BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 7(3)

Molecular docking of azole drugs and their analogs on CYP121 of *Mycobacterium tuberculosis*

Jagadish Chandrabose Sundaramurthi, Swetha Kumar, Kannayan Silambuchelvi, Luke Elizabeth Hanna*

ICMR-Biomedical Informatics Centre, Tuberculosis Research Centre, (ICMR), Chetpet, Chennai-600031, Tamil Nadu, India; Luke Elizabeth Hanna - Email: hannatrc@yahoo.com; Phone: +91-44-2836 9597; Fax: +91-44-2836 2528; *Corresponding Author

Received August 27, 2011; Accepted August 30, 2011; Published September 28, 2011

Abstract:
The *Mycobacterium tuberculosis* genome codes for 20 different cytochromes. These cytochromes are involved in the breakdown of recalcitrant pollutants and the synthesis of polyketide antibiotics and other complex macromolecules. It has been demonstrated that CYP121 is essential for viability of the bacterium by gene knock-out and complementation studies. CYP121 could therefore be a probable target for the development of new drugs for TB. It has been widely reported that orthologs of CYP121 in fungi are inhibited by azole drugs. We evaluated whether these azole drugs or their structural analogs could bind to and inhibit CYP121 of *M. tuberculosis* using molecular docking. Six molecules with known anti-CYP121 activity were selected from literature and PubChem database was searched to identify structural analogs for these inhibitors. Three hundred and fifty seven molecules were identified as structural analogs and used in docking studies. Fifty three molecules were found to be scored better than the azole drugs and five of them were ranked among the top 12 molecules by two different scoring functions. These molecules may be further tested by *in vitro* experimentation for their activity against CYP121 of *M. tuberculosis*.

Keywords: CYP121, *M. tuberculosis,* docking, ketoconazole, azole drugs.

Background:
Tuberculosis caused 1.3 million deaths among HIV-negative people and 0.38 million deaths among HIV-positive people in 2009. An estimated 250,000 TB patients were notified in 2009, to have multi-drug resistant TB (MDR-TB) **[1]**. *M. tuberculosis* makes a lethal combination with HIV, both catalyzing the progression of AIDS and TB respectively **[2, 3]**. There is hence an urgent need for novel therapeutics for combating these diseases. The genome of *M. tuberculosis* codes for about 4000 proteins **[4]** and identification of an ideal target is the most critical task in the process of drug discovery. Bacterial P450s participate in the degradation of xenobiotics, reduction of nitric oxide and antibiotic synthesis. On the other hand, human P450s are intimately associated with drug metabolism and steroid synthesis **[5, 6]**. The genome of *Mycobacterium tuberculosis* encodes 20 different cytochrome P450 enzymes **[4]**. Among them, CYP121 has been demonstrated to be essential for the viability of *M. tuberculosis* **[7]**. Besides, there is no equivalent enzyme in humans. We therefore hypothesized that CYP121 could be a potential drug target for *M. tuberculosis*. Azole drugs have been demonstrated to bind with high affinity to cytochrome P450eryF (CYP107A1) of *Saccharopolyspora erythraea* **[8]** and have been used effectively for the treatment of fungal infections **[9]**. A few studies have examined the action of azole drugs on P450s in *M. tuberculosis*, particularly on CYP121 **[10, 11]** and CYP130 **[12]** and found them to bind to these proteins, although with a weaker affinity. We therefore made an attempt to identify stronger inhibitors of CYP121 of *M. tuberculosis* using molecular docking with structural analogs of the available azole drugs.

Methodology:
Preparation of small molecules library:
Econazole and clotrimazole **[10, 12]**, ketoconazole, miconazole **[12]** and fluconazole **[11]** have been reported to have a possible effect on P450s of *M. tuberculosis*. Tioconazole **[13]** and the

above mentioned drugs have already been demonstrated to have anti-fungal activity. Structural analogs of these six azole molecules were collected from PubChem **[14]** at 95% structural similarity. All these molecules were screened *in silico* for their inhibitory activity against CYP121.

Molecular Docking:
Ten different structures are available in PDB for CYP121 of *M. tuberculosis* (2IJ5, 2IJ7, 3G5F, 3G5H, 3CY1, 3CY0, 3CXV, 3CXX, 3CXZ, 3CXY). We selected 2IJ7 as the PDB file for docking since it has been crystallized in the presence of fluconazole and heme. The atomic coordinates of Cytochrome P450 121 (2IJ7) of *M. tuberculosis* was downloaded from PDB. All ligands and water molecules except those that were part of the active site in the target were removed and hydrogen atoms were added to the protein. CHARMm force field was then applied to the target. The substrate binding site of CYP121 was selected for docking using CDOCKER (Discovery Studio, 2.0) **[15]**.

