Inhibition of *Mycobacterium tuberculosis* RpfB by Natural Compounds : A Computational Study

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Abstract

The majority of the world population (around 25%) has latent *Mycobacterium tuberculosis* (Mtb) infection, of which only 5–10% individual develop into active tuberculosis (TB) and 90–95% continue to have latent tuberculosis infection. This makes it the biggest global health concern. It has been reported that the resuscitation-promoting factor B (RpfB) is an exciting potential target for tuberculosis drug discovery due to its significant role in the reactivation of latent TB infection to active infection. Several attempts have been made to investigate potential inhibitors against RpfB utilising in silico approaches. The present study also utilized computational approach to investigate microbially derived natural compounds against the Mtb RpfB protein that is a very easy and cost effective. This evaluation used structure-based virtual screening, drug-likeness profiling, molecular docking, molecular dynamics simulation, and free-binding energy calculations. Six potential natural compounds, viz. Cyclizidine I, Boremexin C, Xenocoumacin 2, PM-94128, Cutinostatin B and (+)1-O-demethylvariecolorquinone A, were selected, which displayed a potential binding affinity between -52.39 to -60.87 Kcal/mol MMGBSA score and docking energy between -7.307 Kcal/mol to -6.972 Kcal/mol. All the complexes showed acceptable stability (<2.7 Å RMSD) during 100ns MD simulation time except the RpfB protein-xenocoumacin 2 complex. This result exhibited that the selected compounds have high efficiency in inhibiting the Mtb RpfB and can be taken into account for additional *in vitro* and *in vivo* experimental validation.

Introduction

Tuberculosis (TB) is an airborne, contagious infection caused by *Mycobacterium tuberculosis* (Mtb). *Mycobacterium tuberculosis* are generally aerobic, non-spore-forming, non-motile, acid-fast bacilli and have lipid-rich cell wall containing mycolic acid (Kanabalan et al., 2021). This lipid rich cell wall makes it resistant to many antibiotics. There are two different forms of TB infections: latent and active. Latent tuberculosis is a kind of illness in which the body can combat the bacteria and prevent them from invading . Most people approximately 5-10% of the latent tuberculosis have chances of getting active infection due to low immunity. Resuscitation-promoting factors (Rpfs) are the enzyme in the cell wall that alters the cell wall structure and helps in the resuscitation Mtb dormancy. Of these, RpfB has been reported as most potential anti-dormancy factor during resuscitation process. RpfB shows highest complexity in its structure, as it comprises three DUF348 domains and one G5 domain along with the catalytic domain. The G5 domain having adhesive property. DUF348 is commonly interacts with the G5 domain (Ruggiero et al., 2017). The catalytic domain has c-type lysozyme fold and lytic transglycolases, a potential hydrolases, that cleave the peptotidoglycan (PGN) layers. Hence, in order to avoid the resuscitation of dormant Mtb, the catalytic domain of RpfB could be an attractive target to design novel drugs. Nitrophenylthiocyanates (NPT), which has both anti-RpfB and anti-resuscitation properties. However, this is a synthetic inhibitor, which might have toxic side effects to the human (Demina et al., 2009). Therefore, designing of potent inhibitors with less toxicity is very important in the management of LTBI. In view of this, our study employed a biocomputational tool to find many other novel microbially derived compounds that has property to bind and inhibit RpfB protein.

Methodology

The crystal structure of RpfB protein (PDB ID 4KPM) retrieved from RCSB Protein Data Bank (PDB) database (https://www.rcsb.org/) .

4-Benzoyl-2-nitrophenylthiocyanate (Compound CID: 13699065), a well-known inhibitor of the RpfB protein, was selected as the reference compound (Ruggiero et al., 2013).

Natural Product Atlas (NP atlas) database (https://www.npatlas.org/) (van Santen et al., 2022) containing 32554 microbially derived natural compounds were selected for structure-based virtual screening (SBVS) against RpfB protein, were screened using the GLIDE tool in the Schrodinger suite (A et al., 2023). And, conserved amino acid residues **(Glu[292], Tyr[305], Val[309], Gln[310], Phe[311], Asp[312], Thr[315], Gln[347], Ala[351], Trp[352] and Pro[353])** of RpfB protein, which interacts with substrate were chosen for SBVS.

Here, the Ligprep tool was used to generate 173403 conformations for 32554 natural compounds of the NP Atlas library. SBVS was processed through 3 steps. Following sequential steps of HTVS, SP and XP screening, 43 compounds have docking score range of -8.571 to -6.972 kcal/mol, whereas MM/GBSA scores between -18.58 to -60.87 Kcal/mol was obtained.

