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Design and synthesis, biological evaluation of bis-(1,2,3- and 1,2,4)-triazole derivatives as potential antimicrobial and antifungal agents

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ABSTRACT

A new series of bis-1,2,3- and 1,2,4-triazoles (**10a-m)** were designed and efficiently synthesized using methyl salicylate as potential antimicrobial agents. All compounds were characterized by their proton & ^{13}C NMR, IR, Mass spectral data, and elemental analysis. The final compounds **10a-m** were *in vitro* screened for antimicrobial and antifungal activity against gram negative *Pseudomonas aeruginosa*, *Escherichia coli,* gram positive *Bacillus subtilis, Staphylococcus aureus* strains and *Aspergillus niger* & *Saccharomyces cerevisiae.* Majority of the synthesized compounds exhibited potent antimicrobial activity (MIC 3.9 μg/mL) and promising antifungal activity with the zone of inhibition (ZOI) 1.5–8.2 mm. Compounds like **10d** and **10f** exhibited best antimicrobial activity against *S. aureus*. The molecular docking analysis revealed that all the synthesized derivatives shown better binding affinities. Among all, compound **10f** exhibited best scores. Hence, there was an assumption that introduction of *para*-chloro and bromo-phenyl aromatic groups on triazole moiety could result excellent antimicrobial activity. This substantial growth inhibitory activity of bis-1,2,3- and 1,2,4-triazole derivatives suggested these compounds could assist a new way in the development of lead molecules against microbial infection and antimicrobial resistance investigations.

Antimicrobial resistance (AMR) has been a worldwide concern that became a never-ending fight between humans and the micro biome. Microbial infections are estimated to cause 0.7 million deaths annually and increasing on an everyday basis.[1] Combating antimicrobial resistance has become a major confront for global health, food security and development hindrance. The rise in resistance of microorganisms against antimicrobial drugs threatens the successful treatment of increasing bacterial and fungal infections[2] and affects people health all over the world. As per WHO reports on global antimicrobial resistance, it is expected that >10 million people will suffer from the multi-drug resistance infections with an increased number of human mortality rates. Further, it is expected to raise in antimicrobial drug resistance.[3] Based on these study estimations, it is required to strengthen this research field to develop new, relatively effective antimicrobial and antifungal drugs.[4] Standard drug treatments like β-lactams, aminoglycosides, tetracycline's, sulfonamides, quinolines and others developed during the 20th century, failed to control the emerging (AMR) antimicrobial resistance in some aspects by persisting the ineffective transmit to microbes. Therefore, in current medicinal chemistry, there is an urgent requirement of design and developing new strategies to build up a new effective generation of antimicrobial agents with different working mechanisms to suppress the antibiotic resistance for future use. 1,2,3-Triazoles are well-known scaffolds, which are when conjugated with other hetero cyclic moieties exhibit useful biological activities.[5–10] *N*-Containing heterocyclic compounds are a major part of the pharmaceutical, agrochemical and biological fields.[11,12] Among them 1,2,3-triazole and 1,2,4-triazoles exhibit a broad range of biological activities, such as antifungal,[13–15] Insomnia,[16] anticancer,[17] antineoplastic,[18] antimicrobial,[14,19–21,41] antibacterial,[22,30] antioxidant,[23,24,13] anti-inflammatory,[25,26] antiviral,[27,42] antimycobacterial,[28,29]

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anticonvulsant,[31] antidepressant,[32] and anticoagulants[33] activities (Fig. 1).

Stimulated by above findings and also as a part of our enduring research works in lab, we focused to develop novel antimicrobial agents with potent activity. Here, we described the novel synthesis of bis-1,2,3 and 1,2,4-triazole derivatives **10a-m**. All synthesized compounds were characterized by using ^{1}H NMR and ^{13}C NMR, EI-MS, FT-IR, Mass and elementary analyses. All these compounds **10a-m** were then studied for their antimicrobial, antifungal and antioxidant activities (Fig. 2).

The key cyclisation step of compounds **7a-d** was carried out in 2N NaOH solution under reflux temperature for 24hrs to afford the bis-1,2,3 and 1,2,4-triazole-3-thiol **8a-d** in 80–85% yield. The compound **8a** was confirmed by its ^{1}H NMR spectra which showed the characteristic thiol-SH and triazole proton absorptions as singlets at δ 11.56 and at δ 9.27 respectively. The absorption of –OCH_2 protons was appeared at δ 5.50 as a singlet, and a signal due to benzyl –CH_2 protons was observed at δ 4.76 as a singlet. This was further confirmed from its mass spectral data with molecular ion peak at *m/z* 475 $(M + H)^+$.

The final target bis-(1,2,3- and-1,2,4)-triazole derivatives **10a-m** were successfully accomplished in quantitative yields by the reaction of bis-1,2,3-and 1,2,4-triazole-3-thiols **8a-d** with phenacyl bromides **9a-d** in the presence of potassium carbonate in dry acetone at reflux temperature. The compounds **10a-m** were characterized based on their ^{1}H NMR, ^{13}C NMR, Infrared, elemental analysis and mass spectral data. The ^{1}H NMR spectrum of target compound **10a** exhibited singlets at δ 7.78, δ 5.43, δ 5.02 and δ 4.88 due to 1,2,3-triazole proton, –OCH_2 protons, -N-CH_2,and-S-CH_2 protons respectively. The ^{13}C NMR spectrum of compound **10a** given the (>C═O) keto carbon (phenacyl) signal at δ 191.9, –OCH_2 carbon signal at δ 63.2, -N-CH_2 carbon signal at δ 50.1 and -S-CH_2 carbon signal at δ 40.2. The IR spectrum confirmed the presence of characteristic -C═N- & >C═O, keto functionalities by exhibiting absorption bands at 1592 cm^{-1} and at 1729 cm^{-1}. Elemental analysis data was in good agreement with its structure. Mass spectral data derived for compound **10a** has given the $(M + H)^+$ peak at *m/z* 593, which shown a synergic concord with its calculated mass.

