Original Article

Computational Approach for Locating Effective Cyanobacterial Compounds against *Mycobacterium Tuberculosis*

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ABSTRACT

Introduction: *Mycobacterium tuberculosis* has been a grievous pathogen causing staggering infections worldwide; especially its recently drug resistant strains are intractable. The MurA ligase of cell-wall peptidoglycan pathway is the suitable target often used for drug development. **Methods**: A homology model of MurA enzyme of *M. tuberculosis* was generated and validated by a Ramachandran plot for use in molecular docking studies with 13 cyano-compounds, along with 4 first-line anti-tuberculosis drugs, isoniazid, pyrazinamide, ethambutol and rifampicin. **Results**: Docking scores of two most effective cyano-compounds, pitipeptolides F and pitipeptolides D are -13.765 and -13.678 kcal/mol, respectively, whereas that of rifampicin is -9.173 kcal/mol. Computed LD_{50} values of the majority cyano-compounds were 200 mg/kg in mouse models, whereas that of isoniazid is 133 mg/kg. Most cyano-compounds, isoniazid and rifampicin are of the class III toxicity level or slightly toxic. Isoniazid has the highest LC_{50} value around 0.7 mmol against fathead minnow fish, but it is carcinogenic and mutagenic, as known from the computational prediction. **Conclusion**: Effective-most cyano-compounds, pitipeptolides F and pitipeptolides D could be used as alterative/ complementary agents against recently reported drug-resistant strains of *M. tuberculosis*.

Key words: Tuberculosis, MurA enzyme, Cyano-compounds, Antimycobacterial inhibitors, Computational toxicity study, Molecular docking.

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INTRODUCTION

A survey of World Health Organization (WHO) reveals that 9.2 million new tubercle bacillus (TB) or *Mycobacterium tuberculosis* (Mtb) infection cases are recorded globally, of which about 3 million infections from Africa, a 3 million from Southeast Asia and about a 2 million from Western Pacific region are reported. Furthermore, India and China are reported to have the largest total numbers of new cases, while South Africa had the highest rate of new cases.[1,2] Despite the use of the combination-chemotherapy with 4 first-line drugs, isoniazid, pyrazinamide, ethambutol and rifampicin, along with selective second-line drugs in the regular therapy and in the 'directly observed treatment short-course programme', mortality figure is consistently increasing in African and South Asian countries.[3] Furthermore, several second-line drugs, chemotherapeutics (ethionamide, thiacetazone, prothionamide and clofazimine), antibiotics (amikacin, kanamycin, debekacin, cycloserine, capreomycin and viomycin) are not well tolerated.[3] Moreover, multidrug resistant (MDR), resistance to at least isoniazid and rifampicin, extensively drug-resistant (XDR) and extremely drug-resistant (XXDR)

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and ghoulish totally drug-resistant (TDR) tuberculosis cases described as resistant to resistant 'all first-line drugs and second-line drugs' TB strains have emerged, rendering the present module of chemotherapy ineffective at several areas.[4-6] Mainly, Mtb developed resistance to pyrazinamide with alteration(s) in cell wall synthesis.[7,8] In this scenario, the development of a novel well-tolerated and emulating anti-mycobacterial agent, for the control of MDR, XDR or TDR Mtb, is a dire necessity.

Peptidoglycan layer is the major component of Mtb bacterial cell wall providing shape and rigidity, as in eubacteria. It is a homogeneous polymer consisting of sugar and amino acid polymers, N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), cross-linked by trans-peptide bridges, lying exterior to the plasma membrane. The biosynthesis of peptidoglycan layer is catalysed by enzymes, GlmS, GlmM, GlmU, MurA, MurB, MurC, MurD, MurE and MurF, at several coordinated cytoplasmic and periplasmicsteps (Table 1).[9,10] In the cytoplasmic step, the synthesis of UDP-MurNAc from UDP-GlcNAc is mediated by MurA ligase (UDP-N-acetylglucosamine 1-carboxyvinyl transferase), which was used as a suitable target for the drug development attempt.[9-12] Previously for drug development, to address drug resistant strains of the Gram-positive bacterium, *Enterococcus faecalis* as well as, of *M. leprae,* Mur ligases were shown as target enzymes in molecular docking attempts.[13,14] Obviously, an *in silico* computation would help locating a suitable control agent without the hit-and-miss method, which is often followed in drug targeting attempts; *in vivo* attempts would follow by apothecary, after being suitably indicated by computational work. Since, no molecular docking work using any Mur ligase is reported for drug targeting Mtb, this work describes screening of 13 cyano-compounds as possible antimycobacterial agents. As it is, cyanobacteria constitute a unique group of photosynthetic prokaryotes growing in soil, inland and marine waters. Several bioactive cyano-compounds have been isolated and characterized, which could be used in drug discovery, for example, against cancer.[15] Here only 13 cyano-compounds were selected in docking attempts against Mtb MurA enzyme from earlier reports on antimycobacterial activity.[16-18]

