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RESEARCH ARTICLE

Design of Novel HIV-1 Protease Inhibitors with Favorable Oral Properties using a Molecular Modelling Approach

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ABSTRACT:
Acquired immunodeficiency syndrome (AIDS) is a chronic and potentially fatal transmissible disease caused by the Human Immunodeficiency Virus (HIV). Since its discovery in 1981, an estimated 85 million cases and 40 million AIDS related deaths have occurred worldwide. Among the two types of HIV, HIV-1 accounts for over 90% of reported cases. Throughout the years, multiple drugs have been approved for the treatment of AIDS. However, these drugs face many drawbacks such as toxic side effects, non-optimal pharmacodynamic profile and drug resistance due to virus mutation. This study aims to design novel potent HIV-1 protease inhibitors that overcome these drawbacks through molecular modelling methods. Pubchem database was screened for potential lead compounds. Results were filtered through two phases of ADMET and docking studies. Finally, the chosen lead compound was optimized through fragment replacement to obtain the novel inhibitors.

KEYWORDS: AIDS, HIV-1 protease, Virtual screening, ADMET, Lead optimization.

INTRODUCTION:
The World Health Organization estimates that, since 1981, 65 to 113 (average 85.6) million people have been infected with HIV, 32.9 to 51.3(average 40.4) million people have died due to HIV related complications, and 33.1 to 45.7 (average 39) million people were living with HIV at the end of 2022[1]. There are two main types of HIV, the more virulent and infectious type HIV-1 (particularly subtype M) which accounts for almost 90% of global cases, and the less transmissible and less prevalent HIV-2[2,3]. Many efforts were made to eradicate this deadly pandemic, chief of which were antiviral medication. There are currently 24 unique FDA approved drugs for treating HIV infections[4].

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However, these drugs suffer from major drawbacks such as toxic adverse reactions, drug interactions, poor CNS penetrability, low oral bioavailability due to high CYP450 metabolism and possibly the biggest factor; drug resistance due to virus mutation[5,6].

This study aims to design novel potent antiretroviral drugs that overcome these drawbacks using a molecular modelling approach, which was shown to be an effective way in developing novel drugs including anti-HIV medication in multiple studies using various strategies[7-10].

HIV-1 Protease:
Similar to other retroviruses, HIVProtease is one of the three main enzymes necessary for viral replication alongside Integrase and Reverse Transcriptase[11]. It plays a crucial role in producing mature virulent virions[6]. It functions via proteolysis of Gag and Gag-Pol precursor polypeptides to produce the structural components of infectious virions[6,12,13]. Hence, a major class of antiretroviral medications (ART) used to treat HIV infections are HIV Protease inhibitors.

HIV-1 Protease is a homodimer of two identical – 99 amino acid – chains[14]. Two aspartate residues ASP25 and ASP125 (one from each monomer) form the main catalytic active site[6,14]. There are three main regions in the protease structure: the active site, the flexible "flaps", and the dimer interface[14]. Protease crystal structure is shown in figure 1 (PDB: 2IEN)[15].

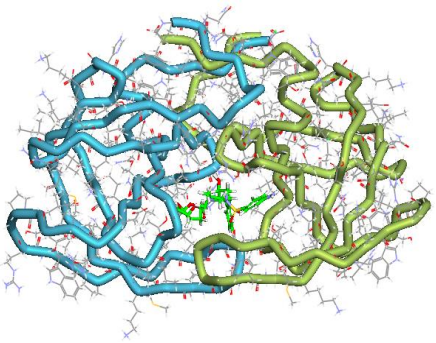


Figure 1: 3D structure of HIV-1 Protease complexed with inhibitor Darunavir (PDB: 2IEN)

Protease Inhibitors (PIs):

PIs function through competitive inhibition of HIV protease by binding to active site residues[16]. Several PIs have been approved for treating HIV infections such as Atazanavir and Darunavir (figure 2). Inhibitor placement in the active site and closing of the flaps renders the protease unable to process its substrates[14]. The hydroxyl group of the inhibitor binds to the catalytic ASP25 and ASP125 residues. Inhibitors also interact with other adjacent residues, namely Gly27, Asp29, Asp30, and Gly48[17]. However, drug-resistance mutations decrease inhibitor-protein affinity through multiple mechanisms, such as changing active site shape which reduces hydrophobic interactions with the inhibitor, or introducing bulkier residues to the active site which increases steric hindrance[5,6,17]. Moreover, drug resistance could be attributed to limited effective drug concentrations in viral reservoirs such as the central nervous system due to low blood brain barrier penetration [6]. In addition to this, some PIs exhibit serious adverse effects including diarrhea, hyperlipidemia, nephrolithiasis, and hepatitis[6,18].

