Molecular Interaction of Novel Compound 2-Methylheptyl Isonicotinate Produced by *Streptomyces* sp. 201 with Dihydrodipicolinate Synthase (DHDPS) Enzyme of *Mycobacterium tuberculosis* for its Antibacterial Activity

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Abstract Antibiotic resistance is a growing problem in multi-drug-resistant tuberculosis which is caused by *Mycobacterium tuberculosis* (MTB). Hence there is an urgent need for designing or developing a novel or potent anti-tubercular agent. The Lysine/DAP biosynthetic pathway is a promising target because of its role in cell wall and amino acid biosynthesis. In our study we performed a molecular docking analysis of a novel antibacterial isolated from *Streptomyces* sp. 201 at three different binding site of dihydrodipicolinate synthase (DHDPS) enzyme of MTB. The molecular docking studies suggest that the novel molecule shows favourable interaction at the three different binding sites as compared to five experimentally known inhibitors of DHDPS.

Keywords DHDPS · Molecular docking · Antibiotic resistance

Introduction

Tuberculosis is a deadly infectious disease which is caused by *Mycobacterium tuberculosis* in humans [1]. Tuberculosis usually attacks the lungs but can also affect other parts of the body. One-third of the world's population is thought to be infected with *M. tuberculosis* [2], and new infections occur at a rate of one per second [3]. In 2007, an estimated 13.7 million people had active TB disease, with 9.3 million new cases and 1.8 million deaths [4]. Hence there is an

urgent need to design or develop a novel or potent antitubercular agents.

For the past few years, Lysine/DAP biosynthetic pathway has been gaining high attention due to its foremost feature in the synthesis of D,L-diaminopimelic acid (meso-DAP) and lysine. Both components are essential for cross-linking peptidoglycan chains to provide strength and rigidity to the bacterial cell wall. [5–7].

Dihydrodipicolinate synthase (DHDPS) catalyses the condensation reaction by means of a ping pong mechanism in which a pyruvate forms a Schiff base with a lysine residue in the active site of the enzyme [8, 9]. In addition to its central role of lysine biosynthesis, DHDPS also delivers the direct precursor of dipicolinic acid which plays a key role in the bacterial sporulation process [10]. Laber et al. [11], also described that in the DHDPS reaction pyruvate binds to the enzyme by forming a Schiff base with the ϵ -amino group of the Lysine residue. It has been observed that *Mycobacterium* cell walls are characterized by an unusual high DAP content. Moreover gene-knock out experiments with *Mycobacterium smegmatis* has demonstrated the essentiality of DAP pathway for the bacteria, where the absence of DAP results in cell lysis and death [12].

In view of its importance, the designing of potential inhibitors against any enzyme of this pathway may display novel classes of antitubercular agents.

The three-dimensional crystal structures of DHDPS from *M. tuberculosis* [13], *Escherichia coli* [14], *Nicotiana sylvestris* [15], *Staphylococcus aureus* [16], *Bacillus anthracis* [17], are available at Protein Data Bank (<http://www.rcsb.org/>). The present study mainly focused on molecular docking studies of a novel compound (2-methylheptyl isonicotinate) isolated from *Streptomyces* sp. 201 [18–21] against DHDPS enzyme of MTB.

