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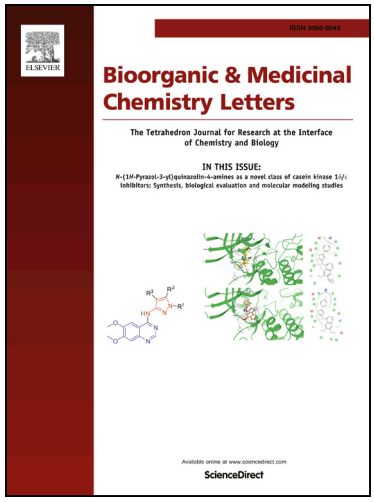

Synthesis and Biological Evaluation of Novel 1,2,3-Triazole derivatives as Anti-Tubercular Agents

Abdul Aziz Ali, Dhrubajyoti Gogoi, Amrita K. Chaliha, Alak K. Buragohain, Priyanka Trivedi, Prakash J. Saikia, Prveen S. Gehlot, Arvind Kumar, Vinita Chaturvedi, Diganta Sarma

PII: S0960-894X(17)30701-1
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.07.008
Reference: BMCL 25121

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 28 March 2017
Revised Date: 9 June 2017
Accepted Date: 3 July 2017

Please cite this article as: Aziz Ali, A., Gogoi, D., Chaliha, A.K., Buragohain, A.K., Trivedi, P., Saikia, P.J., Gehlot, P.S., Kumar, A., Chaturvedi, V., Sarma, D., Synthesis and Biological Evaluation of Novel 1,2,3-Triazole derivatives as Anti-Tubercular Agents, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.07.008

Graphical Abstract

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Synthesis and Biological Evaluation of Novel 1,2,3-Triazole derivatives as Anti-Tubercular Agents

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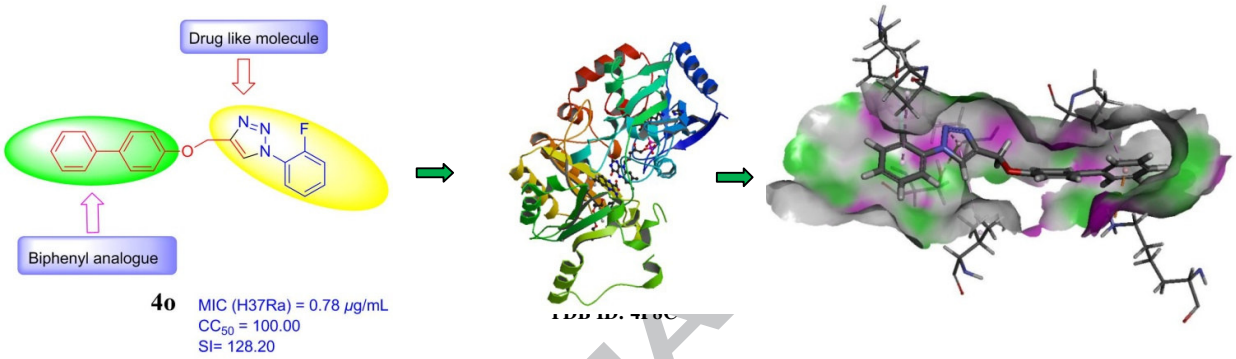
Abdul Aziz Ali[a], Dhrubajyoti Gogoi[b], Amrita K. Chaliha[b], Alak K. Buragohain[b], Priyanka Trivedi[c], Prakash J. Saikia[d], Prveen S. Gehlot[e], Arvind Kumar[e], Vinita Chaturvedi[c,*], Diganta Sarma[a,*]



Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Tuberculosis
1,2,3-Triazoles
Antimycobacterial activity
Cytotoxicity
Molecular docking

ABSTRACT

A library of seventeen novel 1,2,3-Triazole derivatives were efficiently synthesized in excellent yields by the popular 'click chemistry' approach and evaluated in vitro for their anti-tubercular activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177 strain). Among the series, six compounds exhibited significant activity with minimum inhibitory concentration (MIC) values ranging from 3.12 to 0.78 μg/mL and along with no significant cytotoxicity against MBMDMQs (mouse bone marrow derived macrophages). Molecular docking of the target compounds into the active site of DprE1 (Decaprenylphosphoryl-β-D-ribose-2′-epimerase) enzyme revealed noteworthy information on the plausible binding interactions.

