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DRUG DISCOVERY

Antimycobacterial Drug Design: Homology Modeling and Docking Studies on *Mycobacterium tuberculosis* Alanine Racemase

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ABSTRACT

The emergence of drug resistant strains of *Mycobacterium tuberculosis* necessitates the discovery of new molecular scaffolds a priority, and the current situation even necessitates the re-engineering and repositioning of some old drug families to achieve effective control. Due to its essential nature and coupled with the absence of a human homolog, an essential and uniquely prokaryotic enzyme alanine racemase has been pursued as a target for antimycobacterial drug discovery. Biological assembly of obligatory dimer functional unit is modeled. D-Cycloserine is a rigid analog of D-alanine is chosen as scaffold for the rational design of new analogs. In this study, we present a unified approach involving homology modeling and molecular docking studies. Collectively, the results demonstrate the feasibility of using homology modeling and molecular docking studies to obtain novel alanine racemase inhibitor lead compounds that are potentially useful for development as antimycobacterial agents. It should be noted that this computational predicted data should be validated using suitable assays for further consideration.

Keywords: Antimycobacterial drug discovery, Alanine racemase, Homology modeling, Molecular docking

Abbreviations: PDB - Protein Data Bank, kDa-Kilodalton, DOTS - Directly Observed Therapy Short Course, RMS-Root Mean Square, ADME/T - Absorption, Distribution, Metabolism, Excretion and Toxicity

1. INTRODUCTION

Once thought to be on the decline, Tuberculosis (TB) still remains a major global health problem. It ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). TB is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (Mtb). It typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB) (WHO 2012). The global burden of TB remains enormous. In 2011, there were an estimated 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million people died from TB (WHO 2012). This situation is further exacerbated by the emergence of multi-drug resistant form of TB (MDR-TB) and extensively drug-resistant TB (XDR-TB).

Tuberculosis

1. Once thought to be on the decline, tuberculosis still remains a major global health problem today
2. The emergence of multidrug resistant strains and persistence nature of Mycobacterium tuberculosis has caused stringent need to search novel drug targets
3. Non-homologous proteins of metabolic pathways are first preference for effective drug designing to avoid the deceptive targeting and side-effects in host parasite diseases
4. Due to its essential nature, coupled with the absence of a human homolog, an essential and uniquely prokaryotic enzyme alanine racemase has long been an attractive drug target for antimicrobial drug discovery.
5. The developed model showed a good overall structural quality and stereochemical parameters compliance as confirmed by different validation tools
6. Lead molecules were generated and prioritized to follow Lipinski's rule-of-five based on the drug likeliness properties
7. The scheme of the study has designed and identified several novel non-substrate alanine racemase inhibitors

Today's TB drug regimen takes too long to cure, and is too complicated to comply with. Despite the flaws with and growing resistance to current TB treatments, no new TB drugs have been developed in nearly 50 years (Tomioka and Namba 2006; TBAlliance 2012). The poor efficiency of identifying new TB drugs by screening pharmaceutical library collections has been linked to the limited chemical diversity within these collections (Payne, Gwynn et al. 2007). In addition, many new antibiotic candidates are chemical molecules re-engineered from old drug classes discovered decades ago. This approach has identified new TB drugs from existing antibacterial drug classes and either involved the redesign of accessible scaffolds to improve their antimycobacterial potencies or more directly, the repositioning of known antibacterial drugs with good antimycobacterial activity (Koul, Arnoult et al. 2011). During re-engineering of known scaffolds, chemical modifications are introduced into the core structure that may lead to improved bactericidal activities, better resistance profiles, safety, tolerability or superior pharmacokinetic/ pharmacodynamic properties (Koul, Arnoult et al. 2011).

The bacterial cell wall is an ideal target for drug design since similar structures and biosynthetic pathways are absent from mammalian hosts (Feng and Barletta 2003). D-alanine is one of the central molecules of the cross-linking step of peptidoglycan assembly. In an effort to discover new drugs to treat TB we chose Alanine racemase (Alr) as the target of our drug discovery efforts. In Mtb, alanine racemase (EC 5.1.1.1), a pyridoxal 5′-phosphate (PLP)-containing enzyme catalyzes the racemization of L-alanine to D-alanine, which is an essential precursor for the pentapeptide cross-bridge of the peptidoglycan layer in bacterial cell wall (Anthony, Strych et al. 2011). Alr was considered for drug designing due its role in cell wall

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Homology modeling: Predicting the tertiary structure of an unknown based on the known coordinates of a protein to which it is homologous i.e., has a high degree of sequence identity/similarity

Dimer: Dimer is a macromolecular complex formed by two, usually non-covalently bound, macromolecules like proteins. It is a quaternary structure of a protein. A homo-dimer would be formed by two identical molecules and a hetero-dimer would be formed by two different macromolecules

Docking: Docking is an energy-based operation for exploring the binding modes of two interaction molecules. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule.

synthesis, cell wall organization, alanine metabolic process, alanine racemase activity, and pyridoxal phosphate binding. This enzyme is ubiquitous among prokaryotes, and with very few exceptions are absent in eukaryotes, making them a logical target for the development of novel antibiotics (Strych, Penland et al. 2001). The active form of the enzyme is an obligatory dimer containing two monomers of 43 kDa in head-to-tail orientation. Residues from both monomers contribute to the two active sites, where PLP and alanine bind (LeMagueres, Im et al. 2005; Anthony, Strych et al. 2011). Due to its essential nature, coupled with the absence of a human homolog, an essential and uniquely prokaryotic enzyme Alr has long been an attractive drug target and has long been pursued as a target for antimicrobial drug discovery.

The emergence of emergence of MDR-TB and XDR-TB makes the discovery of new molecular scaffolds a priority, and the current situation even necessitates the re-engineering and repositioning of some old drug families to achieve effective control (Koul, Arnoult et al. 2011). Considering the urgency of the situation, drug discovery strategy for the incremental improvements of existing scaffolds is to fill a drug development pipeline is chosen for the purpose of this study. D-Cycloserine (DCS; 4-amino-3-isoxazolidinone) is a rigid analog of D-alanine. It interferes with an early step in bacterial cell wall synthesis in the cytoplasm by competitive inhibition of two enzymes, L-alanine racemase, which forms D-alanine from L-alanine, and D-alanylalanine synthetase, which incorporates D-alanine into the pentapeptide necessary for peptidoglycan formation and bacterial cell wall synthesis (Knox, Law et al. 2011; DrugBank 2012). DCS is effective against mycobacteria and is indicated in the treatment of multidrug-resistant active pulmonary and extra-pulmonary TB in the DOTS-Plus management plan. The potent bactericidal effect of DCS against mycobacteria makes this drug an attractive prototype compound to develop novel antimycobacterial agents (Feng and Barletta 2003). In addition, identification of the lethal target(s) of DCS action would allow for the rational design of new antimycobacterial drugs, structurally related or unrelated to DCS, targeting enzymes of the D-alanine pathway of peptidoglycan biosynthesis (Feng and Barletta 2003). Moreover, this type of inhibitors may weaken the cell wall and act synergistically with other antimicrobial agents (Feng and Barletta 2003).

2. OBJECTIVE AND SCOPE OF THE STUDY

Advances in the identification of new TB drug targets have been driven largely by the availability of the genome sequence of Mtb (Koul, Arnoult et al. 2011). The main objective of the present research is to develop a unified approach involving homology modeling and molecular docking studies on Mtb-Alr. We hope the present work forms the basis for further molecular modeling and biochemical studies on targeting the Mtb-Alr enzyme for therapy, and conclusively reveal a potent lead compound based on best values of docking energy.

3. MATERIALS AND METHODOLOGY

The various computational software programs have been appropriately referred in the framework of the study and are mostly based on Windows or Linux operating system. The methodology pursued is sequentially represented in the research scheme below.

