Int J Pharm Bio Sci 2014 July ; 5 (3) : (B) 31 - 39

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DOCKING STUDIES FOR TUBERCULOSIS TAKING ALANINE RACEMASE AS A RECEPTOR AND A NOVEL PLANT SOURCE QUERCETIN AS A POTENTIAL DRUG SOURCE

PRIYANKA NARAD*, ASHISH JAIN AND ABHISHEK SENGUPTA

Amity Institute of Biotechnology, Amity University, Uttar Pradesh, India.

ABSTRACT

Tuberculosis is known to be a fatal infectious disease causing a large number of deaths every year. The causative agent of TB is the pathogenic bacteria *Mycobacterium tuberculosis*. Although vaccines and drugs have been developed, cases of TB infections are still widespread largely due to development of drug resistant strains of *Mycobacterium tuberculosis* which makes it imperative to search for a novel drug target. Due to its essential nature and the requirement of new drug targets for better anti mycobacterial drug, an essential and uniquely prokaryotic enzyme alanine racemase has been pursued as a target. A scrutinization of the published writings supported the role of mycolic acid a major component of cell wall synthesis of bacteria increasing the novelty of alanine racemase as a drug target for noble drug discovery. Our study is based on designing natural inhibitors taking alanine racemase as a target. The ligands were drawn using Marvin Sketch. The choice of the ligands was based on the criterion that the ligands would be chosen from plant sources to minimize the mutagenic behavior of the selected ligands. Further we have done docking calculations using Molegro Virtual Docker where alanine racemase was used as a receptor and was docked by all the potential ligands including the ones derived from plant sources like kaemferol, mimosine, naphthoquinone, quercetin and xanthone and the traditional drugs used to cure TB. Based on the total energy value calculated on the basis of a scoring function designed it has been seen that Quercitin with cavity -3 (Mol Doc Score -102.489 Kcal/mol) has the best inhibitory effect followed by Mimosine (Mol Doc Score -100.51 Kcal/mol). The results suggest that the energy of the chosen ligand is better than the existing drugs, and can be considered as a novel and effective drug in the specific remedy of pulmonary tuberculosis. This computational predicted data could be further validated using suitable assays for further consideration.

KEYWORDS: Computer-Aided Drug Design, Quercetin, Alanine Racemase, Molagro Virtual Docker

PRIYANKA NARAD
Amity Institute of Biotechnology, Amity University, Uttar Pradesh, India.

***Corresponding author**

INTRODUCTION

The cell wall structure of *Mycobacterium tuberculosis* deserves special attention because it is unique among prokaryotes, and it is a major determinant of virulence for the bacterium[1]. The cell wall complex contains peptidoglycan, but otherwise it is composed of complex lipids. Over 60% of the mycobacterial cell wall is lipid[1]. The lipid fraction of mycobacterium cell wall consists of three major components, mycolic acids, cord factor, and wax-D. Mycolic acids are unique alpha branched lipids found in cell walls of Mycobacterium and Corynebacterium. They make up 50% of the dry weight of the mycobacterial cell envelope[2,3].The putative hypothesis states that they prevent attack of the mycobacteria by cationic proteins, lysozyme, and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum[4,5,6]. Cell wall biosynthesis acts as a good source of target for molecular docking because biosynthetic pathways of eukaryotes do not have any similarity with the prokaryotic organisms. Complex cell wall structure of mycobacterium makes them possible to survive within the human macrophages[7].Alanine racemase is an enzyme which is responsible for the cell wall biosynthesis and hence can act as a potential drug target. d-Alanine (d-ala) is a critical component of peptidoglycan and alanine racemase (Alr), the enzyme responsible for racemization of l-ala into d-ala, is an essential enzyme in most bacteria. Various drugs are available for the treatment of tuberculosis, First line drugs are isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB) etc., and second line drugs are para amino salicylate (PAS), kanamycin and cycloserine (CS)[8,9]. Current TB therapy, also known as directly observed treatment short-course (DOTS) consists of an initial phase of treatment with these 4 drugs for 2 months daily, followed by treatment with INH and RIF for another 4 months (WHO 2000)[10]. The commercial drugs that are available are subjected to disadvantages in their administration which is too long and very expensive. An innovative, approach is needed for new drug designing against novel drug target to fight tuberculosis. d-Cycloserine (DCS), which is a second-line anti-TB drug, is known to interfere with peptidoglycan synthesis by inhibiting Alr and d-ala-d-ala ligase, Ddl[11]. The previous reports have not been able to disentangle which of the the two targets Ddl/Alr are more beneficial for the targeting purposes. The fact that most drugs work on the basic principle of causing an over expression of either of these targets therefore confirms their utility in drug development. However, non-reversibility of inhibition in the presence of d-ala showed that the cellular killing could result from off-target effects. The work carried out was the docking studies of alanine racemase as a receptor for drug targeting. After the review of literature, it was established that compound from plant sources could be used as a potential ligand for the purpose of drug targeting. Computer aided drug designing is useful for identification and analysis of the drug-target interactions. An approach to design the ligands useful for targeting the alanine racemase receptor has been carried out.

