Rational Drug Designing for Drug Target Alanine Racemase (Alr) of Mycobacterium tuberculosis

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Citation

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Abstract

The emergence of multidrug resistant strains and persistence nature of Mycobacterium tuberculosis has caused stringent need to search novel drug targets. Non-homologous proteins of metabolic pathways are first preference for effective drug designing to avoid the deceptive targeting and side-effect in host parasite diseases. In the present study, fourteen unique pathways have been identified throughin-silico comparative metabolic pathways analysis of the host Homo sapiens and the pathogen M. tuberculosis H37Rv. Alanine Racemase (Rv3423c) was considered for drug designing due its role in cell wall synthesis, cell wall organization , alanine metabolic process , alanine racemase activity, and pyridoxal phosphate binding etc. Alanine Racemase (Alr) has crystallographic structure (1XFC) in Protein Data Bank. Ligand library of 50 molecules were designed through Ligand Scout 2.0 and docking studies were performed by the AutoDock 4.0. On the basis of docking energies, a list of top 6 molecules has been proposed which has good compatibility binding affinity with target. The docking studies also suggest that ASP (85), LYS (156), ALA (181) are important determinant residues in binding with ligands, as they are highly involved in hydrogen bond interactions with the ligands.

INTRODUCTION

Re-emergence of multidrug resistant strains of Mycobacterium tuberculosis has caused a serious discussion because tuberculosis continues to be a major cause of morbidity and mortality throughout the world (Aagaard et al.,2003) and infects approximately 36 million people worldwide between 2002 to 2020 (WHO, 2005). TB Structural Genomics Consortium (TBSGC) reported 1,238,518 deaths and 5,308,950 new cases in 2008. The rise in number of patients has caused global concern and stringent need to review various therapeutics aspects of tuberculosis (Zhang et al., 2006). Drugs available for the treatment of tuberculosis, First line drugs are isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB) etc., and second line drugs, para amino salicylate (PAS), kanamycin, cycloserine (CS), ethionamide (ETA), amikacin, capreomycin, thiacetazone, fluoroquinolones. 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). Major drawbacks of current TB therapy are long drugs administration (at least 6-8 months) and very expensive drug combination has to be administered with significant side effects (Chopra et. al., 2003). An innovative, approach is needed for new drug designing against novel drug target to fight tuberculosis.

COMPUTER-AIDED DRUG DESIGN (CADD)

Drug discovery and development is an intense, lengthy and an interdisciplinary endeavor. Drug discovery is started with target identification and lead discovery, followed by lead optimization and pre-clinical in vitro and in vivo studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development. Computer-aided drug designing and bioinformatics approaches are facilitated the drug discovery process. Computer-aided drug design, often called structure based design involves using the biochemical information of ligand-receptor interaction in order to postulate ligand refinements i.e. improvement in binding affinity to receptor. Identification of new lead compounds for new target depends on the information of the target-ligand system, like target and ligand are well known or Target is known but ligand is not known, or ligand is known etc. A large no. of software's is available on different information and different strategy for new lead compounds, like Ligbuilder (Wang et al., 2000), ligand scout 2.0. Compatibility of target and ligand could be performed through docking. Docking is a method which predicts the

preferred orientation of target to ligand when bound to each other to form a stable complex (Lengauer et. al, 1996). Molecular-docking-based virtual screening is an important tool in drug discovery that is used to significantly reduce the number of possible chemical compounds to be investigated. In addition to the selection of a sound docking strategy with appropriate scoring functions, another technical challenge is to in silico screen millions of compounds in a reasonable time. To meet this challenge, it is necessary to use high performance computing (HPC) platforms and techniques. Several commercial as well as Academics docking programs, Glide (Friesner et. Al., 2004), LigandFit, GLOD, M-ZDOCK (G. Costakes) and Autodock (Vaque M, 2006) are available.

