}N_6O$ calculated: 451.2246, found: 451.2250 $(M+H)^+$.

6-(4-((1-(3,4-dichlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10g)

Colorless solid (88%); mp 131-132 °C; IR (KBr, cm^{-1}) 3045, 2825, 1678, 1563, 1356, 775. ^1H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.92 (d, *J* = 9.2 Hz, 1H), 7.78 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 7.4 Hz, 1H), 7.56 – 7.36 (m, 4H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 172.12, 148.46, 144.58, 142.18, 140.84, 137.84, 136.14 134.51, 134.42, 132.26, 130.12, 128.13, 127.69, 126.61,124.82, 124.24, 123.11, 122.64, 120.43, 119.92, 116.26, 58.18, 50.74, 45.27. HRMS: (ESI,m/z) for $C_{26}H_{23}Cl_2N_6$ calculated: 489.1361, found: 489.1370 $(M+H)^+$.

6-(4-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10h)

Colorless solid (95%); mp 162-164 °C; IR (KBr, cm^{-1}) 3063, 2829, 1645, 1576, 1387, 816, 771. ^1H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.46 (m, 8H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 151.76, 144.35, 142.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 116.85, 114.36, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{24}ClN_6$ calculated: 455.1751, found: 455.1758 $(M+H)^+$.

6-(4-((1-(2-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10i)

Pale yellow solid (82%); mp 122-123 °C; IR (KBr, cm^{-1}) 3058, 2838, 1647, 1578, 1355, 787, 741. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.96 (d, *J* = 9.2 Hz, 1H), 7.74 (d, *J* = 7.2 Hz, 1H), 7.68 (d, *J* = 9.2 Hz, 1H), 7.58 – 7.36 (m, 6H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 173.42, 149.76, 142.36, 140.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.42, 128.13, 127.92, 127.69, 126.61, 125.60, 124.72, 123.11, 121.64, 120.43, 119.92, 115.85, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{24}ClN_6$ calculated: 455.1751, found: 455.1758 $(M+H)^+$.

6-(4-((1-(3-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10j)

Colorless solid (94%); mp 121-122 °C; IR (KBr, cm^{-1}) 3059, 2867, 1678, 1566, 1359, 765, 698. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.48 (d, *J* = 8.4 Hz, 1H), 8.44 (d, *J* = 7.6, Hz, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 8.12 –8.00 (s, 2H),7.93 (d, *J* = 9.2 Hz, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 8.81 (d, *J* = 7.6 Hz, 1H), 7.78 – 7.46 (m, 5H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 173.12, 151.76, 144.35, 142.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60, 124.12, 123.11, 122.88 121.64, 120.43, 119.92, 118.85, 116.36, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{24}ClN_6$ calculated: 455.1751, found: 455.1757 $(M+H)^+$.

6-(4-((1-(2-bromophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10k)

Pale brown semi solid (82%); IR (KBr, cm^{-1}) 3059, 2856, 1672, 1567, 1351, 778, 743. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.42 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.96 (d, *J* = 9.2 Hz, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 7.75 (d, *J* = 9.2 Hz, 1H), 7.71 – 7.36 (m, 6H), 3.92 (s, 2H), 3.48 (m, 4H), 2.92 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ

173.42, 150.76, 142.36, 140.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.42, 128.13, 127.92, 127.69, 126.61, 125.60, 124.72, 123.11, 121.64, 120.43, 119.92, 116.85, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{24}BrN_6$ calculated: 499.1245, found: 499.1251 $(M+H)^+$.

6-(4-((1-(4-bromophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10l)

Colorless solid (94%); mp 164-165 °C; IR (KBr, cm^{-1}) 3076, 2868, 1658, 1572, 1388, 836, 776. ^1H NMR (400MHz, $CDCl_3$) δ 8.56 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.48 (m, 8H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 151.76, 148.45, 142.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 118.85, 116.36, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{24}BrN_6$ calculated: 499.1245, found: 499.1251 $(M+H)^+$.

6-(4-((1-(3-chloro-4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10m)

Colorless solid (92%); mp 120-121 °C; IR (KBr, cm^{-1}) 3061, 2858, 1679, 1577, 1354, 778. ^1H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.88 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.65 (d, *J* = 7.4 Hz, 1H), 7.58 – 7.32 (m, 4H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 161.46, 150.44, 144.58, 142.18, 140.84, 137.84, 136.14 135.51, 134.80, 132.26, 130.12, 128.13, 127.69, 126.61, 125.60, 123.11, 122.64, 120.43, 119.92, 118.26, 58.18, 50.74, 45.27. HRMS:(ESI,m/z) for $C_{26}H_{23}ClFN_6$ calculated: 473.1656, found: 473.1662 $(M+H)^+$.

6-(4-((1-(3,4-difluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10n)

Brown colour solid (94%); mp 113-114 °C; IR (KBr, cm^{-1}) 3073, 2854, 1672, 1567, 1355, 778. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.4 Hz, 1H), 8.44 (d, *J* = 7.6, Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.73 (d, *J* = 7.4 Hz, 1H), 7.70 – 7.34 (m, 4H),6.88 (s, 1H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 152.23, 149.58, 148.78, 142.18, 140.84, 137.84, 136.14 134.51, 134.42, 132.26, 130.12, 128.13, 126.61,124.82, 124.24, 123.11, 122.64, 120.43, 119.92, 112.26, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{23}F_2N_6$ calculated: 457.1952, found: 457.1958 $(M+H)^+$.

6-(4-((1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10o)

Colorless solid (93%); mp 148-150 °C; IR (KBr, cm^{-1}) 3069, 2852, 1678, 1541, 1367, 816, 779. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.51 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.90 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.32 (m, 8H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 151.76, 148.45, 142.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 114.85, 108.16, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{24}FN_6$ calculated: 439.2046, found: 439.2039 $(M+H)^+$.

6-(4-((1-(3-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10p)

Yellow solid (85%); mp 130-131 °C; IR (KBr, cm^{-1}) 3058, 2858, 1676, 1562, 1352, 772, 716. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.4 Hz, 1H), 8.36 (d, *J* = 7.2, Hz, 1H), 8.21 (d, *J* = 7.6

Hz, 1H), 8.10 –8.00 (s, 2H),7.98 (d, *J* = 9.6 Hz, 1H), 7.94 (d, *J* = 7.2 Hz, 1H), 8.86 (d, *J* = 7.6 Hz, 1H), 7.76 – 7.39 (m, 5H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 173.93, 150.16, 143.45, 142.58, 138.44, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60,124.89, 124.12,123.11, 122.88 121.64, 121.13, 119.62, 118.42, 116.76, 58.78, 50.64, 45.36. HRMS: (ESI, m/z) for $C_{27}H_{24}F_3N_6$ calculated: 489.2014, found: 489.2008 $(M+H)^+$.

6-(4-((1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (10q)

Pale yellow semi solid (90%); mp N.D.; IR (KBr, cm^{-1}) 3077, 2867, 1658, 1563, 1528, 1386, 1354, 862, 773. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.56 (d, *J* = 8.4 Hz, 1H), 8.4 (d, *J* = 7.6, Hz, 1H), 8.32 (d, *J* = 7.2 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.10 –8.00 (s, 2H),7.93 (d, *J* = 9.2 Hz, 1H), 7.72 (d, *J* = 7.2 Hz, 1H), 7.68 – 7.42 (m, 5H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.52, 150.84, 142.18, 140.84, 137.84, 136.14. 136.08, 135.51, 134.80, 132.26, 129.12, 128.13, 127.62, 126.41, 125.62, 124.11, 123.64, 121.43, 119.92, 118.26, 108.12, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{24}N_7O_2$ calculated: 466.1991, found: 466.1984 $(M+H)^+$.

Synthesis of 11a-b

6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine **(9)** (0.6571 mmol) was dissolved in toluene (5 mL). Then CuTC (0.0657 mmol), and various sulfonyl azide (0.7228 mmol) was added. Resultant mixture was stirred at RT for 1 h. Completion of the reaction was monitored by TLC using 2% MeOH in DCM as mobile phase. After the reaction was complete, saturated aq NH_4Cl (5 mL) was added and the compound was extracted using EtOAc (3 x 5 mL). Combined organic

layers were washed with saturated brine solution, dried over anhydrous Na_2SO_4 and evaporated in vacuo. Column chromatography of the residue using 1–2% MeOH in DCM gave title compounds **11a-b**.

6-(4-((1-(phenylsulfonyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (11a)

Colorless solid (74%); mp 151-152 °C; IR (KBr, cm^{-1}) 3099, 2854, 1645, 1539, 1323, 1356, 1179, 769. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.32 (m, 9H), 3.92 (s, 2H), 3.54 (m, 4H), 2.90 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 174.12, 151.76, 142.18, 139.84, 137.84, 135.51, 134.42, 130.80, 129.47, 128.13, 127.69, 126.61, 124.60, 123.11, 121.64, 120.43, 119.92, 114.85, 108.16, 58.18, 50.74, 45.27. HRMS: (ESI, m/z) for $C_{26}H_{25}N_6O_2S$ calculated: 485.1759, found: 485.1764 $(M+H)^+$.

6-(4-((1-tosyl-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (11b)

Colorless solid (78%); mp 101-102 °C; IR (KBr, cm^{-1}) 3076, 2848, 1656, 1536, 1319, 1364, 1187, 787, 761. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.83 (d, *J* = 9.2 Hz, 2H), 7.72 (d, *J* = 7.6 Hz, 2H), 7.68 – 7.46 (m, 4H), 3.92 (s, 2H), 3.55 (m, 4H), 2.90 (m, 4H), 2.42 (s, 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 172.12, 150.76, 142.35, 140.58, 139.84, 138.84, 135.51, 134.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 118.85, 114.36, 58.18, 50.74, 45.27, 24.64. HRMS: (ESI, m/z) for $C_{27}H_{27}N_6O_2S$ calculated: 499.1916, found: 499.1922 $(M+H)^+$.

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Chapter V

Synthesis of 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine hybrid analogues as anti-tubercular agents

Chapter 5

Synthesis of 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine hybrid analogues as anti-tubercular agents

5.1 Introduction

2-Aminothiazoles are well known potential therapeutic agents viz. antiviral, NPY_5 antagonists, PGE_2 inhibitors, anticancer, opiod receptor agonists, antimycobacterial, anti-inflammatory, antiprion etc., [1-8]. The wide applicability of thiazole framework in drug discovery is attributed to the use of inexpensive starting materials and adoption of simple synthetic strategy to wangle diverse molecules. In particular, nitazoxanide (NTZ) and its active metabolite tizoxanide (TIZ) are known to inhibit replicative and non-replicative TB [9]. Some of the drugs based on aminothiazoles moiety are depicted in **figure 5.1**.

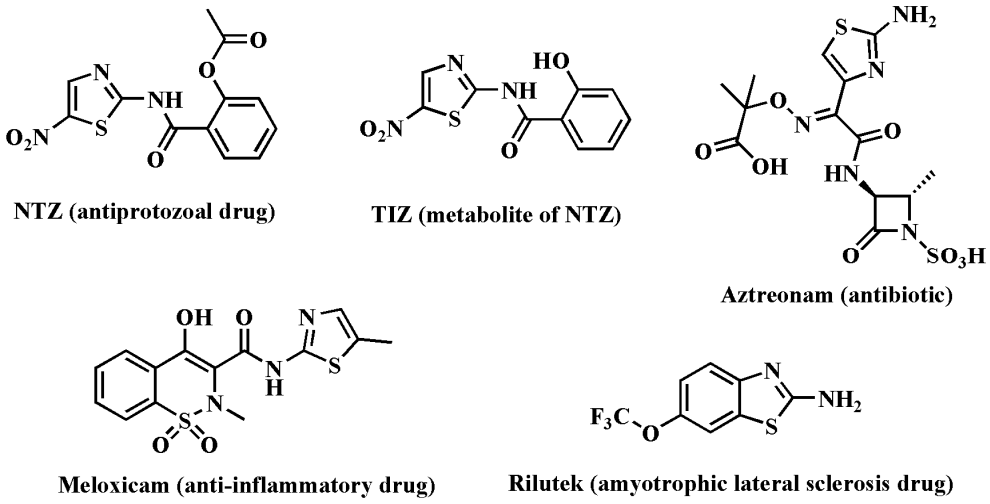


Figure 5.1 Drugs currently in use based on 2-aminothiazole skeleton

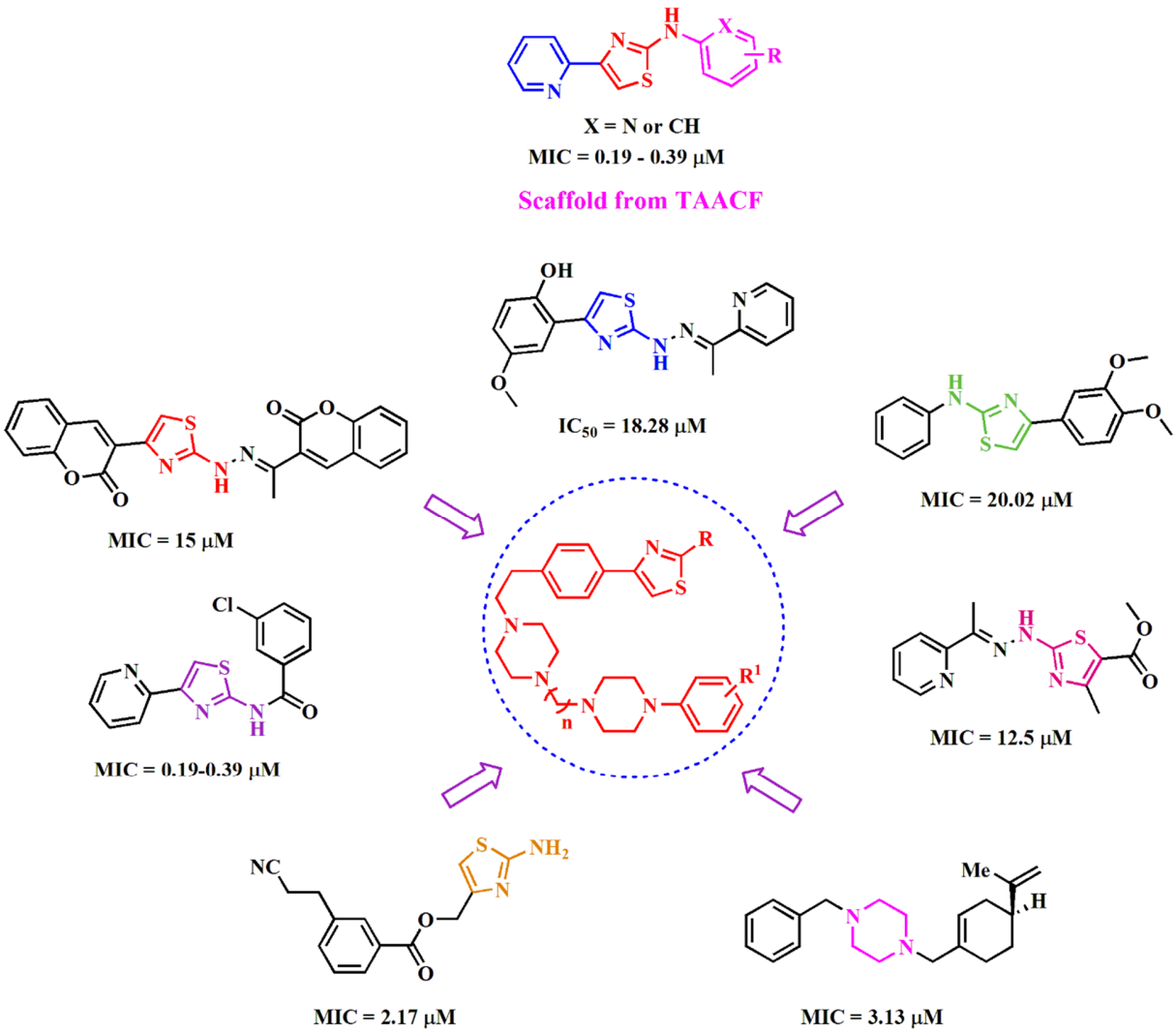


Figure 5.2 Scaffold from TAACF high-throughput screening campaign and design strategy to achieve title compounds.

In addition, Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) and the National Institutes of Health, Molecular Libraries Screening Centres Network disclosed encouraging results from an MTB whole-cell high-throughput assay and identified aminothiazoles as an important core which is depicted in **figure 5.2** [6,10-12]. Few research groups synthesized various 2-(2-hydrazinyl)thiazole derivatives and reported good activity

against MTB $H_{37}Rv$ strain [13-15]. Similarly, Makam et al. reported 2-(2-hydrazinyl)thiazole derivatives which are structurally similar to thiolactomycin using rational hybrid approach with good anti-TB activity [16]. Sridhar et al. synthesized *N*^{1}-hydroxy-*N*-(4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide analogues which inhibit MTB methionine aminopeptidases [17]. Ranjith et al. synthesized (2-aminothiazol-4-yl)methyl 3-(2-cyanoethyl)benzoate and found to exhibit good anti-TB activity than INH against MTB $H_{37}Rv$, *Mycobacterium Smegmatis* (ATCC 19420), *Mycobacterium Fortuitum* (ATCC 19542) and MDR-TB strains [18]. Meissner et al. synthesized 3-bromo-*N*-(4-(pyridin-2-yl)thiazol-2-yl)benzamide derivatives which inhibit MTB $H_{37}Rv$ strain at 0.008 µg/mL [6]. Very recently, Pieroni et al. synthesized *N*-substituted-2-aminothiazole derivatives and clearly briefed the SAR emphasizing on the mandatory of aminomethyl group at second position of thiazole [19].

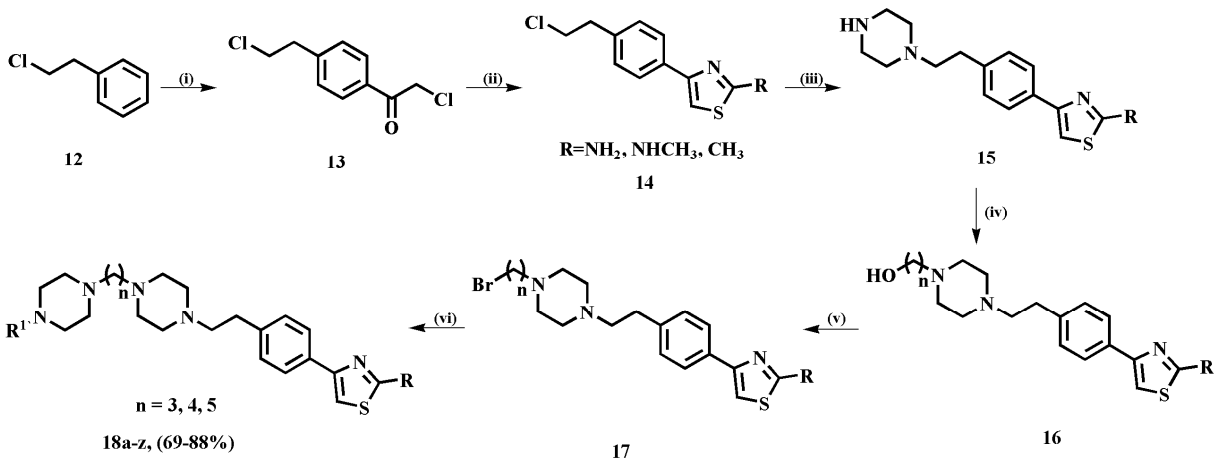
Piperazine linkers have made vast impact on drug discovery processes as they have ability to increase the lipophilicity of the molecule to a greater extent. Bogatcheva et al. synthesized various diamine linked moieties and reported good *in vitro* and *in vivo* anti-tubercular activity against MTB $H_{37}Rv$ strain [20]. Huang et al. synthesized 2-methylbenzothiazoles which inhibited the growth of MTB $H_{37}Rv$ strain at 1.4 µM [21].

With this collective information and emphasizing on molecular hybridization approach [22-24] we drew a synthetic stratagem (**figure 5.2**) to fit all these imperative pharmacophoric groups into one distinct scaffold and synthesized 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine analogues.

5.2 Results and Discussion

5.2.1 Chemistry

We synthesized 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine analogues as sketched in **scheme 5.1**. We adopted reported procedure with slight modification to prepare 4-(4-(2-chloroethyl)phenyl)-2-substitutedthiazole (**14**) [25], then **14** was coupled with anhydrous piperazine in DMF at 100 °C for 3 h to give 1-(4-(2-substitutedthiazol-4-yl)phenethyl)piperazine (**15**). Compound 4-(4-(4-(2-substitutedthiazol-4-yl)phenethyl)piperazin-1-yl)alkan-1-ol (**16**) was obtained by coupling **15** with bromoalkanols in the presence of TEA and DMF at 100 °C for 3 h; further heating **16** at 120 °C with 40% hydrobromic acid (HBr) and tetrabutylammonium bromide (TBAB) for 2 h fetched 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(n-bromoalkyl)piperazine (**17**).



Scheme 5.1 Synthetic strategy to achieve title compounds

Reagents and conditions: (i) $ClCH_2COCl$ (1.2 eq), aluminium chloride (2 eq), DCM 0 ºC - RT, 1h (ii) $RCSNH_2$ (1 eq), acetone, reflux, 3h (iii) anhydrous piperazine (3 eq), TEA (1 eq),

potassium iodide (0.2 eq), DMF, 100 ºC, 3h (iv) bromoalkanols (1.2 eq), TEA (1.5 eq), DMF, 100 ºC, 3h (v) HBr (40% aqueous) (0.6 eq), TBAB (0.3 eq), neat, 120 ºC, 2h (vi) substituted piperazines (1.2 eq), TEA (2 eq), DMF, 120 ºC, 3h.

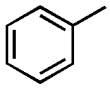
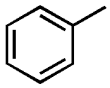
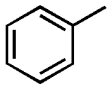
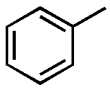
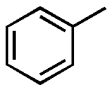
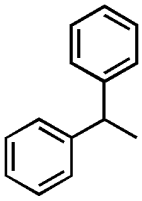
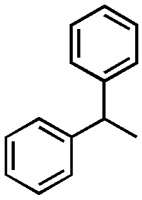
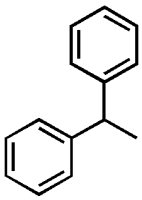
Subsequently various substituted piperazines were coupled with **17** at 120 °C for 3 h using TEA and DMF to achieve title compounds (**18a-z**). All the title compounds displayed multiplet in the range 2.75-2.95 ppm and 3.45-3.65 ppm corresponding to piperazine (-CH_2-) protons. Characteristic aromatic proton (C-5) of thiazole ring resonated in the range 6.50-6.75 ppm. Both analytical and spectral data (^{1}H NMR, ^{13}C NMR, IR and HRMS) of all the synthesized compounds were confirmed and employed further for their antimycobacterial evaluation.

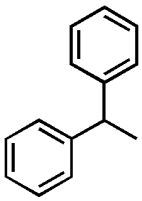
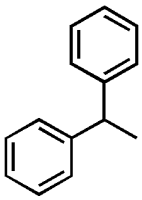
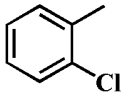
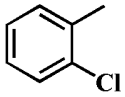
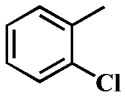
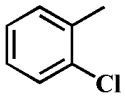
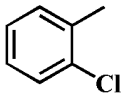
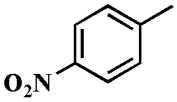
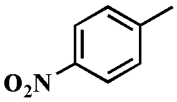
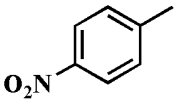
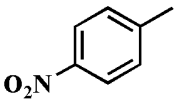
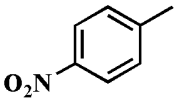
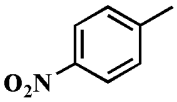
5.2.2 Antimycobacterial activity

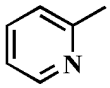
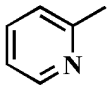
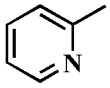
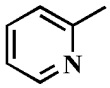
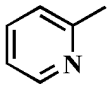
All the synthesized compounds were tested for their ability to inhibit the growth of MTB $H_{37}R_V$ strain by MABA. INH and Rifampicin were used as the positive drug standard. The *in vitro* antimycobacterial test results of final compounds are tabulated in **Table 5.1** as MIC and the activity ranged between 1.56 and >6.25 µg/mL. Compounds with MIC ≤6.25 µg/mL were further subjected to cytotoxicity studies. Amongst the series, compounds **18j**, **18p** and **18r** inhibit 99% growth of MTB H_{37}Rv strain at a concentration 6.25 µg/mL whereas compounds **18a**, **18f**, **18g**, **18n** and **18v** inhibit 99% growth of MTB H_{37}Rv strain at a concentration 3.125 µg/mL. Compound **18h** emerged as the most promising candidate by inhibiting 99% growth of MTB H_{37}Rv strain at a concentration 1.56 µg/mL. Among the synthesized compounds electron releasing groups like phenyl and benzhydryl piperazine with propyl and butyl linker respectively, bearing *N*-methylthiazol-2-amine backbone were found to be inactive (**18c** and **18i**, MIC = 50 µg/mL). Next, keeping propyl linker intact we synthesized 2-methylthiazole moiety bearing

phenyl piperazine (**18e**) which exhibited two fold increase in the activity (MIC = 25 µg/mL). Subsequently an alkyl chain was increased to fetch compound **18b** which exhibited similar

Table 5.1 Antimycobacterial activities of compound **18a-z** against MTB $H_{37}Rv$ strain

Compound No.	R^1	n	R	MIC (µg/mL) against MTB $H_{37}Rv$
18a		3	NH_2	3.125
18b		4	NH_2	25
18c		3	$NHCH_3$	50
18d		4	$NHCH_3$	12.5
18e		3	CH_3	25
18f		3	NH_2	3.125
18g		4	NH_2	3.125
18h		3	$NHCH_3$	1.56

18i		4	$NHCH_3$	50
18j		3	CH_3	6.25
18k		3	NH_2	50
18l		4	NH_2	50
18m		3	$NHCH_3$	12.5
18n		4	$NHCH_3$	3.125
18o		3	CH_3	50
18p		3	NH_2	6.25
18q		4	NH_2	12.5
18r		5	NH_2	6.25
18s		3	$NHCH_3$	12.5
18t		4	$NHCH_3$	50
18u		3	CH_3	50

18v		3	NH_2	3.125
18w		4	NH_2	12.5
18x		3	$NHCH_3$	12.5
18y		4	$NHCH_3$	25
18z		3	CH_3	50
Isoniazid				0.05
Rifampicin				0.1

activity (MIC = 25 µg/mL). With this increased activity, butyl link tailored to phenyl group and *N*-methylthiazol-2-amine backbone leading to **18d**, amplified the activity further by two fold (MIC = 12.5 µg/mL). Switching from aminothiazoles to 2-methylthiazole with benzhydryl-piperazine and propyl linker exhibited significant activity (**18j**, MIC = 6.25 µg/mL). Notably, compounds with phenyl and benzhydryl piperazine tagged 2-aminothiazoles with propyl and butyl linker respectively exhibited good activity (**18a**, **18f** and **18g**, MIC = 3.125 µg/mL). Eventually, compound **18h** comprising *N*-methylthiazol-2-amine attached to benzhydryl piperazine through propyl link emerged as a potential candidate by inhibiting the MTB $H_{37}Rv$ strain at 1.56 µg/mL. In general, compounds with electron withdrawing groups were found to be inactive except compound **18n** bearing *N*-methylthiazol-2-amine attached to 2-chlorophenyl piperazine through butyl link which exhibited good activity (MIC = 3.125 µg/mL). On the other hand, employing 1-(2-pyridyl)piperazine led to compound **18v** consisting 2-aminothiazole

backbone through propyl linker which exhibited good activity (MIC = 3.125 µg/mL). Structure-activity relationship evidently indicates the necessity of 2-aminomethyl group at 2nd position of thiazole which might involve in hydrogen bonding to a greater extent to exhibit good activity.

