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Structure-based virtual screening as a tool for the identification of novel inhibitors against *Mycobacterium tuberculosis* 3-dehydroquinate dehydratase

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ABSTRACT

3-Dehydroquinate dehydratase (DHQase), the third enzyme of the shikimate pathway, catalyzes the reversible reaction of 3-dehydroquinate into 3-dehydroshikimate. The aim of the present study was to identify new drug-like molecules as inhibitors for *Mycobacterium tuberculosis* DHQase employing structure-based pharmacophore modeling technique using an in house database consisting of about 2500 small molecules. Further the pharmacophore models were validated using enrichment calculations, and finally three models were employed for high-throughput virtual screening and docking to identify novel small molecules as DHQase inhibitors. Five compounds were identified, out of which, one molecule (**Lead 1**) showed 58% inhibition at 50 μM concentration in the Mtb DHQase assay. Chemical derivatives of the **Lead 1** when tested evolved top two hits with IC_{50}s of 17.1 and 31.5 μM as well as MIC values of 25 and 6.25 μg/mL respectively and no cytotoxicity up to 100 μM concentration.

1. Introduction

Tuberculosis (TB) is the second greatest killer worldwide due to a single infectious agent being responsible for 1.3 million deaths and 8.6 million people infected in 2012 [1]. According to WHO (World Health Organization), approximately two billion people are latently infected and about 10% of those person could develop active disease during their lifetime. Moreover, 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged between 15 and 44. It was estimated that 530,000 children became ill and 74,000 HIV-negative children died of TB in 2012. Also, TB is a leading killer of people living with HIV, causing one-fifth of all deaths of HIV-infected people. *Mycobacterium tuberculosis* is the main causative agent of TB in humans and the dissemination of multi-drug-resistant strains challenges the existing treatments for TB [1].

Targeting essential metabolic pathways of micro-organisms absent in humans is an attractive strategy for the development of new therapies. The shikimate pathway produces an important precursor of aromatic compounds in bacteria, fungi, plants and apicomplexan parasites [2–5]. The pathway comprises of seven enzymes each catalyzing a separate step that converts erythrose-4-phosphate and phosphoenolpyruvate into chorismate, the precursor for the synthesis of aromatic amino acids, folic acid, ubiquinone, and many other aromatic compounds [2,6,7]. As reported from gene disruption studies, enzymes from the shikimate pathway were found to be essential for *M. tuberculosis* survival [8].

3-Dehydroquinate dehydratase (DHQase) is the third enzyme of the shikimate pathway catalyzing the reversible dehydration of 3-dehydroquinate into 3-dehydroshikimate. There are two forms of DHQase (type I and type II), with distinct structures, but both catalyze the overall reaction [9]. *M. tuberculosis* uses type II DHQase (Mtb DHQase), a 17.79 kDa protein containing 147 amino acids.

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Crystallographic data revealed Mtb DHQase as a homododecamer formed from a tetramer of trimers with the active site found near the C-terminal region of strands β1 and β3 of the parallel β-sheet [9,10]. The present study involved structure based design of Mtb DHQase inhibitors using energy optimized pharmacophore as a query to small molecule database searching and retrieved suitable hits based on pharmacophore fitness, followed by docking in the active site of DHQase protein. Finally, five compounds were short-listed and further evaluated for their *in vitro* DHQase assay.

2. Materials and methods

2.1. Computational details

All the computational studies were carried out on an Intel Core 2 Duo E7400 2.80 GHz capacity processor with memory of 2 GB RAM running with RHEL 5.2 operating system. PHASE 3.3 implemented in the Maestro 9.3 software package (Schrodinger, LLC) was used to generate e-pharmacophore models [11]. Glide energy grid was generated for the prepared protein complex. Binding site was defined by a rectangular box surrounding the X-ray ligand. Ligand was refined using the "Refine" option in Glide, and the option to output Glide XP descriptor information was chosen (Glide v5.7, Schrodinger, LLC, New York, NY). For refinement and docking calculations, the default settings as available in the software package were used.

