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In silico targeting methylerythritol phosphate pathway IspD enzyme of *Mycobacterium tuberculosis* for novel anti-mycobacterial drug discovery

Pratik Kumar[1], Maneesh Kumar[1], Harish Kumar[1], Sindhuprava Rana[2], Jainendra Kumar[1], Ganesh Chandra Sahoo[3*]

[1]Department of Biotechnology, Magadh University, Bodh Gaya, India.
[2]Department of Epidemiology, ICMR National Institute for Research in Environmental Health, Bhopal, India.
[3]Biomedical Informatics Center, ICMR Rajendra Memorial Research Institute of Medical Sciences, Patna, India.

ABSTRACT

The incidence of drug-resistant tuberculosis (TB) is the biggest challenge for the global TB control. Currently, resistance has been detected for almost all key anti-TB drugs. Therefore, an approach for a novel drug discovery with new targets is urgently required. A computational study was carried out to target 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase Isopentenyl pyrophosphate (IspD), an enzyme of the methylerythritol phosphate pathway that is essential for mycobacterial survival. Molecular docking was carried out with the available ligands using Discovery studio version 3.5. Among these ligands, rosuvastatin (RST) emerged as one of the suitable compounds against the enzyme that significantly interacted at the active site of the enzyme with the highest LibDock score of 121.08. Gly16, Arg83, Thr84, and Thy190 are potential amino acid residues which contributed to the protein–ligand interaction. The significant interaction between IspD and RST suggested the potency of the ligand in curing TB.

INTRODUCTION

One-third of the global population has been latently infected with TB (LoBue *et al.*, 2009). The WHO reported that an estimated 10 million people fell ill with TB worldwide, and India accounted for 27% of the global TB cases in the year 2018 (World Health Organization, 2019). TB is predominantly an infectious disease caused by a group of mycobacterial species called *Mycobacterium tuberculosis* complex. It is primarily a pulmonary pathogen but can infect other parts of the human body (Jassal and Bishai, 2009; Kumar *et al.*, 2017c). The incidence of drug resistance against key anti-TB drugs is the leading challenge for the global TB control (Centers for Disease Control and Prevention, 2006; Gandhi *et al.*, 2006; Prasad, 2010; Wright *et al.*, 2009; Zignol *et al.*, 2016). The combination therapy of

anti-TB drugs and directly observed treatment short-course was an excellent initiative to prevent the emergence of drug-resistant TB (Johnson, 2007). However, drug-resistant TB has emerged and its prevalence is increasing day by day. Improper, incomplete, or interrupted treatment courses with anti-TB drugs are the major reasons behind the acquired drug resistance (World Health Organization, 2008; Borrell and Gagneux, 2009; World Health Organization, 2014). This situation leads to MDR-TB, where the bacilli resist both rifampicin and isoniazid, which has been virtually reported in all settings. The second-line anti-TB drug treatments for MDR-TB cases are comparatively less effective with more side effects (Falzon *et al.*, 2017). In 2015, the WHO estimated that there were 580,000 new cases of MDR-TB and that 250,000 MDR-TB deaths occurred globally. The estimated global MDR/RR TB burden was 4.1% for new cases and 19% for previously treated cases for the year 2016 (World Health Organization, 2017). Among there MDR, 8.5% extensively drug resistant TB (bacilli having additional resistance with fluoroquinolones and one of the second line injectable anti-TB drugs) and 22% fluoroquinolones resistant cases have been reported in the global TB report 2018 (World

Corresponding Author
Ganesh Chandra Sahoo, Biomedical Informatics Center, ICMR-Rajendra Memorial Research Institute of Medical Sciences, Patna, India.
E-mail: ganeshiitkgp @ gmail.com

Health Organization, 2018). Human immunodeficiency virus coinfection with TB made the situation comparatively more hazardous than active TB infection alone (Gagneux, 2012).

