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Synthesis, bioevaluation and molecular docking study of new piperazine and amide linked dimeric 1,2,3-triazoles

Tejshri R. Deshmukh[a], Smita P. Khare[a], Vagolu S. Krishna[b], Dharmarajan Sriram[b], Jaiprakash N. Sangshetti[c], Vijay M. Khedkar[d], and Bapurao B. Shingate[a]

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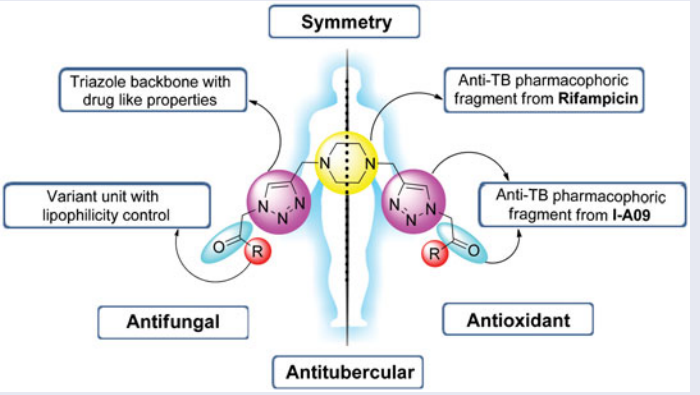
ABSTRACT

In search of more potent new antitubercular agents, a library of novel piperazine tethered dimeric 1,2,3-triazoles were designed by assembling 1,2,3-triazoles and piperazine in a single molecular architectural framework. The titled compounds (**3a–m**) were synthesized by 1,3-dipolar cycloaddition of 1,4-di(prop-2-yn-1-yl)piperazine (**1**) and various azides (**2a–m**) using click chemistry approach with good yields. All the synthesized compounds (**3a–m**) have been screened for their *in vitro* antitubercular, antifungal and antioxidant activities against their respective strains. Among them, **3b**, **3d,** and **3i** have revealed promising antitubercular activity against *Mycobacterium tuberculosis (Mtb)* H37Rv with MIC 12.5 μg/mL. Molecular docking results provided well-clustered solutions to the mode of binding for these molecules into the active site of *Mtb* enoyl reductase (InhA). In addition to this, most of synthesized compounds were found to have potential antifungal as well as antioxidant activity.

GRAPHICAL ABSTRACT



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KEYWORDS

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Introduction

Tuberculosis (TB) is infectious lung disease caused by pathogenic bacterium *Mycobacterium tuberculosis* (*Mtb*).[1] Near about one-third of the world's population is infected by *Mtb* from which only India and China altogether contributes to almost 40% of total new TB cases.[2] According to the World Health Organization (WHO), over 10.4 million new TB cases with 1.4 million TB related deaths have been reported.[3] Although there are several new therapeutic leads in different stages of clinical trials, Bedaquiline is the only new anti-TB drug approved in last half-century by FDA.[4] Hence, there is an urgent need to develop newer chemical entities with promising anti-TB activity to kill TB completely with less side effects in shorter time period.

Immune-compromised individuals like organ transplant recipients as well as the patients of TB, cancer, AIDS, and leukemia are vulnerable to fungal infections.[5] The co-infection of *Mtb* with opportunistic pulmonary fungal pathogen causes infection in the lungs.[6] Most of TB patients with HIV infection are usually infected by the fungal pathogen.[7] It was also observed that the risk of fungal infections was at higher percentage in the patients with MDR-TB.[8] Many physicians fail to diagnose the fungal co-infection with TB because it is hindered by TB and also it does not show any clinical sign of existence.[9] In such cases, the fate of patients may lead to death even after TB was cured completely. Hence, there is a need for such promising anti-TB drugs that kills fungal pathogens too.

Intake of high doses of anti-TB drugs over a long period of time in the body causes pulmonary tissue inflammation in TB patients. It is occurred due to the oxidative stress developed by the free radicals burst from activated macrophages and various anti-TB drugs.[10] In such conditions antioxidants used to prevent the formation of free radicals or scavenge the generation of reactive oxygen species (ROS) which inhibits the pulmonary inflammation.[11] Therefore, if the antitubercular drugs demonstrate antioxidant activity as well then it is possible to avoid the pulmonary tissue inflammation in TB patients.

Nowadays, the 1,2,3-triazoles are being highlighted by many researchers as showing excellent biological as well as physical properties. Among the various triazoles, the 1,4-disubstituted-1,2,3-triazole derivatives have been found to be promising drug core moiety.[12] It is easy to synthesize by Cu (I)-catalyzed 1,3-dipolar cycloaddition of azide and alkyne by using Sharpless and Meldal's greener approach of click chemistry.[13] The 1,2,3-triazoles are found as a bioisoster of amide bond due to which it can easily bind with a high affinity to many biological targets.[14] The 1,2,3-triazole scaffolds have shown various biological activities like antitubercular,[15] anti-HIV,[16] antibacterial,[17] antifungal,[18] antimalarial,[19] antiviral,[20] anticancer,[21] anticonvulsant,[22] antioxidant[23] and anticoccidiostats.[24]

Piperazine bearing molecules have displayed a wide range of biological activities such as anticancer,[25] antitubercular[26] and antifungal.[27] Recently, the piperazine incorporated 1,2,3-triazole derivatives were reported and displays various activities.[28] Literature survey revealed that dimeric 1,2,3-triazoles, piperazine clubbed with 1,2,3-triazole and 1,2,3-triazole with amide linker scaffolds displayed enhanced antitubercular[29–32] activity and representative structures are shown in Figure 1.