5.2.3 Cytotoxicity assay

RAW 264.7 cell lines derived from BALB/c mice maintain many of the properties of macrophages including nitric oxide production and phagocytosis. As MTB generally reside and multiply inside the macrophages, so to carry out the cytotoxicity against RAW 264.7 cells is imperative to scrutinize the selectivity of compounds against MTB rather than host macrophage. Hence the active compounds **18a**, **18f**, **18g**, **18h**, **18j**, **18n**, **18p**, **18r** and **18v** were subjected to *in vitro* cytotoxicity studies by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The IC_{50} and selectivity index (SI) values are tabulated in **Table 5.2**. The SI is defined as the ratio of the IC_{50} to the MIC (MTB H_{37}Rv strain). SI value >10 indicates that the compound may be considered for further studies. Tested active compounds exhibited percentage growth inhibitions of RAW 264.7 cells in the range of 31.76-46.43% at a concentration 50 µg/mL. Among the tested compounds, **18j**, **18p** and **18r** exhibited toxicity (SI $\geq$ 7) and compounds **18a**, **18f**, **18g**, **18n** and **18v** were non-toxic (SI $\geq$ 15). Nevertheless, most active compound **18h** (SI $\geq$ 30) was found to be safe for further studies and emerged as most potent molecule of the series. These encouraging results imply the suitability of compounds in an endeavour to attain lead molecule for further drug development.

Table 5.2 IC_{50} (µg/mL) and selectivity index (SI) values of active compounds against mouse macrophage cell lines (RAW264.7)

Compound No.	MIC (µg/mL) in MTB $H_{37}Rv$	% cell inhibition at 50 µg/mL	IC_{50} (µg/mL) approximation	SI value IC_{50}/MIC
18a	3.125	35.62	>50	>15
18f	3.125	38.42	>50	>15
18g	3.125	46.43	>50	>15
18h	1.56	31.76	>50	>30
18j	6.25	42.8	>50	>7
18n	3.125	40.4	>50	>15
18p	6.25	36.46	>50	>7
18r	6.25	44.6	>50	>7
18v	3.125	38.1	>50	>15

[a]Minimum inhibitory concentration against $H_{37}Rv$ strain of MTB (µg/mL).
[b]Measurement of cytotoxicity in RAW264.7 cells: 50% inhibitory concentrations µg/mL).
[c]Selectivity index: IC_{50} in RAW264.7 cells/MIC against MTB.

5.3 Conclusion

In conclusion, our preliminary anti-tubercular screening results earmarked us to fine-tune the architecture of 2-aminothiazole framework to fetch desired pharmacophoric features that might be explored to evaluate various drug resistant forms of TB. We disclose that integrating *N*-methyl-4-(4-(2-(piperazin-1-yl)ethyl)phenyl)thiazol-2-amine to benzhydryl piperazine through propyl linker emerged as a prospective candidate by inhibiting the MTB $H_{37}Rv$ strain at concentration 1.56 µg/mL (**18h**).

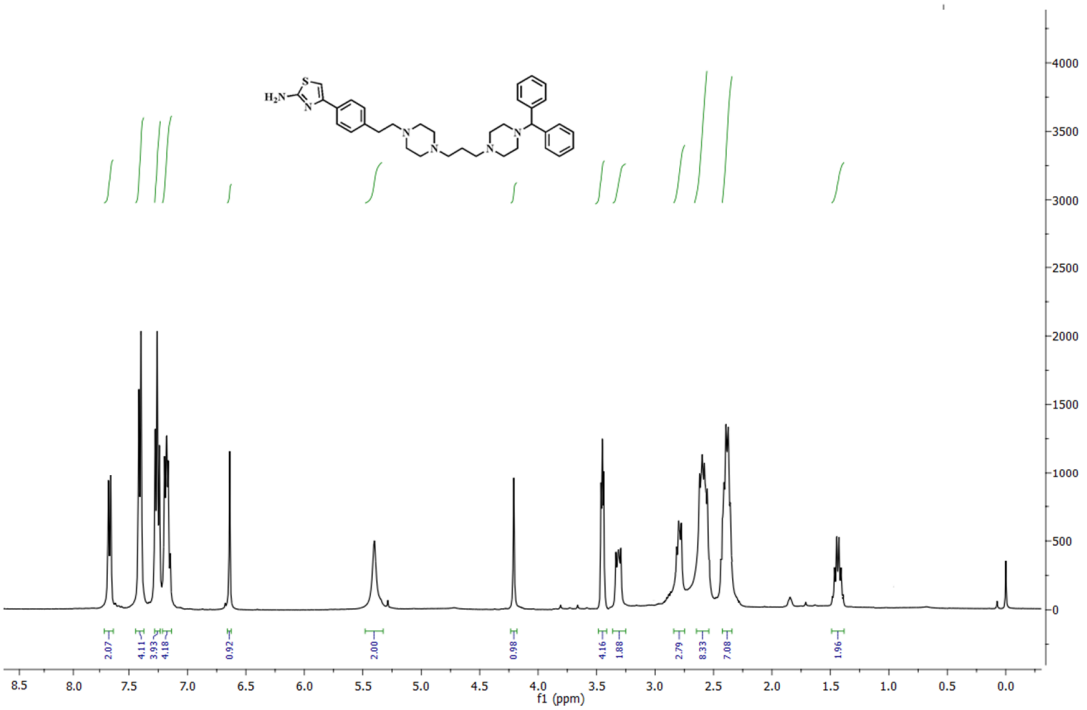
5.4 Experimental section

5.4.1 Materials and methods

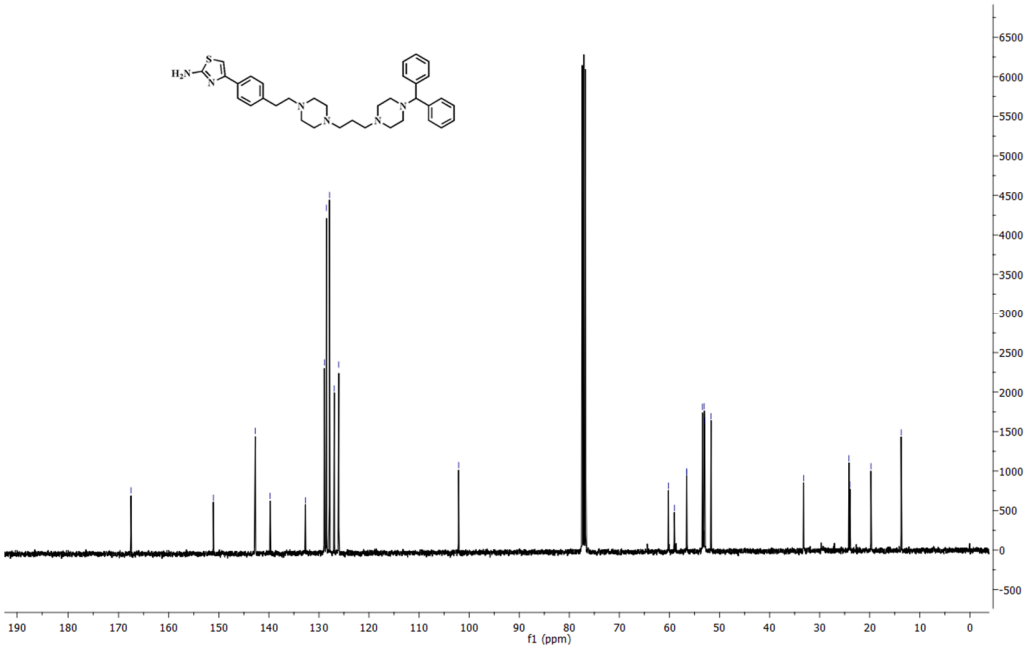
Chemicals and solvents were procured from commercial sources and are analytically pure. Thin-layer chromatography (TLC) was carried out on aluminium-supported silica gel plates (Merck 60 F254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 230-400 mesh). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded at 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) are given in ppm. 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. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Elemental analysis was carried out on Elementar (vario MICRO cube, Hanau, Germany).

Synthesis of title compounds (18a-z)

To a solution of **6** (1 eq) in DMF and TEA (2 eq) was added substituted piperazine (1.2 eq) and heated at 120 °C for 3 h. Completion of the reaction was monitored by TLC using 20% MeOH in DCM as mobile phase. After the reaction was complete, DMF was evaporated under vacuo and added 5 mL of water. Compound was extracted using EtOAc (3 x 5 mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous sodium sulphate and evaporated in vacuo. The crude residue was purified by column chromatography (MeOH/DCM) to get compounds **18a-z**.



^{1}H NMR spectrum of compound **18f**



^{13}C NMR spectrum of compound **18f**

4-(4-(2-(4-(3-(4-phenylpiperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18a)

Off white solid (83%); mp 184-186 °C; IR (KBr, cm^{-1}) 3386, 3023, 2886, 1643, 1592, 1346, 864. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 7.18 Hz, 2H), 7.33 (d, *J* = 7.3 Hz, 2H), 7.21-7.16 (m, 2H), 6.89-6.81 (m, 3H), 6.68 (s, 1H), 5.52 (b, 2H), 3.58-3.51 (m, 4H), 3.37-3.31 (m, 2H), 2.86-2.79 (m, 4H), 2.63-2.55 (m, 8H), 2.42-2.36 (m, 6H), 2.17-2.08 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.61, 152.86, 150.23, 143.65, 133.87, 128.91, 128.12, 127.53, 124.05, 102.11, 100.67, 60.86, 58.92, 56.11, 55.81, 51.34, 32.12, 24.61, 20.19, 12.87. HRMS: (ESI m/z) for $C_{28}H_{39}N_6S$ calculated: 491.2957, found: 491.2983 $(M+H)^+$.

4-(4-(2-(4-(4-(4-phenylpiperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18b)

Beige solid (86%); mp 192-194 °C; IR (KBr, cm^{-1}) 3398, 3042, 2912, 1678, 1611, 1356, 878. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.18 Hz, 2H), 7.29 (d, *J* = 7.3 Hz, 2H), 7.19-7.06 (m, 2H), 6.86-6.79 (m, 3H), 6.64 (s, 1H), 5.49 (b, 2H), 3.53-3.44 (m, 4H), 3.27-3.18 (m, 2H), 2.76-2.68 (m, 4H), 2.58-2.49 (m, 8H), 2.36-2.28 (m, 6H), 1.96-1.88 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.89, 151.83, 150.04, 142.92, 133.09, 128.15, 127.26, 126.28, 124.82, 101.69, 100.07, 59.37, 58.39, 56.81, 55.13, 51.61, 30.72, 25.56, 21.63, 12.06, 11.42. HRMS: (ESI m/z) for $C_{29}H_{41}N_6S$ calculated: 505.3113, found: 505.3133 $(M+H)^+$.

***N*-methyl-4-(4-(2-(4-(3-(4-phenylpiperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)thiazol -2-amine (18c)**

Beige solid (79%); mp 178-180 °C; IR (KBr, cm^{-1}) 3394, 3038, 2891, 1639, 1597, 1359, 872. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.18 Hz, 2H), 7.35 (d, *J* = 7.3 Hz, 2H), 7.27-7.19 (m, 2H), 6.78-6.69 (m, 3H), 6.59 (s, 1H), 4.96 (b, 1H), 3.61-3.54 (m, 4H), 2.92-2.81 (m, 4H), 2.81-2.77 (m, 8H), 2.47 (s, 3H), 2.38-2.31 (m, 8H), 1.79-1.72 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.83, 151.62, 150.02, 144.68, 134.62, 129.26, 128.77, 127.62, 124.33, 102.15, 100.99, 61.83, 59.09, 56.44, 55.22, 51.88, 34.29, 32.14, 24.90, 20.48,11.94. HRMS: (ESI m/z) for $C_{29}H_{41}N_6S$ calculated: 505.3113, found: 505.3129 $(M+H)^+$.

***N*-methyl-4-(4-(2-(4-(4-(4-phenylpiperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18d)**

Pale yellow solid (81%); mp 191-193 °C; IR (KBr, cm^{-1}) 3372, 3041, 2875, 1657, 1588, 1376, 859. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.18 Hz, 2H), 7.42 (d, *J* = 7.3 Hz, 2H), 7.28-7.21 (m, 2H), 6.83-6.77 (m, 3H), 6.61 (s, 1H), 4.82 (b, 1H), 3.58-3.51 (m, 4H), 2.87-2.79 (m, 4H), 2.72-2.67 (m, 8H), 2.51 (s, 3H), 2.37-2.28 (m, 8H), 2.11-2.08 (m, 2H), 1.92-1.88 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.12, 152.41, 151.83, 145.22, 135.21, 128.16, 127.72, 126.15, 124.11, 101.83, 100.54, 61.13, 59.85, 56.05, 55.24, 52.47, 34.25, 32.63, 25.35, 21.37, 12.88, 11.26. HRMS: (ESI m/z) for $C_{30}H_{43}N_6S$ calculated: 519.3270, found: 519.3251 $(M+H)^+$.

1-(4-(2-methylthiazol-4-yl)phenethyl)-4-(3-(4-phenylpiperazin-1-yl)propyl)piperazine (18e)

Off white solid (75%); mp 201-203 °C; IR (KBr, cm^{-1}) 3037, 2871, 1653, 1586, 1367, 891. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 7.18 Hz, 2H), 7.45 (d, *J* = 7.3 Hz, 2H), 7.32-7.25 (m,

2H), 6.73-6.64 (m, 3H), 6.58 (s, 1H), 3.58-3.46 (m, 6H), 2.87 (t, *J* = 4.7 Hz, 2H), 2.72-2.63 (m, 6H), 2.51 (t, *J* = 3.8 Hz, 2H), 2.41 (s, 3H), 2.34-2.29 (m, 8H), 1.86-1.81 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.51, 153.83, 151.53, 146.66, 134.73, 128.16, 127.14, 126.62, 124.94, 102.35, 100.26, 60.83, 58.45, 56.11, 55.35, 52.17, 35.22, 31.62, 26.62, 21.88, 11.83. HRMS: (ESI m/z) for $C_{29}H_{40}N_5S$ calculated: 490.3004, found: 490.3023 $(M+H)^+$.

4-(4-(2-(4-(3-(4-benzhydrylpiperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18f)

White solid (72%); mp 168-170 °C; IR (KBr, cm^{-1}) 3399, 3067, 2896, 1669, 1588, 1355, 876. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (t, *J* = 11.9 Hz, 2H), 7.41 (d, *J* = 7.4 Hz, 4H), 7.26 (t, *J* = 7.1 Hz, 4H), 7.17-7.14 (dd, *J* = 12.2, 7.4 Hz, 4H), 6.66 (s, 1H), 5.34 (b, 2H), 4.21 (s, 1H), 3.42-3.36 (m, 4H), 3.29-3.18 (m, 2H), 2.89-2.81 (m, 4H), 2.58-2.51 (m, 8H), 2.37-2.28 (m, 6H), 1.49-1.42 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.54, 151.09, 142.70, 139.75, 132.70, 128.91, 128.49, 127.90, 126.94, 126.05, 102.11, 60.21, 59.03, 56.55, 56.52, 53.40, 53.06, 52.95, 51.68, 33.21, 24.15, 23.96, 19.76, 13.72. HRMS: (ESI m/z) for $C_{35}H_{45}N_6S$ calculated: 581.3426, found: 581.3418 $(M+H)^+$.

4-(4-(2-(4-(4-(4-benzhydrylpiperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18g)

Beige solid (81%); mp 175-177 °C; IR (KBr, cm^{-1}) 3412, 3039, 2871, 1655, 1578, 1372, 877. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.69 (t, *J* = 11.4 Hz, 2H), 7.39 (d, *J* = 7.1 Hz, 4H), 7.29 (t, *J* = 6.9 Hz, 4H), 7.19-7.16 (dd, *J* = 11.9, 7.6 Hz, 4H), 6.68 (s, 1H), 5.33 (b, 2H), 4.18 (s, 1H), 3.48-3.38 (m, 4H), 3.36-3.27 (m, 2H), 2.92-2.83 (m, 4H), 2.51-2.44 (m, 8H), 2.46-2.32 (m, 6H), 1.92-

1.84 (m, 2H), 1.55-1.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.41, 151.01, 141.66, 138.99, 133.45, 127.78, 127.32, 126.67, 126.23, 126.01, 104.43, 62.77, 60.96, 57.49, 56.83, 54.97, 53.76, 51.87, 50.54, 34.39, 25.75, 23.54, 18.44, 14.56, 13.96. HRMS: (ESI m/z) for $C_{36}H_{47}N_6S$ calculated: 595.3583, found: 595.3577 $(M+H)^+$.

4-(4-(2-(4-(3-(4-benzhydrylpiperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)-*N*-methyl thiazol-2-amine (18h)

Pale yellow solid (88%); mp 193-195 °C; IR (KBr, cm^{-1}) 3397, 3037, 2872, 1653, 1588, 1361, 882. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68-7.65 (t, *J* = 11.4 Hz, 2H), 7.41 (d, *J* = 7.1 Hz, 4H), 7.26 (t, *J* = 6.9 Hz, 4H), 7.12-7.09 (dd, *J* = 11.9, 7.6 Hz, 4H), 6.65 (s, 1H), 5.23 (b, 1H), 4.19 (s, 1H), 3.45-3.39 (m, 4H), 3.36-3.30 (m, 2H), 2.88-2.79 (m, 4H), 2.79 (s, 3H), 2.71-2.67 (m, 3H), 2.62-2.54 (m, 8H), 2.38-2.32 (m, 7H), 1.62-1.58 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.01, 152.67, 141.87, 139.34, 132.12, 128.26, 127.23, 126.77, 125.23, 124.22, 101.82, 61.82, 58.62, 56.66, 55.52, 53.85, 53.06, 52.24, 51.23, 32.77, 29.45 25.72, 24.15, 19.24, 12.72. HRMS: (ESI m/z) for $C_{36}H_{47}N_6S$ calculated: 595.3583, found: 595.3571 $(M+H)^+$.

4-(4-(2-(4-(4-(4-benzhydrylpiperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)-*N*-methyl thiazol-2-amine (18i)

Off white solid (77%); mp 211-212 °C; IR (KBr, cm^{-1}) 3393, 3053, 2882, 1667, 1584, 1367, 889. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67-7.64 (t, *J* = 11.8 Hz, 2H), 7.43 (d, *J* = 7.6 Hz, 4H), 7.33-7.31 (t, *J* = 7.2 Hz, 4H), 7.26-7.23 (dd, *J* = 11.7, 7.9 Hz, 4H), 6.75 (s, 1H), 5.23 (b, 1H), 4.13 (s, 1H), 3.47-3.37 (m, 2H), 2.99-2.91 (m, 4H), 2.63-2.52 (m, 8H), 2.49 (s, 3H), 2.39-2.31 (m, 8H), 2.13-2.09 (m, 2H), 1.75-1.69 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.22, 151.61, 141.42, 138.26,

132.77, 128.15, 127.72, 126.15, 125.01, 124.31, 102.72, 60.62, 58.15, 56.26, 55.15, 53.63, 53.12, 52.67, 50.83, 32.21, 29.12, 25.53, 24.11, 20.28, 12.12, 11.68. HRMS: (ESI m/z) for $C_{37}H_{49}N_6S$ calculated: 609.3739, found: 609.3721 $(M+H)^+$.

1-(4-(2-methylthiazol-4-yl)phenethyl)-4-(3-(4-benzhydrylpiperazin-1-yl)propyl)piperazine (18j)

Brown solid (76%); mp 208-210 °C; IR (KBr, cm^{-1}) 3079, 2934, 1683, 1612, 1378, 883. ^1H NMR (400 MHz, $CDCl_3$) δ 7.65-7.62 (t, *J* = 11.9 Hz, 2H), 7.41 (d, *J* = 7.4 Hz, 4H), 7.26-7.23 (t, *J* = 7.1 Hz, 4H), 7.17-7.14 (dd, *J* = 12.2, 7.4 Hz, 4H), 6.66 (s, 1H), 4.32 (s, 1H), 3.52-3.47 (m, 2H), 2.98-2.92 (m, 4H), 2.75 (s, 3H), 2.61-2.53 (m, 8H), 2.36-2.28 (m, 8H), 1.71-1.66 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.12, 154.86, 143.15, 140.82, 133.34, 129.24, 128.15, 127.26, 126.72, 125.74, 104.89, 61.83, 60.73, 57.83, 57.32, 54.26, 53.54, 52.62, 51.15, 34.63, 25.65 24.72, 21.56, 19.58, 14.22. HRMS: (ESI m/z) for $C_{36}H_{46}N_5S$ calculated: 580.3474, found: 580.3466 $(M+H)^+$.

4-(4-(2-(4-(3-(4-(2-chlorophenyl)piperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18k)

Pale yellow solid (85%); mp 152-154 °C; IR (KBr, cm^{-1}) 3398, 3071, 2905, 1687, 1565, 1405, 877, 763. ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.31-7.28 (dd, *J* = 14.4, 3.8 Hz, 3H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 7.7 Hz, 1H), 6.67 (s, 1H), 5.25 (b, 2H), 3.39-3.29 (m, 2H), 3.22-3.17 (m, 4H), 2.78-2.71 (m, 2H), 2.52-2.44 (m, 12H), 2.36-2.23 (m, 4H), 1.83-1.73 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.03, 158.61, 149.36, 140.71, 133.88, 132.16, 129.82, 128.16, 127.11, 126.47, 123.71, 121.81, 110.88, 61.39, 59.27, 57.72,

55.87, 53.83, 52.04, 50.82, 31.61, 24.76, 18.72, 16.16, 13.67. HRMS: (ESI m/z) for $C_{28}H_{38}ClN_6S$ calculated: 525.2567, found: 525.2558 $(M+H)^+$.

4-(4-(2-(4-(4-(4-(2-chlorophenyl)piperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18l)

Off white solid (71%); mp 148-150 °C; IR (KBr, cm^{-1}) 3382, 3062, 2876, 1676, 1544, 1356, 868, 775. ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.37-7.34 (dd, *J* = 14.4, 3.8 Hz, 3H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 6.68 (s, 1H), 5.27 (b, 2H), 3.42-3.33 (m, 2H), 3.27-3.13 (m, 4H), 2.86-2.72 (m, 2H), 2.61-2.52 (m, 12H), 2.35-2.22 (m, 4H), 1.76-1.62 (m, 2H), 1.47-1.35 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.16, 157.96, 149.13, 141.67, 134.67, 132.84, 129.67, 128.71, 127.82, 126.87, 123.22, 121.64, 110.11, 60.92, 58.61, 57.24, 55.15, 53.72, 52.55, 50.26, 32.82, 25.72, 19.26, 15.17, 12.72, 11.67. HRMS: (ESI m/z) for $C_{29}H_{40}ClN_6S$ calculated: 539.2724, found: 539.2745 $(M+H)^+$.

4-(4-(2-(4-(3-(4-(2-chlorophenyl)piperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)-*N*-methylthiazol-2-amine (18m)

Brown solid (78%); mp 141-143 °C; IR (KBr, cm^{-1}) 3375, 3084, 2862, 1685, 1588, 1332, 856, 761. ^1H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.27-7.23 (dd, *J* = 14.4, 3.8 Hz, 3H), 7.07 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 7.7 Hz, 1H), 6.63 (s, 1H), 5.17 (b, 1H), 3.42-3.34 (m, 2H), 3.29-3.21 (m, 4H), 2.66-2.58 (m, 2H), 2.45-2.39 (m, 12H), 2.36-2.22 (s, 3H), 2.29-2.24 (m, 4H), 1.72-1.67 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.23, 157.42, 148.55, 139.78, 132.11, 131.35, 129.15, 128.66, 127.75, 126.34, 122.86, 120.58, 111.25, 60.36,

59.70, 57.58, 54.44, 52.68, 51.95, 50.26, 32.36, 24.97, 23.32, 18.37, 16.88, 13.37. HRMS: (ESI m/z) for $C_{29}H_{40}ClN_6S$ calculated: 539.2724, found: 539.2728 $(M+H)^+$.

4-(4-(2-(4-(4-(4-(2-chlorophenyl)piperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)-*N*-methyl thiazol-2-amine (18n)

Pale yellow solid (80%); mp 145-147 °C; IR (KBr, cm^{-1}) 3382, 3105, 2877, 1676, 1592, 1355, 859, 776. ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.1 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.32-7.28 (dd, *J* = 14.4, 3.8 Hz, 3H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.61 (s, 1H), 5.22 (b, 1H), 3.39-3.26 (m, 2H), 3.14-2.93 (m, 4H), 2.78-2.71 (m, 2H), 2.59-2.41 (m, 12H), 2.49 (s, 3H), 2.38-2.22 (m, 4H), 2.05-1.91 (m, 2H), 1.55-1.47 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.16, 156.82, 149.62, 139.16, 133.21, 131.83, 129.93, 128.41, 127.18, 126.67, 123.26, 120.11, 110.82, 60.43, 58.34, 56.05, 53.83, 52.22, 51.43, 50.34, 33.66, 24.57, 23.26, 18.82, 16.23, 13.71, 11.78. HRMS: (ESI m/z) for $C_{30}H_{42}ClN_6S$ calculated: 553.2880, found: 553.2859 $(M+H)^+$.

1-(4-(2-methylthiazol-4-yl)phenethyl)-4-(3-(4-(2-chlorophenyl)piperazin-1-yl)propyl) piperazine (18o)

Beige solid (74%); mp 156-158 °C; IR (KBr, cm^{-1}) 3092, 2903, 1696, 1592, 1348, 867, 784. ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 1H), 7.25-7.21 (dd, *J* = 14.4, 3.8 Hz, 3H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 7.7 Hz, 1H), 6.63 (s, 1H), 3.33-3.25 (m, 2H), 3.16-2.99 (m, 4H), 2.86-2.78 (m, 2H), 2.71 (s, 3H), 2.65-2.54 (m, 12H), 2.48-2.36 (m, 4H), 1.46-1.37 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.81, 155.04, 149.09, 139.78, 132.64, 130.63, 129.04, 128.77, 127.62, 126.42, 123.79, 120.43, 111.80, 59.95, 56.35, 53.30, 52.82,

52.53, 50.91, 33.09, 24.24, 23.54, 19.80, 19.32, 13.71. HRMS: (ESI m/z) for $C_{29}H_{39}ClN_5S$ calculated: 524.2615, found: 524.2633 $(M+H)^+$.

4-(4-(2-(4-(3-(4-(4-nitrophenyl)piperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18p)

Yellow solid (69%); mp 186-188 °C; IR (KBr, cm^{-1}) 3376, 3048, 2865, 1656, 1569, 1535, 1378, 1327, 886, 843. ^1H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 9.3 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 3.0 Hz, 2H), 6.77 (d, *J* = 9.3 Hz, 2H), 6.61 (s, 1H), 5.53 (b, 2H), 3.57-3.44 (m, 6H), 2.78-2.71 (m, 2H), 2.66-2.59 (m, 6H), 2.51-2.49 (m, 6H), 2.45-2.23 (m, 4H), 1.53-1.47 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.32, 158.63, 155.37, 138.93, 136.38, 131.70, 129.37, 127.12, 126.49, 111.44, 110.49, 60.48, 58.70, 57.25, 56.42, 54.74, 52.26, 44.96, 33.06, 25.70, 22.47, 18.58, 10.64. HRMS: (ESI m/z) for $C_{28}H_{38}N_7O_2S$ calculated: 536.2808, found: 536.2811 $(M+H)^+$.

4-(4-(2-(4-(4-(4-(4-nitrophenyl)piperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18q)

Yellow solid (71%); mp. 193-195 °C; IR (KBr, cm^{-1}) 3378, 3095, 2878, 1667, 1587, 1546, 1374, 1332, 889, 852. ^1H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 9.3 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 3.0 Hz, 2H), 6.72 (d, *J* = 9.3 Hz, 2H), 6.62 (s, 1H), 5.52 (b, 2H), 3.65-3.56 (m, 6H), 2.71-2.68 (m, 2H), 2.63-2.58 (m, 6H), 2.52-2.49 (m, 6H), 2.43-2.38 (m, 4H), 1.74-1.67 (m, 2H), 1.43-1.37 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.89, 157.75, 154.78, 138.37, 136.70, 131.36, 129.48, 127.70, 126.33, 110.23, 109.60, 60.37, 58.26, 57.27, 56.18, 54.29, 52.80, 44.22,

33.25, 24.32, 23.72, 22.19, 19.49, 11.93. HRMS: (ESI m/z) for $C_{29}H_{40}N_7O_2S$ calculated: 550.2964, found: 550.2954 $(M+H)^+$.