Mycobacteria have MEP pathways that are involved in the production of a group of compounds called isoprenoids. The group consists of more than 40,000 compounds that play a crucial role in the growth and development of living organisms (Li and Wang, 2016). These compounds function as both primary and secondary metabolites (Frank and Groll, 2016; Sacchettini and Poulter, 1997). These isoprenoids are significantly involved in cellular metabolism, such as electron transport system, cell respiration, photosynthesis, membrane biosynthesis, cell signaling, and so on (Bouvier *et al.*, 2005; Heuston *et al.*, 2012). MTB has essential isoprenoids like menaquinone (which participates in oxidative phosphorylation) and polyprenyl phosphate (which synthesizes the components of the cell wall like arabinogalactan and lipoarabinomannan) (Bouvier *et al.*, 2005; Brennan, 2003). These types of isoprenoids are synthesized by polymerization of five carbon isoprene units of IPP and its isomer dimethylallyl pyrophosphate (Rodrıguez-Concepción and Boronat, 2002). The known biosynthesis pathways for isoprenoids in humans and bacteria are different. In humans, it follows the mevalonate pathway, while in bacteria they follow the MEP pathway. Additionally, it is interesting that the enzymes involved in the MEP pathway have no homology with the proteins of humans (Sassetti *et al.*, 2003). Several enzymes are involved in the MEP pathway; however, IPP is observed as one of the essential enzymes for the survival for the mycobacterium (Eoh *et al.*, 2007; Eoh *et al.*, 2009). This detail prompts us to consider the IspD enzyme as an ideal target for inhibiting the mycobacterial MEP pathway and to develop antimycobacterial molecules (Eoh *et al.*, 2009). With regard to MDR-TB, there is a need for a novel drug discovery with a new approach which can be used in the combination therapy to shorten the duration of treatment (Andries *et al.*, 2005; World Health Organization, 2014).

In this study, we have evaluated and analyzed the specific interactions of the IspD enzyme with the available ligands by using the *in silico* molecular docking tool. Despite having fosmidomycin and aryl bis-sulfonamide as efficient inhibitors against IspD (Zhang *et al.*, 2011) and IspF (Thelemann *et al.*, 2015), other molecules are also being considered in this study to observe their possible interactions with MTB IspD.

METHODS AND MATERIALS

PDB file retrieval

The PDB structure of the *M. tuberculosis* IspD enzyme was complex with positive control (CTP) and Mg²⁺ ion. The 3Q7U. pdb was retrieved from the research collaboratory for structural bioinformatics website (Berman *et al.*, 2000). The mycobacterial IspD is a homodimer, wherein each subunit consists of 231 amino acid residues. Each subunit contains two crucial domains, which includes the larger globular domain and the smaller β-domain. Here, the chain A of 3Q7U.pdb file was selected for the

computational study. The β-domains of the monomers are mainly responsible for the formation of the dimer. The attached CTP and Mg²⁺ ion were deleted from the structure, followed by refinement of the protein subunit.

Protein simulation

The catalytic pocket, which actively participates in interaction with ligands, mainly consists of polar amino acid residues. The 3-D structure was further refined by using the CHARMM inbuilt in DSv3.5, which minimizes the energy of the macromolecule (Brooks *et al.*, 1983; Kumar *et al.*, 2017a; Visualizer, 2012). A total of 13 ligands along with one CTP were used for this study. The 3-D structures of these ligands were taken from PubChem in the SDF file (Kim *et al.*, 2016; Rana *et al.*, 2017). The details of the ligands with their PubChem CID codes are listed in Table 1. Further simulation of the protein was carried out by using the SHAKE algorithm. The simulation process was carried out in 2000 steps of the steepest descent minimization techniques at 300K (Kräutler *et al.*, 2001; Kumar *et al.*, 2017b).

Conserved domain identification and binding site

Each protein has its conserved domain in which the ligands show their chemical activities. All the ligands were optimized before docking by using the prepare ligand tool DSv3.5. The ligand-binding site module is an authentic program that can predict and can suitably characterize the binding site module with all its functional residues in the protein by using the DSv3.5. The entire amino acid sequence of 3Q7U_A was selected and with the help of the CHARMM force field, and the protein coordinate was prepared. The binding site for the natural substrate was selected for docking (Kant *et al.*, 2018).

Docking process

Molecular docking was carried out on the prepared protein and stimulated ligands using the DSv3.5 computational package. Docking occurred between the prepared protein and ligands at the defined site (coordinates: X-26.622, Y-4.23, and Z-14.34) by using the LibDock algorithm. The selected ligands were processed and optimized before docking. The protein interacted with each optimized compound in several poses, and their interactions are evaluated in terms of the LibDock score. The highest score for a specific ligand projects the best suited pose. The LibDock possesses specific physicochemical properties of the ligands that direct docking according to the equivalent features present in the protein binding sites. The docking analysis discovered that rosuvastatin (RST) is the best scoring molecule that can effectively inhibit the domain of the IspD enzyme (Kumar *et al.*, 2018a; Kumar *et al.*, 2019; Kumari *et al.*, 2019; Mahato *et al.*, 2017).

