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Discovery of Novel HIV Protease Inhibitors using Modern Computational Techniques

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

Sunday N. Okafor*[1,2], Pavimol Angsantikul[1], Hashim Ahmed[1]

[1]Center for Biomedical Research, Population Council, New York, NY 10065, USA
[2]Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, 41001 Nsukka, Enugu State Nigeria

* Corresponding author: sokafor@popcouncil.org; sunday.Okafor@unn.edu.ng

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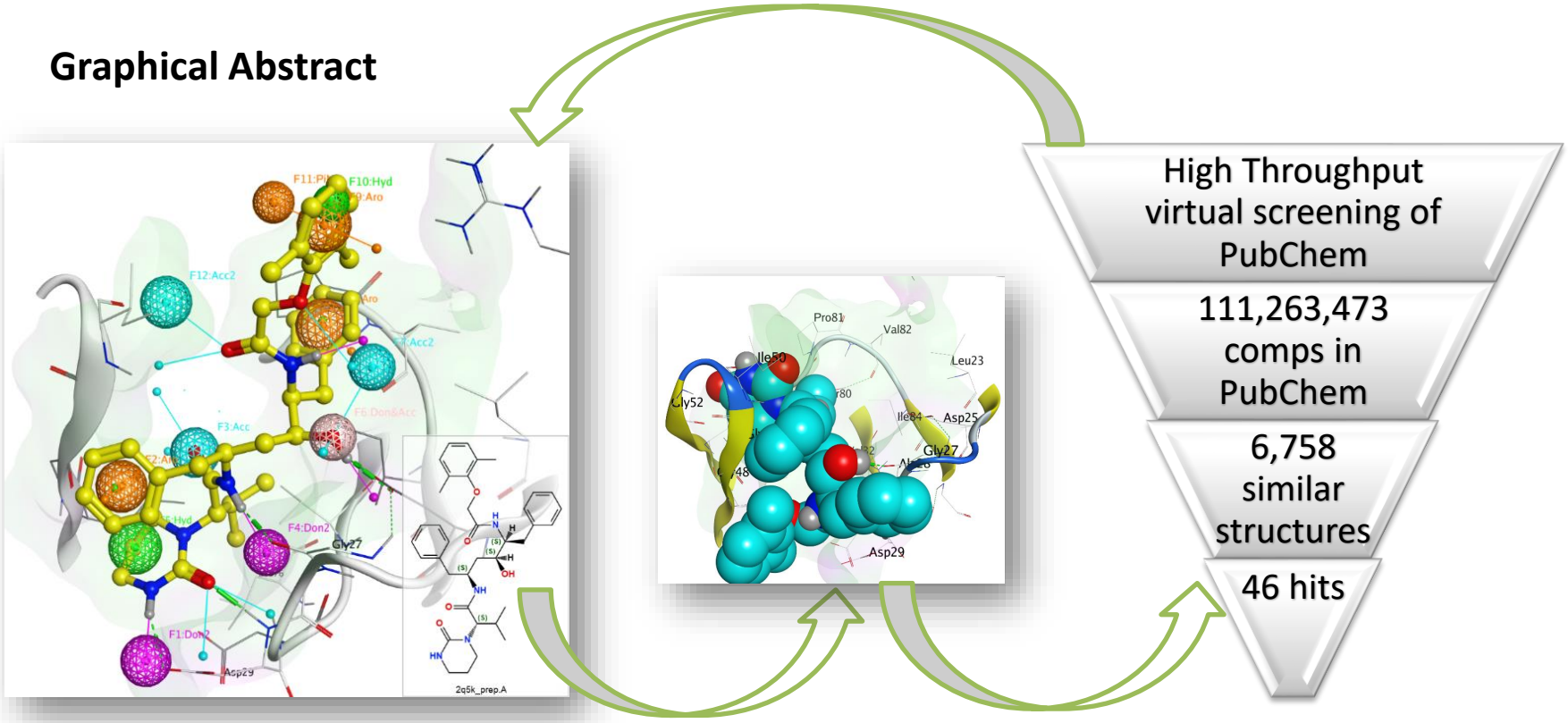
Graphical Abstract



