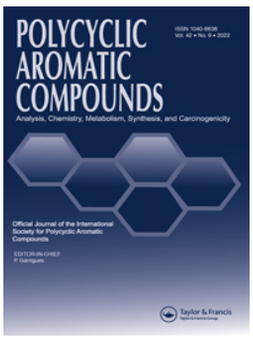

Polycyclic Aromatic Compounds

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[Et_3NH][HSO_4]-Catalyzed One-Pot Solvent Free Syntheses of Functionalized [1,6]-Naphthyridines and Biological Evaluation

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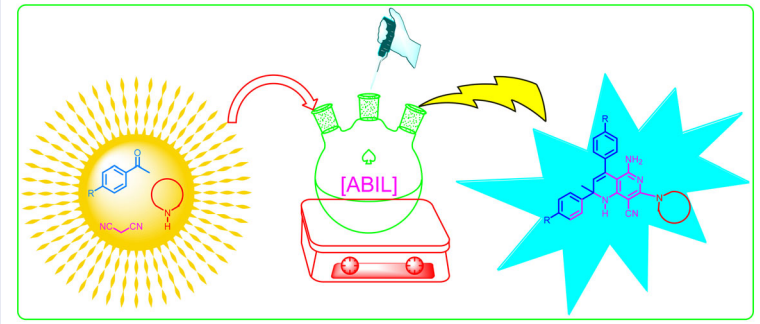
ABSTRACT

We have developed a convenient one-pot multicomponent synthesis of highly functionalized [1,6]-naphthyridines under solvent free condition using [Et_3NH][HSO_4] in excellent yield. This protocol offers several advantages, including short reaction time, simple experimental workup procedure and no toxic byproducts, avoids the use of toxic organic solvents and anhydrous conditions. Further. we have screened the synthesized naphthyridines for *in vitro* antibacterial, antifungal and antioxidant activity. Furthermore, a molecular docking study of these compounds was carried out to investigate their binding pattern with the target, β-Ketoacyl-acyl carrier protein synthase III (FabH). Finally, the ADME parameters for these compounds showed good drug like properties and can be developed as oral drug candidates.



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Antimicrobial; Antioxidant; FabH; ADME; Naphthyridines; [Et_3NH][HSO_4]; multicomponent reactions

Introduction

Naphthyridine is a bicyclic heterocycle containing a pyridine ring fused to that of the dihydropyridine ring. According to literature survey, we found that the methods which are used for the synthesis of the functionalized [1,6]-naphthyridines and their benzo/heterofused analogues involves either multistep sequences or inert atmosphere, lengthy reaction time, expensive catalyst and laborious work up. Being a multifunctional entity, it finds application in nearly every field of laboratory, industrial and medicinal chemistry. Naphthyridines continued to be of great interest due to a wide spectrum of their biological activities such as used in agrochemicals,[1]

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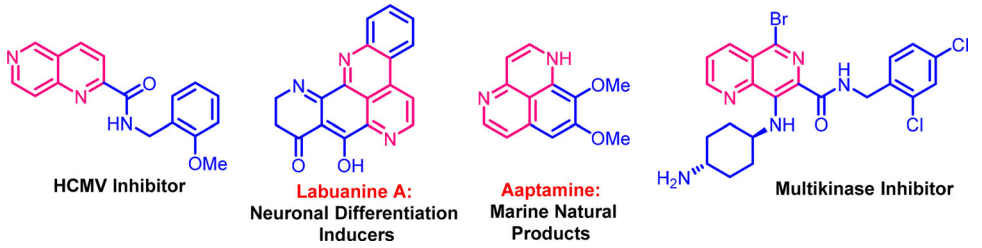


Figure 1. Pharmacologically active naphthyridine based active compounds.

pharmaceuticals,[1] and fluorescent probes.[2] Currently, numerous naphthyridine-containing molecules exhibited medicinal properties for the prevention and treatment of Alzheimer's disease (AD),[3] bacterial infections,[4] heart failure,[5] angiogenic disorder,[6] cancer,[7] parasitic infections[8] and viral infections[9] have been reported. Therefore, the construction of naphthyridines have received increasing attention recently.[10] Human cytomegalovirus (HCMV) is a species-specific DNA virus belonging to the herpesviridae family. The structures of pharmacologically active naphthyridine based compounds are shown in Figure 1.

Nowadays, greener reaction media has gaining more importance to perform organic transformations safely. Ionic liquids referred as 'designer solvent' due to their physical and chemical properties, and can be adjusted by a careful choice of cation and anion. Ionic liquid has been turned to be a kind of promising alternative medium for various chemical processes due to its good solvating capability, non-inflammability, negligible vapor pressure, ease of recyclability, controlled miscibility and high thermal stability.[11] In particular, acidic Bronsted ionic liquids [ABILs] are of special importance, because they possess simultaneously the proton acidity and the characteristic properties of ionic liquids.[12] ABILs offer environmentally friendly catalytic properties due to the combination of the advantages of liquid acids and solid acids, such as uniform acid sites, stability in water and air, easy seperation and reusability. The ionic liquid has been proved to be a very excellent catalyst as well as solvent for many organic transformations.[13]

Triethylammonium Hydrogen Sulfate [Et_3NH][HSO_4] (TEAHS) ionic liquid has been proved to be a very excellent catalyst as well as solvent for many organic transformations such as for the synthesis of quinoline,[13] coumarin,[14] biscoumarins,[15] 1,8-dioxo-octahydroxanthenes,[16] thiazolidine and oxazolidine,[17] hydrazone,[18] 4,4′-(arylmethylene)bis(1*H*-pyrazol-5-ols),[19] functionalized aminoalkyl and amidoalkyl naphthol,[20] *β*-amino carbonyl pyrimidines,[21] xanthene,[22] 3,4-Dihydropyrimidin-2(1*H*)-one[23] derivatives and in hydrolytic reaction,[24] 3,4,5-substituted furan-2(5*H*)-ones[25] and α-amino phosphonates.[26]

The approach to functionalized [1,6]-naphthyridines and their benzo/heterofused analogues presented herein offers an unprecedented coupling which leads to the construction of both the nitrogen containing rings during the synthesis without starting from any nitrogen containing heterocyclic moiety. Recently, Shen and coworkers described the synthesis of naphtho[2,3-b][1,6]naphthyridines catalyzed by acetic acid.[27a] Zhang et. al. described synthesis of 1,6-naphthyridine-2,5-dione derivatives under utrasound irradiation in water with acetic acid as catalyst[27b] and recycle heterogeneous solid acid catalyst.[27c] Vennila et. al. synthesized new 10-methoxy dibenzo[b,h][1,6]naphthyridine carboxylic acid from 3-methoxyaniline by a new route.[27d] A survey of the literature shows that the majority of the strategies involve either multistep sequences,[27e-j] or expensive catalysts,[27g-j,28] inert atmosphere,[27f,g,28a] lengthy reaction time,[27g,h] and laborious workup.[27f-h]

However, [Et_3NH][HSO_4] has not been explored yet for the synthesis of functionalized [1,6]-naphthyridines *via* multicomponent reaction. Therefore, in continuation of our work on the development of novel synthetic methodologies for organic transformations,[26,29] we employed [Et_3NH][HSO_4] as an acidic Bronsted ionic liquid as a green, efficient, and recyclable catalyst as well as a solvent for the synthesis of functionalized [1,6]-naphthyridines. Further, we have screened

the synthesized naphthyridines for *in vitro* antimicrobial and antioxidant activity. In order to rationalize the promsing data obstained from antimicrobial screening and to gain an insight into plausible mechanism of action, a molecular docking study was performed against a critical target, *β*-Ketoacyl-acyl carrier protein synthase III (FabH) which could provide clustered solutions on binding mode and various thermodynamic interactions governing the binding affinity.

