

Virtual Screening of Interaction of rpoB gene to Secondary-Line Anti-Leprosy Drugs for Rifampicin Resistant *M. leprae* using In-Silico Approach

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Abstract

Leprosy, caused by the pathogen *Mycobacterium leprae*, is still a public health threat in some parts of the world. It has been recently noted that several *M. leprae* isolates have developed secondary resistance to WHO recommended MDT especially to its bactericidal drug, Rifampicin. In this work, *in silico* molecular docking was performed using Molegro virtual Docker docking server, in order to find the binding interaction and other properties of fluoroquinolones and minocycline which are used as secondary line drugs for *M. leprae* rpoB gene, resistant to Rifampicin and could be of great help in selection of secondary line of anti-leprosy drugs using rpoB coded protein as a potential target.

Keywords

M. leprae, rpoB, Drug resistance, Rifampicin, Fluoroquinolones, Minocycline, docking, ligand conformations; protein-ligand interactions; ligand-binding site

Introduction

Leprosy, a chronic infectious disease, is caused by an obligate intracellular pathogen- *Mycobacterium leprae* (*M. leprae*). Although the prevalence of this disease has significantly gone down after the introduction of WHO regimen of Multidrug Therapy (MDT), the incidence remains a constant peril with approximately 211, 973 cases reported globally in 2015 out of which 127,334 cases were reported from India alone^{1,2}. Multi-drug therapy (MDT) includes Rifampicin (RIF), Dapsone and Clofazimine in 1982 by WHO³. RIF is being used for the treatment of mycobacterial diseases such as tuberculosis and leprosy due to its efficient antimicrobial action. However, after its use for more than 3 decades' drug resistance to RIF in leprosy is being reported⁴⁻⁸. Considering that the resistance to RIF might pose a problem in the treatment of leprosy, a study on RIF and its interaction with resistant *M. leprae* mutants at the level of structure and function of bacterial DNA-dependent RNA polymerase (RNAP) and its involvement in the modulation of complex physiological pathways will be of utmost importance⁹. Mutations in the rpoB gene encoding the β subunit of RNA polymerase have been reported to result in resistance to RIF in several mycobacterial species including *M. leprae*^{10,11}.

Resistance to RIF is almost entirely coupled to mutations within a 279-bp region of the rpoB gene, called the RIF resistance-determining region (RRDR).

Since the late 1990s, multi-drug resistant (MDR) isolates of *M. leprae*, resistant to RIF and DDS, have emerged, the importance of Ofloxacin has been a focus for the treatment of MDR leprosy¹². Fluoroquinolones (FQs) are bactericidal antibiotics currently in use as second-line drugs in the treatment of TB and leprosy¹³. FQs inhibit type II DNA topoisomerases, including DNA gyrase and topoisomerase IV¹⁴. However, as *M. leprae* possess only DNA gyrase¹⁵, it acts as the sole target for FQs. Recently, use of Ofloxacin has been very common for treatment of other infections and therefore there is an urgent reason to the search for newer potent anti-leprosy drugs and is a challenge for scientific community.

During the past 20 years, a number of newer antimicrobial agents, among quinolones (e.g., Pefloxacin, Ofloxacin, Sparfloxacin, and Moxifloxacin), the macrolides (e.g., clarithromycin), and the tetracyclines (e.g., Minocycline), exhibited fairly good *in vivo* activity against experimental mouse footpad infections with *M. leprae* and in human trials^{16,17}. Among these newer antimicrobial agents, Moxifloxacin has been demonstrated to display very powerful bactericidal activity against *M. leprae* that is virtually identical to that of rifampicin¹⁸.

Minocycline, an alkylated amino-tetracycline, widely used for bacterial infections, has also shown to have bactericidal activity against *M. leprae* foot pad growth in mice¹⁹. Furthermore, *in vitro* studies²⁰ have also shown that minocycline inhibited the metabolic activity of *M. leprae*.

Lacking direct evidence for the mechanisms of *M. leprae*'s resistance to most of the anti-leprosy drugs, our current understanding is based on studies carried out in *M. tuberculosis*²¹ other bacteria, and a few studies with *M. leprae* genes in surrogate hosts²².