MATERIAL AND METHODS

IDENTIFICATION OF UNIQUE PATHWAYS OF BY THE COMPARATIVE STUDY OF

Metabolic pathway of the host H. sapiens and the pathogen M. tuberculosis have been compared using

KEGG database (Kanehisa et al., 2002). Pathways which do not appear in the host but present in the pathogen have been identified as unique pathways. unique pathways were identified in order to design drug candidates for proteins that were present in M. tuberculosis but not in H. sapiens. This will make the drug safer or harmless for H. sapiens.

IDENTIFICATION OF NON-HOMOLOGOUS PROTEINS BY PERFORMING THE BLAST SEARCH

Enzymes of unique pathways as well as enzymes involved in other metabolic pathways under carbohydrate metabolism, amino acid metabolism, lipid metabolism, energy metabolism, vitamin and cofactor biosynthesis and nucleotide metabolism have been identified from the KEGG database. All the proteins of the pathways have been subjected to a BLASTp search against the non-redundant database (Altschul et al., 1997). Though sequence similarity less than 25% implies for low similarity, we adopted a stringent measurement of no similarity for non-homologues proteins (Anishetty et al., 2005).

TARGET CHARACTERIZATION AND LIGAND LIBRARY GENERATION

Selected target were structurally characterized (Active site) through online tools (Pocket finder, p-cats) and offline software (Surface racer). Pocket-Finder finds the active site by scanning a probe radius 1.6 angstoms along all gridlines of grid resolution 0.9 angstroms surrounding the protein. Surface Racer calculates exact accessible surface area, molecular surface area and average curvature of molecular surface for macromolecules. The program also analyzes cavities in the protein interior inaccessible to solvent from outside. Each tool has its unique way of identifying the active site. To obtain more accurate results active site prediction was done on the basis of comparative analysis of results.

Ligand Scout software which automatically calculates a potential pharmacophore by considering the distances and angles between the corresponding chemical functions of the ligand and of the target-protein, was used for ligand generation(G. Wolber, 2005). Further to validate the interaction between the protein and the ligand Ligplot was used. It automatically generates schematic diagrams of protein-ligand interactions for a given PDB file. The interactions shown are those mediated by hydrogen bonds and by hydrophobic contacts.

VIRTUAL SCREENING

Molecular-docking-based virtual screening is an important tool in drug discovery that is used to significantly reduce the number of possible chemical compounds to be investigated. Screenings of best compatible ligand to target were performed through docking by AutoDock. AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. It provides results that are more accurate and reliable.

RESULTS AND DISCUSSION

In present study, fourteen unique pathways with 24 new non-homologous targets were identified through in-silico comparative metabolic pathway analysis of Homo sapiens and M. tuberculosis H37Rv. Pathways which are not present in the Homo sapiens but present in the mycobacterium are designated as unique pathways. Total, 119 metabolic pathways have been found in mycobacterium tuberculosis including 14 unique pathway proteins (Table-1).