Compounds such as kaemferolmimosine, naphthoquinone, quercetin and xanthone were selected for the docking process .Plant sources prove to be a potent source since it reduces the side effects incurred by other available drugs.[12,13]

MATERIALS AND METHODS

I.*STRUCTURE RETRIVAL*

3-Dimensional structure of alanine racemase (PDB ID - 1XFC) was retrieved using Protein Data Bank which could act as target molecule for molecular docking.The structure was viewed using Swiss-PDB Viewer to form a better understanding of the molecule in order to use it as a drug target.

II. ACTIVE SITE PREDICTION

Active site prediction of the receptor molecule was carried out by Molagro Virtual docker.

For active site prediction detect cavities option in molagro virtual docker have been utilized. Five drugs related to the treatment of tuberculosis; alanine recemase inhibitor, were selected and their analogous structures were searched from the Drug Bank. From a list of drugs generated, drugs with approved structure were selected. All ligands in this study are derived from plant source so that the chances of mutagenic behavior of the ligand can be minimized.

III.LEAD COMPOUND VALIDATION

Marvin Sketch was utilized to draw ligand molecule and to determine IUPAC name of different ligand compounds. The ligand molecule was then converted into the PDB file format.

IV. LEAD OPTIMIZATION

The ORISIS Property Explorer have been used to draw chemical structure and to calculate on the server itself various drug relevant properties whenever a structure is valid .Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or poor intestinal absorption is shown in red whereas a green color indicates drug conform behavior.

V. TARGET CHARACTERIZATION AND LEAD GENERATION

Molegro Virtual Docker is selected as the platform to carry put the proposed work. MVD is a cohesive environment which carries out dynamic interaction studies. The characterization of the target and lead generation was effectively carried out using the modules.

VI. SCREENING OF DOCKED COMPLEX

Screenings of different docked complex were performed by molagro virtual docker on the basis of energy as an important constraint of stability. The ligand molecule which shows minimum energy with the receptor molecule was chosen as best drug for the respective target enzymes.

RESULTS AND DISCUSSION

Retrieval of structure of the receptor or target molecule was carried out from Protein Data Bank and prediction of active sites in the target protein was done through molagro virtual docker software. For the prediction of active sites receptor molecule was imported through import molecules function in the file menu and then detect cavities option was utilized in the preparation menu which is depicted in the figure 1