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The crystal structure of *Mtb*-DHDPS (PDB ID:1XXX) is a tetramer comprising of four identical subunits arranged in D2 symmetry. Each monomer comprises an N-terminal (β/α) 8-barrel domain and a C-terminal domain consisting of three α -helices. The residues responsible for substrate binding and catalysis are located in the (β/α) 8-barrel domain. The crystallographic asymmetric unit contains two tetramers of the enzyme. Each tetramer can be described as a dimer of dimers, with the two monomers tightly bound to each other to form the tight dimer, and weaker interactions between the dimeric units [13]. The active site of the enzyme is located at the centre of each monomer, facing the central cavity of the tetramer. It is situated in a pocket at the C-terminal side of the (β/α) 8-barrel, as in all known (β/α) 8-enzymes. The active site centers about Lys171 that forms a Schiff base with the substrate pyruvate. In the *E. coli* enzyme, the equivalent amino acid is Lys161, [11]. Three amino acid residues form the conserved catalytic triad: Tyr143, Thr54, and Tyr117, whereby Tyr117 is contributed from the adjacent monomer across the tight-dimer interface. Both the identity, as well as the relative spatial orientation of these functional groups, is conserved among characterized DHDPS enzymes [13]. There have been reports of experimental procedures for designing of inhibitors against DHDPS but no potent inhibitors have been reported till date [22, 23]. A few work on virtual screening of pyruvate analogs against DHDPS inhibitors has been reported recently [24], but the present work on molecular docking studies against DHDPS enzyme by 2-methylheptyl isonicotinate (2MHI) produced by *Streptomyces* sp. 201 is reported for the first time.

Materials and Method

Generation of Chemical Structures

Two-dimensional and three-dimensional structure of 2MHI isolated from *Streptomyces* sp. 201 was generated using Cambridge Soft ChemOffice 2010 [25]. Additionally we also generated five experimentally known inhibitors i.e., dimethyl-1,4-dihydro-4-oxopyridine-2,6-dicarboxylate, piperidine-2,6-dicarboxylic acid, 1,4-dihydro-4-oxopyridine-2,6-dicarboxylic acid, dimethylpiperidine-2,6-dicarboxylate, pyridine-2,6-dicarboxylic acid of *Mtb* DHDPS, inhibiting the enzyme activity in the range of 24–84% [26] for validation purpose. The energy of the generated chemical structures was minimized using MM2 force field methods [27] and save as SYBL mol2 files.

Filter for Lipinski Rule of Five

The generated structures 2MHI, dimethyl-1,4-dihydro-4-oxopyridine-2,6-dicarboxylate, piperidine-2,6-dicarboxylic

acid, 1,4-dihydro-4-oxopyridine-2,6-dicarboxylic acid, dimethylpiperidine-2,6-dicarboxylate and pyridine-2,6-dicarboxylic acid were subjected to Lipinski's rule of five filters [28, 29] which determine the chemical compound with certain pharmacological or biological activity for an orally active drug in humans.

Antibacterial Assay and MIC Conducted by Bordoloi et al. 2001

A screening work on anti-bacterial assay of 2MHI and Isoniazid—an anti-tuberculosic drug has been conducted by Bordoloi et al. 2001 showing 2MHI having a strong anti-bacterial property against *Bacillus subtilis*, *E. coli*, *Proteus mirabilis*, *Arthrobacter*, *Shigella* and *Klebsiella* species as compared to Isoniazid which is shown in Table 1 [18].

Computation

Molecular docking was carried out using Molegro Virtual Docker (MVD) [30]. MVD is molecular visualization and molecular docking software which is based on a differential evolution algorithm; the solution of the algorithm takes into account the sum of the intermolecular interaction energy between the ligand and the protein and the intramolecular interaction energy of the ligand. The docking energy scoring function is based on the modified piecewise linear potential with new hydrogen bonding and electrostatic terms included. Full description of the algorithm and its reliability compared to other common docking algorithm can be found in reference [31].

The three-dimensional crystal structure of *MTB* DHDPS (PDB ID: 1XXX) determined by X-ray crystallography retrieved from protein databank [13] was imported in the MVD [30] software and for docking purposes, all the 1,587 water molecules, eight DTT molecules, eight Mg^{2+} and eight Cl^- ions were removed. Potential binding sites were predicted using MVD [30] and the binding cavity was set at three different sites, Cavity-1 (X: 0.62, Y: 38.04, Z: 71.26),

Table 1 Minimum inhibitory concentrations required for antibacterial activity of 2-methyl isonicotinate and isoniazid as carried out by Bordoloi et al. 2001