Abstract:

The human immunodeficiency virus type 1 (HIV-1) has continued to be a global concern. With the new HIV incidence, the emergence of multi-drug resistance and the untoward side effects of currently used anti-HIV drugs, there is an urgent need to discover more efficient anti-HIV drugs. Modern computational tools have played vital roles in facilitating drug discovery process. This research focuses on pharmacophore-based similarity search to screen 111,566,735 unique compounds in the PubChem database to discover novel HIV-1 PIs. We used *in-silico* approach involving 3D-similarity search, physicochemical and ADMET evaluations, HIV protease-inhibitor prediction (IC_{50}/percent inhibition), rigid receptor-molecular docking studies and free-binding energy calculations. The 10 FDA approved HIV PIs (saquinavir, lopinavir, ritonavir, amprenavir, fosamprenavir, atazanavir, nelfinavir, darunavir, tipranavir and indinavir) were used as reference. The *in-silico* analysis revealed that fourteen out of the twenty-eight selected optimized hit molecules were within the acceptable range of all the parameters investigated. The hit molecules demonstrated significant binding affinity to the HIV protease (PR) when compared to the reference drugs with the residues ASP25, GLY27, ASP29, ASP30, ILE50 involved in essential hydrogen bonding and π-π stacked interactions, which stabilize the optimized hit molecules in the active binding site of the HIV-1 PR (PDB:2Q5K). HPS/002 and HPS004 are the most promising in terms of IC_{50}/percent inhibition (90.15%) of HIV-1 PR, in addition to their drug metabolism and safety profile. These hit candidates should be investigated further as possible HIV-1 PIs with improved efficacy and low toxicity through *in-vitro* experiments and clinical trial investigation.

Keywords: HIV protease; ADMET; pharmacophore; molecular docking; HIV protease inhibitors

Introduction

- HIV protease (PR) is one of the three enzymes essential in the life cycle of Human immunodeficiency virus (HIV) survival and replication. At some point in the life cycle of the HIV, immature viral particles are produced. These budded immature viral particles that contain catalytically inactive protease cannot undergo maturation to an infective form [1]. The role of PR is basically to catalyze the hydrolysis of Gag and Gag-Pol polyproteins thereby generating mature infectious virions [2]. HIV protease inhibitors work by antagonizing the process that leads to generation of mature infectious virions.
- Over the decades, computer-aided drug design (CADD) has brought tremendous breakthrough in the field of drug discovery. Basically, CADD is used to catalyze and rationalize the drug design process while reducing costs [3].
- Pharmacophore is a molecular framework that carries the essential features (phoros) responsible for a drug's biological activity (pharmacon) [4]. There are so many applications of pharmacophore modeling in drug discovery process ranging from virtual screening to target identification, scaffold hopping, ligand profiling, lead optimization and *de novo* drug design.
- Therefore, this method is widely used tool in CADD and chemoinformatics fields [5].

Methods

- Pharmacophore-based similarity modeling was used to search the PubChem Database
- The Molecular Descriptors Algorithm in Molecular Operating Environment (MOE) was used to evaluate the physicochemical properties of the compounds
- The evaluation of the ADMET parameters were carried out using the pkCSM
- Furthermore, the organ toxicity and toxicity end points were evaluated with ProTox II
- HIVprotI was used in the prediction of the anti-HIV activity (IC_{50} (μM) and the percentage inhibition) of the selected hits.
- Detailed molecular docking studies and the visualization of the molecular interactions were carried out with MOE

