Click Chemistry Inspired Synthesis of Novel Triazolyl Conjugates and their Antimicrobial Activity

THESIS

Submitted in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

by

Poonam Khedar

Under the supervision of

Prof. Anil Kumar

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PILANI (RAJASTHAN) INDIA

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Dedicated to

My family

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI (RAJASTHAN)

CERTIFICATE

This is to certify that the thesis entitled **"Click Chemistry Inspired Synthesis of Novel Triazolyl Conjugates and their Antimicrobial Activity"** submitted by **Mrs. Poonam Khedar** ID No **2011PHXF0024P** for the award of Ph. D. Degree of the Institute embodies the original work done by her under my supervision.

Signature in full of the Supervisor:

Name in capital block letters: **Prof. ANIL KUMAR**

Designation: Associate Professor

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ABSTRACT

Synthesis of nitrogen containing heterocycles especially triazoles has been recognized most elaborated field because of their widespread applications in multidisciplinary fields. 1,2,3-Triazoles are widely used for the synthesis of antimicrobial drugs. The thesis entitled "**Click chemistry inspired synthesis of novel triazolyl conjugates and their antimicrobial activity**" deals with the synthesis of some selected 1,4-disubstituted-1,2,3-triazol-4-yl heterocycles *via* copper catalysed azide-alkyne cycloaddition (CuAAC) and their antimicrobial evaluation. The thesis is divided into six chapters.

The first chapter of thesis describes the brief literature overview on recent progress in the synthesis of 1,4-disubstituted 1,2,3-triazole scaffolds using CuAAC for evaluation of their antimicrobial activity against different type of Gram positive and Gram negative bacteria and fungi. Brief synthetic procedures and their biological applications as antimicrobial derivatives are reviewed in this chapter.

The second chapter of the thesis portrays the synthesis of 1,4-disubstituted 1,2,3-triazoles which are isosterically modified azole heterocycles by CuAAC. The chapter is divided in two parts. In part-A, modified azole isosteres are synthesized in which imidazole ring of Miconazole drug is replaced with 1,2,3-triazole ring in good to excellent yields (80-92%). The synthesized compounds were found to be poor antimicrobial agent which was attributed to their poor solubility. In part-B, piperazine linked 1,4-disubstituted-1,2,3-triazoles were prepared by CuAAC and were evaluated for antibacterial activity against Gram negative (*E. coli, P. putida*) and Gram positive (*S. aureus*) bacteria and for antifungal activity against (*F. oxysporum, F. gramillarium* and *F. monalliforme*) fungi. The compounds showed moderate antimicrobial activity with MIC values ranging 64-128 μg/mL and zone of inhibition from 12 to16 mm. Derivatives having 2-chloro, 4-fluoro substitution on aryl ring were more effective antibacterial agent. The hydrochloric salts of piperazine linked triazoles showed slightly increased antimicrobial activity.

The third chapter of the thesis is focused on the functionalization of imidazo[1,2-*a*]pyridines to get imidazo[1,2-*a*]pyridine linked 1,4-disubstituted 1,2,3-triazoles using click chemistry approach. Imidazo[1,2-*a*]pyridines were synthesized by using simple compounds acetophenones and 2-aminopyridines and were functionalized to get azidomethyl substituted/ terminal alkyne linked imidazo[1,2-*a*]pyridines *via* simple mesylation/Sonagashira coupling reactions which provided imidazo[1,2-*a*]pyridine linked 1,4-disubstituted 1,2,3-triazoles *via* CuAAC in good yield (30-88 %). Synthesized derivatives were screened for antimicrobial activity against different bacteria (*K. pneumonia, P. putida, B. subtilis* and *S. aureus*) and fungi (*C. albicans, A. flavus, F. oxysporum* and *P. citrinum*). The MIC values were observed in the range from 3.8 to 15.2 μg/mL. The generation of reactive oxygen species and live-dead cell essay exposed them as good antimicrobial agent.

The fourth chapter of thesis describes the regio-selective synthesis of hetero-aryl tethered 1,4-disubstituted 1,2,3-triazoles by CuAAC. Thiopyrimidine and *N*-phenylaminopyrimidines linked 1,2,3-triazole derivatives were synthesized starting from 2-thiouracil. Prepared pyrimidine triazoles were evaluated for antibacterial activity against Gram negative (*E. coli, S. typhi, K. pneumoniae, P. putida*) Gram positive (*B. subtilis, S. Aureus*) bacteria which showed that derivative with 2-amino substitution on pyrimidine ring and pyridine substitution on triazole ring were more effective antibacterial agent with MIC value of 6.25 μg/mL and zone of inhibition 18-19 mm against different bacteria. All triazolopyrimidines showed MIC value in the range from 6.25 to 25 μg/mL and zone of inhibition 17-18 mm against different fungi.

The fifth chapter of the thesis describes the synthesis of 1,2,3-triazolyl indoles using CuAAC. The designed strategy resulted in the construction of novel triazole-fused indole frameworks in good yields (73-92%). Synthesized derivatives were screened for antimicrobial action against Gram negative (*S. typhi* and *P. putida*) and Gram positive (*B. subtilis, S. Aureus*) bacteria as well as fungi (*C. albicans, A. flavus*). All the derivatives showed MIC value in the range of 8-64 μg/mL and zone of inhibition 11-18 mm against different bacteria and fungi. Derivatives having electron withdrawing group on indole ring were found to be better antimicrobial agent with MIC value of 4-8 μg/mL. Most effective derivatives were also screened for cell death by AO/Et.Br dual staining and PI essay methods in which compound having 5-bromo substitution on indole and *t*-butyl phenyl substitution on triazole ring emitted maximum red fluorescence. Again RBC haemolysis was also studied for all derivatives in which active compounds showed less than 25% haemolysis.

Finally, overall thesis work is summarized in chapter six along with future scope of the research work.

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LIST OF ABBREVIATIONS / SYMBOLS

Abbreviation/Symbol	Description
α	Alpha
β	Beta
γ	gamma
Δ	Delta
μg	microgram
μL	microliter
mL	milliliter
°C	Degree centigrade
Å	Angstrom
Ac	Acetyl
ACN	Acetonitrile
Ar	Aryl
[bmim]HSO_4	1-Butyl-3-methylimidazolium hydrogen sulfate
Bu	Butyl
t-BuOH	*tert*-Butanol
calcd.	Calculated
^{13}C	Carbon-13
cat.	Catalyst
CMGL	α- Gluco Carboxymethyl glycoside lactone
cp	Cyclopentadienyl
$CDCl_3$	Deuterated chloroform
cfu	Colony forming unit
Conc	Concentration

COSY	Correlation Spectroscopy (NMR)
CuAAC	Copper catalyzed Azide-Alkyne Cycloaddition
D	Debye
d	Doublet
DAPYs	Diarylpyrimidines
DCFH-DA	2', 7'-dichlorofluorescin diacetate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
dd	Doublet of doublet
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMA	*N*,*N*-Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DEAD	Diethyl azodicarboxylate
DMF	*N*,*N*-Dimethylformamide
DMSO-d_6	Deuterated dimethylsulfoxide
DOSY	Diffusion ordered spectroscopy
EC	Effective concentration
EtOAc	Ethyl acetate
Equiv	Equivalent
g	Gram
h	Hours
HPLC	High performance liquid chromatography
HRMS	High Resolution Mass Spectra
IR	Infrared
Hz	Hertz
J	Coupling constant

Lit.	Literature
MBC	Minimum bactericidal concentration
Me	Methyl
MS	Mass spectrometry
mp	Melting point
m	Multiplet
m	Meta
MsCl	Methane sulphonylchloride
mg	Milligram
MHz	Mega hertz
MIC	Minimum inhibitory concentration
min	Minutes
mL	Milliliter
mm	milimetre
mmol	Millimole
μW	Microwave
MRSA	Methicillin resistant *staphylococcus aureus*
N_2	Nitrogen gas
NMR	Nuclear Magnetic Resonance
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
Pn	Pentyl
π	Pi
PDA	Potato dextrose agar
PEG	Polyethylene glycol
Ph	Phenyl

ppm	Parts per million
PS	Polymer supported
%	Percentage
p-TSA	*p*-Toluenesulfonic acid
PTT	Phenyl trimethylammonium tribromide
R	Hydrocarbon
rt	Room temperature
rpm	Rotations per minute
ROS	Reactive oxygen species
s	Singlet
t	Triplet
TBAF	Tetrabutylammonium fluoride
TBTA	Tris(benzyltriazolylmethyl)amine
TBDPSCl	*tert*-Butyldiphenylchlorosilane
THTP	4,5,6,7-Tetrahydrothieno[2,3-*c*]pyridine
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
TEA	Triethyl amine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
$TMSN_3$	Trimethylsilyl azide
OTf	Triflouromethanesulfonate
δ	Parts per million
w	Watt
ZOI	Zone of inibition

Chapter I

Click Chemistry and Antimicrobial Activity of 1,4-Disubstituted 1,2,3-Triazoles

1.1 Introduction

Heterocycles are the compounds which contain hydrocarbons cyclic ring with at least one heteroatom.[1-3] Nitrogen, oxygen and sulphur are most commonly found heteroatom in heterocyclic compounds, among them nitrogen containing heterocycles are predominantly found as a basic part of biomolecules such as haemoglobin, chlorophyll, vitamins, hormones and amino acids etc.[4-6,2] Synthesis of heterocycles is one of the most engaged tool for the construction of natural products and drugs in pharmaceutical as well as in material chemistry. Imidazole, 1,2,4-triazole, 1,2,3-triazole and pyrazole are frequently observed five membered heterocyclic rings in bioactive compounds. Apart from non-existence in nature, 1,2,3-triazoles have got attention due to their widespread applications in pharmaceutical and bio-medical chemistry because of their stability, bio-activity, inertness towards degradation on heating, hydrogen bonding ability and linking property with different heterocycles.[7] Its hydrogen bonding ability can be contributed to its dipole moment 5 D which is like imidazoles and far more than amides. 1,2,3-triazole ring is a planar core structure with three adjacent nitrogen atoms providing three possible positions (1, 4 and 5) for substitutions (**Figure 1.1**). 1,2,3-Triazole is an isostere of 1,2,4-triazole which is present in numerous bioactive compounds especially in antifungal and antibacterial agents. 1,2,3-Triazoles have also caught researcher's attention because of their wide applications in material science, polymer science, synthetic chemistry, peptidomimetic chemistry, medicinal chemistry, agrochemicals, fertilisers etc. [8-12]

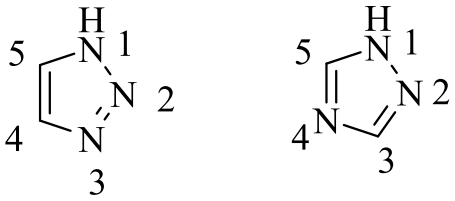


Figure 1.1: Structures of isomeric 1,2,3- and 1,2,4-triazole.

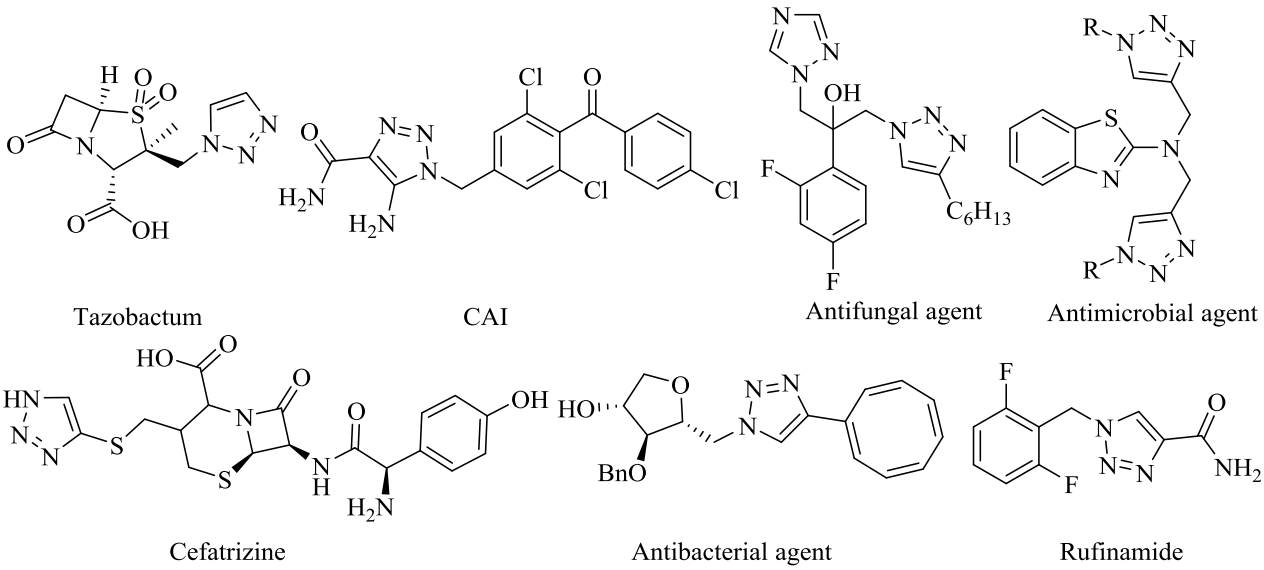
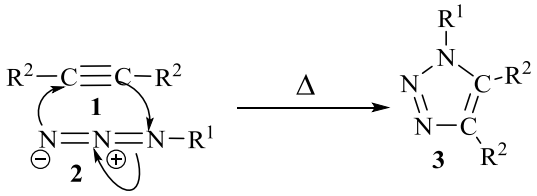


Figure 1.2: 1,2,3-Triazole containing drug analogues.

1.2 Synthesis of 1,2,3-triazoles

1,3-Dipolar cycloaddition reaction also known as Huisgen's cycloaddition is a classical method to synthesize a large variety of five-membered heterocycles (**Scheme 1.1**).[13,14] In this reaction, generally the dipolarophiles are alkenes, alkynes, carbonyls and nitriles while the 1,3-dipole is a three-atom conjugated system with four π-electrons delocalized over the three atoms. Various 1,3-dipoles and dipolarophiles are depicted in **Figure 1.3.**



Scheme 1.1: [3+2] cycloaddition of alkyne and azide.

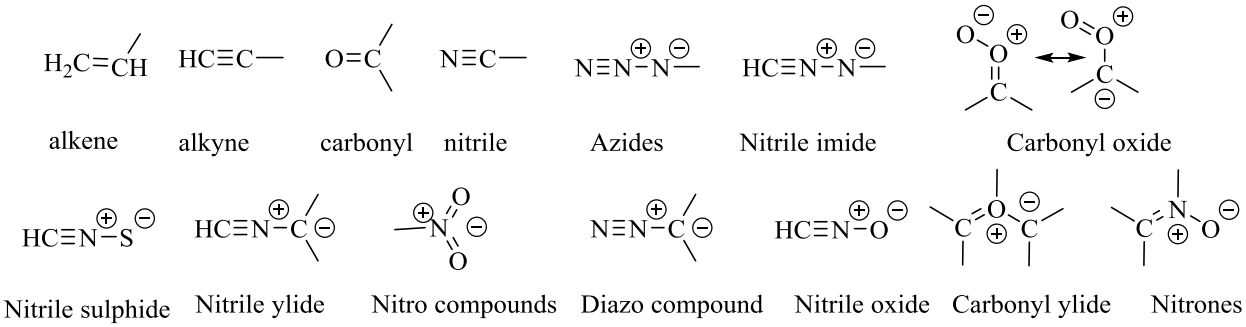
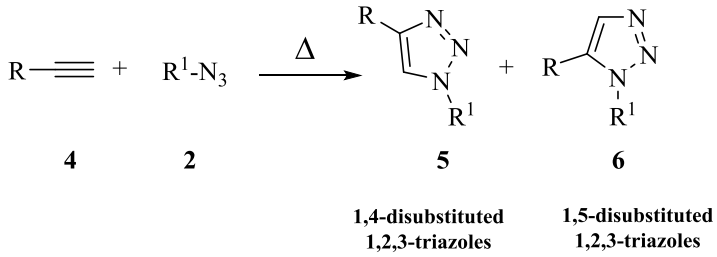


Figure 1.3: Dipoles and dipolarophiles for 1,3-dipolar cycloaddition.

In the synthesis of 1,2,3-triazoles *via* 1,3-dipolar cycloaddition, alkynes act as dipolarophiles and azides as dipole part. When both the motifs are heated simultaneously, they offer 1,2,3-triazoles. Generally when both the azide and alkyne part are symmetrically substituted, we get only one product but if they are not symmetrically substituted, it provides a mixture of 1,4 and 1,5-disubstituted 1,2,3-triazoles (**Scheme 1.2**). This method affords a mixture of regio-isomeric products, requires elevated temperatures, column chromatographic separation, longer reaction time and results in various side products leading to difficulty in isolation.

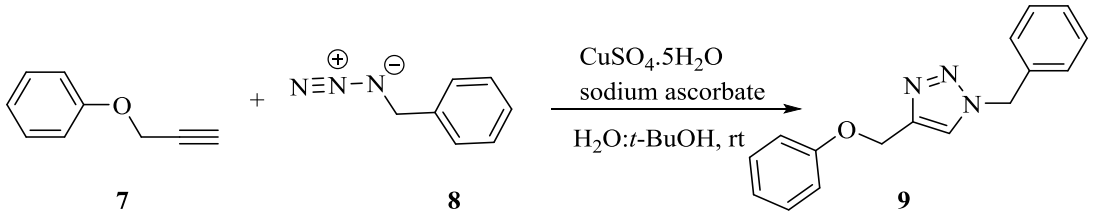


Scheme 1.2: 1,3-Dipolar cycloaddition of azides and alkynes under thermal condition.

Thus, there has been great interest to develop methods to improve the regio-selectivity, yields and reaction conditions for these reactions. Two valuable advances in this are cycloaddition in presence of copper and ruthenium catalysts.

1.2.1 Copper catalyzed azide-alkyne cycloaddition

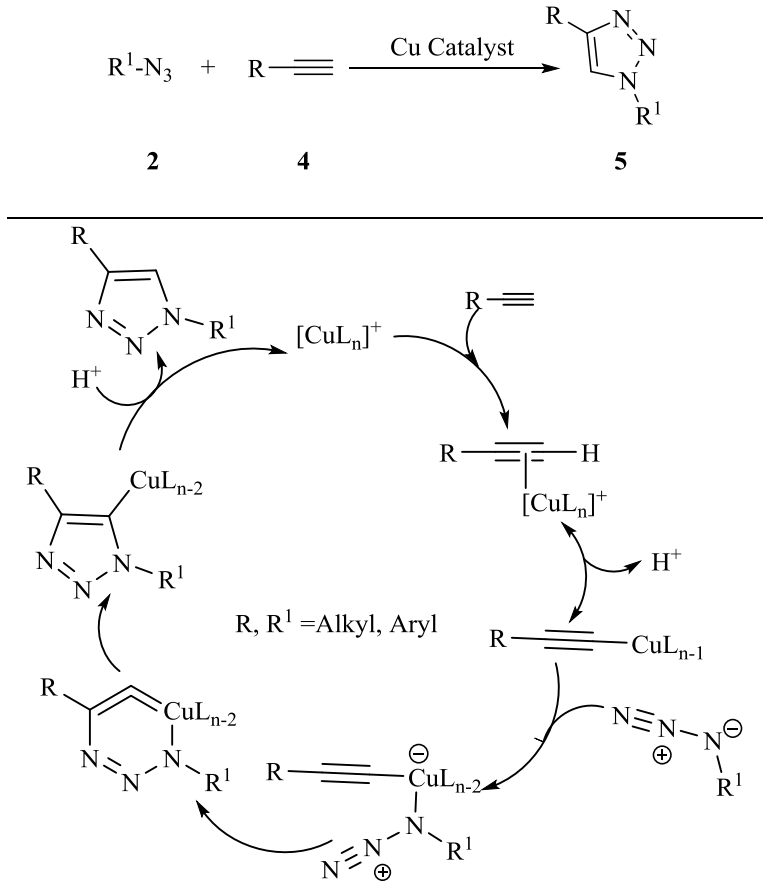
In 2002, Sharpless and Meldal independently reported regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles by cycloaddition of azide and alkyne using $CuSO_4.5H_2O$/ Na-ascorbate and CuI respectively, where Sharpless termed it as 'Copper catalyzed azide-alkyne cycloaddition' (**CuAAC**).[15,16]



Scheme 1.3: Copper catalyzed azide-alkyne cycloaddition.

Despite the apparent simplicity of reaction, its mechanism involves multiple steps involving copper-acetylide coordination complexes. Cu(I) forms a π-complex with acetylene which by replacing most acidic hydrogen of acetylene (terminal hydrogen atom) forms copper acetylide intermediate. Another Cu atom activates the azide by coordinating with nitrogen atom. Consequently, azide interacts with acetylide complex to form copper azide-acetylide

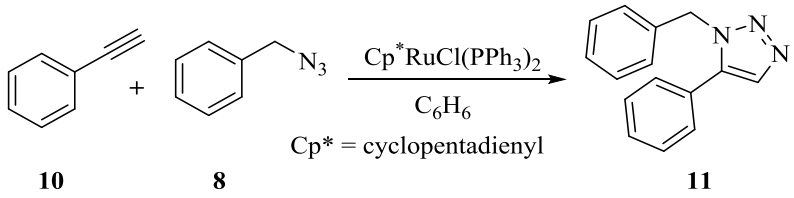
complex. This complex goes to cyclization, protonation followed by dissociation of copper catalyst to result in formation of 1,4-disubstituted triazoles (**Scheme 1.4**).[17]

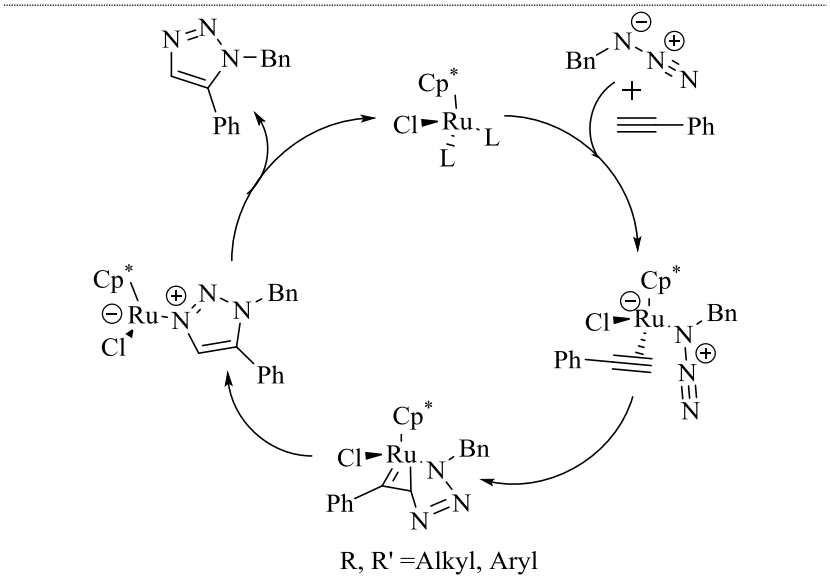


Scheme 1.4: Copper catalyzed azide-alkyne cycloaddition.

1.2.2 Ruthenium catalyzed azide-alkyne cycloaddition

Fokin and co-workers observed first time that when Ru^{II} cyclopentadienyl (Cp) complex is employed as catalyst in cycloaddition of azide and alkyne it results in formation of 1,5-disubstituted 1,2,3-triazoles exclusively (**Scheme 1.5**).[18] It has been proposed that $Ruthenium^{II}$ (Cp) complex undergoes oxidative addition with azide and alkyne. Here, the new C-N bond is formed in between more electronegative carbon of alkyne and terminal nitrogen atom of azide. This intermediate then undergoes reductive elimination resulting into 1,5-disubstituted 1,2,3-triazole (**Scheme 1.5**).[19,20]





Scheme 1.5: Ruthenium catalyzed azide-alkyne cycloaddition.

1.3 Click chemistry

In 1998, K. Barry Sharpless coined the term 'Click chemistry' and fully described it in 2001 which is now a days most widely applied method towards the synthesis of 1,2,3-triazoles.[21] According to him:

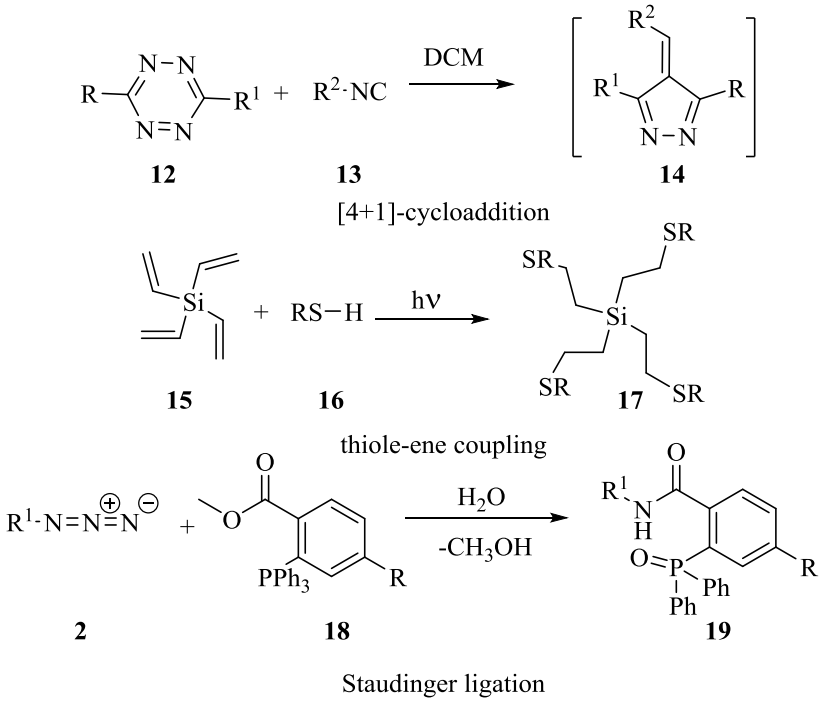
"Click reaction is a set of reactions which are modular, easy to perform, wide in scope, provide high yield of products, have atom economy, not affected by environment i.e. water, air etc., offer very less or no side products those can be removed easily, stereospecific (but not necessarily enantioselective) and could be conducted in one pot using easily removable or environmentally benign solvents.**"**

Generally click reactions are not affected by water hence we can use water as solvent in these type of reactions and work over a wide range of pH from 3-12. Click chemistry plays an important role in heterocyclic synthesis as a large bunch of reactions follows this criterion such as:

- addition to carbon-carbon multiple bonds such as epoxidation, dehydroxylation, aziridination,
- Michael addition i.e. non-aldol type carbonyl chemistry such as formation of thioureas, ureas, hydrazones, amides,
- cycloaddition reactions
- Staudinger ligation,

❖ ring opening of epoxides and aziridines, etc.

Click chemistry is anticipated to join different required substrates with biomolecules or two small molecules to obtain required moiety *via* cycloaddition reactions. In this measure, [4+1] cycloaddition between isonitriles and tetrazines,[22] thiol-ene reaction,[23] diels-alder reaction,[24] Staudinger ligation,[25] [3+2] cycloaddition such as Huisgen's 1,3-dipolar cycloaddition are commonly explored types of click reactions (**Scheme 1.6**). Among them, 3+2 cycloaddition was referred as premier example of click chemistry and is widely explored.[26]



Scheme 1.6: Diversity in click chemistry.

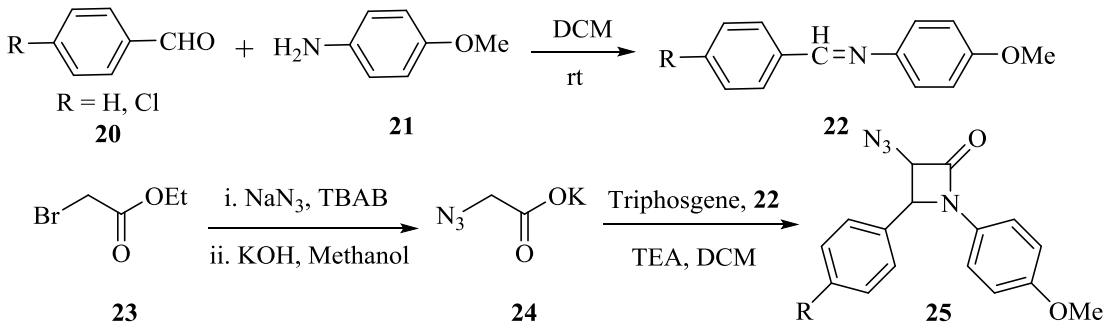
CuAAC is one of the most reliable click reaction for the efficient regioselective preparation of 1,4-disubstituted 1,2,3-triazoles [27,28] from a wide range of substituted alkynes and azides in excellent yields with no side products which cannot be typically attained by the traditional Huisgen's thermal cycloaddition reaction in different solvents.[29] Although Sharpless *et al.* engaged $CuSO_4$ as catalyst in CuAAC which was then reduced by sodium ascorbate into Cu^I, there are diverse methods to obtain Cu^I *i.e.* Cu^I salts as copper iodide,[16] *in-situ* reduction of Cu^{II} salts into Cu^I as copper acetate,[17] copper sulphate and comproportionation (a chemical reaction where two reactant of same element with different oxidation number will form a product with an oxidation number intermediate of two reactant) of Cu^0 and Cu^{II} salts.[30] It is pragmatic that nitrogen-based ligands can stabilize the Cu^I oxidation state under aerobic, aqueous conditions and promote the desired transformation although sometimes steric and

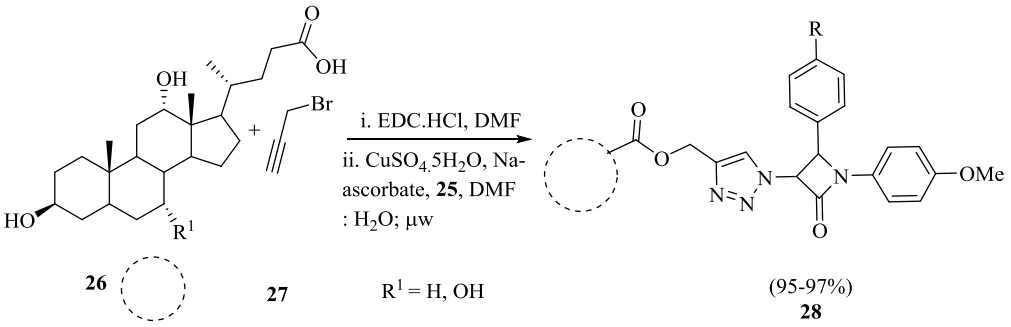
electronic factors may also play an important role in click chemistry.[31] Literature is flooded with reports where 1,2,3-triaozles are functionalised as antiviral, anti-microbial, anti-HIV, anti-bacterial[1] etc.

1.4 Synthesis of antimicrobial 1,4-disubstituted 1,2,3-triazoles *via* CuAAC:

1,2,3-Triazoles are bioisosteres of 1,2,4-triazoles. Several of the 1,2,4-triazole based antimicrobial drugs have developed resistance and thus there have been great attention to find potent antimicrobial agent in recent years. CuAAC have been exclusively explored for the design and synthesis of novel anti-microbial agents.

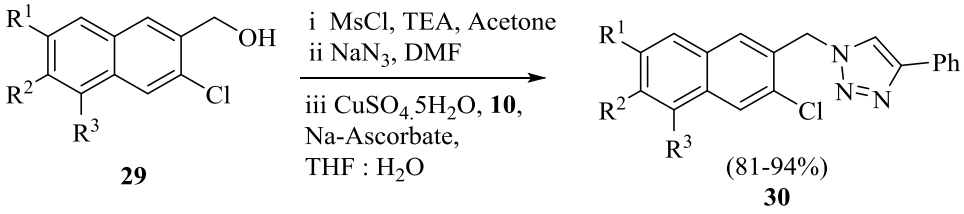
Hazra *et al* has synthesized novel 1,2,3-triazole linked-β-lactum bile acid conjugates utilising CuAAC to access them for their antimicrobial activity.[32] Ethyl bromoacetate **23** was allowed to react with sodium azide followed by saponification of ester functionality with KOH in methanol to accomplish potassium salt of azidoacetic acid **24**. These salts were treated with imine **22** already prepared by reaction of aldehyde and amine to offer azido-β-lactums **25** *via* Staudinger ligation in the presence of triphosgene and triethylamine. **28** was acquired by propargylation of **26** followed by CuAAC with **25** (**Scheme 1.7**). All the accomplished derivatives were screened for in-vitro anibacterial and anti-fungal activity which showed that all azido- β-lactams and steroidal alkynes were inactive but most of the triazole conjugates are proficient for antibacterial and antifungal action. The antibacterial activity of compounds was higher than fluconazole with MIC value 16-32 μg/mL, while againt fungi *Y. lypolitica*, it was only 4 μg/mL for some derivatives.





Scheme 1.7: Synthesis of 1,2,3-triazole tethered β-lactam bile acid conjugates.

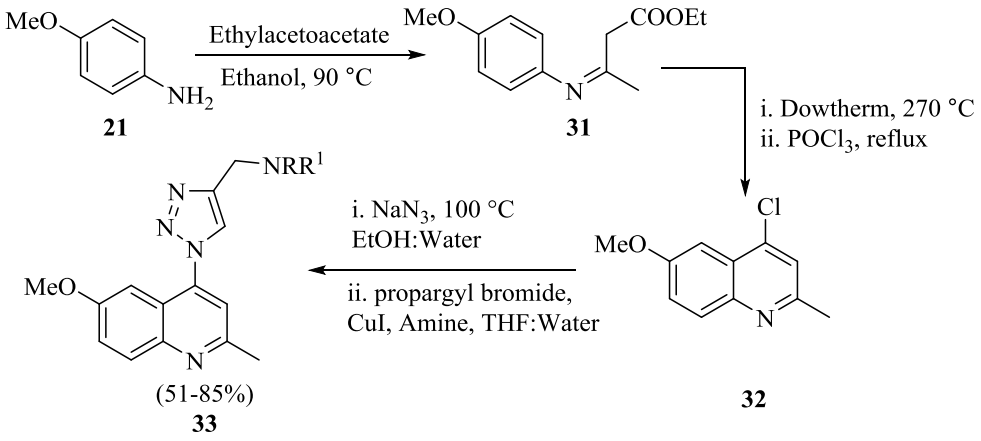
A series of quinoline conjugated triazoles was accomplished by Shingare *et al* in 2010 to assess their biological activity.[33] Accordingly, (2-chloroquinolin-3-yl) methanol **29** were mingled with MsCl in presence of TEA in acetone to furnish (2-chloroquinolin-3-yl)methyl methanesulfonate which on blending up with NaN_3 in DMF shaped into 3-(azidomethyl)-2-chloroquinoline. These azides were transformed to desired motif 2-chloro-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)quinolines **30** on reaction with phenyl acetylene (**Scheme 1.8**). All derivatives were tested for *in vitro* antibacterial and antifungal activity considering zone of inhibition which shows that the synthesized scaffolds are moderate for anti-microbial action. Antibacterial action was performed against *E. coli* and *B. subtilis* which revealed that derivatives with methyl and methoxy substitution were good with zone of inhibiton upto 16 mm having MIC value 10-25 µg/mL. When the scaffolds were screened for antifungal action against *C. albicans* and *A. niger*, again same results obtained having MIC value 10-25 µg/mL with zone of inhibition 14-16 mm.



Scheme 1.8: CuAAC for construction of quinoline conjugated 1,2,3-triazoles.

Adhikari group has exhibited a route towards design and syntheis of *N*-[1-(6-methoxy-2-ethylquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl]methylamines **33** to promote them for evaluation of their antimicrobial activity.[34] 4-Methoxy aniline **21** was reacted with ethylacetoacetate to obtain 3-(4-methoxyphenyl-amio)-but-2-enoic acid ethylester which was consequently tranformed into 6-methoxy-2-methylquinolin-4-ol by heating at 270 °C using Dowtherm. Next, to achieve essential intermediate 4-azido-6-methyl-2-methylquinoline, 6-methoxy-2-

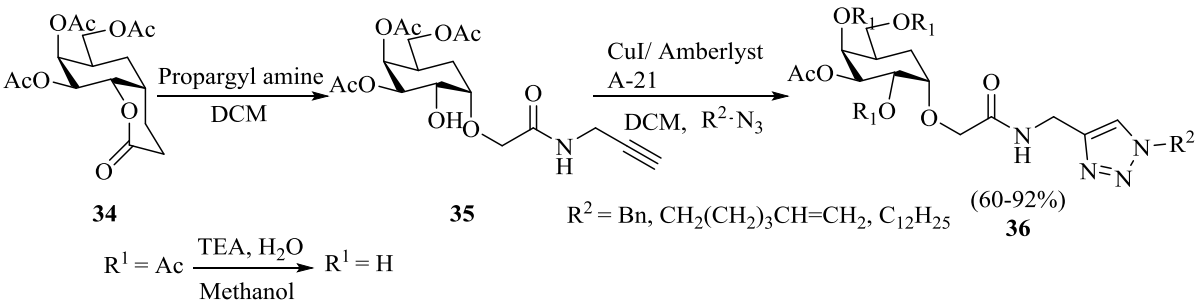
methylquinolin-4-ol was refluxed with $POCl_3$ followed by subsequent nucleophilic substitution with sodium azide on heating in autoclave at 100 °C. Synthesis of targeted compound **33** was accomplished by sequential one pot reaction of intermediate azide with propargyl bromide and secondary amine in presence of TEA and CuI (**Scheme 1.9**). All the imitative derivatives were subjected to *in vitro* antibacterial and antifungal screening against different pathogenic strains *i.e. S. aureus, E. coli, P. aeruginosa, K. pneumoniae, A. flavus, A. fumigates* and *C. albicans* etc. and all the compounds showed good activity against all pathogens. The enhanced antimicrobial action of some composites was ascribed to the active piperazine ring attached to them in place of secondary amine. Compounds bearing methoxy, fluoro and cyclopropyl were also better comparatively with MIC value 6.25 µg/mL.



Scheme 1.9: Syntheis of biologically active *N*-[1-(6-methoxy-2-ethylquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl]methylamines.

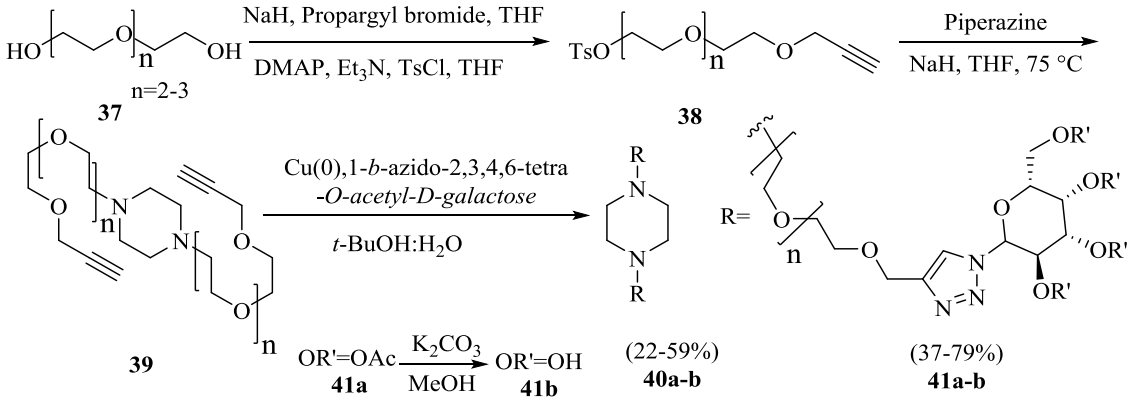
Rauter group has achieved the synthesis of sugars embodying triazole conjugated carbonyl systems initiating with carboxymethyl glycoside conjugates to test their antimicrobial activity.[35] Starting material α- Gluco Carboxymethyl glycoside lactone (CMGL) **34** required for **scheme 1.10** was synthesized by iso-maltulose oxidation followed by acetylation. **34** was reacted with propargyl amine in DCM to open lactone ring. These terminal alkyne containing glycosides **35** were reacted with different alkyl and aryl azide to get 1,2,3-triazole tethered glycosides **36** through click chemistry which was further followed by deprotection of hydroxyl groups (**Scheme 1.10**). All the derivatised compounds were screened for antimicrobial activity against different Gram-positive bacteria *E. faecalis, S. aureus, B. subtilis, B. cereus* and Gram-negative bacteria *E. coli, S. enteritidis* and plant pathogenic fungi *A. niger, F. solani* by calculating zone of inhibition but to discourage it was observed that there is no enhancement in activity even after tethering of 1,2,3-triazoles with

glycosides. Although derivatives bearing dodecyl group could show inhibitory action against *B. cereus* and *B. subtilis* for some extent with zone of inhibition 39 and 40 mm respectively but most of the compounds showed zone of inhibition ≈12mm.



Scheme 1.10: Modification of sugars to assess 1,2,3-triazole conjugated carbonyl glycosides.

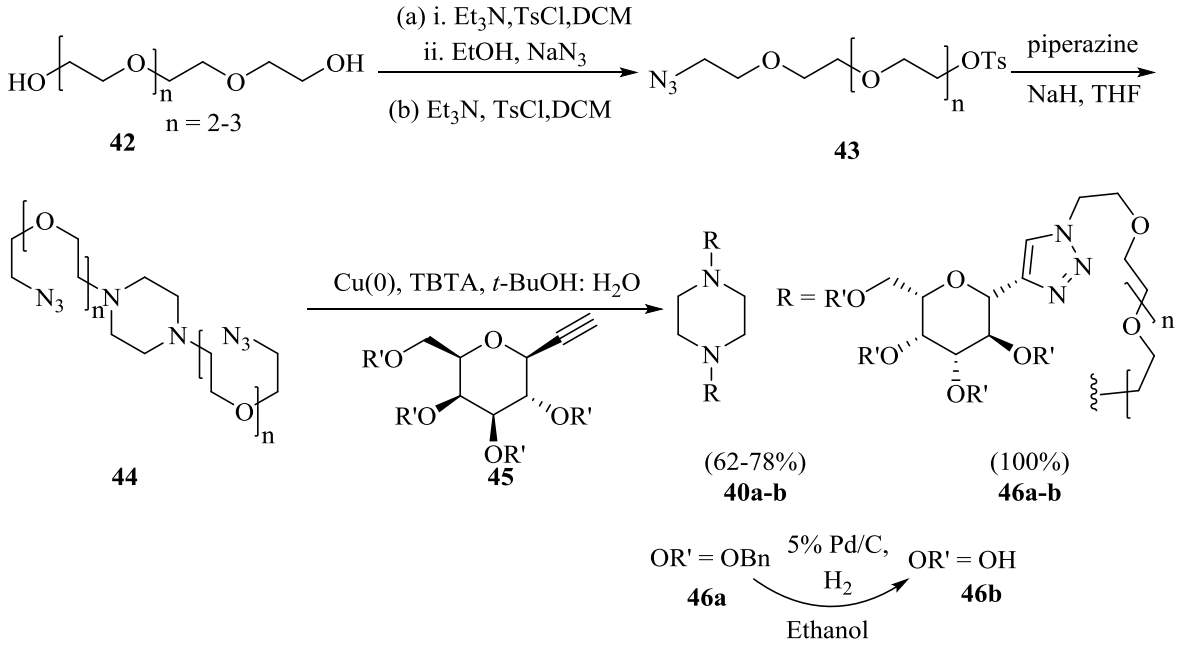
Hughes group demonstrated the synthesis of 1,2,3-triazole linked galactopyranosides by following click chemistry approach.[36] The synthesized galactopyranosides possess piperazine core, poly ethylene glycol (PEG) linker and galactotriazole units. Piperazine was reacted with 2 moles of *O*-propargylated and tosylated 2,2'-oxybis(ethan-1-ol) **38** to obtain **39** which was reacted with 1-β-azido-2,3,4,6-tetra-*O*-acetyl-*D*-galactose via CuAAC to afford **40a (Scheme 1.11)**.



Scheme 1.11: Design and synthesis of 1,2,3-triazole linked galactopyranosides.

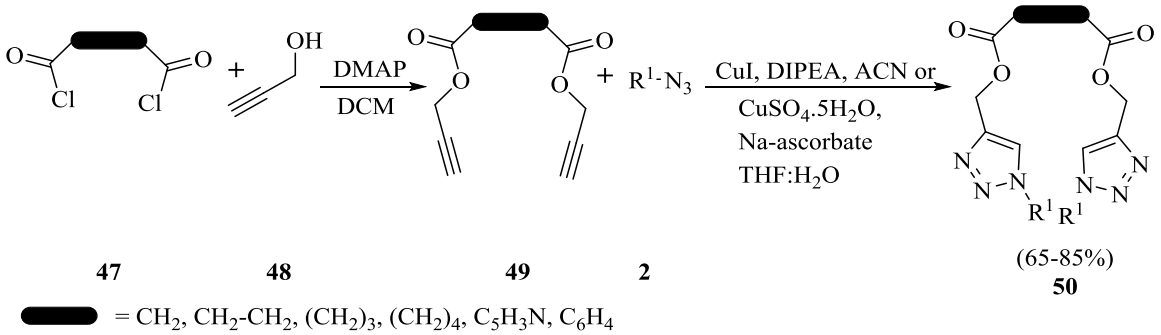
In this contrast, alcoholic group of 2,2'-(ethane-1,2-diylbis(oxy))bis(ethan-1-ol) **42** was transformed into azide and alkyne group was bonded with galactose motif . These scaffolds were binded *via* CuAAC to afford **40a** which on debenzylation afforded **40b**. For, CuAAC, Cu (I) was generated insitu from Cu (0) and tris(benzyltriazolylmethyl)amine (TBTA) (**Scheme 1.12)**. The main purpose to choose these scaffolds is that piperazine motif is found having improved activity against cholera toxin (CT) and PEG shows enhanced solubility in water. The synthesized motifs were tested for inhibition of cholera toxin. It has been

concluded that α-anomer is less potent than β-anomer of galactopyranoside, however there is no much difference in activity. 1,2,3-triazole linked galactopyranosides were more potent towards CT than *m*-nitrophenyl-α-D-galactopyranocides.



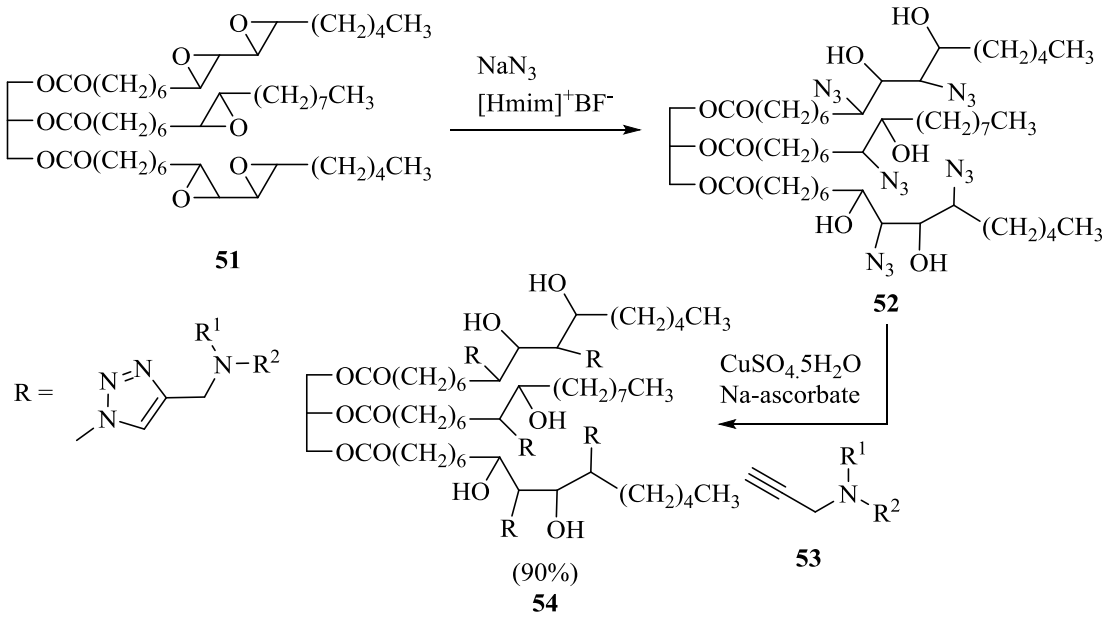
Scheme 1.12: Synthesis of bivalent galactotriazole CT ligands.

Kaushik and co-workers have developed the synthetic strategy for the synthesis of 1,2,3-bistriazoles by reaction of various benzyl azides with bisalkynes.[37] In their approach, acid dichlorides **47** were reacted with propargyl alcohol **48** in presence of DMAP to offer bis alkynes **49** which were subsequently reacted with different azides **2** *via* click reaction in acetonitrile using copper (I) as catalyst and DIPEA as base to get 1,2,3-bistriazoles **50** in good yield (**Scheme 1.13**). All the scaffolds were screened for antibacterial activity against Gram positive *B. subtilis*, Gram negative *E. coli* and antifungal activity against *A. niger* and *C. albicans* which showed that all the synthesized derivatives are moderate to good active having comparable MIC value (0.0123-0.028 μmol/mL) with fluconazole (0.0098 μmol/mL) and norfloxacin (0.0102 μmol/mL). Derivative having pyridinyl spacer with benzyl substitution on triazole ring was best antibacterial agent with MIC value 0.0123 μmol/mL for both bacteria while having methylene linker with benzyl substitution on triazole ring was best antifungal agent with MIC value 0.0140 μmol/mL for both fungi.



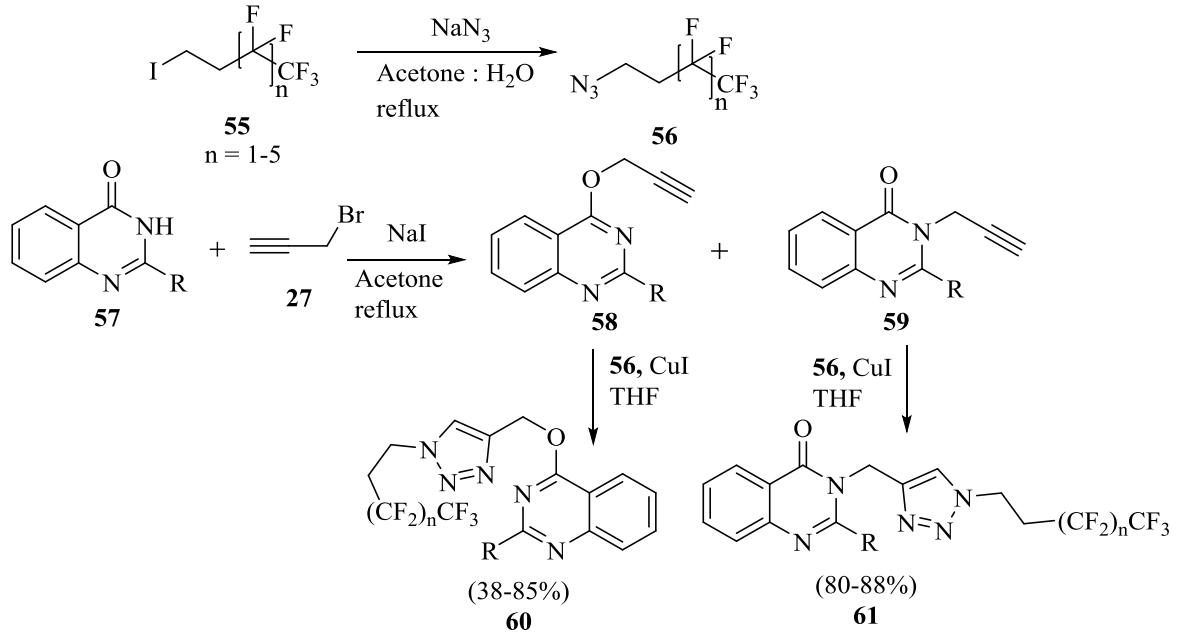
Scheme 1.13: Synthesis of 1,4-disubstituted 1,2,3-bistriazoles.

Yeganeh *et al* have constructed polyurethane derived 12,3-triazole functionalised soyabean oil based polyols to evaluate their biocidal activities.[38] In their approach, epoxidised soyabean oil **51** was treated with sodium azide to provide azide-polyols **52** which were subjected to copper catalyzed azide-alkyne cycloadditon to offer 1,4-disubstituted 1,2,3-triazole functionalised soyabean oil based polyols **54**. Amine activity was also introduced in the scaffolds by reacting hydroxy-azide ligated polyurethanes with tertiary amine embraced alkynes (**Scheme 1.14**). On assessing antibacterial activity against *E. coli*, *S. aureus* and antifungal activity against *C. albicans* through agar diffusion it was observed that tertiary amine embedded 1,2,3-triazole functionalised soyabean oil based polyols are far better than simple triazole based polyols. The activity was calculated on the basis of percentage of microorganism reduction which revealed that amine containing soyabean oil based triazoles showed 100% reduction with zone of inhibition 2.5 mm and 4.7 mm against *S. aureus* and *C. albicans* respectively.



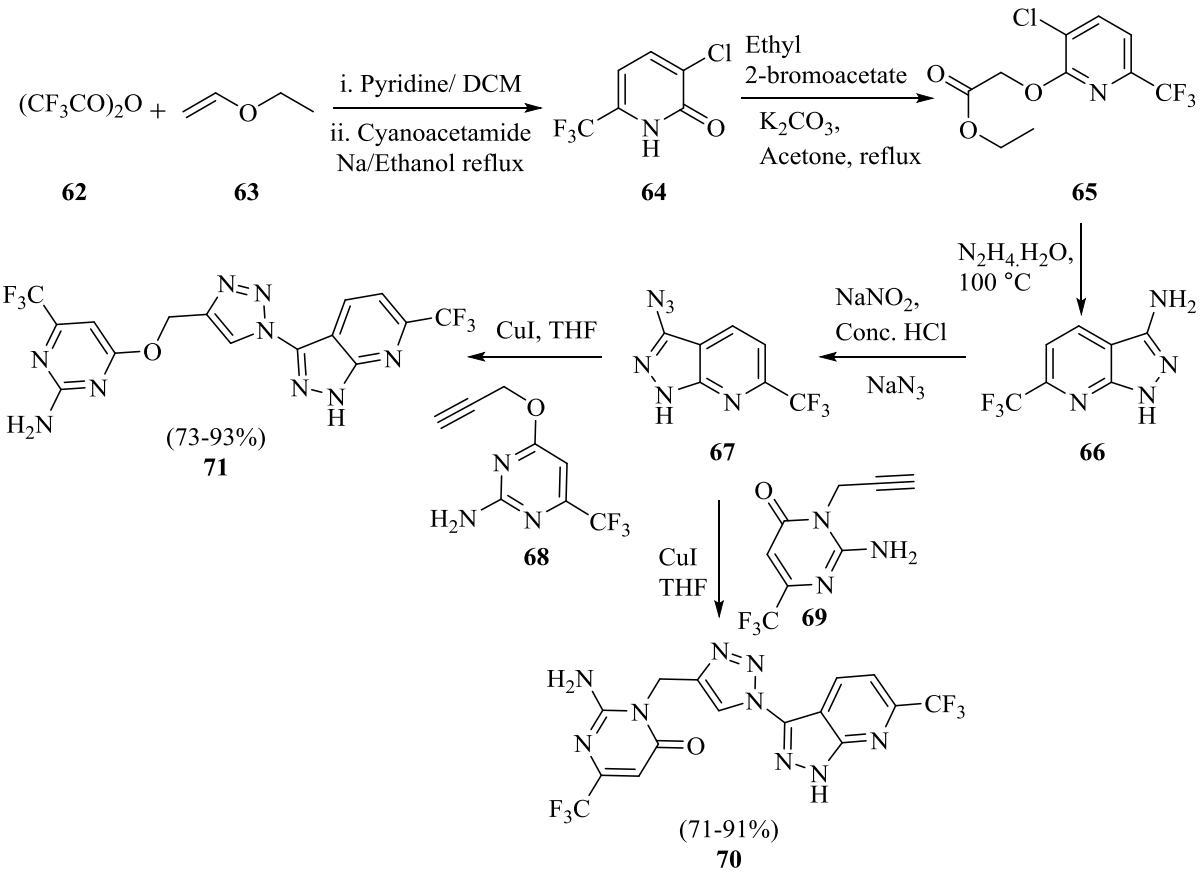
Scheme 1.14: Derivatization of soyabean oil based polyols to get conjugated 1,2,3-triazoles.

Narsaiah and co-workers designed the strategy to produce perfluoroalkyl-1*H*-1,2,3-triazol-4-yl conjugated quinazolines **60** and **61** for examining their antimicrobial activity.[39] In this regard, initially the group theortically predicted the formation of *N*- and *O*- propargylated quinazoline scaffold **58** and **59** from quinazolin-4-ones and then executed experimentally using propargyl bromide and NaI. Simultaneously, perfluoroalkyl azides **56** were assembled by reacting perfluoroalkyliodide **57** with sodium azide. Then propargylated quinazolines were amalgamated with perfluoroalkyl azide to catch aspirating perfluoroalkyl-1*H*-1,2,3-triazol-4-yl conjugated quinazolines **60** and **61** *via* CuAAC (**Scheme 1.15**). The construction was followed by antimicrobial screening against Gram-positive (*B. subtilis, S. aureus, S.epidermidis*), Gram-negative (*P. aeruginosa* and *E. coli*) bacterial and different fungal strains (*C. albicans, S. cerevisiae, R. oryzae, A. niger, A. flavus* and *C. rugosa*) which led to most of the compounds showing promising activity. Compound **60** having trifluoro substitution with chain length of 7 carbon atoms were good antibacterial agents against all bacterial strains except *E. coli* having MIC value 9.375 μg/mL while others were less with MIC value 75-150 μg/mL. In antifungal activity, it was observed that most of the compounds were inactive against *R. oryzae* and *C. rugosa* when 30 μg/mL concentration was used. Most of the compounds were found moderately active when 30 μg/mL concentration was used with zone of inhibition 7-12 mm which increases on increasing concentration. Derivative **61** with trifluoro substitution having chain length of 5 carbon atom was most active (9-13 mm zone of inhibition) but no compound could reach to standard (22-25 mm) Amphotecerin-B.



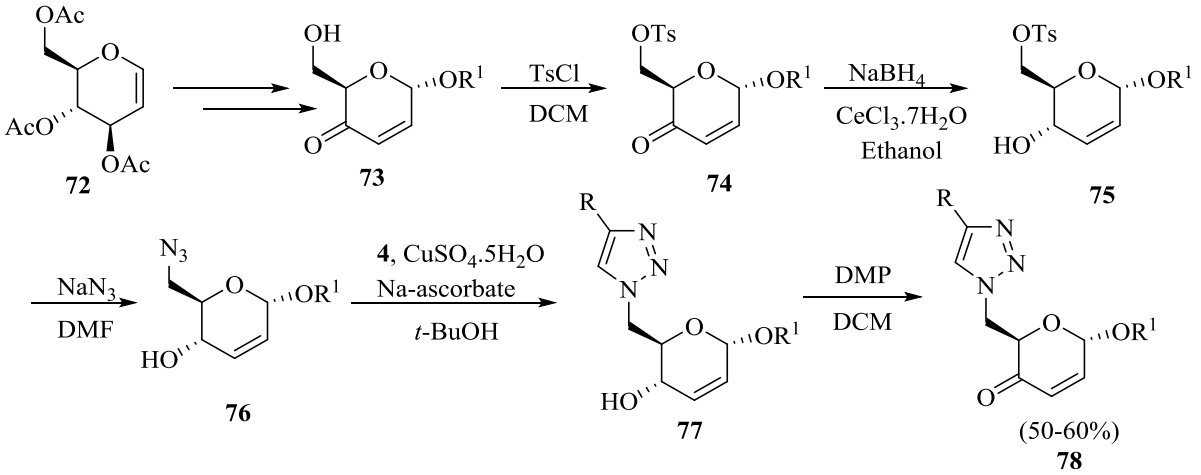
Scheme 1.15: Synthesis of perfluoroalkyl-*1H*-1,2,3-triazol-4-yl conjugated quinazolines.

The same group has made an attempt for erection of pyrazolo[3,4-*b*]pyridine and pyrimidine functionalised triazoles for further evaluation of their anti-microbial activity.[40] In their approach, acylation of vinylethyl ether **63** was done followed by reaction with cyanoacetamide in presence of sodium ethoxide to get 3-cyano-6-trifluromethyl-2-(1*H)*-pyridone **64** which was selectively *O*-alkylated with α-bromoethylacetate using potassium carbonate followed by reaction with hydrazine hydrate to obtain 6-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine **66** which was diazotized to offer 3-azido-6-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridine **67**. **67** was reacted with 2-amino-3-prop-2-ynyl-6-trifluoromethyl-3*H*-pyrimidin-4-one using Sharpless copper catalyzed Click chemistry to gain 1,4-disubstituted pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazoles **70** and **71** (**Scheme 1.16**). In their approach towards anti-microbial study, the group scrutinized MIC values for several bacteria *i.e. M. luteus, S. aureus, B. subtilis, E. coli, P. aeruginosa, K. planticola* and fungi *C. albicans* and observed that *O*-alkylated derivatives were better than *N*-alkylated compounds, the former showed good activity against all microorganism except *C. albicans* with MIC value 7.8-15.6 μg/mL (some >250 μg/mL). Selected compounds were studied for bacterial inhibition and minimum bactericidal concentration (MBC). *O*-alkylated derivative having decyl chain substitution was found most potent against all bacterii (IC_{50} value 3.9±0.14 μg/mL) except *E.coli* for which without alkyl subtitution it was the best with IC_{50} value 7.8±0.11 μg/mL of bacterial inhibition. MBC value for the selected compounds was found 7.8-15.6 μg/mL for different bacteria. No compound could show inhibitory action against *C. albicans.*



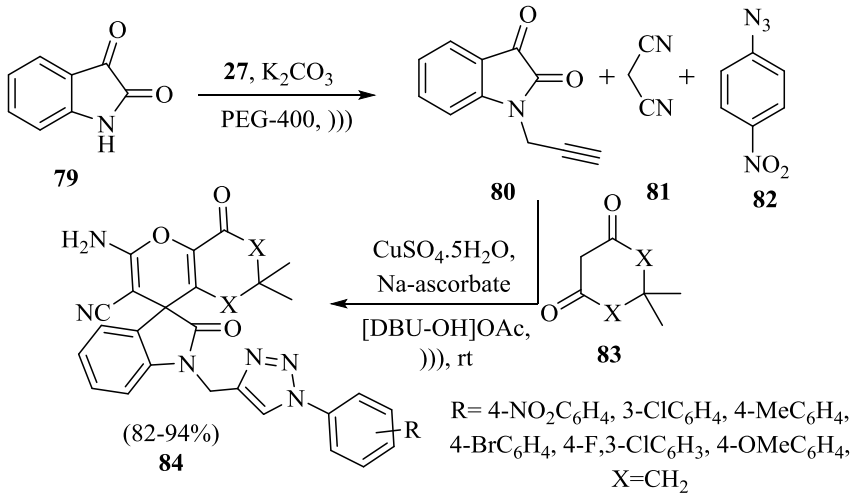
Scheme 1.16: CuAAC to get pyrazolo[3,4-*b*]pyridine and pyrimidine functionalised triazoles.

Prabhakar *et al* performed molecular hybridization based synthesis of 6-triazolyl-2,3,6-trideoxy sugars as novel broad spectrum antimicrobial agents employing click chemistry approach.[41] In their strategy, D-glucal **72** was transformed into 2,3-dideoxy hex-2-enopyranosid-4-uloses **73** whose hydroxyl group at C-6 position was tosylated to furnish 6-*O*-tosyl derivative **74**. Luche reduction of composite **74** delivered 4-hydroxy derivatives **75** which on azidation with NaN_3 in DMF at 120 °C provided 6-azido-4-*O*-hydroxy 2,3,6-trideoxy hex-2-enopyranosides **76** in good yield (**Scheme 1.17**). These azido scaffolds were treated with different terminal alkynes in the key step using click chemistry to offer 6-triazolo derivatives **77** which were oxidized to targeted sugar triazole conjugates **78**. Above mentioned triazole conjugates **77** and **78** were evaluated for *in vitro* antibacterial activity against *S. aureus*, *K. pneumoniae*, *E. coli* and *P. aeruginosa* which revealed them as good antibacterial agents with 90% growth inhibition in concentration 0.78-50 μg/mL.



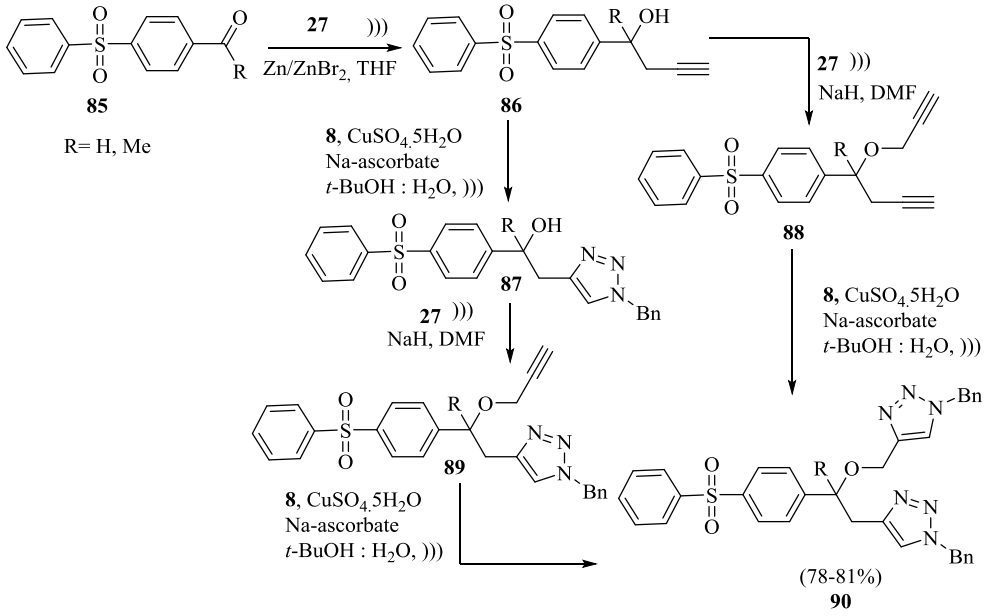
Scheme 1.17: Click chemistry inspired molecular hybridisation of 1,2,3-triazoles and trideoxy sugars.

Besides room temperature stirring, CuAAC could be accomplished under ultrasonic irradiation in benign solvents with enhanced yield in less time. Khurana and colleagues have performed ultrasound promoted synthesis of fluoroscent triazolyl spirooxyindoles.[42] At first, *N* atom of isatin **79** was propargylated under ultarsonic waves using propargyl bromide and PEG 400 as solvent. This propargylated oxyindole **80** was transformed to triazolyl spirooxyindoles **84** using CuAAC under ultrasonic irradiation in one pot synthesis pattern using dimedone **83**, 4-nitrophenylazide **82**, malononitrile **81** and DBU based ionic liquid in ethanol as solvent (**Scheme 1.18**). The reaction was completed within 15 minutes offering excellent yields which was earlier incomplete even after 120 minutes. Antimicrobial activity of all derivatives was inspected against two Gram positive (*S. aureus, B. subtilis*) and two Gram negative (*E. coli, P. aeruginosa*) bacteria which reveal that almost all compounds are good antibacterial agents against Gram positive bacteria with zone of inhibition in range of 13.6-20.6 mm and 15.6-22.3 mm against *S.aureus* and *B. subtilis* respectively but no compound could show activity against Gram negative bacteria. MIC and MBC value were also calculated against Gram positive bacteria, in which it was observed that derivative having *p*-bromo subtitution was best with MIC value 32 and 16 μg/mL against *S. aureus* and *B. subtilis* respectively while MBC value was 64 μg/mL for both the bacterii. The antifungal activity was tested against *C. albicans* and *S. cerevisiae* which showed that all derivatives are good antifungal agents with 12.3-16.6 mm and 13.6-17.6 mm range of zone of inhibition against *C. albicans* and *S. cerevisiae* respectively. Scaffold containing methoxy substitution were best antifungal agent against both bacterii showing MIC value 64 μg/mL for *S. cerevisiae* lower than standard Amphotecerin-B (100 μg/mL).



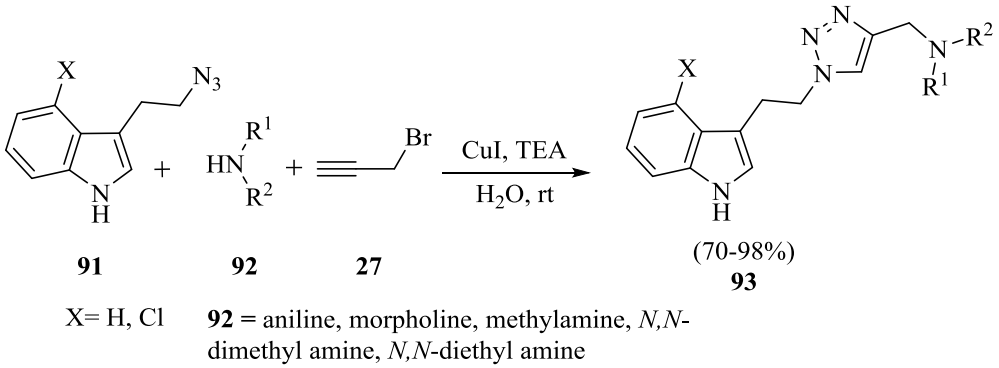
Scheme 1.18: Synthesis of 1,2,3-triazolyl conjugated spirooxyindoles under ultrasound irradiation.

Mady *et al* has made an attempt to synthesize novel 1,2,3-triazole linked diaryl sulfones by exploiting ultrasound assisted CuAAC.[43] In their endeavour, they performed ultra sound mediated Barbier type propargylation of corresponding carbonyl compound **85** using propargyl bromide, zinc and zinc bromide to get **86** which was then transformed to triazoles **87** *via* CuAAC under ultrasonification in excellent yields. Next to this, hydroxyl group in triazolyl sulfones was again propargylated followed by subsequent CuAAC to afford ditriazolyl aryl sulfones **90**. Alternatively, first hydroxyl portion of propargylated aryl sulfones **86** was propargylated follwed by consquent double CuAAC to acheive **90**. All the synthetic moves were performed under ultrasonic irradiation **(Scheme 1.19)**. All the formulated derivatives were tested for antibacterial and antifungal activity by calculating their MIC and zone of inhibition. The MIC value for antibacterial essay was observed 50-200 µg/mL with zone of inhibition 17-25 mm. MIC value for fungi *A. niger* was observed 25 µg/mL with zone of inhibition in the range of 32-34 mm for different scaffolds. The SAR study was also performed for synthesized gibbets. It was revealed that ditriazolyl sulfone scaffolds having methyl substitution are more better in antimicrobial action compared to monotriazolyl sulfones.



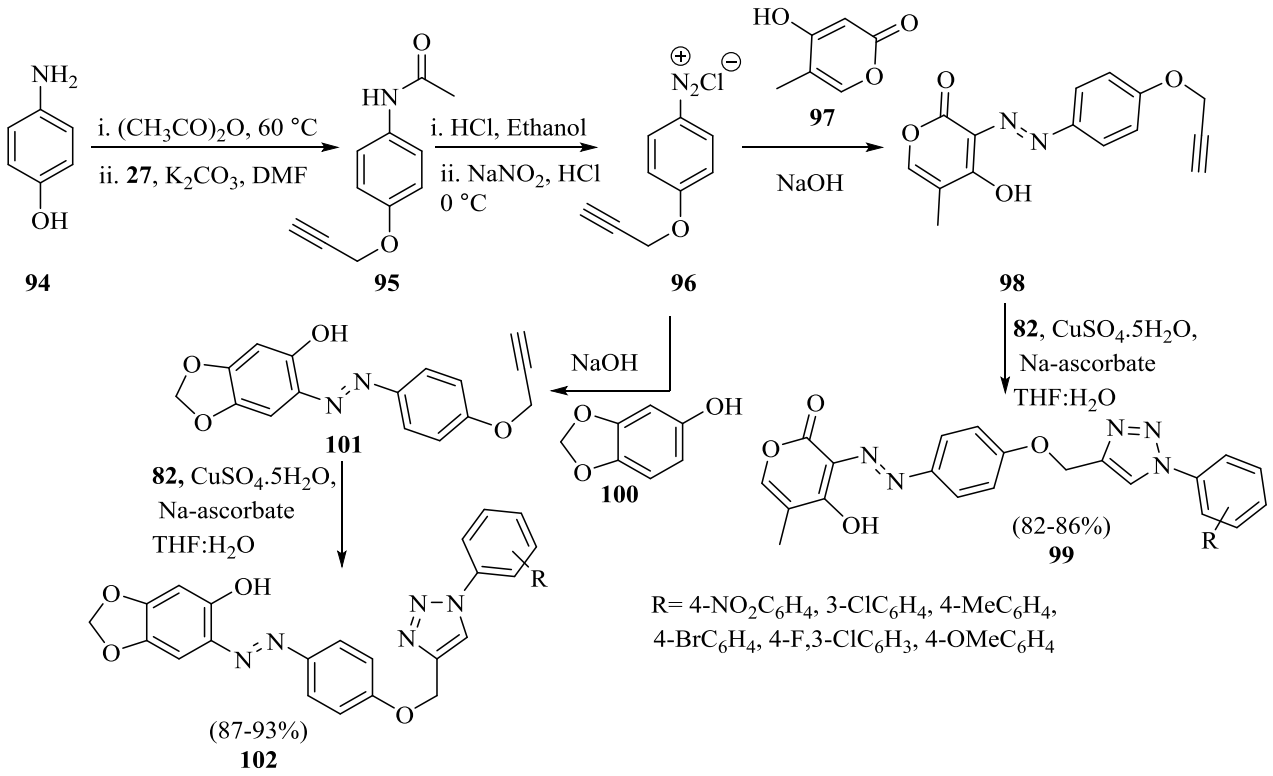
Scheme 1.19: Ultrasonic assisted synthesis of diarylsulfone linked bis-1,2,3-triazoles.

A new series of ethylene spaced indolyltriazoles has been ascribed by Swamy group and scrutinized for their antimicrobial activity.[44] In this approach, they have synthesized triazole ethylene spaced bis heterocycles **93** in one pot manner applying click chemistry approach. Propargyl bromide, indolyl azide **91** and secondary amines **92** were added in one pot manner in water and CuI added as catalyst with TEA. The reaction was completed in 9-15 hours offering good to excellent yields of products (**Scheme 1.20**). All the derived scaffolds were tested for their anti-microbial activity against various Gram positive and Gram negative bacteria *i.e. P. aeruginosa*, *S. aureus, E. coli* and *S. faecalis* and their MIC values were found 15-90 μg/mL in which derivative having chloro substitution on indole ring containing morpholine as amine was found best with MIC value 15-20 μg/mL against different bacteria comparable to standard ciprolfoxacin (12 μg/mL). When antifungal essay was performed for all the scaffolds against *C. albicans* and *A. niger*, it was observed that scaffold containing *N,N*-dimethyl amine motif with no substitution on indole was good antifungal agent with zone of inhibition 19 and 17 mm respectively. All compounds were observed good antifungal agents with zone of inhibition 9-19 mm.



Scheme 1.20: Construction of ethylene spaced triazolylindoles tethered amines.

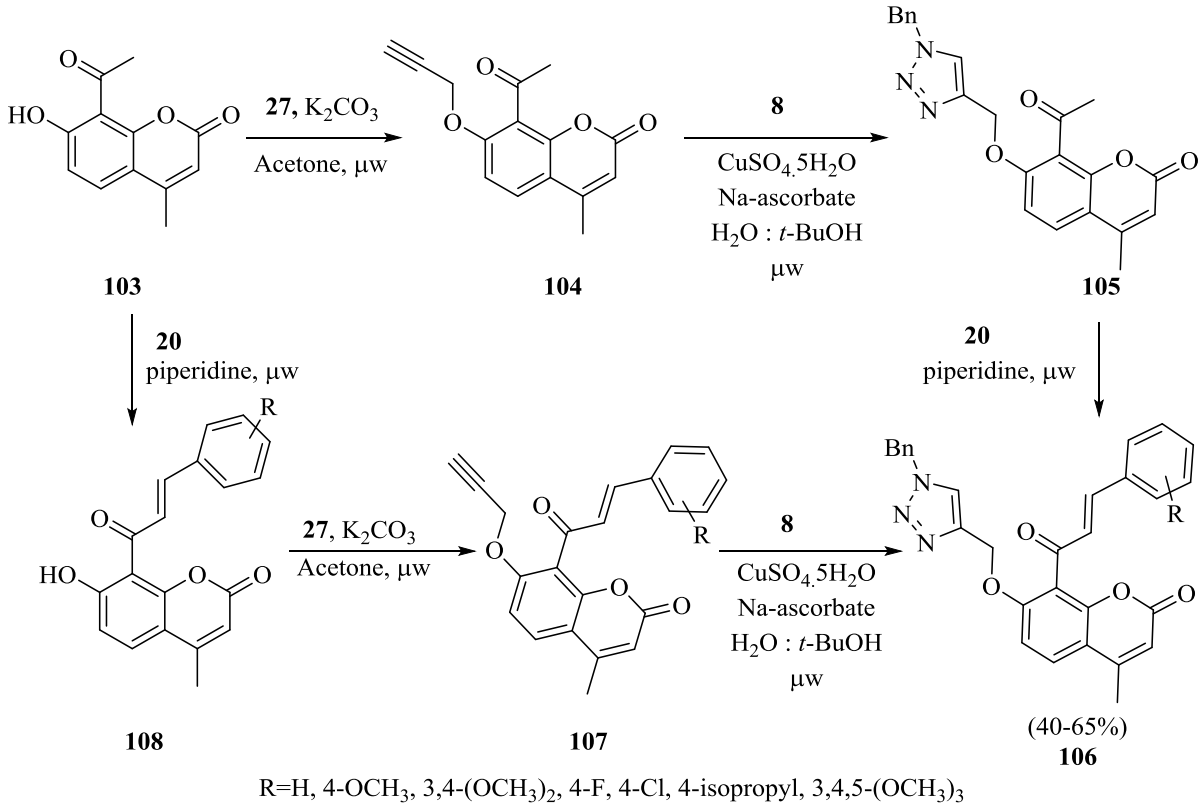
Khurana *et al* developed methodology to test antimicrobial activity of azo dyes. They synthesized triazole ligated azo dyes and evaluated them for their anti microbial activity.[45] *p*-Aminophenol was transformed to **95** by simple reactions which was tranformed into **96** by diazotization. **96** on coupling with 4-hydroxy-5-methyl-2*H*-pyran-2-one or sesamol yielded 4-hydroxy-5-methyl-3{(4-(pro-2-ynyloxy)phenyl)diazenyl-2*H*-pyran-2-one **98** or 6-{(4-(prop-2-ynyloxy)phenyl)diazenyl}benzo-[*d*][1,3]dioxol-5-ol respectively **101**. **98** And **101** were reacted with various aryl azides *via* CuAAC to grant 1,2,3-triazole legated azo dyes **99** and **102 (Scheme 1.21)**. When the derivatives were screened for antibacterial (*S. aureus, B. subtilis, E. coli, P. aeruginosa*) assay, no significant effect was observed of sesamol or pyran ring but derivative containing methoxy substitution were most effective against all bacterii with zone of inhibition 23.3, 24.6 and 16.3 mm for *S. aureus, B. subtilis, E. coli* respectively but no compound was active against *P. aeruginosa*. Most of the compounds exhibited zone of inhibition 14.3-24.6 mm with MIC value 32-256 μg/mL. All the derivatives were evaluated for antifungal activity against *A. niger* and *A. flavus* by calculating percentage growth inhibition which was in the range of 37.7-57.7% again having methoxy substituent most effective.



Scheme 1.21: Synthesis of 1,2,3-triazole tethered azo dyes for antimicrobial evaluation.

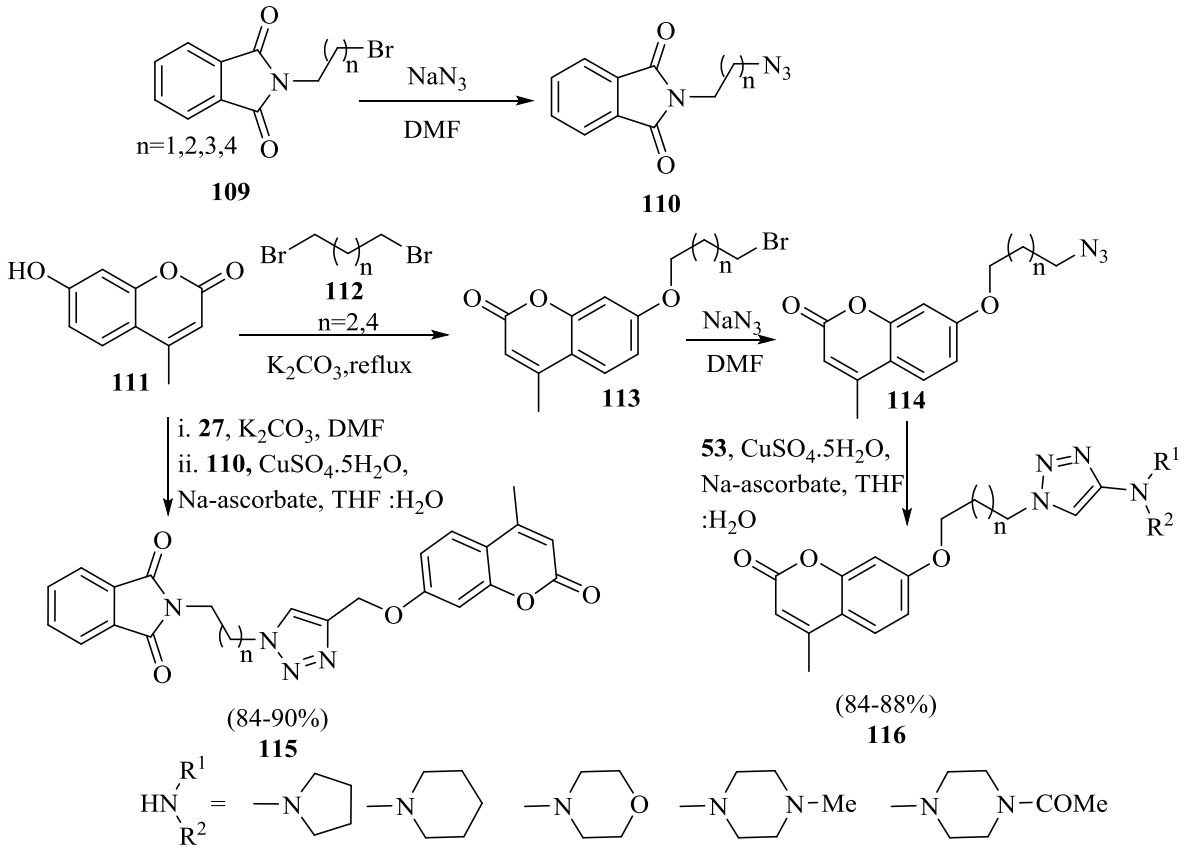
Microwave irradiation is also an alternate for shorter reaction time following environment friendly conditions with high yields of products. Sparkled by the biological activities of chalcones, triazoles and coumarins, Dongamanti and his coworkers proposed a strategy for the synthesis of 1,2,3-triazole linked chalcone-coumarin hybrid scaffolds utilising conventional as well as microwave irradiation.[46] Initially, acetylated 7-hydroxy-4-methylcoumarin **103** was propargylated followed by subsequent click reaction with benzylazide to offer 1,2,3-triazolo linked coumarins **105** under microwave irradiation. Consequent condensation of **105** with arylhalide in presence of piperidine granted desired compound triazole linked coumarin chalcones **106**. Alternatively, first chalcones **108** were constructed followed by propargylation with subsequent CuAAC to offer **106 (Scheme 1.22)**. After the synthesis, the antimicrobial screening of compounds against different Gram positive bacteria (*S. aureus, B. subtilis*), Gram negative (*E. coli, P. aeruginosa*) bacteria and fungal (*A. niger, P. italicum, F. oxysporum*) pathogenic strains was performd by calculating their zone of inhibition (4-28 mm for different bacteria and 8-30 mm for fungi) which revealed that some compounds are enough effective as much as reference amixicillin and micostatin for antibacterial and antifungal activity respectively. Scaffold having *p*-methoxy substitution was best antibacterial agent against all bacteria while 2,4-dimethoxy substituted derivative was best antifungal agent having zone of inhibition (13, 21, 27 mm against *A.*

niger, P. italicum, F. oxysporum respectively) more than control mycostation (12, 20, 25 mm for *A. niger, P. italicum, F. oxysporum* respectively*)*.