This study focusses here the bioinformatics approach for the *in silico* virtual screening of some anti-mycobacterial agents (Ofloxacin, Sparfloxacin, Moxifloxacin, and Minocycline) against rpoB protein in *M. leprae* using Molegro Virtual Docker

2011.5.0 (MVD). Generally, all RRDR mutations are coupled with different level of RIF resistance among clinical isolates. However, the Ser456Leu substitution, a most commonly found mutation, is correlated with a high level of resistance with molecular as well as mouse foot pad studies. Therefore, in the present study we used this mutant sequence to dock with secondary line of drugs to determine the best possible treatment for the RIF resistant *M. leprae*. In the present investigation, we screened fluoroquinolone such as Ofloxacin, Sparfloxacin, and Moxifloxacin and Minocycline based on better Interaction energy and MolDock score, to find out their efficiency on resistant strain through *in silico* study.

Methodology

Ethical Approval

This study was approved by the Ethical Committee of The Leprosy Mission Trust India (TLMTI)

Molecular modeling of protein and ligand

The structures of all five ligands namely Rifampicin, Ofloxacin, Moxifloxacin, Sparfloxacin, and Minocycline (Pubchem CID No. 5381226, 4583, 152946, 60464, and 54675783 respectively) were downloaded from the Pubchem in PDB format. Two forms of *M. leprae* rpoB protein were used in this project. One mutant type (Z-4, Ser456Leu; this mutation is confirmed by mouse foot pad assay to confer high level resistance for RIF. This strain is a kind gift from Dr. M. Matsuoka, Japan) and one wild type. Since to the best of our knowledge, no X-ray crystal structure has been reported for *M. leprae* rpoB, the amino acid sequence (279 residues) of *M. leprae*, wild type rpoB was retrieved from the UniProt protein sequence database (UniProt code: P30760) To generate three-dimensional models, we used a protein threading alignment program I-TASSER²². The I-TASSER algorithm first gives a prediction of the secondary structure from the sequence searched against a PDB library database of solved protein structures to identify the best possible templates. I-TASSER retrieves template proteins with similar folds from the PDB library, is used to build a three-dimensional structure. The confidence score (C-score) was used to evaluate the quality of the generated models. C-scores fall within the range of -5 to 0.95 with scores above -1.78 indicating correct model topology.

Structure Validation

The rpoB model was validated by using the RAMPAGE server which evaluates a protein's 3D-structure based on the stereochemistry quality of torsion angles and geometry²⁴. The Ramachandran plot showed 86.2% feasibility (69aa) in favorable positions and 12.5% (10 residues) in acceptable regions, with just 1.2% in an outlier region (1 residue) for mutant *rpoB* (Z-4), and 82.7% (62 aa) in

favorable regions and 17.3% (13 residues) in acceptable regions with no outliers for wild type, supporting the high quality of the 3D-model (Figure. 2).

Active Site Analysis and Binding Site Identification

Active site identification and cavity volume were measured based on the active site pocket size and shape as defined by MVD. The binding sites for the docking were predicted and cavities were chosen such that the entire receptor molecule was included within the selection grid (Table 1). Computer Atlas of Surface Topography of proteins (CASTp) which provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins has been used to find the pockets to see interacting amino acids in the wild type model. (<https://bioinformatictools.wordpress.com/tag/castp/>)

Results and Discussion

Docking Analysis

Molegro Virtual Docker 2011 5.0 (MVD) is widely used computer based tools to process virtual docking of small molecules (ligand) with enzyme. This program has number of tools to perform the docking process. We used homology modeling, protein threading, molecular docking and dynamics studies to investigate the structural and dynamic features of *M. leprae* rpoB protein of wild and mutant complexes. A three-dimensional model for rpoB was generated using the threading approach. The obtained 3D-model was stereo-chemically evaluated on RAMPAGE server²⁴ which provides a score based on proline and glycine preferential positions according to a Ramachandran plot. For docking analysis, the lowest energy most stable conformations of rpoB wild and mutant type as obtained from Molecular Dynamics simulations due to the stereo chemical quality of the structures.