3.1. Sequence Analysis

The amino acid sequence of Mtb-Alr in FASTA format was retrieved from NCBI (NCBIGenBank: CAB01033.1; Cole, Brosch et al. 1998). The Rv3423c Gene Info, Mtb-Alr protein sequence and Mtb-Alr genomic of Mtb H37Rv were also retrieved from Tuberculosis Data Base for cross verification and validation (Reddy, Riley et al. 2009). Sequence alignment search was done using BLASTP program (version BLASTP 2.2.26) at NCBI (Altschul, Madden et al. 1997). After choosing template with highest similarity to Mtb-Alr sequence, pairwise sequence alignment was performed using programme EMBOSS Needle available at http://www.ebi.ac.uk/Tools/psa/. Multiple sequence alignment is performed using the program ClustalW (version 2) through web form available at http://www.ebi.ac.uk/Tools/msa/ClustalW2/ (Thompson, Gibson et al. 2002; Chenna, Sugawara et al. 2003; Larkin MA 2007; Goujon M 2010).

3.2. Homology Modeling

MODELLER is used for homology or comparative modeling of protein three-dimensional structures (Marti-Renom, Stuart et al. 2000; Fiser and Sali 2003; Eswar, Webb et al. 2006; Eswar, Webb et al. 2007). The three dimensional model of Mtb-Alr was built using the crystal structure coordinates of PDB ID: 1XFC A-chain. All steps of homology modeling were performed using MODELLER 9 v10 software. Atom files with coordinates for the template structures, the alignment file with the alignment of the template structures with the target sequence, and MODELLER commands in a script file were generated and run through MODELLER 9v10. Nearly 100 runs of model building were carried out in order to obtain the most reasonable homology model of Mtb-Alr and a preliminary structural investigation was done on the graphic screen to scrutinize the reliability of the alignment. Structural refinement of the developed model, visualization, superpositions and alignments was performed using UCSF Chimera version 1.6.1 (Pettersen, Goddard et al. 2004; Goddard, Huang et al. 2005; Meng, Pettersen et al. 2006; Goddard, Huang et al. 2007; Yang, Lasker et al. 2011).

3.3. Validation of the Generated Homology Model

The built model of Mtb-Alr was assessed using protein structure and model assessment tools available at SwissModel Workspace over http://swissmodel.expasy.org (Schwede, Kopp et al. 2003; Arnold, Bordoli et al. 2006; Bordoli, Kiefer et al. 2009; Bordoli and Schwede 2012). QMEAN6 score is used to evaluate the generated Mtb-Alr homology model (Benkert, Tosatto et al. 2008; Benkert, Kunzli et al. 2009; Benkert, Tosatto et al. 2009). The atomic empirical mean force potential ANOLEA (Atomic Non-Local Environment Assessment) is used to assess packing quality of the model (Melo, Devos et al. 1997; Melo and Feytmans, 1998). GROMOS is a general-purpose molecular dynamics computer simulation package is used for the analysis of conformations (Christen, Hunenberger et al. 2005). The PROCHECK v.3.5 suite of programs is used to assess the "stereochemical quality" of the built Mtb-Alr homology model available at http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/ (Laskowski 1993; Laskowski, Rullmannn et al. 1996). A set of WHAT IF checks on the Mtb-Alr model was performed using servers at http://swift.cmbi.ru.nl/servers/html/index.html (Vriend, 1990; Hooft, 1996). A set of ProSA checks on Mtb-Alr homology model were performed using server located at https://prosa.services.came.sbg.ac.at/prosa.php (Sippl, 1993; Wiederstein and Sippl 2007).

3.4. The Secondary Structure Analysis

The secondary structure analysis of Mtb-Alr model was carried out through PDBsum online server at http://www.ebi.ac.uk/pdbsum/ (Laskowski, Hutchinson et al. 1997; Laskowski 2001; Laskowski, Chistyakov et al. 2005; Laskowski 2007; Laskowski 2009).

3.5. Domain Analysis and Ligand Binding Site Analysis

For classifying the Mtb-Alr model sequence at superfamily, family and subfamily levels, and predicting the occurrence of functional domains a sequence search of InterProScan (Version 4.8), via InterProScan web server at http://www.ebi.ac.uk/Tools/pfa/iprscan/ is performed (Zdobnov and Apweiler 2001; Quevillon, Silventoinen et al. 2005; Mulder and Apweiler 2007; Kelly, Vincent et al. 2010). For cross verification a Scansite (Ver 2.0) of Mtb-Alr model sequence for Motifs was performed at Scansite web server over http://scansite3.mit.edu/#home (Obenauer, Cantley et

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Table 1 QMEAN6 score of the built Mtb-Alr homology model

Scoring function term	Raw score	Z-score
C-beta interaction energy[a]	-132.48	-0.04
All-atom pairwise energy[b]	-9339.65	-0.05
Solvation energy[c]	-40.39	0.37
Torsion angle energy[d]	-122.09	0.88
Secondary structure agreement[e]	80.6%	-0.11
Solvent accessibility agreement[f]	81.1%	0.04
QMEAN6 score	0.785	0.17

[a] Residue-level, secondary structure specific interaction potential using Cβ atoms as interaction centers. Range 3...25 Å, step size: 1 Å
[b] All-atom, secondary structure specific interaction potential using all 167 atom types. Range 3...20 Å, step size: 0.5 Å
[c] Potential reflecting the propensity of a certain amino acid for a certain degree of solvent exposure approximated by the number of Cβ atoms within a sphere of 9 Å around the centre Cβ.
[d] Extended torsion potential over 3 consecutive residues. Bin sizes: 45 degree for the center residue, 90 degree for the 2 adjacent residues
[e] Agreement between the predicted secondary structure of the target sequence and the calculated secondary structure of the model.
[f] Agreement between the predicted relative solvent accessibility using ACCpro (buried/exposed) and the relative solvent accessibility derived from DSSP (>25% accessibility => exposed)

al. 2003). The prediction of ligand-binding sites was done using 3DLigandSite is a web server at http://www.sbg.bio.ic.ac.uk/3dligandsite (Wass, Kelley et al. 2010).

3.6. NESTS Analysis

Nests analysis is done over ProFunc server available at http://www.ebi.ac.uk/thornton-srv/databases/ProFunc (Pal, Suhnel et al. 2002; Watson and Milner-White 2002).

3.7. Biological Assembly of Obligatory Dimer Functional Unit Structure

The active form of the Alr enzyme is an obligatory dimer containing two monomers of 43 kDa in head-to-tail orientation. Residues from both monomers contribute to the two active sites. Biological assembly of obligatory dimer functional unit from monomer of built homology model is performed using SymmDock Web Server located at http://bioinfo3d.cs.tau.ac.il/SymmDock (Dina Schneidman-Duhovny, Yuval Inbar et al. 2005; Schneidman-Duhovny, Inbar et al. 2005).

3.8. Generating Ligands and Optimization

D-Cycloserine (DCS; 4-amino-3-isoxazolidinone) is a rigid analog of D-alanine is chosen as scaffold for the rational design of new D-cycloserine analog antimycobacterial drugs, structurally related to DCS, and targeting enzymes of the D-alanine pathway of peptidoglycan biosynthesis. In this scenario of designing ligands, all the modifications were done by taking in to consideration of a database of substituents and spacers (linkers) obtained by substructure analysis of a collection of current drugs and development drugs. 100 lead molecules were designed with structural modifications of parent molecule using Molinspiration server located at http://www.molinspiration.com /services/search. html. All these molecules were then analyzed for their ability to follow Lipinski's Rule-of-five by subjecting them to Molinspiration server at http://www.molinspiration.com/cgi-bin/properties. The 25 high ranked lead molecules were prioritized to follow Lipinski's rule-of-five based on the drug likeliness properties.