4-(4-(2-(4-(5-(4-(4-nitrophenyl)piperazin-1-yl)pentyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18r)

Yellow solid (79%); mp 187-189 °C; IR (KBr, cm^{-1}) 3395, 3078, 2865, 1673, 1583, 1554, 1389, 1345, 878, 844. ^1H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 9.3 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 3.0 Hz, 2H), 6.69 (d, *J* = 9.3 Hz, 2H), 6.59 (s, 1H), 5.46 (b, 2H), 3.57-3.45 (m, 6H), 2.65-2.58 (m, 2H), 2.53-2.49 (m, 6H), 2.46-2.43 (m, 6H), 2.41-2.23 (m, 4H), 1.61-1.55 (m, 4H), 1.36-1.25 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.62, 157.38, 155.21, 137.69, 136.75, 131.87, 130.43, 127.26, 126.19, 110.61, 110.04, 61.72, 58.83, 57.86, 56.47, 54.38, 52.23, 45.52, 33.69, 25.68, 24.32, 23.37, 22.93, 19.72, 12.26. HRMS: (ESI m/z) for $C_{30}H_{42}N_7O_2S$ calculated: 564.3121, found: 564.3143 $(M+H)^+$.

***N*-methyl-4-(4-(2-(4-(3-(4-(4-nitrophenyl)piperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl) thiazol-2-amine (18s)**

Yellow solid (73%); mp 181-183 °C; IR (KBr, cm^{-1}) 3383, 3084, 2871, 1682, 1576, 1543, 1377, 1338, 876, 841. ^1H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 9.3 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 3.0 Hz, 2H), 6.75 (d, *J* = 9.3 Hz, 2H), 6.61 (s, 1H), 5.22 (s, 1H), 3.61-3.54 (m, 6H), 2.74-2.70 (m, 2H), 2.67-2.63 (m, 6H), 2.60-2.58 (m, 6H), 2.56 (s, 3H), 2.47-2.41 (m, 4H), 1.67-1.59 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.24, 156.16, 154.27, 138.77, 136.37, 131.48, 130.55, 128.74, 127.33, 111.47, 110.37, 60.16, 59.72, 57.23, 56.16, 54.77, 52.73, 45.48, 33.77,

29.58, 24.37, 23.25, 19.32, 11.69. HRMS: (ESI m/z) for $C_{29}H_{40}N_7O_2S$ calculated: 550.2964, found: 550.2951 $(M+H)^+$.

_N_-methyl-4-(4-(2-(4-(4-(4-(4-nitrophenyl)piperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl) thiazol-2-amine (18t)

Yellow solid (78%); mp 189-191 °C; IR (KBr, cm^{-1}) 3367, 3074, 2885, 1678, 1584, 1539, 1388, 1341, 892, 852. ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 9.3 Hz, 2H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 3.0 Hz, 2H), 6.93 (d, *J* = 9.3 Hz, 2H), 6.63 (s, 1H), 5.26 (s, 1H), 3.42-3.31 (m, 6H), 2.79-2.72 (m, 2H), 2.69-2.61 (m, 6H), 2.56-2.54 (m, 6H), 2.52 (s, 3H), 2.43-2.39 (m, 4H), 2.01-1.92 (m, 2H), 1.46-1.39 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.01, 156.96, 154.25, 139.43, 137.97, 132.94, 130.66, 127.27, 126.16, 112.28, 110.78, 61.82, 59.83, 57.7 4, 56.46, 53.27, 52.49, 45.72, 33.28, 29.39, 24.82, 23.29, 19.77, 13.23, 11.62. HRMS: (ESI m/z) for $C_{30}H_{42}N_7O_2S$ calculated: 564.3121, found: 564.3139 $(M+H)^+$.

1-(4-(2-methylthiazol-4-yl)phenethyl)-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)propyl) piperazine (18u)

Pale yellow solid (73%); mp 177-179 °C; IR (KBr, cm^{-1}) 3086, 2887, 1667, 1576, 1532, 1378, 1345, 887, 849. ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 9.3 Hz, 2H), 7.78 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 3.0 Hz, 2H), 6.81 (d, *J* = 9.3 Hz, 2H), 6.61 (s, 1H), 3.49-3.40 (m, 6H), 2.81-2.79 (m, 2H), 2.76 (s, 3H), 2.63-2.61 (m, 6H), 2.58-2.52 (m, 6H), 2.44-2.39 (m, 4H), 1.46-1.39 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.84, 155.02, 154.85, 139.89, 138.39, 132.61, 129.04, 126.41, 125.96, 112.61, 111.81, 60.06, 59.15, 56.42, 56.31, 53.01, 52.68, 46.99, 33.19, 24.23, 23.94,

19.80, 19.34,13.71. HRMS: (ESI m/z) for $C_{29}H_{39}N_6O_2S$ calculated: 535.2855, found: 535.2862 $(M+H)^+$.

4-(4-(2-(4-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18v)

White solid (84%); mp 175-177 °C; IR (KBr, cm^{-1}) 3382, 3037, 2876, 1665, 1589, 1356, 876. ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (dd, *J* = 4.9, 1.2 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.47-7.45 (ddd, *J* = 8.8, 7.2, 2.0 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 2H), 6.83-6.49 (m, 3H), 5.45 (s, 2H), 3.59-3.51 (m, 4H), 2.81 (dd, *J* = 9.9, 6.3 Hz, 2H), 2.74-2.59 (m, 6H), 2.59-2.48 (m, 8H), 2.47-2.39 (m, 4H),1.82-1.68 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.49, 159.53, 151.14, 147.93, 139.75, 137.48, 132.71, 128.92, 126.05, 113.31, 107.10, 102.11, 60.22, 56.67, 56.60, 53.12, 53.05, 52.96, 45.17, 33.24, 24.14. HRMS: (ESI m/z) for $C_{27}H_{38}N_7S$ calculated: 492.2909, found: 492.2912 $(M+H)^+$.

4-(4-(2-(4-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl)thiazol-2-amine (18w)

Off white solid (77%); mp 182-184 °C; IR (KBr, cm^{-1}) 3376, 3048, 2891, 1672, 1568, 1377, 882. ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (dd, *J* = 4.7, 1.1 Hz, 1H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.61-7.59 (ddd, *J* = 8.1, 6.9, 1.8 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 2H), 6.72-6.56 (m, 3H), 5.39 (s, 2H), 3.55-3.47 (m, 4H), 2.77 (dd, *J* = 9.7, 6.6 Hz, 2H), 2.68-2.54 (m, 6H), 2.49-2.36 (m, 8H), 2.33-2.26 (m, 4H), 1.91-1.72 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.22, 160.23, 152.64, 149.17, 140.88, 139.25, 133.66, 129.34, 127.61, 114.43, 108.19, 103.51, 61.83, 57.37, 56.11, 53.84, 52.83, 51.24,

46.24, 34.77, 26.49, 24.92. HRMS: (ESI m/z) for $C_{28}H_{40}N_7S$ calculated: 506.3066, found: 506.3059 $(M+H)^+$.

***N*-methyl-4-(4-(2-(4-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)piperazin-1-yl)ethyl)phenyl) thiazol-2-amine (18x)**

Beige solid (71%); mp 179-181 °C; IR (KBr, cm^{-1}) 3389, 3053, 2882, 1691, 1584, 1372, 873. ^1H NMR (400 MHz, $CDCl_3$) δ 8.62 (dd, *J* = 4.7, 1.5 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.49-7.47 (ddd, *J* = 8.1, 6.7, 1.7 Hz, 1H), 7.34 (d, *J* = 8.7 Hz, 2H), 6.92-6.53 (m, 3H), 5.37 (s, 1H), 3.54-3.43 (m, 4H), 2.93 (dd, *J* = 9.7, 5.8 Hz, 2H), 2.72-2.63 (m, 6H), 2.53-2.45 (m, 8H), 2.38-2.27 (m, 7H), 1.78-1.66 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.89, 158.95, 152.44, 148.21, 138.62, 138.11, 133.84, 129.15, 127.84, 114.37, 108.26, 103.44, 59.51, 57.72, 55.94, 53.32, 52.73, 51.22, 46.53, 34.35, 29.83, 26.62. HRMS: (ESI m/z) for $C_{28}H_{40}N_7S$ calculated: 506.3066, found: 506.3041 $(M+H)^+$.

***N*-methyl-4-(4-(2-(4-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)piperazin-1-yl)ethyl)phenyl) thiazol-2-amine (18y)**

Off white solid (73%); mp 191-193 °C; IR (KBr, cm^{-1}) 3384, 3077, 2889, 1678, 1557, 1392, 897. ^1H NMR (400 MHz, $CDCl_3$) δ 8.35 (dd, *J* = 5.1, 1.6 Hz, 1H), 7.82 (d, *J* = 8.1 Hz, 2H), 7.72-7.70 (ddd, *J* = 7.6, 6.2, 1.3 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 2H), 6.61-6.54 (m, 3H), 5.41 (s, 1H), 3.53-3.41 (m, 4H), 2.68 (dd, *J* = 9.2, 6.7 Hz, 2H), 2.55-2.47 (m, 6H), 2.43-2.37 (m, 8H), 2.29-2.23 (m, 7H), 1.92-1.86 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.44, 161.56, 151.62, 150.67, 141.34, 140.87, 134.12, 130.73, 128.81, 115.99, 109.44, 104.33, 60.52, 56.84, 55.73, 54.24, 53.45, 51.73,

46.87, 35.41, 27.16, 25.84, 24.21. HRMS: (ESI m/z) for $C_{29}H_{42}N_7S$ calculated: 520.3222, found: 520.3234 $(M+H)^+$.

1-(4-(2-methylthiazol-4-yl)phenethyl)-4-(3-(4-(pyridin-2-yl)piperazin-1-l)propyl)piperazine (18z)

Off white solid (79%); mp 180-182 °C; IR (KBr, cm^{-1}) 3088, 2862, 1661, 1579, 1383, 875. ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (dd, *J* = 4.9, 1.2 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.47-7.45 (ddd, *J* = 8.8, 7.2, 2.0 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 2H), 6.83-6.49 (m, 3H), 5.45 (s, 2H), 3.59-3.51 (m, 4H), 2.81 (dd, *J* = 9.9, 6.3 Hz, 2H), 2.74-2.59 (m, 6H), 2.59-2.48 (m, 8H), 2.47-2.39 (m, 4H), 1.82-1.68 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.49, 159.53, 151.14, 147.93, 139.75, 137.48, 132.71, 128.92, 126.05, 113.31, 107.10, 102.11, 60.22, 56.67, 56.60, 53.12, 53.05, 52.96, 45.17, 33.24, 24.14. HRMS: (ESI m/z) for $C_{28}H_{39}N_6S$ calculated: 491.2957, found: 491.2967 $(M+H)^+$.

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Part-B

Chapter VI

Introduction

Introduction

Chapter 6

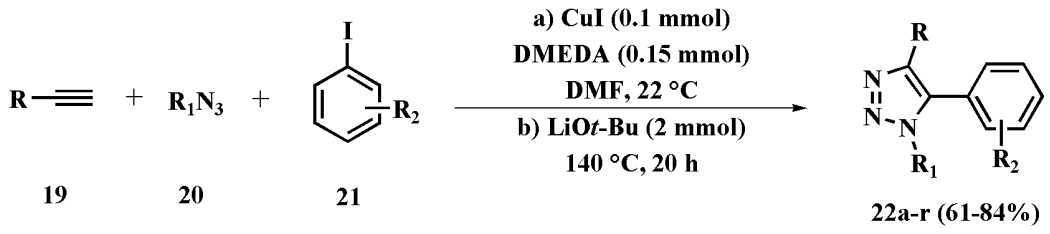
6.1 Introduction

Multi-component reactions (MCRs) turned out to be a potentially more efficient synthetic strategy, which attributes renaissance in organic synthesis to afford sustainable and diversity oriented functionalized molecules. MCRs alleviate synthetic efficiency undeniably to reduce cost, energy, processing time and waste which are instinctive drawbacks involved in the wearying multistep synthesis [1]. Ever since the impetus of rapid development in molecular hybridization strategy, MCRs spearhead facile access of privileged heterocyclic small molecule libraries of agrochemical and drug discovery process which fundamentally rely on complexity of the drug-like molecules to study in depth structure-activity relationships for lead optimisation [2].

Complimentarily copper (I) catalyzed azide-alkyne cycloaddition (CuAAC) gained remarkable attention with momentous traction among scientists owing to their operationally effortless step- and atom-economy reactions to attain diversely engineered molecules with prospective applicability to a great length in materials science and medicinal chemistry [3]. In addition to traditional purpose of copper salts in CuAAC, ligand endorsed Ullmann type cross-coupling approaches for C-C, C-N, C-O, C-S and C-Se bond formation surged the research solving some of the quandaries to a new horizon alongside inter/intra-molecular oxidative C–H/N–H cross-coupling reaction. Thus, the possibility of establishing *N*-heterocyclic core tethering diverse and complex skeleton was realized in an expedient manner [4].

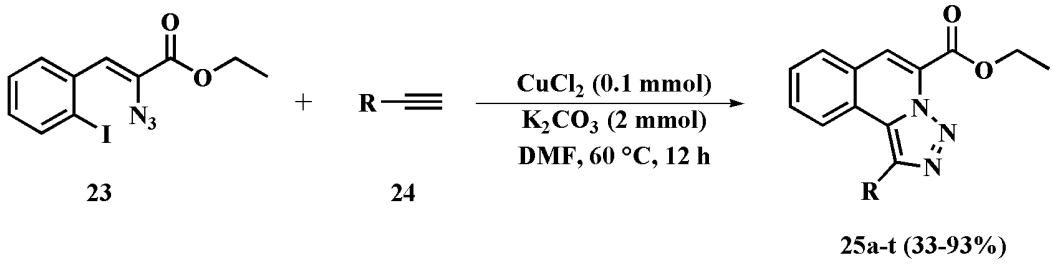
Copper-catalysed reactions are developed to construct C-C and C-heteroatom bonds possessing broad synthetic utility many decades ago [5]. These processes involved stoichiometric amount of copper catalysts and harsh reaction conditions to deliver the product [6]. Copper catalysis have received less attention as palladium catalysis was widely employed. Palladium catalysts are expensive, toxic and suffered yield loss when the substrates had polar functional groups [7]. Many researchers revisited and made significant improvements employing catalytic quantities of [Cu]-species with considerably milder reaction conditions to construct C-C and C-X (X = N, O, S, P) bonds [8,9]. Relatively, copper catalysed reactions are found to be more attractive than palladium catalysed reactions due to the greater extent of functional group tolerance [10]. Most of the copper salts are inexpensive, less toxic compared to palladium catalysts and are complementary to greener conditions [11].

Revitalizing by the catalytic property of copper, the researchers began to scrutinize its multi-catalysis to achieve more than one distinct catalytic process in one-pot. In such operations various research groups have revealed dual catalytic behaviour of copper [12], Ackermann et al. approached one-pot fashion to synthesize trisubstituted 1,2,3-triazoles (**22**) employing acetylenes, substituted azides and various iodoarenes at 22 °C. Initially, the click reaction occurs using CuI (0.01 mmol), DMEDA (0.015 mmol) in DMF to afford 1,4-substituted-1,2,3-triazole, followed by C_5-*H* arylation using *t*-BuOLi (2 mmol) as base at 140 °C for 20 h [12c].



Scheme 6.1

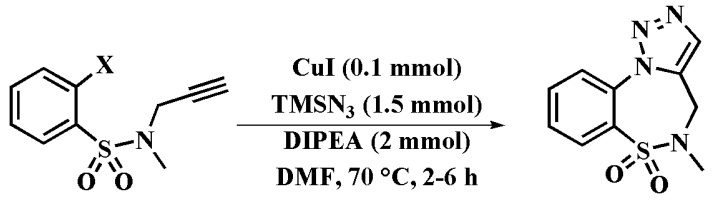
Hu et al. reacted compound **23** with various acetylenes at 60 °C using $CuCl_2$ (0.010 mmol) and K_2CO_3 (2 mmol) in DMF for 12 h to give [1,2,3]triazolo[5,1-*a*]isoquinolines (**25**) in good yield. The reactions proceed via click reaction followed by intramolecular C_5-*H* arylation [12h].



23 **24** **25a-t (33-93%)**

Scheme 6.2

Barange et al. synthesized 1,2,3-triazolothiadiazepine-1,1-dioxide from compound **26** using CuI (0.01 mmol), $TMSN_3$ (1.5 mmol), DIPEA (2 mmol) in DMF at 70 °C for 2-6 h. $TMSN_3$ being the azide source reacts with acetylinic part of compound **26** followed by intramolecular Ullmann type coupling to form N-C bond [12b].



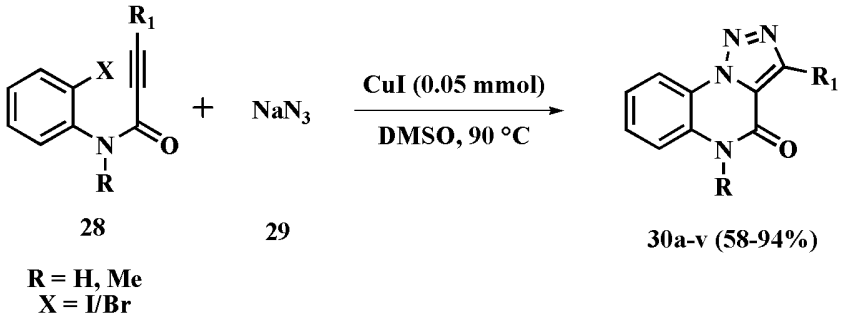
26

X = I/Br

27a-j (50-80%)

Scheme 6.3

Following the similar fashion Yan et al. synthesized [1,2,3]triazolo[1,5-*a*]quinoxalin-4(5*H*)-ones in good yields employing NaN_3 as the azide source [12g].

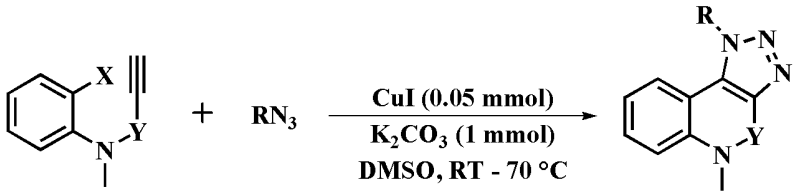


28 **29** **30a-v (58-94%)**

R = H, Me
X = I/Br

Scheme 6.4

With the successful Ullmann type coupling of *N*-(2-haloaryl)propiolamides with NaN_3, same research group in another paper reported copper-catalyzed tandem reaction of *N*-(2-haloaryl)propiolamides with various organic azides for the synthesis of 1*H*-[1,2,3]triazolo[4,5-*c*]quinolin-4(5*H*)-ones derivatives (**33**) in good yields [12d].

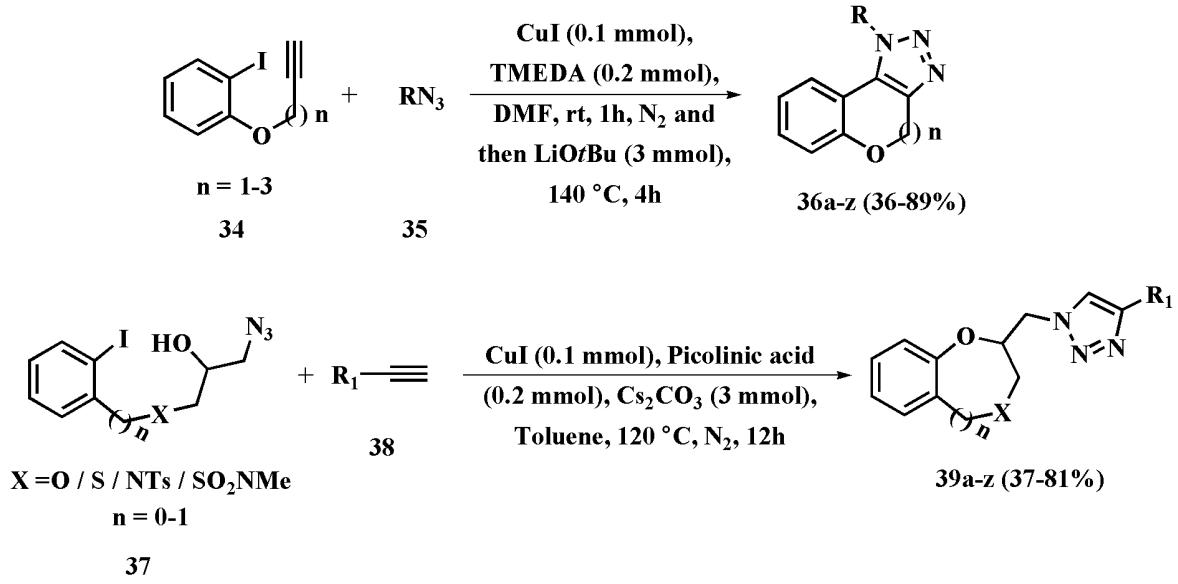


31 **32** **33a-r (51-94%)**

X = I/Br
Y = CO / CH_2

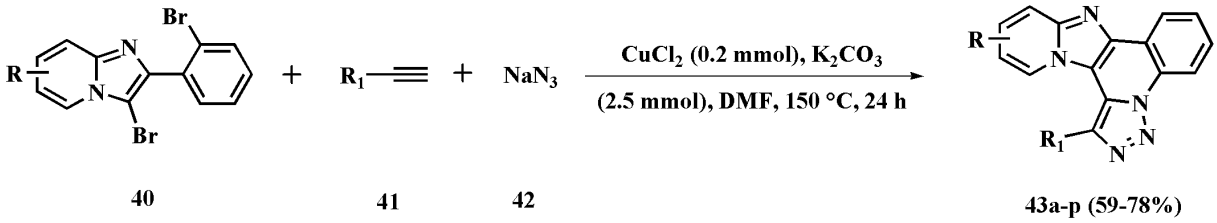
Scheme 6.5

Swamy et al. executed click reaction to afford 1,2,3-triazole framework followed by intramolecular C_5–*H* arylation to generate [6,6]-, [6,7]-, [6,8]-, and [6,9] ring-fused triazoles. In another paper the same group reported the synthesis of benzo-condensed six or seven membered rings containing two hetero-atoms attached to a 1,2,3-triazole framework [12f,13].



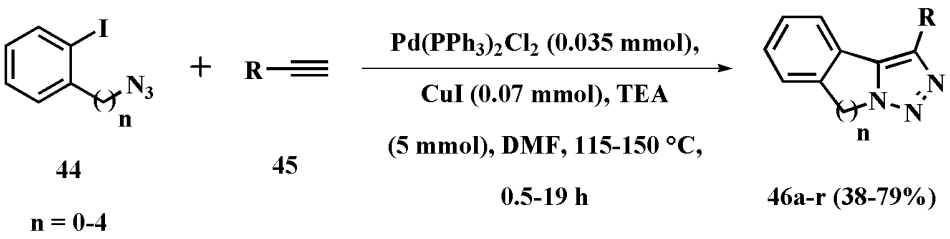
Scheme 6.6

Pericherla et al. employed MCR for regioselective synthesis of 1,2,3-triazole-fused imidazo[1,2-*a*]pyridines. Various 3-Bromo-2-(2-bromophenyl)imidazo[1,2-*a*]pyridines (**40**), substituted acetylenes (**41**) and sodium azide (**42**) in the presence of $CuCl_2$ (0.02 mmol), K_2CO_3 (2.5 mmol) in DMF were heated at 150 °C for 24 h to get the final compounds [12a].



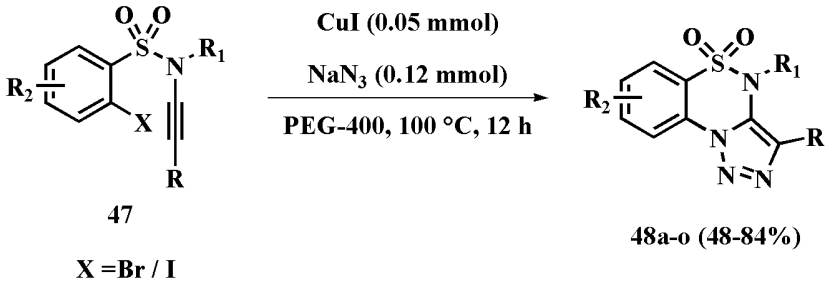
Scheme 6.7

Brahma et al. developed palladium-copper catalysed reactions in one-pot to synthesize 1,2,3-triazole fused with five-, six-, seven- and eight-membered benzoheterocycles, including isoindoline, tetrahydroisoquinoline, benzoazepine and benzoazocine [12i].



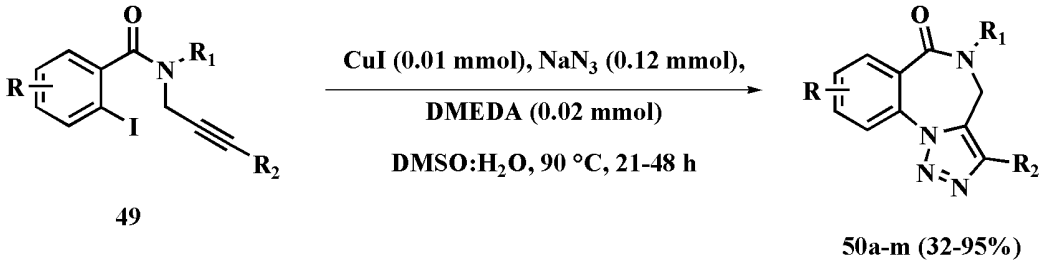
Scheme 6.8

Siva Reddy et al. reported the utility of functionalised ynamides to develop an efficient one-pot approach for the synthesis of fused triazolo 1,2,4-benzothiadiazine-1,1-dioxide derivatives in the presence of CuI (0.05 mmol) using PEG-400 as the solvent medium at 100 °C for 12 h [12e].



Scheme 6.9

Chen et al. synthesized 3-substituted [1,2,3]triazolo[1,5-*a*][1,4]benzodiazepin-6(5*H*)-ones from various 2-iodo-*N*-phenyl-*N*-(3-phenylprop-2-ynyl)benzamides (**49**) and sodium azide in the presence of CuI (0.01 mmol), DMEDA (0.02mol) in DMSO:H_2O (9:1) at 90 °C for 21-48 h [12j].



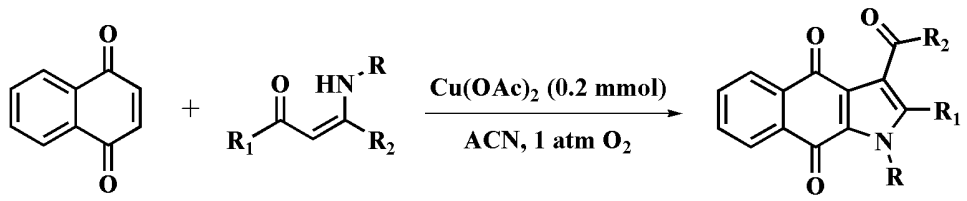
49

50a-m (32-95%)

Scheme 6.10

Having explored the traditional utility of copper salts for click reaction followed by C_5-*H* arylation or N-C bond formation to construct 1,2,3-triazole fused heterocycles, many research group explored copper salts as lewis acid and oxidative catalysts.

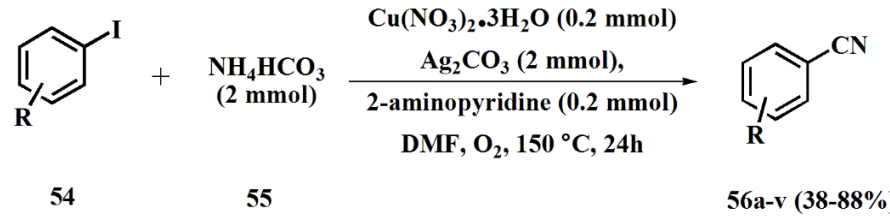
Sun et al. synthesized benzo[*f*]indole-4,9-diones in which copper was a Lewis acid and oxidative catalyst [14].



51 52 53a-z (71-96%)

Scheme 6.11

Pawar et al. illustrated the role of copper both in the generation of cyanide units from DMF–ammonia and in the cyanation of aryl halides [15].



Scheme 6.12

Overwhelmed with these beneficial information we were interested in conjoining MCR and the dual role of copper catalyst to synthesize versatile molecules. Results and discussions are elaborated in the next chapter.

