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In Silico Identification of Drug-Like Inhibitors against *Mtb*-DHDPS: A Shape-Based Approach

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Abstract

Tuberculosis (TB) is now a curable malady, but still million of the people suffer and lots of them dies every year. It means that new and potent drug molecules are required to prevail over multidrug resistant. Towards this direction, four different shape-based strategies (e.g electroshape, spectrophores, shape-IT, and align-IT) were employed to identify novel drug-like inhibitors against dihydodipicolinate synthase (DHDPS) of *Mycobacterium tuberculosis*. The best pose of template mplecule (PUB475318) was used to perform ligand-based search against various libraries of small chemical molecules (e.g., DrugBank, Ligand Expo, ChEMBL, ChEBI, GLASS, HMDB, and ZINC database) that predicted 329 virtual hits. These data sets were further shortened to 295 pro-lead molecules through filtration of Lipinski rule of five. Further, ADMET analysis was carried out that depicted 25 plausible hits following all the criteria of pharmacokinetic analysis that was subsequently reduced to 9 consistent hits after multi-scoring molecular interaction analysis using Biopredicta, MVD, and ADT. Hereafter, 2 out of 9 hits (DB01118 and DB00749) were succeeded as potent pro-lead molecules after comparison of known inhibitors (PUB475318 and CID10367). Moreover, DB01118 was depicted as the best lead molecule after post analysis of MD simulation study of 10 ns.

Keywords: *Mycobacterium tuberculosis*, DHDPS, Docking, Biopredicta, Shape-based approach, MD simulation.

INTRODUCTION

Dihydrodipicolinate synthase (DHDPS) is a key enzyme for the biosynthesis of essential amino acids and several important metabolites in microbes. In the aspartate biosynthetic pathway, numerous important metabolites are synthesized such as diaminopimelate (DAP), S-adenosylmethionine, and Dipicolinate. These metabolites play fundamental roles in essential developmental processes such as bacterial cell wall biosynthesis and virulence factor production. The dipicolinate is a major element of sporulation in gram-positive bacteria (Ragkousi *et al*., 2003) and DAP is necessary for cross-linking of the peptidoglycan polymers in bacterial cell wall synthesis (Van Heijenoort, 2001). The computational approaches such as virtual screening have been successfully used as an efficient alternative to high throughput screening approaches for the discovery and development of new compounds (Kumar *et al*., 2015). In the present scenario, a shape-based screening is considered as one of the important and well-known approaches in the field of CADD. Shape-priority docking method based is also introduced recently, which highlights the importance of shape-based analysis of drug molecules. This approach utilizes the concept of shape and electrostatic potential similarity to select the molecule which may show similar binding mode into the active site (Kirchmair *et al*., 2009). This approach plays a role in the identification of novel inhibitors with high potency (Temml *et al*., 2014; Kumar *et al*., 2013). It can also be used as an alternative for the optimization of more selective and potent new antimicrobial compounds instead of synthesizing new inhibitors.

Inhibition of DHDPS enzyme is a promising drug target strategy against *Mycobacterium tuberculosis* (*Mtb).* Numerous inhibitors against *Mtb*-DHDPS have been identified so for, but quest to find the best is still unexplored. Towards this direction a comparison between experimentally known and predicted inhibitor was made by (Garg *et al.*, 2010) through molecular dynamics simulation study. They proposed that PUB475318 is bestowed better inhibition potential as compared to the previously reported inhibitors of *Mtb*-DHDPS.

In this work, the best binding conformation of PUB475318 (Garg *et al*., 2010) was used for shape-based virtual screening using SwissSimilarity tool (http://www.swisssimilarity.ch/). The top rank hits were further subjected to ADME and toxicity filters. The final filter was based on molecular docking analysis. Each screened molecule carries the characteristics of the highly electronegative groups on both sides separated by an average distance of 6 Å. Finally, the best predicted 6 compounds exhibited minimum three H-bonding interactions with Arg99 and Arg249. Moreover, MD simulations were carried out on selected compound in order to check the stability of ligand and target complex. During the MD simulations, the compounds showed same H-bonding interactions and remained bound to key active residues. These identified hits could be useful for designing the more potent inhibitors against DHDPS family.