Results

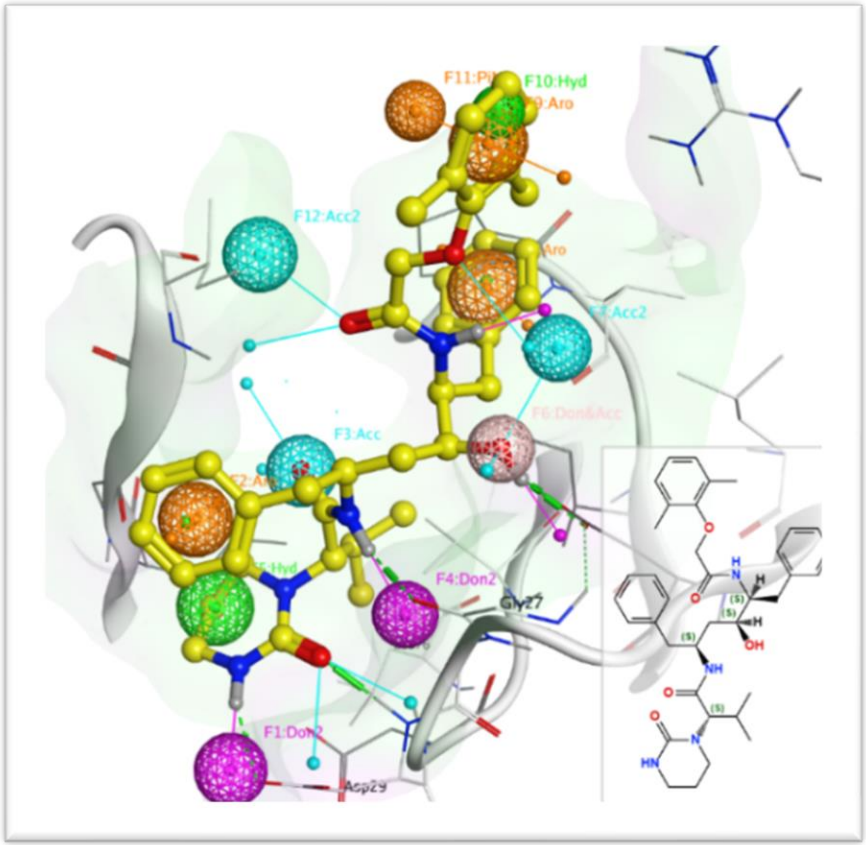


Figure 1: Pharmacophore annotation of lopinavir bound to 2Q5K using unified scheme

