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Insilico studies of 2-methylheptyl isonicotinate produced by *Streptomyces* sps. 201 against dihydrodipicolinate synthase enzyme of *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis is thought to have infected one-third of the world's population and antibiotic resistance is a growing problem in multi-drug-resistant tuberculosis which is caused by *Mycobacterium tuberculosis* (MTB). It has been reported that Mycobacterial cell walls are characterized by high DAP (diaminopimelic acid) content—an intermediate of the (S)-lysine biosynthetic pathway. Hence, the Lysine/DAP biosynthetic pathway is a promising target because of its role in cell wall and amino acid biosynthesis. In this study we performed a molecular docking analysis of a novel antibacterial isolated from *Streptomyces* sps. 201 against dihydrodipicolinate synthase (DHDPS) enzyme of *Mycobacterium tuberculosis*. The docking studies suggest that the novel molecule binds at active site LYS 171 forming a cleft and at other potential ligand binding site exhibiting all the major interactions such as hydrogen bonding, hydrophobic interaction and electrostatic interaction with (THR55, TYR143, ARG148, LYS171, VAL257 and GLY256) residues.

Keywords: DHDPS; Molecular Docking; Hydrogen Bonding

1. INTRODUCTION

Tuberculosis is common and in many cases lethal and infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* [1]. Tuberculosis typically attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air. One-third of the world's population is thought to be infected with *Mycobacterium 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]. Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected. Hence there is an urgent need to design or develop a novel or potent antitubicular agents.

The three-dimensional crystal structures of DHDPS enzyme of *Mycobacterium tuberculosis* (PDB ID: 1XXX) [5], is available at Protein Data Bank (http://www.rcsb.org/). There have been reports of experimental procedures for designing of inhibitors against DHDPS but no potent inhibitors have been reported till date [6-8].

The crystal structure of Mtb-DHDPS enzyme (PDBID: 1XXX) is a tetramer comprising of four identical subunits arranged in D2 symmetry. Each monomer comprises an N-terminal (b/a) 8-barrel domain and a C-terminal domain consisting of three a-helices. The residues responsible for substrate binding and catalysis are located in the (b/a) 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 [5].

The present study mainly focused on molecular docking studies of a novel compound 2-methylheptyl isonicotinate isolated from *Streptomyces* sps. 201 [9-12] against dihydrodipicolinate synthase (DHDPS) enzyme of MTB at the active site residue (LYS-171 which is responsible for substrate binding and catalysis), which results in the formation of hydrogen bonds using Molegro Virtual Docker [13]. A few work of virtual screening of pyruvate analogs against DHDPS inhibitors has been reported [14], here we have reported the molecular docking analysis of

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2-methylheptyl isonicotinate against DHDPS enzyme of MTB.

2. MATERIALS AND METHOD

The 2D structure of 2-methylheptyl isonicotinate (2MHI) isolated form *Streptomyces* sp. 201 was generated using Cambridge Soft ChemOffice 2008 [15] shown in **Figure 1**. The energy of the generated structure of 2MHI was converted to 3D structure using ChemOffice 2008 [15] and minimized to 17.9850 kcal/mol using MM2 force field methods [16] and save as SYBL mol2 files for docking purposes.

The physio-chemical properties of 2-methylheptyl isonicotinate were predicted using Chemoffice 2008 [15] for Lipinski rule of five filters [17]. 2-methyheptyl isonicotinate was again screened for possible side effects and toxic effects using PASS prediction (Prediction of Activity Spectra for Substances) [18].

The three-dimensional crystal structure of MTB DHDPS (PDB ID: 1XXX) retrieved from Protein Databank was imported in the Molegro Virtual Docker [13] and for docking purposes, all the 1587 water molecules, eight DTT molecules, eight Mg^{2+} and eight Cl^{-} ions were removed. MTB DHDPS enzyme consists of 8 chains (A-H), considering that the chains are identical and independent of each other, only chain A from the enzyme was selected and imported in the MVD to perform the docking simulation.

The potential ligand binding sites of the MTB DHDPS were detected using the cavity detection program in MVD 5.0 Molegro Virtual Docker. A cavity having a volume of 43.5 and a surface area of 162.56 was detected which is shown in **Figure 2**. The amino acid residues that lie in the potential ligand binding site are THR54, THR55, TYR143, ILE145, GLY147, ARG148, LYS171, ALA173, GLY194-ALA197, ILE211-ILE214 and CYS248-VAL 257.

The methodology adopted in this work 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 proteinis 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 [19].