The designed final target bis-triazole derivatives (**10a-m)** were synthesized efficiently by following the flexible synthetic approach as detailed in Scheme 1. The synthesis of target compounds **10a-m** was started primarily with propargylation reaction on methyl salicylate **1** by using the propargyl bromide in presence of potassium carbonate base and followed by a solvent i.e., dry dimethyl formamide to render corresponding alkyne compound **2** with a yield of 85%. The structure of alkyne group in compound **2** was confirmed by its spectral data.[34] By using copper (I) catalyzed Huisgene 1,3-dipolar cycloaddition of alkyne **2** with azides **3a-c** effectively produced triazoles **4a-c** in excellent yields.[35] The formation of triazole derivatives **4a** were confirmed by their analytical data. The data depicts the ^{1}H NMR spectrum of triazole **4a** where, it's characteristic triazole proton signal observed as a singlet at δ 8.02, absorption of –OCH_2- two protons were noticeable as a singlet at δ 5.40, then the absorption corresponding to ester –OCH_3 appeared as a singlet at δ 3.89. It is then additionally confirmed by following the mass spectral data which showed up (M + 1) peak at *m/z* 344. The obtained triazoles **4a-c** were then treated with hydrazine hydrate by utilizing the 1,4-dioxane as a solvent under reflux conditions for 24 h to afford benzohydrazides **5a-c** in quantitative yields. The **5a** compound structure was confirmed based on its spectral data where, the singlet –OCH_3 absorption peak δ 3.89 is disappeared in its ^{1}H NMR spectrum and followed by the appearance of two new absorptions in its spectra at δ 4.53 (two protons for a singlet) and δ 9.21 (one proton for a singlet) due to NH_2 and NH respectively. The FT-IR spectrum of the particular compound **5a** showed absorption bands at 3097 cm^{-1} and 3394 cm^{-1} which could be attributed to NH and NH_2 groups. Additionally, the Mass spectral data of compound **5a** is in accordance with its molecular formula which gave its (M + 1) peak at *m/z* 344. The treatment of benzohydrazides **5a-c** with aryl thiocyanates **6a-b** in ethanol under reflux conditions for 12–16hrs afforded the corresponding hydrazine carbothioamide derivatives **7a-d** in good yields. Spectral data revealed the formation of compound **7a** where ^{1}H NMR of compound **7a** exhibited one signal for the proton of –CO-NH- as a singlet at δ 10.07, signal of two protons corresponding to the Ph-CH_2- was observed as a doublet at δ 4.71 and absorption of two protons of O-CH_2- appeared as a singlet at δ 5.40. Its ^{13}C NMR spectrum showed absorption signals of >C═S and >C═O carbons at δ 184.4 and δ 156.0 respectively. FT-IR spectrum of the compound **7a** has shown absorption bands of >C═S and >C═O at 1728 cm^{-1} and 1600 cm^{-1} respectively. It also showed a balanced agreement of elemental analysis and mass spectral data with its calculated mass of compound **7a**.

Antimicrobial activity

The *in vitro* assessment of newly synthesized bis-(1,2,3,-and-1,2,4)-triazole derivatives against two strains of gram-positive and negative *Staphylococcus aureus (S. aureus)* MTCC 96, *Bacillus subtilis (B. subtilis)* MTCC 441 and *Pseudomonas aeruginosa (P. aeruginosa)* MTCC 424, *Escherichia coli (E.coli)* MTCC 443 was performed. The selection of the bacterial strains was based on their marked-up ability in developing drug resistance. Among all, the bacterial growth inhibitory activities of the synthesized compounds have shown favorable inhibition activity against gram positive and gram negative bacteria. Though all compounds showed good inhibition against B. subtilis compounds **10d** and **10f** were endowed with high selectivity against *S. aureus* species (Table 1). Therefore, from the *in vitro* antibacterial assessments, the compounds **10d** and **10f** can be promising drugs when compared with the other existing ampicillin antibiotic drugs (42). Thus the results can suggest these molecules to be a promising prospect of drugs with analogs or derivatives. *In vitro* antimicrobial analysis of synthesized compounds (Table 1) was done by calculating the particular concentration of compound where there is an initiation of bacterial growth inhibition and considered as minimal concentration of compound (MIC) required for bacterial growth inhibition. A comprehensive study of compounds at molecular level interactions with target protein 1HSK (Responsible for the bacterial cell wall biogenesis by involving in the peptidoglycan penta glycine bridge formation) performed using Molegro virtual docking server to supplement the promising biological activity of compounds (Table 1) as better antibacterial agents.

Antifungal activity

Synthesized compounds (**10a-m)** were tested against filamentous fungi Aspergillus niger (*A. niger*) MTCC 404 and yeast Saccharomyces

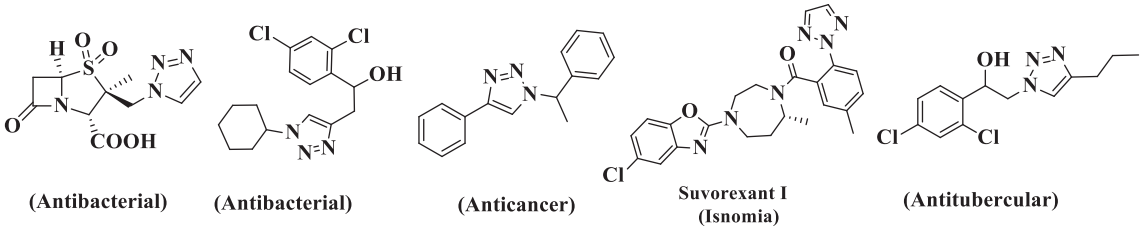


Fig. 1. 1,2,3-Triazole based biologically active agents.

