Synthesis of novel triazole functionalized pyridine derivatives as potential antimicrobial and anti-biofilm agents

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A series of novel 1-substituted (1*H*-1,2,3-triazole-4-yl) methoxy functionalized pyridine derivatives **5** and **6** have been prepared starting from 2(1*H*) pyridone **1** *via* selective *O*-propargylation followed by reaction with diverse substituted azides under Sharpless conditions. All the compounds **5** and **6** have been screened for antimicrobial activity, minimum bactericidal concentration and biofilm inhibition activity. Compounds **5d**, **5l** and **5s** which showed promising activity specifically towards *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* MLS-16 MTCC 2940 have been identified. Further, *in silico* docking studies have been carried out on the inhibition of dehydrosqualene synthase enzyme of *S. aureus*. This is a key enzyme in the biosynthesis of staphyloxanthin, a virulence factor for *S. aureus*. Further, on screening for antioxidant activity, the compounds **5l**, **5q** and **5n** showed promising activity.

Keywords: Pyridine, triazoles, azides, antimicrobial activity, anti-biofilm, Sharpless conditions

It is well documented that several bacterial pathogens have developed antibiotic resistance to various classes of antibiotics since past 30 years. The over use of antimicrobial drugs in clinical practice is one of the major factors for drug resistance. *Staphylococcus aureus* is a harmful and opportunistic human pathogen of significant concern causing a multitude of diseases owing to its exceptional virulence, stress tolerance, and capacity to accumulate antimicrobial resistance as well as to resist hosts' innate immune defence mechanisms[1]. It is a predominant clinically important pathogen causing various community and hospital-acquired infections such as moderate to severe skin infections to potentially fatal diseases like endocarditis, toxic shock syndrome or necrotic pneumonia by evading different antibiotic regimes[2]. In recent years, the methicillin-resistant *S. aureus* (MRSA) has received global attention since it is problematic and poses serious threat to public health in both community and clinical settings involving considerable morbidity and mortality[3]. In view of the rapid increase in the multi-drug resistant (MDR) strains of *Staphylococcus aureus*, the need to search for new antimicrobial drug targets has been initiated.

In the present context, pyridine nucleus present in many biologically active compounds is known to possess anti-tubercular[4], anti-viral[5], insecticidal[6], antimicrobial[7-9], anti-neoplastic[10] and anti-tumor activity[11-13]. A few of pyridine fused derivatives such as quinolones were reported as antibacterial agents[14]. Alternatively, the 1,2,3-triazole scaffold primarily has unique structural features and also exhibits promising biological activity[15-17], which include significant anti-proliferative activity over wide range of human cancer cell lines[18,19]. More recently, several reports have appeared on the synthesis of 1,2,3-triazoles[20], their anti-tubercular[21] and potassium or sodium channel activating activity[22-24].

In continuation to our earlier efforts on the synthesis of potential molecules[25-27], we have designed compounds with pyridine, 1,2,3-triazole and a trifluoromethyl group to conceive a hybrid molecule and accomplished a series of novel 1-substituted (1,2,3-triazol-4-yl) methoxy functionalized pyridine derivatives **5** and **6**. All the products from the **5** and **6** series were screened for antimicrobial activity. The promising compounds were further screened for minimum bactericidal concentration, biofilm inhibition and

antioxidant activities. The compounds **5d**, **5l**, **5s**, **5q**, **5j**, **5n** and **5h** which showed promising activity have been identified. Compounds with fluorine, hydroxy and nitro groups substituted on the phenyl ring at a strategic position of triazole promoted the antioxidant activity. Alternatively, the long alkyl chain substitution in particular position of triazole ring enhanced the antimicrobial activity.

Results and Discussion
Chemistry

The 2-oxo-6(trifluoromethyl)1,2-dihydropyridine-3-carbonitrile **1** (Ref 28) on reaction with propargyl

bromide under basic conditions at RT formed selectively *O*-propargylated derivative 2-*O*-propargyl-3-cyano-6-trifluoromethyl pyridine **2**. Same reaction under reflux conditions resulted in 2-*O*-propargyl-3-carboxamide-6-trifluoromethyl pyridine **3**. Compound **2** was further hydrolyzed to form 2-*O*-propargyl-3-carboxy-6-trifluoromethyl pyridine **4**. Compounds **2**, **3** and **4** were independently reacted with different substituted alkyl, aryl azides under Sharpless conditions and respective 1-substituted (1*H*-1,2,3-triazol-4-yl) methoxy functionalized-6-trifluoromethyl pyridine derivatives **5** and **6** were obtained. The synthetic sequences are outlined in Scheme I and Scheme II.
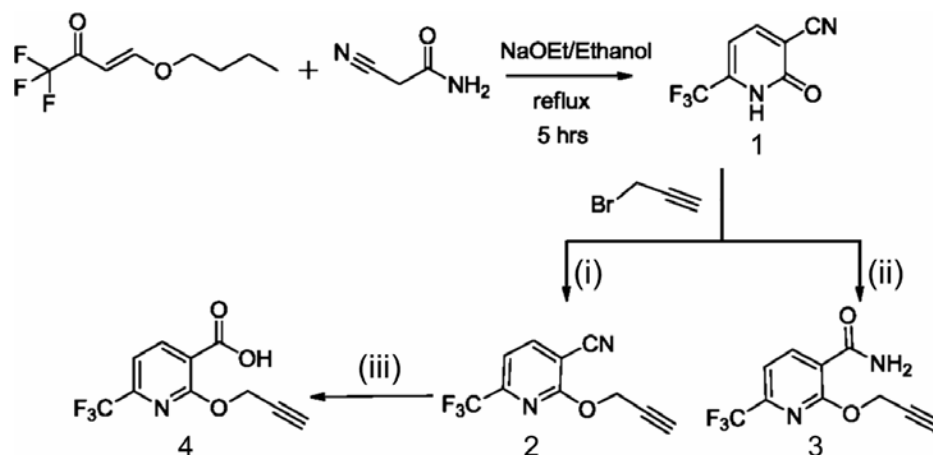
Reagents and conditions: (i) K$_2$CO$_3$, DCM, NaI, Acetone, RT, 4-6 h. (ii) K$_2$CO$_3$, DCM, NaI, Acetone, 4-8 h, reflux. (iii) 10% NaOH, aq.solution, Reflux, 4 h

Scheme I — Synthesis of 2-(prop-2-yn-1-yloxy)-6-(trifluoromethyl) nicotinonitrile or nicotinamide or nicotinic acid
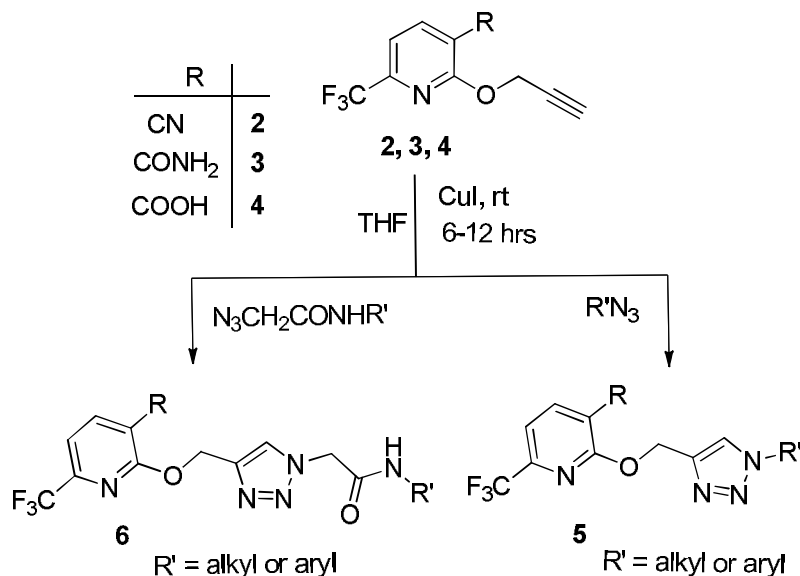
Scheme II — Synthesis of triazole tagged pyridine derivatives

The mechanistic pathway for compound **3** is shown in Figure 1. The products are tabulated in Table I.

Biological activity
Antimicrobial activity for 5 and 6 series compounds

Compounds **5a-t** and **6a-e** were screened for *in vitro* antibacterial activity in comparison to ciprofloxacin as standard against different Gram-positive bacterial strains such as *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS-16 MTCC 2940, *B. subtilis* MTCC 121 and Gram-negative bacterial strains such as *E. coli* MTCC 739, *P. aeruginosa* MTCC 2453 and *K. planticola* MTCC 530. Among all the screened ones, only compounds **5d**, **5l** and **5s** showed promising activity against *M. luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 (a control strain for antibiotic susceptibility testing) and *Staphylococcus aureus* MLS-16 MTCC 2940 (standard MLS model strain resistant to Macrolide-Lincosamide-Streptogramin B (MLS) antibiotics). However, these compounds **5d, 5l** and **5s** did not show activity against *Micrococcus luteus* MTCC 2470 strain even upto the highest tested concentration of 150 µg/mL. The activity data is tabulated in Table II. The compounds **5d**, **5l** and **5s** were further screened for minimum bacterial concentration and they were found to exhibit promising activity at micromolar concentration. The results are shown in Table III.