Table 1: Volume of binding cavities detected

	Volume of the Binding Pocket(in Å)	
	WT	Z-4
Minocycline	24.064	31.744
Ofloxacin	20.48	27.136
Sparfloxacin	18.432	36.352
Moxifloxacin	21.504	31.849

Interaction Energy versus MolDock Score as Scoring Criterion

The binding interactions were characterized by the ligand binding energies against the rpoB model using the MVD. To investigate the effect of the internal energy of the receptor, the docking poses

previously calculated were rescored using the interaction energy between the receptor and the ligand instead of the total energy of the complexes. It was found that Rifampicin, Ofloxacin, Moxifloxacin, Sparfloxacin, and Minocycline have -185.131, -195.938, -195.086, -101.656, and -196.51 kcal/mol of internal energy correspondingly with wild type rpoB, which is much higher than the mutant form. **Table 2** shows the internal and interaction energies including the total, electrostatic and steric for all the residues in the active site of rpoB–inhibitor complex.

Analysis of Binding

Over all, Ofloxacin and Sparfloxacin had a lower binding affinity towards the mutated form of rpoB as indicated by the MolDock score of -80.8705 and -81.575 in comparison to the wild type protein, with which it had a docking score of -78.735 and -71.778 respectively. The docked conformation of mutated Ofloxacin and Sparfloxacin complex is overall similar. The results on the contrary obtained indicated a lower affinity of rpoB for the wild type protein in comparison to the mutant. The interaction energies of wild and mutant rpoB-inhibitors complex, for Moxifloxacin, Minocycline, Sparfloxacin and Ofloxacin (-13.511 and -14.3883), (-28.927 and -31.12), (-19.878 and -24.4193), and (-17.203 and -20.4404) respectively (Figure 3&4). From the results obtained, MolDock score of wild and mutant rpoB-inhibitors complex for Moxifloxacin (-81.576 and -98.043) Minocycline (-67.583 and -81.2871) Sparfloxacin (-71.778 and -81.5758) and Ofloxacin (-78.735 and -80.8705) were observed. These results revealed that Minocycline and Moxifloxacin are the best inhibitors for mutant (resistant) rpoB protein compared with the other drugs used in this study. Docking results demonstrated that hydrogen bond energy also plays a major role in making the binding stable; hydrogen bond interaction can act either as antagonist or agonist for a ligand with receptor. On

analyzing the docking results, hydrogen bond energies were found ranging from -2.1702 to -5.3383 for wild type and Mutant from -1.90915 to -5.443 for various inhibitors. Hence, the hydrogen bond energy interactions contribute extensively in the drug binding to the wild type rpoB protein Table 2.

Comparison between Moxifloxacin and Minocycline for resistant strain

The molecular modeling study revealed that the substitutions in the mutant rpoB protein lead to local perturbations in the protein structure and alter the chemical environment and geometry of the rpoB protein in terms of both shape and size. The resistant strain used in this study showed high sensitivity to both Moxifloxacin and Minocycline. Although Moxifloxacin is a drug of choice but Minocycline works better as anti-mycobacterial agent. This could be correlated with the higher interaction energies between the wild type and mutant rpoB receptors and Minocycline (-28.927 and -31.12kcal/mol) compared to the output from Moxifloxacin (-13.511 and -14.3883kcal/mol). Further, the hydrogen bond energy between Moxifloxacin-rpoB mutant type yielded the -4.199 kcal/mol whereas Minocycline shows a higher value of -5.443 kcal/mol. Figure 5 shows that there was not a large difference between the two drugs for mutant Z-4, and the wild type complexes as both the drugs possessed comparatively higher internal energies and hence showed better affinity for wild type-rpoB. This could also be extended to the observations that not only Moxifloxacin and Minocycline are the only drugs other than rifampicin to be consistently bactericidal against *M. leprae* in clinical trials^{25,26}. Moxifloxacin exhibited good bactericidal activity against multiplying organisms it had very limited sterilizing activity against persisters²⁷ while Minocycline displayed powerful bactericidal activity for such patients²⁸.

