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P1 and P1′ *para*-fluoro phenyl groups show enhanced binding and favorable predicted pharmacological properties: Structure-based virtual screening of extended lopinavir analogs against multi-drug resistant HIV-1 protease

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ABSTRACT

Crystal structure of multidrug-resistant (MDR) clinical isolate 769, human immunodeficiency virus type-1 (HIV-1) protease in complex with lopinavir (LPV) (PDB ID: 1RV7) showed altered binding orientation of LPV in the expanded active site cavity, causing loss of contacts and decrease in potency. In the current study, with a goal to restore the lost contacts, three libraries of LPV analogs containing extended P1 and/or P1′ phenyl groups were designed and docked into the expanded active site cavity of the MDR769 HIV-1 protease. The compounds were then ranked based on three criteria: binding affinity, overall binding profile and predicted pharmacological properties. Among the twelve proposed extensions in different combinations, compound **14** (consists of *para*-fluoro phenyl group as both P1 and P1′ moieties) was identified as a lead with improved binding profile, binding affinity against the MDR protease and favorable predicted pharmacological properties comparable to those of LPV. The binding affinity of **14** against wild type (NL4-3) HIV-1 protease was comparable to that of LPV and was better than LPV against an ensemble of MDR HIV-1 protease variants. Thus, **14** shows enhanced binding affinity by restoring lost contacts in the expanded active site cavity of MDR769 HIV-1 protease variants suggesting that it may have higher potency compared to that of LPV and hence should be further synthesized and evaluated against NL4-3 as well as MDR variants of HIV-1.

1. Introduction

Human immunodeficiency virus type-1 (HIV-1) protease [1] is a homo-dimeric aspartyl protease that is critical for the viral replication and maturation [2,3]. Inhibition of the protease would significantly lower the viral infection within the host as well as between the hosts. Among the various generations of protease inhibitors (PI), Kaletra [4,5] has been a very successful combination of the PIs – lopinavir (LVP) and ritonavir (RTV) [6]. As shown in Fig. 1a, both LPV and RTV have similar functional groups at the P1 and P1′ positions with the transition state mimic hydroxyl group [7–9] in between the P1 and P1′.

Previously it has been shown that severe accumulation of mutations causes loss of binding [10] for LPV against the multi-drug resistant (MDR) clinical isolate 769 [11] HIV-1 protease (PDB ID: 1RV7). LPV shows more contacts with wild type (PDB ID: 1MUI) than with MDR769 HIV-1 protease (PDB ID: 1RV7). The MDR769 HIV-1 protease shows a wide-open conformation of the flaps [12] due to conformational rigidity caused by the accumulation of mutations [13]. A similar trend of wide-open flaps consistent in four variants [14] with the MDR769 background was also reported earlier. Mutations from longer side chain residues to shorter side chain residues cause an overall expansion of the active site cavity, which in turn causes weaker binding of the PI due to loss of contacts [10,15]. Recently it has been shown that a scanning Ala/Phe chemical mutagenesis approach can be effectively used to probe the expanded active site cavity of MDR769 HIV-1 protease with peptides [16]. Based on these structural analyses, it was

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hypothesized that by substituting the P1 and P1′ phenyl groups of LPV with extended analogs of phenyl group one can restore the lost contacts in the expanded active site cavity.

With a goal to restore the lost contacts, 12 extensions were designed as shown in Fig. 1b. The 12 proposed extended analogs were categorized into three libraries. Library #1 (compounds **1**–**12**) consists of asymmetric compounds where the P1′ phenyl group is substituted by the extended analog; library #2 (compounds **13**–**24**) consists of symmetric compounds where both the P1 and P1′ phenyl groups are substituted by the same extended analog and library #3 (compounds **25**–**32**) consists of compounds with combination of extended analogs from libraries 1 and 2 that exhibit improved binding. Virtual screening [17–19] of the compounds was performed using AutoDock-Vina [20] against the MDR769 protease receptor (without ligand) taken from the crystal structures, PDB IDs: 1RV7 and 3OQ7. The compounds were ranked according to their binding affinities (docking score) using the protocol given in Fig. 2 and a lead compound was identified based on a combination of highest binding affinity value, improved overall binding profile and favorable predicted pharmacological properties. The lead compound was further docked into the active site cavity of (i) wild type (NL4-3) HIV-1 protease taken from the crystal structure of NL4-3 HIV-1 protease in complex with LPV [21], (PDB ID: 1MUI), (ii) all the protease–LPV crystal structures available in RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) to date and (iii) an ensemble of MDR variants (PDB IDs: 3OQ7, 3OQA, 3OQD, 3PJ6 and 2RKF). Docking solutions were analyzed in detail. Molecular properties of pharmacological interest, based on Lipinski's rule of five [22], were predicted using molinspiration software (http://www.molinspiration.com) for each of the 32 compounds designed in the current study, in order to verify any violations that may decrease the drug-likeliness of the compound.