Tuberculosis (TB), an infectious terrible disease caused by the acid fast bacillus *Mycobacterium tuberculosis* remains one of the most life threatening diseases to public health globally after human immunodeficiency virus (HIV).[1-2] Normally it attacks the lungs (pulmonary TB) but can attack other organs as well (extrapulmonary TB) and spreads in the air when patients expel bacteria by coughing, sneezing, or spit. According to World Health Organization (WHO) report, 9.6 million new TB cases were estimated and claiming the lives of 1.5 million people in the year 2014 despite the great advances in chemotherapy and the Bacille-Calmette-Guérin (BCG) vaccine.[3] The current standard therapy attributed for TB is a six month regimen, termed DOTS (Directly Observed Therapy, Short-course) in which the initial 2 months include isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (E), followed by a 4 month continuation phase of RIF and INH.[4] Furthermore, TB attracts numerous interest of the scientific community due to high weakness of human immunodeficiency virus (HIV)-infected persons to this disease and the global emergence of multidrug resistant (MDR) defined as resistant to the two most efficient TB drugs, rifampin and isoniazid, and extensively drug-resistant (XDR) strains that are further resistant to the fluoroquinolones and one of the second-line injectable drugs (i.e. amikacin, kanamycin, or capreomycin).[5-7] The confines of long-term oral chemotherapy and scarce compliance to the current treatment regimen, the discovery of bedaquiline in the end of 2012, build a new hope for the treatment of TB and especially MDR-TB.[8] Nevertheless the side effects of bedaquiline such as nausea, joint pain and headache create a risk in clinical use.[9-10] Therefore, there is a still need for the development of new and effective antimycobacterials with reduced toxicity, synthetically feasible, stronger efficacy that function by novel mechanisms of action against emerging MDR and XDR TB bacteria and latent diseases in shorter treatment duration.

1,2,3-Triazoles are five member N-heterocyclic compounds and are stable to metabolic degradation. They are also capable of hydrogen bonding, which can be favorable in the binding of biomolecular targets and can improve the solubility.[11-12] Although absent in nature, the 1,2,3-Triazoles have found a broad spectrum of biological applications such as anti-tubercular,[13] antibacterial,[14] anti-allergic,[15] anti-HIV,[16] anti-fungal,[17-18] anti-inflammatory,[19] anticancer,[20-21] and α-glycosidase inhibitor activities.[22] β-lactum antibiotic Tazobactum, anticancer compound carboxyamidotriazole (CAI), cefatrizine are some drugs available in the market that possess 1,2,3-triazole moiety.[23] Many approaches for the synthesis of 1,2,3-triazoles have been developed so far, in which a typical click reaction, copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) is definitely the most effective strategies.[24] In recent years a wide range of 1,2,3-triazole derivatives were synthesized and reported to exhibit potent antitubercular activity (**Figure 1**), especially benzofuran salicylic acid derivative (I-A09) is a lead antitubercular agent

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presently in clinical evaluations.[25-34] In continuation to our ongoing study aimed towards the development on 1,2,3-triazole synthesis[35-38] and previous research efforts toward the discovery of potent anti-TB agents[39] herein, we wish to disclose a facile click synthesis of a series of novel 1,4-disubstituted 1,2,3-triazole analogues and their antimycobacterial evaluation.

