Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(5):153-159

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development of rifampicin derivatives sensitive to the rpoB mutated *Mycobacterium tuberculosis:* An *insilico* approach

Santosh Kumar K.[1], Sambandam Cecilia[2], Kannan I.[2]*, Premavathi R. K.[2] and Shantha S.[2]

[1]*Tagore Medical College and Hospital, Rathinamangalam, Chennai, India*
[2]*Department of Microbiology, Tagore Medical College and Hospital, Rathinamangalam, Chennai, India*

ABSTRACT

The treatment of tuberculosis (TB) usually consists of administrating series of drugs over a span of six to nine months. The important drugs prescribed to TB patients are Rifampicin and Isoniazid. The activity of rifampicin is attributed to its ability to bind and inhibit the DNA dependent RNA polymerase enzyme of bacteria. Mutations conferring rifampicin resistance (Rif_R) map almost exclusively to the rpoB gene that produces beta subunit of RNA polymerase enzyme. The mutation thus created produces the protein which has less binding affinity for the rifampicin thus escapes the inhibitory action of the rifampicin. Thus in the present study an insilico approach has been done to modify the structure of rifampicin structure in such a way that it can bind effectively to the mutated RNA polymerase enzyme. The three dimensional structure of RNA polymerase of M. tuberculosis and its mutants are predicted by homology modelling by MODELLER software. The predicted 3D structure was then validated with the Ramachandran plot. The derivatives of the rifampicin were prepared in ACD chemsketch software. The protein – ligand docking was performed by Hex version 8.0.0. From the present study it is evident that there is possibility of preparing rifampicin derivatives that can be sensitive for the rifampicin resistant strain of M. tuberculosis. The study also has proposed four rifampicin derivatives based on binding energy values that can be sensitive to the rifampicin resistant strain of M. tuberculosis.

Keywords: Mycobacterium tuberculosis, rifampicin resistance, molecular docking, rifampicin derivatives.

INTRODUCTION

The tuberculosis (TB) continues to be major health throughout the world. In the last report of world health organisation (WHO) in 2013, there were an estimated 8.6 million incident cases of TB in 2012 and 1.3 million deaths were attributed to the disease. More than half a million cases occurred in children and 320,000 deaths were reported among HIV-infected persons [1]. The treatment of TB usually consists of administrating series of drugs over a span of six to nine months [2]. The important drugs prescribed to TB patients are Rifampicin and Isoniazid. The other first-line drugs such as Streptomycin, Pyrazinamide, and Ethambutol have also been used for the treatment of TB [3].

The rate of multi-drug resistant TB strains resistant to at least two of the first-line TB drugs viz. isoniazid and rifampicin has caused a great difficulty in the treatment of TB [4]. The resistance to second-line anti-TB drugs such as fluoroquinolones and at least one of the injectables has been known as extensively drug resistant tuberculosis (XDR-TB) [5].

Rifampicin (Figure 1), one of the key components of antibiotic therapy, is the most important and broad spectrum antibiotics [6]. The activity of rifampicin is attributed to its ability to bind and inhibit the DNA dependent RNA polymerase enzyme of bacteria [7]. Mutations conferring rifampicin resistance (Rif_R) map almost exclusively to the

rpoB gene, that produces beta subunit of RNA polymerase enzyme [8,9]. The mutation thus created produces the protein which has less binding affinity for the rifampicin thus escapes the inhibitory action of the rifampicin [10].