RESULTS AND DISCUSSION

Genetic mutation is assumed to be the reason behind most of the drug resistance cases. For example, rifampicin

targets the β-subunit of the RNA polymerase and eventually blocks transcription. The *rpoB* gene identifies the β-subunit of the RNA polymerase. The specific genetic mutation in the sequence of the *rpoB* gene can make the bacilli resistant to rifampicin. Additionally, antibiotic members of the same group

of drugs having the same target can show a certain level of cross-resistance. Therefore, it is more important to find new targets for drug development against TB. The present research study targeted another enzyme, i.e., IspD, which is essential for bacterial survival.

Table 1. List of ligands used for molecular docking and their highest dock score with IspD.

S.No.	Ligands	CID No.	Dock score
1	CTP	6176	161.03
2	Rosuvastatin	446157	121.08
3	Ketoclomazone	12811046	100.84
4	1H-Pyrrolo(2,3-b)quinoxaline	3046546	97.89
5	9H-Pyrrolo[2,3f]quinoxaline	45121556	86.37
6	D-erythritol 1-phosphate	11820149	84.58
7	But-3-enyl diphosphate	46236598	83.77
8	Pyrrolo[1,2-a]quinoxaline	67476	80.32
9	Propyl Trihydrogen diphosphate	448670	79.86
10	Schembl1651692	46236597	78.82
11	Methyl hydroxytriazaindolizine	75629	76.99
12	Fosmidomycin	572	76.8
13	Sulfanilamide	5333	72.45
14	Triazolopyrimidine derivative	330031	71.3

Modeled protein prediction and assessment using homology modeling approaches

Chain A of the 3-D structure of 3Q7U has been used in this study. The monomer chain A has one active site in which the optimized ligands were docked for their optimum activities. The structure has seven alpha helixes (α), three small 3_{10} helixes (η), ten beta sheets (β), and three flexible loops (FL) (Fig. 1). The sequence of amino acids residues is shown according to Kabsch and Sander's method in Figure 2. The 3D–1D score assessed by Verify3D described 85.84% of the amino acid residues, with an average 3D–1D score of ≥ 0.2. Notably, the overall model excellence was illustrated by the ERRAT plot analysis, which showed that nearly 91.705% of the residues were found below the 95% error cutoff limits (Fig. 3). The 2-D protein structure showed interactions between Gly16, Arg83, Thr84, and Tyr190 with RST (Fig. 4). It also showed weak interactions that probably govern the binding of the RST to the catalytic pocket in IspD. The receptor which binds with ligand formed six intermolecular hydrogen bonds with electrostatic interaction and Van der Waal's
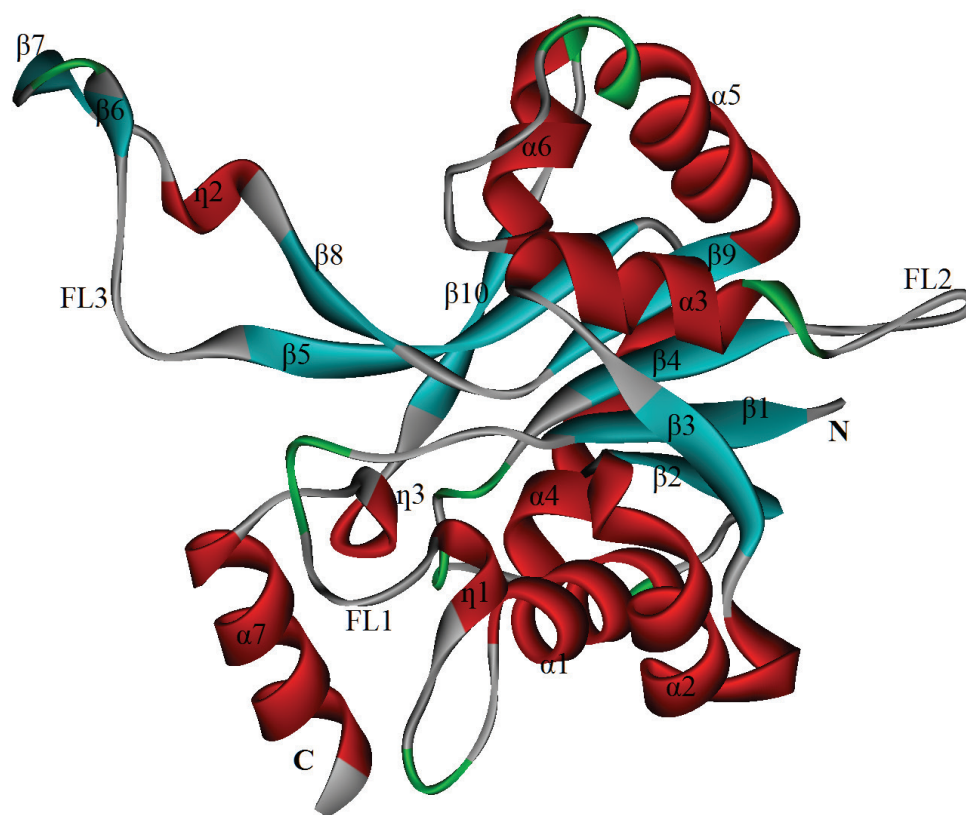