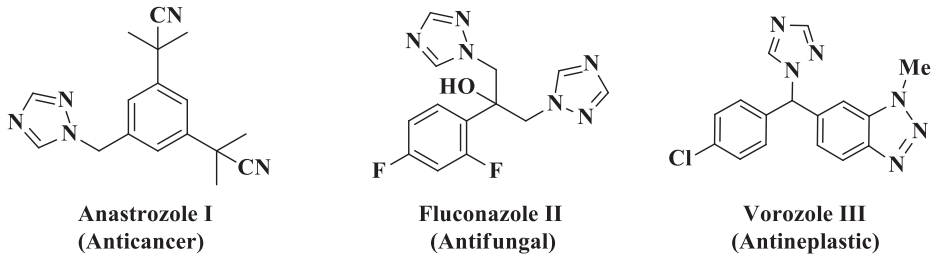
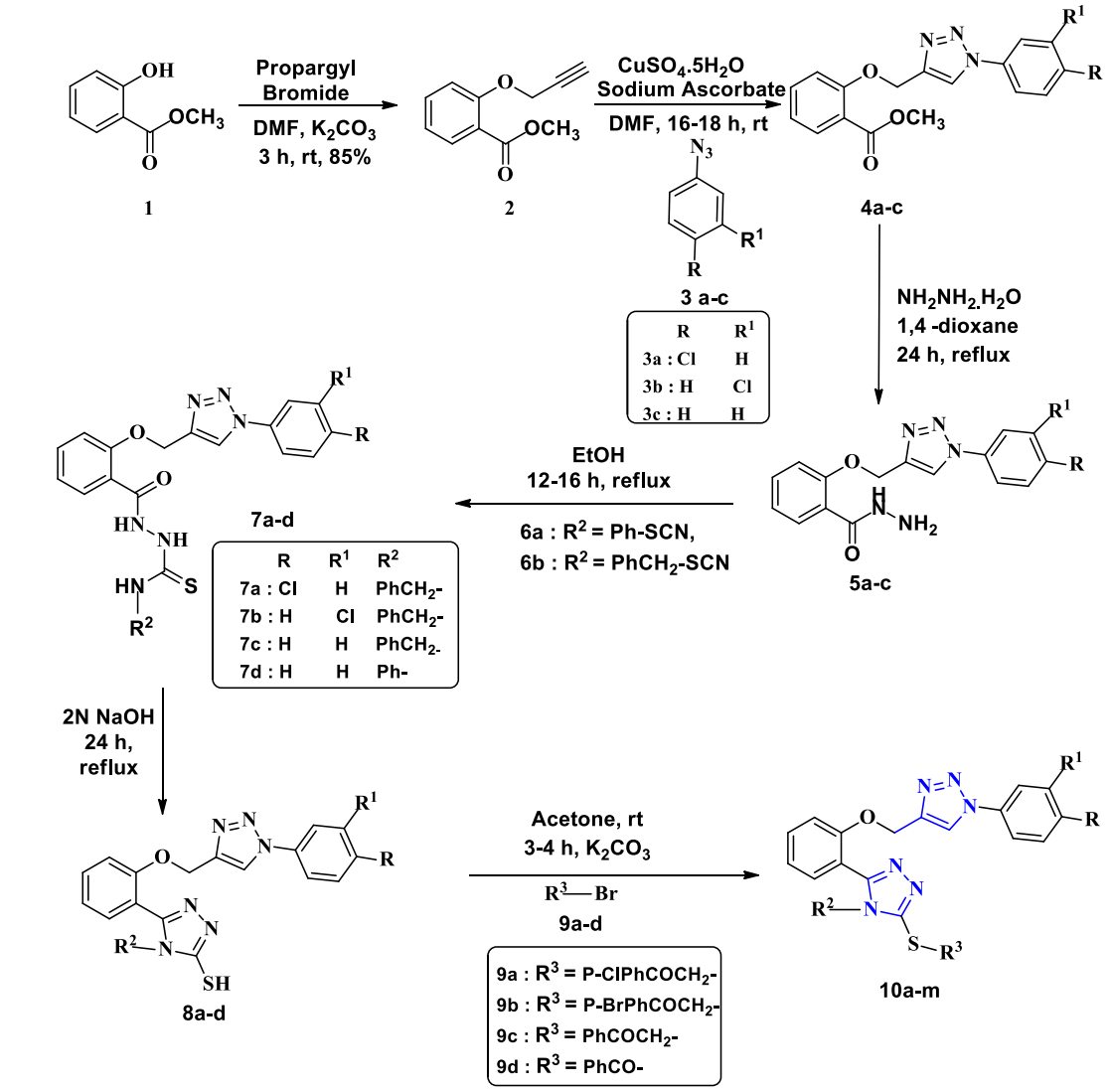


Fig. 2. 1,2,4-Triazole containing biologically active drugs.



R	R¹	R²
3a : Cl	H	
3b : H	Cl	
3c : H	H	

R	R¹	R²
7a : Cl	H	$PhCH_2$-
7b : H	Cl	$PhCH_2$-
7c : H	H	$PhCH_2$-
7d : H	H	Ph-

R	R¹	R²	R³	M.P (°C)	Yield
10a : Cl	H	$PhCH_2$-	PhCOCH2-	170-172	88%
10b : Cl	H	$PhCH_2$-	P-BrPhCOCH$_2$-	176-178	85%
10c : Cl	H	$PhCH_2$-	P-ClPhCOCH$_2$-	172-174	84%
10d . Cl	H	$PhCH_2$-	PhCO-	168-170	80%
10e : H	H	$PhCH_2$-	P-ClPhCOCH$_2$-	156-158	83%
10f : H	H	$PhCH_2$-	P-BrPhCOCH$_2$-	160-152	82%
10g : H	H	$PhCH_2$-	PhCO-	152-154	86%

R	R¹	R²	R³	M.P (°C)	Yield
10h : H	H	Ph-	PhCOCH$_2$-	148-150	80%
10i : H	H	Ph-	P-ClPhCOCH$_2$-	150-152	88%
10j : H	H	Ph-	P-BrPhCOCH$_2$-	162-164	87%
10k : H	H	$PhCH_2$-	m-BrPhCOCH$_2$-	166-168	83%
10l : H	H	$PhCH_2$-	m-ClPhCOCH$_2$-	178-180	82%
10m : H	H	$PhCH_2$-	PhCOCH$_2$-	172-174	86%

Scheme 1. Synthesis of Bis-(1,2,3,- and 1,2,4)- Triazole Derivatives 10a-m.

Table 1
Minimal Inhibitory Concentration (MIC μg/ml).* Antimicrobial activities of synthesized bis-1,2,3-and-1,2,4-Triazole derivatives (**10a-m)** against growth of gram-positive and gram-negative bacterial strains by micro dilution method.