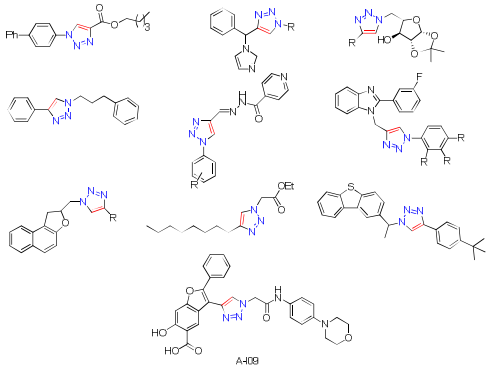
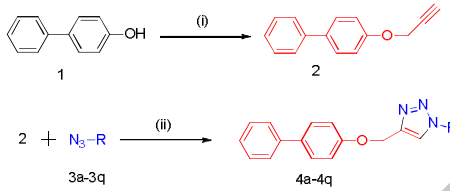


Figure 1. Representative structure of 1,2,3-triazole derivatives that exhibit anti-tubercular activity

The synthetic strategy was initiated with the preparation of azides, one of the coupling partners of Cu-catalyzed azide alkyne cycloaddition reaction. As reported earlier, the benzyl azides were prepared from the corresponding organic bromides by stirring the bromide with NaN_3 in water/acetone (1:3) at room temperature to give the desired azide as yellow oil. Similarly octyl azide was prepared by refluxing1-bromooctane with NaN_3 in acetone/water mixture overnight affording the corresponding azide as colourless oil. Aromatic azides were synthesized by diazotization of the corresponding aromatic amines with $NaNO_2$ followed by addition of NaN_3. Aromatic azides were obtained as yellow oils with yields ranging from 70% to 95%. The azides were used directly in the next step without purification and the chemical structures of the azides were confirmed by analyzing the crude product using FT-IR spectroscopy. A strong absorption band at 2090-2100 cm^{-1}, attributed to the stretching vibrations of the N_3 bond of the azido group. The starting alkyne **2** was prepared by using commercially available 4-phenylphenol, **1** by simple alkylation with propargyl bromide in the presence of K_2CO_3 as a base in N,N-dimethylformamide (DMF) affording the corresponding alkyne in excellent yield as reported in **Scheme 1**. The synthesis of 1,2,3-triazole derivatives were accomplished through Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition reaction between **2** and appropriate azides in presence of CuI as catalyst and $DHQD_2(PHAL)$ as ligand in H_2O/DCM for 0.5 to 2h affording 1,4-disubstituted-1,2,3-triazoles in good to excellent yields as depicted in **Scheme 1** (80-96%). The structures of the products were characterized by thin layer chromatography (TLC), ^1H NMR, ^{13}C NMR, FT-IR and mass spectrometry. IR spectrum of **4c** showed absorption bands at 2990, 1598 and 1049 cm^{-1} indicating the presence of -CH_2, phenyl and C-N groups. The ^1H NMR spectrum of compound **4c** exhibited the presence of one distinctive singlet signal at around δ 5.32 ppm indicating the attachment of methylene group to oxygen respectively. In addition, the appearance of most informative singlet signal around at δ 8.05 ppm confirms the presence of triazole proton. In ^{13}C NMR, the most prominent carbon signals observed around δ 61.9 ppm accounted for the presence of methylene carbon attached to the oxygen to the biphenyl ring. In addition, the characteristic carbon signals appearing around δ 145.2 and 120.6 ppm were assigned to C-4 and C-5 of triazoles ring, while the various aromatic carbons resonated around δ 145.2-114.9 ppm. Further, LC mass spectrum showed [M+1] ion peak at m/z 407.9 which is in agreement with the molecular formula $C_{21}H_{16}BrN_3O$.