Results and discussion

Chemistry

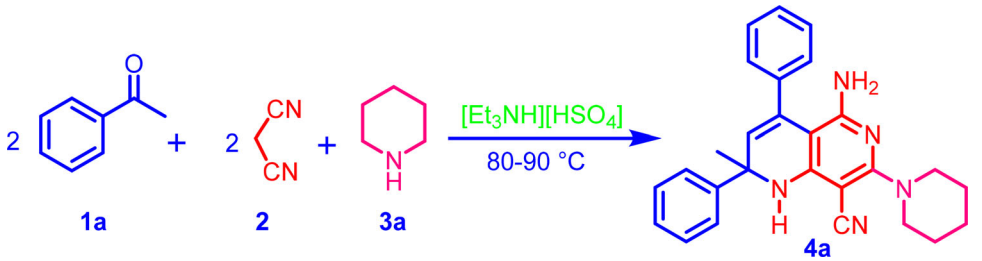
In search of the best experimental reaction conditions, reaction of acetophenone **1a**, malononitrile **2** and piperidine **3a** in ecofriendly solvent free condition using ionic liquid $[Et_3NH][HSO_4]$ at 80-90 °C was considered as a standard model reaction (Scheme 1). Initially, the reaction was carried out in absence of the catalyst, the product **4a** was formed in trace amount (Table 1, entry 1).

To determine the appropriate concentration of the catalyst $[Et_3NH][HSO_4]$, the model reaction at different concentrations of $[Et_3NH][HSO_4]$ such as 5, 10, 15, 20 and 25 mol% has been carried out. The functionalized [1,6]-naphthyridine formed in 60, 80, 85, 93 and 93% yields, respectively in given times (Table 1, entries 2-6). The increase in concentration of catalyst from 20 to 25 mol% does not increase the yield of product. This indicates that, 20 mol% of $[Et_3NH][HSO_4]$ is sufficient for the reaction by considering yield of product.

To evaluate the effect of solvents, dichloromethane (DCM), THF, 1,4-dioxane, toluene, CH_3CN and EtOH were used for the model reaction. It has been observed that, the use of solvents retards the rate of reaction and affords the desired product in lower yields than that for neat reaction condition (Table 2, entry 1-6).

To check the ecofriendliness of $[Et_3NH][HSO_4]$, we recycled the ionic liquid $[Et_3NH][HSO_4]$ for five times Table 3. The reaction proceeded cleanly with good yields (93, 93, 92, 90, 90 and 85%); although a weight loss of ~5% of $[Et_3NH][HSO_4]$ was observed from cycle to cycle due to mechanical loss (Table 3, entries 1-6).

With these optimized reaction conditions for model reaction i.e., 20 mol% $[Et_3NH][HSO_4]$ catalyst, 80-90 °C and solvent-free conditions, we have synthesized a series of functionalized



Scheme 1. Standard model reaction

Table 1. Effect of concentration of catalyst and time[a].

Entry	$[Et_3NH][HSO_4]$ (mol %)	Time (Min)	Yield[b] (%)
1	–	60	Trace
2	5	60	60
3	10	45	80
4	15	15	85
5	20	10	93
6	25	10	93

[a]**Reaction conditions**: Acetophenone **1a** (2 mmol), malononitrile **2** (2 mmol), piperidine **3a** (1 mmol), solvent-free at 80-90 °C.
[b]Isolated yield.

Table 2. Screening of solvents.

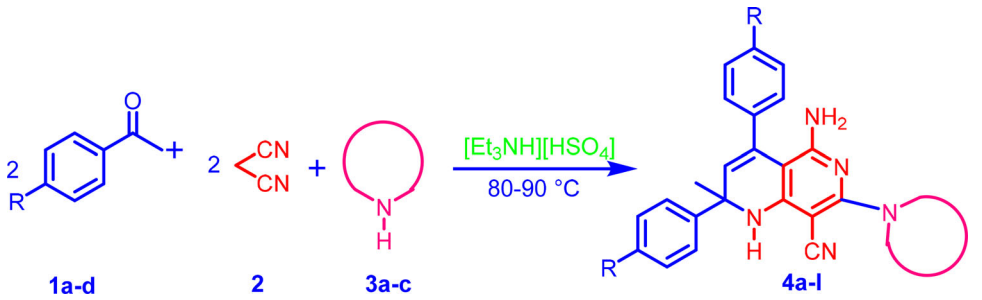
Entry	Solvent	Yield[a] (%)
1	DCM	44
2	THF	46
3	1,4-Dioxane	48
4	Toluene	55
5	Acetonitrile	58
6	Ethanol	60
7	Solvent free	93

Reaction conditions: Acetophenone **1a** (2 mmol), malononitrile **2** (2 mmol), piperidine **3a** (1 mmol) in 20 mol% $[Et_3NH][HSO_4]$.
[a]Isolated yield.

Table 3. Reusability of catalyst for model reaction.

Entry	Run	Time[a] (Min)	Yield[b]
1	Fresh	10	93
2	1	10	93
3	2	10	92
4	3	10	90
5	4	10	90
6	5	15	85

[a]Reaction progress monitered by TLC.
[b]Isolated yield.



Scheme 2. Synthesis of functionalized [1,6]-naphthyridines **4a-l**.

[1,6]-naphthyridines (**4 b-l**) by reacting acetophenones (**1a-d**), malononitrile (**2**) and secondary amines (**3a-c**) in excellent yields (Scheme 2, Figure 2).

The formation of functionalized [1,6]-naphthyridines **4a-l** have been confirmed by physical data[30] and spectroscopic methods such as ^{1}H NMR,^{13}C NMR and mass. According to the ^{1}H NMR spectrum of representative compound **4a**, the singlet observed at δ 1.65 ppm for proton of methyl group, the multiplet observed at δ 1.48-1.55 ppm confirms the six protons from three methylene groups present in piperidine ring and multiplet observed at δ 3.44 ppm for methylene four proton attached to the nitrogen heteroatom. Similarly, broad singlet observed at δ 4.92 ppm for -NH_2 protons. In addition, a singlet observed at δ 5.57 ppm assigned to -NH proton present in [1,6]-naphthyridine and singlet for alkene proton observed at δ 6.74 ppm confirmed the formation of [1,6]-naphthyridine ring. Furthermore, all the aromatic protons appeared at expected chemical shifts and integral values. The synthesis of [1,6]-naphthyridine was further confirmed by ^{13}C NMR spectral data **4a**, in which the carbon signals of methylene group is resonated at δ 24.3 ppm. The signal observed at δ 30.9 ppm indicates the presence of methyl carbon. The signals at δ 48.7 ppm indicate the presence of methylene carbon attached to the nitrogen heteroatom. The signal observed at δ 56.5 ppm indicates the tertiary carbon atom on which methyl and phenyl ring is present. In addition to this the signal observed at δ 118.7 indicates the presence of carbon in -CN group, while all other carbons gave signals at expected values.

