Structure based *de novo* design of IspD inhibitors as anti-tubercular agents

Rohith Anand Varikoti, Rahul P. Gangwal, Gaurao V. Dhoke, R.Venkata Krishnan, Abhay T. Sangamwar*

Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), Sect-67, S.A.S. Nagar, Punjab-160 062, India

* Corresponding author: Tel: 0172-2214682

E-mail: abhays@niper.ac.in

Abstract:

Tuberculosis is one of the leading contagious diseases, caused by *Mycobacterium tuberculosis*. Despite improvements in anti-tubercular agents, it remains one of the most prevalent infectious diseases worldwide, responsible for a total of 1.6 million deaths annually. The emergence of multidrug resistant strains highlighted the need of discovering novel drug targets for the development of anti-tubercular agents. 2-C-methyl-D-erythritol-4-phosphate cytidyltransferase (IspD) is an enzyme involved in MEP pathway for isoprenoid biosynthesis, which is considered as an attractive target for the discovery of novel antibiotics for its essentiality in bacteria and absence in mammals. In the present study, we have employed structure based drug design approach to develop novel and potent inhibitors for IspD receptor. To explore binding affinity and hydrogen bond interaction between the ligand and active site of IspD receptor, docking studies were performed. ADMET and synthetic accessibility filters were used to screen designed molecules. Finally, ten compounds were selected and subsequently submitted for the synthesis and *in vitro* studies as IspD inhibitors.

Keywords: ADMET, Anti-tubercular agents, *De novo* design, IspD inhibitors, Synthetic accessibility

1. Introduction

Mycobacterium tuberculosis (MTB), the causative organism of tuberculosis, is ranked the leading bacterial infectious agent [1, 2]. World Health Organization reported that there were about 6,50,000 cases of multidrug-resistant TB (MDR-TB) present in the world in 2010 and it is estimated that within 2020, approximately 1000 million people will be newly infected, over 150 million people will get sick, and 36 million will die of TB if proper control measures are not established [3]. Furthermore, the emergence of MDR-TB and extensively drug-resistant (XDR) TB is paving the way for almost untreatable tuberculosis. Therefore, the development of new drugs for the treatment of MDR-TB, XDR-TB, and latent TB is a priority task [4, 5]. As these drugs have to be selective for Mycobacteria, effective against drug resistant strains, and compatible with HIV-1 drugs, a novel mode of action is necessitated [6, 7].

Polyprenyl phosphate, an isoprenoid, carries activated sugar for the biosynthesis of arabinogalactan, arabinomannan and lipoarabinomannan (LAM), thereby supporting cell wall synthesis in Mycobacterium [8]. The side chain of menaquinone, a component of the electron transport chain of *M.tuberculosis*, is also derived from polyprenyl diphosphate. Thus, isoprenoids are vital for the survival of *M.tuberculosis*. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are synthesized via the methylerythritol phosphate (MEP) pathway, participate in the generation of isoprenoids in eubacteria [9]. Many enzymes are involved in MEP pathway, of which 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase (IspD) encoded by the Rv3852c gene is vital for the growth of MTB [10]. The IspD protein is a very promising drug target for anti-tuberculosis drugs as no ortholog of MTB IspD exists [10].

In the present study, we have employed structure based *de novo* design approach to design novel and potent inhibitors for IspD. Binding affinity and hydrogen bond interactions between the ligand and active site of IspD were explored by docking studies. Designed molecules were screened for ADMET and SYLVIA score. Finally, ten different molecules were selected as IspD inhibitors and subsequently submitted for synthesis and *in vitro* analysis.

2. Material and Methodology

2.1. *De novo* ligand design by LigBuilder

The overall work flow followed during the designing of IspD inhibitors is as shown in Fig. 1.