2. Materials and methods

2.1. Design and preparation of extended LPV analogs for docking

Extended LPV analogs were designed using ChemDraw and were modeled using the coordinates of LPV taken from the crystal structure, PDB ID: 1MUI, as a template. The compounds were then energy minimized using the Chem3D (http://www.cambridgesoft.com) interface to avoid any bad contacts (steric clashes). Three libraries of compounds were generated. Library#1 consists of asymmetric extensions (compounds **1**–**12**) with an extended phenyl group at P1′ position. Library#2 consists of symmetric extensions (compounds **13**–**24**) with extended phenyl groups at both P1 and P1′ positions. Library#3 consists of combinations of the extensions from libraries 1 and 2 with best binding affinities. The compounds were further prepared for docking using AutoDockTools [23] graphics interface as described previously [15].

2.2. Preparation of the receptor for docking

The crystal structures of MDR769 HIV-1 protease, PDB IDs: 1RV7 and 3OQ7, were chosen as receptors. Coordinates for crystallographic waters and LPV were deleted. Asn25 and Asn125 were modified to Asp25 and Asp125 to represent catalytically active form of the protease followed by the addition of polar hydrogens to the receptor. The three-dimensional docking grid has been generated covering the residues from the active site area, flaps and the 80s loops (Pro79 to Asn83) that are critical in ligand binding. This receptor grid was generated using the AutoDockTools graphics interface and the same grid was used for docking all the compounds one at a time. Vina [20] program was used to perform the fast pace exhaustive conformational searching for each of the designed compounds.

2.3. Virtual screening, ranking and prediction of pharmacological properties for the extended LPV analogs

The binding pose with the highest binding affinity (out of the top five poses) for each compound was chosen and binding orientation was analyzed. Final ranking of the compounds was performed as described in Fig. 2. Molecular properties of pharmacological interest (based on Lipinski's rule of five) for each compound were calculated using molinspiration software (http://www.molinspiration.com).

2.4. Structural analysis

All the protease-drug interactions were calculated as described previously [15]. A cut-off of 3 Å distance between the donor and acceptor, 90° angle for the donor and 60° for the acceptor were used for the calculation of hydrogen bonds. The hydrophobic contacts were computed between the two carbon atoms (one from the protease residue and the other from LPV/LPV analog) within a distance of 4 Å. Figs. 1, 4a and S3 were prepared using ChemDraw Ultra (http://www.cambridgesoft.com); Fig. 5 was generated using the open source molecular graphics program, PyMol (v0.99) (http://www.pymol.org).

3. Results

3.1. Virtual screening of extended LPV analogs

Among the 24 compounds from libraries 1 and 2 used for the virtual screening of the expanded active site cavity of the MDR protease, compound **14** was identified as the lead compound with highest binding affinity and improved overall binding profile. As shown in Fig. 3, a total of 22 extended LPV analogs (**1**–**20**, **22** and **23**) show better binding affinity values compared to that of LPV (−6.7 kcal/mol) screened against the MDR protease. Compounds **24** and **21** showed lowest binding affinity values −6.6 kcal/mol and −5.9 kcal/mol, respectively among the 24 compounds.

3.2. Binding affinities of asymmetric compounds (library–1)

Virtual screening of the asymmetric series of compounds (**1**–**12**) with an extended phenyl analog at the P1′ position yielded three compounds (**1**, **6** and **11**) with highest binding affinity within library #1. Among the asymmetric series, the *para*-methyl extension at P1′ position of LPV (compound **1**) was chosen as the lead compound with best binding affinity and overall improved binding profile. Compounds **6** and **11**, in spite of improved binding, were not chosen due to their relatively higher molecular weight. The highest binding affinity value among this series was −7.7 kcal/mol (compound **1**) and the lowest was −6.9 kcal/mol for the compounds **7**, **9** and **12** (Fig. 3).

3.3. Binding affinities of symmetric compounds (library–2)

The symmetric series of compounds (**13**–**24**) have the same extended phenyl analog at both P1 and P1′ positions on LPV template. Virtual screening of the symmetric series of compounds yielded one compound, **14**, with highest binding affinity. Among the symmetric series, compound **14**, with *para*-fluoro extensions at both P1 and P1′ positions of LPV shows best binding affinity (−7.8 kcal/mol) and was chosen as the lead compound within the library #2. As shown in Fig. 3, the highest and lowest binding affinity values among this series are −7.8 kcal/mol and −5.9 kcal/mol for **14** and **21**, respectively.