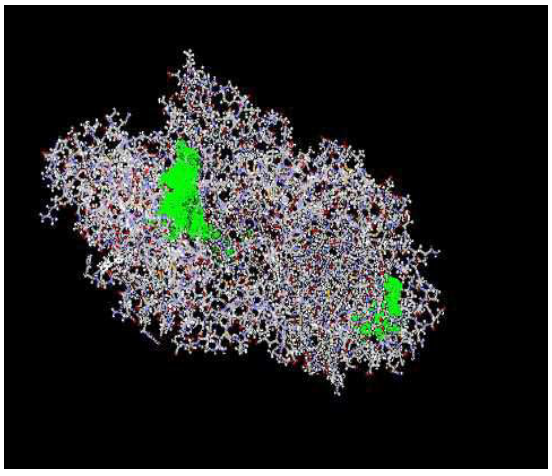
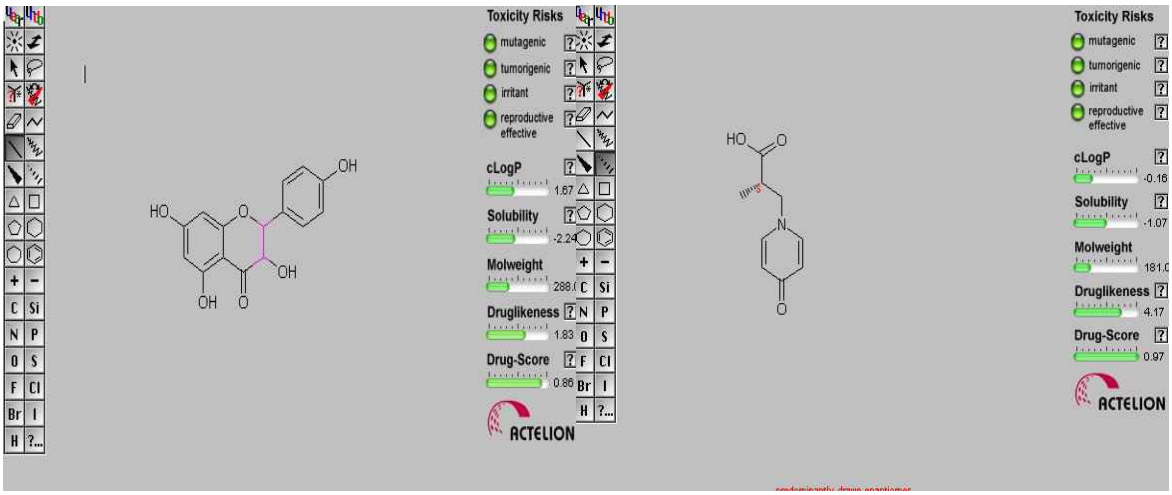


Figure 1
Structure of alanine racemase target molecule with active sites through molagro virtual docker as docking software.

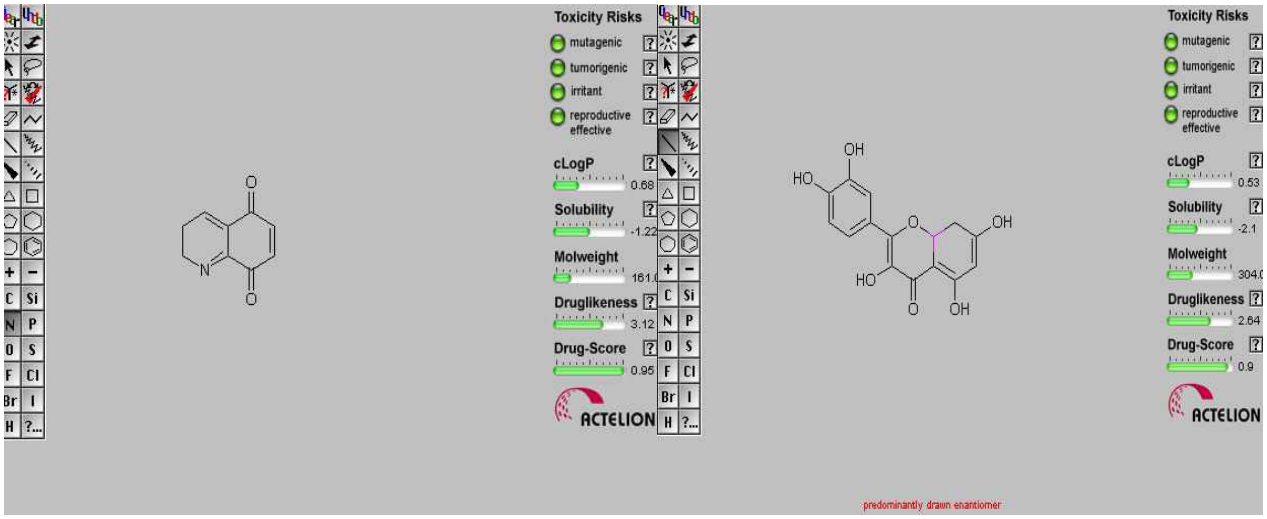
Lead optimization was done by using ORISIS Property explorer. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or poor intestinal absorption are shown in red, whereas a green color indicates drug conform behavior. Green color below in all the parameters shows that chosen ligands can be act as a appropriate drug. All ligands have been chosen from plant sources for easy availability and effective drug like behavior. Kaempferol is a natural flavonol and can be isolated from a variety of plant sources such as tea, broccoli, delphinium, Witch-hazel, grapefruit, cabbage, kale, beans, endive, leek, tomato, strawberries, grapes, brussels sprouts, apples etc. Kaempferol is a yellow crystalline solid with a melting point of 276-278 °C. It is slightly soluble in water but soluble in hot ethanol and diethyl ether. Second ligand mimosine is a non-protein amino acid is found in leaves, pods and seeds of tropical legumes of the genus Leucaena. Third ligand naphthoquinone forms the central chemical structure of many natural compounds, most notably the Vitamin K. 2-Methylnaphthoquinone is a more effective coagulant than vitamin K. Othernaturalnaphtoquinones include juglone, plumbagin, droserone. Fourth ligand xanthones are chemical compounds found naturally in a variety of organic materials. Quercitin is our main ligand which has been our area of interest. It is a flavonoid generated from a plant source and a large range of applications. Lead optimization through ORISIS Property Explorer is shown below. Five ligands or lead molecules discussed above have been optimized through ORISIS Property Explorer tool and green color confirms the low mutagenicity or good intestinal absorption and drug conform behavior.