Figure 1. Three dimensional model of the IspD protein produced by the DSv3.5. α helices are in red, β sheets are in blue, and FL are in grey.

force, which enabled suitable interaction between the protein and the ligand.

Protein–ligand interaction

The in-depth *in silico* analysis revealed the prepared model suitably interacted with the proposed ligands. The protein–ligand interaction was assessed and validated by the DSv3.5. The ligands and protein subunit were optimized, and molecular docking was carried out at the natural active site of the protein. A range of dock scores were observed for each ligand with different poses. The focus was on the ligand with the highest dock score after positive control. We observed that RST had the highest LibDock score of 121.08. RST is a member of the statin family that is used to control the formation of cholesterol in humans. It competitively inhibits the HMG Co-A reductase, a critical enzyme of the mevalonate pathway for isoprenoids production (Prinz *et al.*, 2008). Details of all the studied ligands with their maximum LibDock score are shown in Table 1.

The modeled IspD protein strongly interacted with the RST ligand. The crucial active site residues Gly16, Arg83, Thr84, and Thy190 played a vital role in the protein–ligand stabilization process (Fig. 5). The amino acid residue Gly16 was present in the FL1, and Arg83 and Thr84 were found within the α3-helix. The

surface view of the RST–IspD interactions is shown in Figure 6. Ketoclomazone and 1H-pyrrolo-(2,3-b)-quinoxaline are the other essential compounds with an LibDock score of 100.84 and 97.89, respectively, followed the RST–IspD interaction. The atomic interaction between the RST and IspD is presented in Table 2.

Obiol-Pardo *et al.* (2010) modeled the IspD of *M. tuberculosis* to derive a reliable model for use in the structure-based drug design. Varikoti *et al.* (2012) attempted to structure the *de novo* design of possible inhibitor molecules for docking at the active site of the IspD enzyme. The docking score observed for RST in this study was slightly lesser than that observed for the natural substrate. However, it does not reflect the affinity of RST to be less than CTP for this protein. We used the IspD structure that was already bound to the CTP and then removed the CTP. Therefore, there are conformational changes in the IspD structure that is bound and unbound to CTP. Recently, the growth retarding effect of RST has been observed on the MTB culture (Kumar *et al.*, 2018b).