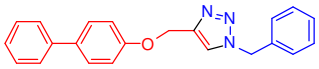
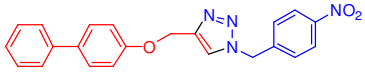
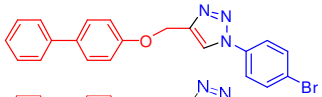
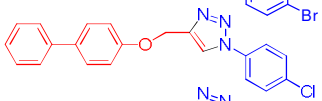
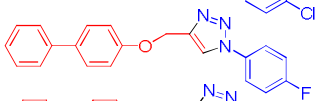
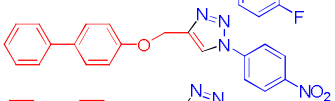
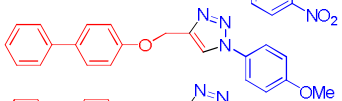
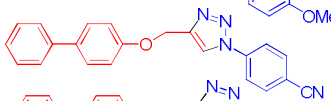
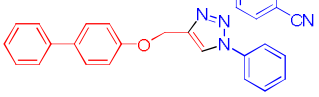
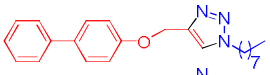
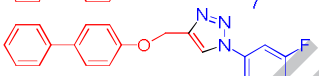
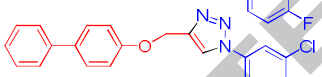
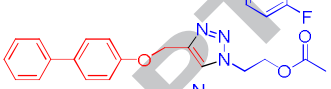
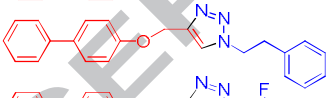
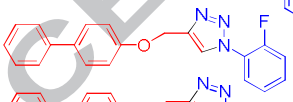
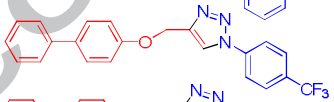
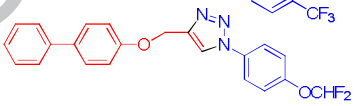
Scheme 1. Reagents and conditions: (i) Propargyl bromide, K_2CO_3, DMF, 90% ; (ii) CuI (1 mol%), $DHQD_2(PHAL)$ (1 mol%), H_2O/DCM (1:1), rt, 0.5-2h, 80-96%.

The synthesized compounds were tested for their ability to inhibit the growth of *M. tuberculosis* H37Ra (ATCC 25177 strain) by Agar-based proportion Assay as shown in **Table 1.** The lowest concentration of a compound up to which there was no visible growth of bacilli was its minimal inhibitory concentration (MIC). Out of seventeen compounds screened for their in vitro anti-tubercular activity against *M.tuberculosis* H37Ra, six compounds were found active with MIC in the range of 3.12-0.78 μg/mL (**Table 1**) and the rest were with MIC >12.5 μg/mL. The compounds with potent MIC of 3.12 and 1.56 μg/mL were also tested for their cytotoxicity against MBMDMQs (mouse bone marrow derived macrophages) and found to be nontoxic on their selectivity index (SI>10, ratio of CC_{50} against mammalian cells and the MIC). Among tested series, compound **4o** with fluoro group at second position on phenyl ring of the triazole derivatives exhibited excellent antimycobacterial activity with MIC= 0.78 μg/mL as compared to the first-line antitubercular drug ethambutol (MIC=2.00 μg/mL) whereas compounds **4p** and **4q** substituted with -CF_3 and –$OCHF_2$ groups at fourth position on phenyl ring of the triazole moiety exhibited moderate to good activities with MIC= 3.12 and 1.56 μg/mL, respectively. The compounds **4k** and **4l** with 3,4-difluoro and 3-chloro-4-fluoro group on the phenyl ring displayed considerable antimycobacterial activity with MIC values of 3.12 and 1.56 μg/mL, respectively. Moreover the compound **4m** with ester group also showed significant activity with MIC=1.56 μg/mL, corroborating its antimicrobial nature. However, the activity is not as profound as that of isoniazid (INH), the most active first-line anti-TB drug.