6.2 References

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Chapter VII

Multicomponent cascade reaction: dual role of copper in the synthesis of 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinoline and their photophysical studies

Chapter 7

Multicomponent cascade reaction: dual role of copper in the synthesis of 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinoline and their photophysical studies

7.1 Introduction

To implement MCR emphasizing dual role of the copper catalyst, we decided to synthesize 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinolines as these two heterocyclic moieties have ample applications. In particular, substituted benzimidazoles and their azino-fused cyclic derivatives have drawn considerable attention of medicinal and organic chemists due to wide range of biological activities [1,2]. Benzo-annulated analogues such as benzimidazo[2,1-*a*]isoquinolines, benzimidazo[1,2-*c*]quinazolines, or benzimidazo[3,2-*a*]quinolinium hydrochloride salts showed interesting biological properties [3]. In particular, benzimidazo[1,2-*a*]quinolines are known for medicinal importance displaying wide biological activities such as antitumor, antiproliferative and DNA binding agents, and in material sciences as chemosensors, fluorescent probes for DNA detection, fluorescent optical whiteners and disperse dyes [4,5].

On the other hand, 1,2,3-triazoles have been postulated to generate a nonclassic bioisostere of amide bond [6,7] which is an essential feature to increase binding affinity towards receptor. Much attention has been paid to click chemistry arena due to easily accessible novel complex and diversified heterocycles using environmentally benign and relatively inexpensive catalyst and starting materials. 1,2,3-Triazoles are identified as antifungal, anticancer, antiprotozoal, antiphotoaging, HIV type-1 protease inhibitor, β-lactum antibiotic, histone deacetylase inhibitor

[8-12] etc. Some of the drugs based on 1,2,3-triazoles which are currently in use are depicted in **figure 7.1**.

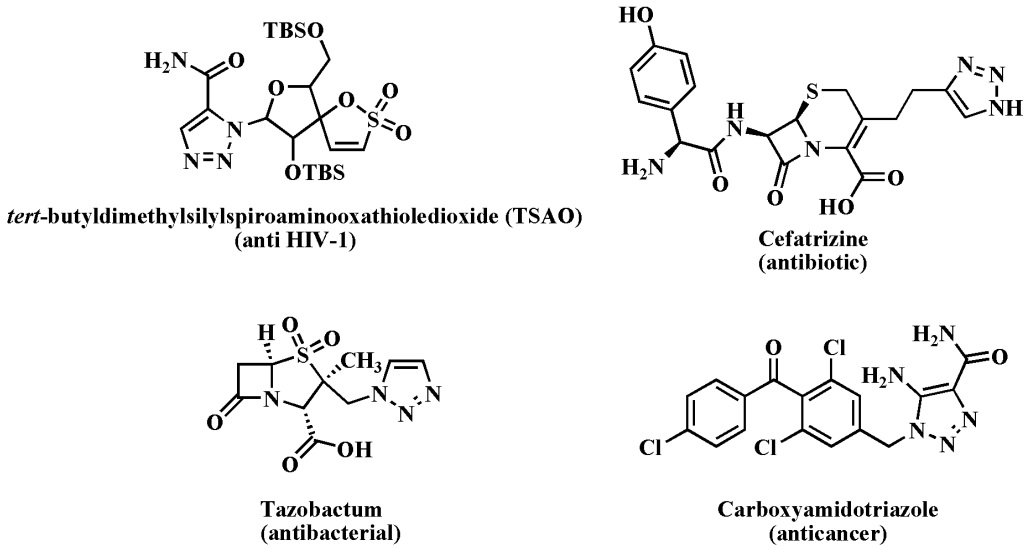
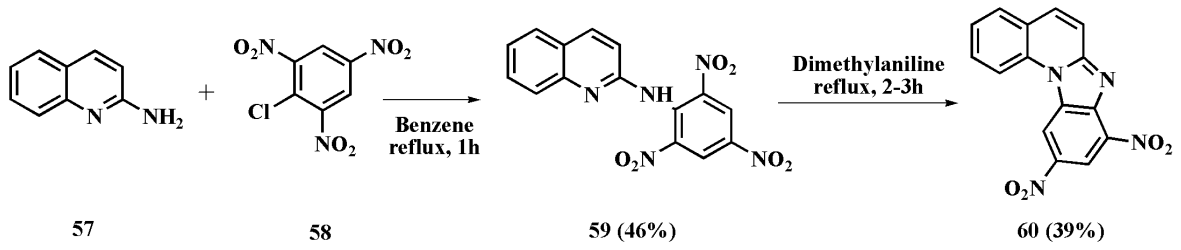


Figure 7.1 Drugs currently in use based on 1,2,3-triazole skeleton

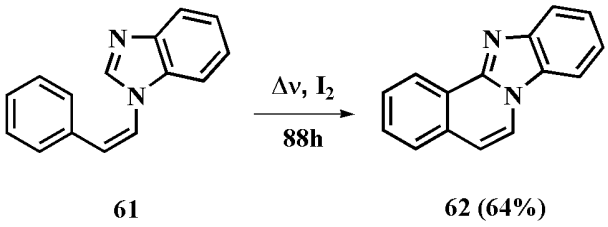
7.2 Literature review

Copious synthetic strategies are available to accomplish this aza-fused heterocycles. For example Morgan et al. were the first to prepare benzimidazo[1,2-*a*]quinoline framework by the classical method from 2-aminoquinoline and picryl chloride [13].



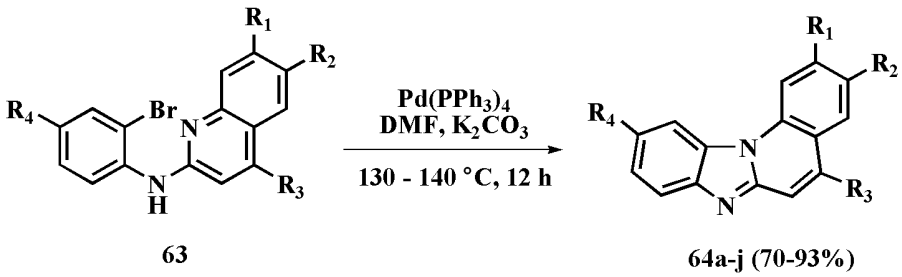
Scheme 7.1 Morgan et al. synthetic method for benzimidazo[1,2-*a*]quinoline.

Cooper et al. reported the synthesis of imidazo[1,2-*a*]quinoline, where photochemical dehydrocyclization takes place by UV irradiation of methanolic solution of styryl-2-benzimidazole [14].



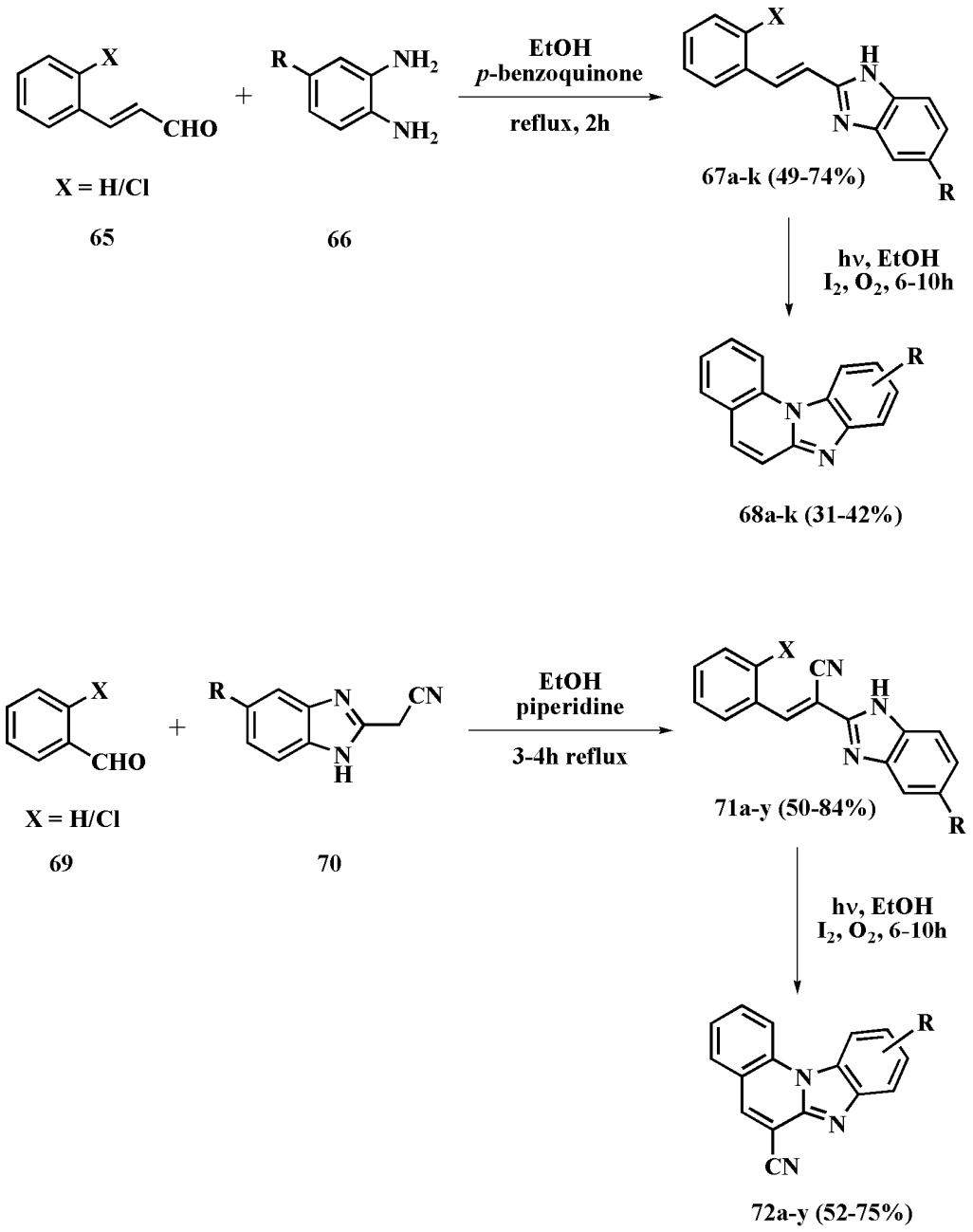
Scheme 7.2 Cooper et al. synthetic method for benzimidazo[1,2-*a*]quinoline.

Venkatesh et al. have developed palladium-catalyzed synthetic method for preparing substituted benzimidazo[1,2-*a*]quinolines starting from phenyl amino substituted quinolines [15].



Scheme 7.3 Venkatesh et al. synthetic method for benzimidazo[1,2-*a*]quinoline.

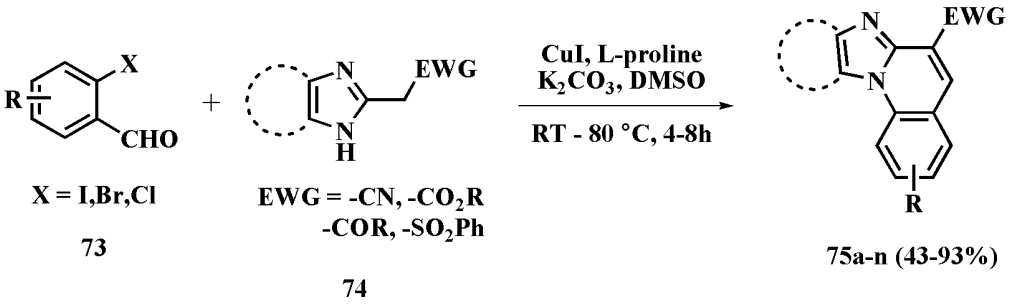
Hranjec et al. have described photochemical dehydrocyclization strategy to synthesize substituted benzimidazo[1,2-*a*]quinolines in moderate yield over two steps [16].



Scheme 7.4 Hranjec et al. synthetic method for benzimidazo[1,2-*a*]quinoline.

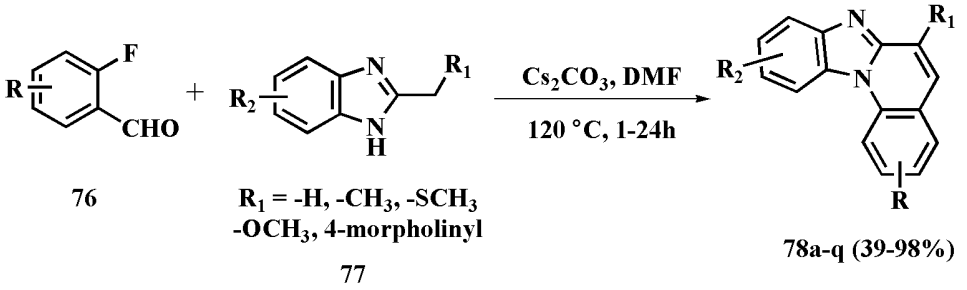
Cai et al. employed less toxic, inexpensive and abundant copper catalyst which was found to be superior to all other previous methods. The reaction proceeds via Ullmann type/Knoevenagel

coupling to synthesize benzimidazo[1,2-*a*]quinolines starting from substituted *ortho*-halo benzaldehydes and electron-withdrawing substituted methylene benzimidazoles [17].



Scheme 7.5 Cai et al. synthetic method for benzimidazo[1,2-*a*]quinoline

Kato et al. developed metal free protocol and synthesized benzimidazo[1,2-*a*]quinolines via S_NAr coupling utilizing substituted *ortho*-fluoro benzaldehydes and substituted methylene benzimidazoles in moderate to good yield [18].



Scheme 7.6 Kato et al. synthetic method for benzimidazo[1,2-*a*]quinoline

In many instances the existing reports involve multi-step synthesis, pre-functionalised synthons to facilitate the key step Knoevenagel condensation and photochemical dehydrocyclization strategy which dramatically suffer from poor yields. As a result, MCR emphasizing the dual role of copper to synthesize 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinolines and exploration of their photophysical properties in the current study was undertaken.

7.3 Results and Discussion

We herein demonstrated the synthesis of such molecules starting from a non-activated readily prepared precursor like 1,2,3-triazole, which was formed *in situ*, and studied its influence in activating a methylene link to undergo Knoevenagel condensation. Hitherto, only a couple of published examples existed based on a click and activate approach to synthesize 1,2,3-triazole tethered 2-quinolinone and iminochromene [19]. Furthermore, advancement of this unique arena is not explored much, so this ingenuity opens the window for chemists to develop 1,2,3-triazole anchored fused heterocycles. Thus, in one synthetic operation we envisioned a three component domino process via click reaction/N–C bond formation/Knoevenagel condensation (**figure 7.2**).

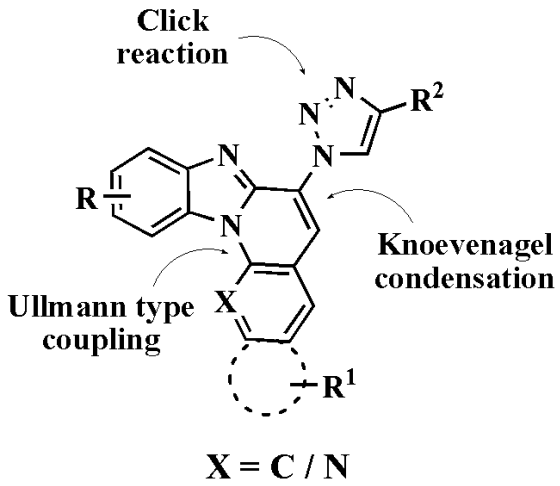
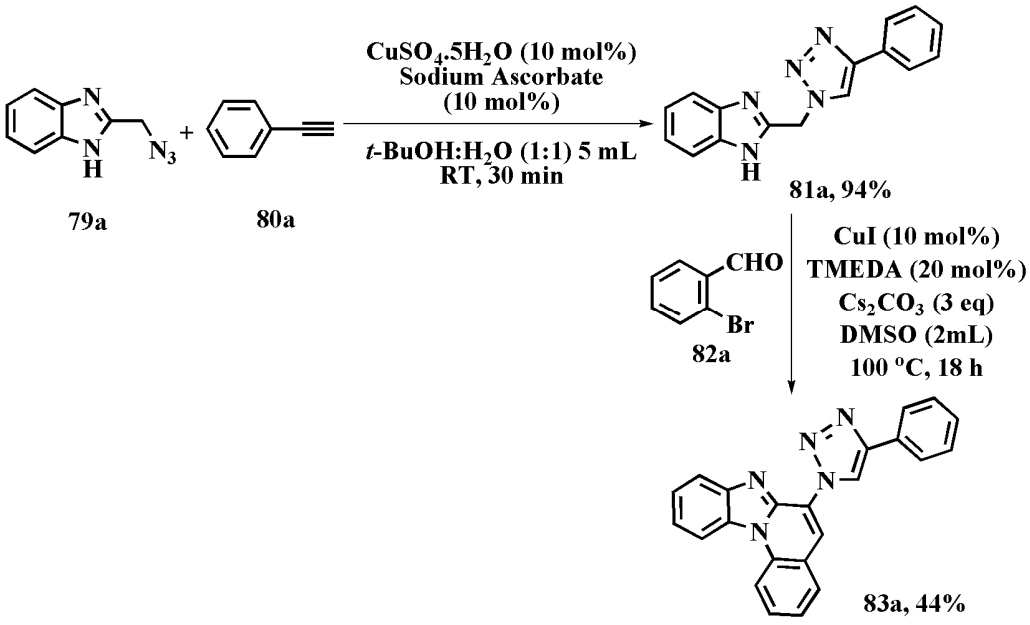


Figure 7.2 Design strategy of 1,2,3-triazole appended benzimidazo[1,2-*a*]quinoline.

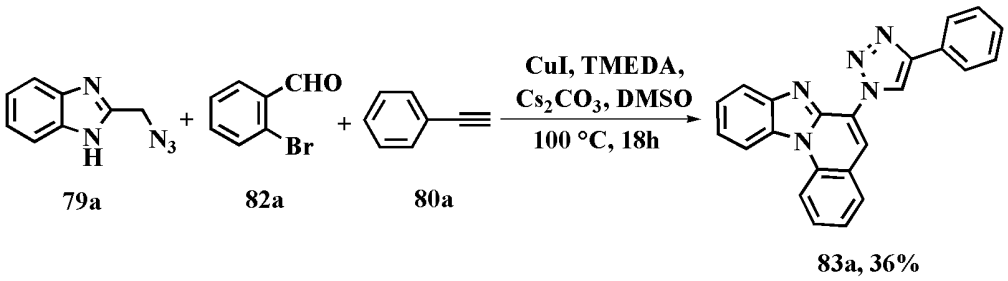
At the outset, we began to assemble 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinolines in a two-step process as depicted in **scheme 7.7**. The reactants 2-(azidomethyl)-1*H*-benzo[*d*]imidazole (**79a**) and phenylacetylene (**80a**) were subjected to [3 + 2] cycloaddition in the presence of $CuSO_4.5H_2O$, sodium ascorbate in *t*-BuOH : H_2O (1 : 1) at RT for 30 min. After completion of the reaction, it was diluted with water and the resultant solid was filtered to yield

2-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-1*H*-benzo[*d*]imidazole (**81a**) in 94% yield. The structure of product **81a** was confirmed from its analytical data. In the ^{1}H NMR spectrum, methylene (–CH_2–) bridged protons resonated at δ 5.89 ppm and a singlet at 8.05 ppm resonated due to the C_5–*H* of 1,2,3-triazole. Later, we treated **81a** with 2-bromobenzaldehyde (**82a**) in the presence of CuI (0.1 mmol)/TMEDA (0.2 mmol)/Cs_2CO_3 (3 mmol) in DMSO at 100 °C for 18 h under N_2 atmosphere. To our delight, we isolated the desired product **83a** in 44% yield. The structure of **83a** was characterized by spectral data. In the ^{1}H NMR spectrum, disappearance of a singlet at δ 5.89 ppm due to methylene (–CH_2–) bridged protons and a broad peak at δ 12.2 ppm due to benzimidazole N–H were noticed. Also a singlet appeared at δ 9.84 ppm for highly deshielded C_5–*H* along with other protons at their respective positions amply warranted the product formation. With this appealing and heartening result in hand, we focused on how to alleviate this sequential transformation.



Scheme 7.7 Synthesis of target molecule over two step procedure.

As a next step, we steered our attempts to carry out MCR by employing 2-(azidomethyl)-1*H*-benzo[*d*]imidazole (**79a**), 2-bromobenzaldehyde (**82a**) and phenylacetylene (**80a**) as condensation partners. These three components were mixed with CuI (0.1 mmol)/TMEDA (0.2 mmol)/Cs_2CO_3 (3 mmol) in DMSO and were heated at 100 °C for 18 h under N_2 atmosphere. After purification, we isolated the desired product in 36% yield and in this process overall four new bonds (1C–C and 3C–N) were formed in a single step (**scheme 7.8**). With this encouraging result, we then focused our attention on optimizing the reaction conditions by screening various parameters such as catalysts, ligands, bases, solvents, and temperature to obtain a satisfactory yield for this MCR and the results are summarized in **Table 7.1**.



Scheme 7.8 Synthesis of target molecule in one-pot.

An augment to the yield (43%) along with accelerating reaction time (8 h) was noticed when the utility of L-proline was tested (**Table 7.1, entry 1**). Among others, 1,10-phenanthroline and 8-hydroxyquinoline, even with longer reaction times, didn't significantly influence the yield (**entries 2 and 3**). Picolinic acid was found to be the ideal ligand because it promulgated the reaction to afford tandem product in 52% yield (**entry 4**). Later, effects of various bases were scrutinized (**entries 5–8**). *t*-BuOK and DBU equally suppressed the reaction yielding a complex reaction mixture and K_3PO_4 required a longer reaction time (10 h) but with significant increase in the yield (**entry 7, 72%**). Gratifyingly, K_2CO_3 emerged to be the best possible base to afford

the product in 89% yield (**entry-8**). Further, the effectiveness of various copper catalysts was examined (**entries 9–11**). CuBr and CuCl were found to give diminished yields, while Cu_2O was found to be detrimental and resulted in a complex reaction mass. Subsequently, the effect of altering polar protic and aprotic solvents was assessed (**entries 12–19**). Polar aprotic solvents in general did not fetch amenable yields, while acetonitrile and water afforded solely triazole **81a**. Nevertheless, polyethylene glycol (PEG-400) at 100 °C for 18 h yielded 86% of product (**entry 15**). Decrease in the yield was observed when lower loadings of CuI (0.05 mmol), picolinic acid (0.1 mmol) (**entry 16**) and K_2CO_3 (2.5 mmol) (**entry 17**) were used. Taking advantage of PEG-400, which is a less toxic and environmentally benign solvent, we attempted the tandem reaction at 120 °C. Strikingly, that condition was revealed to be superior to all other solvent choices, affording the product in 93% yield (**entry 18**). Thus, the catalytic system employing CuI (0.1 mmol), picolinic acid (0.2 mmol) and K_2CO_3 (3 mmol) in PEG-400 at 120 °C for 8 h was revealed to be optimal in realizing this synthetic strategy.

Table 7.1 Optimization of cascade reaction conditions[a]

Entry	Catalyst	Ligand	Base	Solvent	T(°C)/Time	Yield[b]%
1	CuI	(L)-Proline	Cs_2CO_3	DMSO	100/8 h	43
2	CuI	1,10-Phen-anthroline	Cs_2CO_3	DMSO	100/12 h	42
3	CuI	8-Hydroxy-quinoline	Cs_2CO_3	DMSO	100/12 h	46
4	CuI	Picolinic acid	Cs_2CO_3	DMSO	100/8 h	52
5	CuI	Picolinic acid	*t*-BuOK	DMSO	100/8 h	CR[c]

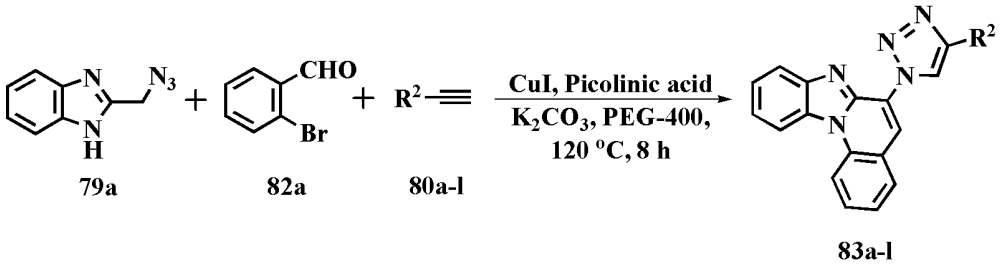
6	CuI	Picolinic acid	DBU	DMSO	100/8 h	CR[c]
7	CuI	Picolinic acid	K_3PO_4	DMSO	100/10 h	72
8	CuI	Picolinic acid	K_2CO_3	DMSO	100/8 h	89
9	CuBr	Picolinic acid	K_2CO_3	DMSO	100/8 h	79
10	CuCl	Picolinic acid	K_2CO_3	DMSO	100/8 h	47
11	Cu_2O	Picolinic acid	K_2CO_3	DMSO	100/8 h	CR[c]
12	CuI	Picolinic acid	K_2CO_3	DMF	100/8 h	45
13	CuI	Picolinic acid	K_2CO_3	DMA	100/8 h	52
14	CuI	Picolinic acid	K_2CO_3	ACN	80/24 h	NR[d]
15	CuI	Picolinic acid	K_2CO_3	PEG	100/18 h	86
16[e]	CuI	Picolinic acid	K_2CO_3	PEG	120/18 h	74
17[f]	CuI	Picolinic acid	K_2CO_3	PEG	120/14 h	84
18	**CuI**	**Picolinic acid**	**K_2CO_3**	**PEG**	**120/8 h**	**93**
19	CuI	Picolinic acid	K_2CO_3	Water	100/24 h	NR[d]

[a]Quantities used: 2-(azidomethyl)-1*H*-benzo[*d*]imidazole (1 mmol), 2-bromobenzaldehyde (1 mmol) and phenylacetylene (1 mmol), catalyst (0.1 mmol), ligand (0.2 mmol), base (3 mmol), solvent (2 mL). [b]yields are for isolated products. [c]complex reaction mixture. [d]no reaction, triazole **81a** remained unconsumed (entry **14** - 81%; entry **19** - 79%). [e]CuI (0.05 mmol), ligand (0.1 mmol). [f]K_2CO_3 (2.5 mmol).

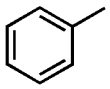
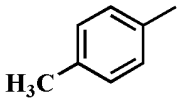
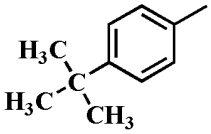
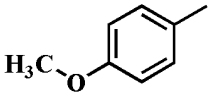
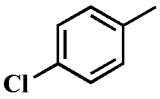
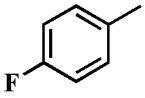
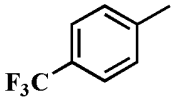
With this fine-tuned MCR, further efforts were streamlined to expand the generality and scope of this methodology by varying different alkynes (**Table 7.2**). Hyperconjugated systems 4-

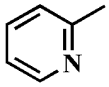
ethynyltoluene and 4-*tert*-butylphenylacetylene reacted smoothly to afford the desired products in 88 and 72% yields, respectively (**83b** and **83c**, **Table 7.2**). Electron-withdrawing phenylacetylenes bearing –Cl, –F and $–CF_3$ groups enhanced the yield of desired product **83e** (75%), **83f** (87%), and **83g** (80%) when compared to electron-rich phenylacetylene **83d** (–OMe, 72% yield). To make this protocol virtually more efficient, we were drawn towards dealing with low boiling aliphatic alkynes. Primarily, cyclopropylacetylene was subjected to MCR following our optimized condition but an unexpectedly lower yield of desired product (**83h**, 43%) was noticed. As the boiling point of the alkyne is low, heating at higher temperature might have resulted in lower yield. In order to circumvent the diminished yield, we slightly modified the procedure by mixing all the components of the reaction and stirred the resultant mixture at 50 °C for 1 h to predominantly favour the click reaction before elevating the operating temperature to 120 °C for 8 h. Encouragingly, the reaction proceeded with ease resulting in an improved yield of product (**83h**, 77%). This modified condition was conveniently employed to yield compound **83i** in 71%. Subsequently, we used ethynyltrimethylsilane as an alkyne partner. As expected, product **83j** was isolated in 88% yield after removal of the trimethylsilyl group due to the basic reaction medium along with higher temperature. Continuing with the aliphatic acetylenes, we decided to explore alkynes bearing an unprotected alcohol functional group; hence we carried the MCR with propargyl alcohol, **79a** and **82a**. To our surprise, the reaction was clean and the desired product **83k** was obtained in 67% yield. Furthermore, to study the reactivity profile with alkyne, 2-ethynylpyridine was subjected to this MCR. As evident, the alkyne smoothly participated in this cascade reaction to afford the tandem product **83l** in 76% yield.