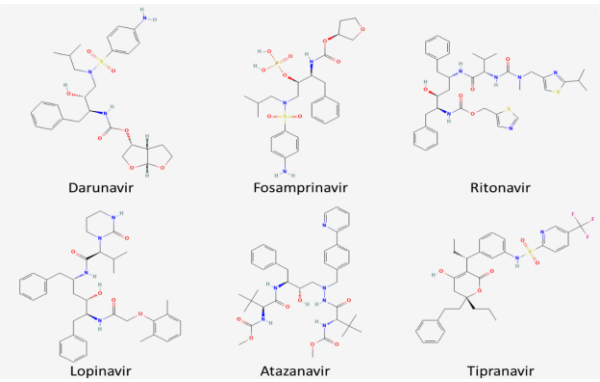


Figure 2:Chemical structure of FDA approved Protease Inhibitors

Design rationale:

In order to overcome the aforementioned challenges in designing potent PIs withfavorable pharmacokinetic profiles, and that are less affected by HIV mutations, several strategies were implemented.Promoting strong interactions with protease backbone in addition to the catalytic ASP25 and ASP125 residueswas shown to be a major strategy in overcoming HIV drug resistance[19,20]. As such, this study will take into account not only binding affinity, but also the number and strength of favorable interactions between the potential novel inhibitors and protein backbone, using molecular docking, in order to predict inhibitor binding poses within protein active site[21,22]. The approved drug Darunavir - a second generation PI - was selected as the basis for screening and as positive control, as it exhibits strong bonding with the catalytic site as well as protease backbone[19] (figure 3). This led to darunavir having high potency against known PI-resistant HIV strains[19,23]. However, darunavir was shown to be implicated in liver toxicity ranging from transient asymptomatic aminotransferase serum elevation to acute and severe hepatitis[18,24]. This study will aim to circumvent this toxicity through in-silico ADMET studies on the new inhibitors while focusing heavily on hepatotoxicity. As previously mentioned, most PIs have low BBB penetrability, causing low CNS drug concentrations, that of which could act as viral reservoirs [5]. As such, blood brain barrier penetration will be a major part of ADMET studies to insure good CNS reachability.Additionally, oral drug likeness will be tested using Lipinski's Rule of Five and Veber Rule[25-28] to insure good oral pharmacokinetic properties.

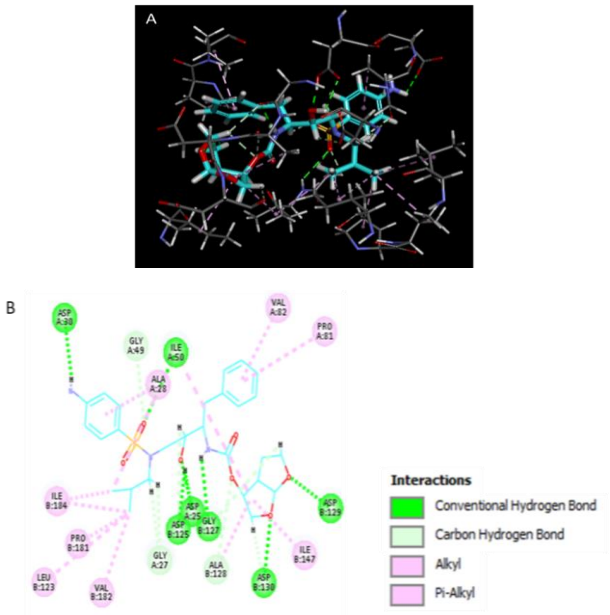


Figure 3: A: 3D representation of darunavir docked into HIV-1 protease active site. B: 2D representation of darunavir interactions with active site residues

MATERIALS AND METHODS:

BIOVIA Discovery Studio software[29-31] was used to perform the molecular modelling studies.