Scoring Docked Molecules:
In order to enhance the accuracy of the prediction, the docked poses were ranked using two different scoring functions namely PLP1 and Ligscore2, since these two methods of scoring are reported to be better than several other methods **[16]**. Molecules that scored best by both methods were identified as potential leads for tuberculosis drug discovery.

Results and Discussion:
The density of cytochrome in *M. tuberculosis* is 200 fold more than in humans **[17]**. The preponderance of P450 *in M. tuberculosis* suggests that these enzymes could have important cellular functions in the pathogen. Since the inhibitory role of azole drugs on cytochrome P450s of other organisms has been widely demonstrated, we screened six azole drugs and their respective structural analogs for their effect on CYP121 of *M. tuberculosis* using *in silico* methods. The six azole molecules (econazole, clotrimazole, ketoconazole, miconazole, tioconazole and flucanozole) were first docked on to CYP121 and scored using PLP1 as well as LigScore2. Ketoconazole was found to have the best score by both methods. Therefore, ketoconazole was chosen as the control molecule for our analysis. Three hundred and fifty seven molecules were collected from PubChem as structural analogs for the above six azole molecules at 95% similarity. Each of these analogs were docked onto CYP121 and scored using PLP1 and LigScore 2. Of the 357 molecules, 60 had a better docking score than ketoconazole when PLP1 scoring function was applied and 115 molecules scored better than ketoconazole using LigScore2.

Fifty three molecules were consistently picked up by both methods as better molecules than ketoconazole, as they possibly have better binding affinity to CYP121 than ketoconazole. Of the 53 molecules, 43 were found to be structural analogs of ketoconazole and 6, 2 and 2 were analogs of Econazole, Miconazole and Tioconazole, respectively. None of the analogs of Clotrimazole and Fluconazole scored better than ketoconazole. The top ten molecules ranked by PLP1 and LigScore2 with corresponding energy scores are listed in **Table 1 (see Supplementary material)**. We have used Chemical ID (CID) given in PubChem for identification of the molecules in our analysis. Four of the 53 molecules (CID: 21443149, 21499732, 12854724 and 20406023) were found to be among the top ten ranking molecules identified by both PLP1 and LigScore2. One molecule (CID: 20519316) which scored as third best by the PLP1 scoring function was placed in the 12th rank by LigScore2. The short-listed five molecules were subjected to energy minimization using steepest descent method. A significant difference in the binding energy was observed after energy minimization was carried out. **Figure 1** shows a representative structure after energy minimization. Seward *et al.* (2006) reported a set of ten amino acids to be part of the active site of CYP121 **[11]**. It was found that all the ten amino acids comprising the active site of CYP 121 were present in the docked poses of two molecules (21499732 and 20406023) and nine residues were present in the docked pose of the molecule (12854724), showing the effective binding of these molecules to the active site of CYP121. We therefore propose that these five molecules could serve as potential leads for novel drug discovery for tuberculosis.

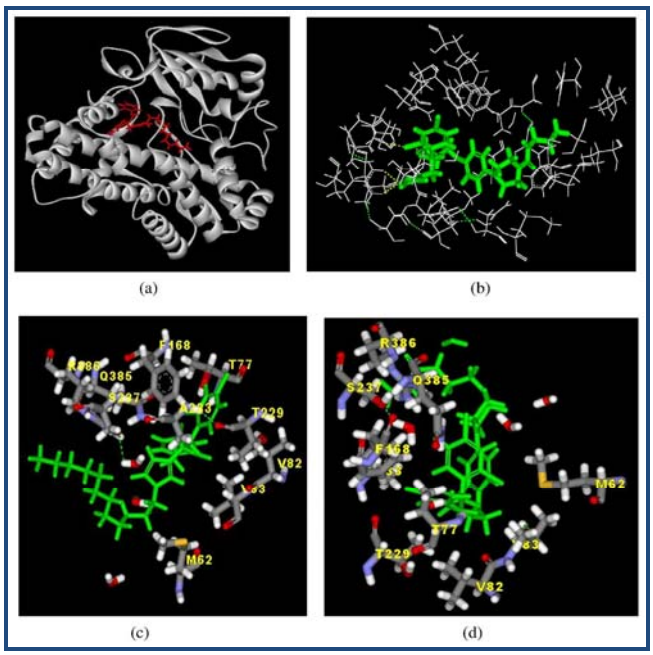

Figure 1: The docked poses of CYP121 with three representative molecules after energy minimization. a) The protein CYP121 is shown in solid ribbon display with a molecule CID: 21499732 binding in its active site. b) The hydrogen bonds between the molecule (CID: 12854724) and the protein are shown in yellow color. c) and d) Residues present in the active site of the CYP121 that interact with small molecules CID: 21499732 and CID: 20406023 respectively, are highlighted.

