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Screening and Identification Potent Inhibitors of LAM protein involved in *Mycobacterium tuberculosis*

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ABSTRACT

Mycobacterium tuberculosis, the etiological agent of tuberculosis, spreads by aerosol, mainly infecting alveolar macrophages, by which the bacterium is ingested. Through inhibition of macrophage functions, the bacterium modulates the host immune response. The best-characterized virulence factor of *M. tuberculosis*, lipoarabinomannan (LAM), is an abundant glycolipid, which is attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor and extends through the cell wall of the bacterium. During uptake of mycobacteria, LAM interacts with the cell membrane of the host macrophage via specific receptors, including the macrophage mannose receptor and complement receptor and can then be detected at multiple sites in the cell. The host cell exports LAM from the phagosome in an exocytosis-like manner, eliciting responses in bystander cells. Host-directed therapies for tuberculosis have been much written about lately. If such therapies are developed, their use is likely to be most significant in patients with multidrug-resistant and extensively drug-resistant tuberculosis. The current work presented in this article could point to *in silico* identification the inhibitors of LAM protein by molecular docking approach to development novel strategies evaluating structure-based drug designing.

Keywords : *M. tuberculosis,* Molecular docking, *in silico,* LAM, multidrug Resistant

I. INTRODUCTION

Tuberculosis is a leading infectious cause of morbidity and mortality in adults worldwide, killing about 1.7 million people in 2016, most of them in low- and middle-income countries. HIV/AIDS is the most important factor predisposing to TB infection and mortality in parts of the world where both infections are prevalent. Tuberculosis properly refers only to disease caused by Mycobacterium tuberculosis (for which humans are the main reservoir). Similar disease occasionally results from the closely related mycobacteria, M. bovis, M. africanum, and M. microti—which together with M. tuberculosis are known as the Mycobacterium tuberculosis complex. M. tuberculosis usually enters the alveolar passages of exposed humans in an aerosol droplet, where its first contact is thought be with resident macrophages, but it is also possible that bacteria can be initially ingested by alveolar epithelial type II pneumocytes (1). Mycobacteria are speculated to have existed as early as 150 million years ago, in the Jurassic period (2), and today they are ubiquitous, occurring in every habitat and ecosystem of the world, perhaps except for the polar regions. Tuberculosis results almost exclusively from inhalation of airborne particles (droplet nuclei) containing M. tuberculosis. This cell type is found in greater numbers than macrophages in alveoli, and M. tuberculosis can infect and grow in these pneumocytes ex vivo(3).They disperse primarily through coughing, singing, and other forced respiratory maneuvers by people who have active

pulmonary or laryngeal TB and whose sputum contains a significant number of organisms (typically enough to render the smear positive). People with pulmonary cavitary lesions are especially infectious because of the high number of bacteria contained within a lesion. Droplet nuclei (particles < 5μ in diameter) containing tubercle bacilli may remain suspended in room air currents for several hours, increasing the chance of spread. However, once these droplets land on a surface, it is difficult to resuspend the organisms as respirable particles. Although such actions can resuspend dust particles containing tubercle bacilli, these particles are far too large to reach the alveolar surfaces necessary to initiate infection. Contact with fomites do not appear to facilitate spread. In addition, dendritic cells play a very important role in the early stages of infection since they are much better antigen presenters than are macrophages (4) and presumably play a key role in activating T cells with specific M. tuberculosis antigens). Since dendritic cells are migratory, unlike differentiated macrophages they also may play an important role in dissemination of M. tuberculosis. (5) How contagious patients with untreated active pulmonary TB are varies widely. Certain strains of M. tuberculosis are more contagious, and patients with positive sputum smears are more contagious than those with positive results only on culture. Patients with cavitary disease are more contagious than those without. Environmental factors also are important. Transmission is enhanced by frequent or prolonged exposure to untreated patients who are dispersing large numbers of tubercle bacilli in overcrowded, poorly ventilated enclosed spaces; consequently, people living in poverty or in institutions are at particular risk. Health care practitioners who have close contact with active cases have increased risk. Thus, estimates of contagiousness vary widely; some studies suggest that only 1 in 3 patients with untreated pulmonary TB infect any close contacts; the WHO estimates that each untreated patient may infect 10 to 15 people per year. However, most of those who are infected do not develop active disease. (6).The current (recommended) treatment for drug‐sensitive TB, also called "first‐line" TB treatment, was developed over 40 years ago and requires that multiple drugs be taken, often daily, for six to nine months. This drug treatment can cure active, drug‐sensitive TB, as long as treatment is completed properly, with no interruptions. It can also spread to other parts of the body, like the brain and spine. A type of bacteria called Mycobacterium tuberculosis causes it. Mycobacterium tuberculosis and Mycobacterium leprae, the causative agents of tuberculosis and leprosy, respectively, produce large quantities of lipoarabinomannan (LAM), a highly immunogenic, cell wall-associated glycolipid. This molecule has been previously reported to be a potent inhibitor of gamma interferon-mediated activation of murine macrophages. Studies of the mechanism by which this mycobacterial glycolipid down-regulates macrophage effector functions provide evidence that LAM acts at several levels and that it can (i) scavenge potentially cytotoxic oxygen free radicals, (ii) inhibit protein kinase C activity, and (iii) block the transcriptional activation of gamma interferon-inducible genes in human macrophage-like cell lines. These results suggest that LAM can inhibit macrophage activation and triggering and cytocidal activity and that it may represent a chemically defined virulence factor contributing to the persistence of mycobacteria within mononuclear phagocytes.