Protein preparation:

First, the RCSB protein database[32,33] was screened for an appropriate crystal structure of HIV-1 protease. A wildtype HIV-1 protease bound to Darunavir (PDB: 2IEN[15]) was chosen as it has a high resolution of 1.3A^o and the appropriate ligand. In order for the crystal structure to be used it must first be prepared using appropriate software. The automatic protein preparation wizard in Discovery Studio was used to prepare the crystal structure of 2IEN. Protein loops were built. Hydrogen atoms were added at the physiological pH level (pH=7.4). Structure minimization was carried out using the CHARMmforcefield[34] and all water molecules were deleted.

Database Screening and ligand preparation:

Darunavir was selected as positive control and basis to screen the Pubchem compound database[35]. A Tanimoto[36] threshold of 70% was used to screen for similar compounds.The retrieved compounds were then prepared using the ligand preparation wizard in Discovery studio. Hydrogen atoms were added and atomic valences were corrected. Tautomers, isomers and ionized states were generated within a pH range of (6.5-8.5).

ADMET studies:

The prepared compounds were filtered through Lipinski's Rule of Five and Veber rule for oral drug likeness. No more than one violation was allowed. The molecular properties and ADMET descriptors were calculated for the filtered compounds which in turn were filtered once more through BBB penetration, intestinal absorbance levels and hepatotoxicity. Only compounds with medium or higher BBB penetration, moderateor higherintestinal absorbance and no predicted hepatotoxicity were retained (table 1).

Table 1: BBB penetration and intestinal absorption levels and their corresponding descriptor

BBB Penetration level	Intestinal absorption level
0 (very high)	0 (good)
1 (high)	1 (moderate)
2 (medium)	2 (poor)
3 (low)	3 (very poor)
4 (undefined)	

Molecular docking:

The remaining compounds were docked through a two-step process. In both cases, the binding site sphere was determined using the crystalized ligand as the center, and the sphere size was set to 11A^o. Darunavir was used as positive control. The first docking process was conducted using the Libdockhigh-throughputscreening protocol[37], which uses polar and apolar features (Hotspots) to define ligand-receptor interactions and scoring in order to dock a large number of compounds in a short amount of time.Number of Hotspots was set to 100, docking tolerance was set to 0.25 and docking preference was set to high quality. Compounds that exhibit relatively similar orhigher Libdock score than darunavir were then subjected to the second phase of docking using the CDOCKER protocol[38] which is a more refined grid-based docking method that employs the CHARMmforcefield for ligand-receptor complex minimization and pose scoring, and as such is more reliable and precise in determining ligand-receptor binding affinity.Random conformations and orientations to refine were both set to 10 and simulated annealing was set to true. The docking procedure was validated by redocking darunavir within the protein crystal structure. RMSD of the docked pose relative to the crystalized pose was 0.62A^o, which indicates good docking precision.

Lead determination:

The best scoring compounds with similar or higher CDOCKER score compared to darunavir were selected for pose interaction analysis. The Analyze ligand poses wizard was used to better analyze ligand-receptor interactions. The compound that formed the highest number of strong hydrogen bonds with active site residues and exhibited a high docking score was selected as the lead compound to be used for the design of novel inhibitors.

Lead optimization:

The selected lead compound was then optimized through substituent fragment replacement to better improve receptor affinity and pharmacokinetic profile[39,40]. The Discovery Studio extensive fragment libraries were used to generate new ligands. The generated ligands were filtered using the previous workflow through ADMET studies with the addition ofAmes mutagenicity[41]. Finally, the generated compounds were subjected to docking simulations in order to obtain the final compounds which exhibit high binding affinity and favorable pharmacokinetics properties.

RESULTS AND DISCUSSION:

Database screening and ligand preparation:

The Pubchem compound database was screened using the Tanimoto similarity search with a threshold of 70% of similarity to darunavir. 20931 compounds were retrieved from the database and prepared using the ligand preparation wizard.

ADMET studies:

The prepared compounds were filtered using Lipinski's Rule of Five and Veber rule with a maximum of one allowed violation resulting in the elimination of 13894 compounds and retaining 7037 compounds. Afterwards, ADMET descriptors were calculated. The remaining 7037 compounds were filtered through BBB penetration and intestinal absorption levels. Only compounds with medium or higher BBB penetration and moderate or higher intestinal absorption and no hepatotoxicity were retained, resulting in a further6569compounds being eliminated and468 compounds retained for docking studies.