3.9. Protein-ligand Docking

The docking of ligands to the active site of Mtb-Alr model was performed using AutoDock Vina software. AutoDock Vina (version is 1.1.2) is a new open-source program for drug discovery, molecular docking and virtual screening, offering multi-core capability, high performance and enhanced accuracy and ease of use (Trott and Olson 2010). AutoDock Vina automatically calculates the grid maps and clusters the results in a way transparent to the user. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Using the software, polar hydrogen atoms were added to the Mtb-Alr protein and its nonpolar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The grid box with a dimension of 20 x 20 x 20 points was used around the catalytic triad to cover the entire enzyme binding site and accommodate ligands to move freely. The 25 high ranked DCS lead molecules were chosen for docking simulations on to Mtb-Alr model.

4. RESULTS AND DISCUSSION

The amino acid sequence of Mtb-Alr obtained from NCBI comprising of 408 amino acids and bearing a gi. No1449364 (NCBIGenBank: CAB01033.1; Cole, Brosch et al. 1998). The BLAST-P tool was performed by selecting database as PDB alone, and has resulted in a total of 56 blast hits over the query sequence with varied

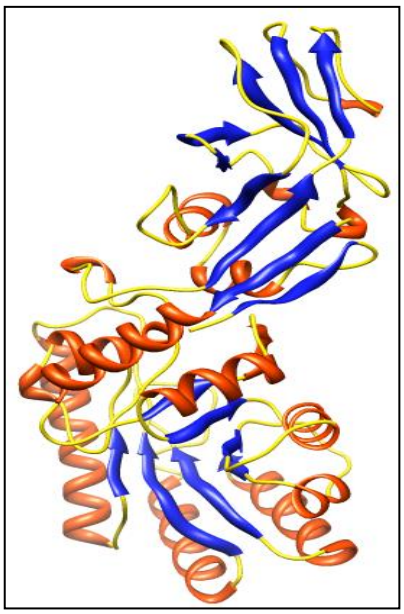

Figure 1
The built 3-D model of Mtb-Alr showing different secondary structure conformations. The helix is represented in orange, the strands in blue, and loops in yellow.

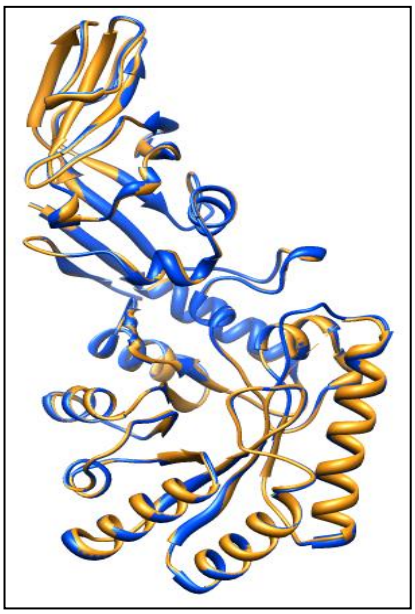

Figure 2
Superposition of crystal structure of template 1XFC-A with built 3-D model of Mtb-Alr. The backbone of 1XFC-A model is in orange and the built 3-D model of Mtb-Alr is in blue

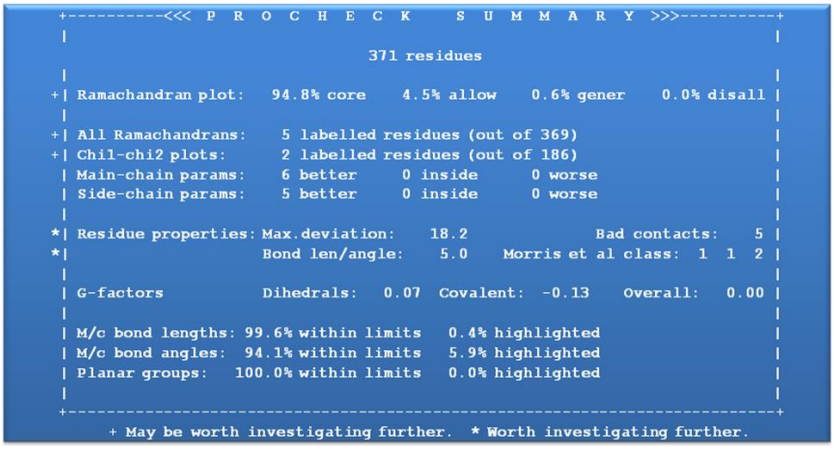

Figure 3
Summary of PROCHECK analysis

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Table 2 Results of WHAT IF checks of Mtb	
Part 1: Structure Z-scores	
1st generation packing quality	-0.615
Ramachandran plot appearance	0.939
chi-1/chi-2 rotamer normality	-0.573
Backbone conformation	-0.770
Part 2: RMS Z-scores	
Bond lengths	0.960
Bond angles	1.198
Omega angle restraints	0.664
Side chain planarity	0.300
Improper dihedral distribution	1.051
Inside/Outside distribution	0.946

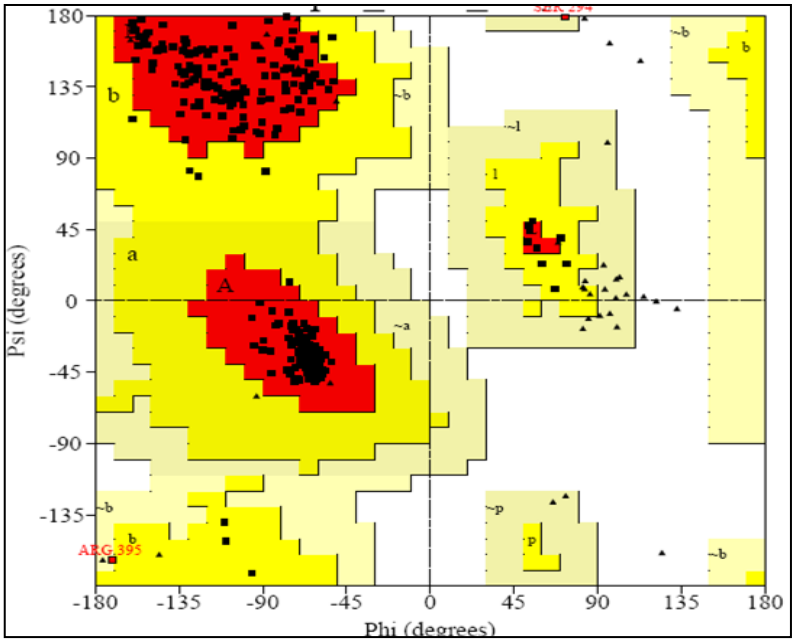

Figure 4
Ramachandran plot for Mtb-Alr model from PROCHECK analysis

alignment values. Specific hits were from Type III Pyridoxal 5-phosphate (PLP)-dependent enzyme alanine racemase super family. These are proteins of fold type III PLP-dependent enzymes that play essential roles in the interconversion between L- and D-alanine, which is an essential component of the peptidoglycan layer of bacterial cell walls. In this case, PDB-ID: 1XFC-A, PDB-ID: 1VFH-A and PDB-ID: 2DY3-A were three high-scoring database matches that align to most of the query sequence. 1XFC-A, 1VFH-A and 2DY3-A showed a query coverage of 94%, 89% and 88% respectively. Among the obtained BLAST P results, the chain A crystal structure of alanine racemase at 1.9 A resolution with PDB ID: 1XFC-A was identified to possess highest similarity to Mtb-Alr, and hence it was chosen as a template.