Nuclear magnetic resonance (NMR) or X-ray crystallography structure of Mtb MurA enzyme is not yet reported and that being unavailable in Protein Data Bank (PDB), in this work a homology model of Mtb MurA is generated and validated by a Ramachandran plot. It was further used as the drug-target in docking study with cyano-compounds, in comparison to 4 first-line anti-tuberculosis drugs and antibiotic, isoniazid, pyrazinamide, ethambutol and rifampicin, individually. Toxicity study is an essential corollary, before the pre-clinical stage of a recommended chemical, which is predicted suitable as drugable agent computationally. It is anticipated that the most effective cyano-compound determined herein could be used as new anti-tuberculosis drug.

MATERIALS AND METHODS

Homology modeling of *M. tuberculosis* MurA ligase

The amino acid sequence of Mtb MurA ligase was retrieved from the UniProtKB database (http://www.uniprot.org/). The 3-Dstructure of MurA ligase was modelled using the MurA of the bacterium, *Listeria monocytogenes* as the suitable template, with the help of tools, BLASTp (http://blast.ncbi.nlm.nih.gov/Blast), Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2), CS-BLAST (http://toolkit.tuebingen.mpg.de/cs_blast), I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/)

Table 1: Family of muramidase (Mur) ligases involved in peptidoglycan synthesis of *Mycobacterium tuberculosis*.

Gene	Function	EC no.
MurA	UDP-*N*-acetylglucosamine 1-carboxyvinyltranferase	2.5.1.7
MurB	UDP-*N*-acetylmuramate dehydrogenase	1.1.1.158
MurC	UDP-*N*-acetylmuramate-alanine ligase	6.3.2.8
MurD	UDP-*N*-acetylmuramylalanine-D-glutamate ligase	6.3.2.9
MurE	UDP-*N*-acetylmuramyl-tripeptide synthetase	6.3.2.13
MurF	UDP-MurNac-pentapeptide sythetase	6.3.2.15
MurG	UDP-*N*-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphosphpryl-decaprenol-N-acetylglucosamine transferase	2.4.1.227
MurI	Glutamate recemase	5.1.1.3

EC no., Enzyme commission number

and RaptorX (http://raptorx.uchicago.edu/).The secondary structure and protein sequence analyses of Mtb MurA were predicted by tools, PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/), SOPMA tool (http://nhjy.hzau.edu.cn/) and ProtParam (http://web.expasy.org/protparam/).

Model validation

The homology model of Mtb MurA ligase was generated by the MODELLER 9.14 software (academic version; http//:www.salilab.org/modeler.[19] Based on the lowest value of normalized discrete optimized protein energy (DOPE) of 5 generated homology models, the suitable 3-D model was selected and validated using the tool, Structure Analysis and Verification Server (SAVES) (http://services.mbi.ucla.edu/) with programmes, PROCHECK, VERIFY3D, ERRAT and PROVE, to verify bond length values, dihedral and torsion angles attained a stable configuration, by generating a Ramachandran plot. Moreover, Ramachandran plot analysis (http://mordred.bioc.cam.ac.uk/), WHAT IF (http://swift.cmbi.ru.nl/), MolProbity (http://molprobity.biochem.duke.edu/) and ProSA-web (https://prosa.services.came.sbg.ac.at/) were too used for checking the reliability of the generated homology model of MurA ligase. Furthermore, the model was subjected to energy minimization by the Swiss-Pdb Viewer (http://spdbv.vital-it.ch/) software, for maintaining its stability before a docking attempt.

Molecular docking

In molecular docking, the possibility of blocking the target protein/receptor, involved in a particular disease by a ligand in inhibiting its active site(s), is assessed. A ligand gets attached onto the larger molecule, the receptor in a specific orientation with generation of an energy value, the docking score (kcal/mol.), specific for the particular protein-ligand complex. The most effective ligand is selected from the minimum docking score of a complex, among several ligands and similar receptor complexes.