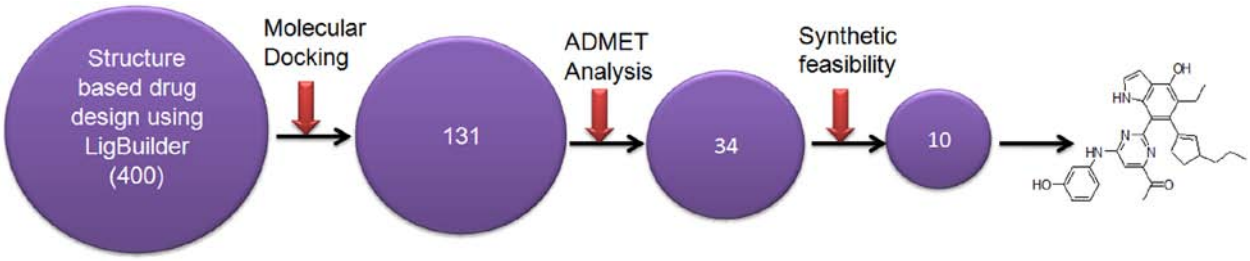

Fig. 1. The work flow followed during the designing of IspD inhibitors.

LigBuilder1.2[11] follows genetic algorithm to design ligands within the active site. It is based on 3D structure of the target protein. The binding affinities were scored using empirical scoring function. The bioavailability of the designed ligands was predicted using a set of chemical rules. The fitness score of the molecule is influenced by binding affinity and chemical viability. New molecules were designed from seed structure(s) within the active site of IspD. Pocket, grow and process modules were used for the analysis of the active site of the target and also in the preparation of input files for the designing of inhibitors. We set the parameters as MW: 200-500; logP: 5 to 8; Hbond donor: 1-5; Hbond acceptor: 1 to 10, for designing the novel ligands.

2.2. Molecular docking

Molecular docking studies were carried out to calculate binding affinities, to screen and to investigate the intermolecular interactions between ligands and active site residues of target protein. The binding affinities of known inhibitors were compared and, the new ligands were screened based on their interactions with amino acids of the active site. Input files for molecular docking analysis were generated using Protein Preparation Wizard and LigPrep modules. Hydrogen atoms were added to target, the protonation states for histidine residues were optimized and the hydrogen atom was minimized up to 0.30Å RMSD using the Protein Preparation Wizard. A grid of 10Å around the ligand centre was generated by Receptor Grid Generation tool using the prepared protein structure [12]. Extra precision (XP) Glide algorithm was validated by re-docking the co-crystallized inhibitor into the grid of target. Energy minimization of designed ligands was done using LigPrep with the OPLS_2005 force field [13]. Using the grid of the target protein, the XP mode of docking was repeated for each designed hit molecule using the grid of the target protein, as described above. The default settings for scaling the van der Waals radii were selected: a scaling factor of 0.8 and a partial charge cut-off of 0.25 with no constraints were defined during docking process [14].

2.3. Synthesis accessibility prediction

Synthesis accessibility of the designed compounds is a major factor to be determined in *de novo* designing. SYLVIA[15] software was used to determine the synthesis accessibility of our prioritized newly generated compounds. SYLVIA scores of compound range from 1 (easy to synthesize) to 10 (difficult to synthesize) based on synthesis accessibility. Different structural complexity measures, different measures of similarity to available starting material and retro synthetic reaction fitness were considered in this scoring function.

2.6. Similarity search

To confirm novelty in the screened hits similarity search was performed using PubChem and SciFinder scholar search tools. The compounds which showed partial similarity to hits were subjected to screening process using the molecular docking studies with the same parameters which were used to select the hit molecules from the virtual hits.