Table 7.2 Screening of diverse acetylenes for the MCR[a]



79a 82a 80a-l 83a-l

Compound	R^2	Yield[b]
83a		93%
83b		88%
83c		72%
83d		72%
83e		75%
83f		87%
83g		80%
83h		77%
83i		71%

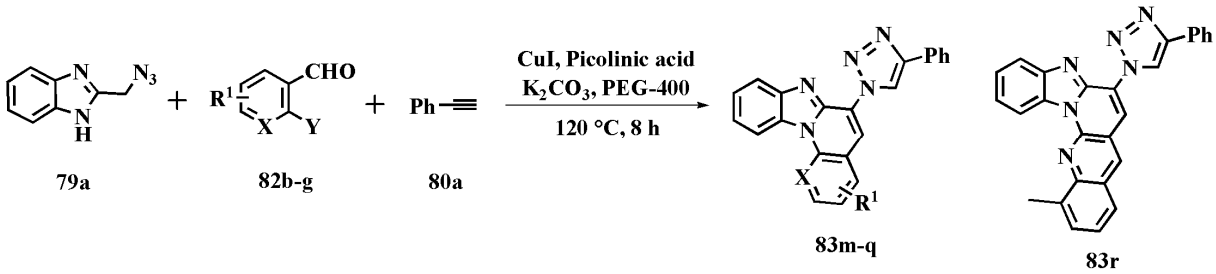
83j	**H**	88%
83k	**HOH_2C**	67%
83l		76%

[a]Quantities used: **79** (1 mmol), **82** (1 mmol), **80** (1 mmol), CuI (0.1 mmol), picolinic acid (0.2 mmol), K_2CO_3 (3 mmol), PEG-400 (2 mL). [b]Yields are for isolated products.

We then proceeded to evaluate the scope of substituted 2-bromobenzaldehydes (**Table 7.3**). 2-bromobenzaldehyde with one electron-rich group (–OMe) at C_5 position furnished a tandem product in good yield (**83m**, 64%), compared to 2-bromobenzaldehyde with two methoxy groups at C_4 and C_5 positions (**83n**, 52%). Also, 2-bromo-5-chlorobenzaldehyde furnished the tandem product **83o** in good yield (67%). However, 2-bromobenzaldehyde with an electron-withdrawing group (–F) at C_5 position was found to favour the tandem reaction to provide **83p** in 89% yield. As an extension, we tested our optimized condition on ortho-halo (hetero)aryl carboxaldehyde moieties to generate benzimidazo[1,2-*a*][1,8]naphthyridine derivatives. Such compounds have ample application in photophysical and biological properties such as a fluorescent chemodosimeter for detecting anionic species, OLEDs, and cannabinoid receptors [20]. Typically, 2-bromo-3-pyridinecarboxaldehyde reacted smoothly to deliver desired product in 81% yield (**83q**). Conversely, when 2-chloro-8-methylquinoline-3-carboxaldehyde was employed, decrease in the yield was noticed (**83r**, 60%). It is also worth mentioning that **83q** and **83r** took a shorter reaction time (6 h) primarily attributing to the reactivity of heteroaryl halides

by a nucleophilic aromatic substitution (S_NAr) reaction via an addition-elimination mechanism [21].

Table 7.3 Screening of diverse 2-bromo/chloro(hetero)aryl aldehyde for the MCR[a]

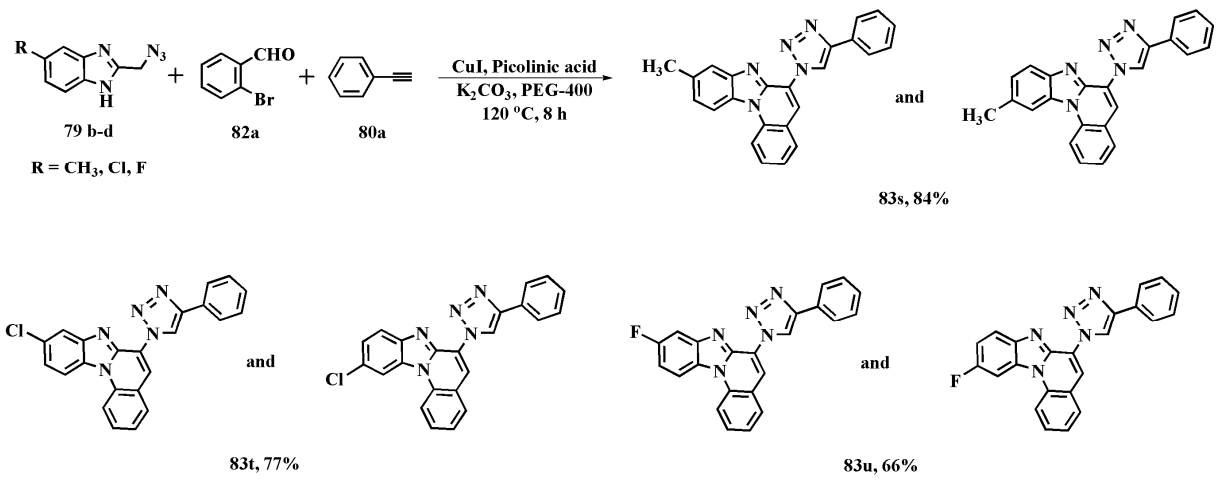


Compound	R^1	X	Y	Yield[b]
83m	5-OMe	CH	Br	64%
83n	4,5-$(OMe)_2$	CH	Br	52%
83o	5-Cl	CH	Br	67%
83p	5-F	CH	Br	89%
83q	H	N	Br	81%
83r	--	N	Cl	60%

[a]Quantities used: **79** (1 mmol), **82** (1 mmol), **80** (1 mmol), CuI (0.1 mmol), picolinic acid (0.2 mmol), K_2CO_3 (3 mmol), PEG-400 (2 mL). [b]Yields are for isolated products.

Eventually, we turned our attention to explore the reactivity profile of substituted 2-(azidomethyl)-1*H*-benzo[*d*]imidazole. Substituents like $–CH_3$, –Cl and –F reacted smoothly to furnish the desired product in good yield (**Table 7.4**: 84%, **83s**; 77%, **83t**; 66%, **83u**). This indicated that the electronic effect on a benzimidazole core doesn't impede the reactivity profile. By virtue of its reactivity, products were found to exist as mixtures of regioisomers in 1:1 ratio with substituents at C_9 and C_{10} positions (position numbering on structures).

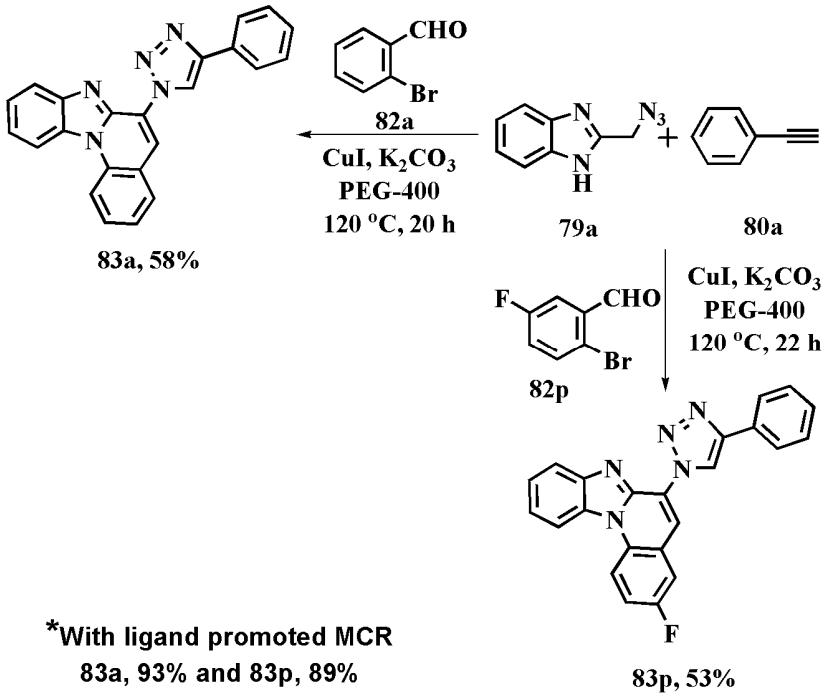
Table 7.4 Screening of diverse 2-(azidomethyl)-1*H*-benzo[*d*]imidazoles for the MCR[a]



[a]Quantities used: **79** (1 mmol), **82** (1 mmol), **80** (1 mmol), CuI (0.1 mmol), picolinic acid (0.2 mmol), K_2CO_3 (3 mmol), PEG-400 (2 mL).

Furthermore, Verma et al. accounted for the utilization of benzotriazole based ligands with copper catalysis to synthesize various nitrogen heterocycles [22]. Inspired by the remarkable ligand properties of the 1,2,3-triazole framework, we thus attempted the MCR employing a ligand-free condition (**scheme 7.9**). Accordingly, **79a** and **80a** was coupled with 2-bromobenzaldehyde and 2-bromo-5-fluorobenzaldehyde. The former reaction took 20 h to

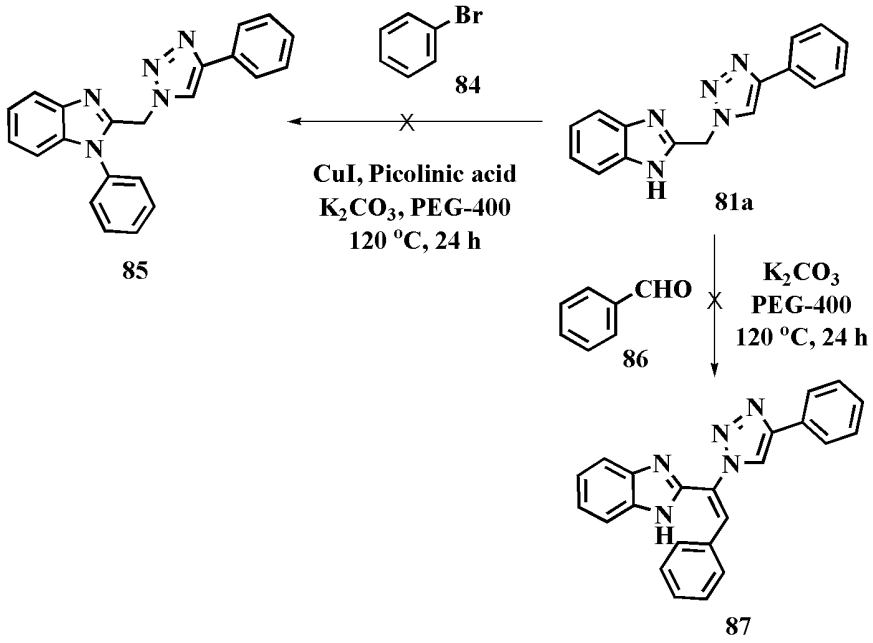
provide **83a** in 58% yield and the latter took 22 h to provide **83p** in 53% yield. Nevertheless, a ligand-promoted reaction fetched the desired product in an appreciable yield (**Table 7.2**, **83a**, 93% and **Table 7.3**, **83p**, 89%) in comparison to a ligand-free reaction with a shorter reaction time of 8 h. Hence, we implemented picolinic acid as a ligand to accelerate the reaction for the synthesis of 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinoline analogues.



Scheme 7.9 MCR employing ligand-free condition.

To investigate the reaction sequence for formation of tandem products, we carried out two parallel reactions (**scheme 7.10**). In first instance we performed *N*-arylation of **81a** with bromobenzene and in the second instance we carried out Knoevenagel condensation of **81a** with benzaldehyde employing the optimized reaction condition. Despite prolonged heating for 24 h, we did not notice *N*-arylated product in the former reaction and Knoevenagel condensed product in the latter reaction, intermediate **81a** remained unconsumed. The failure of *N*-arylation could

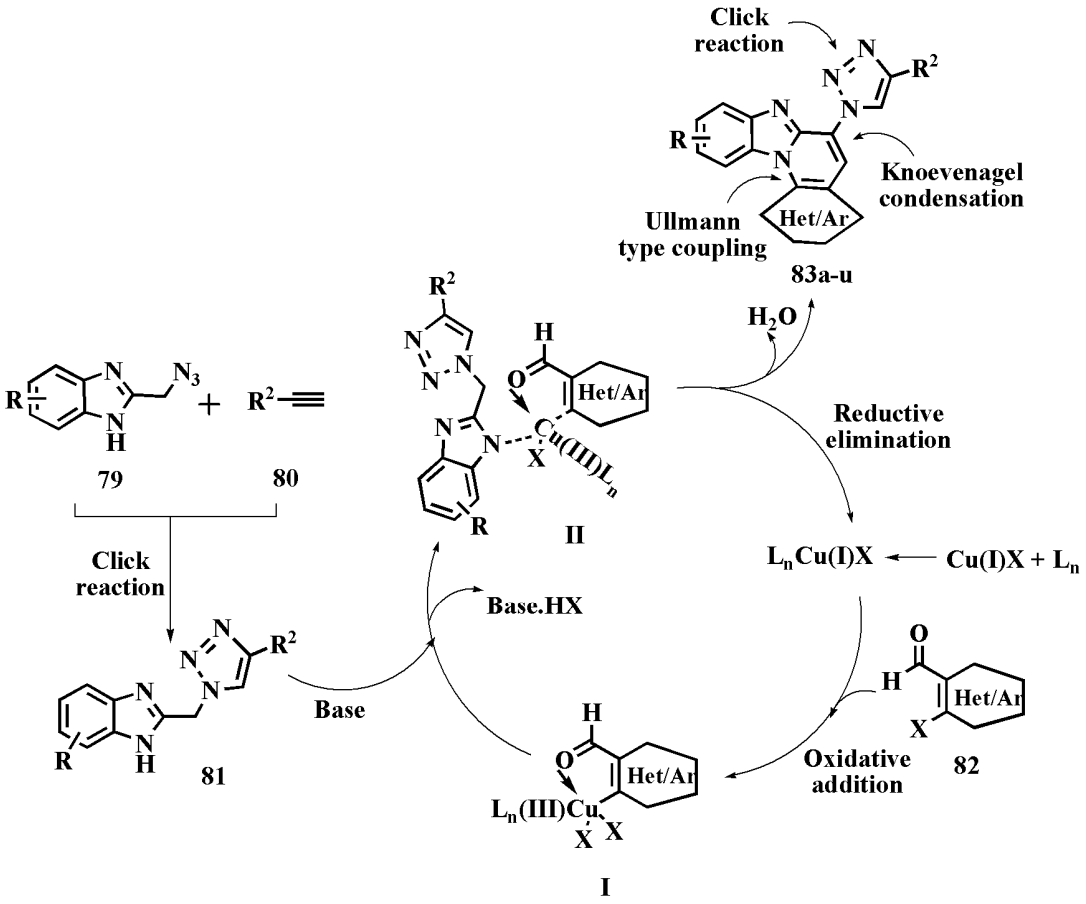
be attributed to steric effects rising from the methylene triazole and destabilizing the copper complex or the absence of a suitable coordinating group at *ortho* position of bromobenzene. In the latter case, the methylene group sandwiched between 1,2,3-triazole and benzimidazole heterocycles was not active enough to take part in a Knoevenagel condensation apparently due to a less acidic nature.



Scheme 7.10 Control experiments.

Facts from the control experiment suggested that the bottleneck for this tandem process would be carbonyl group assisted *N*-arylation [21b,23] followed by Knoevenagel condensation. The plausible mechanistic pathway for this MCR based on the outcome of control experiments and literature precedents is deduced (**scheme 7.11**). Primarily, a click reaction between **79** and **80** resulted in the formation of compound **81** with augments in methylene group reactivity. On the other hand, an ortho-halo (hetero)aryl carbonyl compound forms a complex with the copper catalyst through oxidative addition to furnish intermediate **I**. Compound **81** upon deprotonation

in the presence of base and metallation with the intermediate **I** would provide the intermediate **II**. Ultimately, reductive elimination of **II** furnishes the desired tandem product **83** through a domino process of *N*-arylation-Knoevenagel condensation to complete the catalytic cycle.



Scheme 7.11 Plausible mechanistic pathway for the formation of tandem product.

From the literature, a plethora of benzimidazo[1,2-*a*]quinoline core has been established as good fluorophore [5c,5d]. Additionally, tailoring a 1,2,3-triazole moiety onto existing fluorophores has endowed remarkable photophysical properties [4d,24]. We therefore opted to examine the UV and fluorescence properties of a diversely oriented 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinoline backbone and the spectral properties are summarized in **Table 7.5**. The quantum

yield was determined in $CHCl_3$ solution (within a maximum absorbance close to 0.1) and was calculated relative to the quantum yield of standard compound quinine sulphate in 0.1 M H_2SO_4 ($Ø_F$ = 0.54) at 25 °C [25]. Newly synthesized fluorophores could function as versatile feedstock for biological applications owing to high extinction coefficients (>150 nm). Considering electronic effects on the 1,2,3-triazole moiety (**83a–g** and **83l**) introduced by the substitution on the phenyl group, both electron-donating and withdrawing substituents resulted in modest quantum yields. Among the aliphatic systems (**83h–k**), the cyclopropyl group leading to compound **83h** resulted in a prejudicial quantum yield, whereas employing an acyclic n-butyl group affording compound **83i** resulted in a cumulative quantum yield. An electron donating substituent with a dimethoxy group on the quinoline fragment resulted in an improved quantum yield in comparison to electron withdrawing substituents (**83o** and **83p**). An enhanced quantum yield was observed upon hopping to heterocyclic moieties (**83q** and **83r**). Eventually, though an electron withdrawing (–F) substituent (**83u**) is present on the benzimidazole core, this molecule possessed high quantum yield of 0.21, probably due to the contribution from a lone pair of electrons on the –F substituent and presence of a rich π-electron system. Absorption and emission spectra of **83q**, **83r**, and **83u** in $CHCl_3$ at 25 °C are shown in **figure 7.3**.

Table 7.5 Spectral properties of fluorophores **83a–u**[a]

Compound	λ_{max} (nm)	λ_{em} (nm)	$\varepsilon \times 10^3$ ($dm^3\ mol^{-1}\ cm^{-1}$)	$Ø_F$[b]	Stokes Shift (nm)
83a	370, 266, 242	427	29.4, 100.8, 134.7	0.08	185
83b	368, 264, 228	429	16.3, 72.4, 83.9	0.07	201
83c	370, 266, 250	427	16.0, 86.6, 75.0	0.10	161

83d	370, 268, 242	428	26.1, 110.4, 103.3	0.09	160
83e	370, 264, 246	428	22.1, 79.0, 75.9	0.09	164
83f	368, 266, 241	427	20.6, 82.7, 87.1	0.08	186
83g	370, 268, 242	428	16.4, 56.4, 60.1	0.09	186
83h	228	428	79.3	0.02	200
83i	366, 268, 242	423	23.3, 89.1, 72.5	0.11	155
83j	366, 266, 242	424	4.4, 17.3, 16.0	0.08	158
83k	368, 272, 242	426	14.1, 47.0, 43.3	0.08	154
83l	370, 266, 242	429	19.1, 65.9, 68.8	0.10	187
83m	240	440	140.8	0.03	200
83n	384, 274, 242	421 444	50.0, 120.1, 163.9	0.13	202
83o	376, 270, 242	434	12.7, 50.4, 59.3	0.05	192
83p	376, 264, 246	433	8.6, 36.0, 34.6	0.07	169
83q	378, 250	439	22.9, 71.1	0.15	189
83r	376, 274, 242	432 458	32.2, 98.0, 98.4	0.15	216
83s	372, 268, 242	434	19.8, 88.6, 111.0	0.12	192
83t	368, 268, 248	425	21.7, 73.9, 78.9	0.06	177
83u	368, 266, 248	423	11.4, 25.5, 25.0	0.21	157

[a]Measured in $CHCl_3$ at 25 °C. [b]Measured with quinine sulphate in 0.1M H_2SO_4 as standard.

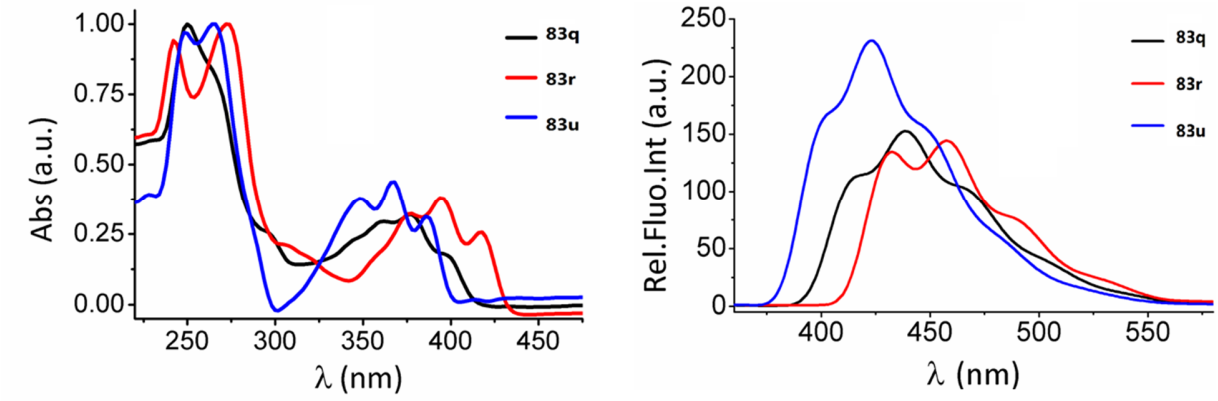


Figure 7.3 Absorption and emission spectra of **83q**, **83r**, and **83u** in $CHCl_3$ at 25 °C.

7.4 Conclusion

In conclusion, the present MCR deals with a facile and direct avenue to synthesize versatile 1,2,3-triazole anchored benzimidazo[1,2-*a*]quinolines. An ease of operation employing a dual catalytic system with low catalyst loading and PEG-400 as an environmentally benign solvent crafts this protocol to be more appealing and synthetically efficient. The domino process revealed good tolerance to various substrates affording desired products in moderate to good yields. It is apparent that click and activate approach could serve as a novel platform to engineer an immensely decorated 1,2,3-triazole anchored nitrogenous fused heterocyclic framework. The 1,2,3-triazole motif was revealed to be moderately electron withdrawing in activating the adjacent methylene linker to undergo a Knoevenagel condensation. Owing to the high extinction coefficients (>150 nm), these molecules could function as versatile feedstock for biological and fluorescent applications.

7.5 Experimental Section

7.5.1 Methods and materials

Chemicals and solvents were procured from commercial sources and are analytically pure. 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 230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded at 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) are given in ppm. Some of the compound's ^{1}H and ^{13}C NMR spectra were recorded by addition of 10 mL of formic acid or trifluoroacetic acid to the $CDCl_3$ solution due to the solubility issues. IR spectra were recorded on a FT-IR spectrometer (Shimadzu) 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. HRMS were recorded on a QSTAR XL hybrid MS/MS mass spectrometer. UV-Visible absorption spectra were recorded using a Jasco V-650 spectrophotometer and fluorescence spectra were recorded using a Jasco FP-6300 spectrofluorometer.

Synthesis of substituted 2-(azidomethyl)-1*H*-benzo[*d*]imidazole

Step 1: Substituted 2-(chloromethyl)-1*H*-benzo[*d*]imidazole was prepared according to the literature procedure [19]. Substituted *o*-phenylenediamine (0.05 mol), chloroacetic acid (0.075 mol) and 4N hydrochloric acid (50 mL) was heated under reflux for 45 minutes. The mixture was allowed to stand overnight, diluted with 100 mL of water, cooled and neutralized with sodium bicarbonate. The resultant solid was filtered, washed with cold water and dried over vacuum. The crude product was taken as such for the step 2 without further purification.

Step 2: Substituted 2-(azidomethyl)-1*H*-benzo[*d*]imidazole was prepared according to the literature procedure [19]. Substituted 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (0.05 mol) and NaN_3 (0.055 mol) in DMSO (40 mL) was stirred at room temperature. The reaction was monitored by TLC. After completion, diluted with 100 mL of water and extracted with diethyl ether (10 mL x 3). The combined organic extracts were washed with brine, and dried over anhydrous Na_2SO_4. After the organic solvent was removed under reduced pressure, the residue was purified by column chromatography to provide the title compound.

2-(azidomethyl)-1*H*-benzo[*d*]imidazole (79a)

Off-white solid (75%); mp 120-121 °C; IR ν_{max} (KBr) 2103, 1433, 1309, 1271, 1031, 997, 747 cm^{-1}; Characterization details (^1H and ^{13}C NMR) correlate with the literature report [19].

2-(azidomethyl)-5-methyl-1*H*-benzo[*d*]imidazole (79b)

Beige solid (82%); mp 102-103 °C; IR ν_{max} (KBr) 2173, 2103, 1450, 1326, 1280, 1254, 1188, 1140, 1027, 801 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.2 Hz, 1H), 7.38 (s, 1H), 7.11 (dd, *J* = 8.4, 1.3 Hz, 1H), 4.73 (s, 2H), 2.47 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.64, 138.19, 137.02, 133.14, 124.67, 115.33, 114.66, 48.45, 21.81.

2-(azidomethyl)-5-chloro-1*H*-benzo[*d*]imidazole (79c)

Light brown solid (72%); mp 112-113 °C; IR ν_{max} (KBr) 2178, 2105, 1424, 1318, 1276, 1061, 1023, 800 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.30 – 7.26 (m, 1H), 4.79 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.57, 132.46, 132.28, 130.44, 127.04, 115.43, 114.39, 45.65.

2-(azidomethyl)-5-fluoro-1*H*-benzo[*d*]imidazole (79d)

Light brown solid (68%); mp 81-82 °C; IR ν_{max} (KBr) 2186, 2101, 1445, 1328, 1256, 1139, 1027, 860, 809 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (m, 1H), 7.21 (m, 1H), 6.98 (m, 1H), 4.69 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.62 (d, $^1J_{CF}$ = 240.38 Hz), 150.00, 138.31, 134.99, 116.04 (d, $^3J_{CF}$ = 10.1 Hz), 111.59 (d, $^2J_{CF}$ = 25.25 Hz), 101.21 (d, $^2J_{CF}$ = 27.27 Hz), 48.39.

Synthesis of 6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (81a)

2-(azidomethyl)-1*H*-benzo[*d*]imidazole **79a** (3 mmol), phenylacetylene **80a** (3 mmol), $CuSO_4.5H_2O$ (0.1 mmol), sodium ascorbate (0.2 mmol) and *t*-BuOH:H_2O (1:1, 5mL) were added into a 10 mL round bottom flask. The reaction mixture was stirred at room temperature for 30 min. Reaction progress was monitored by TLC. After completion, the reaction mass was diluted with water (10 mL). Resultant precipitate was filtered and dried to obtain analytical pure product **81a**.

Colorless solid (94%); mp 209-210 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 8.05 (s, 1H), 7.79-7.77 (d, *J* = 7.8 Hz, 3H), 7.42-7.38 (t, *J* = 7.5 Hz, 2H), 7.36-7.29 (m, 2H), 7.27-7.25 (dd, *J* = 5.8, 2.8 Hz, 2H), 5.89 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.96, 130.24, 128.70, 128.10, 125.52, 120.44, 48.03; HRMS (ESI, m/z): Calcd for $C_{16}H_{14}N_5$ $[M+H]^+$ 276.1249, found 276.1251.