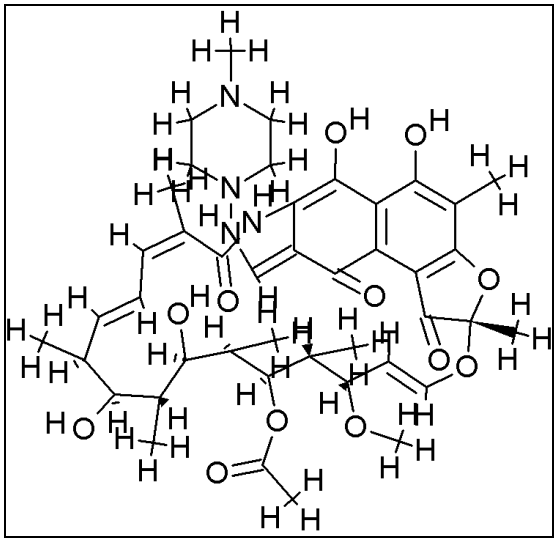

Figure 1: Rifampicin

The change in the amino acid residue(s) in the rifampicin binding site of the mutated RNA polymerase enzyme is the key to the rifampicin resistance of the *M. tuberculosis.* The change thus occurred never allows the rifampicin to bind to the RNA polymerase there by escapes its inhibition. Thus in the present study an *insilico* approach has been made to modify the structure of rifampicin structure in such a way that it can bind effectively to the mutated RNA polymerase enzyme.

EXPERIMENTAL SECTION

The work was performed in the bioinformatics facility of Department of Microbiology, Tagore Medical College and Hospital.

Prediction of three dimensional structures of proteins

The three dimensional structure of beta sub unit of RNA polymerase of *M. tuberculosis* and its mutants are predicted by homology modelling. It is the method to determine 3D structure of protein with the help of 3D structure of homologous proteins. Softwares used were Modeller 9.11 and Easy modeller 2.0 GUI [11]. First the primary structure of beta sub unit of RNA polymerase of *M. tuberculosis* was retrieved from UniProtKB database (www.uniprot.org/help/uniprotkb). The primary structure in FASTA format was submitted in BLASTp (*blast.ncbi.nlm.nih.gov/*) to find the homologous proteins. The proteins of low e value were selected for further study. The 3D structures of the homologous proteins (Table 1) were retrieved from RCSB database (*www.rcsb.org/*). The 3D structures of homologous proteins were submitted along with the primary structure of beta sub unit of RNA polymerase of *M. tuberculosis* to Modeller software through GUI Easy Modeller. The predicted 3D structure was then validated with the Ramachandran plot. It was also further validated in ProQ online tool (www.sbc.su.se/~bjornw/ProQ*).* The primary structures of beta sub unit of RNA polymerase of various rifampicin resistant strains of *M. tuberculosis* were also collected from uniprotKb. The name of the various strains with the details of the mutation is given in Table 2. These are also subjected to homology modelling to develop the 3D structure.

Table 1: The name of the homologous proteins and their RCSB code

S. No.	Name of the protein	RCSB code
1.	*Thermus aquaticus* core RNA Polymerase	1HQM
2.	Recombinant *Thermus aquaticus* RNA Polymerase	2GHO
3.	*Thermus aquaticus* RNA Polymerase holoenzyme	1L9U

Table 2: The name of the various strains with the details of the mutation

Position(s)	Length	Description of mutation and the name of the strain
423 – 423	1	V → A in strain: vr1; rifampicin-resistant.
436 – 436	1	L → P in strain: vr2; rifampicin-resistant.
437 – 437	1	S → T in strain: vr3; rifampicin-resistant.
438 – 441	4	QFMD → H in strain: RJ49; rifampicin-resistant.
438 – 438	1	Q → L in strain: vr4; rifampicin-resistant.
439 – 439	1	F → V in strain: RJ37; rifampicin-resistant.
440 – 443	4	Missing in strain: RJ55; rifampicin-resistant.
441 – 441	1	D → V in strain: vr3; rifampicin-resistant.
449 – 452	4	LTHK → WPQ in strain: RJ48; rifampicin-resistant.
451 – 451	1	H → D in strain: vr5; rifampicin-resistant.
451 – 451	1	H → L in strain: SP28; rifampicin-resistant.
451 – 451	1	H → N in strain: vr6; rifampicin-resistant.
451 – 451	1	H → P in strain: vr8; rifampicin-resistant.
451 – 451	1	H → Q in strain: vr1; rifampicin-resistant.
451 – 451	1	H → R in strain: vr7; rifampicin-resistant.
456 – 456	1	S → L in strain: vr11 and RJ37; rifampicin-resistant.
456 – 456	1	S → Q in strain: vr9; rifampicin-resistant.
456 – 456	1	S → W in strain: vr10; rifampicin-resistant.
458 – 458	1	L → P in strain: vr12 and SP22; rifampicin-resistant.