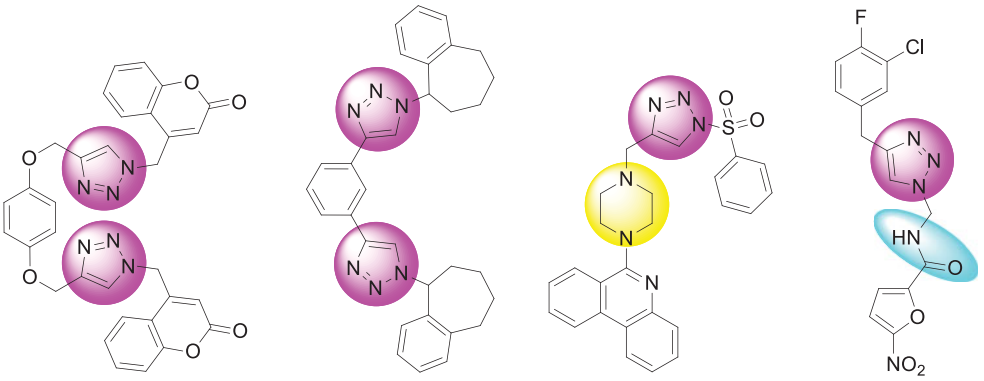


Figure 1. Antitubercular agents bearing 1,2,3-triazole, amide linker, dimeric triazole and piperazine scaffolds.

Most of the receptors in human body have shown to possess two binding sites as they possess symmetrical structures and show better binding affinity with dimeric drugs as compared to monomers. Beddell and coworkers described the use of hemoglobin as a drug-receptor model to explain that symmetrical receptors interact with drugs or hormone molecules, having symmetrical features.[33] Literature reveals that dimeric compounds show better bioactivities than monomers.[34] Therefore, to enhance the bioactivity, most researchers are taking interest in the synthesis and bioevaluation of dimeric compounds.

By considering the therapeutic significance of the above and in continuation of our earlier work on chemistry and biology of 1,2,3-triazole based compounds[35–45] herein, we would like to report the synthesis of novel dimeric 1,2,3-triazoles with piperazine scaffold and their antitubercular, antifungal and antioxidant activity evaluations. In addition to this, we have also performed molecular docking study for newly synthesized compounds.

Results and discussion

Chemistry

The designed scaffold was originated from molecular hybridization of pharmacophoric fragments of antitubercular agents[31,46] as shown in Figure 2. The design of newly synthesized piperazine and amide linked dimeric 1,2,3-triazoles was broadly divided into four segments. Substituted 1,2,3-triazolyl acetamide is a bioactive fragment from I-A09 and its analogs derived from the respective appended aromatic/cyclic/aliphatic groups as variant unit contributes as most active pharmacophoric unit in the designed strategy. In addition to this, the piperazinyl fragment from rifampicin also helps to enhance the pharmacophoric property. The lipophilicity control in the designed strategy could be accomplished with the substitutional variant unit of respective aromatic/cyclic/aliphatic moieties. The main backbone of the design was the two 1,2,3-triazolyl units situated in symmetrical manner and may exhibit drug-like properties.

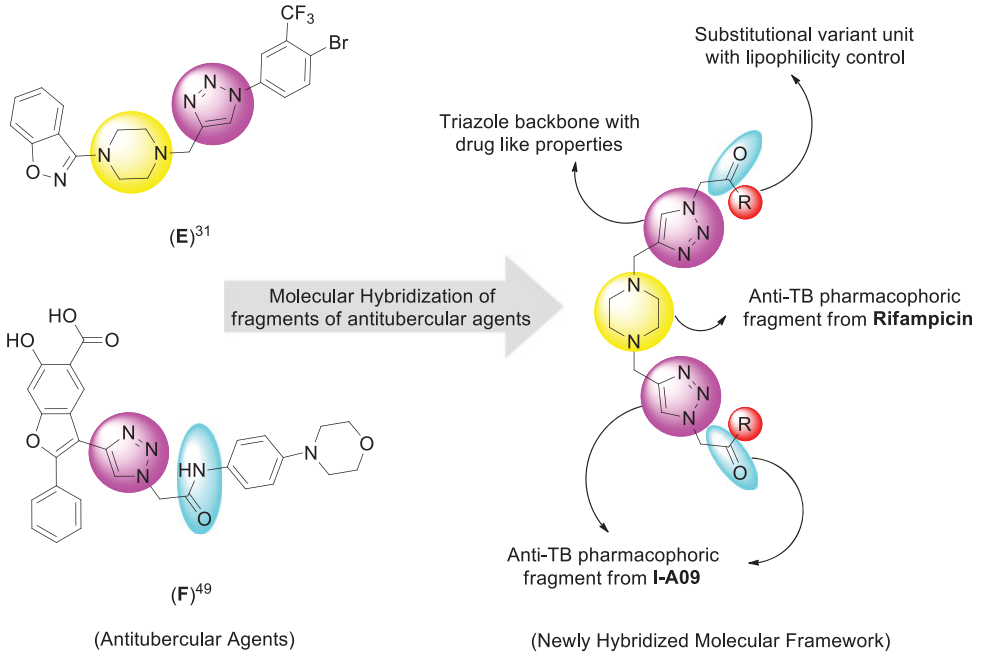
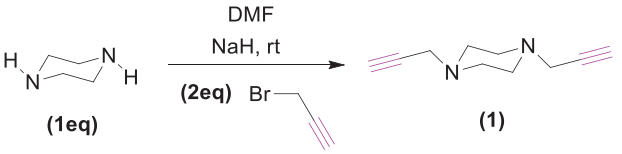


Figure 2. Design strategy for the synthesis of piperazine and amide linked dimeric 1,2,3-triazoles.