The quality of Pairwise Sequence Alignment between the target and template sequences is the most important factor determining the accuracy of the homology model. The pairwise sequence alignment has shown the overall identity as 384/408 (94.1%), similarity: 384/408 (94.1%), gaps: 24/408 (5.9%) and score: 1961.0 inferring a good homology. There were few gaps and variations in alignment of sequences which correspond to the sequences at the loops of structures. Multiple sequence alignment is also an important step for phylogenetic analysis, which aims to model the substitutions that have occurred over evolution and derive the evolutionary relationships between sequences. The outcome of multiple sequence alignment is presented a close evolutionary relationship with PDB ID: 1XFC-A, 1VFH-A and 2DY3-A.

MODELLER is used for homology or comparative modeling is a fully automated and constructs energy minimized protein models by satisfaction of spatial restraints extracted from the template PDB file 1XFC-A. The built 3-D model of Mtb-Alr showing different secondary structure conformations is shown in figure 1. Superposition of crystal structure of 1XFC-A with built 3-D model of Mtb-Alr is shown in figure 2. The percent identity of built 3-D homology model of Mtb-Alr compared to 1XFC-A when divided by longer sequence length was 98.65% and while divided by shorter sequence length was 100%. Therefore the built 3-D homology model of Mtb-Alr can thus be characterized as a good theoretical model for further analysis.

Evaluation of model quality is a crucial step in homology modeling (Rodriguez, Chinea et al. 1998; Chivian and Baker 2006; Gao, Xu et al. 2009). The assessment of protein structures is a delicate matter. Currently, there is not a single method able to consistently and accurately predict the three-dimensional structure of a protein. The results of QMEAN6 score of the Mtb-Alr model are shown in Table 1.

QMEAN6 is a composite scoring function which is able to derive both global (i.e. for the entire structure) and local (i.e. per residue) error estimates on the basis of one single model. QMEAN6 is a reliability global score for the whole model reflecting the predicted model reliability ranging from 0 to 1. The quality estimate ranges between 0 and 1 with higher values for better models. QMEAN6 score of the built Mtb-Alr model is 0.785 indicating optimal model reliability (Benkert, Tosatto et al. 2008; Benkert, Kunzli et al. 2009; Benkert, Tosatto et al. 2009). ANOLEA performs energy calculations on a protein chain, evaluating the "Non- Local Environment" (NLE) of each heavy atom in the molecule, and GROMOS computer simulation package studies the biomolecular systems and can be applied to the analysis of conformations obtained by experiment or by computer simulation. ANOLEA and GROMOS results noticeably indicate the predominance of the negative energy values for a given amino acid representing favorable energy environment, and thus indicating a favorable packing quality the models.

PROCHECK is to assess how normal, or conversely how unusual, the geometry of the residues in a given protein structure is, as compared with stereochemical parameters derived from well-refined, high-resolution structures. PROCHECK was used to obtain Ramachandran plot for Mtb-Alr homology model to understand the distribution of Ø/ψ angles of amino acids. The results are shown in the figure 3 and figure 4. 94.8 % of the amino acids are in the most favored regions, 4.5 % are in the additional allowed regions, 0.6 % are in the generously allowed region and 0.0% in the disallowed regions. Further the parameters of PROCHECK have given an insight of main-chain properties and five side chain parameters of Mtb-Alr model which has shown to contain no bad contacts and all the stereochemical parameters are in par with the standard values. 99.6% of main chain bond lengths, 94.1% main chain bond angles 100.0% of planar groups are within limits. The overall G-value was observed to be within the permitted range with G-factors for dihedrals as 0.07, for covalent as -0.13 and overall is 0.00. All these results of PROCHECK demonstrated a positive indication of the reliability of Mtb-Alr homology model for docking studies.

The results of WHAT IF checks on the Mtb-Alr model are shown in Table 2.The first part of the table shows a number of global quality indicators. The second part of the table mostly gives an impression of how well the model conforms to common refinement restraint values. The distribution of residue types over the inside and the outside of the protein is normal with RMS Z-score of 0.946. Structural average packing environment was satisfactory .The structural average packing score is-0.746 and is within normal ranges. No missing atoms were detected in residues and all expected atoms are present in residues. All parameters are within the range of templates, and can be inferred that the developed model is of good quality. The ProSA z-score indicates overall model quality and its values are displayed in a plot that contains the z-scores of all experimentally determined protein chains in current PDB. ProSA-web analysis of Mtb-Alr model is represented as figure 5, and has shown the Z-score value of -8.67 and is in the range of native conformation of similar size template PDB:1XFC-A which is-8.3. PDBsum secondary structure

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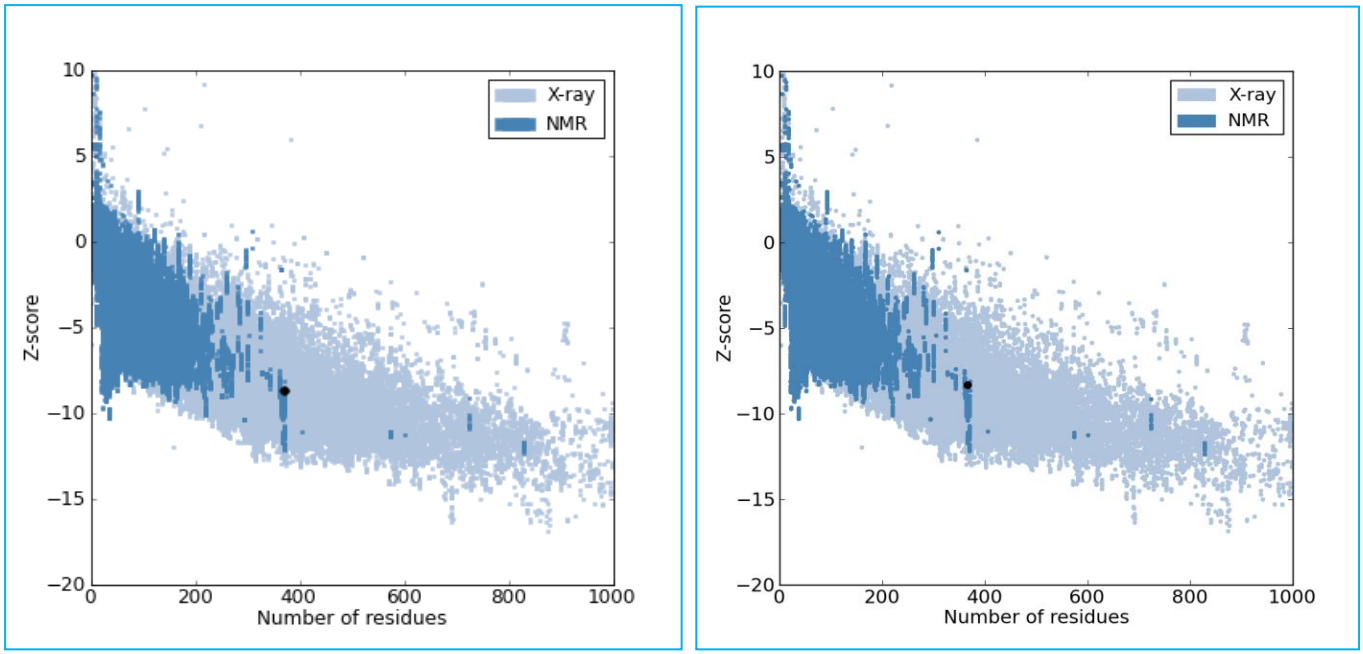

Figure 5

ProSA-web analysis:Z-score plot of Mtb-Alr Model (left) and Z-score plot of PDB:1XFC-A (Right)