Molecular docking:

First, the remaining 468compounds were docked into the prepared HIV-1 protease (PDB: 2IEN) using the Libdock protocol. Darunavir was docked using the same parameters which resulted in a Libdock score of 167. Since Libdock is a high-throughput screening protocol, and as such has relatively low precision, a threshold score of 140was used to separate compounds with relatively similar or higher binding affinity to darunavir to be carried out to the next round of docking using CDOCKER protocol.158 compounds were then docked using CDOCKER. 80 compounds successfully docked, 9 of which showed good CDOCKER score compared to darunavir (-CDOCKER ENERGY = 46.91), and were subsequently subjected to pose analysis to determine hydrogen bond formation with active site residues. Results are shown in table 2.

Lead determination:

In order to determine the best compound to act as lead to design the new inhibitors, binding site analysis was performed for the 9 top scoring compounds. Compounds 4225196 and 3607048 formed strong hydrogen bonding only with ASP25, while compound 30211636 formed strong hydrogen bonds with both ASP25 and ASP125 through its amide group (figure 4). Moreover, compound 30211636 formed strong pi-anion and attractive charge bonds with ASP125 through its toluoyl ring and protonated sulfonamide nitrogen respectively. This together with its good intestinal absorbance andacceptable BBB penetration levels, as well as its overall favorable oral drug likeness made compound 30211636 a good candidate to become the lead compound for designing novel inhibitors.

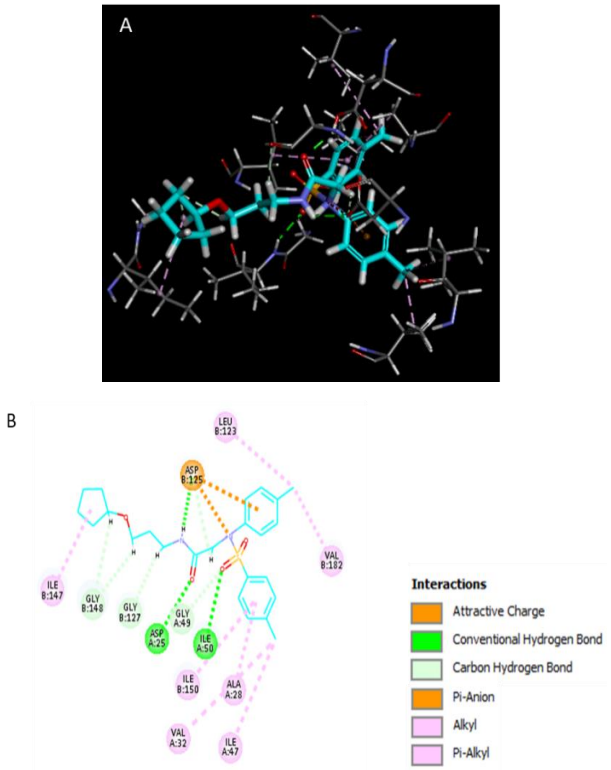


Figure 4: A: 3D representation of compound 3021166 docked into HIV-1 protease active site. B: 2D representation of compound 30211636 interac`tions with active site residues.

Table 2: Docking results and ADMET descriptors of the compounds after second round of docking:

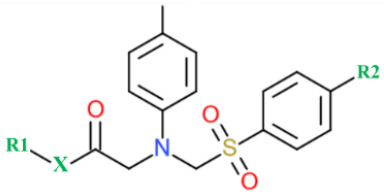
Compound	-CDOCKER Energy	Number of hydrogen bonds*	Libdock score	BBB penetration level	Human intestinal absorption level	ROF and VR violations**
3607048	48.29	2 (1)	170.77	2	0	1
4225196	46.64	4 (1)	159.87	2	0	0
6700271	46.53	4 (0)	171.57	2	0	1
4335840	46.28	3 (0)	176.66	2	0	1
30211636	45.93	3 (2)	151.23	2	0	0
4338769	45.63	4 (0)	171.02	2	0	1
5056073	45.43	3 (0)	167.52	2	0	1
60343417	45.39	4 (0)	155.75	1	0	0
3464898	44.24	3 (0)	179.16	1	0	1
Darunavir	46.91	7 (3)	167.65	4	2	3