Biofilm inhibition activity for short-listed compounds

Biofilms are structured bacterial communities embedded in a self-produced polymeric matrix that serves to protect the colonized niche from

competitors[29,30]. The biofilms protect the cells not only from the host immune response but also exhibit increased tolerance to antibiotics, biocides, stress and thus they pose a challenge[31]. Biofilm formation is a ubiquitous defence mechanism and has been shown as

Table I — Synthesis and physical properties of compounds **5a-t** and **6a-e**

| S. No.	Compd	R	R′	Yield (%)	m.p. (°C)
1	**5a**	4-F, 3-ClC$_6$H$_3$	CN	82	139-42
2	**5b**	4-FC$_6$H$_4$	CN	87	136-39
3	**5c**	4-OCH$_3$C$_6$H$_4$	CN	80	120-23
4	**5d**	C$_8$H$_{17}$	CN	91	Liquid
5	**5e**	4-Br, 2-OCF$_3$C$_6$H$_3$	CN	81	113-16
6	**5f**	4-NO$_2$C$_6$H$_4$	CN	70	168-71
7	**5g**	2-CF$_3$C$_6$H$_4$	CN	88	137-40
8	**5h**	4-FC$_6$H$_4$	CONH$_2$	82	170-73
9	**5i**	3OCH$_3$C$_6$H$_4$	CONH$_2$	76	127-30
10	**5j**	3-CF$_3$C$_6$H$_4$	CONH$_2$	88	128-31
11	**5k**	4-NO$_2$C$_6$H$_4$	CONH$_2$	86	211-14
12	**5l**	4-OH, 3-NO$_2$C$_6$H$_3$	CONH$_2$	77	202-205
13	**5m**	4-Br,2-OCF$_3$C$_6$H$_3$	COOH	74	138-41
14	**5n**	3-OCH$_3$C$_6$H$_4$	COOH	76	128-31
15	**5o**	3-OCF$_3$C$_6$H$_4$	COOH	78	118-21
16	**5p**	4-OH, 3-NO$_2$C$_6$H$_3$	COOH	80	208-11
17	**5q**	4-FC$_6$H$_4$	COOH	68	210-13
18	**5r**	C$_6$H$_5$	COOH	77	203-206
19	**5s**	C$_8$H$_{17}$	COOH	89	Liquid
20	**5t**	4-CH (CH$_3$)$_2$, 2-Br C$_6$H$_3$	COOH	68	108-11
21	**6a**	4-OCH$_3$C$_6$H$_4$	CN	95	120-23
22	**6b**	C$_6$H$_{11}$	CN	88	158-61
23	**6c**	3-ClC$_6$H$_4$	CONH$_2$	89	209-12
24	**6d**	C$_5$H$_4$N	CONH$_2$	81	172-75
25	**6e**	4-CH$_3$C$_6$H$_4$	CONH$_2$	88	205-208
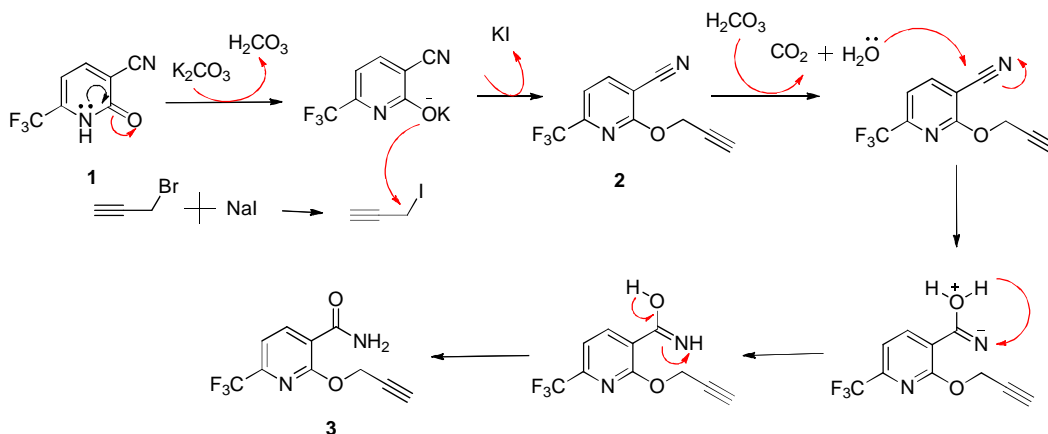
Figure 1 — Schematic representation of compound **3**

Table II — Antimicrobial activity for **5** and **6** series compounds

Test Compd	MIC values (µg/mL)		
	Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940
5d	18.75	9.37	9.37
5l	37.50	9.37	9.37
5m	–	37.50	37.50
5o	–	75.0	150
5s	–	9.37	18.75
Ciprofloxacin	0.58	0.58	0.58

Table III — Minimum bactericidal concentration for compounds **5d**, **5l** and **5s**

Test Compd	Minimum bactericidal concentration (MBC, µg/mL)		
	Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940
5d	18.7	9.37	4.6
5l	18.7	9.37	4.6
5s	>150	18.7	18.7
Ciprofloxacin	1.17	1.17	0.58

a primary element in the antibiotic resistance and can cause serious chronic infections in humans *via* hospital and community environments[32]. According to the US National Institute of Health report, >80% of the bacterial infections are due to biofilms and among the several bacterial strains encountered in clinical environments, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* are known to form biofilms[33]. These bacteria colonize the surfaces of various medical devices and implants such as stents, heart valves, vascular grafts and catheters by bacterial adhesion and biofilm formation[34]. Significant efforts were made in the recent past towards understanding the biology of biofilms and the search for novel inhibitors for the control of biofilm formation and biofilm related cellular processes[35,36]. In the present study, based on the promising antimicrobial activity results, three compounds (**5d**, **5l** and **5s**) were short-listed and screened for biofilm inhibition activity against *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* MLS-16 MTCC 2940 in comparison to erythromycin as standard control drug. It was noticed that these strains formed biofilms at either the bottom of the well or at the air-liquid interface[37]. The results suggested that all these three compounds (**5d**, **5l** and **5s**) exhibited promising biofilm inhibition activity with biofilm inhibition concentration of 4.6, 4.6 and 9.37 µg/mL,

Table IV — Biofilm inhibition activity for compounds **5d**, **5l** and **5s**

Test Compd	IC$_{50}$ values (µg/mL)		
	Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940
5d	9.37 ± 0.23	4.6 ± 0.32	4.6 ± 0.18
5l	18.7 ± 0.26	4.6 ± 0.38	4.6 ± 0.16
5s	>150	9.37 ± 0.21	9.37 ± 0.42
Erythromycin	0.32 ± 0.14	0.29 ± 0.12	0.31 ± 0.22
Ciprofloxacin	0.23 ± 0.11	0.31 ± 0.12	0.18 ± 0.09

respectively. The results are shown in Table IV. From a structure-activity relationship perspective, it was observed that the synthesized compounds with pyridine scaffold has various substituents such as *n*-octyl chain for compound **5d**, 4-hydroxy-3-nitro phenyl for compound **5l**, 4-bromo-2-trifluoro-methoxy phenyl for compound **5m**, 3-trifluoro-methoxy phenyl for compound **5o** and *n*-octyl chain substituent on the triazole ring for compound **5s** which have electron donating and/or electron withdrawing properties that have contributed to the antimicrobial and/or anti-biofilm activities.

From a mechanistic perspective, these test compounds (**5d**, **5l** and **5s**) caused the disruption and detachment of the biofilm from the surface due to the destabilization of the EPS produced by the *Micrococcus luteus* and *Staphylococcus aureus* test cultures, which might have caused the dispersal of the bacterial cells from the biofilm. The dispersed cells were more susceptible to these test compounds and resulted in microbial cell death which was quantified as a decrease in the microbial population as compared to the untreated biofilms. Some of the published reports illustrate the potential of few small molecule scaffolds such as halogenated furanones[38], sponge-derived natural alkaloid derivatives like oroidin and bromoageliferin[39-42], 2-aminoimidazoles and imidazo-pyridinium salts[43], dichlorocarbazol derivative[44], and triazole derivatives[45,46] which caused the disruption of bacterial chemical signaling and biofilm formation in some pathogenic bacteria. These molecules also structurally resembled bacterial acyl-homoserine lactone (AHL) quorum-sensing molecules[47,48] and effectively interfered with the quorum signaling, the subsequent gene expression and the swarming phenotype[49-51].

Antioxidant activity for 5 and 6 series compounds

The predominant reactive oxygen species (ROS) generated by cell metabolism or by exogenous factors such as hydrogen peroxide (H_2O_2), hydroxyl radical

(OH) and the superoxide anion radical ($\cdot O_2^-$). Free radicals are chemical species containing one or more unpaired electrons, most of them are unstable and capable of abstracting electrons from other molecules. These free radicals play essential functional roles in cell signaling, apoptosis and gene expression. On the other hand, excessive free radical attack can damage DNA, proteins and lipids, resulting in diseases like cancer, neurological degeneration and arthritis, as well as the process of aging[52,53]. Thus, the prevention of oxidative stress related diseases has been tentatively achieved by the identification of promising antioxidant compounds that can scavenge ROS and reactive nitrogen species (RNS) and thus avoid radical-induced oxidative damage. Considering these facts, the compounds **5a**-**t** and **6a-e** were assayed for antioxidant activity based on DPPH assay and α-tocopherol was used as standard control. The compounds **5l**, **5n** and **5q** exhibited promising radical scavenging activity, while compounds **5h**, **5j** and **5p** showed moderate antioxidant activity. Among the tested ones, the compounds **5l** and **5q** were equipotent to the standard. In the present study, the relationship with different substituted phenyl moieties on the triazole ring and the antioxidant potentials were found on some promising compounds (**5l**, **5n** and **5q**), which rely mainly on their electron-rich moieties, which hold high resonance stability. Thus, from a structure-activity relationship perspective, the pyridine derivatives have 4-hydroxy-3-nitro phenyl substituent for compound **5l** and 4-fluoro phenyl substituent on the triazole ring of the triazole-tagged pyridine scaffold for compound **5q** have electron donating and/or electron withdrawing groups that contribute to the promising antioxidant activity. The results are tabulated in Table V. Some of the earlier studies also indicated that triazole-tagged heterocyclic derivatives exhibited promising antioxidant activities[54-56].