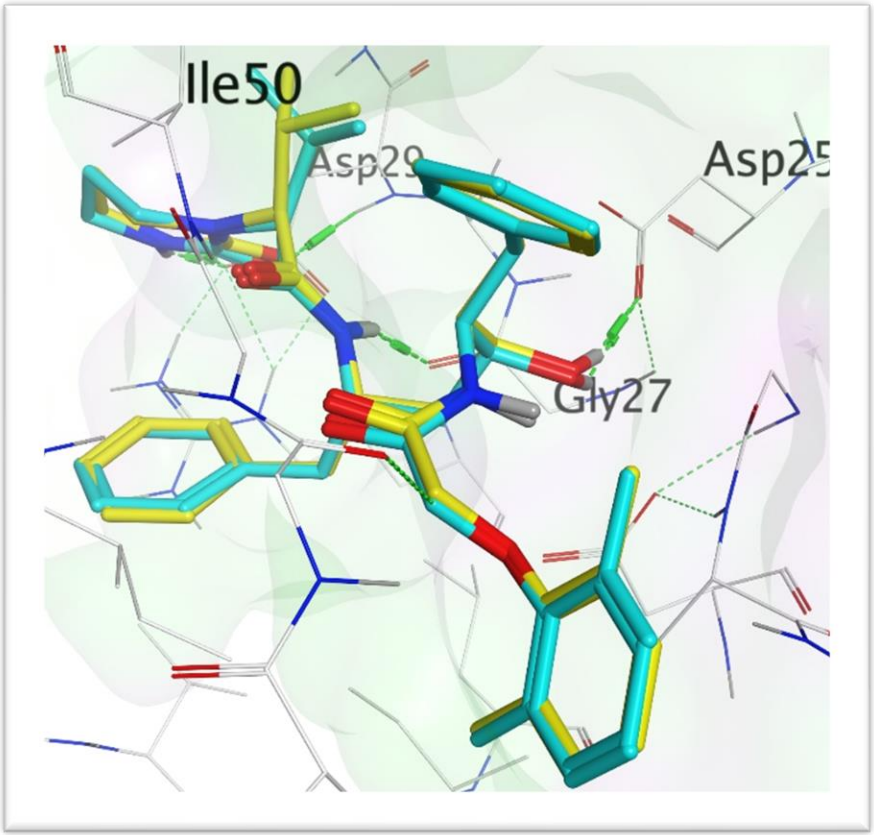


Figure 2: Validation of the docking protocol with 2Q5K bound to lopinavir. The docked lopinavir (cyan) was overlaid on the co-crystallized lopinavir (yellow)

Table 1: Binding Free energy (kcal/mol) and the HIV protease inhibitory activity of selected hits from different reference drugs

S/N	Comp Code	ΔG (kcal/mol)	IC_{50} (µM)	% Inhibition
1	PC_HPS/002	-8.24	48.9	90.13
2	PC_HPS/004	-7.88	48.9	90.13
3	PC_HPS/006	-8.63	31.17	59.80
4	PC_HPS/007	-8.57	36.33	60.78
5	PC_HPS/008	-8.54	14.58	59.48
6	PC_HPS/009	-7.31	-14.7	65.58
7	Lopinavir	-7.80	203.69	90.12
8	Ritonavir	-8.35	24.99	53.74
9	Fosamprenavir	-6.88	39.79	65.65

Table 2: Absorption and Distribution (pkCSM)

		Absorption							Distribution			
S/N	Comp Code	Water solubility	Caco2 perm	IntestineAbs	Skin Perm.	P-GPS	P-GP1I	P-GP2I	VDss	FU	BBB perm.	CNS perm.
2	PC_HPS/002	-4.625	0.271	61.515	-2.735	Yes	Yes	Yes	-0.399	-0.399	-0.712	-3.11
4	PC_HPS/004	-4.625	0.271	61.515	61.515	Yes	Yes	Yes	-0.399	0.012	-0.712	-3.11
7	PC_HPS/006	-4.11	0.737	71.415	-2.735	Yes	Yes	Yes	0.302	0.048	-1.844	-3.642
8	PC_HPS/007	-3.209	0.464	69.849	-2.735	Yes	Yes	Yes	1.105	1.105	-1.789	-3.895
9	PC_HPS/008	-4.232	0.561	79.662	-2.735	Yes	Yes	Yes	0.273	0	-1.678	-3.23
11	PC_HPS/009	-3.315	0.356	66.625	-2.741	Yes	Yes	No	0.639	0.093	-1.081	-3.461
5	Lopinavir	-2.892	1.497	76.395	-2.735	No	No	No	0.011	0.381	-1.525	-1.418
10	Ritonavir	-3.358	0.377	69.45	-2.735	Yes	Yes	Yes	0.429	0	-1.665	-3.295
15	Fosamprenavir	-3.239	0.201	76.433	-2.735	Yes	Yes	No	0.228	0.12	-1.816	-4.025

Table 3: Metabolism and Excretion (pkCSM)

		Metabolism							Excretion	
S/N	Comp Code	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Total Clearance	Renal OCT2 substrate
2	PC_HPS/002	No	Yes	No	No	No	No	Yes	0.429	Yes
4	PC_HPS/004	No	Yes	No	No	No	No	Yes	0.429	No
7	PC_HPS/006	No	Yes	No	No	No	No	Yes	1.122	No
8	PC_HPS/007	No	Yes	No	No	No	No	Yes	1.202	No
9	PC_HPS/008	No	Yes	No	Yes	Yes	No	Yes	0.374	No
11	PC_HPS/009	No	Yes	No	No	No	No	Yes	1.021	No
5	Lopinavir	No	No	Yes	No	No	No	No	-114.036	No
10	Ritonavir	No	Yes	No	No	Yes	No	Yes	0.564	No
15	Fosamprenavir	No	Yes	No	No	No	No	Yes	0.282	No