Finally, the screened poses were utilised in order to calculate the binding free energy following default parameters of the (OPLS-2005) force field (Jorgensen & Tirado-Rives, 1988) in the Prime MM/GBSA (Molecular Mechanics/Generalised Born Surface Area) method (Jacobson et al., 2002).

Potential lead compounds were sorted in accordance with characteristics including drug-likeness, pharmacokinetics, physicochemical, and medicinal chemistry friendliness using the swiss ADME web tool (Daina et al., 2017).

Molecular dynamics simulation for selected complexes at 100ns was carried out utilising the academic package of Desmond-maestro 2020–4.

The calculation of molecular mechanics/generalized born surface area (MM/GBSA) was carried out using Prime MM/GBSA module in Schrödinger-Maestro using the default parameters of MM/GBSA protocol (*Schrödinger Release 2020-4: Prime, Schrödinger, LLC, New York, NY, 2020*, n.d.).

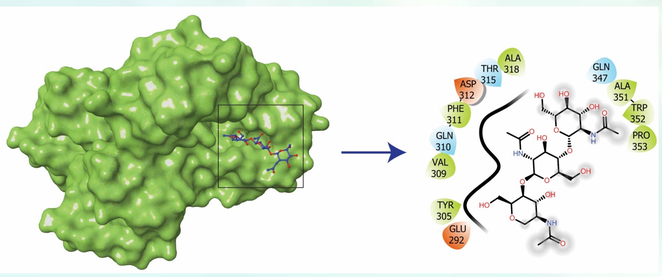

Figure 1. 3D structure of RpfB protein in complex with triacetyl-beta-chitotriose and it's 2D interaction in the active site of RpfB.

Results and Discussion

Table 1. List of top six selected virtual screened natural compounds against RpfB protein of *Mycobacterium tuberculosis*.

Title	Compound Name	Docking score (Kcal/mol)	MM/GBSA ΔG Bind (Kcal/mol)
NPA022565	Cyclizidine I	-7.307	-60.87
NPA031636	Boremexin C	-7.609	-60.33
NPA015250	Xenocoumacin 2	-7.553	-59.14
NPA008908	PM-94128	-7.894	-59.11
NPA014415	Cutinostatin B	-7.378	-57.01
NPA028225	(+)1-O-demethylvariecolorquinone A	-6.972	-53.57
CID: 13699065	4-Benzoyl-2-nitrophenylthiocyanate	-9.163	-44.42

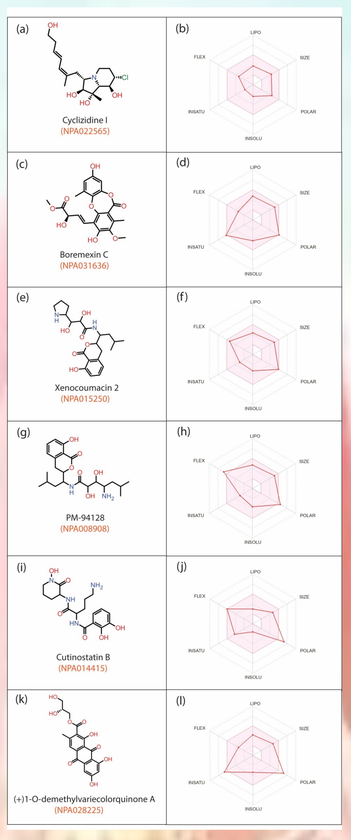

Figure 2. 2-Dimensional structures and ADME profiling of the selected microbially derived natural compounds, i.e., (**a**) Cyclizidine I, (**b**) Boremexin C, (**c**) Xenocoumacin 2, (**d**) PM-94128, (**e**) Cutinostatin B (**f**) (+) 1-O-demethylvariecolorquinone A.

