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Research Article

DOCKING BASED PHARMACOPHORE MODEL FOR *MYCOBACTERIUM TUBERCULOSIS* PEPTIDE DEFORMYLASE INHIBITORS AND ITS APPLICATION IN DRUG DESIGNING

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ABSTRACT

Objective: Peptide deformylase (PDF) is a metalloprotease enzyme playing important role in protein synthesis. This enzyme is encoded by def gene and its essentiality makes it as attractive drug-target in drug discovery process. The objective of this study was to explore the computational approaches to investigate PDF inhibitors.

Methods: In this study, we have explored the binding mode of *Mycobacterium tuberculosis* (*M.tb*) PDF inhibitors using AutoDock and identified the key residues involved in bonding. We have also screened the Zinc database and identified potential hits with more potent binding energy as compared to previously known inhibitors.

Results: Our study suggested that Val50 and Leu107 are the key residues involve in H-bonding. We have also identified two hydrophobic pockets and two electron acceptor regions using docking based pharmacophore features. Among the various docking based descriptors, binding free energy and unbound system energy showed a correlation value 0.14 and 0.65 with inhibitory activity. However, removal of two outliers identified by clustering technique showed the improvement in correlation value 0.44, and 0.89 with inhibitory activity.

Conclusion: This study will be useful in future for better inhibitor designing and understanding the ligand protein interactions against mtb PDF protein.

INTRODUCTION

Mycobacterium tuberculosis, a causative agent of tuberculosis infected one-third of population globally [1]. The long treatment time and high use of antibiotics leads to the emergence of resistance strains of M.tb like multiple drug-resistant (MDR), extensive drug-resistant (XDR) [2]. Emerging resistance and HIV/M.tb co-infection imposes burden to scientific as well as pharmaceutical companies [3]. Therefore, efforts are now focusing on searching new drug-target as well as new chemical entity. Peptide deformylase (PDF) a metalloprotease, identified by gene knock out experiment act as drug target essential for growth and survival of M.tb [4]. This enzyme catalyzes the hydrolytic removal of the N-terminal formyl group from nascent proteins [5, 6]. The structure of catalytically active enzyme in the nickel (Ni2+) bound form in complex with inhibitor was described [7]. In the past, many Structure-Activity relationships (SAR) have been reported for designing PDF inhibitors [8-11]. In case of M.tb, only single crystal structure 3E3U bound with an inhibitor in presence of Ni2+ ion has been reported in RCSB protein data bank. A docking based approach for ePDF have also been reported [12] but so far docking based approach has not applied for mPDF for identification/prediction of inhibitors. In this study, we explored the binding mode of PDF inhibitors in the vicinity of active site and also the key residues involve in the interaction. Best of author knowledge, this is the first report on in silico study of mPDF inhibitors.

MATERIALS AND METHODS

Dataset

Although, numbers of inhibitor have been reported against different bacterial PDF enzymes [17-24]. In this study, we have used the 14 inhibitors identified against mPDF under the same experimental conditions. All these compounds were downloaded from PubChem BioAssay (AID-411947) in SDF file [13]. Initially, these were converted into 3D structure using open babel software [14], followed by energy minimize using obminimize program with MMFF94s force field along with addition of hydrogen atoms.

Receptor and Ligand Preparation

The mPDF crystal structure (PDB-ID 3E3U) was taken from PDB database (www.rcsb.org). Since the protein is a homo-trimeric structure, therefore chain A is kept, while the B and C chains are deleted; the water and ligand were also removed. The PDF is a metalloprotease enzyme and its activity require the heavy metal Ni^{2+}, therefore the metal ion was kept in enzyme structure for docking. Next, step involves the addition of hydrogen atoms, assigning charge, merging non-polar hydrogen atoms and defining AD4 atom types using AutoDock4.0.1 [15]. A grid with dimensions 40 x 40 x 40 and spacing 0.375 Å was defined using Autogrid feature of AutoDock. The Ni initial parameters (are set as r = 1.170 Å, q = +2.0 and van der Waals well depth of 0.100 kcal/mol) reported by Musiani [16] as the AutoDock default values. For conformational and orientation search of inhibitors Lamarckian genetic algorithm (LGA) is used with number of conformation set to 30, while keeping rest of parameters as default.

Zinc Database Screening

We have used most potent mPDF inhibitor (CID- 44570532) as query to search Zinc database using four-pharmacophore features (1 hydrophobic region, 3 electron donor) with maximum hit per molecule set to 1 and maximum RMSD value 0.5 [Figure-1]. Based on these criteria, we identified 551 compounds most similar to query ligand. Next, these ligands were docked into the active site of PDF (as described above).

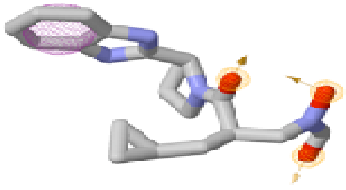

Fig.1: Represent the pharmacophore features of compound (CID- 44570532) used for searching the Zinc database.

RESULTS

For the docking of 14 inhibitors, *M. tuberculosis* PDF crystal structure stored in the PDB file 3E3U was retrieved. The crystal structure of PDF consisted of three similar chains (A, B, and C) with inhibitor bound at active site [25]. The water molecules and inhibitor were removed using PYMOL software [26] and chain A was considered for the docking purpose. In order to validate our docking methodology, crystal bound ligand were taken and energy minimized and dock in the active site of PDF enzyme.