Table 4: The results of the toxicity evaluation with pkCSM

S/N	Comp Code	AMES toxicity	Max. human tolerated dose	hERG I inhibitor	hERG II inhibitor	(LD_{50}) mol/kg	(LOAEL) log mg/kg_bw/day	Hepato-toxicity	Skin Sensitization	T.Pyriformis toxicity	Minnow toxicity
2	PC_HPS/002	No	-0.254	No	Yes	2.38	2.979	Yes	No	0.285	-1.219
4	PC_HPS/004	No	-0.254	No	Yes	2.674	3.842	Yes	No	0.285	-1.219
6	PC_HPS/005	No	-0.22	No	Yes	2.818	3.631	Yes	No	0.285	4.55
7	PC_HPS/006	No	-0.224	No	Yes	3.206	2.454	Yes	No	0.285	1.04
8	PC_HPS/007	No	-0.3	No	Yes	2.714	2.126	Yes	No	0.285	1.197
9	PC_HPS/008	No	-0.308	No	Yes	2.661	2.169	Yes	No	0.286	1.535
11	PC_HPS/009	No	0.021	No	No	2.578	1.744	Yes	No	0.285	-3.865
5	Lopinavir	No	-0.297	No	Yes	2.382	5.949	Yes	No	0.286	-1.501
10	Ritonavir	No	0.096	No	Yes	2.703	2.231	Yes	No	0.285	1.787
15	Fosamprenavir	No	-0.029	No	No	2.396	2.151	Yes	No	0.285	-4.393

Table 5: The results of the Toxicity test with ProToxII

S/N	Comp Code	LD_{50} mg/kg	Toxici-ty class	Average similarity %	Prediction accuracy %	Hepato-toxicity	Carcino-genicity	Immuno-toxicity	Muta-genicity	Cyto-toxicity	Aroma-tase	Estrogen receptor-α	Androgen receptor	PPAR-γ
2	HPS/002	5000	V	58.87	67.38	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
4	HPS/004	5000	V	58.87	67.38	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
7	HPS/006	800	IV	42.36	54.26	Inactive	Inactive	Inactive[a]	Inactive	Inactive	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
8	HPS/007	1000	IV	42.53	54.26	Inactive	Inactive	Inactive[a]	Inactive	Inactive	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
9	HPS/008	500	IV	40.28	54.26	Inactive	Inactive	Inactive[a]	Inactive	Inactive	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
11	HPS/009	300	III	48.58	54.26	Inactive	Inactive	Inactive[a]	Inactive[a]	Inactive	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
5	Lopinavir	5000	V	59.88	67.38	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
10	Ritonavir	1000	IV	42.32	54.26	Active[a]	Inactive	Inactive[a]	Inactive[a]	Inactive	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]
15	Fosampren avir	300	III	43.99	54.26	Inactive[a]	Inactive	Inactive[a]	Inactive	Inactive	Inactive[a]	Inactive[a]	Inactive[a]	Inactive[a]

[a]High probability (≥0.70); Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma

Table 6: Binding interactions of the hit compounds with 2Q5K

S/N	Compound	Ligand	Receptor	Interaction	Distance (A)	E (kcal/mol)
1.	HPS/002	O 1	O GLY 27	H-donor	2.94	-1.9
		C 21	O GLY 27	H-donor	3.21	-0.2
		C 58	OD2 ASP 30	H-donor	3.60	-0.2
		O 6	N ASP 29	H-acceptor	2.94	-1.6
		6-ring	CA GLY 27	pi-H	4.28	-0.2
		6-ring	CG1 ILE 50	pi-H	4.24	-0.4
2.	HPS/004	C 21	O GLY 27	H-donor	3.52	-0.2
		O 3	N ILE 50	H-acceptor	3.37	-0.8
		O 6	N ASP 29	H-acceptor	3.22	-2.4
		6-ring	CA ILE 47	pi-H	4.54	-0.2
		6-ring	N GLY 48	pi-H	3.85	-1.2
3.	HPS/006	S 1	OG1 THR 80	H-donor	3.73	-1.2
		N 10	O GLY 27	H-donor	3.69	-0.2
		C 29	OD1 ASP 25	H-donor	3.12	-2.6
		S 1	CD1 ILE 54	H-acceptor	4.11	-0.2
		O 4	CA GLY 49	H-acceptor	3.30	-0.6
4.	HPS/007	C 20	OD1 ASP 25	H-donor	3.78	-0.2
		C 37	OD1 ASP 25	H-donor	3.09	-0.2
		C 47	O LEU 24	H-donor	3.55	-0.3
		C 72	OD2 ASP 30	H-donor	3.87	-0.2
		C 75	O GLY 48	H-donor	3.44	-0.2
		S 2	CG1 ILE 50	H-acceptor	4.49	-3.3
		O 7	CA GLY 49	H-acceptor	4.49	-0.5
		5-ring	NH1ARG 8	pi-cation	4.59	-0.5
		6-ring	N ASP 29	pi-H	4.22	-0.2
		6-ring	N ASP 30	pi-H	4.84	-0.5
		5-ring	CA GLY 49	pi-H	3.89	-0.2
		5-ring	CB PRO 81	pi-H	3.82	-0.6