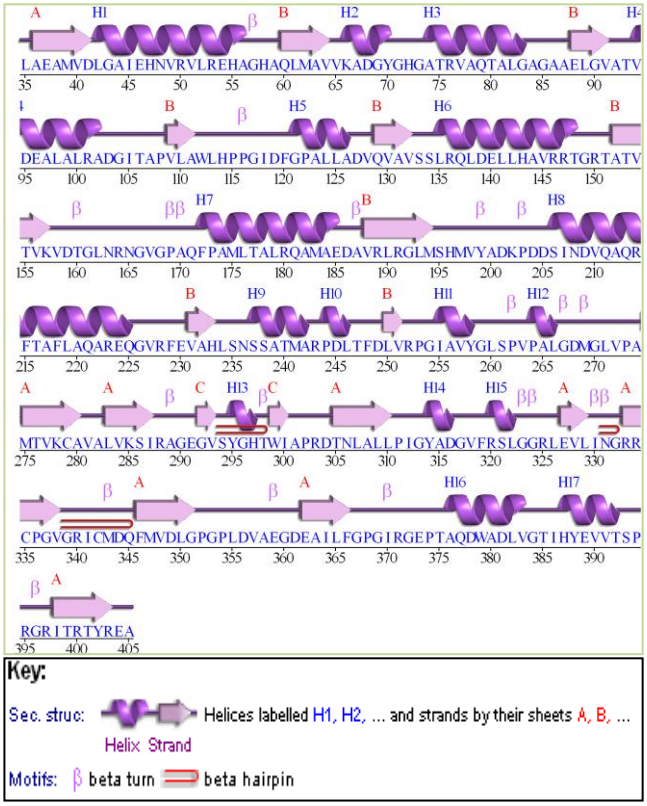

Figure 6

Analysis of PDBSum: Wiring diagram of Mtb-Alr Secondary structure

summary indicated a total of 371 residues distributed as 88 residues (23.7%) as strands, 113 residues (30.5%) as alpha helix , 19 residues (5.1%) as 3-10 helix and 151 residues (40.7%) as other secondary structures. Wiring diagram of Mtb-Alr secondary structure is shown as figure 6.

InterProScan has identified the built model sequence with Alanine racemase signature as evident by InterPro entry accession number IPR000821. The Alr, N-terminal domain is identified at 6-228 residues by InterPro entry accession number IPR001608. The Alr, C-terminal domain is identified at 240-368 residues by InterPro entry accession number IPR011079. The Alr, pyridoxal-phosphate attachment site is identified at 29-39 residues by InterPro entry accession number IPR020622. The PLP-binding barrel superfamily signature is identified at 7-240 residues by superfamily HMM library identifier SSFS1419. The results of Scansite were aligned with the InterProScan, and Alr, N-terminal domain is identified at 6-228 residues and Alr, C-terminal domain is identified at 240-368 residues. 3DLigandSite utilizes protein-structure prediction to provide structural models for proteins that have not been solved. Ligands bound to structures similar to the query are superimposed onto the model and used to predict the binding site. The predicted binding site for Mtb-Alr homology model is of the residues Val 64, Lys 66, Tyr 70, Trp 112, His 196, Tyr 199, Asn 236, Ser 237, Arg 252, Pro 253, Gly 254, Ile 255 and Tyr 388. The predicted bindings site us shown in the figure 7.

ProFunc is a web server for predicting the likely function of proteins whose 3D structure is known but whose function is not (Pal, Suhnel et al. 2002; Watson and Milner-White 2002).Nests are structural motifs that are found in functionally important regions of protein structures. 16 nests were located in this built model chain with the residue conservation score of 1.0. The conservation score ranges from 0.0, signifying that the residue is not at all conserved, to 1.0, which indicates it is perfectly conserved. While five residue ranges demonstrated the nest score above 2.0. A nest score above 2.0 is suggestive of the nest being a functionally significant one. The five residues are residue ranges are Asp268-Gly270 (Asp268, Met269 Gly270) with nest score 4.0 , Pro368-Arg371 (Pro368, Gly369, Ile370, Arg371) with nest score 3.89, Gly71-Gly73 (Gly71, His72, Gly73) with nest score 2.0, Thr160-Asn165 (Thr160, Gly161, Leu162, Asn163, Arg164 , Asn165) with nest score 2.0 and Gly324-Leu326 (Gly324,Arg 325, Leu326) with nest score 2.0. Collectively, the developed model showed a good overall structural quality and stereochemical parameters compliance as confirmed by different validation tools. SymmDock is an algorithm for prediction of complexes with Cn symmetry by geometry based docking. Given the structure of the asymmetric unit of the multimer complex, SymmDock predicts the structure of the entire complex. The output of the method is a list of complexes that fulfill the cyclic symmetry constraints. The symmetry constraints were cross verified with that of PDB ID-1XFC Biological assembly.

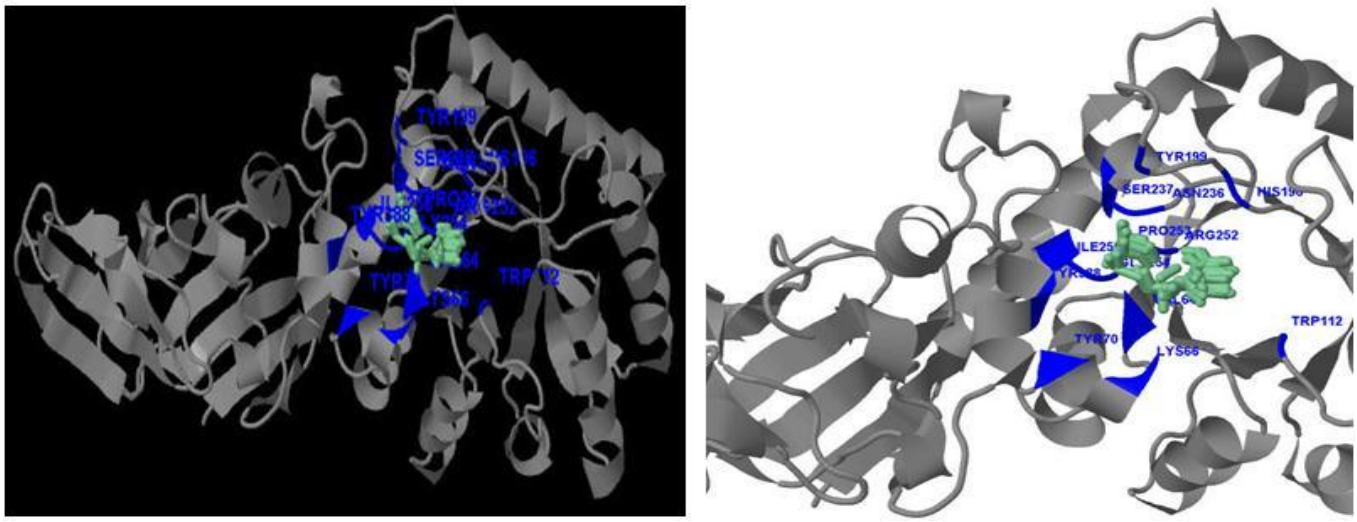

Figure 7
Predicted binding site of Mtb-Alr

Table 4 Results of multiple docking runs performed with AutoDock Vina over Mtb-Alr and DCS lead molecules

Ligand	Binding Affinity (kcal/mol)	RMSD/UB[a]	RMSD/LB[b]
DCS 1	-4.2	11.73	10.9
DCS 2	-4.3	12.53	11.76
DCS 3	-4.4	11.65	11.06
DCS 4	-4.4	9.61	9.00
DCS 5	-4.5	13.53	12.99
DCS 6	-4.6	11.25	10.56
DCS 7	-4.6	10.70	10.42
DCS 8	-4.6	15.09	14.26
DCS 9	-4.6	14.23	13.79
DCS 10	-4.7	14.07	13.62
DCS 11	-4.7	6.25	5.63
DCS 12	-4.8	17.99	16.90
DCS 13	-4.9	12.90	12.44
DCS 14	-5.1	8.64	8.21
DCS 15	-5.3	7.42	5.87
DCS 16	-5.5	16.59	15.95
DCS 17	-5.5	5.83	4.81
DCS 19	-5.6	9.40	8.84
DCS 22	-5.9	12.84	11.82
DCS 21	-6.5	10.39	9.32
DCS 25	-6.5	13.07	11.95
DCS 20	-7.3	10.59	9.38
DCS 23	-7.7	8.71	7.88
DCS 24	-7.8	7.74	6.34
DCS 25	-9.1	7.26	6.60