Molecular modeling studies for the potent compounds 5d, 5l and 5s

Some of the recent studies have focused on targeting staphyloxanthin, a golden triterpenoid carotenoid pigment that functions as a virulence factor for *S. aureus*[57]. The biosynthetic pathway of this pigment proceeds through the head to head condensation of two molecules of farnesyl diphosphate to synthesize the C_{30} isoprenoid, presqualene diphosphate, which is then converted to dehydrosqualene by the dehydrosqualene synthase (CrtM) enzyme of *S. aureus*[57,58]. Subsequent enzymatic dehydrogenation steps through

Table V — Antioxidant activity of compounds **5a-t** and **6a-e**

Test Compd	EC_{50} ($\mu g\ mL^{-1}$) (Mean ± S.D.)
5a	58.9 ± 0.26
5b	125.4 ± 0.41
5h	25.1 ± 0.28
5j	33.4 ± 0.31
5l	10.9 ± 0.20
5n	16.4 ± 0.19
5p	48.5 ± 0.41
5q	11.2 ± 0.24
5r	93.4 ± 0.33
5s	50.2 ± 0.25
6b	128.2 ± 0.32
6c	152.3 ± 0.52
6d	96.3 ± 0.36
6e	145.4 ± 0.48
α-Tocopherol	10.6 ± 0.18

this precursor molecule creates the conjugated chromophore responsible for the golden colour of the end product, staphyloxanthin[59]. This pigment acts as an antioxidant and protects the bacterium against oxidative stress due to host immune defense by quenching reactive oxygen species and killing the neutrophils[60]. Three phosphosulfonates such as BPH-652, BPH-698 and BPH-700 were reported to inhibit the CrtM enzyme[57]. Recent studies have shown that zaragozic acid blocks *S. aureus* staphyloxanthin and biofilm formation[61,62].

In the present study, three promising derivatives (**5d**, **5l** and **5s**) have been identified as potent compounds based on biological studies. These were subjected to molecular docking studies on the protein ligands (2ZCS and 2ZCQ) of CrtM enzyme of *S. aureus*. It was observed that the amino acid residues present in the active binding site of the protein ligands 2ZCS and 2ZCQ of CrtM can frequently interact with compounds **5d**, **5l** and **5s**, and that these residues are responsible for the selectivity of *S. aureus* dehydrosqualene synthase inhibitors. The docked poses of all the compounds with 2ZCS and 2ZCQ are shown in Figure 2 and Figure 3, respectively, and clearly demonstrate the binding positions of the ligands with the protein. Analysis of the receptor/ligand complex models generated after successful molecular docking of all the synthesized compounds with the dehydrosqualene synthase inhibitor was done based on the parameters such as hydrogen bond distance, amino acid interactions, binding energy and orientation of the docked compound within the active site. As a general rule, in most of the potent antibacterial agents,
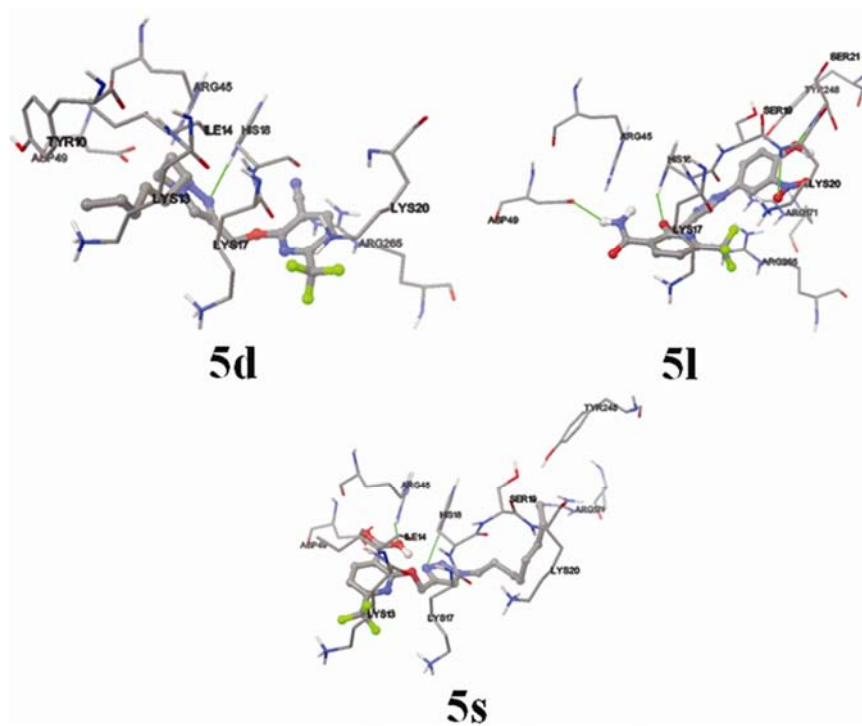
Figure 2 — Compounds **5d**, **5l** and **5s** docked to the active site of *Staphylococcus aureus* dehydrosqualene synthase (PDB ID: 2ZCS)

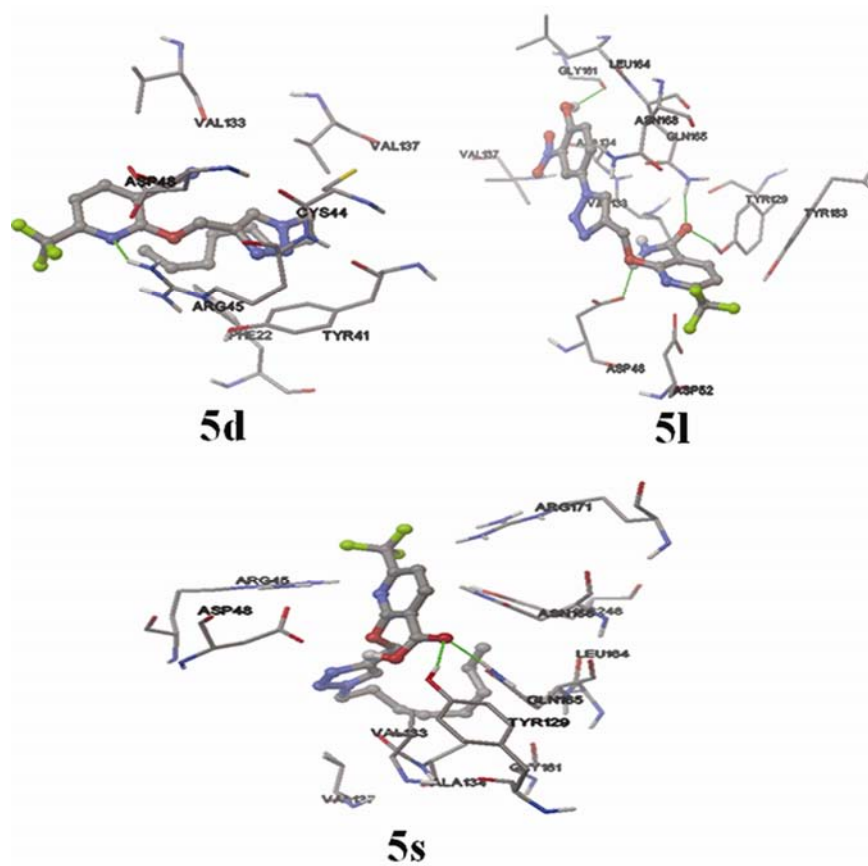

Figure 3 — Compounds **5d**, **5l** and **5s** docked to the active site of *Staphylococcus aureus* dehydrosqualene synthase (PDB ID: 2ZCQ)

both hydrogen bond and hydrophobic interactions between the compound and the active sites of the receptor have been found to be responsible for mediating the biological activity. In case of the template PDB 2ZCS, among the amino acids interacting with the ligand, His18 showed interaction with all the three compounds. The compound **5d** interacted with His18 only. Compound **5l** showed the highest number of amino acid interactions, His18, Ser19, Asp49, and Tyr248. Compound **5s** showed interaction with Arg45 along with His18. Whereas, in case of the template PDB 2ZCQ, the compound **5d** interacted with Arg45 only. Compound **5l** showed highest number of amino acid interactions with Asp48, Tyr129, Gly161 and Gln165. While compound **5s** showed interaction with Tyr129 and Gln165. The binding energy and the amino acids interacting with the ligand are shown in Table VI and Table VII. Further, the mode of binding of acetate and squalestatin analogs on CrtM enzyme of *S. aureus* was also examined based on molecular docking studies[63]. In this study, it was observed that His18, Arg45, Asp48, Asp52, Tyr129, Gln165, Asn168 and Asp172 residues showed a comparative high frequency of interaction with the tested analogs. Some other studies also reported that biphosphonates[64] and zaragozic acids[65] are potent dehydrosqualene synthase inhibitors which have been confirmed based on docking studies.