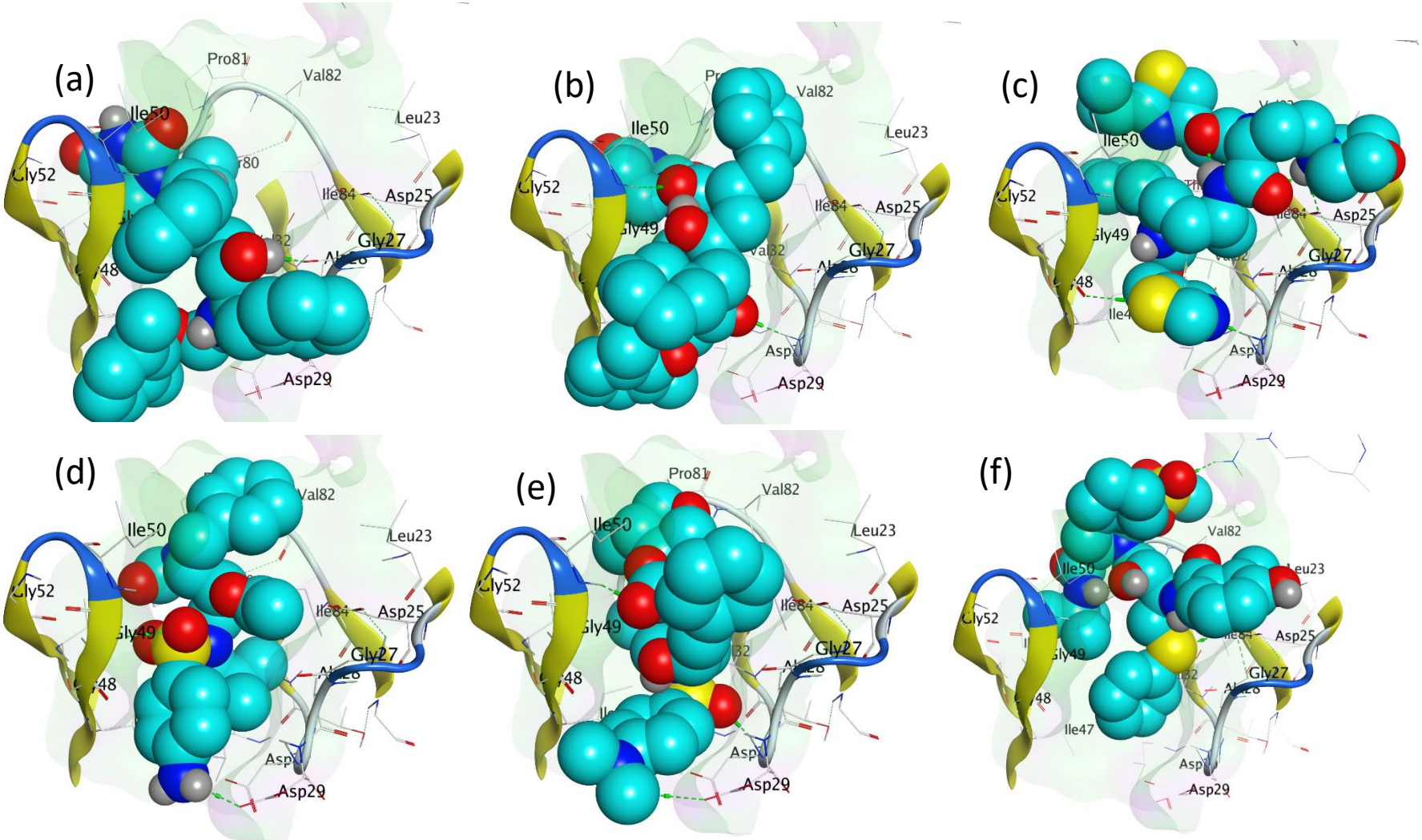


Figure 4: The docking poses of (a) HPS/002 (b) HPS/004 (c) HPS/006 (d) HPS/007 (e) HPS/008 (f) HPS/009 in the binding cavity of 2Q5K

Discussion

- The physicochemical properties of the compounds which are important factors in the determination of the solubility, permeability, and bioavailability of orally delivered drug. Our molecules were within the range of Lipinski's rule. Traditionally, therapeutics have been small molecules that fall within the Lipinski's rule of five
- The binding free energy, ΔG (kcal/mol) of the hit molecules is lower than the reference drug used (table 1). The low ΔG (high negative values) is an indication of high binding affinity of these molecules with HIV protease (2Q5K).
- Molecular docking simulation clearly demonstrated significant chemical interactions of the atoms of the ligands and the amino acids residues of the HIV protease (table 6 and figure 4).
- All the hit molecules formed prominent hydrogen bond with at least two catalytic residues (ASP 25, GLY 27 and ASP 29) in the floor of the active site.
- The carbonyl and the hydroxy group of HPS/002, through Hydrogen bond interactions, combined with the O GLY 27 and N ASP 29 respectively.

- It was observed that HPS/007 has the highest binding affinity (-8.57 kcal/mol). In addition to the chemical interactions with the residues in the catalytic active site, the two thiazole rings contributed significantly to this high binding affinity of HPS/007 with the HIV protease.
- The results of HIV protease inhibitory activity – IC_{50} (µM) and % inhibition of the selected hits from different reference drugs using HIVprotI are shown in table 1. The fourteen selected hit molecules showed significant activity against HIV-1 protease with IC_{50} range of -2.52 – 48.90 µM and % inhibition range of 52.71 – 90.13%
- HPS/002 has a comparable % inhibition of 90.13% to its reference drug - lopinavir (90.12%) and significantly higher % inhibition than ritonavir (53.74 %) and fosamprenavir (65.65%). However, it showed lower and better IC_{50} of 48.90µM than lopinavir (203.69), ritonavir (24.99) and fosamprenavir (39.79 µM)
- The pharmacokinetic and pharmacodynamic profiles are usually used to assess the safety and efficacy of a drug during the drug development process. Despite how promising a drug candidate may be, the ADMET drug properties determine the extent it can be useful as drug. The ADME results are shown in tables 2 – 3.

- All the hit molecules have high GI absorption (poorly absorbed <30%), denoting an increase in permeability.
- For a given compound a logBB > 0.3 is considered to readily cross the blood-brain barrier while molecules with logBB < -1 are poorly distributed to the brain. Similarly, compounds with a logPS > -2 are considered to penetrate the central nervous system (CNS) while those with logPS < -3 are considered as unable to penetrate the CNS.
- All the hit molecules, including the reference drugs are not readily able to cross the blood-brain barrier (BBB) or to penetrate the central nervous system (CNS), except lopinavir, which can penetrate the CNS but was not readily able to cross the BBB
- While some of the hit molecules have high volume of distribution at steady state (VDss), others have low to moderate VDss.
- CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 play key roles in drug metabolism. The results suggest that except for lopinavir, HPS/015, HPS/019, HPS/024 and HPS/028, all the other hit molecules and reference drugs inhibit the CYP3A4 sub-enzymes of cytochrome P450 (CYP) – table 3. CYP3A4 is responsible for metabolizing ~50% of all drugs by itself.