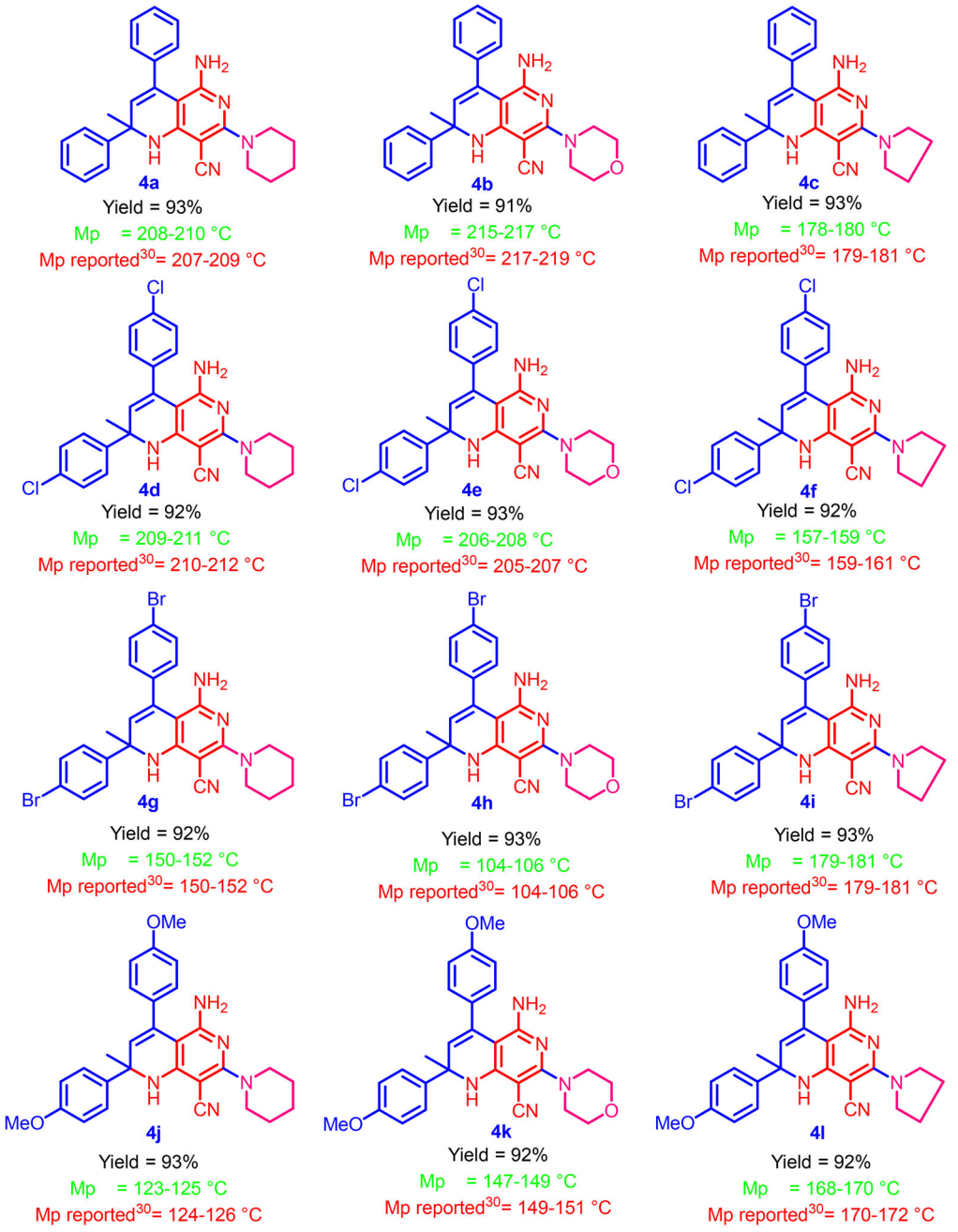


Figure 2. Structures, yields and melting point of the [1,6]-naphthyridines (**4a-l**).

Biological evaluation

Antibacterial activity

The functionalized [1,6]-naphthyridines **4a-l** were screened for antibacterial activity against the two Gram positive and two Gram negative bacterial strains and results are shown in Table 4.

For bacterial strain *S. aureus*, the compounds **4 g**, **4 h**, and **4j** shows excellent inhibitory activity with MIC value 4 µg/mL, which is equivalent to the clinical drug ampicilin (MIC 4 µg/mL).

Table 4. *In vitro* antimicrobial (MIC) and antioxidant activities (IC_{50}) of **4a-l** (μg/mL).

	Gram + ve bacteria		Gram -ve bacteria		Antifungal activity			
Compound	*SA*	*ML*	*EC*	*PF*	*CA*	*FO*	*AF*	DPPH IC_{50}
4a	16	32	32	32	16	32	64	21.3
4b	8	16	32	32	16	32	64	27.3
4c	16	8	16	8	16	16	64	22.1
4d	8	8	16	8	32	16	32	18.1
4e	8	16	4	4	16	64	32	19.3
4f	8	16	8	4	16	32	16	18.9
4g	4	32	4	8	16	16	16	16.1
4h	4	8	32	8	32	64	64	16.3
4i	8	8	32	32	16	32	32	16.4
4j	4	16	8	16	16	16	16	25.3
4k	8	8	4	8	64	16	16	30.2
4l	8	8	4	16	64	64	64	20.3
Ampicilin	4	16	4	2	–	–	–	–
Kanamycin	2	2	2	2	–	–	–	–
Miconazole	–	–	–	–	16	16	16	–
Fluconazole	–	–	–	–	2	2	4	–
BHT	–	–	–	–	–	–	–	16.5

SA: Staphylococcus aureus; ML: Micrococcus luteus; EC: Escherichia coli; PF: Pseudomonas fluorescens; CA: Candida albicans; FO: Fusarium oxysporum; AF: Aspergillus flavus; BHT: Butylated Hydroxy Toluene.

For bacterial strain *M.luteus*, compounds **4c**, **4d**, **4 h**, **4i**, **4k** and **4 l** exhibit two-fold antibacterial activity with MIC value 8 μg/mL and compounds **4 b**, **4e**, **4f** and **4j** with MIC value 16 μg/mL exhibited equivalent activity as compared to the clinical drug ampicilin (MIC 16 μg/mL). For bacterial strain *E. coli* compounds **4e**, **4 g**, **4k** and **4 l** with MIC value 4 μg/mL exhibited equivalent activity as compared to the clinical drug ampicilin (MIC 4 μg/mL) and for *P. fluorescens*, all the synthesized compounds exhibited moderate antibacterial activity compared to the standard antibacterial drugs.