Table 2: Total Docking Energies between Wild and Mutant type RpoB with Inhibitors

Energy Change in Kcal/mol	WT				Z-4			
	Internal Energy	Interaction Energy	Hydrogen bond Energy	MolDock Score	Internal Energy	Interaction Energy	Hydrogen bond Energy	MolDock Score
Minocycline	-196.51	-28.927	-4.0049	-67.583	-112.407	-31.12	-5.443	-81.2871
Ofloxacin	-195.938	-17.203	-2.1702	-78.735	-101.311	-20.4404	-3.93097	-80.8705
Sparfloxacin	-101.656	-19.878	-5.3383	-71.778	-105.575	-24.4193	-1.90915	-81.5758
Moxifloxacin	-195.086	-13.511	-2.5648	-81.576	-112.432	-14.3883	-4.19972	-98.043

The new drugs for treatment in leprosy are mainly emerging from Tuberculosis research. In vitro

studies of the anti-mycobacterial activities of various quinolones have revealed the following. FQs

exhibit fairly potent anti-*M. tuberculosis* and in vitro anti-*M. tuberculosis* activities of new Quinolones', as determined by their MICs, are in the following order Sitafloxacin=Gatifloxacin =Moxifloxacin = Sprafloxacin> Levofloxacin = Ciprofloxacin > Ofloxacin. Their therapeutic efficacies against *M. tuberculosis* infection were in the order Moxifloxacin= Sprafloxacin= Levofloxacin>Ofloxacin>>pefloxacin²⁹.

In a few clinical trials in *M. leprae* infected mouse the order of *in vivo* activity was as follows Ethionamide <clarithromycin = minocycline = ofloxacin <rifampicin = moxifloxacin³⁰ which are much in concordance with the *in silico* results of the present study. The docking scores confirmed that Moxifloxacin had a better killing activity than both Sparfloxacin and Ofloxacin against *M. leprae*. Rise in Quinolone resistance is a concern, as ofloxacin is widely used for ordinary infections; and so suggests that quinolones may not be a suitable component of future development of anti-leprosy regimens.

This was strikingly evident from the study at The Leprosy Mission hospitals in India, where resistance to Ofloxacin has been frequently noted⁸. Therefore, studies on the efficacy of alternatives drugs are, therefore and search for newer drugs will be a major area for research. *In silico* analysis on these compounds helps us to understand the binding potential of compounds with the target enzyme of *M.leprae*.

Figure 1: Binding Interactions of RpoB (mutant strain, Z-4) with a) Ofloxacin; b) Sparfloxacin; c) Minocycline; d) Moxifloxacin