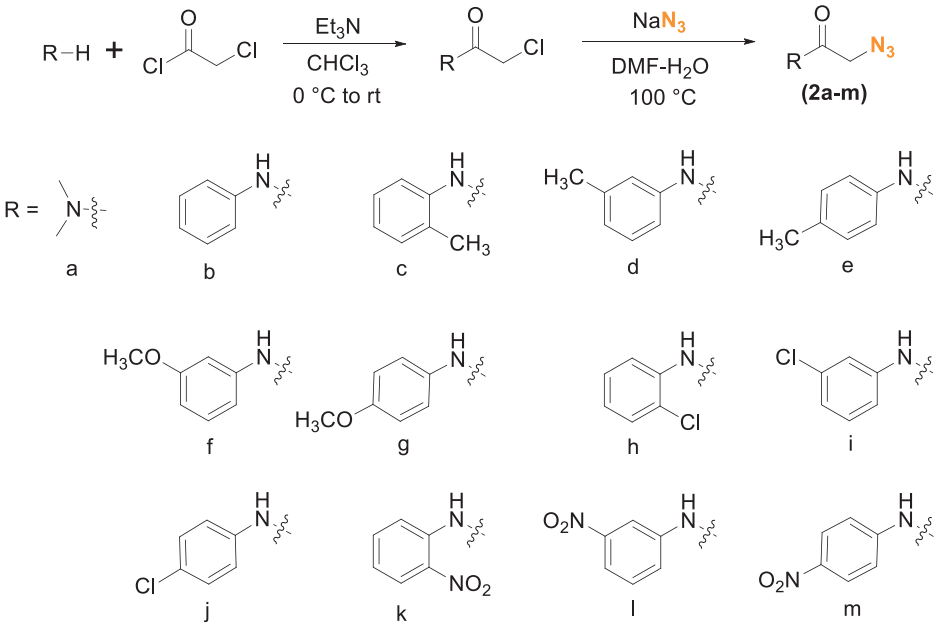


Scheme 1. Synthesis of piperazine and amide linked dimeric 1,2,3-triazoles (**3a–m**).

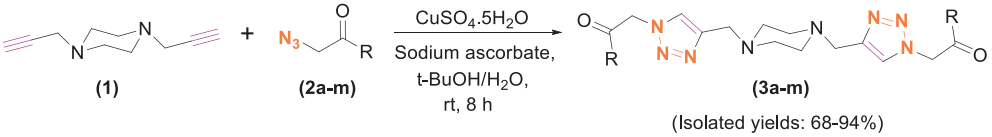
The synthetic route of new piperazine and amide linked dimeric 1,2,3-triazoles (**3a–m**) is depicted in Scheme 1. We have freshly synthesized various azides and dialkyne as a starting material for the synthesis of target compounds. The synthesis of one of the starting compounds, 1,4-di(prop-2-yn-1-yl)piperazine (**1**) is described in Scheme 2. In first step, piperazine was allowed to react with propargyl bromide in the presence of NaH as a base in *N,N*-dimethylformamide (DMF) at room temperature to obtain key intermediate 1,4-di(prop-2-yn-1-yl)piperazine (**1**) in a good yield.[47]

Various substituted azides (**2a–m**) were freshly prepared by reported procedure[48] as shown in Scheme 3. These azides (**2a–m**) were obtained from corresponding amines when reacts with chloroacetyl chloride in presence of triethylamine as a base using chloroform as a solvent at 0 °C gives corresponding substituted chloroacetyl amide intermediates. These intermediates on further reaction with sodium azide in DMF:H_2O (3:1) at 100 °C resulted in the corresponding azides (**2a–m**) in good yields.

After the synthesis of all the starting materials, the targeted compounds (**3a–m**) were synthesized by 1,3-dipolar cycloaddition of 1,4-di(prop-2-yn-1-yl)piperazine (**1**) with freshly prepared azides (**2a–m**) in presence of catalytic amount of $CuSO_4.5H_2O$ and



Scheme 2. Synthetic route for azides (**2a–m**) from respective amines.



Scheme 3. Synthesis of 1,4-di(prop-2-yn-1-yl)piperazine (**1**).

sodium ascorbate as a source of copper (I) in *t*-BuOH/H_2O (1:2) for 8 h at room temperature in good to excellent yields (Scheme 1).

All the newly synthesized compounds have been characterized using physical data, ^{1}H NMR, ^{13}C NMR, and HRMS spectral analysis. One of the representative ^{1}H NMR spectrum of compound (**3d**), displays peaks, at δ 4.79, 5.52, 8.70, and 10.46 ppm as a single, due to the O–CH_2, N–CH_2, triazolyl–H, and amido N–H, respectively. The presence of two characteristic carbon signals is observed at δ 21.7 and 175.0 ppm in ^{13}C NMR spectrum of (**3d**) owing to the signals of methylene groups of piperazine and amide group, respectively. The HRMS spectrum further strengthens the structure assigned to (**3d**) as it displays $M+H^+$ ion peak at *m/z* 515.2636 for the molecular formula $C_{26}H_{31}N_{10}O_2$ and equivalent to the calculated $M+H^+$ ion peak at 515.2632.

Biological assay

In vitro *antitubercular activity*

These piperazine tethered dimeric 1,2,3-triazoles (**3a–m**) were tested for their *in vitro* antitubercular activity against *M. tuberculosis* H37Rv (ATCC 27294) by using microplate

Almar Blue assay (MABA)[49] for the determination of MIC in triplicates. The MIC values (μg/mL) of compounds (**3a–m**) have been compared with standard drugs (rifampicin, isoniazid, ethambutol, and ciprofloxacin) and the results are given in Table 1.

It has been observed that the dimeric triazoles were displayed good to moderate antitubercular activity with MIC values ranging from 12.5 to 25 μg/mL. Among the newly synthesized compounds, **3b**, **3d,** and **3i** were exhibited good antitubercular activity with MIC value 12.5 μg/mL. Further, the compounds **3a** and **3m** were also showed moderate antitubercular activity against *Mtb* with MIC value 25 μg/mL. The remaining compounds of the series having MIC values >25 μg/mL, that are considered to be inactive against *Mtb*. It was observed that inhibition depends upon substituent group attached to triazole ring. The compounds **3b**, **3d,** and **3i** with substituent group R containing phenyl ring with H, 3-CH_3 and 3-Cl groups respectively, that might be one of the reasons for better antitubercular activity as compared to other compounds of the series. Whereas, the compounds **3a** and **3m** with substituent group R bearing dimethyl and 4-NO_2 group respectively, exhibits moderate antitubercular activity.