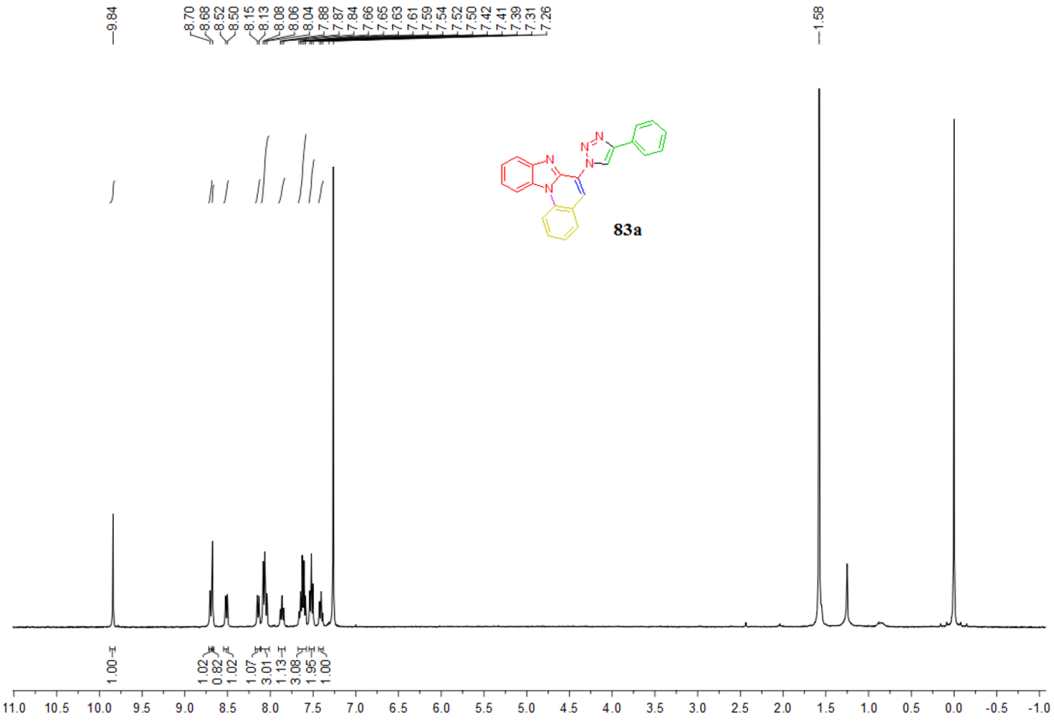
Synthesis of 6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83a)

An oven dried 10 mL round bottom flask was charged with 2-(azidomethyl)-1*H*-benzo[*d*]imidazole **79a** (1 mmol), 2-bromobenzaldehyde **82a** (1 mmol), phenylacetylene **80a** (1

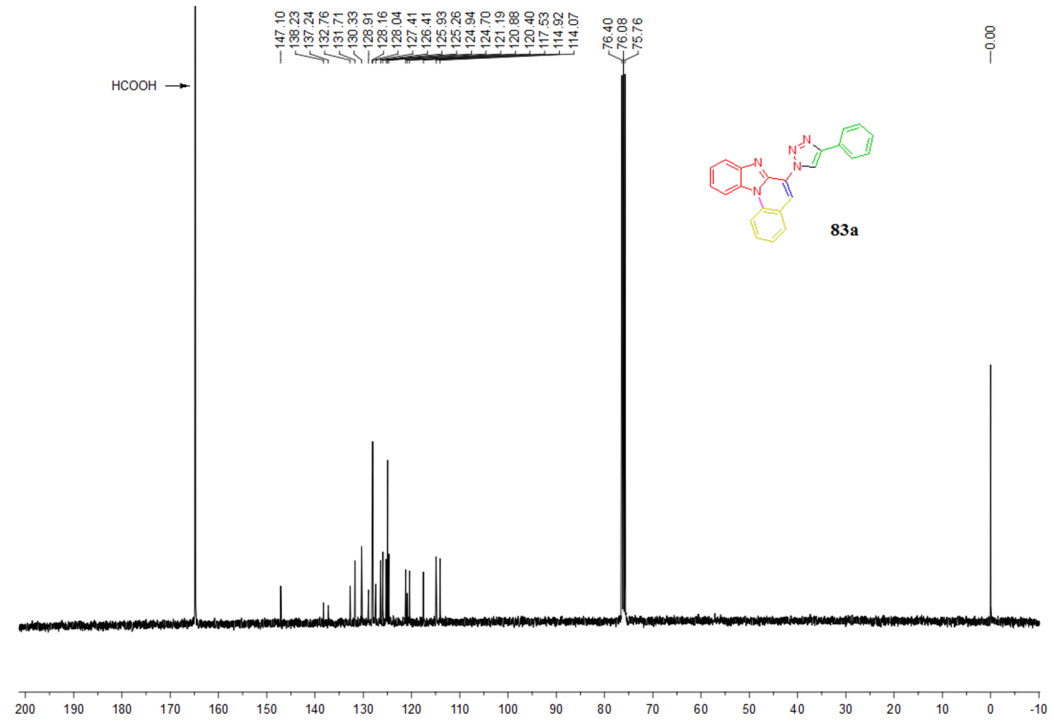
mmol), K_2CO_3 (3 mmol), CuI (0.1 mmol), picolinic acid (0.2 mmol) and PEG-400 (2 mL). The resulting mixture was stirred at 120 °C under N_2 atmosphere. Reaction progress was monitored by TLC. After completion, the reaction mass was allowed to cool to ambient temperature, diluted with water (5 mL) and extracted with DCM (3 × 5 mL). The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude material was purified by column chromatography using eluent ($CHCl_3$/EtOAc = 50/1) to obtain desired tandem product **83a** in 93% yield (335 mg). The compounds **83b-g** and **83k-u** were prepared following the same protocol.

Synthesis of substituted 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinolines 83h-j

An oven dried 10 mL round bottom flask was charged with 2-(azidomethyl)-1*H*-benzo[*d*]imidazole **79a** (1 mmol), 2-bromobenzaldehyde **82a** (1 mmol), low boiling substituted acetylene **80h-j** (1 mmol), K_2CO_3 (3 mmol), CuI (0.1 mmol), picolinic acid (0.2 mmol) and PEG-400 (2 mL). The resulting mixture was stirred under N_2 atmosphere at 50 ºC for 1 h before elevating the operating temperature to 120 ºC for 8 h. Reaction progress was monitored by TLC. After completion, the reaction mass was allowed to cool to ambient temperature, diluted with water (5 mL) and extracted with DCM (3 × 5 mL). The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude material was purified by column chromatography using eluent ($CHCl_3$/EtOAc = 50/1) to obtain desired tandem product **83h-j**.



^{1}H NMR spectrum of compound **83a**



^{13}C NMR spectrum of compound **83a**

6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83a)

Colorless solid (93%); mp 216-217 °C; IR ν_{max} (KBr, cm^{-1}) 3033, 1538, 1453, 1397, 1245, 1202, 1016, 898, 756; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.70 (d, *J* = 8 Hz, 1H), 8.68 (s, 1H), 8.51 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 6.9 Hz, 1H), 8.06 (t, *J* = 8.5 Hz, 3H), 7.90 – 7.83 (m, 1H), 7.69 – 7.56 (m, 3H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.10, 138.23, 137.24, 132.76, 131.71, 130.33, 128.91, 128.16, 128.04, 127.41, 126.41, 125.93, 125.26, 124.94, 124.70, 121.19, 120.88, 120.40, 117.53, 114.92, 114.07. HRMS (ESI, m/z): Calcd for $C_{23}H_{16}N_5$ $[M+H]^+$ 362.1406, found 362.1410.

6-(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83b)

Beige solid (88%); mp 252-253 °C; IR ν_{max} (KBr, cm^{-1}) 3154, 1541, 1467, 1398, 1246, 1078, 1018, 813, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 8.61 (d, *J* = 9.6 Hz, 2H), 8.44 (d, *J* = 7.7 Hz, 1H), 8.10 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.98 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 2H), 7.83 – 7.79 (ddd, *J* = 8.5, 7.3, 1.5 Hz, 1H), 7.63 – 7.53 (m, 3H), 7.34 – 7.29 (m, 2H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.82, 144.24, 141.58, 138.11, 134.60, 131.24, 130.46, 130.31, 129.54, 127.71, 125.97, 125.12, 124.97, 124.93, 123.74, 122.20, 121.49, 121.13, 121.02, 115.06, 114.15, 21.38. HRMS (ESI, m/z): Calcd for $C_{24}H_{18}N_5$ $[M+H]^+$ 376.1562, found 376.1569.

6-(4-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83c)

Beige solid (72%); mp 216-217 °C; IR ν_{max} (KBr, cm^{-1}) 3055, 1541, 1467, 1395, 1245, 1199, 1075, 1015, 821, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 8.63 (d, *J* = 7.6 Hz, 2H), 8.45 (d, *J* = 7.6 Hz, 1H), 8.11 (m, 1H), 7.99 (dd, *J* = 7.4, 2.9 Hz, 3H), 7.84 – 7.78 (m, 1H), 7.63 – 7.53

(m, 5H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.35, 147.66, 144.12, 141.37, 134.41, 131.07, 130.30, 130.18, 127.73, 125.81, 125.78, 125.03, 124.85, 124.75, 123.66, 122.06, 121.57, 121.02, 120.72, 114.90, 114.04, 34.74, 31.37. HRMS (ESI, m/z): Calcd for $C_{27}H_{24}N_5$ $[M+H]^+$ 418.2032, found 418.2028.

6-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83d)

Beige solid (72%); mp 215-216 °C; IR ν_{max} (KBr, cm^{-1}) 3051, 1563, 1498, 1465, 1247, 1022, 818, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (s, 1H), 8.64 (d, *J* = 8.5 Hz, 1H), 8.61 (s, 1H), 8.47 (d, *J* = 7.6 Hz, 1H), 8.11 (d, *J* = 7.1 Hz, 1H), 8.02 - 7.97 (m, 3H), 7.85 – 7.81 (t, *J* = 7.9 Hz, 1H), 7.65 – 7.55 (m, 3H), 7.05 (d, *J* = 9.6 Hz, 2H), 3.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.78, 147.60, 144.26, 141.52, 134.69, 131.21, 130.43, 130.24, 127.36, 125.11, 124.95, 124.91, 123.73, 123.25, 122.18, 121.09, 121.00, 120.97, 115.04, 114.26, 114.15, 55.37. HRMS (ESI, m/z): Calcd for $C_{24}H_{18}N_5O$ $[M+H]^+$ 392.1511, found 392.1516.

6-(4-(4-chlorophenyl)-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83e)

Colorless solid (81%); mp 278-279 °C; IR ν_{max} (KBr, cm^{-1}) 3046, 1542, 1468, 1247, 1198, 1017, 821, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.76 – 8.64 (m, 2H), 8.51 (d, *J* = 6.8 Hz, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 8.05 (d, *J* = 7.9 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 2H), 7.89 – 7.85 (t, *J* = 6.7 Hz, 1H), 7.65 – 7.59 (dd, *J* = 14.5, 7.0 Hz, 3H), 7.48 (d, *J* = 8.3 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.68, 136.22, 134.42, 133.31, 132.37, 132.10, 130.91, 128.37, 128.32, 128.10, 127.53, 127.37, 126.27, 125.47, 121.69, 119.64, 118.91, 115.76, 115.64, 115.03. HRMS (ESI, m/z): Calcd for $C_{23}H_{15}ClN_5$ $[M+H]^+$ 396.1016, found 396.1021.

6-(4-(4-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*] quinoline (83f)

Beige solid (87%); mp 292-293 °C; IR ν_{max} (KBr, cm^{-1}) 3052, 1562, 1495, 1400, 1226, 1019, 827, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 8.70 (d, *J* = 8.6 Hz, 1H), 8.67 (s, 1H), 8.52 (d, *J* = 7.5 Hz, 1H), 8.14 (d, *J* = 8.9 Hz, 1H), 8.08 – 8.02 (m, 3H), 7.87 (t, *J* = 7.9 Hz, 1H), 7.68 – 7.58 (m, 3H), 7.23 – 7.18 (t, *J* = 8.7 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.06 (d, $^{1}J_{CF}$ = 250.48 Hz), 147.70, 138.29, 135.62, 133.64, 133.46, 131.69, 129.50, 128.50, 127.98 (d, $^{3}J_{CF}$ = 9.09 Hz), 127.85, 127.77, 126.60, 124.44, 122.50, 120.85, 117.45, 116.39, 116.17 (d, $^{2}J_{CF}$ = 25.25 Hz), 115.61. HRMS (ESI, m/z): Calcd for $C_{23}H_{15}FN_5$ $[M+H]^+$ 380.1311, found 380.1315.

6-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83g)

Colorless solid (80%); mp 283-284 °C; IR ν_{max} (KBr, cm^{-1}) 3054, 1620, 1541, 1397, 1254, 1114, 1015, 826, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 8.65 (d, *J* = 8.8 Hz, 2H), 8.46 (d, *J* = 7.7 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 2H), 8.09 (d, *J* = 5.9 Hz, 1H), 8.01 (d, *J* = 1.2 Hz, 1H), 7.84 – 7.80 (m, 1H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.59 – 7.54 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9, 163.9, 132.3, 131.0, 129.7, 128.9, 127.2, 126.5, 125.4, 123.8, 122.5, 121.1, 114.0, 55.6. HRMS (ESI, m/z): Calcd for $C_{24}H_{15}F_3N_5$ $[M+H]^+$ 430.1280, found 430.1278.

6-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83h)

Pale green solid (77%); mp 194-195 °C; IR ν_{max} (KBr, cm^{-1}) 3081, 1540, 1397, 1243, 1027, 893, 813, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.62 (d, *J* = 8.5 Hz, 1H), 8.52 (s, 1H), 8.45 (dd, *J* = 7.4, 1.4 Hz, 1H), 8.09 (dd, *J* = 7.2, 1.6 Hz, 1H), 8.00-7.95 (m, 1H), 7.84 – 7.77 (m, 1H), 7.63 – 7.54 (m, 3H), 2.20 – 2.11 (m, 1H), 1.08 – 1.04 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.08, 143.12, 140.58, 133.38, 130.08, 129.22, 129.07, 123.97, 123.80, 122.59, 121.11, 119.97,

119.84, 113.89, 113.02, 6.75, 5.89. HRMS (ESI, m/z): Calcd for $C_{20}H_{16}N_5$ $[M+H]^+$ 326.1406, found 326.1402.

6-(4-butyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83i)

Colorless solid (71%); mp 130-131 °C; IR ν_{max} (KBr, cm^{-1}) 3052, 1539, 1453, 1399, 1224, 1035, 889, 814, 756; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.62 (d, *J* = 8.5 Hz, 1H), 8.54 (s, 1H), 8.45 (d, *J* = 8.2 Hz, 1H), 8.11 – 8.06 (m, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.84 – 7.78 (m, 1H), 7.63 – 7.53 (m, 3H), 2.95 – 2.89 (m, 2H), 1.88 – 1.80 (dt, *J* = 13.0, 7.6 Hz, 2H), 1.55 – 1.45 (m, 2H), 1.03 – 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.43, 144.19, 141.70, 134.45, 131.16, 130.30, 130.14, 125.09, 125.03, 124.89, 123.65, 122.97, 122.21, 121.03, 120.95, 114.97, 114.11, 31.59, 25.58, 22.50, 13.93. HRMS (ESI, m/z): Calcd for $C_{21}H_{20}N_5$ $[M+H]^+$ 342.1719, found 342.1721.

6-(1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83j)

Pale yellow solid (88%); mp 212-213 °C; IR ν_{max} (KBr, cm^{-1}) 3059, 1544, 1466, 1395, 1252, 1216, 1078, 1010, 737; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.59 (s, 1H), 8.68 (d, *J* = 8.5 Hz, 1H), 8.62 (s, 1H), 8.51 – 8.48 (dd, *J* = 7.1, 1.8 Hz, 1H), 8.11 – 8.08 (dd, *J* = 7.0, 2.2 Hz, 1H), 8.04 – 8.01 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.98 (d, *J* = 1.1 Hz, 1H), 7.88 – 7.83 (m, 1H), 7.64 – 7.58 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.19, 141.58, 134.57, 133.81, 131.17, 130.42, 130.38, 125.98, 125.12, 124.96, 123.75, 122.08, 121.34, 121.07, 115.02, 114.11. HRMS (ESI, m/z): Calcd for $C_{17}H_{12}N_5$ $[M+H]^+$ 286.1093, found 286.1097.

(1-(benzimidazo[1,2-*a*]quinolin-6-yl)-1*H*-1,2,3-triazol-4-yl) methanol (83k)

Beige solid (67%); mp 236-237 °C; IR ν_{max} (KBr, cm^{-1}) 3302, 3074, 1540, 1401, 1202, 1039, 848, 756; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.55 (s, 1H), 8.69 (d, *J* = 8.5 Hz, 1H), 8.60 (s, 1H), 8.50 (d, *J* = 8.5 Hz, 1H), 8.10 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.89 - 7.85 (t, *J* = 7.9 Hz, 1H), 7.69 – 7.55 (m, 3H), 5.01 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.32, 147.32, 139.98, 134.10, 132.70, 131.31, 130.14, 127.25, 127.07, 126.78, 126.26, 125.56, 124.64, 122.12, 118.79, 115.94, 114.94, 55.00. HRMS (ESI, m/z): Calcd for $C_{18}H_{14}N_5O$ $[M+H]^+$ 316.1198, found 316.1195.

6-(4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)benzoimidazo[1,2-*a*]quinoline (83l)

Beige solid (66%); mp 248-249 °C; IR ν_{max} (KBr, cm^{-1}) 3048, 1599, 1545, 1470, 1399, 1213, 1020, 789, 757, 733; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.12 (s, 1H), 8.75 (d, *J* = 4.8 Hz, 1H), 8.65 (d, *J* = 8.6 Hz, 1H), 8.63 (s, 1H), 8.47 (d, *J* = 7.6 Hz, 1H), 8.32 (d, *J* = 7.9 Hz, 1H), 8.13 (d, *J* = 7.5 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.90 – 7.81 (m, 2H), 7.64 – 7.57 (m, 3H), 7.34 – 7.31 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.49, 143.82, 143.48, 134.22, 133.39, 131.74, 130.04, 128.60, 127.80, 127.24, 126.69, 126.02, 125.88, 124.32, 122.21, 121.58, 118.38, 116.21, 115.22. HRMS (ESI, m/z): Calcd for $C_{22}H_{15}N_6$ $[M+H]^+$ 363.1358, found 363.1362.

3-methoxy-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83m)

Pale green solid (64%); mp 254-255 °C; IR ν_{max} (KBr, cm^{-1}) 3061, 1539, 1482, 1454, 1404, 1236, 1019, 806, 768, 739, 694; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.57 (s, 1H), 8.53 (d, *J* = 8.9 Hz, 1H), 8.40 (d, *J* = 8.1 Hz, 1H), 8.13 – 8.05 (m, 3H), 7.64 – 7.51 (m, 4H), 7.45 – 7.36 (m, 3H), 3.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.98, 148.26, 137.29, 136.14,

129.32, 129.20, 129.07, 128.10, 128.00, 127.73, 126.28, 126.02, 125.87, 123.88, 122.63, 121.23, 120.78, 117.57, 117.41, 115.13, 111.66, 55.99. HRMS (ESI, m/z): Calcd for $C_{24}H_{18}N_5O$ $[M+H]^+$ 392.1511, found 392.1509.

2,3-dimethoxy-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83n)

Beige solid (76%); mp 280-281 °C; IR ν_{max} (KBr, cm^{-1}) 3064, 1536, 1468, 1389, 1270, 1017, 759; ^1H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 8.46 (s, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 8.08 – 8.04 (td, *J* = 7.9, 0.9 Hz, 3H), 7.93 (s, 1H), 7.63 – 7.57 (m, 1H), 7.54 – 7.49 (td, *J* = 8.1, 4.8 Hz, 3H), 7.43 – 7.37 (m, 1H), 7.21 (s, 1H), 4.14 (s, 3H), 4.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.91, 148.38, 147.43, 136.16, 133.72, 128.43, 128.30, 128.15, 127.73, 127.11, 126.36, 125.05, 124.97, 119.62, 117.08, 116.27, 115.91, 114.23, 109.25, 97.23, 55.95, 55.55. HRMS (ESI, m/z): Calcd for $C_{25}H_{20}N_5O_2$ $[M+H]^+$ 422.1617, found 422.1620.

3-chloro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83o)

Beige solid (60%); mp 271-272 °C; IR ν_{max} (KBr, cm^{-1}) 3058, 1535, 1448, 1402, 1205, 1015, 796, 738, 688; ^1H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1H), 8.57 (d, *J* = 7.7 Hz, 2H), 8.39 (m, 1H), 8.12 (m, 1H), 8.06 (dd, *J* = 5.2, 3.2 Hz, 2H), 7.97 (d, *J* = 2.4 Hz, 1H), 7.78 – 7.75 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.66 – 7.57 (m, 2H), 7.55 – 7.49 (m, 2H), 7.44 – 7.38 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.05, 140.58, 139.88, 132.41, 131.87, 131.80, 130.22, 129.83, 129.17, 129.11, 128.60, 126.82, 126.06, 125.37, 123.89, 123.52, 123.29, 121.84, 119.69, 116.96, 114.46. HRMS (ESI, m/z): Calcd for $C_{23}H_{15}ClN_5$ $[M+H]^+$ 396.1016, found 396.1014.

3-fluoro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83p)

Pale green solid (89%); mp 312-313 °C; IR ν_{max} (KBr, cm^{-1}) 3032, 1539, 1451, 1402, 1203, 1013, 916, 760; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H), 8.91 (s, 1H), 8.89 (d, *J* = 3.9 Hz, 1H), 8.65 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 7.7 Hz, 1H), 7.94 – 7.81 (m, 4H), 7.73 – 7.65 (m, 2H), 7.39 – 7.30 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.38 (d, $^1J_{CF}$ = 252.5 Hz), 147.87, 136.17, 132.97, 128.85, 128.79, 128.31, 128.18, 126.86, 126.40, 126.07, 125.13, 123.48 (d, $^3J_{CF}$ = 9.09 Hz), 121.30 (d, $^2J_{CF}$ = 26.26 Hz), 120.36, 119.74, 118.09 (d, $^3J_{CF}$ = 9.09 Hz), 116.05, 115.49 (d, $^2J_{CF}$ = 24.24 Hz), 114.74. HRMS (ESI, m/z): Calcd for $C_{23}H_{15}FN_5$ $[M+H]^+$ 380.1311, found 380.1314.

6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83q)

Colorless solid (81%); mp 315-316 °C; IR ν_{max} (KBr, cm^{-1}) 3050, 1530, 1449, 1200, 1014, 903, 766; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.55 (d, *J* = 8.0 Hz, 1H), 9.22 (d, *J* = 3.2 Hz, 1H), 9.14 (s, 1H), 9.11 (s, 1H), 8.77 (d, *J* = 7.2 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.99 – 7.86 (m, 3H), 7.66 – 7.54 (m, 2H), 7.19 (d, *J* = 3.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.24, 147.85, 142.97, 138.87, 136.46, 132.54, 128.72, 128.56, 128.21, 127.85, 126.80, 126.49, 125.25, 125.06, 123.21, 120.12, 119.54, 118.24, 116.67, 114.92. HRMS (ESI, m/z): Calcd for $C_{22}H_{15}N_6$ $[M+H]^+$ 363.1358, found 363.1355.

12-methyl-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzo[*g*]benzimidazo[1,2-*a*][1,8]naphthyridine (83r)

Green solid (60%); mp 316-317 °C; IR ν_{max} (KBr, cm^{-1}) 3045, 1610, 1534, 1476, 1402, 1208, 1015, 765; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (d, *J* = 7.2 Hz, 1H), 9.26 (s, 1H), 9.19 (s, 1H),

9.14 (s, 1H), 8.15 (d, *J* = 6.8 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.98 – 7.94 (m, 3H), 7.79 – 7.72 (m, 3H), 7.50-7.44 (m, 3H), 3.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.58, 147.27, 141.43, 140.95, 138.17, 136.61, 134.17, 134.06, 129.64, 129.17, 129.00, 128.75, 128.09, 127.69, 127.55, 126.91, 126.86, 125.94, 120.46, 120.21, 118.20, 116.36, 115.22, 18.66. HRMS (ESI, m/z): Calcd for $C_{27}H_{19}N_6$ $[M+H]^+$ 427.1671, found 427.1676.

9-methyl-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline and 10-methyl-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83s)

(for regioisomeric mixture = 1:1) Beige solid (84%); mp 242-243 °C; IR ν_{max} (KBr, cm^{-1}) 3055, 1539, 1469, 1396, 1199, 1014, 766, 692; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 0.5H), 9.81 (s, 0.5H), 8.66 – 8.58 (m, 2H), 8.32 (d, *J* = 8.6 Hz, 0.5H), 8.25 (s, 0.5H), 8.11 – 8.06 (m, 2H), 8.04 – 7.97 (m, 1.5H), 7.90 (s, 0.5H), 7.83 (m, 1H), 7.61 – 7.50 (m, 3H), 7.47 – 7.38 (m, 2H), 2.71 (s, 1.5H), 2.63 (s, 1.5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.13, 148.04, 138.22, 136.11, 133.84, 133.55, 132.60, 132.30, 131.18, 131.10, 130.27, 129.19, 129.14, 129.09, 128.78, 128.55, 128.45, 127.92, 127.34, 126.81, 126.58, 125.99, 125.87, 125.38, 122.09, 121.80, 121.46, 121.28, 118.14, 117.81, 115.84, 114.62, 114.51, 22.27, 21.60. HRMS (ESI, m/z): Calcd for $C_{24}H_{18}N_5$ $[M+H]^+$ 376.1562, found 376.1565.

9-chloro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline and 10-chloro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83t)

(for regioisomeric mixture = 1:1) Off white solid (77%); mp 278-279 °C; IR ν_{max} (KBr, cm^{-1}) 3057, 1539, 1395, 1201, 1017, 765; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 0.5H), 9.74 (s, 0.5H), 8.67 (s, 0.5H), 8.65 (s, 0.5H), 8.54 (dd, *J* = 16.8, 8.5 Hz, 1H), 8.45 (d, *J* = 1.7 Hz, 0.5H), 8.37 (d,

J = 8.9 Hz, 0.5H), 8.09 – 8.00 (m, 4H), 7.85 (dd, *J* = 14.0, 6.6 Hz, 1H), 7.64 – 7.57 (m, 1.5H), 7.56 – 7.48 (m, 2.5H), 7.41 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.55, 148.52, 138.97, 138.93, 134.51, 134.46, 134.43, 133.96, 133.85, 133.13, 133.10, 132.34, 131.75, 129.82, 129.63, 129.22, 129.09, 128.14, 128.07, 127.85, 127.04, 126.02, 122.48, 122.44, 121.02, 120.93, 120.80, 120.61, 118.35, 118.33, 116.99, 116.63, 116.31, 116.27, 115.60. HRMS (ESI, m/z): Calcd for $C_{23}H_{15}ClN_5$ $[M+H]^+$ 396.1016, found 396.1019.

9-fluoro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline and 10-fluoro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzimidazo[1,2-*a*]quinoline (83u)

(for regioisomeric mixture = 1:1) Colorless solid (66%); mp 266-267 °C; IR ν_{max} (KBr, cm^{-1}) 3035, 1541, 1476, 1398, 1203, 1016, 762, 692; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 0.5H), 9.76 (s, 0.5H), 8.68 (s, 0.5H), 8.63 (s, 0.5H), 8.60 (d, *J* = 8.5 Hz, 0.5H), 8.49 (d, *J* = 8.5 Hz, 0.5H), 8.42 (dd, *J* = 9.2, 4.4 Hz, 0.5H), 8.17 (dd, *J* = 9.6, 2.3 Hz, 0.5H), 8.08 – 8.01 (m, 3.5H), 7.88 – 7.83 (m, 1H), 7.76 (dd, *J* = 9.0, 2.5 Hz, 0.5H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.51 (td, *J* = 7.6, 1.5 Hz, 2H), 7.43 – 7.31 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160 (d, $^1J_{CF}$ = 253.51 Hz), 158.98 (d, $^1J_{CF}$ = 245.43 Hz), 147.54, 140.85, 140.67, 135.95, 133.68, 133.62, 132.81, 132.54, 131.34, 131.29, 129.96, 129.84, 129.34, 129.31, 129.16, 128.34, 128.29, 126.96, 126.87, 126.78, 126.33, 126.04, 122.42, 122.08, 122.03, 121.68, 121.61, 120.03, 119.93, 116.18, 116.07, 115.92, 115.66, 115.58, 115.50, 114.21, 113.96, 104.84, 104.56, 102.19, 101.89. HRMS (ESI, m/z): Calcd for $C_{23}H_{15}FN_5$ $[M+H]^+$ 380.1311, found 380.1315.