METHODOLOGY

Retrieval of protein 3D structure

The crystal structure (3D) of *Mtb*-DHDPS (PDB ID: 1XXX) was extracted from RCSB Protein Data Bank. The coordinates of the chloride ion, magnesium ion, 2, 3-dihydroxy-1, 4-dithiobutane (DDT), and water molecules were removed to prepare the protein for molecular docking. The protein was energetically minimized using the CHARMm force field.

Retrieval of ligands 3D structure

3D structures of ligands were retrieved from the PubChem database of NCBI. The structures of PUB475318-shape based similar compounds were extracted from the Swiss Similarity web tool for low to ultra high-throughput ligand-based virtual screening database. By applying CHARMm force, ligands were energetically minimized.

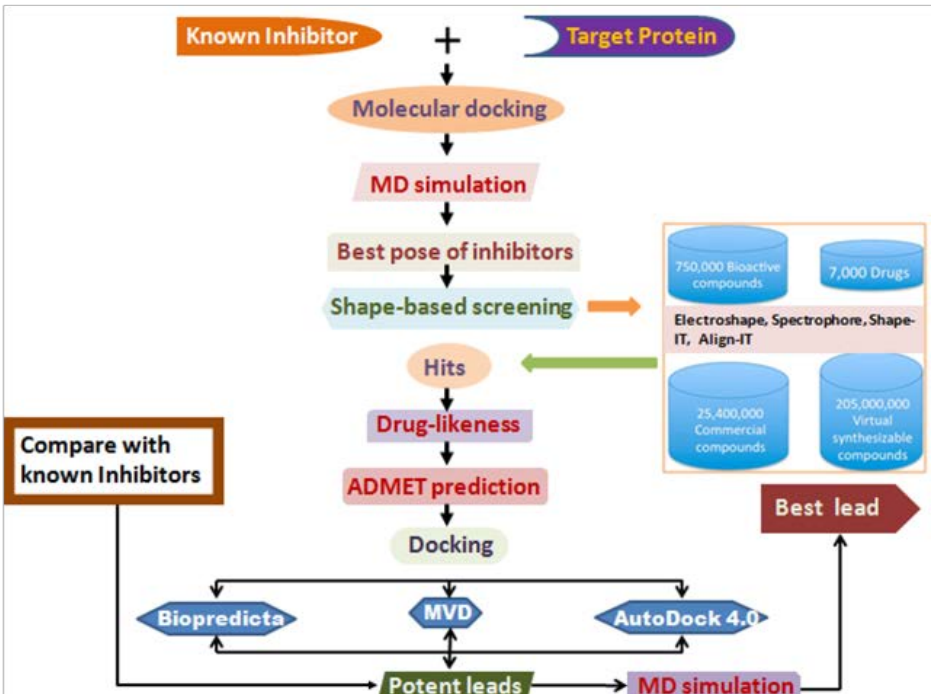

Figure 1. Flow chart of methodology.

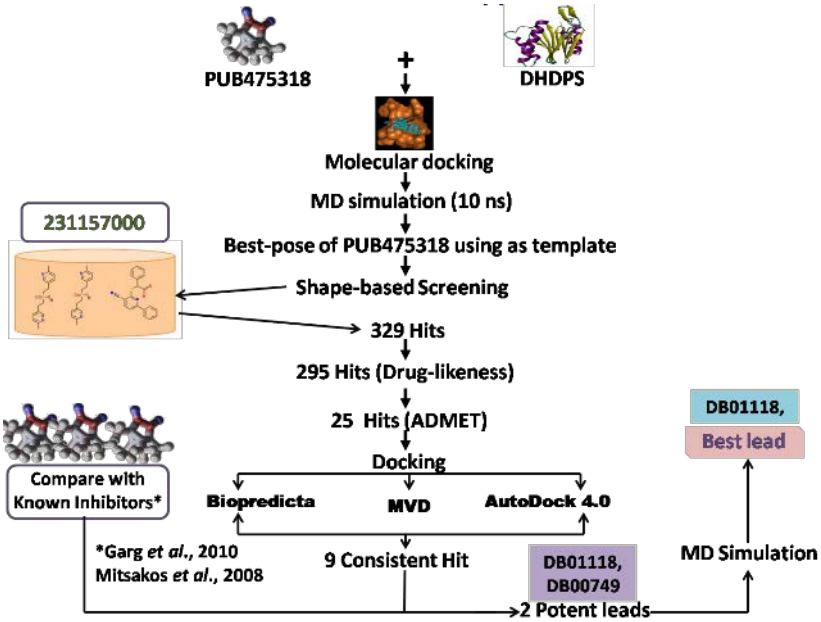

Figure 2. Flow chart of shape-based outcome.