CONCLUSION

The current work suggests that RST is a potent molecule for binding the enzyme IspD to other vital molecular interactions at its active site. However, at present, new drugs for TB treatment
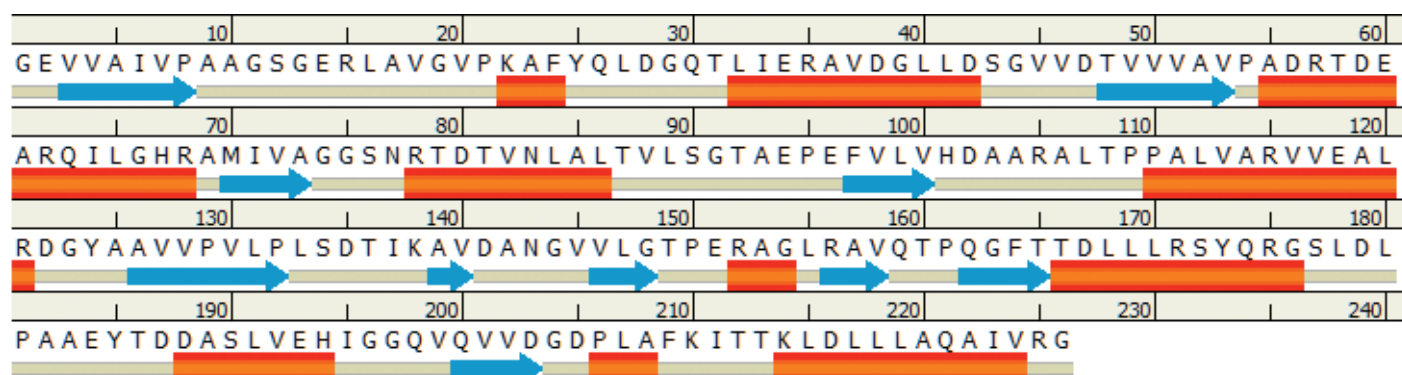

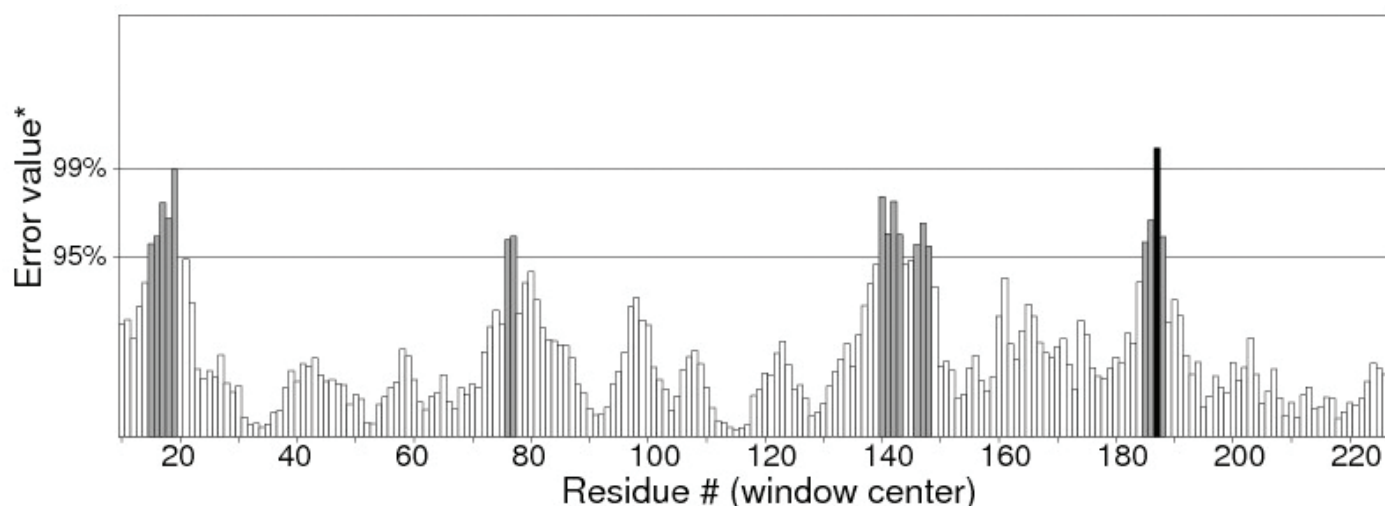**Figure 2.** Amino acid sequence of 3Q7U.