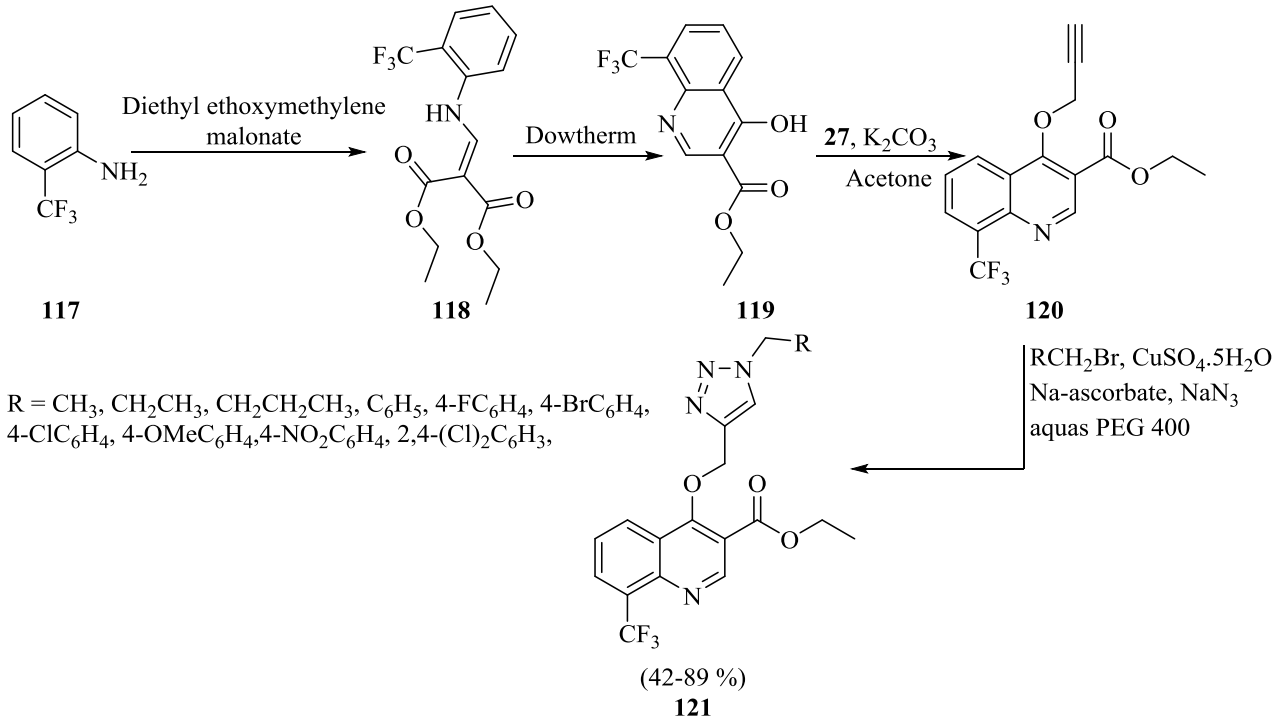
Scheme 1.22: Microwave irradiated synthesis of 1,2,3-triazole linked chalcone-coumarin hybrid scaffolds.

Jain group has developed methodology for the incorporation of 1,2,3-triazoles, heterocylic amines, coumarin along with a lippophilic spacer in single molecular framework to inspect their antimicrobial activity.[47] *N*-(bromoalkyl)isoindoline-1,3-dione **109** on reaction with sodium azide provided azide **110** which by CuAAC with *O*-propargylated **111** offered **115**. **111** was alkylated with 1,4-dibromobutane and consequently transformed into azide **114** which on CuAAC with **53** yielded **116** (**Scheme 1.23**). All the gibbets were tested for antifungal activity (*A. niger, A. fumigatus, A. flavus, C. albicans*) using standard miconazole and for antibacterial activity against Gram positive (*S. aureus, B. subtilis, S. epidermidis*) and Gram negative (*E. coli, P. aeruginosa, S. typhi, K. pneumoniae*) bacteria. It was observed that derivative **116** tethered with morpholine or *N*-acetyl piperazine were most compelling antibacterial agent having 11.7-17.1 mm zone of inhibition. While for antifungal essay derivative **115** with alkyl chain length of 5 C-atom was most effective against *A. fumigatus* (23.4 mm) and almost all scaffolds are good antifungal agents with zone of inhibition 11.9-23.4 mm for different fungi.



Scheme 1.23: 1,2,3-Triazoles as a linker for tethering coumarin with amines.

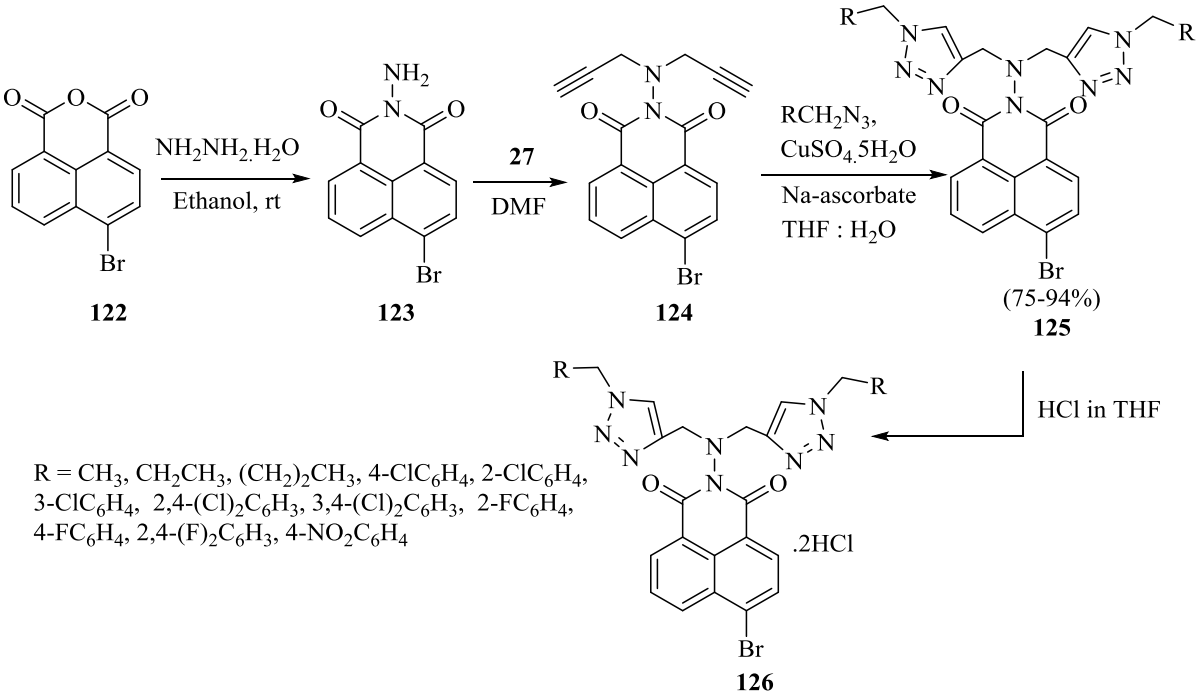
Hegde *et al* have made an attempt to synthesize heterocyclic triazoles by constructing 8-trifluoromethylquinoline 1,2,3-triazole scaffolds.[48] In their approach, intermediate **119** was synthesized by Gould-Jacob method which was then propargylated with propargyl bromide to get **120**. The CuAAC of **120** with different alkyl, aryl and benzyl azides supplied different 1,2,3-triazole linked 8-trifluoromethylquinolines **121 (Scheme 1.24)**. All the manufactured derivatives were screened for antibacterial and antifungal action against *E. coli, B. subtilis, P. aeruginosa* and fungal strains *A. flavus, C. keratinophilum* and *C. albicans* by well plate method using ciprofloxacin and fluconazole as standards. The screening was done at 0.5 mg/mL and 1.0 mg/mL concentration of scaffolds in DMSO which revealed that all of the compounds are antibacterial agents to some extent but motif containing chloro substitution were far better antibacterial agents with zone of inhibition 14 mm, 8 mm and 10 mm against *E. coli, B. subtilis* and *P. aeruginsoa* respectively at the concentration of 0.5 µg/mL. Same type of results were obtained in antifungal activity with chloro substitution having zone of inhibition 5-9 mm while scaffold having alkyl chain or methoxy substitution showed no or negligible activity.



117 **118** **119** **120** **121**

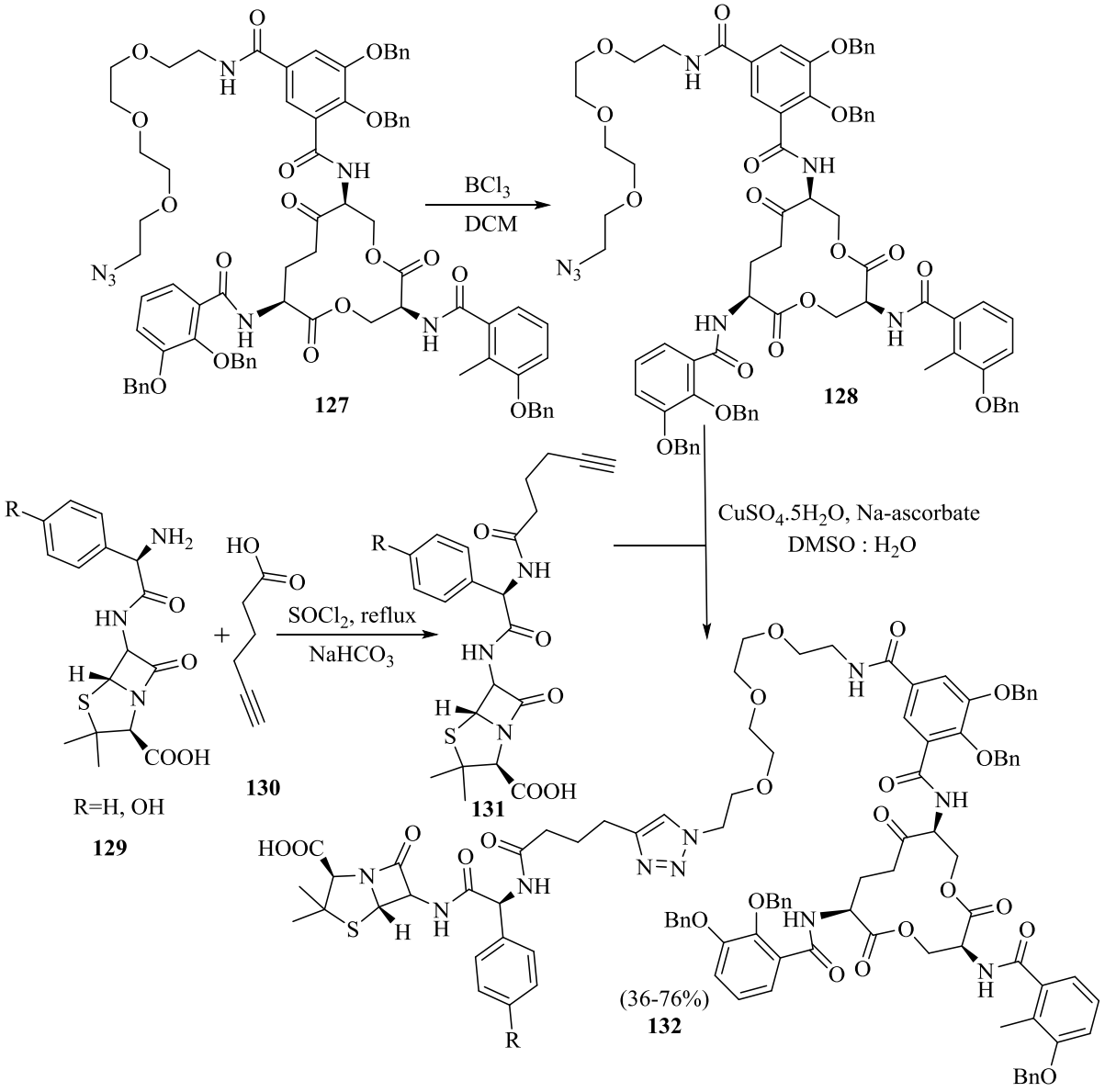
Scheme 1.24: Heterocyclic synthesis of 8-trifluoromethylquinoline-1,2,3-triazole scaffolds.

Zhou and co-workers produced 1,2,3-triazole incorporated naphthalimides for their antimicrobial study.[49] On reaction of hydrazine hydrate with 4-bromo-1,8-naphthalic anhydride **122**, they got *N*-amino-naphthalimide **123** which on propargylation with propargyl bromide issued diprop-2-ynylamino-naphthalimide **124**. Compound **124** was adapted into triazole-fused naphthalimides **125** using copper sulfate and sodium-ascorbate as catalyst (**Scheme 1.25**). The synthesized triazoles were also transformed into their salts **126**. The constructed designs employed for antimicrobial essay against four Gram positive, four Gram negative and three fungal strains in 2-512 μg/mL concentrations which showed that most of the compounds were inactive against fungal strains although unsubstituted naphthilimide containing triazoles with heptyl chain substitution and its salt showed good activity against *A. flavus* with concentration 16 μg/mL far better than fluconazole (256 μg/mL). Most of the compounds found weakly active against bacterial strains with MIC value >512 μg/mL but scaffolds with chloro and fluoro substitution were very good antibacterial agent against *E. coli* with MIC value 1 μg/mL (2,4-dichloro derivative of **134**) far better than standard Chloromycin (16 μg/mL) and Norfloxacin (2 μg/mL). It was also observed that salts were more better in antimicrobial activity than the corresponding compound.



Scheme 1.25: Synthesis of 1,2,3-triazole incorporated naphthalimides.

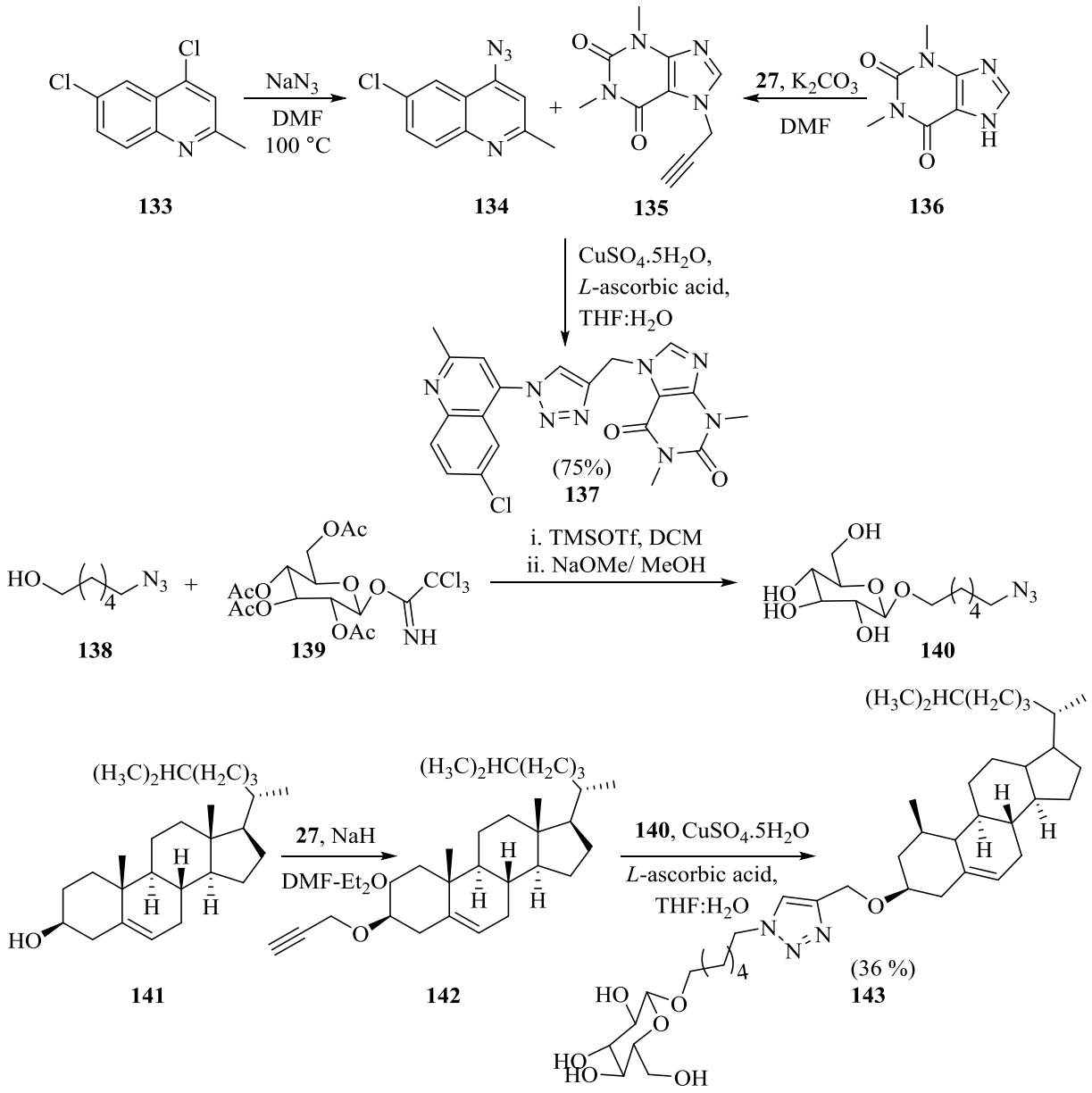
Conjugation of two different scaffold can lead to higher antimicrobial activity. By this method, we can attain the characteristics of two scaffolds simultaneously which would increase their activity in a positive mode. Nolan group envisoned the bioconjugation of enterobactin with ampicillin *via* a polyethylene based glycol-1,2,3-triazole conjugate to examine the antibacterial activity against *E. coli*.[50] Ampicillin **129** on refluxing with hex-5-ynoic acid **130** in $SOCl_2$ created amide link offering alkyne attached ampicillin **131** (**Scheme 1.26**). Subsequently, deprotection of catechol part of enterobactin-azide **127** using BCl_3, followed by CuAAC with ampicillin derivative **131** offered 1,4-disubstituted 1,2,3-triazole linked ampicillin-enterobactin conjugate **132**. These scaffolds were tested for antibacterial activity agaisnt 6 different types of pathogen *E. coli* using 10-fold dilution method which showed enhanced antibacterial activity against all six *E. coli* pathogens with 1000 times decrease in MIC value 10 nM which was 1 μM for Enterobactin and Ampicillin.



Scheme 1.26: Conjugation of Enterobactin with Ampicillin *via* polyethylene based glycol-1,2,3-triazole conjugates.

Based on the same methodology of bioconjugation, Sayed Aly synthesized three different series of 1,2,3-triazoles ligated chalcone, cholesterol and theophylline via CuAAC.[51] Cholesterol **141** was *O*-propargylated while *N*-propargylation was carried out for theophylline **136**. Azidolysis of chloroquinoline **133** was processed to get azidoquinoline **134** while spacer linker azidohexanol **138** was reacted with **139** to get azides **140**. Above mentioned azides and alkynes were linked *via* CuAAC offering various triazoles **137** and **143** (**Scheme 1.27**) which were screened for antimicrobial activity aganst *E.coli, S. aureus, A. flavus* and *C. albicans* using Ampicillin and Amphotecerin-B as control ending into the conclusion that triazole motif with glucopyranocylhexyl and cholesteryl group is more

effective for antibacterial action having 18% more zone of inhibition than control ampicillin but it was 12% less active against *C. albicans* than Amphotecerin B.

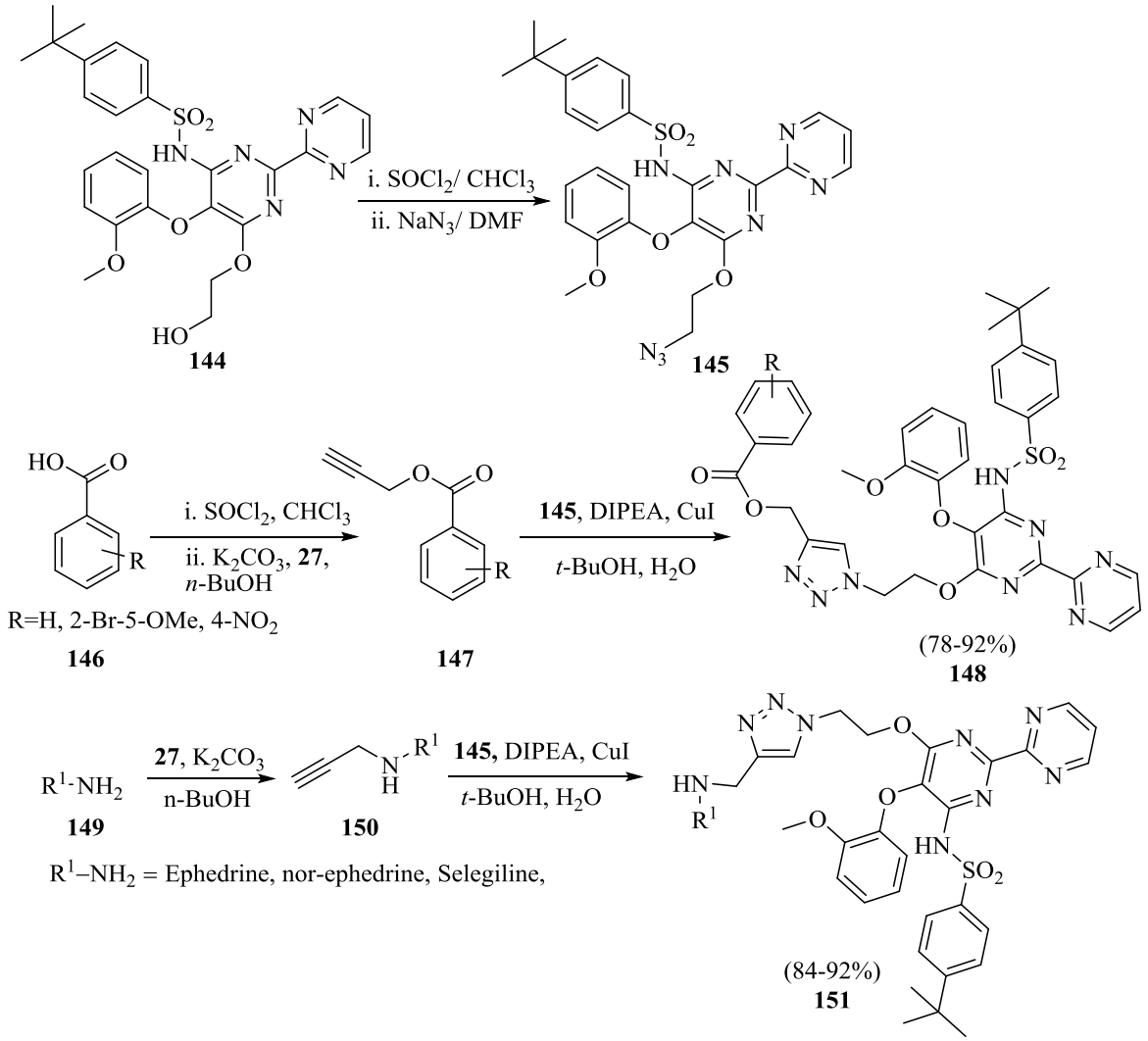


133 **134** **135** **136** **137** **138** **139** **140** **141** **142** **143**

Scheme 1.27: Synthesis of 1,2,3-triazole ligated chalcones, cholesterols and theophyllines.

Rajendran *et al* have designed and synthesized bosentan ligated 1,2,3-triazoles and further examined their antimicrobial activity.[52] In their approach, bosentan **144** was reacted with thionyl chloride followed by azidation to yield azido-bosentan **145**. Simultaneously, terminal amino alkynes were prepared by reacting amine **149** with propargyl bromide in presence of base or transforming acids **146** into acid chloride followed by propargylation. These terminal alkynes **147** and **150** were reacted with bosentan affixed azides employing click chemistry approach which offered bosentan 1,2,3-triazoles **148** and **151** respectively **(Scheme 1.28)**.

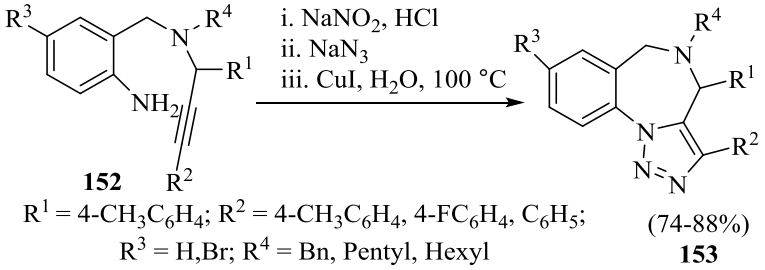
The synthesized scaffolds were subjected to antimicrobial screening against a vast series of Gram positive and Gram-negative bacteria and two fungi by calculating their zone of inhibition. It was observed that scaffold having selegiline substitution were inactive against any bacteria or fungi while scaffold **148** with methoxy substitution were best antibacterial agent with zone of inhibition 17-20 mm against different bacteria but it was inactive against *S. typhaemurium*. The MIC values were observed 31.25-500 µg/mL. Scaffold having methoxy substitution was also comparable (ZOI 15-22 mm) for different bacteria while it was the best antifungal agent against *C. albicans* (16 mm), *M. pachydermatis* (12 mm). Most of the derivatives were inactive against fungi.



Scheme 1.28: CuAAC to get bosentan ligated 1,2,3-triazoles.

A new series of 1,4-disubstituted 1,2,3,-triazolo[1,5-*a*]benzodiazepines has been synthesized by Perumal *et al* by sequential diazotization, azidation and cycloaddition reaction in a one pot fashion.[53] The sequential A^3-coupling of *N*-Benzyl-1-(2-nitrophenyl)methanamine, aromatic

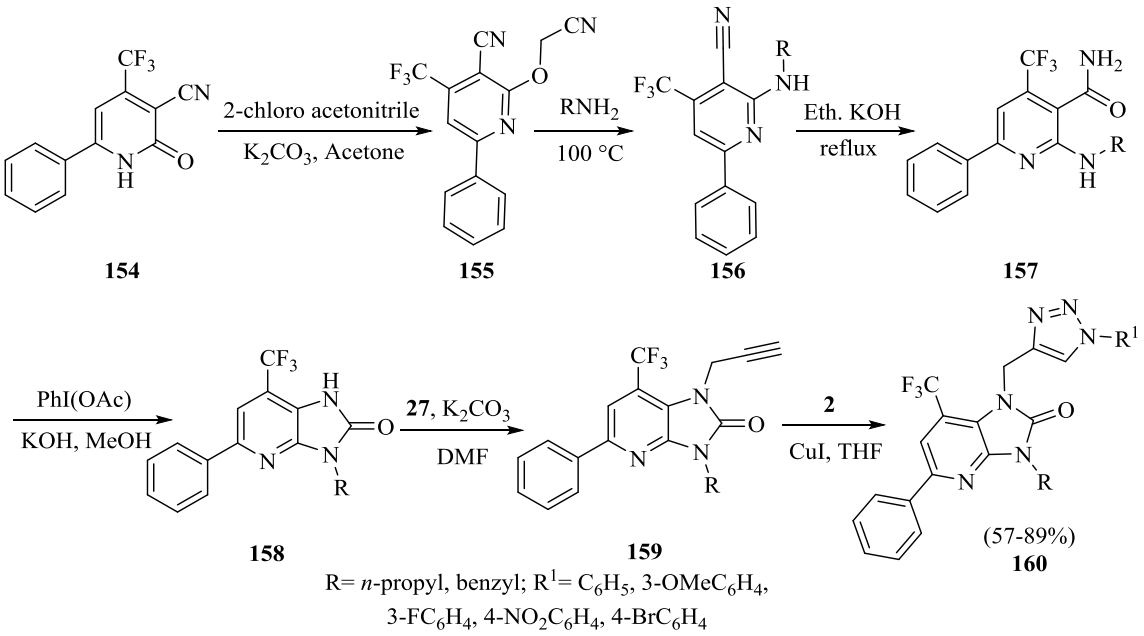
aldehyde and alkyne followed by reduction of nitro group supplied 2-amino-*N*-benzylpropargylamine **152**. These 2-amino-*N*-benzylpropargylamine were subjected to diazotization with sodium nitrite in dil. HCl to get diazonium salt which on subsequent azidation followed by intramolecular CuAAC under thermal conditions lead to desired annulated 1,4-disubstituted 1,2,3,-triazolo[1,5-*a*]benzodiazepines **153** (**Scheme 1.29**). The synthesized annulated compounds were examined for their antimicrobial activty against a vast series of four Gram positive, five Gram negative bcteria and two fungi in which it was found that almost all derivatives are good antibacterial agents with zone of inhibition 10-26 mm for different bacterii but scaffold with 4-chlorophenyl and 4-bromophenyl subtitution (R^2) showed no inhibition for any bacteria. The MIC values were found very high (31.25-250 µg/mL) in comparison of standard 6.25 µg/mL. On antifungal screening against *C. albicans* and *M. pachydermatis*, most of the compounds were inactive while some derivatives were moderately active with zone of inhibition 10-15 mm very less than standard ketoconazole 28 and 26 mm respectively.



Scheme 1.29: One pot synthesis of 1,4-disubstituted 1,2,3,-triazolo[1,5-*a*]benzodiazepines.

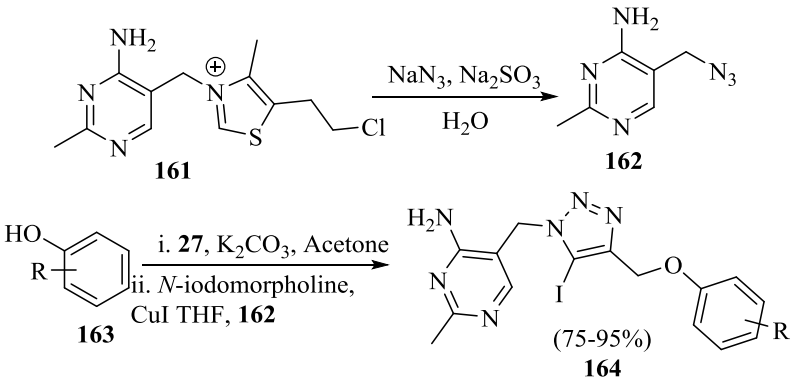
Banda and collagoue has reported the creation of 1,2,3-triazole functionalised imidazo[4,5-b]pyridine-2-(3*H*)-one to ascertain their antimicrobial activity.[54] 2-Oxo-6-phenyl-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile **154** was customized to 2-(cyanomethoxy)-6-phenyl-4-(trifluoromethyl)nicotinonitrile **155** on reaction with 2-chloroacetinitrile followed by amination with primary amines to grant 2-amino/alkylamino-6-phenyl-4-(trifluoromethyl)nicotinonitrile **156**. On hydrolysis with ethanolic KOH, **156** was tranformed into amides which dealed with hypervalent iodine to offer imidazo[4,5-*b*]pyridine-2(3*H*)-one **158** by Hoffmann type rearrangement. Propargylation of imidazo[4,5-*b*]pyridine-2(3*H*)-one and consequent CuAAC proffered desired composite 1,2,3-triazole functionalised imidazo[4,5-*b*]pyridine-2-(3*H*)-one **160** (**Scheme 1.30**). Derivatives of **160**were exploited for antimicrobial activity against Gram positive *S.aureus, B. subtilis, M. luteus* and Gram negative *K. planticola, E. coli, P. aeruginosa* and fungi *C. albicans* using

neomycin and miconazole as standard respectively for antibacterial and antifungal activity but no compound could show any significant antibacterial/ antifungal activity comparable to standard neomycin or miconazole.



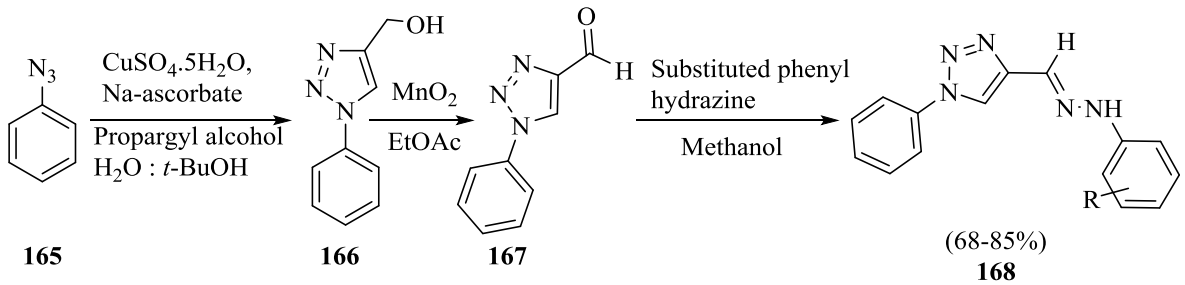
Scheme 1.30: Synthesis of 1,2,3-triazole functionalised imidazo[4,5-*b*]pyridine-2-(3*H*)-one.

Wan and co-workers established the design to synthesize 5-iodo-1,4-disubstituted 1,2,3-triaozle frameworks for screening their antifungal activity (**Scheme 1.31**).[55] Azide **162** was acquired by reaction of sodium azide with thiamine chloride **161** in presence of sodium sulfite in water. The required iodoalkynes were synthesized by propargylation of different phenols **163** followed by iodination with *N*-iodomorpholine. The CuAAC of thiamine azide and iodo-alkynes overtured 5-iodo-1,4-disubstituted-1,2,3-triazoles **164** which were tested for antifungal activity against *G. zeae, R. solani, B. cinerea* and *A. solani.* All the compounds were screened at 100 μg/mL concentration for inhibitory potency which revealed that most of the compounds were good fungicidal agent against *R. solani* and *B. cinerea* by showing inhibitory potency 92-100%. Halo substituted derivatives were wide antifungal agent and showed inhibiton against all pathogenic strains. Derivatives containing cyano or bromo substitution displayed inhibition in low concentration and showed 66-87% inhibitory potency with 12.5 μg/ml concentration.



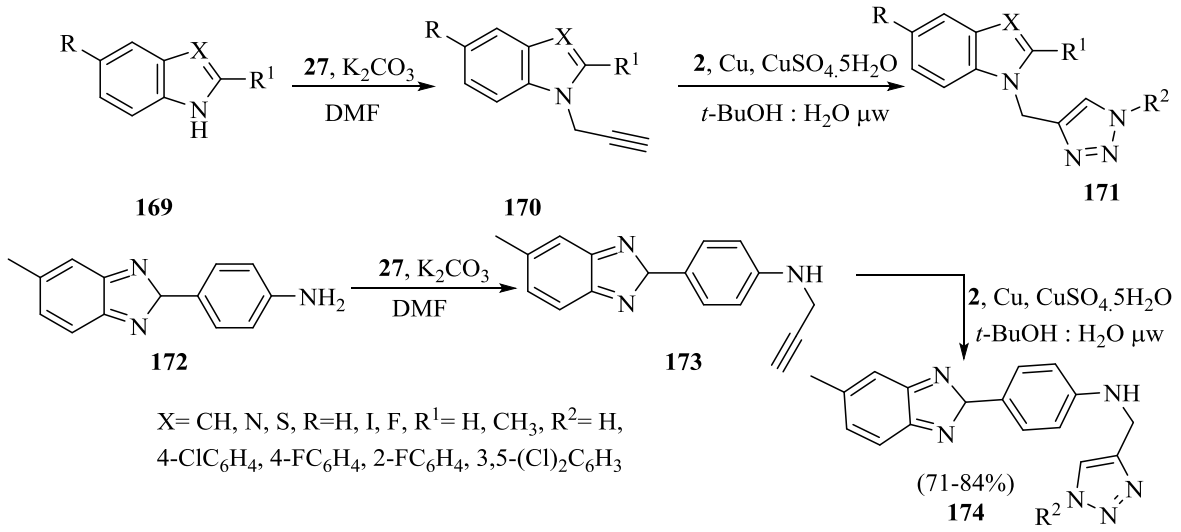
Scheme 1.31: Synthesis of pyrimidine containing 5-iodo-1,4-disubstituted-1,2,3-triazoles.

Ye and collaborators have developed a series of 1,2,3-triazolyl phenyl hydrazone analogoues for evaluation of their antifungal activity against some plant pathogenic fungi.[56] Aromatic azides **165** were diretly reacted with propargyl alcohol to avoid degradation via CuAAC which provided regioselectively 1,4-disubstituted 1,2,3-triazoles **166**. Next, alcohols were oxidised to aldehydes **167** using MnO_2 in ethylacetate. These triazole containing aldehydes were reacted with different substituted phenyl hydrazines to get 1,2,3-triazole tethered phenylhydrazones **168 (Scheme 1.32)**. After the successful synthesis of scaffolds, all the motifs were inspected for their antifungal activity against various plant pathogenic fungi such as *R. solani, S. sclerotiorum, F. graminearum* and *P. capsici* which showed that most of the compounds had considerable activity against *R. solani* with (median effective concentration) EC_{50} values lower than 1 μg/mL derivatives. Derivatives bearing chloro, fluoro substitutions showed inhibitory action against all fungi. Motif containing fluoro substitution on one aryl ring and chloro substitution on another aryl ring inhibited *S. sclerotiorum, F. graminearum, P. capsici* with EC_{50} values having 2.28, 1.01 and 1.85 μg/mL respectively. Derivatives bearing fluoro substitution on both aryl ring was most potent with EC_{50} value 0.61 μg/mL against *F. graminearum.*



Scheme 1.32: CuAAC to assess 1,2,3-triazole attached phenyl hydrazones.

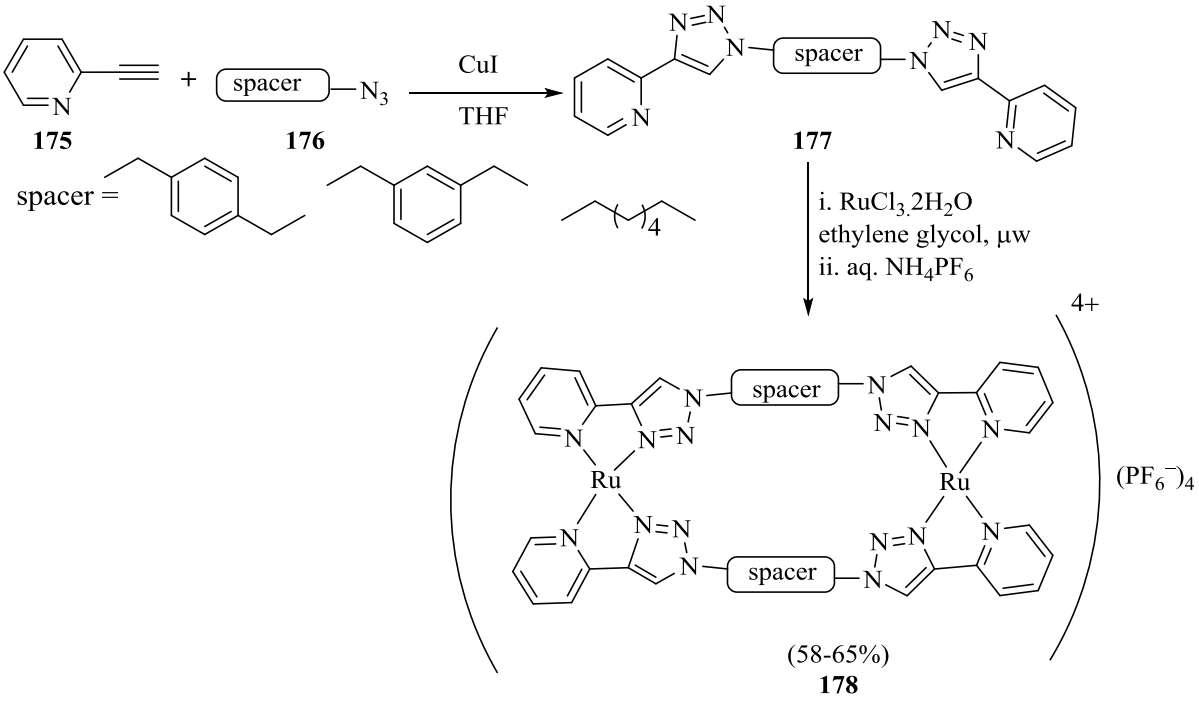
Based on the concept of molecular hybridisation, Malic with his colleague developed a strategy to synthesize 1,2,3-triazole benzofused heterocycles containing benzothiazole as a potent antimicrobial agent.[57] Nitrogen atom of indole, benzamidazole and benzothiazole **169** was propargylated followed by CuAAC, to purvey 1,2,3-triazole ligated indole, benzamidazole **171**. The same course of propargylation was utilised for amine of 2-(4-aminophenyl)benzamidazole **172** followed by consequent CuAAC to furnish 1,2,3-triazolo fused aminophenyl benzamidazoles **174** (**Scheme 1.33**). All the synthesized derivatives were scrutinized for antibacterial activity against Gram positive *S. aureus, P. pneumoniae* and Gram negative bacteria *E.coli, H. influenzae* and *M. catarrhalis* using azithromycin as control. MIC values were calculated for all the scaffolds and it was found that most of the scaffolds were inactive *against S. aureus,P. pneumoniae*, *E.coli* and *H. Influenzae*. Some of the compounds were potent against *M. catarrhalis* especially chloro and fluoro substituted scaffolds with MIC value 0.5 and 1 μg/mL. Based on the lipophilicity results the enhanced antibacterial activity of some compounds was attributed to enhanced membrane permability of those compounds.



Scheme 1.33: Molecular hybridization to get benzo- fused heterocyclic triazoles.

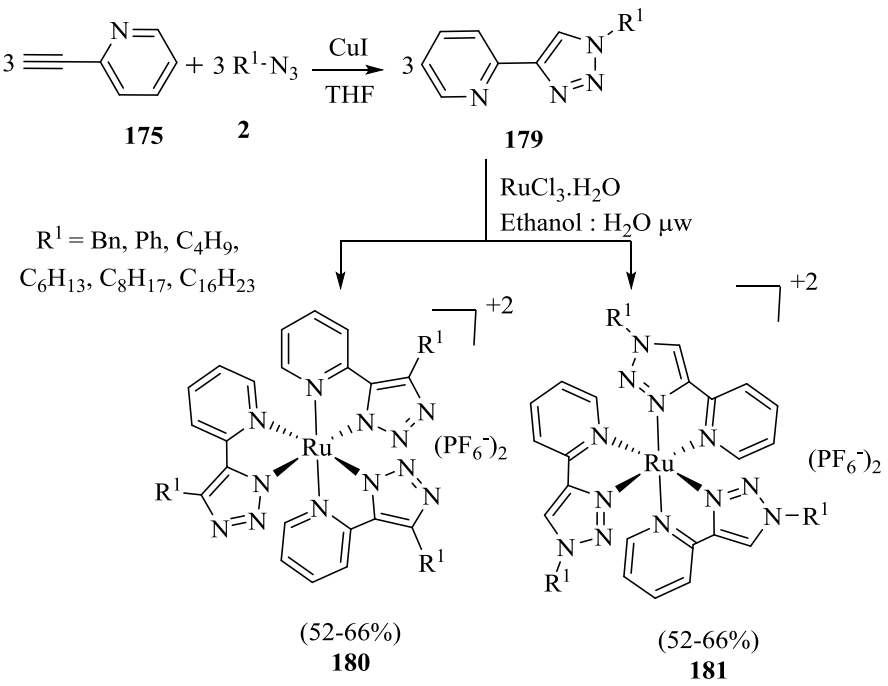
Crowley *et al* have developed new ruthenium(II) triply stranded helicate from a bis-bidentate pyridyl-1,2,3-triazole ligand with spacer and $RuCl_3$ and evaluated their antimicrobial activity.[58] Initially, 2-acetyl pyridines **175** were reacted with different diazidoalkanes and diazidoarenes to get bis-bidentate clicked pyridyl-1,2,3-triazoles **177** *via* CuAAC which were used as ligand to react with $RuCl_3$ under microwave irradiation to yield ruthenium(II)triply stranded pyridyl-1,2,3-triazole ligated complex **178** (**Scheme 1.34**). The developed helicates were characterised by IR, UV-vis, ^1H, ^{13}C, ^1H DOSY NMR specroscopic methods. The

synthesized helicates and free ligands (pyridyl-1,2,3-triazole ligated spacers) were studied for antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria which revealed that ruthenium complexes are weak antibacterial agent with zone of inhibiiton 7-9 mm; also their MIC values were high 256 μg/mL comparably to zentamicin (zone of inhibition 22-25 mm, MIC value >0.5 μg/mL).



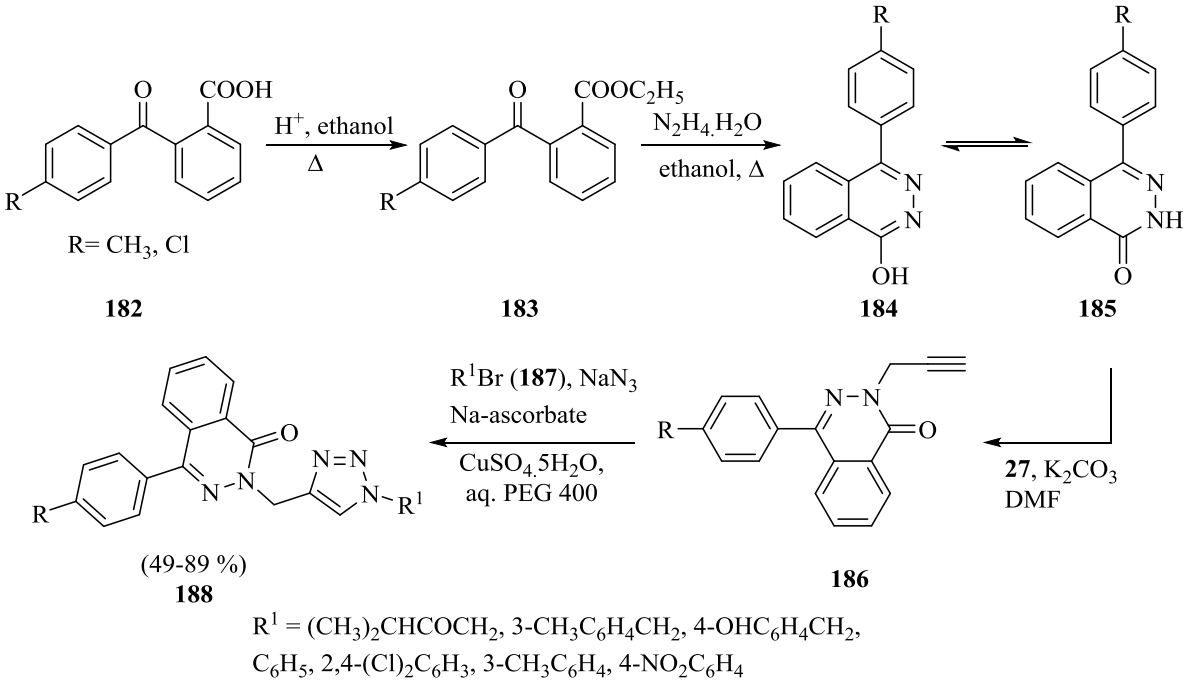
Scheme 1.34: Microwave irradiated complex formation of pyridyl-1,2,3-triazolyl spacers with Ruthenium (II) metal ion.

Other report by Crowley and co-workers has revealed the synthesis of tris(homoleptic) ruthenium (II) complex of 2-(1-R-1*H*-1,2,3-triazol-4-yl)pyridine complexes to test antibacterial activity against various pathogenic bacterias.[59] In this regard, 2-ethynylpyridine **175** was coupled with alkynes to get 1,4-disubstituted-1,2,3-triazoles **179** which were consequently subjected to microwave irradiation with ruthenium (III)chloride to grant desired complex **180** and **181 (Scheme 1.35)**. All the derivatized scaffolds were screened against Gram positive *S. aureus* and Gram negative *E. coli*, which revealed that the synthesized scaffolds are far better in action towards Gram-positive bacteria with zone of inhibition 10-21 mm (MIC value 4-128 μg/mL) but futile for Gram-negative bacteria. The *Mer*-Derivative with hexyl substitution was most effective with zone of inhibition 21 mm having MIC value 8 μg/mL. No compound show any inhibition against *E .coli*.



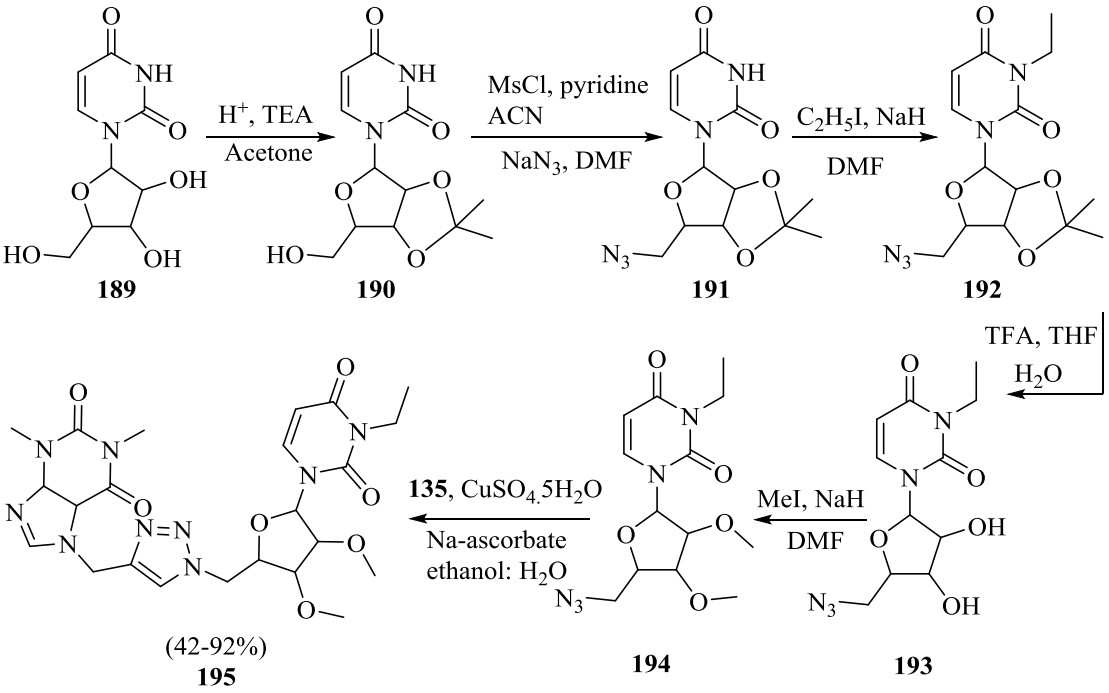
Scheme 1.35: Complex formation of pyridinyl-1,2,3-triazoles with Ruthenium (II) for microbial evaluation under microwave irradiation.

Kalluraya with his coworkers developed strategy to construct derivatives of 1,2,3-triazole ligated pthallazines for evaluation of their antimicrobial activity.[60] Esterification of 2-(4-methyl/chloro-benzyloxy)benzoic acid **182** followed by hydrazinolysis fabricated 4-(4-methyl/chloro-phenyl)pthalazine-1-ol **184** which was on propargylation by condensing with propargyl bromide endeavored 4-(4-chloro/methylphenyl)-2-prop-2-yn-1-ylpthalazin-1(2*H*)-one **186**. Regioselective one pot copper catalyzed azide –alkyne cycloaddition of **186** with various benzyl/phenacyl/alkylbromide, sodium azide led to 1,2,3-triazole ligated pthalazine composites **188** (**Scheme 1.36**). The synthesized derivatives were screened for antibacterial activity against *E. coli, P. aeruginosa* and *B. subtilis* by calculating zone of inhibition which was 8-12 mm for different derivatives. Scaffolds having dichloro substitution were best for antibacterial action against all bacterii with 19.4, 16.3, 18.5 mm zone of inhibition respectively. On analysis of antifungal activity of synthesized scaffolds, most of the derivatives were inactive against *A. flavus, C. keratinophilum* and *C. albicans* but derivative containing 2,4-dichlorobenzyl substitution on triazole ring and 4-chloro substitution on pthalazine ring was best with zone of inhibition 10-16 mm for different fungi.



Scheme 1.36: Construction of pthallazine ligated 1,2,3-triazole derivatives.

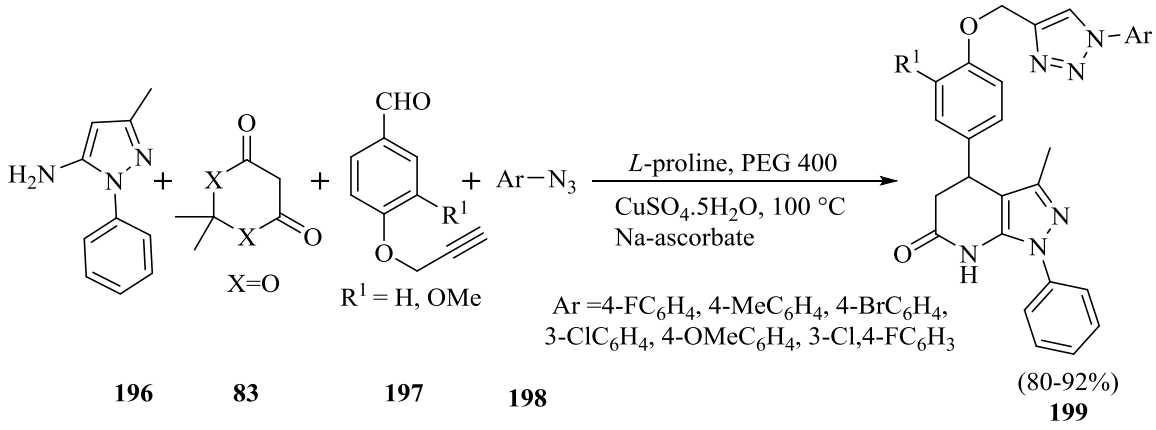
Murugulla and co-workers designed and synthesized theophylline containing 1,2,3-triazoles to check their antimicrobial activity.[61] Uridine **189** was protected in acidic media followed by mesylation and consequent azidation to endow uridinyl azide **194**. Next, theophylline alkyne **135** and uridinyl azide **194** were reacted in presene of catalytic amount of $CuSO_4$ and Na-ascorbate to get theophylline linked uridinyl 1,2,3-triazoles **195** (**Scheme 1.37**). These scaffolds were tested for antibacterial activity against Gram positive *S. aureus, B. cereus* and Gram negative *E. coli, P. aeruginosa* and for antifungal activity against fungi *C. albicans* by serial dilution method using ciprofloxacin as standard drug. It was observed that derivatives having alkyl chain, acetamido group or carboxylate group were better in inhibitory action in which compound having alkyl chain showed inhibitory action with MIC value 0.0156, 0.0625 and 0.0625 mg/mL against *S. aureus, E. coli* and *P. aureginosa* respectively. Among all the tested derivatives, compound bearing triazole protected nucleosides having carboxylate chain showed best inhibitory action for *C. albicans* with MIC value 0.0156 mg/mL.



Scheme 1.37: Synthesis of theophylline containing 1,2,3-triazoles for biological evaluation.

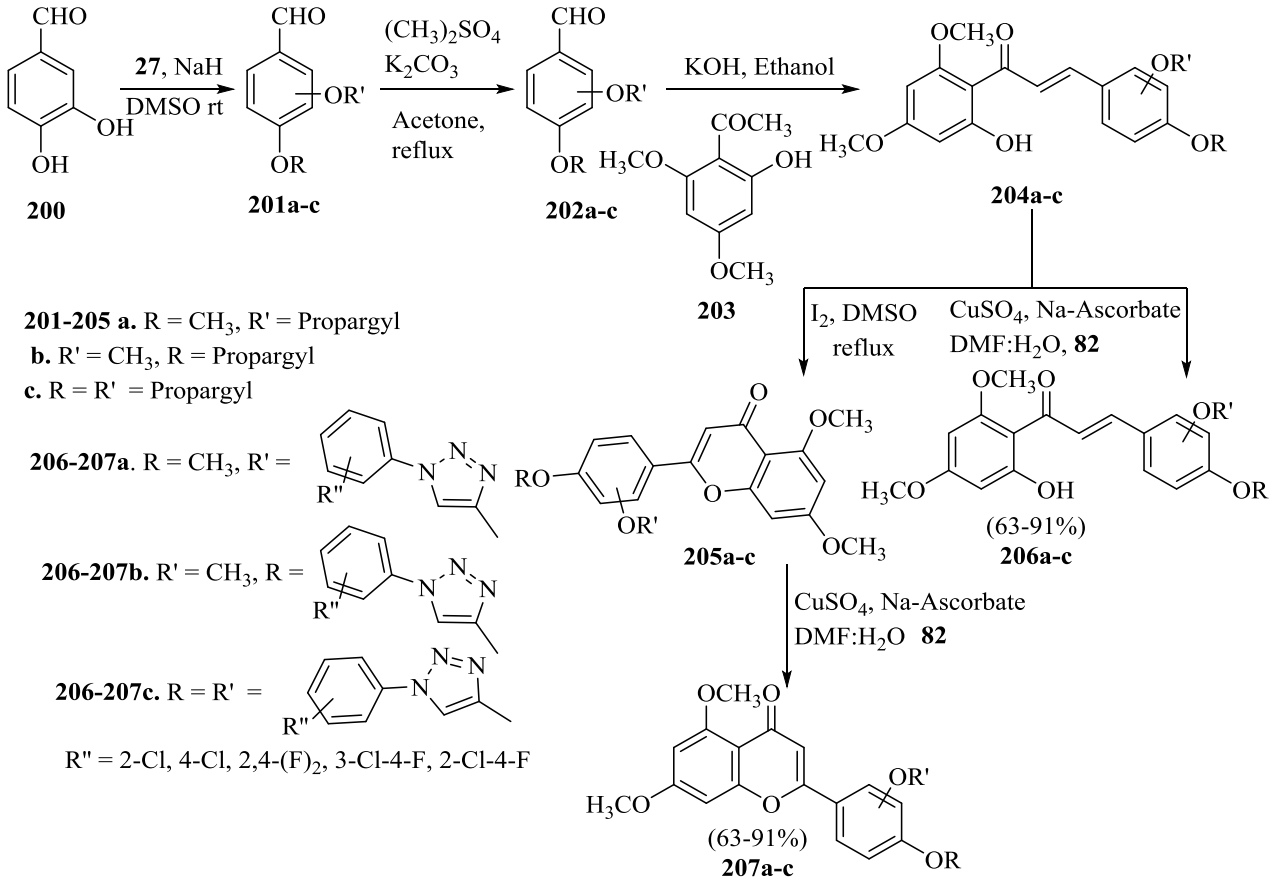
Khurana group has synthesized molecular hybrids containing pyrazole, pyridinone and 1,2,3-triazoles using click chemistry approach by one pot multi-component reaction.[62] The reaction was performed in a one pot four component condensation manner with Meldrum's acid **83**, substituted aryl azides, 4-(prop-2-yn-1-loxy)aryl aldehyde **197** and 3-methyl-1-phenyl-1*H*-pyrazol-5-amine **196**. All scaffolds were reacted in a one pot manner using copper sulfate and sodium ascorbate in presence of *L*-proline as a basic organocatalyst to afford 4-(4-((1-aryl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-6(7*H*)-one **199** at 100 °C. The path involves a series of CuAAC reaction, Knoevenagel condensation followed by Michael addition with 3-methyl-1-phenyl-1*H*-pyrazol-5-amine and subsequent cyclisation to yield **199 (Scheme 1.38)**. All the scaffolds were tested for antimicrobial activity against two Gram negative (*E. coli, P. aeruginosa*), four Gram-positive (*S. aureus, S. epidermidis, B. subtilis, B. cereus*) bacteria which revealed that the derivatives of designed scaffold are inactive against Gram negative bacteria but showed good activity against Gram positive bacteria with zone of inhibition 14.3-23.6 mm for different bacteria. For most of the compounds MIC values were found 28-256 μg/mL and MBC value 16-128 μg/mL except *S. aureus* having MBC value 16-256 μg/mL.and antifungal activity against two yeast strains. When antifungal study was performed against *C. albicans* and *S. cerevisiae*, the diameter of growth inhibition was found 12.3-16.3 and 14.3-16.6 mm respectively for both fungi having MIC value 64-256 μg/mL. It was concluded that

compound with methyl and methoxy substitutions were better in their antimicrobial action having highest zone of inhibition against all Gram positive bacteria and fungi. Apoptosis study was also performed for derivatives.



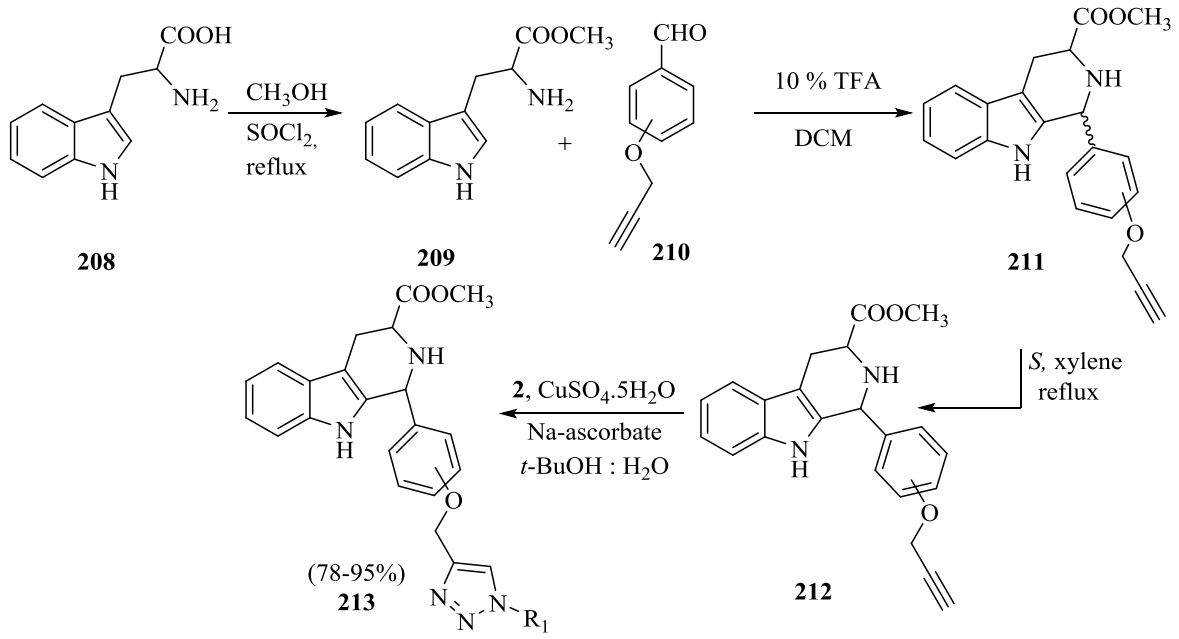
Scheme 1.38: Synthesis of 1,2,3-triazole tethered pyrazolo pyridinones by one pot four-component reaction.

Recently, a facile route towards combining two types of pharmacophores in one moiety *i.e.* 1,2,3-triazole linked flavones and chalcones employing click chemistry was established by Kant and coworkers.[63] Initially, 3,4,-dihydroxy benzaldehyde **200** was propargylated **201a-c** as required. Methylation of propargylated benzaldehyde was carried out using dimethylsulphate, K_2CO_3 to afford **202a-c** which was amalgamated with **203** to obtain chalcone **204a-c.** Flavones **205a-c** were procured by cyclisation of chalcone **204a-c** using I_2, DMSO. These synthesized flavones and chalcones were reacted with different substituted azides **82** to get 1,2,3-triazole linked flavones **206a-c** and chalcones **207a-c** (**Scheme 1.39**). All the synthesized motif were screened for *in vitro* antibacterial (*E. coli, S. aureus, E. faecalis, P. aeruginosa, K. pneumoniae*) and antifungal (*C. albicans, C. neoformans, A. niger, A. fumigatus*) activity and it was observed that most of the compounds showed better antimicrobial activity towards different bacteria and fungi with MIC value 6.25->100 μg/mL. They concluded that chalcone **206a** linked with 2-Cl-4-F azide was most potent towards antibacterial activity (MIC value 6.25 μg/mL) while 2,4-difluoroazide linked chalcone **206c** (MIC value 6.25-12.5 μg/mL) were more potent against fungi. The group also studied antiplasmodial and cytotoxic study of synthesized 1,2,3-triazoles linked flavones and chalcones.



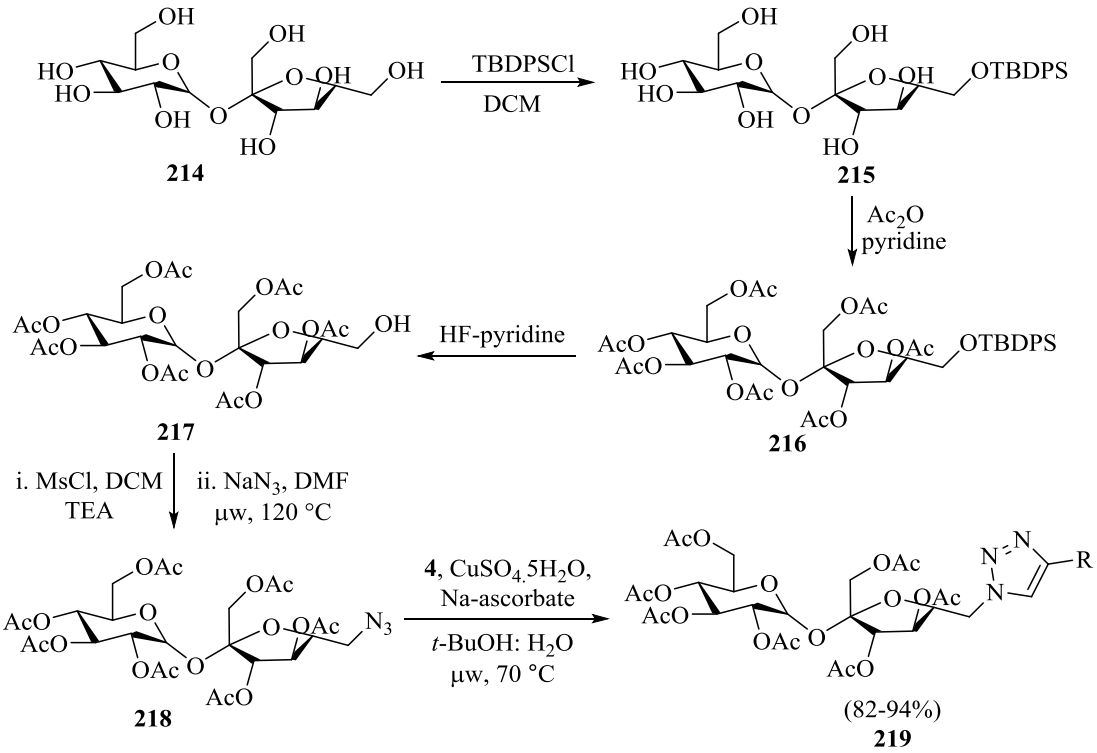
Scheme 1.39: Synthesis of 1,2,3-triazole linked chalcone and flavones hybrids.

Very recently, Salehi *et al* has envisioned the synthesis of 1,2,3-triazole tethered β-carboline derivatives from *L*-tryptophan by Pictet-Spengler reaction followed by 1,3-dipolar cycloaddition.[64] In their approach, esterified *L*-tryptophan **209** was treated with *O*-propargylated benzaldehydes **210** *via* Pictet-Spengler reaction to yield 1,2,3,4-tetrahydro-β-carboline scaffolds **211**. Oxidation of **211** with sulphur lead to **212** which on CuAAC with different aryl azides provided desired motif **213** (**Scheme 1.40**). All the scaffolds were screened for their antibacterial activity against five Gram negative and Gram positive bacteria using chloramphenicol and cefixime as standard antibiotics. Although, most of the compounds are not good against *S. aureus, E. coli, B. cereus* having MIC value 16-128 μg/mL but derivatives having bromo substitution are potent against *E. faecium* with MIC value 8 μg/mL better than reference cefixime having 32 μg/mL inhibition.



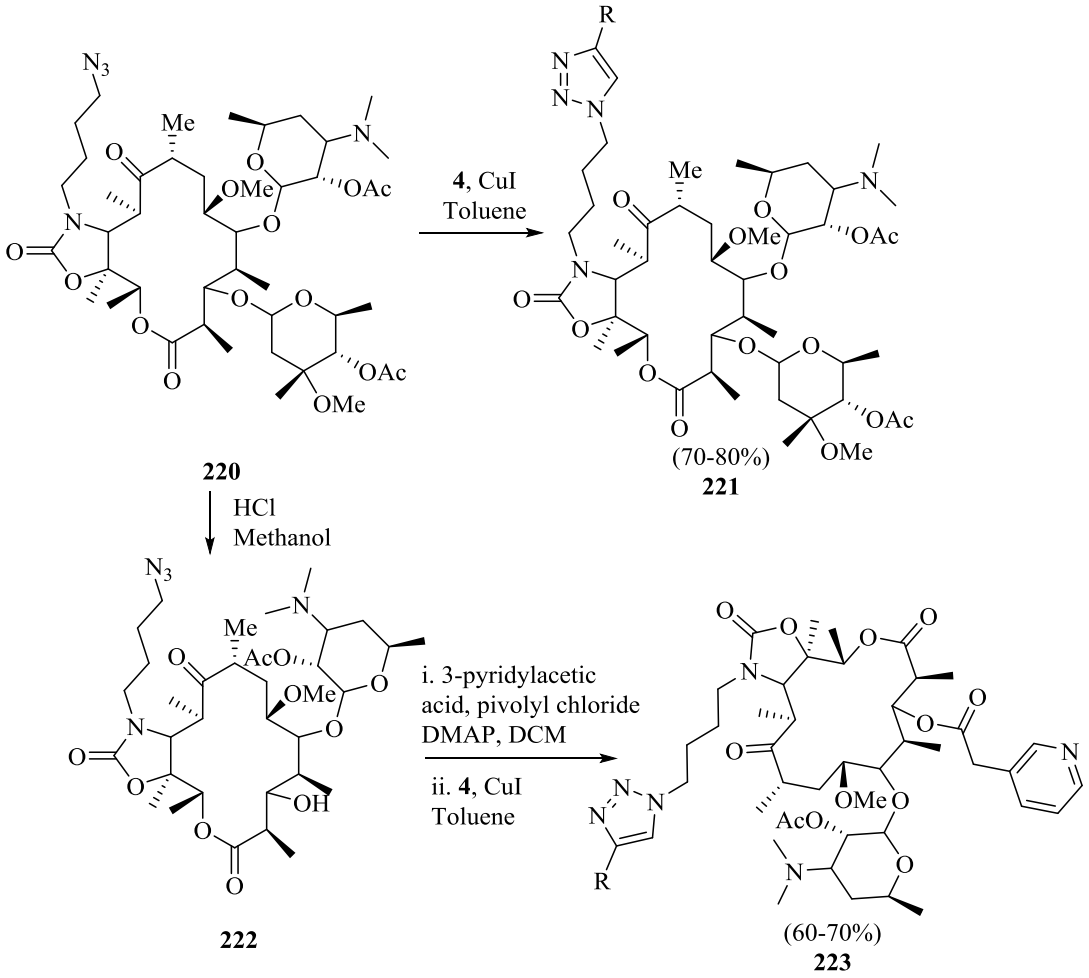
Scheme 1.40: Derivatisation of β-Carbolines to get β-Carboline triazoles for anti-antimicrobial activity.

Barros and colleague envisioned the synthesis of novel 1,2,3-triazoles embedded sugar derivatives under microwave irradiation.[65] In their approach, 6-*O*-*tert*-butyldiphenylsilyl-sucrose **215** was afforded by reaction of sucrose **214** with *tert*-butyldiphenylchlorosilane (TBDPSCl). Hydroxyl groups of **215** were protected by treating with acetic anhydride in pyridine followed by selective deprotection of silyloxy group with HF-pyridine in THF resulting in **217**. Hydroxyl group of **217** was transformed into azide by reaction with methane sulfonyl chloride followed by azidation to get **218**. The latter was treated with different azides *via* click chemistry approach through copper catalyst to provide 1,2,3-triazole embedded sugars **219** (**Scheme 1.41**). All derivatives were screened for antibacterial activity against *S. aureus, B. cereus, E. coli, M. flavus, P. aereuginosa* and *S. typhaemurium* and it was observed that all the motifs are good against various clinical and food contaminant antimicrobials with MIC value 1.1-38 μM and MBC value 2.2-57.2 μM.[60,66] Derivative containing alkyl chain substitution was most effective with MIC value 1.1-4 μM against different bacteria. When the designed scaffold was screened for antifungal activity against various fungi, the MIC values were observed 0.6-26 μM and MFC value 2.2-39 μM for different fungi. Derivative having bromo substitution was most effective against different fungi with MIC value 0.6-4.8 μM. It was concluded that triazole ring significantly increases the antimicrobial activity with alkyl or halo substitutions against different bacteria and fungi.



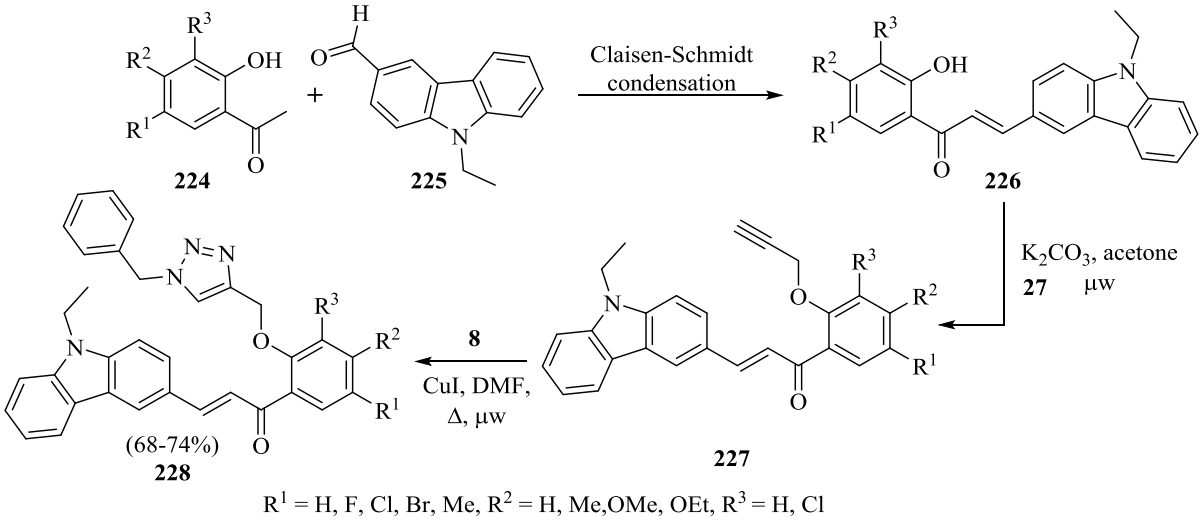
Scheme 1.41: Microwave assisted synthesis of 1,2,3-triazole embedded sugars.

Pereira with his coworkers visualized the synthesis of 1,2,3-triazole tethered 14-member macrolides (a class of natural products that consist of large number of macrocyclic lactones attached with de-oxy sugars as cladinose, desosamine etc.) having a cladinose and 3-pyridyl acetate group at third position of macrolide to scrutinize their antibacterial activity.[67] Azide containing macrolide tethered cladinose **220** was reacted with substituted acetylenes in presence of copper (I) catalyst *via* click chemistry to afford 4-aryl-1,2,3-triazoles **221**. The hydroxyl protecting group **220** was removed and reacted with 3-pyridyl acetic acid and pivolyl chloride catalyzed by DMAP to get 3-pyridyl ligated azido-macrolides **222** which on CuAAC afforded 3-pyridyl ligated 4-aryl-1,2,3-triazoles **223 (Scheme 1.42)**. After the successful synthesis of triazole tethered macrolide conjugates, their antibacterial activity was screened against *S. aureus, S. pneumoniea, S. pyogenes* and *E. faecalis* which revealed that scaffold **221** having 2,4-dichloro or pyridinyl substitution on triazole ring were good antibacterial agents with MIC value 0.06-1 μg/mL. Scaffold **223** was found better than **221** in which also derivative with *p*-aminophenyl substitution was most effective against all bacteria with MIC value 0.06-0.5 μg/mL. Most of the compounds exhibited moderate activity with MIC value 0.06-8 μg/mL but no derivative was better than fluoro-ketolide which was used as standard in *in vitro* and *in vivo* antibacterial screening.



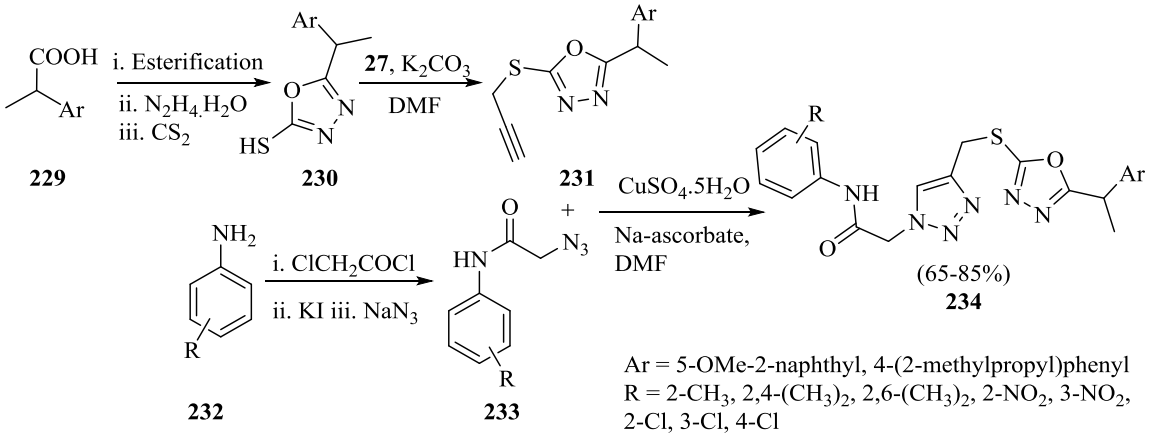
Scheme 1.42: Synthesis of 1,2,3-triazolo macrolides.

Dongamanty *et al* have incorporated the synthesis of 1,2,3-triazole tethered carbazole chalcone motif and evaluated their antimicrobial action.[68] In their approach, initially Claisen-schmidt condensation of 2-hydroxyacetophenone **224** with 9-ethyl-9*H*-carbazole-3-carbaldehyde **225** was performed to get corresponding chalcones **226** which were subsequently propargylated with propargyl bromide. The desired product 1,2,3-triazole tethered carbazole chalcones **228** were obtained by click reaction of propargylated carbazole containing chalcones **227** with different benzyl azides in presence of copper iodide under microwave irradiation (**Scheme 1.43**). After the successful synthesis of derivatives, all motifs were inspected for their antibacterial activity against *E. coli* and *S. aureus* using ampicillin as standard. It was observed that derivative containing electron withdrawing substitution (chloro, bromo, nitro) were better antibacterial agent with zone of inhibition 10-12 mm (50 μg/mL) which increases on increasing concentration. While evaluating antifungal activity against *A. niger* and *C. metapsilosis* zone of inhibition was observed in the range of 8-13 mm (50 μg/mL) which increases on increasing concentration.



Scheme 1.43: Molecular hybridization of chalcones, carbazoles and 1,23-triazoles.

Pal along with his coworkers has synthesized 1,2,3-triazole bearing 1,3,4-oxadiazole derivatives and evaluated them for antibacterial activity.[69] 2-Aryl propionic acid **229** was transformed to oxadiazoles **230** which were selectively *S*-propargylated to get **231**. Simultaneously, amine **232** was transformed to azide as shown in **Scheme** 1.**44.** CuAAC of azide **231** and alkyne **233** lead to desired motif 1,2,3-triazole substituted 1,3,4-oxadiazoles **234** (**Scheme 44**). All scaffolds were tested for antibacterial activity against Gram-positive *S. aureus, B. subtilis, S. epidermidis* and Gram negative *P. aeruginosa, K. pneumoniae* and *E. coli* using amikacin as positive control and their zone of inhibition was calculated. Most of the derivatives were found moderate antibacterial agents with zone of inhibition 10-15 mm. Scaffold having chloro substitution was most potent antibacterial agent against all bacteria with zone of inhibition 13-15 mm.



Scheme 1.44: Synthesis of 1,2,3-triazole bearing 1,3,4-oxadiazoles.

1.5 Conclusion

Thus, a remarkable recent development shows an augmented interest in the chemistry of 1,4-disubstituted 1,2,3-triazoles which might be due to their wide applications in antimicrobial activity. This outline presents the various reports published for the synthesis of different heterocycles linked 1,4-disubstituted 1,2,3-triazoles in last decade. Because of the simplicity in synthesis and significant activity of heterocycles tethered 1,2,3-triazoles, they are highly warrented. It is quite evident that there is large demand for these compounds, which are sought for the synthesis of different heterocyclic substituted 1,2,3-triazoles for their antimicrobial exploration.

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Chapter II

Design, Synthesis and Antimicrobial Activity of 1,2,3-Triazoles as Analogues of Azole drugs

Part-A: Synthesis of 1,2,3-Triazoles Analogues of Miconazole and Evaluation of their Antimicrobial Activities

2.1 Introduction

Carbocyclic compounds containing at least one of the carbon atoms replaced by other atom (commonly N, S, O) are documented as heterocycles which are of immense significance in organic synthesis and medicinal chemistry.[1-4] Among them, nitrogen containing heterocycles are fabulous as they outline main building block of a vast number of natural products and display a broad range of biological properties.[5,3,6,7] Nitrogen containing heterocycles especially azoles have found significance in treatment of fungal infection. In this context, 1,2,4-triazoles are one of the most reliable heterocycles as they inhibit the action of fungal infection with higher selectivity by blocking the enzymes essential for biosynthesis of fungi. Because of their stability up to a wide range of temperature, triazoles are most commonly used in invasive fungal infection such as candidiasis,[8] cryptococcal meningitis[9] and aspergillosis[8].

1,2,3-Triazoles are isostere of 1,2,4-triazoles and have now gained interest to be used as linker[10] to connect two different scaffold by 1,3-dipolar cycloaddition; which are expected to show enhanced activity owing to innovative functionalized drugs.[11-14] They are also used as isostere of peptide bond in glycosides and of various azoles in drugs such as Miconazole, Econazole etc. without altering their bio-chemical properties.[15-18] Their isosteric property with increased activity could be attributed to their greater solubility than normal aromatic rings.[19] Hence they are reliable to be used as isostere and as a linker in medicinal chemistry to design novel compounds.

2.1.1 Azole based antimicrobial drugs

Imidazoles, azepines, pyrrole, indole, pyridine and pyrimidine are the most common heterocyclic rings found in natural products and synthetic drugs.[20] Owing to their high therapeutic index, azoles are extensively used as the first-line drugs in clinic for the treatment of fungal infections. Azoles are most widely used antifungal drugs for systemic fungal infections.[21,47,38] Imidazoles (econazole, ketoconazole) and 1,2,4-triazoles (fluconazole, itraconazole) are common azole motifs recommended as broad spectrum antifungal agents.[48] The paramount advantage of azole drugs over Amphotericin B is due to their low toxicity. Also, azole drugs are far better over echinocandin antifungal agents due to their oral

administration.[21] Despite this, prolonged therapy with azole antifungal agents leads to severe resistance, which limits their use. Therefore, discovery of new efficient antifungal agents are necessary for the treatment of these infections. Miconazole (**1**), Econazole (**2**) and Isoconazole (**3**) (**Figure 2.1**) are well known antifungal drugs having imidazole moiety. In addition, several privileged structural motifs (**4, 5, 6**) were developed to reveal the action of these drugs towards microorganisms.