Figure 1

Table -1 show unique pathways of mycobacterium tuberculosis with reference to

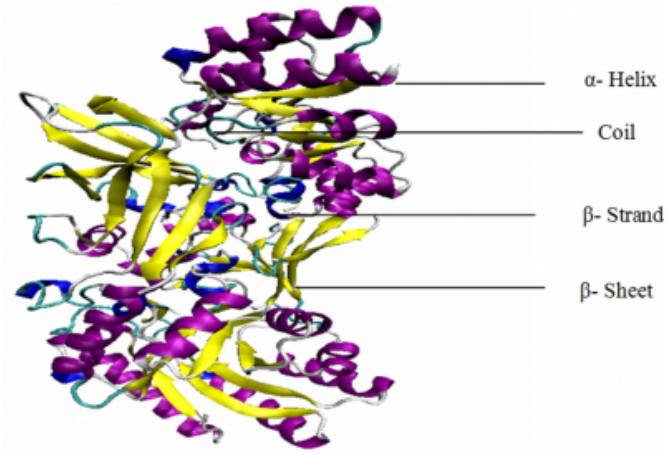
No.	Pathways ID	Name of pathways	Enzyme Accession no.
1	mtu00311	Penicillin and cephalosporin biosynthesis	Rv2068c#
2	mtu00351	1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) degradation	Rv2951c#
3	mtu00362	Benzoate degradation via hydroxylation	Rv2951c#
4	mtu00473	D-Alanine metabolism	Rv3423c(alr), Rv2981c(ddl)#
5	mtu00523	Polyketide sugar unit biosynthesis	Rv3465
6	mtu00540	Lipopolysaccharide biosynthesis	Rv2611c(rmlB), Rv0113(gmhA)
7	mtu00621	Biphenyl degradation	Rv3536c#, Rv3469c(mhpE)#,
8	mtu00622	Toluene and xylene degradation	Rv3536c(rmlB)#, Rv3469c(mhpE)#, Rv3568c(bphC)#
9	mtu00642	Ethylbenzene degradation	Rv0111#, Rv0129c(fbpC)#, Rv0133#,Rv0228#,Rv0262c (aac)#, Rv0517#, Rv1254#, Rv2524c(fas)#, Rv3034c#, Rv3804c(fbpA)#
10	mtu01053	Biosynthesis of siderophore group nonribosomal peptides	Rv2386c(mbtI)#, Rv2378c(mbtG)#
11	mtu02020	Two-component system - General	Rv2498c(citE)#
12	mtu00628	Fluorene degradation	Human homologs of enzymes reported
13	mtu00629	Carbazole degradation	Human homologs of enzymes reported
14	mtu00631	1, 2-Dichloroethane degradation	Human homologs of enzymes reported

Some target ORFs are repeated due to linked between more than one pathways. Targets marked with a (#) symbol are from unique pathways

Non-homologous proteins are first preference for effective drug designing to avoid the deceptive targeting and side-effect. Alanine Racemase (Rv3423c) has been considered for drug designing due its role in cell wall synthesis, cell wall organization, alanine metabolic process, alanine racemase activity, and pyridoxal phosphate binding etc. and its structure is available in Protein Data Bank (1XFC). Characterization of structure is very important for rational drug designing.PDB structure of ALR protein is shown in Figure1.

Figure 2

Figure -1 shows the structure of Alr (1FXC) from PDB data Bank through VMD software



Prediction of active site residues (ALA39, LYS42) of target protein were done through comparative analysis of POCKET FINDER, SURFACE RACER-4.0 and LIGPLOT software results (Figure-2). The comparative results of softwares are shown in table-2.

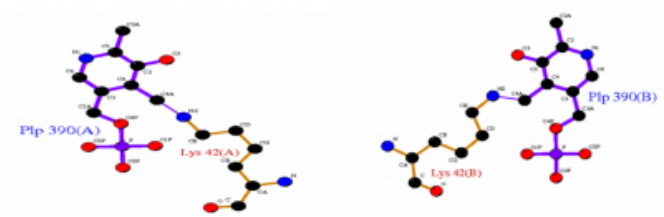
Figure 3

Table-2 Comparative results of softwares and analysis for Active-site prediction

Comparative analysis of softwares results for Active-site identification			
POCKET FINDER	SURFACE RACER-4.0	LIGPLOT	Amino acids
29(ARG),33(GLY) 39(ALA), 42 (LYS) Site Volume: 138 Å3	39(ALA), 42 (LYS), 46(TYR),52(ARG) Site Volume: 174 Å3	42(LYS)	ALA(39) and LYS(42)

Figure 4

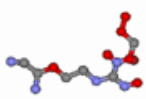
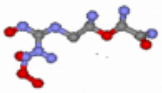
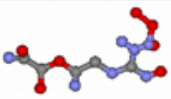
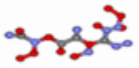
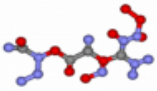
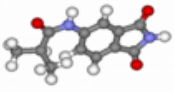
Figure -2 shows the active site residue (LYS- 42) through LIGPLOT software.