- All except HPS/002 are organic cation transporter 2 (OCT2) non-substrate. OCT2 is a renal uptake transporter that plays a critical role in disposal and renal clearance of drugs and endogenous compounds [6]. This evaluation has provided insightful information on drug clearance and potential contraindications of OCT2 transporter drug candidates.
- The LD_{50} (mg/kg body weight) is the median lethal dose at which 50% of test subjects die upon exposure to a compound. None of the hits is fatal when swallowed as shown in table 5. Some may be toxic when swallowed while others may be harmful when swallowed.
- Almost all the hits are found to be inactive, with high probability, to hepato-toxicity, carcinogenicity, immunotoxicity, cytotoxicity, aromatase, estrogen receptor-α, androgen receptor and peroxisome proliferator activated receptor gamma (table 5).
- Disruption of the androgen system is associated with decreased sperm count, increased infertility [7], and diabetes mellitus [8] and other endocrine disorders.

- The AMES tests (table 4) indicates that the hit molecules do not have potential mutagenic tendencies
- None of the hit molecules and the reference drugs showed toxicity against *T. pyriformis.* While some hit molecules may be associated with minnow toxicity, others are not (table 4).
- All the hit molecules showed values less than the maximum human-tolerated dose (0.477 log mg/kg/day), indicating no possible dose related toxicity.
- None of the hit molecules, including the reference drugs are considered a likely inhibitor of hERGI. HPS/002, HPS/004, HPS/007, HPS/023, HPS/027 and some other hit molecules are considered possible hERGII inhibitors
- Human ether-a-go-go-related gene (HERG) expression in tumor cells accelerates cell proliferation [9], and inhibition of HERG currents has been shown to reduce cell proliferation [10].
- In the light of the foregoing discussions, we observe that majority of the hit molecules-predicted toxicities (tables 4 and 5) maintain a relatively lower acute toxicity risk compared to reference drugs.

Conclusions

- Pharmacophore-guided 3D-similarity search, ADMET profiling, molecular docking studies, and *in silico* evaluation of anti-HIV activity were carried out on PubChem database containing 111,566,735 compounds to evaluate potential new antiviral agents against HIV-1 protease.
- The *in-silico* analysis revealed that fourteen (HPS/002, HPS/004, HPS/006, HPS/007, HPS/008, HPS/009, HPS/010, HPS/011, HPS/012, HPS/013, HPS/014, HPS/018, HPS/020, HPS/024) out of the twenty-eight selected optimized hit molecules were within the acceptable range of all the parameters investigated, such as physicochemical and ADMET parameters, the predicted IC_{50}/percent inhibition of HIV PR protein, docking scores, and free binding energies.

- There are clear indications from the docking results that residues ASP25, GLY27, ASP29, ASP30, ILE50 involved in essential hydrogen bonding and π-π stacked interactions stabilized the optimized hit molecules from PubChem in the active binding site of the HIV-1 PR (2Q5K), thereby playing vital roles for the observed anti-HIV activity.
- Out of the fourteen hit candidates, HPS/002 and HPS004 have been found to be most promising in terms of IC_{50}/percent inhibition of HIV-1 PR, in addition to their drug metabolism and safety profile.
- We therefore propose that these fourteen hit molecules with non-toxic and good bioavailability predicted qualities, with emphasis on HPS/002 and HPS/004, should be investigated further as possible PR inhibitors through wet lab experiments and clinical trial investigation.
- This is on the premise that computational studies alone are not sufficient by itself in drug discovery process.

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