Lipophilicity is an important physicochemical property which may lead to improve therapeutic success and quality of drug.[50] In the discovery of any drug, the assessment of lipophilicity is based on Log*P*/*C*Log*P* values. Therefore, to study the correlation of antitubercular activity of synthesized compounds with lipophilicity parameters like Log*P* and *C*Log*P* were calculated using ChemBiodraw ultra 12.0 software and the results are displayed in Table 1. Compounds may show better lipophilicity when value of Log$P \leq 5$. It was found that all synthesized compounds having values of Log*P* and *C*Log*P* are in acceptable range. Hence, all the compounds show excellent lipophilic property which helps to accelerate the rate of therapeutic success.

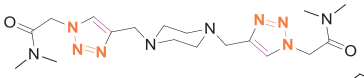
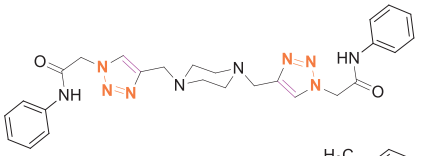
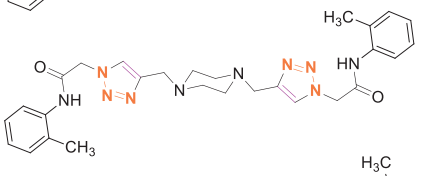
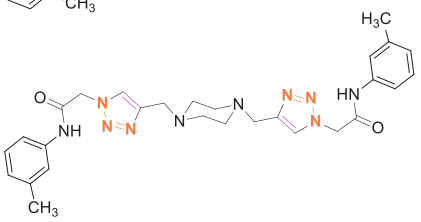
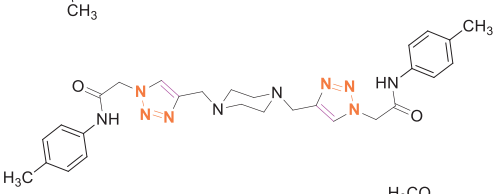
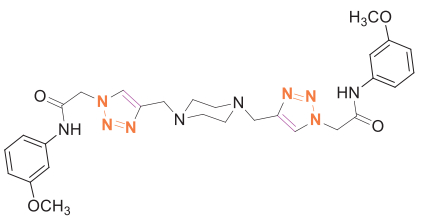
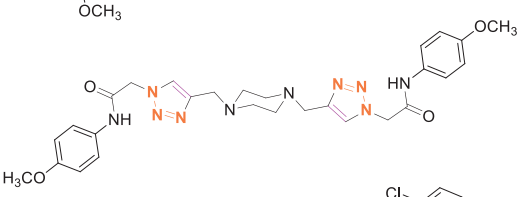
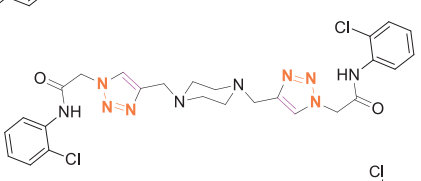
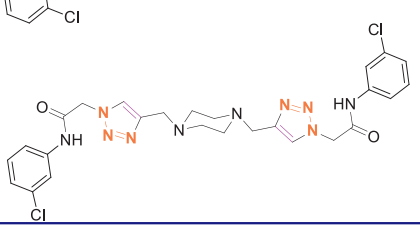
In vitro *antifungal activity*

All these newly synthesized compounds (**3a–m**) were also screened for their *in vitro* antifungal activity against five human pathogenic fungal strains. The obtained screening results were summarized in Table 2.

According to this, most of the synthesized compounds were shown good to excellent antifungal activity against the tested strains. The compound **3g** was found to be most potent antifungal agent against all the tested strains as compared to standard drug miconazole. It shows excellent activity against *C. albicans* and *Aspargillus niger* with MIC 12.5 μg/mL and proved as two-fold more potent antifungal agent than standard miconazole. In this case, the presence of 4-OCH_3 group may influence the activity. The compound **3l** was also displayed more potent or equipotent activity as compared to miconazole. Out of five tested fungal stains, this compound was found to be active against four strains. The presence of 3-NO_2 group on compound **3l** may influence the activity.

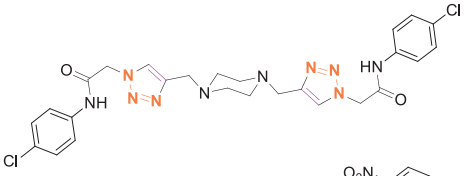
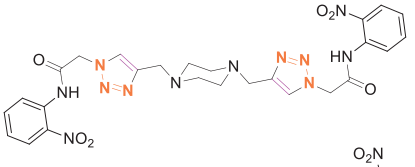
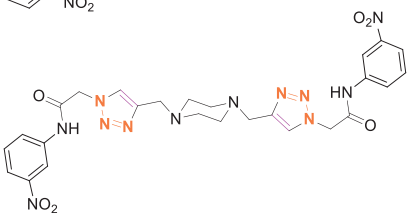
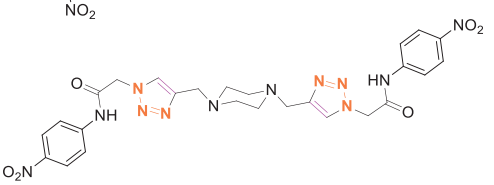
The compounds **3e**, **3h,** and **3m** possess good antifungal activity against at least two fungal stains whereas compounds **3d**, **3e,** and **3k** also show equipotent antifungal activity against only one of the tested stains. All these compounds show either more potent or equipotent antifungal activity in comparison with miconazole, which might be due to presence of variant groups in the molecular framework. Results show that the active

Table 1. *In vitro* antitubercular evaluation of compounds (**3a–m**) against *M. tuberculosis* H37Rv.