Experimental Section

All the chemicals (analytical grade) used in the reactions were from Sigma-Aldrich, St. Louis, MO, USA and Finar Chemicals Ltd., Ahmedabad, India. Melting points of all the compounds was recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV 300 MHz instrument in DMSO-d_6 or CDCl$_3$ using TMS as an internal standard. Electron Spray Ionisation (ESI) and High-Resolution Mass Spectra (HRMS) were recorded on QSTARXL hybrid MS/MS system (Applied Bio-systems, USA) under electrospray ionization. All the reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F$_{254}$ (mesh); spots were visualized with UV light. Merck silica gel (60-120 mesh) was used for column chromatography.

Table VI — Molecular docking studies on CrtM (dehydrosqualene synthase) (PDB ID: 2ZCS) from *Staphylococcus aureus*

Compd	CD[a]	DC[b] (kcal/mol)	BE[c] (Kl) (µM)	AA[d]
5d	56.026, 5.999, 60.238	−4.53	475.75	His18
5l	56.026, 5.999, 60.238	−6.55	15.86	His18, Ser19, Asp49, Tyr248
5s	56.026, 5.999, 60.238	−4.68	372.6	His18, Arg45

[a]Coordinates, [b]Dissociation Constant, [c]Binding energy and [d]Amino acids interacting with the ligand

Table VII — Molecular docking studies on CrtM (dehydrosqualene synthase) (PDB ID: 2ZCQ) from *Staphylococcus aureus*

Compd	CD[a]	DC[b] (kcal/mol)	BE[c] (Kl) (µM)	AA[d]
5d	16.937, 47.554, 41.956	−5.96	634.45	Arg45
5l	16.937, 47.554, 41.956	−6.28	24.88	Asp48, Tyr129, Gly161, Gln165
5s	16.937, 47.554, 41.956	−4.97	227.38	Tyr129, Gln165

[a]Coordinates, [b]Dissociation Constant, [c]Binding energy and [d]Amino acids interacting with the ligand

General procedure for the preparation of 2-oxo-6-trifluoromethyl-1,2-dihydro-pyridine-3-carbonitrile, 1

Cyanoacetamide (2.35 g, 0.028 mol) was taken in ethanol (50 mL) containing sodium ethoxide (shining sodium metal was dissolved in anhydrous ethanol at 0°C, 0.87 g, 0.038 mol) and raised the temperature up to 60°C for 30 min, cooled to RT and 4-butoxy-1,1,1-trifluoro-but-3-en-2-one (5.0 g, 0.025 mol) was added drop-wise for 20 min. Reaction was allowed to reflux for 5 h and the overall reaction was monitored by TLC. After completion of the reaction, it was neutralized with 15% HCl solution, residue was extracted with ethyl acetate, dried over anhydrous sodium sulphate and distilled under vacuum, the obtained residue was purified using 60-120 mesh silica gel column chromatography. Compound was eluted with 25% ethyl acetate in *n*-hexane (1:3). Yield 70.4% (Pale yellow solid). m.p.210-11°C. FTIR (KBr): 2230 (CN), 1672 cm^{-1} (C=O); ^{1}H NMR: (DMSO-d_6, 300 MHz) δ ppm: 7.26 (d, J = 7.74, 1H, =CH-), δ 8.18 (d, J = 7.55, 1H, =CH-); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 99.20 (*C*-CN), 110.06 (Ar-C), 113.70 (CN), 119.52 (q, J = 275.09 Hz) (CF$_3$), 144.58 (Ar-C), 146.81

(q, J = 35.21 Hz) (C-CF$_3$), 163.58 (C=O); ESI-MS: m/z 189 (M+1), 211 (M + Na).

Procedure for the preparation of 2-(prop-2-yn-1-yloxy)-6-(trifluoromethyl) nicotine nitrile, 2

2-Oxo-6-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile **1** (5.0 g, 0.03 mol) and potassium carbonate (7.3 g, 0.05 mol) were taken in dry acetone (50 mL), stirred for 30 minutes at RT, followed by the addition of propargyl bromide (3.1 g, 0.03 mol) then catalytic amount of sodium iodide (NaI) was added. The mixture was continuously stirred for 6 h at RT. The progress of the reaction was monitored by TLC, and after completion of the reaction, acetone was removed under reduced pressure. The residue was treated with ice cold water (40 mL) and the aqueous layer was extracted twice with ethyl acetate (2 × 40 mL). The combined organic phases were dried over Na$_2$SO$_4$ and evaporated on rotavapor. The resulted residue was purified using 60-120 mesh silica gel column chromatography. Yield 81% (Yellow liquid). FTIR (Neat): 2236 (CN), 2129 (C≡C), 1583 cm^{-1} (C=N); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 2.48 (t, 1H, J = 2.26 Hz, C≡C-H), 5.14 (d, 2H, J = 2.26 Hz, OCH$_2$), 7.41 (d, 1H, J = 7.55 Hz, Ar-H), 8.10 (d, 1H, J = 7.55 Hz, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 55.53 (O-CH$_2$), 75.90 (Acetylene-C), 76.89 (Acetylene-C), 100.68 (C-CN) 113.51 (Ar-C), 113.80 (CN), 120.27 (q, J = 273.99 Hz) (CF$_3$), 144.93 (Ar-C), 148.81 (q, J = 34.11 Hz) (C-CF$_3$), 162.30 (Ar-C-O); ESI-MS: m/z 227 (M+1); HRMS: m/z Calcd for C$_{10}$H$_6$F$_3$N$_2$O ([M+H]$^+$): 227.0124. Found: 227.0124.

Procedure for the preparation of 2-(prop-2-yn-1-yloxy)-6-(trifluoromethyl)nicotinamide, 3

2-Oxo-6-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile **1** (5.0 g, 0.03 mol) and potassium carbonate (7.3 g, 0.05 mol) were taken in dry acetone (50 mL), followed by the addition of propargyl bromide (3.1 g, 0.03 mol), then catalytic amount of sodium iodide (NaI) was added. The mixture was continuously stirred for 6 to 10 h at reflux temperature. After completion of the reaction, the residue was treated with ice cold water. The solution was extracted with ethyl acetate, dried over anhydrous sodium sulphate and concentrated. The resulted residue was purified using 60-120 mesh silica gel column chromatography. Yield 74% (Pale yellow solid). m.p.143-45°C. FTIR (KBr): 3459, 3173 (amide, NH$_2$), 2131 (C≡C), 1693 (amide, CO), 1616 cm^{-1} (C=N); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 2.56 (t, 1H, J = 2.20 Hz, C≡C-H), 5.20

(d, 2H, J = 2.20 Hz, OCH$_2$), 6.10 (br, s, 1H, CONH$_2$), 7.48 (d, 1H, J = 7.72 Hz, Ar-H), 7.68 (br, s, 1H, CONH$_2$), 8.71 (d, 1H, J = 7.72 Hz, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 54.29 (O-CH$_2$), 75.56 (Acetylene-C), 76.77 (Acetylene-C), 113.99 (Ar-C), 118.80 (C-CO), 119.98 (q, J = 273.99 Hz) (CF$_3$), 142.77 (Ar-C), 146.29 (q, J = 34.11 Hz) (C-CF$_3$), 158.31 (Ar-C-O), 163.02 (C=O); ESI-MS: m/z 245 (M+1); HRMS: m/z Calcd for C$_{10}$H$_8$F$_3$N$_2$O$_2$ ([M+H]$^+$): 245.0243. Found: 245.0231.

Procedure for the preparation of 2-(prop-2-yn-1-yloxy)-6-(trifluoromethyl)nicotinic acid, 4

2-(Prop-2-yn-1-yloxy)-6-(trifluoromethyl) nicotinonitrile **2** (100 mg, 0.4 mmol) was taken in 10% sodium hydroxide solution and refluxed for 4 h. After completion of the reaction, was diluted with cold water and neutralised with 1 M hydrochloric acid and the separated solid was collected by filtration. Yield 75% (White solid). FTIR (KBr): 3432 (OH), 2134 (C≡C), 1740 (CO), 1560 (C=N), 1446 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 2.58 (t, 1H, J = 2.26 Hz, C≡C-H), 5.23 (d, 2H, J = 2.26 Hz, OCH$_2$), 7.49 (d, 1H, J = 7.55 Hz, Ar-H), 8.59 (d, 1H, J = 7.55 Hz, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 54.36 (O-CH$_2$), 75.86 (Acetylene-C), 76.17 (Acetylene-C), 114.02 (Ar-C), 118.67 (Ar-C), 120.13 (q, J = 273.29 Hz) (CF$_3$), 142.59 (Ar-C), 146.25 (q, J = 34.17 Hz) (C-CF$_3$), 158.61(Ar-C-O), 163.55 (C=O); ESI-MS: m/z 246 (M+1); HRMS: m/z Calcd for C$_{10}$H$_7$F$_3$NO$_3$ ([M+H]$^+$): 246.1541. Found: 246.1534.

General procedure for the preparation of (1H-1,2,3-triazol-4-yl)methoxy functionalized pyridine derivatives 5 and 6

2-(Prop-2-ynyloxy)-6-(trifluoromethyl) nicotinonitrile **2** or nicotinamide **3** or nicotinic acid **4** (0.4 mmol) and catalytic amount of copper iodide (0.02 mmol) were taken in dry tetrahydrofuran, stirred for 30 minutes followed by addition of different alkyl azides (or) substituted aryl azides (or) 2-azido-N-substituted aryl acetamides. The stirring was continued for 6-12 h at RT and after completion of the reaction, the reaction mixture was filtered for the separation of copper iodide (CuI), then tetrahydrofuran was removed under vacuum. The residue was purified by column chromatography using n-hexane and ethyl acetate as eluents.

