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Computational Screening-Based Drug Designing Against Cyclopropane Mycolic Acid Synthase 1 (Cmaa1) Protein of *Mycobacterium Tuberculosis*

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Abstract:
Mycolic acids are an important component of cellular wall in *Mycobacterium tuberculosis*. CmaA1, one of the mycolic acid cyclopropane synthases which is responsible for cis-cyclopropanation at the distal position of α-mycolates. It has been experimentally shown that over-expression of the protein CmaA1 makes the bacteria resistant to hydrogen peroxide, suggesting that the cyclopropanation at the distal positions may be an important adaptation of *M. tuberculosis* against oxidative stress. In this study, Computer Aided Drug Designing approach was employed by considering CmaA1 as target. Three small molecules i.e a1, a2 and a3) were found to be interacting with the active site of the CmaA1 with the highest binding affinity -10.17, -10.17, and -10.12 respectively.

Keywords: *Mycobacterium tuberculosis*, cyclopropane mycolic acid synthase 1, Virtual screening, AutoDock

Introduction

Mycolic acids constitute significant constituents of the cellular wall in *Mycobacterium tuberculosis*. Numerous investigations suggest that functional moieties within the acyl chain of mycolic acids play a crucial role in the pathogenicity and persistence of the bacterium. Three distinct mycolic acid cyclopropane synthases, namely PcaA, CmaA1, and CmaA2, have been identified as the enzymes responsible for these specific modifications of mycolic acids[1].

The cell wall of *Mycobacterium tuberculosis* comprises three types of mycolic acids: α-mycolates, keto-mycolates, and methoxy-mycolates. Among these, α-mycolates are the most abundant and significant, typically consisting of carbon chains averaging 70-80 atoms in length[2,3]. Plethora of studies has shown that in pathogenic *Mycobacterium tuberculosis*, the majority of unsaturated α-mycolates undergo cyclopropanation of the double bonds. This process is mediated by a family of enzymes known as cyclopropane synthases, which utilize S-adenosyl-L-methionine (SAM) as the methyl donor. These enzymes catalyze the cyclopropanation at specific positions on the unsaturated mycolates[1,4-6] and essential for the proper functioning of mycolic acids[7]. CmaA1 is an

enzyme classified as a mycolic acid cyclopropane synthase, specifically facilitating cis-cyclopropanation at the distal position of α-mycolates[4,8].

The high-resolution crystal structure of *M. tuberculosis* CmaA1 has been previously published[9]. The computer aided Drug Designing approach more efficiently uses the crystal structures which propels the *in-silico* screening of chemical compound libraries[10-11]. Therefore, in this study, we attempted to identify compounds that were capable of interacting with the mycobacterial CmaA1 active site by combining through a considerably large virtual compound library using in silico SBDS with an intent to examine the antimycobacterial activity of the selected compounds.

Materials and methods

3D structure of CmaA1

The crystal structure of cyclopropane mycolic acid synthase 1 (CmaA1) was obtained from RCSB PDB database (1KPH) [9,12]. The structure related information was collected from RCSB PDB database. The information related to sequence and functional properties were retrieved from UniProt database with id P9WPB7.

Small molecule library preparation

In this study, small molecules from seven databases (NCI, ASINEX, Chembridge, InterBioscreen, LifeChemicals, MayBridge, and Garlic compounds) underwent a three-level screening process. Initially, Lipinski's rule of five and ADMET prediction were employed, with compounds passing Lipinski's rule further evaluated for ADMET properties using the Discovery Studio 3.5 suite. Subsequently, toxicity of all molecules was predicted, and prior to virtual screening, small molecules were prepared using Discovery Studio Suite (3.5). A detailed description of this process is available in our previous study[13].

Small molecule library screening

Virtual screening plays a vital role for assessing the binding affinity of potential lead compounds against the target protein of interest. In this study, virtual screening was carried out using the Raaccon platform and AutoDock tools in together[14]. Gaestier charges were added to all small molecules and ten confirmations were generated for each small molecule.