Antifungal activity

In case of antifungal activity, all the synthesized [1,6]-naphthyridines **4a-l** shows good to moderate activity against all the tested fungal strains (Table 4).

Compounds **4a**, **4 b**, **4c**, **4e**, **4f**, **4 g**, **4i** and **4j** with MIC value 16 μg/mL exhibited equivalent activity compared with the standard drug miconazole against the fungicidal strain *C. albicans*. Compounds **4c**, **4d**, **4 g**, **4j** and **4k** with MIC value 16 μg/mL exhibited equivalent activity compared with the standard drug miconazole against the fungicidal strain *F. oxysporum*. Compounds **4f**, **4 g**, **4j** and **4k** with MIC value 16 μg/mL exhibited equivalent activity compared to the standard antibacterial drug miconazole for the fungicidal strain *A. flavus*.

Antioxidant activity

All the synthesized compounds **4a-l** shows moderate antioxidant activity as compared to the standard drug BHT (Table 4). The compounds **4 g** (IC_{50}= 16.1 μg/mL), **4 h** (IC_{50}= 16.3 μg/mL) and **4i** (IC_{50}= 16.4 μg/mL) have shown excellent activity as compared to standard drugs BHT (IC_{50}= 16.5 μg/mL). Remaining compounds exhibit good to moderate antioxidant activity as compared to standard drugs BHT.

Computational study

Molecular docking

In an effort to elucidate the plausible mechanism for antimicrobial activity demonstrated by the naphthyridines investigated herein and guide further SAR, molecular docking was performed

Table 5. Molecular docking study results- Glide score, Glide energy, H- bond and π-π stacking.

Comp	Glide Score	Glide energy (Kcal/mol)	H-bond (Å)	π-π / cation-π stacking(Å)
4a	−7.001	−39.539	Gly152(2.104)	Arg249(2.507), Phe213(2.501)/Arg249(2.507)
4b	−7.015	−40.353	Gly152(2.067), Arg151(2.055)	Arg249(2.09), Phe213(2.47), Trp32(2.34)/ Arg249(2.09)
4c	−7.128	−43.49	Gly152(2.048)	Arg249(2.264), Phe213(2.572) /Arg249(2.264)
4d	−7.024	−42.043	Gly152(2.140)	Arg249(1.891), Phe213(2.471), Trp32(2.549)/ Arg249(1.891)
4e	−8.640	−49.152	Gly152(2.067), Arg151(2.111)	Arg249(2.053), Phe213(2.471), Trp32(2.582)/ Arg249(2.053)
4f	−8.102	−46.223	Gly152(2.160)	Arg249(1.878), Phe213(2.415), Trp32(2.626)/ Arg249(1.878)
4g	−8.635	−49.095	Gly152(2.072)	Arg249(1.997), Phe213(2.496), Trp32(2.564)/ Arg249(1.997)
4h	−8.164	−46.336	Gly152(1.979), Arg151(2.118)	Arg249(2.188), Phe213(2.458), Trp32(2.598)/ Arg249
4i	−7.101	−41.869	Gly152(2.086)	Arg249(1.921), Phe213(2.462), Trp32(2.622)/ Arg249(1.921)
4j	−8.197	−46.117	Gly152(1.9777)	Arg249(2.214), Phe213(2.487), Trp32(2.58)/ Arg249(2.214)
4k	−8.194	−46.423	Gly152(1.959), Arg151(2.067)	Arg249(2.275), Phe213(2.441), Trp32(2.602)/ Arg249(2.275)
4l	−8.139	−46.904	Gly152(2.0998)	Arg249(2.03), Phe213(2.472)/Arg249

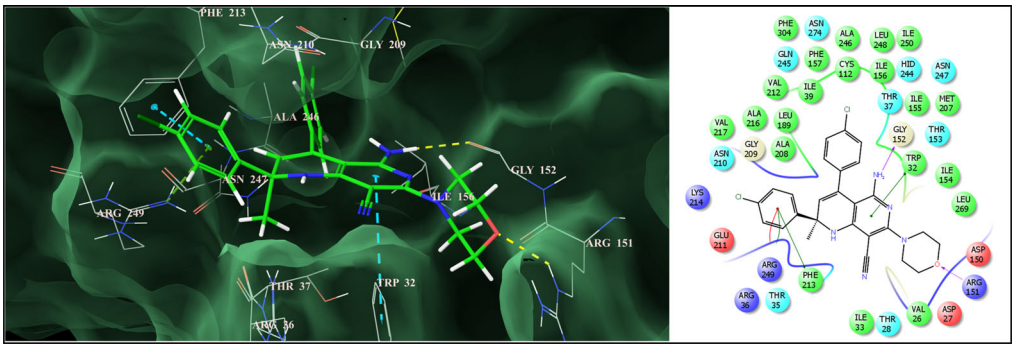


Figure 3. Binding mode of **4e** into the active site of beta-ketoacyl-acyl carrier protein synthase III (on right side: the pink lines represent the hydrogen bonding interactions; the green lines represent π-π stacking interaction while red line represent cation-π stacking interaction).

against β-ketoacyl-acyl carrier protein synthase III (FabH) (PDB code: 1HNJ) using the standard protocol implemented in the GLIDE (Grid-based LIgand Docking with Energetics) program of the Schrodinger Molecular modeling package (Schrodinger, LLC, New York, NY, 2018).[31] FabH is a condensing enzyme that plays an essential and regulatory role in bacterial fatty acid biosynthesis wherein it initiates the fatty acid elongation cycles and is involved in the feedback regulation of the biosynthetic pathway *via* product inhibition. FabH catalyzes the condensation of CoA-attached acetyl group and an ACP-attached malonyl group, yielding acetoacetyl-ACP as its final product. The essentiality of FabH for bacterial viability and due to their central roles in the fatty acid biosynthetic pathway qualifies FabH as an excellent molecular target.[32]

All the naphthyridines were observed to be nicely bound to the active site of FabH with excellent binding affinity (average Glide docking score of −7.778 and Glide binding energy of −44.795 kcal/mol) and engaged in several close interactions (Table 5).

A detailed investigation of the per-residue interactions for one of most active analogs **4e** showed that it could snugly fit into the active site of FabH through an extensive network of steric and electrostatic interactions (Figure 3).