Structures of 13 cyano-compounds and 4 on-going anti-tuberculosis drugs were retrieved from the chemical database, PubChem (https://pubchem.ncbi.nlm.nih.gov/search/ search.cgi) and the DrugBank (http://www.drugbank.ca/), respectively, for docking study. The retrieved structures of compounds/ drugs were saved into dot mol or '.mol' and dot pdb or '.pdb' file formats for use in molecular docking, which were done by using AutoDock Vina and PatchDock softwares, as reported.[20,21] Docking studies with receptor enzyme MurA and individual ligands as cyano-compounds and 4 first-line anti-tuberculosis drugs were attempted. PyMOL (https://www.pymol.org/) and LigPlot (http://www.ebi.ac.uk/thornton-srv/software/ LIGPLOT/#) softwares were used to visualize the generated 3-D model of MurA ligase and its interactions with ligands in docking study.

In silico toxicity prediction

The *in silico* toxicity prediction remains an advanced approach to rationalize the process of preclinical drug development, by reducing the associated time and costs on animal experiments. Here, the ProTox (http://tox.charite.de/tox) web server was used in evaluating LD_{50} values of oral toxicity and the toxicity class of the used cyano-compounds. Additionally, the chemoinformatic tool LAZAR (Lazy Structure-Activity Relationships) (http://lazar.in-silico.de/predict) was used for assessing carcinogenic and mutagenic natures, along with assessing possible LC_{50} value and the daily recommended dose of each cyano-compound used herein and anti-tuberculosis drugs. LAZAR provides 85 % mutagenicity and carcinogenicity up to 95% predictions for a query compound. Moreover, recommended daily dose of a query compound, herein a cyano-compound is calculated by comparison with data of available training set or known drugs approved by Food and drug administration.

RESULTS

Homology modeling of *M. tuberculosis* MurA ligase

After retrieval of target sequence of Mtb MurA ligase, 6 bioinformatics tools were used to find out the suitable template for modeling a 3-D structure. Based on the higher level of sequence similarity and identity, the structure of *L. monocytogenes* MurA ligase was selected as the suitable template with 48 % identity long with query coverage at 99 % for the homology modeling. The template modeling or TM score of MurA was 0.97 obtained through the TM-align programme of I-TASSER. The TM score was seen lying between 0.0 and 1.0 (and is > 0.5), which signifies that the MurA structure and selected template were in the same fold. The secondary structure of MurA ligase was predicted as 38.52 % helix, 19.62 % extended strand, 9.57 % beta-turn and 32.30 % random coil, by the SOPMA tool, which supported the stability of the protein due to the presence of indicated high percentage of helix. The generated homology model as 3-D structure of MurA ligaseof *M. tuberculosis* with helix, sheet and loop regions in three different colors is presented by PyMOL software (Figure 1).The predicted molecular weight of MurA is 44063.5 kd, theoretical isoelectric point is

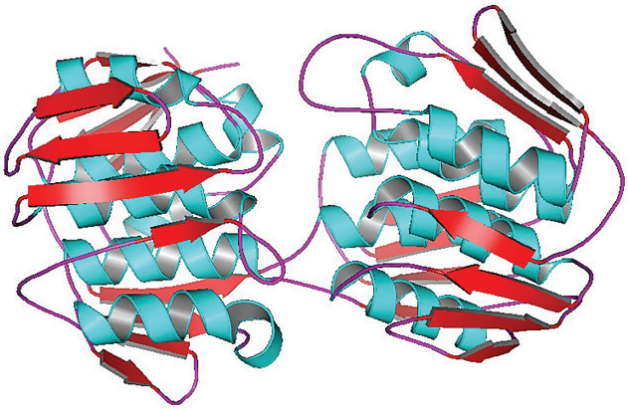


Figure 1: Generated homology model as 3-D structure of MurA ligaseof *M. tuberculosis* by PyMOL visualizer software. In this structure, sky blue color stretches are helix regions, red stretches indicate sheets and violet pink stretches indicate loops.