Bacterium	MIC (Ug/ml)	
	2MHI	Isoniazid
<i>B. subtilis</i>	50	150
<i>E. coli</i>	70	250
<i>Arthrobacter</i> spp.	40	150
<i>P. mirabilis</i>	40	200
<i>Shigella</i> spp.	60	300
<i>Klebsiella</i> spp.	50	250

Table 2 Molecular docking poses score of 2MIH as compared to experimentally known inhibitors of DHDPS against three different binding sites (Cavity-1, 2 and 3) of *M. Tuberculosis* DHDPS enzyme

SN	Compounds	Cavity-1			Cavity-2			Cavity-3		
		Rerank pose score ^a	H-bonding (kJ/mol)	E _{Total} (kJ/mol)	Rerank pose score	H-bonding (kJ/mol)	E _{Total} (kJ/mol)	Rerank pose score	H-bonding (kJ/mol)	E _{Total} (kJ/mol)
1.	2-methylheptyl isonicotinate	-60.25	-4.45	-65.25	-35.48	-2.64	-67.53	-54.03	-6.38	-80.11
2.	dimethyl-1,4-dihydro-4-oxopyridine-2,6-dicarboxylate	-53.84	-8.71	-68.54	102.23	-5.23	-19.8	93.48	0.28	-1.85
3.	piperidine-2,6-dicarboxylic acid	-44.65	-8.54	-44.7	65.69	-4.28	-4.12	-40.56	-2.5	-43
4.	1,4-dihydro-4-oxopyridine-2,6-dicarboxylic acid	-44.41	-7.22	-20.72	170.32	-10.63	-27.13	-41	-8.88	-15.44
5.	Dimethylpiperidine-2,6-dicarboxylate	-26.17	-10.21	-41.46	48.67	-1.01	-11.08	-29.19	-8.69	-27.98
6.	pyridine-2,6-dicarboxylic acid	101.19	-3.77	-17.57	84.98	-4.03	-18.2	-37.63	-5.05	-36.64

^a The rerank score is a linear combination of E-inter (steric, Van der Waals, hydrogen bonding, electrostatic) between the ligand and the protein, and E-intra (torsion, *sp*²-*sp*², hydrogen bonding, Van der Waals, electrostatic) of the ligand weighted by pre-defined coefficients

Fig. 1 **a** Predicted bonded interactions (green dashed lines) between 2MHI (yellow) and Thr55, Arg148 and Gly256 residues (Cavity 1) of *M. tuberculosis* DHDPs and **b** predicted non bonded electrostatic interaction between 2MHI and the residues at the active site. (Color figure online)

Cavity-2 (X: 0.65, Y: 35.58, Z: 61.6)3 and Cavity-3(X:–4.73, Y:35.78, Z:64.51).

The methodology adopted in this study to determine the potential binding sites is a grid-based cavity prediction algorithm. First, a discrete grid with a resolution of 0.8 Å covering the protein is created with a sphere of radius 1.4 Å is placed and checked whether the sphere will overlap with any of the spheres determined by the Van der Waals radii of the protein atoms. Second, each accessible grid point is checked for whether it is part of a cavity or not. The final step is to determine the connected regions. Two grid points are connected if they are neighbours. The cavities found are then ranked according to their volume [31]. Bond flexibility of the ligands was set and the side chain flexibility of the protein for

Fig. 2 **a** Predicted bonded interactions (green dashed lines) between 2MHI (yellow) and Ser58 and Tyr116 residues (Cavity-2) of *M. tuberculosis* DHDPs and **b** predicted non bonded electrostatic interaction between 2MHI and the residues at the active site. (Color figure online)

cavity-1 (Thr54, Thr55, Tyr143, Arg148, Lys171) cavity-2 (Gly53, Thr54, Pro59, Val113, Thr116, Arg148) and cavity3 (Thr54, Thr55, Pro59, Arg158, Gly256, Val 257, Gln258) were set with a tolerance of 1.10 and strength of 0.90 for docking simulations. RMSD threshold for multiple cluster poses was set at 2.00 Å. The docking algorithm was set at a maximum iteration of 1,500 with a simplex evolution size of 50 and a minimum of 10 runs.