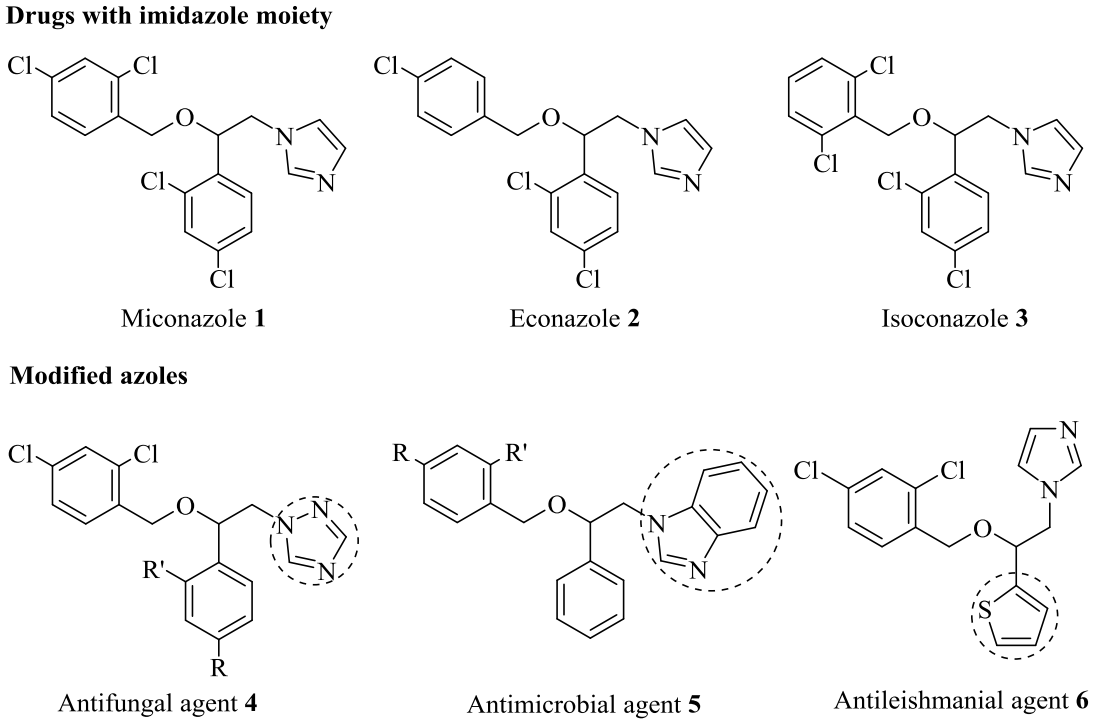
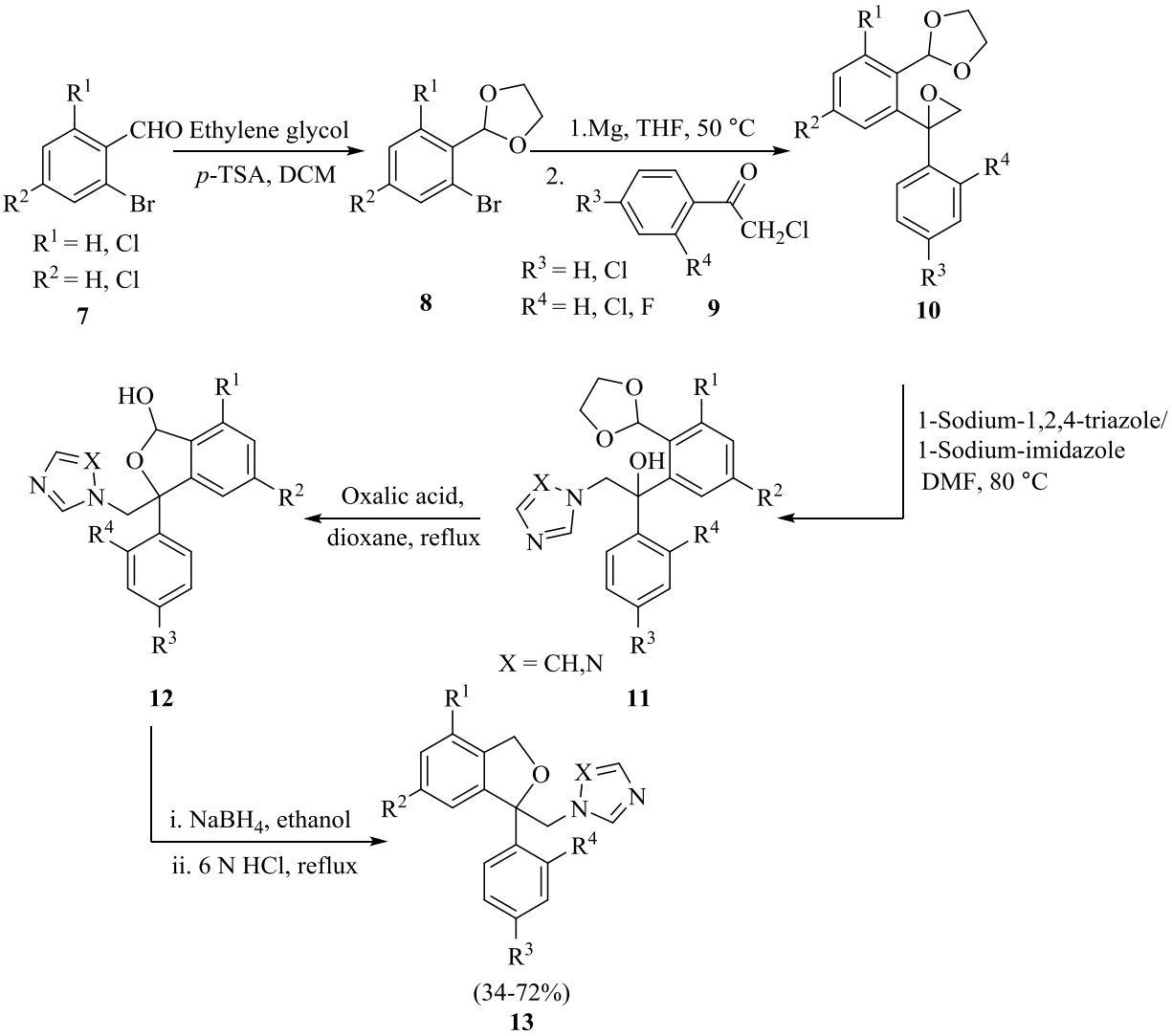


Figure 2.1: Structures of some antifungal azoles.

The 1,2,3-triazoles have been effectively used as an attractive linker to connect two pharmacophores to give an innovative bifunctional drug. The triazole group is not only a linker but also interacts with biological targets through hydrogen bonding and dipole interactions and imparts synergic effect to biological activities. A plethora of literature reports are available where 1,2,3-triazoles are used as linker to synthesize novel antifungal agents.

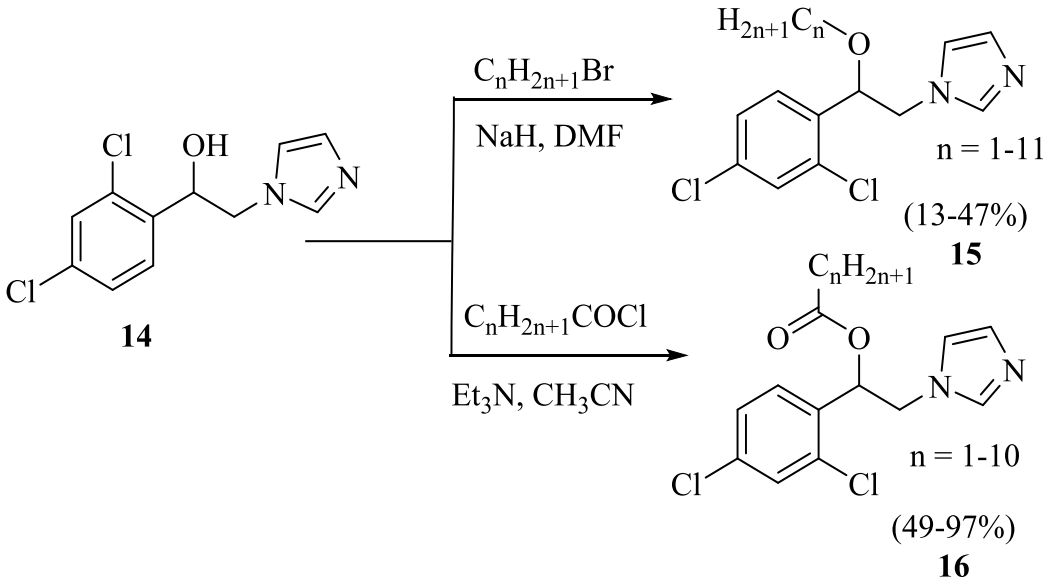
Lovey and co-workers constructed conformationally constrained Miconazole analogous having isobenzofurans in place of aryl ring and the synthesized compounds were screened for antifungal potency.[22] Cyclic acetal **8** has been synthesized using ethylene glycol and 2-bromobenzaldehyde in the presence of *p*-TSA. Grignard reaction of these cyclic acetals using 4-chlorophenacyl chloride afforded **10**. Addition of 1,2,4-triazoles/ imidazoles lead to analogue **13** of Miconazole drug having isobenzofurans in place of *O*-arylated ring (**Scheme**

2.1). Antifungal activity of the derivatives was performed against dermatophytes and Candida species and most of the synthesized compounds were reported to show enhanced antifungal activity but none was found to be orally active.



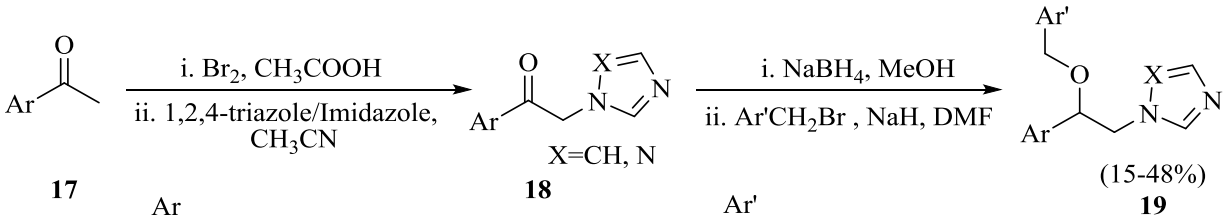
Scheme 2.1: Isobenzofuran as synthetic analogue of Miconazole.

Caujolle *et al* have synthesized ample number of aliphatic ethers and esters which closely resembled with antifungal azole drugs such as Miconazole and Econazole having aliphatic chains in place of *O*-arylated rings.[23] *O*-alkylation and *O*-acylation of 1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethan-1-ol has been accomplished to get the desired products **15, 16,** respectively (**Scheme 2.2**). The synthesized motifs were screened for antifungal efficiency and their hydrophobic character with lipophilicity was evaluated. The evaluated activity revealed that the antifungal action was related to the chain length and the derivative with chain length of six carbon atoms was most promising.



Scheme 2.2: Synthesis of aliphatic ethers and esters of 1-(2,4-dichlorophenyl)-2-(1*H*-imidazolyl)ethanol.

In this context, the same group swapped imidazole moiety in azole series of antifungal drugs with 1,2,4-triazole (**19**) and studied their lipophilicity and antifungal activity against *C. albicans, C. glabrata, C. krusei*.[24] Bromination of 2,4-disubstituted ketones **17** and subsequent condensation with 1,2,4-triazoles propagated **18**. Reduction of **18** and then etherification with 2,4-dichlorobenzylbromides provided target compound **19** (**Scheme 2.3**). They concluded that the antifungal action depends upon the aromatic ring and positions of substituent and 2,4-dichloro derivative was privileged structural motifs for bio-activity.



17

Ar
2,4-dichlorophenyl
3,4-dimethoxyphenyl
1-Naphthyl
2-Naphthyl
2-Benzofuryl
3,4-methylenedioxyphenyl

18

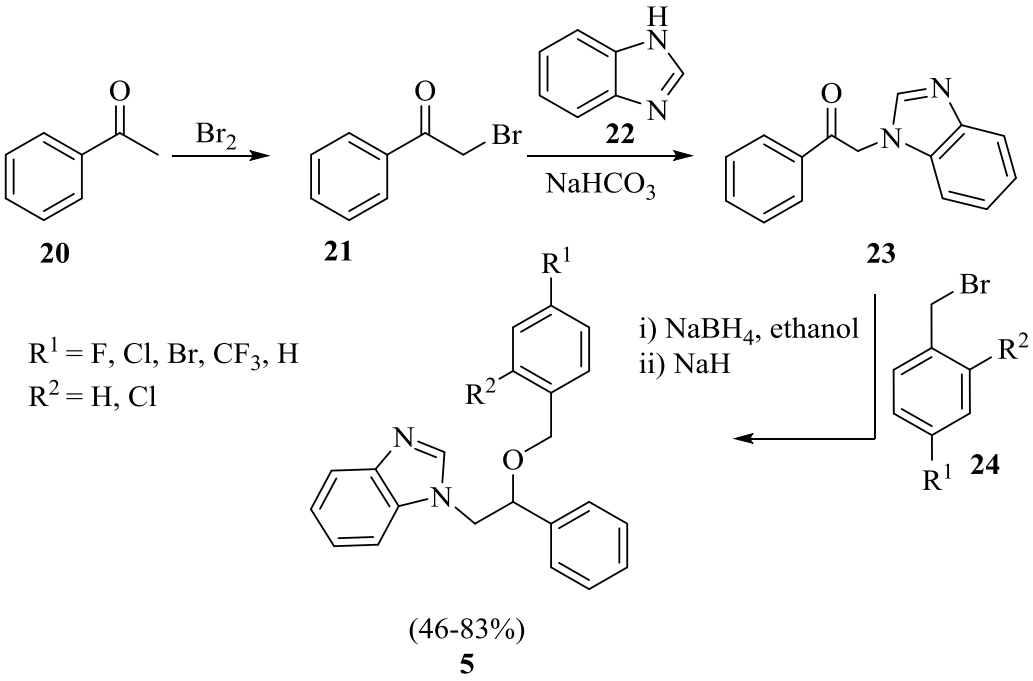
Ar'
3,4-dimethoxyphenyl
1-Naphthyl
2-Naphthyl
2-Benzofuryl
2,4-dichlorophenyl
3,4-methylenedioxyphenyl

19

Scheme 2.3: Synthesis of 1-aryl 2-(1*H*-azolyl)ethanols.

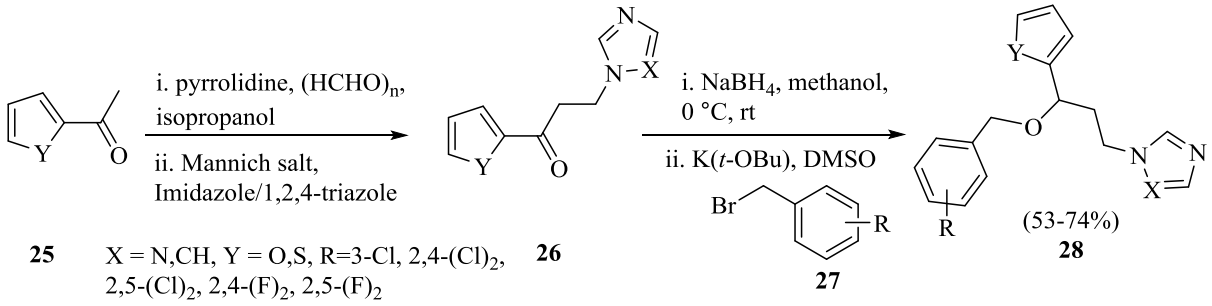
Similarly, Guven's group synthesized azole antifungal drugs by supplimenting imidazole moiety with benzimidazole (**5**) as depicted in **scheme 2.4** and studied their *in-vitro* antibacterial as well as antifungal action against *S. aureus*, Methicillin-resistant *S. aureus, E. coli, C. albicans, C. krusei*. They concluded that derivative with 2,6-dichlorophenyl group

was the most potent compound for antibacterial action towards Gram-positive bacteria *S. aureus* and fungi *C. albicans, C. krusei* with MIC value 3.12 µg/mL, 12.5 µg/mL and 12.5 µg/mL; respectively. None of the synthesized derivatives was active against *E. coli*. Notably, the synthesized compounds were more potent towards anti-bacterial rather than antifungal action.[25]



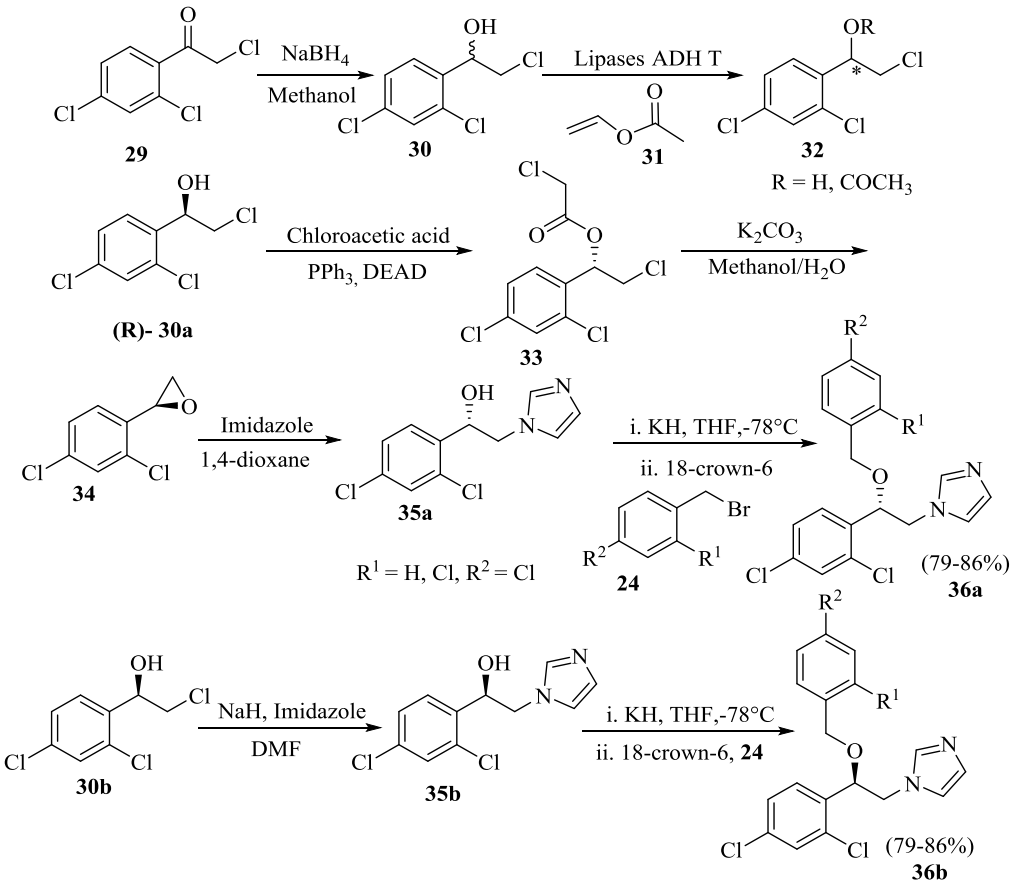
Scheme 2.4: Synthesis of benzimidazole fused Miconazole analogs.

Bhandari and colleagues replaced the aryl ring of these azole drugs with thiophene (**27**) and synthesized a series of compounds to test *in vitro* activity against *Leishmania donovani*.[26] The group proved that these new molecules were more persuasive against leishmania disease than Miconazole. Mannich reaction was accomplished on 2-acetyl thiophene or 2-acetyl furan (**25**) employing pyrollidine and paraformaldehyde. Consequent, replacement of pyrrolidine with imidazole/1*H*-1,2,4-triazole scaffolds resulted in corresponding azolyl ketones **26**. Reduction of **26** and successive etherification with substituted benzyl halides furnished analogue **28** (**Scheme 2.5**). The synthesized derivatives were evaluated against *L. donovani*, in which it was observed that derivative with 3-chlorobenzyloxy moiety was most potent with IC_{50} of 3.04 mM.



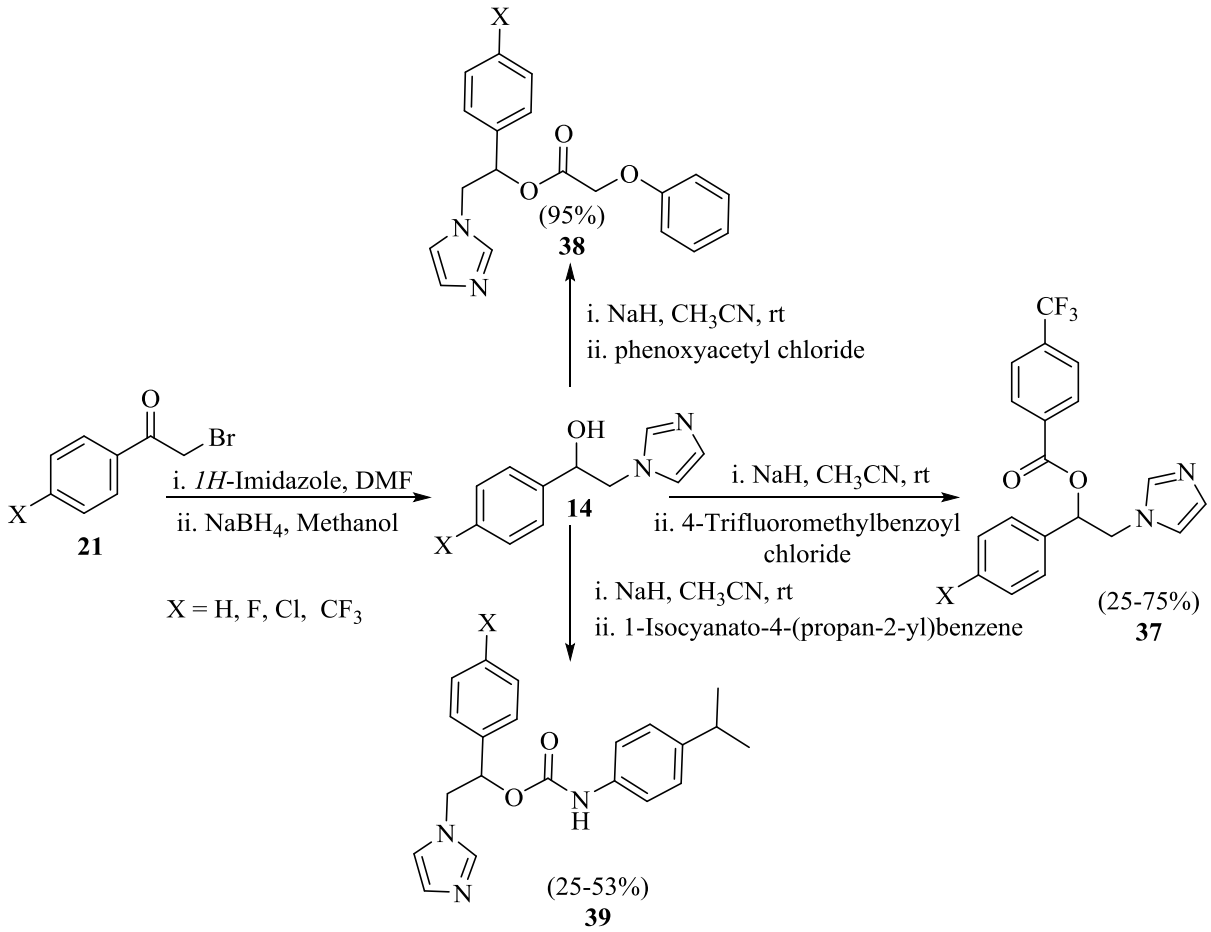
Scheme 2.5: Isosteric modification of azole drugs with thiophene.

Gotor group proposed the asymmetric chemoenzymatic synthesis of Miconazole and Econazole drug enantiomers using lipases and alcohol dehydrogenase enzymes (**Scheme 2.6**).[27] Biological evaluation was performed for both the synthesized enantiomers and racemates. They concluded that (R)-Miconazole is responsible for the activity of Miconazole. In case of Econazole, the (R)-enantiomer (**36a)** resulted in better activity against *C. krusei* while for *C. neoformans*, the (S)-enantiomer (**36b**) was found to show better activity.



Scheme 2.6: Chemoenzymatic asymmetric synthesis of enantiomers of Miconazole (**1**) and Econazole (**2**) drugs.

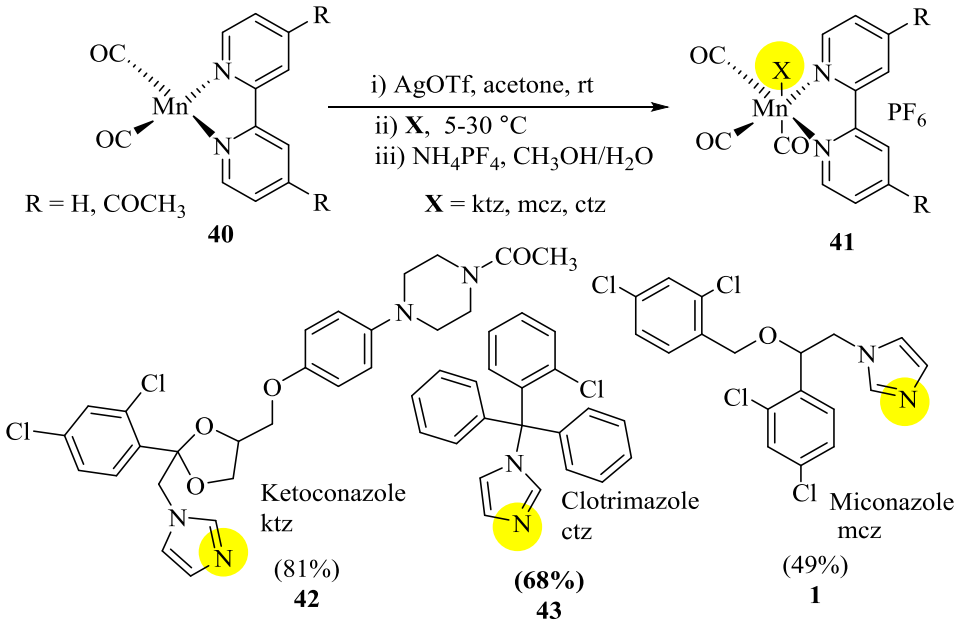
A new series of aromatic ester and carbamate derivatives of 2-(1H-imidazolyl)-1-phenylethanol was developed by Scipione and co-workers.[28] 2-Bromo-1-phenylethanone **21** on treatment with imidazole and consequent reduction afforded alcohol **14**. The synthesized alcohol was reacted under suitable conditions to get desired esters **37**, **38** and carbamates **39,** beside this they also separated enantiomer of ester **37** (**Scheme 2.7**). These synthesized derivatives were screened against *C. albicans* and various *non-albicans C.* species such as *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei* etc. It was observed that ester derivatives with biphenyl ring were more potent than fluconazole and (-) enantiomer of same ester derivative showed 500 times better activity towards C. *krusei* as compared to the (+) enatiomer.



Scheme 2.7: Preparation of 2-(1H-imidazolyl)-1-phenylethanol esters and carbamates.

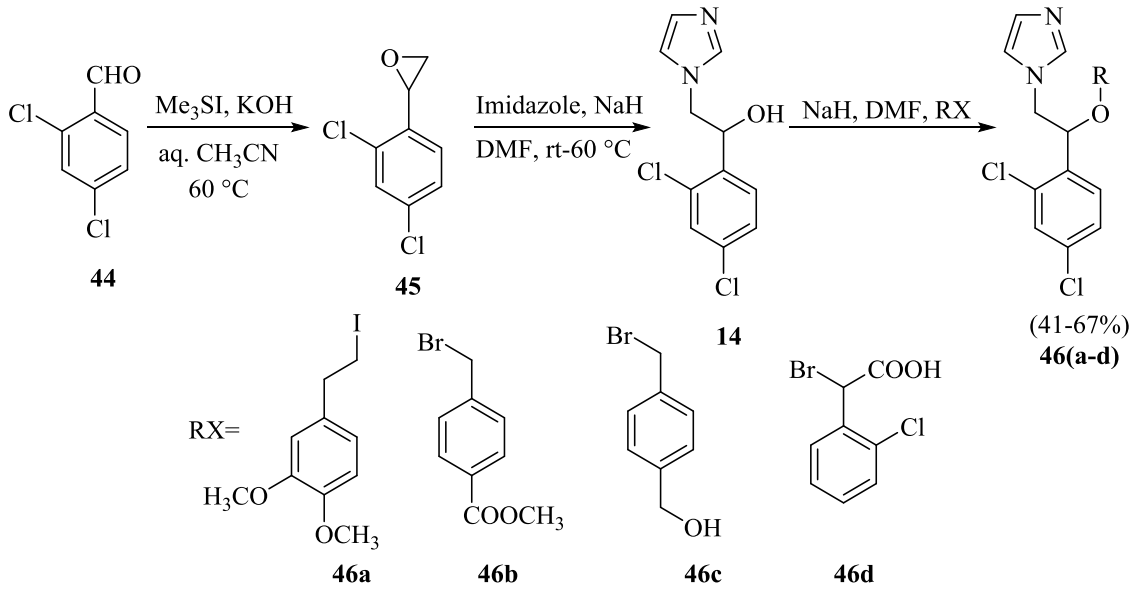
Simpson *et al*. inserted different drugs such as Miconazole, Ketoconazole and Clotrimazole in Manganese(I) tricarbonyl complexes (**41**) to study the effect of metal complex on the activity of these drugs and found that metal coordinated drugs have significantly improved biological activity (**Scheme 2.8**).[29] The synthesized motifs were screened against eight gram-positive and gram-negative bacterial strains and found that the clotrimazole complex showed

submicromolar activity against *S. aureus* and *S. epidermis* with MIC value 0.625 µg/mL.



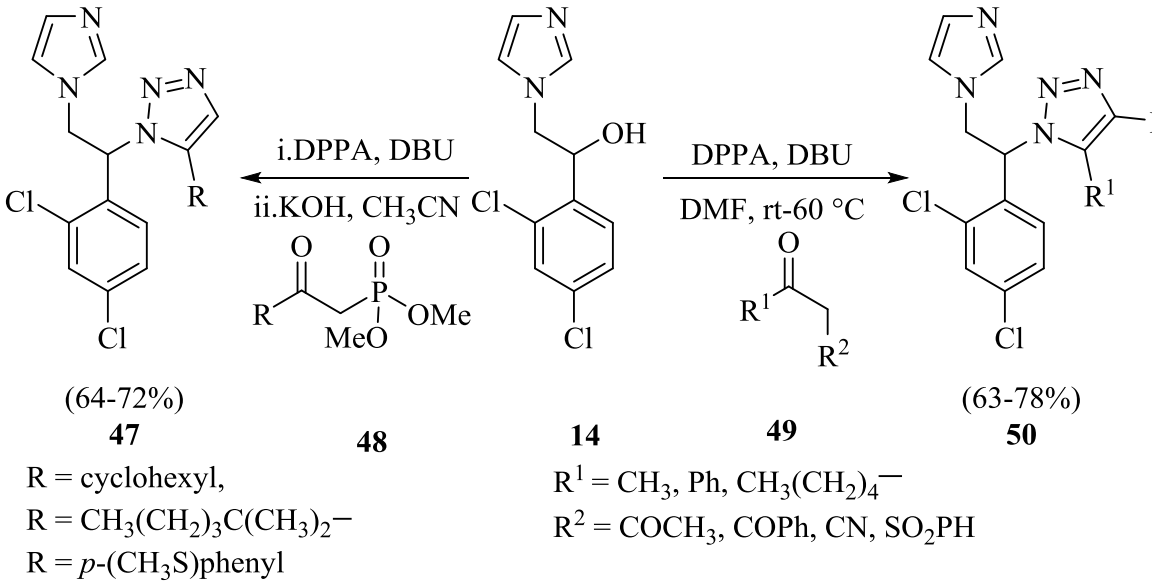
Scheme 2.8: Synthesis of metal tricarbonyl complexes containing azole drugs as ligand.

Davir group has synthesized novel Miconazole analogues and screened them for filamentous fungi and different Candida species.[30] Corey-Chavkowsky epoxidation of 2,4-dichlorobenzaldehyde **44** with trimethylsulfonium iodide in acetonitrile afforded corresponding epoxide **45**. Epoxide ring opening of **45** with imidazole using NaH procured alcohol **14**. The reaction of alcohol **14** with different alkyl halides led to Miconazole analogues **46a-d.** It was observed that analogue **46c** *O*-arylated with 4-methylhydroxy benzyl group was most potent against filamentous fungi as well as against Candida species (**Scheme 2.9**).



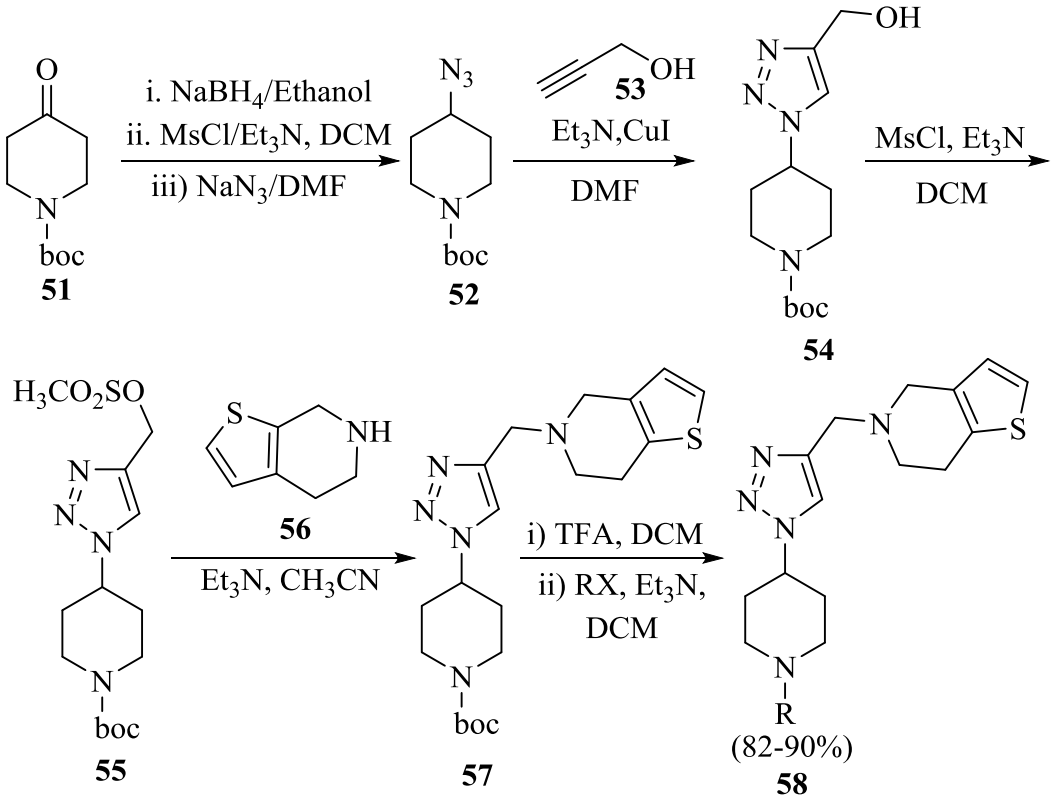
Scheme 2.9: Synthetic route towards novel Miconazole analogue.

In this context, the same group extended their work by synthesizing Miconazole analogue having 1,5-disubstituted and 1,4,5-trisubstituted triazoles scaffolds in place of *O*-benzylated ring *via* azide-enolate 1,3-dipolar cycloadition (**Scheme 2.10**).[31] Alcohol **14** was transformed into azide using DPPA/DBU and was further reacted with enolates to achieve desired product **47** and **50**. The synthesized motifs were screened against different fungi and it was found that Miconazole analogue with 1,5-disubstituted triazoles **50** could be considered as a potential drug candidates.



Scheme 2.10: Synthesis of 1,5-disubstituted and 1,4,5-trisubstituted triazoles as Miconazole analogues.

Shinde group has incorporated piperidine, thienopyridine and 1,2,3-triazoles in one structural motif.[32] *N*-protected 4-azido piperidine **52** was cyclised to triazoles **54** *via* CuAAC using propargyl alcohol. Sulphonylation of **54** was carried out utilizing MsCl and nucleophilic substitution on *O*-mesylated scaffold with 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (THTP) **56** afforded **57**. Deprotection of **57** using trifluoroacetic acid yielded **58** (**Scheme 2.11**). All the synthesized motifs were evaluated for *in vitro* antifungal activity against *C. albicans, F. oxysporum, A. flavus, A. niger* and *C. neoformans*. *C. neoformans* showed unprecedented resistance to the compounds upto 150 μg/mL and most of the compounds were moderately active against fungi. Also, structure activity relationship of constructed scaffolds was studied by employing different substitutions.

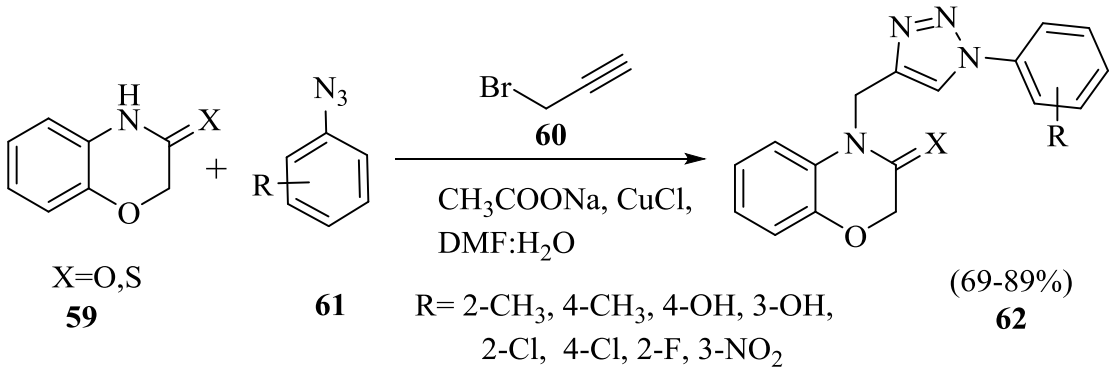


R = H, CH_3, C_2H_5, $COCH_3$, $C_6H_5CH_2$, 4-$CH_3C_6H_5SO_2$, COC_6H_5, 4-ClC_6H_5CO, CH_3SO_2, COC_2H_5, boc = butoxycarbonyl

Scheme 2.11: Synthesis of 5-((1-(piperidin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine scaffolds.

1,2,3-Triazole merged 2-*H*-1,4-benzoxazin-3(4*H*)-one **62** derivatives were constructed by Li and co-workers and evaluated for antifungal action against plant pathogenic fungi, *R. cerealis* and *C. capsici*.[33] Onepot CuAAC of 2-*H*-1,4-benzoxazin-3(4*H*)-one/2-*H*-1,4-benzoxazin-3(4*H*)-thione **59**, propargyl bromide and various substituted aryl azides **61** utilising CuCl as catalyst afforded **62** in good yields (69-89%) as delineated in **Scheme 2.12**. All the

synthesized scaffolds were tested for *in vitro* antifungal activity against *R. cerealis* and *C. capsii* at 20 µg/mL concentration. Among all the tested derivatives, sulphide linked benzoxazine compound having fluoro substitution on phenyl ring was the best antifungal agent with 59 and 68% inhibition against *Rhizoctonia cerealis* and *C. capsici* repectively. Most of the compounds revealed 30-68 % inhibitory action.



Scheme 2.12: Synthesis of 1,2,3-triazole linked benzooxazinone and thiones.

1,2,3-triazole derivatives have been screened for antifungal studies because of their low toxicity and broad antifungal spectrum. [34,35] They are also known to block the biosynthesis of ergosterol by displacing lanosterol from lanosterol 14-demethylase, which is essential component of fungal cell membrane.[36,37] Thus, 1,2,3-Triazoles have proved to be one of the attractive heterocyclic compounds because of their wide range of biological applications. [38,39] In addition to antimicrobial activity, these heterocycles have displayed various biological activities such as anticancer,[40] anti-HIV activity,[41] Src-kinase inhibition,[42] antimicrobial activity,[43] and selective β_3 adrenergic receptor agonism.[44] Triazoles are also useful in industries as dyes, agrochemicals, photographic materials, photostabilizers and corrosion inhibition.[45] Because of their diverse application, synthesis of novel triazoles molecules is an area of current interest.

Triazole conjugated compounds may lead to molecules with improved pharmacological properties in high efficiency under simple reaction conditions. Copper-catalyzed cycloaddition reaction between a terminal alkyne and an azide has been used as a powerful tool for bioconjugation.[46] This approach has wide potential for drug discovery by putting forth multiple arrays of compounds with diverse substitution patterns. Therefore exploring the synthesis of novel 1,4-disubstituted-1,2,3-triazole derivatives is desirable in organic synthesis.

We hypothesized that systematic alteration of the imidazole moiety of azole drugs with the bioisoster 4-substituted-1,2,3-triazoles having hydrophobic groups (**65**) may enhance the potency and lead to new class of azoles with better antifungal activity since triazoles are also known as antifungal agents. Owing to our continuing interest in the synthesis of some pharmacologically important heterocycles, herein, we describe a facile preparation of new class of "drug like" 1,4-disubstituted-1,2,3-triazoles.

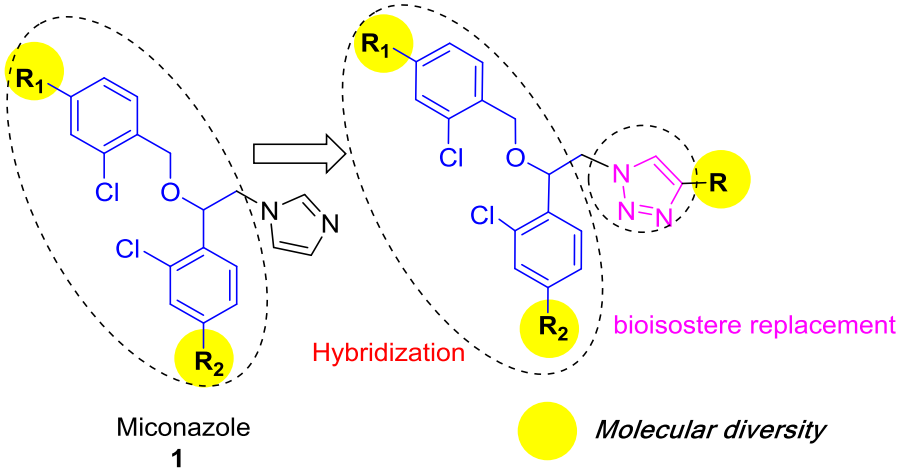
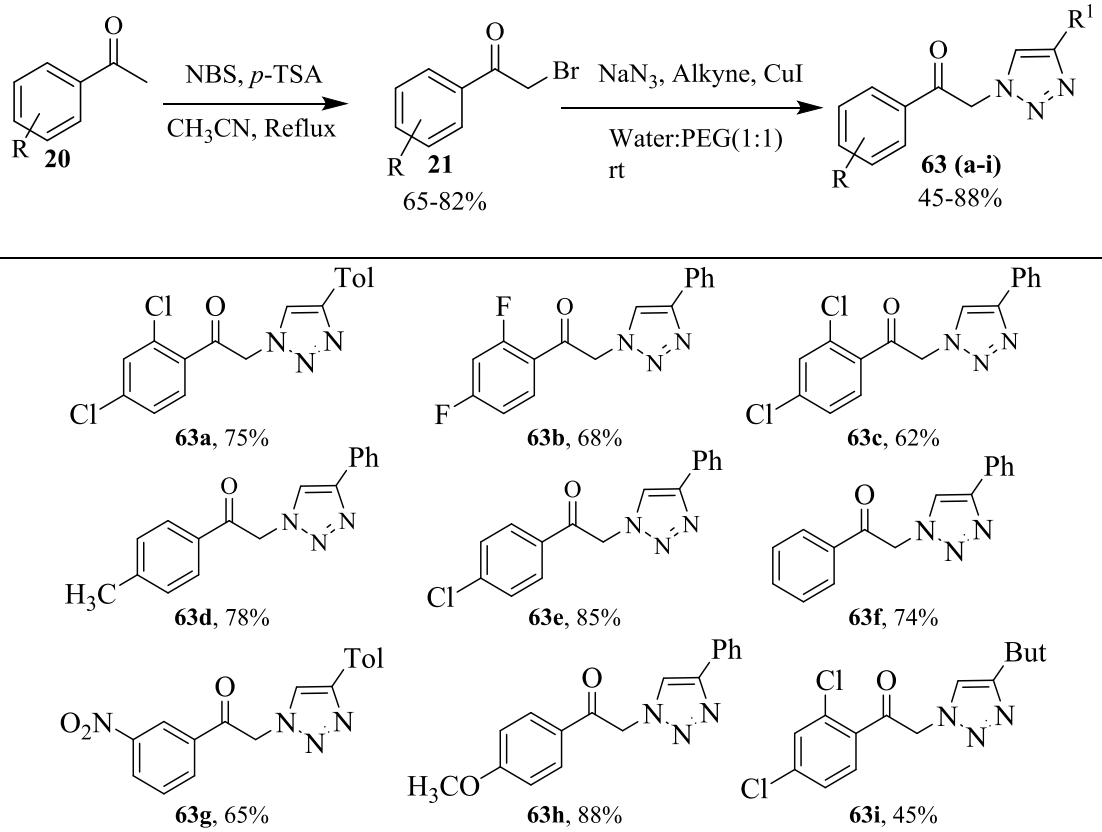


Figure 2.2: Designing triazole isostere of Miconazole drug by molecular hybridization of different pharmacophore units.

2.2 Results and Discussions

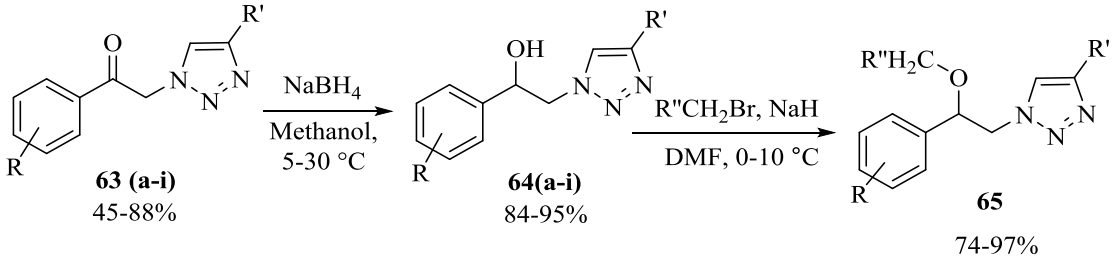
Initially, α-bromoketones (**21a-i**) were synthesized from various ketones as shown in **scheme 2.13**. For this, ketones **20** were treated with *N*-bromosuccinimide (NBS)/*p*-toluenesulphonic acid (*p*-TSA) in acetonitrile. We also screened other brominating reagents such as phenyl trimethylammonium tribromide (PTT), $CuBr_2$, but NBS/*p*-TSA was found to be most suitable to furnish the corresponding α-bromoketones. In the next step, **21** was reacted in a one-pot reaction with NaN_3, and alkynes in PEG-400/water (1:1, *v/v*) in the presence of 10 mol % of CuI. When **21a** was treated with *p*-tolylacetylene (1 equiv.) in PEG-400/H_2O (1:1, *v/v*) with CuI (0.1 equiv.) as catalyst at room temperature for 2 h, it furnished the corresponding triazole **63a** in 75% yield (**Scheme 2.13**). Next, we synthesized other α-triazoloketones (**63b-i**) using CuAAC by varying different alkynes in good to excellent yields (45-88 %). The details of structure and yield are given in **scheme 2.13**. The structure of **63a** was confirmed by IR, ^1H & ^{13}C NMR and mass spectroscopy data. In ^1H NMR, C_5-proton of triazolyl ring resonated around δ 7.90 and protons of α-methylene group to keto appeared as doublet around δ 5.23. In ^{13}C NMR spectra α-methylene carbon appeared around *δ* 51, C_5-carbon of

triazole appeared around δ 122 and carbonyl carbon appeared around δ 192 along with other carbons. In ESI-MS a peak appeared at *m/z* = 255.0037 for $[M + H]^+$ ion of **63a**. The IR spectra of **21** exhibited a strong band at about 1685 cm^{-1} for carbonyl group. The spectral data were in well agreement with the reported in literature.[42]



Scheme 2.13: Synthesis of α-triazoloketones.

After the successful synthesis of **63a**, it was reduced with sodium borohydride in methanol to give corresponding alcohol (**64a**) in excellent yields (95%). The alkylation of alcohol (**64a**) with benzyl bromide in the presence of sodium hydride resulted in target compound (**65aa**) in 87% yield (**Table 2.1**).



Scheme 2.14: Synthesis of **65** emplyoing click chemistry approach.

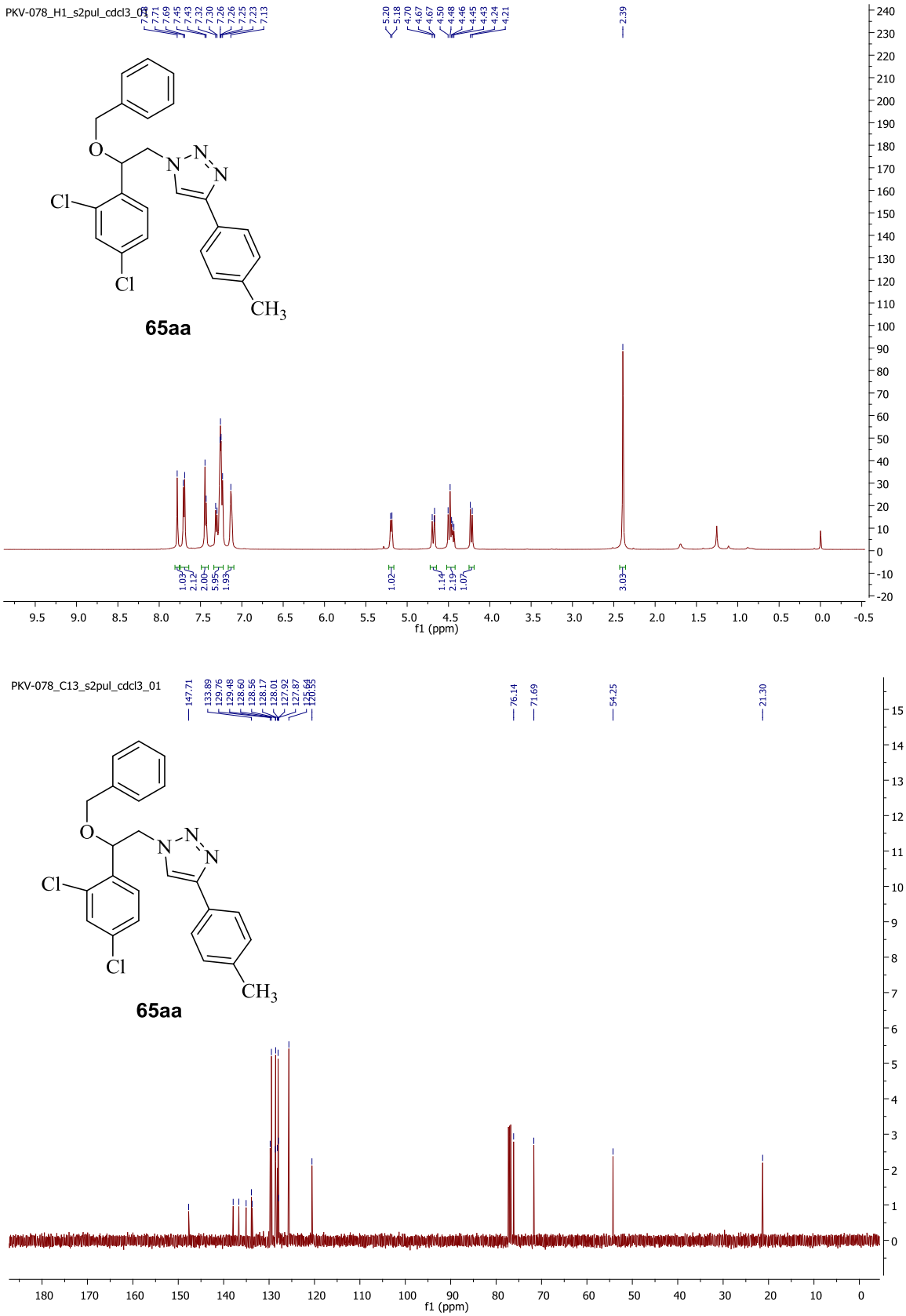


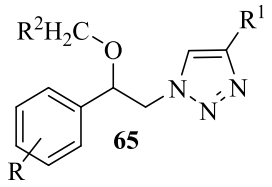
Figure 2.3: ^{1}H and ^{13}C NMR spectra of **65aa.**

The structure of **65aa** was elucidated by ^{1}H, ^{13}C NMR and mass data. In the ^{1}H NMR spectrum of **65aa**, a singlet appeared at δ 7.78 for C_5-proton of triazolyl ring, a doublet of

doublet at δ 5.19 for $OCHCH_2$ protons and two doublet of doublets at δ 4.67 and 4.22 for NCH_2CH protons along with other protons. In ^{13}C NMR of **65aa** the C_4-carbon on the triazolyl ring, C_5-carbon, $OCHCH_2$, OCH_2, $OCHCH_2N$ resonated at δ 147.71, δ 120.55, δ 76.14, δ 71.69 and δ 54.25, respectively (**Figure 2.3**).

An array of novel triazole derivatives **65aa-gc** have been synthesized by varying aliphatic and aromatic substituted acetylenes, substituted acetophenones and benzyl bromides (**Table 2.1**). The overall yield of compound **65** is very good and method is high yielding, simple, convenient and generalized to give a diverse library of drug like molecules. The structures of all the triazoles derivatives (**65**) were characterized by IR, ^1H NMR and ^{13}C NMR spectroscopic data.

Table 2.1. Synthesis of library of 1,4-disubstituted-1,2,3-triazoles.



65

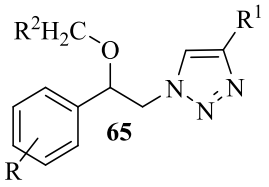
Sr. No.	Compound	R	R^1	R^2	Yield (%)
1	**65aa**	2,4-$(Cl)_2$	4-$CH_3C_6H_4$	Ph	87
2	**65ab**	2,4-$(Cl)_2$	4-$CH_3C_6H_4$	4-ClC_6H_4	86
3	**65bb**	2,4-$(F)_2$	C_6H_5	4-ClC_6H_4	86
4	**65cc**	2,4-$(Cl)_2$	C_6H_5	2-Cl,4-FC_6H_3	88
5	**65ac**	2,4-$(Cl)_2$	4-$CH_3C_6H_4$	2-Cl,4-FC_6H_3	86
6	**65db**	4-CH_3	C_6H_5	4-ClC_6H_4	86
7	**65ec**	4-Cl	C_6H_5	2-Cl,4-FC_6H_3	88
8	**65fc**	H	C_6H_5	2-Cl,4-FC_6H_3	90
9	**65eb**	4-Cl	C_6H_5	4-ClC_6H_4	87
10	**65fb**	H	C_6H_5	4-ClC_6H_4	93
11	**65gb**	3-NO_2	C_6H_5	4-ClC_6H_4	95
12	**65hb**	4-OCH_3	C_6H_5	4-ClC_6H_4	88
13	**65gd**	3-NO_2	C_6H_5	2-F,4-BrC_6H_3	93
14	**65hd**	4-OCH_3	C_6H_5	2-F,4-BrC_6H_3	97
15	**65hc**	4-OCH_3	C_6H_5	2-Cl,4-FC_6H_3	91
16	**65ga**	3-NO_2	C_6H_5	C_6H_5	81
17	**65ea**	4-Cl	C_6H_5	C_6H_5	82

18	**65da**	4-CH_3	C_6H_5	C_6H_5	87
19	**65fa**	H	C_6H_5	C_6H_5	80
20	**65ha**	4-OCH_3	C_6H_5	C_6H_5	85
21	**65he**	4-OCH_3	C_6H_5	H	89
22	**65ce**	2,4-$(Cl)_2$	C_6H_5	H	89
23	**65ae**	2,4-$(Cl)_2$	4-$CH_3C_6H_4$	H	86
24	**65bd**	2,4-$(F)_2$	C_6H_5	2-F,4-BrC_6H_3	82
25	**65dd**	4-CH_3	C_6H_5	2-F,4-BrC_6H_3	82
26	**65ba**	2,4-$(F)_2$	C_6H_5	C_6H_5	85
27	**65ca**	2,4-$(Cl)_2$	C_6H_5	C_6H_5	84
28	**65de**	4-CH_3	C_6H_5	H	80
29	**65cb**	2,4-$(Cl)_2$	C_6H_5	4-ClC_5H_4	90
30	**65bc**	2,4-$(F)_2$	C_6H_5	2-Cl,4-FC_6H_3	85
31	**65gc**	3-NO_2	C_6H_5	2-Cl,4-FC_6H_3	92

2.2.1 Antifungal activity

Antifungal activity of **65aa-gc** was evaluated against *F. oxysporum*, *F. gramillarium* and *F. Monalliforme.* All the compounds were evaluated at the concentrations ranging from 0.5µg/ mL to 128 µg/ mL and scored for MIC as the level of growth inhibition of the microorganisms compared with carbendazim as positive control. The data of antibacterial activities are depicted in table **2.2**. It was found that most of the compounds did not show any activity towards fungi. For all of the compouns the MIC value value was >128 and also zone of inhibition was less than 12. This could be attributed to the lesser solubility of compounds in water.

Table 2.2: Antifungal activities of **65.**



65

Compound*	*F. oxysporum*		*F. gramillarium*		*F. monalliforme*	
	ZOI (mm)	MIC (µg/ml)	ZOI (mm)	MIC (µg/ml)	ZOI (mm)	MIC (µg/ml)
65aa	12	128	12	128	11	128
65cc	11	128	<10	128	11	128
65ac	12	128	10	128	11	128
65db	12	128	-[a]	-[a]	-[a]	-[a]
65ec	12	128	11	128	12	>128
65fc	11	128	11	128	12	128
65gd	12	128	-[a]	-[a]	-[a]	-[a]
65ga	12	128	11	128	-[a]	-[a]
65ea	11	128	11	128	10	>128
65fa	11	128	-[a]	-[a]	-[a]	-[a]
65ha	-[a]	-[a]	-[a]	-[a]	12	128
65he	11	128	11	>128	12	>128
65ca	11	128	11	128	<10	128
65de	11	128	-[a]	-[a]	-[a]	-[a]
65cb	12	128	12	>128	12	>128
Carbendazim	20	16	19	16	19	16

*Remaining compounds were inactive against all three fungi. [a]No activity was observed.

2.3 CONCLUSIONS

In conclusion, we have synthesized a series of novel 1,4-disubstituted-1,2,3-triazole analogues of well-known azole antifungal agents. 1,3-dipolar cycloaddition of azide and alkyne lead to 1,4-disubstituted 1,2,3-triazole derivatives in high yields. The present method is easy, practical and highly efficient. The synthesized compounds have been evaluated for their antifungal activity but poor activity was observed for most of the compounds which can be attributed to their lesser solubility in water.

2.4 Experimental Section

General information

All chemicals were obtained from commercial suppliers and used without further purification. The melting points were determined using EZ-Melt automated melting point apparatus and were not corrected. Reactions were monitored by using thin layer chromatography (TLC) on 0.2 mm silica gel F254 plates (Merck). IR spectra were recorded on Shimadzu FT-IR Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE DMX-500 spectrometry at 500MHz and 125 MHz respectively. All chemicals were obtained from commercial suppliers and used without further purification.

Synthesis of 2-bromo-1-phenylethanone (21): A solution of acetophenone (120 mg, 1.0 mmol), *N*-bromosuccinimide (178 mg, 1.0 mmol) and *p*-toluenesulphonic acid (260 mg, 1.5 mmol) in acetonitrile (7 mL) was stirred for 4 h at reflux temperature. The completion of reaction was confirmed by TLC. After completion, reaction mass was allowed to come to ambient temperature and evaporated. The residue was diluted with water and extracted into ethyl acetate. Organic layer was dried over anhydrous sodium sulfate and evaporated to remove the volatiles. The crude compound was recrystallized from hexanes.

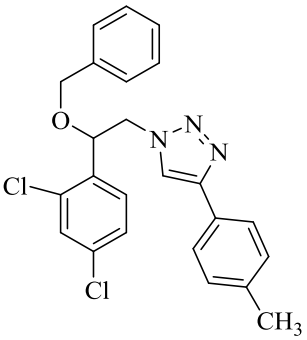
Synthesis of 1-phenyl-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethanone (63a): To a slurry of phenacyl bromide (**21**) (200 mg, 1.0 mmol), phenylacetylene (102 mg, 1.0 mmol), sodium azide (98 mg, 1.5 mmol) in water: PEG-400 (1:1, 10 mL) was added cuprous iodide (10 mg, 0.05 mmol) and the mixture was stirred for 2 h at 25-30 °C. Water was added and extracted into ethyl acetate. Organic layer was dried over anhydrous sodium sulfate and evaporated to remove the volatiles. The crude compound was purified by column chromatography (25% EtOAc/Hexanes).

Synthesis of 1-phenyl-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethanol (64a): To a stirred solution of ketone (**63**) (263 mg, 1.0 mmol) in methanol at 0 °C was slowly added sodium borohydride

(37 mg, 1.0 mmol) in portions and the reaction mass was stirred at 25-30 °C for 16 h. The volatiles were evaroated and the residue was diluted with water, followed by acidification with 2M HCl (pH = 3-4) and extracted into ethyl acetate. Organic layer was dried over anhydrous sodium sulfate and evaporated the volatiles. The crude compound was washed with ether and dried the solids under vacuum.

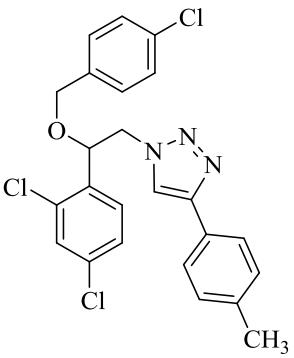
Synthesis of 1-(2-(benzyloxy)-2-phenylethyl)-4-phenyl-1*H*-1,2,3-triazole (65aa): To a stirred solution of alcohol (**64a**, 265 mg, 1.0 mmol), benzyl bromide (190 mg, 1.1 mmol) in DMF (10 mL) at 0 °C slowly added sodium hydride (1.2 mmol) and stirred the reaction mass for 2 h at 25-30 °C. Added water and ethyl acetate separated the layers. Organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated the volatiles. The crude compound was purified by column chromatography (10% EtOAc/Hexanes).

1-(2-(Benzyloxy)-2-(2,4-dichlorophenyl)ethyl)-4-*p*-tolyl-1*H*-1,2,3-triazole (65aa):



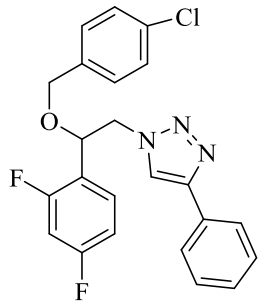
Yield: 381 mg (87%) (28%)*; white solid; mp 142-145 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.70 (d, J = 7.8 Hz, 2H), 7.44 (d, J = 6.9 Hz, 2H), 7.34 – 7.20 (m, 6H), 7.16 – 7.09 (m, 2H), 5.19 (dd, J = 8.2, 2.1 Hz, 1H), 4.67 (dd, J = 8.2, 2.1 Hz, 1H), 4.54 – 4.38 (m, 2H), 4.22 (dd, J = 8.2, 2.1 Hz, 1H), 2.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.71, 137.90, 136.66, 135.05, 133.89, 133.74, 129.76, 129.48, 128.60, 128.56, 128.17, 128.01, 127.92, 127.87, 125.64, 120.55, 76.14, 71.69, 54.25, 21.30; FT-IR (KBr) in cm^{-1}: 3147, 2883, 1940, 1587, 1494, 1230, 1099, 1045, 790.

1-(2-(4-Chlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-4-*p*-tolyl-1*H*-1,2,3-triazole (65ab):



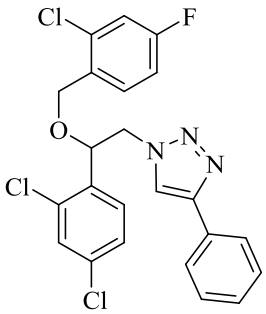
Yield: 405 mg (86%) (30%); off white solid; mp 132-134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.70 (d, J = 7.7 Hz, 2H), 7.47 – 7.40 (m, 2H), 7.32 (d, J = 8.3 Hz, 1H), 7.28 – 7.18 (m, 4H), 7.04 (d, J = 7.9 Hz, 2H), 5.18 (dd, J = 8.2, 2.2 Hz, 1H), 4.67 (dd, J = 14.2, 2.3 Hz, 1H), 4.51 – 4.39 (m, 2H), 4.18 (d, J = 11.7 Hz, 1H), 2.40 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.77, 138.04, 135.18, 135.14, 133.99, 133.73, 133.64, 129.82, 129.56, 129.28, 128.73, 128.52, 128.01, 127.71, 125.60, 120.47, 76.21, 70.83, 54.20, 21.33; FT-IR (KBr) in cm^{-1}: 3050, 2954, 2918, 1556, 1494, 1469, 1417, 1120, 1014, 800, 760.

1-(2-(4-Chlorobenzyloxy)-2-(2,4-difluorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65bb):



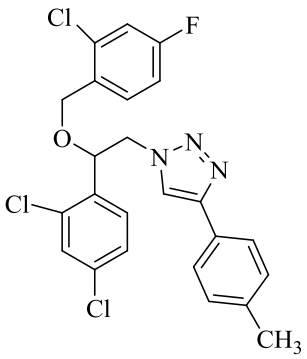
Yield: 365 mg (86%) (45%); white solid; mp 100-102 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.77 (m, 3H), 7.47 – 7.42 (m, 2H), 7.41 – 7.30 (m, 2H), 7.24 – 7.19 (m, 2H), 7.07 – 7.00 (m, 2H), 6.97 – 6.84 (m, 2H), 5.08 (dd, *J* = 8.5, 3.4 Hz, 1H), 4.68 (dd, *J* = 14.2, 3.4 Hz, 1H), 4.58 (dd, *J* = 14.2, 8.5 Hz, 1H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.20 (d, *J* = 11.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.63, 135.25, 133.93, 130.53, 129.22, 129.01, 128.96, 128.89, 128.71, 128.18, 125.67, 120.96, 112.27, 112.24, 112.10, 112.07, 104.63, 104.43, 104.23, 73.62, 70.69, 54.65; FT-IR (KBr) in cm^{-1}: 3116, 3082, 2866, 1620, 1504, 1427, 1286, 1228, 1139, 1047, 879, 767.

1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65cc):



Yield: 418 mg (88%) (30%); white solid; mp 147-148 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.80 (d, *J* = 7.4 Hz, 2H), 7.48 – 7.40 (m, 4H), 7.37 – 7.30 (m, 2H), 7.17 (dd, *J* = 8.5, 6.1 Hz, 1H), 7.03 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.91 – 6.86 (m, 1H), 5.23 (dd, *J* = 8.5, 2.8 Hz, 1H), 4.72 (dd, *J* = 14.3, 2.9 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.46 (dd, *J* = 14.3, 8.5 Hz, 1H), 4.32 (d, *J* = 12.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.22, 161.22, 147.65, 135.24, 133.67, 133.57, 131.21, 131.14, 130.58, 129.84, 128.83, 128.47, 128.13, 127.96, 125.67, 120.84, 117.10, 116.91, 114.26, 114.09, 76.71, 68.38, 54.23; FT-IR (KBr) in cm^{-1}: 3155, 3061, 2943, 1600, 1564, 1494, 1238, 1091, 1012, 869, 759, 692.

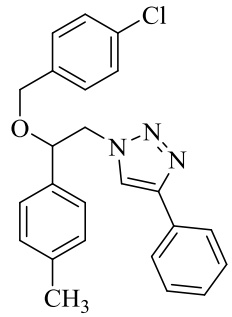
1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-4-*p*-tolyl-1*H*-1,2,3-triazole (65ac):



Yield: 421 mg (86%) (35%); off white solid; mp 154-156 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.50 – 7.43 (m, 2H), 7.33 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.28 – 7.23 (m, 2H), 7.17 (dd, *J* = 8.5, 6.1 Hz, 1H), 7.04 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.89 (td, *J* = 8.3, 2.6 Hz, 1H), 5.23 (dd, *J* = 8.5, 2.9 Hz, 1H), 4.72 (dd, *J* = 14.3, 2.9 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.46 (dd, *J* = 14.3, 8.5 Hz, 1H), 4.32 (d, *J* = 12.0 Hz, 1H), 2.40 (s, 3H);

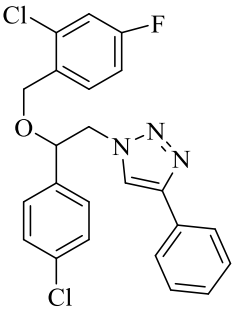
^{13}C NMR (125 MHz, DMSO-d_6) δ 158.45, 156.46, 142.98, 133.22, 130.46, 129.62, 128.92, 128.86, 126.43, 126.36, 125.68, 125.65, 125.07, 124.75, 123.73, 123.20, 123.02, 120.83, 115.74, 112.33, 112.13, 109.51, 109.34, 71.98, 63.60, 49.45, 16.54; FT-IR (KBr) in cm^{-1}: 3159, 3024, 2891, 1600, 1469, 1238, 1093, 1012, 867, 786.

1-(2-(4-Chlorobenzyloxy)-2-*p*-tolylethyl)-4-phenyl-1*H*-1,2,3-triazole (65db):



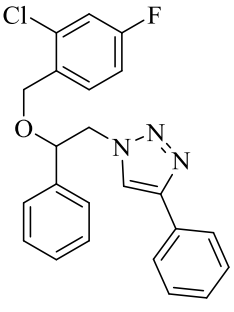
Yield: 346 mg (86%) (41%) white solid; mp 135-137 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 – 7.77 (m, 3H), 7.47 – 7.41 (m, 2H), 7.37 – 7.31 (m, 1H), 7.29 – 7.18 (m, 6H), 7.05 – 6.99 (m, 2H), 4.77 – 4.71 (m, 1H), 4.64 – 4.58 (m, 1H), 4.55 – 4.49 (m, 1H), 4.44 (dd, J = 11.8, 1.6 Hz, 1H), 4.16 (dd, J = 11.8, 1.8 Hz, 1H), 2.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.42, 138.86, 135.88, 134.48, 133.66, 130.71, 129.75, 129.14, 128.86, 128.61, 128.08, 126.73, 125.67, 121.18, 79.97, 70.03, 56.21, 21.23; FT-IR (KBr) in cm^{-1}: 3116, 3032, 2868, 1608, 1487, 1230, 1105, 1012, 825, 765, 696.

1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(4-chlorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65ec):



Yield: 400 mg (88%) (47%); white solid; mp 134-136 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 – 7.77 (m, 3H), 7.47 – 7.38 (m, 4H), 7.38 – 7.32 (m, 3H), 7.17 – 7.11 (m, 1H), 7.05 – 7.01 (m, 1H), 6.91 – 6.85 (m, 1H), 4.81 (dd, J = 8.6, 3.1 Hz, 1H), 4.68 – 4.59 (m, 1H), 4.54 – 4.47 (m, 2H), 4.30 (d, J = 12.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.14, 161.15, 147.53, 136.01, 134.89, 131.03, 130.96, 130.59, 129.32, 128.84, 128.13, 128.07, 125.65, 121.15, 117.07, 116.87, 114.17, 114.00, 79.89, 67.86, 55.99; FT-IR (KBr) in cm^{-1}: 3120, 3088, 2872, 1600, 1492, 1460, 1234, 1091, 817, 696.

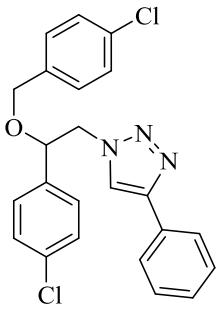
1-(2-(2-Chloro-4-fluorobenzyloxy)-2-phenylethyl)-4-phenyl-1*H*-1,2,3-triazole (65fc):



Yield: 367 mg (90%) (46%); white solid; mp 147-149 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 3H), 7.49 – 7.31 (m, 8H), 7.21 – 7.15 (m, 1H), 7.04 (d, J = 8.5 Hz, 1H), 6.89 (t, J = 8.2 Hz, 1H), 4.84 (dd, J = 8.7, 2.2 Hz, 1H), 4.67 (dd, J = 14.0, 2.2 Hz, 1H), 4.60 – 4.47 (m, 2H), 4.33 (d, J = 11.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.07, 161.08, 147.45, 137.50, 134.33, 130.98, 130.91, 130.71, 129.06, 129.02,

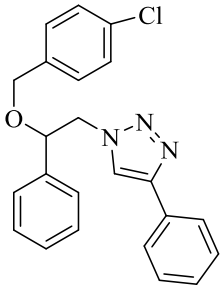
128.82, 128.05, 126.72, 125.65, 121.19, 117.00, 116.80, 114.10, 113.93, 80.58, 67.72, 56.19; FT-IR (KBr) in cm^{-1}: 3116, 3089, 875, 1492, 1440, 1259, 1109, 1085, 904, 862, 694.

1-(2-(4-Chlorobenzyloxy)-2-(4-chlorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65eb):



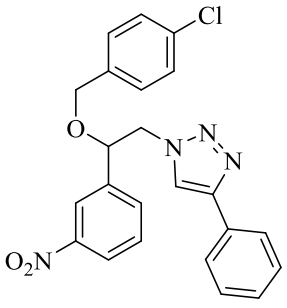
Yield: 370 mg (87%) (47%); white solid; mp 114-116 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (dd, J = 8.2, 1.0 Hz, 2H), 7.78 (s, 1H), 7.48 – 7.39 (m, 4H), 7.38 – 7.33 (m, 1H), 7.32 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 4.77 (dd, J = 8.9, 3.6 Hz, 1H), 4.61 (dd, J = 14.1, 3.7 Hz, 1H), 4.51 (dd, J = 14.1, 8.9 Hz, 1H), 4.44 (d, J = 11.7 Hz, 1H), 4.17 (d, J = 11.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.53, 136.05, 135.43, 134.87, 133.88, 130.54, 129.34, 129.14, 128.89, 128.70, 128.18, 128.10, 125.67, 121.14, 79.43, 70.34, 55.98; FT-IR (KBr) in cm^{-1}: 3115, 3086, 2875, 1492, 1220, 1087, 831, 765, 694.

1-(2-(4-Chlorobenzyloxy)-2-phenylethyl)-4-phenyl-1*H*-1,2,3-triazole (65fb):



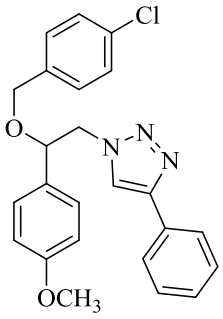
Yield: 362 mg (93%) (48%); white solid; mp 125-127 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, J = 7.8 Hz, 2H), 7.78 (s, 1H), 7.48 – 7.31 (m, 8H), 7.22 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 8.2 Hz, 2H), 4.79 (dd, J = 9.0, 3.4 Hz, 1H), 4.64 (dd, J = 14.1, 3.5 Hz, 1H), 4.54 (dd, J = 14.1, 9.1 Hz, 1H), 4.46 (d, J = 11.7 Hz, 1H), 4.18 (d, J = 11.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.44, 137.56, 135.77, 133.72, 130.66, 129.15, 129.08, 128.99, 128.87, 128.63, 128.10, 126.76, 125.67, 121.19, 80.15, 70.21, 56.18; FT-IR (KBr) in cm^{-1}: 3116, 3086, 2914, 1598, 1492, 1197, 1089, 1014, 810, 740.

1-(2-(4-Chlorobenzyloxy)-2-(3-nitrophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65gb):



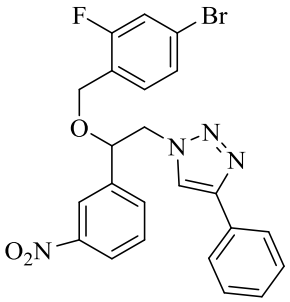
Yield: 412 mg (95%) (35%); off white solid; mp 117-119 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.26 – 8.21 (m, 1H), 7.82 (s, 1H), 7.80 (dd, J = 8.3, 0.9 Hz, 2H), 7.67 (d, J = 7.7 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.44 (t, J = 7.6 Hz, 2H), 7.39 – 7.32 (m, 1H), 7.22 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.2 Hz, 2H), 4.94 (dd, J = 8.7, 3.5 Hz, 1H), 4.66 (dd, J = 14.2, 3.6 Hz, 1H), 4.55 (dd, J = 14.2, 8.8 Hz, 1H), 4.48 (d, J = 11.7 Hz, 1H), 4.22 (d, J = 11.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.78, 147.67, 139.96, 134.97, 134.09, 132.81, 130.40, 130.21, 129.22, 128.92, 128.78, 128.28, 125.69, 123.94, 121.60, 121.21, 79.13, 70.92, 55.71; FT-IR (KBr) in cm^{-1}: 3089, 2873, 1523, 1489, 1359, 1228, 1078, 821, 690.

1-(2-(4-Chlorobenzyloxy)-2-(4-methoxyphenyl) ethyl)-4-phenyl-1*H*-1,2,3-triazole (65hb):



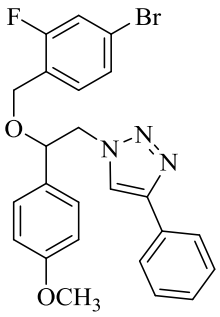
Yield: 368 mg (88%) (57%); white solid; mp 111-113 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 – 7.78 (m, 2H), 7.77 (s, 1H), 7.44 (t, J = 7.6 Hz, 2H), 7.37 – 7.31 (m, 1H), 7.31 – 7.27 (m, 2H), 7.21 (d, J = 8.4 Hz, 2H), 7.05 – 7.00 (m, 2H), 6.97 – 6.92 (m, 2H), 4.72 (dd, J = 8.9, 3.8 Hz, 1H), 4.63 – 4.49 (m, 2H), 4.42 (d, J = 11.8 Hz, 1H), 4.15 (d, J = 11.8 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.07, 147.40, 135.89, 133.65, 130.70, 129.40, 129.13, 128.86, 128.61, 128.08, 128.06, 125.66, 121.15, 114.44, 79.65, 69.90, 56.20, 55.34; FT-IR (KBr) in cm^{-1}: 3118, 3088, 3005, 2926, 2837, 1608, 1514, 1427, 1210, 1176, 1031, 815, 673.

1-(2-(4-Bromo-2-fluorobenzyloxy)-2-(3-nitrophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65gd):



Yield: 462 mg (93%) (35%); ash colour solid; mp 119-121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (s, 1H), 8.16 (d, J = 8.1 Hz, 1H), 7.75 (s, 1H), 7.72 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 6.9 Hz, 1H), 7.53 (t, J = 7.9 Hz, 1H), 7.37 (t, J = 7.6 Hz, 2H), 7.31 – 7.25 (m, 1H), 7.13 – 7.04 (m, 2H), 6.93 (t, J = 7.9 Hz, 1H), 4.86 (dd, J = 8.7, 3.1 Hz, 1H), 4.59 (dd, J = 14.2, 3.3 Hz, 1H), 4.49 – 4.39 (m, 2H), 4.22 (d, J = 11.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.77, 139.77, 132.75, 131.41, 131.37, 130.42, 130.19, 130.15, 128.89, 128.23, 127.68, 127.65, 125.68, 123.98, 121.55, 121.06, 119.32, 119.12, 79.53, 65.03, 55.69; FT-IR (KBr) in cm^{-1}: 3126, 3034, 2881, 1525, 1485, 1346, 1230, 1095, 879, 692.

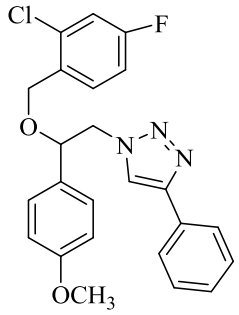
1-(2-(4-Bromo-2-fluorobenzyloxy)-2-(4-methoxyphenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65hd):



Yield: 467 mg (97%) (58%); off white solid; mp 118-119 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 – 7.69 (m, 3H), 7.37 (t, J = 7.6 Hz, 2H), 7.30 – 7.21 (m, 3H), 7.12 – 7.04 (m, 2H), 6.93 (t, J = 7.9 Hz, 1H), 6.88 (d, J = 8.6 Hz, 2H), 4.65 (dd, J = 9.0, 3.5 Hz, 1H), 4.53 (dd, J = 14.1, 3.6 Hz, 1H), 4.47 – 4.35 (m, 2H), 4.17 (d, J = 12.0 Hz, 1H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.11, 147.45, 131.25, 131.22, 130.71,

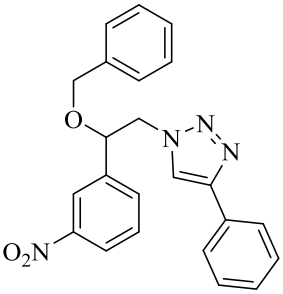
129.21, 128.83, 128.04, 128.01, 127.48, 127.45, 125.66, 123.64, 122.14, 121.03, 119.16, 118.96, 114.44, 80.01, 64.02, 64.00, 56.18, 55.34; FT-IR (KBr) in cm^{-1}: 3115, 3086, 2843, 1608, 1573, 1485, 1251, 1097, 873, 694.

1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(4-methoxyphenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65hc):



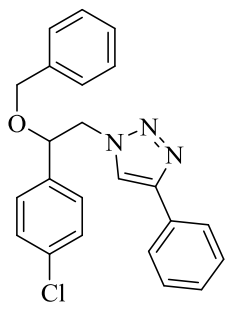
Yield: 397 mg (91%) (57%); white solid; mp 131-133 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.78 (m, 3H), 7.43 (t, J = 7.7 Hz, 2H), 7.34 (t, J = 8.1 Hz, 3H), 7.19 – 7.12 (m, 1H), 7.03 (dd, J = 8.4, 2.4 Hz, 2H), 6.96 (d, J = 8.3 Hz, 1H), 6.88 (td, J = 8.1, 2.3 Hz, 1H), 4.76 (dd, J = 9.0, 3.5 Hz, 1H), 4.62 (dd, J = 14.2, 3.3 Hz, 1H), 4.56 – 4.45 (m, 2H), 4.29 (d, J = 12.1 Hz, 1H), 3.84 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.03, 161.04, 160.07, 147.42, 134.30, 134.22, 131.09, 131.06, 130.94, 130.87, 130.71, 129.33, 128.83, 128.05, 125.65, 121.21, 116.98, 116.79, 114.42, 114.09, 113.92, 80.07, 67.41, 56.23, 55.34; FT-IR (KBr) in cm^{-1}: 3113, 3061, 2920, 2848, 1608, 1514, 1440, 1257, 1180, 1033, 904, 696.

1-(2-(Benzyloxy)-2-(3-nitrophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65ga):



Yield: 324 mg (81%) (31%); pale yellow liquid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.24 (s, 1H), 8.18 – 8.15 (m, 1H), 7.79 (s, 1H), 7.76 – 7.72 (m, 2H), 7.60 (dd, J = 6.6, 1.1 Hz, 1H), 7.52 (t, J = 7.9 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.32 – 7.25 (m, 1H), 7.23 – 7.17 (m, 3H), 7.08 – 7.02 (m, 2H), 4.87 (dd, J = 8.7, 3.5 Hz, 1H), 4.60 (dd, J = 14.2, 3.6 Hz, 1H), 4.52 – 4.42 (m, 2H), 4.19 (d, J = 11.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.78, 147.62, 140.16, 136.42, 132.83, 130.43, 130.12, 128.87, 128.63, 128.30, 128.23, 127.99, 125.74, 123.86, 121.65, 121.28, 78.95, 71.76, 55.81; FT-IR (KBr) in cm^{-1}: 3140, 2947, 2870, 1537, 1348, 1095, 1014, 763, 698.

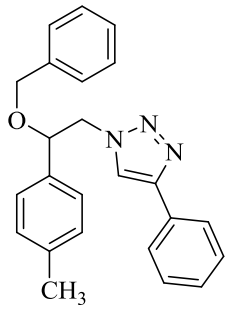
1-(2-(Benzyloxy)-2-(4-chlorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65ea):



Yield: 318 mg (82%) (44%); white solid; mp 114-116 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 – 7.78 (m, 3H), 7.49 – 7.38 (m, 4H), 7.37 – 7.32 (m, 3H), 7.30 – 7.23 (m, 3H), 7.12 (dd, J = 5.9, 2.3 Hz, 2H), 4.79 (dd, J = 8.8, 3.5 Hz, 1H), 4.62 (dd, J = 14.1, 3.5 Hz, 1H), 4.54 – 4.46 (m, 2H), 4.21 (d, J = 11.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.94,

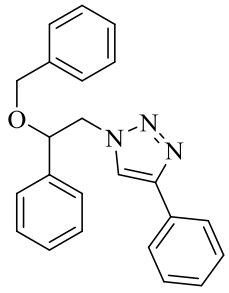
136.30, 134.73, 130.67, 129.27, 128.84, 128.53, 128.14, 128.10, 128.07, 127.90, 125.70, 121.24, 121.18, 79.28, 71.15, 56.04; FT-IR (KBr) in cm^{-1}: 3124, 3088, 2872, 1608, 1492, 1442, 1303, 1112, 1080, 831, 694.

1-(2-(Benzyloxy)-2-*p*-tolylethyl)-4-phenyl-1*H*-1,2,3-triazole (65da):



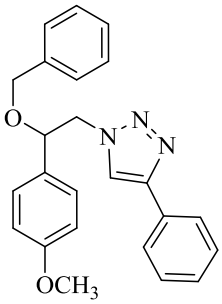
Yield: 321 mg (87%) (41%); white solid; mp 147-150 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.86 – 7.78 (m, 3H), 7.43 (t, J = 7.4 Hz, 2H), 7.38 – 7.20 (m, 8H), 7.14 – 7.08 (m, 2H), 4.75 (dd, J = 8.9, 2.9 Hz, 1H), 4.62 (dd, J = 14.0, 2.8 Hz, 1H), 4.57 – 4.45 (m, 2H), 4.19 (d, J = 11.5 Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 147.39, 138.74, 137.38, 134.72, 130.83, 129.72, 128.83, 128.48, 128.02, 127.92, 126.78, 125.71, 121.32, 79.84, 70.84, 56.29, 21.27; FT-IR (KBr) in cm^{-1}:3116, 3089, 2868, 1610, 1514, 1440, 1199, 1099, 975, 824, 765, 694.

1-(2-(Benzyloxy)-2-phenylethyl)-4-phenyl-1*H*-1,2,3-triazole (65fa):



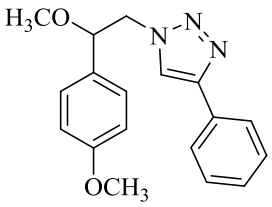
Yield: 284 mg (80%) (41%); white solid; mp 120-122 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 – 7.81 (m, 3H), 7.49 – 7.31 (m, 8H), 7.30 – 7.21 (m, 3H), 7.20 – 7.05 (m, 2H), 4.80 (d, J = 8.9 Hz, 1H), 4.71 – 4.59 (m, 1H), 4.60 – 4.46 (m, 2H), 4.22 (d, J = 11.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.79, 137.26, 130.72, 129.02, 128.88, 128.81, 128.47, 128.04, 127.94, 127.91, 126.79, 125.71, 121.29, 80.02, 71.03, 56.27; FT-IR (KBr) in cm^{-1}: 3124, 3097, 2870, 1494, 1467, 1111, 1028, 744, 732.