2-((1-(3-Chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl) nicotinonitrile, 5a:
Yield 82% (White solid). m.p.139-42°C. FTIR (KBr):

2233 (CN), 1588 (C=N), 1508 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 5.76 (s, 2H, OCH$_2$), 7.32 (t, 1H, J = 8.61 Hz, Ar-H), 7.44 (d, 1H, J = 7.65 Hz, Ar-H), 7.59-7.64 (m, 1H, Ar-H), 7.84-7.87 (m, 1H, Ar-H), 8.12 (d, 1H, J = 7.65 Hz, Ar-H) 8.16 (s, 1H, triazole-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 60.96 (O-CH$_2$), 100.85 (Ar-C), 113.43 (CN), 113.59 (d, J = 23.05 Hz) (Ar-C), 117.69 (Ar-C), 120.21 (d, J =7.68 Hz) (Ar-C), 120.41 (q, J = 274.98 Hz) (CF$_3$), 122.93 (triazole-C), 133.28 (Ar-C), 142.98 (triazole-C), 145.04 (Ar-C), 148.53 (q, J = 36.22 Hz) (*C*-CF$_3$), 157.97 (d, J = 252.48 Hz) (Ar-C-F), 162.77 (Ar-C-O); ESI-MS: *m/z* 398 (M+1), 420 (M +23); HRMS: *m/z* Calcd for C$_{16}$H$_9$F$_4$ClN$_5$O ([M+H]$^+$): 398.0231. Found: 398.0238.

2-((1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)meth-hoxy)-6(trifluoromethyl)nicotinonitrile, 5b: Yield 87% (Dark brown solid). m.p.136-39°C. FTIR (KBr): 2232 (CN), 1590 (C=N), 1517 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 6.76 (s, 2H, OCH$_2$), 7.20-7.29 (m, 2H, Ar-H), 7.42 (d, 1H, J = 7.55 Hz, Ar-H), 7.66-7.74 (m, 2H, Ar-H), 8.11 (d, 1H, J = 7.55 Hz, Ar-H), 8.15 (s, 1H, triazole-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 60.96 (O-CH$_2$), 100.77 (Ar-C), 113.47 (CN), 113.51 (Ar-C), 116.67 (d, J = 23.05 Hz) (Ar-C), 120.36 (q, J = 274.48 Hz) (CF$_3$), 122.40 (d, J = 8.23 Hz) (Ar-C), 122.90 (triazole-C), 132.93 (Ar-C), 142.66 (triazole-C), 145.02 (Ar-C), 148.42 (q, J = 35.67 Hz) (*C*-CF$_3$), 162.36 (d, J = 249.19 Hz) (Ar-*C*-F), 162.76 (Ar-C-O); ESI-MS: *m/z* 364 (M+1), 386 (M +23); HRMS: *m/z* Calcd for C$_{16}$H$_{10}$F$_4$N$_5$O ([M+H]$^+$): 364.0816. Found: 364.0809.

2-((1-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6(trifluoromethyl)nicotinonitrile, 5c: Yield 80% (Ash solid). m.p.120-23°C. FTIR (KBr): 2237 (CN), 1591 (C=N), 1521 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 3.87 (s, 3H, OCH$_3$), 5.75 (s, 2H, OCH$_2$), 7.03 (d, 2H, J = 9.06 Hz, Ar-H), 7.41 (d, 1H, J = 7.55 Hz, Ar-H), 7.62 (d, 2H, J = 9.06 Hz, Ar-H), 8.09 (d, 1H, J = 7.55 Hz, Ar-H), 8.11 (s, 1H, triazole-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 55.49 (O-CH$_3$), 61.03 (OCH$_2$), 106.11 (Ar-C), 113.42 (CN), 114.76 (2Ar-C), 120.55 (q, J = 275.24 Hz) (CF$_3$), 122.81 (triazole-C), 130.48 (2Ar-C), 137.67 (Ar-C), 142.469 (triazole-C), 144.97 (Ar-C), 148.62 (q, J = 36.02 Hz) (*C*-CF$_3$), 160.48 (Ar-*C*-OCH$_3$), 162.80 (Ar-*C*-O); ESI-MS: *m/z* 376 (M+1), 398 (M +23); HRMS: *m/z* Calcd for C$_{17}$H$_{13}$F$_3$N$_5$O$_2$ ([M+H]$^+$): 376.1015. Found: 376.1012.

2-((1-Octyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotinonitrile, 5d: Yield 91% (Orange liquid). FTIR (Neat): 2237 (CN), 1590 (C=N),

1463 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 0.87 (t, J = 6.79 Hz, 3H, CH$_3$), 1.18-1.37 (m, 10H, -CH$_2$-), 1.83-1.96 (p, 2H, -CH$_2$-), 4.35 (t, 2H, J = 7.55 Hz, NCH$_2$) 5.67 (s, 2H, OCH$_2$), 7.39 (d, J = 7.55 Hz, 1H, Ar-H), 7.71 (s, 1H, triazole-H), 8.08 (d, 1H, J = 7.55 Hz, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 13.97 (alip-C), 22.51 (alip-C), 26.39 (alip-C), 28.86 (alip-C), 29.64 (alip-C), 30.14 (alip-C), 31.61 (alip-C), 50.42 (N-CH$_2$), 61.35 (O-CH$_2$), 100.81(Ar-C) , 113.31 (Ar-C), 113.50 (CN), 120.39 (q, J = 275.19 Hz (CF$_3$), 124.09 (triazole-C), 141.86 (triazole-C), 144.85 (Ar-C), 148.62 (q, J = 35.42 Hz (*C*-CF$_3$), 162.94 (Ar-*C*-O); ESI-MS: *m/z* 382 (M+1), 404 (M +23); HRMS: *m/z* Calcd for C$_{18}$H$_{23}$F$_3$N$_5$O ([M+H]$^+$): 382.1849. Found: 382.1843.

2-((1-(4-Bromo-2-(trifluoromethoxy)phenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoro methyl)nicotin-onitrile, 5e: Yield 81% (White solid). m.p.113-16°C. FTIR (KBr): 2232 (CN), 1592 (C=N), 1553 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 5.79 (s, 2H, OCH$_2$), 7.42 (d, 1H, J = 7.55 Hz, Ar-H), 7.62-7.67 (m, 2H, Ar-H), 7.75-7.80 (m, 1H, Ar-H), 8.11 (d, J = 7.55 Hz, 1H, Ar-H), 8.20 (s, 1H, triazole-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 60.86 (O-CH$_2$), 100.87 (*C*-CN), 113.35 (CN), 113.51 (Ar-C), 119.92 (q, J = 261.88 Hz) (O-*C*F$_3$), 120.35 (q, J = 274.54 Hz) (*C*F$_3$-C), 123.44 (triazole-C), 124.99 (Ar-C), 125.85 (Ar-C), 127.49 (Ar-C), 128.67 (Ar-C), 131.24 (Ar-C), 140.88 (Ar-C), 142.54 (triazole-C), 145.01 (Ar-C), 148.60 (q, J = 36.32 Hz) (*C*-CF$_3$), 162.74 (Ar-*C*-O); ESI-MS: *m/z* 508 (M+1); HRMS: *m/z* Calcd for C$_{17}$H$_9$BrF$_6$N$_5$O$_2$ ([M+H]$^+$): 508.1532. Found: 508.1525.

2-((1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)meth-hoxy)-6-(trifluoromethyl)nicotinonitrile, 5f: Yield 70% (Pale yellow solid). m.p.168-71°C. FTIR (KBr): 2237 (CN), 1593 (C=N), 1534 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 5.78 (s, 2H, OCH$_2$), 7.44 (d, 1H, J = 7.72 Hz, Ar-H), 7.97 (d, 2H, J = 8.83 Hz, Ar-H), 8.12 (d, 1H, J = 7.72 Hz, Ar-H), 8.31 (s, 1H, triazole-H), 8.43 (d, 2H, J = 8.83 Hz, Ar-H); ESI-MS: *m/z* 391 (M+1), 413 (M +23); HRMS: *m/z* Calcd for C$_{16}$H$_{10}$F$_3$N$_6$O$_3$ ([M+H]$^+$): 391.0580. Found: 391.0567.

6-(Trifluoromethyl)-2-((1-(2-(trifluoromethyl)-phenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) nicotin onitrile, 5g: Yield 88% (White solid). m.p.137-40°C. FTIR (KBr): 2239 (CN), 1594 (C=N), 1490 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 5.77 (s, 2H, OCH$_2$), 7.42 (d, 1H, J = 7.55 Hz, Ar-H), 7.66-7.73 (m, 2H, Ar-H), 7.94-8.03 (m, 2H, Ar-H), 8.10 (d, 1H, J = 7.55

Hz, Ar-H), 8.24 (s, 1H, triazole-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 60.96 (O-CH$_2$), 100.90 (Ar-C), 113.44 (Ar-C), 113.60 (CN), 117.42 (Ar-C), 120.42 (q, J = 274.61 Hz) (CF_3-C), 122.76 (triazole-C), 123.46 (Ar-C), 124.07 (q, J = 272.97 Hz) (CF$_3$), 125.57 (Ar-C), 130.62 (Ar-C), 132.42 (q, J = 33.48 Hz) (C-CF$_3$), 137.05 (Ar-C), 143.08 (triazole-C), 145.05 (Ar-C), 148.55 (q, J = 36.04 Hz) (C-CF$_3$), 162.80 (Ar-C-O); ESI-MS: m/z 414 (M+1), 436 (M+23); HRMS: m/z Calcd for C$_{17}$H$_{10}$F$_6$N$_5$O ([M+H]$^+$): 414.0784. Found: 414.0772.