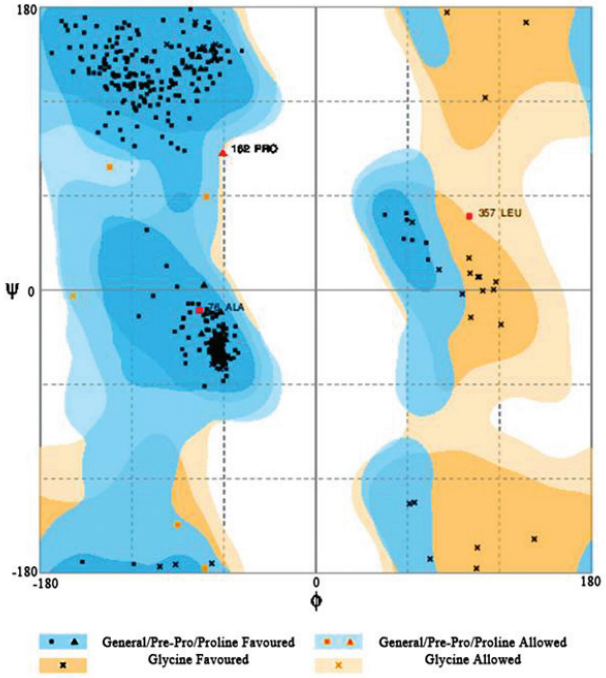


Figure 2: Ramachandran plot statistics of the generated model of Mtb MurA enzyme. The plot was calculated with the Ramachandran plot analysis program, where 98.1 % amino acids are in favoured region 1.2 % amino acids are in allowed region and 0.7 % amino acids are in outlier region.

5.30 and the instability index is 28.49 (<40). As a result, instability index value and a higher proportion of the peptide as helix indicated that the protein is more stable in nature, determined by the ProtParam tool. In total numbers of negative charged residues are 49 % and the positive charged residues are 34 %, which indicated the MurA enzyme as acidic in nature.

Model validation

The Ramachandran plot statistics indicated that, 98.1 % residues were in the most favored region, 1.2 % residues were in allowed region and only 0.7 % residues were in the disallowed region (Figure 2). Thus, the generated model of MurA was geometrically and stereo-chemically acceptable. Further, knowledge-based energy curves were generated for the target with Z-score values (MurA Z-score: -9.95), and for the template (3R38 Z-score: -10.92) by using ProSA-web (Supplementary Figure 1). The Z-score and energy curve also indicated that the MurA model was within the Z-score range of experimentally determined NMR solved protein structure; and the Z-score of template was less different to the modeled MurA structure; ProSA-web confirmed the quality and consistency of the model that is acceptable. Data regarding the validation of the generated MurA model was validated.

Molecular docking

Thirteen cyano-compounds and 4 anti-tuberculosis drugs were docked individually against generated target, the Mtb MurA enzyme. Effective docking score values of 4 anti-tuberculosis drugs are as given against each (kcal/mol), -9.173 (rifampicin) > -7.865 (ethambutol) > -7.568 (isoniazid) > -6.981 (pyrazinamide) (Table 2). Among cyano-compounds, pitipeptolides F with docking score value, -13.765 was the most effective cyano-compound against Mtb MurA enzyme. Effective docking score values of 13 cyano-compounds are as given against each (kcal/mol): -13.765 (pitipeptolides F)

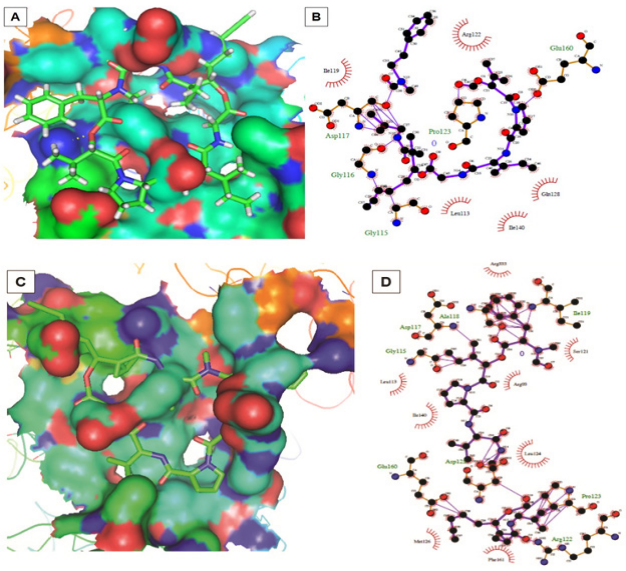


Figure 3: Interaction of the target Mtb MurA ligase with two effective cyano-compounds in docking attempts, visualized by softwares PyMOL and LigPlot. A and B represent two surface interaction-views of pitipeptolides D. And C and D represent surface interaction-views of MurA ligase with pitipeptolides F.