Compound Sl. No	Gram positive bacteria (MIC μg/ml)		Gram negative bacteria (MIC μg/ml)	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
	MTCC 96	MTCC 441	MTCC 424	MTCC 443
10a	9.7 ± 0.03	5.2 ± 0.00	9.0 ± 0.00	10.0 ± 0.11
10b	8.5 ± 0.06	4.4 ± 0.05	8.1 ± 0.03	9.0 ± 0.05
10c	8.0 ± 0.05	4.0 ± 0.05	8.2 ± 0.03	8.5 ± 0.11
10d	4.1 ± 0.05	6.5 ± 0.05	7.3 ± 0.03	6.1 ± 0.08
10e	9.5 ± 0.05	5.0 ± 0.05	8.8 ± 0.03	9.1 ± 0.05
10f	3.9 ± 0.05	4.2 ± 0.05	8.8 ± 0.03	4.5 ± 0.05
10g	8.9 ± 0.06	4.0 ± 0.05	8.7 ± 0.05	10.0 ± 0.05
10h	9.8 ± 0.02	4.9 ± 0.05	9.1 ± 0.03	9.8 ± 0.05
10i	8.4 ± 0.05	4.0 ± 0.11	7.9 ± 0.06	>45.4 ± 0.56
10j	7.9 ± 0.05	4.1 ± 0.05	7.5 ± 0.05	10.0 ± 0.06
10k	8.7 ± 0.03	4.7 ± 0.05	8.5 ± 0.05	9.0 ± 0.05
10l	8.0 ± 0.05	4.1 ± 0.05	7.5 ± 0.05	>40.8 ± 0.60
10m	7.9 ± 0.05	5.0 ± 0.05	7.5 ± 0.05	10.0 ± 0.11
Ampicillin	10.0 ± 0.08	10.0 ± 0.06	10.0 ± 0.08	3.9 ± 0.03

Tale 2
Zone of inhibition (mm)* at specified concentrations in parenthesis.

Comp Sl. No	*Aspergillusniger* MTCC 404			*Saccharomyces cerevisiae* MTCC 1344		
	ZOI-mm (Compound Concentration-μM)					
10a	6.0 ± 0.00 (16.8)	8.0 ± 0.05 (25.2)	8.2 ± 0.05 (33.7)	5.8 ± 0.05 (16.8)	5.7 ± 0.05 (25.2)	7.0 ± 0.00 (33.7)
10b	2.0 ± 0.57 (14.8)	2.0 ± 0.48 (22.2)	3.0 ± 0.11 (29.7)	1.5 ± 0.14 (14.8)	2.0 ± 0.00 (22.2)	3.0 ± 0.11 (29.7)
10c	2.0 ± 0.03 (15.9)	2.0 ± 0.06 (23.9)	3.0 ± 0.11 (31.8)	2.0 ± 0.00 (15.9)	2.0 ± 0.00 (23.9)	3.0 ± 0.11 (31.8)
10d	5.3 ± 0.11 (17.2)	6.0 ± 0.00 (25.9)	7.6 ± 0.06 (34.5)	5.0 ± 0.00 (17.2)	7.0 ± 0.05 (25.2)	7.4 ± 0.05 (34.5)
10e	1.5 ± 0.11 (16.8)	3.0 ± 0.11 (25.2)	3.0 ± 0.11 (33.7)	2.0 ± 0.00 (16.8)	3.0 ± 0.11 (25.2)	3.0 ± 0.11 (33.7)
10f	5.0 ± 0.00 (15.6)	7.4 ± 0.05 (23.4)	7.6 ± 0.08 (31.2)	6.0 ± 0.05 (15.6)	6.5 ± 0.05 (23.4)	7.0 ± 0.00 (31.2)
10g	5.5 ± 0.05 (18.3)	6.8 ± 0.00 (27.5)	7.0 ± 0.00 (36.6)	6.0 ± 0.08 (18.3)	6.8 ± 0.05 (27.5)	6.7 ± 0.05 (36.6)
10h	2.1 ± 0.03 (18.3)	3.0 ± 0.11 (27.5)	3.0 ± 0.11 (36.6)	1.5 ± 0.14 (18.3)	2.0 ± 0.00 (27.5)	3.0 ± 0.11 (36.6)
10i	2.1 ± 0.03 (17.2)	3.0 ± 0.11 (25.9)	3.0 ± 0.11 (34.5)	2.0 ± 0.00 (17.2)	3.0 ± 0.11 (25.9)	3.0 ± 0.11 (34.5)
10j	1.5 ± 0.05 (16)	2.0 ± 0.06 (24)	3.0 ± 0.11 (32.1)	2.0 ± 0.00 (16)	2.0 ± 0.00 (24)	3.0 ± 0.11 (32.1)
10k	7.3 ± 0.06 (14.9)	7.0 ± 0.08 (22.3)	7.5 ± 0.05 (29.8)	6.2 ± 0.05 (14.9)	6.6 ± 0.05 (22.3)	7.4 ± 0.05 (29.8)
10l	2.0 ± 0.05 (15.9)	2.0 ± 0.06 (23.9)	3.0 ± 0.11 (31.8)	1.5 ± 0.03 (15.9)	2.0 ± 0.00 (23.9)	3.0 ± 0.11 (31.8)
10m	2.0 ± 0.00 (16.8)	3.0 ± 0.11 (25.2)	3.0 ± 0.11 (33.7)	2.0 ± 0.00 (16.8)	3.0 ± 0.11 (25.2)	3.0 ± 0.11 (33.7)
Miconazole	8.0 ± 0.11 (24)	10.0 ± 0.11 (36)	12.0 ± 0.05 (48)	8.0 ± 0.11 (24)	9.0 ± 0.00 (36)	12.0 ± 0.05 (48)

cerevisiae (*S. cerevisiae*) MTCC 1344 strains for antifungal activity. Our studies identified **10a, 10d, 10f** and **10k** compounds could show efficient antifungal efficacy among all the other synthesized compounds (Table 2).

Antifungal activity of Bis-1,2,3 and-1,2,4-Triazole derivatives (**10a-m)** against *A. niger* MTCC 404 and *S. cerevisiae* MTCC 1344 upon incubation after 2 days at 37 °C followed by cross streak plate method.

Antioxidant activity

On considering the DPPH free radical scavenging assay principle, it was demonstrated that all synthesized compounds (**10a-m)** were exhibited negligible potency in clearing the free radicals in the reaction mixture (Data not shown in manuscript).

Molecular docking analysis

Molecular docking is an *in silico* tool that follows the manual protocol to mimic ligand interactions with receptors to find the binding site and its affinity towards active site conformational changes. During bacterial inhibition, the existing β-lactam antibiotics are the often targets more abundantly with more than one penicillin binding protein by their close structural association. Studies on cell wall biosynthesis inhibitors noted that, there is an analogous observation between β-lactam antibiotics and the sugar-amino acid backbone. Soon after the bacterial cell wall biosynthesis mechanism considers these β-lactam antibiotics as their own and continues the formation of penta glycine peptidoglycan layers. In view of these aspects, the current study focused on synthesis of potent non-β-lactam derivatives called bis-1,2,3 and 1,2,4-triazoles, which can be successfully and likely targets the MurB protein effectively to inhibit the bacterial growth in various strains to combat the drug-resistance.