2-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotinamide, 5h: Yield 82% (White solid). m.p.170-73°C. FTIR (KBr): 3421, 3458 (amide, NH$_2$), 1677 (CO), 1587 (C=N), 1545 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.80 (s, 2H, OCH$_2$), 7.21 (m, 2H, Ar-H), 7.44-7.59 (m, 2H, Ar-H), 7.69-7.82 (m, 3H, Ar-H), 8.34 (d, 1H, J = 7.36 Hz, Ar-H), 8.66 (d, 1H, J = 7.36 Hz, Ar-H); ESI-MS: m/z 382 (M+H), 404 (M +Na); HRMS: m/z Calcd for C$_{16}$H$_{12}$F$_4$N$_5$O$_2$ (M+H): 382.0921. Found: 382.0920.

2-((1-(3-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotina mide, 5i: Yield 76% (Brown solid). m.p.127-30°C. FTIR (KBr): 3437, 3452 (amide, NH$_2$), 1690 (CO), 1578 (C=N), 1555 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.79 (s, 3H, OCH$_3$), 5.70 (s, 2H, OCH$_2$), 6.78-6.95 (m, 2H, Ar-H), 7.12-7.27 (m, 2H, Ar-H), 7.29-7.43 (m, 2H, Ar-H), 7.65 (br, s, 1H, CONH$_2$), 8.22 (s, 1H, Ar-H), 8.57 (d, 1H, J = 7.55 Hz, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 54.74 (O-CH$_3$), 59.46 (O-CH$_2$), 105.41 (Ar-C), 111.43 (Ar-C), 113.65 (Ar-C), 113.75 (Ar-C), 119.05 (Ar-C), 120.27 (q, J = 272.33 Hz) (CF$_3$), 122.22 (triazole-C), 129.75 (Ar-C), 136.89 (Ar-C), 141.99 (triazole-C), 142.68 (Ar-C), 146.34 (q, J = 35.76 Hz) (C-CF$_3$), 158.88 (Ar-C-OCH$_3$), 159.66 (Ar-C-O), 162.86 (C=O); ESI-MS: m/z 394 (M+H), 416 (M +Na); HRMS: m/z Calcd for C$_{17}$H$_{15}$F$_3$N$_5$O$_3$ (M+H): 394.1121. Found: 394.1124.

6-(Trifluoromethyl)-2-((1-(3-(trifluoromethyl)-phenyl)-1H-1,2,3-triazol-4-yl)methoxy) nicotinamide, 5j: Yield 88% (White solid). m.p.128-31°C. FTIR (KBr): 3448, 3475 (amide, NH$_2$), 1685 (amide, CO), 1590 (C=N), 1548 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.71 (s, 2H, OCH$_2$), 7.13 (br, s, 1H, CONH$_2$), 7.41 (d, 1H, J = 7.74 Hz, Ar-H), 7.56-7.72 (m, 3H, Ar-H), 7.92 (br, s, 1H, CONH2), 8.0 (s, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 8.56 (d, 1H, J = 7.74 Hz,

Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 58.81 (O-CH$_2$), 113.08 (Ar-C), 115.66 (Ar-C), 118.39 (q, J = 273.99 Hz) (CF$_3$), 119.05 (Ar-C), 119.53 (q, J = 274.54 Hz) (CF$_3$), 121.73 (triazole-C), 122.21 (Ar-C), 123.82 (Ar-C), 129.46 (Ar-C), 130.26 (q, J = 33.04 Hz) (C-CF$_3$), 135.75 (Ar-C), 141.75 (Ar-C), 141.97 (triazole-C), 144.89 (q, J = 34.66 Hz) (C-CF$_3$), 158.23 (Ar-C-OCH$_3$), 162.30 (C=O); ESI-MS: m/z 432 (M+H), 454 (M +Na); HRMS: m/z Calcd for C$_{17}$H$_{12}$F$_6$N$_5$O$_2$ (M+H): 432.0889. Found: 432.0875.

2-((1-(4-Nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotinamide, 5k: Yield 86% (White solid). m.p.211-14°C. FTIR (KBr): 3444, 3478 (amide, NH$_2$), 1691 (amide, CO), 1598 (C=N), 1555 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.77 (s, 2H, OCH$_2$) 7.53 (d, 1H, J = 7.74 Hz, Ar-H), 7.70 (br, s, 2H, CONH$_2$), 8.16 (d, 2H, J = 9.06 Hz, Ar-H), 8.44 (d, 2H, J = 9.06 Hz, Ar-H), 8.57 (d, 1H, J = 7.74 Hz, Ar-H) , 8.,83 (s, 1H, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 58.94 (O-CH$_2$), 113.35 (Ar-C), 119.47 (Ar-C), 122.00 (triazole-C), 124.27 (Ar-C), 139.83 (Ar-C), 142.14 (Ar-C), 142.54 (triazole-C), 145.89 (Ar-C), 158.42 (Ar-C-OCH$_3$), 162.57 (C=O); ESI-MS: m/z 409 (M+H), 431 (M +Na); HRMS: m/z Calcd for C$_{16}$H$_{12}$F$_3$N$_6$O$_4$ (M+H): 409.0866. Found: 409.0862.

2-((1-(4-Hydroxy-3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl) nicotina mide, 5l: Yield 77% (yellow solid). m.p.203-205°C. FTIR (KBr): 3402, 3340, (amide, NH$_2$), 1660, (CO), 1611 (C=N), 1589 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.68 (s, 2H, OCH$_2$), 7.27 (d, 1H, J = 8.87 Hz, Ar-H), 7.39-7.65 (m, 4H, Ar-H), 7.89-7.97 (m, 1H, Ar-H), 8.36 (s, 1H, Ar-H), 8.47-8.60 (m, 2H, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 58.52 (O-CH$_2$), 112.79 (Ar-C), 115.44 (Ar-C), 119.19 (Ar-C), 119.28 (Ar-C), 119.18 (q, J = 275.64 Hz) (CF$_3$), 121.41 (triazole-C), 121.71 (Ar-C), 125.63 (Ar-C), 126.71 (Ar-C), 134.45 (Ar-C), 141.24 (Ar-C), 141.46 (triazole-C), 144.28 (q, J = 36.86 Hz) (C-CF$_3$), 151.23 (Ar-C-OH), 157.92 (Ar-C-O), 162.07 (C=O); ESI-MS: m/z 425 (M+H), 447 (M +Na); HRMS: m/z Calcd for C$_{16}$H$_{12}$F$_3$N$_6$O$_5$ (M+H): 425.0679. Found: 425.0675.

2-((1-(4-Bromo-2-(trifluoromethoxy)phenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluo romethyl) nicotinic acid, 5m: Yield 74% (White solid). m.p.138-41°C. FTIR (KBr): 3155 (OH), 1706 (CO), 1599 (C=N), 1558 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.74 (s, 2H, OCH$_2$), 7.36-7.42 (d, 1H, J = 7.74 Hz,

Ar-H), 7.54-7.58 (m, 2H, Ar-H), 7.65-7.77 (m, 2H, Ar-H), 8.23 (d, 1H, J = 7.74, Ar-H), 8.32-8.39 (m, 1H, Ar-H); ESI-MS: m/z 527 (M+H), 549 (M+Na); HRMS: m/z Calcd for $C_{17}H_{10}F_6N_4O_4Br$ (M+H): 526.9784. Found: 526.9773.

2-((1-(3-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotinic acid, 5n: Yield 76% (Brown solid). m.p.128-31°C. FTIR (KBr): 3421 (OH), 1719 (CO), 1602 (C=N), 1503 (C=C), 1054 cm^{-1} (C-F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.80 (s, 3H, -OCH$_3$), 5.65 (s, 2H, OCH$_2$), 6.82-7.09 (m, 1H, Ar-H), 7.12-7.54 (m, 4H, Ar-H) 8.15-8.55 (m, 2H, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 55.47 (O-CH$_3$), 59.60 (O-CH$_2$), 105.74 (Ar-C), 112.00 (Ar-C), 113.95 (Ar-C), 114.39 (Ar-C), 119.58 (Ar-C), 120.82 (q, J = 274.54 Hz) (CF$_3$), 123.34 (triazole-C), 130.77 (Ar-C), 137.44 (Ar-C), 142.65 (Ar-C), 142.98 (triazole-C), 145.51 (q, J = 34.66 Hz) (*C*-CF$_3$), 160.11 (*C*-OCH$_3$), 160.20 (Ar-*C*-O), 164.85 (C=O); ESI-MS: m/z 395 (M+H); HRMS: m/z Calcd for $C_{17}H_{14}F_3N_4O_4$ (M+H): 395.0961. Found: 395.0954.

2-((1-(3-Trifluoromethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl) nicotinic acid, 5: Yield 78% (Brown solid). m.p.118-21°C. FTIR (KBr): 3415 (OH), 1730 (CO), 1604 (C=N), 1555 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.62 (s, 2H, OCH$_2$), 7.04-7.13 (m, 2H, Ar-H), 7.20 (d, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.57-7.67 (m, 1H, Ar-H) 8.27-8.39 (m, 3H, Ar-H); ESI-MS: m/z 449 (M+H); HRMS: m/z Calcd for $C_{17}H_{11}F_6N_4O_4$ (M+H): 449.0897. Found: 449.0890.