Generation of rifampicin derivatives

The structure of rifampicin is obtained from pubchem database in sdf format. The structure was converted to MDL format in Open Babel software (www.vcclab.org/lab/babel/start.html). The derivatives of the rifampicin were prepared in ACD chemsketch software. ACD/ChemSketch is the powerful all-purpose chemical drawing and graphics package from ACD/Labs developed to draw the desired molecules and to store it in various desired formats. It also helps to generate IUPAC names and to calculate certain chemical properties of the chemicals. The pharmocophore region of Rifampicin molecule is constituted by a β lactam ring which was maintained in the preparation of rifampicin derivatives. Hence only side chain modifications were performed to prepare the library of ligands for docking with the receptor RNA polymerase enzyme. A total of 120 derivatives were prepared and all the prepared compounds were saved in MDL format. Finally all the compounds were converted to pdb format by Open Babel software.

Protein – Ligand docking

The protein – ligand docking was performed by Hex version 8.0.0. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules [12,13]. Hex can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result[21].

The parameters used for the docking process were:

1. Correlation type – Shape + Electrostatics
2. FFT Mode - 3D
3. Post Processing- MM Energies
4. Grid Dimension - 0.6
5. Receptor range – 180
6. Ligand range – 180
7. Twist range – 360
8. Distance Range – 40

RESULTS AND DISCUSSION

Three structure prediction

The primary structure of beta subunit of RNA polymerase of *M. tuberculosis* was retrieved from UniprotKB database. The primary structure in FASTA format is shown in Figure 2. It consists of 1172 aminoacids.

- >sp|P9WGY9|RPOB_MYCTU DNA-directed RNA polymerase subunit beta OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) GN=rpoB PE=1 SV=1
- MLEGCILADSRQSKTAASPSPSRPQSSSNNSVPGAPNRVSFAKLREPLEVPGLLDVQTDSF
EWLIGSPRWRESAAVNPVGGLEEVLYELSPIEDFSGSMSLSFSDPRFDDVKAPVDECKDKD
MTYAAPLFVTAEFINNNTGEIKSQTVMDFMMTEKGTFIINGTERVVVSQLVRSPGVYFDET
IDKSTDKTLHSVKVIPSRGAWLEFDVDKRDTVGVRIDRKRRQVTVLLKALGWTSEQIVERF
GFSEIMRSTLEKDNTVGTDEALLDIYRKLRPGEPPTKESAQTLLENLFFKEKRYDARVGRYKV
NKKLGLHVGEPITSSTLTEEDVVATIEYLVRLHEGQTTMTVPGGVEVPVETDDIDHFGNRRL
RTVGELIQNQIRVGSRMERVVRERMTTQDVEAITPQTLINIRPVVAAIKEFFGTSQLSQFM
DQNNPLSGLTHKRRLSALGPGGLSRERAGLEVRDVHPSHYGRMCPIETPEGPNIGLIGSLS
VYARVNPFGFIETPYRKVVDGVVSDEIVYLTADEEDHVVAQANSPIDADGRFVEPRVLVRR
KAGEVEYVPSSEVDYMDVSPRQMVSVATAMIPFLEHDDANRALMGANMQRQAVPLVR
SEAPLVGTGMELRAAIDAGDVVVAEESGVIEEVSADYITVMHDNGTRRTYRMRKFARSNH
GTCANQCPIVDAGRVEAGQVIADGPCTDDGEMALGKNLLVAIMPWEGHNYEDAIILSNR
LVEEDVLTSIHIEEHEIDARDTKLGAEEITRDIPNISDEVLADLDERGIVRIGAEVRDGDILVGK
VTPKGETELTPEERLLRAIFGEKAREVRDTSLKVPHGESGKVIGIRVFSREDEDELPAGVNEL
VRVYVAQKRKISDGDKLAGRHGNKGVIGKILPVEDMPFLADGTPVDIILNTHGVPRRMIG
QILETHLGWCAHSGWKVDAAKGVPDWAARLPDELLEAQPNAIVSTPVFDGAQEAELQG
LLSCTLPNRDGDVLVDADGKAMLFDGRSGEPFPYPVTVGYMYIMKLHHLVDDKIHARSTG
PYSMITQQPLGGKAQFGGQRFGEMECWAMQAYGAAYTLQELLTIKSDDTVGRVKVYEAI
VKGENIPEPGIPESFKVLLKELQSLCLNVEVLSSDGAAIELREGEDEDLERAAANLGINLSRN
ESASVEDLA