A significant newtwork of van der Waals interactions were observed with Asn247(-2.479 Kcal/mol), Gly209(-2.887 Kcal/mol), Met207(-3.179 Kcal/mol), Ile156(-2.643 Kcal/mol), Gly152(-2.615 Kcal/mol), Thr37(-1.127 Kcal/mol) and Trp32(-4.727 Kcal/mol) residues through the 5-amino-2-methyl-1,2-dihydro-[1,6]naphthyridine nucleus while the morpholine side chain exhibited a similar chain of interactions with Arg151(-2.939 Kcal/mol), Thr28(-1.115 Kcal/mol), Asp27(-1.032 Kcal/mol) and Val26(-1.543 Kcal/mol) residues. Even the 2,4-bis-(4-chloro-phenyl) side chain also showed significant van der Waals interactions with Arg249(-2.276 Kcal/mol),

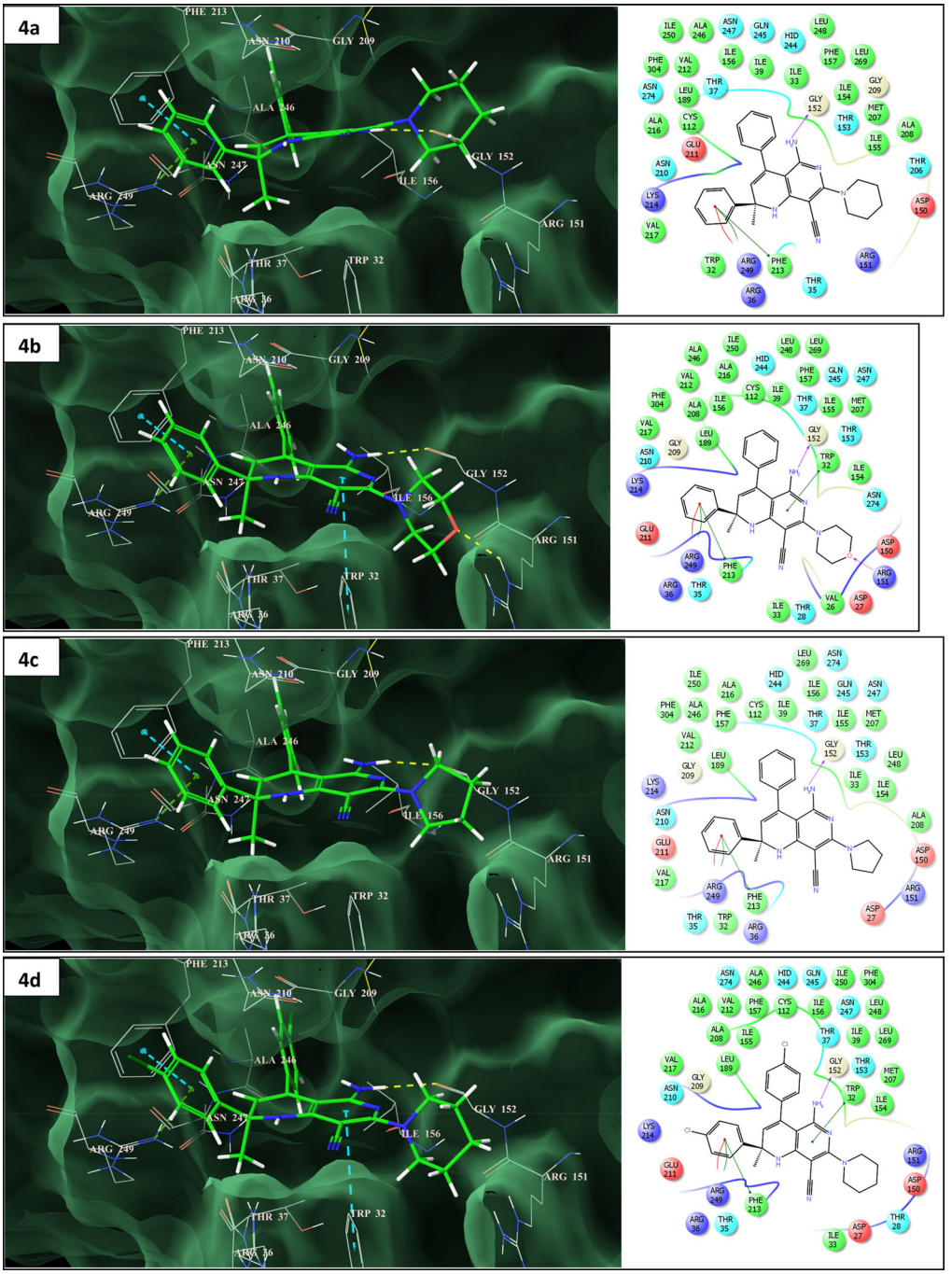


Figure 4. Binding mode of naphthyridines into the active site of Beta-Ketoacyl-Acyl Carrier Protein Synthase III (on right side: the pink lines represent the hydrogen bonding interactions; the green lines represent π-π stacking interaction while red line represents cation-π stacking interaction).

Ala246(-1.569 Kcal/mol), Lys214(-1.015 Kcal/mol), Phe213(-5.249 Kcal/mol), Val212(-1.044 Kcal/mol), Asn210(-4.644 Kcal/mol) and Arg36(-3.542 Kcal/mol) residues of the active site. The enhanced binding affinity of **4e** is also attributed to significant electrostatic interactions observed with Arg249(-2.704 Kcal/mol), Lys214(-1.088 Kcal/mol), Gly152(-1.531 Kcal/mol) and Arg151(-