2-((1-(4-Hydroxy-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl) nicotinic acid, 5p: Yield 80% (Orange solid). m.p.208-11°C. FTIR (KBr): 3266 (OH), 1709 (CO), 1593 (C=N), 1542 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.81 (s, 2H, OCH$_2$), 5.71 (br, s, 1H, OH), 7.27-7.46 (m, 2H, Ar-H), 7.97-8.11 (m, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 8.34-8.53 (m, 2H, Ar-H), 10.80 (br, s, 1H, OH); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 55.26 (O-CH$_2$), 116.20 (Ar-C), 116.04 (Ar-C), 119.76 (Ar-C), 120.87 (triazole-C), 121.01 (q, J = 272.89 Hz) (CF$_3$), 122.05 (Ar-C), 128.07 (Ar-C), 128.78 (Ar-C), 133.67 (Ar-C), 142.33 (triazole-C), 144.01 (q, J = 34.66 Hz) (*C*-CF$_3$), 149.10 (Ar-C), 153.27 (Ar-*C*-OH), 160.37 (Ar-*C*-O), 164.60 (C=O); ESI-MS: m/z 426 (M+H), 448 (M +Na); HRMS: m/z Calcd for $C_{16}H_{11}F_3N_5O_6$ (M+H): 426.0644. Found: 426.0640.

2-((1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotinic acid, 5q: Yield 68% (Pale yellow solid). m.p.210-13°C. FTIR (KBr): 3447 (OH), 1698 (CO), 1597 (C=N), 1514 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.72 (s, 2H, OCH$_2$), 7.20-7.31 (m, 2H, Ar-H), 7.40 (d, 1H, J = 7.59 Hz, Ar-H), 7.53 (br, s, 1H, Ar-H) 7.69-7.79 (m, 2H, Ar-H), 8.27 (s, 1H, Ar-H), 8.36 (d, 1H, J = 7.59, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 58.37 (O-CH$_2$), 112.07 (Ar-C), 115.07 (Ar-C), 119.32 (q, J = 274.54 Hz) (CF$_3$), 120.81 (Ar-C), 121.51 (triazole-C), 131.60 (Ar-C), 141.24 (Ar-C), 141.94 (triazole-C), 144.95 (q, J = 35.76 Hz) (*C*-CF$_3$), 159.15 (d, J = 250.25 Hz) (Ar-C-F), 162.06 (Ar-C-O), 163.40 (C=O); ESI-MS: m/z 383 (M+H), 405 (M+Na); HRMS: m/z Calcd for $C_{16}H_{11}F_4N_4O_3$ (M+H): 383.0761. Found: 383.0761.

2-((1-Phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotinic acid, 5r: Yield 77% (Purple solid). m.p.203-206°C. FTIR (KBr): 3444 (OH), 1698 (CO), 1598 (C=N), 1514 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.73 (s, 2H, OCH$_2$), 7.19-7.33 (m, 2H, Ar-H), 7.40 (d, 1H, J = 7.55 Hz, Ar-H), 7.47-7.54 (m, 2H, Ar-H), 7.69-7.82 (m, 2H, Ar-H) 8.27 (s, 1H, Ar-H) 8.37 (d, 1H, J = 7.55, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 59.26 (O-CH$_2$), 112.61 (Ar-C), 115.56 (Ar-C), 115.93 (Ar-C), 118.19 (q, J = 274.54 Hz) (CF$_3$), 121.45 (Ar-C), 121.56 (Ar-C), 122.00 (triazole-C), 132.30 (Ar-C), 142.00 (Ar-C), 142.96 (triazole-C), 146.39 (q, J = 35.76 Hz) (*C*-CF$_3$), 159.67 (Ar-C), 160.06 (Ar-C), 162.96 (Ar-C-O), 164.31 (C=O); ESI-MS: m/z 365 (M+H); HRMS: m/z Calcd for $C_{16}H_{12}F_3N_4O_3$ (M+H): 365.0431. Found: 365.0425.

2-((1-Octyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl)nicotinic acid, 5s: Yield 89% (Brown liquid). FTIR (Neat): 3442 (OH), 1716 (CO), 1596 (C=N), 1470 cm^{-1} (C=C); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 0.84-0.90 (m, 5H, -CH$_2$-CH$_3$), 1.21-1.35 (m, 6H, 3×CH$_2$) 1.85-1.93 (m, 4H, 2× CH$_2$), 3.35 (t, 2H, -NCH$_2$), 5.75 (s, 2H, OCH$_2$), 7.43 (d, 1H, J = 7.62 Hz, Ar-H), 7.53 (br, 1H, OH), 7.73 (s, 1H, Ar-H), 8.50 (d, 1H, J = 7.62 Hz, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 14.00 (alip-C), 22.53 (alip-C), 26.38 (alip-C), 28.97 (alip-C), 29.64 (alip-C), 30.07 (alip-C), 31.62 (alip-C), 50.52 (N-CH$_2$), 60.24 (O-CH$_2$), 104.36 (Ar-C), 113.85 (Ar-C), 120.34 (q, J = 274.43 Hz) (CF$_3$), 124.42 (triazole-C), 143.78 (triazole-C), 147.23 (q, J = 34.57 Hz) (*C*-CF$_3$), 160.70 (Ar-C-O), 164.91 (C=O); ESI-MS: m/z 401 (M+H), 423 (M+Na); HRMS: m/z Calcd for $C_{18}H_{24}F_3N_4O_3$ (M+H): 401.1795. Found: 401.1788.

2-((1-(2-Bromo-4-isopropylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl) nicotinic acid, 5t: Yield 68% (Orange solid). m.p.108-11°C. FTIR (KBr): 3415 (OH), 1703 (CO), 1577 (C=N), 1513 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.21 (d, 6H, J = 6.98 Hz, 2CH$_3$), 2.84-2.97 (m, 1H, -CH-), 5.68 (s, 2H, OCH$_2$), 7.27 (d, 1H, J = 7.55 Hz, Ar-H), 7.34-7.43 (m, 3H, Ar-H) 7.52 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 8.11 (d, 1H, J = 7.55 Hz, Ar-H); ESI-MS: m/z 486 (M+H), 507 (M+Na); HRMS: m/z Calcd for C$_{19}$H$_{17}$F$_3$N$_4$O$_3$ (M+H): 485.1707. Found: 485.1707.

2-(4-(((3-Cyano-6-(trifluoromethyl)pyridin-2-yl)-oxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-methoxy-phenyl)acetamide, 6a: Yield 89% (Yellow solid). m.p.120-23°C. FTIR (KBr): 3293 (Amide-NH), 2238 (CN), 1690 (CO), 1595 (C=N), 1513 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.73 (s, 3H, OCH$_3$), 5.26 (s, 2H, NCH$_2$), 5.67 (s, 2H, OCH$_2$) 7.68-7.87 (m, 2H, Ar-H), 7.42-.7.53 (m, 3H, Ar-H), 8.06 (s, 1H, Ar-H), 8.22 (d, 1H, J = 7.55 Hz, Ar-H), 10.10 (br, s, 1H, NH); ESI-MS: m/z 433 (M+H); HRMS: m/z Calcd for C$_{19}$H$_{16}$F$_3$N$_6$O$_3$ (M+H): 433.1230. Found: 433.1216.

2-(4-(((3-Cyano-6-(trifluoromethyl)pyridin-2-yl)-oxy)methyl)-1H-1,2,3-triazol-1-yl)-N-cycl ohexyl-acetamide, 6b: Yield 88% (Yellow solid). m.p.158-61°C. FTIR (KBr): 3344 (Amide-NH), 2240 (CN), 1658 (CO), 1589 (C=N), 1562 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.84-1.46 (m, 6H, 3×CH2), 1.52-1.98 (m, 4H, CH$_2$), 3.72 (m, 1H, -CH-) 5.08 (s, 2H, NCH$_2$), 5.70 (s, 2H, OCH$_2$) 7.49 (d, 1H, J = 7.74 Hz, Ar-H), 7.74, (br, 1H, NH) 8.02 (d, 1H, J = 7.74 Hz, Ar-H), 8.22 (s, 1H, Ar-H); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 23.93 (CH$_2$-CH$_2$-CH$_2$), 24.58 (CH$_2$-CH$_2$-CH$_2$), 31.77 (CH$_2$-CH(NH)-CH$_2$), 47.77 (N-CH), 51.66 (N-CH$_2$), 60.21 (O-CH$_2$), 99.95 (Ar-C), 112.89 (CN), 113.90 (Ar-C), 120.59 (q, J = 273.89 Hz) (CF$_3$), 125.34 (triazole-C), 140.78 (triazole-C), 144.67 (Ar-C), 147.06 (q, J = 35.36 Hz) (C-CF$_3$), 161.98 (Ar-C-O), 163.22 (C=O); ESI-MS: m/z 409 (M+H), 431 (M+Na); HRMS: m/z Calcd for C$_{18}$H$_{20}$F$_3$N$_6$O$_2$ ([M+H]$^+$): 409.1594. Found: 409.1585.

2-((1-(2-((3-Chlorophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluor omethyl)nicotin-amide, 6c: Yield 89% (White solid). m.p.209-12°C. FTIR (KBr): 3405, 3342, (amide, NH$_2$), 1661, 1651 (amide, CO), 1606 (C=N), 1598 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.23 (s, 2H, NCH$_2$), 5.67 (s, 2H, OCH$_2$) 6.88-7.29 (m, 2H, Ar-H), 7.30-.7.56 (m, 3H, Ar-H), 7.57-7.79 (m, 2H, Ar-H), 7.99 (br, s, 1H, CONH$_2$), 8.58 (br, s, 1H, CONH$_2$) , 10.31 (br, s, 1H, NH); ESI-MS: m/z 455 (M+H), 477 (M +Na); HRMS: m/z Calcd for C$_{18}$H$_{15}$ClF$_3$N$_6$O$_3$ (M+H): 455.0821. Found: 455.0825.

