

ANTI-TUBERCULOSIS DRUGS AGAINST MYCOBACTERIUM TUBERCULOSIS PknB AND MUTANTS L33D/ D76A – A COMPARATIVE DOCKING STUDY

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ABSTRACT

Objective: *Mycobacterium tuberculosis*, the causative agent of dreadful disease tuberculosis causes two million deaths per year. PknB a class of transmembrane Ser/Thr protein kinase plays crucial role in bacterial viability and regulation in cell wall synthesis. Though anti-tuberculosis drugs are more effective in tuberculosis treatment, but due to mutations drug resistance is commonly noticed in many cases.

Methods: In order to study the inhibition activity of anti-tuberculosis drugs with PknB, we performed virtual screening and molecular docking analysis with native PknB and two mutants L33D/ D76A. Virtual screening was done with four drugs in AutoDock Vina and initial screening was done based on binding affinity.

Results: Rifampicin and ethambutol showed more binding affinities towards mutant strains rather than native PknB. Molecular docking studies on screened drugs clearly showed inhibition activity against PknB mutant strains with least binding energy and more hydrogen bonds.

Conclusion: Comparative docking studies on PknB native and mutant strains may provide novel hints for the drug design on *Mycobacterium tuberculosis* PknB for potent inhibitors and overcome the drug resistance.

Keywords: Tuberculosis, PknB, Native, Mutant and Molecular docking.

INTRODUCTION

Tuberculosis is the primary cause of mortality due to an infectious disease in the world today. The causative agent of tuberculosis is the intracellular pathogen *M.tuberculosis* [1]. Recent studies have focused on finding new pathways vulnerable to inhibition by small molecules and previously unexploited by drug discovery efforts [2]. The inhibition of signaling pathways both in *M.tuberculosis* and the host may yield new classes of drug targets and a large amount of recent studies are focused on developing this further [3].

M.tuberculosis, a pathogen able to adapt to the changing environmental conditions requires an efficient way of sensing and transducing extracellular signals[4]. For cell signals, the regulation of cell growth and cell division involving the reversible phosphorylation on serine/threonine residues is critical in the bacterium [5]. Kinases are attractive drug targets due to the range of crucial cellular processes in which they are involved. Previous studies have focused on the potential of PknB and PknG as drug targets in *M.tuberculosis*, although the majority has reported significantly less impressive activity against whole cells than against the purified protein in vitro [6]. In exponential growth stage of *M.tuberculosis*, PknB is predominantly expressed and PknB overexpression affects cell wall synthesis and cell division [7].

PknB, a transmembrane Ser/Thr protein kinase is considered as suitable target for tuberculosis[8]. PknB is predicted to consist of 626 amino acids with a transmembrane segment dividing the protein into an N-terminal intracellular domain and a C-terminal

extracellular domain. The N-terminal domain of PknB includes a kinase domain and juxtamembrane linker of 52 residues[9].

MATERIALS & METHODS

Dataset

Comparative molecular docking analysis was carried out with crystal structure of *Mycobacterium tuberculosis* PknB native (PDB ID: 1MRU) and mutants L33D/D76A (PDB ID: 3ORI /3ORM). The ligands AGS and Mg⁺² ions were removed from the PDB file of native. Protein molecule was prepared for docking analysis by adding kollman charges to both native and mutants protein and saved in PDBQT format. The dataset of four first line anti-tuberculosis drugs were selected from PubChem database. Gasteiger charges were added to the ligands and saved in PDBQT format.

Virtual screening

Virtual screening was performed in AutoDock Vina using PyMOL plugin (Table 1). Vina using input files in PDBQT format of both receptor and ligand. The volume of the box fixed to 27000Å to have large search space and box parameters of centre covered the active sites of ATP binding and size 60x60x60. The average accuracy of binding prediction was increased by assume docking as a stochastic global optimization of the scoring function, pre calculating grid maps and precalculating the interaction between every atom type pair at every distance. The parameter exhaustiveness made search algorithm better for accurate binding prediction. The results of Vina showed the binding affinity in kcal/mol.

Table 1: Virtual screening results from AutoDock Vina.

S. No.	Compound	Native PknB Binding affinity (kcal/mol)	Mutant L33D PknB Binding affinity (kcal/mol)	Mutant D76A PknB Binding affinity (kcal/mol)
1	Isoniazid	-4.0	-3.6	-3.8
2	Rifampicin	-8.6	-9.2	-9.7
3	Pyrazinamide	-3.9	-3.2	-3.5
4	Ethambutol	-3.7	-4.2	-4.0

Table 2: Molecular docking results of drug rifampicin from AutoDock

S. No.	PknB	Hydrogen bonds	Ligand interaction	Inhibition constant (nM)
1	Native	1	ARG161 NH1....O	562
2	Mutant L33D	4	VAL95N....O, GLN181OE1....H, TYR182 OH....O, ARG137 NE....O	480
3	Mutant D76A	4	VAL 95 O.....H, GLU 93 O.....N, GLN181 O.....N, ARG137 NE.....O,	468

Table 3: Molecular docking results of drug ethambutol from AutoDock

S. No.	PknB	Hydrogen bonds	Ligand interaction	Inhibition constant (nM)
1	Native	2	THR179 OG1....O, GLY 218 O.....H	460
2	Mutant L33D	3	VAL95O....N, GLN181OE1....H, TYR182 OH....O	420
3	Mutant D76A	5	VAL 95 O.....H, GLU 93 O.....N, GLN181 O.....N, ARG137 NE.....O, ARG137 NH2.....O	416

Molecular docking

Molecular docking in AutoDock 4.0 was performed for screening the drug rifampicin (Table 2) and ethambutol (Table 3) to confirm the binding mode and binding energy with PknB. AutoGrid program with searching function and AutoDock program using scoring function were run in order to obtain the docked complex of given input. The final best conformation of docked complex was selected based on least binding energy.