Entry	Structures	MIC (μg/mL)	Log*P*[a]	C Log*P*[b]
3a		25	−1.63	−1.8236
3b		12.5	1.23	1.0272
3c		>25	2.2	0.7252
3d		12.5	2.2	2.0252
3e		>25	2.2	2.0252
3f		>25	0.97	1.178
3g		>25	0.97	1.178
3h		>25	2.34	1.2684
3i		12.5	2.34	2.9684

(*continued*)

Table 1. Continued.

Entry	Structures	MIC (μg/mL)	Log*P*[a]	*C*Log*P*[b]
3j		>25	2.34	2.9684
3k		>25	–	0.6972
3l		>25	–	1.6172
3m		25	–	1.6172
Isoniazid		0.1	–	–
Rifampicin		0.2	–	–
Ethambutol		1.56	–	–
Ciprofloxacin		1.56	–	–

a and b calculated using ChemBioDraw Ultra 12.0.

Table 2. *In vitro* antifungal and antioxidant activity of synthesized compounds (**3a–m**).

	(MIC) in μg/mL against fungal strains					
Compounds	CA	FO	AF	AN	CN	IC_{50} (μg/mL) antioxidant activity
3a	25	50	100	175	25	39.74 ± 0.95
3b	75	37.5	50	100	62.5	11.17 ± 0.89
3c	150	50	100	175	*	31.04 ± 0.24
3d	50	75	25	75	25	11.34 ± 0.45
3e	25	50	50	100	25	10.12 ± 0.25
3f	100	150	175	*	62.5	12.85 ± 0.27
3g	12.5	25	12.5	12.5	25	38.62 ± 0.31
3h	37.5	25	37.5	37.5	12.5	41.37 ± 0.08
3i	75	100	150	150	*	10.64 ± 0.29
3j	125	150	125	*	175	24.54 ± 0.20
3k	25	37.5	50	100	62.5	25.09 ± 0.77
3l	25	50	12.5	25	25	13.98 ± 0.44
3m	62.5	25	25	25	50	11.17 ± 0.35
Miconazole	25	25	12.5	25	25	NT
BHT	NT	NT	NT	NT	NT	16.47 ± 0.18

CA: *Candida albicans* (NCIM 3471); FO: *Fusarium oxysporum* (NCIM 1332); AF: *Aspergillus flavus* (NCIM 539); AN: *Aspergillus niger* (NCIM 1196); CN: *Cryptococcus neoformans* (NCIM 576); NT: not tested;
*No activity up to 200 mg/mL; BHT: butylated hydroxy toluene.

compounds will serve as an important scaffold for further designing and development of new antifungal agents in future.

Antioxidant activity

Generally, due to intake of higher doses of antitubercular drugs by the patient of tuberculosis over a long period of time leads to develop oxidative stress which may result in pulmonary tissue inflammation.[10] DPPH radical scavenging activity[11] is the most commonly used method for screening antioxidant activities of the various natural as well as synthetic antioxidants. The IC_{50} of BHT (butylated hydroxyl toluene) used for comparison and the results were summarized in Table 2. It was observed that most of the synthesized compounds show excellent antioxidant activity with promising lower IC_{50} values in comparison with standard antioxidant agent, BHT. The compounds **3e** and **3i** were displayed excellent radical scavenging activity with IC_{50} 10.12 and 10.64 µg/mL, respectively and found as most potent antioxidant agents. Both these compounds **3e** and **3i** were also found to be more potent than standard antioxidant agent, BHT. In addition to this, the compounds **3b**, **3d**, and **3m** were also displayed excellent radical scavenging activity with IC_{50} values ranges 11.17–11.34 µg/mL and found to be more potent than BHT. According to Table 2, the compounds **3f** and **3l** also acts as better antioxidant agents than BHT, as both having IC_{50} values 12.85 and 13.98 µg/mL, respectively. Whereas, remaining compounds were observed as less potent than BHT.

Computational study

Molecular docking study

Promising level of anti-tubercular activity produced by the piperazine tethered dimeric 1,2,3-triazoles (compounds **3b**, **3d,** and **3i**) prompted us to perform molecular docking studies to identify their potential biological target and elucidate the plausible mechanism by which these compounds inhibit the pathogen. It was performed using with *Glide* (Grid-Based Ligand Docking With Energetics) program.[51] Among the array of crucial targets, mycobacterial enoyl reductase (InhA) enzyme has recently drawn significant attention of medicinal chemists as a potential target to combat the pathogen. In addition, several reports have demonstrated the potential of the triazole scaffold to inhibit mycobacterial enoyl reductase (InhA).[52] The enoyl-ACP (CoA) reductase (FabI/ENR/InhA) plays a crucial role in the fatty acids elongation cycle contributing to mycolic acid biosynthesis through the mycobacterial type II fatty acid biosynthesis pathway. Its inhibition is shown in the fast-growing model organism *Mycobacterium smegmatis* to inhibit mycolic acid synthesis and induce cell lysis.[53] Therefore, impaired action of InhA leads to the loss of cell integrity and consequently to cell death. These reports served as the basis for selecting enoyl-ACP reductase as the target to investigate the binding mode of the compounds and to gain an insight into the ligand-protein interactions.

The theoretical predictions from the molecular docking study showed that all the five compounds (**3b**, **3d,** and **3i**) could successfully dock into the active site of *Mtb* enoyl reductase (InhA) with varying degree of affinities. Their complexation with the receptor

was stabilized by formation of several bonded and non-bonded interactions. A detailed per-residue interaction analysis between these molecules and the residue forming the active site of the enzyme was carried out to identify the most significantly interacting residues and the type of thermodynamic elements governing their binding to the target. The intermolecular interaction energy values (Glidescore, Glide energy) along with per-residue interactions obtained from the docking calculation are summarized in Table 3.

