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In silico screening of novel inhibitors of M17 Leucine Amino Peptidase (LAP) of *Plasmodium vivax* as therapeutic candidate

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ABSTRACT

M17 LAP (Leucine Amino Peptidase) plays an important role in the hydrolysis of amino acids essential for growth and development of *Plasmodium vivax* (Pv), the pathogen causing malaria. In this paper a homology model of PvLAP was generated using MODELLER v9.15. From different *in-silico* methods such as structure based, ligand based and *de novo* drug designing a total of 90 compounds were selected for docking studies. A final list of 10 compounds was prepared. The study reported the identification of 2-[(3-azaniumyl-2-hydroxy-4-phenylbutanoyl) amino]-4-methylpentanoate as the best inhibitor in terms of docking score and pharmacophoric features. The reliability of the binding mode of the inhibitor is confirmed by molecular dynamics (MD) simulation study with GROMACS software for a simulation time of 20 ns in water environment. Finally, *in silico* ADMET analysis of the inhibitors using MedChem Designer v3 evaluated the drug likeness of the best hits to be considered for industrial pharmaceutical research.

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1. Introduction

After *Plasmodium falciparum*,*Plasmodium vivax* (Pv) is the second most important malaria species producing an extremely debilitating relapsing disease mainly in the Middle East, Asia and Western pacific regions [1]. Although the mortality rate is lower than that of *P. falciparum* infection, there is evidence for development of drug resistance and its morbidity is often underestimated. The parasite has a complex life cycle with the persistent liver hypnozoite stages producing relapses for up to 5 years [2]. The asexual intra-erythrocytic stage of parasite development is responsible for clinical symptoms. This stage carries out host haemoglobin degradation pathway and release of amino acids which is utilized to maintain the osmotic pressure within the infected erythrocyte, and prevents the premature cell lysis during parasite growth [3,4]. The catabolism of host cell haemoglobin to peptide fragments occurred within digestive vacuole and transported towards parasitic cytosol where the peptide fragments get degraded into free amino acids by aminopeptidase [3–5]. M17 leucine aminopeptidase belongs to the metalloaminoeptidase family and played a significant role in the catalysis of the terminal stage of haemoglobin degradation and are critical to this stage [4]. The activity of aminopeptidase in the parasitic cytosol are optimum at neutral pH and have preference for synthetic substrates containing leucine and alanine, the most abundant amino acids of human haemoglobin; 24% approximately, at the N-terminus [3].

Leucine aminopeptidase is a catalytically active homodimer, neutral aminopeptidase of molecular weight approximately 67.8 kDa (PF14_0439) [3,4]. The potent and slow binding inhibitor of LAP, bestatin [(2S, 3*R*)-3-amino-2-hydroxy-4-phenyl-butanoyl]-L-leucine is a dipeptide immunopotentiator that was first isolated from a culture filtrate of *Streptomyces olivoreticuli* [6,7]. Bestatin successfully inhibit the growth of *P. falciparum*, *in-vitro* and *in-vivo* culture with inhibition constant (Ki) of 25 nM and an IC_{50} of 14.87 μM [8].

Inhibitory activities of bestatin and its various synthetic analogues against *P. falciparum* aminopeptidase are reported in many literatures [7,8]. But, potent inhibitors of *P. vivax* M17 leucine aminopeptidase are to be developed. Here, we made an *in-silico* approach for virtual screening, pharmocophore mapping and *de novo* drug designing for identification of potential inhibitors against *P. vivax* M17 LAP. Combination of structure-based and ligand-based approach along with ADMET prediction study is a best way to identify lead compounds in the drug discovery process [9]. In our work, we tried to utilize a combination of these methods to identify novel and potential compound against PvLAP.

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2. Materials and methods

2.1. Homology modeling

In the lack of experimentally solved 3D structure of *P. vivax* M17 leucine aminopepetidase, we tried to make the homology model of *P. vivax* LAP protein. The amino acid sequence of *P. vivax* LAP was retrieved from NCBI (GenBank: EDL46203) in FASTA format. The sequence is 621 amino acids in length. The protein sequence was subjected to BLASTp search [10] against PDB database. BLASTp resulted with 3KQX-Chain A, crystal structure of *P. falciparum* protease having target-template identity of 73% with an e-value of 0.0. The alignment of target and template was generated using Praline program [11]. We build the homology model using MODELLER v9.15 [12] followed by sequential loop refinement using loop refinement tool provided by the software. The modeled structure validation was done using tools and servers like PROCHECK [13], VERIFY 3D [14], ProSA [15], ERRAT [16].