II. MATERIAL AND METHODS

1. Retrieval of LAM protein structure- The 3D structure of the LAM protein was retrieved from RCSB Protein Data Bank (PDB). The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids where the data is typically

obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy (7). RasMol is a computer program written for molecular graphics visualization intended and used mainly to depict and explore biological macromolecule structures. The retrieved 3D structure of LAM protein was visualized using RasMol visualization tool (8)

2. Retrieval and identification of natural lead compounds - The natural compounds were retrieved from PubChem database along with their SDF structures and SMILES. PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information. The simplified molecular-input line-entry system (SMILES) is a specification in the form of a line notation for describing the structure of chemical species using short ASCII strings. Structure Data Format (SDF) is a chemical file formats to represent multiple chemical structure records and associated data fields. SDF was developed and published by Molecular Design Limited (MDL) (9)

3. Screening by Lipinski rule of five -The compounds were screened for properties using Lipinski rule of five. The molecules were subjected to a DruLito tool, an offline tool to predict the drug likeness of the lead compounds. (10)

4. Screening by ADME properties- Swiss ADME was used to predict ADME (Absorption, Distribution, Metabolism and Elimination) properties of drug like molecules. SwissADME is web tool that gives free access to a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness (11)

5. Test for toxicity- The toxicity of the compounds were predicted using the admetSAR online server.The ADMET structure-activity relationship server, entitled admetSAR, is a comprehensive knowledge and tool for predicting ADMET properties of drug candidates and environmental chemicals (12)

6. Molecular Docking- The molecular docking of the lead compounds against the LAM protein was performed by using HEX and AutoDock offline tool. Hex is the first Fourier transform (FFT)-based protein docking server (13). It is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate protein-ligand docking, assuming the ligand is rigid. AutoDock is a molecular modeling simulation software. It is especially effective for protein-ligand docking. AutoDock 4 is available under the GNU General Public License. (14)

III. RESULTS AND DISCUSSION

The 3D structure of the LAM protein was retrieved (PDB ID: 3MH8) from RCSB PDB database. Protein Data Bank (PDB) is a repository of 3-D structural data of biomacromolecules. The active site of LAM protein was predicted by using CASTp (Computed Atlas of Surface Topography of proteins) server. The identification of active sites is often the starting point for protein function, annotation and structure-based drug design. Further molecule was visualized using Rasmol software package. [Fig. no.1] The natural compounds showing anti tuberculosis activity was obtained by literature survey. The surveyed lead compounds for targeting LAM protein were retrieved from PubChem database along with their SMILES and 2D SDF structures for further analysis. The retrieved lead compounds are shown in Table No 1. These retrieved compounds were now proceeded to anylysis Lipinski Rule of 5. Further these compounds were