[a] Root Mean Square Deviation/Upper Bound
[b] Root Mean Square Deviation/Lower Bound

AutoDock:
AutoDock is an automated procedure for predicting the interaction of ligands with biomacromolecular targets

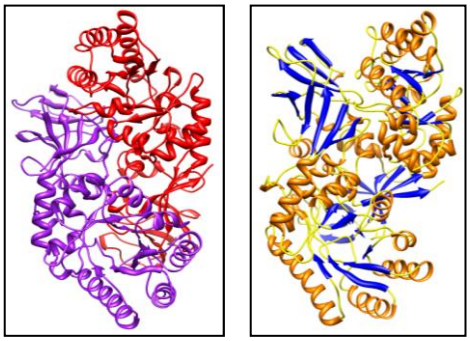

Figure 8
3-D Biological assembly of obligatory dimer functional unit represented as ribbon. Chain A is represented in violet color and chain B as red color. Secondary structure conformations are represented in the following color code: helix is represented in orange, the strands in blue, and loops in yellow

Biological assembly of obligatory dimer functional unit is shown in figure 8.

The 25 high ranked lead molecules were prioritized to follow Lipinski's rule-of-five based on the drug likeliness properties is listed in table 3. Docking of small molecule compounds onto the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure based drug design process. A docking study usually starts with the definition of a binding site, in general a restricted region of the protein. The results of multiple docking runs are automatically analyzed and a ranked list of the docked poses is generated in terms of predicted binding affinity in kcal/mol. The results of multiple docking runs performed with AutoDock Vina over Mtb-Alr and DCS lead molecules is represented in Table 4. All the lead molecules have shown interactions with the Mtb-Alr active site amino acids determined earlier. These interactions may be due to the formation of H-bonds or by the establishment of vanderwalls forces. DCS analog 25 (DCS 25) achieved a good convergence with the best-docked conformations having the lowest binding compared to other series of DCS inhibitor analogs with lowest binding energy -9.1 Kcal/mol.

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Table 3 DCS lead molecules – Molecular properties including Rule of 5 parameters and molecular drug-likeness

Ligand	Formula	Canonical SMILES	IUPAC Name	Mi Log P[a]	TPSA[b]	N atoms	Mol weight	noN	nOHNH	n Voilations[c]	n rotb[d]	Volume[e]
DCS1	C6H11NO2	CC1C(OC(=O)C1N)C	(3R,4S,5S)-3-amino-4,5-dimethyloxolan-2-one	-1.393	52.328	9	129.159	3	2	0	0	124.655
DCS2	C5H8N2O2	CNC1CC(=O)NC1=O	3-(methylamino)pyrrolidine-2,5-dione	-1.356	58.196	9	128.131	4	2	0	1	114.755
DCS3	C4H6N2O2	CON1C=CNC1=O	3-methoxy-1H-imidazol-2-one	-0.403	47.03	8	114.104	4	1	0	1	98.925
DCS4	C5H7NO2	CNC1=CCOC1=O	4-(methylamino)-2H-furan-5-one	-0.943	38.332	8	113.116	3	1	0	1	102.943
DCS5	C5H7NOS	COC1=CSC=C1N	4-methoxythiophen-3-amine	0.993	35.257	8	129.184	2	2	0	1	111.59
DCS6	C3H6N4O	C1(C(=O)NC(=N1)N)N	2,4-diamino-1,4-dihydroimidazol-5-one	-2.013	93.508	8	114.108	5	5	0	0	95.844
DCS7	C3H4N2OS	C1=C(C(=O)NS1)N	4-amino-1,2-thiazol-3-one	-0.078	58.885	7	116.145	3	3	0	0	90.013
DCS8	C6H12N2O	CCN1CCC(C1=O)N	3-amino-1-ethylpyrrolidin-2-one	-0.965	46.332	9	128.175	3	2	0	1	128.643
DCS9	C4H9N3O	C1CN(C(=O)N1)CN	1-(aminomethyl)imidazolidin-2-one	-1.096	58.359	8	115.136	4	3	0	1	107.657
DCS10	C3H8N2O2	CC(C(=O)NO)N	2-amino-N-hydroxypropanamide	-1.692	75.349	7	104.109	4	4	0	1	96.716
DCS11	C4H10N4O	CNC1C(=O)NNN1C	1-methyl-5-(methylamino)triazolidin-4-one	-1.196	56.39	9	130.151	5	3	0	1	120.717
DCS12	C10H10N2O2S	CN1C(=O)N(C(=O)S1)CC2=CC=CC=C2	4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione	1.155	44.01	15	222.269	4	4	0	2	188.228
DCS13	C6H9NOS	CCOC1=C(C=CS1)N	2-ethoxythiophen-3-amine	1.565	35.257	9	143.211	2	2	0	2	128.391
DCS14	C10H9N3O5	C1C(C(=O)NO1)NC=C2C=C(C=CC2=O)[N+](=O)[O-]	4-[[(Z)-(3-nitro-6-oxocyclohexa-2,4-dien-1-ylidene)methyl]amino]-1,2-oxazolidin-3-one	-0.165	113.254	18	251.198	8	2	0	3	201.922
DCS15	C5H10N2O	C1CC(C(=O)C1N)N	2,5-diaminocyclopentan-1-one	-2.389	69.117	8	114.148	3	4	0	0	110.613
DCS16	C12H14N2O5S	CC1=CC=C(C=C1)S(=O)(=O)NC2CON(C2=O)C(=O)C	N-(2-acetyl-3-oxo-1,2-oxazolidin-4-yl)-4-methylbenzenesulfonamide	-0.026	92.783	20	298.32	7	1	0	3	243.523
DCS17	C3H6N2O2	C1C(C(=O)NO1)N	(4R)-4-amino-1,2-oxazolidin-3-one	-1.898	64.355	7	102.093	4	3	0	0	87.082
DCS18	C3H6N2O2	C1C(C(=O)NO1)N	(4S)-4-amino-1,2-oxazolidin-3-one	-1.898	64.355	7	102.093	4	3	0	0	87.082
DCS19	C7H12N2O2S	CCCN1C(=O)N(C(=O)S1)CC	4-ethyl-2-propyl-1,2,4-thiadiazolidine-3,5-dione	0.815	44.01	12.0	188.252	4	0	0	3	166.984
DCS20	C6H10N2OS2	CCN1C(=S)N(SC1=O)CC	2,4-diethyl-3-sulfanylidene-1,2,4-thiadiazolidin-5-one	0.655	26.939	11	190.293	3	0	0	2	159.06
DCS21	C8H14N2O2S	CCCCN1C(=O)N(SC1=O)CC	4-butyl-2-ethyl-1,2,4-thiadiazolidine-3,5-dione	1.374	44.01	13	202.279	4	0	0	4	183.786
DCS22	C7H12N2O2S	CCN1C(=O)N(SC1=O)C(C)C	4-ethyl-2-propan-2-yl-1,2,4-thiadiazolidine-3,5-dione	0.675	44.01	12	188.252	4	0	0	2	166.769
DCS23	C7H10N2O4S	CCOC(=O)CN1C(=O)N(SC1=O)C	ethyl 2-(2-methyl-3,5-dioxo-1,2,4-thiadiazolidin-4-yl)acetate	-0.202	70.315	14	218.234	6	0	0	4	178.15
DCS24	C6H12N4O4	C(C1C(=O)NC(C(=O)N1)CON)ON	3,6-bis(aminooxymethyl)piperazine-2,5-dione	-2.9	128.71	14	204.186	8	4	0	4	172.371
DCS25	C11H12N2O2S	CCN1C(=O)N(C(=O)S1)CC2=CC=CC=C2	4-benzyl-2-ethyl-1,2,4-thiadiazolidine-3,5-dione	1.531	44.01	16	236.296	4	0	0	3	205.03