1-(2-(Benzyloxy)-2-(4-methoxyphenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65ha):



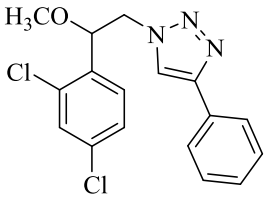
Yield: 327 mg (85%) (54%); white solid; mp 123-125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 – 7.76 (m, 3H), 7.45 – 7.40 (m, 2H), 7.37 – 7.28 (m, 3H), 7.27 – 7.23 (m, 3H), 7.15 – 7.06 (m, 2H), 6.97 – 6.92 (m, 2H), 4.73 (dd, J = 8.8, 3.5 Hz, 1H), 4.65 – 4.57 (m, 1H), 4.56 – 4.43 (m, 2H), 4.19 (d, J = 11.6 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.00, 147.38, 137.40, 130.84, 129.68, 128.81, 128.46, 128.09, 128.00, 127.89, 125.70, 121.26, 114.40, 79.52, 70.73, 56.27, 55.33; FT-IR (KBr) in cm^{-1}: 3125, 2881, 1610, 1514, 1301, 1111, 1031, 829, 694.

1-(2-Methoxy-2-(4-methoxyphenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65he):



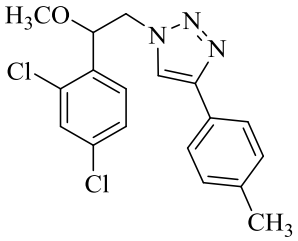
Yield: 275 mg (89%) (57%); yellow solid; mp 110-112 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 – 7.81 (m, 3H), 7.42 (t, J = 7.7 Hz, 2H), 7.33 (t, J = 7.4 Hz, 1H), 7.27 – 7.23 (m, 2H), 6.95 – 6.90 (m, 2H), 4.61 – 4.51 (m, 2H), 4.50 – 4.43 (m, 1H), 3.82 (s, 3H), 3.20 (s, 3H);^{13}C NMR (125 MHz, $CDCl_3$) δ 159.91, 147.42, 130.80, 129.61, 128.78, 127.99, 127.92, 125.70, 121.09, 114.29, 81.87, 56.75, 56.27, 55.30; FT-IR (KBr) in cm^{-1}: 3113, 2983, 1614, 1504, 1301, 1109, 1028, 821, 694.

1-(2-(2,4-Dichlorophenyl)-2-methoxyethyl)-4-phenyl-1*H*-1,2,3-triazole (65ce):



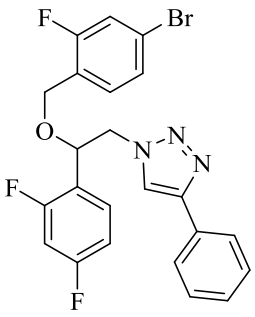
Yield: 310 mg (89%) (28%); colourless liquid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.76 (d, J = 7.2 Hz, 2H), 7.38 – 7.31 (m, 3H), 7.28 – 7.16 (m, 3H), 4.92 (dd, J = 7.9, 2.9 Hz, 1H), 4.62 (dd, J = 14.2, 2.9 Hz, 1H), 4.34 (dd, J = 14.2, 7.9 Hz, 1H), 3.17 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 142.89, 130.19, 128.99, 128.89, 125.94, 125.00, 124.05, 123.53, 123.33, 123.07, 120.98, 116.15, 73.77, 52.81, 49.43; FT-IR (KBr) in cm^{-1}: 3147, 2893, 1585, 1469, 1230, 1047, 866, 694.

1-(2-(2,4-Dichlorophenyl)-2-methoxyethyl)-4-*p*-tolyl-*1H*-1,2,3-triazole (65ae):



Yield: 312 mg (86%) (25%); pale yellow liquid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.66 (d, J = 7.8 Hz, 2H), 7.37 – 7.35 (m, 1H), 7.24 – 7.18 (m, 2H), 7.18 – 7.11 (m, 2H), 4.93 (dd, J = 7.9, 2.7 Hz, 1H), 4.64 – 4.57 (m, 1H), 4.38 – 4.29 (m, 1H), 3.17 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.72, 137.92, 134.92, 133.74, 133.67, 129.73, 129.47, 128.28, 127.84, 127.81, 125.64, 120.53, 78.54, 57.55, 54.17, 21.28; FT-IR (KBr) in cm^{-1}: 3116, 2873, 1485, 1222, 1120, 1097, 871, 694.

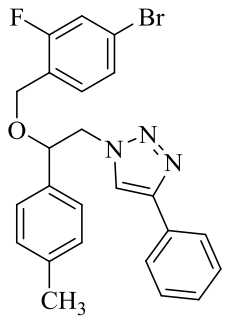
1-(2-(4-Bromo-2-fluorobenzyloxy)-2-(2,4-difluorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65bd):



Yield: 400 mg (82%) (43%); white solid; mp 120-121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 – 7.76 (m, 3H), 7.45 (t, J = 7.5 Hz, 2H), 7.42 – 7.31 (m, 2H), 7.23 – 7.11(m, 2H), 7.06 – 6.97 (m, 1H), 6.99 – 6.84 (m, 2H), 5.08 (d, J = 8.4 Hz, 1H), 4.74 – 4.65 (m, 1H), 4.60 – 4.51 (m, 1H), 4.49 (d, J = 11.8 Hz, 1H), 4.28 (d, J = 11.8 Hz, 1H); ^{13}C NMR

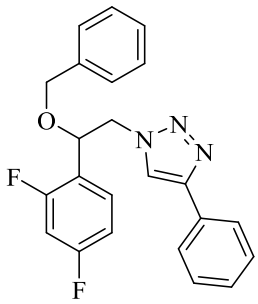
(125 MHz, $CDCl_3$) δ 159.52, 147.65, 131.38, 131.34, 130.56, 128.87, 128.15, 127.62, 127.59, 125.67, 123.01, 120.88, 119.25, 119.05, 112.27, 112.10, 104.64, 104.44, 104.23, 74.02, 64.80, 54.63; FT-IR (KBr) in cm^{-1}: 3113, 2861, 1614, 1504, 1226, 1122, 966, 767, 694.

1-(2-(4-Bromo-2-fluorobenzyloxy)-2-*p*-tolylethyl)-4-phenyl-1*H*-1,2,3-triazole (65dd):



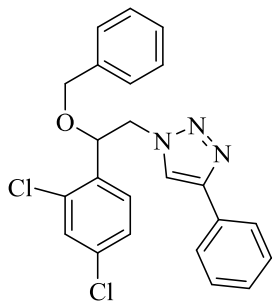
Yield: 382 mg (82%) (39%); white solid; mp 139-140 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 – 7.75 (m, 3H), 7.48 – 7.39 (m, 2H), 7.37 – 7.32 (m, 1H), 7.30 – 7.22 (m, 4H), 7.20 – 7.09 (m, 2H), 7.00 (t, J = 7.9 Hz, 1H), 4.73 (dd, J = 9.1, 3.4 Hz, 1H), 4.62 (dd, J = 14.2, 3.4 Hz, 1H), 4.53 – 4.43 (m, 2H), 4.24 (d, J = 12.0 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.75, 154.74, 142.71, 134.16, 129.54, 126.51, 126.47, 125.99, 125.00, 124.08, 123.28, 122.73, 122.70, 121.92, 120.92, 119.00, 118.88, 117.47, 117.39, 116.30, 114.41, 114.21, 75.58, 59.40, 59.38, 51.43, 16.48; FT-IR (KBr) in cm^{-1}: 3116, 2873, 1485, 1392, 1222, 1097, 871, 694.

1-(2-(Benzyloxy)-2-(2,4-difluorophenyl)ethyl)-4-phenyl-*1H*-1,2,3-triazole (65ba):



Yield: 332 mg (85%) (45%); white solid; mp 102-104 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.73 (d, J = 7.9 Hz, 2H), 7.41 – 7.34 (m, 4H), 7.33 – 7.22 (m, 2H), 7.21 – 7.16 (m, 1H), 7.05 (dd, J = 5.5, 2.3 Hz, 2H), 6.85 (t, J = 8.2 Hz, 1H), 6.82 – 6.77 (m, 1H), 5.02 (dd, J = 8.3, 3.1 Hz, 1H), 4.61 (dd, J = 14.1, 3.3 Hz, 1H), 4.50 (dd, J = 14.1, 8.4 Hz, 1H), 4.42 (d, J = 11.5 Hz, 1H), 4.17 (d, J = 11.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.62, 147.59, 136.76, 130.66, 129.05, 129.01, 128.97, 128.93, 128.81, 128.54, 128.13, 128.09, 127.94, 125.71, 121.04, 112.17, 112.00, 111.97, 104.55, 104.34, 104.14, 73.51, 71.54, 54.72; FT-IR (KBr) in cm^{-1}: 3115, 2860, 1504, 1288, 1138, 1029, 854, 694.

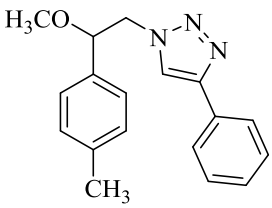
1-(2-(Benzyloxy)-2-(2,4-dichlorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65ca):



Yield:356 mg (84%) (25%); off white solid; mp 105-106 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 – 7.77 (m, 3H), 7.48 – 7.39 (m, 4H), 7.37 – 7.30 (m, 2H), 7.29 – 7.21 (m, 3H), 7.17 – 7.09 (m, 2H), 5.20 (d, J = 6.3 Hz, 1H), 4.70 (dd, J = 14.1, 1.9 Hz, 1H), 4.54 – 4.43 (m, 2H), 4.23 (d, J = 11.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.64, 136.63, 135.08, 133.83, 133.73, 130.67, 129.77, 128.80, 128.59, 128.56,

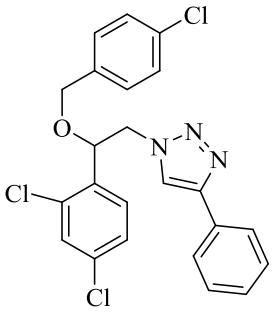
128.18, 128.08, 128.01, 127.93, 125.72, 120.91, 76.12, 71.70, 54.28; FT-IR (KBr) in cm^{-1}: 3113, 3086, 2883, 1587, 1462, 1226, 1103, 1028, 800, 694.

1-(2-Methoxy-2-*p*-tolylethyl)-4-phenyl-1*H*-1,2,3-triazole (65de):



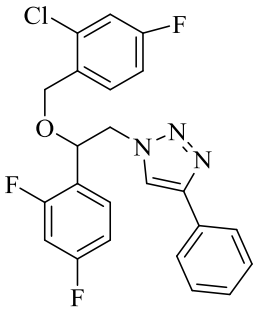
Yield: 234 mg (80%) (38%); pale green solid; mp 143-145 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 – 7.77 (m, 3H), 7.42 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 7.3 Hz, 1H), 7.27 – 7.16 (m, 4H), 4.65 – 4.50 (m, 2H), 4.48 – 4.42 (m, 1H), 3.20 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.43, 138.58, 134.65, 130.81, 129.59, 128.79, 127.99, 126.60, 125.71, 121.12, 82.17, 56.87, 56.28, 21.19; FT-IR (KBr) in cm^{-1}: 3116, 2866, 1608, 1487, 1230, 1085, 825, 694.

1-(2-(4-Chlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65cb):



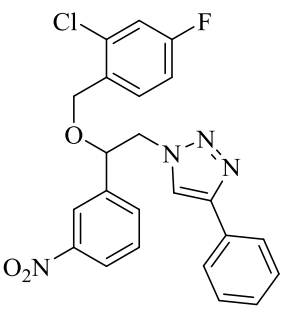
Yield: 412 mg (90%) (27%); off white solid; mp 108-110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 – 7.66 (m, 3H), 7.45 – 7.36 (m, 4H), 7.32 – 7.25 (m, 2H), 7.14 (d, J = 7.4 Hz, 2H), 6.97 (d, J = 7.5 Hz, 2H), 5.11 (d, J = 6.9 Hz, 1H), 4.61 (d, J = 14.2 Hz, 1H), 4.48 – 4.32 (m, 2H), 4.11 (d, J = 11.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.69, 135.22, 135.12, 134.02, 133.73, 133.62, 130.53, 129.82, 129.27, 128.87, 128.73, 128.52, 128.17, 128.00, 125.69, 120.78, 76.20, 70.86, 54.23; FT-IR (KBr) in cm^{-1}: 3111, 2862, 1585, 1489, 1228, 1087, 800, 696.

1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(2,4-difluorophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65bc):



Yield: 376 mg (85%) (45%); off white solid; mp 106-108 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 – 7.78(m, 3H), 7.48 – 7.37 (m, 3H), 7.36 – 7.29(m, 1H), 7.17 (t, J = 6.6 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.98 – 6.85 (m, 3H), 5.13 (d, J = 8.1 Hz, 1H), 4.71 (d, J = 14.1 Hz, 1H), 4.63 – 4.49 (m, 2H), 4.35 (d, J = 11.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.18, 147.62, 131.10, 131.03, 130.56, 128.83, 128.13, 125.66, 120.97, 117.08, 116.88, 114.22, 114.06, 112.19, 112.05, 104.63, 104.42, 104.22, 74.12, 68.20, 54.66; FT-IR (KBr) in cm^{-1}: 3169, 2877, 1604, 1494, 1234, 1111, 891, 694.

1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(3-nitrophenyl)ethyl)-4-phenyl-1*H*-1,2,3-triazole (65gc):



Yield: 415 mg (92%) (27%); pale yellow liquid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.25 (d, J = 7.1 Hz, 1H), 7.88 – 7.76 (m, 3H), 7.74 – 7.67 (m, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.43 (d, J = 7.0 Hz, 2H), 7.39 – 7.28 (m, 1H), 7.20 – 7.12 (m, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.94 – 6.81 (m, 1H), 4.98 (d, J = 7.5 Hz, 1H), 4.68 (d, J = 13.8 Hz, 1H), 4.61 – 4.49 (m, 2H), 4.36 (d, J = 11.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.28, 148.78, 147.67, 139.90, 132.80, 131.27, 131.20, 130.43, 130.17, 128.88, 128.24, 125.67, 123.98, 121.58, 121.20, 117.18, 116.98, 114.32, 114.16, 79.62, 68.45, 55.75; FT-IR (KBr) in cm^{-1}: 3155, 2929, 1525, 1240, 1082, 912, 812, 667.

*Yields reported in second parenthesis are overall Yield.

2.4.1 Antifungal Assay: Antifungal activities of chemically synthesized compounds were determined by agar well diffusion method at a concentration 128 µg /mL for all the compounds. For the experimental work, a microcentrifuge tube of 1.5 mL size was filled with 1 ml autoclaved distilled water and a loopful of freshly cultured fungus was properly mixed. 100 µL of this solution was uniformly spread on the PDA plate. After some time wells of 9 mm diameter were prepared by the sterile metallic borer and 100 µL of compound solution was added in respective wells. Plates were incubated at 28 °C for 4 days under dark conditions. Mean diameter of inhibition zone was measured to determine the antifungal activity. The experiment was performed in triplicates. Further, MIC assay of all the compounds were calculated at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, 128.0 µg/ mL concentrations. Tubes containing 10 mL of sterilized czapek dox broth medium was inoculated with 100 µL of freshly grown culture. Appropriate amount of compound was added to achieve the desired concentrations. The tubes were incubated at 28 °C for 4 days under dark conditions and carefully observed for the presence of turbidity. Carbendazim was used as positive control.

Part-B: Synthesis of Piperazine-Triazole Derivatives and Evaluation of their Antimicrobial Activities

2.5 Introduction

Piperazine is commonly found along with azole moiety in several antifungal drugs such as itraconazole and ketoconazole. In recent years, 1,2,3-triazoles gained the interest owing to their broad range of biological activities such as anti-HIV,[41] anti-inflammatory,[49] anti-malarial,[50] anti-viral,[51] and anticancer activities.[52,53] Now a days, these aza-heterocyclic motifs are widely studied as antifungal agents owing to their low toxicity and broad antifungal spectrum.[54-56] Moreover, moderate dipole character, rigidity, non-toxicity, increased solubility in water compared to normal aromatic compounds, hydrogen bonding ability and stability under different conditions have made 1,2,3-triazoles reliable and tolerable to wide variety of functional groups with enhanced biological activities.[57,58,11]

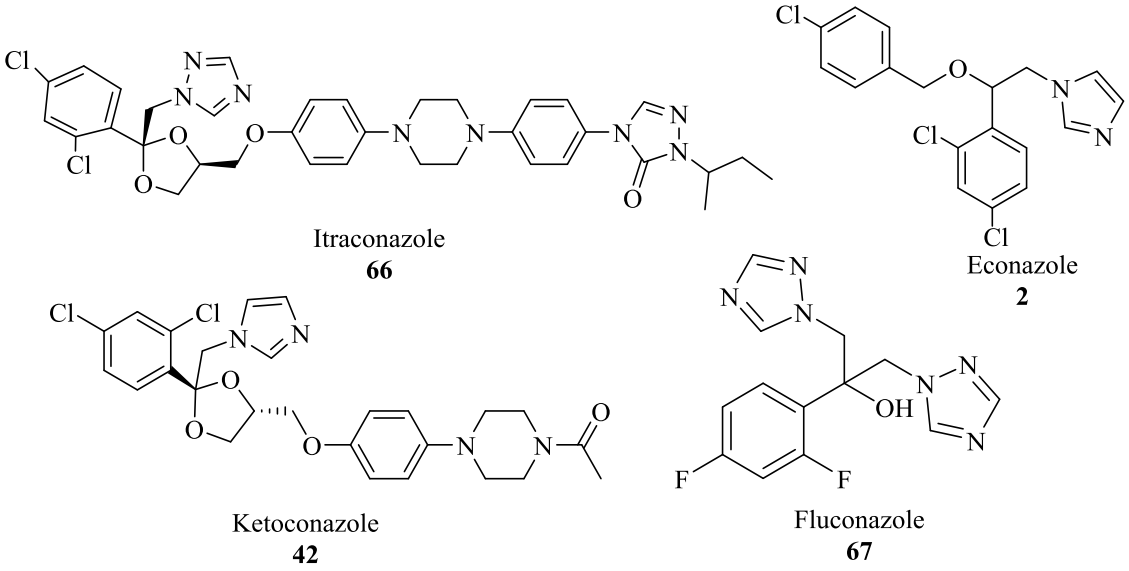


Figure 2.4: Azole containing antifungal drugs.

The bioisosteric replacement of imidazole and 1,2,4-triazole motif in drug molecules is a remarkable concept for the discovery and development of novel drug molecules possessing enhanced antifungal or antibacterial activity.[24-26] Fluconazole is also a bioisostere of these drugs recommended as first line antifungal drug by World Health Organization.[59]

Another approach for generating novel drug molecules is the fusion of two or more pharmacophores in a single molecular framework. In this context, copper-catalyzed azide–alkyne 1,3-dipolar cycloaddition (CuAAC) 'click reaction' is one of the most reliable and straightforward way for conjugation of two pharmacophores.

In part A of this chapter, we have synthesized 1,2,3-triazole analogue of Miconazole and found that they exhibited poor antifungal activity. The poor activity of these compounds was attributed to their lower solubility. We intended to improve the solubility of these compounds by incorporating piperazine moiety. Inspired by the click chemistry and prompted by the chemotherapeutic importance of 1,2,3-triazoles as bioisostere in drugs and hybridization approach, we envisioned the synthesis of novel piperazine-triazole derivatives as antimicrobial agents (**Figure 2.5**). A key feature of our designed strategy is the replacement of imidazole or 1,2,4-triazole with bioisostere 1,2,3-triazole unit and hybridization of piperazine and alkoxy units. Herein, we wish to report the synthesis and antimicrobial activities of some novel piperazine-triazole derivatives **68**.

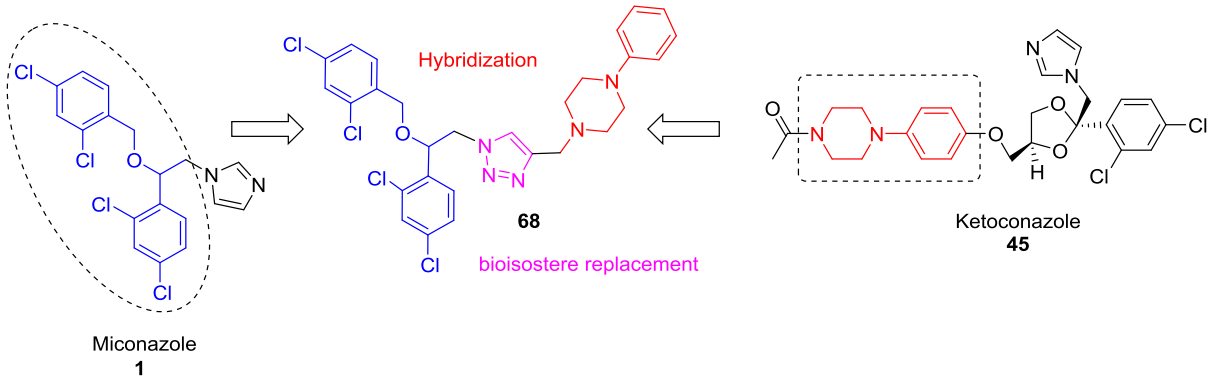
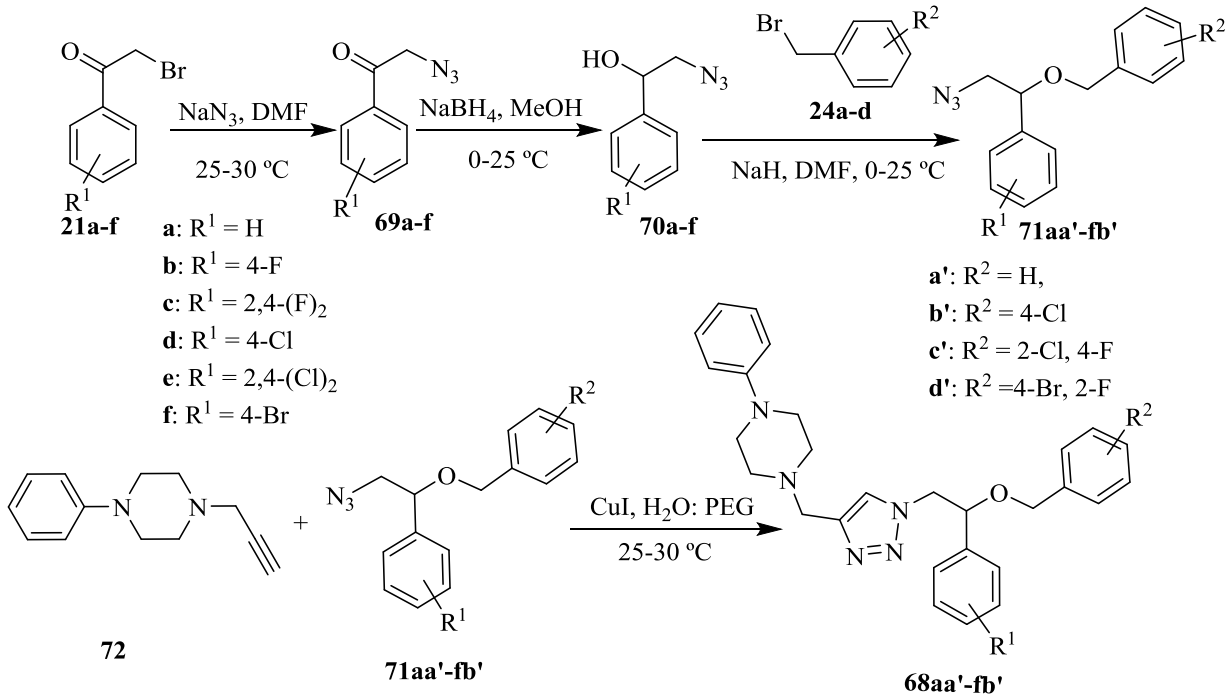


Figure 2.5: Rational for design of piperazine-triazole derivatives

2.5 Results and discussion

Synthetic route for the targeted 1,2,3-triazoles (**68aa'-fd'**) is outlined in **scheme 2.15**. α-Haloketones (**21a-f**) were prepared by reacting acetophenones with *N*-bromosuccinimide in presence of *p*-toluenesulfonic acid.[60] These phenacyl bromides (**21a-f**) were treated with NaN_3 to afford 2-azido-1-phenylethanones (**69a-f**). The azide substitution is a straightforward reaction with excellent yields and they were subsequently reduced by $NaBH_4$ to give the corresponding alcohols (**70a-f**). The high purity of reduced products (**70a-f**) after regular work-up allowed us to move to next step without further purification. Benzylation of these alcohols with various substituted benzyl bromides (**24a'-d'**) using sodium hydride resulted in (2-azido-1-(benzyloxy)ethyl)benzenes (**71aa'-fb'**). While synthesizing an array of azides (**71aa'-fb'**), it was realized that substituents of phenyl ring on alcohols (**70a-f**) or benzyl bromides (**24a'-d'**) did not affect the yields to the great extent. Simultaneously, propargylation of 1-phenylpiperazine was achieved by reacting with propargyl bromide using K_2CO_3 to obtain 1-phenyl-4-(prop-2-ynyl)piperazine (**72**) in excellent yield. The targeted 1,2,3-triazoles (**68aa'-fb'**) were achieved by copper catalyzed Huisgen 1,3-dipolar

cycloaddition of azides (**71aa'-fb'**) with alkyne (**72**) also known as click reaction (**Scheme 2.15**). An array of compounds (**68aa'-fb'**) were synthesized in good to excellent yields using aforementioned synthetic route. To obviate the solubility issues for biological screening, we also prepared the salts of these novel piperazine linked 1,4-disubstituted 1,2,3-triazoles by passing dry HCl gas.



Scheme 2.15: Synthesis of piperazine-triazole derivatives.

The azole antifungal drugs in use contain halo-substituted aryl groups such as 2,4-dichloro, 2,4-difluoro substituted benzyl rings, thus we mainly focused to synthesize target molecules of halo-substituted rings. The yields and physical data of all the synthesized compounds are shown in **Table 2.3**. All the synthesized compounds were well characterized by spectroscopic data such as ^{1}H NMR and ^{13}C NMR. In the ^{1}H NMR spectra of **68aa'-fb'** a characteristic singlet peak for triazole C_4-proton (C-H) appeared at δ ~7.51 along with the two triplets δ ~3.18 (t, J = 4.8 Hz, 4H) and δ ~2.64 (t, J = 4.8 Hz, 4H) for piperazine protons. ^{13}C NMR of these motifs further confirmed the assigned structures.

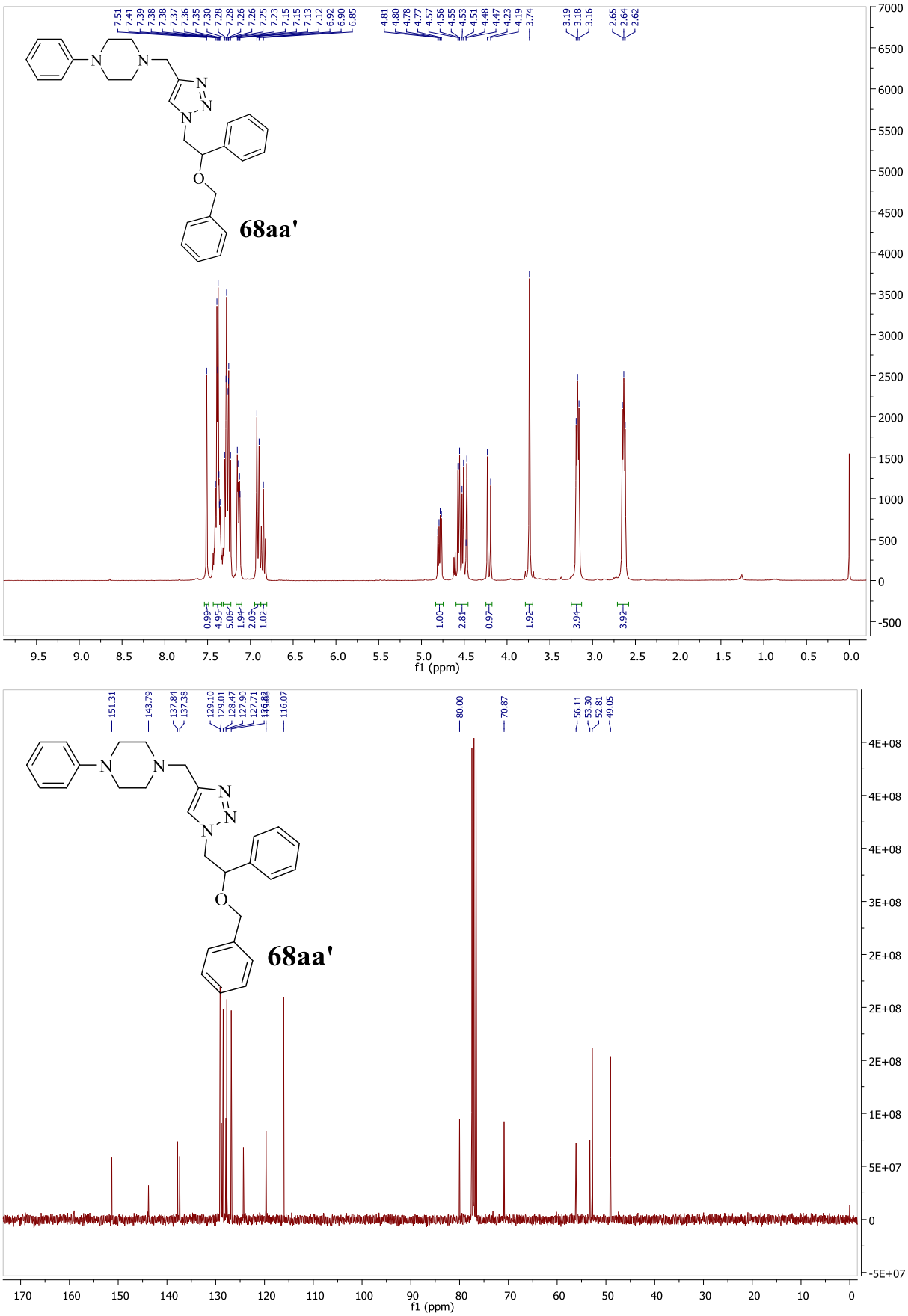
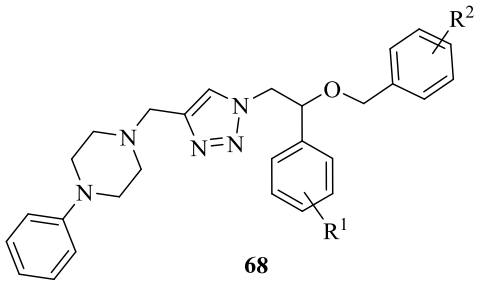


Figure 2.6: ^{1}H and ^{13}C NMR spectra of 1-((1-(2-(benzyloxy)-2-phenylethyl)-1H-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (**68aa'**).

Table 2.3: Synthesis of piperazine-triazole derivatives **68**



68

Entry	R^1	R^2	Product	Yield[a] (Purity)[b] (%)	mp (°C)
1	H	H	**68aa'**	89 (99.4)	82-83
2	H	4-Cl	**68ab'**	88 (96.9)	83-84
3	H	2-Cl, 4-F	**68ac'**	96 (96.9)	86-87
4	H	2-F, 4-Br	**68ad'**	89 (93.1)	86-87
5	4-F	H	**68ba'**	78 (95.0)	114-115
6	4-F	4-Cl	**68bb'**	73 (98.0)	112-113
7	$2,4\text{-}(F)_2$	4-Cl	**68cb'**	80 (99.2)	91-92
8	$2,4\text{-}(F)_2$	2-Cl, 4-F	**68cc'**	81 (98.1)	99-100
9	4-Cl	4-Cl	**68db'**	72 (96.0)	128-129
10	4-Cl	2-Cl, 4-F	**68dc'**	99 (97.7)	112-113
11	4-Cl	2-F, 4-Br	**68dd'**	98 (96.8)	92-94
12	$2,4\text{-}(Cl)_2$	4-Cl	**68eb'**	63 (99.3)	88-89
13	$2,4\text{-}(Cl)_2$	2-Cl, 4-F	**68ec'**	81 (98.4)	104-105
14	$2,4\text{-}(Cl)_2$	2-F, 4-Br	**68ed'**	68 (97.0)	91-92
15	4-Br	4-Cl	**68fb'**	67 (94.4)	85-86

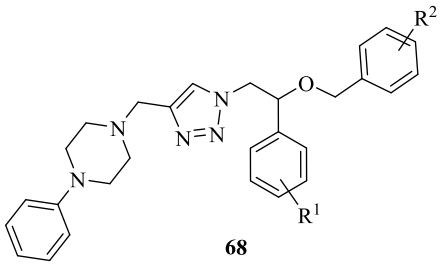
[a]Isolated yield. [b]Determined by HPLC analysis using Waters Sunfire C-18 column and acetonitrile as solvent.

2.5.1 Biological activity

The synthesized compounds **68aa′-fb′** were screened for antibacterial activities against Gram negative *E. coli* and *P. putida*, Gram positive *S. Aureus.* All compounds were evaluated at the concentrations ranging from 0.5µg/mL to 128µg/mL and scored for MIC as the level of

growth inhibition of the microorganisms compared with chloramphenicol. The data of antibacterial activities are depicted in table 2.4. Compound **68ac'** with phenyl and 4-fluoro-2-chlorobenzyloxy group showed highest activity against both Gram positive and Gram negative bacteria (**Table 2.4, entry 3**).

Table 2.4: Antibacterial activities of **68** and their HCl salts.[a,b]



68

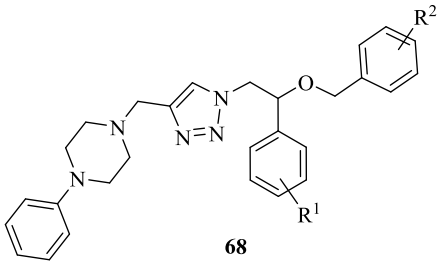
Compound	*E.coli*		*P. putida*		*S. aureus*	
	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
68aa'	15 (**20**)	128 (**64**)	15 (**20**)	128 (**64**)	14 (**17**)	128 (128)
68ab'	15 (16)	128 (128)	15 (16)	128 (128)	14 (16)	128 (128)
68ac'	**16 (16)**	**64 (>64**)	**16 (16)**	**64 (64**)	**14 (14)**	**64** (>128)
68ba'	14 (**18**)	128 (**64**)	14 (**18**)	128 (**64**)	14 (16)	128 (128)
68bb'	15 (17)	128 (128)	15 (17)	128 (128)	14 (17)	128 (128)
68cc'	14 (17)	128 (128)	14 (17)	128 (128)	13 (17)	128 (128)
68eb'	14 (14)	128 (128)	14 (14)	128 (128)	13 (14)	128 (128)
68ec'	14 (16)	128 (128)	14 (16)	128 (128)	12 (16)	128 (128)
68ed'	14 (15)	128 (128)	14 (15)	128 (128)	13 (15)	128 (128)
68fb'	15 (17)	128 (128)	15 (15)	128 (128)	14 (14)	128 (128)
Chloramphenicol	22	16	24	16	23	16

[a]The values in brackets are for the HCl salt of the compound. [b]No activity was observed for **68ad'**, **68cb'**, **68db'**, **68dc'**, **68dd'**.

Further, antifungal activity of **68aa′-fb′** and its HCl salts was evaluated against *F. oxysporum*, *F. gramillarium* and *F. monalliforme.* All the compounds were evaluated at the concentrations ranging from 0.5 µg/mL to 128 µg/mL and scored for MIC as the level of growth inhibition of the microorganisms compared with carbendazim as positive control. The data of antibacterial activities are depicted in **table 2.5**. It was found that **68aa′** was more potent among all compounds in neutral form towards *F. oxysporum* and *F. gramillarium* but

it did not show any activity towards *F. monalliforme*. On the other hand, **68ac′** showed most significant activity towards all of the three fungi both in salt form as well as in neutral form. It is worth noting that most of the compounds were inactive towards *F. monalliforme* fungi in both forms. Compound **68ad′**, **68dc′**, **68fb′** did not show any antifungal activity in salt form but in neutral form they showed moderate activity towards *F. oxysporum* and *F. gramillarium*. Most of the other compounds were moderately active towards *F. oxysporum* and *F. gramillarium.*

Table 2.5: Antifungal activities of **68** and their HCl salts.[a]



68

Compound	*F. oxysporum*		*F. gramillarium*		*F. monalliforme*	
	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
68aa'	15 (15)	64 (128)	15 (15)	64 (128)	-[b] (12)	-[b] (128)
68ab'	14 (14)	128 (128)	14 (14)	128 (128)	14 (-)	128 (-[b])
68ac'	16 (16)	128 (64)	16 (16)	128 (64)	14 (14)	128 (128)
68ad'	12 (-[b])	128 (-[b])	14 (-[b])	128 (-)	-[b]	-[b]
68ba'	13 (12)	128 (128)	13 (12)	128 (128)	-[b]	-[b]
68bb'	13 (13)	128 (128)	13 (13)	128 (128)	11 (-[b])	128 (-[b])
68cb'	12 (15)	128 (128)	12 (15)	>128 (128)	-[b] (14)	-[b] (>128)
68cc'	15 (15)	128 (128)	15 (-[b])	128 (-)	12 (-[b])	128 (-[b])
68db'	12 (12)	128 (128)	12 (12)	128 (128)	-[b]	-[b]
68dc'	14 (15)	128 (128)	14 (15)	128 (128)	-[b] (12)	-[b] (128)
68dd'	15 (-[b])	128 (-[b])	15 (-[b])	128 (-)	-[b]	-[b]
68eb'	14 (15)	128 (128)	14 (15)	128 (128)	-[b]	-[b]
68ec'	14 (12)	128 (128)	14 (12)	128 (128)	-[b] (12)	-[b] (128)
68ed'	11 (12)	128 (128)	11 (-[b])	128 (-[b])	-[b]	-[b]
68fb'	15 (-[b])	128 (-[b])	15 (-[b])	128 (-[b])	-[b]	-[b]
Carbendazim	20	16	19	16	19	16

[a]The values in brackets are for the HCl salt of the compound. [b]No activity was observed.

2.6 Conclusions

In conclusion, we have synthesized a series of novel piperazine-1,2,3-triazoles derivatives, which entailed the bioisosteric replacement of the imidazole scaffold and hybridization of two drug scaffolds by employing the click chemistry. The yield of the compounds was good to excellent and all the synthesized compounds were of high purity. The compounds were evaluated for antibacterial activities against Gram negative (*E. coli* and *P. putida*), and Gram positive *S. aureus* bacteria as well as for fungicidal activities against *F. oxysporum, F. gramillarium* and *F. monalliforme* fungi. Compound **68ac′** exhibited moderate but promising antibacterial activity against Gram negative bacteria and fungicidal activity against *F. oxysporum and F. gramillarium*. The structure-activity relationship data provide insights for further optimization of the piperazine-triazole derivatives in discovery of antimicrobial agents.

2.7 Experimental

General information: All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined in open capillary tubes on an EZ-Melt automated melting point apparatus and are uncorrected. Reactions were monitored by using thin layer chromatography (TLC) on 0.2 mm silica gel F_{254} plates (Merck). The chemical structures of final products were characterized by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) determined at room temperature with a Bruker Avance DMX-300 spectrometer at 300 MHz and 75 MHz respectively. Chemical shifts were reported in parts per million (ppm) using deuterated solvent peak or Tetramethylsilane (internal) as the standard.

Synthesis of 2-azido-1-phenylethanol (70a): 2-Bromo-1-phenylethan-1-one was synthesized as mentioned in part A of this chapter. A solution of 2-bromo-1-phenylethan-1-one (5 g, 25 mmol) and NaN_3 (2.4 g, 37 mmol) in DMF (50 mL) was stirred at room temperature (25 °C) for 12 h. After completion of the reaction, the reaction mass was diluted with cold water and extracted into ethyl acetate. Organic layer was dried over anhydrous sodium sulphate and evaporated to remove the volatiles. The crude residue was purified by column chromatography to obtain 2-azido-1-phenylethanone (**69a**, 4.02 g) as transparent liquid. Further, to the stirred solution of ketone (**69a**) (160 mg, 1.0 mmol) in methanol at 0 °C was slowly added sodium borohydride (37 mg, 1.0 mmol) in portions and stirred the reaction mass at 25-30 °C for 1.5 h. Evaporated the volatiles and residue was diluted with water, followed by acidification with 2 M HCl (pH ~ 3-4) and extraction with ethyl acetate. Organic layer dried over anhydrous sodium sulphate and evaporated the volatiles.

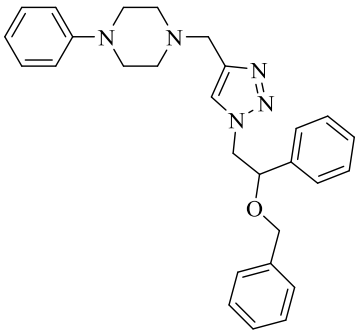
Synthesis of (2-azido-1-(benzyloxy)ethyl)benzene (71a): To a stirred solution of alcohol (**70a**) (163 mg, 1.0 mmol), benzyl bromide (190 mg, 1.1 mmol) in DMF (10 mL) at 0 °C slowly added sodium hydride (29 mg, 1.2 mmol) and stirred the reaction mass for 2 h at 25-30 °C. On completion of reaction, water and ethyl acetate were added and organic layer was separated. Organic layer was washed with water, dried over anhydrous sodium sulphate and evaporated the volatiles. The crude compound was purified by column chromatography.

Preparation of 1-((1-(2-(benzyloxy)-2-phenylethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenyl piperazine (68aa'): Mixture of (2-azido-1-(benzyloxy)ethyl)benzene (250 mg, 1.0 mmol), 1-phenyl-4-(prop-2-ynyl)piperazine **72** (240 mg, 1.2 mmol) and CuI (10 mg, 0.05 mmol) in water: PEG 400 (1:1 *v/v*) (10 mL) was stirred at rt for 5 h. After confirmation of completion of reaction by TLC, water was added and extracted into ethyl acetate. Separated

the organic layer, dried over sodium sulphate and evaporated the volatiles. The crude residue was purified by column chromatography (EtOAc:hexanes, 1:3).

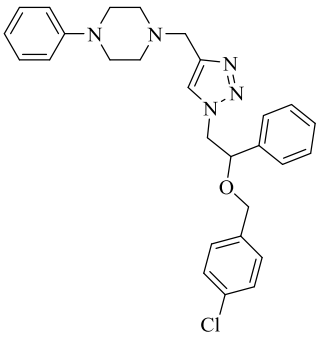
General procedure for HCl salt formation of 68: Free bases, were dissolved in CH_2Cl_2 and treated with dry HCl gas, which was generated *in situ*. After 15 min, the solids were filtered and dried under vacuum to afford quantitative yields of corresponding HCl salts of **68aa'** to **68fb'**.

1-((1-(2-(Benzyloxy)-2-phenylethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68aa'):



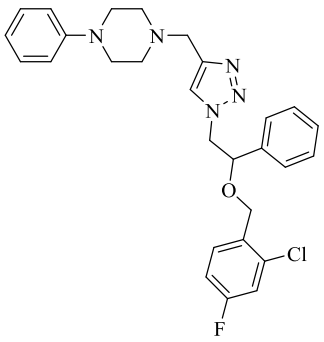
Yield: 403 mg (89%); white solid; mp 82-83 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.44 – 7.34 (m, 5H), 7.28 (dd, *J* = 7.0, 5.1 Hz, 4H), 7.23 (s, 1H), 7.14 (dd, *J* = 7.3, 1.9 Hz, 2H), 6.91 (d, *J* = 7.9 Hz, 2H), 6.85 (s, 1H), 4.79 (dd, *J* = 8.4, 4.3 Hz, 1H), 4.58 – 4.50 (m, 2H), 4.47 (d, *J* = 2.8 Hz, 1H), 4.21 (d, *J* = 11.6 Hz, 1H), 3.74 (s, 2H), 3.22 – 3.13 (m, 4H), 2.68 – 2.58 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.31, 143.79, 137.84, 137.38, 129.10, 129.01, 128.85, 128.47, 127.90, 127.71, 126.82, 124.32, 119.68, 116.07, 80.00, 70.87, 56.11, 53.30, 52.81, 49.05.

1-((1-(2-(4-Chlorobenzyloxy)-2-phenylethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68ab'):



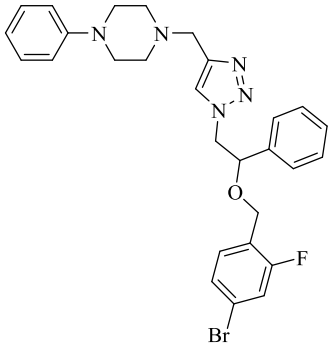
Yield: 430 mg (88%); yellow solid; mp 83-83.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.43 – 7.32 (m, 5H), 7.30 – 7.25 (m, 3H), 7.24 (d, *J* = 1.8 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 7.9 Hz, 2H), 6.85 (t, *J* = 7.3 Hz, 1H), 4.78 (dd, *J* = 8.2, 4.5 Hz, 1H), 4.55 (dd, *J* = 6.3, 5.2 Hz, 2H), 4.44 (d, *J* = 11.8 Hz, 1H), 4.22 – 4.13 (m, 1H), 3.74 (s, 2H), 3.21 – 3.13 (m, 4H), 2.69 – 2.58 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.27, 143.85, 137.61, 135.89, 133.67, 129.12, 129.07, 128.98, 128.97, 128.63, 126.78, 124.23, 119.72, 116.08, 80.15, 70.08, 56.04, 53.28, 52.81, 49.06.

1-((1-(2-(2-Chloro-4-fluorobenzyloxy)-2-phenylethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68ac'):



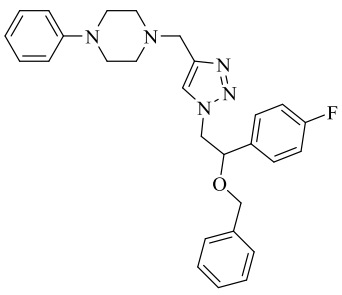
Yield: 485 mg (96%); white solid; mp 86-87 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.43 – 7.36 (m, 5H), 7.26 (dd, *J* = 8.7, 7.2 Hz, 3H), 7.22 – 7.18 (m, 1H), 7.07 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.97 – 6.89 (m, 3H), 6.85 (t, *J* = 7.3 Hz, 1H), 4.82 (dd, *J* = 8.4, 4.3 Hz, 1H), 4.55 (dd, *J* = 14.0, 5.4 Hz, 2H), 4.47 (d, *J* = 12.1 Hz, 1H), 4.31 (d, *J* = 12.1 Hz, 1H), 3.73 (s, 2H), 3.21 – 3.12 (m, 4H), 2.66 – 2.59 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.71, 160.40, 151.27, 143.85, 137.53, 134.21, 134.07, 131.13, 131.09, 130.77, 130.65, 129.11, 129.06, 129.00, 126.74, 124.25, 119.71, 117.04, 116.71, 116.06, 114.15, 113.87, 80.62, 67.69, 56.05, 53.26, 52.80, 49.04.

1-((1-(2-(4-Bromo-2-fluorobenzyloxy)-2-phenylethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68ad'):



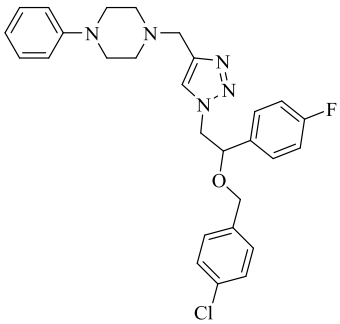
Yield: 490 mg (89%); white solid; mp 86-86.6 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.38 (q, *J* = 7.4 Hz, 6H), 7.27 (d, *J* = 8.7 Hz, 2H), 7.24 – 7.15 (m, 2H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 2H), 6.85 (t, *J* = 7.2 Hz, 1H), 4.79 (dd, *J* = 8.5, 4.0 Hz, 1H), 4.59 – 4.42 (m, 3H), 4.26 (d, *J* = 12.0 Hz, 1H), 3.74 (s, 2H), 3.26 – 3.12 (m, 4H), 2.70 – 2.59 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.13, 158.78, 151.27, 143.82, 137.39, 131.14, 131.08, 129.11, 129.08, 129.02, 127.51, 127.46, 126.73, 124.14, 123.84, 123.64, 122.21, 122.09, 119.71, 119.22, 118.90, 116.09, 80.54, 64.27, 64.23, 56.03, 53.27, 52.81, 49.04.

1-((1-(2-(Benzyloxy)-2-(4-fluorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68ba'):



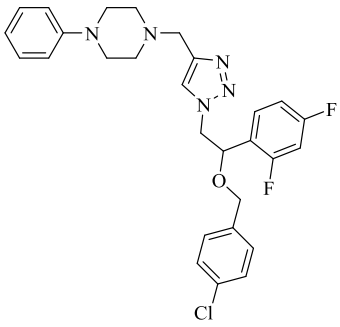
Yield: 273 mg (58%); brown solid; mp 114-115 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.30 (ddd, *J* = 12.1, 7.0, 3.8 Hz, 6H), 7.24 (s, 1H), 7.11 (dt, *J* = 14.8, 5.4 Hz, 4H), 6.89 (dd, *J* = 11.9, 7.7 Hz, 3H), 4.83 – 4.75 (m, 1H), 4.58 – 4.44 (m, 3H), 4.21 (d, *J* = 11.7 Hz, 1H), 3.91 (d, *J* = 2.3 Hz, 2H), 3.29 – 3.21 (m, 4H), 2.87 – 2.74 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.78, 141.48, 137.11, 133.47, 133.43, 129.19, 128.62, 128.51, 127.98, 127.69, 125.45, 120.28, 116.38, 116.17, 115.89, 79.04, 70.83, 56.10, 52.26, 52.11, 48.28, 29.69.

1-((1-(2-(4-Chlorobenzyloxy)-2-(4-fluorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68bb'):



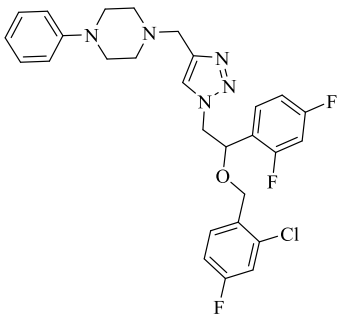
Yield: 370 mg (73%); yellow solid; mp 112-113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.47 – 7.27 (m, 5H), 7.24 – 7.18 (m, 1H), 7.08 (t, *J* = 8.7 Hz, 5H), 6.90 (d, *J* = 7.1 Hz, 3H), 4.81 (d, *J* = 5.7 Hz, 1H), 4.55 (d, *J* = 5.7 Hz, 2H), 4.40 (d, *J* = 11.8 Hz, 1H), 4.27 – 4.07 (m, 3H), 3.37 (s, 4H), 3.02 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.60, 161.32, 150.27, 139.65, 135.71, 133.62, 133.15, 129.27, 129.00, 128.66, 128.58, 126.58, 120.73, 116.64, 116.23, 115.94, 79.05, 70.01, 56.13, 52.20, 52.04, 47.77, 29.69.

1-((1-(2-(4-Chlorobenzyloxy)-2-(2,4-difluorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68cb'):



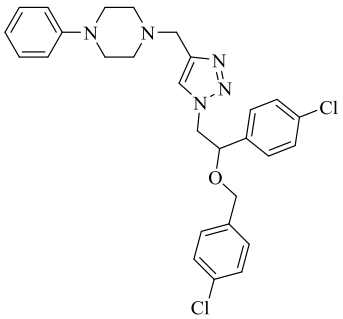
Yield: 420 mg (80%); yellow solid; mp 91-92 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.39 – 7.32 (m, 1H), 7.28 (dd, *J* = 7.8, 6.0 Hz, 4H), 7.24 (d, *J* = 1.9 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 6.95 – 6.89 (m, 4H), 6.88 – 6.82 (m, 2H), 5.07 (dd, *J* = 7.9, 4.1 Hz, 1H), 4.66 – 4.56 (m, 2H), 4.44 (d, *J* = 11.8 Hz, 1H), 4.22 (d, *J* = 11.8 Hz, 1H), 3.74 (s, 2H), 3.23 – 3.14 (m, 4H), 2.67 – 2.60 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.24, 144.05, 135.38, 133.89, 129.12, 129.03, 128.71, 124.01, 120.59, 120.40, 119.75, 116.09, 112.30, 111.97, 104.77, 104.43, 104.09, 73.73, 70.61, 54.54, 53.21, 52.79, 49.03.

1-((1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(2,4-difluorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68cc'):



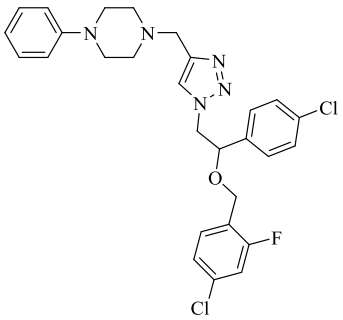
Yield: 440 mg (81%); white solid; mp 99-100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.36 (dd, *J* = 14.7, 8.3 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.23 – 7.19 (m, 1H), 7.08 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.99 – 6.82 (m, 7H), 5.12 (dd, *J* = 7.9, 3.9 Hz, 1H), 4.67 – 4.57 (m, 2H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.35 (d, *J* = 12.0 Hz, 1H), 3.74 (s, 2H), 3.23 – 3.13 (m, 5H), 2.70 – 2.58 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.24, 144.05, 135.38, 133.89, 129.12, 129.03, 128.71, 124.01, 120.59, 120.40, 119.75, 116.09, 112.30, 111.97, 104.77, 104.43, 104.09, 73.73, 70.61, 54.54, 53.21, 52.79, 49.03.

1-((1-(2-(4-Chlorobenzyloxy)-2-(4-chlorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68db'):



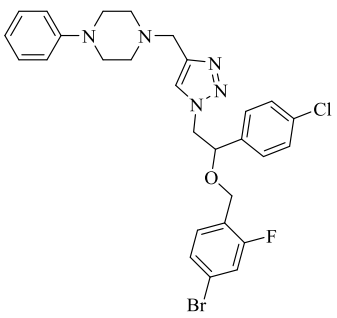
Yield: 272 mg (52%); brown solid; mp 128-129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.34 – 7.28 (m, 2H), 7.18 (dd, *J* = 9.6, 5.2 Hz, 7H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 7.9 Hz, 2H), 6.78 (t, *J* = 7.3 Hz, 1H), 4.70 (dd, *J* = 7.3, 5.4 Hz, 1H), 4.47 – 4.43 (m, 2H), 4.35 (d, *J* = 11.8 Hz, 1H), 4.10 (d, *J* = 11.8 Hz, 1H), 3.67 (s, 2H), 3.15 – 3.08 (m, 4H), 2.62 – 2.51 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.23, 143.88, 136.12, 135.56, 134.84, 133.82, 129.32, 129.12, 128.99, 128.70, 128.13, 124.26, 119.75, 116.09, 79.41, 70.22, 55.81, 53.23, 52.81, 49.03.

1-((1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(4-chlorophenyl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68dc'):



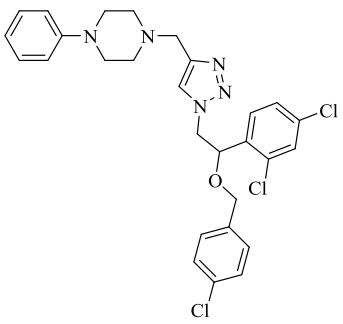
Yield: 534 mg (99%); off-white solid; mp 112-113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.41 – 7.36 (m, 2H), 7.32 – 7.26 (m, 4H), 7.21 (dt, *J* = 8.6, 4.0 Hz, 2H), 7.08 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.98 – 6.89 (m, 4H), 6.85 (t, *J* = 7.3 Hz, 1H), 4.82 (dd, *J* = 7.6, 5.0 Hz, 1H), 4.53 (dd, *J* = 6.3, 2.2 Hz, 2H), 4.49 – 4.44 (m, 1H), 4.31 (d, *J* = 12.0 Hz, 1H), 3.73 (s, 2H), 3.22 – 3.14 (m, 5H), 2.66 – 2.59 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.80, 160.48, 151.24, 143.95, 136.06, 134.88, 134.29, 134.16, 130.83, 130.80, 130.71, 129.31, 129.11, 128.10, 124.23, 119.73, 117.12, 116.79, 116.06, 114.23, 113.95, 79.92, 67.85, 55.82, 53.23, 52.81, 49.03.

1-((1-(2-(4-Bromo-2-fluorobenzyloxy)-2-(4-chlorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68dd') :



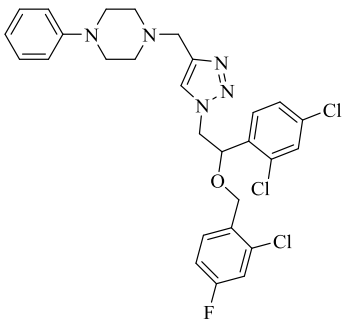
Yield: 572 mg (98%); brown solid; mp 92-94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.31 – 7.25 (m, 5H), 7.23 – 7.16 (m, 2H), 7.04 (t, *J* = 7.9 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 2H), 6.85 (t, *J* = 7.3 Hz, 1H), 4.78 (dd, *J* = 8.0, 4.5 Hz, 1H), 4.51 (t, *J* = 5.9 Hz, 2H), 4.43 (d, *J* = 11.8 Hz, 1H), 4.25 (d, *J* = 11.9 Hz, 1H), 3.74 (s, 2H), 3.25 – 3.16 (m, 4H), 2.70 – 2.60 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.16, 158.81, 151.22, 143.80, 135.91, 134.92, 131.20, 131.13, 129.33, 129.12, 128.09, 127.58, 127.53, 124.20, 123.52, 123.33, 122.43, 122.31, 119.75, 119.30, 118.97, 116.11, 79.82, 64.40, 55.80, 53.22, 52.83, 48.99.

1-((1-(2-(4-Chlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68eb'):



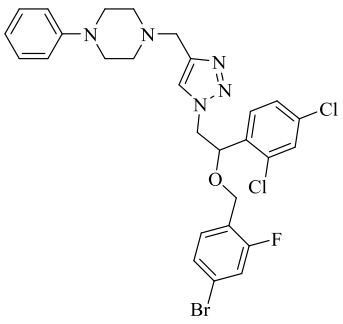
Yield: 350 mg (63%); white solid; mp 88-89 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.36 (d, *J* = 2.0 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.20 (q, *J* = 8.5 Hz, 7H), 7.00 (d, *J* = 8.4 Hz, 3H), 6.85 (d, *J* = 7.9 Hz, 3H), 6.77 (t, *J* = 7.3 Hz, 1H), 5.11 (dd, *J* = 8.1, 3.3 Hz, 1H), 4.57 (dd, *J* = 14.2, 3.3 Hz, 1H), 4.46 – 4.38 (m, 2H), 4.35 (d, *J* = 2.9 Hz, 1H), 4.12 (d, *J* = 11.8 Hz, 1H), 3.67 (s, 2H), 3.16 – 3.07 (m, 5H), 2.61 – 2.54 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.24, 144.16, 135.26, 135.21, 133.95, 133.79, 133.67, 129.83, 129.11, 128.73, 128.57, 127.99, 123.85, 119.73, 116.09, 76.21, 70.74, 54.13, 53.27, 52.86, 49.04.

1-((1-(2-(2-Chloro-4-fluorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68ec'):



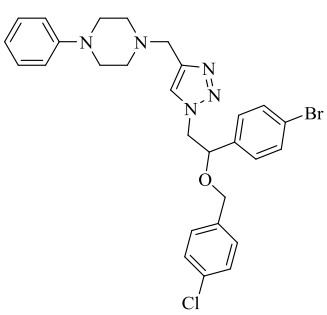
Yield: 465 mg (81%); white solid; mp 104-105 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.43 (dd, *J* = 10.9, 5.1 Hz, 2H), 7.34 – 7.25 (m, 3H), 7.24 – 7.20 (m, 1H), 7.08 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.00 – 6.89 (m, 3H), 6.85 (t, *J* = 7.3 Hz, 1H), 5.23 (dd, *J* = 8.1, 3.1 Hz, 1H), 4.67 (dd, *J* = 14.2, 3.2 Hz, 1H), 4.57 – 4.42 (m, 2H), 4.33 (d, *J* = 12.0 Hz, 1H), 3.74 (s, 2H), 3.26 – 3.14 (m, 4H), 2.73 – 2.59 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.23, 144.12, 135.24, 133.73, 133.58, 131.00, 130.88, 130.51, 129.84, 129.11, 128.51, 127.95, 123.89, 119.73, 117.14, 116.81, 116.07, 114.32, 114.04, 68.34, 54.14, 53.27, 52.86, 49.02, 29.69.

1-((1-(2-(4-Bromo-2-fluorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68ed'):



Yield: 420 mg (68%); yellow solid; mp 91-92 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.47 – 7.36 (m, 2H), 7.27 (t, *J* = 12.8 Hz, 5H), 7.07 (t, *J* = 7.7 Hz, 1H), 6.99 – 6.78 (m, 4H), 5.19 (d, *J* = 5.5 Hz, 1H), 4.65 (d, *J* = 14.1 Hz, 1H), 4.44 (t, *J* = 11.2 Hz, 2H), 4.26 (d, *J* = 11.7 Hz, 1H), 3.75 (s, 2H), 3.22 (s, 5H), 2.67 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.18, 158.84, 151.23, 144.06, 135.27, 133.76, 133.46, 131.34, 131.27, 129.83, 129.11, 128.48, 127.99, 127.66, 127.61, 123.84, 123.24, 123.04, 122.61, 122.49, 119.71, 119.30, 118.98, 116.10, 64.97, 64.92, 54.13, 53.29, 52.90, 49.01, 29.70.

1-((1-(2-(4-Bromophenyl)-2-(4-chlorobenzyloxy)ethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-4-phenylpiperazine (68fb'):



Yield: 340 mg (60%); yellow solid; mp 85-86 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 – 7.51 (m, 2H), 7.46 (s, 1H), 7.27 (dd, *J* = 6.8, 1.5 Hz, 4H), 7.24 – 7.18 (m, 2H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.85 (t, *J* = 7.3 Hz, 1H), 4.76 (dd, *J* = 7.3, 5.4 Hz, 1H), 4.58 – 4.48 (m, 1H), 4.42 (d, *J* = 11.8 Hz, 1H), 4.17 (d, *J* = 11.8 Hz, 1H), 3.77 (d, *J* = 11.7 Hz, 2H), 3.24 – 3.14 (m, 4H), 2.74 – 2.60 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.20, 143.77, 136.64, 135.53, 133.83, 132.28, 129.12, 128.99, 128.70, 128.44, 124.30, 122.98, 119.78, 116.11, 79.44, 70.24, 55.74, 53.14, 52.75, 48.98.

2.8.1 Antibacterial essay: Antibacterial assay of the compounds was performed using agar well diffusion method. For the experimental work, Muller-Hilton (Himedia, India) agar medium was prepared and sterilized by autoclaving at 121°C at 15 psi for 15 min. The medium was poured into sterile petri dishes, under aseptic conditions using laminar air flow chamber. After the solidification of medium, the suspension of the test organism (10^6 cfu/ mL) was spread onto the individual media plates using a sterile glass spreader. After some time well size of 9 mm diameter was made by the sterile metallic borer and the solution of working compound of concentration 128 µg/mL was poured into the wells. The experiment was performed in triplicates of each compound. Zone of inhibition assay was performed at 128 µg/ml concentration for all the compounds using well diffusion method. The plates were incubated at 37 °C for 18-24 h under dark conditions. The determination as whether the organism is susceptible, intermediate or resistant was made by measuring the size of zone of inhibition in comparison with standard antibiotic. MIC assay was performed to determine the lowest concentrations of compound necessary to inhibit a test organism. MIC values were evaluated for all the compounds using broth micro dilution method. Assay was carried out for the compounds at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, 128.0 µg/ mL concentrations. A set of tubes containing Muller Hilton broth medium with different concentrations of compound were prepared. The tubes were inoculated with bacterial cultures (10^6 cfu/mL) and incubated on a rotary shaker (180 rpm) at 37 °C for 18-24 h under dark conditions. MIC values were defined as lowest concentrations of compound that prevented the visible growth of the bacteria after the incubation period. All the experiments were performed in triplicates. Tetracyclin was used as a positive control at a concentration of 18 µg/mL.

2.8.2 Antifungal assay: Antifungal activities of chemically synthesized compounds were determined by agar well diffusion method at a concentration 128 µg/mL for all the compounds. For the experimental work, an eppendroff tube of 1.5 mL size was filled with 1 mL autoclaved distilled water and a loopful of freshly cultured fungus was properly mixed. 100 µL of this solution was uniformly spread on the PDA plate. After some time wells of 9 mm diameter was prepared by the sterile metallic borer and 100 µL of compound solution was added in respective wells. Plates were incubated at 28 °C for 4 days under dark conditions. Mean diameter of inhibition zone was measured to determine the antifungal activity. The experiment was performed in triplicates. MIC assay of all the compounds was performed at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, 128.0 µg/mL concentrations. Tubes containing 10 mL of sterilized czapeks dox broth medium was inoculated with 100 µL of freshly grown culture. Appropriate amount of compound was added to achieve the desired concentrations. The tubes were incubated at 28 °C for 4 days under dark conditions and carefully observed for the presence of turbidity. Carbendazim was used as positive control.

2.9 References

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Chapter III

Synthesis and Antimicrobial Study of Imidazo[1,2-*a*]pyridine 1,2,3-Triazole Derivatives

3.1 Introduction

Imidazole ring fused with pyridine moiety sharing one nitrogen and carbon atom are known as imidazopyridines which are important biologically active nitrogen containing bicyclic aromatic heterocycles (**Fig. 3.1**).[1] There are different types of imidazopyridines on the basis of fusion of imidazole and pyridine ring such as imidazo[1,2-*a*]pyridine, imidazo[1,5-*a*]pyridine etc. Among them, imidazo[1,2-*a*]pyridine is an important class of fused aza heterocyclic compounds widely present in the area of natural products and pharmaceuticals. This core ring system is present in numerous drug molecules such as Alpidem (for treatment of anxiety), Saripidem and Nicopidem (anxiolytic), Zolpidem (for treatment of insomnia), Zolimidine (peptic ulcer), Olprinone (cardiotonic agent), GSK812397 (HIV infection), Rifaximin (antibiotic) and YM529 (**Fig. 3.2**).[2] Imidazo[1,2-*a*]pyridines have received substantial attention in the field of pharmaceutical industry due to their pronounced biological activities. Compounds with imidazo[1,2-*a*]pyridine scaffold have been shown to possess broad range of pharmacological properties including antimicrobial,[3] antifungal,[4] antiviral,[5] anticancer,[6-7] antirhinoviral,[8] antitubercular,[9] antiulcer,[10-12] antiprotozoal,[13] γ-aminobutyric acid (GABA) inhibition,[14] antibacterial,[15] insulin-like growth factor-1 receptor (IGF-1R) inhibition,[16] HIF-1α prolyl hydroxylase inhibition,[17] K^+-stimulated ATPase inhibition,[18,19] *Mycobacterium tuberculosis* glutamine synthetase inhibition,[20] bone resorption inhibition[21,22] and selective cyclin dependent kinase inhibition[23,24] etc. Beside this, because of their CNS activities imidazo[1,2-*a*]pyridines are used as sedatives, hypnotics and anxiolytic etc. without any considerable side effect.[25]

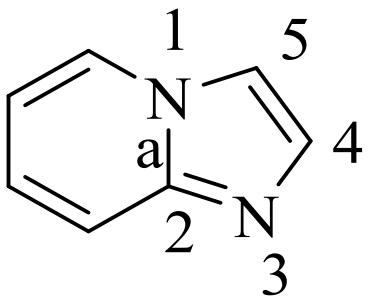


Figure 3.1: Structure of imidazo[1,2-*a*]pyridine.

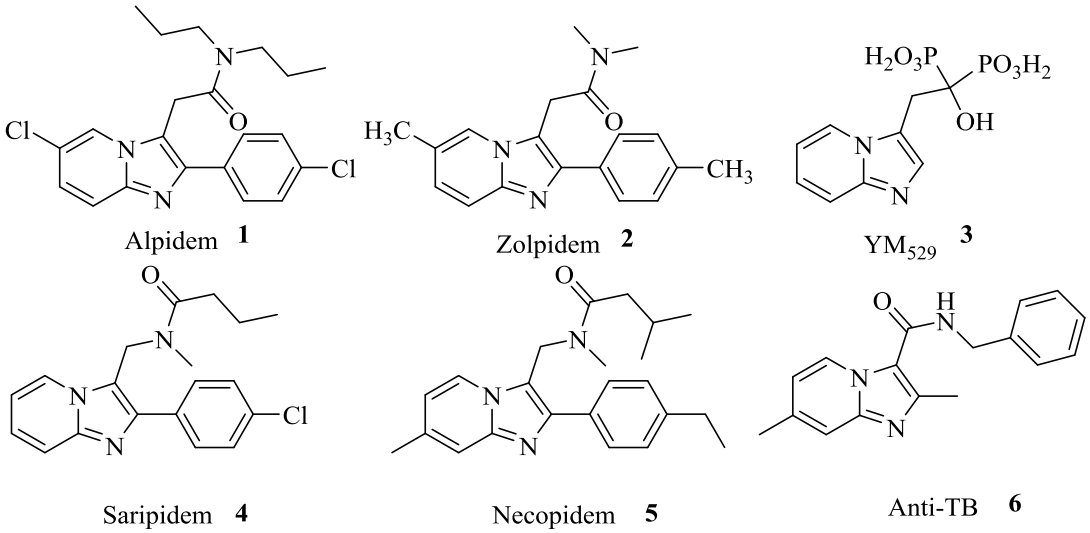
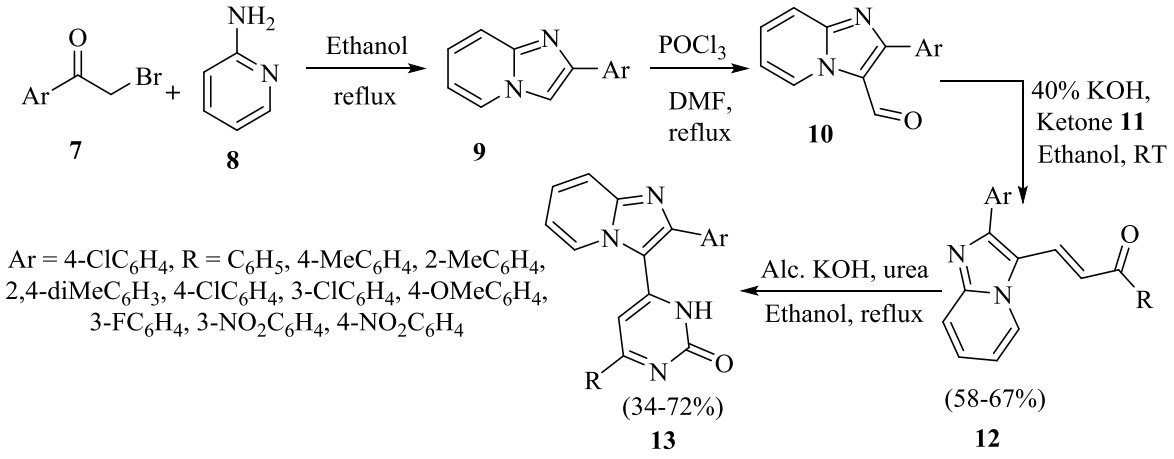


Figure 3.2: Chemical structure of some pharmacologically important imidazo[1,2-*a*]pyridines.

A vast series of literature is available on the synthesis of imidazo[1,2-*a*]pyridines and their biological applications such as antibacterial, antifungal, antiviral, GABA inhibitor etc. Herein, we have summarized the reports on antimicrobial applications of imidazo[1,2-*a*]pyridine motif over last decade.

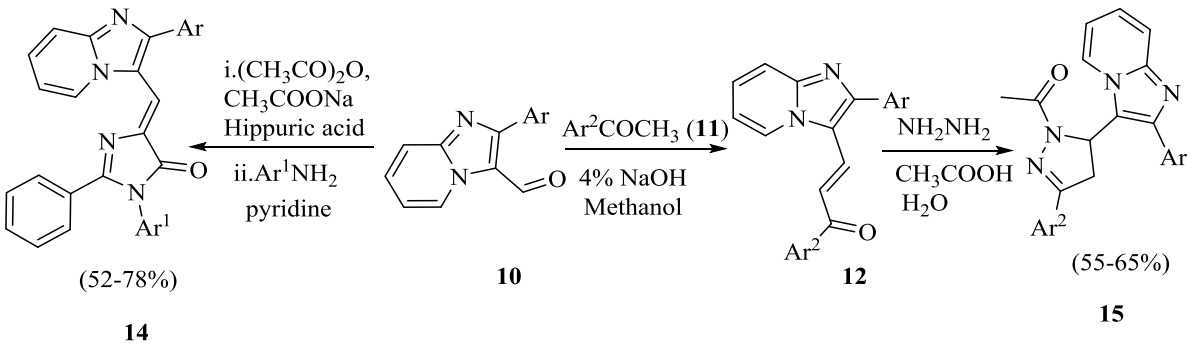
3.1.1 Imidazo[1,2-*a*]pyridines as antimicrobial agents

Joshi and coworkers synthesized imidazo[1,2-*a*]pyridine nucleus bearing chalcone and oxypyrimidine ring to evaluate their antimicrobial activity against various Gram positive and Gram negative bacteria and fungi *A. niger*.[26] Reaction of phenacyl bromide 7 with 2-aminopyridine **8** offered imidazo[1,2-*a*]pyridine **9** which was subsequently formylated to obtain **10**. Reaction of **10** with different ketones in presence of alkaline ethanol lead to enones **12** which were cyclised to oxopyrimidines **13** by treating with urea in basic media (Scheme 3.1). Synthesized enone and pyrimidine tethered imidazo[1,2-*a*]pyridines **12** and **13** were tested for antibacterial and antifungal activity. It was observed that compounds bearing electron withdrawing group showed better activity than having electron donating group against all tested bacteria showing zone of inhibition up to 22 mm; however, most of the compounds did not show any activity against fungi *A. niger*.



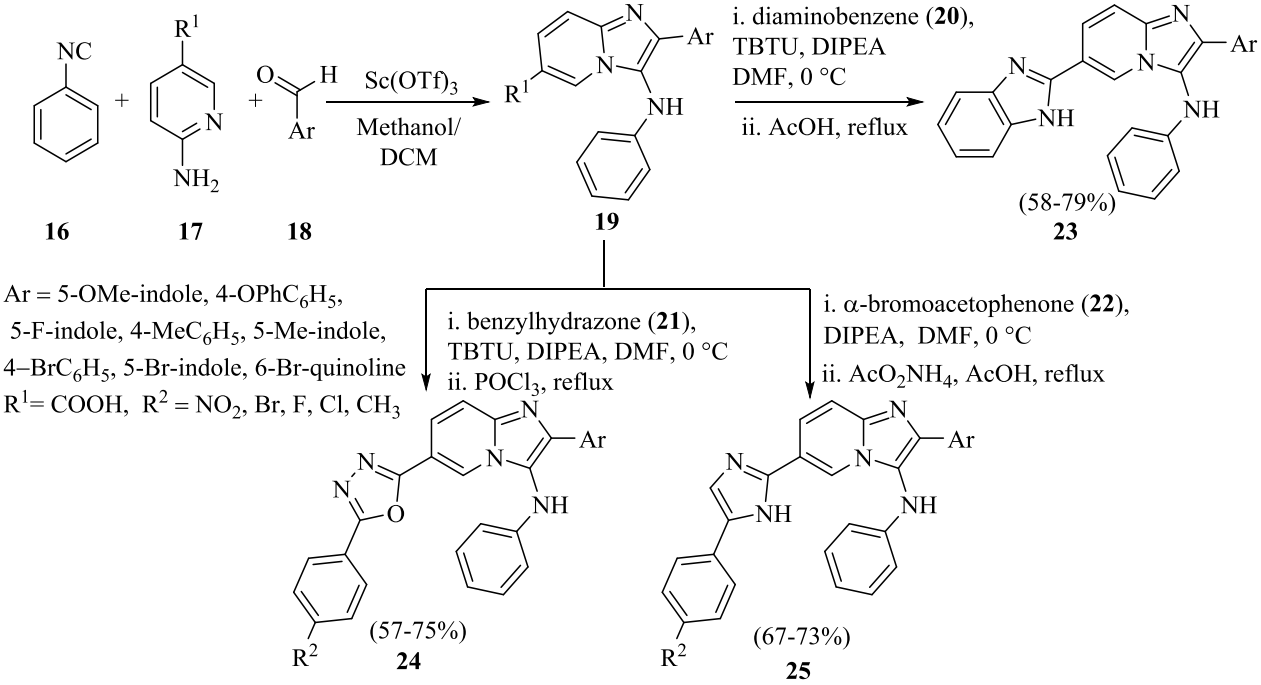
Scheme 3.1: Synthesis of imidazo[1,2-*a*]pyridines bearing enone **12** and oxypyrimidines **13**.

In continuation of their research on imidazo[1,2-*a*]pyridine nuclei as antimicrobial agents, the same group developed pyrazoline and imidazolinone linked imidazo[1,2-*a*]pyridine scaffolds for evaluation of their antimicrobial inhibition.[27] Chalcones **12** were synthesized by Claisen condensation of **10** with different ketones in presence of catalytic alkali. Heterocyclisation of **12** with hydrazine hydrate in presence of catalytic amount of acetic acid yielded pyrazoline tethered imidazo[1,2-*a*]pyridines **15**. Carbaldehyde **10** on Erlenmeyer synthesis with hippuric acid in presence of sodium acetate and acetic anhydride provided azalactones which were transformed into imidazolinone tethered imidazo[1,2-*a*]pyridines **14** on reaction with various amines in presence of pyridine (**Scheme 3.2**). All the synthesized structural motifs were screened for antimicrobial activity against five different pathogenic Gram positive, Gram negative bacteria and fungi *A. niger* at the concentration of 40 µg/mL. It was observed that zone of inhibition increases up to 21 mm when electron withdrawing substitution is there on phenyl ring attached to pyrazoline ring. All derivatives exhibited zone of inhibition in range of 21-10 mm for different bacteria while it was 22-15 mm against *A. niger* for most of the compounds.



Scheme 3.2: Synthesis of pyrazoline and imidazolinone linked imidazo[1,2-*a*]pyridines.

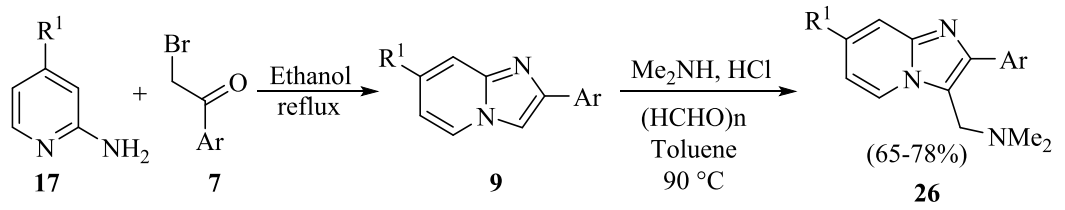
Al-Tel and colleague synthesized polyfunctional imidazo[1,2-*a*]pyridine scaffolds for the identification of a potent antimicrobial agent.[28] In the synthetic methodology, they performed classical [4+1]-cycloaddition *via* Groebke Blackburn reaction of an aldehyde **18**, aminopyridine derivative **17** and isocyanide **16** in the presence of $Sc(OTf)_3$ to provide imidazo[1,2-*a*]pyridine **19** which was coupled with different scaffolds such as diaminobenzene, benzylhydrazone and α-bromoacetophenone to yield desired motifs **23, 24** and **25** (**Scheme 3.3**). These scaffolds were tested for antibacterial activity against six Gram-positive and Gram-negative bacterial strains which ended into broad spectrum antibiotic with MIC value varying from >127 μg/mL to 0.5 μg/mL. All the scaffolds were especially more potent against *S. aureus* with MIC values <0.5 μg/mL. When the scaffolds were tested for antifungal activity against *C. albicans, C. parapsilosis, A. flavus* and *M. gypseum*, almost all compounds showed strong antifungal activity with MIC values ranging 19.65-1.11 μg/mL.



Scheme 3.3: Synthesis of imidazo[1,2-*a*]pyridine tethered heterocycles as antimicrobial agents.

Desai and coworkers reported synthesis and biological evaluation of *N*,*N*-dimethyl amine substituted imidazo[1,2-*a*]pyridines **26** (Scheme 3.4).[29] At first, imidazo[1,2-*a*]pyridines were constructed by reacting 2-aminopyridine with different α-bromoacetophenones which were then subjected to one pot multicomponent Mannich reaction with *N*,*N*-dimethylamine, paraformaldehyde in toluene to give **26** (Scheme 3.4). Synthesized compounds were evaluated for their antibacterial and antifungal activity and it was observed that compounds with electron withdrawing group showed better antibiotic activity as compared to the compounds with

electron donating substitution such as hydroxyl group. Also compounds bearing chloro, fluoro, bromo substitutions were found to have better antifungal activity against *A. clavatus*. Compound with 2,4-difluroaryl substitution was most potent against *A. niger* with MIC value 50 µg/mL.

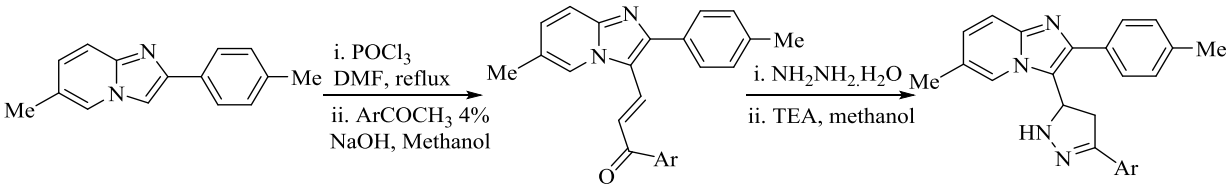


17 **7** **9** **26**

R^1 = CH_3, Ar = 2-OHC_6H_4, 2,4-diOHC_6H_3, 4-OH-3-$NO_2C_6H_3$, 3-ClC_6H_4, 2-BrC_6H_4, 3-Cl-4-FC_6H_3, 2,4-diOH-5-$NO_2C_6H_2$, 3-Br-2-FC_6H_4, 2-Br-4-$NO_2C_6H_4$, 2,3-diFC_6H_3, 2,4-diFC_6H_3, 2,6-diFC_6H_3,

Scheme 3.4: Synthesis of *N*,*N*-dimethyl amine substituted imidazo[1,2-*a*]pyridine compounds.

Shah *et al* described synthesis of pyrazoline linked imidazo[1,2-*a*]pyridines (**28**) by the reaction of chalcones **27** with hydrazine hydrate in presence of triethyl amine base in methanol in good yields (**Scheme 3.5**).[30] Synthesized compounds were screened for antimicrobial activity against different bacterias such as *S. aureus, B. subtilis, P. aeruginosa, E. coli* and fungi *A. niger*. Compounds bearing electron withdrawing groups were found to be far better in inhibitory action against different bacteria with zone of inhibition upto 22 mm comparable to ciprofloxacin (17 mm) and amoxicillin (24 mm).

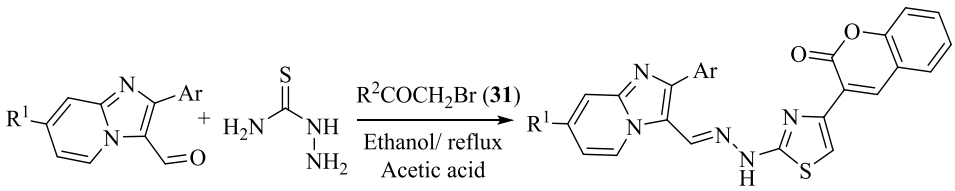


9 **27** **28**

Ar = 4-$OCH_3C_6H_4$, 4-ClC_6H_4, C_6H_5, 2,4-diClC_6H_3, 4-OH-3-$OCH_3C_6H_3$, 4-BrC_6H_4, 2-OHC_6H_4, 4-OHC_6H_4, 4-$NH_2C_6H_4$, 2-C_4H_3S, 4-$NO_2C_6H_4$, 4-MeC_6H_4

Scheme 3.5: Synthesis of pyrazoline linked imidazo[1,2-*a*]pyridines.

Bavantula *et al* synthesized imidazo[1,2-*a*]pyridine incorporated thiazolyl coumarins (**32**) *via* one pot multicomponent condensation reaction between carbaldehyde **29**, 3-(2-bromoacetyl)-2H-chromen-2-ones **31** and thiosemicarbazide **30** in the presence of catalytic amount of acetic acid in ethanol under reflux condition (**Scheme 3.6**).[31]All the synthesized compounds showed moderate antimicrobial activity with MIC values greater than 150 µg/mL against Gram positive and Gram negative bacteria but none of the derivative exhibited good antifungal activity.