The results are depicted in Figure-2.

1.Kaempferol **2.Mimosine**



3.Napthoquinone **4.Quercetin**



5.Xanthone

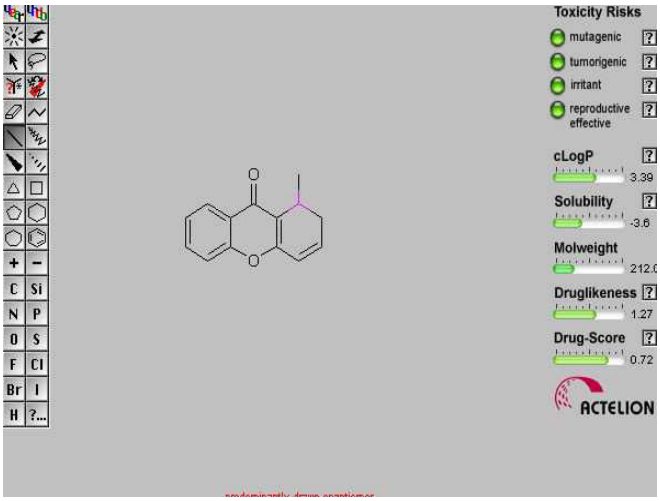


Figure – 2
Five ligand or lead molecules which have been optimized through ORISIS Property explorer tool.

Docking was carried out by using molagro virtual docker. Table-1 shows the result of docking process. The table depicts the description of the docking complex, the cavity number, Moldoc score, Rerank score, hydrogen bond and affinity score. As outlined in the table, the target protein-quercetin complex has the best score of -102.489.

Table 1
This table highlights the docking parameter and the cavity with minimum energy and maximum interaction affinity

S.No	Docking complex	Cavity	MolDoc score	Rerank score	H Bond	Affinity
1.	Target protein + Kaemferol	Cavity-3	-98.4804	-86.7282	-4.5392	-22.192
2.	Target protein + Mimosine	Cavity-1	-100.51	-71.129	-16.66	-25.8941
3.	Target protein + Naphthoquinone	Cavity-3	-85.6506	-59.747	-2.5	-19.562
4.	Target protein + Quercetin	Cavity-3	-102.489	-82.4983	-3.6922	-19.309
5.	Target protein + Xanthone	Cavity-3	-85.129	-73.195	0	-22.773

Drugs that are conventionally used shows less MolDoc score and Rerank score as compared to the quercetin ligand selected in this study which justifies that quercetin can be act as a potent drug molecule in the future for the treatment of tuberculosis. Two conventionally used drug for the treatment of tuberculosis have also been selected and their docking parameter are listed in the table-2.Isoniazid is used as an antibacterial drug as per the dose prescribed for administration. Its chemical nomenclature is Isonicotinic acid hydrazide. The second drug that was selected for a parallel comparison is Rifampin. It comes under the category of broad spectrum drugs and has demonstrated activity against several types of infections.Rifampin is a semi synthetic antibiotic produced from *Streptomyces mediterrane.*