[a]Octanol/water partition coefficient
[b] Topological polar surface area
[c] Number of Lipinski "Rule of 5" violations
[d] Number of Rotatable Bonds
[e]Molecular volume

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5. CONCLUSION

The emergence of emergence of MDR-TB and XDR-TB makes the discovery of new molecular scaffolds a priority. Considering the urgency of the current situation which even necessitates the re-engineering and repositioning of some old drug families, drug discovery strategy for the incremental improvements of existing scaffolds is to fill a drug development pipeline is chosen for the purpose of this study. Due to its essential nature, coupled with the absence of a human homolog, an essential and uniquely prokaryotic enzyme Alr has long been an attractive drug target and has long been pursued as a target for antimycobacterial drug discovery. D-Cycloserine is a rigid analog of D-alanine is chosen as scaffold for the rational design of new D-cycloserine analog antimycobacterial drugs targeting enzymes of the D-alanine pathway of peptidoglycan biosynthesis. This study highlights the feasibility of obtaining novel Alr inhibitor lead compounds by incremental improvements of existing scaffolds is to fill a drug development pipeline. For the rational structure-based design of drugs, knowledge of the three-dimensional structure of the target protein is indeed inevitably required. Thus, referring to suitable reference structures, well spread in sequence and folding space, it will become increasingly possible to generate realistic models for any given protein sequence using comparative modelling techniques. This technique can be considered sufficiently mature. The developed Mtb-Alr homology model showed good overall structural quality and was confirmed using several different validation tools. The scheme of the study has designed and identified several alanine racemase inhibitors. The inhibitors analogs were designed and docked to find out the most favorable binding analogue. Based on the molecular docking results and Lipinki's values, 4-benzyl-2-ethyl-1,2,4-thiadiazolidine-3,5-dione was confirmed as a promising lead compound. Further, bioassay and pre-clinical analysis of this compound is necessary to accurately understand its molecular mechanism of action and pharmacological efficiency to conclusively state it as an anti-mycobacterial analogue. The combination of genomics and bioinformatics has the potential to generate the information and knowledge that will enable the conception and development of new therapies and interventions needed to treat TB and will facilitate a more rational, and directional approach to search for new drug targets.

SUMMARY OF THE RESEARCH

1. This study highlights the feasibility of obtaining novel alanine racemase inhibitor lead compounds by incremental improvements of existing scaffolds is to fill anti-TB drug development pipeline.
2. The developed model showed good overall structural quality and was confirmed using several different validation tools.
3. The scheme of the study has designed and identified several novel non-substrate alanine racemase inhibitors.

FUTURE ISSUES

1. Computational predicted data should be validated using suitable functional assays for further consideration.
2. Pre-clinical analyses of designed analogues are necessary to accurately understand its molecular mechanism of action and pharmacological efficiency to conclusively state them as an anti-mycobacterial analogue
3. Calculate ADME/T properties of the designed ligands using the ADME/T tools in future
4. Further research is necessary to assess the lethal targets in the D-alanine branch of peptidoglycan biosynthesis in mycobacteria. This information is necessary for the development of new antimycobacterial agents targeting the D-alanine pathway.

DISCLOSURE STATEMENT

This research is a part of the doctoral research and its curriculum of the corresponding author and in not affiliated to the professional employment. There is no financial support for this research work.

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REFERENCES

1. Altschul, S. F., T. L. Madden, et al. (1997). "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res 25(17): 3389-402.
2. Anthony, K. G., U. Strych, et al. (2011). "New classes of alanine racemase inhibitors identified by high-throughput screening show antimicrobial activity against Mycobacterium tuberculosis." PLoS One 6(5): e20374.
3. Arnold, K., L. Bordoli, et al. (2006). "The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling." Bioinformatics 22(2): 195-201.
4. Benkert, P., M. Kunzli, et al. (2009). "QMEAN server for protein model quality estimation." Nucleic Acids Res 37(Web Server issue): W510-4.
5. Benkert, P., S. C. Tosatto, et al. (2008). "QMEAN: A comprehensive scoring function for model quality assessment." Proteins 71(1): 261-77.
6. Benkert, P., S. C. Tosatto, et al. (2009). "Global and local model quality estimation at CASP8 using the scoring functions QMEAN and QMEANclust." Proteins 77 Suppl 9: 173-80.
7. Bordoli, L., F. Kiefer, et al. (2009). "Protein structure homology modeling using SWISS-MODEL workspace." Nat Protoc 4(1): 1-13.
8. Bordoli, L. and T. Schwede (2012). "Automated protein structure modeling with SWISS-MODEL Workspace and the Protein Model Portal." Methods Mol Biol 857: 107-36.
9. Chenna, R., H. Sugawara, et al. (2003). "Multiple sequence alignment with the Clustal series of programs." Nucleic Acids Res 31(13): 3497-500.
10. Chivian, D. and D. Baker (2006). "Homology modeling using parametric alignment ensemble generation with consensus and energy-based model selection." Nucleic Acids Res 34(17): e112.
11. Christen, M., P. H. Hunenberger, et al. (2005). "The GROMOS software for biomolecular simulation: GROMOS05." J Comput Chem 26(16): 1719-51.
12. Cole, S. T., R. Brosch, et al. (1998). "Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence." Nature 393(6685): 537-44.
13. Dina Schneidman-Duhovny, Yuval Inbar, et al. (2005). "PatchDock and SymmDock: servers for rigid and symmetric docking." Nucleic Acids Research= 33(suppl 2): W363-W367.
14. DrugBank. (2012). "DrugBank database of Cycloserine. Accession Number:DB00260.", from http://www.drugbank.ca/drugs/DB00260.
15. Eswar, N., B. Webb, et al. (2006). "Comparative protein structure modeling using Modeller." Curr Protoc Bioinformatics Chapter 5: Unit 5 6.
16. Eswar, N., B. Webb, et al. (2007). "Comparative protein structure modeling using MODELLER." Curr Protoc Protein Sci Chapter 2: Unit 2 9.
17. Feng, Z. and R. G. Barletta (2003). "Roles of Mycobacterium smegmatis D-alanine:D-alanine ligase and D-alanine racemase in the mechanisms of action of and resistance to the peptidoglycan inhibitor D-cycloserine." Antimicrob Agents Chemother 47(1): 283-91.