subjected to DruLiTo druglikeness software package. This tool predict Molecular weight, H bond donor, H bond acceptor, log P and rotatable bonds from SDF structure file which shown in Table No 2. From the Table No 2 the compounds which followed the Lipinski rule were tested for their toxicity by an online sever, admetSAR. The ADMET structure-activity relationship server, entitled admetSAR, is a comprehensive knowledge and tool for predicting ADMET properties of drug candidates and environmental chemicals. In our server, over 200,000 ADMET annotated data points for about 96 thousand of unique compounds have been manually curated from large literatures. The admetSAR server provides a user-friendly interface to easily search for chemical profiles. From the above compounds, Piperine, Quinolone, Coumarin, Licochalcone A, KauraneBerberine, Thiolactomycin, Ascididemin, Calanolide A, Phloretin, Limonenewere found to be non- toxic and are the lead candidates for molecular docking. Further these compounds which are non-toxic were checked for their pharmacokinetic properties, synthetic accessibility, GI absorption and the ability to inhibit CYP3A4 and bioavailability score. The compounds piperine, quinolone, shinolone, thiolactomycin, tryptharin have high GI absorption and a reasonable synthetic accessibility and bioavailability score and don't inhibit CYP3A4. The results of SwissADME are shown in Table No 3. The above compounds which passed the Lipinski rule, pharmacokinetic properties and toxicity were proceeded for docking against LAM protein by HEX and Autodock4. The docking score of piperine is less and the docking score of limonene is more. Therefore, the conformation is stable of piperine. The docking score of these compounds are shown in Table No 4. Autodock was performed of these compounds against LAM protein to obtain the binding energies. Piperine showed the lowest binding energy with the binding score of -7.15 while quinolone showed the highest binding energy score of -4.8. Therefore, piperine was a novel drug candidate as compared to Quninolone, Coumarin, Thiolactomycin and Limonene. The results showing binding energies of the compounds Piperine, coumarin and thiolactomaycin by Autodock are shown Table No 4 and the binding complexes are shown in Figure 2, 3, 4 and 5

Table 1. Retrieved compounds from PubChem

Sr. No	Compound name	PubChem ID
1	Phenazine	4757
2	Clofazimine	2794
3	Piperine	638024
4	Mycin	131752015
5	Quinolone	11820
6	Pyridomycin	3037036
7	Coumarin	323

8	Quinquangulin	3008110
9	Rubrofusarin	72537
10	(R)-Shinanolone	12315505
11	Chabamide	53243800
12	Bakuchiol	5468522
13	Licochalcone A	5318998
14	Mycothiol	441148
15	Grisellimycin	72535
16	Kaurane	12304767
17	Berberine	2353
18	Thiolactomycin	135403829
19	Tryptanthrin	73549
20	Asciddemin	189219
21	Calanolide A	64972
22	Oleanolic acid	10494
23	Ursolic acid	64945
24	Phloretin	4788
25	Limonene	22311

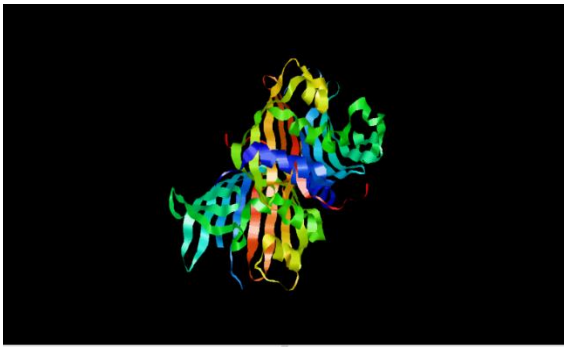

Fig. 1 : LAM 3D structure representation by Rasmol

Table 2 : Compounds which followed Lipinski Rule of Five

Compound name	Molecular weight	Log P	H bond acceptor	H bond donor
Phenazine	180.07	0.51	2	0
Clofazimine	427.12	4.724	4	1
Piperine	285.14	2.518	4	0
Quinolone	159.07	0.633	2	0
Coumarin	146.04	1.022	2	0
Quinquangulin	286.08	1.509	5	2
Rubrofusarin	272.07	1.328	5	2
(R)-Shinanolone	192.08	0.147	3	2
Licochalcone A	338.15	3.733	4	2
Kaurane	274.27	10.044	0	0
Berberine	336.12	2.473	4	0
Thiolactomycin	210.07	2.458	2	1
Tryptanthrin	248.06	0.446	4	0
Ascididemin	283.07	-0.198	4	0
Calanolide A	370.18	3.712	5	1
Phloretin	274.08	1.803	5	4
Limonene	136.13	3.729	0	0