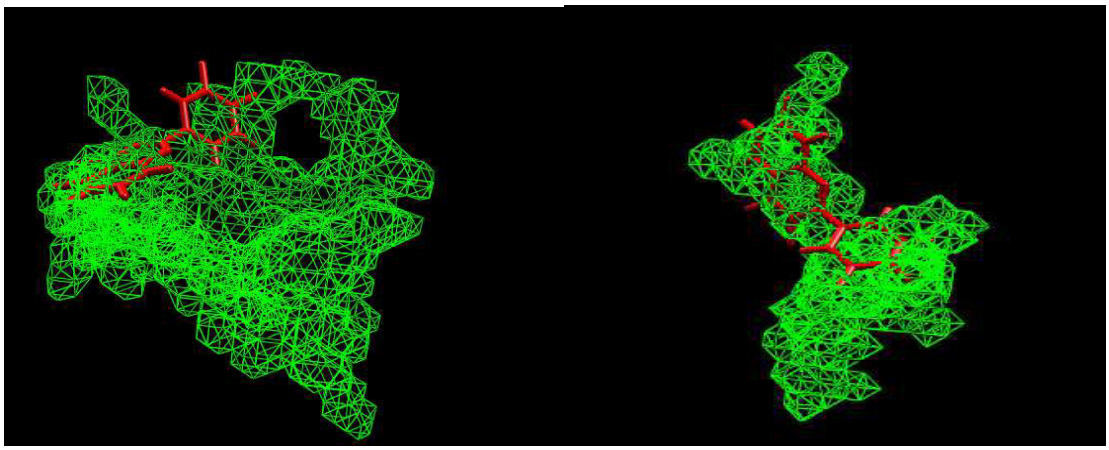
Table-2
Binding energy in terms of MolDoc score and affinity between conventionally used drug molecule and target protein:

S.No	Docking complex	Cavity	MolDoc score	Rerank score	H Bond	Affinity
1.	Target protein + isoniazid	Cavity-3	-89.154	-80.226	-3.5392	-20.523
2.	Target protein + rifampin	Cavity-1	-83.234	-73.543	-11.54	-22.654

The comparative analysis between the traditional drugs and the chosen ligand quercetin proves that quercetin can be a more effective drug for the treatment of tuberculosis because quercetin shows effective binding energy as compared to previously used drugs.

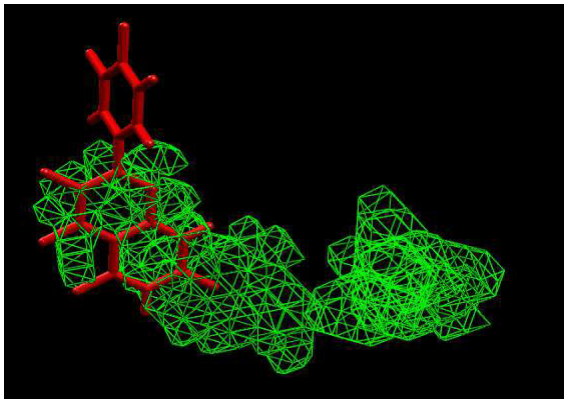
Quercetin

Name	Ligand	MolDockScore	Rerank Score	HBond	Name	Ligand	MolDockScore	Rerank Score	HBond
☐ [01] UNK...	UNK_0	-100.101	-78.5356	-12.0015	☑ [00] UNK...	UNK_0	-93.2261	-75.4454	-6.2446



CAVITY -1 **CAVITY -2**

Name	Ligand	MolDockScore	Rerank Score	HBond
☐ [00] UNK...	UNK_0	-102.489	-82.4983	-3.6922



CAVITY-3

Figure -3

Quercetin as a lead compound shows minimum energy and maximum MolDoc score among all the five ligands and their docked structure were predicted through molsgro virtual docker.

The result of docking studies shows that out of five ligands namely kaemferol ,mimosine naphthoquinone ,quercetin and xanthone; quercetin is found to have maximum stability with the target molecule and can be act as a potent drug against tuberculosis. Isoniazid and rifampin are two conventionally used potent drugs for the treatment of tuberculosis but in docking studies quercetin shows more effective interaction with the alanine racemase than the conventionally used drugs. Active sites in alanine racemase (Fig 1) were predicted with help of molagro virtual docker to which ligand molecules were docked and significant inferences are drawn. Lead optimization through ORISIS Property Explorer tool (Fig 2)confirm the drug like behavior of ligand molecule .When docking were performed quercetin shows maximum stability and high docking score (Fig 3) from the other selected and previously used lead molecule this confirm the characteristics of quercetin as a potent drug for the treatment of tuberculosis by targeting the alanine racemase enzyme which is responsible for cell wall synthesis of mycobacterium –causative agent of tuberculosis.