The lowest energy docked conformation (Fig. 3) of **3b** was found to be tightly bound to the active site of InhA at the same co-ordinates as the co-crystallized native ligand with the central piperazine nucleus engaging in a significant van der Waals interactions with Lys165 (−1.997 kcal/mol), Ala191 −1.020 kcal/mol), Met161 (−2.482 kcal/mol), Asp148 (−2.05 kcal/mol), Met147 (−2.539 kcal/mol), Ile21 (−2.497 kcal/mol) and Ile16 (−1.245 kcal/mol) residues; the triazole pharmacophores showed van der Waals interactions with Ile194 (−1.831 kcal/mol), Pro193 (−2.576 kcal/mol), Tyr158 (−3.441 kcal/mol), Met103 (−2.03 kcal/mol), Ser94 (−3.076 kcal/mol), Ala22 (−2.076 kcal/mol), Ser20 (−2.469 kcal/mol).

Similarly the *N*-phenylacetamide groups flanking the piperazine nucleus also showed favorable van der Waals interactions via Leu218 (−2.664 kcal/mol), Ile215 (−2.582 kcal/mol), Met199 (−3.424 kcal/mol), Ala157 (−2.164 kcal/mol), Met155 (−2.393 kcal/mol), Phe149 (−2.83 kcal/mol), Gly96 (−2.277 kcal/mol), Ile95 (−2.506 kcal/mol), Phe41 (−2.933 kcal/mol), Ile15 (−2.537 kcal/mol) and Gly14 (−2.91 kcal/mol) residues of active site. On the other hand, Arg195 (−1.235 kcal/mol), Gly192 (−2.817 kcal/mol), Glu169 (−2.300 kcal/mol) and Ser20 (−2.356 kcal/mol) residue interacted electrostatically with triazole pharmacophore while Asp148 (−2.759 kcal/mol) got engaged in similar type of contact with piperazine scaffold.

The enhanced binding affinity of **3b** has also attributed to two prominent hydrogen-bonding interaction observed first between Gly14 residue and the amine group substituted (–NH–) linker of the *N*-phenylacetamide group with a bonding distance of 2.12 Å while second H-bond was observed between the Lys165 residue and piperazine nitrogen with a bonding distance of 2.61 Å. Furthermore, a close π–π stacking interactions observed between the triazole ring and Tyr158 residue (1.907 Å) contributed significantly to the stability of **3b** within the active site of InhA. These type of hydrogen bonding and the π–π stacking interactions "anchor" the ligand into the active site of the enzyme facilitating the steric and electrostatic interactions.

A similar binding mode and network of interactions was observed for **3d** and **3i** (Figs. 4 and 5) as well. The per-residue ligand interaction analysis suggests that steric complementarity with the active site residues of InhA governed the mechanical interlocking of these molecules reflected in the relatively higher contribution of favorable van der Waals interactions over the other components contributing to the binding affinity

(In Figs. 3–5, the green lines on right side signify π–π stacking interactions while the pink lines represent the hydrogen bonding interactions.)

In silico *ADME prediction*

we have also predicted in *silico* ADME properties of newly synthesized piperazine tethered dimeric 1,2,3-triazoles (**3a–m**). The results of ADME predictions were summarized

Table 3. Quantitative per-residue interaction analysis of the molecular docking study on *Mtb* InhA for the most active piperazine tethered dimeric 1,2,3-triazoles.

Code	*M. tb H37Rv* MIC (μg/ml)	Docking score	Glide interaction energy (kcal/mole)	Per-residues interactions			
				Van der Waals (kcal/mol)	Coulombic (kcal/mol)	H-bonds (Å)	Π–π Stacking (Å)
3b	12.5	−10.914	−65.793	Leu218 (−2.664), Ile215 (−2.582), Met199 (−3.424), Ile194 (−1.831), Pro193 (−2.576), Ala191 (−1.020), Lys165 (−1.997), Met161 (−2.482), Tyr158 (−3.441), Ala157 (−2.164), Met155 (−2.393), Phe149 (−2.83), Asp148 (−2.05), Met147 (−2.539), Met103 (−2.03), Gly96 (−2.277), Ile95 (−2.506), Ser94 (−3.076), Phe41 (−2.933), Ala22 (−2.076), Ile21 (−2.497), Ser20 (−2.469), Ile16 (−1.245), Ile15 (−2.537), Gly14 (−2.91)	Arg195 (−1.235), Gly192 (−2.817), Glu169 (−2.300), Asp148 (−2.759), Ser20 (−2.356)	Gly14 (2.12), Lys165 (2.61)	Tyr158 (1.907)
3d	12.5	−10.451	−64.197	Leu218 (−1.883), Ile215 (−1.348), Met199 (−2.111), Ile194 (−1.068), Pro193 (−2.015), Ala191 (−1.052), Lys165 (−1.838), Met161 (−1.240), Tyr158 (−2.012), Ala157 (−1.924), Met155 (−2.112), Phe149 (−2.969), Asp148 (−1.646), Met147 (−2.856), Ile122 (−1.083), Met103 (−1.901), Gly96 (−2.389), Ile95 (−2.176), Ser94 (−3.255), Phe41 (−2.372), Ile21 (−2.734), Ser20 (−2.116), Ile16 (−1.434), Gly14 (−2.638)	Glu219 (−1.461), Lys165 (−5.25), Ile95 (−1.144), Ser94 (−1.547)	Gly96 (2.42)	Tyr158 (2.116), Phe149 (2.778), Phe41 (2.784)
3i	12.5	−10.585	−64.189	Leu218 (−2.274), Ile215 (−2.799), Met199 (−3.411), Ile194 (−1.116), Pro193 (−1.667), Ala191 (−0.999), Lys165 (−1.689), Met161 (−1.142), Tyr158 (−1.112), Ala157 (−1.935), Pro156 (−0.931), Met155 (−1.467), Phe149 (−2.946), Asp148 (−1.514), Met147 (−2.903), Met103 (−1.588), Phe97 (−1.211), Gly96 (−2.563), Ile95 (−2.343), Ser94 (−3.382), Phe41 (−2.529), Ile21 (−2.855), Ser20 (−1.995), Ile16 (−1.122), Gly14 (−2.8)	Ser20 (−2.161), Ile95 (−1.593)	Ser20 (2.72), Gly96 (2.24)	–