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Drug discovery, 2013, 3(8), 27-36,

18. Fiser, A. and A. Sali (2003). "ModLoop: automated modeling of loops in protein structures." Bioinformatics 19(18): 2500-1.
19. Gao, X., J. Xu, et al. (2009). "Predicting local quality of a sequence-structure alignment." J Bioinform Comput Biol 7(5): 789-810.
20. Goddard, T. D., C. C. Huang, et al. (2005). "Software extensions to UCSF chimera for interactive visualization of large molecular assemblies." Structure 13(3): 473-82.
21. Goddard, T. D., C. C. Huang, et al. (2007). "Visualizing density maps with UCSF Chimera." J Struct Biol 157(1): 281-7.
22. Goujon M, M. H., Li W, Valentin F, Squizzato S, Paern J, Lopez R (2010). "A new bioinformatics analysis tools framework at EMBL-EBI (2010)." Nucleic acids research 38 Suppl 695-9.
23. Hooft.R.W.W., V. G., Sander.C, and Abola. E.E (1996). "WHAT_CHECK (verification routines from WHAT IF) Errors in protein structures." Nature. 381: 272.
24. Kelly, R. J., D. E. Vincent, et al. (2010). "IPRStats: visualization of the functional potential of an InterProScan run." BMC Bioinformatics 11 Suppl 12: S13.
25. Knox, C., V. Law, et al. (2011). "DrugBank 3.0: a comprehensive resource for 'omics' research on drugs." Nucleic Acids Res 39(Database issue): D1035-41.
26. Koul, A., E. Arnoult, et al. (2011). "The challenge of new drug discovery for tuberculosis." Nature 469(7331): 483-90.
27. Larkin MA, B. G., Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ and Higgins DG (2007). "ClustalW and ClustalX version 2 (2007)." Bioinformatics 23(21): 2947-2948.
28. Laskowski, R. A. (2001). "PDBsum: summaries and analyses of PDB structures." Nucleic Acids Res 29(1): 221-2.
29. Laskowski, R. A. (2007). "Enhancing the functional annotation of PDB structures in PDBsum using key figures extracted from the literature." Bioinformatics 23(14): 1824-7.
30. Laskowski, R. A. (2009). "PDBsum new things." Nucleic Acids Res 37(Database issue): D355-9.
31. Laskowski, R. A., V. V. Chistyakov, et al. (2005). "PDBsum more: new summaries and analyses of the known 3D structures of proteins and nucleic acids." Nucleic Acids Res 33(Database issue): D266-8.
32. Laskowski, R. A., E. G. Hutchinson, et al. (1997). "PDBsum: a Web-based database of summaries and analyses of all PDB structures." Trends Biochem Sci 22(12): 488-90.
33. Laskowski, R. A., MacArthur, M.W., Moss, D.S. and Thornton, J.M. (1993). "'PROCHECK: A program to check the stereochemical quality of protein structures' " J. Appl. Cryst 26: 283-291.
34. Laskowski, R. A., J. A. Rullmannn, et al. (1996). "AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR." J Biomol NMR 8(4): 477-86.
35. LeMagueres, P., H. Im, et al. (2005). "The 1.9 A crystal structure of alanine racemase from Mycobacterium tuberculosis contains a conserved entryway into the active site." Biochemistry 44(5): 1471-81.
36. Marti-Renom, M. A., A. C. Stuart, et al. (2000). "Comparative protein structure modeling of genes and genomes." Annu Rev Biophys Biomol Struct 29: 291-325.
37. Melo, F., D. Devos, et al. (1997). "ANOLEA: a www server to assess protein structures." Proc Int Conf Intell Syst Mol Biol 5: 187-90.
38. Melo, F. and E. Feytmans (1998). "Assessing protein structures with a non-local atomic interaction energy." J Mol Biol 277(5): 1141-52.
39. Meng, E. C., E. F. Pettersen, et al. (2006). "Tools for integrated sequence-structure analysis with UCSF Chimera." BMC Bioinformatics 7: 339.
40. Mulder, N. and R. Apweiler (2007). "InterPro and InterProScan: tools for protein sequence classification and comparison." Methods Mol Biol 396: 59-70.
41. NCBIGenBank:CAB01033.1. "ALANINE RACEMASE ALR [Mycobacterium tuberculosis H37Rv]." from http://www.ncbi.nlm.nih.gov/protein/1449364.
42. Obenauer, J. C., L. C. Cantley, et al. (2003). "Scansite 2.0: Proteome-wide prediction of cell signaling interactions using short sequence motifs." Nucleic Acids Res 31(13): 3635-41.
43. Pal, D., J. Suhnel, et al. (2002). "New principles of protein structure: nests, eggs--and what next?" Angew Chem Int Ed Engl 41(24): 4663-5.
44. Payne, D. J., M. N. Gwynn, et al. (2007). "Drugs for bad bugs: confronting the challenges of antibacterial discovery." Nat Rev Drug Discov 6(1): 29-40.
45. Pettersen, E. F., T. D. Goddard, et al. (2004). "UCSF Chimera--a visualization system for exploratory research and analysis." J Comput Chem 25(13): 1605-12.
46. Quevillon, E., V. Silventoinen, et al. (2005). "InterProScan: protein domains identifier." Nucleic Acids Res 33(Web Server issue): W116-20.
47. Reddy, T. B., R. Riley, et al. (2009). "TB database: an integrated platform for tuberculosis research." Nucleic Acids Res 37(Database issue): D499-508.
48. Rodriguez, R., G. Chinea, et al. (1998). "Homology modeling, model and software evaluation: three related resources." Bioinformatics 14(6): 523-8.
49. Schneidman-Duhovny, D., Y. Inbar, et al. (2005). "Geometry based flexible and symmetric protein docking." Proteins 60: 224-231.
50. Schwede, T., J. Kopp, et al. (2003). "SWISS-MODEL: An automated protein homology-modeling server." Nucleic Acids Res 31(13): 3381-5.
51. Sippl, M. J. (1993). "Recognition of errors in three-dimensional structures of proteins." Proteins 17(4): 355-62.
52. Strych, U., R. L. Penland, et al. (2001). "Characterization of the alanine racemases from two mycobacteria." FEMS Microbiol Lett 196(2): 93-8.
53. TBAlliance. (2012). "Global Alliance for TB Drug Development - The TB Pandemic." 2012, from http://www.tballiance.org/.
54. Thompson, J. D., T. J. Gibson, et al. (2002). "Multiple sequence alignment using ClustalW and ClustalX." Curr Protoc Bioinformatics Chapter 2: Unit 2 3.
55. Tomioka, H. and K. Namba (2006). "Development of antituberculous drugs: current status and future prospects." Kekkaku 81(12): 753-74.
56. Trott, O. and A. J. Olson (2010). "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading." J Comput Chem 31(2): 455-61.
57. Vriend.G (1990). "WHAT IF: a molecular modelling and drug design program." J. Mol. Graph 8: 52-56.
58. Wass, M. N., L. A. Kelley, et al. (2010). "3DLigandSite: predicting ligand-binding sites using similar structures." Nucleic Acids Res 38(Web Server issue): W469-73.
59. Watson, J. D. and E. J. Milner-White (2002). "A novel main-chain anion-binding site in proteins: the nest. A particular combination of phi,psi values in successive residues gives rise to anion-binding sites that occur commonly and are found often at functionally important regions." J Mol Biol 315(2): 171-82.
60. WHO (2012). Global tuberculosis report 2012. Geneva , Switzerland, World Health Organization.
61. Wiederstein, M. and M. J. Sippl (2007). "ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins." Nucleic Acids Res 35(Web Server issue): W407-10.
62. Yang, Z., K. Lasker, et al. (2011). "UCSF Chimera, MODELLER, and IMP: An integrated modeling system." J Struct Biol 179(3): 269-78.
63. Zdobnov, E. M. and R. Apweiler (2001). "InterProScan--an integration platform for the signature-recognition methods in InterPro." Bioinformatics 17(9): 847-8.

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RELATED RESOURCES

1. TB database: an integrated platform for tuberculosis research at http://www.tbdb.org/
2. TB Structural Genomics Consortium at http://www.webTB.org
3. The TubercuList database at http://www.tuberculist.epfl.ch
4. The Wellcome Trust Sanger Institute genomic research centre at http://www.sanger.ac.uk/
5. ExPASy SIB Bioinformatics Resource Portal at http://us.expasy.org/
6. European Bioinformatics Institute databases and tools at http://www.ebi.ac.uk/
7. NCBI databases at http://www.ncbi.nlm.nih.gov/sites/gquery?itool=toolbar

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