* Number of total hydrogen bonds and number ofhydrogen bonds formed with residues ASP25 and ASP125 between parenthesis.** Number of Lipinski's rule of five and Veber rule violations

Lead optimization:

The 3-cyclopentoxy-N-propyl substituent (R1) was chosen for fragment replacement. This is due to it not forming strong bonds with the receptor. Additionally, the amide nitrogen was replacedin some cases to prevent intramolecular hydrogen bond formation with sulfonamide group in case of no hydrogen bond forming with ASP125. On the other hand, while the 4-toluoyl substituentof the sulfonamide does not form strong bonding with the receptor, it is important for the right placement and orientation of the molecule in the active site pocket, as deleting this group caused the compound to dock in a less optimal orientation. As such, only the para methyl substituent (R2) was changed. Using Discovery Studio's extensive fragment libraries,1228 unique new ligands were generated and subsequently filtered through Lipinski's rule of five, Veber rule, BBB penetration and human intestinal absorption levels, hepatotoxicity and Ames mutagenicity, resulting in 18ligands remaining. Molecular docking simulation was carried out on the remaining ligands using the CDOCKER protocol. Chemical structure of the top 12 compounds are shown in table 3. Docking resultsof the top 12 compounds are shown in table 4.

Table 3: Chemical structure of the top 12 new inhibitors



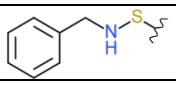
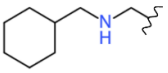
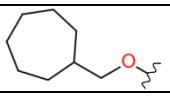
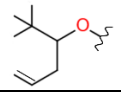
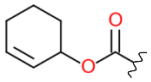
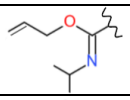
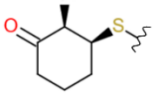
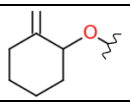
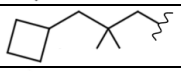
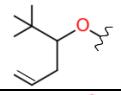
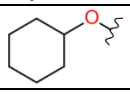
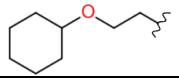
Compound	R1	R2	X
11a		$-CH_3$	-NH-
11b		$-CH_3$	-NH-
11c		$-CH_3$	-NH-
11d		$-CH_3$	-NH-
11e		$-CH_3$	-NH-
11g		$-CH_3$	-NH-
11s		$-CH_3$	-NH-
12c		$-CH_3$	$-CH_2-$
13a		-OH	$-CH_2-$
13d		-OH	$-CH_2-$
14e		$-CH_3$	$>C=CH_2$
15e		$-CH_3$	-S-

Table 4: Docking results and ADMET descriptors of the top 12 new compounds:

Compound	-CDOCKER Energy	Number of hydrogen bonds*	Number of favorable interactions**	BBB penetration level	Human intestinal absorption level	ROF and VR violations***
11a	61.58	4 (1)	19	2	0	0
11b	59.28	3 (2)	18	2	0	0
13a	56.27	6 (2)	15	1	0	1
11g	54.28	3 (1)	19	2	0	0
13d	54.14	6 (2)	15	2	0	0
11s	54.12	2 (1)	15	2	0	0
15e	53.71	3 (1)	17	1	0	1
11c	53.65	3 (2)	18	2	0	0
14e	49.78	2 (1)	19	1	0	1
11d	49.63	2 (1)	24	2	0	0
11e	47.62	4 (1)	17	2	0	0
12c	46.88	3 (1)	17	1	0	0

* Number of total hydrogen bonds and number of hydrogen bonds formed with residues ASP25 and ASP125 between parenthesis. **Number of favorable interactions with backbone. *** Number of Lipinski's rule of five and Veber rule violations