CONCLUSION

In the present study alanine racemase has been considered for drug designing due its role in cell wall synthesis, cell wall organization, alanine metabolic process, and alanine racemase activity. The protein were inhibited or stimulated by the ligand by molecular docking (matching) mechanisms. The beat docking results is the most important step in the drug designing. The ligand which shows lowest energy, high MolDoc score and have the molecular weight, H-bond, log P as per the Lipinski's rule of 5 was considered as a best ligand for inhibiting the activity of alanine racemase . Docking studies were performed by the molagro virtual docker. On the basis of docking energies, quercetin has good compatibility binding affinity with target. These findings can be subjected to further validation using the experimental evidence.

CONFLICT OF INTEREST

The above manuscript has no declared conflict of interest.

REFERENCES

1. Awasthy D, Bharath S, Subbulakshmi V, Sharma U. Microbiology. 2012 Feb;158(Pt 2):319-27. doi: 10.1099/mic.0.054064-0. Epub 2011 Nov 10.
2. Anthony K. G.,Strych U., Yeung K. R., Shoen C. S., Perez O., Krause K. L.,Cynamon M. H., Aristoff P. A., Koski R. A. (2011). New classes of alanine racemase inhibitors identified by high-throughput screening show antimicrobial activity against Mycobacterium tuberculosis
3. Bruning J. B., Murillo A. C., Chacon O., Barletta R. G., Sacchettini J. C.(2011). Structure of the Mycobacterium tuberculosis D-alanine:D-alanine ligase, a target of the antituberculosis drug D-cycloserine. Antimicrob Agents Chemother 55, 291–301.
4. S.K. Kushwaha, P. Chauhan, M. jha, S. Shrivastava: Rational Drug Designing for Drug Target Alanine Racemase (Alr) of Mycobacterium tuberculosis. The Internet Journal of Laboratory Medicine. 2009 Volume 3 Number 2.
5. Boissier, F., Bardou, F., Guillet, V., Uttenweiler-Joseph, S., Daffe, M., Quemard, A., Mourey, L., Journal: (2006) J.Biol.Chem. 281: 4434-4445
6. Jayakanthan M, Wadhwa G, Madan Mohan T, Ponnusamy Balasubramaniun L, Sundar D. Computer Aided Drug Design for Cancer-Causing H-Ras p21 MutantProtein. Letters in Drug Design and Discovery, Volume 6, Issue1/2009.
7. Golden, MP; Vikram, HR (2005). "Extrapulmonary tuberculosis: an overview.". American family physician 72 (9): 1761–8
8. Southwick, Frederick (10 December 2007). "Chapter 4: Pulmonary Infections". Infectious Diseases: A Clinical Short Course, 2nd ed.. McGraw-Hill Medical Publishing Division. p. 104. ISBN 0071477225
9. Steen A., Palumbo E., Deghorain M., Cocconcelli P. S., Delcour J., Kuipers O. P., Kok J., Buist G., Hols P.(2005). Autolysis of Lactococcus lactis is increased upon d-alanine depletion of peptidoglycan and lipoteichoic acids. J Bacteriol 187, 114–124.
10. Kim MG, Strych U, Krause K, Benedik M, Kohn H. Evaluation of Amino-substituted Heterocyclic Derivatives as Alanine Racemase Inhibitors. Medicinal Chemistry Research. 2003;12:130–138.
11. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, et al. (1997) Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol 35: 907–914.

12. Bifani PJ, Mathema B, Kurepina NE, Kreiswirth BN (2002) Global dissemination of the Mycobacterium tuberculosis W-Beijing family strains. Trends Microbiol 10: 45–52.
13. Narad P, Malpani M, Chakraborty V, Sengupta A (2012) Comparative Homology Modelling an Docking of Lamin A molecule and its incidence in Progeria, Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 1164 – 1170.
14. Kaufmann S. H., Hussey G., Lambert P. H.(2010). New vaccines for tuberculosis. Lancet 375, 2110–2119.
15. 15 .Strych U., Penland R. L., Jimenez M., Krause K. L., Benedik M. J.(2001). Characterization of the alanine racemases from two mycobacteria. FEMS Microbiol Lett 196, 93–98.