Molecular docking provides a potent tool to different type of interactions that govern the binding of a molecule to the biological receptor. Therefore, with the aim of rationalizing the observed antitubercular results and to investigate the possible interactions of the studied compounds (**4k-4q**), molecular docking study was performed with the target enzyme, Decaprenylphosphoryl-β-D-ribose-2′-epimerase, DprE1(PDB ID: 4P8C) of *Mycobacterium tuberculosis* using CHARMm based docking software (CDOCKER) available at the BIOVIA Discovery Studio v4.5 platform. DprE1 is a flavin adenine dinucleotide (FAD) dependent oxidoreductase; the key enzyme involved in the biosynthesis of decaprenylphosphoryl-D-arabinose (DPA), which is the only known donor of D-arabinofuranosyl residues for the synthesis of arabinogalactan, a basic precursor for the survival of mycobacteria.[40] The binding interactions of docked compounds with the DprE1 enzyme are shown in **Figure 2**. In this study, 3D structure of *M. tuberculosis* DprE1 complexed with Y22 (6-(trifluoromethyl)-3-{[4 ((trifluoromethyl)benzyl]amino}quinoxaline-2-carboxylic acid) was selected as target protein and acquired from Protein Data Bank. The optional binding site was also computed at the Y22 and compounds were docked with the receptor. Docking

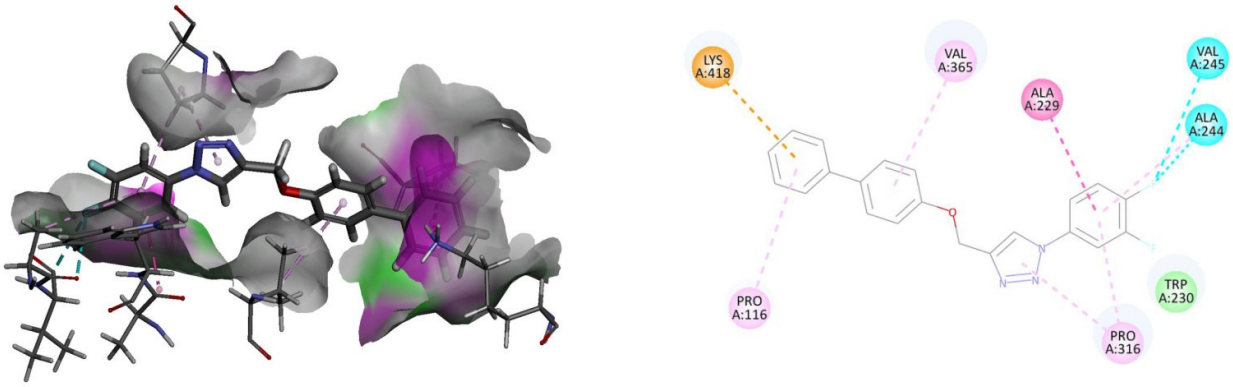
performance was accessed based on the CDOCKER docking score.

Table 1. Biological Activity Studies of compounds **4a-4q** Using Copper-Catalyzed Click Chemistry

Compounds	Structure	C log P[a]	MIC (μg/ml)	CC_{50}[b]	Selectivity Index (SI)
4a		4.81	>25.00	ND	ND
4b		4.55	>25.00	ND	ND
4c		6.29	>25.00	ND	ND
4d		6.14	>25.00	ND	ND
4e		5.57	>25.00	ND	ND
4f		5.37	>25.00	ND	ND
4g		5.49	>25.00	ND	ND
4h		5.09	>25.00	ND	ND
4i		5.26	>25.00	ND	ND
4j		6.74	>25.00	ND	ND
4k		5.70	3.12	100.00	32.05
4l		6.34	1.56	100.00	64.10
4m		3.35	1.56	100.00	64.10
4n		5.14	>25.00	ND	ND
4o		5.57	0.78	100.00	128.20
4p		6.44	3.12	100.00	32.02
4q		5.93	1.56	100.00	32.05
	Isoniazid	-	0.025	100.00	4000.00
	Ethambutol	-	2.00	100.00	50.00

[a]CLog P calculated using Chemdraw Ultra 12.0 software by Cambridge Soft.[b]CC_{50} were evaluated towards Mouse bone marrow macrophages.
ND - not done.