MurB (UDP-*N*-acetylenol pyruvylglucosamine reductase) (PDB ID: **1HSK)** is an attractive target, facilitates a key role in bacterial infections by involving in cell wall biosynthesis. It plays a vital role in peptidoglycan biosynthesis mediated with a catalyzing step in continuous process of cytoplasmic and membrane-bound reactions. It mediates in amino sugar metabolism to covert the lipid-I to lipid-II molecule in cell wall biosynthesis. It helps in reducing the EP-UNAG (enolpyruvyl-uridinediphosphate *N*-acetyl glucosamine) as an intermediate to relieve m-A2pm (UNAM-pentapeptide) protein to synthesize cell wall precursor called UNAM (uridine-diphosphate *N*-acetyl Muamic acid). In order to know the binding affinity interactions, the XRD model of *S. aureus N*-acetyl enolpyruvylglucosaminereductase (MurB) co-crystal with FAD (PDB ID: **1HSK**) was chosen with high resolution of 2.3 Å (Benson et al., 2001). In dealing with these ligand synthesis and treatment, it directs bacteria to generate a successful loose imperfect cell wall, which can be easily destructed by regular or novel antibiotics alone or in combination therapy to advance antibiotic-resistant bacterial growth. To the model

Table 3
Docking scores for synthesized compounds against MurB protein **(PDB ID: 1HSK)**.

Ligand	MolDock Score 1HSK	Rerank Score 1HSK
10a	−205.22	−126.57
10b	−196.55	−116.80
10c	−215.24	−114.72
10d	−204.68	−123.06
10e	−189.45	−131.51
10f	−209.12	−143.99
10g	−225.51	−180.47
10h	−203.06	−144.94
10i	−205.58	−121.77
10j	−202.82	−17.60
10k	−213.56	−91.50
10l	−206.30	−78.33
10m	−213.30	−47.42

PDB's we have screened the Bis-1,2,3-and-1,2,4-Triazole derivatives at the binding site specific region of flavin adenine dinucleotide (FAD) and it is observed that all **10a-10m** showed better binding affinities with potent MolDock score and Rerank score (Table 3) but **10f** ligand performed a strong binding affinity and its activity was confirmed by experimental *in vitro* approach. The docking results of 10th series compounds with MurB were regarded as good in relation to *in vitro* antibacterial MIC values (as shown in Table 1).

The target binding site of FAD is fully saturated with polar group amino acids (SER, GLN, ASN, TYR). This formulates the newly synthesized bis-1,2,3-and1,2,4-Triazole derivatives to form hydrogen bond interactions with target as proton donors or acceptors. In present study, all compounds displayed a high moldock score with maximum number of $\pi$and hydrogen interactions at binding pocket. On top of it, the Pi-alkyl and pi-pi, pi-sigma along with other hydrogen interactions are prevalently found in docking result of ligand–protein structures. These interactions would contribute a close proximal spatial orientation like FAD and their interactions with identical amino acid residues on same positions like FAD. The whole synthesized ligands **(10a-m)** have shown a good docking score along with strong binding affinities towards MurB as shown in Table 3. 2D-visualization of **10f** with **1HSK** (Fig. 3) shown an additional H-bond interactions with active site residues. In comparison with the present interactions and earlier literature findings, we noted that the MurB protein receptor with ligand binding residue position atoms at SER82 and 238, ARG188, GLY81 and GLU308 are effective target sites responsible for receptor active site conformational changes. The key interaction residues at the binding site are ARG 225, 310, ILE84, 140, GLY146, 153 and PRO141. The active site of MurB was identified from the earlier studies and also confirmed in Protein ligand server: https://projects.biotec.tu-dresden.de/plip-web/plip/index for identifying the hydrophobic interaction (Pro 141, Leu 231, Phe 274) and water bridges (Gly 146, Ser 143 and Tyr 77) in 1HSK crystal structure bound with FAD (Flavin-Adenine Dinucleotide) as inhibitor. Our docking also showed a broad vicinity of molecular binding at the same active site as shown in 2D and 3D-images (Fig. 3), and the synthesized compounds have also shown analogous interactions as found in model PDB structure. By following the principles and parameters obtained from the electronic properties & geometry, it is recommended that the compound with electrostatic interaction plays a key fundamental role in attracting aromatic and amino acid residues.[36]

Moreover, it is well acknowledged that the collection and display of heteroatoms, penta-membered rings like triazoles and pyrazoles were regarded as potential scaffolds in developing antibacterial affinity with continuation of -N and -O atomic residues in their aromatic rings. The phenyl groups in molecule showed strong π-cation interaction with ARG 188 and 225, π–alkyl interactions were observed with ALA, ILE and PHE at 154, 84 and 997 positions, π –lone pair stabilizing interaction were observed with SER 238. The phenyl aromatic rings linked to the triazole group of compounds showed many π interactions. The increase in protein–ligand interactions at aromatic amino acid residues raises the stability of newly derived ligand binding site conformations.

Structure-activity relationship (SAR) studies

From our previous studies, triazole derivatives are reported with synthetic feasibility and shown numerous antibacterial activities, focused on developing bis-triazole moieties as a lead scaffold in the present study. In addition to this, the collection of aromatic penta rings like triazole and pyrazole groups, heteroatoms acknowledged a potent framing in antibacterial action with fixing nitrogen and oxygen atoms in their aromatic rings. The synthesized series of compounds **(10a-10m)** showed better docking scores with good synergy in-*vitro* biological activity. 10f showed better activity against *S. aureus* MTCC 96 (MIC 3.9 ± 0.05 μg/ml). It is noticed that–Chloro and –Bromo halogenic substitutes at -para and -meta positions of aryl group attached to $-CH_2CO$ (i.e., $-R^3$) bestowed with pretty good biological inhibitory activities when compared with $-CH_2COPh$ and –COPh assembly alone. Compounds with benzyl group substitutions at $-R^2$ positions improved bacterial growth inhibition compared with phenyl group replacing alone. Few compounds with the halo group (–Cl) in place of –R group also showed slightly potent inhibitory activity than compounds devoid of halo group at $-R^3$ position. Hence, the introduction of more halogens to the aryl group attached to triazole moiety enhanced the antibacterial efficacy. On the contrary, few compounds have shown a least activity against *S. aureus* even by presence of halogen's could assess the discrepancy among binding at various active sites. It may also infer that, larger the