Drug-likeness prediction

Lipinski rule of five (RO5) was employed to predict the drug likeness of ligands. RO5 includes molecular mass (< = 500 Dalton), high lipophilicity (Log p < = 5) H-bond donors (< = 5), H-bond acceptors (< = 10) and molar refractivity (40-130). These filtrations ensure drug-likeness for molecules obeying two or more features of RO5 (Hamzeh-Mivehroud *et al.*, 2016; El-Telbany *et al.*, 2017).

Docking simulations

BioPredicta tool of VlifeMDS package (Junaid *et al.* 2016), MVD (http://www.clcbio.com) and AutoDock Tools 4.0 were used for molecular interaction studies of ligands and protein.

BioPredicta

It employed Genetic algorithm (GA), Piecewise Linear Pairwise Potential (PLP) and Grid algorithms energy minimization by using MMFF force fields. The Dock scoring function was used to assess the binding efficacies of ligands. This scoring function takes into account the terms for van der Walls interaction, hydrophobic effects, hydrogen bonding and deformation penalty.

Molecular Virtual Docker

It integrates highly efficient PLP and MolDock scoring function for molecular docking. Docking parameters and other required parameters were set to default values (Shaheen *et al.* 2015). MolDock-rerank score was further employed to judge the binding affinity of ligands.

AutoDock Tools

Polar H-atoms, Kollman united atom and atom type parameters were added and further, non-polar H-atoms were merged during generation of the protein pdbqt file. During preparation of ligand pdbqt file, polar H-atoms added, non-polar H-atoms merged, number of torsions, and rotatable bonds were defined. Cubic volume of 40 × 40 × 40 Å 3 with 0.408 Å grid points spacing and X: 240.7, Y: 51.50, Z: 78.02 centre coordinates was set to cover the entire active site and accommodate ligand to move freely. Lamarckian genetic algorithm was employed for the receptor-fixed ligand-flexible docking calculations. The conformer having lowest free energy of binding (ΔG) was considered for further analysis Khan *et al*, 2011; Khan *et al*, 2013; Khan *et al*, 2015; Rehman *et al*, 2016; Khan *et al*, 2017; Sharma *et al*, 2019).

MD simulation

Molecular dynamics (MD) simulation of protein-ligand docked complex was performed using GROMACS 5.0.5 (Spoel *et al.,* 2005) software with amber99SB-ildn (Lindorff-Larsen *et al*., 2010) force field. Topology files for the ligand molecules were generated using antechamber program with GAFF force field (Wang *et al*., 2004). Protein-ligand complex was solvated in cubic box with TIP3P (Jorgensen *et al*., 1983) water model molecules as solvent. Periodic boundary conditions were used during MD simulation. Bond lengths were constrained using LINCS algorithm *(*Hess *et al*., 1997). Seven sodium ions (Na+) were added to neutralize the system. The particle mesh Ewald method was used for electrostatic calculations. Energy minimization of system was performed using steepest descent algorithm with tolerance value of 100 kJ mol^{-1} nm^{-1}. Energy minimization was followed by equilibration using NVT and NPT ensemble for 500 ps. Finally, 10 ns production MD was performed for the system, with trajectories generated every 2 femto second (fs), and snapshots saves every 2 pico second (ps) (Tripathi *et al*.,2015). Gromacs utility commands gmxrms, gmxrmsf, gmxsasa,gmx gyrate and gmxhbondwere used to analyze root mean square deviation (RMSD), root mean square fluctuations (RMSF), ssolvent accessible surface area (SASA), radius of gyration (Rg) and number of hydrogen bonds formed between protein and ligands, respectively. Plots were generated using GRACE plotting software (http://plasma-gate.weizmann.ac.il/Grace/). Figure was generated using PyMol.