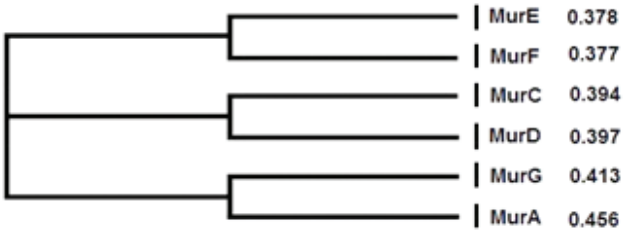


Figure 4: Phylogenetic tree of MurA, MurC, MurD, MurE, MurF and MurG of *M. tuberculosis* ATCC 25618/H37Rv.

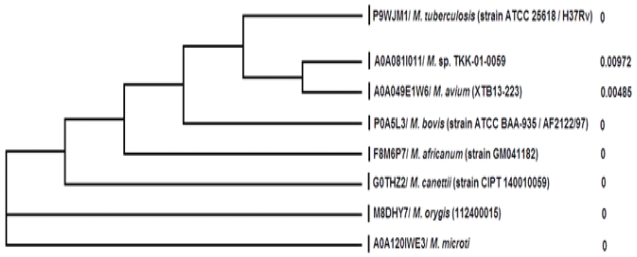
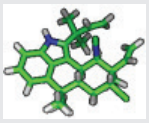
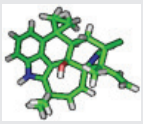
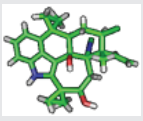
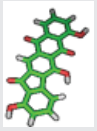
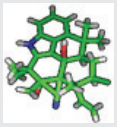
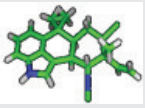
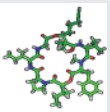
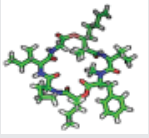
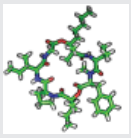
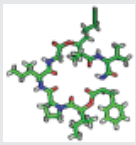
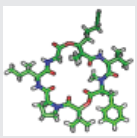
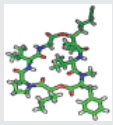
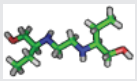
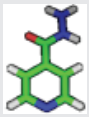
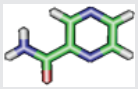
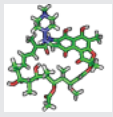


Figure 5: Phylogenetic tree was constructed using MurA enzyme of 8 species of *Mycobacterium*.

Table 2: Three-D structures and relevant information of antimycobacterial cyano-compounds and their docking scores (kcal/mol) against MurA.

Cyano-compound; chemical class	Source organisms; Reference	3-D structure	Information	Docking score
Ambiguine A isonitrile; indole *alkaloid*	*Fischerella ambigua*; [22]		Compound ID:21610112 MW:406.99074g/mol MF:$C_{26}H_{31}ClN_2$ H-bd:1 H-ba:1	-9.193
Ambiguine K isonitrile; indole *alkaloid*	*Fischerella ambigua*; [16]		Compound ID: 44139368 MW: 420.97426 g/mol MF: $C_{26}H_{29}ClN_2O$ H-bd:2 H-ba:2	-9.079
Ambiguine M isonitrile; indole *alkaloid*	*Fischerella ambigua*; [16]		Compound ID: 44139369 MW: 438.98954g/mol MF: $C_{26}H_{31}ClN_2O_2$ H-bd:3 H-ba:3	-9.377
Eucapsitrione; anthraquinone derivative	*Eucapsis* sp. and *Fischerella ambigua*; [17]		Compound ID: 46919489 MW: 358.3005 g/mol MF: $C_{21}H_{10}O_6$ H-bd:3 H-ba:6	-10.361
Fischambiguine B; indole *alkaloid*	*Fischerella ambigua*; [22]		Compound ID: 71452317 MW: 436.97366g/mol MF: $C_{26}H_{29}ClN_2O_2$ H-bd: 2 H-ba: 3	-9.625
Hapalindole A; alkaloid;	*Hapalosiphon fontinalis* and *Fischerella* sp.; [23]		PubChem ID:185159 MF:$C_{21}H_{23}ClN_2$ MW:338.87372 [g/mol] H-bd:1 H-ba:1	-9.891
Pitipeptolides A; cyclic depsipeptide	*Lyngbya majuscula*; [24]		Compound ID: 11803484 MW: 808.015 [g/mol] MF: $C_{44}H_{65}N_5O_9$ H-bd: 3 H-ba: 9	-12.672
Pitipeptolides B; cyclic depsipeptide	*Lyngbya majuscule*; [24]		Compound ID: 10819182 MW: 810.03088 [g/mol] MF: $C_{44}H_{67}N_5O_9$ H-bd: 3 H-ba: 9	-12.175

Continued...