3. Results and Discussion

3.1 Design of new ligands

In our attempt of structure based *de novo* design of novel IspD inhibitors, we referred to the insights of molecular docking and pharmacophoric features. LigBuilder was implemented for designing of novel and potent inhibitors in the active site of IspD. It helps in analyzing the active site of the receptor and to derive the key interaction sites, which were further used for designing of new molecules based on the seed structure using grow strategy. From the literature source we found most of the anti-tubercular agents to be less potent against whole cells when compared to that against the purified protein *in vivo* [16]. This difference in activity might be due to the cell wall permeability factor in the whole cells. Thus, it is necessary that inhibitors have high permeability and there by its potency against whole cells will increase. Therefore, we have set logP values to 5-8. Genetic algorithm population size was set to 30000, and 200 genetic generations were carried out for designing new inhibitors. Fig. 2 shows the seed structure (aminopyrimidine) with growing points. A total of, 400 new compounds were generated by the grow strategy of LigBuilder from seed structures.

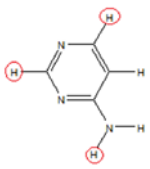

Fig. 2. The seed structure along with growing points used for designing novel IspD inhibitors

3.2. Molecular docking

The molecular docking analysis was performed using Glide5.5[14] to prioritize the designed virtual hits and subsequently to identify the false positives hits. All designed compounds were docked into the active site of IspD, and top 131 virtual hits have shown the similar binding pattern as that of the co-crystallized ligand. Fig. 3 shows the docking of the top virtual hit (compound 1) with docking score of -7.559 into the active site. It has shown binding to the hinge region by forming H-bonds with the peptide backbone of Arg20 and Lys215. It has also shown additional hydrogen bond with Thr86. Top virtual hits were showing numerous π-π interactions, most notably with Lys27 and Arg83 residue in the hydrophobic pocket.

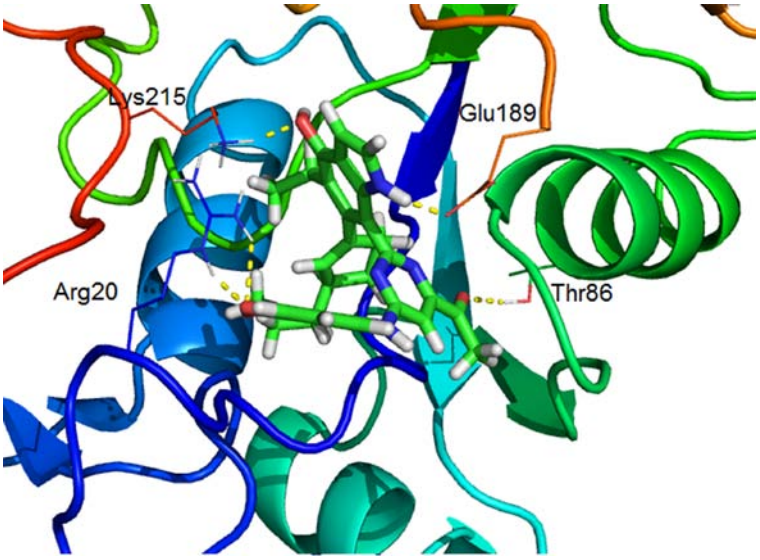

Fig. 3. The binding pocket of IspD (PDB ID: 2XWN) with docked conformation of the top virtual hit.

The molecules were further screened for ADMET properties using Accelrys Discovery Studio2.5[17] and Derek2.0 [18]. The synthetic accessibility of 34 prioritized designed virtual hits after ADMET analysis was evaluated by SYLVIA software. 26 compounds were showing SYLVIA score below 6, which suggests that all these compounds are easy to synthesize. After carefully analyzing the ADMET properties of 26 compounds obtained from docking and synthetic accessibility analysis, we finally considered 10 hits, which were not showing any toxicity or less toxicity as predicted by DEREK (Fig. 4). The Glide score, SYLVIA score, and LogP values of top ten virtual hits were as shown in Table 1.

Table 1. The Glide Score, SYLVIA Score and LogP values of top ten virtual hits.