Binding free energy for the protein-ligand docked complex was calculated using *g_mmpbsa*tool (Kumari *et al.,* 2014). Molecular mechanics Poission-Boltzmann surface area (MMPBSA) method was utilized to calculate the binding free energy between protein and ligand. MMPBSA calculations were performed using 50 snapshots (one every 10 ps) taken from the last 1ns of molecular dynamics trajectory (Kumari *et al*., 2014; Tripathi *et al*.,2015).

Shape-based screening

The approach utilizes the concept of shape and electrostatic potential similarity to select new molecules which may show similar binding modes into the active site. This is an effective tool for identification and optimization of novel inhibitors with high potency and more selectivity (Zoete *et al*., 2016). The Shape Based score was used to rank the screened molecules. The best 329 hits from approved, experimental, withdrawn, investigational in Swiss Similarity web tool for ligand based virtual screening (Zoete *et al*., 2016). A flow chart of the virtual screening used in this approach as shown in Figure 1.

RESULTS AND DISCUSSION

Binding mode analysis

Four different shape-based approaches (e.g., Electroshape, Spectrophores, Shape-IT, and Align-IT) (Zoete *et al*., 2016) were employed to identify potential bioactive analogs from different categories (e.g., Approved: 1'516; Experimental: 4'788; Investigational: 504, and Withdrawn: 161 molecules) of drug-like databases using energetically stable pose of the template (PUB475318). First two methods allow screening via 3D similarity without superimposing the molecular coordinates of template structure. While last two methods perform screening through superimposing the molecular coordinates excluding the position of pharmacophoric features.. It was ensued from outcome of the study that most of the predicted lead molecules exhibited similar functional groups but different scaffolds. Lead molecules having diverse scaffolds showed better propensity towards the molecular interactions with target protein. Flow chart of shape-based outcome is shown in Figure 2.

Toxicity and ADME studies

The ADME and toxicity (carcinogenicity, mutagenicity, and hepatotoxicity) of screened molecules were predicted. Non-toxic molecules were filtered using carcinogenicity and mutagenicity rat models. ADME properties [AlogP98, absorption (95% and 99% level), polar surface area, blood brain barrier, solubility, and hepatotoxicity] were calculated for Non-toxic molecules from ADME descriptor tool. A total of 25 compounds are chosen for further analysis.

Molecular docking studies

Molecular docking studies were performed for the best binding conformation predication of screened molecules into the active site of *Mtb*-DHDPS using AutoDock4.0 program. The 25 molecules were employed in molecular docking studies to explore the binding mode. Total 9 molecules exhibited good binding free energies as well as the required H-bonding interactions with active site residues Thr54, Thr55, Arg148 Tyr143, and Lys171 (Table 1).

Table 1. Molecular interactions of top predicted hits and their comparison with known inhibitors

S. No.	Compound ID	Biopredicta (Docking Score)	AutoDock Tools 4.0 (ΔG)	MVD 5.5 (MolDock Score)	No. of H-bonds
1.	DB05074	-8.697655	-12.49	-134.923	4
2.	DB13660	-7.222914	-9.08	-86.159	2
3.	DB01118	-8.727655	-12.56	-135.403	5
4.	DB03284	-7.671286	-9.56	-89.259	2
5.	DB08207	-7.976235	-10.32	-107.462	1
6.	DB07843	-7.897710	-10.18	-105.916	1
7.	DB01228	-7.861614	-10.09	-105.718	3
8.	DB02312	-7.961257	-10.21	-106.278	3
9.	DB00126	-7.034956	-8.92	-100.899	2
10.	PUB475318#	-8.506021	-12.34	-134.323	3
11.	CID10367*	-6.634203	-9.34	-100.894	4

#predicted inhibitor (Garg *et al*., 2010)
*experimentally known inhibitor (Mitsakos *et al*., 2008)

DB01118 occupied the space nearer to the active site of *Mtb*-DHDPS and showed consistency in docking results followed by DB05074 and template (PUB475318). These molecules have similar structure as PUB475318 and followed the same interactions, which is found in PUB475318 during molecular docking and MD simulation studies. This is non derivatives of cefmetazole, and hence, they can be chosen for DHDPS inhibitors and to modulate for lead optimization. Molecular interaction of DB01118, DB05074 and template (PUB475318) are respectively shown in Figure 3, 4 and 5.