Figure 2: Primary sequence of beta subunit of DNA dependent RNA polymerase of *M. tuberculosis*

The 3D structure of beta subunit of RNA polymerase was successfully predicted by homology modelling. The predicted 3D structure is given in Figure 3. The structure is stored in pdb format for further use.

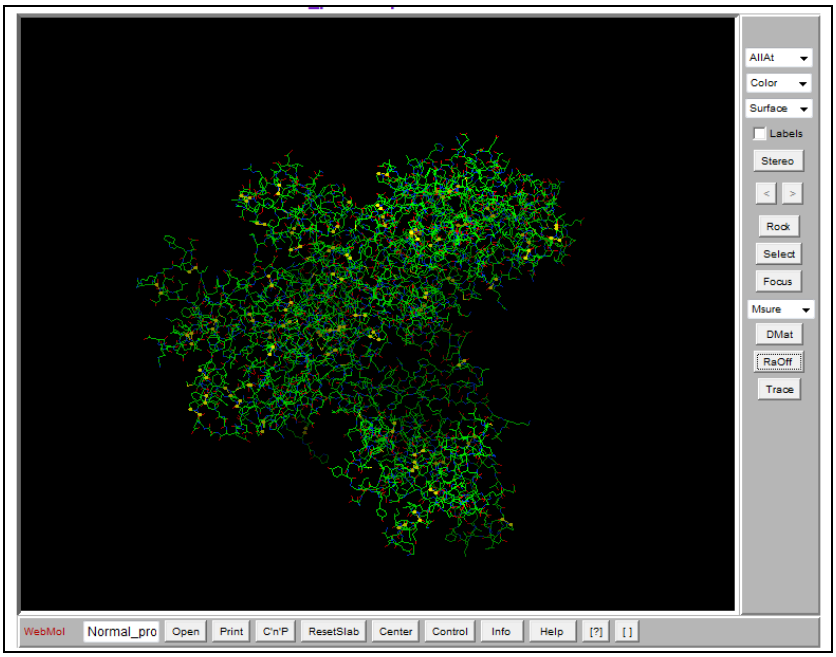

Figure 3: Three dimensional structure of beta subunit of DNA dependent RNA polymerase of *M. tuberculosis* viewed by pymol molecular viewer

Similarly the 3D structures of other 19 mutant proteins were also successfully determined by homology modelling and were stored in pdb format.

Validation of the structure

The Ramachandran plot was generated from the predicted 3D structure of beta subunit of RNA polymerase to validate it. The Ramachandran plot is shown in Figure 4. From the figure it is seen that the most of the residues clustered tightly in the most-favoured regions with very few outliers showing that the predicted structure is good.

The predicted structure was further validated by ProQ online tool. The predicted LGscore and Maxsub are 7.433 and 0.602 respectively. The values obtained shows that the predicted structure is extremely good model.