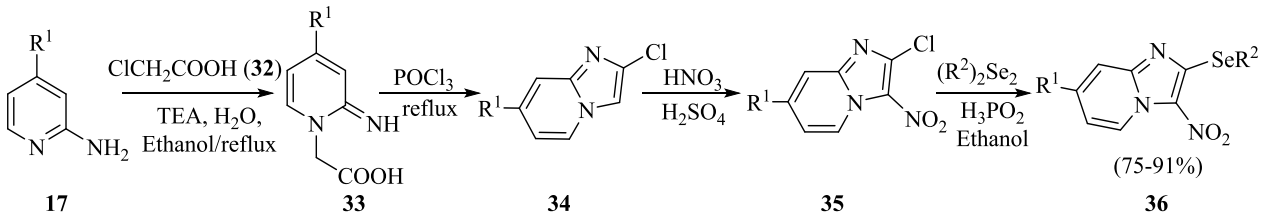


29 30 32

R^1 = H, CH_3, R^2 = Coumaryl, Naphthocoumaryl, Ar = 4-BrC_6H_4, 4-ClC_6H_4, 4-$NO_2C_6H_4$, 4-$OCH_3C_6H_4$

Scheme 3.6: Synthesis of imidazo[1,2-*a*]pyridine linked thiazolyl coumarins.

Based on synergic effect concept, Sharma *et al* synthesized imidazo[1,2-*a*]pyridine linked organoselenium compounds and examined for their antimicrobial activity against various pathogenic bacteria and fungi.[32] For the synthesis of aspirated compound, 2-aminopyridine **17** was condensed with 2-chloroacetic acid **32** which on refluxing with phosphorus oxychloride offered 2-chloroimidazo[1,2-*a*]pyridine **34** *via* intra-molecular cyclization. Nitration of **34** gave **35** which on reaction with organoselenide ion resulted into formation of **36** (Scheme 3.7)**.** All the synthesized compounds were examined for their antimicrobial activity against *E. coli, A. fumigates, C. krusei* and *A. niger*. Imidazo[1,2-*a*]pyridine linked selenide ion tethered with non-substituted aryl and pyridine ring are better in inhibitory action with MIC value 2.48 μg/mL and 10.41 μg/mL, respectively against *E. coli*. Also, the compounds were evaluated for synergic effect using mixture of organoselenide and Kanamycin/ Rifampycin for *E.coli* and with fluconazole for *C. krusei*. The MIC values were found to be lower under synergic condition. When the derivatives were tested for antifungal activity against *A. fumigates, A. niger, C. krusei* and *C. parapsilosis*, only compound with pyridyl ring tethered selenide showed MIC values 9.96, 19.93, 9.96 and 19.93 μg/mL, respectively. All other compounds were not active against tested fungi.



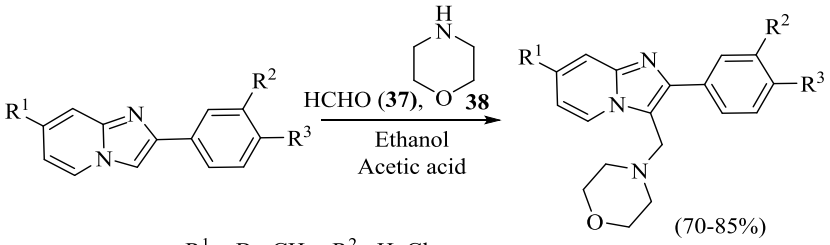
17 33 34 35 36

R^1 = H, CH_3 R^2 = C_6H_5, C_5H_4N, 2-MeC_6H_4, 4-MeC_6H_4, 4-$OMeC_6H_4$, 2,4,6-$(CH_3)_3C_6H_2$, Naphthyl, 4-(N,N-$(CH_3)_2$N)C_6H_4

Scheme 3.7: Synthesis of imidazo[1,2-*a*]pyridine linked organoselium compounds.

Bodke *et al* synthesized morpholine linked imidazo[1,2-*a*]pyridines to evaluate their biological activity (Scheme 3.8).[33] Imidazo[1,2-*a*]pyridines **9** were reacted with morpholine **38** and formaldehyde **37** in presence of catalytic amount of acetic acid to yield 3-(morpholin-4-

ylmethyl)-imidazo[1,2-*a*]pyridines **39**. The synthesized derivatives were screened for their antimicrobial evaluation against different bacteria and fungi. Compounds bearing electron withdrawing group were more effective against both bacteria and fungi. Antimicrobial activity was found to be concentration dependent. It was also observed that derivatives possessing bromo and nitro substitution were active against all bacterial and fungal strains *i.e. S. aureus, P. putida, E. coli, M. griseous, C. albicans* etc. Compounds bearing methyl substitution on pyridyl ring were completely inactive against fungi *M. griseous.*

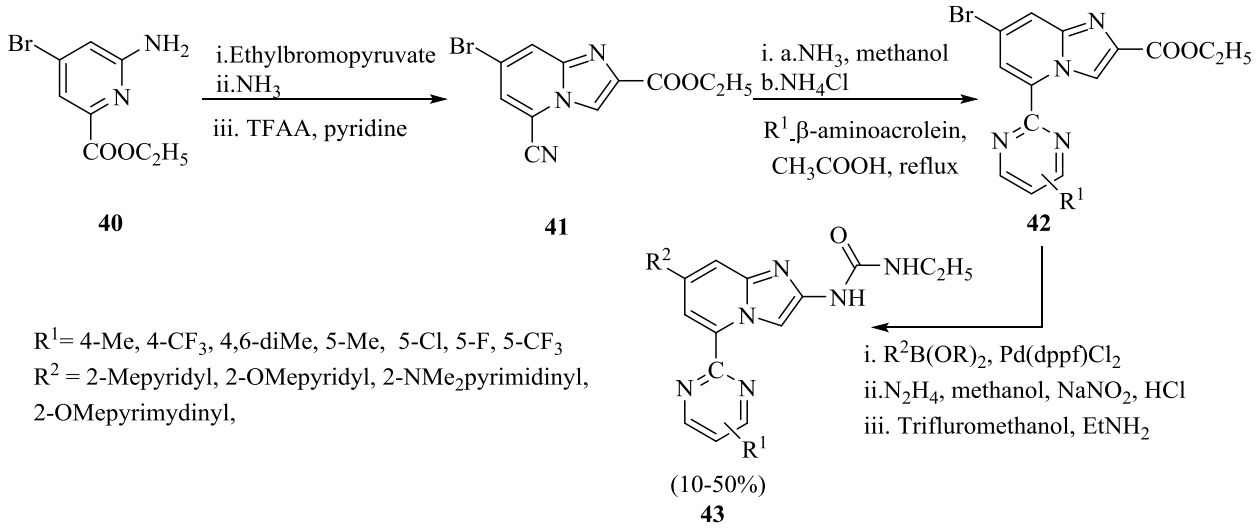


9 R^1 = Br, CH_3, R^2 =H, Cl; R^3= Cl, Br, NO_2, CN

39

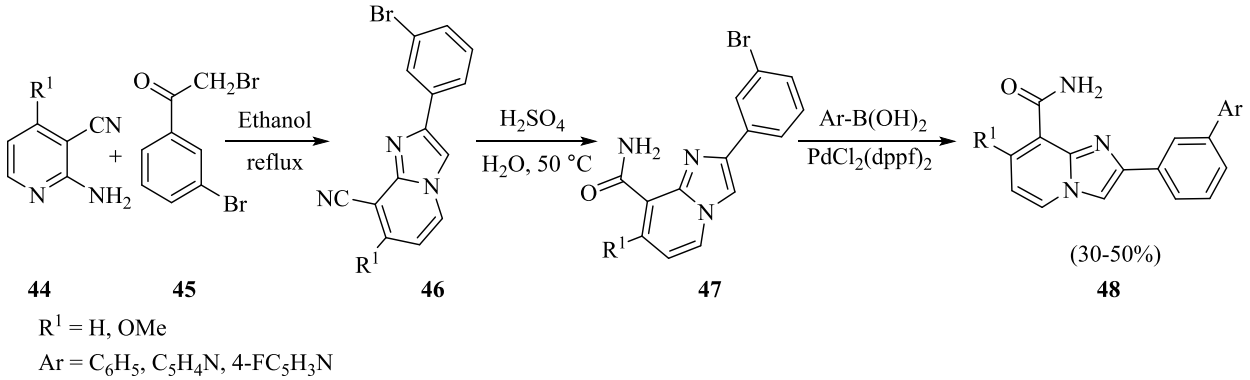
Scheme 3.8: Synthesis of imidazo[1,2-*a*]pyridines.

Starr and colleagues visualized the importance of pyrimidine tethered imidazo[1,2-*a*]pyridines as antibacterial agents targeting ATPase domains of DNA gyrase and topoisomerase IV.[15] Initially, the synthesis of 2-ethoxycarbonyl imidazo[1,2-*a*]pyridine was carried out by reaction of 2-amino-4-bromo-6-ethoxycarbonylpyridine **40** with ethyl bromopyruvate using ethanol as solvent. Selective ammonolysis of diethyl ester using ammonia at C5 position followed by dehydration with TFAA offered nitrile **41**. **41** under basic conditions presented methyl imidate derivative which on reflux with ammonium chloride in methanol offered amidine intermediate that was transformed into pyrimidines **42** by reaction with appropriate 1,3-dicarbonyl synthons. Sequential Suzuki coupling followed by hydrazinolysis, Curtius rearrangement and consecutive aminolysis with ethylamine provided the desired scaffold **43** (**Scheme 3.9**). All the synthesized scaffolds were evaluated for microbial inhibition against five gram positive bacteria which showed moderate to good inhibition.



Scheme 3.9: Access towards the synthesis of pyrimidines tethered imidazo[1,2-*a*]pyridines.

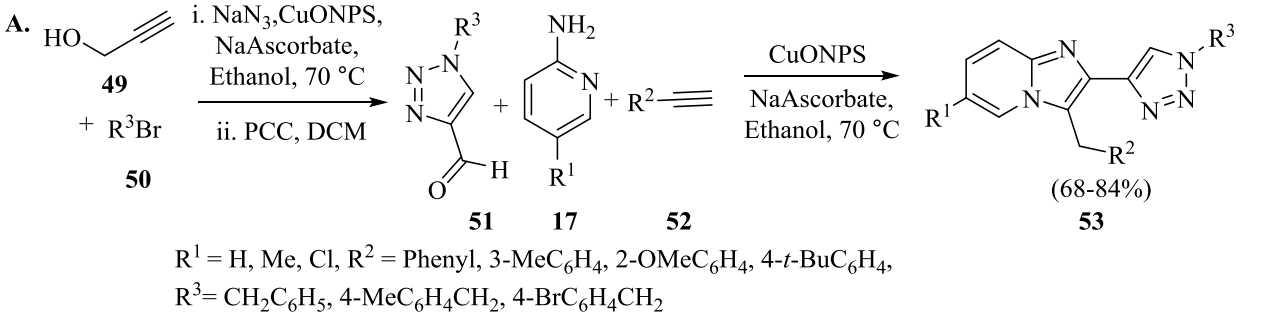
Ramachandran and his group designed and synthesized imidazo[1,2-*a*]pyridine-8-carboxamides **48** for evaluation of their antibacterial activity. 2-Amino-3-cyanopyridine **44** on reaction with 2-bromo-1-(3-bromoophenyl)ethanone **45** in ethanol furnished imidazo[1,2-*a*]pyridine **46**.[34] The nitrile group of **46** on hydrolysis using concentrated sulphuric acid yielded corresponding amide **47**. Suzuki reaction of **47** using boronic acids gave desired product **48** in good yield (**Scheme 3.10**). All the compounds were screened against various Gram positive and Gram negative bacteria but none of the compounds showed good inhibitory action. However, these compounds were found to be good inhibitors of *M. tuberculosis*. Based on these literature reports, it was concluded that imidazo[1,2-*a*]pyridines scaffold is of considerable importance in pharmacological chemistry from the point view of antimicrobial activity.

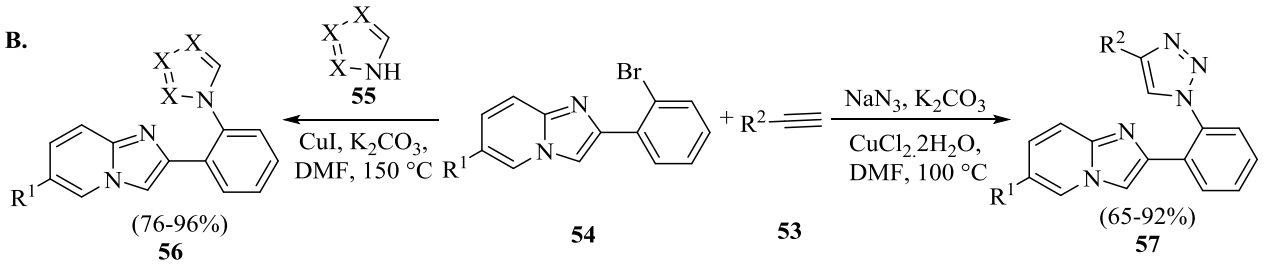


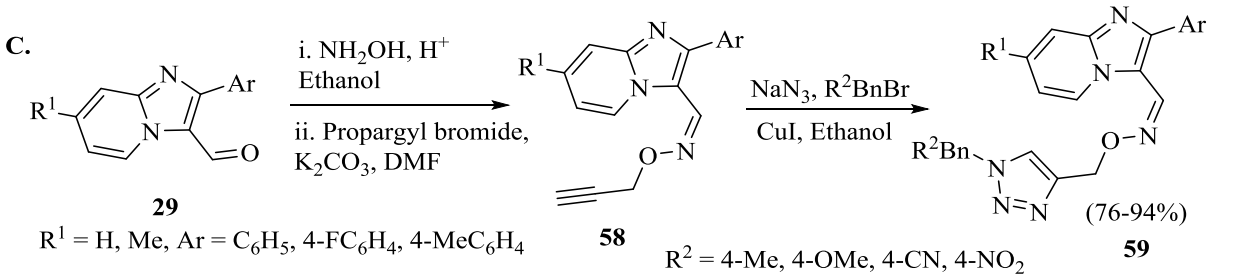
Scheme 3.10: Stepwise synthesis of imidazo[1,2-*a*]pyridine-8-carboxamides.

On the other hand, 1,4-disubstituted 1,2,3-triazoles are also found to show antimicrobial activity.[35-38] There are reports where triazole tethered imidazo[1,2-*a*]pyridines are synthesized by different methodologies. An array of 2-triazolylimidazo[1,2-*a*]pyridine **53** was constructed

in one-pot three component manner using 1-alkyl-1,2,3-triazole-4-carbaldehyde, amidine and terminal alkynes in presence of 5 mol % copper nanoxide (CuONPS) as catalyst and 10 mol % of sodium ascorbate (Scheme 3.11A).[39] Our group has synthesized azole substituted imidazo[1,2-*a*]pyridines **56** and **57** *via* copper catalysed Ullmann type *C-N* coupling (Scheme 3.11B).[40] Adhikari group also synthesized 1,2,3-triazole linked suitably substituted imidazo[1,2-*a*]pyridines **59** and evaluated them for antipileptic activity[25] (Scheme 3.11C). Although, there are reports on the synthesis of different type of 1,2,3-triazole linked imidazo[1,2-*a*]pyridines but no report is available on their antimicrobial activity besides having both the structural motif parts as biologically active.







Scheme 3.11: Synthesis of 1,2,3-triazolyl imidazo[1,2-*a*]pyridines.

Owing to our continuing interest in the synthesis of novel triazolyl heterocyclic compounds[41,42] and the pharmacological records of these privileged motifs prompted us to explore synthesis of their conjugates. Herein, we describe the facile preparation of 3-(triazol-1-yl)methyl-*H*-

imidazo[1,2-*a*]pyridines *via* copper catalyzed 1,3-dipolar cycloaddition of (imidazo[1,2-*a*]pyridine-3-yl)azidomethane and terminal alkynes and their structural isomer 3-(triazol-4-yl) -imidazo[1,2-*a*]pyridines (**Figure 3.3**). We have evaluated all the derivatives for their antibacterial and antifungal activity. Biofilm inhibition study was performed against *S. aureus*. To the best of our knowledge, this is the first report on synthesis of 3-(triazol-1-yl)methyl-imidazo[1,2-a]pyridines and 3-(triazol-4-yl)-imidazo[1,2-*a*]pyridines *via* CuAAC and their antimicrobial evaluation.

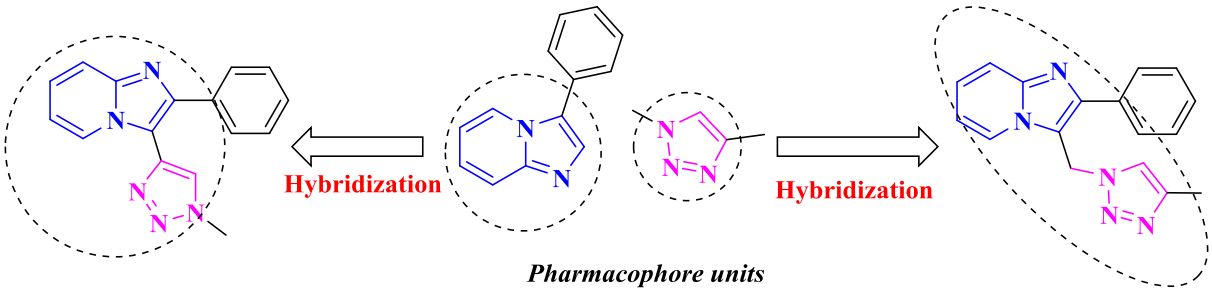
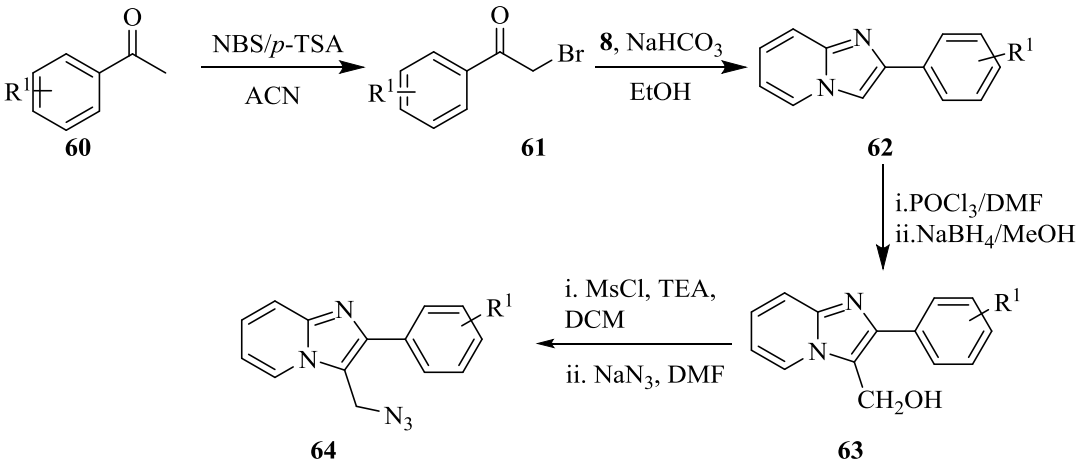


Figure 3.3: Molecular hybridization of imidazo[1,2-*a*]pyridine and 1,2,3-triazole ring.

3.2 Results and discussion

Aiming to the synthesis of the 3-(azidomethyl)-imidazo[1,2-*a*]pyridine intermediate **64,** we first performed the bromination of acetophenones (**60**). Initially, acetophenones were treated with *N*-bromosuccinimide (NBS)/*p*-toluenesulphonic acid (*p*-TSA) in acetonitrile to get α-bromoacetophenones (**61**). Among tested brominating agents phenyltrimethylammonium tribromide (PTT), $CuBr_2$ and NBS/*p*-TSA, latter was found to be most suitable to furnish the corresponding α-bromoketones **61**. The physical and spectroscopic data of **61** were in agreement with reported literature values. The α-bromoacetophenones (**61**) were reacted with 2-aminopyridine to get corresponding imidazo[1,2-*a*]pyridines (**62**) following the literature procedure.[14] These imidazo[1,2-*a*]pyridines were converted to 2-(imidazo[1,2-*a*]pyridin-3-yl)ethanol (**63**) by formylation using $POCl_3$/DMF at 80 °C followed by reduction with $NaBH_4$ (**Scheme 3. 12**). For converting **63** to **64**, initially we tried reaction with PBr_3 in DCM followed by sodium azide, but it did not give the desired product. Next, we tried to convert alcohol functionality to mesyl group by reaction with mesyl chloride in DCM. The reaction proceeded well but we could not isolate the product due to unstability of mesyl derivative (decomposes into starting material). We therefore did *in situ* mesylation followed by reaction with sodium azide to get compound **64**. With further optimization of reaction conditions, the best results were obtained by adding sodium azide *in situ* after one hour of mesylation. The *in situ* generation of organic azides **64** minimizes hazards derived from the isolation, reduces reaction

time and waste generation due to an additional synthetic step. The structure of **64** was ascertained by spectroscopic data. A characteristic peak appeared at 2080 cm^{-1} in IR spectra for the azide group. A singlet at δ = 6.05 for CH_2N_3 protons in ^1H NMR along with other protons and a signal at δ = 41 ppm in ^{13}C NMR spectra along with other carbon signals for CH_2N_3 was in consistent with expected structure of the protons. We then synthesized different 3-(azidomethyl)-imidazo[1,2-*a*]pyridines **64** using these conditions (**Scheme 3.12**).



Scheme 3.12: Synthesis of 3-(azidomethyl)-imidazo[1,2-*a*]pyridines **64**.

Synthesis of 3-(triazol-1-yl)methyl-imidazo[1,2-*a*]pyridines (**65**) was achieved by click reaction of **64** with terminal alkynes (**53**). Initially, reaction of **64** with 4-methylphenylacetylene (**53a**) was selected as model reaction. The reaction of **64** (1 mmol) with 4-methylphenyl acetylene (1.1 mmol) in the presence of 10 mol% CuI in PEG-400: water[43] (10 mL, 1: 1 *v/v*) at room temperature gave **65b** in 50 % yield. The structure of **65b** was characterized by NMR and mass spectroscopic data. In the ^1H NMR a characteristic singlet appeared at δ = 7.57 ppm for C_5-*H* of triazolyl ring and a singlet at δ = 6.02 ppm for N-CH_2 along with other protons. In the ^{13}C NMR spectra N-CH_2 carbon appeared at δ = 44.04 ppm, C_4 of triazole ring appeared at δ = 148.64 ppm and C_5 at δ = 123.88 ppm along with other carbons. In ESI-MS a peak appeared at *m/z* = 366.1637 for $[M + H]^+$ of **65b**. Absence of peak in the region of 2080 cm^{-1} in IR spectra further confirmed the structure. Use of the PEG-400/water (1: 1 *v/v*) appeared to be the most promising reaction media. We also performed reaction for longer time, but complete conversion was not achieved even after 16 hours of reaction. Next we performed the reaction at higher temperature (60 °C) and under μw heating. Moderate heating, microwave irradiation and other modification in reaction conditions such as change in catalyst loading & concentration did not improve the yield of final product. Thus we selected

reaction at room temperature in PEG-400: water (1: 1 *v/v*) using 5 mol % of CuI as optimized reaction condition for the click reaction.

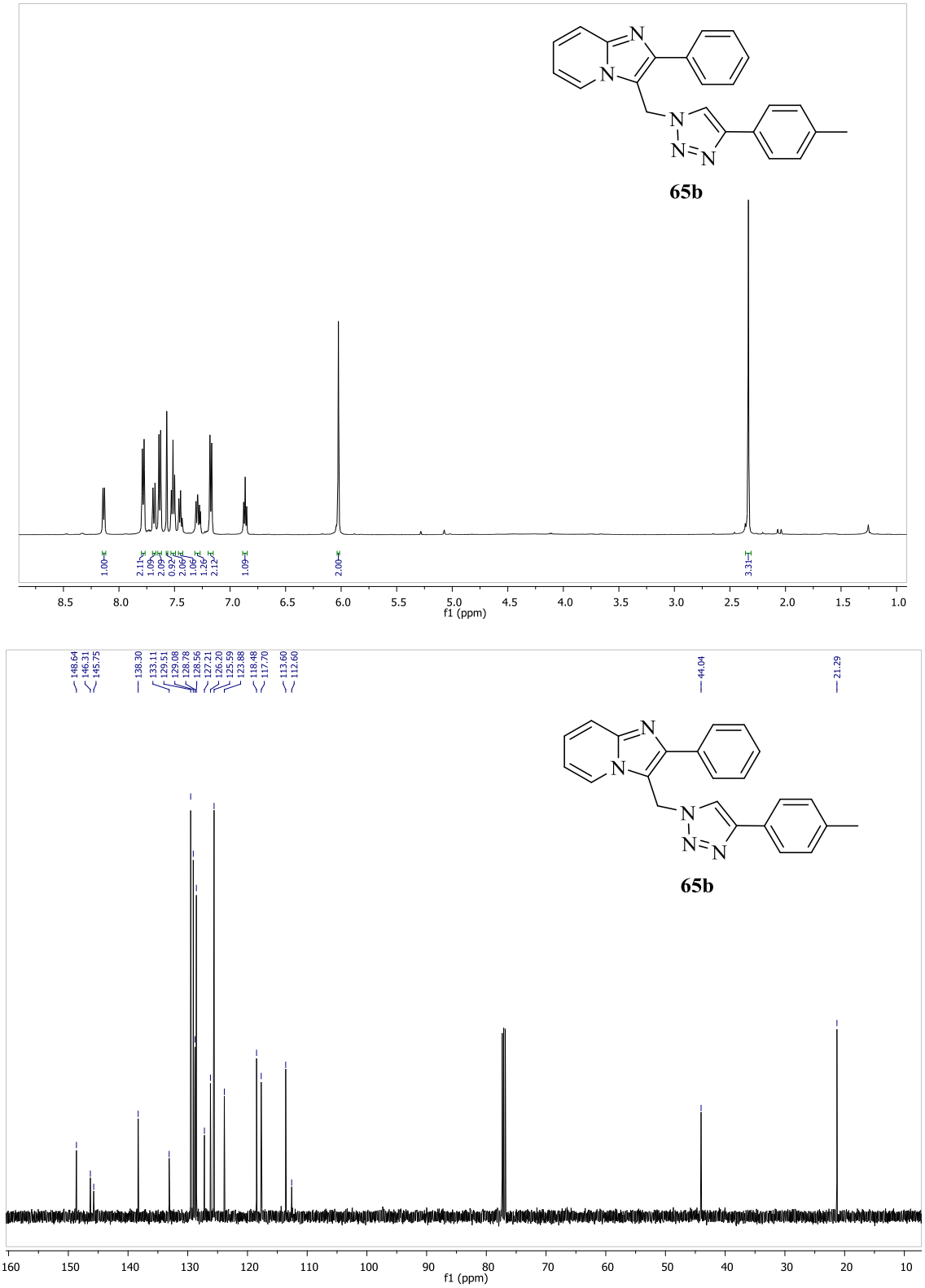
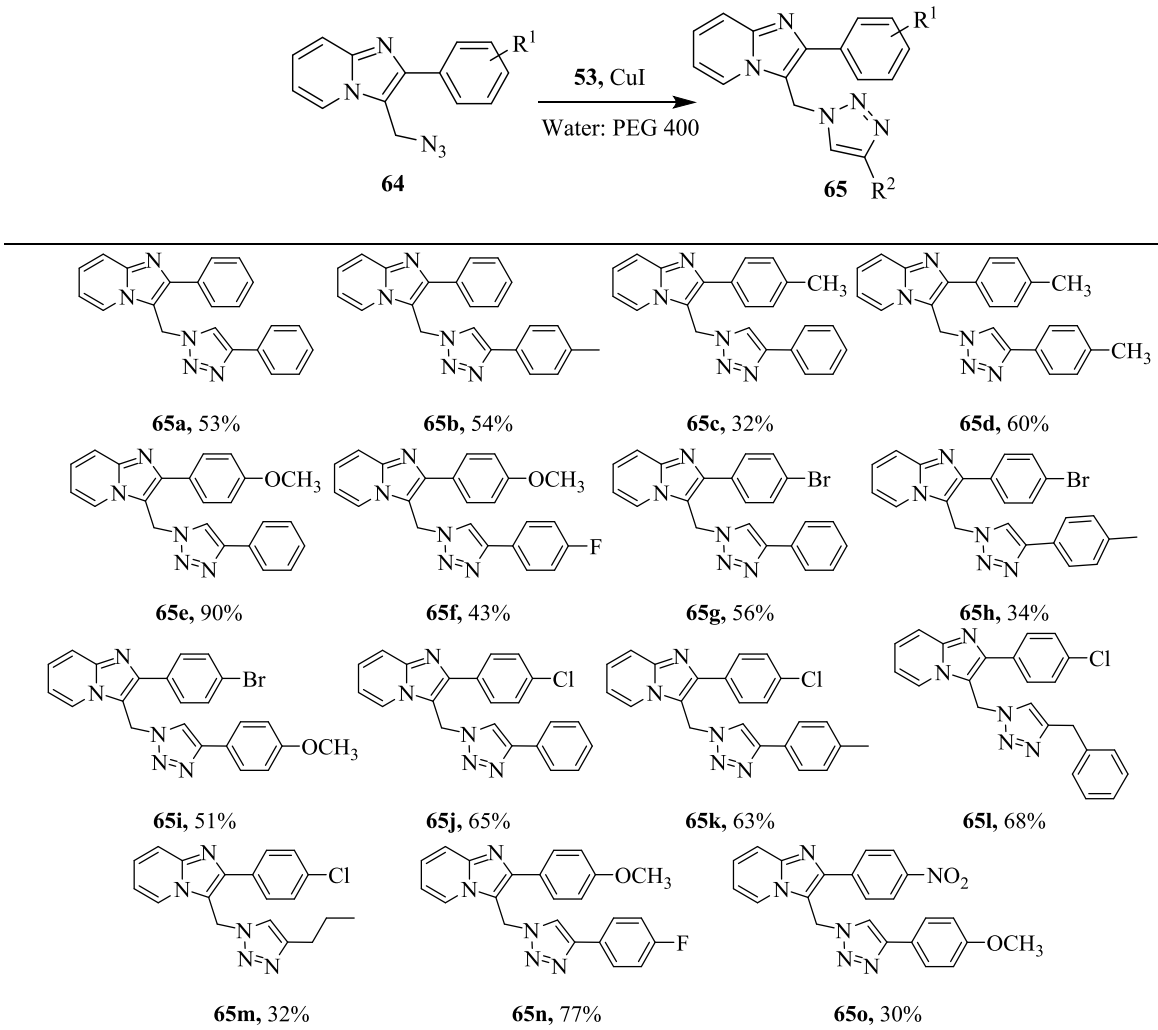


Figure 3.4: ^{1}H and ^{13}C NMR spectra of **65b.**

With the optimized protocol in hand, we next set out to explore its scope. To our delight, different (imidazo[1,2-*a*]pyridinyl)azidomethanes (**64a-g**) and alkynes (**53a-e**) underwent smooth reactions to give 3-(triazol-1-yl)methyl-imidazo[1,2-*a*]pyridines **65a-o** in good to excellent yield (25-85%, Scheme 3.13) at room temperature in 5-7 h. All the compounds were of high purity and gave satisfactory spectroscopic data. The structure of products was confirmed as 1,4-disubstituted 1,2,3-triazole and not as 1,5-disubstituted 1,2,3-triazole by ^{1}H NMR and ^{13}C NMR spectroscopic data.



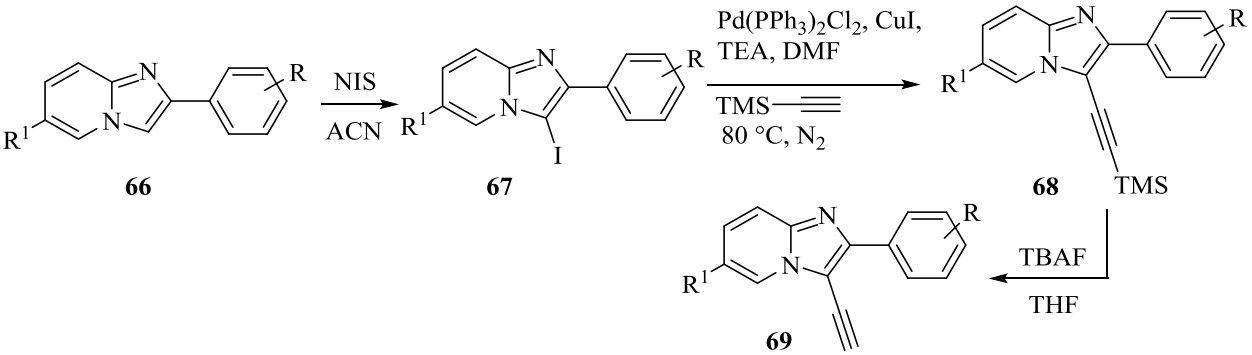
Scheme 3.13.Synthsis of 3-(triazol-1-yl)methyl-imidazo[1,2-*a*]pyridines derivatives **65a-o** *via* click chemistry.

The aryl alkynes are found to give better yields than aliphatic alkynes. Azidomethylimidazo[1,2-*a*]pyridine (**64**) having 2-aryl substituted with electron withdrawing group gave better yield of the corresponding product **65**, however, solubility of these compounds makes the isolation difficult. It is worth to mention that when **64** was treated with propiolic acid it did not result in formation of desired product. The mechanism of reaction is

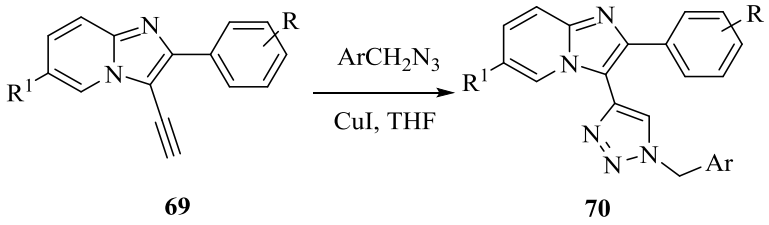
believed to be same as reported for copper(I) catalyzed 1,3-cycloaddition of alkyne and azide.[44] The Cu(I) activated alkyne rapidly couple with azide (**64**) *via* the 1,3-dipolar cycloaddition, and replacement of Cu(I) by a proton will regenerate the copper(I) catalyst and give coupled product. The tolerance of different substituent such as methyl, methoxy, fluoro, chloro, bromo, and nitro in this protocol provides the opportunity for further chemical manipulations in 3-(triazol-1-yl)methyl-imidazo[1,2-*a*]pyridines and thus novel therapeutically important heterocycles can be generated. The synthetic utility of this protocol becomes more attractive from green chemistry view as the click reaction is performed in PEG-water as reaction medium.

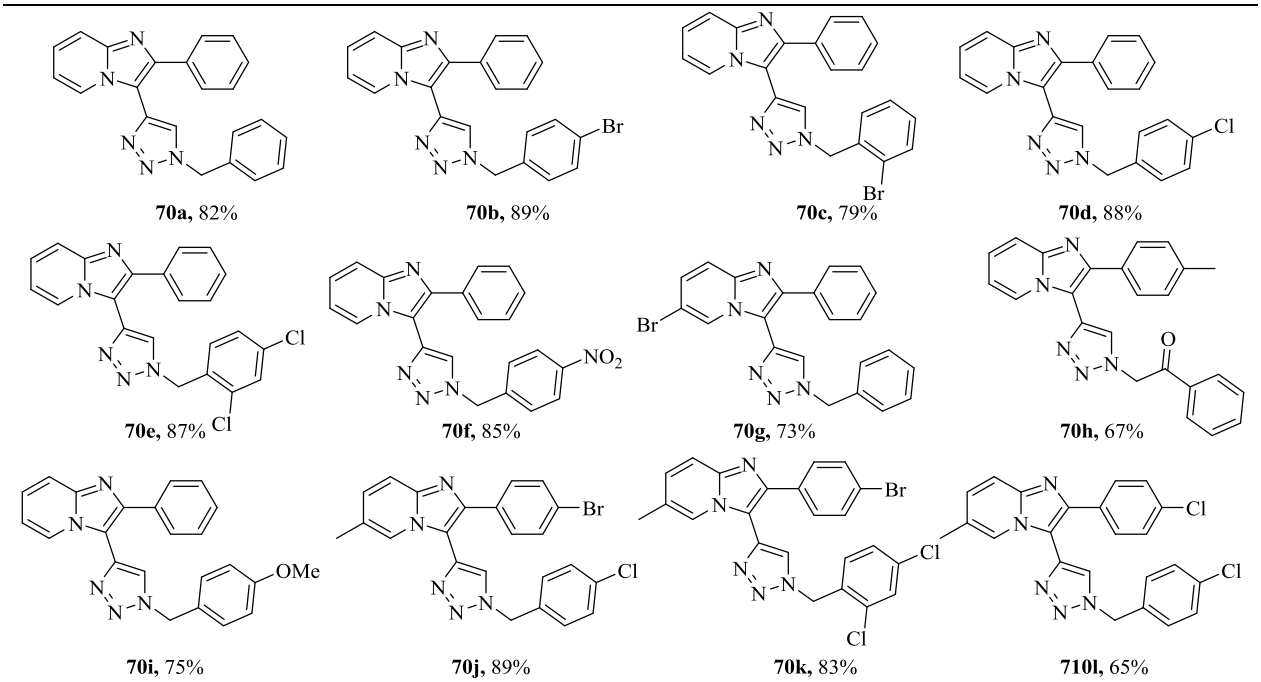
After the successful synthesis of **65a-o,** we planned to synthesize isomeric triazole by adding alkyne part to imidazopyridine nucleus followed by reaction with different benzyl azides. For this purpose, 3-acetylene substituted imidazo[1,2-*a*]pyridines **69** were synthesized from imidazo[1,2-*a*]pyridines **66** (Scheme 3.13). Iodination of imidazo[1,2-a]pyridine **66** was executed using *N*-iodosuccinimide in acetonitrile to give 3-iodo-2-phenylimidazo[1,2-*a*]pyridine **67** which was consequently coupled with trimethylsilylacetylene (TMSacetylene) *via* Sonagashira reaction to give **68**. Desilylation of 2-(4-methoxyphenyl)-3-((trimethylsilyl)ethynyl)imidazo[1,2-*a*]pyridine was carried out using TBAF/THF under nitrogen atmosphere which provided 3-ethynyl-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine **69** within 15 minutes. The structure of alkyne **69** was confirmed by spectroscopic data. A characteristic peak was observed at 3332 cm^{-1} for alkyne in IR spectrum. After the successful synthesis of alkyne **69**, it was reacted with benzyl azide using CuI/THF to get triazole 3-(1-benzyl-1H-1,2,3-triazol-4-yl)-2-(4-methoxyphenyl) imidazo[1,2-*a*]pyridine **70i**. For example, 1 Equivalent of 3-ethynyl-2-(4-methoxyphenyl) imidazo[1,2-*a*]pyridine **69** was reacted with benzyl azide (1equiv.) using 10 mol % CuI as catalyst and THF as solvent at room temperature for 10 hours to give 3-(1-benzyl-1H-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine **70i** in 82% yield. The structure of compound **70i** was confirmed by NMR and mass spectroscopic data. In ^{1}H NMR a characteristic singlet appeared at δ = 7.43 ppm for C_5-*H* of triazolyl ring and a singlet at δ = 5.56 ppm for N-CH_2 along with other protons. In ^{13}C NMR spectra N-CH_2 carbon appeared at δ = 54.24 ppm, methoxy carbons at δ = 53.25 ppm, C_4 of triazole ring appeared at δ = 144.52 ppm and C_5 of triazole ring appeared at δ = 133.49 ppm along with other carbons. In ESI-MS a peak appeared at *m/z* = 382.1562 $[M + H]^+$ ion of **70i**. Absence of peak in the region of 2080 cm^{-1} in IR spectra further confirmed the transformation of azide into triazole. The reaction was performed under different solvent conditions such as DMF, DMSO,

water, water: PEG 400 (1:1 *v/v*), ethanol, acetonitrile but use of the THF appeared to be the most promising reaction media as changing another solvent could not enhance yield of products. Although DMF was also a good reaction media but isolation of product was difficult. Thus, we selected reaction at room temperature in THF using 10 mol % of CuI as optimized reaction condition for this click reaction.



Scheme 3.14: Synthetic route for 3-ethynyl-2-arylimidazo[1,2-*a*]pyridine **69**.





Scheme 3.15: Synthesis of triazolo-imidazo[1,2-*a*]pyridines **70.**

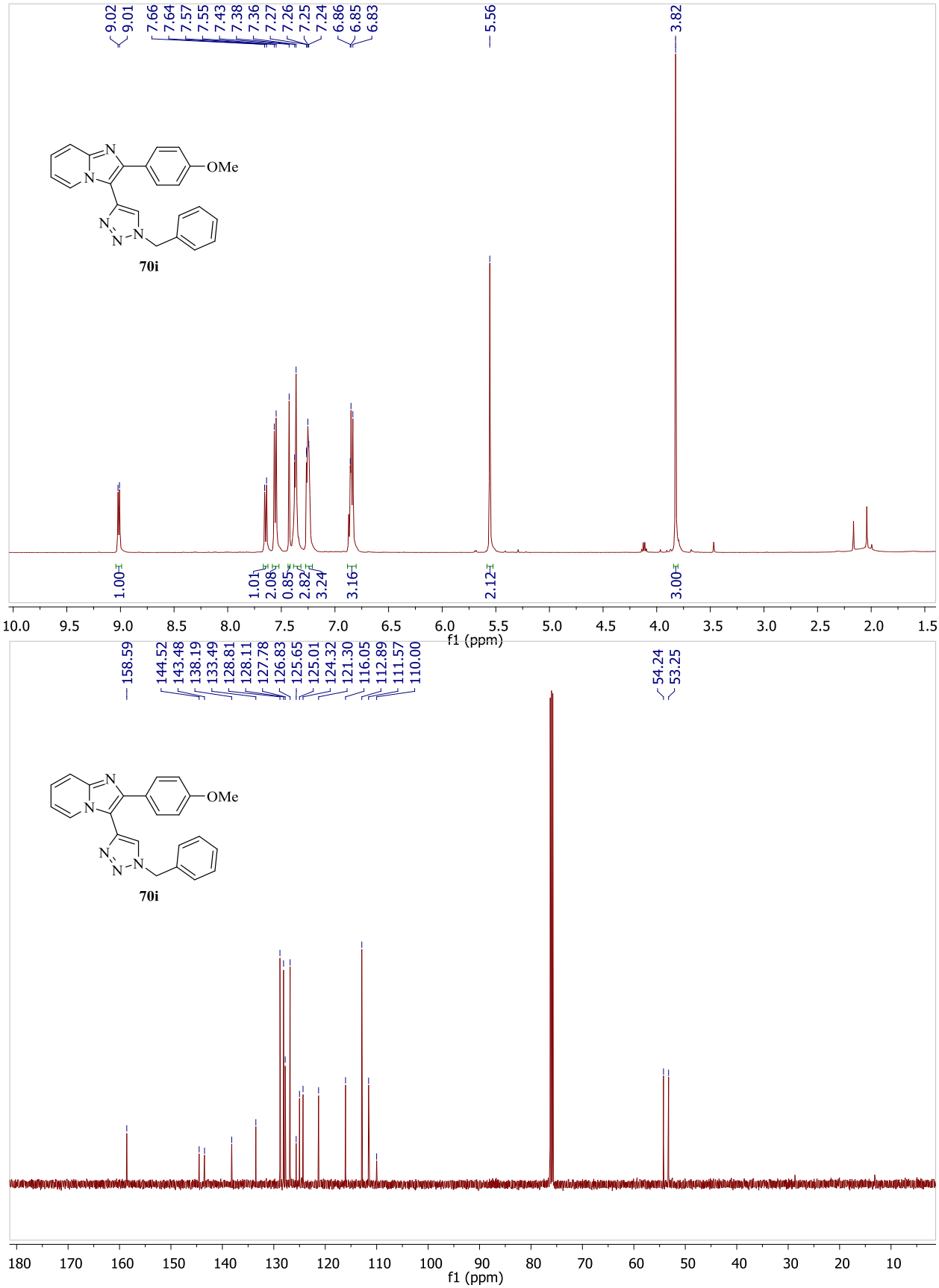


Figure 3.5: ^{1}H and ^{13}C NMR spectra of **70i.**

After the successful synthesis of **70i**, we prepared a series of triazolyl-imidazo[1,2-*a*]pyridines by varying substituted Imidazopyridines and alkynes. Different functional groups were very well tolerated under these conditions. All the synthesized compounds were very well characterised by ^{1}H NMR and ^{13}C NMR data.

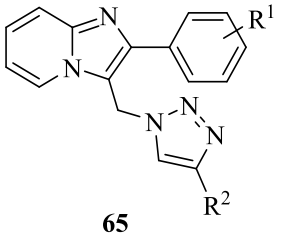
3.2.1 Biological activity

The synthesized compounds triazolyl-imidazo[1,2-*a*]pyridines (**65a-65o)** and their isomeric triazoles (**70a-70l**) were screened for antibacterial activities against Gram negative *K. pneumoniae* and *P. putida* and Gram positive *B. subtilis*, *S. aureus.* All compounds were evaluated at the concentrations ranging from 0.5 μg/mL to 128 μg/mL and scored for MIC as the level of growth inhibition of the microorganisms using ciprofloxacin as standards *via* agar well diffusion method. DMSO was used as solvent control. The data of antibacterial activities are depicted in table **3.1**. It was observed that when triazole motif was tethered with imidazopyridine nucleus *via* methylene linker, the antibacterial activity was less compared to alkyne linked imidazopyridines. For compounds **65a-65o**, most of the compounds showed inhibitory action with MIC values 7.6 μg/mL (**Table 3.1,** entry **65a**, **65b**, **65c**, **65f**, **65m**, **65n**, **65o**) while rest is having comparable more MIC value 15.6 μg/mL (**Table 3.1**, entry **65d**, **65e**, **65g**, **65h**, **65i**, **65k**, **65l**). Even after this concentration, no compound could show zone of inhibition in the range of reference ciprofloxacin having ≈23 mm. But, when alkyne part was attached with imidazopyridine, the isomeric triazolyl imidazopyridines were found to show good inhibitory action against all the bacteria with less MIC in comparison of ciprofloxacin (6.25 μg/mL). Compound **70a**, **70d** and **70l** showed MIC value 3.8 μg/mL with zone of inhibition 18-19 mm while other compounds were moderate with MIC value 7.6 and 15.2 μg/mL. The zone of inhibition was found to have 13-16 mm. Based on these results, we can say that imidazopyridine scaffold having simple aryl ring or with chloro substitution are better in antibacterial action rather than methyl, methoxy or bromo substitution. Also, species linked with electron donating group showed less zone of inhibiton in comparison of derivatives with electron withdrawing substitution such as bromo, nitro etc.(Table 3.1, entry 6,7). 4-chlorobenzyl group showed highest activity against both gram positive and gram negative bacteria (**Table 3.1, entry 70d, 70l**). Compound **70a** has both unsubstituted phenyl ring while **70d** has a 4-chlorophenyl and phenyl ring.

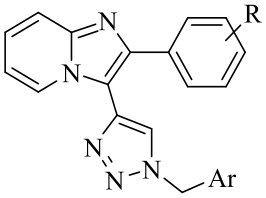
Further, antifungal activity of **65a-65o** and its isomeric triazoles **70a-70l** was evaluated against *C. albicans, A. flavus, F. oxysporum* and *P. citrinum.* All the compounds were evaluated at the concentrations ranging from 0.5 μg/mL to 128 μg/mL and scored for MIC as the level of

growth inhibition of the microorganisms compared with Amphotecerin B (MIC 30 μg/mL) as positive control. The data of antifungal activities are depicted in **table 3.2**. Again, methylene linked triazolylimidazo[1,2-*a*]pyridines showed less inhibitory action compared to directly tethered triazolyl-imidazo[1,2-*a*]pyridines. Compound **65i**, having bromo substituion on phenyl ring and methylsubstituted triazole did not show any inhibitory action against any fungi and **65l** containing benzyl susbtitution on triazole ring was inactive against *F. oxysporum* and *P. citrinum.* Also, **65m** having alkyl substitution on triazole ring was inactive against *C. albicans* and *F. oxysporum.* But to our delight, almost all alkyne linked triazolyl imidazopyridine showed moderate antifungal activity. It was found that **70d** was most potent among all compounds against all fungi *C. albicans, A. Flavus, F. oxysporum* and *P. citrinum.* All other scaffolds have comparable MIC values (15.2 μg/mL, 30.4 μg/mL and 60.8 μg/mL) with zone of inhibition 13-16 mm showing moderate activity against all fungi. It was concluded that directly tethered triazolyl-imidazo[1,2-*a*]pyridine scaffolds containing chloro substitution are better both in antibacterial as well as antifungal action.

Table 3.1: Antibacterial activities of **65** and **70**.



65



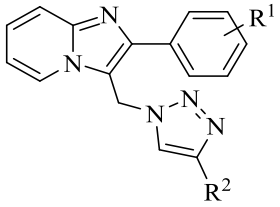
70

Compound no.	*K. pneumoniae*	*P. putida*	*B. subtilis*	*S. aureus*	
	MIC(ZOI)	**MIC(ZOI)**	**MIC(ZOI)**	**MIC(ZOI)**	**Biofilm inhibition (%)±SD**
65a	7.6(16)	7.6(15)	30.4(14)	30.4(14)	48±3
65b	7.6(17)	7.6(17)	15.2(15)	15.2(14)	34±2
65c	7.6 (17)	7.6 (16)	7.6 (16)	7.6 (16)	32±2
65d	15.2(14)	7.6(15)	15.2(14)	7.6(16)	28±3
65e	15.2 (14)	15.2(14)	15.2(14)	15.2(14)	43±2
65f	7.6(16)	15.2(15)	15.2(13)	7.6(15)	39±4

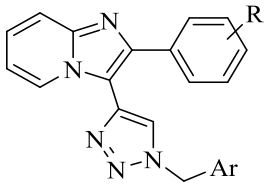
65g	15.2(14)	7.6(16)	15.2(14)	7.6(15)	51±2
65h	15.2(14)	15.2(13)	15.2(13)	15.2(14)	51±4
65i	15.2 (15)	15.2 (15)	15.2 (14)	15.2 (14)	41±3
65j	60.8(13)	60.8(13)	60.8(13)	60.8(12)	52±4
65k	15.2(15)	30.4(14)	30.4(14)	15.2(14)	33±3
65l	15.2 (14)	15.2 (14)	15.2 (14)	15.2 (14)	47±4
65m	7.6 (15)	15.2 (14)	7.6(14)	7.6 (13)	37±4
65n	7.6 (16)	7.6 (16)	7.6 (15)	7.6 (15)	44±1
65o	60.8(12)	60.8(12)	30.4(13)	30.4(13)	57±4
70a	3.8 (19)	3.8 (18)	3.8 (18)	7.6 (18)	55±3
70b	15.2 (14)	15.2 (14)	30.4 (13)	15.2 (14)	45±2
70c	15.2 (14)	15.2 (15)	15.2 (14)	15.2 (14)	61±1
70d	3.8(19)	3.8(19)	3.8(18)	3.8(18)	49±3
70e	7.6(16)	15.2(13)	7.6(14)	7.6(15)	30±3
70f	7.6 (16)	7.6 (16)	7.6 (15)	7.6 (16)	48±4
70g	15.2 (15)	15.2 (15)	15.2 (15)	15.2 (15)	65±2
70h	30.4 (13)	30.4 (14)	30.4 (14)	30.4 (14)	35±4
70i	7.6 (16)	7.6 (15)	7.6 (15)	7.6 (15)	63±3
70j	15.2(14)	15.2(14)	15.2(14)	15.2(14)	37±4
70k	7.6 (16)	7.6 (15)	7.6 (15)	7.6 (15)	50±2
70l	3.8(18)	3.8(19)	3.8(18)	3.8(18)	46±1
Ciprofloxacin	6.25 (23)	6.25 (22)	6.25 (23)	6.25(23)	100±3

MIC = Minimum inhibitory concentrations (µg/ml), ZOI = Zone of inhibition (mm)

Table 3.2: Antifungal activities of **65** and **70**.



65



70

Compound No.	*C. Albicans*	*A. flavus*	*F. oxysporum*	*P. citrinum*
	MIC(ZOI)	**MIC(ZOI)**	**MIC(ZOI)**	**MIC(ZOI)**
65a	60.8(13)	60.8(12)	60.8(13)	60.8(13)
65b	15.2(14)	60.8(13)	60.8(13)	30.4(15)
65c	7.6(16)	7.6(16)	7.6(15)	7.6(16)
65d	15.2(14)	15.2(14)	30.4(13)	15.2(14)
65e	15.2 (14)	30.4(14)	15.2(14)	15.2(14)
65f	60.8(15)	30.4 (15)	30.4(13)	15.2(15)
65g	15.2(14)	15.2 (15)	60.8(14)	60.8 (13)
65h	15.2(14)	15.2(13)	15.2(13)	15.2(14)
65i	-	-	-	-
65j	30.4(14)	30.4(13)	30.4(15)	60.8(12)
65k	>15.2(15)	>30.4(12)	30.4(12)	60.8 (13)
65l	30.4(12)	30.4(12)	-	-
65m	-	60.8 (13)	-	15.2(14)
65n	15.2(13)	15.2(13)	15.2(14)	15.2(14)
65o	30.4(14)	30.4(14)	15.2(15)	30.4(13)
70a	15.2 (16)	7.6 (15)	7.6 (15)	30.4 (16)
70b	15.2 (14)	15.2 (14)	30.4 (13)	15.2 (14)
70c	15.2 (14)	15.2 (15)	30.4 (13)	30.4 (13)

70d	7.6(17)	15.2(18)	7.6(17)	7.6(18)
70e	15.3(15)	30.4(14)	15.2(14)	15.2(15)
70f	15.2 (13)	7.6 (14)	15.2 (13)	15.2 (14)
70g	30.4 (15)	15.2 (14)	15.2 (15)	15.2 (14)
70h	15.2 (15)	15.2 (14)	15.2 (15)	15.2 (15)
70i	15.2 (15)	7.6 (15)	7.6 (16)	15.2 (15)
70j	30.4(13)	30.4(12)	7.6 (14)	7.6 (14)
70k	60.8 (14)	60.8 (13)	15.2 (14)	15.2 (15)
70l	15.2 (14)	15.2 (16)	60.8(13)	30.4 (14)
Amphotericin B	30 (23)	30(22)	30(23)	30(22)

MIC = Minimum inhibitory concentrations (µg/ml), ZOI = Zone of inhibition (mm)

3.2.2 Biofilm inhibition

Biofilm are complex communities of bacteria that are self-produced matrix of polysachharides, proteins and extracellular DNA responsible for bacterial resistance towards antibiotics. It is known that mostly bacteria acquire resistance to antibiotic drugs *via* formation of extracellular biofilm hence an antibiofilm approach will be a promising method to handle the aforementioned problem. Based on this, all above synthesized triazolyl-imidazo[1,2-*a*]pyridine derivatives were evaluated for biofilm inhibition activity to explore the possible role of synthesized derivatives in inhibition of biofilm formation in *S. aureus*. The biofilm inhibition study was performed using crystal violet assay retention method using ciprofloxacin as standard. This method is based on the hypothesis that higher the biofilm formation, greater the extent of absorption of crystal violet, lesser is the effectiveness of compounds. The percentage of inhibiton for both type of triazole-imidazo[1,2-*a*]pyridines is shown in figure **3.6**. The results showed that these compounds have moderate to good anti-biofilm activity.

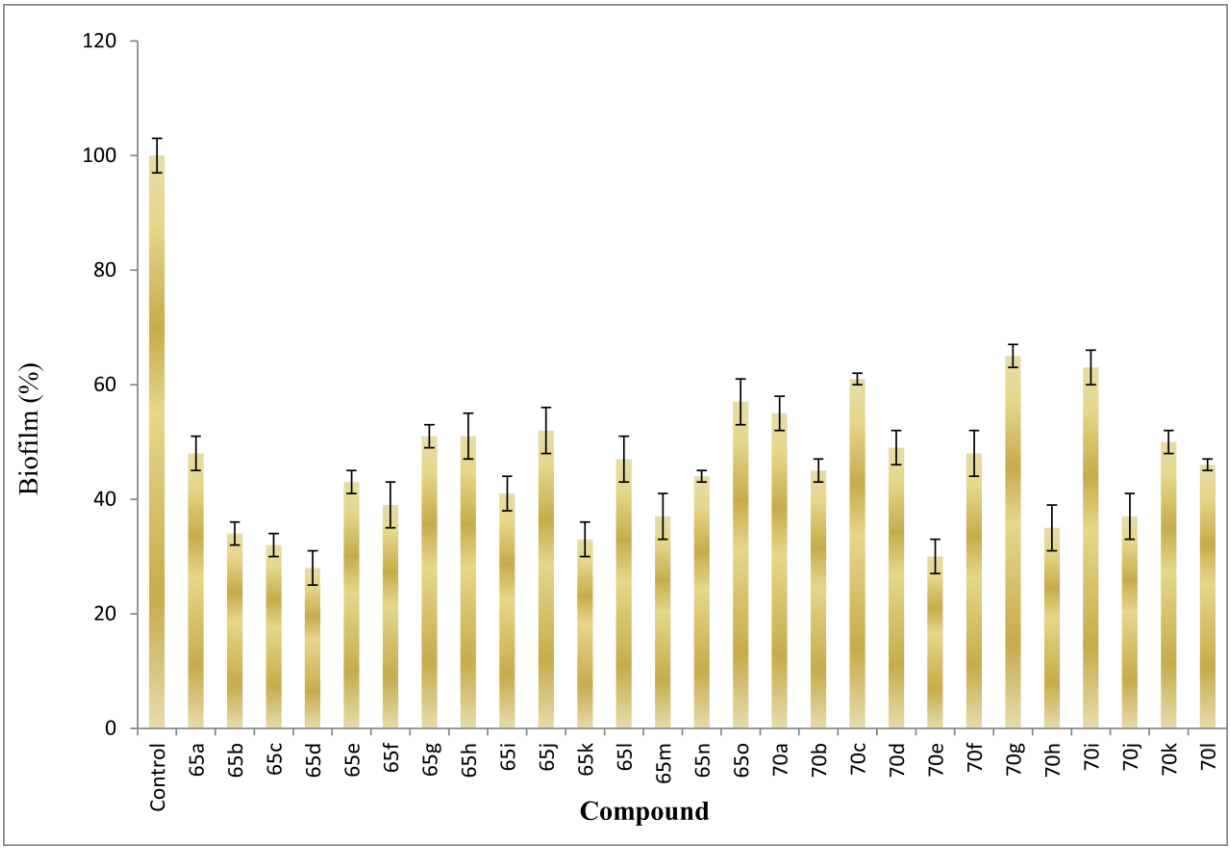


Figure 3.6: Biofilm inhibition of *S. aureus* by different triazolyl-imidazo[1,2-*a*]pyridine derivatives (**65 and 70**).

3.2.3 Evaluation of ROS production

The protective effect of representative compounds i.e. **70a, 70d, 70l** and **70f** against 2', 7'-dichlorofluorescin diacetate (DCFH-DA) oxidation was determined in bacterial cells by fluorescence microscopy. The fluorescent dye 2', 7'-dichlorofluorescein diacetate (DCFH-DA), is a specific oxidation-sensitive fluorescent probe commonly used to evaluate the total reactive oxygen species (ROS) formation. After being taken by cells, it is hydrolysed to dichlorodihydrofluorescin (DCFH) which is trapped within the cell. In our assay, bacterial cell was treated with the selected compounds **70a**, **70d**, **70l** and **70f** for 2 h and observed for the fluorescence intensity under the EPI-fluorescence microscope. The probe DCFH is non-fluorescent dye, but is oxidized to the highly fluorescent DCF by intracellular H_2O_2 or nitric oxide (the action of cellular ROS). The bacterial culture was grown up to mid-log phase and then treated with **70a**, **70d**, **70f** and **70l** followed by incubation with 2 µM of DCFH-DA for 30 min at room temperature. After that, the fluorescence of DCF was measured at 530 nm after excitation at 485 nm (**Figure 3.7**). It was observed that **70a**, **70d** and **70l** markedly increases the ROS production as shown in figure 3.7. It can be concluded that *p*-chloro substitution on

benzyl ring significantly increases the ROS production while presence of nitro group revealed antioxidant activity.

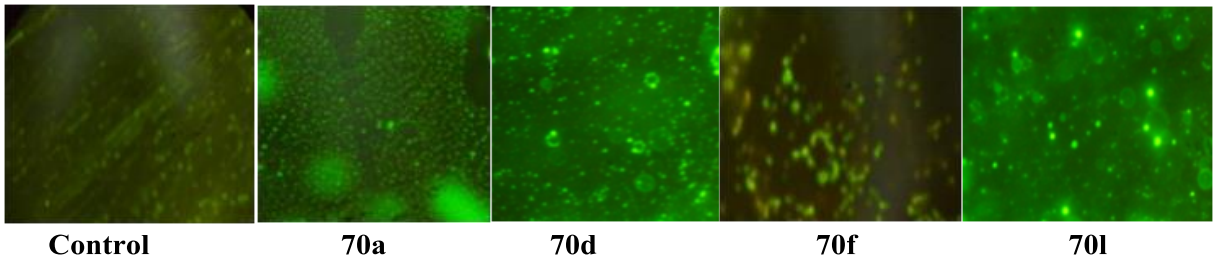


Figure 3.7: Incubation of ROS in bacterial cells with DCFH-DA.

3.2.4 Bactericidal activity:

The differentiation between live and dead cells caused by efficient antimicrobial compounds **70a**, **70d**, **70f** and **70l** was evaluated by acridine orange and ethidium bromide (AO/EtBr) dual staining assay. The dye AO can enter inside the living cells and binds with the living cells DNA to emit green fluorescence, whereas EtBr enters only through modified cell membrane of dead cells and emit red fluorescence. It is evident from Figure 3.8 that untreated bacterial cells displayed green fluorescence while the cells treated with compounds **70a**, **70d**, **70l**, **70f** exhibited red fluorescence along with minor green fluorescence (**Figure 3.8**). The red fluorescence was completely absent from the control sample. Appearance of red fluorescence from the compound treated bacterial cells illustrates the bactericidal potential of compounds. These observed results therefore reveal that selected compounds caused the cell damage by making loss of membrane integrity.

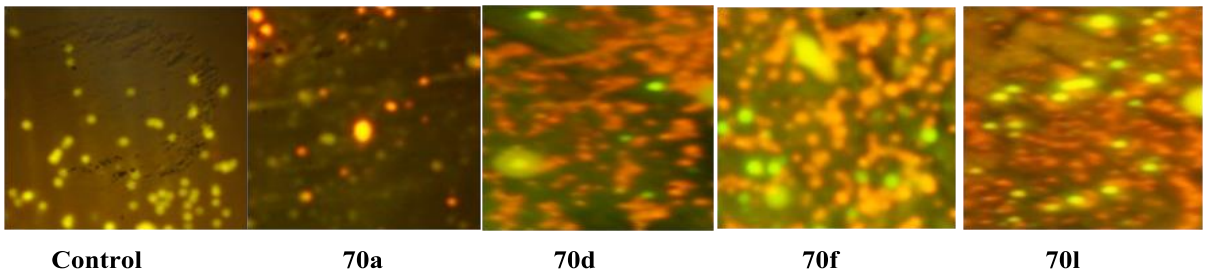


Figure 3.8: Bactericidal potency of triazolo-imidazopyridines repectively for control, **70a**, **70d**, **70f** and **70l**.

3.3 Conclusion

In summary, we have successfully developed a novel and highly efficient method towards the synthesis of 3-(triazol-1-yl)methyl-*H*-imidazo[1,2-*a*]pyridines and 3-(triazol-4-yl)-imidazo[1,2-*a*]pyridines. Two series of triazolo-imidazo[1,2-*a*]pyridines with different functionalities such as fluoro, chloro, bromo, methyl, methoxy were prepared and evaluated for their antimicrobial

activity against Gram positive and Gram negative bacteria and for antifungal activity. It was observed that 3-(triazol-4-yl)-imidazo[1,2-*a*]pyridines are better in antimicrobial action than 3-(triazol-1-yl)methyl-*H*-imidazo[1,2-*a*]pyridines. Compounds bearing simple aryl ring or chloro substituted aryl ring are good antibacterial as well as antifungal agent. Also, biofilm inhibition was performed against *S. aureus* which shows that derivative **65g** bearing bromo substitution on imidazo[1,2-*a*]pyridine part exhibits biofilm inhibition upto 65 percent.

3.4 Experimental Section

General information: All chemicals were obtained from commercial suppliers and used without further purification. The melting points were determined using EZ-Melt automated melting point apparatus and were not corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE DMX-500 and Bruker AVANCE DMX-400.

Synthetic procedure for intermediates and imidazo[1,2-*a*]pyridine-triazoles is given below:

2-Bromo-1-phenylethanone (61): A solution of acetophenone (1.20 g, 10 mmol), *N*-bromosuccinimide (1.77 g, 10 mmol) and *p*-toluenesulphonic acid (2.58 g, 15 mmol) in acetonitrile (35 mL) was stirred for 4 h at reflux temperature. After completion, reaction mass was allowed to cool to ambient temperature and evaporated. The residue was diluted with water and extracted into ethyl acetate. Organic layer was dried over anhydrous sodium sulphate and the volatiles were evaporated. The crude compound was recrystallized from hexanes.

2-Phenylimidazo[1,2-*a*]pyridine (62): To a mixture of 2-bromo-1-phenylethanone (**61**) (1.9 g, 10 mmol) and 2-aminopyridine (0.9 g, 10 mmol) in ethanol (35 mL) added sodium bicarbonate (1.26 g, 15 mmol) and stirred at reflux temperature for 5 h. After completion, reaction mass was allowed to cool to ambient temperature and evaporated. Water was added to the residue and extracted into ethyl acetate twice. Organic layer was dried over anhydrous sodium sulphate and the volatiles were removed by evaporation on rotary evaporator. Crude compound was forwarded to next step without further purification.

2-Phenylimidazo[1,2-*a*]pyridine-3-carbaldehyde: In a 50 mL round bottom flask added $POCl_3$ (1.3 mL, 13 mmol) in DMF (15 mL) at 0 °C. After occurance of yellow-orange colour in solution, added 2-phenylimidazo[1,2-*a*]pyridine (1.94 g, 10mmol). Stirred reaction mass at 80 °C for 1.5 h and allowed it to cool to ambient temperature after completion of reaction. Reaction mass was neutralized with saturated $NaHCO_3$ (20mL) solution. Extracted into ethyl acetate (100 mL), dried organic layer over sodium sulphate and evaporated the volatiles.

(2-Phenylimidazo[1,2-*a*]pyridin-3-yl)methanol (63): To a stirred solution of 2-phenylimidazo[1,2-*a*]pyridine-3-carbaldehyde (2.2 g, 10 mmol) in methanol (25 mL) was added $NaBH_4$ (0.494 g, 12 mmol) and stirred at rt for 1 h, evaporated the volatiles. Residue was diluted with water and neutralized with 2M HCl. Compound was extracted with ethylacetate (2 × 20 mL), dried over sodium sulphate and volatiles were evaporated. Compound was used for next step without further purification.

3-(Azidomethyl)-2-phenyl-imidazo[1,2-*a*]pyridine (64): To a stirred solution of (2-phenylimidazo[1,2-*a*]pyridin-3-yl)methanol (2.2 g, 10 mmol) in DCM (25 mL) added triethylamine (1.8 mL, 13 mmol) followed by methane sulfonyl chloride (1 mL, 13 mmol) at 0 °C and stirred the reaction mass at rt for 1 h. Volatiles were evaporated and crude was dissolved in DMF (15 mL), added sodium azide (0.84 g, 13 mmol) and stirred at rt for 14 h. After completion of reaction, water (50 mL) was added and extracted in ethyl acetate (2 × 25 mL). Organic layer was dried over sodium sulphate and concentrated on a rotary evaporator. Crude compound was purified by column chromatography (15 % Ethyl acetate / Hexane).

2-Phenyl-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65): Mixture of 3-(azidomethyl)-2-phenyl-1*H*-imidazo[1,2-*a*]pyridine (100 mg, 0.40 mmol), phenylacetylene (62 mg, 0.6 mmol) and CuI (4 mg, 0.02 mmol) in water: polyethylene glycol (1:1 v/v) (2 mL) stirred at rt for 5 h. After completion of the reaction as indicated by TLC, water was added and extracted into ethyl acetate (2 × 10 mL). Organic layer was dried over sodium sulphate and evaporated the vlatiles. Crude was purified by column chromatography using 30 % ethyl acetate / hexane as eluent.

3-Iodo-2-phenylimidazo[1,2-*a*]pyridine (66): 2-Phenylimidazo[1,2-*a*]pyridine (1.94 g, 10mmol) was dissolved in acetonitrile (30 mL) at room temperature, *N*-iodosuccinimide (3 g,13mmol) was added and reaction mixture was stirred at room temperature for 1 h. The completion of reaction was confirmed by TLC. After completion of reaction, reaction mass was concentrated and neutralized with 10 % sodium thiosulphate solution. Product was extracted in ethyl acetate and organic layer was dried over sodium sulphate, evaporated the volatiles. The product was used for next step without further purification.

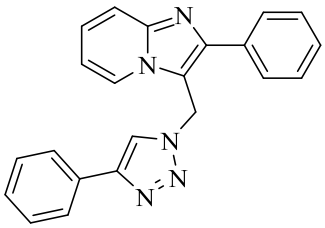
2-Phenyl-3-((trimethylsilyl)ethynyl)imidazo[1,2-*a*]pyridine (68): In a dry 100 mL round bottom flask dissolved 3-iodo-2-phenylimidazo[1,2-*a*]pyridine (3.2 g, 10 mmol) in DMF (40 mL), added TEA (15 mL) to it and degassed with nitrogen for 3 minutes. To the reaction mixture, CuI (19 mg, 0.1mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.1mmol) and TMS-acetylene (1.18 g, 12 mmol) were added, degassed and stirred at 80 °C for 30 minutes. After completion of reaction, reaction mixture was cooled to room temperature, added water and extracted with ethylacetate (2 × 60 mL). Dried organic layer by adding sodium sulphate and pure compound was isolated by column chromatography.

3-Ethynyl-2-phenylimidazo[1,2-*a*]pyridine (69): 68 (2.9 g, 10 mmol) was dissolved in THF (40 mL) under nitrogen atmosphere and kept on stirring at 0 °C, TBAF (1 Molar solution in

THF, 14 mL, 15 mmol) was added slowly *via* syringe under nitrogen atmosphere and reaction mixture was stirred at room temperature for 15 minutes. After confirmation of reaction completion by TLC, reaction mass was concentrated and purified by column chromatography on silica gel using ethyl acetate and hexane (25 % *v/v*) as eluent.

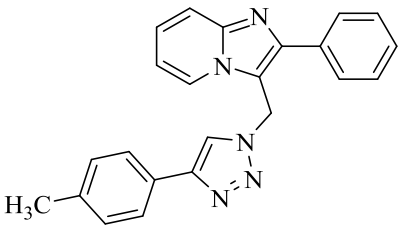
3-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine (70): In a 10 mL round bottom flask dissolved **69** (100 mg, 0.4 mmol) in THF, added benzyl azide (80 mg, 0.6 mmol), CuI (8 mg, 0.04 mmol) and stirred at room temperature for 10 h. After completion, added water and extracted into ethyl acetate. Organic layer was dried over sodium sulphate and evaporated the volatiles. Crude was purified by column chromatography using ethyl acetate and hexane (1:3) as eluent.

2-Phenyl-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine(65a):



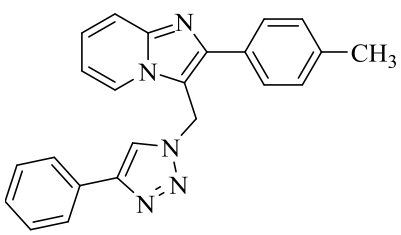
Yield: 61 mg (43%); yellow solid; mp 148-150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.79 (d, J = 6.5 Hz, 2H), 7.75 (d, J = 7.9 Hz, 2H), 7.71 (d, J = 8.3 Hz, 1H), 7.61 (s, 1H), 7.52 (t, J = 7.0 Hz, 2H), 7.45 (t, J = 7.3 Hz, 1H), 7.37 (t, J = 7.6 Hz, 2H), 7.30 (t, J = 7.3 Hz, 2H), 6.87 (t, J = 6.7 Hz, 1H), 6.04 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.58, 145.81, 133.17, 130.03, 129.08, 128.84, 128.78, 128.54, 128.42, 126.16, 125.70, 123.85, 118.82, 117.83, 117.79, 113.62, 44.11; MS (ESI): *m/z* 352.1569 $(M+H)^+$.

2-Phenyl-3-((4-*p*-tolyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65b):



Yield: 67 mg (46%); yellow solid; mp 164-165 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, J = 6.7 Hz, 1H), 7.78 (d, J = 7.9 Hz, 2H), 7.68 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 7.9 Hz, 2H), 7.57 (s, 1H), 7.51 (t, J = 7.6 Hz, 2H), 7.45 (t, J = 7.3 Hz, 1H), 7.29 (dd, J = 8.4, 7.5 Hz, 1H), 7.17 (d, J = 7.9 Hz, 2H), 6.86 (t, J = 6.8 Hz, 1H), 6.02 (s, 2H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.64, 146.31, 145.75, 138.30, 133.11, 129.51, 129.08, 128.78, 128.56, 127.21, 126.20, 125.59, 123.88, 118.48, 117.70, 113.60, 112.60, 44.04, 21.29. MS (ESI): *m/z* 366.1669 $(M+H)^+$.

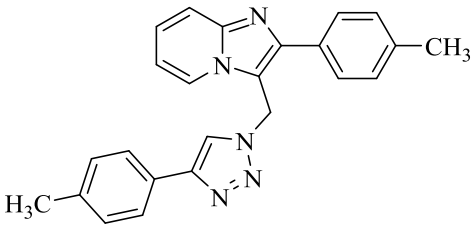
3-((4-Phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-2-*p*-tolyl-imidazo[1,2-*a*]pyridine (65c):



Yield: 45 mg (32%); off-white solid; mp 190-191 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 6.8 Hz, 1H), 7.74 (d, J

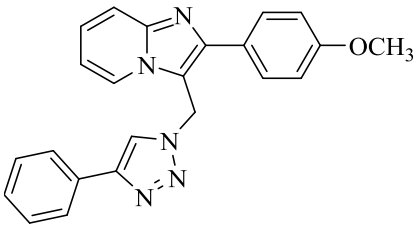
= 7.5 Hz, 2H), 7.71 – 7.67 (m, 3H), 7.57 (s, 1H), 7.38 (d, *J* = 7.4 Hz, 1H), 7.35 (d, *J* = 4.5 Hz, 2H), 7.33 (s, 1H), 7.30 (dd, *J* = 7.0, 4.2 Hz, 2H), 6.86 (t, *J* = 6.8 Hz, 1H), 6.06 (s, 2H), 2.44 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.58, 146.64, 145.86, 138.73, 130.36, 130.09, 129.79, 128.82, 128.37, 125.93, 125.69, 123.70, 118.70, 117.77, 113.42, 112.17, 111.31, 44.16, 21.34; MS (ESI): *m/z* 366.1615 $(M+H)^+$.

2-*p*-Tolyl-3-((4-*p*-tolyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65d):



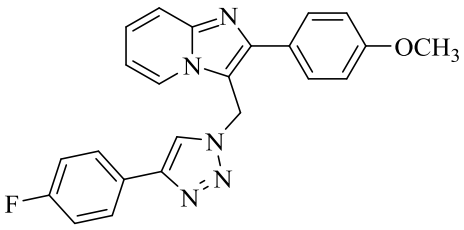
Yield: 72 mg (50%); off-white solid; mp 192-193 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, *J* = 6.7 Hz, 1H), 7.71 – 7.67 (m, 3H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.53 (s, 1H), 7.34 (d, *J* = 7.9 Hz, 2H), 7.30 – 7.26 (m, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 6.85 (t, *J* = 6.8 Hz, 1H), 6.04 (s, 2H), 2.44 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.64, 146.60, 145.84, 138.70, 138.26, 130.39, 129.78, 129.49, 128.37, 127.27, 125.90, 125.58, 123.73, 118.35, 117.75, 113.38, 112.22, 44.12, 21.34, 21.26; MS (ESI): *m/z* 380.1799 $(M+H)^+$.

2-(4-Methoxyphenyl)-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65e):



Yield: 116 mg (85%); yellow solid; mp 128-130°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, *J* = 6.8 Hz, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.76 – 7.73 (m, 2H), 7.71 (d, *J* = 8.7 Hz, 2H), 7.59 (s, 1H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.33 – 7.28 (m, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 6.88 (t, *J* = 6.9 Hz, 1H), 6.02 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.20, 148.59, 146.01, 145.64, 129.96, 129.86, 129.47, 128.83, 128.44, 126.39, 125.72, 123.67, 118.77, 117.46, 115.52, 114.59, 113.71, 55.38, 44.08; MS (ESI): *m/z* 382.1482 $(M+H)^+$.

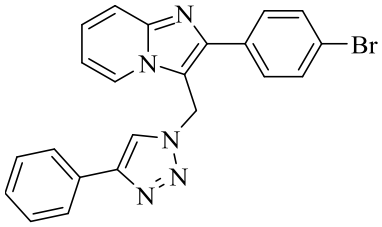
3-((4-(4-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-(4-methoxyphenyl)-imidazo[1,2-a]pyridine (65f):



Yield: 53 mg (37%); yellow liquid; ^{1}H NMR (500 MHz, CDCl3) δ 8.13 (d, J = 6.7 Hz, 1H), 7.73 (dd, *J* = 7.9, 5.4 Hz, 5H), 7.55 (s, 1H), 7.34 – 7.29 (m, 1H), 7.07 (dd, *J* = 9.4, 8.2 Hz, 4H), 6.89 (t, *J* = 6.7 Hz, 1H), 6.04 (s, 2H), 3.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.19,

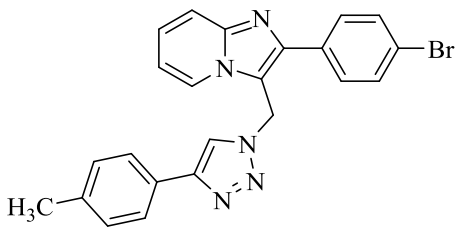
147.75, 145.63, 130.90, 129.77, 128.82, 127.52, 127.45, 126.26, 125.24, 123.66, 118.49, 117.54, 115.92, 115.75, 114.58, 113.62, 55.40, 44.14; MS (ESI): m/z 400.1522 $(M+H)^+$.

2-(4-Bromophenyl)-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65g):



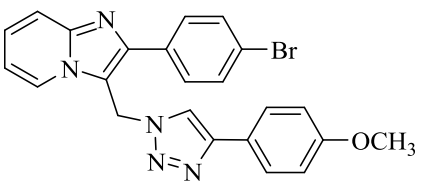
Yield: 65 mg (50%); off-white solid; mp 187-188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 6.7 Hz, 1H), 7.77 (d, J = 7.3 Hz, 2H), 7.75 – 7.67 (m, 4H), 7.60 (s, 1H), 7.44 – 7.38 (m, 2H), 7.34 (dd, J = 14.8, 7.2 Hz, 2H), 7.28 (d, J = 1.2 Hz, 1H), 6.93 (t, J = 6.7 Hz, 1H), 6.06 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.72, 145.91, 145.29, 132.31, 132.15, 130.04, 129.95, 128.87, 128.51, 126.42, 125.72, 123.82, 123.20, 118.67, 117.86, 113.80, 112.60, 43.97; MS (ESI): *m/z* = 430.0737 $[M + H]^+$ 432.0653 $[M + H + 2]^+$.

2-(4-Bromophenyl)-3-((4-*p*-tolyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65h):



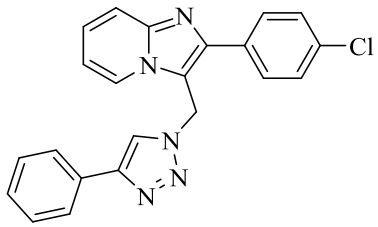
Yield: 36 mg (26%); yellow solid; mp 196-198 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.56 (d, J = 6.5 Hz, 1H), 7.85 (d, J = 7.7 Hz, 2H), 7.71 (t, J = 7.9 Hz, 5H), 7.42 – 7.36 (m, 1H), 7.22 (d, J = 7.8 Hz, 2H), 7.04 (t, J = 6.7 Hz, 1H), 6.16 (s, 2H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.61, 147.09, 137.76, 133.35, 132.16, 130.56, 129.93, 129.84, 128.14, 127.37, 126.57, 125.64, 125.40, 122.03, 121.43, 117.44, 113.50, 43.52, 21.28; MS (ESI): *m/z* = 444.0725 $[M + H]^+$ 446.0745 $[M + H + 2]^+$.

2-(4-Bromophenyl)-3-((4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65i):



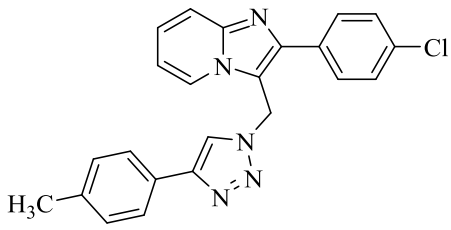
Yield: 61 mg (44%); yellow-brown solid; mp 169-171 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.69 (dd, J = 3.3, 1.1 Hz, 1H), 7.68 – 7.66 (m, 5H), 7.50 (s, 1H), 7.35 – 7.31 (m, 1H), 6.92 (d, J = 1.7 Hz, 1H), 6.90 (t, J = 3.2 Hz, 2H), 6.02 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.80, 148.58, 132.29, 132.07, 131.15, 130.03, 128.64, 127.04, 126.45, 123.85, 123.18, 122.62, 117.84, 117.80, 114.24, 113.81, 105.43, 55.32, 43.90; MS (ESI): *m/z* 460.0626 $(M+H)^+$ 462.0688 $[M + H + 2]^+$.

2-(4-Chlorophenyl)-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65j):



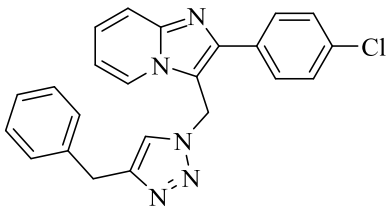
Yield: 81 mg (59%); white yellow solid; mp 172-174 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, J = 5.7 Hz, 1H), 7.76 – 7.71 (m, 4H), 7.70 (d, J = 8.8 Hz, 1H), 7.59 (s, 1H), 7.50 (d, J = 7.9 Hz, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.31 (d, J = 9.2 Hz, 2H), 6.89 (t, J = 6.8 Hz, 1H), 6.02 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.66, 134.88, 131.78, 129.97, 129.73, 129.31, 128.85, 128.47, 127.39, 126.27, 125.70, 123.79, 118.69, 117.87, 115.74, 113.70, 43.98, 29.69; MS (ESI): *m/z* 385.0843 $(M+H)^+$.

2-(4-Chlorophenyl)-3-((4-*p*-tolyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65k):



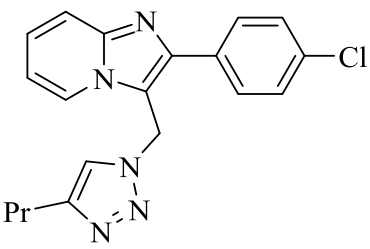
Yield: 82 mg (58%); white solid; mp 218-219 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, J = 6.5 Hz, 1H), 7.74 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 9.0 Hz, 1H), 7.64 (d, J = 8.1 Hz, 2H), 7.53 (s, 1H), 7.51 (d, J = 8.3 Hz, 2H), 7.34 – 7.28 (m, 1H), 7.20 – 7.16 (m, 2H), 6.89 (t, J = 6.8 Hz, 1H), 6.02 (s, 2H), 2.35 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.76, 145.31, 138.39, 134.88, 131.81, 129.74, 129.52, 129.31, 128.42, 127.14, 126.25, 125.60, 123.80, 123.73, 118.28, 117.86, 113.68, 43.95, 21.27; MS (ESI): *m/z* 400.1335 $(M+H)^+$.

3-((4-Benzyl-1*H*-1,2,3-triazol-1-yl)methyl)-2-(4-chlorophenyl)-imidazo[1,2-*a*]pyridine (65l):



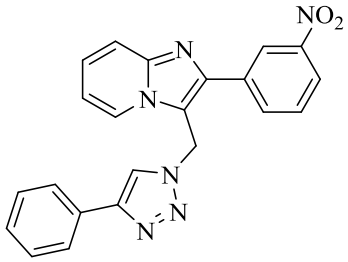
Yield: 80 mg (57%); brown liquid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 6.7 Hz, 1H), 7.70 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 7.31 (dd, J = 8.4, 7.4 Hz, 1H), 7.26 – 7.25 (m, 1H), 7.24 (s, 1H), 7.19 (t, J = 5.5 Hz, 3H), 7.05 (s, 1H), 6.89 (t, J = 6.8 Hz, 1H), 5.91 (s, 2H), 4.02 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.54, 145.06, 138.60, 134.83, 131.65, 130.58, 129.77, 129.38, 129.23, 128.63, 128.59, 126.59, 126.27, 123.86, 120.64, 117.76, 113.63, 43.75, 32.17; MS (ESI): *m/z* 400.0976 $(M+H)^+$.