Results and Discussion

Importance of CmaA1 for drug designing

CmaA1, one of the mycolic acid cyclopropane synthases which is responsible for cis-cyclopropanation at the distal position of α-mycolates. It has been experimentally shown that over expression of the protein CmaA1 makes the bacteria resistant to hydrogen peroxide, suggesting that the cyclopropanation at the distal positions may be an important adaptation of *M. tuberculosis* against oxidative stress[4,8]. Hence CmaA1 is an important target for anti-tuberculosis therapy[14-15].

3D structure and active site, virtual screening

The 3D structure of the CmaA1 was downloaded from the RCSB PDB database for virtual screening and the binding site residues of S-adenosyl-L-homocysteine active compound with CmaA1 was considered as active site for drug designing[9]. The S-adenosyl-L-homocysteine compound was actively forming Hydrogen bonds with Glu-124, Trp-123, Leu-95, Thr-94, Gln-99, Gly-74, Tyr-33, Ser-34, Gly-72, Ile-136 and Hydrophobic interactions with Trp-123, Leu-95, Phe-142 residues of CmaA1. The docking grid box was prepared with the vicinity of the above-mentioned

residues for performing the virtual screening. For each compound 10 active docking confirmations were obtained.

Highest binding energy and interactions

Three small molecules, designated as a1, a2, and a3, were identified to actively bind to the binding site cavity, exhibiting the highest binding affinities of -10.17, -10.17, and -10.12, respectively. A stringent cut-off for binding affinity, specifically greater than -10 kcal/mol, was employed to select the most promising candidates. Compound a1 establishes Hydrophobic interactions with Val-71, Leu-95, Ala-138 and Phe-142; hydrogen bonds with Leu-93, Glu-124 and His-141; and Salt bridges with His-8 and Arg-146 residues. Compound a2 establishes Hydrophobic interactions with Val-71, Leu-93, Leu-95, Ala-138 and Phe-142; hydrogen bonds with Thr-94, Leu-95 and Glu-124; and Salt bridges with His-8 residues. Compound a3 establishes Hydrophobic interactions with Val-71, Leu-93, Leu-95, Ala-138 and Phe-142; hydrogen bonds with Leu-93, Thr-94, Leu-95 and Glu-124; and Salt bridges with His-8 residues (Table 1). The binding site residues of the three compounds (a1, a2 and a3) exhibit similarity to the residues in the S-adenosyl-L-homocysteine binding site. Apart from these all-docked structures were clustered in one orientation (Figure 1).

Table 1: Binding site residues of compound a1, a2 and a3 with CmaA1 active site

Name	Binding Energy	Interacting residues after docking		
		Hydrophobic Interactions	Hydrogen Bonds	Salt Bridges
1363 (a1)	-10.17	Val-71, Leu-95, Ala-138, Phe-142	Leu-93, Glu-124, His-141	His-8, Arg-146
1813 (a2)	-10.17	Val-71, Leu-93, Leu-95, Ala-138, Phe-142	Thr-94, Leu-95, Glu-124	His-8
1339 (a3)	-10.12	Val-71, Leu-93, Leu-95, Ala-138, Phe-142	Leu-93, Thr-94, Leu-95, Glu-124	His-8

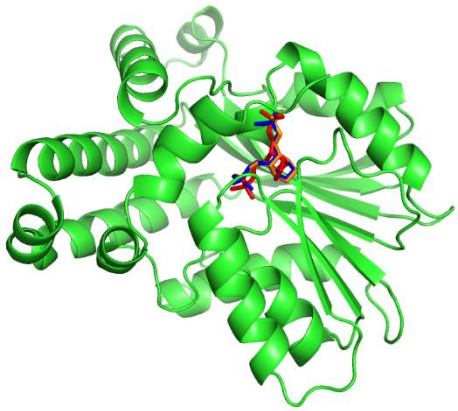

Figure 1: The superimposed structure of docked compound a1(red), a2 (orange) and a3 (blue)

Conclusion

CmaA1 is a critical target for anti-tuberculosis therapy. In this study, we identified three compounds, designated as a1, a2, and a3, which effectively bind to the active site of CmaA1. These compounds are promising candidates for further screening through *in vitro* and *in vivo* validation.

Conflict of Interest: Nil

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