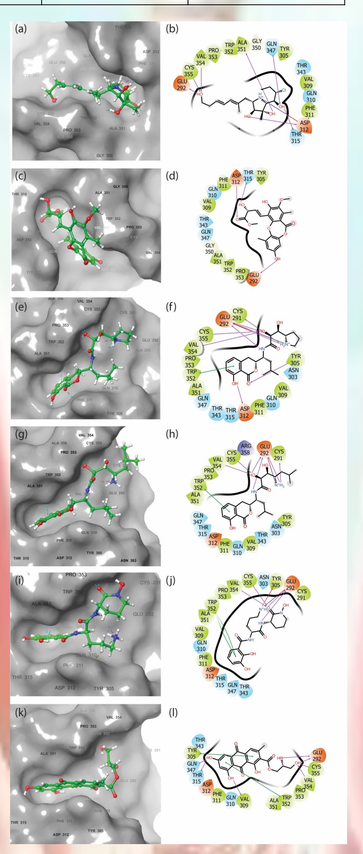

Figure 3. 3-D and 2-D docked complexes poses of the selected microbially derived natural compounds, Whereas in 2-D structures, H-bond formation (pink arrows), hydrophobic interaction (green), π–π stacking (green), π–cation interaction (red), polar residue (blue), negative residue (red), glycine (grey) and Salt bridge (red and blue) interactions are logged for docked complexes of RpfB protein with selected natural compounds.

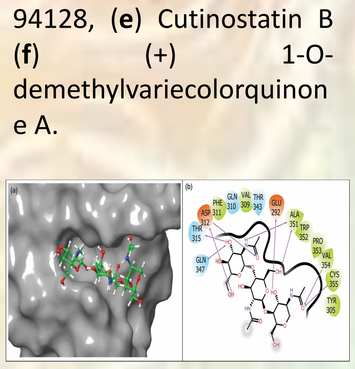

Figure 4. 3D and 2D docked complex poses of the reference complex, i.e. RpfB- 4-Benzoyl-2-nitrophenylthiocyanate complex. In 2D structures, hydrogen bond formation (pink arrows), hydrophobic (green), polar (blue) and red (negative) interactions are logged for docked complex.

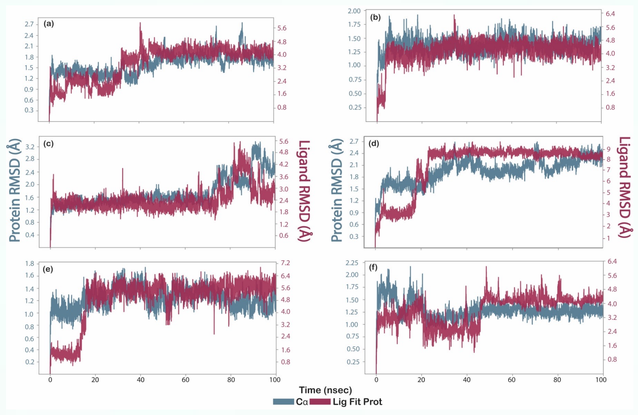

Figure 5. RMSD plots for the backbone atoms of RpfB protein and selected microbially derived natural compounds, fit on selected target protein were extracted from 100 ns MD simulation trajectories of different docked complexes.

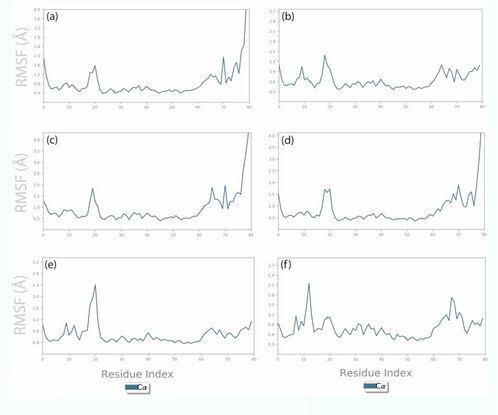

Figure 6. RMSF plot generated for the RpfB protein docked with selected natural compounds, i.e. (**a**) Cyclizidine I, (**b**) Boremexin C, (**c**) Xenocoumacin 2, (**d**) PM-94128, (**e**) Cutinostatin B, and (**f**) (+) 1-O- demethylvariecolorquinone A.

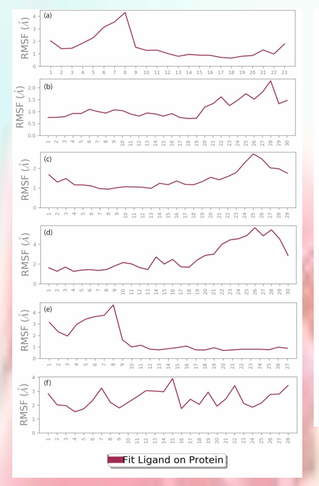

Figure 7. RMSF plot generated for the docked natural compounds, i.e. (**a**) Cyclizidine I, (**b**) Boremexin C, (**c**) Xenocoumacin 2, (**d**) PM-94128, (**e**) Cutinostatin B, and (**f**) (+) 1-O-demethylvariecolorquinone A, fit in the RpfB protein during 100 ns molecular dynamics simulation interval.