2-(4-Chlorophenyl)-3-((4-propyl-1*H*-1,2,3-triazol-1-yl)methyl)-imidazo[1,2-*a*]pyridine (65m):



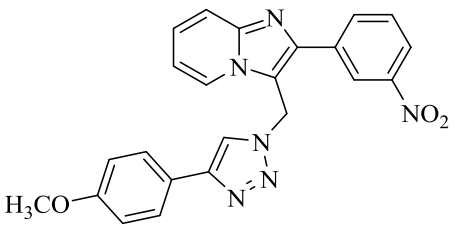
Yield: 27 mg (22%); yellow solid; mp 124-125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 6.8 Hz, 1H), 7.70 (t, J = 8.9 Hz, 3H), 7.49 (d, J = 8.4 Hz, 2H), 7.33 – 7.29 (m, 1H), 7.10 (s, 1H), 6.89 (t, J = 6.7 Hz, 1H), 5.95 (s, 2H), 2.63 (t, J = 7.6 Hz, 2H), 1.64 – 1.62 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.30, 143.00, 134.80, 131.84, 129.74, 129.25, 126.16, 125.89, 125.41, 123.85, 119.79, 117.80, 113.56, 43.68, 27.65, 22.55, 13.73; MS (ESI): *m/z* 352.0935 $(M+H)^+$.

2-(3-nitrophenyl)-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)imidazo[1,2-*a*]pyridine (65n):



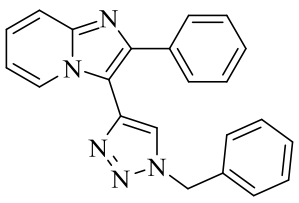
Yield: 96 mg (71%); off-white solid; mp 230-232°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (s, 1H), 8.69 (s, 2H), 8.37 (s, 1H), 8.27 (d, J = 7.3 Hz, 1H), 7.83 (d, J = 6.9 Hz, 4H), 7.47 – 7.39 (m, 3H), 7.31 (t, J = 6.9 Hz, 1H), 7.09 (d, J = 5.4 Hz, 1H), 6.26 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 148.63, 147.00, 134.58, 130.89, 129.33, 128.47, 126.94, 125.69, 124.49, 123.18, 123.05, 122.01, 121.97, 117.74, 113.83, 43.46; MS (ESI): *m/z* 397.1246 $(M+H)^+$.

3-((4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-(3-nitrophenyl)–imidazo[1,2-*a*] pyridine (65o):



Yield: 25 mg (17%); pale yellow solid; mp 207-208 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.38 (d, J = 6.3 Hz, 1H), 8.33 (d, J = 8.2 Hz, 1H), 8.20 (dd, J = 7.7, 1.0 Hz, 1H), 7.73 (d, J = 9.3 Hz, 5H), 7.39 – 7.35 (m, 1H), 6.97 (t, J = 6.8 Hz, 1H), 6.94 (d, J = 8.5 Hz, 2H), 6.03 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.84, 148.62, 146.04, 135.26, 134.64, 134.46, 131.09, 130.23, 127.07, 126.72, 124.29, 123.35, 123.29, 122.58, 118.62, 118.08, 117.99, 114.29, 113.99, 55.32, 43.78; MS (ESI): *m/z* 427.1374 $(M+H)^+$.

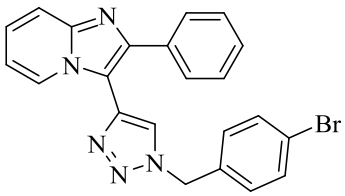
3-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine (70a):



Yield: 132 mg (82%); white solid; mp 140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.45 (d, J = 7.0 Hz, 1H), 7.72 – 7.66 (m, 3H, 7.45 – 7.39 (m, 3H), 7.38 – 7.33 (m, 6H), 6.99 (dd, J = 9.8, 3.8 Hz, 1H),

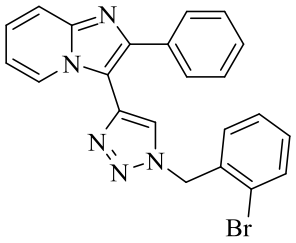
5.74 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.76, 145.16, 144.00, 136.82, 136.44, 134.43, 129.27, 128.84, 128.63, 128.42, 128.23, 128.18, 127.53, 126.26, 126.08, 125.57, 117.35, 113.46, 111.66, 53.53; MS (ESI): *m/z* 352.1484 $(M+H)^+$.)

3-(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine (70b):



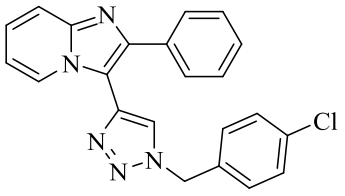
Yield: 175 mg (89%); white solid; mp 161-162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.45 (s, 1H), 7.71 – 7.66 (m, 3H), 7.63 (d, J = 8.4 Hz, 2H), 7.41 – 7.29 (m, 6H), 7.00 (td, J = 6.8, 0.9 Hz, 1H), 5.72 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.13, 143.97, 136.85, 135.83, 134.36, 132.20, 130.51, 128.88, 128.45, 128.24, 126.33, 126.10, 125.61, 121.93, 117.32, 113.49, 111.61, 52.81; MS (ESI): *m/z* = 430.0591 $[M + H]^+$ 432.0579 $[M + H + 2]^+$.

3-(1-(2-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine(70c):



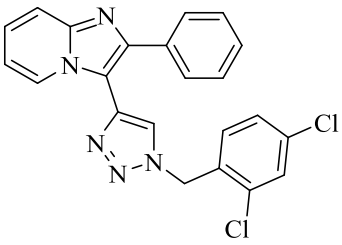
Yield: 156 mg (79%); white solid; mp 182-183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, *J* = 6.9 Hz, 1H), 8.41 (s, 1H), 7.75 – 7.63 (m, 4H), 7.46 (td, *J* = 7.5, 1.0 Hz, 1H), 7.39 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.37 – 7.31 (m, 4H), 7.25 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.00 (td, *J* = 6.8, 0.9 Hz, 1H), 5.80 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.16, 144.02, 136.61, 135.18, 134.39, 133.44, 130.97, 128.96, 128.86, 128.79, 128.42, 128.23, 127.58, 126.57, 126.28, 125.55, 125.28, 123.32, 117.37, 113.48, 111.55, 53.79; MS (ESI): *m/z* = 430.0577 $[M + H]^+$ 432.0579 $[M + H + 2]^+$.

3-(1-(4-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine (70d):



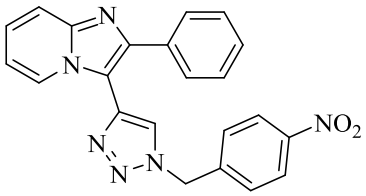
Yield: 155 mg (88%); white solid; mp 176-177 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.47 – 8.44 (m, 1H), 7.72 – 7.68 (m, 2H), 7.67 (d, *J* = 1.4 Hz, 1H), 7.51 – 7.47 (m, 2H), 7.40 – 7.34 (m, 6H), 6.99 (td, *J* = 6.8, 1.1 Hz, 1H), 5.74 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.15, 144.03, 136.88, 135.41, 134.42, 133.37, 130.20, 129.26, 128.86, 128.43, 128.24, 126.26, 126.07, 125.59, 117.35, 113.45, 111.59, 52.75; MS (ESI): *m/z* 387.1084 $(M+H)^+$.

3-(1-(2,4-Dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine (70e):



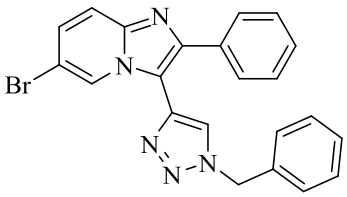
Yield: 167 mg (87%); white solid; mp 170 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, J = 6.9 Hz, 1H), 8.43 (s, 1H), 7.74 (d, J = 2.1 Hz, 1H), 7.72 – 7.67 (m, 3H), 7.54 (d, J = 2.1 Hz, 1H, 7.51 (d, J = 2.1 Hz, 1H), 7.39 (dd, J = 7.5, 2.3 Hz, 2H), 7.36 – 7.33 (m, 2H), 7.00 (t, J = 6.8 Hz, 1H), 5.82 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 136.66, 134.53, 134.41, 134.26, 132.73, 132.44, 129.69, 128.86, 128.43, 128.39, 128.23, 126.52, 126.28, 125.56, 117.38, 113.47, 50.98; MS (ESI): *m/z* 421.0688 $(M+H)^{+}$.

3-(1-(4-Nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)-2-phenylimidazo[1,2-*a*]pyridine (70f):



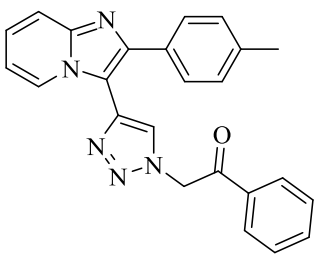
Yield: 154 mg (85%); white solid; mp 136 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 1H), 8.47 (d, J = 6.9 Hz, 1H), 8.33 – 8.25 (m, 2H), 7.72 – 7.67 (m, 3H), 7.58 (d, J = 8.8 Hz, 2H), 7.42 – 7.33 (m, 4H), 7.00 (td, J = 6.8, 1.1 Hz, 1H), 5.92 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.73, 145.19, 144.09, 143.82, 136.97, 134.35, 129.36, 128.92, 128.47, 128.25, 126.43, 126.37, 125.63, 124.42, 117.34, 113.51, 111.48, 52.66; MS (ESI): *m/z* 397.1335 $(M+H)^{+}$.

3-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-6-bromo-2-phenylimidazo[1,2-*a*]pyridine (70g):



Yield: 105 mg (73%); brown solid; mp 167-168 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.26 (s, 1H), 7.61 (d, J = 6.3 Hz, 2H), 7.56 (d, J = 9.4 Hz, 1H), 7.42 – 7.35 (m, 4H), 7.35 – 7.30 (m, 4H), 7.27 – 7.24 (m, 2H), 5.56 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.14, 144.01, 138.55, 134.29, 133.79, 129.18, 128.92, 128.91, 128.56, 128.46, 127.89, 126.25, 122.41, 117.84, 112.01, 107.59, 54.36; MS (ESI): *m/z* = 430.0590 $[M + H]^{+}$ 432.0568 $[M + H + 2]^{+}$.

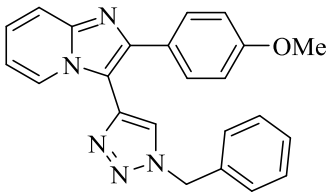
1-Phenyl-2-(4-(2-(*p*-tolyl)imidazo[1,2-*a*]pyridin-3-yl)-1*H*-1,2,3-triazol-1-yl)ethan-1-one (70h):



Yield: 113 mg (67%); brown solid; mp 141 °C; 1H NMR (500 MHz, CDCl3) δ 9.06 (s, 1H), 8.16 – 7.91 (m, 2H), 7.76 (d, J = 4.7 Hz, 1H), 7.72 – 7.60 (m, 3H), 7.60 – 7.51 (m, 2H), 7.34 – 7.24 (m, 2H), 7.24 – 7.16 (m, 2H), 6.91 (d, J = 3.3 Hz, 1H), 5.90 (s, 2H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.75, 138.23, 134.71, 133.80, 130.75,

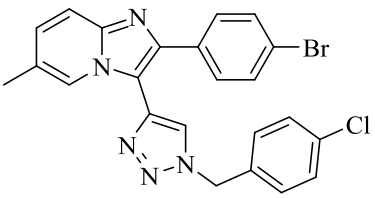
129.36, 129.28, 129.21, 128.60, 128.11, 126.14, 125.86, 125.79, 124.48, 116.99, 112.97, 55.65, 21.34; MS (ESI): *m/z* 394.1672 $(M+H)^+$.

3-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine (70i):



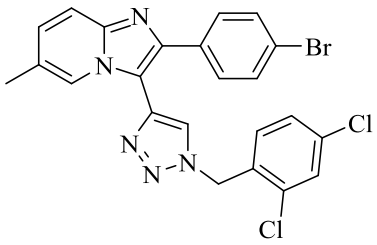
Yield: 115 mg (75%); yellow solid; mp 154-155 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (d, *J* = 6.9 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.43 (s, 1H), 7.36 (t, *J* = 8.9 Hz, 3H), 7.26 (dd, *J* = 8.3, 2.5 Hz, 3H), 6.94 – 6.76 (m, 3H), 5.56 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.59, 144.52, 143.48, 138.19, 133.49, 128.81, 128.11, 127.78, 126.83, 125.65, 125.01, 124.32, 121.30, 116.05, 112.89, 111.57, 110.00, 54.24, 53.25; MS (ESI): *m/z* 382.1582 $(M+H)^+$.

2-(4-Bromophenyl)-3-(1-(4-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-6-methylimidazo[1,2-*a*]pyridine (70j):



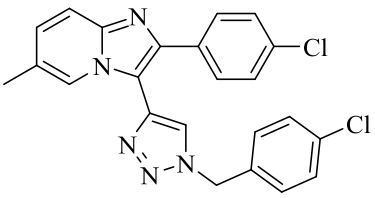
Yield: 136 mg (89%); white solid; mp 162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.36 (d, *J* = 7.0 Hz, 1H), 7.61 (d, *J* = 8.6 Hz, 2H), 7.55 (t, *J* = 8.4 Hz, 2H), 7.51 – 7.44 (m, 3H), 7.42 – 7.34 (m, 2H), 6.84 (dd, *J* = 7.1, 1.5 Hz, 1H), 5.73 (s, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.61, 143.58, 142.50, 137.08, 136.78, 135.38, 133.81, 133.37, 131.82, 130.26, 130.13, 129.93, 129.27, 129.03, 125.87, 124.89, 121.61, 116.03, 115.62, 111.34, 52.76, 21.25; MS (ESI): *m/z* = 478.0335 $[M + H]^+$ 480.0356 $[M + H + 2]^+$.

2-(4-Bromophenyl)-3-(1-(2,4-dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-6-methylimidazo [1,2-*a*]pyridine (70k):



Yield: 136 mg (83%); white solid; mp 221 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H), 8.34 (d, *J* = 7.0 Hz, 1H), 7.75 (d, *J* = 1.9 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.5 Hz, 2H), 7.52 (d, *J* = 2.0 Hz, 1H), 7.47 (s, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 6.87 (d, *J* = 7.1 Hz, 2H), 5.81 (s, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 137.17, 134.50, 134.23, 132.74, 132.43, 131.82, 130.11, 129.68, 128.42, 126.40, 124.86, 121.63, 119.57, 116.10, 115.63, 50.97, 21.26; MS (ESI): *m/z* = 511.9982 $[M + H]^+$ 513.9943 $[M + H + 2]^+$.

3-(1-(4-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-2-(4-chlorophenyl)-6-methylimidazo[1,2-*a*]pyridine (70l):



Yield: 106 mg (65%); white solid; mp 215 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.57 (dd, J = 8.5, 6.6 Hz, 3H), 7.43 – 7.35 (m, 3H), 7.29 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.15 (dd, J = 9.1, 1.4 Hz, 1H), 5.56 (s, 2H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.83, 143.37, 139.02, 135.09, 134.03, 132.91, 132.85, 129.82, 129.44, 129.32, 128.97, 128.72, 123.51, 122.76, 122.42, 116.63, 111.08, 53.63, 18.41; MS (ESI): *m/z* 435.0822 $(M+H)^+$.

3.4.1 Antibacterial assay: The synthesized compounds were tested for antibacterial activity against four Gram negative (*P. putida, K. pneumoniae*) and two Gram positive (*B. subtilis, S. aureus*) bacteria as per the following standard method (NCCL, 1993). The tested bacterial cultures were freshly cultured and inoculated into the Luria-bertani broth medium and kept for overnight at 37 °C with shaking at 150 rpm. Meanwhile, autoclaved Luria-bertani agar medium was equipped and poured into sterile glass petri-dishes (90 mm) under aseptic conditions. After solidification of medium, 100 µL culture of each test strain (10^7 cfu/mL) was spread over it using a sterile glass spreader and left for 15 min for complete adsorption. After adsorption, wells of size 6 mm diameter were made by the sterile metallic borer and the solution of compounds with different concentration were poured into the wells. The compounds to be tested were dissolved in DMSO (Merck, India). After incubation at 37 °C for 24 h, the diameter of the zone of inhibition was measured in comparison with standard antibiotic 'ciprofloxacin'. Solvent, DMSO was used as negative control while antibiotic 'ciprofloxacin' was used as positive control. For the MIC assay, test compounds were prepared in the concentrations of 8, 16, 32, 64, 128 and 256 µg/mL in DMSO and serial diluted test samples of each compound (150 µL) were added in 96 well micro-trays. The same amount of test microorganism was added to micro-trays well to obtain a final volume of 300 µL and incubated at 37 °C for 24 h. MIC value is defined as the lowest concentration of compound that inhibit the visible growth of bacteria (OD_{600} less than 0.06). Each assay was performed in duplicate sets.

3.4.2 Antifungal assay: Antifungal activities of test compounds were determined by agar well diffusion method against the fungal strains *C. albicans, A. flavus, F. oxysporum* and *P.*

citrinum. For the experimental work, a loopful of each strain was grown in Potato dextrose broth (PDB, Himedia, India) medium at 28 °C for 4-5 days. Following optimal growth of each fungal strain, 100 µL of culture was uniformly spread on the potato dextrose agar medium plate. Following adsorption, wells of 6 mm was prepared by the sterile metallic borer and solution of working compound of different concentration was poured into the wells. Plates were incubated at 28 °C for 4-5 days under dark conditions. Mean diameter of inhibition zone was measured to determine the antifungal activity. For the MIC assay, sterile test tubes containing 5 mL of sterilized czapeks dox broth medium was inoculated with 100 µL of freshly grown culture of each test strain and appropriate amount of compound was added to achieve the desired concentrations. The tubes were incubated at 28 °C for 5 days under dark conditions and carefully observed for the presence of turbidity. Amphotericin B was used as positive control. The experiment was performed in duplicate sets.

3.4.3 Evaluation of ROS production: The fluorescent dye 2', 7'-dichlorofluorescein diacetate (DCFH-DA), commonly used as probe to evaluate the reactive oxygen species (ROS) formation. In our assay, we treat the bacterial cell with the selected compounds **70a**, **70d**, **70f** and **70l** for 2 h and observed for the fluorescence intensity under the epi-fluorescence microscope. The probe DCFH is non-fluorescent dye, but is oxidized to the highly fluorescent DCF by intracellular H_2O_2 or nitric oxide. The bacterial culture was grown up to mid-log phase and then treated with 2 µM of DCFH2-DA for 30 min at room temperature. Following treatment the fluorescence of DCF was measured at 530 nm after excitation at 485 nm.

3.4.4 Live-dead bacterial screening: To discriminate the live and dead bacterial cells, the overnight grown culture of *P. putida* (10^7 cfu mL^{-1}) was treated with selected compounds **70a**, **70d**, **70f** and **70l** at 3×MIC for 4 h. The working solution of acridine orange (AO, 15 µg mL^{-1}) and ethidium bromide (EtBr, 50 µg mL^{-1}) were prepared in the PBS buffer (1X pH 7.2). After the compounds treatment, 5 µL each of acridine orange and ethidium bromide was added to 500 µL of *P. putida* culture following the standard protocol of Jakopec et al. (2006) with minor modifications. The suspension was centrifuged at 5,000 g for 10 min and supernatant was discarded. The cell pellet was washed with 1X PBS buffer three times to remove any traces of unbound dyes. Washed cell pellet was streaked on the glass slide with a cover slip on top of it and viewed under epi-fluorescence microscope (Olympus-CKX41, Olympus, Japan) at intensity between 450 and 490 nm using 100X objective lens and 10X eyepiece lens.

3.4.5 Biofilm inhibition assay: The pathogenic bacterial strain *S. aureus* was cultured in tryptic-soy broth medium and optical density of bacterial suspension was adjusted to 1×106 cfu/mL. The synthesized compounds having concentrations 50 µg/mL were mixed to the grown bacterial culture and properly mixed. The aliquots of 100 µL was distributed in to the 96-well polystyrene micro-titer plates (Tarson, India) and kept under static condition at 37 ºC for 24 h. The medium was discarded with the micro pipettes and the plate was washed with PBS buffer (1X, pH 7.2) to wash away the non-adherent bacterial culture. The well of micro-titer plates were stained with 100 µL of 0.1 % crystal violet solution for 30 min at room temperature. After incubation, the staining solution was discarded and wells were washed with autoclaved Milli-Q water and kept for air drying at room temperature. The stained biofilm were solubilized with 100 µL of 95% ethanol and absorbance was taken at 540 nm. Blank wells were taken as background control. All the experiments were carried out in triplicates and the values are indicated as mean ± SD.

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Chapter IV

Synthesis and antimicrobial activity of pyrimidine-triazole derivatives

4.1 Introduction

Heterocyclic compounds containing pyrimidine framework constitute an important class of natural and synthetic compounds. Pyrimidine based chemical architectures exhibit wide range of pharmacological activities including anti-malarial,[1] anti-viral,[2] anti-bacterial,[3] anti-cancer[4] and anti-HIV[5] activity. Substituted pyrimidines are important molecular frameworks in biological entities,[6] material science,[7] synthetic chemistry[8] and co-ordination chemistry[9]. Transformation of arylpyrimidines to diaryl pyrimidines (DAPYs) has led to highly potent non-nucleoside reverse transcriptase inhibitors (NNRTI) with nanomolar EC_{50} values against wild-type and drug-resistant HIV-1 strains.[10,11] Rilpivirine and Etavirine are DAPYs which are used as drug for HIV (**Figure 4.1**). Excellent pharmacological profile of DAPYs has encouraged medicinal chemists to synthesize new NNRTI agents.[12]

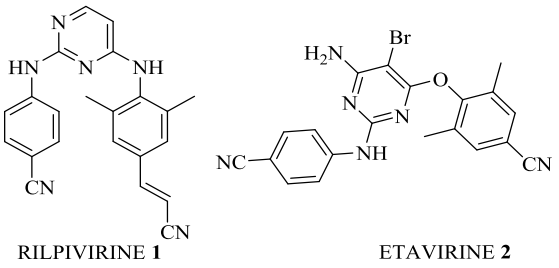


Figure 4.1: Diaryl substituted Pyrimidine drugs.

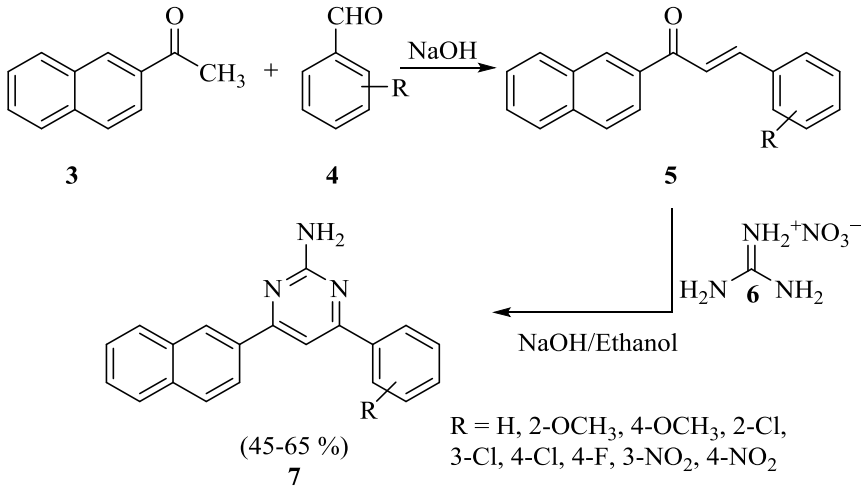
Some of the recent reports on the antibacterial, antifungal and HIV inhibitory activity of amino-pyrimidines and DAPYs are summarized below.

4.2 Bioactive pyrimidine derivatives

4.2.1 Pyrimidine derivatives as antimicrobial agent

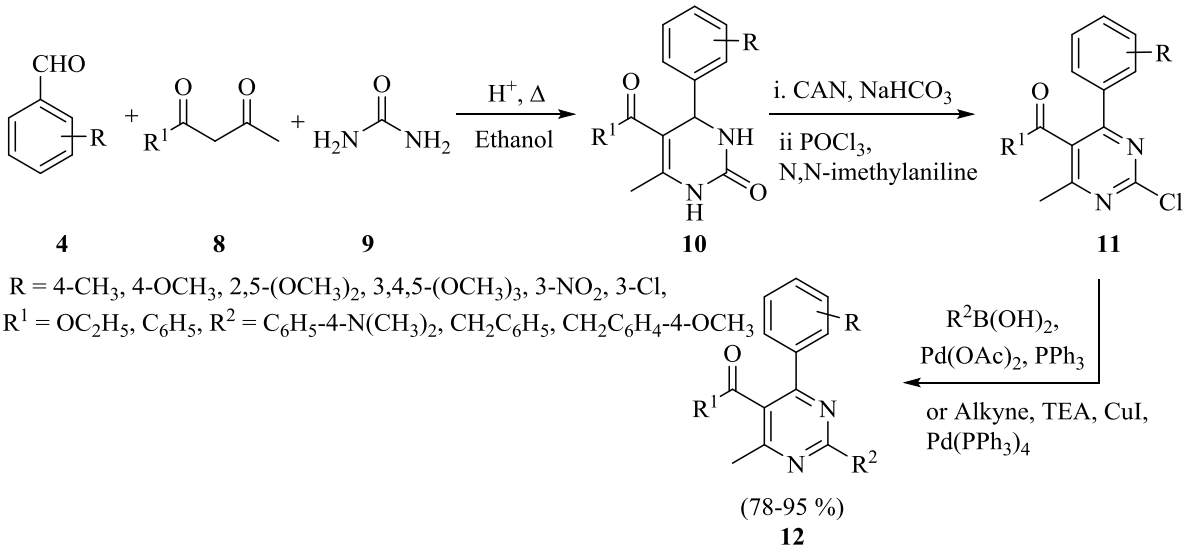
A series of naphthyl aminopyrimidines (**5**) was synthesized by Nagarazan and co-workers to examine their antimicrobial activity.[13] Claisen-Schmidt condensation of acetylated naphthalene with benzaldehyde produced chalcones **5** which on treatment with guanidine nitrate in basic media gave naphthyl bearing aminopyrimidines **7** (**Scheme 4.1**). All synthesized compounds were tested for antimicrobial activity against Gram positive *S. aureus, P. aeruginosa* and Gram negative *K. pneumoniae, E. coli* and fungi *T. tonsurans, M. gypseum*. All the screened derivatives showed MIC values 2.55-60 μg/mL and 2.22-25 μg/mL against different bacteria and fungi, respectively less MIC value against Gram negative bacteria in comparison of Gram positive bacteria. Among all derivatives, napthyl-4-

aryl-2-aminopyrimidine compounds having 4-Cl and 4-F substitution in the aryl ring at C-4 were found to be better antimicrobial agents against both bacteria and fungi.



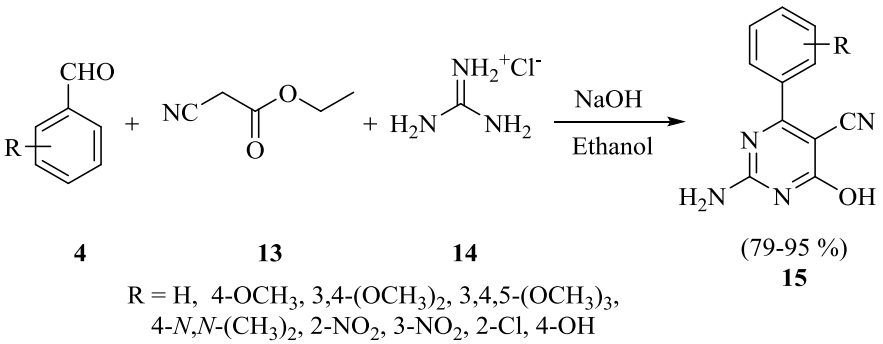
Scheme 4.1: Synthesis of naphthyl-pyrimidines as antimicrobial agents.

Srinivasan and co-workers have synthesized tetrasubstituted pyrimidines and evaluated their antimicrobial activity.[14] Biginelli reaction of benzaldehyde **4**, ethyl acetoacetate **8** and urea **9** provided pyrimidines-2-ones **10** which were subsequently oxidized followed by chlorination to get 2-chloropyrimidine derivatives **11**. Suzuki or Sonagshira reaction was performed on **11** to get desired tetrasubstituted pyrimidines **12** (**Scheme 4.2**). All the compounds were evaluated for their antibacterial activity against Gram positive *S.aureus* and Gram negative *E. coli* bacteria and for antifungal activity against *C. albicans, C. neoformans, Y. lipolitica* and *F. oxysporum*. MIC (50 and 90% inhibition) values were calculated and it was observed that the derivative having methoxy substitutions showed better antifungal activity against all fungi with MIC_{50} value ranging between 4-64 μg/mL. Methoxy substituted derivatives were also effective against *S.aureus* (MIC_{50} 8 μg/mL) but pyrimidines with phenyl group were found better against *E. coli* (MIC_{50} 8 μg/mL).



Scheme 4.2: Sequential synthesis of tetrasubstituted-pyrimidines.

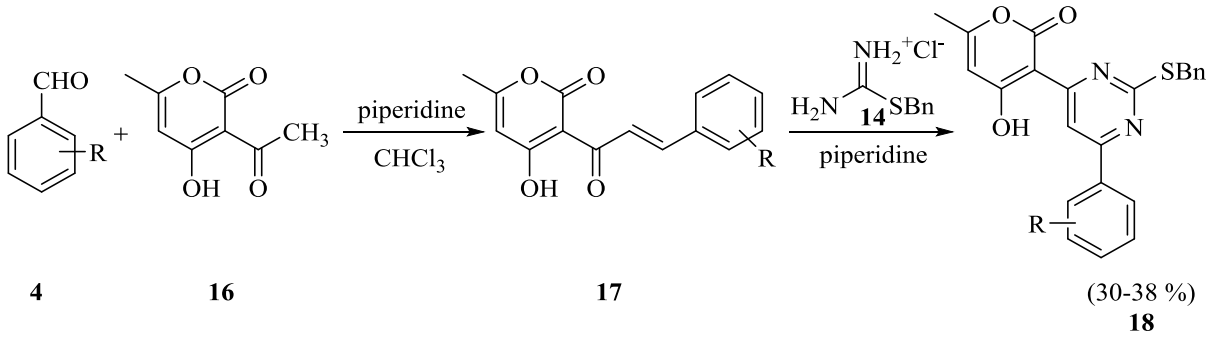
Anbhule and colleagues designed a simple approach for the synthesis of 2-amino-5-cyano-6-hydroxyl-4-arylpyrimidines **15** *via* one-pot multicomponent condensation reaction of aromatic aldehyde **4**, ethyl cyanoacetate **13** and guanidine hydrochloride **14** under basic conditions (**Scheme 4.3**).[15] A series of pyrimidines derivatives was synthesized and all the compounds were evaluated for their antibacterial activity against Gram positive *S. aureus* and Gram negative *E. coli* by measuring zone of inhibition with varying concentration from 50-750 ppm. It was observed that inhibitory action increases with increasing concentration although most of the compounds were inactive upto 100 ppm against both bacteria. Pyrimidine with 4-phenyl group was most effective against *S. aureus* (ZOI 10-18 mm under different concentrations) while compound with 4-(*N*,*N*-dimethyl)aminophenyl group was found to be most effective against *E. coli* (ZOI 7-12 mm).



Scheme 4.3: Synthesis of 2-amino-5-cyano-6-hydroxyl-4-arylpyrimidines.

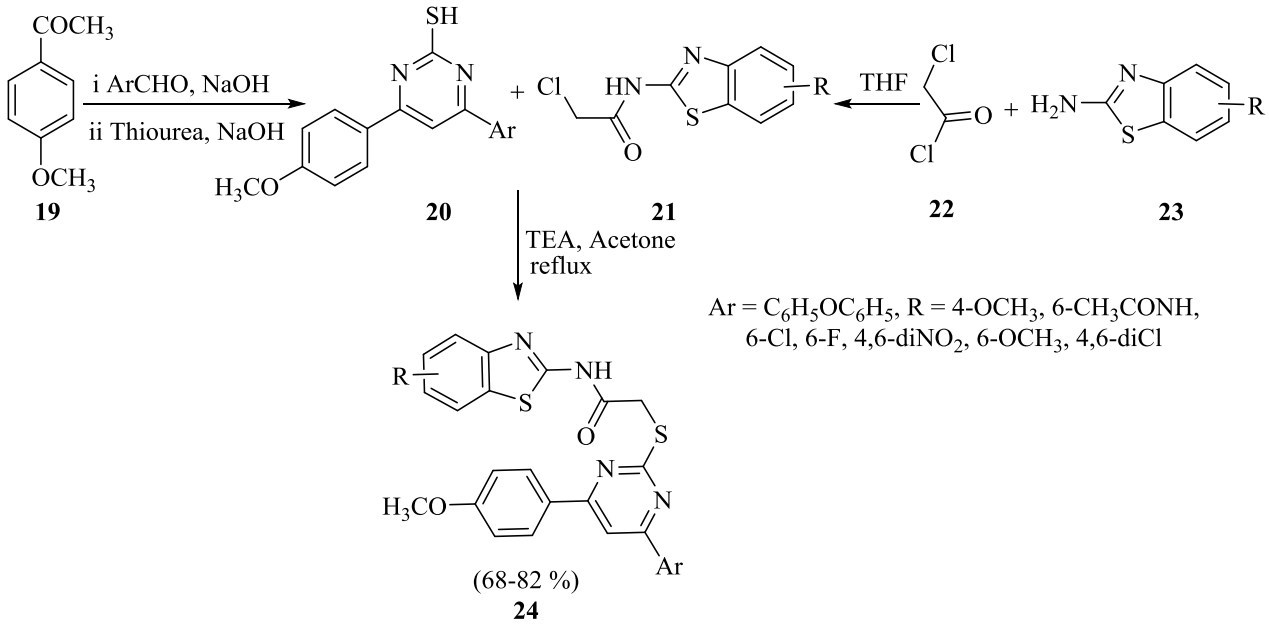
Kaur *et al* have synthesized a series of pyranyl side chain bearing thiopyrimidine derivatives for evaluation of their antibacterial activity.[16] Initially, ketone **16** was reacted with aldehyde

in basic condition to get chalcones **17** which were transformed into desired pyranyl tethered 2-benzylthiopyrimidines **18** (**Scheme 4.4**). All the synthesized compounds were evaluated for their antibacterial activity against *B. subtilis* and *P. aeruginosa* using ciprofloxacin (10 μg/mL, 22 and 30 mm zone of inhibition, respectively) as standard. The derivatives were screened upto 500 μg/mL concentration. Derivative having thienyl or fluoro substitution were found to be best antibacterial agents among all of the screened compounds with 12 and 11 mm zone of inhibition against *B. subtilis* and 7 and 9 mm zone of inhibition against *P. aeruginsoa* when 500 μg/mL concentration was used.



Scheme 4.4: Construction of pyranyl tethered thiopyrimidine scaffolds.

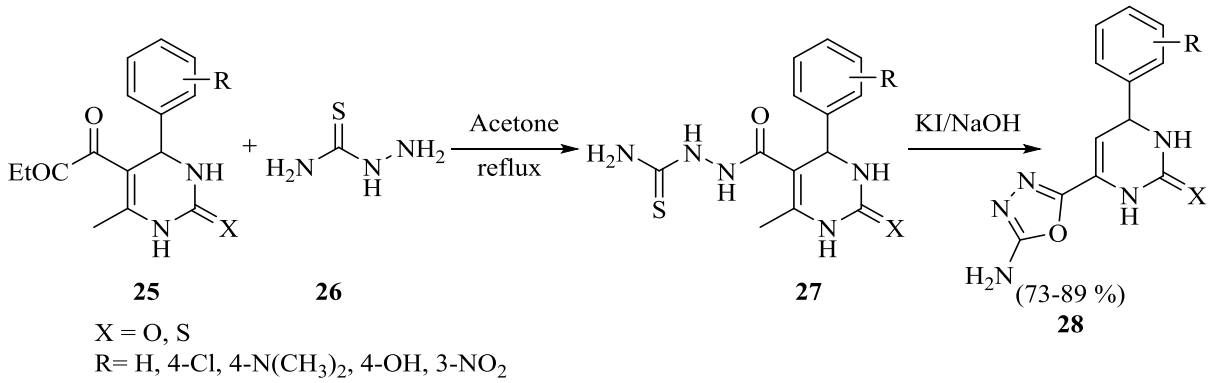
Gupta and co-workers have synthesized thiazolyl acetamide substituted pyrimidines and evaluated their antimicrobial activity.[17] Initially, Chalcones were constructed by aldol condensation of 4-methoxyacetophenone with *m*-phenoxybenzaldehyde which were transformed into pyrimidines **20** on treatment with thiourea. Pyrimidines **20** on reaction with substituted benzothiazolyl chloramides **21** offered thiazolyl acatamide substituted pyrimidines **24** (**Scheme 4.5**). All the thiazolylacetamide-pyrimidines were evaluated for antibacterial activity against Gram positive *S. aureus, S. pyogenes* and Gram negative *E. coli, P. aeruginosa* as well as for antifungal activity against *C. albicans* and *A. niger*. The MIC values were calculated and it was found that most of the compounds showed moderate to poor activity with MIC value 25-1000 μg/mL. Pyrimidine derivative with 4-methoxy substitution on benzothiazole ring was most potent with MIC value 25-100 μg/mL against different bacteria and with dichloro substitution was best antifungal agent among all synthesized compounds with MIC value 200 and 250 μg/mL against *E. coli* and *P. aeruginosa*, respectively.



19 **20** **21** **22** **23** **24**

Scheme 4.5: Synthesis of thiazolyl acetamide linked pyrimidines.

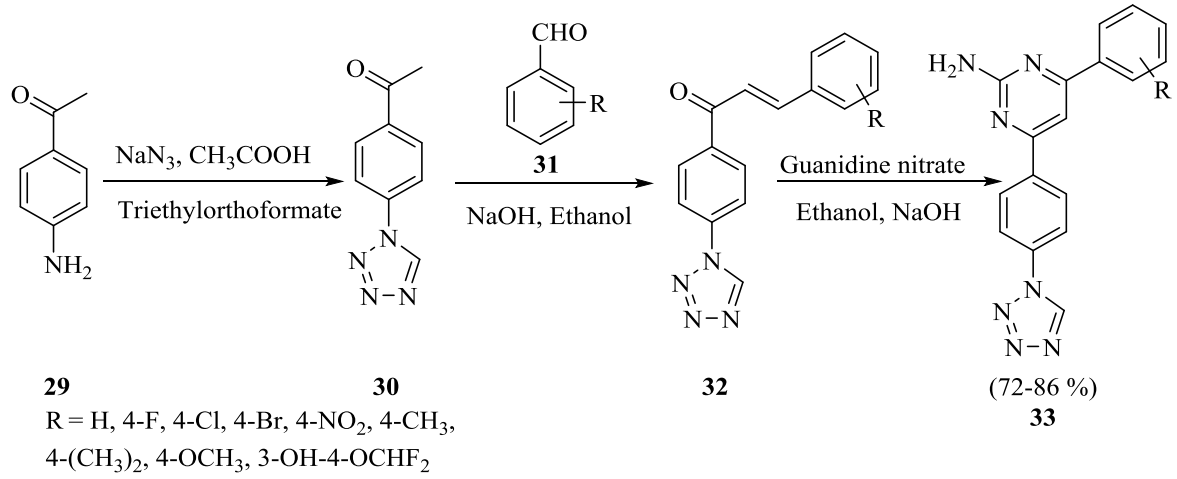
Andrews group has synthesized 1,3,4-oxadiazole bearing pyrimidines and evaluated them for antifungal activity.[18] Initially, pyrimidine ethyl ester **25** was synthesized by Biginelli reaction of benzaldehyde, ethyl acetoacetate and urea which were subsequently condensed with thiosemicarbazide **26** to achieve carbothioamide pyrimidines **27**. On reaction with KI/NaOH, **27** provided desired motif 1,3,4-oxadiazole tethered pyrimidines **28** (**Scheme 4.6**). All synthesized derivatives were screened for antifungal activity against *C. albicans*, *P. species* and *A. niger* using Amphotecerin-B as reference at 10 µg/mL concentration. Zone of inhibition was calculated and it was observed that most of the compounds were moderate antifungal agent with zone of inhibition 5-12 mm. Derivative with phenyl ring was best antifungal agent against *C. albicans* with zone of inhibition 23 mm.



25 **26** **27** **28**

Scheme 4.6: Synthesis of pyrimidine based aminooxadiazoles.

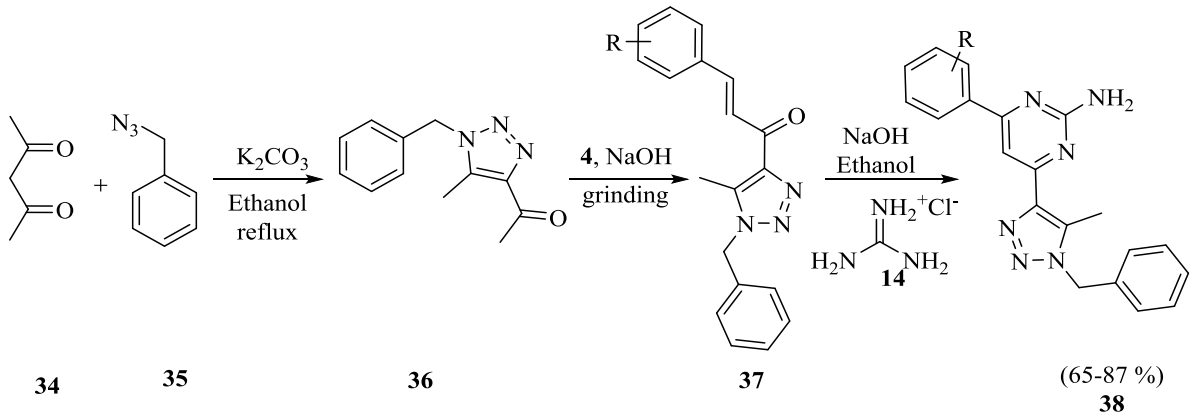
Gopalkrishnan *et al* have synthesized tetrazole bearing aminopyrimidines and examined them for their antifungal activity.[19] *p*-Aminoacetophenone **29** were transformed into tetrazolo acetophenone **30** which were consecutively transformed to chalcones **32** by reaction with aldehyde **31**. Desired tetrazolo pyrimidines **33** were acheived by the reaction of these chalcones with guanidine nitrate under basic condition (**Scheme 4.7**). The antifungal activity of synthesized compounds was tested by calculating their MIC values against *C. albicans, S. cerevisiae, A. niger* and *A. fumigates* using fluconazole as positive control. Derivatives substituted with 4-hydroxy and 3,4-difluromethoxy substitutions were found to show broad spectrum antifungal activity with MIC value (<0.16-0.625 μg/mL) which was comparable to fluconazole (<0.16-2.5 μg/mL). Derivative having 3-chloro substitution was least active against *S. cerevisiae* with MIC value 40 μg/mL). All the tested compounds were moderate antifungal agents with MIC value 0.16-2.5 μg/mL.



Scheme 4.7: Synthesis of tetrazole linked aminopyrimidines.

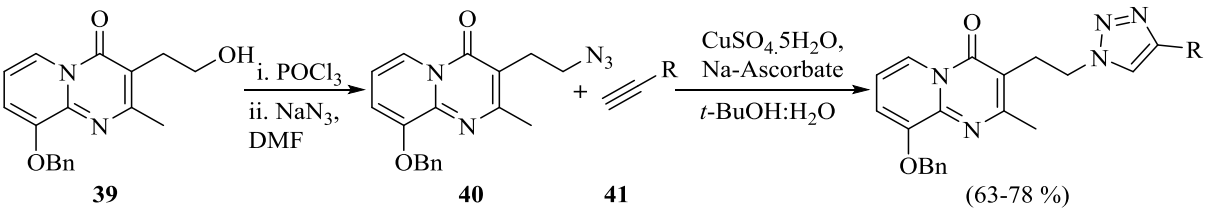
Ponnuswamy and co-workers envisioned the synthesis of 1,2,3-triazole linked 2-aminopyrimidines to determine their antibacterial activity.[20] Initially, benzyl azide **35** was refluxed with acetylacetone **34** in ethanol to give 1,4,5-trisubstituted 1,2,3-triazoles **36** which were consequently transformed into triazolyl chalcones **37** by condensation with aldehyde. These chalcones were afterwards transformed into triazolylpyrimidines **38** on refluxing with base and guanidine hydrochloride in ethanol/water (**Scheme 4.8**). All the synthesized compounds were evaluated for *in vitro* antibacterial activity against *S.aureus, P. aeruginosa* and *K. pneumoniae* bacteria by calculating their MIC value. Most of the compounds were either found to be inactive against *S. aureus* or their MIC values (35 μg/mL) were higher than standard tetracycline (15 μg/mL). Derivatives having *p*-methoxy, *o*-methoxy, *p*-fluoro, 2,4-difluro, 2,4-dimethoxy and *p*-bromo substitution were better antibacterial agent than standard

(10 µg/mL) against *P. aeruginosa* with MIC value 2 µg/mL while compound with *p*-methoxy substitution was most potent with MIC value equal to tetracycline (2 µg/mL) for *K. pneumoniae*. It was concluded that methoxy and fluoro substitution increased antibacterial activity.



Scheme 4.8: Synthesis of 1,2,3-triazole linked 2-aminopyridines.

A series of triazole fused pyimidine motifs was constructed by Sabarinathan *et al* to evaluate their antibacterial activity.[21] They started the synthesis of desired scaffold by chlorinating **39** with $POCl_3$ which was consequently converted into azide **40**. This azide bearing pyrimidine scaffold **40** was reacted with different alkynes applying CuAAC to obtain desired 1,2,3-triazolo pyrimidines **42** (**Scheme 4.9**). After the successful synthesis, all the derivatives were tested for their antibacterial activity against *S. aereus, B. subtilis, E. coli, P aerugenosa.* The zone of inhibition of all derivatives for different bacterii was measured for 500 µg/mL and 1000 µg/mL concentration using amikacin as positive control. Derivative with 2-bromo-5-methoxy substitution was found to be the most potent among all compounds against both Gram positive bacteria while derivative having methylene hydroxy group attached to triazole ring was most potent against Gram negative bacteria. All the compounds showed zone of inhibiton in the range of 7-17 mm which was less than amikacin (24-27 mm).



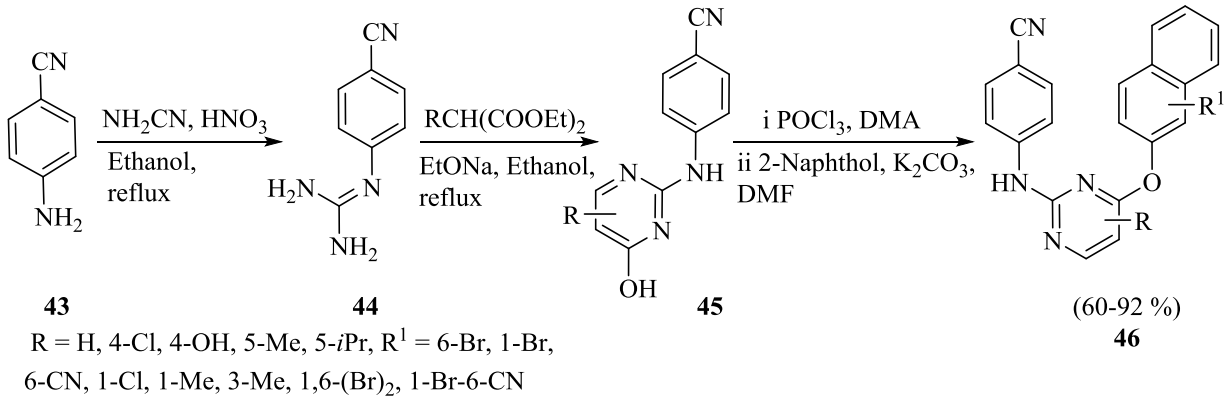
R = CH_2OH, C_5H_{11}, $C_6H_{10}OH$, $C_6H_5COOCH_2$, 3-MeC_6H_4COO, 5-OCH_3-2-BrC_6H_3COO, 3,4-$(OMe)_2C_6H_3COO$, 4-$NO_2C_6H_4COO$

Scheme 4.9: CuAAC for construction of triazolo-aminopyrimidines.

4.2.2 Diaryl pyrimidines as anti-HIV agent

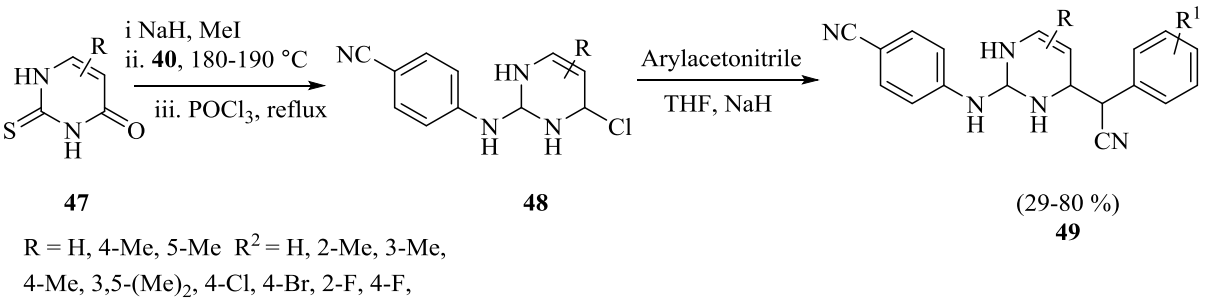
DAPYs are an important class of NNRTIs to specially inhibit HIV1 reverse transcriptase inhibitor and mutant strains because of their conformational flexibility, positional adaptability and hydrogen bonding ability.[22] Most employed DAPYs Etavirine and Rilpivirine have been approved by U. S. Food and Drug Administration in 2008 and 2011, respectively.[23,24] Some of the reports on anti-HIV action of DAPYs are summarized here:

Diaryl pyrimidines with naphthyl substitution **46** were developed by Chen group for the evaluation of their non-nucleoside inhibition action against HIV-1 reverse transcriptase.[25] Diethylmalonate derivatives were reacted with 2-(4-cyanophenyl)guanidine **44** in the presence of sodium ethoxide to provide 4-(pyrimidin-2-ylamino)benzonitriles **45** which were chlorinated with $POCl_3$ and consequent treatment with 2-naphthol derivatives in DMF under nitrogen atmosphere offered desired naphthyl substituted diarylpyrimidines **46** (**Scheme 4.10**). Most of the synthesized compounds were found to have excellent activity towards the inhibition of wild type HIV-1.



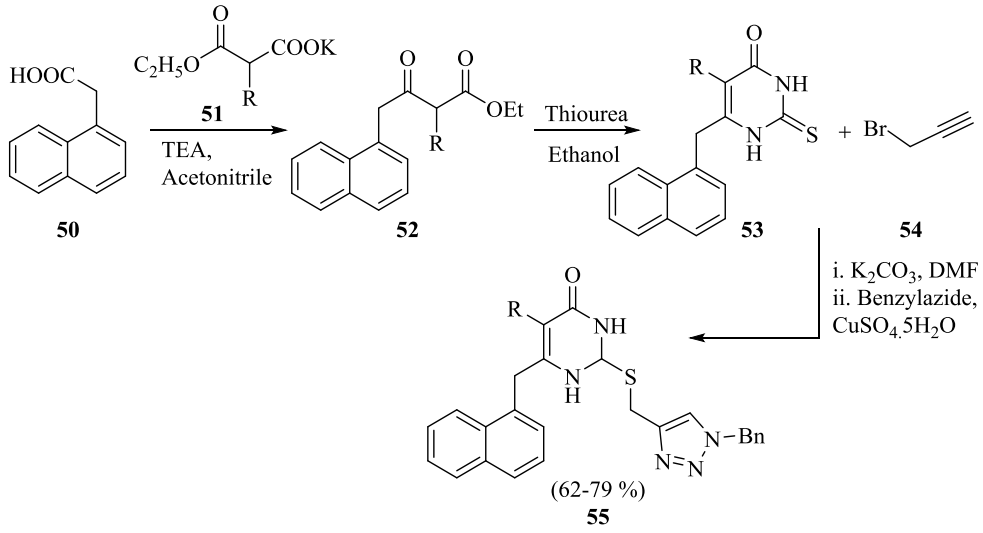
Scheme 4.10: Diaryl substituted 2-aminopyrimidines as potential HIV-1 inhibitor.

The same group has also synthesized diaryl pyrimidines **49** as non-nucleoside HIV-1 inhibitor. 2-Thiouracil **44** was converted to 4-chloro-2-aminopyrimidines **48** by *S*-allylation, substitution followed by chlorination which was transformed into **49** by C-C coupling in presence of sodium hydride (**Scheme 4.11**)**.** All the compounds were tested against MT-4 cells for inhibitory action against different HIV strains and it was observed that derivative having 5-Me, 6-F substitution on aryl ring at C-4 position showed highest potency with EC_{50} value of 1.8 nm.



Scheme 4.11: Synthesis of diarylpyrimidines.

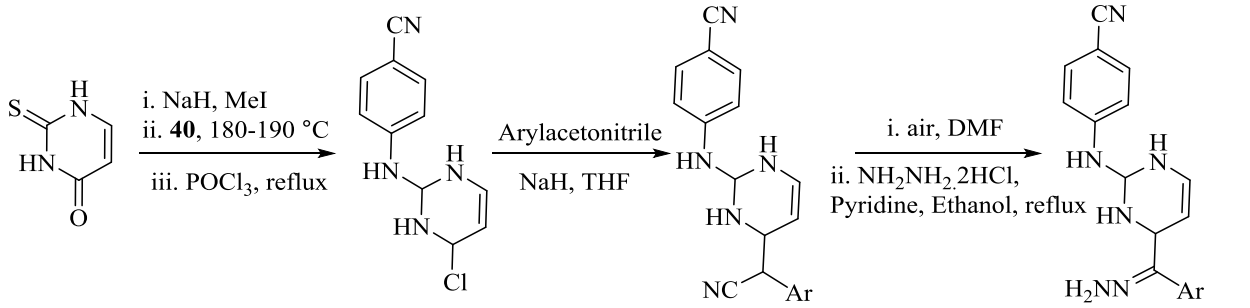
Zhan and Liu group has synthesized naphthyl substituted triazolyl pyrimidines to evaluate their anti-HIV activity.[27] Naphthalen-1-yl acetic acid **50** was transformed into β-ketoesters **52** which offered naphthalene tethered thiopyrimidines **53** on reaction with thiourea. *S*-atom of thiopyrimidines **53** was propargylated which was sequentially transformed into naphthalene tethered triazolyl pyrimidines **55** by CuAAC reaction with azides (**Scheme 4.12**). All derivatives were tested against different HIV strains for inhibitory action, cytotoxicity and selectivity values using nevarapine, azidothymidine, delavirdine and efavirniz as reference drugs. It was concluded that compound with methyl and acetoxy substitution were the most potent inhibitor.



Scheme 4.12: Synthesis of naphthyl substituted triazole linked 2-thiopyrimidines.

Chen group in the continuation of their work on diaryl pyrimidines, has synthesized diaryl pyrimidines with ketone hydrazone substitution on methylene linker and further evaluated their anti-viral activity against HIV-1 in MT-4 cells.[10] **57** Was transformed into **58** by reacting with 2-arylacetonitriles which were further converted into the corresponding ketones on keeping in air using DMF as solution. Afterwards, reaction with hydrazine provided diaryl pyrimidinone hydrazones **59** (**Scheme 4.13**). Most of the synthesized diaryl pyrimidinone

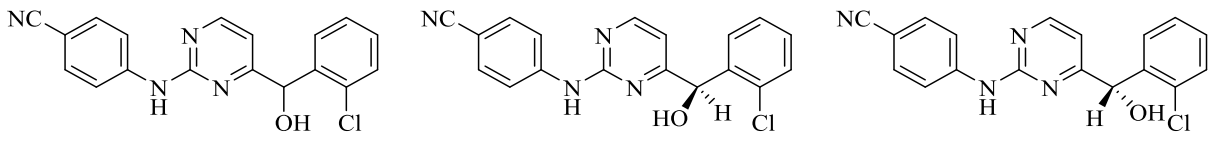
hydrazones illustrated good activity against wild-type HIV-1 virus with EC_{50} in the range of 1.7-3.2 nm. Also, excellent selectivity with broad spectrum activity against wild type HIV-1 and HIV-1 double mutated strain RES056 was observed for infected over uninfected cells. The SAR study and molecular modelling of the derivatives was also performed.



Ar = C_6H_5, 2-$CH_3C_6H_4$, 3-$CH_3C_6H_4$, 4-$CH_3C_6H_4$, 2-F-C_6H_4, 3-F-C_6H_4, 4-F-C_6H_4, 2-ClC_6H_4,3-ClC_6H_4, 4-ClC_6H_4, 2-BrC_6H_4, 4-BrC_6H_4, 4-$OCH_3C_6H_4$, 2,4-$(Cl)_2C_6H_3$, 3,4-$(F)_2C_6H_3$, 2,5-$(F)_2C_6H_3$, 1-Naphthyl, 2-Naphthyl

Scheme 4.13: Synthesis of hydrazone ligated diaryl aminopyrimidines.

Chen group has also isolated enantiomers **60a** and **60b** from the racemic mixture of diaryl pyrimidines **60** and evaluated them as non-nucleoside HIV-1 reverse transcriptase inhibitor by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazoliumbromide method (**Figure 4.2**).[28] Drugs nevirapine, zidovudine, delavirdine, etavirine were used as reference drugs for anti-HIV activities in MT-4 cells infected with WT HIV-1 and HIV-2. It was observed that these both these isomers were more potent than drugs nevirapine and delavirdine with EC50 value 65.3 nm and 5.3 nm respectively for (R) and (S)-enantiomer and SI value of 6801. It was concluded that inhibitory action of the racemate was due to the (R)-enantiomer.

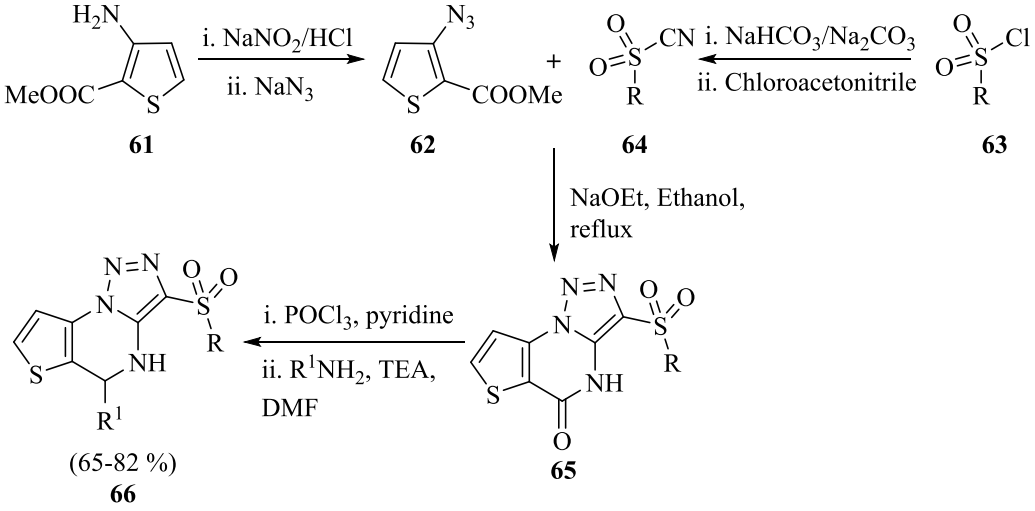


Racemate 60 | **(R)-60a** | **(S)-60b**

Figure 4.2: Chiral aminopyrimidines for evaluation of their anti-HIV activity.

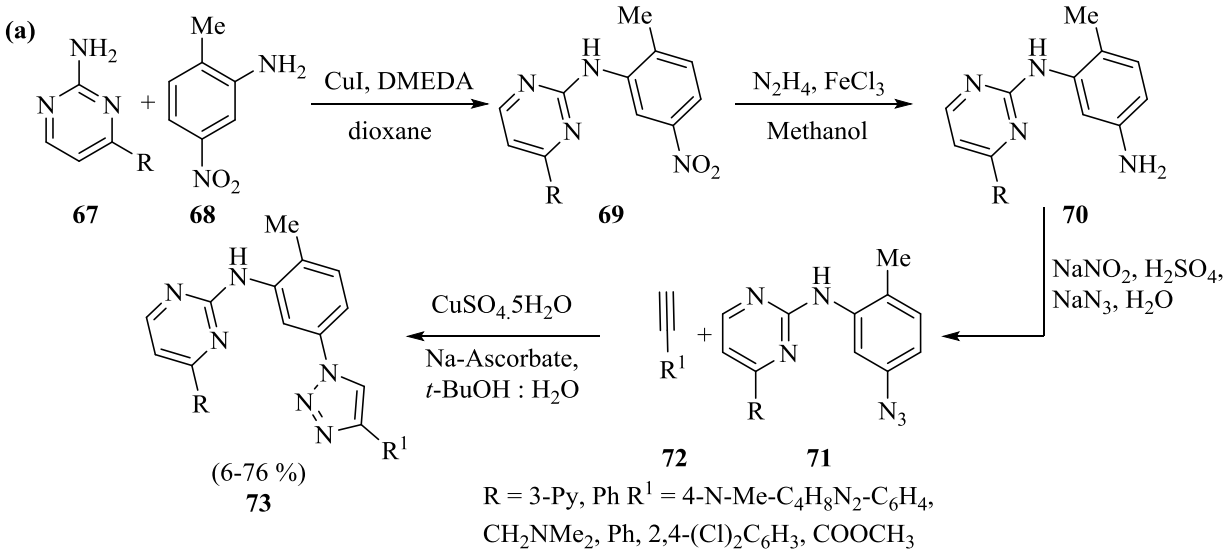
Lee group has synthesized triazolothienopyrimidines (**66**) and evaluated them for anti-HIV activity.[29] Amine group of **61** was converted into azide to obtain **62** and chlorosulphone (**63**) was transformed into sulphone nitriles **64**. Both **62** and sulphone nitriles **64** were reacted in the presence of sodium ethanoate to get triazolotheienopyrimidine-2-ones **65** which were subsequently transformed into amine substituted triazolothienopyrimidines **66** by reaction

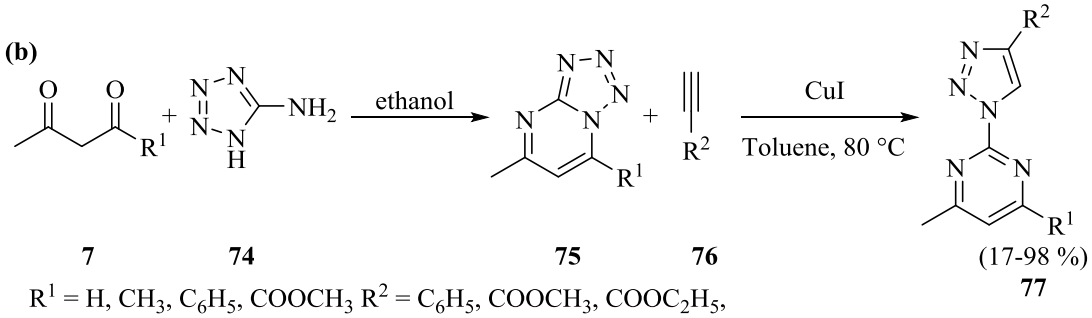
with $POCl_3$ followed by substituion with amines (**Scheme 4.14**). All the compounds were tested for anti-HIV activity and exhibited significant inhibition of HIV-1 replication.

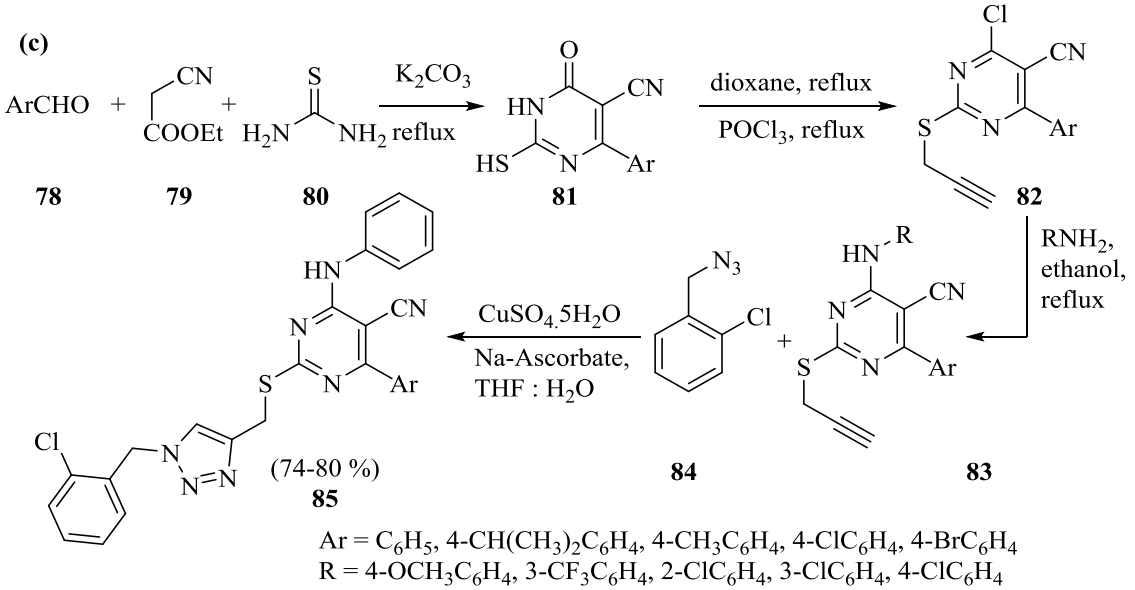


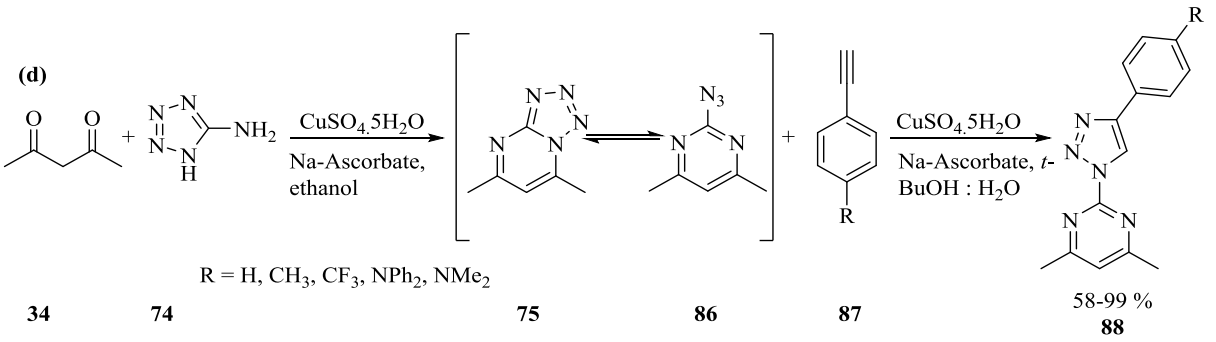
Scheme 4.14: Synthesis of triazolothienopyrimidines.

A vast series of literature supports that many research groups have performed the molecular hybridization of diarylpyrimidines with 1,2,3-triazoles to get different triazole-pyrimidine conjugates and thus synthesized conjugates are used for different purposes. Passarella and co-workers synthesized *N*-[2-Methyl-5-(triazol-1-y)phenyl]pyrimidines-2-amine **73** utilising CuAAC for the evaluation of their inhibitory action against Bcr-Abl (**Scheme 4.15a**).[30] Weigelt et al have synthesized triazole pyrimidines **77** from tetrazolopyrimidines (**Scheme 4.15b**).[31] Liu group designed the synthesis of novel 1,2,3-triazolo pyrimidines **85** for studying their anti-cancer activity (**Scheme 4.15c**).[32] Baudequin group synthesized 2-triazolopyrimidines **88** and studied their photo-physical properties (**Scheme 4.15d**).[33] With this much literature support, CuAAC is seldom executed to get triazole tethered pyrimidines and no report is available on the antimicrobial activity of this particular group.


(a)
CuI, DMEDA
dioxane
N_2H_4, $FeCl_3$
Methanol
$NaNO_2$, H_2SO_4,
NaN_3, H_2O
$CuSO_4.5H_2O$
Na-Ascorbate,
t-BuOH : H_2O
67
68
69
70
71
72
73
(6-76 %)
R = 3-Py, Ph R^1 = 4-N-Me-$C_4H_8N_2$-C_6H_4,
CH_2NMe_2, Ph, 2,4-$(Cl)_2C_6H_3$, $COOCH_3$


(b)
ethanol
CuI
Toluene, 80 °C
7
74
75
76
77
(17-98 %)
R^1 = H, CH_3, C_6H_5, $COOCH_3$ R^2 = C_6H_5, $COOCH_3$, $COOC_2H_5$,
$CH_2C_6H_5$, 2-$CH_3C_6H_4$, 3-$CH_3C_6H_4$, 4-$CH_3C_6H_4$


(c)
ArCHO
K_2CO_3
reflux
dioxane, reflux
$POCl_3$, reflux
RNH_2,
ethanol,
reflux
$CuSO_4.5H_2O$
Na-Ascorbate,
THF : H_2O
78
79
80
81
82
83
84
85
(74-80 %)
Ar = C_6H_5, 4-$CH(CH_3)_2C_6H_4$, 4-$CH_3C_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4
R = 4-$OCH_3C_6H_4$, 3-$CF_3C_6H_4$, 2-ClC_6H_4, 3-ClC_6H_4, 4-ClC_6H_4



Scheme 4.15: Synthesis of bioactive triazole tethered 2-thio/amino pyrimidines.

On the other hand, molecular hybridisation plays crucial role in medicinal chemistry as it offers a new class of drugs by mimicking the properties of parent nuclei.[34,35] The hybrid motif is designed with the purpose of proper binding with reaction centre and may have increased activity towards the infectious cell or tissue. In this regard, 1,2,3-triazoles have got attention of medicinal chemists owing to their easy accessibility *via* copper-catalysed azide-alkyne cycloaddition (CuAAC) and their wide range of biological activities such as antibacterial, anti-HIV, Src-kinase inhibition etc.[36,37] 1,2,3-Triazoles have been explored as isostere of imidazole and 1,2,4-triazoles for designing antimicrobial and antifungal agents.[38,39] A great deal of effort has been made for bio-conjugation of two different pharmacologically active scaffolds to achieve enhanced activity using CuAAC.[40]

The promising activities shown by pyrimidine based scaffolds and success of click chemistry in molecular hybridization motivated us to design novel triazolo-pyrimidine hybrids (**Figure 4.3**) as potential antimicrobial and anti-HIV agents. Herein, we report our results on synthesis of 2-methylthio-4-triazolo-pyrimidines and 1-arylamino-4-triazolo-pyrimidines and their anti-bacterial, anti-fungal, and anti-HIV activities.

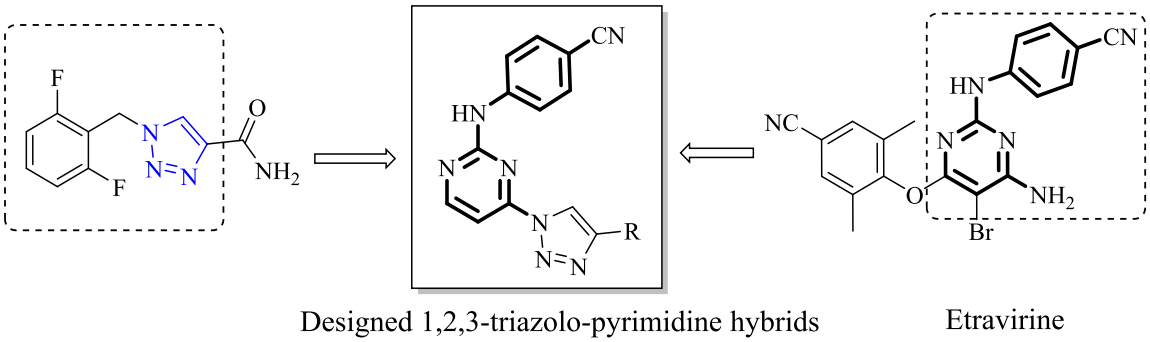
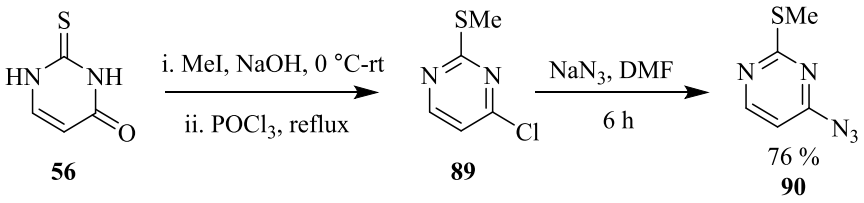


Figure 4.3: Structure of drugs containing pyrimidine motif and 1,2,3-triazole and design of new 1,2,3-triazolo-pyrimidine hybrids.

4.3 Results and discussions

The substrate, 4-azido-2-(methylthio)pyrimidine (**90**) was synthesized from 2-thiouracil by the conventional three-step sequence according to a reported procedure.[11,41] Initially, 2-thiouracil (**56**) was alkylated with methyl iodide to afford 2-(methylthio)pyrimidin-4(5*H*)-one which on reaction with $POCl_3$ resulted in the formation of 4-chloro-2-(methylthio)pyrimidines (**89**). Reaction of **89** with NaN_3 in DMF at room temperature (25-30 °C) afforded **90** after 6 h in overall 76% yield.

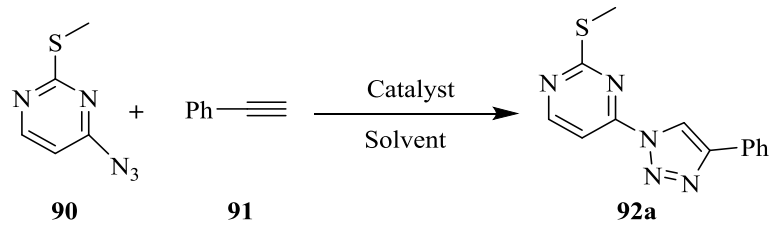


Scheme 4.16: Synthetic route for 4-azido-2-(methylthio)pyrimidine.

Next, we optimized conditions for CuACC click reaction of **90** with phenylacetylene (**91**) to give 2-(methylthio)-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)pyrimidines (**92a**) (**Table 4.1**). Reaction of **65** with phenylacetylene was performed with CuI (10 mol %) in different solvents at room temperature (Table 4.1, entries 1-10). Best yield of **92a** (93%) was obtained in water: PEG-400 (1: 1 *v/v*) (Table 4.1, entry 10). Screening of Cu(II) catalysts such as $CuSO_4.5H_2O$, $Cu(OAc)_2.H_2O$, $CuCl_2$, and $Cu(OTf)_2$ in the presence of sodium ascorbate in DMF and water: PEG-400 (entries 11-18) revealed that CuI (10 mol %) was the suitable catalyst for this transformation in water: PEG-400.

With the optimized conditions in hand, we then synthesized an array of 2-methylthio-4-triazolo-pyrimidines (**92a-92n**) by using different aliphatic and aromatic alkynes. The yields and structure of all the synthesized compounds are given in Scheme **4.17**. It was observed that there was not much difference in reactivity of aliphatic alkynes and aromatic alkynes. Heteroaryl alkynes also provided good yield (80%) of corresponding triazolo-pyrimidine **92f**. All the synthesized compounds were characterized by the ^{1}H NMR, ^{13}C NMR and HRMS analysis. A characteristic singlet peak for triazole C_4-proton (C-H) appeared at ~8.76 ppm along with the two doublets at ~8.69 (d, J = 5.4 Hz, 1H) and ~7.83 (d, J = 5.4 Hz, 1H) ppm for pyrimidine protons while –SMe protons appeared at ~2.65 ppm in the ^{1}H NMR spectra of **92a-n**. Similarly, in ^{13}C NMR a characteristic peak appeared at ~104 ppm for C_5-triazole carbon along with peak for all other carbons. Representative ^{1}H NMR and ^{13}C NMR spectra of compound **92f** are given in figure **4.4**.

Table 4.1: Optimization of reaction conditions[a]



90 **91** **92a**

S. No.	Catalyst	Solvent	Time (h)	Yield (%)[b]
1.	CuI	Methanol	13	30
2.	CuI	THF	5	85
3.	CuI	DCM	17	76
4.	CuI	DMF	6	90
5.	CuI	Water	13	Traces
6.	CuI	PEG 400	13	Traces
7.	CuI	DMSO	13	67
8.	CuI	Acetonitrile	6	79
9.	CuI	Water : Ethanol	13	Traces
10.	CuI	Water : PEG 400	4	93
11.	$Cu(OAc)_2.H_2O$, Na-Ascorbate[c]	DMF	7	82
12.	$Cu(OAc)_2.H_2O$, Na-Ascorbate	Water : PEG 400	8	87
13.	$CuCl_2$, Na-Ascorbate	DMF	13	20
14.	$CuCl_2$, Na-Ascorbate	Water : PEG 400	13	27
15.	$CuSO_4.5H_2O$, Na-Ascorbate	DMF	8	87
16.	$CuSO_4.5H_2O$, Na-Ascorbate	Water : PEG 400	10	82
17.	$Cu(OTf)_2$, Na-Ascorbate	DMF	13	Traces
18.	$Cu(OTf)_2$, Na-Ascorbate	Water : PEG 400	13	Traces

[a]Reaction conditions: **90** (1.0 mmol), phenylacetylene (1.1 mmol), catalyst (10 mol %), solvent 5 mL, [b]Isolated yield, [c](20 mol %).

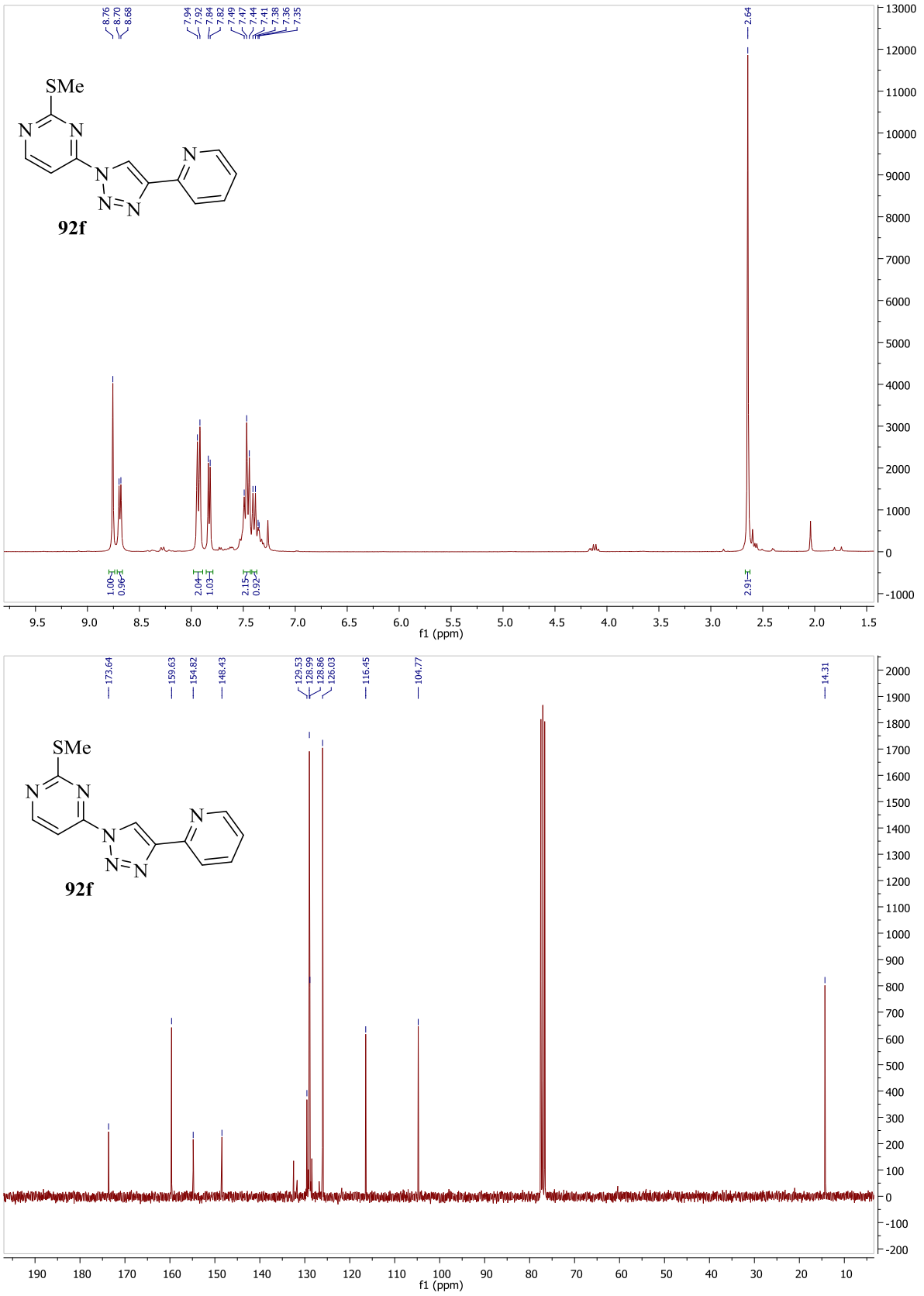
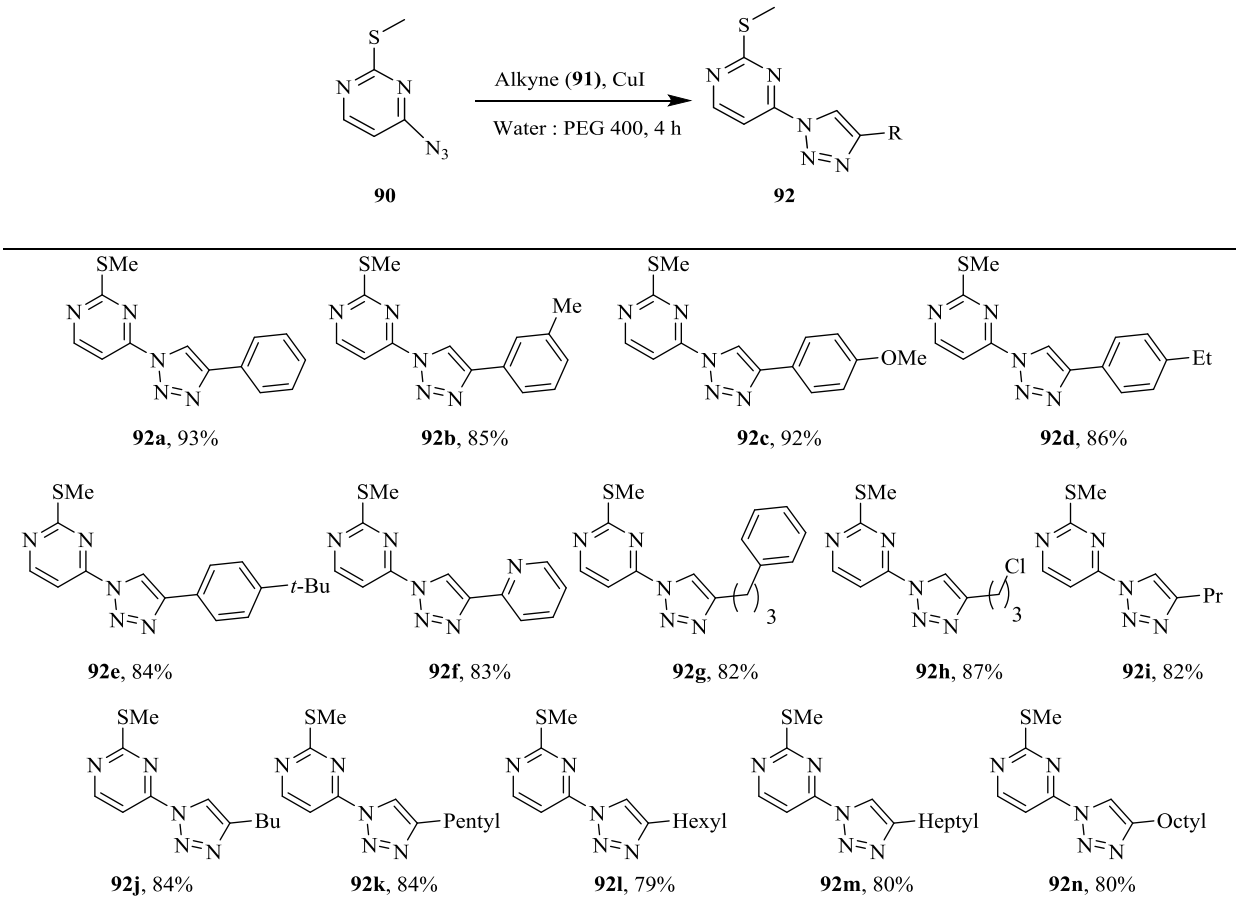


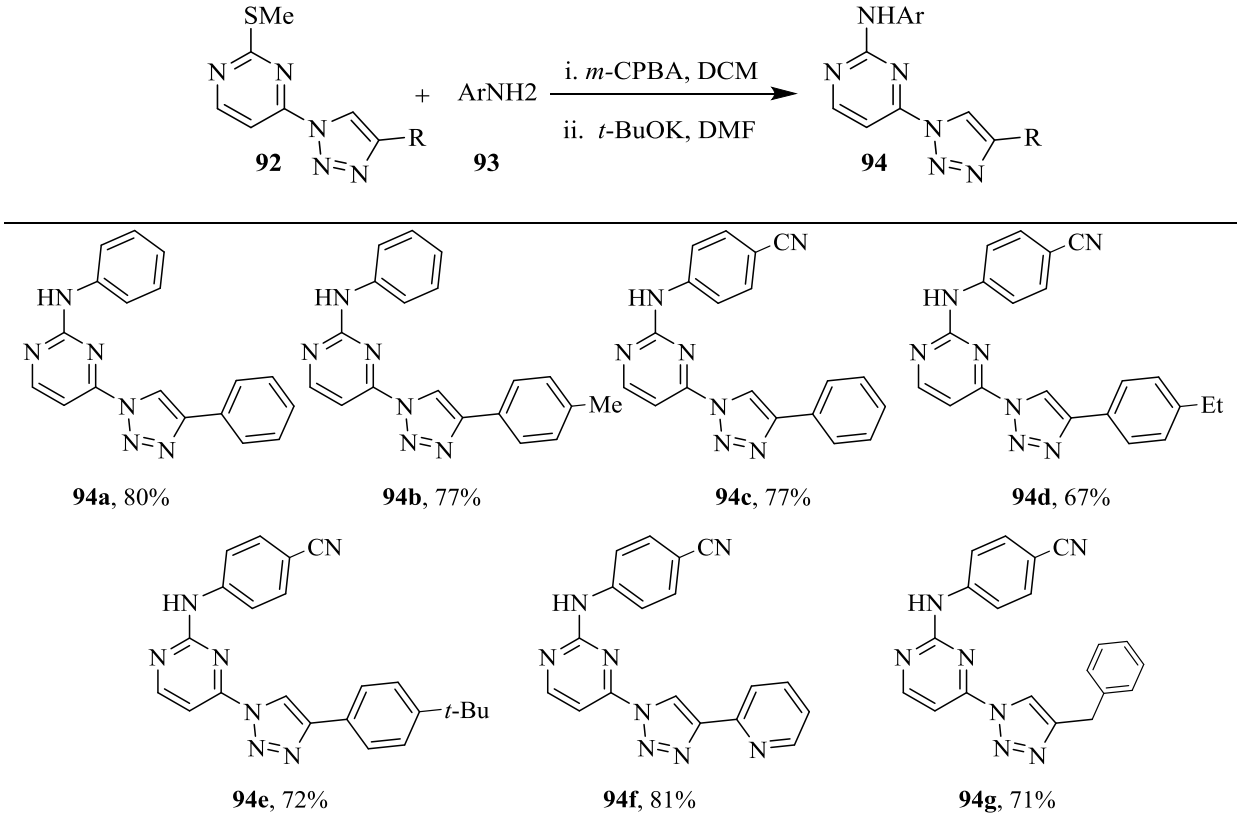
Figure 4.4: ^{1}H and ^{13}C NMR spectra of **92f**.