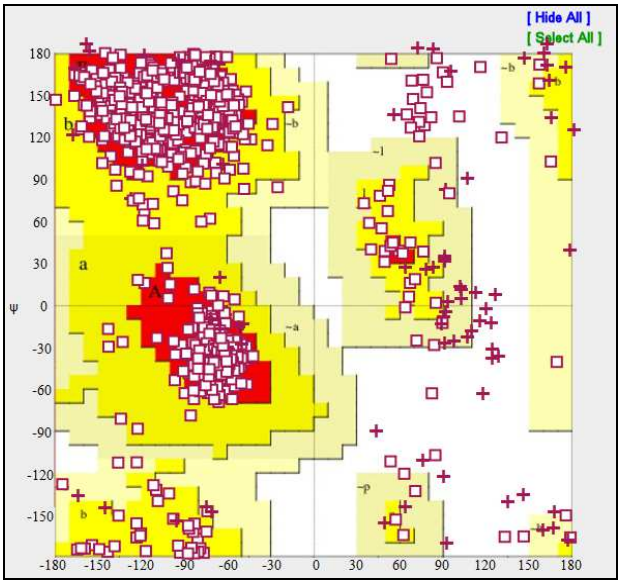

Figure 4: Ramachandran plot of three dimensional structure of beta subunit of DNA dependent RNA polymerase of *M. tuberculosis*

Similarly the 3D structures of all the mutated proteins were validated and were found to be good.

Molecular docking

First the rifampicin was docked with the normal beta subunit of RNA polymerase to find its efficacy of binding. The docking pose of the rifampicin- beta subunit of RNA polymerase is shown in Figure 5. Its energy value was -324.72 which shows a very good binding.

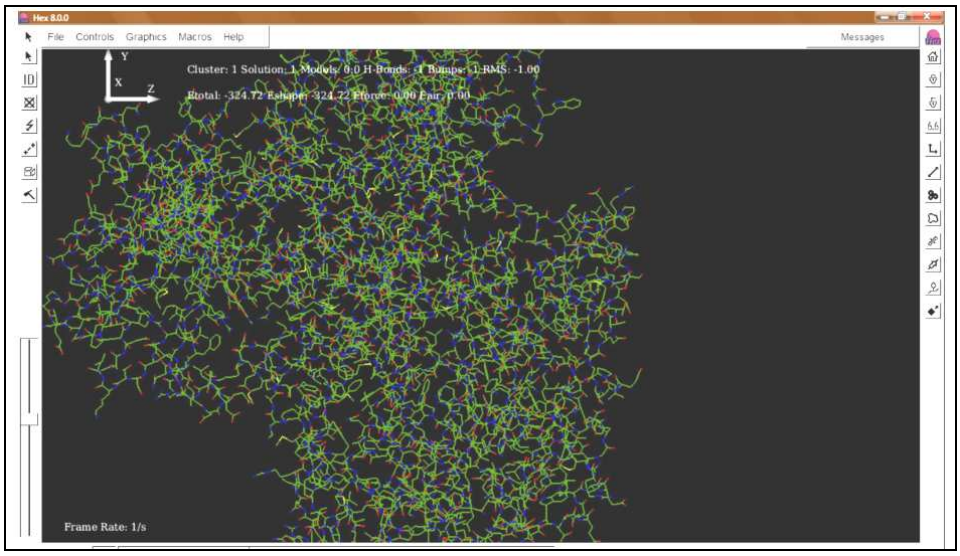

Figure 5: Docking pose of rifampicin- beta subunit of RNA polymerase performed in Hex 8.0

Similarly rifampicin and its 120 derivatives were docked with all the 19 mutated beta subunit of RNA polymerase. As expected the rifampicin showed a poor binding energy with the all the mutated beta subunit of RNA polymerase. Among the 120 rifampicin derivatives, four derivatives showed good binding energy with all 19 mutated beta subunit of RNA polymerase. The results are showed in Table 3.

Table 3: The mean docked energy values of the rifampicin and the selected four derivatives with all 19 mutated beta subunit of RNA polymerase enzyme

S. No.	Ligand	Mean E value (K. Cal.) for 19 mutated RNA polymerase enzyme
1.	Rifampicin	-28.6 ± 7.2
2.	Derivative 1	-304.2 ± 34.1
3.	Derivative 2	-312.8 ± 44.6
4.	Derivative 3	-293.7 ± 49.4
5.	Derivative 4	-306.8 ± 37.7

The four rifampicin derivatives are simple substitutions of functional groups of rifampicin. The derivatives and the modifications from parent rifampicin are shown in Figure 6.