Table 3 : Results of SwissADME

Compound name	GI absorption	CYP3A4 inhibitor	Synthetic Accessibility	Bioavailability Score
Piperine	High	No	2.92	0.55
Quinolone	High	No	1.39	0.55
Coumarin	High	No	2.74	0.55
Thiolactomycin	High	No	4.17	0.55

Table 4 : Docking score of compounds

Compound name	Autodock	HEX
Piperine	-7.15	-208.5
Quinolone	-4.8	-149.9
Coumarin	-5.11	-142.6
Thiolactomycin	-5.57	-159.8

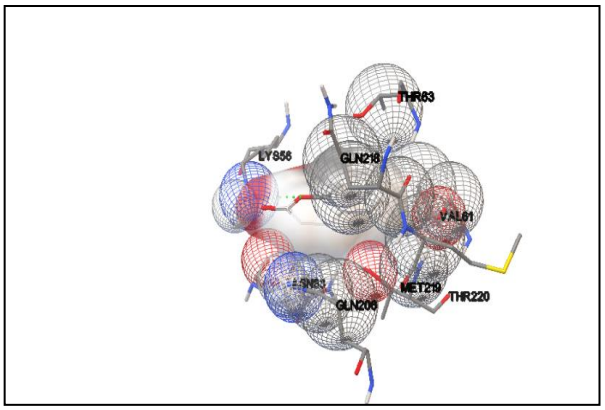

Figure 2. Piperine

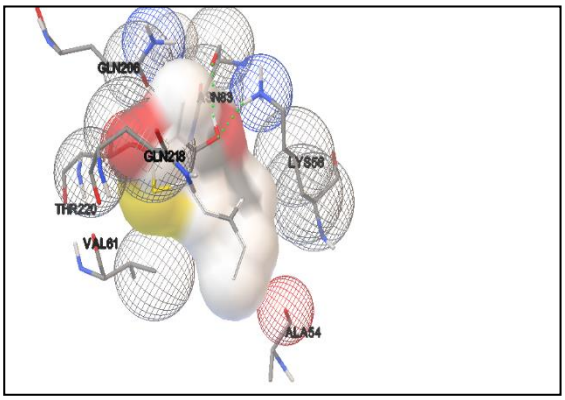

Figure 3. Coumarin

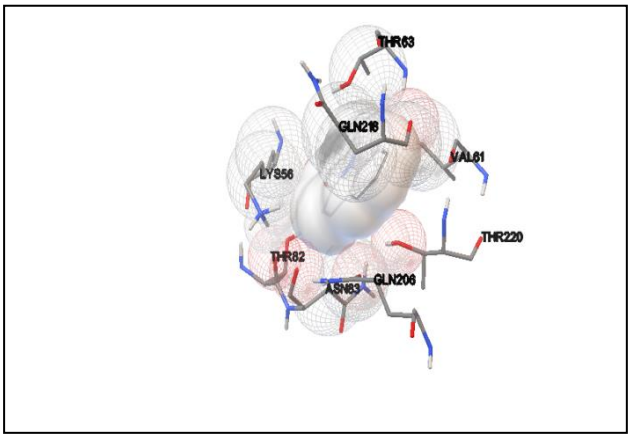

Figure 4. Thiolactomycin

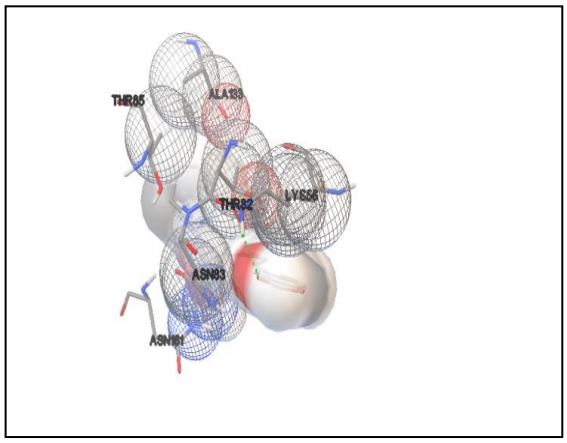

Figure 5. Quinolone

IV. CONCLUSION

3D structure of the LAM protein was downloaded from RCSB PDB having PDB ID- 3MH8.Identification of lead compounds and natural compounds showing anti tuberculosis was done by literature survey and their structures in 2D SDF format was retrieved for further analysis. These compounds were checked for its Lipinski Properties, Pharmacokinetic properties and its toxicity with the help of DruLiTo tool, SwissADME online tool and ADMETsar online server respectively. Piperine, Quinolone, Coumarin, Licochalcone A, KauraneBerberine, Thiolactomycin, Ascididemin, Calanolide A, Phloretin, Limonene were found to be non- toxic and compounds piperine, quinolone, shinolone, thiolactomycin, tryptharin have high GI absorption and a reasonable synthetic accessibility and bioavailability score and don't inhibit CYP3A4. Molecular docking was performed by HEX and AutoDock. The docking score of piperine is less and the docking score of Thiolactomycin is more. Therefore, the conformation is stable of piperine. Autodock was performed of these compounds against LAM protein to obtain the binding energies. Piperine showed the lowest binding energy with the binding score of -7.15 while quinolone showed the highest binding energy score of -4.8. Therefore, piperine was a novel drug candidate as compared to Quninolone, Coumarin and Thiolactomycin. It is concluded that Piperine, which