Scheme 4.17: Synthesis of 2-methylthio-4-triazolo-pyrimidines (**92**).

The aforementioned 2-methylthio-4-triazolo-pyrimidines (**92**) were then converted to 1-arylamino-4-triazolo-pyrimidines (**94a-h**) *via* oxidation of the thio group to sulfone with *m*-CPBA followed by nucleophilic substitution with aryl amine in the presence of potassium *tert*-butoxide in DMF (**Scheme 4.18**). Overall good to excellent yields of 1-arylamino-4-triazolo-pyrimidines were obtained (**Scheme 4.18**). Synthesis of **94g** was achieved by first reacting 2-(methylthio)pyrimidin-4(5*H*)-one with 4-cyanoaniline at 180 °C to give 1-(4-cyanophenyl)aminopyrimidin-4(*5H*)-one followed by reaction with $POCl_3$, NaN_3/DMF [11] and 3-phenyl-1-propyne in the presence of CuI (10 mol%). The ^{1}H NMR and ^{13}C NMR data of all the synthesized compounds were well in agreement with the structure of the compounds. In the ^{1}H NMR spectra of **94** the characteristic singlet peak for triazole C_4-proton appeared at ~9.10 ppm and a singlet at ~10.11 ppm along with all peaks for other protons. The peak owing to methyl protons attached with sulphur atom disappeared. In the ^{13}C NMR of **94**, C1-

carbon of pyrimidines ring appeared ~161 ppm along with other carbons. A representative ^{1}H NMR and ^{13}C NMR spectra of compound **94a** is given in figure **4.5**.



Scheme 4.18: Preparation of *N*-phenyl-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrimidin-2-amine.

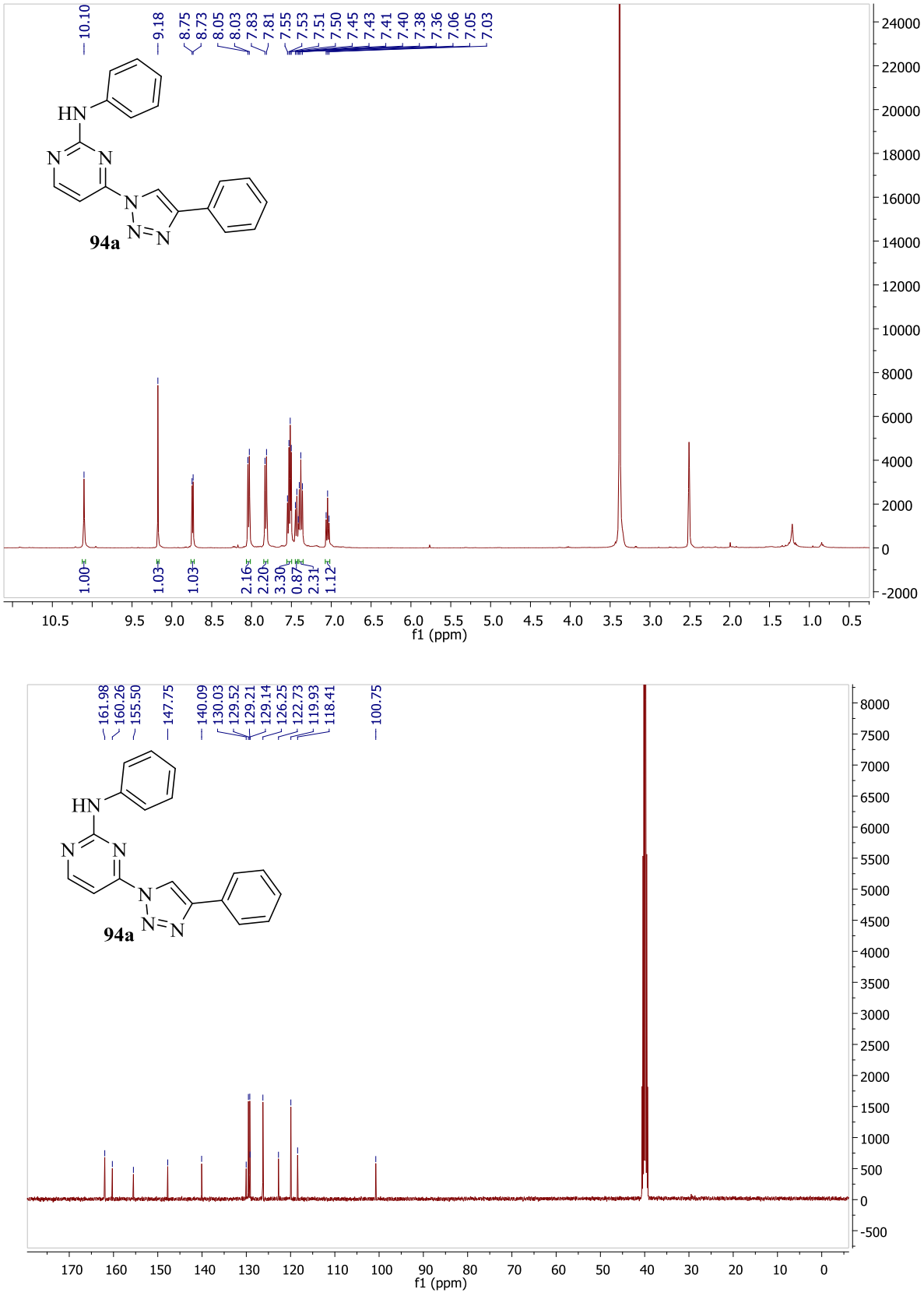
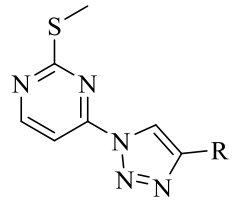


Figure **4.5:** ^{1}H and ^{13}C NMR spectra of **94a**.

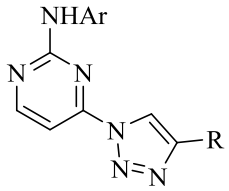
4.4 Antimicrobial activity

The synthesized compounds **92a-92n** and **94a-94g** were screened for antibacterial activities against Gram negative *E. coli, S. typhi, K. pneumoniae* and *P. putida*, Gram positive *B. subtilis* and *S. Aureus* bacteria. All compounds were evaluated at the concentrations ranging 0.5-200 μg/mL and scored for MIC as the level of growth inhibition of the microorganisms compared with Ciprofloxacin. Zone of inhibition was also calculated for all of the derivatized scaffolds. The data of antibacterial activities are depicted in **table 4.2**. The most effective data is shown in bold. Compound **92g** exhibited promising activity against Gram negative bacteria *S. typhi* and *P. putida* (MIC values ranging between 6.25 and 12.5 μg/mL) and moderate activity against *E. coli* and *K. pneumonia*. Further, compound **94c** showed excellent antimicrobial activity against *S. typhi*, *P. putida* and *K. pneumoniae* with (MIC values of 6.25 μg/mL) and moderate activity against *E. coli* (MIC 25 μg/mL) (Table 4.2, entry 18). Compound **94f** was found to be active against both Gram negative bacteria and Gram positive bacteria (MIC values ranging between 6.25 and 12.5 μg/mL) (Table 4.2, entry 21). These values are comparable with the positive control. All other compounds showed weak or poor activity towards both Gram positive and Gram negative bacteria and compound **92i** did not show any action towards Gram positive bacteria (Table 4.2, entry 9). Active compound **92g** contains a (3-phenyl)propyl group at C-4 position of triazole and compound **94c** and **94f** has (4-cyanophenyl)amino at C-1 of pyrimidine ring and tolyl and 2-pyridyl group at C-4 position of triazole, respectively. Kinetic assay of most potent compounds **94f**, **94c** and **92g** was also performed against all four Gram negative bacteria (**Figure 4.6**). It was observed that bactericidal activity increases with concentration and derivative **94f** was most effective against all bacteria showing ≈90 % bactericidal action when 50 μg/mL concentration was used. The results suggested that the antibacterial activity of these derivatives was also markedly influenced by their structural properties.

Table 4.2: Antibacterial activity of **92** and **94**.



92



94

Compound	*S. typhi*		*P.putida*		*E. coli*		*K. pneumoniae*		*B. subtilis*		*S. aureus*	
	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
92a	13	50	12	50	12	>50	14	>50	13	>25	13	>25
92b	13	50	13	50	14	50	14	50	12	50	14	50
92c	12	200	12	200	13	>100	13	>100	13	>100	12	>100
92d	13	>50	14	>50	14	>50	13	100	13	100	13	100
92e	14	25	15	25	14	50	13	50	14	>25	14	>25
92f	14	50	12	50	12	>25	13	>25	13	50	14	50
92g	16	6.25	17	6.25	16	>25	15	>25	15	50	14	>25
92h	15	12.5	15	12.5	15	12.5	15	25	14	25	14	12.5
92i	12	100	12	100	13	100	13	100	-[c]	-[c]	-[c]	-[c]
92j	14	>50	13	>50	13	100	14	100	13	>50	13	>50
92k	15	25	15	25	15	25	14	>25	14	>25	15	25
92l	14	50	15	50	14	50	14	>50	14	>50	13	50
92m	14	>50	14	>50	14	>50	13	100	13	>50	14	>50
92n	13	>100	12	>100	12	>100	13	100	13	100	13	100
94a	13	50	14	50	14	>50	12	>50	12	100	13	100
94b	13	50	14	50	15	>50	13	>50	13	100	12	100
94c	19	6.25	18	6.25	16	25	18	6.25	15	>25	15	>25
94d	14	>25	14	>25	14	50	13	50	13	50	12	>50

94e	14	>12.5	15	>12.5	15	>12.5	14	25	14	25	14	25
94f	18	6.25	18	6.25	18	12.5	17	6.25	17	12.5	17	12.5
94g	14	>25	14	>25	13	>25	14	50	15	50	13	100
Ciprofloxacin	22	6.25	21	6.25	20	6.25	20	6.25	22	6.25	21	6.25

[a]Zone of inhibition (mm), [b]Minimum inhibitory concentration (ug/ mL), -[c]No activity was observed.

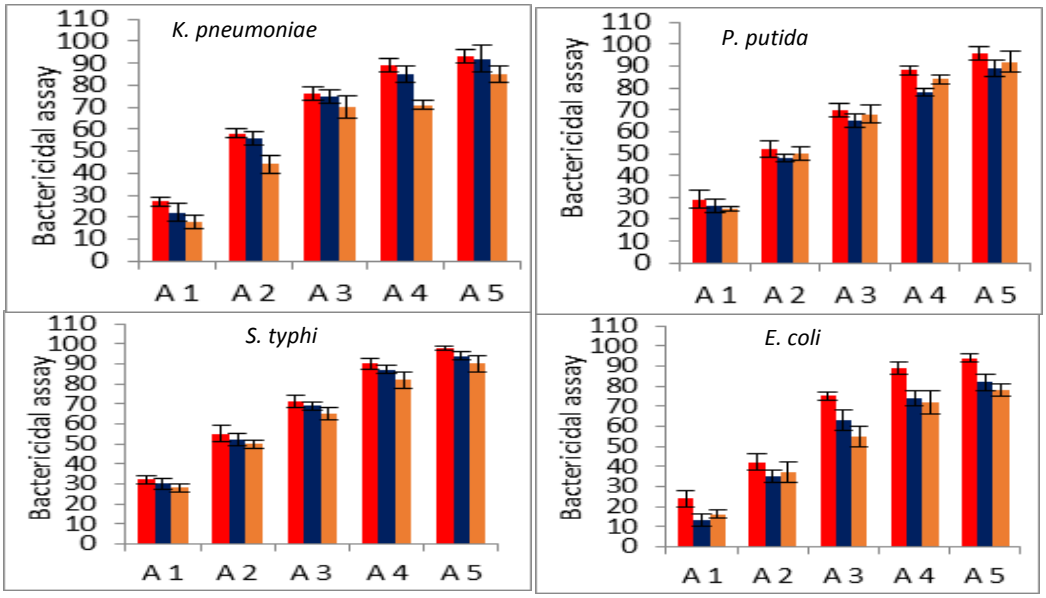
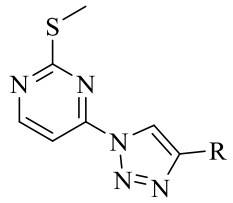


Figure 4.6: Kinetic essay of **94f** (red), **94c** (blue) and **92g** (orange). A1, A2, A3, A4, A5 represent the respective compound concentrations of 3.125, 6.25, 12.5, 25, and 50 µg/ml.

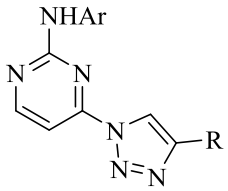
Further, antifungal activity of **92a-92n** and **94a-94g** was evaluated against *C. albicans, A. Flavus and F. oxysporum.* All the compounds were evaluated at the concentrations ranging from 0.5 µg/mL to 128 µg/mL and scored for MIC as the level of growth inhibition of the microorganisms compared with Amphotecerin B as positive control. The data of antifungal activities are depicted in table 4.3. It is noticeable from the initial screening that the hybrids **92g**, **92h**, **94c** and **94f** displayed fair to good activity against all three fungi tested with MIC values ranging from 6.25 to 25 µg/mL and ZOI of 17-18. These values are comparable with the positive control. The most effective data is shown in bold. All compounds were found to be moderate to good antifungal agents with zone of inhibition 11-18 mm. It was found that **92h** and **94c** were more potent among all compounds towards all three fungi. Compond **92h** containing 3-chloropropyl group attached with triazole part and having 2-thiomethylpyrimidine, showed MIC value of 6.25 µg/mL with zone of inhibition 17-18 mm while **94c** having 4-methylphenyl ring attached with triazole and 4-aminobenzonitrile group with pyrimidine ring showed MIC value 6.25-12.5 µg/mL with zone of inhibition 17-18 mm

against different fungi. It is worth to note that most of the compounds were moderately active towards all three fungi and comparable in concentration with the positive control (**Table 4.3**).

Table 4.3: Antifungal activity of triazole-pyrimidnines **92** and **94**.



92



94

Compound	*C. albicans*		*A. flavus*		*F. oxysporum*	
	ZOI[a]	MIC[b]	ZOI	MIC	ZOI	MIC
92a	13	100	14	100	14	100
92b	14	100	13	100	13	100
92c	14	>100	14	>100	13	>100
92d	12	>200	12	>200	11	>200
92e	14	100	14	>100	13	>100
92f	14	>100	14	>100	13	>100
92g	15	25	15	25	14	>25
92h	**17**	**6.25**	**18**	**6.25**	**18**	**>6.25**
92i	14	>100	13	>100	13	>100
92j	14	100	13	>100	13	>100
92k	14	50	13	50	14	50
92l	13	100	13	100	14	100
92m	13	>100	13	>100	13	>100
92n	12	>100	12	>100	14	>100
94a	13	>100	14	>100	13	>100
94b	13	>50	13	>50	14	100

94c	**18**	**>6.25**	**17**	**>6.25**	**17**	**12.5**
94d	14	50	14	50	14	50
94e	15	50	14	50	14	>50
94f	16	12.5	16	12.5	15	25
94g	15	50	14	50	14	>50
Amphotericin B	23	30	23	30	22	30

[a]Zone of inhibition, [b]Minimum inhibitory concentration (ug/ mL).

4.4 Conclusion

In summary, we have prepared 2-thiomethyl 1,2,3-triazolo-pyrimidines and 1-arylamino-1,2,3-triazolo-pyrimidines in good to excellent yields using CuAAC reaction in water: PEG-400. Compounds from both the series have been evaluated for *in vitro* antimicrobial activity against different Gram positive and Gram negative bacterial strains and compound **92g**, **94c** and **94f** were found to have excellent antimicrobial activity. Compound **92h** and **94c** also showed good anti-fungal activity.

4.5 Experimental:

General information: All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined in open capillary tubes on an EZ-Melt automated melting point apparatus and are uncorrected. Reactions were monitored by using thin layer chromatography (TLC) on 0.2 mm silica gel F254 plates (Merck) and detected under UV light. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. High-resolution mass spectra (HRMS) were obtained on a quadrupole time of flight (qTOF) mass spectrometer. Silica gel (100–200 mesh) was used for column chromatography. Chemical shifts were reported in parts per million (ppm) using deuterated solvent peak or tetramethylsilane (internal) as the standard.

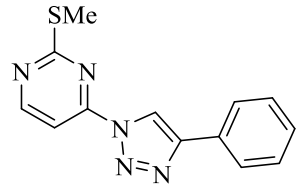
Preparation of 4-chloro-2-(methylthio)pyrimidine (89): NaOH (0.4 g, 10 mmol) was added portion-wise to a suspension of 2-thiouracil (1.28 g, 10 mmol) in H_2O (25 mL) at rt. After the reaction mixture was stirred for 30 min, methyliodide (1 mL, 15 mmol) was added and stirred at rt for 24 h. White precipitate occurred which was filtered and washed with water, dried it and refluxed it with $POCl_3$ (1.2 mL, 13 mmol) for 45 min. The mixture was poured into 100 mL ice-water and stirred at room temperature for 1 h. The resulting precipitate was filtered, washed with water, dried and used for next step without further purification.

Preparation of 4-azido-2-(methylthio)pyrimidine (90): 4-Chloro-2-(methylthio)-pyrimidines (1.6 g, 10 mmol) was dissolved in DMF (15 mL) and sodium azide (0.65 g, 10 mmol) was added and stirred at rt for 6 h. Added water to reaction mass and washed with ethylacetate (2×30 mL). Dried the organic layer over sodium sulphate and concentrated it. The product was used for next step without further purification.

Preparation of 2-(methylthio)-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)pyrimidines (92a): To a 10 mL round bottom flask was added water : PEG-400 (5 mL, 1:1 *v/v*), 4-azido-2-(methylthio)pyrimidines **90** (167 mg, 1 mmol), alkyne **91** (1.1 mmol) and CuI (19 mg). The reaction mass was stirred at room temperature for 4 h. The progress of the reaction was monitored by thin layer chromatography. Reaction mixture was added to separating funnel and extracted by ethyl acetate (10 mL × 2). Combined organic layer was dried over sodium sulphate and purified by column chromatography over silica gel 100-200 mesh using hexane/ ethyl acetate as eluent.

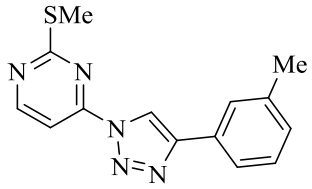
Preparation of *N*-phenyl-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)pyrimidin-2-amine (94a): Compound **92a** (0.66 mmol) was dissolved in dichloromethane (5 mL) in a 50 mL round bottom flask and cooled to 0 °C. *m*-Chloroperbenzoic acid (339 mg, 1.98 mmol) was added to the cooled solution and reaction mass stirred by bringing reaction temperature to room temperature for 4 h. The reaction mass was concentrated on rotary evaporator, added water (5 mL) and extracted by ethyl acetate (2 × 10 mL). Organic layer was dried over sodium sulphate, and concentrated. The product was then dissolved in DMF at 0 °C and potassium *tert*-butoxide (168 mg, 1.5 mmol) was added slowly. After 15 minutes, arylamine (0.73 mmol) was added and stirred at room temperature for 16 h. The progress of the reaction was monitored by thin layer chromatography. Reaction mixture was added to separating funnel and extracted by ethyl acetate (10 mL × 2). Combined organic layer was dried over sodium sulphate and purified by column chromatography over silica gel 100-200 mesh using hexane/ ethyl acetate as eluent.

2-(Methylthio)-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)pyrimidines (92a):



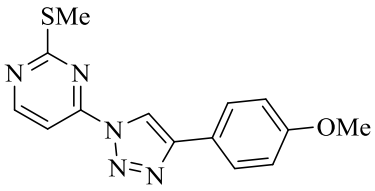
Yield: 250 mg (93%); white solid; mp 178 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.69 (d, *J* = 5.3 Hz, 1H), 7.93 (d, *J* = 7.4 Hz, 2H), 7.83 (d, *J* = 5.4 Hz, 1H), 7.47 (t, *J* = 7.4 Hz, 2H), 7.40 (d, *J* = 7.2 Hz, 1H), 2.64 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.64, 159.63, 154.82, 148.43, 129.53, 128.99, 128.86, 126.03, 116.45, 104.77, 14.31; HRMS (ESI) *m/z*: calcd for $C_{13}H_{12}N_5S$ 270.0735, found 270.0765 $[M + H]^+$.

2-(Methylthio)-4-(4-(*m*-tolyl)-1H-1,2,3-triazol-1-yl)pyrimidine (92b):



Yield: 240 mg (85%); white solid; mp 130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.70 (d, *J* = 5.4 Hz, 1H), 7.84 (d, *J* = 5.4 Hz, 1H), 7.78 (s, 1H), 7.72 (d, *J* = 7.7 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 7.6 Hz, 1H), 2.66 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.60, 159.60, 154.80, 148.52, 138.71, 129.64, 129.37, 128.89, 126.65, 123.13, 116.38, 104.76, 21.47, 14.34; HRMS (ESI) *m/z*: calcd for $C_{14}H_{14}N_5S$ 284.0964, found 284.0962 $[M + H]^+$.

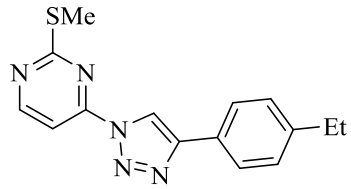
4-(4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2-(methylthio)pyrimidines (92c):



Yield: 275 mg (92%); white solid; mp 184-185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 5.6 Hz, 1H), 8.69 (s, 1H),

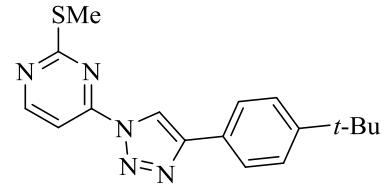
7.86 (dd, *J* = 12.3, 7.0 Hz, 3H), 7.01 (d, *J* = 8.7 Hz, 2H), 3.88 (s, 3H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.56, 160.11, 159.56, 154.84, 148.30, 127.38, 122.14, 115.50, 114.39, 104.75, 55.38, 14.33; HRMS (ESI) *m/z*: calcd for $C_{14}H_{14}N_5OS$ 300.0914, found 300.0914 $[M + H]^+$.

4-(4-(4-Ethylphenyl)-1*H*-1,2,3-triazol-1-yl)-2-(methylthio)pyrimidines (92d):



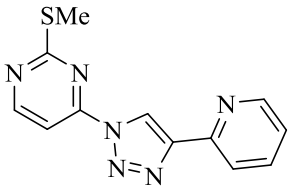
Yield: 255 mg (86%); yellow solid; mp 138-139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.72 (d, *J* = 5.4 Hz, 1H), 7.87 (dd, *J* = 6.7, 4.5 Hz, 3H), 7.33 (d, *J* = 8.2 Hz, 2H), 2.73 (q, *J* = 7.6 Hz, 2H), 2.67 (s, 3H), 1.30 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.60, 159.60, 154.86, 148.55, 145.23, 128.51, 126.92, 126.04, 116.07, 104.79, 28.75, 15.52, 14.34; HRMS (ESI) *m/z*: calcd for $C_{15}H_{16}N_5S$ 298.1121, found 298.1119 $[M + H]^+$.

4-(4-(4-(*Tert*-butyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-(methylthio)pyrimidines (92e):



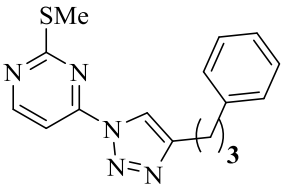
Yield: 273 mg (84%); pale yellow solid; mp 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.71 (d, *J* = 5.4 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 5.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 2.67 (s, 3H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.58, 159.59, 154.86, 152.09, 148.45, 126.68, 125.93, 125.79, 116.10, 104.80, 34.78, 31.29, 14.34; HRMS (ESI) *m/z*: calcd for $C_{13}H_{12}N_5S$ 326.1434, found 326.1434 $[M + H]^+$.

2-(Methylthio)-4-(4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)pyrimidines (92f):



Yield: 224 mg (83%); off white solid; mp 186-187 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1H), 8.73 (d, *J* = 5.4 Hz, 1H), 8.68 (d, *J* = 4.3 Hz, 1H), 8.29 (d, *J* = 7.9 Hz, 1H), 7.88-7.87 (m, 1H), 7.86 (d, *J* = 5.4 Hz, 1H), 7.34 (dd, *J* = 6.8, 5.3 Hz, 1H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.93, 159.74, 154.85, 149.73, 149.28, 148.93, 137.07, 123.51, 120.77, 119.09, 104.73, 14.37; HRMS (ESI) *m/z*: calcd for $C_{12}H_{11}N_6S$ 271.0760, found 271.0759 $[M + H]^+$.

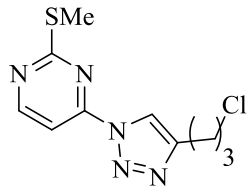
2-(Methylthio)-4-(4-(3-phenylpropyl)-1*H*-1,2,3-triazol-1-yl)pyrimidines (92g):



Yield: 232 mg (82%); yellow solid; mp 56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 5.4 Hz, 1H), 8.32 (s, 1H), 7.81 (d, *J* = 5.4 Hz,

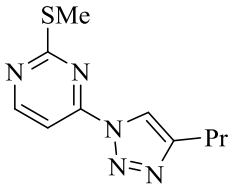
1H), 7.36 – 7.29 (m, 2H), 7.23 (d, *J* = 7.7 Hz, 3H), 2.89 – 2.84 (m, 2H), 2.78 – 2.72 (m, 2H), 2.64 (s, 3H), 2.15 – 2.06 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.49, 159.56, 154.91, 148.94, 141.62, 128.50, 128.41, 125.96, 118.09, 104.66, 35.30, 30.76, 25.09, 14.29; HRMS (ESI) *m/z*: calcd for $C_{16}H_{18}N_5S$ 312.1277, found 312.1277 $[M + H]^+$.

4-(4-(3-Chloropropyl)-1*H*-1,2,3-triazol-1-yl)-2-(methylthio)pyrimidines (92h):



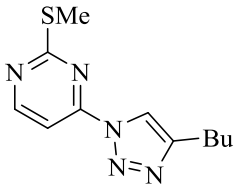
Yield: 234 mg (87%); off-white solid; mp 81-82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 5.3 Hz, 1H), 8.47 (s, 1H), 7.81 (d, *J* = 5.3 Hz, 1H), 3.63 (t, *J* = 5.7 Hz, 2H), 3.01 (s, 2H), 2.63 (s, 3H), 2.26 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.58, 159.64, 154.85, 147.41, 118.54, 104.65, 44.01, 31.59, 22.66, 14.30; HRMS (ESI) *m/z*: calcd for $C_{10}H_{13}ClN_5S$ 270.0575, found 270.0569 $[M + H]^+$.

2-(Methylthio)-4-(4-propyl-1*H*-1,2,3-triazol-1-yl)pyrimidines (92i):



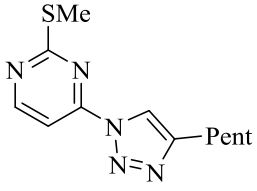
Yield: 192 mg (82%); yellow sticky liquid, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, *J* = 5.4 Hz, 1H), 8.31 (s, 1H), 7.79 (d, *J* = 5.4 Hz, 1H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.63 (s, 3H), 1.87 – 1.69 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.45, 159.51, 154.94, 149.18, 118.00, 104.65, 27.56, 22.42, 14.25, 13.71; HRMS (ESI) *m/z*: calcd for $C_{10}H_{14}N_5S$ 236.0964, found 236.0962 $[M + H]^+$. 2.63 (s, 3H), 1.87 – 1.69 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 4H).

4-(4-Butyl-1*H*-1,2,3-triazol-1-yl)-2-(methylthio)pyrimidines (92j):



Yield: 209 mg (84%); yellow solid; mp 55 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, *J* = 5.4 Hz, 1H), 8.30 (s, 1H), 7.78 (d, *J* = 5.4 Hz, 1H), 2.81 (t, *J* = 7.7 Hz, 2H), 2.63 (s, 3H), 1.80 – 1.66 (m, 2H), 1.51 – 1.35 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.45, 159.50, 154.95, 149.41, 117.92, 104.65, 31.22, 25.26, 22.23, 14.25, 13.78; HRMS (ESI) *m/z*: calcd for $C_{11}H_{16}N_5S$ 250.1121, found 250.1120 $[M + H]^+$.

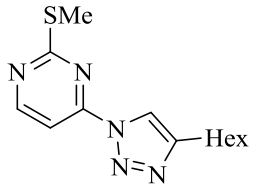
2-(Methylthio)-4-(4-pentyl-1*H*-1,2,3-triazol-1-yl)pyrimidines (92k):



Yield: 221 mg (84%), yellow solid; mp 89 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, *J* = 5.4 Hz, 1H), 8.30 (s, 1H), 7.79 (d, *J* = 5.4 Hz, 1H), 2.84 – 2.77 (m, 2H), 2.63 (s, 3H), 1.82 – 1.68 (m, 2H), 1.43 – 1.34 (m, 4H), 0.97 – 0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.45, 159.50, 149.45, 117.92, 107.96, 104.67, 31.34, 28.83, 25.56, 22.39, 14.26, 13.98; HRMS

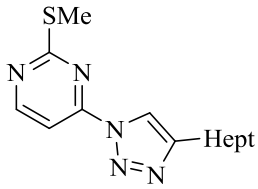
(ESI) *m/z*: calcd for $C_{12}H_{18}N_5S$ 264.1277, found 264.1276 $[M + H]^+$.

4-(4-Hexyl-1*H*-1,2,3-triazol-1-yl)-2-(methylthio)pyrimidines (92l):



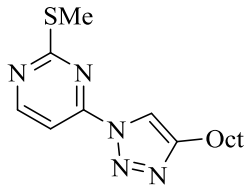
Yield: 218 mg (79%); yellow sticky solid; mp 56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 5.4 Hz, 1H), 8.31 (s, 1H), 7.79 (d, *J* = 5.4 Hz, 1H), 2.81 (t, *J* = 7.7 Hz, 2H), 2.63 (s, 3H), 1.78 – 1.69 (m, 2H), 1.43 – 1.36 (m, 2H), 1.32 (td, *J* = 7.1, 3.9 Hz, 4H), 0.89 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.43, 159.50, 154.94, 149.44, 117.92, 104.65, 31.54, 29.12, 28.85, 25.59, 22.56, 14.27, 14.08; HRMS (ESI) *m/z*: calcd for $C_{13}H_{20}N_5S$ 278.1434, found 278.1430 $[M + H]^+$.

4-(4-Heptyl-1*H*-1,2,3-triazol-1-yl)-2-(methylthio)pyrimidines (92m):



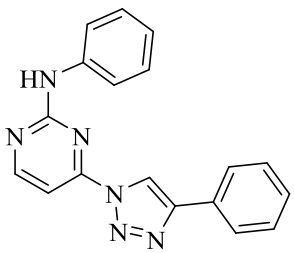
Yield: 232 mg (80%), yellow solid, mp 62 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, *J* = 5.4 Hz, 1H), 8.30 (s, 1H), 7.79 (d, *J* = 5.4 Hz, 1H), 2.80 (t, *J* = 7.7 Hz, 2H), 2.63 (s, 3H), 1.79 – 1.69 (m, 2H), 1.44 – 1.24 (m, 8H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.45, 159.49, 154.96, 149.44, 117.90, 104.66, 31.72, 29.15, 29.13, 29.00, 25.59, 22.62, 14.25, 14.07; HRMS (ESI) *m/z*: calcd for $C_{14}H_{22}N_5S$ 292.1590, found 292.1590 $[M + H]^+$.

2-(Methylthio)-4-(4-octyl-1*H*-1,2,3-triazol-1-yl)pyrimidines (92n):



Yield: 244 mg (80%); off-white solid; mp 67 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, *J* =5.3 Hz, 1H), 8.34 (s, 1H), 7.81 (d, *J* = 5.3 Hz, 1H), 2.81 (t, *J* = 7.5 Hz, 2H), 2.64 (s, 3H), 1.87 – 1.66 (m, 2H), 1.43 – 1.25 (m, 10H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.44, 159.50, 154.96, 149.48, 117.95, 104.67, 31.85, 29.32, 29.20, 29.19, 29.16, 25.60, 22.66, 14.27, 14.11; HRMS (ESI) *m/z*: calcd for $C_{15}H_{24}N_5S$ 306.1747, found 306.1739 $[M + H]^+$.

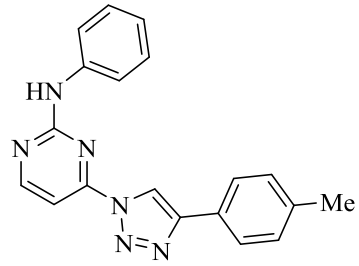
***N*-phenyl-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)pyrimidin-2-amine (94a):**



Yield: 242 mg (77%); pale yellow solid; mp 243-244 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1H), 9.18 (s, 1H), 8.74 (d, *J* = 5.3 Hz, 1H), 8.04 (d, *J* = 7.2 Hz, 2H), 7.82 (d, *J* = 7.7 Hz, 2H), 7.56 – 7.48 (m, 3H), 7.44 (d, *J* = 7.4 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 2H), 7.05 (t, *J* = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.98, 160.26, 155.50, 147.75, 140.09, 130.03, 129.52, 129.21, 129.14, 126.25, 122.73,

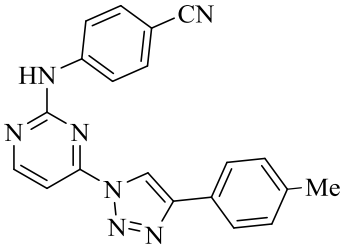
119.93, 118.41, 100.75; HRMS (ESI) *m/z*: calcd for $C_{18}H_{15}N_6$ 315.1354, found 315.1323 $[M + H]^+$.

_N_-phenyl-4-(4-(_p_-tolyl)-1_H_-1,2,3-triazol-1-yl)pyrimidin-2-amine (94b):



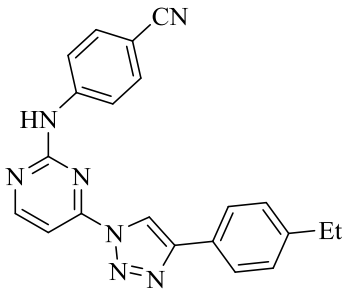
Yield: 262 mg (80%); pale yellow solid; mp 248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.10 (s, 1H), 8.74 (s, 1H), 7.91 (d, *J* = 7.3 Hz, 2H), 7.81 (d, *J* = 7.4 Hz, 2H), 7.49 (d, *J* = 2.7 Hz, 1H), 7.39 – 7.35 (m, 2H), 7.32 (d, *J* = 7.4 Hz, 2H), 7.04 (t, *J* = 6.1 Hz, 1H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.93, 160.25, 155.50, 147.81, 140.07, 138.59, 130.06, 129.21, 127.23, 126.17, 122.72, 119.91, 117.93, 100.74, 21.38; HRMS (ESI) *m/z*: calcd for $C_{19}H_{17}N_6$ 329.1509, found 329.1502 $[M + H]^+$.

4-((4-(4-(_p_-Tolyl)-1_H_-1,2,3-triazol-1-yl)pyrimidin-2-yl)amino)benzonitrile (94c):



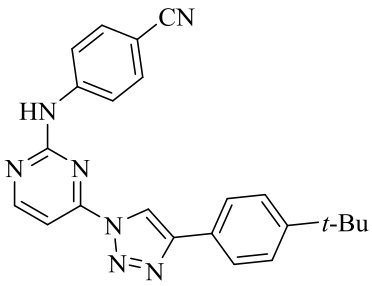
Yield: 282 mg (80%); white solid; mp 223-224 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.60 (s, 1H), 9.12 (s, 1H), 8.81 (d, *J* = 5.0 Hz, 1H), 8.03 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 5.1 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.02, 159.67, 155.49, 147.93, 144.56, 138.63, 133.68, 130.02, 127.15, 126.19, 119.88, 119.41, 117.97, 103.74, 102.16, 21.37; HRMS (ESI) *m/z*: calcd for $C_{20}H_{16}N_7$ 354.1462, found 354.1459 $[M + H]^+$

4-((4-(4-(4-Ethylphenyl)-1_H_-1,2,3-triazol-1-yl)pyrimidin-2-yl)amino)benzonitrile (94d):



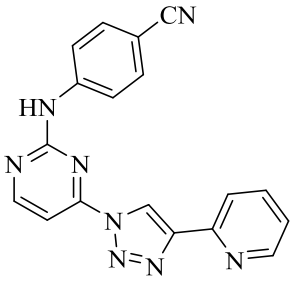
Yield: 264 mg (72%); off-white solid; mp 230-231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 9.14 (s, 1H), 8.81 (d, *J* = 5.3 Hz, 1H), 8.03 (d, *J* = 8.6 Hz, 2H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.82 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 5.3 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 2.67 (q, 2H), 1.22 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.71, 159.43, 155.35, 148.16, 145.02, 143.87, 133.44, 132.88, 128.39, 126.87, 125.85, 119.36, 119.08, 116.09, 114.05, 104.41, 101.43, 28.54, 15.42; HRMS (ESI) *m/z*: calcd for $C_{21}H_{18}N_7$ 368.1618, found 368.1617 $[M + H]^+$.

4-((4-(4-(4-(*Tert*-butyl)phenyl)-1*H*-1,2,3-triazol-1-yl)pyrimidin-2-yl)amino)benzonitrile (94e):



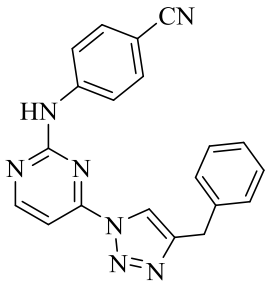
Yield: 284 mg (72%); white solid; mp 185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 9.14 (s, 1H), 8.81 (d, *J* = 5.3 Hz, 1H), 8.02 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 5.3 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.04, 159.66, 155.48, 151.74, 147.88, 144.58, 133.68, 127.17, 126.25, 126.08, 119.90, 119.41, 118.07, 103.74, 102.16, 34.93, 31.49; HRMS (ESI) *m/z*: calcd for $C_{23}H_{22}N_7$ 396.1931, found 396.1929 $[M + H]^+$.

4-((4-(4-(Pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)pyrimidin-2-yl)amino)benzonitrile (94f):



Yield: 275 mg (81%); yellow solid; mp 134 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H), 8.89 (d, *J* = 5.4 Hz, 1H), 8.73 – 8.67 (m, 1H), 8.20 – 8.15 (m, 2H), 7.98 (td, *J* = 7.7, 1.8 Hz, 2H), 7.92 (d, *J* = 5.4 Hz, 1H), 7.45 (ddd, *J* = 7.6, 4.8, 1.1 Hz, 1H), 7.40 – 7.34 (m, 1H), 6.64 – 6.56 (m, 1H), 6.15 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.16, 161.32, 154.86, 153.51, 153.49, 150.40, 149.11, 148.74, 137.96, 133.92, 124.31, 120.79, 120.33, 113.89, 105.74; HRMS (ESI) *m/z*: calcd for $C_{18}H_{13}N_8$ 341.1258, found 341.1252 $[M + H]^+$.

4-((4-(4-Benzyl-1*H*-1,2,3-triazol-1-yl)pyrimidin-2-yl)amino)benzonitrile (94g):



Yield: 243 mg (67%); off-white solid; mp 240-241 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.60 (s, 1H), 9.18 (s, 1H), 8.83 (d, *J* = 5.3 Hz, 1H), 8.68 (d, *J* = 16.4 Hz, 1H), 8.17 (s, 1H), 8.00 (d, *J* = 8.8 Hz, 3H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 5.4 Hz, 1H), 7.50 (d, *J* = 22.8 Hz, 1H), 3.51 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.21, 159.67, 144.53, 137.89, 133.64, 120.25, 120.22, 120.19, 119.84, 119.44, 103.91, 102.26, 98.88, 70.24; HRMS (ESI) *m/z*: calcd for $C_{20}H_{16}N_7$ 354.1462, found 354.1462 $[M + H]^+$.

4.5.1 Antibacterial assay[42]

The synthesized compounds were tested for antibacterial activity against four Gram negative (*Salmonella typhi, Pseudomoas putida, Escherichia coli, Klebsiella pneumoniae*) and two Gram positive (*Bacillus subtilis, Staphylococcus aureus*) bacteria as per the following

standard method (NCCL, 1993). The tested bacterial cultures were freshly cultured and inoculated into the Luria-bertani broth medium and kept for overnight at 37 °C with shaking at 150 rpm. Meanwile, autoclaved Luria-bertani agar medium was equipped and poured into sterile glass petri-dishes (90 mm) under aseptic conditions. After solidification of medium, 100 µl culture of each test strain (10^7 CFU/mL) was spread over it using a sterile glass spreader and left for 15 min for complete adsorption. After adsorption, wells of size 6 mm diameter were made by the sterile metallic borer and the solution of compounds with different concentration were poured into the wells. The compounds to be tested were dissolved in DMSO (Merck, India). After incubation at 37 °C for 24 h, the diameter of the zone of inhibition was measured in comparison with standard antibiotic 'ciprofloxacin'. Solvent DMSO was used as negative control while antibiotic 'ciprofloxacin' was used as positive control. For the MIC assay, test compounds were prepared in the concentrations of 8, 16, 32, 64, 128 and 256 µg/mL in DMSO and serial diluted test samples of each compound (150 µl) were added in 96 well micro-trays. The same amount of test microorganism was added to micro-trays well to obtain a final volume of 300 µl and incubated at 37 °C for 24 h. MIC value is defined as the lowest concentration of compound that inhibit the visible growth of bacteria (OD_{600} less than 0.06). Each assay was performed in duplicate sets.

4.5.2 Antifungal assay[42]

Antifungal activities of test compounds were determined by agar well diffusion method against the fungal strains *Candida albicans*, *Aspergillus flavus* and *Fusarium oxysporum*. For the experimental work, a loopful of each strain was grown in Potato dextrose broth (PDB, Himedia, India) medium at 28 °C for 4-5 days. Following optimal growth of each fungal strain, 100 µl of culture was uniformly spread on the potato dextrose agar medium plate. Following adsorption, wells of 6 mm was prepared by the sterile metallic borer and solution of working compound of different concentration was poured into the wells. Plates were incubated at 28 °C for 4-5 days under dark conditions. Mean diameter of inhibition zone was measured to determine the antifungal activity. For the MIC assay, sterile test tubes containing 5 mL of sterilized czapeks dox broth medium was inoculated with 100 µl of freshly grown culture of each test strain and appropriate amount of compound was added to achieve the desired concentrations. The tubes were incubated at 28 °C for 5 days under dark conditions and carefully observed for the presence of turbidity. Amphotericin B was used as positive control. The experiment was performed in duplicate sets.

4.7 References

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Chapter V

Synthesis and antimicrobial study of indolyl-triazole derivatives

5.1 **Introduction**

Indoles are bicyclic aromatic heterocycles consisting of a six membered benzene ring fused with five-membered nitrogen containing pyrole ring which forms basic unit in a large number of naturally occurring compounds.[1,2] Indole skeleton is considered as most attractive heterocyclic framework with a wide range of biological and medicinal activities which is lavishly found in natural products as well as therapeutic agents.[3-9] Among them, 3-substituted indoles are chief building block in pharmaceutical chemistry as well as in natural products.[10-21] Indole is basic structural motif in many compounds *i.e*. plant hormone auxin,[22] β-blocker pindolol,[23] CDK-4 inhibitor,[24] tyr-kinase inhibitor,[25,26] anti-inflammatory drug indomethacin[27] and natural hallucinogen dimethyltryptamine[28]. Besides this, indole moiety is also found in heterocycles with diverse pharmacological properties like analgesic,[29] melatonin antagonist,[30,31] anti-allergic,[32] antibacterial,[33] antifungal,[34] antiviral,[35] antimalarial,[36] anti-histamine,[37] anti-cancer,[38,39] cardio-vusculant[40] and anti-oxidant[41,42] etc. Because of their wide applications in medicinal and pharmaceutical chemistry, a large number of indole containing heterocycles have been synthesized and used in different bio-medical applications (**Figure 5.1**).

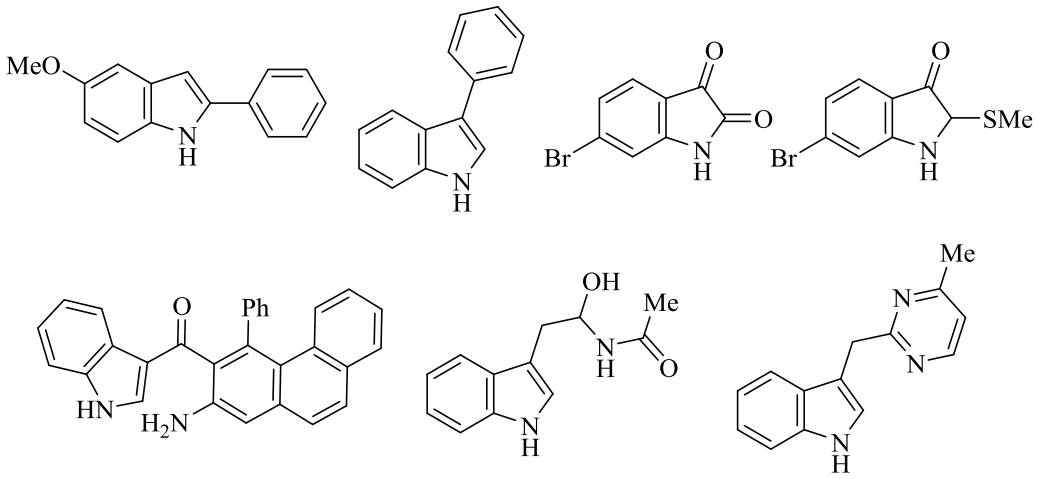
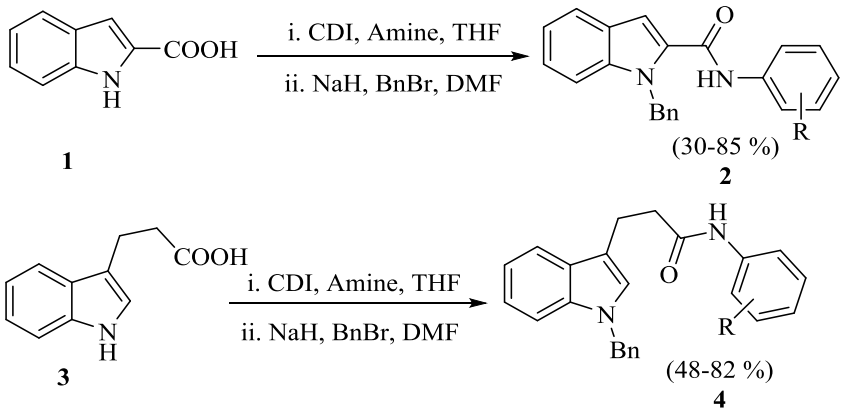


Figure 5.1: Structure of some antibacterial indole scaffolds.

A large number of reports on biological activity of indoles supports them to be privileged heterocyclic framework. Different indole derived heterocycles have been used as antimicrobial, antifungal and antibacterial agents (Figure 5.1). A brief overview of some recent reports on the antimicrobial activity of indole based heterocycles is described below:

Olgen group have constructed a series of novel indole carboxamide **2** and propanimide **4** using indole-2-carboxylic acid to evaluate them for antimicrobial activity (**Scheme 5.1**).[43-45] After the successful synthesis of derivatives, antimicrobial screening was done against *S.*

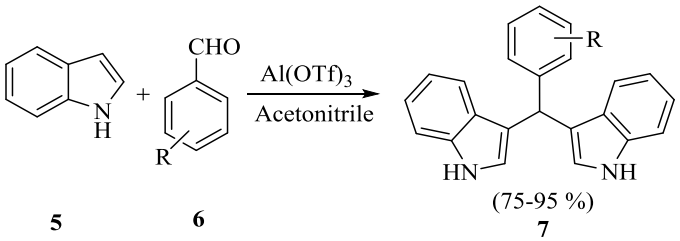
aureus, B. subtilis, E. coli and *A. niger* and their zone of inhibition was calculated. All compounds exhibited zone of inhibition 10-22 mm against bacteria and fungi. When selected derivatives were utilized to calculate MIC value, none of the compounds showed comparable MIC value with ampicillin against bacteria. While slight inhibition was seen against *C. albicans* with MIC range 6.56-13.12 µg/mL. SAR study revealed that position of substituent strongly affected the antimicrobial action.



CDI = 1,1'-carbonyldiimidazole, R = 4-Cl, 3-Cl-4-F,
BnBr = $C_6H_5CH_2Br$, 4-$FC_6H_4CH_2Br$

Scheme 5.1: Synthesis of indolyl-carboxamides.

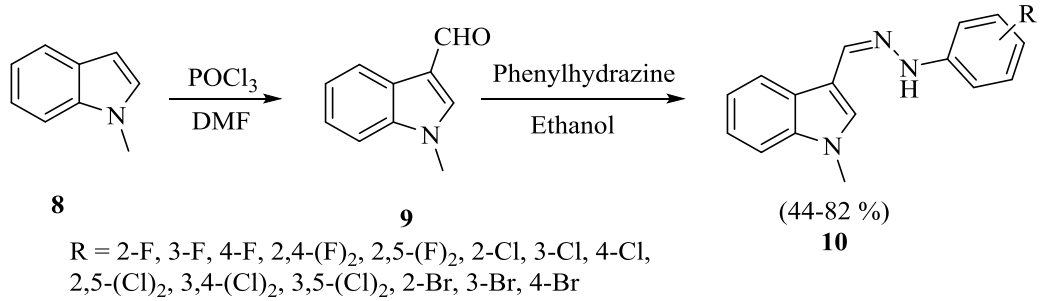
Kamal *et al* have synthesized bis-indolylmethanes **7** by the reaction of indoles with different aldehydes in the presence of aluminium catalyst (**Scheme 5.2**).[46] The synthesized derivatives were screened for antimicrobial activity against a series of bacteria and fungi and their zone of inhibition was calculated at the concentration of 100 and 150 µg/mL. Most of the compounds were found inactive against *K. pneumoniae*. Scaffold having nitrofuryl or thienyl group showed better antibacterial activity with zone of inhibition 16-19 mm. All compounds were most active against *S. aureus* among all bacteria. All compounds were also tested against fungal strains *C. albicans, R. orygae* and *A. niger*. Among all derivatives, only 5-nitrosubstituted derivative showed good activity against all fungi, while all other compounds were inactive.



R = H, 2-Cl, 4-Cl, 4-Br, 2-NO_2, 3-NO_2, 4-NO_2, 4-CH_3, 4-OCH_3, 2-OH.
4-OH, 3,4-$(Cl)_2$, 3,4,5-$(OCH_3)_3$, 2-Thienyl, 5-Nitrofuryl, 5-Nitrothienyl

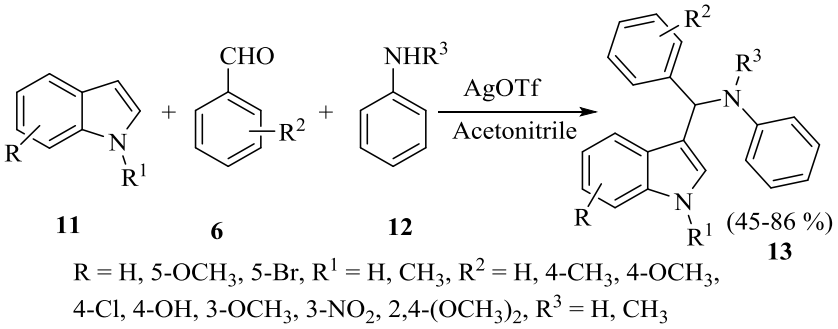
Scheme 5.2: Synthesis of bis-indolyl methanes.

Suzen and coworkers synthesized 1-methylindole-3-carbaldehyde hydrazone derivatives **10** to evaluate them against multi-drug resistant bacteria.[47,48] 1-Methylindole was formylated followed by reaction with different hydrazines to get indole-hydrazone derivatives **10** (**Scheme 5.3**). The synthesized series of indolyl-hydrazones was tested against Gram positive *S. aureus, B. subtilis, MRSA* and Gram negative *E. coli* and fungi *C. albicans*. Their MIC value was calculated using ampicillin, ciprofloxacin and fluconazole as standard drugs. All the scaffolds showed MIC value 6.25-100 μg/mL against Gram positive, 50-100 μg/mL against Gram-negative bacteria and 3.125-25 μg/mL against fungi *C. albicans*. It was observed that scaffolds having halo (difluoro or dichloro) substitutions were better antimicrobial agent against Gram-positive bacteria and fungi.



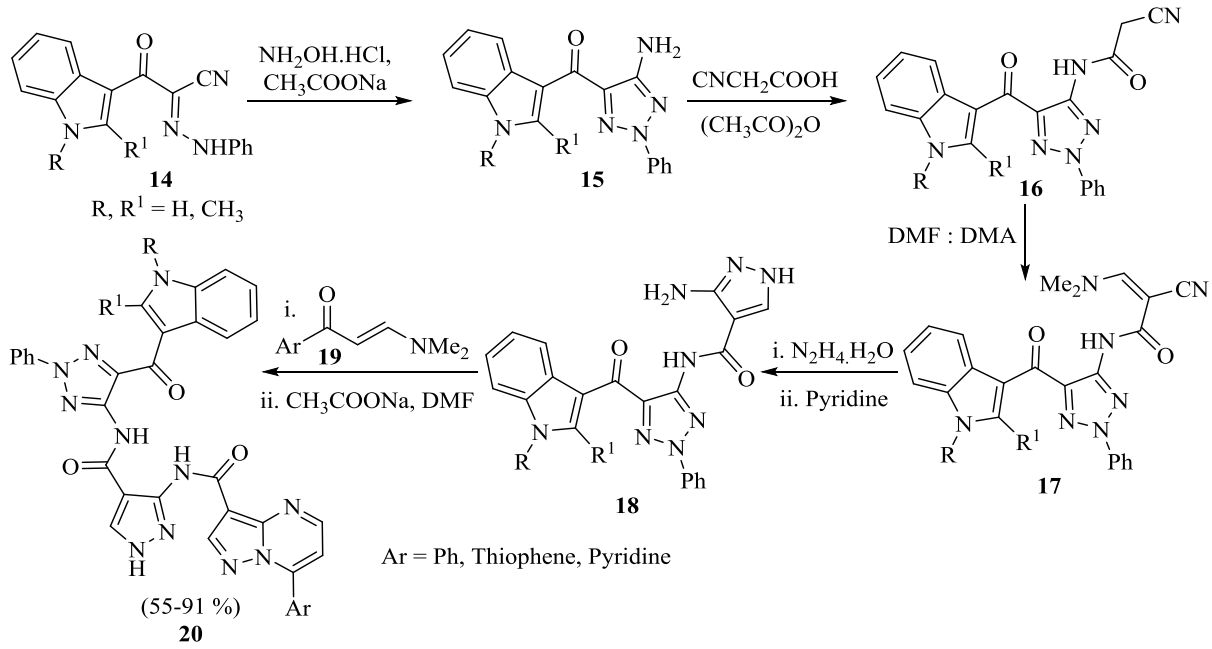
Scheme 5.3: Synthesis of indolyl-hydrazone derivatives.

Kumar *et al* have developed one pot multicomponent synthesis of 3-aminoalkylated indoles **13** by reaction of substituted indoles **11** with aldehydes and amines using silver triflate as catalyst (**Scheme 5.4**).[49] The synthesized derivatives were evaluated for antibacterial activity against Gram positive and Gram negative bacteria *B. subtilis, S. aureus* and *E. coli* and their MIC values and zone of inhibition was calculated. All the derivatives showed zone of inhibition 10-18 mm with MIC values 64-128 μg/mL against different bacteria. All the synthesized derivatives showed good activity against *B. subtilis*. Derivatives having bromo substitution on indole and methoxy substitution on aldehyde were found to be best antibacterial agents.



Scheme 5.4: One-pot multi-component synthesis of 3-aminoalkylated indoles.

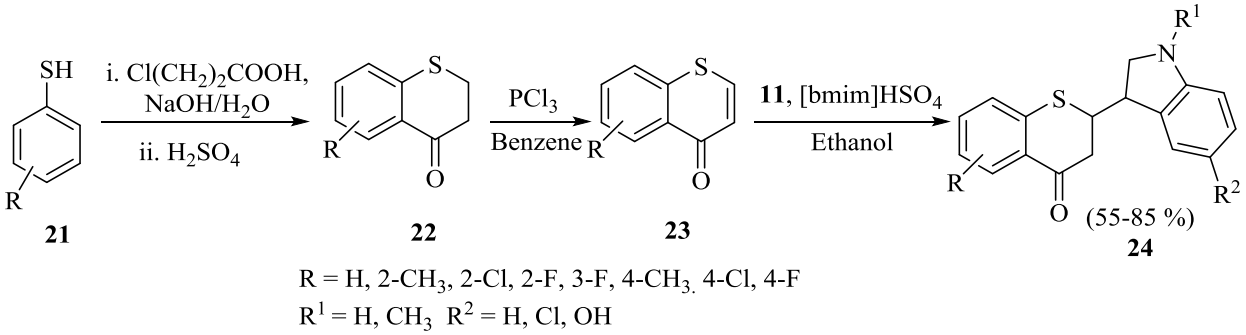
Behbehani and colleagues synthesized indole containing pyrazolo-pyrimidine scaffolds **20** from 2-arylhydrazononitriles **14** and evaluated them for their antimicrobial activity.[50] Indole **14** was reacted with hydroxylamine hydrochloride to yield 2-aryl-1,2,3-triazol-5-amines **15** which were transformed to cycanoacetamides **16** by reacting with 2-cyanoacetic acid. Cyanoacetamide **16** was reacted with DMF/DMA to convert to corresponding enamine **17**. Enamine **17** was further reacted with hydrazine hydrate to convert to corresponding pyrazole **18**. Reaction of indolyl-aminopyrazole **18** with enaminones **19** under thermal/microwave irradiation yielded pyrazolo-pyrimidines **20** (**Scheme 5.5**). All the synthesized compounds were evaluated for antimicrobial activity against Gram positive (*B. subtilis, S. aureus*), Gram negative (*E. coli*) bacteria and yeast *C. albicans* and their zone of inhibition were calculated. 1,2,3-Triazole containing indoles were found to be broad spectrum antimicrobial agents with zone of inhibition 6-13 mm while pyrazole containing derivatives were active only against *B. subtilis* with zone of inhibition 11.2 mm. Pyrazolo-pyrimidine tethered indoles were moderately active antimicrobial agents with zone of inhibition 4-8 mm.



Scheme 5.5: Synthesis of indolyl-pyrazolo-pyrimidine derivatives.

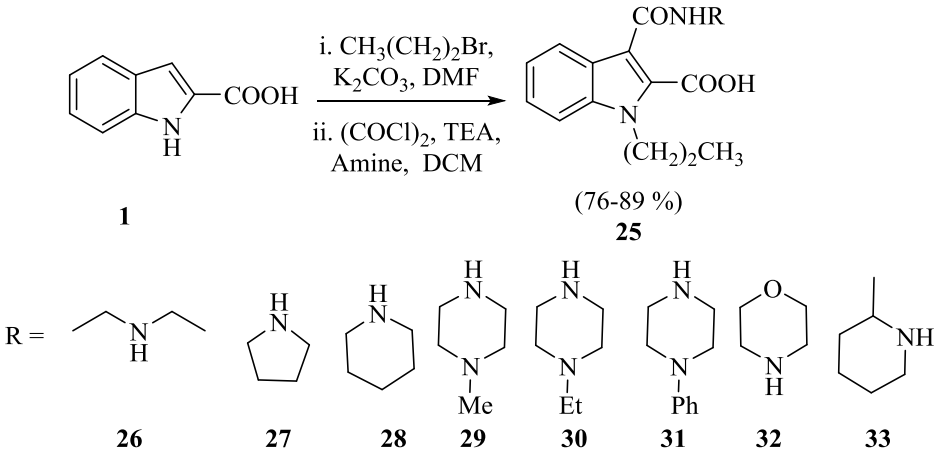
Song group has reported the ionic liquid catalysed synthesis of 2-(indole-3-yl)-thiochroman-4-ones *via* Michael addition.[51] In their approach, thiophenol **21** was reacted with 3-chloropropionic acid in presence of base followed by acid catalysed cyclisation to yield thio-chromane-4-ones which were transformed into thiochormones **23** by reacting with PCl_3. Michael addition of **23** with indoles in the presence of [bmim]HSO_4 lead to desired 2-(indole-3-yl)-thiochroman-4-ones **24** (**Scheme 5.6**). After successful synthesis, all compounds were screened for antifungal activity against *C. albicans, C. neoformans, M. racemosa, M.*

gypseum and *E. floccosum* by calculating their MIC values using Amphotecerin B and Fluconazole as positive control. It was observed that most of the compounds were better than fluconazole with MIC value 4-32 µg/mL while rest were found to show moderate activity with MIC value 64-128 µg/mL. Compounds with electron withdrawing group substitution such as chloro, fluoro on aryl ring of thiochromone were better antifungal agents.



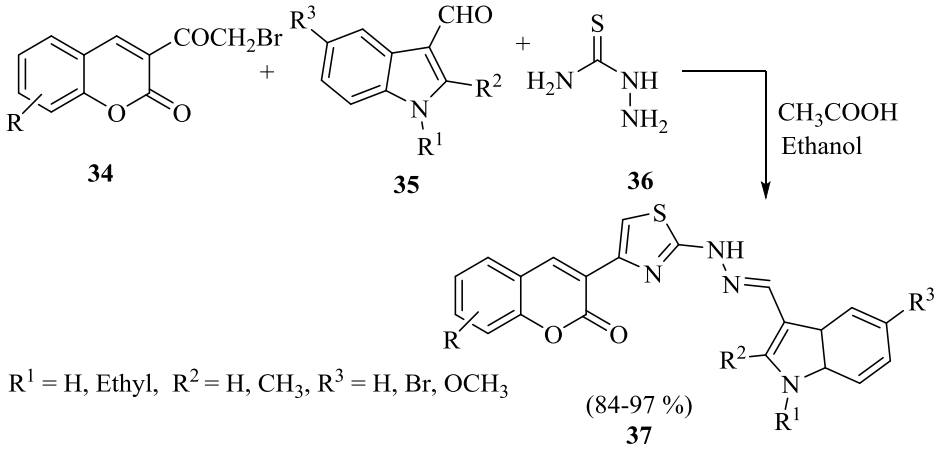
Scheme 5.6: Synthesis of 2-(indole-3-yl)-thiochroman-4-ones.

G. N. Raju and co-workers synthesized indole-2-carboxylic acid derivatives to evaluate their antimicrobial activity.[52] Indole-2-carboxylic acid was *N*-propylated followed by reaction with oxalyl chloride and secondary amines in the presence of triethylamine to yield a series of indole-2-carboxylic acid derivatives **25** (**Scheme 5.7**). All the synthesized compounds were evaluated for antibacterial activity against Gram positive bacteria *S. aureus, S. pyogenes*, Gram negative bacteria *E. coli* and *P. aeruginosa* by calculating their MIC values using Ampicillin as standard. The derivative with pyrollidine substitution was most effective against *S. aureus* (MIC value 50 µg/mL) while diisopropylamine containing motif was better against *E. coli*. All compounds were found to be moderately active with MIC value 50-500 µg/mL. When the scaffolds were screened against fungal strains *C. albicans* and *A. niger*, they showed moderate activity with MIC value 200-1000 µg/mL.



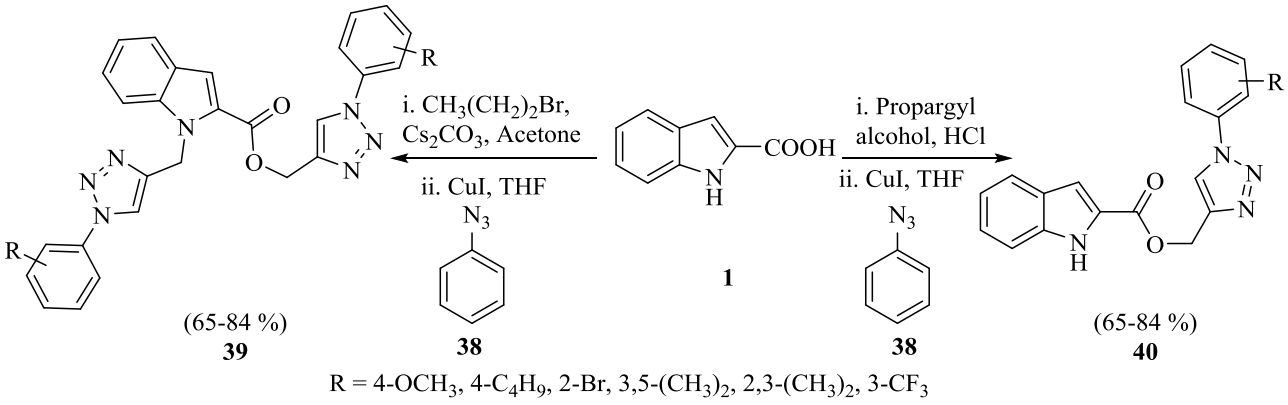
Scheme 5.7: Synthesis of indole-2-carboxylic acids derivatives.

Bavantula and colleagues have performed one pot multicomponent synthesis of indole tethered thiazolylcoumarins and evaluated their antibacterial activity.[53] One pot reaction of indole-3-carbaldehyde **35**, 2-(2-bromoacetyl)-chromene-2-ones **34** and thiosemicarbazide **36** in presence of catalytic amount of acetic acid resulted in the formation of thiazolyl coumarins **37** (**Scheme 5.8**). A series of derivatives was synthesized and evaluated for antibacterial activity against *B. subtilis* (Gram positive) and *E. coli* (Gram negative) bacteria using Streptomycin as standard by calculating their zone of inhibition. It was observed that compounds without any substitution on coumaryl and indole ring showed best antibacterial activity with zone of inhibition 18 and 15 mm against *B. subtilis* and *E. coli*, repectively. All the derivatives were good antibacterial agents with zone of inhibition 10-18 µg/mL.



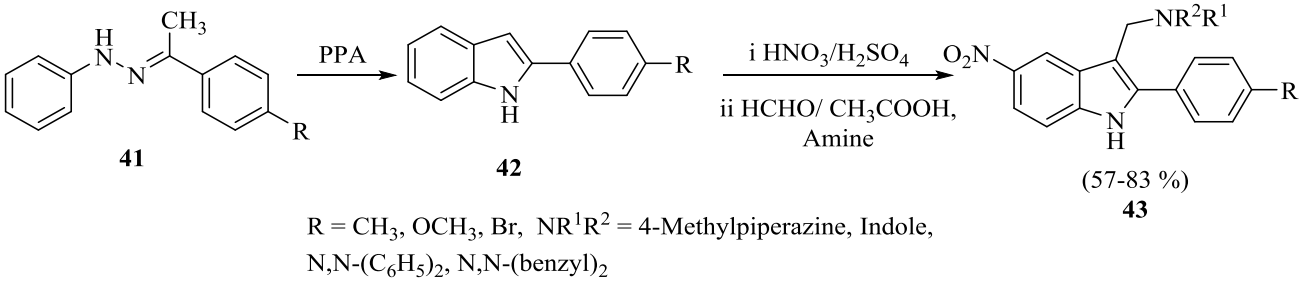
Scheme 5.8: Synthesis of indole tethered thiazolylcoumarins.

Nagavelli with his coworkers has transformed indole-2-carboxylic acid to mono and bis-1,4-disubstituted-1,2,3-triazole tethered indoles.[54] Compound **39** and **40** were obtained by propargylation of indole-2-carboxylic acid which were reacted with different phenyl azides *via* CuAAC (**Scheme 5.9**). All derivatives were screened for *in vitro* antibacterial activity against Gram positive (*S. aureus* and *B. subtilis*) and Gram negative *E. coli* and *P. vulgaris* by calculating their MIC values. Most of the compounds were poor antibacterial agent with MIC value >150 µg/mL but derivative having electron withdrawing substitution i.e. trifluoromethyl group on phenyl ring and bromo substitution on indole ring were best antibacterial agents with MIC value 1.17-4.68 µg/mL.



Scheme 5.9: Synthesis of mono- and bis-1,4-disubstituted-1,2,3-triazolyl indoles.

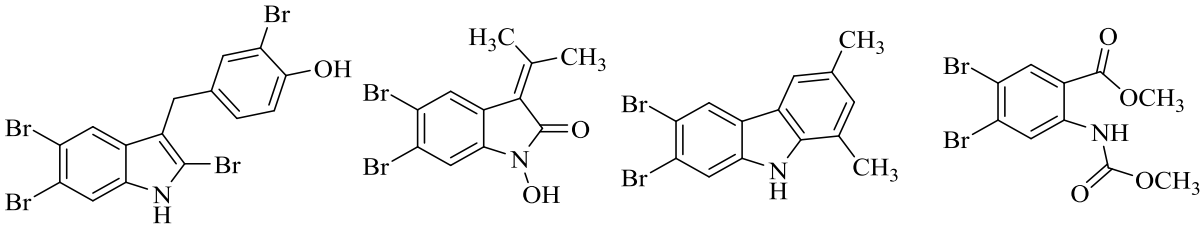
Kapoor group has synthesized Mannich bases of 2,5-disubstituted indoles **43** to evaluate their antimicrobial activity.[55] Phenyl hydrazine was reacted with different acetophenones to get substituted acetophenone phenylhydrazones **41** which were cyclised to indoles **42** in the presence of phosphoric acid. Nitration of **42** resulted in 5-nitro-2-arylindoles which were then reacted with secondary amine and formaldehyde to get corresponding Mannich base substituted nitro indoles **43** (**Scheme 5.10**). The synthesized series was examined for their antimicrobial activity against Gram positive *S. aureus, B, subtilis,* Gram negative *E. coli, P. aeruginosa* and fungal strains *C. albicans* and *A. niger*. All scaffolds were observed to be good antimicrobial agents with MIC value 1.46-2.44 µg/mL which was in accordance to standard ciprofloxacin (2.33 µg/mL) and fluconazole (1.99 µg/mL). All the compounds were found most effective against *S. aureus* and motif with *p*-Br or *p*-methoxy substitution were the best.



Scheme 5.10: Synthesis of Mannich bases of 2,5-disubstituted indoles.

Zhang *et al* have isolated four brominated indole alkaloids **44-47** from *Laurencia similis* and tested them for their antibacterial activity (**Figure 5.2**).[18] Among all four isolated alkaloids, two are naturally occurring alkaloids while two were isolated first time (**46, 47**). All four isolated alkaloids were tested for their antibacterial activity against Gram positive *S. aureus, B. subtilis, B. thuringensis* and four Gram negative *P. lachrymans, A. tumefaciens, X.*

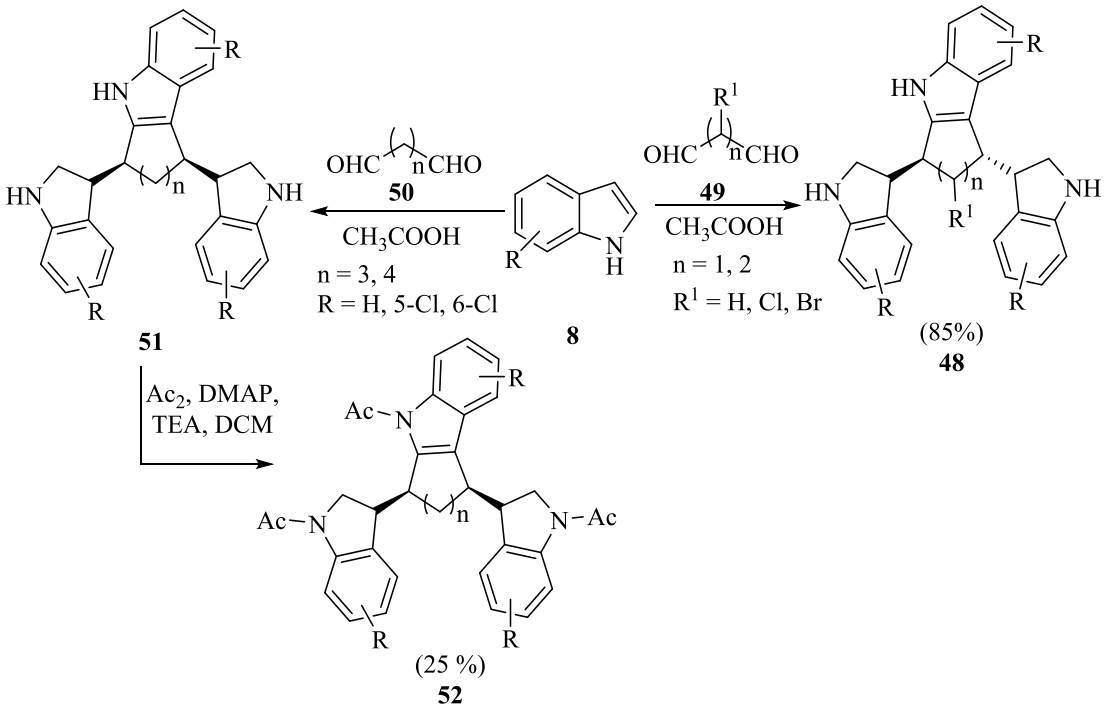
vesicatoria and *R. solanacearum* and their MIC values were calculated. Compound **44** showed broad spectrum antibacterial activity against Gram positive and Gram negative bacteria with MIC values 2-8 μg/mL while compound **45** was moderately active with MIC value 12.5-50 μg/mL. Compound **46** and **47** were inactive with MIC value >250 μg/mL.



44 **45** **46** **47**

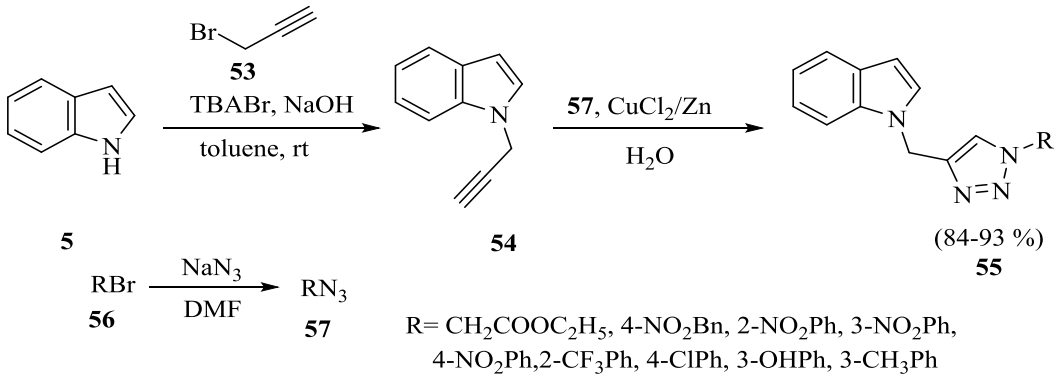
Figure 5.2: Natural brominated indole alkaloids isolated from *Laurencia similis*.

Hilgeroth and colleague have reported synthesis of bis(indolyl) substituted cycloalkane annelated indoles to inspect their antibacterial action against *S. aureus* and MRSA.[16] Dialdehydes with 1 or 2 methylene group between them (**49** and **50**) were reacted with indole to get *trans*-bis indolyl-cycloalkane linked indoles **48** while longer chain between aldehyde lead to *cis*-isomer **51**. *N*-Acylation of indoles **51** was also performed to get **52** (**Scheme 5.11**). All compounds were evaluated for antibacterial activity against different *S. aureus* variants and MRSA variants. Their MIC values were calculated as the minimum concentration which completely inhibits the growth of bacterii. All the compounds showed good antibacterial activity with MIC value 3.125-12.5 μg/mL, however *N*-alkylation of indole lead to decreased activity with MIC value of 50-100 μg/mL for most of the compounds.



Scheme 5.11: Synthesis of bisindolyl- substituted cycloalkene-annelated indoles.

Xu group has prepared novel indole derivatives linked with 1,2,3-triazoles *via* CuAAC.[56] Indole **5** was reacted with propargyl bromide **53** to give *N*-propargylindoles **54**, these propargylated indoles **54** were reacted with different substituted azides **57** in the presence of $CuCl_2$/Zn in water to get corresponding 1,2,3-triazole linked indoles **55** (**Scheme 5.12**). The synthesized compounds were screened for antifungal activity against *C. capsici* and *C. physalospora* at 20 μg/mL concentration and the inhibitory action against *C. capsici* was observed upto 83% for some derivatives. Compounds without substitution or having *o*-substitution with electron withdrawing group were far better in activity.



Scheme 5.12: Synthetic route for 1,2,3-triaozle linked indole derivatives *via* CuAAC.

Shrinizadeh[57] *et al* have evaluated antimicrobial activity of indole-3-aldehyde and 5-bromoindole-3-aldehyde hydrazide and hydrazones against *S. aureus, MRSA, E. coli, B.*

Subtilis and *C. albicans*. It was found that compounds with halogenated phenyl ring were better in antimicrobial action against *MRSA* and *S. aureus* relative to Ampicillin.

In continuation of our interest in synthesis of 1,4-disubstituted 1,2,3-triazolyl heterocycles and wide applicability of indoles and 1,2,3-triazoles, herein, we have synthesized 3-(1,4-disubstituted 1,2,3-triazol-4-yl)methyl-indoles. All the synthesized derivatives were well characterized by spectroscopic data and their antimicrobial activities were evaluated along with live-dead cell study, cell-death by PI assay and haemolysis study.

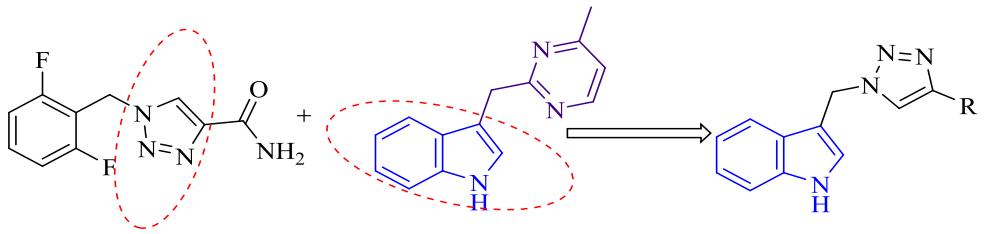
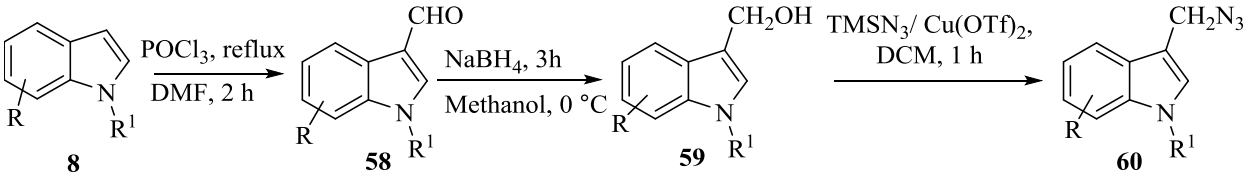


Figure 5.3: Rational for designing new triazolyl-indoles.

5.2 Results and discussions

Aiming to synthesize desired triazolyl-indoles, indoles were converted to (1*H*-indol-3-yl)methanol (**59**) by formylation using $POCl_3$/DMF at 80 °C followed by reduction with $NaBH_4$ (**Scheme 5.13**). For converting **59** to **60**, initially we tried to convert alcohol functionality to mesyl group by reaction with mesyl chloride in DCM. We performed *in situ* mesylation followed by reaction with sodium azide *in situ* after one hour of mesylation to get compound **60**. The *in situ* generation of organic azides **60** minimizes hazards derived from the isolation, avoids the time-consuming and waste generation of an additional synthetic step. Alternatively, direct conversion of alcohol was performed using $Cu(OTf)_2$ and $TMSN_3$ in DCM. This method is found better than earlier and azidation was performed using copper (II) triflate and $TMSN_3$. The structure of **60** was ascertained by spectroscopic data. A characteristic peak appeared at 2080 cm^{-1} in IR spectra for the azide group. We then synthesized different 3-(azidomethyl)-indoles **60** using these conditions.

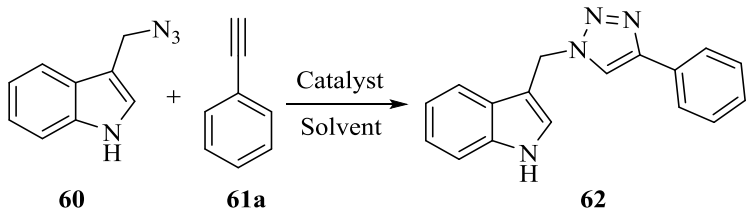


Scheme 5.13: Synthesis of 3-(azidomethyl)-1H-indole.

Synthesis of 3-(triazol-1-yl)methyl-indoles was achieved by click reaction of **60** with terminal acetylenes **61**. Initially, the reaction of **60** with phenyl acetylene was selected as model reaction to optimize reaction conditions. Results of optimization reactions are shown in Table

5.1. In the absence of copper catalyst, reaction failed to furnish desired product and starting materials were recovered (**Table 5.1**, entry 7). Different solvents and catalysts were screened and CuI (10 mol %) in THF at room temperature was found best to give 3-(triazol-1-yl) methyl-indole **62a** in 90% yield as white solid.

Table 5.1: Optimization of reaction conditions for the synthesis of triazole indoles **62**.