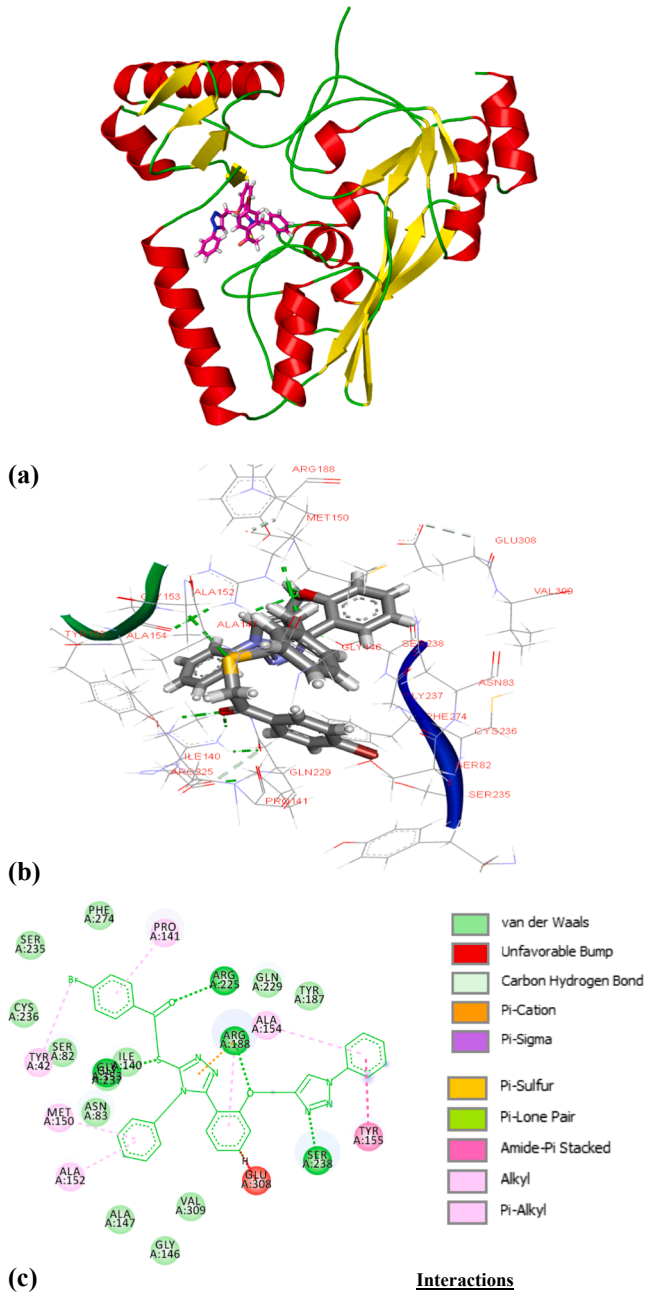


Fig. 3. *In silico* docking analysis depicts the interaction of **10a-m** derivatives with target MurB protein **(PDB ID: 1HSK)** in protein–ligand complex.[37,38] 3D and 2D visualization of **10f** molecular interaction with target MurB Protein (PDB ID: **1HSK). (a)** Secondary structure and 3D-cartoon-ribbon view of **10f** compound with **1HSK. (b)** 3D enlarged stick view of compound **10f** (green colour) binding with **1HSK** (Grey colour) **(c)** 2D dock pose and ligand interaction of **10f** molecule with MurB protein showing molecular binding sites in active regions.

number of substituents might interfere with the surrounding residues and disturb the sidechain's suitable confirmation within the active site.

In silico ADME evaluation

All the synthesized compounds **(10a-m)** were analyzed for toxicity and other ADMET properties by using an online web server called SwissADME (http://www.swissadme.ch) to identify the leads as bestowed molecules for clinical trials and other management. The compound toxicity was also established by ProTox-II chemical toxicity predictions (http://tox.charite.de/protox_II/). After the performance of synthesized compounds with final conventional biological actions, the 10^{th} series of compounds were then assessed for the physicochemical parameters along with the pharmacokinetic properties and drug-likeness with an aid of freely available SwissADME web source.[39,40]

As acknowledged in the model study, the compounds were stand alone as a potent satisfactory with their performance in bioavailability (oral). Furthermore, in combination of topological surface polarity (TPSA), lipophilic nature, Molecular Weight and flexibility, solubility, saturation shown better drug likeness. The synthesized all compounds have showed an acceptable range of the above listed parameters and are graphically displayed in radar plots as mentioned below Fig. 4. Ability of compounds for drug-likeness was determined by number of free rotatable bonds and Lipinski's rule along with Eagan's, Veber's rules. Thus, these compounds were fulfilled with good pharmacokinetic profiles with satisfied criterion of drug-likeness. In comparison with above all synthesized compounds, the specific molecules like **10a, 10d, 10f** and **10k** were identified as prominent antibacterial agents. Hence, they were also characterized for further pharmacokinetic analysis for future drug development.

Radars illustrating oral bioavailability and the drug-likeness of the compounds

The pink area in radar figure represents the appropriate range of physicochemical space for optimal oral bioavailability (SIZE: MWt, LIPO: Lipophilicity, INSOLU: Solubility, POLAR: TPSA, INSATU: Insaturation, FLEX: Flexivbility). Here in fig. 4, the red line represents the ability range of compounds tested. The Table 4 shows the outcome of listed properties and suggested with no significant violations of Lipinski's rule and the calculated physicochemical & pharmacokinetic descriptors are found to be within the projected thresholds (Drug-likeness acceptance, No. of Violations. Table 5 and predicted hERG-liability Table 6).

Discussion

In Summary, we have designed and synthesized a novel series of bis-(1,2,3-and 1,2,4)-triazole derivatives **10a-m**. All of these compounds were characterized by using their spectral data like ^{1}H & ^{13}C NMR, Mass, FT-IR and elemental analysis and further investigated for their antioxidant, antifungal and antimicrobial activities and derived a noticing wide range of antibacterial activity in **10d** (R = Cl-Ph) and **10f** (R_3 = p-BrPhCOCH$_2$-S-). Hence, both of these compounds were found to be effective against microbial growth. The rise of interest in inhibiting the MurB protein by binding analysis gives a clear glance in developing the inhibitors against antimicrobial resistance. Our findings suggested that amongst all the synthesized compounds **10a** (R = Cl-Ph), **10d** (R = Cl-Ph), **10f** (R_3 = p-BrPhCOCH$_2$-S-) and **10k** (R_3 = m-BrPhCOCH$_2$-S-) were the major compounds exhibited enhanced antifungal activity. From the principle of DPPH-free radical scavenging assay, it is depicted that none