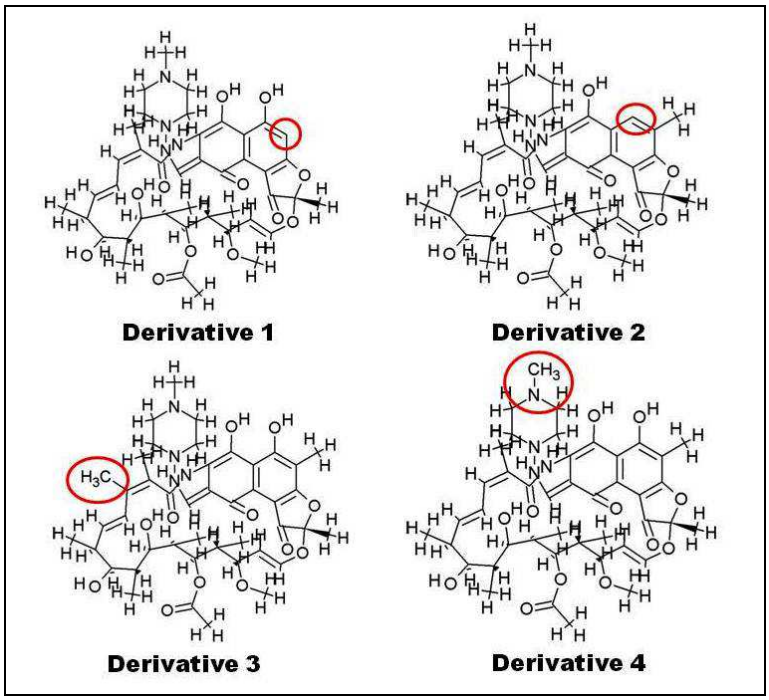

Figure 6: Rifampicin derivatives having good binding energy to the mutated beta subunit of RNA polymerase enzyme of *M. tuberculosis*

CONCLUSION

From the present study it is evident that there is possibility of preparing rifampicin derivatives that can be sensitive for the rifampicin resistant strain of *M. tuberculosis*. The study also has proposed four rifampicin derivatives that can be sensitive to the rifampicin resistant strain of *M. tuberculosis*.

REFERENCES

[1] World Health Organization. *Global Tuberculosis Report* **2013**; WHO/HTM/TB/2013.11: Geneva, Switzerland, 2013.
[2] ED Chan, Iseman MD. *British Medical J* **2002**, 325, 1282-1286.
[3] A Van rie, Enarson D. *The Lancet* **2006**, 368(9547), 1554–1556.
[4] Prasad R. *Indian J Tub*. **2005**, 52,121-31.
[5] NS Shah, Wright A, Bai GH. *Emerg Infect Dis* **2007**, 13(3), 380–387.
[6] Sensi, P. *Rev. Infect. Dis.* **1983**, 3, 402–406.
[7] Hartmann G, Honikel KO, Knusel, F, Nuesch J. *Biochim. Biophys. Acta* **1967**, 145, 843–844.
[8] Ramaswamy S, Musser, JM. *Tuber. Lung Dis.* **1998**, 79, 3–29.
[9] Heep M, Rieger U, Beck D, Lehn N. *Antimicrob. Agents and Chemotherapeutics* **2000**, 44, 1075–1077.
[10] Telenti A, Imboden P, Marchesi F, Lowrie, D, Cole S, Colston, MJ, Matter L, Schopfer K, Bodmer T. *Lancet* **1993**, 341, 647–550.

[11] Eswar N, Marti-Renom MA, Webb B, Madhusudhan MS, Eramian D, Shen M, Pieper U, Sali A. Comparative Protein Structure Modeling With MODELLER. Current Protocols in Bioinformatics, John Wiley & Sons, Inc., Supplement 15, 5.6.1-5.6.30, **2006**.
[12] Ritchie D. W., Evaluation of Protein Docking Predictions using Hex 3.1 in CAPRI rounds 1-2, Proteins, Structure, Fucntion and Genetics, Wiley-liss Inc.
[13] Ritchie D. W. and Kemp G. J. L., *Struct. Funct. Genet.* **2000**, 39, 178-194.