is a natural substance found in black pepper shows anti tuberculosis activity and is a novel drug candidate for drug designing.

V. REFERENCES

[1]. Mehta, P. K., C. H. King, E. H. White, J. J. Murtagh, Jr., and F. D. Quinn. 1996. Comparison of in vitro models for the study of Mycobacterium tuberculosis invasion and intracellular replication. Infect. Immun. 64:2673-2679.

[2]. Hayman J. 1984. Mycobacterium ulcerans: an infection from Jurassic time? Lancet ii:1015–1016.

[3]. Bermudez, L. E., and J. Goodman. 1996. Mycobacterium tuberculosis invades and replicates within type II alveolar cells. Infect. Immun. 64:1400-1406.

[4]. Tascon, R. E., C. S. Soares, S. Ragno, E. Stavropoulos, E. M. Hirst, and M. J. Colston. 2000. Mycobacterium tuberculosis-activated dendritic cells induce protective immunity in mice. Immunology99:473-480.

[5]. Gonzalez-Juarrero, M., and I. M. Orme. 2001. Characterization of murine lung dendritic cells infected with Mycobacterium tuberculosis. Infect. Immun. 69:1127-1133.

[6]. Pawlowski A, Jansson M, Sköld M, Rottenberg ME, Källenius G. PLoSPathog. 2012 Feb;8(2):e1002464. doi: 10.1371/journal.ppat.1002464. Epub 2012 Feb 16.

[7]. Helen M. Berman,Tammy Battistuz, T. N. Bhat,c Wolfgang F. Bluhm, Philip E. Bourne, etal, 2002. The Protein Data Bank.

[8]. Roger Sayle and E. James Milner-White, 1995. RasMol: A Program for Fast Realistic Rendering of Molecular Structures with Shadows.

[9]. Sunghwan Kim, Thiessen, Evan E. Bolton et al., 2015. PubChem Substance and Compound databases.

[10]. Bickerton, G.R., et al. (2012) Quantifying the chemical beauty of drugs. Nat. Chem., 4, 90-98.

[11]. Antoine Daina, Olivier Michielin & Vincent Zoete, 2017. SwissADME: a free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules.

[12]. Congying Xu., et al., 2015. In Silico Prediction of Chemical Ames Mutagenicity.

[13]. Gary Macindoe, Lazaros Mavridis, et al. 2010. HexServer: an FFT-based protein docking server powered by graphics processors.

[14]. Oleg Trott and Arthur J. Olson, 2011. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading.

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