Table 2: Cont'd				
Pitipeptolides C; cyclic depsipeptide	*Lyngbya majuscule*; [18]		Compound ID: 15479259 MW: 812.04676 [g/mol] MF: $C_{44}H_{69}N_5O_9$ H-bd: 3 H-ba: 9	-12.988
Pitipeptolides D; cyclic depsipeptide	*Lyngbya majuscule*; [18]		Compound ID: 54597385 MW: 793.98842 [g/mol] MF: $C_{43}H_{63}N_5O_9$ H-bd: 4 H-ba: 9	-13.678
Pitipeptolides E; cyclic depsipeptide	*Lyngbya majuscule*; [18]		Compound ID: 54597438 MW: 793.98842 [g/mol] MF: $C_{43}H_{63}N_5O_9$ H-bd: 3 H-ba: 9	-13.152
Pitipeptolides F; cyclic depsipeptide	*Lyngbya majuscule*; [18]		Compound ID: 54597439 MW: 793.98842 [g/mol] MF: $C_{43}H_{63}N_5O_9$ H-bd: 3 H-ba: 9	-13.765
Scytoscalarol; terterpene	*Scytonema* sp.; [22]		Compound ID: 49775750 MW: 415.655 [g/mol] MF: $C_{26}H_{45}N_3O$ H-bd: 3 H-ba: 2	-11.710
Ethambutol*	-		DrugBank ID: DB00330 MW: 204.30976 [g/mol] MF: $C_{10}H_{24}N_2O_2$ H-bd:4 H-ba: 4	-7.065
Isoniazid*	-		DrugBank ID: DB00951 MW: 137.13928 [g/mol] MF: $C_6H_7N_3O$ H-bd: 2 H-ba: 3	-7.568
Pyrazinamide*	-		DrugBank ID: DB00339 MW: 123.1127 [g/mol] MF: $C_5H_5N_3O$ H-bd: 1 H-ba: 3	-6.981
Rifampicin*	-		DrugBank ID: DB01045 MW: 822.94022 [g/mol] MF: $C_{43}H_{58}N_4O_{12}$ H-bd: 6 H-ba: 15	-9.173

*, antibiotics, H-ba, hydrogen-bond acceptor; H-bd, hydrogen-bond donor; MF, molecular formula; MW, molecular weight.

> -13.678 (pitipeptolides D) > -13.152 (pitipeptolides E) > -12.988 (pitipeptolides C) > -12.672 (pitipeptolides A) > -12.175 (pitipeptolides B) > -11.710 (scytoscalarol) > -10.361 (eucapsitrione) > -9.891 (hapalindole A) > -9.625 (fischambiguine B) > -9.377 (ambiguine M isonitrile) > -9.193 (ambiguine A isonitrile) > -9.079 (ambiguine K isonitrile) (Table 3). Moreover, all used cyano-compounds had the higher docking score values in comparison to those of TB-drugs used. Interactions of cyano-compounds, pitipeptolides F and pitipeptolides D with Mtb MurA during docking are elucidated (Figure 3).

In silico toxicity prediction

According to ProTox web server, the decreasing order of LD_{50} values (mg/kg) are cited against each compound: rifampicin (3222), pyrazinamide (1800), scytoscalarol (1600), ethambutol (998), eucapsitrione (385), six pitipeptolides A, B, C, D, E, F (200, each), isoniazid (133), ambiguine M isonitrile (136), and fischambiguine B (136), ambiguine A isonitrile (110) and hapalindole A

Table 3: *In silico* toxicity assessment of cyano-compounds along with those of anti-TB drugs as references, with maximum FDA recommended daily dose (mmol).