MD simulations

The RMSD profile of protein and ligand were analyzed. Figure 6a depicts the RMSD of protein backbone atoms taking initial protein structure as reference. Protein RMSD achieved stability within initial 1 ns and showed stability in the trajectory with average value of (1.22 ± 0.14 Å). Similarly, ligand showed stable RMSD with average value (1.88 ± 0.20 Å) (Figure 6b). From the RMSD analysis it can be inferred that protein-ligand complex was showing stable conformation during MD simulation of 10 ns.

Root Mean Square Fluctuation (RMSF) of the protein backbone atoms showed small values (0.5 -1 Å), corresponding to the stability in the protein structure in molecular dynamics trajectories (Figure 6c). Similarly, the radius of gyration (Rg) (Figure 6d) represents the compactness of protein showed stability in the protein with average value of 18.08 ± 0.08 Å. Further, solvent accessible surface area (SASA) (Figure 6e) analysis of protein also represented stable surface area of the protein with average value 122.15 ± 1.70 nm^2. 2-3 hydrogen bonds were formed between the protein-ligand complexes during production MD, showing stability of ligand binding (Figure 6f).

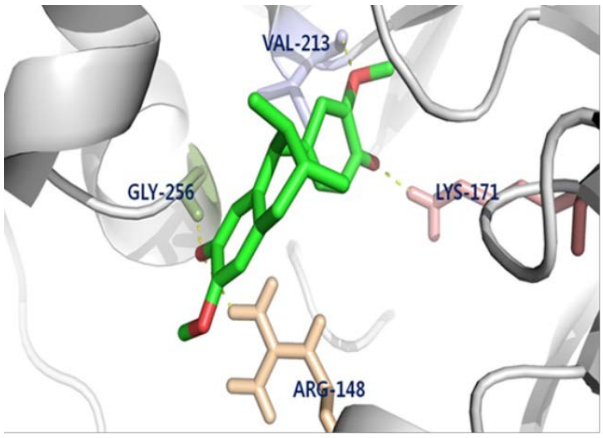

Figure 3. Docked complex of DB01118 with DHDPS.

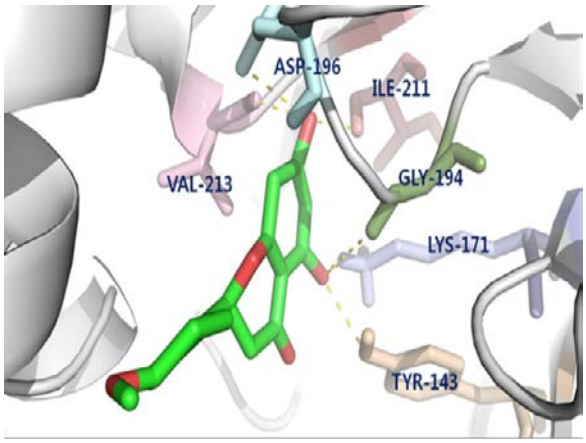

Figure 4. Docked complex of DB05074 with DHDPS.

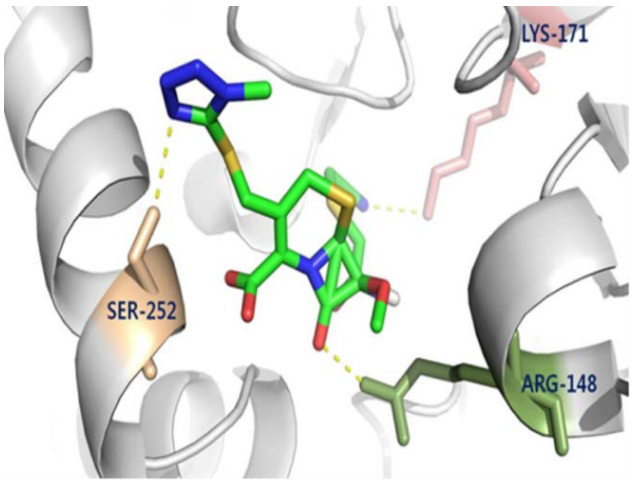

Figure 5. Docked complex of PUB475318 with DHDPS.