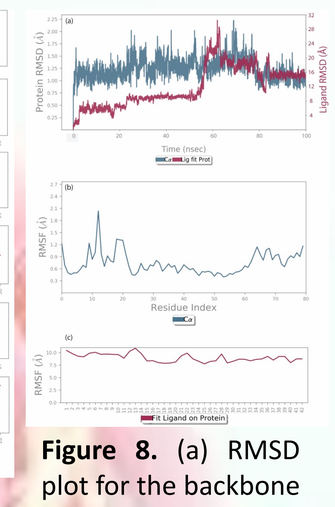

Figure 8. (a) RMSD plot for the backbone atoms of RpfB protein in complex with the reference molecule, i.e. 4-Benzoyl-2-nitrophenylthiocyanate, (b) RMSF plot generated for the RpfB docked with reference ligand, (c) RMSF plot generated for the docked reference compound fit in the RpfB protein during 100 ns molecular dynamics simulation interval.

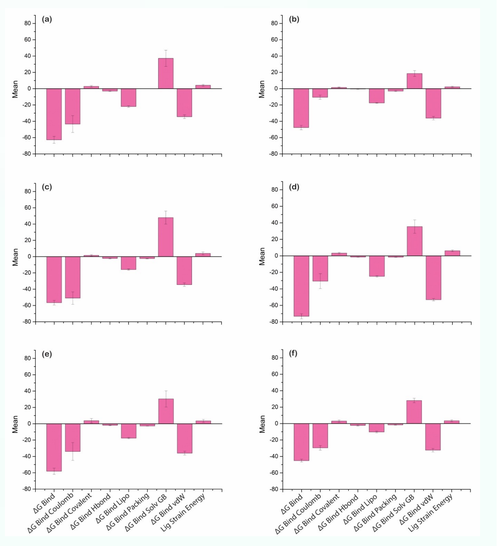

Figure 9. Calculated net Binding free energy and energy components values for RpfB protein complex with selected microbially derived natural compounds, i.e., (**a**) Cyclizidine I, (**b**) Boremexin C, (**c**) Xenocoumacin 2, (**d**) PM-94128, (**e**) Cutinostatin B and (**f**) (+) 1-O-demethylvariecolorquinone A, snapshots extracted from last nine ns MD simulation trajectory.

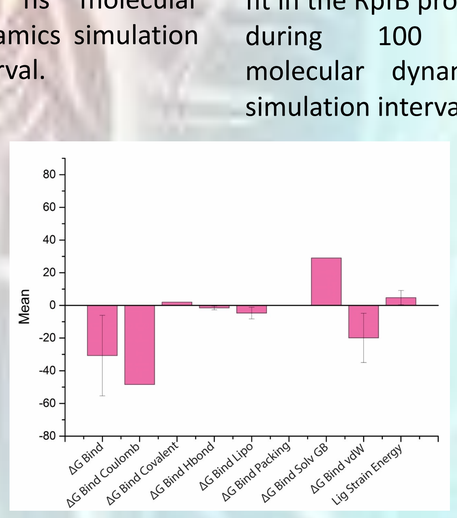

Figure 10. Calculated net Binding free energy and energy components values for RpfB protein complex with 4-Benzoyl-2-nitrophenylthiocyanate reference molecule snapshots extracted from last 9 ns MD simulation trajectory.

Conclusion & Future Aspects

This study used the NP Atlas library to assess the potential of natural substances against the RpfB protein using virtual screening, molecular dynamics, binding free energy and drug-likeness profiling. Here, six compounds were selected based on MMGBSA score through SBVS of natural compounds against RpfB protein. Moreover, MDS further revealed the stability of complexes with respective compounds. According to the analysis of docked complexes, specific natural compounds occupied the active site by forming Hydrogen bonds, Hydrophobic bonds, π–π stacking, π–cation interaction, and Polar, Negative, Glycine, and Salt bridges interactions with RpfB protein residues. Finally, these outcomes validated the Cyclizidine I, Boremexin C, Xenocoumacin 2, PM-94128, Cutinostatin B and (+)1-O-demethylvariecolorquinone A as potential inhibitors of RpfB and can be used for further in vitro and in vivo study to develop anti-dormant drug of TB for the complete elimination of tuberculosis.

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