Cyano-compound/ Anti-TB drug	Fish toxicity LC_{50}	Mouse carcinogenicity; mutagenicity	LD_{50}; toxicity class	Recommended daily dose
Ambiguine A isonitrile	0.0034082947466186	Non-carcinogen; non-mutagenic	110; III	0.00174816485794716
Ambiguine K isonitrile	0.00154367939121187	Non-carcinogen; non-mutagenic	60; III	0.00227899315840001
Ambiguine M isonitrile	0.00738241180993074	Non-carcinogen; non-mutagenic	136; III	0.00594116677457886
Eucapsitrione	Not predicted	Non-carcinogen; mutagenic	385; IV	Not predicted
Fischambiguine B	0.00699000000000007	Non-carcinogen; non-mutagenic	136; III	0.00591692988293637
Hapalindole A	0.00248004404055083	Non-carcinogen; non-mutagenic	110; III	0.00178878082978523
Pitipeptolides A	0.0231924977492949	Non-carcinogen; non-mutagenic	200; III	0.00236752726254867
Pitipeptolides B	0.0183395882632407	Non-carcinogen; non-mutagenic	200; III	0.00236752726254867
Pitipeptolides C	0.0242958427200449	Non-carcinogen; non-mutagenic	200; III	0.00196920696078117
Pitipeptolides D	0.0163871000518421	Non-carcinogen; non-mutagenic	200; III	0.00211958401295321
Pitipeptolides E	0.0231924977492949	Non-carcinogen; non-mutagenic	200; III	0.00174822126733245
Pitipeptolides F	0.020663724682756	Non-carcinogen; non-mutagenic	200; III	0.00224508680995582
Scytoscalarol	Not predicted	Carcinogen; non-mutagenic	1600; IV	0.00811053697038394
Ethambutol	Not predicted	Non-carcinogen; non-mutagenic	998; IV	0.0490197349457053
Isoniazid	0.700645136095585	Carcinogen; mutagenic	133; III	-1.1371618461445
Pyrazinamide	Not predicted	Non-carcinogen; non-mutagenic	1800; IV	-0.613181576657421
Rifampicin	0.00552411213689556	Non-carcinogen; Not predicted	3322; V	0.012826812250905

Note: FDA, Food and drug administration; Toxicity (mg/kg) class I: fatal if swallowed, $LD_{50} \leq 5$; and likewise, class II, fatal: $5 \leq 50$; class III, toxic: $50 \leq 300$; class IV, harmful: $300 \leq 2000$; class V, may be harmful: $2000 \leq 5000$; and class VI, non-toxic: > 5000.

(110) and ambiguine A isonitrile (60) (Table 3; Suppl. Fig. 2). Among cyano-compounds and 4 anti-TB drugs, ambiguine A isonitrile, ambiguine K isonitrile, ambiguine M isonitrile, fischambiguine B, hapalindole A, pitipeptolides A, pitipeptolides B, pitipeptolides C, pitipeptolides D, pitipeptolides E, pitipeptolides F and isoniazid are in class III of toxicity compounds (LD_{50} value with $50 \leq 300$ mg/kg, and increase in the toxicity class is marked by decreased toxic effects); and eucapsitrione, scytoscalarol, ethambutol and pyrazinamide are in class IV of toxicity compounds (LD_{50} would be $300 \leq 2000$ mg/kg in this class); and only rifampicin is in class V toxicity compound (LD_{50} would be $2000 \leq 5000$ mg/kg in this class) (Table 3). Moreover, isoniazid and 11 cyano-compounds would be in class III toxic compound, but none of the later is carcinogenic. Thus, in comparison to isoniazid, LD_{50} values of most cyano-compounds are less toxic; and thus those could be promoted as drugs due to lesser levels of toxicity (Table 3). According to LAZAR tool,used13 cyano-compounds and the rest of the 3 anti-tuberculosis drugs are arranged according to fish toxicity (in decreasing order of LC_{50} value): pitipeptolides C, pitipeptolides A, pitipeptolides E, pitipeptolides F, pitipeptolides B, pitipeptolides D,ambiguine M isonitrile, fischambiguine B, rifampicin, ambiguine A isonitrile, hapalindole A, and ambiguine K isonitrile (Table 3, Supplementary Figure 3). And the rest 4 compounds, eucapsitrione, scytoscalarol,

ethambutol and pyrazinamide were not predicted any bio-toxicity by the toxicity analyzer tool, LAZAR. Among 13 cyano-compounds, eucapsitrione is mutagenic and scytoscalarol is carcinogenic in nature. Here, isoniazid has the highest LC_{50} value around 0.7 mmol against fathead minnow fish in comparison to other anti-TB drugs and the used cyano-compounds, but it has carcinogenic and mutagenic activity as indicated by the computational prediction (Table 3). Moreover, cited cyano-compounds could be promoted as new anti-TB agents based on findings of molecular docking, LD_{50}, LC_{50} and toxicity class level.