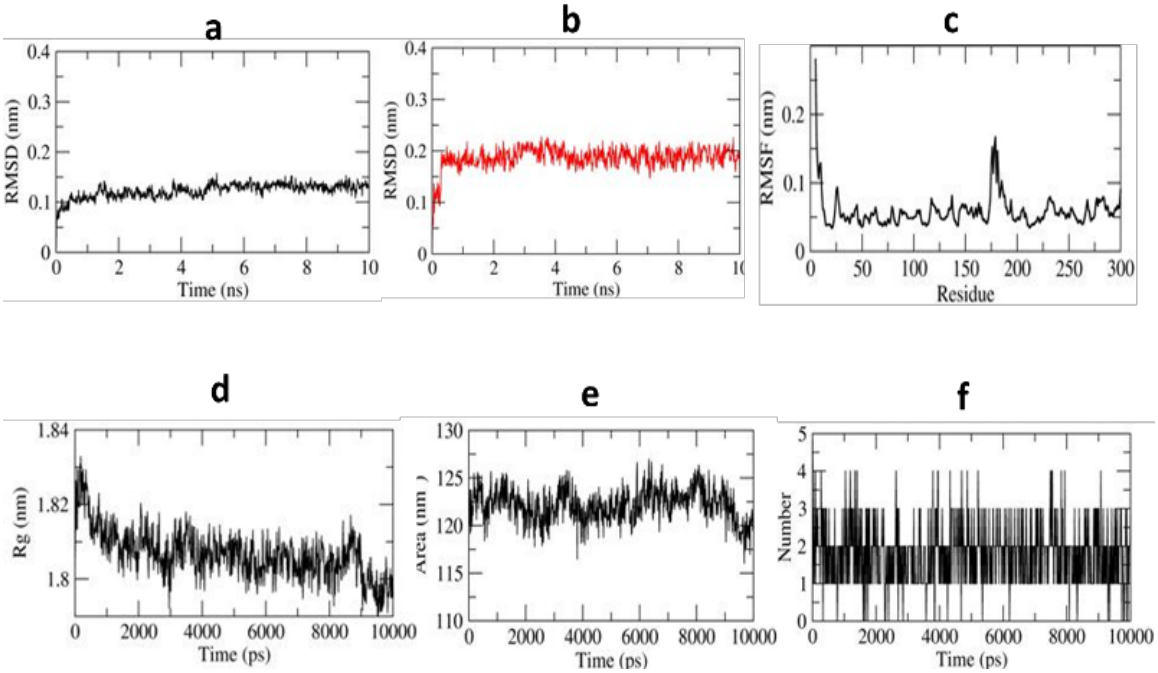

Figure 6. MD simulation: **(a)** rmsd trajectory of protein, **(b)** rmsd trajectory of DB01118, **(c)** RMSF of protein backbone, **(d)** radius of gyration, **(e)** SASA of protein, and **(f)** H-bond formation stability.

Binding free energy was calculated for the protein-ligand complex (Table 2). The ligand showed good binding free energy of -45.32±9.38 kJ/mol. From the whole MD simulation it can be ascertained that the ligand was showing good binding with protein and was in stable conformation at binding site.

CONCLUSION

The molecular docking studies on shape-based virtual screening compound of DB01118 indicate that the docked complex showed favorable molecular interaction as comapred to template molecule (PUB475318) and the experimentally known inhibitors blocking at the active site of *Mtb*-DHDPS enzyme. Besides, there are reports of PUB475318 and its derivatives showing strong anti-bacterial property against Mtb. The compounds used in the present docking study could also possess anti-bacterial activity against Mtb since they are shape-based compound of PUB475318 at 90% similarity. Moreover, another significatnt observation from the ligand–protein interaction analysis between the top docking hits and the active site residues of DHDPS enzyme reveals that the interaction with Arg148 play an imperative role in stable binding.

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Conflict Of Interest

The authors declare no conflict of interest and disclosures associated with the manuscript

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