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ORIGINAL ARTICLE

Molecular designing and Virtual Screening Based Drug design for MABA Enzyme of Mycobacterium Tuberculosis

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ABSTRACT

Objective: Computer Aided Drug Designing of the NAP binding site of MabA enzymes.

Methodology: Mycobacterium tuberculosis causes global infectious disease tuberculosis, which has remained among the top 10 causes of death worldwide. The emerged multidrug-resistant TB (MDR TB) and extensively drug-resistant TB (XDR TB) makes this problem more complex. β-ketoacyl-ACP reductase (MabA), a member of the type-II fatty acid elongation system (FAS-II) is primarily involved in the creation of very long chain fatty acid derivatives. These derivatives are essential precursors for mycolic acids, primary constituents of M. tuberculosis cell wall. Here we have adopted the computational approach followed by ligand molecular modelling, ligand screening, virtual screening and binding pattern analysis in search of potential new lead compounds for Mycobacterium tuberculosis MabA protein.

Results: The ligands were screened using an integrated computational protocol that relies on virtual screening and ADMET analysis methods. In this study, we found two compounds, i.e., Compound a1 and a2, which showed the highest binding energy and established the hydrogen bond with the MabA enzyme.

Conclusion: Computer Aided Drug Designing approach revealed that two compounds (a1 and a2) had the potential to interact with the NAP binding site of MabA enzymes.

Keywords: β-ketoacyl-ACP reductase, MabA, Computer Aided Drug Designing, ADMET

INTRODUCTION

The infectious disease known as tuberculosis (TB), is caused by the bacteria Mycobacterium tuberculosis (Mtb), and is one of the main causes of death around the world. As per the worldwide tuberculosis report, there were a projected 1.3 million fatalities caused by tuberculosis among HIV-negative people in the year 2021. This number was seen to be 1.3 million in 2020 and 1.2 million in 2019 [1]. In addition to large numbers of infections and deaths, the blooming of multi-drug-resistance-TB (MDR-TB) together with extensively-drug-resistant-TB (XDR-TB) and Rifampicin-resistant-TB (RR-TB) are causing this more contagious and complex. Therefore, there is an urgent need to screen/identify novel drug candidates targeting Mtb to combat this situation.

Mycobacterium tuberculosis has an intricate cell wall that is made up of four different types of biomolecules: mycolic acids, lipoarabinomannan, arabinogalactan, and peptidoglycans[2]. Mycolic acids, the long-chain fatty acids (C_{74} – C_{90}), are the building blocks of mycobacterial cell walls and play a crucial role in stabilizing the cell envelop permeability and bacterial survival[3]. The biosynthesis of mycolic acids generally relies on two canonical enzymatic systems; fatty acid synthase-I (FAS-I) and FAS-II systems[4]. The FAS-I system in Mtb is composed of a large multifunctional protein complex that synthesizes short-chain fatty acids ($C_{16/18}$ – $C_{24/26}$). Subsequently, the FAS-II system (which is comprised of a group of four enzymes and incapable of de novo synthesis) elongates short-chain fatty acids ($C_{16/18}$, generated by FAS-I) to long-chain meromycolic acid (C_{56}) and then forms mycolic acid by Claisen condensation reaction[5].

In the FAS-II system, the β-ketoacyl-ACP synthase III (FabH) primes the fatty acid biosynthesis by catalyzing the condensation of malonyl-ACP and palmitoyl-CoA. Once the β-ketoacyl-ACP is generated, the NADPH-dependent β-ketoacyl-ACP reductase (MabA/FabG1) reduces to β-hydroxyacyl-ACP. Sequentially, the β-hydroxyacyl-ACP dehydratase complex (HadAB/BC) dehydrates β-hydroxyacyl-ACP into trans-2-enoyl-ACP, which then is reduced by NADH-dependent enoyl-ACP reductase (InhA) to produce an acyl-ACP with two additional carbons and meromycolic acid. Consequently, two β-ketoacyl-ACP synthases (KasA/B) catalyze acyl-ACP and malonyl-ACP, and then the cycle continues iteratively to produce an elongated acyl-chain. Altogether, InhA, MabA, HasB, and KasA are reported to be critical for bacterial growth[6].

