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Ligand based virtual screening and biological evaluation of inhibitors of chorismate mutase (Rv1885c) from *Mycobacterium tuberculosis* H37Rv

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Abstract—We have identified new lead candidates that possess inhibitory activity against *Mycobacterium tuberculosis* H37Rv chorismate mutase by a ligand-based virtual screening optimized for lead evaluation in combination with in vitro enzymatic assay. The initial virtual screening using a ligand-based pharmacophore model identified 95 compounds from an in-house small molecule database of 15,452 compounds. The obtained hits were further evaluated by molecular docking and 15 compounds were short listed based on docking scores and the other scoring functions and subjected to biological assay. Chorismate mutase activity assays identified four compounds as inhibitors of *M. tuberculosis* chorismate mutase (MtCM) with low K_i values. The structural models for these ligands in the chorismate mutase binding site will facilitate medicinal chemistry efforts for lead optimization against this protein.
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Tuberculosis (TB) kills more than two million people a year worldwide (http://www.avert.org/tuberc.htm).[1] The emergence of multiple-drug-resistant TB and its synergism with HIV is a burgeoning threat which compels characterization of new enzyme targets and the development of new drugs (http://www.avert.org/tuberc.htm).

Chorismate mutase (EC 5.4.99.5) catalyzes the Claisen rearrangement of chorismate to prephenate (Fig. 1) in the shikimate pathway which leads to the synthesis of the aromatic amino acids phenylalanine and tyrosine. This is the single known example of an enzyme catalyzing a pericyclic reaction.

Shikimate pathway for the biosynthesis of aromatic compounds is evidently present in bacteria, fungi, and plants but absent in animals. Therefore, chorismate mutase is a novel target for generation of antibiotics, fungicides, and herbicides.[2]
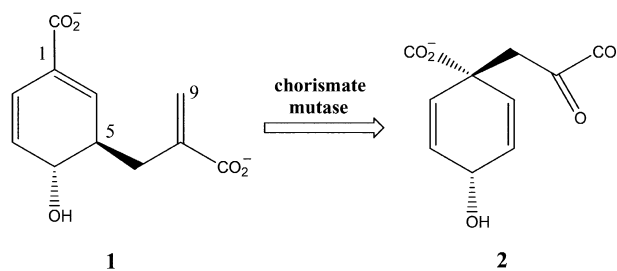

Figure 1. Claisen rearrangement of chorismate to prephenate.

Mycobacterium tuberculosis H37Rv genome contains two genes (Rv1885c and Rv0948c) responsible for chorismate mutase activity.[3] The protein encoded by Rv1885c has been characterized as a mono-functional chorismate mutase (MtCM) having an N-terminal signal sequence (1–33 residues). While the shikimate pathway is commonly present in the cytoplasm of bacteria and higher plants, MtCM is secreted out of the cell to provide support to *M. tuberculosis* in aromatic amino acid deficient medium.[4] It has also been proposed recently that MtCM might interact with the host macrophages and might be important in virulence.[5] On the other hand, the protein encoded by Rv0948c is a bifunctional

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chorismate mutase-prephanate dehydratase, that lacks a signal sequence and is, therefore, restricted to the cytoplasm. Moreover, it has been shown that Rv1885c is the major contributor toward the CM activity of the cell, while Rv0948c is only a minor contributor.[3] For our study we have used the recently solved crystal structure of MtCM (Rv1885c) (PDB ID—2F6L).[6]