(a)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ALA A:244
PRO A:116
TRP A:230
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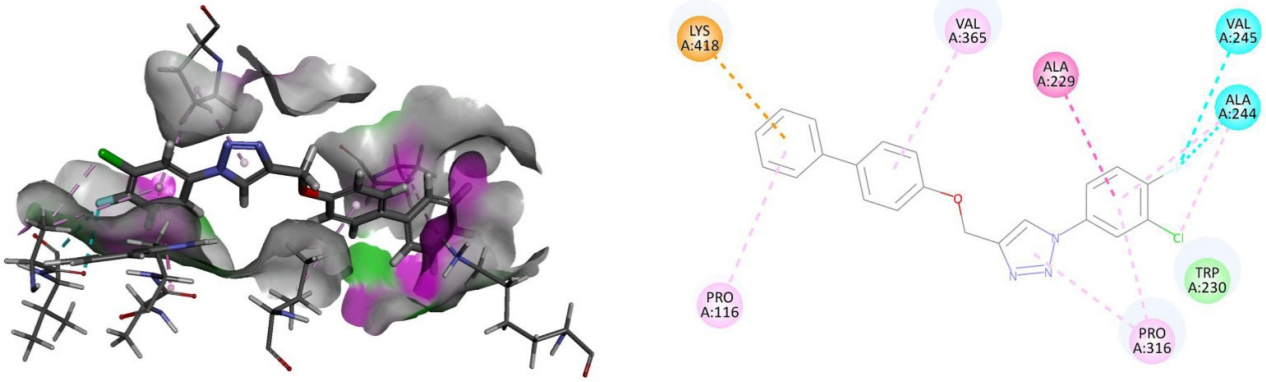
(b)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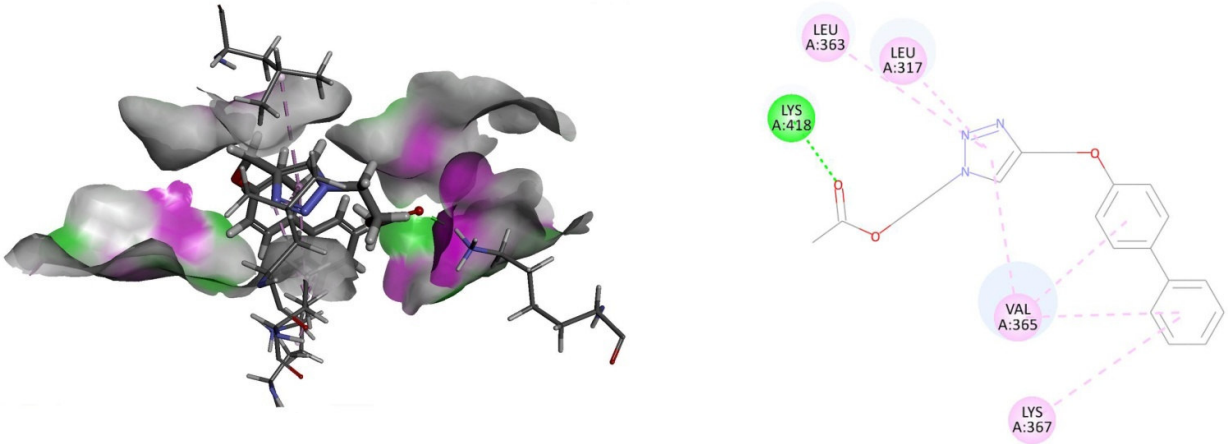
ALA A:229
VAL A:245
ALA A:244
PRO A:116
TRP A:230
PRO A:316