2-((1-(2-Oxo-2-(pyridin-3-ylamino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoro methyl) nicotin-amide, 6d: Yield 81% (Brown solid). m.p.172-75°C. FTIR (KBr): 3441, 3277, (amide, NH$_2$, NH), 1677, 1624 (amide, CO), 1592 (C=N), 1547 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.32 (s, 2H, NCH$_2$), 5.73 (s, 2H, OCH$_2$), 6.86 (br, s, 1H, CONH$_2$), 7.28 (br, s, 1H, Ar-H), 7.46 (d, 1H, J = 8.06 Hz, Ar-H), 7.75 (br, s, 1H, CONH$_2$), 8.05 (s, 1H, Ar-H), 8.14 (m, 1H, Ar-H), 8.35 (br, s, 1H, Ar-H), 8.64 (d, 1H, J = 8.06 Hz, Ar-H), 8.74 (br, s, 1H, Ar-H),), 10.49 (br, s, 1H, NH); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 51.09 (N-CH$_2$), 58.90 (O-CH$_2$), 112.88 (Ar-C), 118.92 (Ar-C), 119.47 (q, J = 273.70 Hz) (CF$_3$), 124.87 (triazole-C), 140.50 (Ar-C), 141.58 (triazole-C), 144.82 (q, J = 32.42 Hz) (C-CF$_3$), 158.24 (Ar-C-O), 162.29 (NH-C=O), 163.12 (NH$_2$-C=O); ESI-MS: m/z 422 (M+H), 444 (M+Na); HRMS: m/z Calcd for C$_{17}$H$_{15}$F$_3$N$_7$O$_3$ (M+H): 422.1183. Found: 422.1169.

2-((1-(2-Oxo-2-(p-tolylamino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(trifluoromethyl) nicotin-amide, 6e: Yield 88% (White solid). m.p.205-208°C. FTIR (KBr): 3400, 3300, 3259 (amide, NH$_2$, NH), 1674, 1661 (amide, CO), 1557 (C=N), 1513 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.30 (s, 3H, -CH$_3$), 5.27 (s, 2H, -NCH$_2$), 5.71 (s, 2H, OCH$_2$) 7.05-7.24 (m, 3H, Ar-H), 7.41-.7.52 (m, 3H, Ar-H), 7.72 (br, s, 1H, CONH$_2$), 8.06 (s, 1H, triazol-H), 8.62 (d, 1H, J = 7.74 Hz, Ar-H) , 10.11 (br, s, 1H, NH); ^{13}C NMR (CDCl$_3$, 75 MHz): δ 19.36 (CH$_3$), 51.30 (N-CH$_2$), 59.03 (O-CH$_2$), 112.99 (Ar-C), 118.33 (Ar-C), 118.91 (Ar-C), 119.60 (q, J = 273.99 Hz) (CF$_3$), 124.86 (triazole-C), 127.83 (Ar-C), 131.98 (Ar-C), 134.31 (Ar-C), 140.55 (triazole-C), 141.73 (Ar-C), 145.00 (q, J = 35.21 Hz) (C-CF$_3$), 158.36 (Ar-C-O), 162.17 (NH-C=O), 162.45 (NH$_2$-C=O); ESI-MS: m/z 435 (M+Na), 457 (M +23); HRMS: m/z Calcd for C$_{19}$H$_{18}$F$_3$N$_6$O$_3$ (M+H): 435.1387. Found: 435.1376.

Antimicrobial activity assay

The antimicrobial activity of the test compounds was determined using well diffusion method[66] against different pathogenic reference strains procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded

on the surface of the media Petri plates, containing Muller-Hinton agar with 0.1 mL of previously prepared microbial suspensions individually containing 1.5×10^8 cfu mL^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized derivatives at a dose range of $150 - 0.58$ µg well^{-1} were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solution of ciprofloxacin at a dose range of 150 - 0.58 µg well^{-1} and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 37°C for bacterial strains and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All experiments were carried out in duplicate and mean values are represented.

Minimum bactericidal concentration (MBC) assay

The minimum bactericidal assay[67] was performed in sterile 2.0 mL microfuge tubes against a panel of pathogenic bacterial strains, including *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* MLS-16 MTCC 2940 were cultured overnight in Mueller Hinton broth. Serial dilutions of test compounds were prepared in Mueller Hinton broth with different concentrations ranging from 0 to 150 µg mL^{-1}. To the test compounds, 100 µL of overnight cultured bacterial suspensions were added to achieve a final concentration of 1.5×10^8 cfu mL^{-1} (equal to 0.5 McFarland standard) and incubated at 37°C for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 µL of suspension from the tubes onto Mueller Hinton agar plates and were incubated for 24 h at 37°C to observe the growth of test organisms. MBC is the lowest concentration of test compound required to kill a particular bacterium strain. All the experiments were carried out in triplicates and the values are indicated as mean ± S.D.

Biofilm inhibition assay

The test compounds were screened in sterile 96 well polystyrene microtiter plates using the modified biofilm inhibition assay[68,69] against a panel of pathogenic bacterial strains including *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* MLS-16 MTCC 2940, which were cultured overnight in tryptone soy broth

(supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 250 µg mL^{-1} were mixed with the bacterial suspensions having an initial inoculum concentration of 5×10^5 CFU/mL. Aliquots of 100 µL were distributed in each well and then incubated at 37°C for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Each well of the microtiter plate was stained with 100 µL of 0.1% crystal violet solution followed by 30 min incubation at RT. Later the crystal violet solution from the plates was discarded, thoroughly washed with distilled water 3 to 4 times and air dried at RT. The crystal violet stained biofilm was solubilised in 95% ethanol (100 µL) and the absorbance was recorded at 540 nm using TRIAD multimode reader (Dynex Technologies, Inc, Chantilly, VA, USA). Blank wells were employed as background check. The inhibition data were interpreted from the dose-response curves, where biofilm inhibition concentration is defined as the inhibitor concentration required for inhibiting the biofilm formation under the above assay conditions. All the experiments were carried out in triplicate and the values are indicated as mean ± S.D.

Antioxidant activity assay

The antioxidant activity of synthesized derivatives was assessed on the basis of the free radical scavenging effect of the stable 1,1-diphenyl-picrylhydrazyl (DPPH) following a previously described method[70] with some modifications. The diluted working solutions of the synthesized compounds were prepared in methanol (5, 10, 20, 40, 60 and 180 mg mL^{-1}). One mL of methanol solution of DPPH (0.002%) was mixed with 1 mL solution of test compound. The mixture was shaken vigorously and left to stand in dark for 30 min. Absorbance of the resulting solution was measured at 517 nm in a Lambda 25 UV-Vis spectrophotometer (Perkin-Elmer, Shelton, CT, USA). α-Tocopherol was used as positive control. The radical scavenging ability is measured as a decrease in the absorbance of DPPH. Lower absorbance of the reaction mixture has indicated higher free radical scavenging activity. DPPH radical scavenging activity is calculated using the following formula:

DPPH radical scavenging activity (%) = [(absorbance of control – absorbance of test sample) / (absorbance of control)] × 100

Radical scavenging potential was expressed as EC_{50} value which represents the test compound

concentration at which 50% of the DPPH radicals were scavenged. All the experiments were carried out in triplicate and the values are indicated as mean ± S.D.

Molecular docking studies for the promising compounds

The molecular docking studies were performed using Autodock tools (ADT)[71,72] version 1.5.6 and Autodock version 4.2. All the ligands were sketched and energy minimization was performed using the SYBYL programming package, version 6.7 (Tripos Associates, St. Louis, USA), on a Silicon Graphics workstation. All the molecules were minimized by adding Gasteiger-Hückel charges. The searching grid was extended above the preferred target proteins. Initially, the crystal structures of *S. aureus* dehydrosqualene synthase (CrtM) complexed with bisphosphonate derivatives, BPH-652 (PDB ID: 2ZCQ) and BPH-700 (PDB ID: 2ZCS) having a resolution of 2.38 Å were retrieved from RCSB protein data bank. Before docking all these protein crystal structures were cleaned by removing the water molecules and hydrogen atoms were added to these target proteins for correct ionization and tautomeric states of amino acid residues. Torsions were set to all the ligands. All the promising leads (**5d, 5l** and **5s**) identified based on antimicrobial and antibiofilm assays were docked to the target protein complexes (2ZCQ and 2ZCS) with the molecules considered as a rigid body and the ligand being flexible. Grid box was generated and x,y,z (16.937, 47.554, 41.956) coordinates were added. The search was extended over the whole receptor protein used as blind molecular docking. The search was carried out with the Lamarckian Genetic Algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations.

Conclusions

A series of novel 1-substituted (1*H*-1,2,3-triazol-4-yl)methoxy functionalized pyridine derivatives were prepared and evaluated for antimicrobial, minimum bactericidal concentration (MBC), biofilm inhibition and antioxidant activities and the compounds **5d**, **5l**, **5s**, **5q**, **5j**, **5n** and **5h** which showed promising activity have been identified. Moreover, the docking studies suggested that the synthesized potent compounds were involved in the binding with the amino acid residues which constitute the active site and play an important role in the functionality of dehydrosqualene synthase of *S. aureus*. Further, *in vitro* and *in vivo* studies with these compounds with

respect to inhibition of dehydrosqualene synthase (CrtM) of *S. aureus* could provide more insight into the mechanism of action of these inhibitors.

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