Conclusion:
Molecular docking studies have helped in the identification of potential small molecules for drug discovery for various diseases **[18]**. Recently Izumizono *et al.* (2011) demonstrated the potential of docking in short-listing potential candidates and subsequently confirmed its efficiency by *in vitro* testing on *M. tuberculosis* **[19]**. We employed the same principle of molecular docking and followed it up with the use of stringent scoring functions to enhance the accuracy of our results. The set of molecules identified by us in this study are very likely to serve as potential leads in the search for new drugs against tuberculosis.

Acknowledgement:
We acknowledge the Indian Council of Medical Research for providing the Biomedical Informatics facility.

References:

[1] http://www.who.int/tb/publications/2010/en/index.html.
[2] Perkins MD & Cunningham J. *J Infect Dis*. 2003 **196**: S15 [PMID: 17624822]
[3] Toossi Z. *J Infect Dis*. 2003 **188**: 1146 [PMID: 14551885]
[4] Cole ST *et al*. *Nature* 1998 **393**: 537 [PMID: 9634230]
[5] Munro AW & Lindsay JG. *Mol Microbiol*. 1996 **20**: 1115 [PMID: 8809764]
[6] Munro AW *et al*. *Biochem Soc Trans*. 2003 **31**: 625 [PMID: 12773169]
[7] McLean KJ *et al*. *J Biol Chem*. 2008 **283**: 33406 [PMID: 18818197]
[8] Leys D *et al*. *J Biol Chem*. 2003 **278:** 5141 [PMID: 12435731]
[9] http://www.drugbank.ca/
[10] Ahmad Z *et al*. *FEMS Microbiol Lett*. 2005 **251**: 19 [PMID: 16143463]
[11] Seward HE *et al*. *J Biol Chem*. 2006 **281**: 39437 [PMID: 17028183]
[12] Ouellet H *et al*. *J Biol Chem*. 2008 **283**: 5069 [PMID: 18089574]
[13] Marriott MS. *J Gen Microbiol*. 1980 **117**: 253 [PMID: 6993625]
[14] http://pubchem.ncbi.nlm.nih.gov/
[15] Wu G *et al*. *J Comput Chem*. 2003 **24**: 1549 [PMID: 12925999]
[16] Cheng T *et al*. *J Chem Inf Model*. 2009 **49**: 1079 [PMID: 19358517]
[17] McLean KJ *et al*. *Trends Microbiol*. 2006 **14**: 220 [PMID: 16581251]
[18] Kolb P *et al*. *Curr Opin Biotechnol*. 2009 **20**: 429 [PMID: 19733475]
[19] Izumizono Y *et al*. *Eur J Med Chem*. 2011 **46**: 1849 [PMID: 21397998]

Edited by P Kangueane
Citation: Sundaramurthi *et al.* Bioinformation 7(3): 130-133 (2011)

BIOINFORMATION

open access

Supplementary material:

Table 1: List of highly ranked molecules by scoring functions PLP1 and LigScore2.

S.No	CID	-PLP1 Score	PLP1 Rank	LigScore2 Score	LigScore2 Rank
1	13388752	137.04	1	7.57	21
2	13388716	129.64	2	7.54	23
3	20519316	129.29	3	7.9	12
4	13388820	128.98	4	7.44	27
5	21443149	126.07	5	8.21	4
6	21499732	125.02	6	8.35	1
7	12854724	124.52	7	8.23	3
8	20519309	123.69	8	7.57	20
9	13388695	123.67	9	7.06	48
10	20406023	122.94	10	8.11	6
11	18648552	122.94	10	7.17	42
12	21499673	110.72	43	8.35	2
13	21499733	118.2	23	8.18	5
14	21499675	111.37	40	8.04	7
15	13388774	121.88	14	8	8
16	13388770	119.09	21	7.96	9
17	18596344	121.74	15	7.95	10