7.6 References

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Chapter VIII

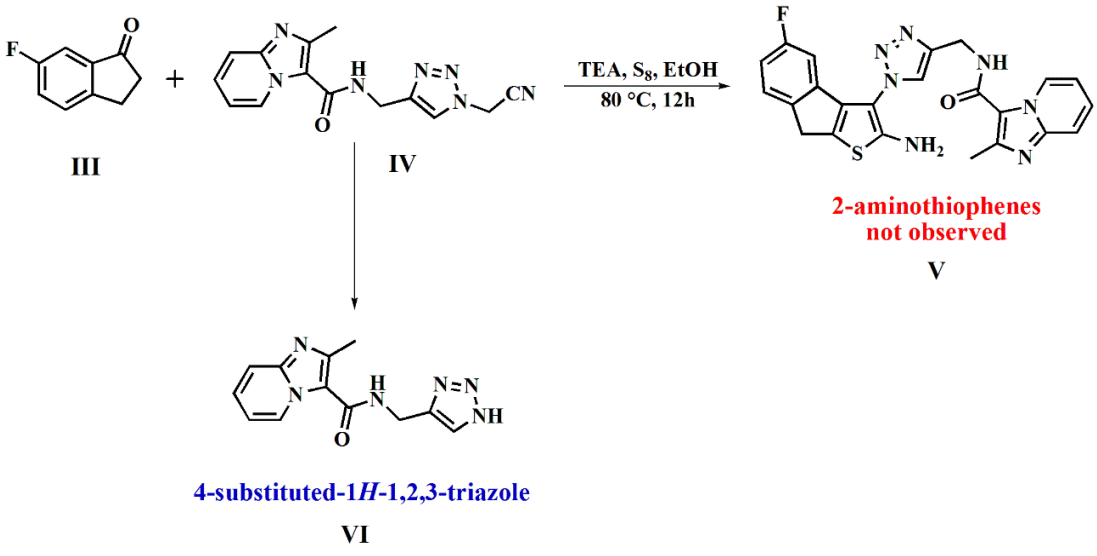
One-pot synthesis of 4-substituted-1*H*-1,2,3-triazole employing sulfur to deprotect methylene nitrile group

Chapter 8

One-pot synthesis of 4-substituted-1*H*-1,2,3-triazole employing sulfur to deprotect methylene nitrile group

8.1 Introduction

In the previous chapter we have successfully demonstrated the synthesis of 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinoline employing click and activate strategy. In continuation to explore the above strategy, we intended to synthesize 1,2,3-triazole tethered 2-aminothiophene framework employing Gewald type reaction as they possess ample applications in medicinal chemistry arena [1]. We subjected compound **III** and **IV** in the presence of TEA, S_8 in EtOH at 80 °C for 12 h. Surprisingly, compound corresponding to the structure **VI** was isolated instead of compound **V (Scheme 8.1)**.



Scheme 8.1 Gewald reaction attempted for the synthesis of 1,2,3-triazole tethered-2-aminothiophene.

From the literature we found that 4-substituted-1*H*-1,2,3-triazole skeleton has attractive medicinal properties like antiviral [2], antimalarial [3], anticancer [4], angiogenesis inhibition [5], anti-inflammatory [6], histone deacetylase inhibition [7], antibacterial [8], anxiolytic [9], and as CO_2 probing agents [10].

Plethora of synthetic strategies are available to synthesize this heterocycle. In general, trimethylsilyl azide or sodium azide is employed as azide source along with the synthons which are acetylene equivalents **figure 8.1** [11].

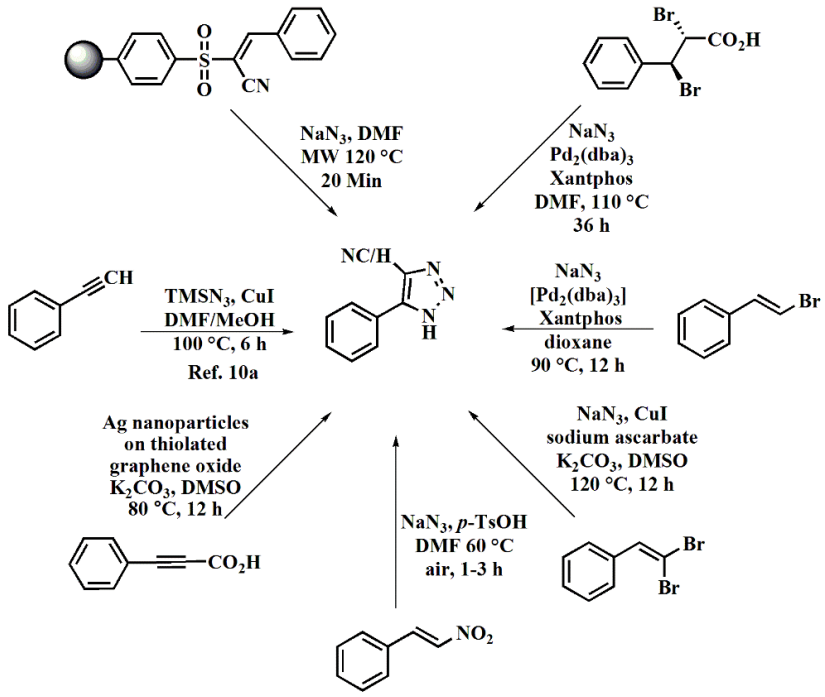


Figure 8.1 Various synthons of acetylene equivalents

Ueda et al. synthesized 1*H*-1,2,3-triazole employing terminal acetylenes, trimethylsilyl azide, CuI in a mixture of DMF and methanol (4:1) solvent at 100 °C for 6-20 h in about 69-86% [11a].

Kim et al. developed decarboxylative strategy employing aryl alkynyl carboxylic acids, NaN_3, K_2CO_3, Ag-decorated graphene oxide catalyst in DMSO at 80 °C for 12 h to obtain 1*H*-1,2,3-triazole in 50-75% yield [11b].

Quan et al. subjected arylnitroolefins, NaN_3, *p*-TsOH in DMF at 60 °C for 1-3 h in the presence of air as an oxidant to afford 1*H*-1,2,3-triazoles in 66-97% yield [11c].

Wang et al. utilized 1,1-dibromoalkenes, NaN_3, K_2CO_3, CuI, sodium ascorbate in DMSO at 120 °C for 12 h to obtain 1*H*-1,2,3-triazole in 61-86% yield [11d].

Barluenga et al. explored the reaction between alkenyl halides, NaN_3, $Pd_2(dba)_3$ and xantphos in dioxane at 90 °C for 10-24 h to obtain 1*H*-1,2,3-triazole in 45-94% yield [11e].

Zhang et al. treated *anti*-3-aryl-2,3-dibromopropanoic acids, NaN_3, $Pd_2(dba)_3$ and Xantphos in DMF at 110 °C for 36 h to obtain 1*H*-1,2,3-triazole in 50-71% yield [11f].

Gao et al. chose solid phase studies and designed polystyrene/1% divinylbenzene sodium sulfinate resin. The sulfinate was treated with α-bromoketones, amides and esters followed by Knoevenagel condensation with aldehydes. The resultant polymer-supported vinyl sulfone, NaN_3, DMF were subjected to MW irradiation at 120 °C for 20 Min to yield 37-78% of desired 1*H*-1,2,3-triazoles [11g].

On the other hand, azides are tagged to labile groups to afford *N*-protected 1,2,3-triazoles. These labile groups are then readily cleaved by acid, base or UV irradiation to afford 1*H*-1,2,3-triazoles **figure 8.2** [12].

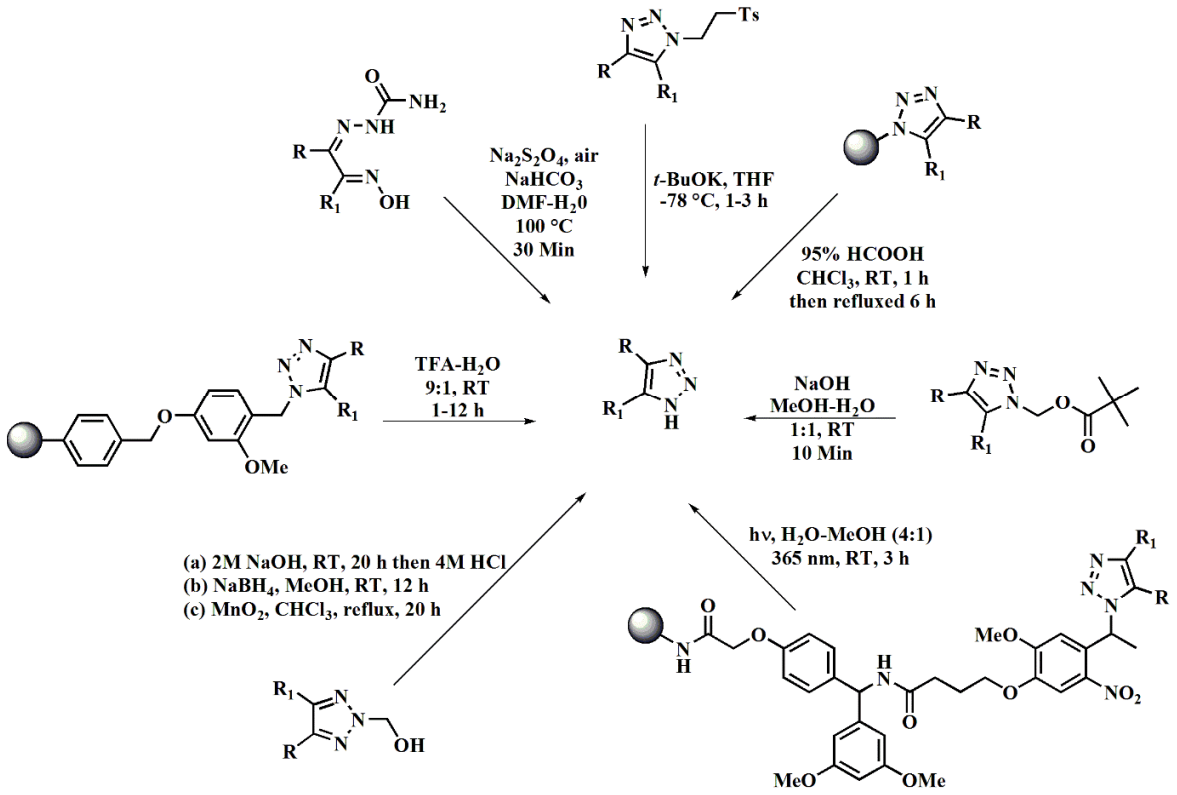


Figure 8.2 Synthesis of 4-substituted-1*H*-1,2,3-triazoles employing various deprotecting groups.

Harju et al. carried out solid phase synthesis of 1,2,3-triazoles prepared from the 2-methoxy resin or from the Wang resin with the 4-hydroxybenzyl substituent at nitrogen. The resultant *N*-substituted-1,2,3-triazoles upon treating with mixture of TFA-H_2O (9:1) at RT for 1-12 h afforded 1*H*-1,2,3-triazoles in 20-58% yield [12a].

Kalisiak et al. developed base promoted deprotection of *N*-hydroxymethyl triazoles. 2M NaOH was treated with *N*-hydroxymethyl triazoles and stirred for 12-20 h at RT to get 1*H*-1,2,3-triazoles in 83-99% yield [12b].

Qvortrup et al. utilized solid-supported photolabile azido-linker to construct *N*-substituted 1,2,3-triazoles. The resultant triazole was treated with H_2O-MeOH (4:1) mixture and irradiated UV radiation (365 nm) for 3 h at RT. 1*H*-1,2,3-triazoles were obtained in 52-95% yield [12c].

Loren et al. subjected *N*-methylpivalate protected triazoles with NaOH in MeOH-H_2O (1:1) for 10 min at RT to afford 1*H*-1,2,3-triazoles in 58-97% yield [12d].

Molteni et al. carried out the reaction of MeOPEG-supported-1,2,3-triazoles in $CHCl_3$ and 95% formic acid at RT for 1 h, and then refluxed for 6 h. 1*H*-1,2,3-triazoles were obtained in 71-85% [12e].

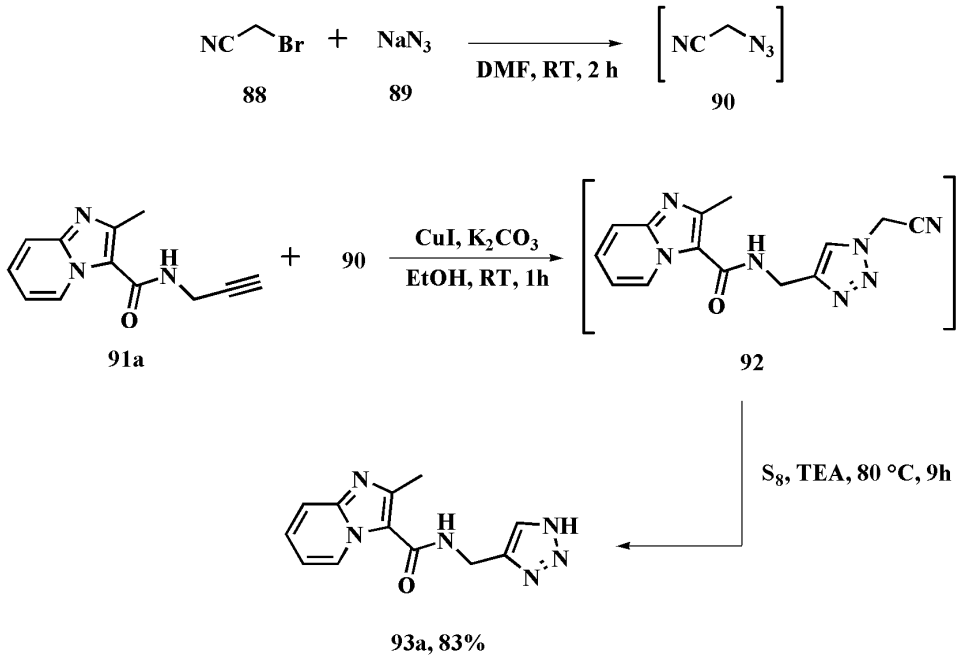
Yap et al. explored β-tosylethyl (TSE) group as useful synthon in the preparation of 1*H*-1,2,3-triazoles. *t*-BuOK in THF was treated with TSE-protected triazole at -78 °C. The resultant solution was slowly warmed to 0 °C over a period of 1-3 h to afford 61-93% [12f].

He et al. explored the synthesis of 1*H*-1,2,3-triazoles without employing azides, acetylene equivalents and metal catalization. Arylglyoxaldoxime semicarbazone in DMF was treated with sodium bicarbonate under the O_2 atmosphere followed by addition of sodium dithionite and

water was added. Resulting mixture was heated at 100 ℃ for 10-20 min. The yields varied in the range 69-98% [12g].

Most of the existing reports deal with prerequisite acetylene and azide synthons which are prepared from special reagents. Employing strong acids, bases, oxidants, higher temperature, and longer reaction time makes them disadvantageous. Thus an operationally simpler system to construct the 1*H*-1,2,3-triazoles was developed employing elemental sulfur as key reagent in the deprotection process of methylene nitrile group (**scheme 8.2**).

8.2 Results and Discussion



Scheme 8.2 Synthetic strategy for the synthesis of 4-substituted-1*H*-1,2,3-triazoles

As a model reaction, a screw top vial was charged with bromoacetonitrile (1 mmol), sodium azide (1 mmol) and DMF (0.2 mL). After stirring at room temperature for 2 h, the reaction

mixture was diluted with 0.8 mL of EtOH followed by the addition of **91a** (1 mmol), CuI (0.1 mmol) and K_2CO_3 (1 mmol). The resultant mixture was stirred for 1 h at RT to form triazole **92**. Then elemental sulfur (1 mmol) was added and continued stirring at 80 °C. After completion of the reaction as indicated by the TLC, volatiles were removed under vacuo and the resultant crude was chromatographed to get desired N-((1*H*-1,2,3-triazol-4-yl)methyl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (**93a**) in 83% yield. With this encouraging result, we then focused our attention on optimizing the reaction conditions by screening various parameters such as catalysts, bases, solvents, and temperature to obtain a satisfactory yield for this one-pot reaction and the results are summarized in **Table 8.1**.

Table 8.1 Optimization of one-pot reaction conditions[a]

Entry	Copper source	Solvent (0.2 mL:0.8 mL)	Base	Sulfur source	T (°C)/ Time	Yield[b] %
1	CuI	DMF + EtOH	K_2CO_3	S_8	80/9 h	83
2	CuI	DMF + EtOH	Cs_2CO_3	S_8	80/5 h	75
3	CuI	DMF + EtOH	*t*-BuOK	S_8	80/7 h	CR[c]
4	CuI	DMF + EtOH	DIPEA	S_8	80/8 h	74
5	CuI	DMF + EtOH	DBU	S_8	80/9 h	79
6	CuI	DMF + EtOH	TEA	S_8	80/7 h	91
7	CuI	DMF + EtOH	TEA	$Na_2S.9H_2O$	80/7 h	CR[c]
8	CuI	DMF + EtOH	TEA	$Na_2S_2O_3.5H_2O$	80/7 h	CR[c]
9	CuI	DMF + EtOH	TEA	NaSH	80/7 h	CR[c]
10	CuI	DMF (1 mL)	TEA	S_8	80/12 h	62

11	CuI	H_2O + EtOH	TEA	S_8	80/8 h	45
12	CuI	DMSO (1 mL)	TEA	S_8	80/9 h	54
13	CuI	DMF + PEG	TEA	S_8	80/10 h	41
14	CuI	DMAc (1 mL)	TEA	S_8	80/8 h	68
15	CuI	DMF + ACN	TEA	S_8	80/8 h	78
16	CuI	DMF + *i*-PrOH	TEA	S_8	80/6 h	83
17	CuI	DMF + *t*-BuOH	TEA	S_8	80/6 h	74
18	CuI	DMF + MeOH	TEA	S_8	80/6 h	80
19	CuBr	DMF + EtOH	TEA	S_8	80/6 h	72
20	CuCl	DMF + EtOH	TEA	S_8	80/6 h	76
21	$Cu(OAc)_2$	DMF + EtOH	TEA	S_8	80/6 h	61
22	CuI	DMF + EtOH	TEA	S_8	90/6 h	82
23	CuI	DMF + EtOH	TEA	S_8	70/7 h	79

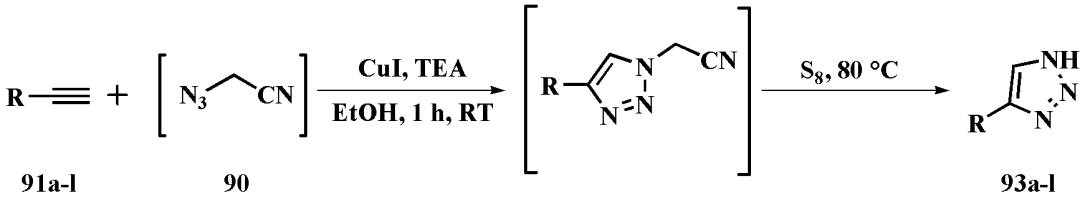
[a]Quantities used: **91** (1 mmol), bromoacetonitrile (1 mmol), NaN_3 (0.1 mmol), catalyst (0.1 mmol), base (3 mmol), sulfur source (1 mmol), solvent (1 mL). [b]yields are for isolated products. [c]complex reaction mixture.

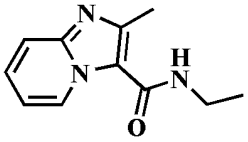
Employing Cs_2CO_3 lower yield of the product (**Table 8.1, entry 2**) was noticed. Complex reaction was observed when *t*-BuOK was used as a base. Hopping to organic bases DIPEA and DBU didn't influence the yield of the product much. Nevertheless, TEA was found to be an efficient base to afford the product in 91% yield. Next, various sulfur sources ($Na_2S.9H_2O$, $Na_2S_2O_3.5H_2O$ and NaSH) suppressed the reaction yielding a complex reaction mixture (**entry 7-9**). Further, the effectiveness of mixture of solvents was tested and found to be ineffective (**entry**

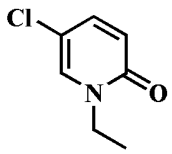
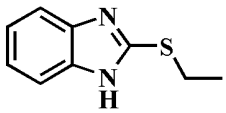
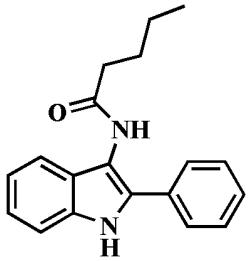
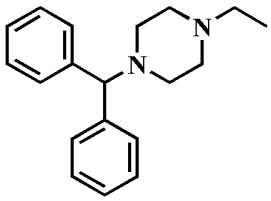
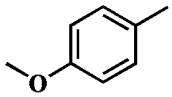
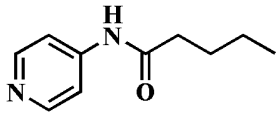
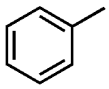
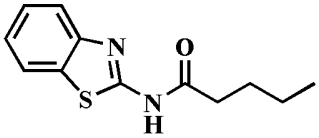
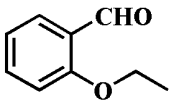
10-18). Subsequently, the catalytic activity of copper salts was assessed (**entries 19–21**) and found to afford diminished yields. Decrease in the yield was observed when the reaction was performed with CuI (0.1 mmol), S_8 (1 mmol), TEA (3 mmol) at 90 °C and 70 °C (**entries 22–23**). Thus, the catalytic system employing CuI (0.1 mmol), S_8 (1 mmol), TEA (3 mmol) in DMF-EtOH (1:4) at 80 °C for 7 h emerged to be optimal in realizing this synthetic strategy (**entry 6**).

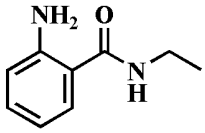
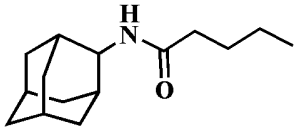
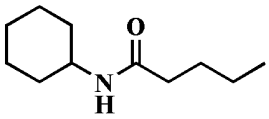
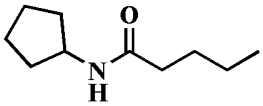
With this fine-tuned one-pot strategy, further efforts were put forth to expand the generality and scope of this methodology by varying different alkynes (**Table 8.2**). Acetylene linked to the heterocyclic compounds took 5-7 h for the reaction to complete affording desired compound in good yield (**93a-i**). Further to test the functional group tolerance we employed acetylenes bearing aldehyde and amine functional group. Reaction was smooth enough to yield the desired compound in good yield (**93j** & **93k**). Next we attempted to synthesize acetylenes linked to adamantyl, cyclohexyl and cyclopentyl systems. The reaction was found to be efficient affording the desired product (**93a-i, Table 8.2**).

Table 8.2 Screening of diverse acetylenes for the one-pot reaction[a]



Compound	R	Time	Yield[b]
93a		7 h	91%

93b		7 h	86%
93c		7 h	64%
93d		7 h	77%
93e		5 h	86%
93f		5 h	85%
93g		7 h	62%
93h		5 h	89%
93i		7 h	74%
93j		5 h	87%

93k		5 h	79%
93l		7 h	72%
93m		7 h	81%
93n		7 h	63%

[a]Quantities used: **91** (1 mmol), **88** (1 mmol), **89** (0.1 mmol), CuI (0.1 mmol), TEA (3 mmol), S_8 (1 mmol), DMF:EtOH (1:5), 80 °C. [b]yields are for isolated products.

8.3 Conclusion

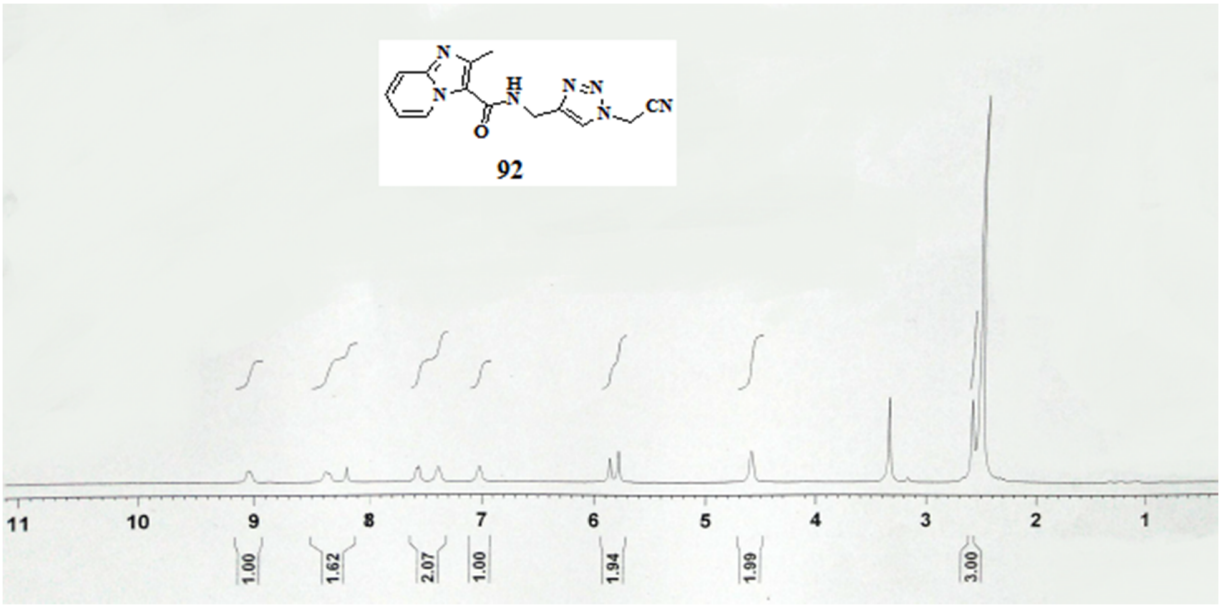
In conclusion, an operationally simpler system to construct the 1*H*-1,2,3-triazoles was developed employing elemental sulfur as a key reagent in the deprotection process of methylene nitrile group. The strategy revealed good tolerance to various substrates affording desired products in moderate to good yields.

8.4 Experimental Section

8.4.1 Materials and methods

Chemicals and solvents were procured from commercial sources and are analytically pure. 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 230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded at 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) are given in ppm. IR spectra were recorded on a FT-IR spectrometer (Shimadzu) 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. HRMS were recorded on a QSTAR XL hybrid MS/MS mass spectrometer.