Visualization and analysis

The H-bond interaction was analyzed using PyMOL tool. The number of H-bonds is calculated between atoms of protein-drug complex. Based on the number of hydrogen bonds the effective binding was confirmed (Fig 1 & 2).

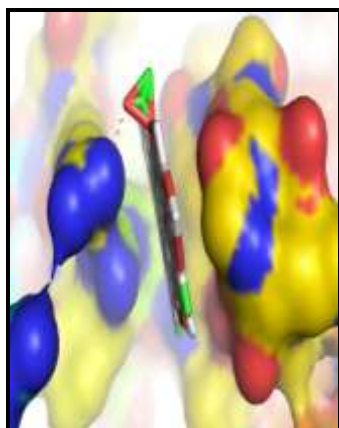


Fig. 1A: Docked complex of rifampicin with native. Hydrogen bond visualized in red dots

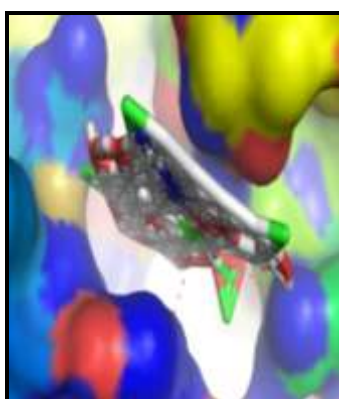


Fig. 1B: Docked complex of rifampicin with mutant L33D. Hydrogen bond visualized in red dots

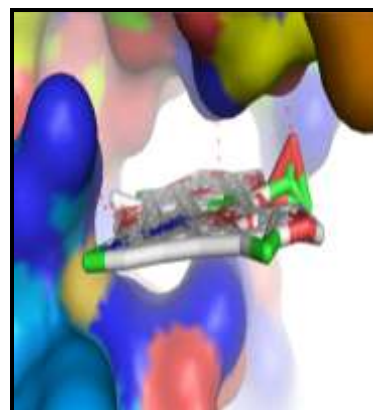


Fig. 1C: Docked complex of rifampicin with mutant D76A. Hydrogen bond visualized in red dots

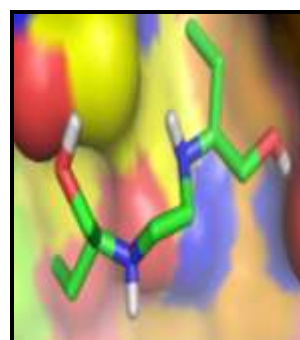


Fig. 2A: Docked complex of ethambutol with native. Hydrogen bond visualized in red dots.

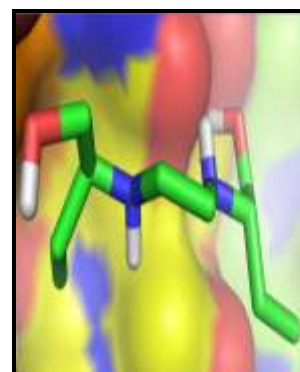


Fig. 2B: Docked complex of ethambutol with mutant L33D. Hydrogen bond visualized in red dots.

RESULTS & DISCUSSION

Comparative docking analysis with native and mutant is a feasible method to study the inhibition. The virtual screening results of anti-tuberculosis drugs showed the binding affinities towards native PknB and mutants. From Vina results, the drug rifampicin ethambutol docked well with mutants with least binding affinity than native. The other drugs isoniazid and pyrazinamide were better towards native but not much effective on mutants. Rifampicin was more effective with binding affinity in range -9 kcal/mol. Molecular docking was performed for screened two drugs and the results showed the binding energy and binding mode of the complex. The docking results of rifampicin were more effective on mutants with four hydrogen bonds and showed least inhibition constant. The key residues Val95 and Glu93 of *M.tuberculosis* PknB were also involved in the interaction which proved the inhibition activity of drug towards mutants. Like rifampicin, ethambutol also showed better inhibition activity on mutants with three and four hydrogen bonds respectively and with least inhibition constant. Overall, the anti-tuberculosis drugs rifampicin and ethambutol have tendency to inhibit mutant L33D/ D76A PknB effectively. Development of multiple drug resistance and continual mutation to the virulent strains of *Mycobacterium* species has urged the significance of research in this field of study[10].

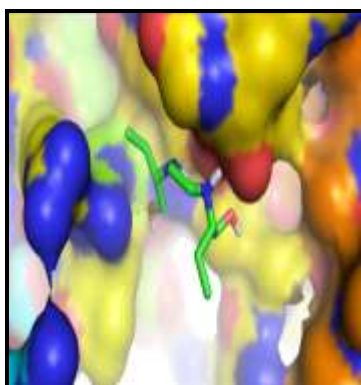


Fig. 2C: Docked complex of ethambutol with mutant D76A. Hydrogen bond visualized in red dots.

CONCLUSION

Significant research and development had reduced the spread of infection and mortality rate of tuberculosis; however, it still continues to remain as a major issue all over the world. Extensive research is focused on the structural aspect to determine potential

targets for drug development. Protein modeling is found to be a very significant and most reliable method for research and also shows promising results.

Our study concluded that the screened drugs may be suitable to overcome the drug resistance of bacteria due to mutation. Our computational studies concluded that the novel approach on *M.tuberculosis* PknB-drug complex towards mutants can be used for the treatment of tuberculosis.

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