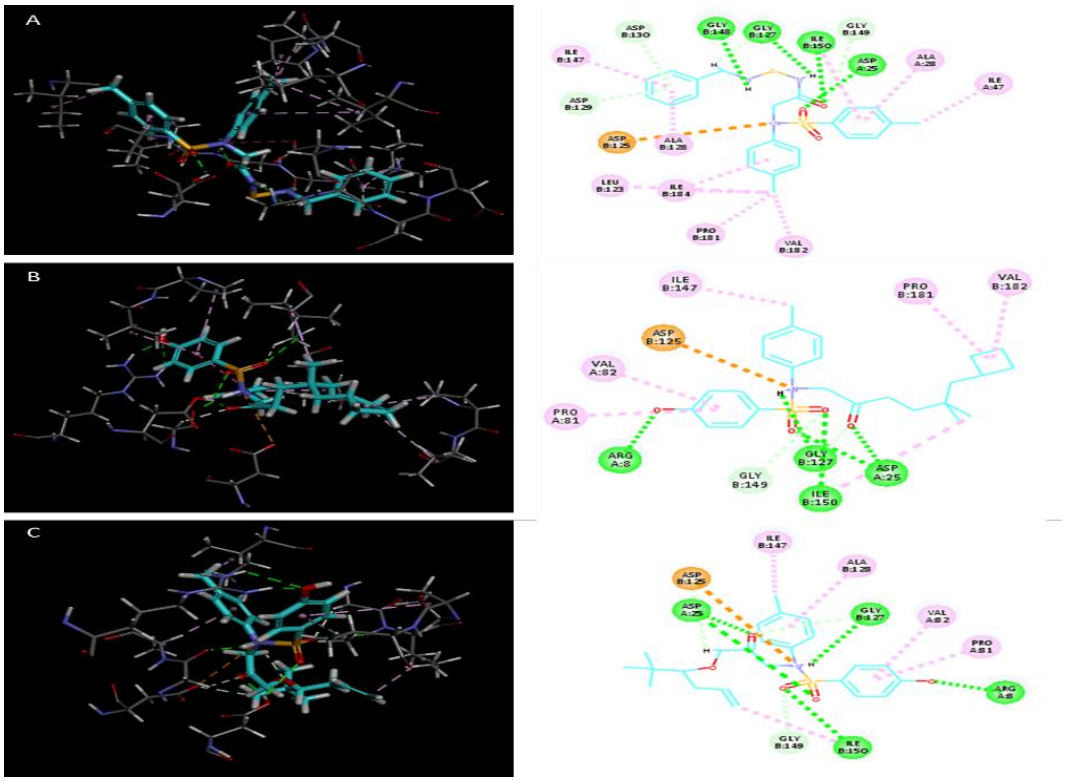


Figure 5: A: 2D and 3D representation of compound 11a docked into HIV-1 Protease. B: 2D and 3D representation of compound 13a docked into HIV-1 Protease. C: 2D and 3D representation of compound 13d docked into HIV-1 Protease

All compounds exhibited high affinity to the active site with favorable interactions ranging from hydrogen bonds, Pi-cation, Pi-anion andattractive charge, and all compounds had good predicted intestinal absorption while having no predicted hepatotoxicity and mutagenic properties. Compound 11a had the highest CDOCKER score (figure 5A) with medium BBB penetration. Compounds 13a and 13d had the largest number of hydrogen bonds formed with catalytic residues and protein backbone (figures 5B and 5C), while having high and medium BBB penetration respectively. Compound 13a also formed two hydrogen bonds with ASP25 through its carbonyl and sulfonamide groups and attractive charge bond with ASP125 through its protonated sulfonamide nitrogen. Moreover, compound 13a also formed hydrogen bonds with both protease chains namely ARG8, GLY127 and ILE150 residues. These interactions entail high affinity to catalytic site and protein backbone which could circumvent the reduced affinity caused by viral mutation.

CONCLUSION:

In this study, a molecular modelling method was implemented to design novel HIV-1 protease inhibitors. In order to overcome the various challenges facing anti-HIV medications, such as drug resistance, toxic side effects and low CNS reachability, different strategies were implemented. First, a suitable lead compound was identified from the Pubchem database through Tanimoto similarity search, ADMET studies, two-step molecular docking simulations and pose analysis. Afterwards, the lead compound was optimized through substituent fragment replacement. The resulting compounds exhibited high affinity and favorable interactions with active site residues.Moreover, they had good oral pharmacokinetic profiles with no predicted hepatotoxicity or mutagenicity. Compound 13a exhibited high affinity, multiple strong hydrogen bonds with catalytic site and protein backbone, while having high predicted blood brain barrier penetration.

ABBREVIATIONS:

ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral medication
BBB	Blood Brain Barrier
CNS	Central Nervous System
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
PI	Protease Inhibitor

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