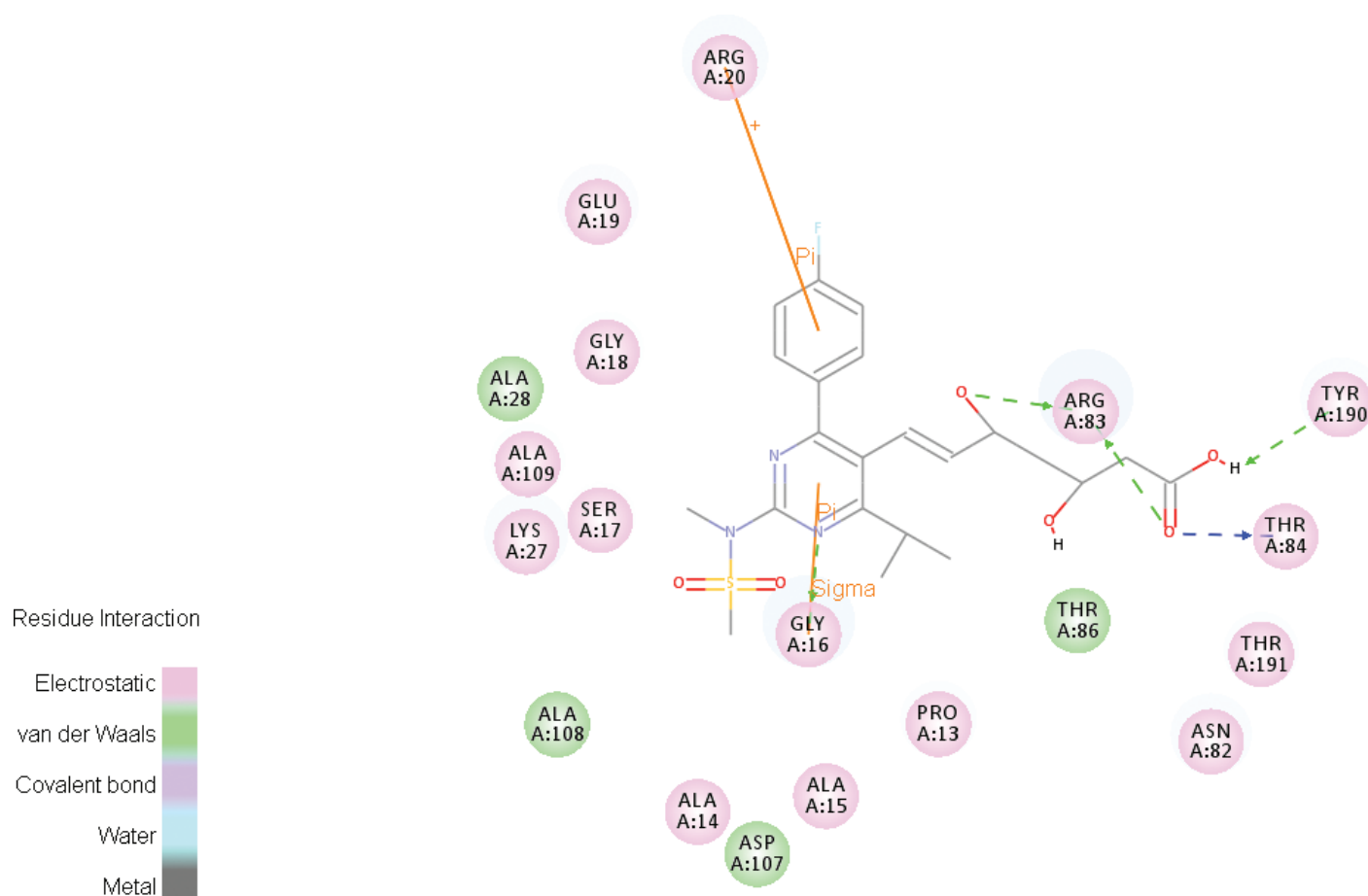**Figure 3.** Overall quality checked by ERRAT.