Ligand library were designed through Ligand Scout 2.0 and ACDLABS 10.0 ChemSketch. Total 50 ligand molecules were generated including Ligand scout generated ligands. Docking was performed by the AutoDock4.0. On the basis of docking energies, a list of top 6 molecules has been proposed which has good compatibility binding affinity with target in table-3.

Figure 5

Table-3 shows list of selected ligands from generated library after docking

S. N.	Ligand SMILE Notation	Structure
Ligand-1	Br.ON(O/C=[O+]\O)C(/NCC[O+](O)C([NH3+])C=N)=[NH+]\O	
Ligand-2	O.OC(=N)C([NH3+])[O+](O)C(N)CN/C(=[NH+]\O)N(N)N[O+]=O	
Ligand-3	O.OC([O+](O)C(N)CN/C(=[NH+]\O)N(N)N[O+]=O)C(=N)O	
Ligand-4	O.N=C(O)[NH+](OO)[O+](N)C(O)C[O+](NO)C(=[NH2+])N(O)N[O+]=O	
Ligand-5	O.ON(N[O+]=O)/C([O+](NO)C(N)C(O)[O+](N)[NH+](NN)C(=N)O)=[N+](/N)N	
Ligand-6	O=C(Nc1ccc2C(=O)NC(=O)c2c1)C(C)C	

Best confirmations of these results are shown in table-4. Each of these confirmation results calculated from the ligand with the help of Autodock are shown below. Lowest

negative energies of these confirmations have shown best binding of ligands to target proteins shown in table-3.

Figure 6

Table-4 shows binding energy list of selected ligand and their interacting amino-acids

S. N.	Best binding Confirmation out of10	Binding energy Kcal/mol	H bonding involves in interaction
Ligand-1	4	-6.35	rigid22:A:ALA156:HN, rigid22:A:VAL181:HN
Ligand-2	1	-6.55	rigid32:A:ARG85:HH21, rigid32:VAL181:HN
Ligand-3	4	-9.06	Rigid38:A:ALA156:HN, rigid38:A:VAL181:HN
Ligand-4	8	-7.11	Rigid40:A:ARG85:HH11,rigid40:A:ARG85:HH21 Rigid40:A:VAL181:HN
Ligand-5	6	-5.14	Rigid42:A:ALA156:HN, rigid42:A:VAL:181:HN
Ligand-6	10	-6.84	Rigid47:A:ARG85:HH21, rigid47:A:ALA156:HN

CONCLUSION

In the present study, comparative metabolic pathway analysis of the host H. sapiens and the pathogen M. tuberculosis has been performed. Fourteen unique pathways of Mycobacterium tuberculosis have been identified with the comparative study to Homo sapiens. Identification of non-homologous proteins has been done by the BLAST similarity search. Alanine Racemase (Rv3423c) has been considered for drug designing due its role in cell wall synthesis, cell wall organization, alanine metabolic process, alanine racemase activity, and pyridoxal phosphate binding etc. Ligand library of 50 molecules were designed through Ligand Scout 2.0 and docking studies were performed by the AutoDock 4.0. On the basis of docking energies, a list of top 6 molecules has been proposed which has good compatibility binding affinity with target. Designed ligand are interacting with ASP (85), LYS (156), ALA (181) residues of protein structure.

FUTURE DIRECTION

Potential target identification of mycobacterium is on high demand due to re-emergence of drug resistance mycobacterium strains. Comparative study of metabolic pathways is a good approach for the identification of mycobacterium tuberculosis but it still need refining with more high level and number of cutoffs. Proposed ligand molecules need further studies like- molecular interaction of ligand with targets, toxicity prediction, drug likeness, etc.

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