Structure Relatedness of 2MHI

Additionally we performed a chemical structure search of 2MHI at the NCBI Pubchem database [32, 33] to study the relatedness of the compound and its antibacterial activity as

Fig. 3 **a** Predicted interactions (green dashed lines) between 2MHI (yellow) and active site residues Arg148 and Gly 256 (Cavity-3) of *M. tuberculosis* DHDPS and **b** predicted non bonded electrostatic interaction between 2MHI and the residues at the active site. (Color figure online)

compared to already experimentally verified compounds which is reported in the database. The search parameters were set at 95% similarity subjected to Lipinski rule of five filters [28, 29].

Table 3 Lipinski Rule of filter for the six compounds used in our study

SN	Compounds	HBA	HBD	Mol Wt	cLogP	Rot B
1	2MHI	3	0	235.15	4.34	8
2	Dimethyl-1,4-dihydro-4-oxypyridine-2,6-dicarboxylate	5	1	211.05	−1.17	4
3	Piperidine-2,6-dicarboxylic acid	5	3	173.07	−1.49	2
4	1,4-Dihydro-4-oxypyridine-2,6-dicarboxylic acid	5	3	183.02	−1.61	2
5	Dimethylpiperidine-2,6-dicarboxylate	5	0	199.08	−4.53	2
6	Pyridine-2,6-dicarboxylic acid	5	2	167.02	1.07	2

Results and Discussion

The six compounds docked at the various binding sites were ranked based on the top poses score as shown in Table 2. Both bonded and non-bonded interactions are observed between 2MHI and Thr55, Arg148 and Gly256 residues (Cavity-1) shown in Fig. 1a, b, Ser58 and Tyr116 (Cavity-2) shown in Fig. 2a, b and Arg148 and Gly 256 (Cavity-3) shown in Fig. 3a, b. Cavity-1 showed the maximal interaction with three residues (Thr55, Arg148 and Gly256) with DHDPS enzyme while Cavity-2 (Ser58 and Tyr116) and Cavity-3 (Arg148 and Gly 256) with only two residues.

Docking of these compounds into the predicted binding site (Cavity 1, 2 and 3) of *M. tuberculosis* DHDPS reveals that 2MHI has a comparatively higher rerank pose score and a favourable total energy (E_{Total}) than the known inhibitors of *M. tuberculosis* DHDPS (Table 2).

Thus the novel isolate from *Streptomyces* sp. 201 could provide structural leads for more potent DHDPS inhibitors. Moreover 2MHI does not violate Lipinski rule of five as shown in Table 3 where the rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion (“ADME”) [33]. Additionally the ground work on screening of antibacterial assay conducted by Bordoloi et al. 2001 also proved that it has a strong anti-bacterial activity [18].

Additionally, the chemical structure search at the NCBI PubChem database also resulted in 14 similar compounds at a similarity of 95% in which 3 compounds (Compounds ID: CID 9856112, CID 531657, CID 531656) has already tested bioassay data on MIC against M.tb.

Conclusion

To conclude, the molecular docking studies shows that 2MHI is a good inhibitor against DHDPS enzyme forming favourable interaction at three different binding sites as compared to other experimentally known inhibitors of *M. tuberculosis* DHDPS. Additionally the compound does not violate Lipinski rule of five to be an orally active drug.

Additionally we have also presented a ground work on screening of antibacterial assay conducted by Bordoloi et al. 2001 showing a strong anti-bacterial activity. Though experimental studies are required to mark it as a lead compound for the development of novel drugs against *M. tuberculosis*, we conclude that the molecular interaction of novel compound 2MHI produced by *Streptomyces* sp. 201 with DHDPS enzyme of *M. tuberculosis* at three different binding site against DHDPS enzyme of *M. tuberculosis* support experimental testing of the compound.

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