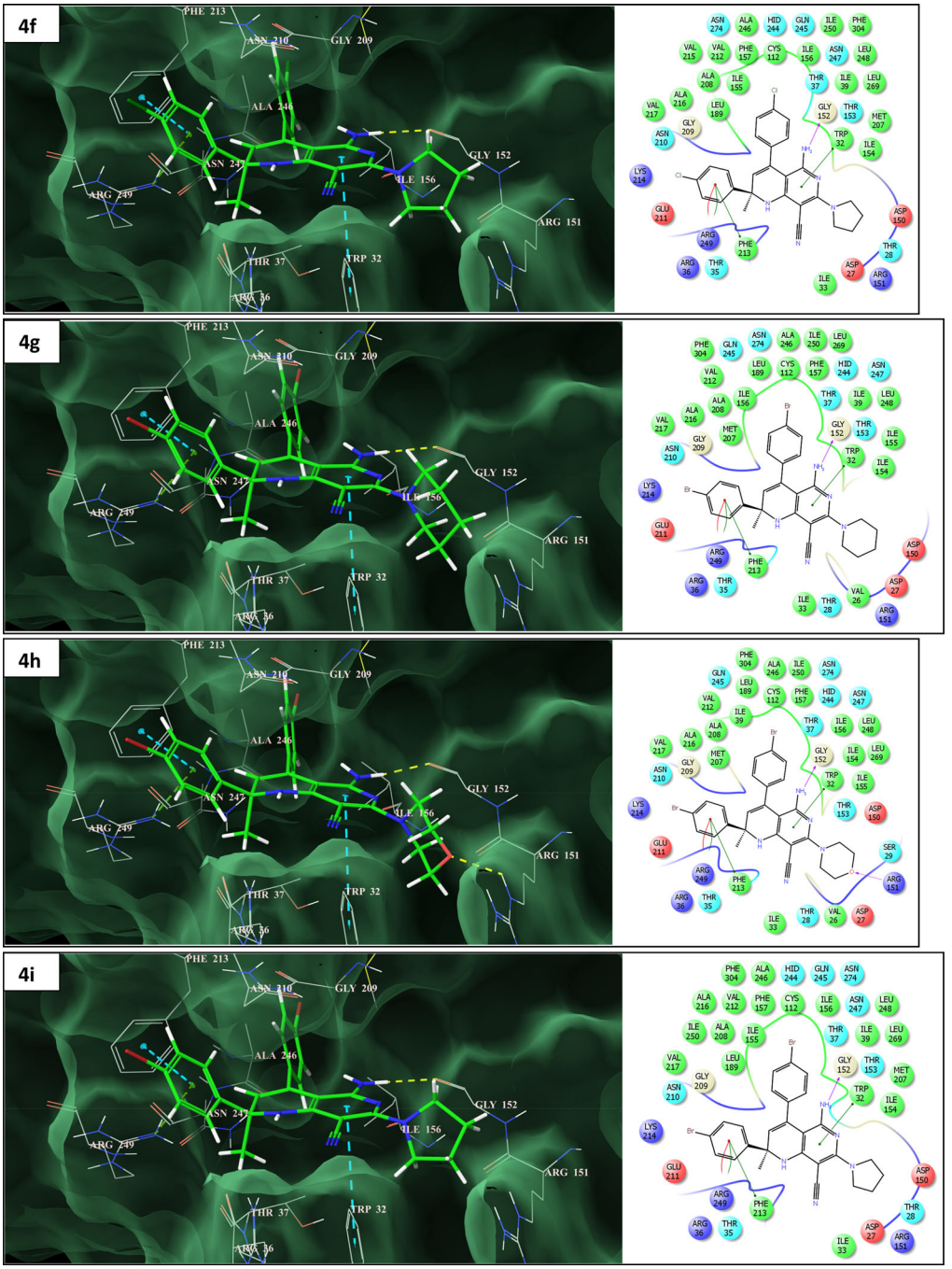


Figure 4. Continued

3.678 Kcal/mol) residues. Furthermore, it was observed to be stabilized into the active site through two prominent hydrogen bonding interactions: first through the amino group (-NH_2) of the naphthyridine ring with Gly152(2.067 Å) and second through the oxygen atom of the morpholine side chain with Arg151(2.111 Å). The compound has also exhibited significant π-π stacking interactions through Arg249(2.053 Å), Phe213(2.471 Å) and Trp32(2.582 Å) as well as a cation-π

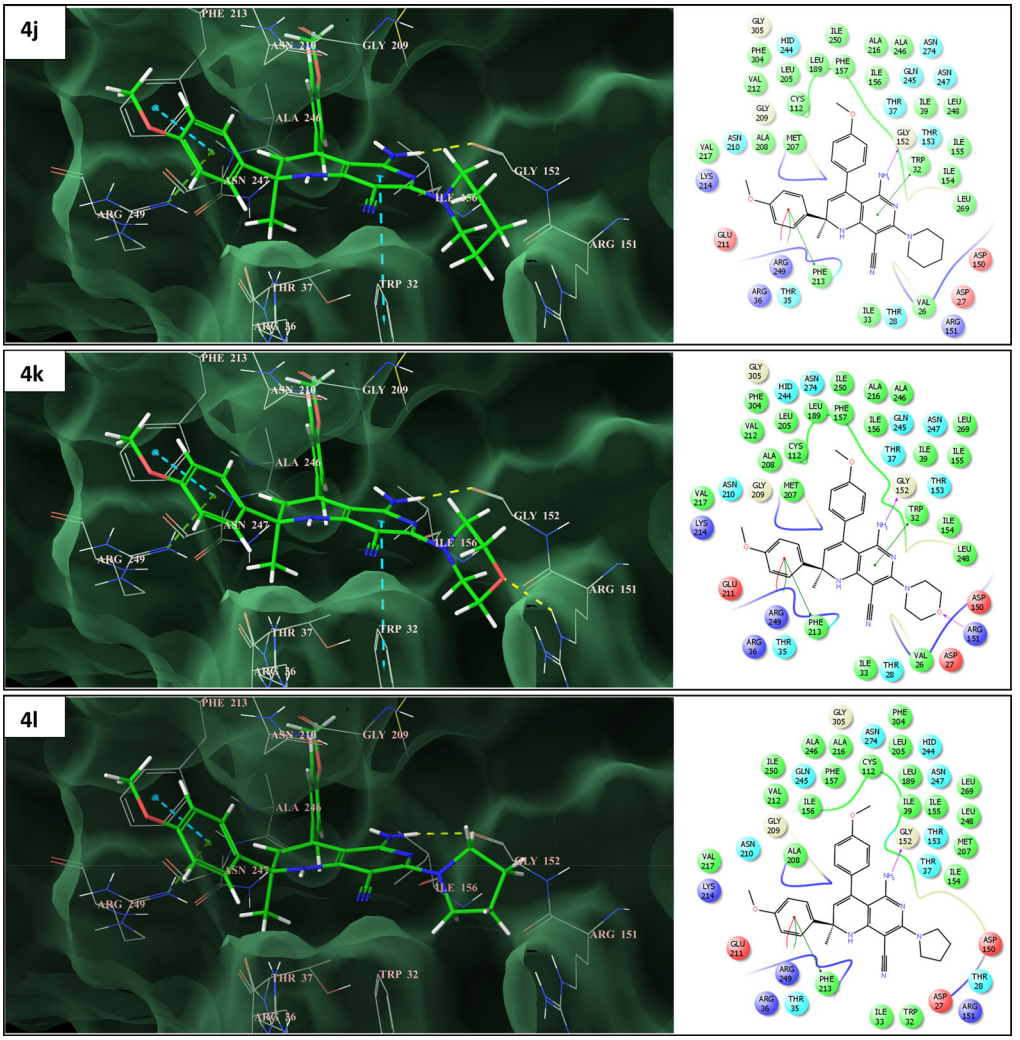


Figure 4. Continued

stacking interaction through Arg249(2.053 Å). Such hydrogen bonding and π stacking interactions serves as an anchor to stabilize the ligand into the 3 D space of the active site and also facilitate the non-bonded (steric and electrostatic) interactions. A similar network of bonded and non-bonded interactions were observed for the other naphthyridines (Figure 4) as well indicating that these molecules could exhibit ther antimicrobial action through inhibiting FabH and could be optimized further to arrive at selective and potent antimicrobial agents.

In silico ADME prediction

The success of a drug is determined not only by good efficacy but also by an acceptable ADME (absorption, distribution, metabolism and excretion) profile. A computational study of all the synthesized **4a-l** was performed for prediction of ADME properties and the value obtained is presented in Table 6. It is observed that, the compounds exhibited a good % ABS (% absorption) ranging from 72.54 to 82.10% (Table 6). Furthermore, only compounds **4 g** and **4i** violated

Table 6. Molecular properties of compounds **4a-4l**.