We have employed an integrated database screening strategy involving two popular 3D-database screening approaches: pharmacophore hypothesis based 3-D database search and protein structure-based docking approach. Since there are no known inhibitors of MtCM, we developed the ligand-based pharmacophore model based on the substrate chorismic acid and three aza inhibitors (Fig. 2) of chorismate mutase from *Saccharomyces cerevisiae*,[7] as the active sites of these two proteins are quite similar.[6] The pharmacophore model was derived by means of a genetic algorithm similarity program GASP.[8] The program employs a genetic algorithm for determining the correspondence between functional groups in the superimposed ligands and the alignment of these groups in a common geometry for receptor binding. Pharmacophore model generated by GASP consists of positions and tolerance for four acceptor sites, AA1–AA4 (Fig. 3). This model was used to perform a pharmacophore search of 3-D compound database to identify 'hits' that satisfy the chemical and the geometrical requirements using UNITY module of Sybyl7.1. The CDRI small molecule repository[9] and database consist of 15,659 molecules out of which 15,452 conform to the modified Lipinski's rule of 5.[10] Only the filtered subset of 15,452 molecules was used for the screening. Virtual screening with UNITY using ligand-based pharmacophore model yielded 95 hits that met the specified requirements. Finally, protein structure-based molecular docking was used to dock each 'hit' to the active site of chorismate mutase and
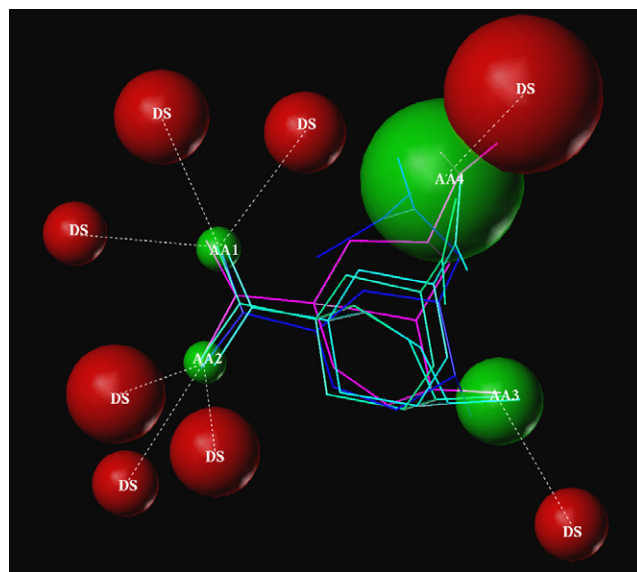

Figure 3. The GASP model for Chorismate mutase inhibitors containing four acceptor atoms shown in green spheres. Red spheres represent the donor sites. The sphere sizes indicate query tolerances.

to rank the binding affinities. FlexX [11] based molecular docking study was carried out to perform scoring and ranking of the hits obtained from database searching. FlexX method of molecular docking involves incremental construction of ligands from smaller fragments in the cavity of a receptor. All the hits obtained in database searching were docked into the inhibitor binding site in the X-ray crystal structure of MtCM (PDB Accession No. 2F6L). The active site in the MtCM was searched using SiteID module of Sybyl7.1[12] and the probable active site determination was accomplished based on previously reported structural information.[6] For a comparative analysis of the hits obtained in database
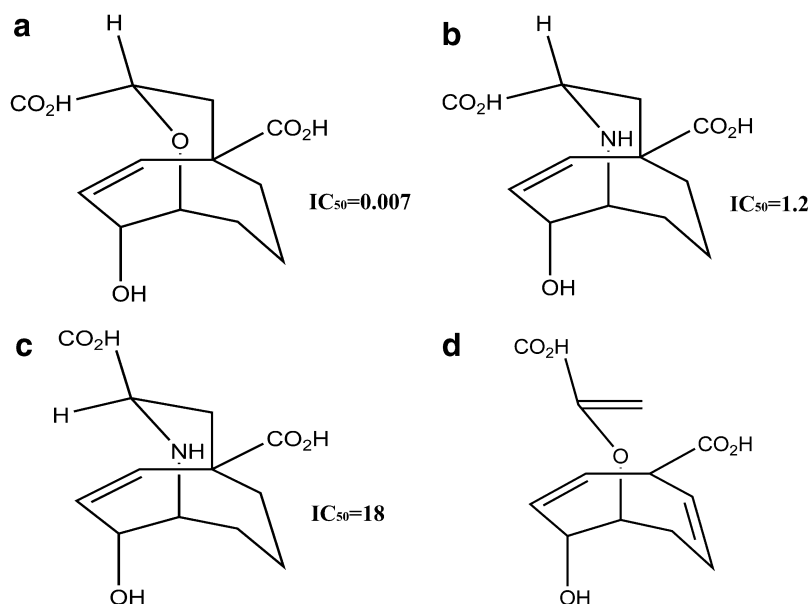

Figure 2. *Saccharomyces cerevisiae* chorismate mutase inhibitors (a–c) and the substrate chorismic acid (d) used for pharmacophore model generation.