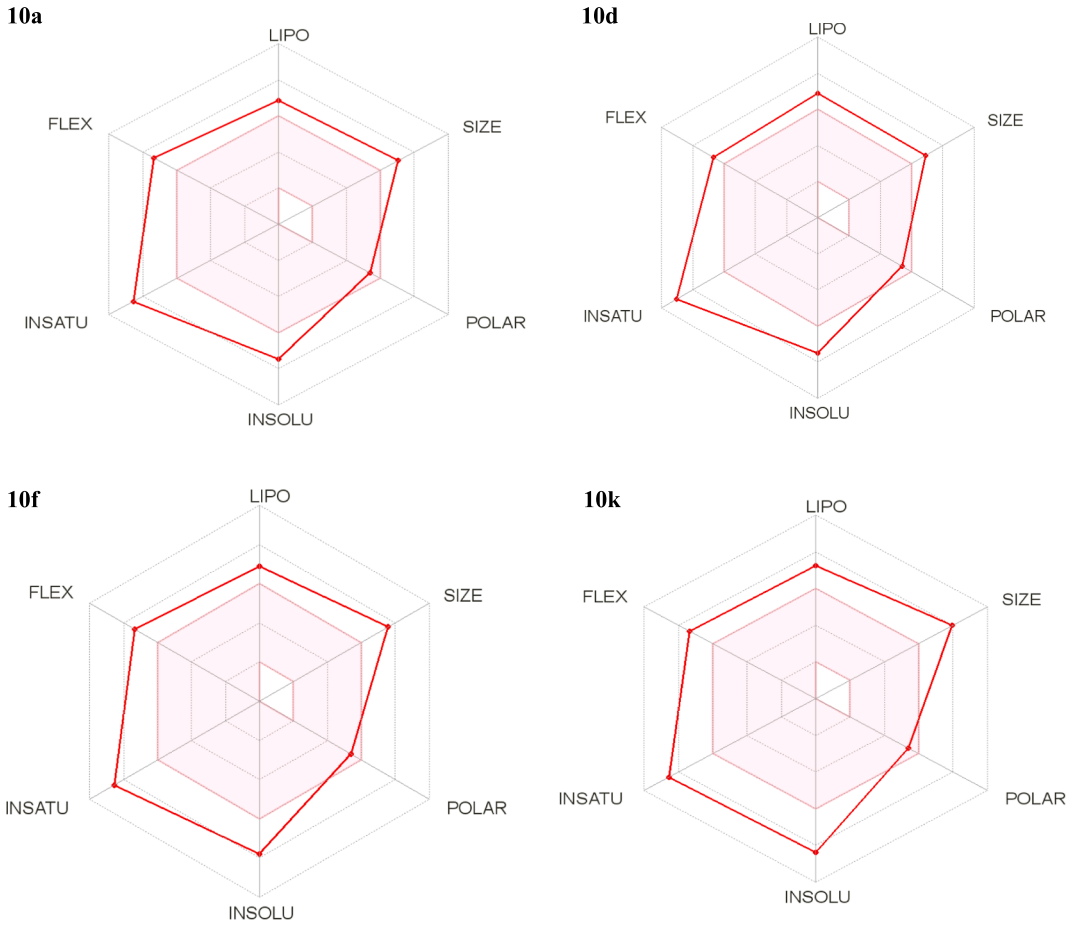


Fig. 4. Drug likeness in 10a, 10d, 10f and 10k.

Table 4
List of calculated physicochemical properties using SwissADME web server.

Comp	MW [a] (g/mol)	ClogP$^{b}_{o/w}$	MlogP[c]	nHBA[d]	nHBD[e]	nRB[f]	TPSA[g](Å^2)	logS[h]
10a	593	5.56	4.87	6	0	11	113.02	−7.48
10d	579.07	5.41	4.68	6	0	10	113.02	−7.49
10f	637.55	5.68	4.96	6	0	11	113.02	−7.80
10k	671	6.20	5.41	6	0	11	113.02	−8.39

a-Molecular weight; b, c-Lipophilicity; d-No. of H-bond acceptors; e-No. of H-bond donors; f-No. of rotatable bonds; g-Topological surface area; h-Solubility.

Table 5
Determination of Drug-likeness acceptance, No. of Violations.

Comp	Lipinski	Veber	Egan	Bio-availability score	GI absorption	BBB permeant	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
10a	No (2)	N0 (1)	N0 (1)	0.17	Low	No	No	Yes	Yes	No	Yes
10d	No (2)	Yes	N0 (1)	0.17	Low	No	No	Yes	Yes	No	Yes
10f	No (2)	N0 (1)	N0 (1)	0.17	Low	No	No	Yes	Yes	No	Yes
10k	No (1)	N0 (1)	No (1)	0.17	Low	No	No	Yes	Yes	No	Yes

Table 6
Predicted hERG-liability of prominent molecules from LabMol web server for application capability.

Comp	Potential cardiotoxic	Confidence	Applicability domain (AD)	Weak or Moderate
10a	+	60%	Yes (Value = 0.27 and limit = 0.26)	60%
10d	+	70%	Yes (Value = 0.28 and limit = 0.26)	50%
10f	+	70%	Yes (Value = 0.28 and limit = 0.26)	50%
10k	+	60%	Yes (Value = 0.27 and limit = 0.26)	50%

of the compounds exhibited their ability in clearing the free radicals in the reaction mixture. From our current *in silico* studies, the best dock scored compounds were further visualized for their active binding regions in target protein and later confirmed that 10f (R_3 = p-BrPhCOCH$_2$-S-) is the potent compound which can specifically bind to active site specific region of MurB protein and proved to have a better inhibition of bacterial cell wall biogenesis by *in vitro* confirmation with minimal microbial growth experiments. Apart from the insight into the rise of synthesized compounds as *antibac*terial agents against developing microbial resistance the compounds were also analyzed with better target binding towards bacterial viability responsive MurB. Compounds with potent inhibitory activities towards bacterial and fungal growths were further evaluated for their ADME and physicochemical properties. Upon keen observation of the obtained results, it is proved that the synthesized triazole derivatives can be a considerable moiety for the development and discovery of novel therapeutic drugs against bacterial resistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

KRA (Corresponding author), SB perceived the idea and provided critical inputs to the concept. KRA and SB planned the experiment, SB and RD, KD generated synthesis data. Molecular docking and Biological assays were carried out by SSR (Co-Corresponding author) and AAG. All the authors KRA, SB, SSR, AAG, RD, DK, PMR, JV, BK, and BVK contributed to analyze, interpret data and wrote the manuscript. All authors contributed to the final reading and approved the submitted revised version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128004.