Increasing evidence suggests that most small-molecule inhibitors are synthesized to inhibit the activity of specific receptors associated with the Mtb cell wall biosynthesis. For example, the anti-tuberculosis drug isoniazid and two other drugs (ethionamide and prothionamide) inhibit InhA[7]. MabA is an essential gene that resides adjacent to InhA in the Mtb genome and plays a crucial role in the second step of the fatty acid biosynthesis elongation cycle. Further, the structural similarity of MabA with InhA, and its affinity for long-chain substrates, paves the way towards developing a MabA-specific substrate analog. In this study, we have adopted virtual-based approaches to dig out potential lead compounds for Mtb-MabA, which may lead to developing anti-mycobacterial drug candidates soon.

METHODS

Wild type MabA 3D structure: The protein sequence of MabA was retrieved from the UniProt database (P9WGT3). A multiple template approach was adopted to remodel the MabA structure MODELLER[8] software was used to generate the 3D structure of the protein. We used both 1UZL (wt) and 1UZN (mt) structures for remodeling of MabA WT structure. A total of one hundred different 3D models were created, and the one with the highest DOPE score was selected. Further, the selected model was refined using the Galaxy Refine server[9].

Preparation of compound library: Small molecules (1,792,771) used for virtual screening in this investigation were obtained from seven different databases i.e., NCI, ASINEX, Chembridge, InterBioscreen, LifeChemicals, MayBridge and Garlic compounds. The small molecules were screened using a three-level method. The small molecules were scrutinized for the first level based on Lipinski's five rules. Further, the screened compounds were filtered by calculating ADMET properties using the ADMET predictor module implemented in Discovery Studio3.5 (DS) computer suite (Accelrys Inc., SanDiego, CA, USA). The ADMET properties such as blood-brain barrier penetration (BBB), hepatotoxicity, aqueous solubility, cytochromeP450 (CYP450) 2D6 inhibition, plasma protein binding (PPB), human intestinal absorption (HIA) was calculated to select the compounds.

Additionally, the toxicity of all ligands was predicted through Toxtree software[10]. Only low and intermediate classes are taken for further study. Again, these ligands were tested for the mutagenicity module of the discovery test for DS 3.5. The compounds that passed mutagenicity module test were prepared with "prepare ligand" module in DS 3.5. Parameter values for "Change Ionization, Generate Tautomers and Generate Isomers"

were false. The prepared ligands were further taken for virtual screening.

Virtual Screening: In the virtual screening approach, the ligand molecules were screened against the MabA protein. Racoon module was used for virtual screening, which is in-built with Autodock Vina[11,12]. The compounds with binding energy (cut off: -8.0 kcal/mol) were categorized based on their chemical properties, ligand efficiency, and ligand binding pattern to ensure their stability in the binding cavity.

RESULTS

3D modeling of MabA: A significant challenging problem in characterizing the protein functions has been studied through both the sequence and structural levels. The three-dimensional structure of a protein and its surface topography can deliver vital information to understanding the role of protein. Hence, the structural information of Fabg1 (MabA) was collected from the protein databank (PDB) database. Wild type and mutant structure of MabA protein were retrieved from the PDB database. As the WT structure is incomplete, the 3D structure was remodeled using the MODELLER software. The comparative modeling approach was employed to determine the wild-type structure's absolute coordinate. The mutant structure of MabA (1UZN) was used as a template to determine the whole wild-type structure.

Virtual screening and Classification of ligands: Cohen-Gonsaud et al. (2002) have demonstrated that MabA inhibitors can be designed to interact with apo-form and lock it; to instigate accurate conformational change and inhibit its function[13]. The nicotinamide-adenine-dinucleotide phosphate (NAP) binding site of the MabA (Asn24, Arg25, Ile27, Ala36, Ala37, Arg47, Asp61, Val62, and Gly90) was identified using the protein Ligand Interaction Profiler (PLIP) software[14]. Based on the binding site residues, a grid was generated to determine xyz coordinates around the enzyme's binding site. Altogether, 1792771 ligands were downloaded from different databases and subjected to Lipinski Rule and ADMET filtration process. In this process, 92356 retains and subjected to mutagenicity and toxicity test. This filtration process generated 3700 ligands prepared by Discovery studio and kept for virtual screening analysis. After the virtual screening, the best ligand was selected based on the binding energy score of the ligand molecule. We have selected the top 3 ligands which having binding energy ≥ -8. Additionally, we checked the ligand efficiency score and binding pattern of the ligand molecules binding to the MabA protein cavity (Table 1).