Molecular docking was carried out using Molegro Virtual Docker (MVD) (Molegro APS: MVD 5.0). 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

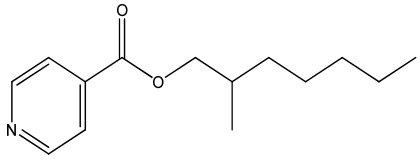

Figure 1. 2D structure of 2-methylheptyl isonicotinate.

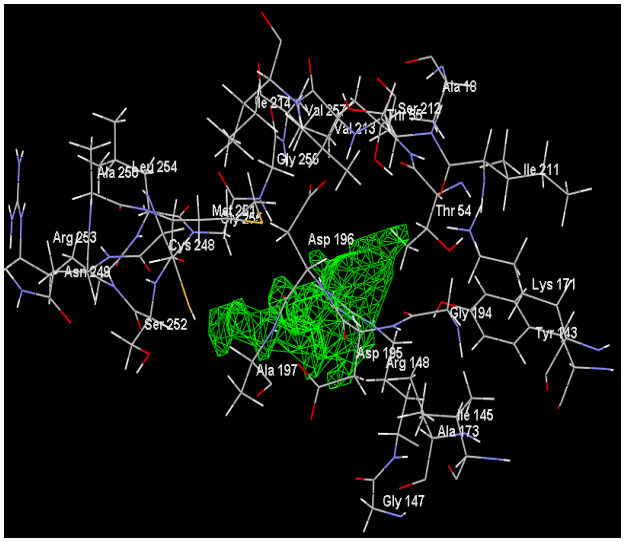

Figure 2. Potential ligand binding sites predicted using Molegro Virtual Docker.

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 (PLP) 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 [19,20].

3. RESULTS AND DISCUSSION

Molecular docking was carried out and the docking score and protein-ligand interaction energy are shown in **Table 1** which shows that there has been a strong inter action between the ligand and the receptor indicating a

Table 1. Docking Score of 2MHI against MTB DHDPS enzyme.

MolDock Score	−79.42
Docking Score	−85.19
Protein-Ligand Interaction Energy	−88.91
Hydrogen Bonding Energy	−2.34
Internal Energy of the Ligand	9.45

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binding affinity. It is also observed that 2MHI was docked into the active site of MTB DHDPS (shown in **Figure 3**). Molegro Virtual Docker uses the MolDock docking engine to predict protein-lignad interactions. MolDock is based on a new hybrid search algorithm, called guided differential evolution [20]. The guided differential evolution algorithm combines the differential evolution (DE) optimization technique with a cavity prediction algorithm which is dynamically used during the docking process [19].

The docking results shows 2-methylheptyl isonicotinate bound tightly at the active site of MTB DHDPS. 2MHI was found lying deep into the cavity/potential ligand binding site exhibiting molecular interactions such as hydrogen bonding, hydrophobic interaction (**Figure 4**) and electrostatic interactions (**Figure 5**).

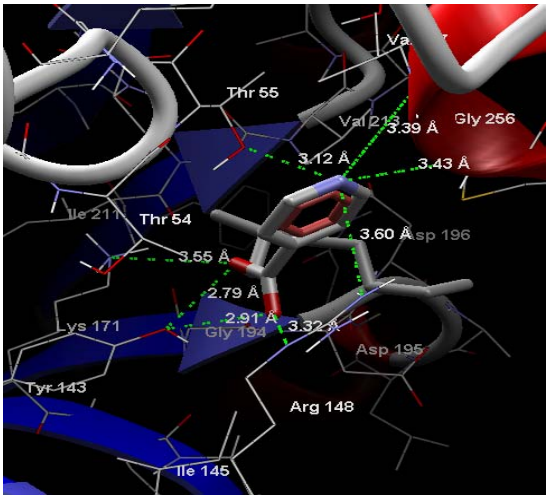

Figure 3. Predicted bonded interactions (green dashed lines) between 2MHI and Thr55, Tyr143, Tyr148, Lys171, Gly256 and Gly257 residues of *M. tuberculosis* DHDPS enzyme.

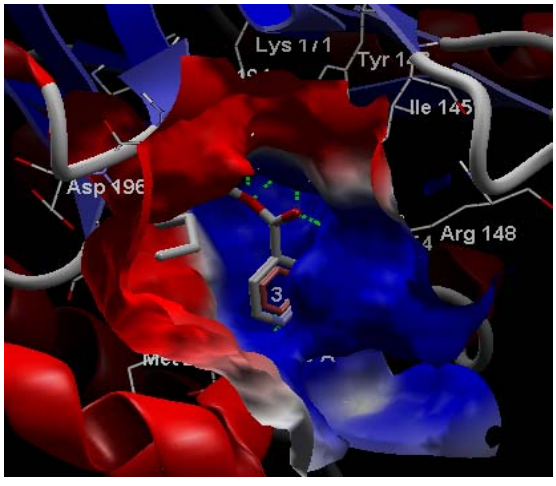

Figure 4. Predicted hydrophobic interaction between 2MHI and the residues at the active site region.