DISCUSSION

Using computational tools, effective-most cyano-compounds, pitipeptolides F and pitipeptolides D were selected and used as alterative/ complementary agents against recently reported drug-resistant strains of *M. tuberculosis*. Computation biology is a system of advanced tools by which, a putative drug target is identified, rather predicted against the 3-D structure of a key target protein of a disease, which may be even generated by homology modeling, as here done for Mtb MurA, using the *L. monocytogenes* MurA structure as the template. Indeed in molecular docking, the target enzyme is blocked by the ligand that would be the probable future drug. Indeed, cyano-compounds from the archaic bacterial group are known to be unique compounds with several therapeutic activities — antibacterial,[16,25] antifungal[26,27] and anticancer efficacies.[15,28] This work computationally describes that two cyano-compounds, pitipeptolides F and D as the most effective antimycobacterial agents, in comparison to the presently used individual first-line anti-TB drugs. The *in silico* attempt of prediction of bio-activity of cyano-compounds included toxicity predictions with fish and mouse models herein. Moreover, isoniazid has been experimentally proved as carcinogenic *in vivo* mouse model too,[29] corroborating the *in silico* prediction presented here.

During a drug development cascade for a disease, screening of a large number of characterized lead compounds are done in pharmacology and medicinal chemistry. But a compound to be designated as drug has to pass through the first stage of pre-clinical trial to four subsequent stages of clinical trials. As it is known, drug candidates entering clinical trials have been estimated having an 8 % chance of becoming marketed drugs with about a 20 % chance of failure due to high levels of host toxicity.[30] Blithely, modern computational predictions on drugable compounds have advantages in the drug development cascades, of saving the time in the *in vitro* and tiresome *in vivo* experiments, which are mandatory for the computationally recommended specific chemical intended as a future drug; nevertheless, animal trials are the essential corollary work in drug development.

CONCLUSION

The putative drug target, MurA enzyme of Mtb was generated by homology modeling and Ramachandran plot statistics proved that the generated MurA model was geometrically and stereo chemically acceptable. Molecular docking attempts could help locating comparatively more effective agents against the target enzyme MurA than the presently used first-line drugs and antibiotics in TB-chemotherapy. Indeed, cyanobacteria possess unique secondary metabolites with antimycobacterial potency evaluated by docking studies, and the indicated compounds are less toxic than isoniazid. Both molecular docking and toxicity studies indicated that two cyano-compounds, pitipeptolides F and D could be promoted as the effective agents against *M. tuberculosis*.

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Conflict of Interest

The authors have declared no conflict of interest.

ABBREVIATION USED

DOPE: discrete optimized protein energy; **LAZAR:** Lazy Structure-Activity Relationships**; MDR:** multidrug resistant; **Mtb:** *Mycobacterium tuberculosis*; **NMR:** nuclear magnetic resonance; **SAVES:** structure analysis and verification server; **TB:** tubercle bacillus; **TDR:** Totally drug-resistant; **WHO:** World Health Organization; **XDR:** extensively drug-resistant; **XXDR:** extremely drug-resistant.

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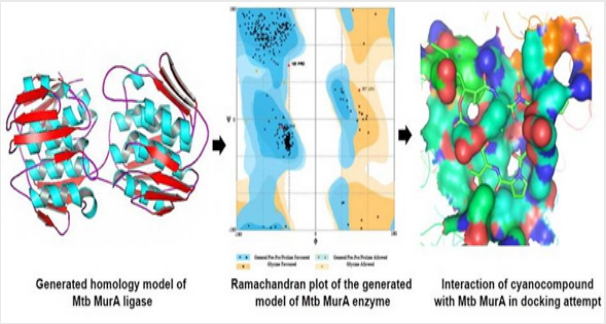
PICTORIAL ABSTRACT



SUMMARY

- *Mycobacterium tuberculosis* causes staggering infections worldwide; particularly its recently drug resistant strains are intractable. Locating or developing a newer controlling antimycobacterials is call of the day.
- The MurA ligase of cell-wall peptidoglycan pathway is the suitable target often used for drug development, and a homology model of MurA enzyme of *M. tuberculosis* was generated and validated by a Ramachandran plot.
- Molecular docking studies with 13 cyano-compounds, along with 4 first-line anti-tuberculosis drugs, isoniazid, pyrazinamide, ethambutol and rifampicin were done for locating effective antimycobacterials.
- Toxicity study is an essential corollary, before the pre-clinical stage of a recommended chemical as a future, which is predicted using tools, ProTox and LAZAR, computationally; both cyano-compounds, pitipeptolides F and pitipeptolides D could be taken as suitable as prospective antimycobacterial agents.

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