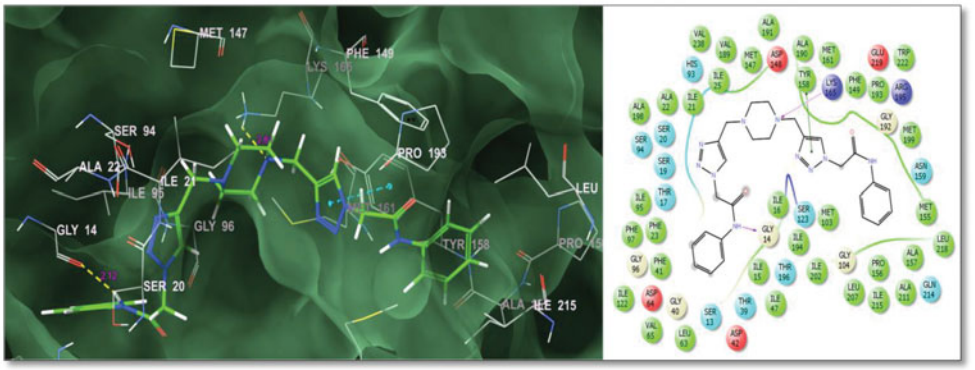


Figure 3. Binding mode of **3b** into the active site of *Mtb* InhA.

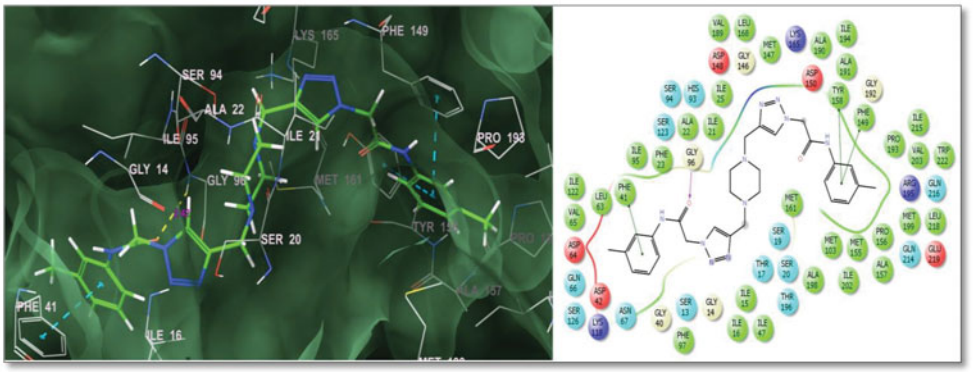


Figure 4. Binding mode of **3d** into the active site of *Mtb* InhA.

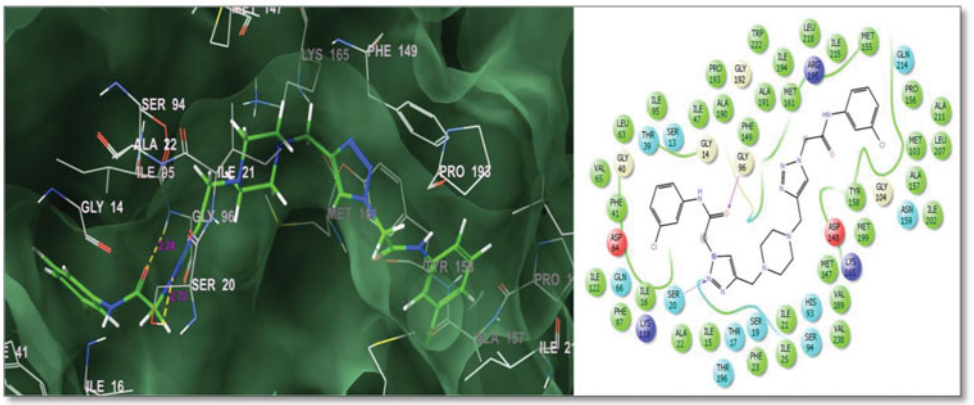


Figure 5. Binding mode of **3i** into the active site of *Mtb* InhA.

in Table 4. In this study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (mi Log*P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface

Table 4. Pharmacokinetic parameters for in *silico* ADME prediction.

Entry	% ABS[a]	TPSA[b] (A^2)	n-RO TB[c]	MV[d]	MW[e]	miLog[f]	n–ON[g]	n–OH NH[h]	Lipinski violations[i]	Drug likeness model score
Rule	–	–	–	–	<500	≤5	<10	<5	≤1	–
3a	71.56	108.53	8	388.48	418.50	−2.84	12	0	1	0.21
3b	65.49	126.11	10	464.29	514.58	0.95	12	2	2	1.05
3c	65.49	126.11	10	497.41	542.64	1.75	12	2	2	1.25
3d	65.49	126.11	10	497.41	542.64	1.80	12	2	2	0.83
3e	65.49	126.11	10	497.41	542.64	1.84	12	2	2	1.26
3f	59.12	144.58	12	515.38	574.64	1.01	14	2	2	0.85
3g	59.12	144.58	12	515.38	574.64	1.06	14	2	2	1.38
3h	65.49	126.11	10	491.36	583.47	2.21	12	2	2	1.46
3i	65.49	126.11	10	491.36	583.47	2.25	12	2	2	0.94
3j	65.49	126.11	10	491.36	583.47	2.30	12	2	2	1.97
3k	33.87	217.76	12	510.96	604.58	0.77	18	2	2	0.43
3l	33.87	217.76	12	510.96	604.58	0.82	18	2	2	0.24
3m	33.87	217.76	12	510.96	604.58	0.86	18	2	2	0.55