Entry	% ABS	TPSA (A^2)	n-ROTB	MV	MW	log p	n-ON	n-OHNH	Lipinski violations	Drug-likeness model score
Rule	–	–	–	–	<500	≤5	<10	<5	≤1	-
4a	82.10	77.97	3	398.85	421.55	4.07	5	3	0	0.41
4b	78.91	87.21	3	391.03	423.52	3.01	6	3	0	0.10
4c	82.10	77.97	3	382.04	407.52	3.57	5	3	0	0.42
4d	82.10	77.97	3	425.92	490.44	5.43	5	3	1	0.74
4e	78.91	87.21	3	418.10	492.41	4.37	6	3	0	0.44
4f	82.10	77.97	3	409.12	476.41	4.92	5	3	0	0.77
4g	82.10	77.97	3	434.62	579.34	5.69	5	3	2	0.45
4h	78.91	87.21	3	426.80	581.31	4.63	6	3	1	0.16
4i	82.10	77.97	3	417.81	565.31	5.18	5	3	2	0.48
4j	75.72	96.44	5	449.94	481.60	4.18	7	3	0	0.41
4k	72.54	105.67	5	442.12	483.57	3.12	8	3	0	0.12
4l	75.72	96.44	5	433.13	467.57	3.68	7	3	0	0.41

Cpd, Compounds, % ABS: percentage absorption, TPSA: topological polar surface area, n-ROTB: number of rotatable bonds, MV: molecular volume, MW: molecular weight, milogP: logarithm of partition coefficient of compound between n-octanol and water, n-ON acceptors: number of hydrogen bond acceptors, n-OHNH donors: number of hydrogen bonds donors.

Lipinski's rule of five (*log p*). All the tested compounds followed the criteria for orally active drug and therefore, these compounds may have a good potential for eventual development as oral agents.

Conclusions

We have developed a convenient one-pot multicomponent synthesis of highly functionalized [1,6]-naphthyridines under solvent free condition using $[Et_3NH][HSO_4]$ in high yields. We have screened the synthesized naphthyridines for *in vitro* antimicrobial and antioxidant activity. This solvent-free domino reaction proceeded smoothly in good to excellent yields and offered several other advantages including short reaction time, simple experimental workup procedures and no toxic byproducts, avoids the use of catalyst, toxic organic solvents and anhydrous conditions.

Molecular docking analysis revealed that these naphthyridines exhibited excellent binding affinity toward crucial microbial target *β*-Ketoacyl-acyl carrier protein synthase III (FabH) engaging in several close and significant bonded and non-bonded interactions. Furthermore, analysis of the ADME parameters for synthesized compounds showed good drug like properties and can be developed as oral drug candidate. The *in silico* results were found to be in harmony with experimentally observed MIC results which provide a strong platform to optimize this scaffold to arrive at selective and potent antimicrobial agents targeting FabH.

Experimental

Synthesis of $[Et_3NH][HSO_4]$

The synthesis of ionic liquid was carried out in a 100 mL round-bottom flask, which was immersed in a recirculating heated water-bath and fitted with a reflux condenser. Sulfuric acid (98%) (1.96 g, 0.02 mol) was added drop wise from triethylamine (2.02 g, 0.02 mol) stirring at 60 °C for 1 h. After the addition, the reaction mixture was stirred for an additional period of 1 h at 70 °C to ensure the reaction had proceeded to completion. The traces of water were removed by heating the residue at 80 °C in high vacuum until the weight of the residue remains constant.

Triethylammonium hydrogen sulfate $[Et_3NH][HSO_4]$: ^{1}H NMR (300 MHz, DMSO d_6): d (ppm) 1.15-1.19 (t, 9H), 3.04-3.12 (m, 6H), 8.98 (s, 1H); ^{13}C NMR (75 MHz, DMSO d_6): d (ppm) 8.88, 46.40.

General procedure for preparation of [1,6]-naphthyridines (4a-l)

A mixture of ketone **1a-d** (2 mmol), malononitrile **2** (2 mmol) and amine **3a-c** (1 mmol) in 20 mol% $[Et_3NH][HSO_4]$ were heated at 80-90 °C for 10-15 minutes. The reaction was monitored by TLC using ethyl acetate:hexane as a solvent system. The reaction mixture was quenched with crushed ice and extracted with ethyl acetate (2 × 25 mL). The organic extracts were washed with brine (2 × 25 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to afford the corresponding crude compounds. The obtained crude compounds were recrystallized using ethanol-ethylacetate solvent system. The residual ionic liquid was washed with diethyl ether, dried under vacuum at 100 °C and reused for subsequent reactions. The recovered ionic liquid could be used for 5 times without much loss of catalytic activity.

5-Amino-2-methyl-2,4-diphenyl-7-piperidin-1-yl-1,2-dihydro-[1,6]-naphthyridine-8-carbonitrile (4a): A mixture of acetophenone **1a** (2 mmol), malononitrile **2** (2 mmol) and piperidine **3a** (1 mmol) in 20 mol% $[Et_3NH][HSO_4]$ were heated at 80-90 °C for 10 min to give [1,6]-naphthyridine **4a** in 93% yield as white solid. Mp 207-209 °C (recrystallized from EtOH-EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.55 (*bs*, 6*H*), 1.65 (*s*, 3*H*), 3.44 (*s*, 4*H*), 4.92 (*bs*, 2*H*), 5.57 (*s*, 1*H*), 6.74 (*s*, 1*H*), 7.14-7.23 (*m*, 3*H*), 7.27-7.38 (*m*, 5*H*) and 7.43 (*d*, 2*H*, $J = 7.5$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 24.3, 25.7, 30.9, 48.7, 56.5, 68.6, 90.6, 118.7, 124.7, 126.6, 126.9, 127.8, 127.9, 128.2, 128.5, 132.6, 138.8, 148.7, 154.7, 154.8 and 161.6.

5-Amino-2-methyl-7-morpholin-4-yl-2,4-diphenyl-1,2-dihydro-[1,6]-naphthyridine-8-carbonitrile (4b): A mixture of acetophenone **1a** (2 mmol), malononitrile **2** (2 mmol) and morpholine **3b** (1 mmol) in 20 mol% $[Et_3NH][HSO_4]$ were heated at 80-90 °C for 10 min to give [1,6]-naphthyridine **4b** in 91% yield as cream colored solid. Mp 217-219 °C (recrystallized from EtOH-EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.66 (*s*, 3*H*), 3.34-3.37 (*m*, 2*H*), 3.47-3.52 (*m*, 2*H*), 3.62 (*bs*, 4*H*), 5.02 (*bs*, 2*H*), 5.60 (*s*, 1*H*), 6.88 (*s*, 1*H*), 7.14-7.23 (*m*, 3*H*), 7.27-7.35 (*m*, 5*H*) and 7.43 (*d*, 2*H*, $J = 7.8$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 30.9, 48.2, 56.5, 66.1, 69.2, 91.0, 124.7, 126.6, 127.4, 128.0, 128.3, 128.5, 132.4, 154.7, 154.8 and 161.6.