References

1 World health organization, Global antimicrobial report, 2019, only available online: https://www.who.int/health-topics/antimicrobial-resistance. (accessed December, 27, 2019).
2 Jagadale S, Chavan A, Shinde A, Sisode V, Bobade V, Mhaske P. *Med Chem Res*. 2020:29.
3 Li D, Qiao SY, Yi GF, et al. *Asian-Aust J Anim Sci*. 2000;13(2):213.
4 Ferreira M, Pinheiro L, Santos-Filho O, et al. *Med Chem Res*. 2014:23.
5 Hong M-C, Hsu DI. Bounthavong, Ceftolozane/tazobactam: a novel antipseudomonal cephalosporin and β-lactamase-inhibitor combination. *Infect Drug Resist*. 2013;6:215–223.
6 Shaikh MH, Subhedar DD, Khan FAK, Sangshetti JN, Shingate BB. *Chin Chem Lett*. 2016;27:295.
7 Dofe VS, Sarkate AP, Lokwani DK, Kathwate SH, Gill CH. *Res Chem Intermed*. 2017;43:15.
8 Lauria A, Delisi R, Mingoia F, et al. *Eur J Org Chem*. 2014;2014:3289.
9 Jadhav RP, Raundal HN, Patil AA, Bobade VD. *J Saudi Chem Soc*. 2017;21:152.
10 Brik A, Alexandratos J, Lin Y-C, et al. *Chem Biol Chem*. 2005;6:1167.
11 Battaglia U, Moody CJ. *J Nat Prod*. 1938;2010:73.
12 Moulin A, Bibian M, Blayo A-L, El Habnouni S, Martinez J, Fehrentz J-A. *Chem Rev*. 1809;2010:110.
13 Bitla S, Sagurthi SR, Dhanavath R, et al. *J Mol Struct*. 2020;1220, 128705.
14 Shi Y, Zhou CH, Zhou XD, Geng RX, Ji QG. Synthesis and antimicrobial evaluation of coumarin-based benzotriazoles and their synergistic effects with chloromycin and fluconazole. *xuexuebao = Actapharmaceutica Sinica*. 2011;46:798.
15 Al-Soud YA, Al-Dweri MN, Al-Masoudi NA. *Farmaco*. 2004;59:775.
16 Wallace DJ, Mangion I, Coleman P. In *Comprehensive Accounts of Pharmaceutical Research and Development: From Discovery to Late-Stage Process Development Volume 1. American Chemical Society*. 2016;1239:1.
17 Lønning P, Geisler J, Dowsett M. *Breast Cancer Res Treat*. 1998;49:S53.
18 Goss PE. *Breast Cancer Res Treat*. 1998;49(Suppl 1):S59.
19 Sumangala V, Poojary B, Chidananda N, Fernandes J, Kumari NS. *Arch Pharmacal Res*. 1911;2010:33.
20 Glowacka IE, Grzonkowski P, Lisiecki P, Kalinowski L, Piotrowska DG. *Arch Pharm*. 2019;352, e1800302.
21 Patil BS, Krishnamurthy G, Shashikumar ND, Lokesh MR, BhojyaNaik HS. *J Chem*. 2013;2013, 462594.

22 Foroumadi A, Mansouri S, Kiani Z, Rahmani A. *Eur J Med Chem*. 2003;38:851.
23 Hameed A, Hassan F. *Book*. 2014:253.
24 Ünver Y, Deniz S, Çelik F, Akar Z, Küçük M, Sancak K. *J Enzyme Inhib Med Chem*. 2016;31:89.
25 Mullican MD, Wilson MW, Conner DT, Kostlan CR, Schrier DJ, Dyer RD. *J Med Chem*. 1993;36:1090.
26 Tozkoparan B, Küpeli E, Yeşilada E, Ertan M. *Bioorg Med Chem*. 1808;2007:15.
27 Todoulou OG, Papadaki-Valiraki AE, Ikeda S, De Clercq E. *Eur J Med Chem*. 1994;29:611.
28 Küçükgüzel I, Küçükgüzel SG, Rollas S, Kiraz M. *Bioorg Med Chem Lett*. 2001;11:1703.
29 Gumrukcuoglu N, Ugras S, Ibrahim Ugras H, Cakir U. *J Appl Polym Sci*. 2011;2012:123.
30 Gao F, Wang T, Xiao J, Huang G. *Eur J Med Chem*. 2019;173:274.
31 Kelley JL, Koble CS, Davis RG, McLean EW, Soroko FE, Cooper BR. *J Med Chem*. 1995;38:4131.
32 Chiu SHL, Huskey SEW. *Drug Metab Dispos*. 1998;26:838.
33 Khalid W, Badshah A, Khan AU, Nadeem H, Ahmed S. *Chem Cent J*. 2018;12:11.
34 Mandl, F. A.; Kirsch, V. C.; Ugur, I.; Kunold, E.; Vomacka, J.; Fetzer, C.; Schneider, S.; Richter, K.; Fuchs, T. M.; Antes, I.; Sieber, S. A. AngewandteChemie (International ed. in English) 2016, 55, 14852.
35 Kim S, Cho SN, Oh T, Kim P. *Bioorg Med Chem Lett*. 2012;22:6844.
36 Zhou H-X, Pang X. *Chem Rev*. 2018;118:1691.
37 Lipeeva, A. V.; Zakharov, D. O.; Burova, L. G.; Frolova, T. S.; Baev, D. S.; Shirokikh, I. V.; Evstropov, A. N.; Sinitsyna, O. I.; Tolsikova, T. G.; Shults, E. E. In Molecules (Basel, Switzerland) 2019, 24.
38 Daina A, Michielin O, Zoete V. *Sci Rep*. 2017;7:42717.
39 Jiang J, Hou Y, Duan M, et al. *Bioorg Med Chem Lett*. 2020, 127660.
40 Gokarn R, et al. Antimicrobial study of Shadguna Rasa sindura. *J Indian Sys Medicine*. 2015;3(3):136.
41 Shi Y, Zhou CH. *Bioorg Med Chem*. 2011;21:956.
42 Ferreria M, Pinheiro L, Santos-Filho O, et al. *Med Chem Res*. 2014;23:1501.