2.2. Protein and ligand preparation

The crystal structure of Mtb DHQase bound to inhibitor (PDB ID: 2Y71) with a resolution of 2.0 Å was retrieved from PDB (protein data bank) and was prepared using the Protein Preparation Wizard of Schrödinger Suite 9.3 [12]. The protein was subjected to many steps such as addition of hydrogens, bond order and formal charge corrections, adjustment of tautomerization and ionization states of protein, etc. The water molecules were removed from the protein and the hydrogen bonding network was optimized by reorienting the hydroxyl and thiol groups in the protein residues. Finally the protein was subjected to energy minimization using OPLS_2005 (optimized potential for liquid simulations) force field. The reference ligand was also retrieved from PDB which had a reported K_i value of approximately 45 nM [13] and was subjected to energy minimization using impact of Schrodinger suite version 9.3. The ligand was minimized using 500 cycles each of SD (steepest descent) and CG (conjugate gradient). The interactions of the ligand with the protein residues in the active site were visualized using ligand interaction diagrams in Schrodinger suite version 9.3

2.3. Glide XP (extra-precision) docking

The prepared crystal structure of protein was used for grid generation, specifying the refined X-ray ligand as a reference to identify the active site. The docking was done by Glide XP (extra precision) protocol where the output gives a docking pose with XP glide score with RMSD (root mean square deviation) to crystal ligand at active site. The score can be considered as a total of binding energies of ligand with the protein (bonding and non-bonding interactions). For pharmacophore hypothesis generation, the energies of hydrophobic interactions, cation–π and π–π interactions were given out in XP descriptor file. The XP Glide scoring function was used to order the best ranked compounds and the specific interactions like π–cation and π–π stacking were analyzed using XP visualizer in Glide module.

2.4. E-pharmacophore generation

The e-pharmacophore hypothesis was created for the ligand by using the xpdes results of the Glide XP output in the docking post-processing tool of the scripts module. Starting with the refined crystal ligand, pharmacophore sites were automatically generated with Phase (Phase, v3.0, Schrodinger, LLC, New York, NY) [14,15] using the default set of six chemical features: hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic (H), negative ionizable (N), positive ionizable (P), and aromatic ring (R). Hydrogen bond acceptor sites were represented as vectors along the hydrogen bond axis in accordance with the hybridization of the acceptor atom. Hydrogen bond donors were represented as projected points located at the corresponding hydrogen bond acceptor positions in the binding site. The ligand was docked with Glide XP and the pose was refined. The Glide XP scoring terms were computed, and the energies were mapped onto atoms. The pharmacophore sites were generated, and the Glide XP energies from the atoms that comprised each pharmacophore sites were summed up. These sites were then ranked based on the individual energies, and the most favorable sites were selected for the pharmacophore hypothesis [16]. This pharmacophore model was then used as query for virtual screening.

2.5. Dataset preparation

BITS-Pilani *in house* database containing 2500 compounds was considered for the e-pharmacophore based screening. All the compounds were prepared using LigPrep module [17] of Schrodinger 9.3 so as to generate high quality structures with appropriate ionization states, tautomers, and ring conformations and stereochemistry. Epik was employed for adjustment of tautomerization and ionization states of molecules at pH 7.0 ± 2.0. Ligand conformers were generated for all the database molecules using ConfGen [17]. All the molecules were energy minimized and filtered using the OPLS_2005 force field, by means of a distance dependent dielectric constant with a prefactor of 4. A maximum of 32 conformations for every ligand were generated and carried forward for virtual screening.

2.6. E-pharmacophore validation

For the fraction of known actives recovered when the database was screened, enrichment factor (EF) was employed. In this study, decoy set consisted of 1000 molecules with an average molecular weight of 400 kDa which were available for download (http://www.schrodinger.com/glidedecoyset). In decoy set further 15 known active molecules of DHQase inhibitors were included for validation. For this, we focused primarily on EF (1%), the enrichment in the top 1% of the decoys, and along with goodness of fit (GH), % actives, % yield were calculated using the following equations:

$$\mathrm{EF} = \frac{H_a \times D}{H_t \times A} \tag{1}$$

$$\mathrm{GH} = \left(\left(\frac{H_a}{4H_tA}\right) \times (3A + H_t)\right) \times \left(1 - \left(\frac{H_t - H_a}{D - A}\right)\right) \tag{2}$$

$$\%\,\mathrm{Yield} = \left[\left(\frac{H_a}{H_b}\right) \times 100\right] \tag{3}$$

$$\%A = \left[\left(\frac{H_a}{A}\right) \times 100\right] \tag{4}$$

where 'H_t' was total number of compounds in the hit list, 'H_a' was the total number of active molecules in the hit list, 'A' was the total number of actives in the decoy set, 'D' was the total number of molecules in the decoy set.