(c)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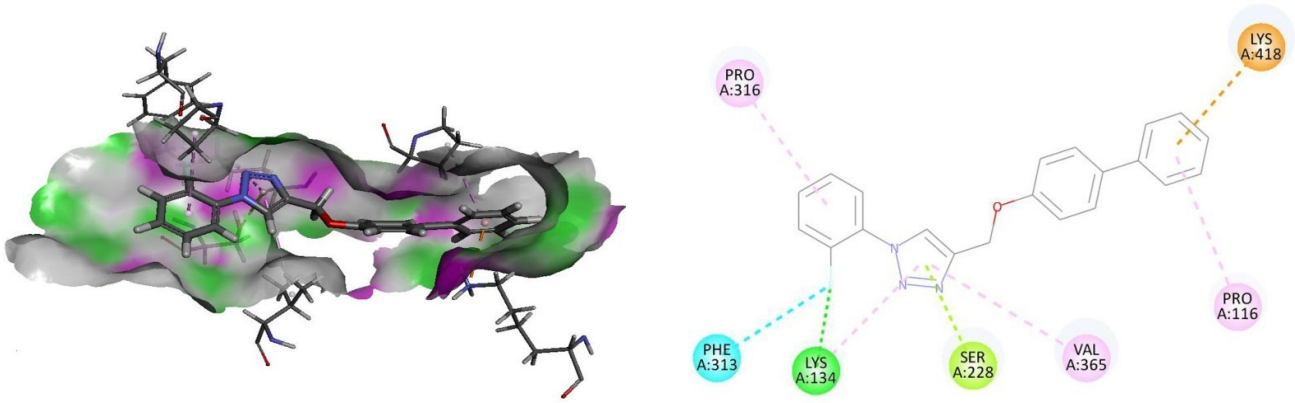
LEU A:317
LYS A:418
VAL A:365
LYS A:367

(d)



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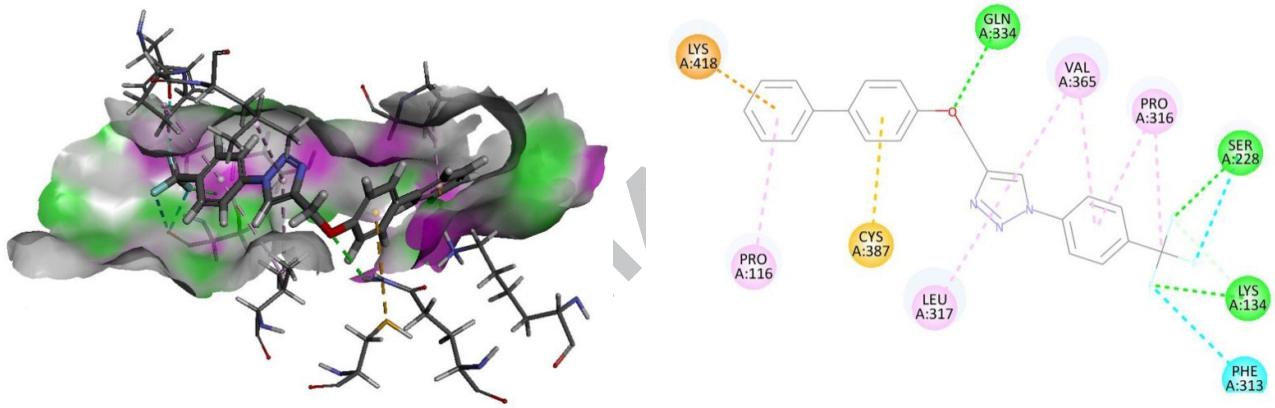