5-Amino-2-methyl-2,4-diphenyl-7-pyrrolidin-1-yl-1,2-dihydro-[1,6]-naphthyridine-8-carbonitrile (4c): A mixture of acetophenone **1a** (2 mmol), malononitrile **2** (2 mmol) and pyrrolidine **3c** (1 mmol) in 20 mol% $[Et_3NH][HSO_4]$ were heated at 80-90 °C for 15 min to give [1,6]-naphthyridine **4c** in 93% yield as light yellow solid. Mp 179-181 °C (recrystallized from EtOH-EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.65 (*s*, 3*H*), 1.77-1.86 (*m*, 4*H*), 3.46-3.49 (*m*, 2*H*), 3.58-3.61 (*m*, 2*H*), 4.83 (*bs*, 2*H*), 5.52 (*s*, 1*H*), 6.40 (*s*, 1*H*), 7.14-7.23 (*m*, 3*H*), 7.27-7.36 (*m*, 5*H*) and 7.44 (*d*, 2*H*, $J = 7.8$ Hz,). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 25.1, 31.0, 48.6, 56.5, 66.0, 89.7, 119.4, 124.8, 126.0, 127.9, 128.0, 128.3, 128.5, 132.8, 139.2, 148.8, 155.0 and 157.4.

5-Amino-2,4-bis-(4-chloro-phenyl)-2-methyl-7-morpholin-4-yl-1,2-dihydro-[1,6]naphthyridine-8-carbonitrile (4e): A mixture of 4-chloroacetophenone **1b** (2 mmol), malononitrile **2** (2 mmol) and morpholine **3b** (1 mmol) in 20 mol% $[Et_3NH][HSO_4]$ were heated at 80-90 °C for 15 min to give [1,6]-naphthyridine **4e** in 93% yield as cream colored solid. Mp 205-207 °C (recrystallized from EtOH-EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.65 (*s*, 3*H*), 3.35-3.39 (*m*, 2*H*), 3.49-3.55 (*m*, 2*H*), 3.58-3.64 (*m*, 4*H*), 5.18 (*bs*, 2*H*), 5.63 (*s*, 1*H*), 7.00 (*s*, 1*H*), 7.22 (*d*, 2*H*, $J = 8.1$ Hz), 7.36 (*t*, 4*H*, $J = 7.6$ Hz) and 7.44 (d, 2*H*, $J = 8.7$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 30.4, 48.1, 56.2, 66.1, 68.9, 98.8, 117.7, 118.4, 126.8, 127.1, 128.0, 128.1, 129.8, 121.2, 132.0, 132.3, 137.0, 147.4, 154.7 and 161.6. HRMS calculated $[M + H]^+$ for $C_{26}H_{24}N_5OCl_2$: 492.0915, found: 492.0905, $[M + Na]^+$ for $C_{26}H_{23}N_5OCl_2Na$: 515.0703, found: 515.0693.

5-Amino-2,4-bis-(4-chloro-phenyl)-2-methyl-7-pyrrolidin-1-yl-1,2-dihydro-[1,6]-naphthyridine-8-carbonitrile (4f): A mixture of 4-chloroacetophenone **1b** (2 mmol), malononitrile **2** (2 mmol) and pyrrolidine **3c** (1 mmol) in 20 mol% $[Et_3NH][HSO_4]$ were heated at 80-90 °C for 15 min to give [1,6]-naphthyridine **4f** in 92% yield as white solid. Mp 159-161 °C (recrystallized from EtOH-EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.64 (*s*, 3*H*), 1.79-1.85 (*m*, 4*H*), 3.49 (*bs*, 2*H*), 3.58 (*bs*, 2*H*), 4.95 (*bs*, 2*H*), 5.54 (*s*, 1*H*), 6.57 (*s*, 1*H*), 7.21 (*d*, 2*H*, $J = 8.1$ Hz), 7.35 (*t*,

4*H*, $J = 8.1$ Hz) and 7.43 (*d*, 2*H*, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 25.1, 30.5, 48.5, 56.1, 65.9, 89.5, 119.2, 126.8, 128.1, 128.2, 129.8, 131.2, 132.3, 137.5, 147.5, 154.8, 154.9 and 157.5. LCMS calculated $[M + H]^+$ for $C_{26}H_{24}N_5Cl_2$: 476.15, found: 476.20,

Experimental protocol for biological activity

Antibacterial activity

Theantimicrobial susceptibility testing of newly synthesized compounds were performed *in vitro* against bacterial strains *viz*., Gram-positive *Staphylococcus Aureus* (ATCC No. 29737), *Micrococcus Luteus* (ATCC No. 398) and Gram-negative *Escherichia Coli* (NCIM No. 2256) and*Pseudomonas Fluorescens* (NCIM No. 2173) respectively, to find out minimum inhibitory concentration (MIC).[33] The MIC was defined as the lowest concentrations of compound that completely inhibit the growth of each strain. Serial two-fold dilutions of all samples were prepared in triplicate in micro titer plates and inoculated with suitably prepared cell suspension to achieve the required initial concentration. Serial dilutions were prepared for screening. Dimethylsulfoxide (DMSO) was used as solvent control. Ampicilin, kanamycin & chloramphenicol were used as a standard antibacterial drug. The concentration range of tested compounds and standard was 128-0.5 µg/mL. The plates were incubated at 37 °C for all micro-organisms; absorbance at 595 nm was recorded to assess the inhibition of cell growth after 24 h. The compounds which are showing promising antibacterial activity were selected for MIC studies. The MIC was determined by assaying at 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 µg/mL concentrations along with standards at the same concentrations.

Antifungal activity

The antifungal activity was evaluated against different fungal strains such as *Aspergillus Niger* (NCIM No. 1196), *Penicillium Chrysogenum* (NCIM No. 723) and C*urvularia Lunata* (NCIM No. 1131).[33] Fluconazole, miconazole and amphotericin B were used as standard drugs for the comparison of antifungal activity. The plates were incubated at 37 °C for all micro-organisms; absorbance at 410 nm was recorded to assess the inhibition of cell growth after 48 h. The lowest concentration inhibiting growth of the organisms was recorded as the MIC. DMSO was used as a solvent or negative control. In order to clarify any effect of DMSO on the biological screening, separate studies were carried out with solutions alone of DMSO and showed no activity against any microbial strains. The compounds which are showing promising antifungal activity were selected for MIC studies. The MIC was determined by assaying at 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 µg/mL concentrations along with standards at the same concentrations.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of some compounds were measured from the bleaching of the purple colored methanol solution of DPPH.[34] The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (5, 10, 25, 50 and 100 µg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. The reaction mixture was incubated at 37 °C. The scavenging activity on DPPHwas determined by measuring the absorbance at 517 nm after 30 min. All tests were performed in triplicate and the mean values were entered. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation

$$\%\ \text{of scavenging} = \left[(A_{\text{control}} - A_{\text{sample}})/(A_{\text{sample}} \times 100)\right]$$

Where, A_{control} is the absorbance of the control (DPPH radical without test sample)

A_{sample} is the absorbance of the test sample (DPPH radical with test sample). The control contains all reagents except the test samples.

ADME prediction

In the present study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog *P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five[35] using Molinspiration online property calculation toolkit.[36] Absorption (% ABS) was calculated by: % ABS = 109-(0.345 × TPSA).[37] Drug-likeness model score (a collective property of physic-chemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value) was computed by MolSoft software.[38] A molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: miLog *P* (octanol-water partition coefficient) $\leq$ 5, molecular weight $\leq$ 500, number of hydrogen bond acceptors $\leq$ 10 and number of hydrogen bond donors $\leq$ 5.[39]

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Disclosure statement

No potential conflict of interest was reported by the authors.

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