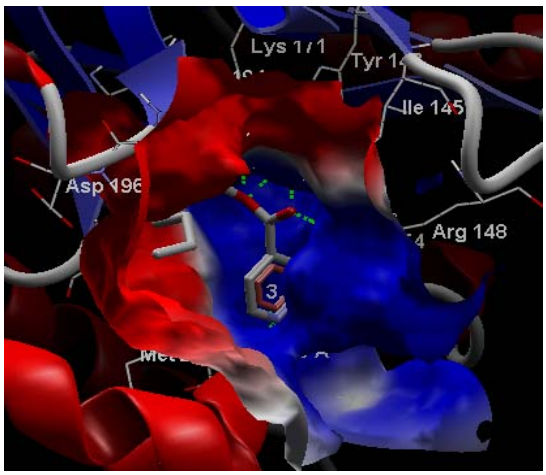

Figure 5. Predicted electrostatic interaction between 2MHI and the residues at the active site region.

The docking analysis also shows the formation of eight interactions with six amino acid residues THR55, TYR143, ARG148, LYS171, VAL257 and GLY256 respectively of MTB DHDPS (shown in **Figure 3**).

In the present study, the MolDock docking score were used. The MolDock scoring function (MolDock Score) used in MVD 5.0 is derived from the PLP scoring functions originally proposed by Gehlhaar *et al.* [21,22] and later extended by Yang *et al.* [23]. The MolDock scoring function further improves these scoring functions with a new hydrogen bonding term and new charge schemes. The docking scoring function, E_{score}, is defined by the following energy terms:

$$E_{score} = E_{inter} + E_{intra}$$

where E_{inter} is the ligand protein interaction energy.

We have also carried out a detailed analysis of docking in terms of protein-ligand interaction energy. The ligand-protein interaction energy analysis (both electrostatic and H-bond) were calculated in order to get a better understanding of the variations between the binding mode of 2MHI and the molecular factors responsible for the activity. **Table 2** enlists the protein-ligand interaction energy calculation including the residues present, interacting atom of the protein and the ligand, protein-ligand interaction distances, ligand atom energies, Hbond and electrostatic energy and Epair which is shown in **Table 2**.

Additionally, the physio-chemical properties predicted using Chemoffice 2008 [19] showed that 2MHI does not violate Lipinski rule of five [21] having a molecular weight of 235.52, 3 hydrogen bond acceptor atom, no hydrogen bond donor atom and LogP of −3.41 which is good enough to be an orally active drug. Moreover the molecule shows a drug likeness of 0.722 from the PASS prediction [22].

Thus the novel isolate form *Streptomyces* sp. 201 could provide a structural leads for DHDPS inhibitor or a novel class of anti-tubercular agent.

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Table 2. Protein-ligand interaction analysis.

ID	Amino Acid	Protein Atom	Ligand Atom	Interaction Distance	Ligand Atom Energies	Hbond & Electrostatic Energy[#]	EPair[*]
0	THR 55	O(OG1)	N(4)	3.12	–8.59	–2.38	–9.62
1	ARG148	N(NH2)	N(4)	3.59	–8.59	–0.00	–9.62
2	GLY256	N	N(4)	3.43	–8.59	–0.29	–9.62
3	VAL257	N	N(4)	3.39	–8.59	–0.45	–9.62
4	TYR143	O(OH)	O(7)	2.91	–4.44	–0.23	–4.59
5	ARG148	N(NE)	O(7)	3.32	–4.44	–1.37	–4.59
6	TYR143	O(OH)	O(8)	2.79	–7.33	–2.5	–6.66
7	LYS171	N(NZ)	O(8)	3.55	–7.33	–0.24	–6.66

[#]Hydrogen bonding and strong electrostatic interaction energy; [*]E-pair-Steric and Hydrogen bonding energy between a ligand atom and a receptor atom.

4. CONCLUSION

The *insilico* studies of novel isolate 2-methylheptyl isonicotinate from *Streptomyces* sps. 201 showed that 2MHI is a good inhibitor of MTB DHDPS enzmye forming both bonded and non-bonded interactions at the active site of the enzyme. The compound showed a strong binding affinity to MTB DHDPS in terms of docking score and hydrogen bonding energy. Additionally the compound does not violate Lipinski rule of five to be an orally active drug and showed a drug likeness of 0.722 using PASS prediction. 2MHI could be a lead molecule or a novel class of potential antitubercular agent. Hence the present study concludes that 2MHI could be an anti-tubercular agent and supports for experimental testing of the compounds.

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