[a]Percentage absorption.
[b]topographical polar surface area.
[c]number of rotatable bonds.
[d]molecular volume.
[e]molecular weight.
[f]lipophilicity.
[g]no. of hydrogen bond acceptors.
[h]no. of hydrogen bond acceptors.
[i]number of violations.

area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five[54] of all the newly synthesized compounds using Molinspiration online property calculation toolkit.[55] Absorption (% ABS) of all the derivatives of the series was calculated by using following formula.

$$\%\,\mathrm{ABS} = 109 - (0.345 \times \mathrm{TPSA}) \quad [56]$$

Drug-likeness model score (a collective property of physico-chemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value) of each and every compound was computed by MolSoft software.[57] A molecule is considered to develop an orally active drug if the drug violates only one of the Lipinski's rule of five having following four valid criteria's: miLog*P* (octanol-water partition coefficient) ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10 and number of hydrogen bond donors ≤5.

It was observed that all the synthesized compounds exhibited moderate to good % ABS ranging from 33.87 to 71.56%. A molecule is considered to develop an orally active drug if the drug violates only one of the Lipinski's rule of five. From the Table 4, it was observed that most of the property predictions were found in an acceptable range and possess average to good potential to develop an orally active drug molecules.

Conclusion

In conclusion, new piperazine tethered dimeric 1,2,3-triazoles (**3a–m**) were synthesized and evaluated against *Mtb* H37Rv strain. The compounds **3 b**, **3d** and **3i** have displayed significant antitubercular activity with MIC value 12.5 µg/mL. Furthermore, antifungal activity of the newly synthesized compounds were also evaluated against five human

pathogenic fungal strains. Among the series, compounds **3g** and **3l** were displayed excellent antifungal activity as compared to standard drug. In addition to this, all the compounds were also examined for antioxidant activity and DPPH radical scavenging assay revealed that the compounds **3b**, **3d**, **3e**, **3i**, and **3m** possess more potent antioxidant activity than BHT. Piperazine tethered dimeric 1,2,3-triazoles offers an attractive lead series for the discovery of novel antitubercular, antifungal and antioxidant agents. Furthermore, molecular docking investigation for the most active compounds into the active site of InhA provided well-clustered solutions to the mode of binding into the active site of mycobacterial InhA. The computational results correlated with the observed experimental values in good agreement suggesting that these molecules could serve as pertinent starting point for further optimization and development as InhA target specific antitubercular agents.

Experimental

Materials and methods

All the chemicals were of laboratory grade, purchased from commercial suppliers Spectrochem, Avra, Alfa Aesar and Sigma Aldrich and were used without further purification. Purity and completion of reaction time of all synthesized compounds were monitored by thin layer chromatography (TLC) using silica gel 60-F_{254} precoated on aluminum sheets as an adsorbent, Merck, Germany and visualization was accomplished by iodine/ultraviolet light. Melting points of all the synthesized compounds were determined in an open capillary tube method and are uncorrected. ^{1}H NMR spectra were recorded on a Brukar DRX-400 and 400 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard and chemical shifts are in δ(ppm). The splitting pattern abbreviations are designed as singlet (s); doublet (d); double doublet (dd); triplet (t); quartet (q) and multiplet (m). ^{13}C NMR spectra were recorded on a Brukar DRX-75 and 100 MHz NMR in DMSO-d_6. High-resolution mass spectra (HRMS) were obtained using HRMS-ESI-Q-Time of flight LC/MS instrument.

General procedure for the synthesis of piperazine incorporated dimeric 1,2,3-triazoles (3a–m)

In a round bottom flask 1,4-di(prop-2-yn-1-yl)piperazine **1** (0.01 mol) and the newly synthesized azides **2a–m** (0.02 mol) were allowed to react in the presence of aqueous solution of $CuSO_4.5H_2O$ and sodium ascorbate *t*-BuOH/H_2O (1:2) at room temperature for 8 h. The same experimental procedure of click reaction we have used in our earlier research work.[58,59] The progress of the reaction was monitored by TLC using ethyl acetate: hexane (3:7) as a solvent system. After completion of the reaction, the reaction mixture was poured on crushed ice. The obtained solid products (**3a–m**) were filtered, dried and crystallised in ethanol-DMF.

Synthesis of 2,2′-(4,4′-(piperazine-1,4-diylbis(methylene))bis(1H-1,2,3-triazole-4,1-diyl))bis (N-phenylacetamide) (3b)

The compound (**3b**) was obtained by Cu (I)-catalyzed 1,3-dipolar cycloaddition reaction between 1,4-di(prop-2-yn-1-yl)piperazine (**1**) and azide (**2b**) as dark greenish solid;

Yield: 94%; Mp: >270 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ_H ppm) 2.95 (s, 4H, piperazinyl-H), 3.02 (s, 4H, piperazinyl-H), 4.78 (s, 4H, piperazinyl N–CH_2), 5.38 (s, 4H, triazolyl N–CH_2), 7.14–7.63 (m, 10H, Ar–H), 8.68 (s, 2H, triazolyl–H), 10.58 (s, 2H, amido N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ_C ppm) 24.5, 70.2, 72.8, 116.9, 119.3, 133.1, 133.5, 135.1, 138.5, 162.0; HRMS (ESI) calcd. for $C_{26}H_{31}N_{10}O_2$ $M+H^+$: 515.2632; found 515.2636.

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Disclosure statement

The authors declare no competing interest.

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