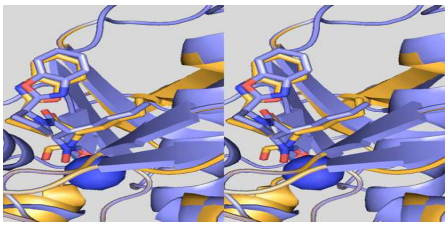

Fig. 2: showing the stereo view of superimposed docked structure yellow color over crystal structure in blue color with RMSD value 1.08.

As shown in Figure-2, the docked ligand binds the same conformation as crystal structure with RMSD value 1.08.

Active Site Analysis and Pharmacophore Identifications

Next step is to docked all other ligands into the active site of PDF enzyme to search for binding mode of these inhibitors. By analyzing the active site of mPDF enzyme docked with inhibitors, we observed that the binding pattern of all the compounds is more or less same [Figure-3]. The numbers of residues participating in the H-bonding with inhibitors varies from 3-5.

As shown in Table-1, nearly all the inhibitors shows the H-bond with Leu107 as well as Val50, indicates that these two interactions plays a major role in binding and could be used as pharmacophore features. As evident from Figure-4, there are two hydrophobic pocket and two site occupied by electronegative O atom that can serve as H-acceptor sites.

While finding the relationship of inhibitory activity with binding energy we observed a correlation value 0.14. Next, we do the clustering of these 14 inhibitors using LibMCS software [27] and found that the two compounds namely 23652895, and 23652829 falls as outliers [Figure-5]. Therefore, after removing these two compounds the correlation score increases from 0.14 to 0.44 (irrespective of sign). Similarly, the correlation value with unbound system energy increases significantly up to 0.89, after the removal of two compounds as mentioned above.

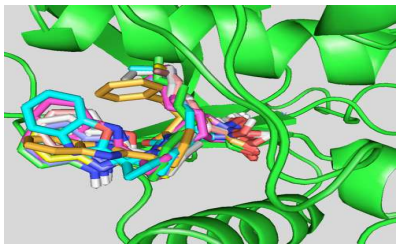

Fig. 3: Depict the binding mode of all 14 inhibitors in the active site of mPDF enzyme.

Table 1: Showing the *M.tb* PDF inhibitors with their compound ID, binding free energy (BFE) and potential H-bonding interaction.

CID	IC_{50}	BFE	H-bond
44570532	8	-6.44	Val50, Gln56, Asn48, Leu107
16220165	10	-5.8	Glu149, Asn48, Gln56, Leu107, Val50
25134267	13	-5.91	Leu107, Val50, Gln56
44570570	13	-5.2	Leu107, Val50, Gln56
44570531	15	-5.94	Val50, Gln56, Asn48
44570609	18	-7.78	Glu149, Asn48, Gln56, Leu107, Val50
44570571	21	-5.9	Leu107, Val50, Gln56
44570530	24	-5.99	Leu107, Val50, Gln56, Glu149
44570569	28	-5.56	Leu107, Val50, Gln56, Glu149
44570610	49	-7.46	Leu107, Val50, Gln56, Glu149
44570533	68	-6.68	Leu107, Val50, Gln56, Glu149
44570572	161	-6.89	Leu107, Val50
23652895	202	-4.88	Leu107, Val50, Gln56
23652829	803	-5.46	Leu107, Val50, Gln56

Table 2: Represent the most potent 15 predicted inhibitors of PDF with their predicted free energy of finding.

ID	-ΔG	ID	-ΔG
ZINC12411200	-7.95	ZINC03428383	-7.17
ZINC03310666	-7.83	ZINC03359116	-7.14
ZINC22063970	-7.43	ZINC03347694	-7.08
ZINC71388930	-7.36	ZINC13533683	-7.09
ZINC71388931	-7.27	ZINC15824439	-6.91
ZINC13058519	-7.25	ZINC03308738	-6.87
ZINC22063967	-7.22	ZINC03363002	-6.84
ZINC38653916	-7.19		

DISCUSSION

This study describes the binding mode of mPDF inhibitors using docking study. We have identified the pharmacophore features using docking based conformation of ligands. These pharmacophore features will be helpful in searching the similar ligands to identify potential structure based inhibitors having these types of features. Our finding also suggested the role of key residue Val50 and Leu107 in H-bonding and will be helpful in further for designing better inhibitors.

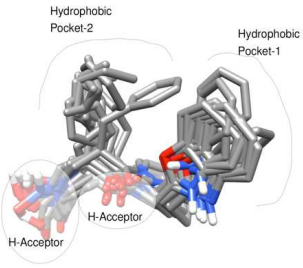

Fig. 4: Depict the Pharmacophore features of docked ligands marked by hydrophobic pocket 1 and 2 as well as two hydrogen acceptor sites.

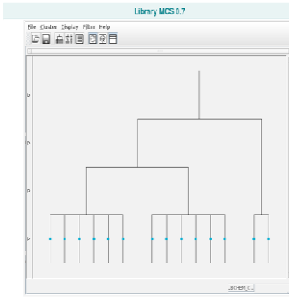

Fig. 5: shows the clustering of all 14 inhibitors, the last two compounds are different from the others.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PP and VD designed and performed the experiments and also analyzed the data. SS, RR, MC and TR wrote the manuscript. This manuscript has been seen and approved by all authors.

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List of abbreviations used

M.tb: mycobacterium tuberculosis, mPDF: mycobacterium Peptide deformylase, ePDF: *e.coli* peptide deformylase, val: valine, leu: leucine, HIV: human immunodeficiency virus, H-bonding: hydrogen bonding

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