Figure 4. The two-dimensional IspD–RST interaction.
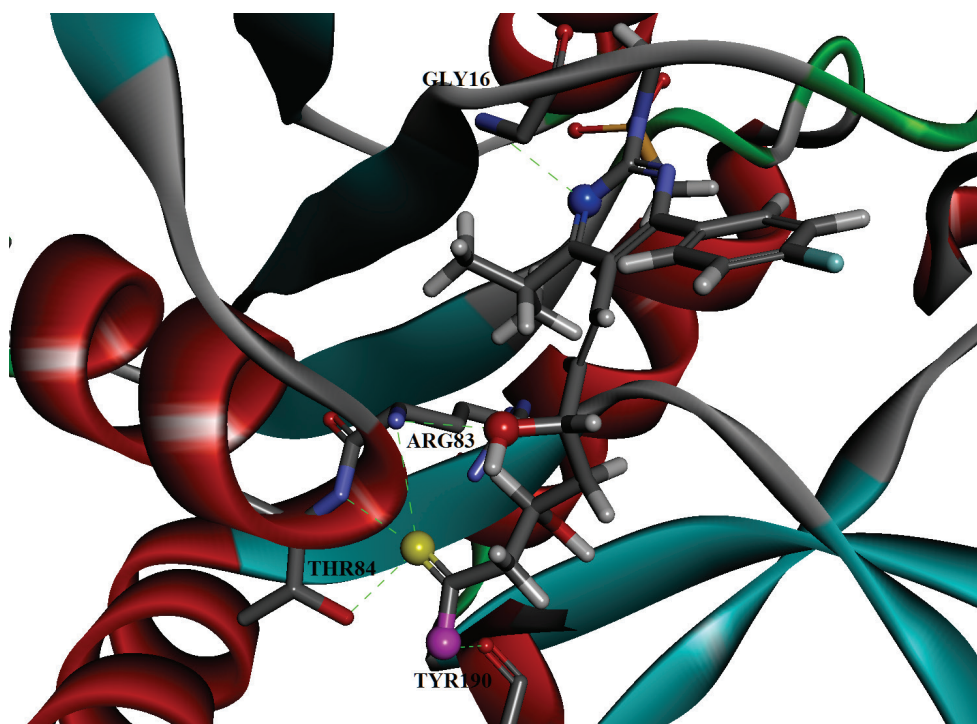

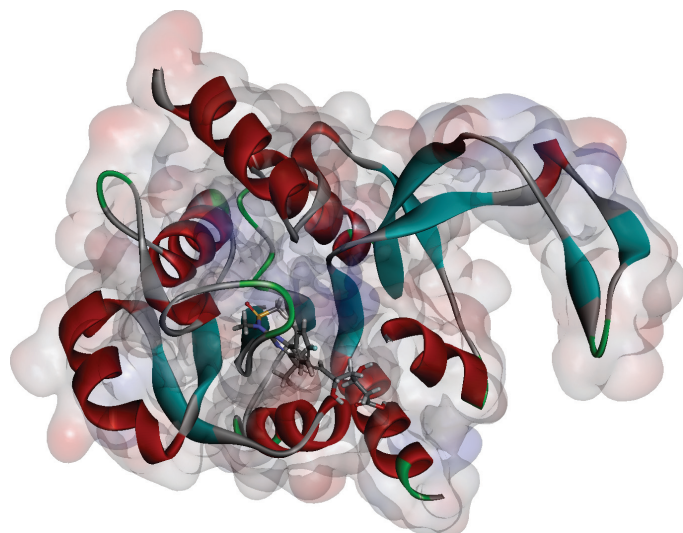**Figure 5.** Protein–ligand interaction of IspD–RST showing intermolecular H-bonding.

Figure 6. Interactions of IspD and RST (surface view).

Table 2. Estimated intermolecular H-bonding between RST and IspD.

Hydrogen bonding	Donor	Acceptor
A:GLY16:N – 446157:N9	N	N9
A:ARG83:N – 446157:O3	N	O3
A:ARG83:N – 446157:O8	N	O8
A:THR84:N – 446157:O8	N	O8
A:THR84:O – 446157:O8	O	O8
A:TYR190:O – 446157:H36	O	H36

are urgently required due to the rapid emergence of drug resistance against available anti-TB drugs and identification of RST, or its structural analog, as a novel anti-TB agent, can play a key role in eliminating TB worldwide. However, the study on the binding RST to other sites of the enzyme and further *in vitro* evaluations will be the future prospects of research.

ACKNOWLEDGMENTS

ABBREVIATIONS

CID: Compound identifier; DSv3.5: Discovery studio version 3.5; IPP: Isopentenyl pyrophosphate; IspD: 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; MDR-TB: Multidrug-resistant TB; MEP: Methylerythritol phosphate; MTB: *Mycobacterium tuberculosis*; RNA: Ribonucleic acid; RST: Rosuvastatin; TB: Tuberculosis; WHO: World Health Organization. PDB: Protein Data Bank; CHARMM: Chemistry at Harvard Macromolecular Mechanics; SDF: Spatial Data File; HMG: 3-hydroxy-3-methyl-glutaryl.

CONFLICT OF INTEREST

Authors declared that there are no conflicts of interest.

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