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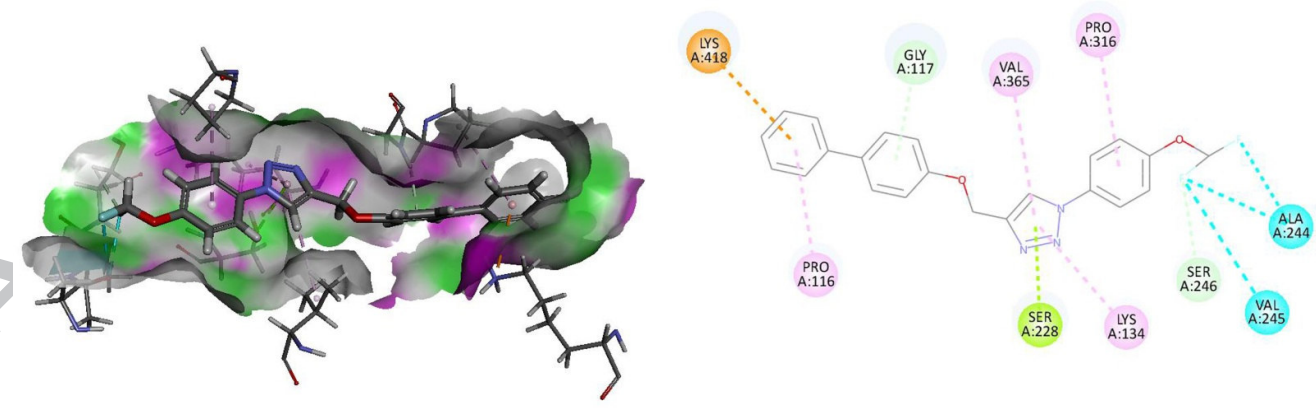

Figure 2. Binding modes of the compounds **4k** (a), **4l** (b), **4m** (c), **4o** (d), **4p** (e) and **4q** (f) with the active sites of DprE1 from docking results.

Herein, the CDOCKER Energy scores were estimated for all the six molecules and compared with Y22. The Energy scores were computed in the range of 7.807 kcal/mol to 20.963 kcal/mol, the Y22 was found to have highest energy score of 22.940 kcal/mol indicating the importance of theses six hits as potential DprE1 inhibitors (**Table s1**, supporting information). All the compounds were found to have optimum binding orientation with the catalytically important amino acids such as Lys418 and Val365. The compound **4o** was found to have molecular interactions with Pro316, Lys418, Pro116, Val365, Ser228, Lys134, Phe313 and indicating its novelty as novel *M. tuberculosis* DprE1 inhibitor. Compounds **4k**, **4l**, **4m**, **4p** and **4q** are also found to be suitable inhibitors for *M. tuberculosis* DprE1. The overall computational results of this investigation well established the novelty of the series of 1,4-disubstituted 1,2,3-triazole derivatives as potential *M. tuberculosis* DprE1 inhibitors.

In summary, we demonstrated the synthesis of a series of novel 1,4-disubstituted 1,2,3-triazole analogous derived from 4-phenyl phenol followed by their in vitro anti-TB activities. The simplicity, readily obtainable reactants and reagents, and reasonably good yields (80–96%) make this synthetic method more attractive and efficient. In addition, the synthesized compounds emerged as most promising antimycobacterial agents against *M. tuberculosis* H37Ra (ATCC 25177 strain) with negligible cytotoxicity. Among all the 17 synthesized compounds, six compounds displayed MIC values below 6.25 μg/ mL, better or comparable activity to the first line anti-TB drugs. Moreover molecular docking studies of these derivatives disclosed a high binding affinity into the active site of DprE1 (Decaprenylphosphoryl-β-D-ribose-2′-epimerase) enzyme. These findings suggest that the newly designed compounds may be considered as potential targets for anti-tubarcular drug discovery.

Acknowledgement

This work was supported by the Council of Scientific and Industrial Research (CSIR), New Delhi, India with research grant No. 02(0154)/13/EMR-II, respectively. A.A.A thanks CSIR, New Delhi for Senior Research Fellowship. We are grateful to the Department of Science and Technology for financial assistance under DST-FIST programme and UGC, New Delhi for Special Assistance Programme (UGC-SAP) to the Department of Chemistry, Dibrugarh University.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/

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