Compound	Glide Score	SYLVIA Score	LogP
1	-7.827	5.88	5.52
2	-6.854	4.76	5.43
3	-6.773	5.69	5.19
4	-6.764	5.68	5.16
5	-6.611	5.88	5.87
6	-6.526	5.03	5.41
7	-6.466	4.94	5.26
8	-6.337	5.69	5.29
9	-6.314	5.99	6.06
10	-6.164	4.64	6.25

The PubChem search tool was used to confirm the novelty of the designed hits. The docking scores for the virtual hits were far superior to that of the partially similar compounds. Molecular docking studies also proved that these compounds were not suitable to form hydrogen bond interactions with the critical amino acid residues like Arg20 and Lys215. The novelty of the top virtual hit was further confirmed by the SciFinder scholar search tool. Hence, we suggest that these top virtual hits with diverse scaffolds are novel as IspD inhibitors.

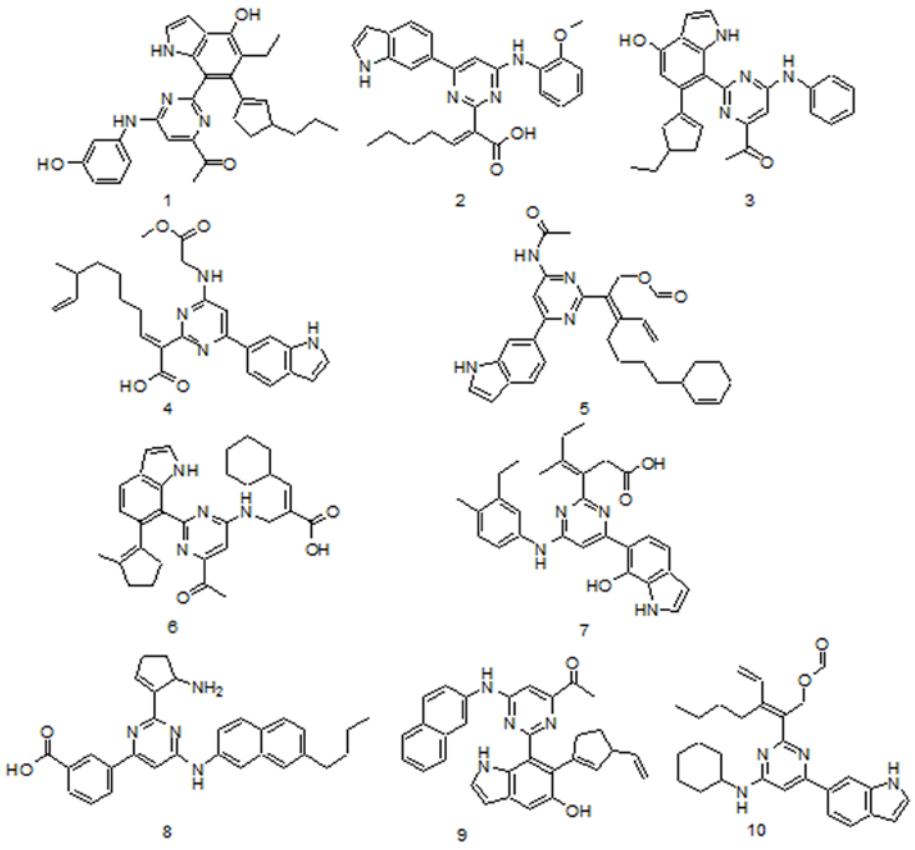

Fig. 4. The chemical structure of the top ten designed virtual hits.

4. Conclusions

In this study, we applied structure based drug design approach to develop IspD inhibitors as anti-tubercular agent using LigBuilder1.2. The designed virtual hits were selected as positive hits based on the molecular docking analysis. ADMET and SYLVIA score analysis were performed to identify top virtual hits as IspD inhibitors. Novelty of top designed virtual hits was confirmed with PubChem and SciFinder scholar search tools. Combining all these results, ten virtual hits were presented as possible lead candidates to be used as novel and potent IspD inhibitors. Further *in vitro* testing of hit compounds would be necessary to confirm the success rate of this work and to optimize the hits subsequently.

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