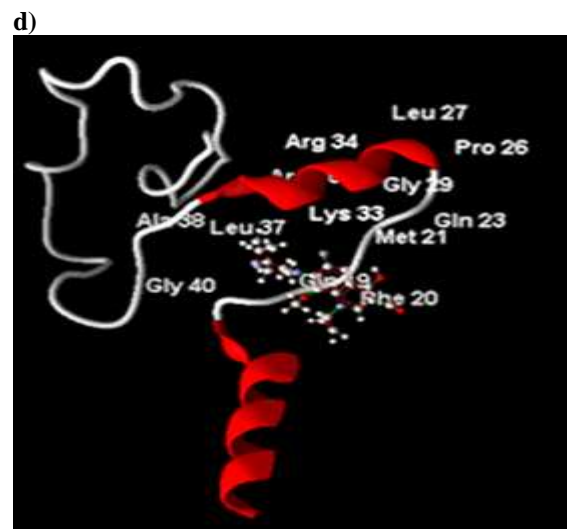
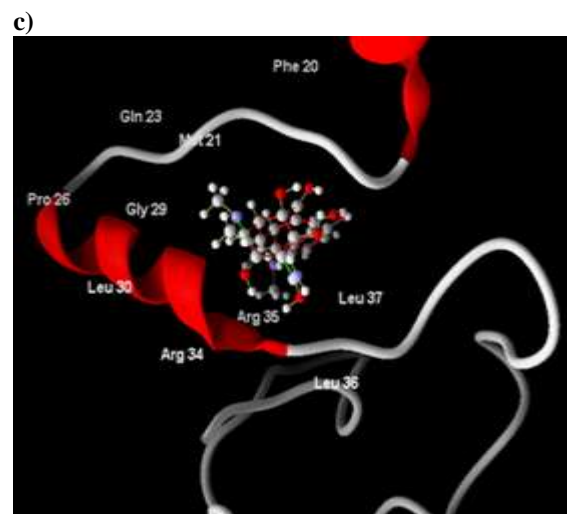
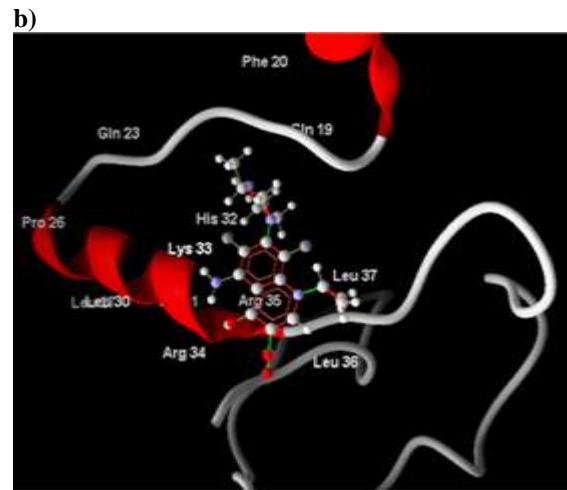
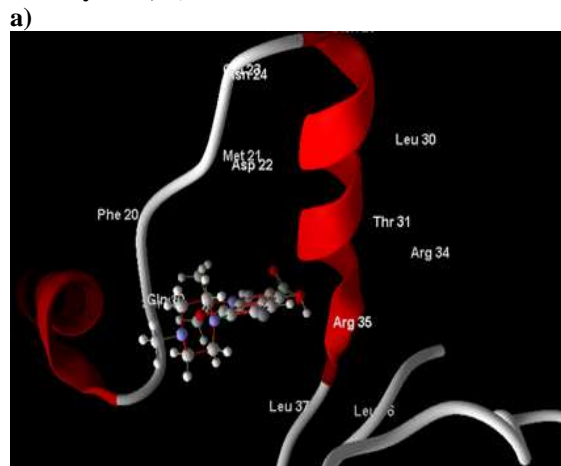
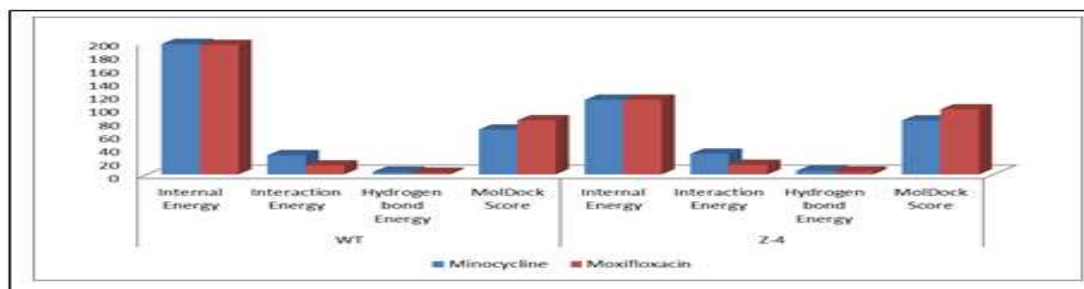


Figure 2: Graph showing comparison between Docking Energies of the Wild Type (WT) and Mutant RpoB (Z-4) with Minocycline and Moxifloxacin



Conclusion

In conclusion, we are suggesting that, a fast, efficient and promising alternative bioinformatics based procedure for the virtual screening and discovery of best drug for proteins is very essential for determining its anti-*M. leprae* activity. We hence conclude that determining the *in silico* predictions by the binding efficacies of inhibitory compounds with DRDR region of new *M. leprae* isolates can be a fast method to select the drug of choice for an effective line of in treatment of leprosy for its further eradication.

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Conflict of Interest: None to declare.

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