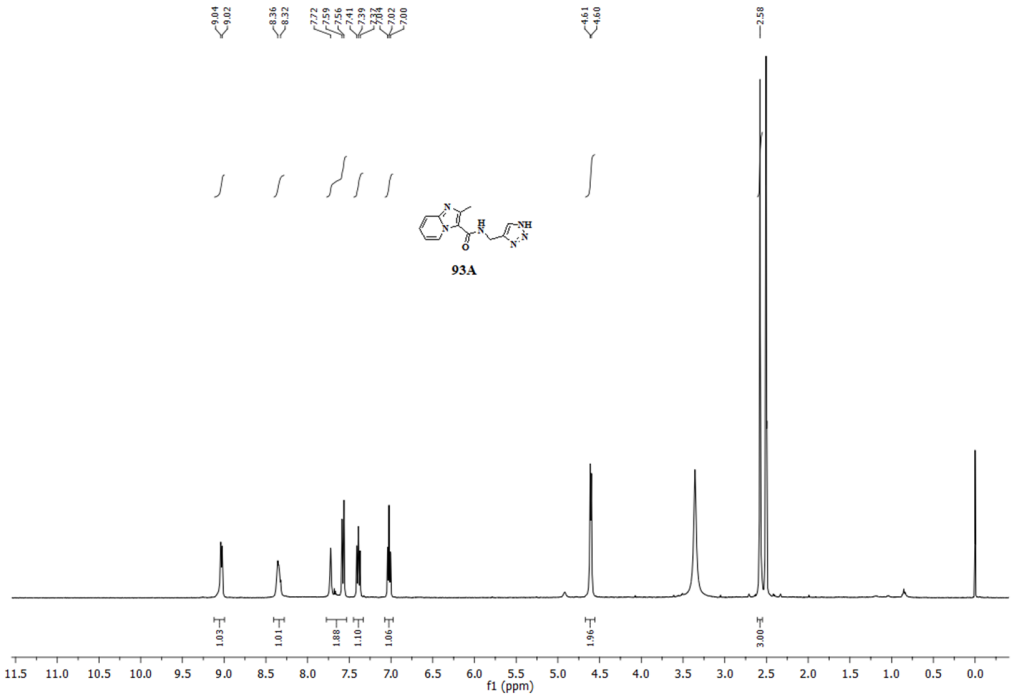


^{1}H NMR spectrum of compound **92**

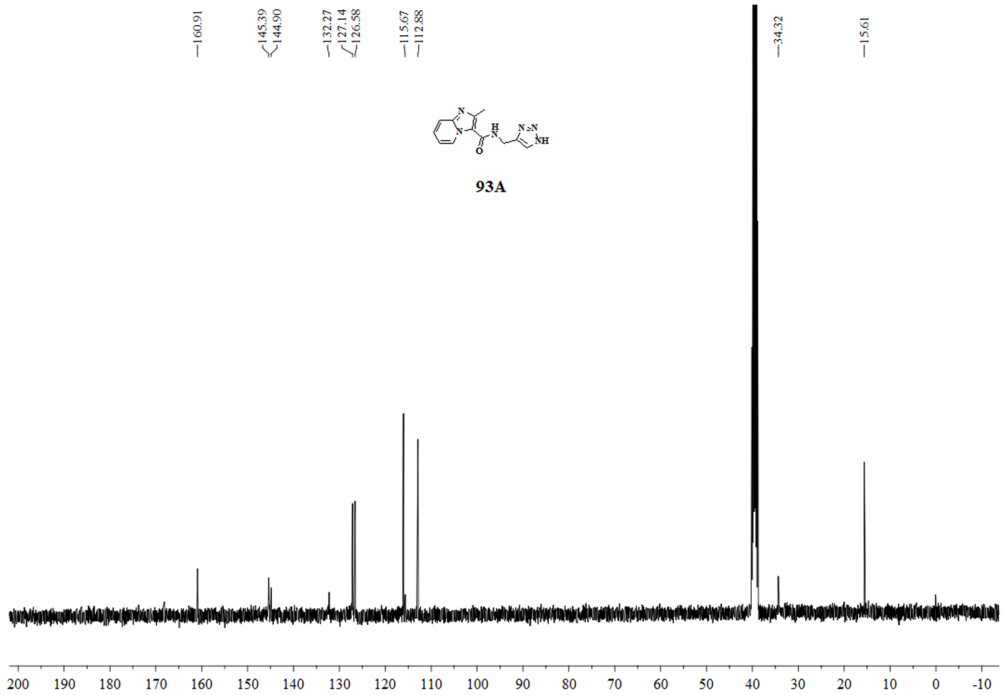
Synthesis of 4-substituted-1*H*-1,2,3-triazole (93a-n)

A screw capped vial was charged bromoacetonitrile **88** (1 mmol), sodium azide **89** (1 mmol) and DMF (0.2 mL). After stirring at room temperature for 2 h, the reaction mixture was diluted with 0.8 mL of EtOH followed by the addition of **91a-n** (1 mmol), CuI (0.1 mmol) and TEA (1 mmol). The resultant mixture was stirred for 1 h at RT to form triazole **92a-n**. Then elemental sulfur (1 mmol) was added and continued stirring at 80 °C. After completion of the reaction as indicated by the TLC, reaction mass was allowed to cool to ambient temperature, diluted with water (5 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layer was dried with

anhydrous Na_2SO_4 and evaporated to dryness. The crude material was purified by column chromatography to obtain desired 4-substituted-1*H*-1,2,3-triazole (**93a-n**).



^{1}H NMR spectrum of compound **93a**



^{13}C NMR spectrum of compound **93a**

***N*-((1*H*-1,2,3-triazol-4-yl)methyl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (93a)**

Beige solid (91%); mp 223-225 °C; IR ν_{max} (KBr, cm^{-1}) 3635, 3420, 3041, 2243, 1672, 742; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, *J* = 6.7 Hz, 1H), 8.34 (d, *J* = 13.3 Hz, 1H), 7.81 – 7.51 (m, 2H), 7.46 – 7.31 (m, 1H), 7.02 (t, *J* = 7.3 Hz, 1H), 4.60 (d, *J* = 5.6 Hz, 2H), 2.58 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.91, 145.39, 144.90, 132.27, 127.14, 126.58, 115.67, 112.88, 34.32, 15.61. HRMS (ESI, m/z): Calcd for $C_{12}H_{13}N_6O$ $[M+H]^+$ 257.1151, found 257.1109.

1-((1*H*-1,2,3-triazol-4-yl)methyl)-5-chloropyridin-2(1*H*)-one (93b)

Beige solid (86%); mp 175-177 °C; IR ν_{max} (KBr, cm^{-1}) 3089, 2917, 1669, 823; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.87 (s, 1H), 8.08 (d, *J* = 2.8 Hz, 1H), 7.71 (s, 1H), 7.50 (dd, *J* = 9.7, 2.8 Hz, 1H), 6.45 (d, *J* = 9.7 Hz, 1H), 5.16 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.52, 143.67, 142.08, 131.98, 123.56, 118.56, 118.33, 47.04. HRMS (ESI, m/z): Calcd for $C_8H_8ClN_4O$ $[M+H]^+$ 211.0387, found 211.0314.

2-(((1*H*-1,2,3-triazol-4-yl)methyl)thio)-1*H*-benzo[*d*]imidazole (93c)

Beige solid (64%); mp 148-150 °C; IR ν_{max} (KBr, cm^{-1}) 3046, 2943, 1644, 743; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.80 (s, 1H), 12.62 (s, 1H), 7.71 (s, 1H), 7.46 (d, *J* = 4.0 Hz, 2H), 7.13 (d, *J* = 8.9 Hz, 2H), 4.63 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.22, 143.90, 138.45, 132.38, 124.33, 115.42, 35.07. HRMS (ESI, m/z): Calcd for $C_{10}H_{10}N_5S$ $[M+H]^+$ 232.0657, found 232.0648.

***N*-(2-phenyl-1*H*-indol-3-yl)-3-(1*H*-1,2,3-triazol-4-yl)propanamide (93d)**

Beige solid (77%); mp 209-210 °C; IR ν_{max} (KBr, cm^{-1}) 3472, 3088, 2907, 1683, 743; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 9.47 (s, 1H), 7.74 (m, 3H), 7.46 (dd, *J* = 16.4, 8.9 Hz, 2H), 7.34 (dd, *J* = 17.8, 7.8 Hz, 2H), 7.22 (d, *J* = 7.5 Hz, 1H), 7.10 (dd, *J* = 16.8, 9.4 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 3.02 (t, *J* = 6.9 Hz, 2H), 2.77 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.86, 143.67, 134.64, 131.12, 130.03, 129.01, 128.55, 127.97, 127.07, 126.8, 123.4, 121.56, 119.06, 116.78, 112.73, 111.93, 36.78, 31.23. HRMS (ESI, m/z): Calcd for $C_{19}H_{18}N_5O$ $[M+H]^+$ 332.1511, found 332.1524.

1-((1*H*-1,2,3-triazol-4-yl)methyl)-4-benzhydrylpiperazine (93e)

Dark brown solid (86%); mp 81-83 °C; IR ν_{max} (KBr, cm^{-1}) 3083, 2897, 1677, 1653, 746, ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.87 (s, 1H), 7.69 (s, 1H), 7.40 (d, *J* = 7.4 Hz, 4H), 7.27 (t, *J* = 7.5 Hz, 4H), 7.16 (t, *J* = 7.2 Hz, 2H), 4.25 (s, 1H), 3.60 (m, 2H), 3.45-3.25 (s, 4H), 2.25-2.22 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 143.45, 142.76, 132.45, 130.22, 128.40, 126.39, 74.56, 55.31, 53.67, 51.78. HRMS (ESI, m/z): Calcd for $C_{20}H_{24}N_5$ $[M+H]^+$ 334.2032, found 334.2018.

4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (93f)

White solid (85%); mp 164-165 °C; IR ν_{max} (KBr, cm^{-1}) 3821, 3713, 2893, 1678, 1517, 1463, 1099, 759; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1 H), 7.79 (d, J = 8.4 Hz, 2 H), 7.01 (d, J = 8.8 Hz, 2 H), 3.77 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.4, 144.9, 127.1, 122.8, 114.4, 55.2; HRMS (ESI, m/z): Calcd for $C_{11}H_{14}N_5O$ $[M+H]^+$ 176.0824, found 176.0809.

***N*-(pyridin-4-yl)-4-(1*H*-1,2,3-triazol-4-yl)butanamide (93g)**

Beige solid (62%); mp 185-187 °C; IR ν_{max} (KBr, cm^{-1}) 3481, 3214, 2922, 1696, 1660; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.59 (s, 1H), 10.30 (s, 1H), 8.41 (d, *J* = 5.8 Hz, 2H), 7.56 (d, *J* = 6.2 Hz, 3H), 2.70 (t, *J* = 7.5 Hz, 2H), 2.40 (t, *J* = 7.4 Hz, 2H), 1.99 – 1.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.43, 156.77, 150.34, 143.78, 132.34, 110.59, 36.41, 32.45, 26.68. HRMS (ESI, m/z): Calcd for $C_{11}H_{14}N_5O$ $[M+H]^+$ 232.1198, found 232.1182.

4-phenyl-1*H*-1,2,3-triazole (93h)

White solid (89%); mp 144-145 °C; IR ν_{max} (KBr, cm^{-1}) 3854, 3746, 2852, 1699, 1558, 1456, 1081, 765; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.48-7.38 (m, 2H), 7.37-7.27 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.3, 130.4, 129.0, 128.4, 128.2, 125.6; HRMS (ESI, m/z): Calcd for $C_{11}H_{14}N_5O$ $[M+H]^+$ 146.0718, found 146.0781.

***N*-(benzo[*d*]thiazol-2-yl)-4-(1*H*-1,2,3-triazol-4-yl)butanamide (93i)**

Brown solid (74%); mp 154-155 °C; IR ν_{max} (KBr, cm^{-1}) 3462, 3273, 2902, 1648, 1618, 739; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.68 – 7.51 (m, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.30 (t, *J* = 7.4 Hz, 1H), 2.84 – 2.65 (m, 2H), 2.61-2.56 (m, 2H), 2.09 – 1.88 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.55, 173.45, 149.38, 143.59, 132.67, 126.77, 125.97, 124.66, 122.52, 121.67, 36.77, 32.54, 26.49. HRMS (ESI, m/z): Calcd for $C_{13}H_{14}N_5OS$ $[M+H]^+$ 288.0919, found 288.0978.

2-((1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (93j)

Brown liquid (87%); IR ν_{max} (KBr, cm^{-1}) 3239, 2902, 1708, 1672, 768; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.04 (s, 1H), 10.35 (s, 1H), 7.69 (dd, *J* = 14.5, 8.0 Hz, 2H), 7.49 – 7.38 (m, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 5.39 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.39, 161.58, 143.33, 136.75, 131.23, 128.44, 127.74, 122.27, 115.37, 73.22. HRMS (ESI, m/z): Calcd for $C_{10}H_{10}N_3O_2$ $[M+H]^+$ 204.0773, found 204.0791.

***N*-((1*H*-1,2,3-triazol-4-yl)methyl)-2-aminobenzamide (93k)**

Brown solid (79%); mp 138-140 °C; IR ν_{max} (KBr, cm^{-1}) 3458, 3386, 3321, 3146, 2911, 1651, 752; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.67 (s, 1H), 8.74 (t, *J* = 5.4 Hz, 1H), 7.67 (s, 1H), 7.51 (d, *J* = 7.0 Hz, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.51 (t, *J* = 7.4 Hz, 1H), 4.48 (d, *J* = 5.7 Hz, 2H), 3.37 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.88, 148.23, 142.83, 134.99, 132.87, 129.33, 119.44, 118.44, 116.64, 44.39. HRMS (ESI, m/z): Calcd for $C_{10}H_{12}N_5O$ $[M+H]^+$ 218.1042, found 218.1087.

***N*-(adamantan-2-yl)-4-(1*H*-1,2,3-triazol-4-yl)butanamide (93l)**

Brown solid (72%); mp 188-190 °C; IR ν_{max} (KBr, cm^{-1}) 3233, 2913, 1655, 1611, 1602, 1453; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (s, 1H), 7.24 (s, 1H), 2.59 (m, 2H), 2.04 (t, *J* = 7.4 Hz, 2H), 1.99 (m, 3H), 1.90 (m, 6H), 1.82 – 1.72 (m, 2H), 1.60 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 142.9, 131.0, 48.2, 40.5, 37.8, 36.4, 32.7, 31.7, 25.8. HRMS (ESI, m/z): Calcd for $C_{16}H_{25}N_4O$ $[M+H]^+$ 289.2028, found 289.2071.

***N*-cyclopentyl-4-(1*H*-1,2,3-triazol-4-yl)butanamide (93m)**

Yellow liquid (81%); IR ν_{max} (KBr, cm^{-1}) 3239, 2918, 1653, 1612, 1588, 1423; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.53 (s, 1H), 7.65 (s, 1H), 7.56 (s, 1H), 3.51 (d, *J* = 7.5 Hz, 1H), 2.70 – 2.55 (m, 2H), 2.07 (t, *J* = 7.3 Hz, 2H), 1.86 – 1.74 (m, 2H), 1.68 (t, *J* = 15.0 Hz, 4H), 1.54 (d, *J* = 12.2 Hz, 1H), 1.30 – 1.04 (m, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.67, 143.23, 132.59, 48.58, 37.45, 34.22, 32.87, 28.76, 25.33, 22.40. HRMS (ESI, m/z): Calcd for $C_{12}H_{21}N_4O$ $[M+H]^+$ 237.1715, found 237.1769.

***N*-cyclohexyl-4-(1*H*-1,2,3-triazol-4-yl)butanamide (93n)**

Yellow liquid (63%); IR ν_{max} (KBr, cm^{-1}) 3244, 2921, 1646, 1609, 1597, 1444; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.53 (s, 1H), 7.76 (s, 1H), 7.56 (s, 1H), 4.05 – 3.88 (m, 1H), 2.61 (dd, *J* = 14.3, 6.9 Hz, 2H), 2.07 (t, *J* = 7.4 Hz, 2H), 1.88 – 1.70 (m, 4H), 1.68 – 1.55 (m, 2H), 1.53 – 1.41 (m, 2H), 1.39 – 1.29 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.46, 143.59, 132.04, 55.39, 37.44, 34.85, 32.19, 26.41, 25.83. HRMS (ESI, m/z): Calcd for $C_{11}H_{19}N_4O$ $[M+H]^+$ 223.1559, found 223.1523.

8.5 References

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Chapter IX

Summary and Conclusion

Summary and Conclusion Chapter 9

PART-A

Rapid spread of MDR and XDR and the recently identified TDR TB strains have been reported. As a result, population of TB patients caused by MTB are increasing in an alarming rate, revealing ineptitude of currently available medicine in the market. Treatment of drug resistant TB is prolonged and expensive, possesses toxicity and adverse reactions are sometimes severe and irreversible. Hence we designed the compounds emphasizing molecular hybridisation approach to merit cost effective and reduced treatment time. Active core of existing antitubercular molecules were identified and made an attempt to tailor them in a single entity anticipating improved drug like features. Three series were synthesized, characterized by ^{1}H, ^{13}C, LCMS, HRMS and evaluated for their antimycobacterial activity.

In **chapter 3**, twenty two ciprofloxacin analogues were synthesized and evaluated for their antimycobacterial activity against MTB $H_{37}Rv$ strain. Amongst, the synthesized compounds, **3p** exhibited 99% inhibition of MTB $H_{37}Rv$ strain with MIC **32 µg/mL**. Compounds **3f** and **3j-m** were significantly active against MTB with MIC **16 µg/mL.** Compound **3c** and **3d** exhibited good activity with MIC **8** and **4 µg/mL** respectively. The anti-tubercular SAR profile suggests that tailoring benzyl and acetyl group by means of appropriate substituent or functional group might provide an insight to obtain the lead compound.

In **chapter 4** we have two schemes. In **scheme 1**, seventeen phenanthridine analogues were synthesized and evaluated for their antimycobacterial activity against MTB $H_{37}Rv$ strain. MTB activity profile of the synthesized compounds point out that further activity can be enhanced by opting appropriate substituent on the phenyl ring at the 4^{th} position of 6-(piperazin-1-yl)phenanthridine. Incorporating pyridine (**8j**, MIC = 1.56 µg/mL) and pyrimidine (**8k**, MIC = 1.56 µg/mL) ring drew a significant attention to employ other heterocycles as well. Nitro group at the *para* position on the phenyl ring has greatly increased the activity of the compound (**8e**, MIC = 1.56 µg/mL).

From the **scheme 2**, preliminary anti-tubercular screening results drive us to engineer the chemical structure of phenanthridine derivative to generate essential pharmacophoric features that could lead to the synthesis of a promising candidate to develop anti-tubercular agent. We discovered that incorporating sulfonyl group in the moiety (**11a**, MIC = 1.56 µg/mL) plays a pivotal role in the activity profile. These findings unfold the possibility of employing various functional groups on this derivative.

In **chapter 5**, twenty six aminothiazole analogues were synthesized and evaluated for their antimycobacterial activity. We found that integrating *N*-methyl-4-(4-(2-(piperazin-1-yl)ethyl)phenyl)thiazol-2-amine to benzhydryl piperazine through propyl linker emerged as a prospective candidate by inhibiting the MTB $H_{37}Rv$ strain at concentration 1.56 µg/mL (**18h**).

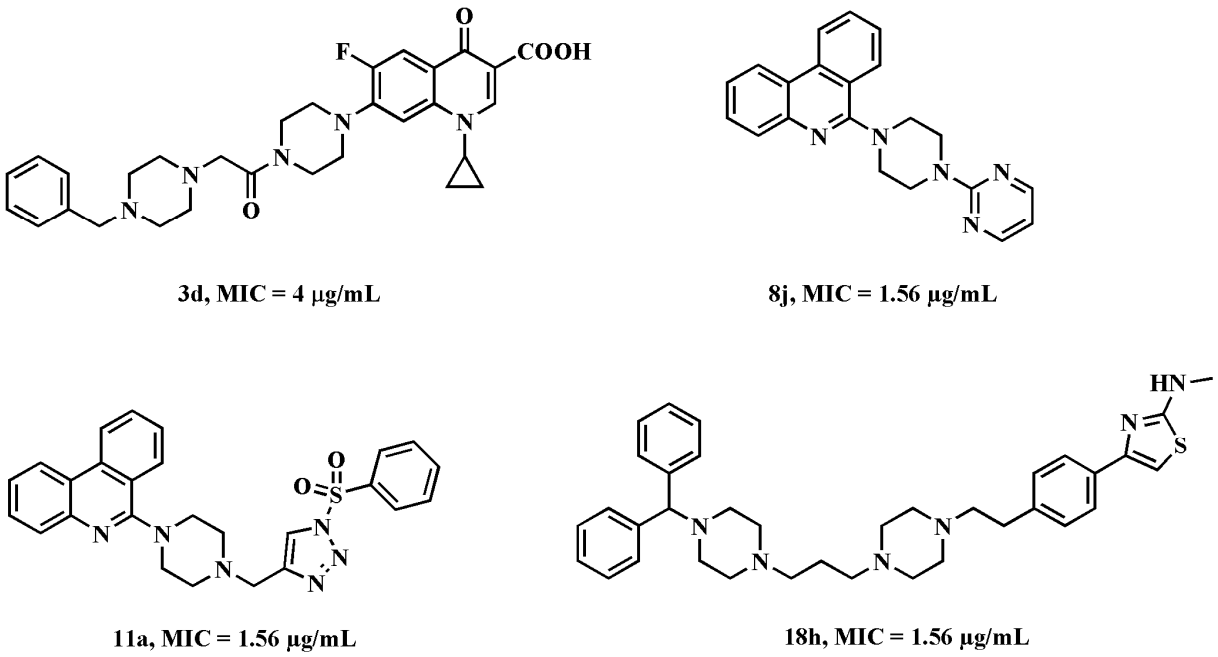

Figure 9.1: Active structures of synthesized compounds

In conclusion, the active compounds depicted in **figure 9.1** found to be promising lead compounds. Structural optimization with suitable substituents could help us to obtain hit compounds for further development to combat TB.

PART B

In **chapter 7**, twenty one 1,2,3-triazole anchored benzimidazo[1,2-*a*]quinolines were synthesized employing click and activate strategy. Quantum yields of synthesized compounds were determined in $CHCl_3$ solution (within a maximum absorbance close to 0.1) and was calculated relative to the quantum yield of standard compound quinine sulphate in 0.1 M H_2SO_4 ($Ø_F$ = 0.54). Amongst the synthesized compounds, **83u** possessed high quantum yield of 0.21,

probably due to the contribution from a lone pair of electrons on the –F substituent and presence of a rich π-electron system. Owing to the high extinction coefficients (>150 nm), synthesized molecules could function as versatile feedstock for biological and fluorescent applications.

In **chapter 8**, fourteen 4-substituted-1*H*-1,2,3-triazoles were synthesized employing elemental sulfur to deprotect the methylene nitrile group. The strategy revealed good tolerance to various substrates affording desired products in moderate to good yields.

In conclusion, click and activate strategy could serve as an important platform to generate immensely decorated 1,2,3-traizole tethered framework. Preliminary fluorescent studies provide insights to further explore the skeleton towards material and biological applications. Further, methylene nitrile revealed to be an excellent alternative as a protecting group.

Future perspectives

- Library of compounds synthesized emphasizing on hybridization approach found to be fruitful in identifying the potential hit molecules. This strategy could be effectively extended to develop promising lead molecules.

- As the antimycobacterial results are encouraging, *in vivo* studies need to be performed to understand the pharmacodynamic and pharmacokinetic profile of the potent analogues for lead optimization.

- Extensive studies on mechanism of action need to be carried out to identify the target site.

- Detailed toxicity profile of active compounds need to be carried out.

- 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinolines were found to be good fluorogenic substrates. Exploring on materials and biological applications could fetch interesting results.

- As 4-substituted-1*H*-1,2,3-triazoles have potential material and biological applications, synthesized compounds could be explored to identify the active molecules.

- Mechanism of deprotection process of methylene nitrile group needs to be identified.

Appendix

List of Publications

From thesis work:

1) **Nagesh**, **H.N**., Mahalakshmi Naidu, K., Harika Rao, D., Padma Sridevi, J., Sriram, D., Yogeeswari, P., Chandra Sekhar, K.V.G. Design, synthesis and evaluation of 6-(4-((substituted-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine analogues as antimycobacterial agents. ***Bioorg. Med. Chem. Lett. 2013, 23, 6805-6810***.

2) **Nagesh**, **H.N**., Suresh, N., Mahalakshmi Naidu, K., Arun, B., Sridevi, J.P., Sriram, D., Yogeeswari, P., Chandra Sekhar, K.V.G. Synthesis and evaluation of anti-tubercular activity of 6-(4-substitutedpiperazin-1-yl) phenanthridine analogues. ***Eur. J. Med. Chem.* 2014, 74, 333-339**.

3) **Nagesh**, **H.N**., Suresh, A., Sairam, S.D.S.S., Sriram, D., Yogeeswari, P., Chandra Sekhar, K.V.G. Design, synthesis and antimycobacterial evaluation of 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine hybrid analogues. ***Eur. J. Med. Chem.* 2014, 84, 605-613**.

4) Suresh, N., **Nagesh**, **H.N**., Renuka, J., Rajput, V., Sharma, R., Khan, I.A., Chandra Sekhar, K.V.G. Synthesis and evaluation of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-substitutedpiperazin-1-yl)acetyl) piperazin-1-yl)quinoline-3-carboxylic acid derivatives as anti-tubercular and antibacterial agents. ***Eur. J. Med. Chem.* 2014, 71, 324-332**.

5) **Nagesh**, **H.N**., Suresh, A., Nagarjuna Reddy, M., Suresh, N., Subbalakshmi, J., Chandra Sekhar, K.V.G. Multicomponent cascade reaction: Dual role of copper in the synthesis of 1,2,3-triazole tethered benzimidazo[1,2-*a*]quinoline and their photophysical studies. ***RSC Adv.* 2016, 6, 15884-15894**.

6) **Nagesh**, **H.N**., Srinivas Rao, S., Suresh, A., Chandra Sekhar, K.V.G. One-pot synthesis of 4-substituted-1*H*-1,2,3-triazole employing sulfur to deprotect methylene nitrile group. (Manuscript under preparation).

Other publications:

1) Suresh, N., **Nagesh**, **H.N**., Anil Kumar, Shirazi, A.N., Parang, K., Chandra Sekhar, K.V.G. Synthesis of novel ciprofloxacin analogues and evaluation of their anti-proliferative effect on human cancer cell lines. ***Bioorg. Med. Chem. Lett.*** **2013, 23, 6292-6295**.

2) Chandra Sekhar, K.V.G., Rao. V.S., Tara Sasank, T. V. N. V., **Nagesh**, **H.N**., Suresh, N, Mahalakshmi Naidu, K., Suresh, A., Synthesis of 3,5-diarylisoxazoles under solvent-free conditions using iodobenzene diacetate. ***Chin. Chem. Lett.*** **2013, 24, 1045 -1048**.

3) **Nagesh**, **H.N**., Suresh, N., Bhanu Prakash, G.V.S., Gupta, S., Rao, J.V., Chandra Sekhar, K.V.G. Design, synthesis and biological evaluation of novel phenanthridinyl piperazine triazoles via click chemistry as anticancer agents. ***Med. Chem. Res.*** **2015, 24, 523-532**.

4) Mahalakshmi Naidu, K., **Nagesh**, **H.N**., Manjeet, S., Sriram, D., Yogeeswari, P., Chandra Sekhar, K.V.G. Novel amide and sulphonamide derivatives of 6-(piperazin-1-yl)phenanthridine as potent *Mycobacterium tuberculosis* $H_{37}Rv$ inhibitors. ***Eur. J. Med. Chem.*** **2015, 92, 415-426**.

Papers presented at Conferences

1) **Nagesh**, **H.N**., Suresh, N., Mahalakshmi Naidu, K., Rao, V.S., Chandra Sekhar, K.V.G. Synthesis of phenanthridinyl piperazines as novel Dopamine D_2 antagonists. 14^{th} CRSI National Symposium on Chemistry, CSIR-NIIST, Thiruvananthapuram, February 3-$5^{th,}$ 2012.

2) **Nagesh**, **H.N**., Mahalakshmi Naidu, K., Suresh, N., Chandra Sekhar, K.V.G. Design and synthesis of novel phenanthridine derivatives as anticancer agents. 19th ISCB International Conference (ISCBC – 2013), Mohanlal Sukhadia University Udaipur, March 2-5th, 2013.

3) **Nagesh**, **H.N**., Mahalakshmi Naidu, K., Suresh, A., Subbalakshimi, J., Sriram, D., Yogeeswari, P., Chandra Sekhar, K.V.G. Design, synthesis and anti-tubercular evaluation of novel benzo[*d*]isoxazole hybrid analogues. International conference on drugs for the Future: Infectious Diseases, Antimicrobial Drug Discovery: Challenges and perspectives DFID-2014, NIPER, Hyderabad, March 27-28th, 2014.

4) **Nagesh**, **H.N**., Suresh, A., Sriram, D., Yogeeswari, P., Chandra Sekhar, K.V.G. A new hybrid approach for design, synthesis and evaluation of substituted phenethylthiazole derivatives as antitubercular agents. 15th Tetrahedron Symposium - Asian Edition on Challenges in Bioorganic and Organic Medicinal Chemistry organised by Elsevier at Singapore Expo, Singapore, October 28 -31st, 2014.

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 after 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 one 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 research interest are synthetic medicinal chemistry and drug design. As investigator, he successfully completed major research projects funded by UGC, DST and DBT. 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 chemist, Indian association of chemistry teachers etc.

Biography of Mr. Nagesh H.N

Mr. Nagesh H.N completed his B.Sc (Physics, Chemistry and Mathematics) in 2004 from University of Mysore. He completed his M.Sc (Inorganic chemistry) in 2006 from University of Mysore. He worked as a Senior Research Associate in Syngene International Limited for four years nine months. He joined as a project fellow (UGC) in BITS Pilani, Hyderabad campus in 2011-April under the supervision of Prof. K.V.G. Chandra Sekhar. He was awarded DBT travel grant for international travel support for attending the 15^{th} Tetrahedron symposium in Singapore. He has published five scientific papers in international journals and presented six papers at national and international conferences.