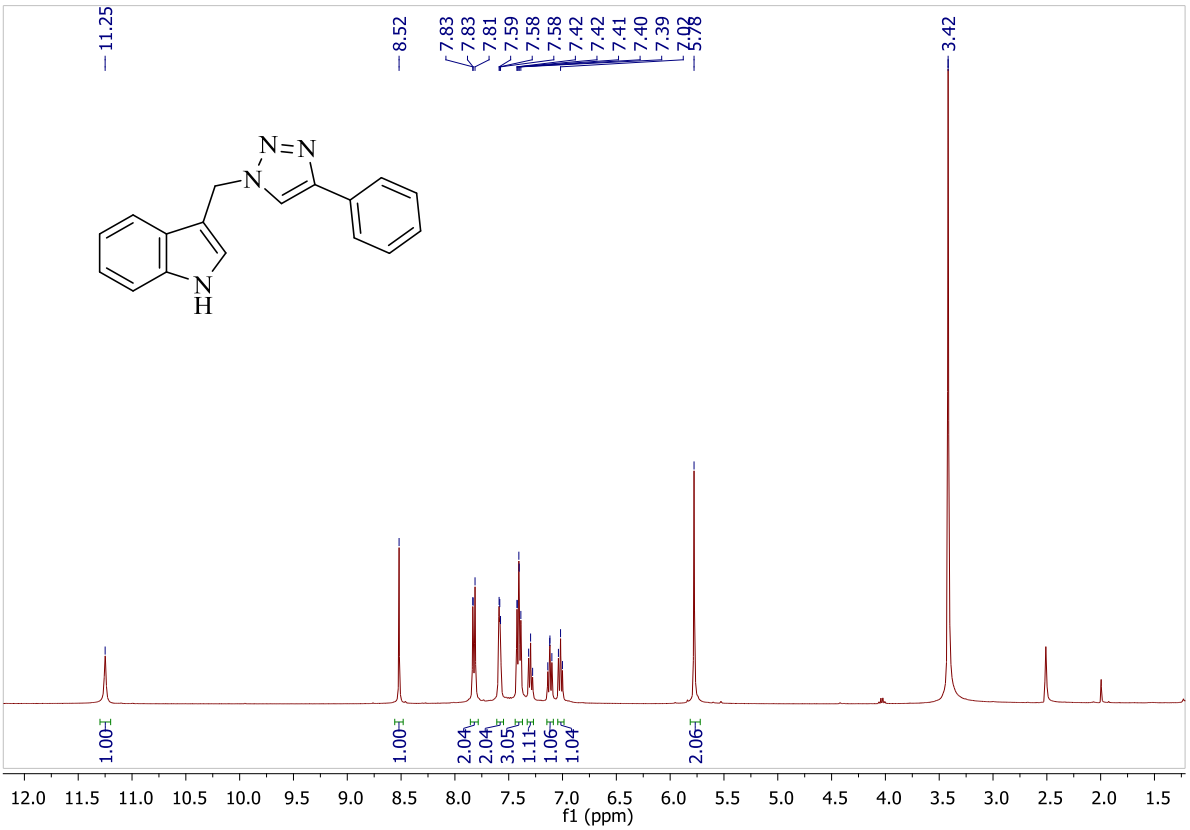


60 **61a** **62**

Entry	Catalyst (10 mol %)	Solvent	Time (h)	Yield (%)
1.	$Cu(SO_4).5H_2O$, Na-Ascorbate	THF	8	82
2.	$Cu(SO_4).5H_2O$, Na-Ascorbate	ACN	8	72
3.	$Cu(SO_4).5H_2O$, Na-Ascorbate	DMF	6	80
4.	$CuCl_2$, Na-Ascorbate	THF	7	45
5.	$CuCl_2$, Na-Ascorbate	ACN	7	43
6.	$CuCl_2$, Na-Ascorbate	DMF	6	47
7.	-	DMF	15	No reaction
8.	CuI	Water	13	Not isolated
9.	CuI	Water : *t*-BuOH	8	62
10.	CuI	*t*-BuOH	8	45
11.	CuI	Water : PEG 400	7	56
12.	CuI	THF	7	90
13.	CuI	ACN	8	78
14.	CuI	DMF	8	84

The structure of **62a** was confirmed by NMR and mass spectroscopic data. All protons and carbons were located at their respective positions in ^1H and ^{13}C NMR spectra (**Figure 5.4**). In ^1H NMR a characteristic singlet appeared at δ = 8.52 ppm for C_5-*H* of triazolyl ring and a

singlet at δ = 5.78 ppm for N-CH_2 along with other protons. N-H peak was observed at δ = 11.25 ppm. In ^{13}C NMR spectra N-CH_2 carbon appeared at 45.84 ppm, C_4 of triazole ring appeared at 136.77 ppm and C_5 at 129.29 ppm along with other carbons. Absence of peak in the region of 2080 cm^{-1} in IR spectra further confirmed the structure of **62a**.



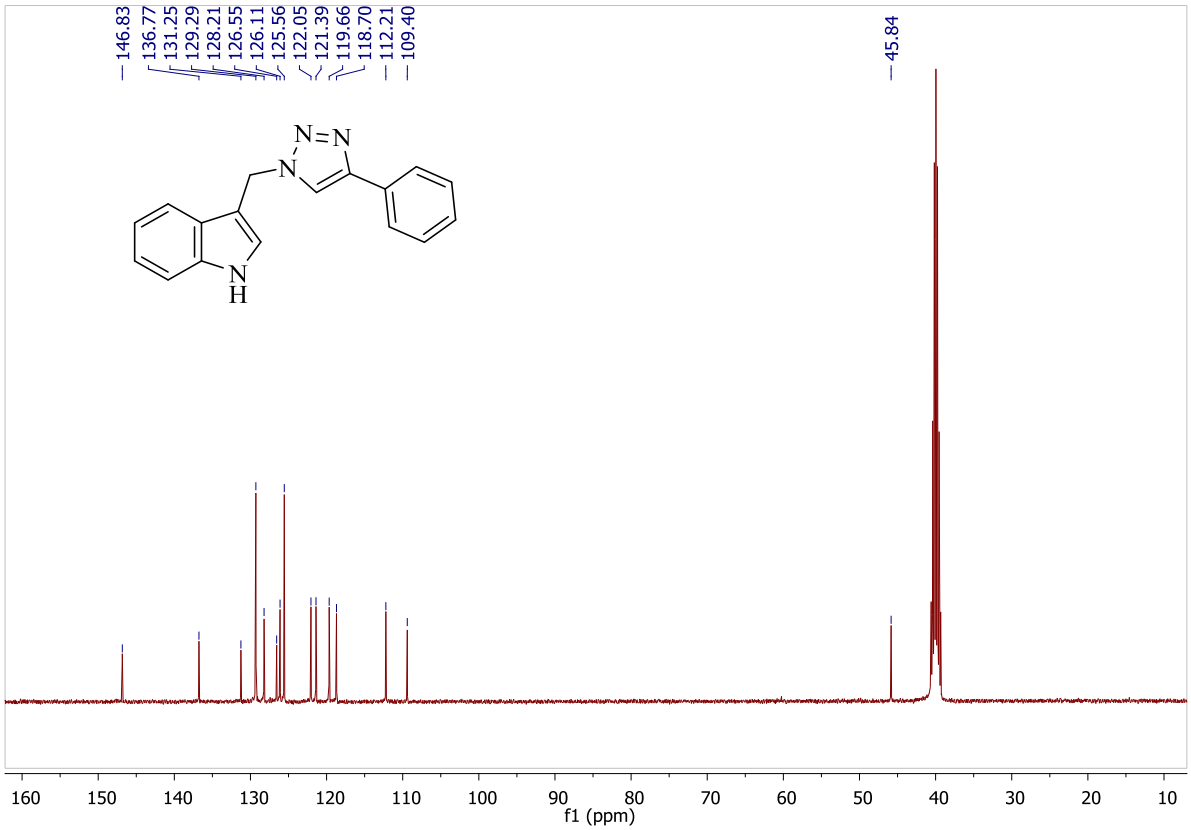
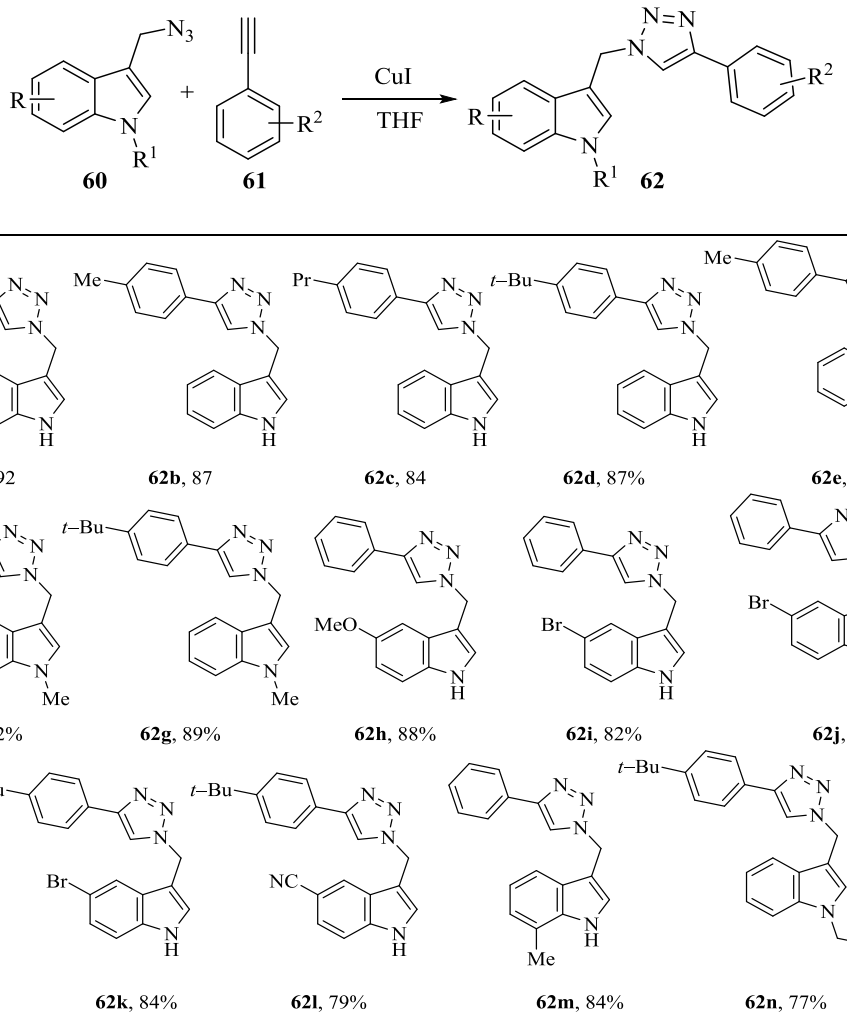


Figure 5.4: ^{1}H and ^{13}C-NMR spectra data of **62a**.

Having optimized reaction conditions, we investigated the scope of this reaction and the results are summarized in **Scheme 5.14**. To our delight, different 3-(azidomethyl)-indoles and alkynes underwent smooth reactions to give 3-(triazol-1-yl)methyl-indole **62a-n** in good to excellent yield (73-92%) at room temperature in 7-9 h. For example, phenylacetylenes with electron donating substituent such as 4-*tert*-butyl and 4-methyl produced the corresponding 1,2,3-triazole-fused indoles (**62b-e** and **62g**, **62k**, **62l** and **62n**) in good yields (80-84 %). Similarly, different substituted indoles were transformed to 1,2,3-triazole-fused methyl-indoles in good yields (**Scheme 5.14**). The tolerance of different substituent such as methyl, bromo, *t*-butyl and nitrile in this protocol provides the opportunity for further chemical manipulations in 3-(triazol-1-yl)methylindoles and thus novel therapeutically important heterocycles can be generated. All the compounds were of high purity and gave satisfactory spectroscopic data. The structure of products was confirmed as 1,4-disubstituted 1,2,3-triazole and not as 1,5-disubstituted 1,2,3-triazole by ^{1}H NMR and ^{13}C NMR spectroscopic data.



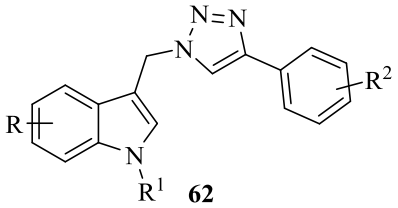
Scheme 5.14: Synthesis of 3-(1,2,3-triazol-4-yl)methylindoles.

5.2.1 Antimicrobial activity

The synthesized compounds **62a-62n** were screened for antibacterial activities against Gram negative *S. typhi* and *P. putida*, Gram positive *B. subtilis, S. Aureus* bacteria. All compounds were evaluated at the concentrations ranging from 0.5 μg/mL to 128 μg/mL and scored for MIC as the level of growth inhibition of the microorganisms compared with ciprofloxacin. The data of antibacterial activities are depicted in **Table 5.2**. The most effective data is shown in bold. Compound **62i and 62k** with bromo substitution on indole ring and phenyl/ 4-*t*-butylphenyl substitution on triazole part showed highest activity against Gram negative as well as Gram positive bacteria with MIC value ≥4 μg/mL, (**Table 5.2**, entry 9 and 10). Derivative **62b** with 4-methyl substitution on triazole ring also showed good antibacterial activity with comparable MIC value (8 μg/mL) with ciprofloxacin (6.25 μg/mL). Zone of inhibition was also calculated for all of the derivative scaffolds against aforementioned bacteria. Compound **62i and 62k** with bromo substitution on indole ring and phenyl/ 4-*t*-butyl

phenyl substitution on triazole part showed highest activity against Gram negative as well as Gram positive bacteria with zone of inhibition 17-19 mm. All scaffolds are good broad spectrum antibacterial agent with zone of inhibiton 12-19 mm (**Table 5.2**).

Table 5.2 : Antibacterial activity of 62a-n.



62

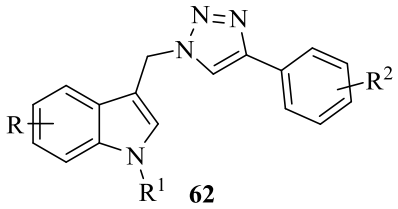
Compound	*S. typhae*		*P. putida*		*B. subtilis*		*S. aureus*	
	MIC	**ZOI**	**MIC**	**ZOI**	**MIC**	**ZOI**	**MIC**	**ZOI**
62a	16	15	32	13	>16	14	>16	14
62b	8	18	8	17	>8	18	>8	17
62c	16	15	16	15	>16	14	16	15
62d	32	13	32	14	32	13	>32	13
62e	32	13	>32	13	32	14	32	13
62f	64	13	64	14	128	12	128	12
62g	16	15	>16	14	16	14	>16	13
62h	32	14	32	15	>32	13	>32	14
62i	4	18	4	19	>4	17	4	18
62j	4	19	>4	18	4	19	4	19
62k	16	15	>16	14	16	15	16	14
62l	8	18	8	18	8	17	8	17
62m	>16	13	>16	13	>16	14	>16	13
62n	16	15	>16	14	16	15	>16	14
Ciprofloxacin	6.25		6.25		6.25		6.25	

MIC = Minimum inhibitory concentrations (µg/mL), ZOI = Zone of inhibition (mm).

Further, antifungal activity of **62a-n** was also evaluated against *C. albicans* and *A.* Flavus. All the compounds were evaluated at the concentrations ranging from 0.5 µg/mL to 128 µg/mL and scored for MIC as the level of growth inhibition of the microorganisms compared with Amphotecerin B as positive control. The data of antifungal activities are depicted in **Table 5.3**. All scaffolds showed MIC values 16-64 µg/mL which was 30 µg/mL for Amphotecerin B, hence they can be considered as good antifungal agent. Derivative **62f** with *N*-methyl substitution exhibited MIC value 128 µg/mL against *C. albicans*. It was found that derivative with electron donating substitution on triazole phenyl ring and electron withdrawing substitution on indole ring are good antimicrobial agents. Zone of inhibition was also calculated for all of the derivative scaffolds against aforementioned fungi. Zone of inhibition

was also measured against fungi *C. albicans* and *A. flavus*. All scaffolds are moderate to good antifungal agent with zone of inhibition 11-18 mm. Derivative **62b** was considered as best antifungal agent with zone of inhibition 18 and 17 mm against *C. albicans* and *A. flavus* respectively. Derivative **62i** and **62j** were also good antifungal agents with zone of inhibition 15-18 mm.

Table 5.3 : Antifungal activity of 62a-n.



62

Compound	*C. albicans*		*A. flavus*	
	MIC	**ZOI**	**MIC**	**ZOI**
62a	32	14	>32	13
62b	16	18	16	17
62c	>32	14	>32	14
62d	>32	14	64	12
62e	64	13	64	12
62f	128	12	>64	11
62g	32	14	>32	14
62h	64	13	64	14
62i	16	18	32	15
62j	16	17	>16	16
62k	64	12	>64	13
62l	32	15	32	14
62m	64	12	>32	13
62n	32	14	>32	13
Amphotecerin-B	30		30	

MIC = Minimum inhibitory concentrations (µg/mL), ZOI = Zone of inhibition (mm).

After evaluating antibacterial and antifungal activity, we went for kinetic assay of most potent compounds **62b**, **62i**, **62j** and **62l** against all four bacteria. It was observed that bactericidal activity increases with concentration and derivative **62i** was most effective against all bacteria showing ≈90 % bactericidal action when 50 µg/mL concentrations was used (**Figure 5.5**).

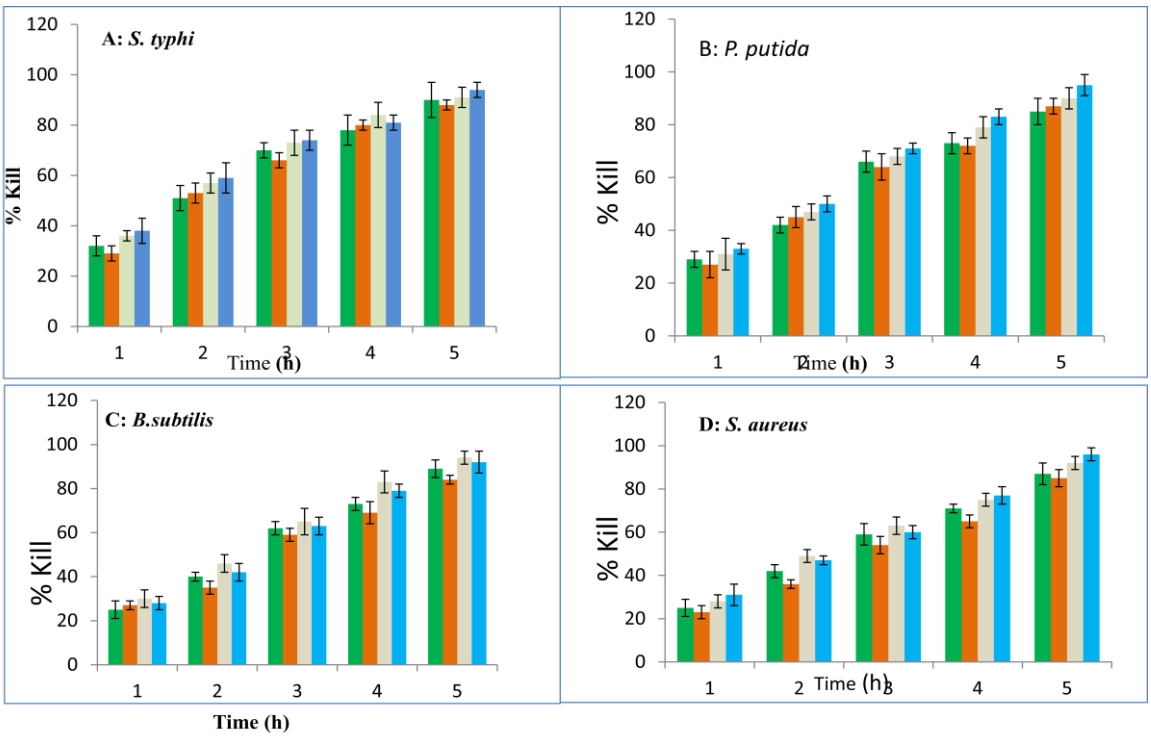


Figure 5.5: Kinetics essay of **62l** (green), **62b** (orange), **62j** (white), **62i** (blue) against *S. typhae*, *P. putida*, *B. subtilis* and *S. aureus* respectively.

5.2.2 Study of live and dead cells

The differentiation between live and dead cells caused by efficient antimicrobial compounds **62b, 62i, 62j** and **62l** was evaluated by AO/Et.Br dual staining assay. The dye AO can enter inside the living cells and bind with the living cells DNA to emit green fluorescence, whereas Et.Br enters only through modified cell membrane of dead cells and emit red fluorescence. It is evident from Figure 5.6 that untreated bacterial cells displayed green fluorescence while the cells treated with compounds **62b, 62i, 62j** and **62l** exhibited red fluorescence along with minor green fluorescence. The red fluorescence was completely absent from the control sample. Appearance of red fluorescence from the compound treated bacterial cells illustrates the bactericidal potential of compounds. These observed results therefore reveal that selected compounds caused the cell damage by making loss of membrane integrity.

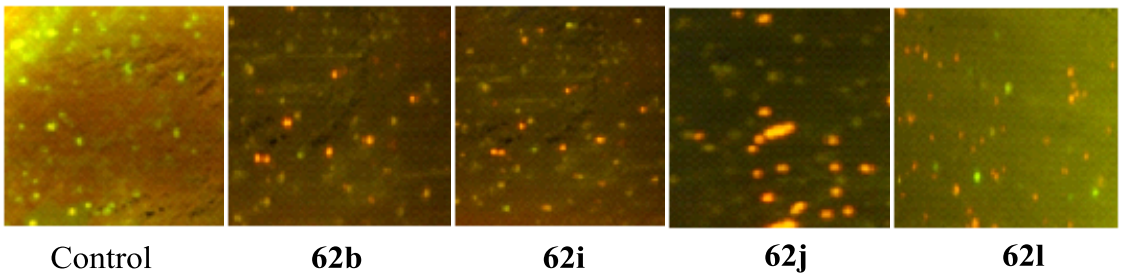


Figure 5.6: Study of live and dead cell by AO/Et.Br dual staining assay.

5.2.3 Propidium iodide (PI) staining study

Propidium iodide (PI) dye penetrates only the damaged or compromised membrane and is used as a probe to detect the dead cells. The dye PI intercalated with double-stranded DNA and subsequent fluorescence detection allowed assessment of the number of non-viable/dead cells. The compounds **62b, 62i, 62j** and **62l** which showed excellent antibacterial activity was further tested for their effect with PI staining. The microscopic images on compounds treated bacterial culture showed significant cell death at 2× MIC, which is similar to the number of cells positive for PI in cell treated with *t*-BHP.

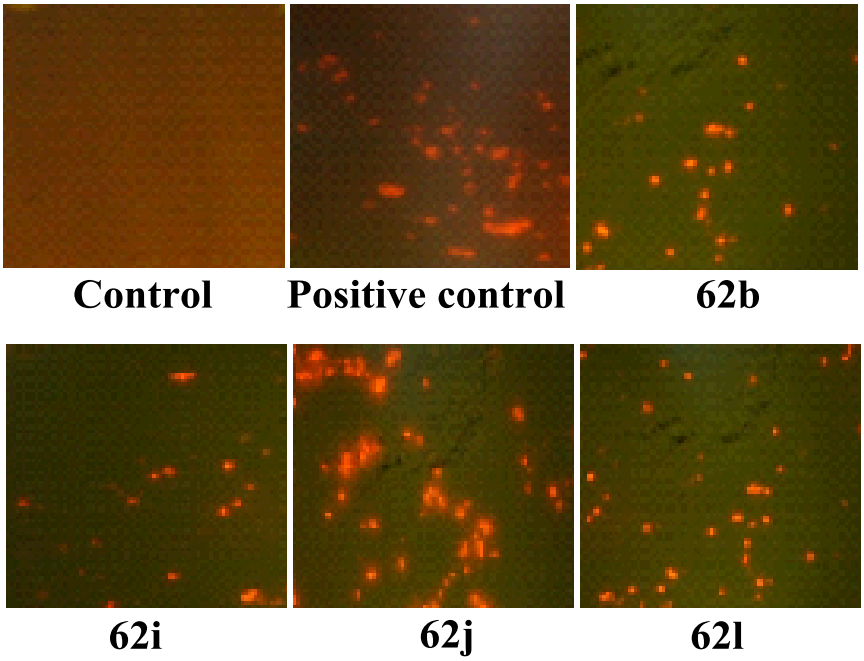


Figure 5.7: Cell-death study by PI essay of selected compounds.

5.2.4 Haemolysis evaluation:

The evaluation of the haemolytic activity is to check the damage caused by synthesized compounds to the membranes of RBCs (erythrocytes). It is an additional tool to verify the importance of synthesized compounds against red blood cells and may also give an idea whether to promote such compounds to the next drug level. The haemolytic activity can occur by several mechanisms, from increased permeability of cell membranes to complete cell lysis. The level of haemolysis in the positive control (Triton-×100) was >95%, however, the synthesized compounds showed only 3-27% haemolysis. The observed results illustrate that these compounds caused the minimum damages to RBC.

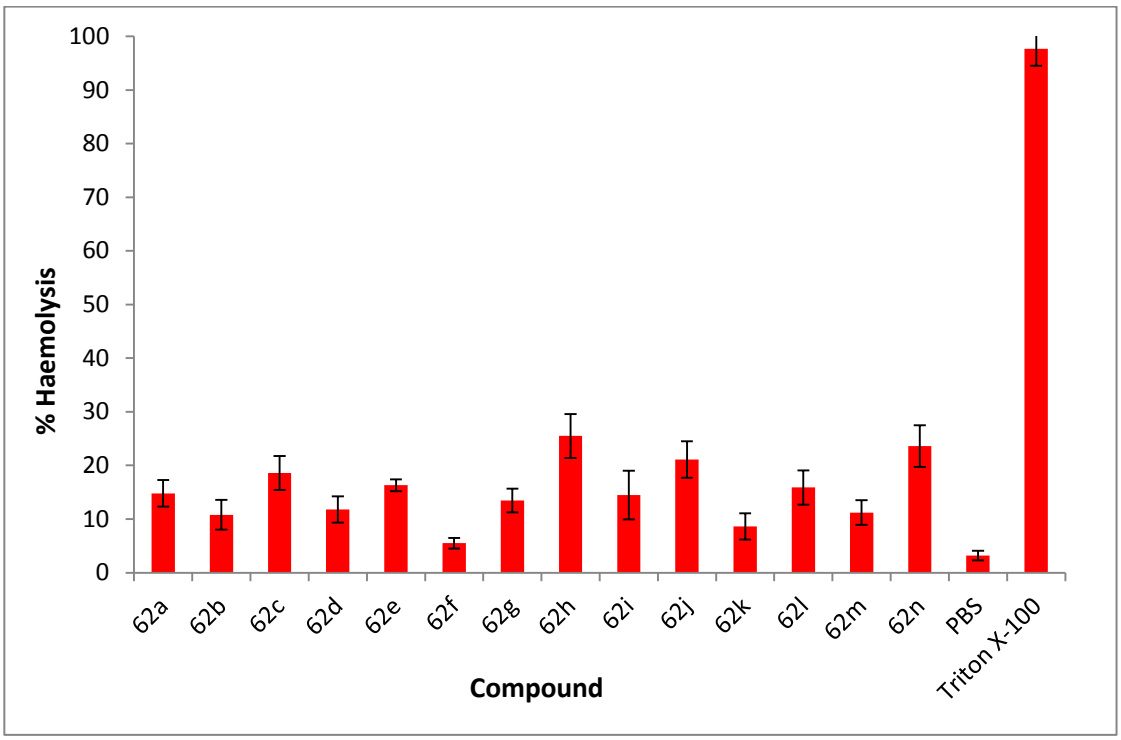


Figure 5.8: Percentage heamolysis performance of triazolyl-indole derivatives.

5.3 **Conclusion**

In summary, we have prepared a novel series of triazolyl-indoles **62a-n** in good to excellent yield using CuAAC. All synthesized compounds were examined for antimicrobial evaluation against different Gram positive, Gram negative bacteria and fungi which showed that triazolyl-indoles are good antimicrobial agents having comparative MIC value and zone of inhibition with control. Derivatives having electron withdrawing substitution (**62b**, **62i**, **62j** and **62l**) on indole ring were found to be better antimicrobial agent with MIC value 4-8 µg/mL. Most effective derivatives were also screened for cell death by AO/Et.Br dual staining and PI essay methods in which compound **62j** emitted maximum red fluorescence. Again RBC haemolysis was also studied for all derivatives in which active compounds showed less than 25% haemolysis.

5.4 Experimental

General information: All chemicals were obtained from commercial suppliers and used without further purification. The melting points were determined using EZ-Melt automated melting point apparatus and were not corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 spectrometry at 400MHz and 100 MHz respectively.

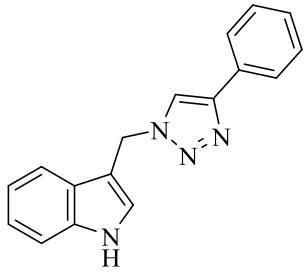
Synthesis of 1*H*-indole-3-carbaldehyde: In a 50 mL round bottom flask, $POCl_3$ (1.1 mL, 11.1 mmol) was added in DMF (10 mL) and cooled to 0 °C. After 15 minutes, indole (1 g, 8.5 mmol) was added to it. Reaction mass was stirred at 80 °C for 1.5 h and allowed it to cool to ambient temperature. Reaction mass was neutralized with saturated $NaHCO_3$ solution (20 mL). Extracted into ethyl acetate (2 × 20 mL), dried organic layer over sodium sulphate and evaporated the volatiles. Compound was used for next step without further purification.

Synthesis of (1*H*-indol-3-yl)methanol : To a stirred solution of 1*H*-indole-3-carbaldehyde (1 g, 6.9 mmol) in methanol (10 mL) was added $NaBH_4$ (0.31 g, 8.3 mmol) and stirred at rt for 1 h. After stoppimg reaction, evaporated the volatiles. Residue was diluted with water and neutralized with 2M HCl. Added ethyl acetate (2 × 20 mL) to extract the compound, dried organic layer over sodium sulphate and evaporated the volatiles. Compound was used to next step without further purification.

Synthesis of 3-(azidomethyl)-1H-indole: To a stirred solution of *1H*-indol-3-yl)methanol (1 g, 6.8 mmol) in DCM (10 mL) was added $Cu(OTf)_2$ (18 mg, 0.05 mmol) and azidotrimethylsilane ($TMSN_3$) (1.4 mL, 10.2 mmol) and stirred the reaction mixture at room temperature for 1h. On completion of reaction, volatiles were evaporated. Added water and ethyl acetate to the residue. Separated the organic layer and dried over sodium sulphate. Evaporated the volatiles and crude compound was purified by column chromatography (15 % EtOAc/ Hexane).

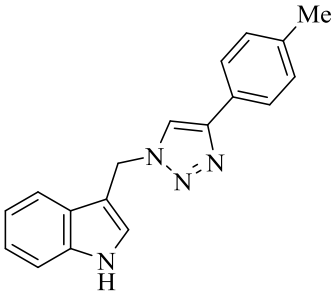
Synthesis of 3-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)-1H-indole: Mixture of 3-(azidomethyl)-1H-indole (172 mg, 1.0 mmol), phenylacetylene (122 mg, 1.2 mmol) and CuI (19 mg, 0.10 mmol) in water: polyethylene glycol (1:*1 v/v*) (4 mL) was stirred at rt for 5 h. After completion, added water and extracted into ethyl acetate (2 × 10 mL). The organic layer was separated and dried over sodium sulphate followed by evaporation of volatiles. Crude was purified by column chromatography (1:4 EtOAc/Hexanes).

3-((4-Phenyl-1H-1,2,3-triazol-1-yl)methyl)-1H-indole (62a)



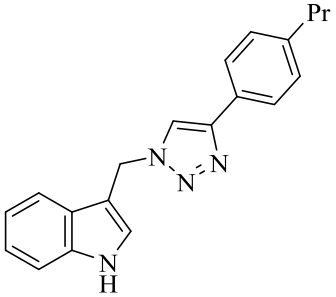
Yield: 252 mg (92%); brown solid; mp 137 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 8.52 (s, 1H), 7.84-7.80 (m, 2H), 7.62 – 7.54 (m, 2H), 7.47 – 7.38 (m, 3H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.12 (dd, *J* = 7.9, 7.2 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 5.78 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.83, 136.77, 131.25, 129.29, 128.21, 126.55, 126.11, 125.56, 122.05, 121.39, 119.66, 118.70, 112.21, 109.40, 45.84.

3-((4-(*p*-Tolyl)-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62b)



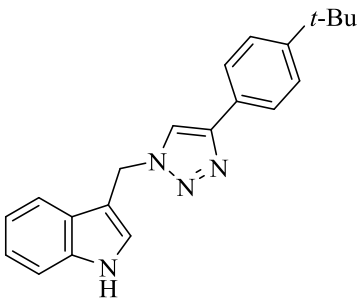
Yield: 250 mg (87%); yellow solid; mp 134 °C^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.65 (d, *J* = 8.1 Hz, 2H), 7.62 (s, 1H), 7.58 – 7.55 (m, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.32 (d, *J* = 2.5 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.21 – 7.12 (m, 3H), 5.75 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.85, 137.91, 136.45, 129.45, 127.80, 126.24, 125.57, 124.61, 122.91, 120.55, 119.08, 118.51, 111.69, 109.14, 46.03, 21.28.

3-((4-(4-Propylphenyl)-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62c)



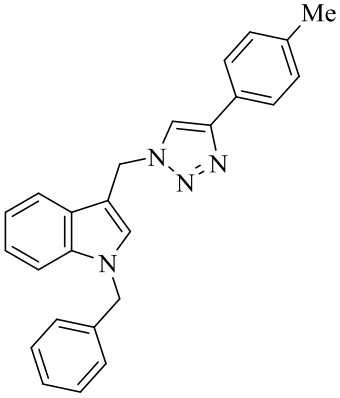
Yield: 265 mg (84%); brown solid; mp 148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.67 (d, *J* = 8.1 Hz, 2H), 7.62 (s, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.33 (d, *J* = 2.3 Hz, 1H), 7.30 – 7.27 (m, 1H), 7.21 – 7.14 (m, 3H), 5.77 (s, 2H), 2.59 (t, *J* = 7.1 Hz, 2H), 1.70 – 1.58 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.89, 142.72, 136.44, 128.87, 128.07, 126.23, 125.57, 124.52, 122.93, 120.57, 119.07, 118.52, 111.65, 109.23, 46.04, 37.79, 24.48, 13.79.

3-((4-(4-(*tert*-Butyl)phenyl)-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62d)



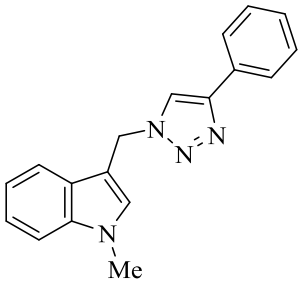
Yield: 287 mg (87%); white solid; mp 144 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.62 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.43 – 7.38 (m, 2H), 7.28 (s, 1H), 7.18 – 7.13 (m, 2H), 5.77 (s, 2H), 1.33 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.11, 147.77, 136.38, 127.84, 126.21, 125.66, 125.39, 124.37, 122.99, 120.63, 119.07, 118.58, 111.57, 109.42, 46.02, 34.63, 31.28.

1-Benzyl-3-((4-(*p*-tolyl)-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62e)



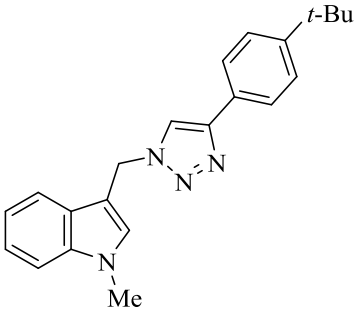
Yield: 300 mg (80%); yellow solid; mp 140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.75 – 7.68 (m, 3H), 7.62 (d, J = 7.9 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.35 – 7.28 (m, 2H), 7.27 – 7.20 (m, 4H), 7.16 – 7.10 (m, 2H), 7.07 – 7.02 (m, 1H), 5.78 (s, 2H), 5.43 (s, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.90, 138.42, 137.51, 136.59, 129.85, 129.56, 129.04, 128.97, 128.47, 127.90, 127.59, 127.40, 127.15, 125.51, 122.31, 121.06, 119.99, 119.14, 110.97, 109.39, 49.56, 45.58, 21.28.

1-Methyl-3-((4-phenyl-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62f)



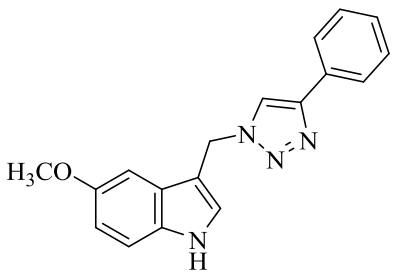
Yield: 235 mg (82%); white solid; mp 120 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.84 – 7.80 (m, 2H), 7.61 (d, J = 7.9 Hz, 1H), 7.54 (s, 1H), 7.46 – 7.37 (m, 3H), 7.32 – 7.27 (m, 1H), 7.21 – 7.15 (m, 1H), 7.09 – 7.03 (m, 1H), 5.77 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.84, 137.19, 131.24, 130.14, 129.29, 128.22, 126.86, 125.56, 123.97, 122.96, 122.14, 121.41, 119.80, 118.92, 111.39, 110.47, 108.62, 45.59, 32.93.

3-((4-(4-(*tert*-Butyl)phenyl)-*1H*-1,2,3-triazol-1-yl)methyl)-1-methyl-*1H*-indole (62g)



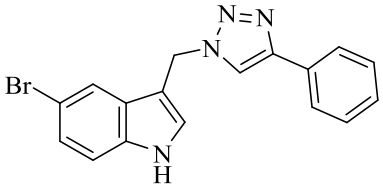
Yield: 306 mg (89%); white solid; mp 142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.2 Hz, 2H), 7.63 (s, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.43 – 7.35 (m, 3H), 7.34 – 7.28 (m, 1H), 7.21 (s, 1H), 7.16 (t, J = 7.4 Hz, 1H), 5.76 (s, 2H), 3.83 (s, 3H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.03, 147.72, 137.22, 128.89, 127.94, 126.76, 125.63, 125.38, 122.52, 120.18, 119.02, 118.68, 109.69, 107.80, 45.87, 34.63, 32.95, 31.29.

5-Methoxy-3-((4-phenyl-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62h):



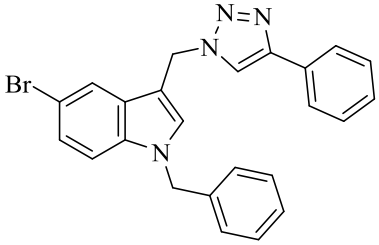
Yield: 267 mg (88%); yellow solid; mp 60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.76 (d, J = 7.3 Hz, 2H), 7.65 (s, 1H), 7.38 (t, J = 7.3 Hz, 2H), 7.34 – 7.27 (m, 3H), 6.97 (s, 1H), 6.90 (dd, J = 8.8, 1.9 Hz, 1H), 5.73 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.69, 147.79, 131.52, 130.61, 128.80, 128.11, 126.72, 125.67, 125.60, 125.22, 119.44, 113.27, 112.53, 108.73, 99.96, 55.85, 46.15.

5-Bromo-3-((4-phenyl-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62i):



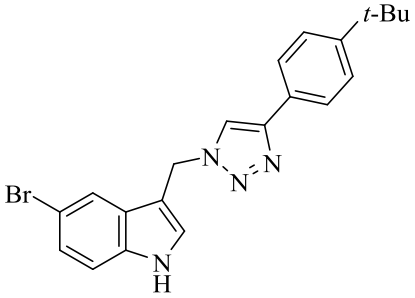
Yield: 290 mg (82%); off-white solid; mp 149 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 8.55 (s, 1H), 7.82 (d, *J* = 8.3 Hz, 2H), 7.70 – 7.60 (m, 2H), 7.45 – 7.36 (m, 3H), 7.35 – 7.27 (m, 1H), 7.21 – 7.16 (m, 1H), 5.79 (d, *J* = 20.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.86, 135.43, 131.21, 129.31, 129.24, 128.45, 128.25, 127.77, 125.57, 125.49, 124.57, 121.46, 121.09, 114.28, 112.33, 109.35, 45.39.

1-Benzyl-5-bromo-3-((4-phenyl-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62j):



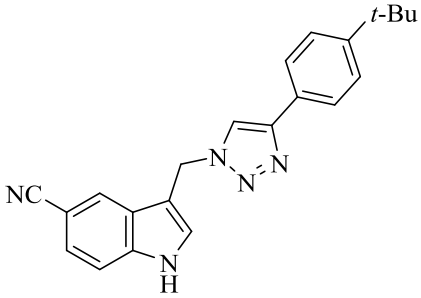
Yield: 372 mg (84%); off-white solid; mp 103 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.77 (s, 1H), 7.47 (s, 1H), 7.46 – 7.43 (m, 1H), 7.43 – 7.38 (m, 2H), 7.35 – 7.29 (m, 4H), 7.28 – 7.23 (m, 3H), 7.21 (d, *J* = 7.2 Hz, 2H), 5.78 (s, 2H), 5.44 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.86, 138.06, 135.30, 131.17, 131.09, 129.33, 129.09, 129.03, 128.29, 128.01, 127.56, 127.36, 125.59, 124.83, 121.53, 113.15, 112.82, 109.25, 49.70, 45.19.

5-Bromo-3-((4-(4-(tert-butyl)phenyl)-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62k):



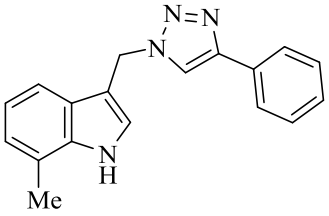
Yield: 343 mg (84%); off-white solid; mp 174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 8.50 (s, 1H), 7.80 (s, 1H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.63 (d, *J* = 2.1 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.41 – 7.34 (m, 2H), 5.75 (s, 2H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.71, 146.84, 140.25, 135.42, 128.45, 127.73, 126.04, 125.36, 124.56, 121.13, 120.68, 114.27, 112.32, 109.40, 45.36, 34.79, 31.51.

3-((4-(4-(*tert*-Butyl)phenyl)-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole-5-carbonitrile (62l):



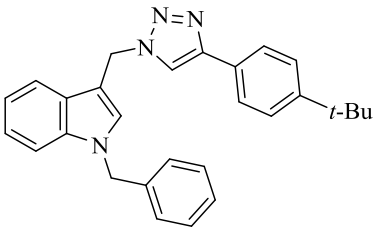
Yield: 280 mg (79%); white solid; mp 197 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 7.96 (s, 1H), 7.71 (d, *J* = 8.7 Hz, 2H), 7.51 – 7.45 (m, 3H), 7.42 (d, *J* = 8.3 Hz, 3H), 7.28 (s, 1H), 5.78 (s, 2H), 1.33 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.47, 148.15, 138.06, 127.45, 126.52, 126.01, 125.86, 125.79, 125.45, 124.20, 120.18, 119.13, 112.66, 110.65, 103.85, 45.56, 34.67, 31.26.

7-Methyl-3-((4-phenyl-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62m):



Yield: 240 mg (84%); brown solid; mp 83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.78 – 7.72 (m, 2H), 7.65 (s, 1H), 7.41 (dd, *J* = 6.8, 2.2 Hz, 1H), 7.40 – 7.35 (m, 2H), 7.33 (d, *J* = 2.5 Hz, 1H), 7.32 (t, *J* = 1.3 Hz, 1H), 7.31 – 7.27 (m, 1H), 7.08 (t, *J* = 5.9 Hz, 1H), 5.76 (s, 2H), 2.53 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.76, 136.08, 130.65, 128.75, 128.04, 125.83, 125.68, 124.34, 123.44, 120.99, 120.82, 119.43, 116.13, 109.56, 46.18, 16.64.

1-Benzyl-3-((4-(4-(*tert*-butyl)phenyl)-*1H*-1,2,3-triazol-1-yl)methyl)-*1H*-indole (62n):



Yield: 323 mg (77%); white solid; mp 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.3 Hz, 1H), 7.63 (s, 1H), 7.56 (d, *J* = 10.8 Hz, 2H), 7.46 – 7.40 (m, 4H), 7.38 – 7.32 (m, 5H), 7.28 (d, *J* = 2.7 Hz, 2H), 7.20 – 7.14 (m, 2H), 5.74 (s, 2H), 5.35 (s, 2H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.08, 136.80, 128.94, 128.88, 128.18, 127.96, 126.99, 126.81, 125.65, 125.59, 125.42, 122.76, 120.46, 120.28, 119.02, 118.86, 110.18, 108.57, 50.24, 45.94, 34.64, 31.30.

5.4.1 **Antibacterial assay**

The synthesized compounds were tested for antibacterial activity against four Gram negative (*S. typhi, P. putida)* and two Gram positive (*B. subtilis, S. aureus*) bacteria as per the following standard method (NCCL, 1993). The tested bacterial cultures were freshly cultured and inoculated into the Luria-bertani broth medium and kept for overnight at 37 °C with shaking at 150 rpm. Meanwhile, autoclaved Luria-bertani agar medium was equipped and poured into sterile glass petri-dishes (90 mm) under aseptic conditions. After solidification of medium, 100 µL culture of each test strain (10^7 cfu/mL) was spread over it using a sterile glass spreader and left for 15 min for complete adsorption. After adsorption, wells of size 6 mm diameter were made by the sterile metallic borer and the solution of compounds with different concentration were poured into the wells. The compounds to be tested were dissolved in DMSO (Merck, India). After incubation at 37 °C for 24 h, the diameter of the zone of inhibition was measured in comparison with standard antibiotic 'ciprofloxacin'. Solvent, DMSO was used as negative control while antibiotic 'ciprofloxacin' was used as positive control. For the MIC assay, test compounds were prepared in the concentrations of 8, 16, 32, 64, 128 and 256 µg/mL in DMSO and serial diluted test samples of each compound (150 µL) were added in 96 well micro-trays. The same amount of test microorganism was

added to micro-trays well to obtain a final volume of 300 µl and incubated at 37 °C for 24 h. MIC value is defined as the lowest concentration of compound that inhibit the visible growth of bacteria (OD_{600} less than 0.06). Each assay was performed in duplicate sets.

5.4.2 Antifungal assay

Antifungal activities of test compounds were determined by agar well diffusion method against the fungal strains *C. albicans* and *A. flavus*. For the experimental work, a loopful of each strain was grown in Potato dextrose broth (PDB, Himedia, India) medium at 28 °C for 4-5 days. Following optimal growth of each fungal strain, 100 µL of culture was uniformly spread on the potato dextrose agar medium plate. Following adsorption, wells of 6 mm was prepared by the sterile metallic borer and solution of working compound of different concentration was poured into the wells. Plates were incubated at 28 °C for 4-5 days under dark conditions. Mean diameter of inhibition zone was measured to determine the antifungal activity. For the MIC assay, sterile test tubes containing 5 mL of sterilized czapeks dox broth medium was inoculated with 100 µL of freshly grown culture of each test strain and appropriate amount of compound was added to achieve the desired concentrations. The tubes were incubated at 28 °C for 5 days under dark conditions and carefully observed for the presence of turbidity. Amphotericin B was used as positive control. The experiment was performed in duplicate sets.

5.4.3 Live-dead bacterial screening

To discriminate the live and dead bacterial cells, the overnight grown culture of *P. putida* (10^7 cfu mL^{-1}) was treated with selected compounds 62b, 62i, 62j, 62l at three times concentration of MIC for 4 h. The working solution of acridine orange (AO, 15 µg mL^{-1}) and ethidium bromide (Et.Br, 50 µg mL^{-1}) were prepared in the PBS buffer (1X pH 7.2). After the compounds treatment, 5 µL each of acridine orange and ethidium bromide was added to 500 µL of *P. putida* culture following the standard protocol of Jakopec et al. (2006) with minor modifications. The suspension was centrifuged at 5,000 g for 10 min and supernatant was discarded. The cell pellet was washed with 1X PBS buffer three times to remove any traces of unbound dyes. Washed cell pellet was streaked on the glass slide with a cover slip on top of it and viewed under epi-fluorescence microscope (Olympus-CKX41, Olympus, Japan) at intensity between 450 and 490 nm using 100X objective lens and 10X eyepiece lens.

5.4.4 Propidium iodide (PI) assay

The freshly grown bacterium *P. putida* was inoculated into the sterile nutrient-broth medium and kept in a rotary shaker (150 rpm) till the optical density (OD) of culture reached up to 0.8 (A600). The culture was treated with selected compounds with double concentration of the MIC value for that compound for 4h. After the treatment, the culture was centrifuged at 5,000 g for 10 min and the cell pellet was stained with 1 mg/mL of propidium iodide (PI) (Sigma-Aldrich, USA). The stained colony was streaked on the clean glass-slide and covered with a glass-slip. The population of PI-positive bacterial cells was compared with untreated cells by epi-fluorescence microscopy. During the experiment, bacterial culture treated with *tert*- butyl hydroperoxide (*t*-BHP) which was taken as positive control.

5.4.5 Haemolytic assay

All the chemically compounds were also tested for hemolytic activity following the standard protocol.[58] The healthy human blood samples were collected from localized hospital at BITS Pilani and washed with sterile phosphate buffer saline (PBS, 1X pH 7.2) solution at least three times. After washing, blood samples were centrifuged at 3,500 rpm for 5 min at room temperature and settled RBC was suspended in PBS buffer of 100 µL. The RBC suspension of 100 µL was mixed with 500 µL buffer solution containing 100 µg/mL of each compound in 1% (v/v) DMSO in PBS buffer. DMSO 1% (v/v) in PBS buffer was used as negative control, whereas 1% Triton-×100 as positive control. The samples were incubated at room temperature for 3h. Following incubation, samples were centrifuged at 8,000 rpm and absorbance of supernatant was read at 540 nm. The % haemolysis was calculated following the equation:

[% Haemolysis = (A540 test sample - A540 negative control/A540 positive control - Absorbance 540 negative control)×100], Each sample was analyzed in duplicate sets.

5.5 References

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Chapter VI

Conclusions

Summary

5.1 General Conclusions

Click chemistry is the term used to describe reactions which are high yielding, wide in scope, need no column chromatography, have atom economy, require less time and are simple to perform. Copper catalysed azide-alkyne cycloaddition (CuAAC) is one of the premier examples of click chemistry which offers 1,4-disubstituted 1,2,3-triazoles. Exclusive regioselectivity, simple reaction condition, high yields and wide substrate scope are some important characteristics which made CuAAC as an important method for the synthesis of 1,2,3-triazoles. 1,2,3-Triazoles are of great importance in organic chemistry because of their easy accessibility, multiple biological properties and their applications in different areas such as agrochemical, medicines, dyes, corrosion inhibition, photostabilizer, photographic material, herbicide and fungicide etc. 1,2,3-Triazoles bind with different heterocycles without altering their physical properties and they have the ability to bind two different heterocycles to bring their properties simultaneously. Also, 1,2,3-triazoles possess different biological activities such as antibacterial, antifungal, anti-HIV and anti-allergic. They have been widely explored for conjugation of different bioactive heterocycles.

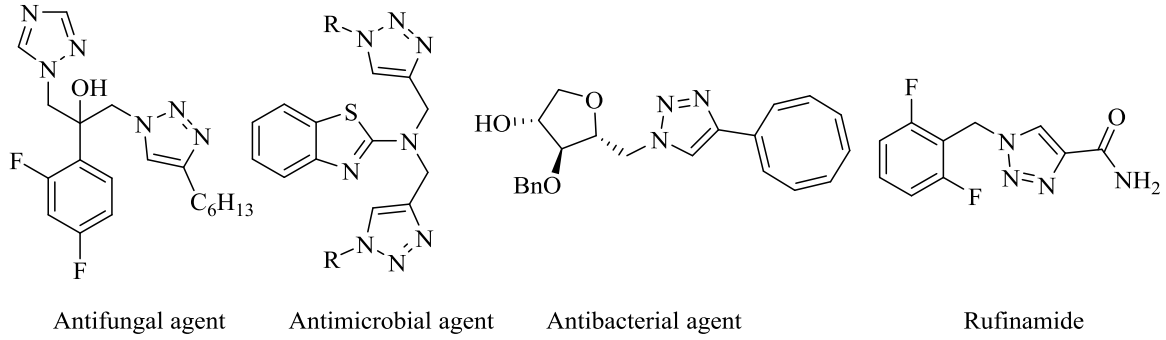


Figure 1: Some biologically active 1,4-disubstituted 1,2,3-triazole containing drugs.

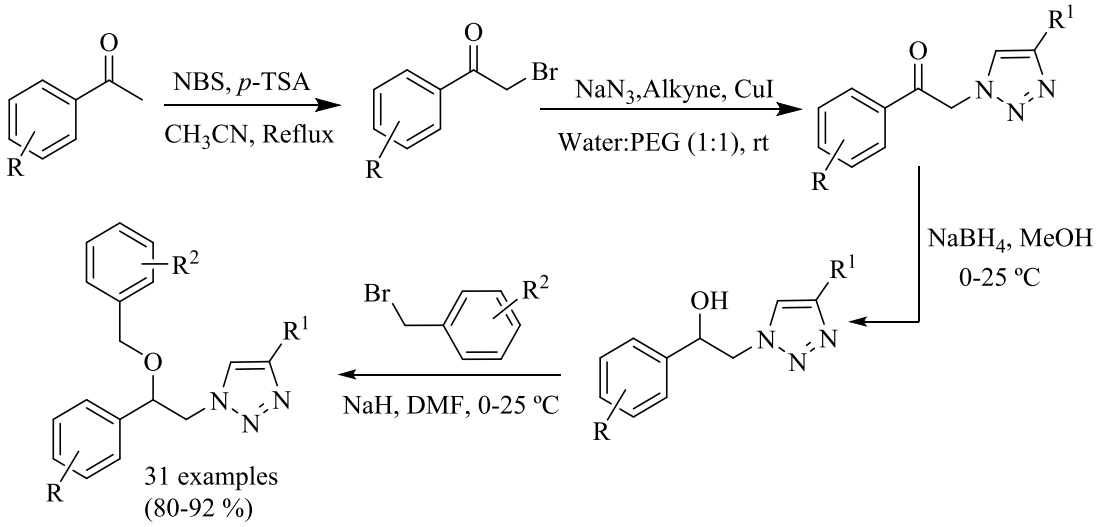
The current thesis entitled "**Click chemistry inspired synthesis of novel triazolyl conjugates and their antimicrobial activity**" deals with the synthesis of some novel 1,4-disubstituted 1,2,3-triazolyl heterocycles by employing copper catalysed azide-alkyne cycloaddition - a click chemistry approach and their biological activity as antimicrobial agents. Mainly, the thesis is focused on the synthesis and antimicrobial evaluation of 1,2,3-triazolyl linked aryl/ heterocyclic rings. The thesis is divided mainly into five chapters.

5.2 Specific Conclusions

The thesis entitled "**Click chemistry inspired synthesis of novel triazolyl conjugates and their antimicrobial activity**" is divided in five chapters. A brief overview of these chapters is discussed below.

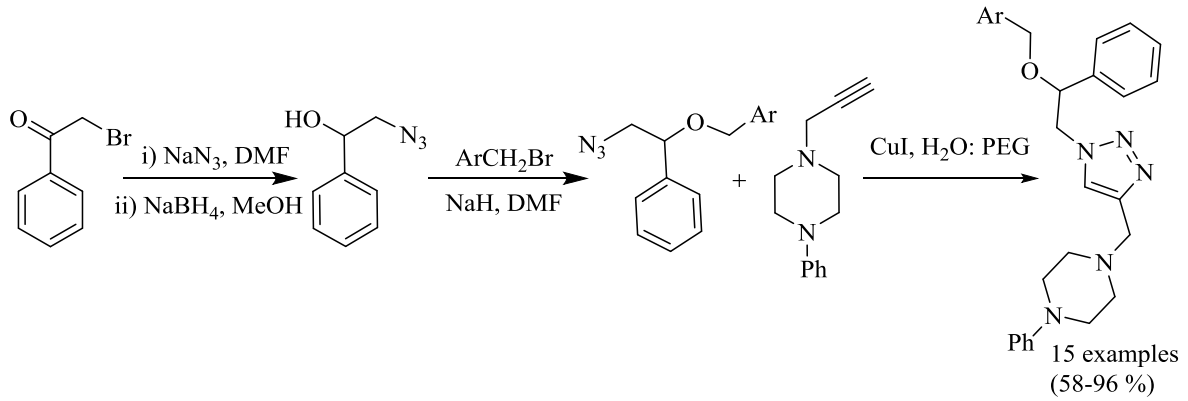
The first chapter of thesis presents a brief literature overview on chemistry of copper catalysed synthesis of 1,2,3-triazoles and their antimicrobial activity. Details on regioselective synthesis of 1,4 and 1,5 disubstituted 1,2,3-triazoles *via* click chemistry is described. A review of reports is presented with schematic explanation of synthetic procedures *via* conventional methods, ultrasonic radiation and microwave irradiation. Simultaneously, their antibacterial and antifungal activity is also described briefly which explains the utilization of corresponding triazoles in pharmacological research. 1,2,3-Triazoles could increase antimicrobial action by linking two different heterocycles or on tethering with heterocycles. Overall, this chapter provides a brief idea on synthetic protocols and antimicrobial behaviour of 1,4-disubstituted 1,2,3-triazoles reported in recent years.

The second chapter of the thesis is focused on the synthesis of 1,2,3-triazole as analogues of azole drug by isosteric replacement of azole ring. A brief introduction is provided on significance of azole drugs such as Miconazole, Econazole as antibacterial and antifungal agents. In this chapter, 1,2,3-triazolyl analogues of azole drugs are synthesized using CuAAC. The chapter is divided in two parts. In part-A, imidazole ring of Miconazole drug is replaced with isosteric 1,2,3-triazole ring using CuAAC reaction. A series of benzyloxy aryl 1,4-disubstituted 1,2,3-triazoles was synthesized in good yields (80-92%) (Scheme1). After synthesizing Miconazole isosteric derivatives, their antibacterial and antifungal activity was performed but all the compounds were found to show poor activity and this may be attributed to the poor solubility of the synthesized compounds.



Scheme 1: Synthesis of novel drug like molecule *via* CuAAC.

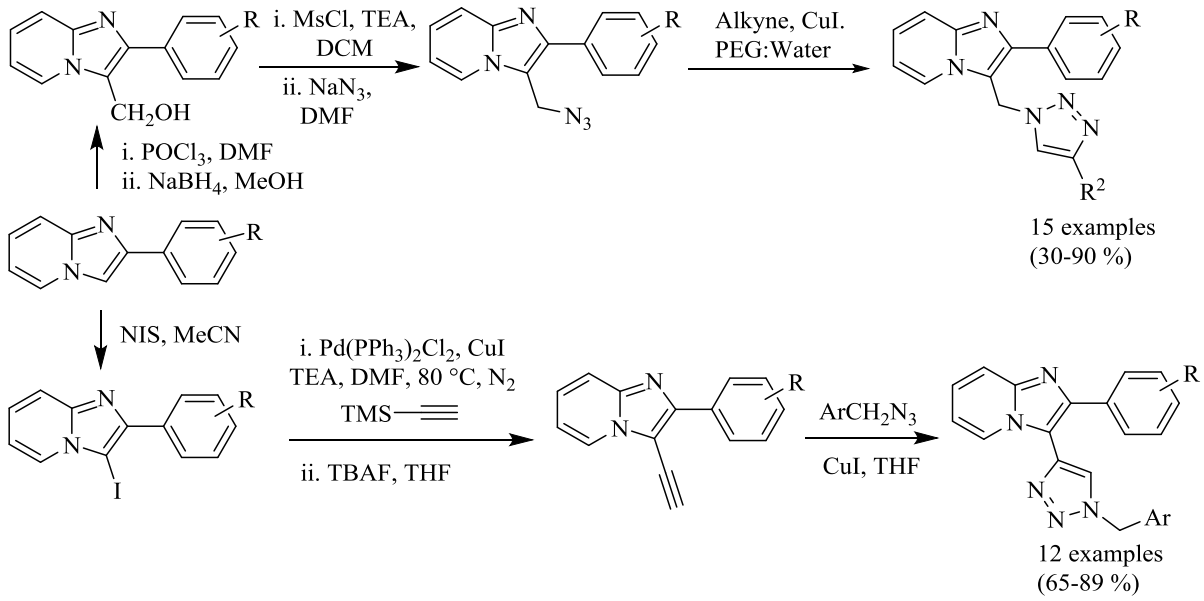
In part B, piperazine-triazole derivatives have been prepared using CuAAC reaction in good to excellent yields (58-96 %) (Scheme 2). It was envisioned that the solubility of 1,2,3-triazoles can be improved by tethering piperazine ring. The synthesized compounds were also converted to their hydrochloric salts. All the derivatives and their salts were evaluated for antibacterial and antifungal activity against different Gram positive, Gram negative bacteria and yeast strains. The minimum inhibitory concentrations and zone of inhibition was calculated for each derivative which showed that the synthesized compounds are moderate antimicrobial agents with MIC value of 64-128 μg/mL and zone of inhibition 14-19 mm. The MIC and zone of inhibition were considerably more for the hydrochloric salt than its corresponding compound.



Scheme 2: Synthesis of piperazine-triazole derivatives

In the third chapter of the thesis novel imidazo[1,2-*a*]pyridine-1,2,3-triazole derivatives have been synthesized and evaluated for their antimicrobial activity. Two series of triazolyl imidazo[1,2-*a*]pyridines *i.e.* 3-(triazol-1-yl)methyl-imidazo[1,2-*a*]pyridines and 3-(triazol-4-

yl)-imidazo[1,2-*a*]pyridines have been synthesized *via* CuAAC (Scheme 3). The compounds were obtained in good to excellent yields (32-90%). Imidazo[1,2-*a*]pyridines were synthesized by reaction of acetophenones and 2-aminopyridines in the presence of CuI following literature procedure. Imidazo[1,2-*a*]pyridine with azidomethyl or acetylene substitution at C-3 position were prepeared by sequential reactions as shown in scheme 3. Reaction of these functionalized imidazo[1,2-a]pyridines with alkynes or azides under CuACC reaction condition provided imidazo[1,2-*a*]pyridine linked 1,4-disubstituted 1,2,3-triazoles in good to excellent yields.

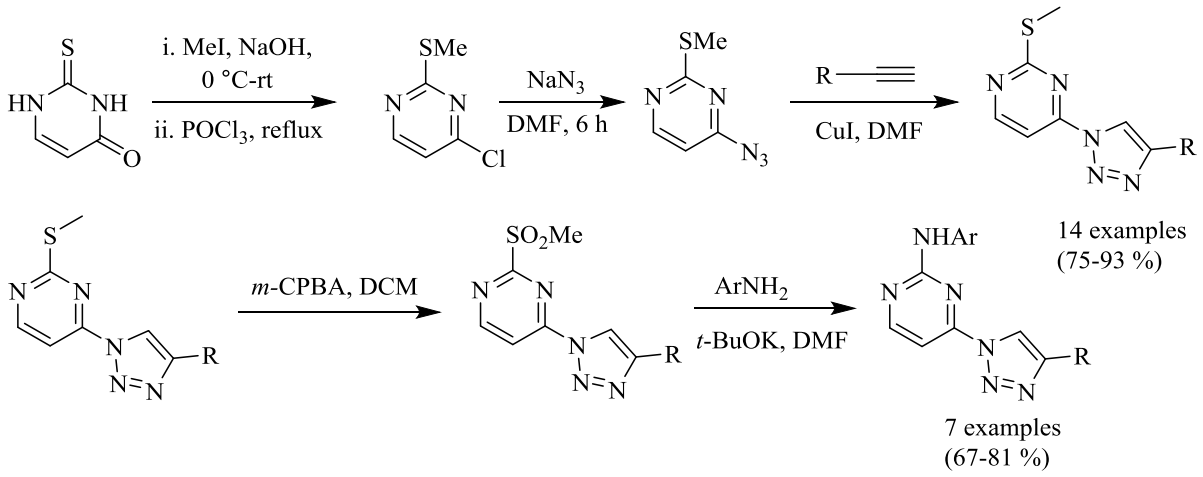


Scheme 3: Synthesis of triazole-imidazo[1,2-*a*]pyridines.

The synthesized derivatives were screened for antimicrobial activity against different bacteria (*K. pneumoniae, P. putida, B. subtilis* and *S. aureus*) and fungi (*C. albicans, A. flavus, F. oxysporum* and *P. citrinum*). The compounds showed good antimicrobial activity with zone of inhibition 12-17 mm against different bacteria and fungi. The MIC value was observed in the range of 3.8-15.2 µg/mL for various bacteria and fungi. The compounds were also evaluated for their biofilm inhibition study against *S. aureus*. ROS study of representative compounds was also performed to check the protective effect against 2′,7′-dichlorofluorescein diacetate (DCFH-DA) oxidation in bacterial cells by fluorescence microscopy. Acridine orange and ethidium bromide (AO/Et.Br) dual staining assay were performed to differentiate the effect caused by the most active compounds on live and dead cells.

Chapter four of the thesis describes the synthesis of pyrimidine-1,2,3-triazolyl derivatives and evaluation of their antimicrobial activity. Two series of pyrimidine-triazole derivatives *viz* thiopyrimidine-1,2,3-triazole derivatives and *N*-phenylaminopyrimidine-1,2,3-triazole

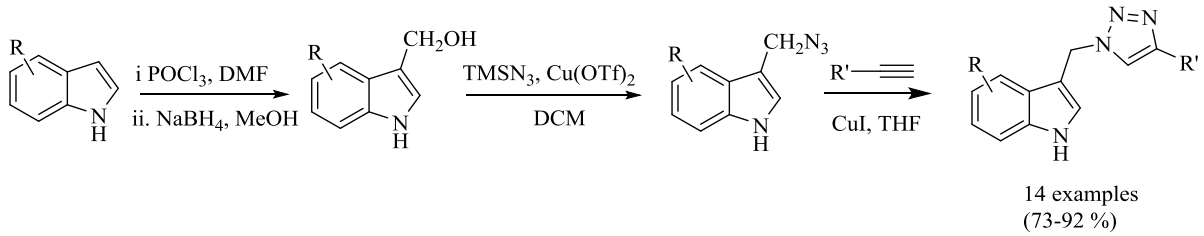
derivatives were synthesized starting from 2-thiouracil in good to excellent yields (67-91%) using CuAAC reaction conditions. Initially, 2-thiouracil was transformed to 2-thiomethyl-4-azidopyrimidines through sequence of reactions shown in scheme 4. Reaction of 2-thiomethyl-4-azidopyrimidines with alkynes in the presence of CuI in DMF gave corresponding thiopyrimidine-1,2,3-triazoles (72-91%). Further, *N*-phenylaminopyrimidine-1,2,3-triazole derivatives were prepared in good yields (67-81%) by oxidation of thiomethyl of thiopyrimidine-1,2,3-triazoles with *m*-CPBA followed by nucleophilic substitution with anilines.



Scheme 4: Synthesis of pyrimidine-triazole derivatives.

All the synthesized pyrimidine-triazole derivatives were evaluated for antibacterial activity against Gram negative (*E. coli, S. typhi, K. pneumoniae* and *P. putida*), Gram positive (*B. subtilis, S. Aureus*) bacteria. Derivatives with 2-amino substitution on pyrimidine ring and pyridine substitution on triazole ring were found to be more effective with MIC value 6.25 µg/mL and zone of inhibition 18-19 mm against different bacteria. The compounds were also evaluated for antifungal activity against *C. albicans, A. Flavus and F. oxysporum* and they showed MIC value 6.25-25 µg/mL and zone of inhibition 17-18 mm.

Chapter five of the thesis is focused on synthesis of novel indolyl-triazole derivatives and evaluation of their antimicrobial activity against different bacterial and fungal strain. Indoles were transformed into indolyl-triazole derivatives by a sequence of reactions *viz* formylation, reduction, azidation and CuAAC reaction (Scheme 5). A series of 14 indolyl-triazole derivatives was prepared in good to excellent yield.



Scheme 5: Synthesis of novel tndolyl-triazole derivatives *via* CuAAC.

Synthesized derivatives were screened for antimicrobial action against Gram negative (*S. typhi* and *P. putida*), Gram positive (*B. subtilis, S. Aureus*) bacteria and *C. albicans* and *A.* Flavus fungi. Indolyl-triazole derivatives showed MIC value 8-64 µg/mL and zone of inhibition 11-18 mm against different bacteria and fungi. Derivatives having electron withdrawing substitution on indole ring were found to be better antimicrobial agent with MIC value 4-8 µg/mL. Kinetic study of most potent compound was done against Gram positive and Gram negative bacterial strains. Ethidium bromide (AO/Et.Br) dual staining assay and propium-iodide essay were performed to differentiate the effects caused by the agents on live and dead cells. Haemolytic activity of the synthesized compounds was also checked against RBC membranes (erythrocytes) to mark the damage caused by the compounds and to further promote them up to drug level.

Future scope of the work

1,2,3-Triazoles have high dipole moment and they participate actively in hydrogen bond formation as well as in dipole–dipole interactions. This framework is stable against acidic and basic hydrolysis which makes them structural bioisosteres of the amide bond, azoles, acyl phosphate and *trans*-olefinic moieties. In addition, 1,2,3-triazoles are stable against oxidative and reductive conditions which results in high aromatic stabilisation and relative resistance to metabolic degradation. CuAAC click reactions has become as widely accepted approach for the regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles.

The thesis mainly focused on synthesis of 1,4-disubstituted-1,2,3-triazoles using CuAAC reaction and evaluation of their antimicrobial activity. The synthesized derivatives were found to show moderate to good antimicrobial activity. The lead compounds can be taken from these studies for further SAR study. Simultaneously, these novel 1,2,3-triazole derivatives could be evaluated for other biological activities such as anti-HIV agents. Especially, *N*-phenylaminopyrimidine-1,2,3-triazole derivatives prepared in chapter IV are analogues of diarylpyrimidines which have been found to be active anti-HIV agents.

Appendices

Appendices

List of Publications *[A-1]*

1. Kasiviswanadharaju Pericherla, **Poonam Khedar**, Bharti Khungar and Anil Kumar, Click chemistry inspired structural modification of azole antifungal agents to synthesize novel 'drug like' molecules, *Tetrahedron Letters* **2012**, *53*, 6761-6764.
2. Kasiviswanadharaju Pericherla, **Poonam Khedar**, Bharti Khungar and Anil Kumar, One-pot sequential C–N coupling and cross dehydrogenative couplings: synthesis of novel azole fused imidazo[1,2-*a*]pyridines, *Chemical Communication* **2013**, *49*, 2924-2926.
3. Kasiviswanadharaju Pericherla, Pinku Kaswan, **Poonam Khedar**, Bharti Khungar, Keykavous Parang and Anil Kumar, Copper catalyzed tandem oxidative C–H amination/cyclizations: Direct access to imidazo[1,2-*a*]pyridines, *RSC Advances* **2013**, *3*, 18923-18930.
4. **Poonam Khedar**, Kasiviswanadharaju Pericherla, and Anil Kumar, Exploration of the CuAAC reaction for the synthesis of novel 3-(triazol-1-yl)-methylimidazo[1,2-*a*]-pyridines, *Synlett* **2012**, *53*, 2609-2614.
5. **Poonam Khedar**, Kasiviswanadharaju Pericherla and Anil Kumar, Copper triflate: an efficient catalyst for the direct conversion of secondary alcohols into azides, *Synlett* **2014**, 515-518.
6. **Poonam Khedar**, Kasiviswanadharaju Pericherla, Rajnish Prakash Singh, Prabhat Nath Jha and Anil Kumar, Click chemistry inspired synthesis of piperazine-triazole derivatives and evaluation of their antimicrobial activities, *Medicinal Chemistry Research* **2015,** 3117-3126.

Exploration of the CuAAC Reaction for the Synthesis of Novel 3-(Triazol-1-yl)-methyl-imidazo[1,2-*a*]pyridines

Poonam Khedar, Kasiviswanadharaju Pericherla, Anil Kumar*

Department of Chemistry, Birla Institute of Technology and Science, Pil
Fax +91(1596)244183; E-mail: anilkumar@bits-pilani.ac.in

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SYNLETT 2012, 23, 2609–2614
Advanced online publication: 18.10.2012
DOI: 10.1055/s-0032-1317379; Art ID: ST-2012-D0714-L

Abstract: The archetypical CuAAC click chemistry is explored to assemble diverse 3-(triazol-1-yl)methyl-imidazo[1,2-*a*]pyridines. The approach is simple, general, and environmentally benign to generate a library of novel triazolo-imidazo[1,2-*a*]pyridine derivatives in good yield (30–90%).

Key words: azides, click chemistry, imidazo[1,2-*a*]pyrimidine 1,2,3-triazole, 3-(triazolyl)methyl-imidazo[1,2-*a*]pyridine

The term 'click chemistry' was first introduced by Sharpless to describe a set of reactions that are high yield-

tuberculosis,[30,31] carbonic anhydrase,[32] c-Src kinase,[33] GSK-3,[34] and indoleamine 2,3-dioxygenase 1 inhibition.[35] They have been widely employed in the pharmaceutical, agrochemical, polymer, and materials field. Synthesis of triazolo-conjugated heterocycles through molecular-hybridization approach has attracted attention from many research groups because it may lead to molecules with improved pharmacophoric properties.[36,37] Therefore exploring the synthesis of novel imidazo[1,2-*a*]pyridine-triazole derivatives is desirable in organic synthesis.

Copper Triflate: An Efficient Catalyst for Direct Conversion of Secondary Alcohols into Azides

Poonam Khedar, Kasiviswanadharaju Pericherla, Anil Kumar*

Department of Chemistry, Birla Institute of Technology and Science, Pilani 333031 , India
Fax +91(1596)244183; E-mail: anilkumar@pilani.bits-pilani.ac.in

Received: 22.10.2013; Accepted after revision: 09.12.2013

SYNLETT 2014, 25, 0515–0518
Advanced online publication: 16.01.2014
DOI: 10.1055/s-0033-1340550; Art ID: ST-2013-D0992-L

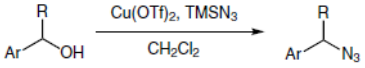
Abstract: A simple, practical, and efficient strategy has been demonstrated for the direct synthesis of organic azides from alcohols using azidotrimethylsilane ($TMSN_3$) as azide source in the presence of copper(II) triflate [$Cu(OTf)_2$]. A variety of alcohols was converted into the corresponding azides in good to excellent yields. The formation of an intermediate carbocation was confirmed by the synthesis of bis(diphenylmethyl) ether.

Key words: azides, azidotrimethylsilane, copper triflate, Lewis acid

Organic azides are a versatile class of compounds frequently used in synthetic organic chemistry.[1–4] They are useful precursors for the preparation of amines,[5,6] nitrenes,[7] and heterocyclic compounds. For example, glyco-

and NaN_3/CCl_4–DMF.[28] Although the reported methods are effective, they suffer from limitations such as inaccessible and expensive reagents and long reaction times as well as cumbersome separation from the generated $Ph_3P{=}O$ and unreacted Ph_3P.

Thus, in this communication we wish to report our preliminary results for the direct conversion of alcohols into azides using azidotrimethylsilane ($TMSN_3$) and copper(II) triflate [$Cu(OTf)_2$, Scheme 1].



Scheme 1 One-pot synthesis of azide from benzyl alcohols

Tetrahedron Letters 53 (2012) 6761–6764

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Click chemistry inspired structural modification of azole antifungal agents to synthesize novel 'drug like' molecules

Kasiviswanadharaju Pericherla, Poonam Khedar, Bharti Khungar, Anil Kumar *

Department of Chemistry, Birla Institute of Technology and Science, Pilani 333 031, India

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Keywords:
Click chemistry
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Drug like
One-pot reaction

ABSTRACT

A new class of 'drug like' 1,4-disubstituted-1,2,3-triazoles is synthesized using one-pot reaction of sodium azide, α-bromo ketones, and alkynes in PEG-400/water (1:1, v/v) under the click chemistry reaction condition followed by reduction of keto group and alkylation. The method is simple, efficient and gives good yield of novel 1,2,3-triazole derivatives.

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One-pot sequential C–N coupling and cross dehydrogenative couplings: synthesis of novel azole fused imidazo[1,2-*a*]pyridines†

Kasiviswanadharaju Pericherla, Poonam Khedar, Bharti Khungar and Anil Kumar*

Cite this: *Chem. Commun.*, 2013, **49**, 2924

Received 25th December 2012,
Accepted 20th February 2013

DOI: 10.1039/c3cc39206f

www.rsc.org/chemcomm

An efficient one-pot protocol has been developed using sequential C–N coupling and intramolecular dehydrogenative cross-couplings for the synthesis of azole fused imidazo[1,2-a]pyridine derivatives in good yields (62–78%).

of synthetic simplicity and efficiency, atom economy, and environmental benefits.[6–9] It avoids pre-functionalization of substrates and minimizes by-product formation.

A number of novel methodologies have been established to

RSC Advances

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Copper catalyzed tandem oxidative C–H amination/cyclizations: Direct access to imidazo[1,2-a]pyridines†

Cite this: *RSC Advances*, 2013, **3**, 18923

Kasiviswanadharaju Pericherla,[a] Pinku Kaswan,[a] Poonam Khedar,[a] Bharti Khungar,[a] Keykavous Parang[b] and Anil Kumar*[a]

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Accepted 6th August 2013

DOI: 10.1039/c3ra43889a

www.rsc.org/advances

A simple and convenient strategy is described for the synthesis of imidazo[1,2-a]pyridines *via* inexpensive copper-catalyzed tandem imine formation and intramolecular aerobic oxidative C–H bond amination/cyclizations. An array of imidazo[1,2-a]pyridines were prepared by the reaction of readily available acetophenones and 2-aminopyridines in good to excellent yields (48–92%). The scope of the method was validated by a single step synthesis of Zolimidine, a drug used for peptic ulcers, in 61% yield.

Med Chem Res (2015) 24:3117–3126
DOI 10.1007/s00044-015-1361-5

MEDICINAL CHEMISTRY RESEARCH

CrossMark

ORIGINAL RESEARCH

Click chemistry inspired synthesis of piperazine-triazole derivatives and evaluation of their antimicrobial activities

Poonam Khedar[1] · Kasiviswanadharaju Pericherla[1] · Rajnish Prakash Singh[2] · Prabhat Nath Jha[2] · Anil Kumar[1]

Received: 17 June 2014 / Accepted: 10 March 2015 / Published online: 27 March 2015

Abstract A series of novel piperazine-1,2,3-triazole derivatives, which entailed the bioisosteric replacement of the imidazole moiety and hybridization of two drug scaffolds was prepared by employing the regioselective copper (I)-catalysed azide-alkyne 1,3-dipolar cycloaddition reaction. The synthesized compounds were evaluated for antibacterial activities against Gram-negative (*E. Coli* and *P. Putida*), Gram-positive *S. Aureus* bacteria and fungicidal activities against *F. oxysporum, F. gramillarium* and *F. monalliforme* fungi. Compound **7ac′** exhibited moderate but promising antibacterial activity against Gram-negative bacteria and fungicidal activity against *F. oxysporum and F. gramillarium.*

(Karyotakis and Anaissie, 1994). Piperazine is also found along with azoles moieties in several antifungal drugs such as itraconazole and ketoconazole. In recent years, 1,2,3-triazoles gained the interest owing to their broad range of biological activities such as anti-HIV (Alvarez *et al.*, 1994), anti-inflammatory (de Oliveira Assis *et al.*, 2012), anti-malarial (Manohar *et al.*, 2011), anti-viral (Ferreira *et al.*, 2014), and anticancer activities (Kamal *et al.*, 2008; Yan *et al.*, 2010). Particularly, these aza-heterocyclic motifs were widely studied as antifungal agents due to their low toxicity and broad antifungal spectrum (Agalave *et al.*, 2011; Jiang *et al.*, 2011; Pore *et al.*, 2012). Moderate dipole character, rigidity, and hydrogen-bonding ability and sta-

List of Oral/poster presented in conferences *[A-3]*

Oral presentations

1. **Poonam Khedar,** Kasiviswanadharaju Pericherla and Anil Kumar, Direct conversion of secondary alcohols into azide using $Cu(OTf)_2/TMSN_3$, NCRDCS 2014, Guru Jambheshwar University of Science and Technology, Hisar, Haryana (February 25-26, **2014**).
2. **Poonam Khedar,** Kasiviswanadharaju Pericherla and Anil Kumar, Direct conversion of secondary alcohols into azide using $Cu(OTf)_2/TMSN_3$, FCASI-2017, Dept. of Chemistry, University of Rajasthan, Jaipur (March13-14, **2015**).

Poster presentations

3. Kasiviswanadharaju Pericherla, **Poonam Khedar**, Bharti Khungar and Anil Kumar, One-pot three component synthesis of substituted imidazo[1,2-*a*]pyridines catalyzed by $Yb(OTf)_3$ and their anti cancer activities, National Symposium on Recent Trends in Chemical Science and Technology, Department of Chemistry, IIT Patna (March 3-4, **2012**).
4. Kasiviswandharaju Pericherla, **Poonam Khedar**, Bharti Khungar and Anil Kumar, Synthesis of substituted 1-amidomethyl imidazo[1,2-*a*]pyridines catalyzed by $Yb(OTf)_3$, National Symposium on Recent Trends in Chemical Sciences, Department of Chemistry, BITS Pilani, Pilani (March 25, **2012**).
5. **Poonam Khedar**, Kasiviswanadharaju Pericherla, Anil Kumar, Click chemistry inspired synthesis of 1,2,3 triazolylimidazo[1,2-a]pyridines, National Symposium on Recent Trends in Chemical Science and Technology, Department of Chemistry, IIT Patna (March 3-4, **2012**).
6. **Poonam Khedar**, Kasiviswanadharaju Pericherla, Anil Kumar, Click chemistry inspired synthesis of 1,2,3-triazolylimidazo[1,2-*a*]pyridines, National Symposium on Recent Trends in Chemical Sciences, Department of Chemistry, BITS Pilani, Pilani (March 25, **2012**).
7. **Poonam Khedar**, Kasiviswanadharaju Pericherla and Anil Kumar, Synthesis of novel 1-((1-(2-aryloxy-2-arylethyl)-1H-1,2,3-triazol-4-yl)methyl)-4-arylpiperazines, Recent Advances and Current Trends in Chemical and Biological Sciences, 19th ISCBC-2013, Mohanlal Sukhadia University, Udaipur (March, 2-5, **2013**).

8. Pinku Kaswan, Kasiviswanadharaju Pericherla, **Poonam Khedar** and Anil Kumar, Copper catalyzed tandem oxidative C–H amination/oxidative cyclizations: direct access to imidazo[1,2-*a*]pyridines, 16th CRSI National Symposium in Chemistry, Department of Chemistry, IIT Bombay, Mumbai (February, 7-9, **2014**).
9. **Poonam Khedar** and Anil Kumar, Synthesis and biological activity of amino-pyrimidine triazoles, National conference on organic chemistry and sustainable development, Dept. of Chemistry, BITS Pilani, Pilani (August, 29-30, 2016).
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BRIEF BIOGRAPHY OF THE CANDIDATE *[A-4]*

Poonam Khedar obtained her M.Sc. in Organic Chemistry from Govt. Morarka College, Jhunjhunu, University of Rajasthan, India during 2008-10. In August 2011, she joined Department of Chemistry, BITS Pilani for PhD program under the guidance of Prof. Anil Kumar with the financial assistance from the Institute. In December 2012, She was selected for UGC-BSR fellowship, simultaneously, in December 2012, she cleared the Joint CSIR-UGC NET and was awarded Junior Research Fellowship (JRF) by UGC, New Delhi. She has published six research articles in peer reviewed international journals and presented papers in seven national/international conferences/symposiums.

In July, 2015 she was selected as PGT-Chemistry in Jawahar Navodaya Vidyalaya under MHRD and continued her Ph.D. work as part-time scholar. Her research interest lies in the synthesis of bio-active molecules by employing simple and greener methodologies.

BRIEF BIOGRAPHY OF THE SUPERVISOR *[A-5]*

Dr. Anil Kumar is Associate Professor of Chemistry at the Birla Institute of Technology and Science, Pilani. He obtained his PhD degree from Department of Chemistry, University of Delhi, Delhi, India under the guidance of Professor SMS Chauhan in 2004. During his doctoral studies he worked on development of heterogeneous catalyst for organic synthesis with emphasis on green chemistry. He was postdoctoral fellow at Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, USA in Prof. Keykavous Parang group during May 2004 to April 2006. In his postdoctoral studies he has worked on synthesis of novel Src kinase inhibitory agents and solid phase synthesis. He joined Department of Chemistry, Birla Institute of Technology and Science, Pilani, India as Assistant Professor in 2006 and was promoted to Associate Professor in February 2013. He was appointed as Associate Dean, Work Integrated Learning Programmes (WILP) in May 2014 and Head of Department of Chemistry in September 2014. He has visited University of Rhode Island, Kingston, USA as visiting scientist and Acadia University, Wolfville, Canada as Harrison McCain visiting professor.

Dr. Kumar is recipient of Harrison McCain Foundation award from Acadia University, Canada for 2012, ISCB Young Scientist award in Chemical Sciences from Indian Society of Chemists and Biologists, Lucknow for 2013 and Dr. Aravind Kumar memorial award from Indian Council of Chemist for 2014. He has 17 year of research experience and 11 year of teaching experience. He has published over 140 research papers in international journals of repute in the area of synthetic organic chemistry, green chemistry and medicinal chemistry and contributed two book chapter and one US patent He has participated in several national and international symposia/conferences and delivered more than 35 invited lectures. He has guided six PhD students and currently supervising eight PhD students. He is editor for Canadian Chemical Transactions and member of editorial advisory board for The Open Catalysis Journal. He has completed four research projects as Principle Investigator and one as Co-PI sponsored by DST, CSIR and UGC. Currently, he has one major project from SERB and one industry project from Ranbaxy in collaboration with Prof. Dalip Kumar. He has also served as a reviewer for several journals. He is life member of Chemical Research Society of India, Bangalore; Indian Society of Chemists and Biologists, Lucknow and Indian Council of Chemists, Agra.

His research interest lies in transition metal catalyzed C−H activation/functionalization, tandem reactions, green chemistry, ionic liquids and medicinal chemistry.