2.2. Database search

Different compound databases were searched for availability of bestatin and its synthetic analogues compounds. For our study, ZINC [17], PubChem [18], ChemSpider [19] and DrugBank [20] were considered as a dockable database. The ZINC database search reported 21 compounds including bestatin with 80% structural similarity. 16 compounds were screened from PubChem database similarity search. The ChemSpider compound database query search provided no significant hits. The DrugBank database sequence similarity search revealed 3 small molecules as possible inhibitor against PvLAP. The overall database search listed a total of 40 small molecules, structurally similar with bestatin. These compounds were manually checked for any kind of redundancy among databases and finally 40 compounds were considered for molecular docking simulation study.

We used ISIS Draw 2.3 software for sketching some analogues of bestatin reported in literature [7] and converted them to their 3D representation by using Chemsketch 3D viewer of ACDLABS 12.0. Ligprep v2.5 [LigPrep, Schrödinger, LLC, New York] was used for energy minimization and final preparation of ligands.

2.3. De novo *drug design*

e-LEA3D server was used for designing of *de novo* lead compound considering bestatin as parent compound. 3D coordinates of PvLAP protein was given as input along with default PLANTS docking parameters [21]. The binding site residue was defined with binding radius 10 Å, weight in final score 1 and molecular weight less than 499.

2.3.1. Structure-based virtual screening

DOCK Blaster server performs structure-based virtual screening against the ZINC chemical database [17]. The final modeled structure of PvLAP was subjected to structure based virtual screening of potential lead compounds from the ZINC database. The sequential screening procedure of DOCK Blaster resulted with 200 lead compounds docked in the active site of the target protein. The compounds were filtered and arranged orderly based on Lipinski's rule of five.

2.3.2. Virtual screening based on structure-based pharmacophore model

ZINCPharmer server [22] was used for virtual screening based on structure-based pharmacophore model generated using LigandScout v3.0 software package [23]. The reference 3D pharmacophore model for PvLAP was generated using complex PDB structure of PfLAP and bestatin (PDB ID: 3KR4). Initially, the generated model was associated with poor performance without any hits. Therefore, we manually tried to adjust these features. The ligand-receptor interacting residues are kept in account during the adjustment of the pharmacophoric model. The generated pharmacophoric model was subjected to ZINCPharmer server as a reference for virtual screening against purchasable compounds of ZINC database. Filters were applied that included maximum 0.7 Root Mean Square Deviation (RMSD), rotatable bond cut-off less than 10, molecular weight in the range of 180–500 Da to get best hits from ZINC database [24,25].

2.3.3. Virtual screening based on ligand-based pharmacophore model

In this screening method bestatin was given as input query in the ZINCPharmer database. Initially, the pharmacophoric feature of bestatin reported with no significant hits. These features were manually modified that include two hydrophobic features, an aromatic feature, a hydrogen bond acceptor, a hydrogen bond donor, a positively charged and a negatively charged feature. Similar filters were applied, as in structure-based pharmacophore model and searched the ZINC database that have best similarity match with the pharmacophoric feature of bestatin to screen purchasable compounds from the ZINC database having similar pharmacophoric features.

2.4. Molecular docking studies

To confirm the binding mode of compounds undertaken in our search with PvLAP protein, molecular docking studies was carried out using AutoDock v4.2.6 software [26]. The docking parameters were defined as coordinates of the center of binding site with $x = 87.277$, $y = 75.241$, $z = 25.563$ and binding radius = 0.375 Å. Rigid docking was performed using Genetic Algorithms and keeping other docking parameters in default. The binding modes of top 10 hits based on the binding energy were re-confirmed using FlexX docking software provided by Lead-IT software package [27]. The active site was defined including all active site residues within a 6.50 Å radius sphere and the docking parameters were kept in default.

2.4.1. Cross-docking analysis

Different docking procedures and algorithms would give rise to different docking results even for same protein and ligand. Cross-docking analysis could be a way to find similar conformations between docked compounds from different docking procedures based on the RMSD of docked conformation of each ligand. So, RMSD values of docking pose of 10 compounds were evaluated.

2.5. Molecular dynamics (MD) simulation

Molecular Dynamics simulation study of the best identified compound was performed to confirm the binding mode determined from molecular docking study using GROMACS v5.0 software package [28]. The topology file of the ligand was prepared using PRODRG server [29] and topology file of protein coordinates was prepared using pdb2gmx tool provided by GROMACS along with the use of GROMOS96 43a1 force field [30]. A protein centered cubic box was defined with a grid cell distances 36 nm X 36 nm X36 nm. The protein-ligand complex is solvated with 216 SPC water and a total of 42,952 solvent molecules were filled in the defined box. To keep the system neutralized 8 Na^+ ions were added. The Particle-Mesh-Ewald (PME) method [31] was used for calculation of long-range electrostatic interactions. A 50,000 steps energy minimization was performed with the steepest descent method. Berendsen thermostat Temperature coupling and Parrinello-Rahman pressure

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