Table 1: Docking results of screened compounds and detailed molecular interactions with MabA

Ligand Id	Binding Energy (kcal/mol	Ligand Efficiency	Interaction with amino acid			
			Hydrophobic Bonds	Hydrogen Bond	Salt Bridge	Pi-Cation Interactions
NAP bound crystal structure				Asn24, Ile27, Arg47, Asp61, Val62 and Gly90	Arg25 and Arg47	Arg47
NAP (Re Docked)	-8.23	-0.27		Thr21, Asn24, Arg25, Ile27, Arg47, Leu91, Thr188, Asp189 and Met190		
3447 (a1)	-8.38	-0.38	Val62	Ala89 and Leu91	Arg47	Arg47
3446 (a2)	-8.07	-0.36	Ile27 and Val62	Ala89 and Leu91	Arg47	Arg47
1065 (a3)	-8	-0.57	Val62, Ala111, Ala116, Phe137 and Val160		Arg47 and Lys157	

DISCUSSION

Significance of MabA as an attractive drug target: MabA, is a strong candidate for the rational development of anti-mycobacterial drugs because of its general specificities. Cohen-Gonsaud et al. (2002)[13] proposed three 3D structures (wildtype-1UZL, mutant2_Apo-1UZM, mutant2_Holo-1UZN). Poncet-Montange et al. (2007) [15] proposed the inactive triple mutant structure, which showed more negligible conformational dynamics in the protein form of the wild-type enzyme. Yet, four crystal structures have been published for MabA, which facilitates the structure-based drug design process. The wild-type crystal structure of MabA was not complete because this protein is not stable at room temperature[13]. In previous studies in silico approach was considered for the lead discovery of MabA enzyme [16,17]. Here, we remodelled the wild-type structure based on structural properties and considered it for computer-aided drug design studies.

The binding free energies obtained for NAP, a1, a2, and a3 with MabA were -8.23, -8.38, -8.07, and -8 kcal/mol, respectively. The docked ligand molecule was superimposed by PyMol software for 3D visualization (Figure 1). The binding energy of a2 with MabA was found to be higher than NAP, and other compounds' binding energy was also comparable with NAP (-8.23 kcal/mol). Redocked NAP established hydrogen bonds with the amino acid residues Thr21, Asn24, Arg25, Ile27, Arg47, Leu91, Thr188, Asp189, and Met190. However, Compound a1 and a2 established hydrogen bonds with Ala89 and Leu91. Leu91 residues established hydrogen bonds and were found to be shared among NAP, a1 and a2. Compound a3 did not show any hydrogen bonds with MabA, and it established hydrophobic amino acid residues Val62, Ala111, Ala116, Phe137, Val160, and salt bridge interaction with amino acid residues Arg47 and Lys157.

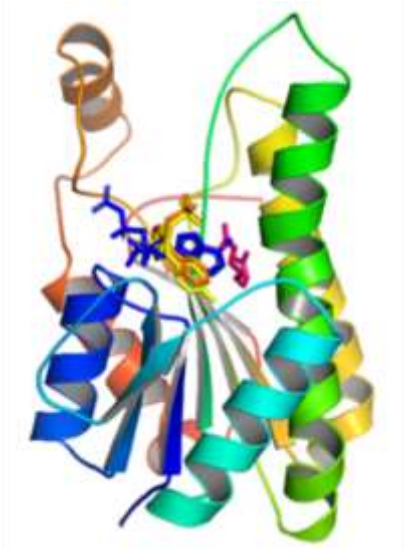

Figure 1: Superimposed 3D structure of the docked ligands with MabA enzyme (NAP-Blue, a1- Yellow, a2-Orange, a3- Red)

CONCLUSION

This Computer Aided Drug Designing approach revealed that two compounds (a1 and a2) had the potential to interact with the NAP binding site of MabA enzymes. The binding energy of screened compounds with MabA is comparable to the binding energy of NAP.

Conflict of interest: Nil

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