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Evaluation of novel Saquinavir analogs for resistance mutation compatibility and potential as an HIV-Protease inhibitor drug

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Abstract:
A fundamental issue related to therapy of HIV-1 infection is the emergence of viral mutations which severely limits the long term efficiency of the HIV-protease (HIV-PR) inhibitors. Development of new drugs is therefore continuously needed. Chemoinformatics enables to design and discover novel molecules analogous to established drugs using computational tools and databases. Saquinavir, an anti-HIV Protease drug is administered for HIV therapy. In this work chemoinformatics tools were used to design structural analogs of Saquinavir as ligand and molecular dockings at AutoDock were performed to identify potential HIV-PR inhibitors. The analogs S1 and S2 when docked with HIV-PR had binding energies of -4.08 and -3.07 kcal/mol respectively which were similar to that for Saquinavir. The molecular docking studies revealed that the changes at N2 of Saquinavir to obtain newly designed analogs S1 (having N2 benzoyl group at N1) and S2 (having 3-oxo-3phenyl propanyl group at N2) were able to dock with HIV-PR with similar affinity as that of Saquinavir. Docking studies and computationally derived pharmacodynamic and pharmacokinetic properties' comparisons at ACD/I-lab establish that analog S2 has more potential to evade the problem of drug resistance mutation against HIV-1 PR subtype-A. S2 can be further developed and tested clinically as a real alternative drug for HIV-1 PR across the clades in future.

Keywords: HIV protease, Drug design, Drug resistance, Lead identification, Molecular Docking, Pharmacokinetics, Saquinavir.

Background:
The UNAIDS World AIDS Day Report-2012 declares that of the 34 million HIV-infected patients about 1.7 million died of AIDS in 2011-12 **[1, 2].** HIV infection is being treated by blocking steps in the life cycle of the virus from entry through multiplication. There are 33 singular antiretroviral drugs approved by FDA for the treatment of HIV infection, a combination of which is used in highly active anti-retroviral therapy (HAART) **[3, 4].** Presently 10 HIV Protease (HIV-PR) inhibitors including Saquinavir are being used as drugs to disrupt enzyme HIV Protease essential for virus replication cycle **[3-5].** The drugs in use become ineffective due to resistance by HIV-mutants and there is therefore always a need to develop new drug molecules. Structure-based pharmacophore designing provides a platform to design novel lead molecules that may have potential as drugs against HIV-PR. The addition of new classes of drugs is also important to tackle the rapid emergence of resistant protease variants that develop a chain of mutations and result in limiting the long term efficiency of these drugs **[6-9].** Largely most of the studies with HIV-drugs and their resistance have been carried out in HIV-1 subtype B **[10]** whereas there are very few studies that have been carried out in HIV-1 subtype A. It is the HIV-PR sequence, protein-structure or binding dynamics of its ligands

that are fundamental to a protease inhibitor design **[6].** This work is an attempt to study the binding thermodynamics of protein HIV-1 PR subtype-A (PDB ID: 3ixo) with the reference drug Saquinavir and its designed structural analogs as ligands to elucidate if any of these have potential to be a possible HIV-1 PR-inhibitor and if they are competent to evade the reported drug resistance mutations in HIV-1 **[11].** Herein we designed two structural analogs of Saquinavir and analyzed them by docking with the enzyme HIV-1 PR subtype A. These two Saquinavir analogs were compared with the parent drug molecule for binding dynamics with the reported drug-resistant mutation sites in literature. The pharmacokinetic attributes and toxicity of the analogs have also been tested *in silico*.

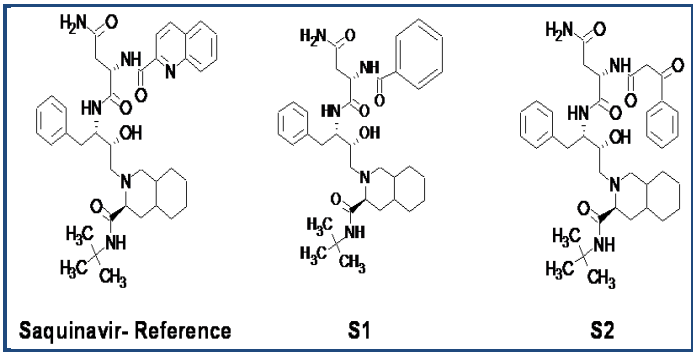

Figure 1: Structure of the reference drug, Saquinavir and structural analogs S1 to S2 with IUPAC names designed and tested for inhibitor qualities where. A: N1- {(2S,3R)-4-[(3S)-3-(tert-butylcarbamoyl) Saquinavir Reference: A-octahydroisoquinolin-2 (1H)-yl]-3-hydroxy-1-phenylbutan-2-yl}-N2- (quinolin-2-ylcarbonyl)-L-aspartamide-methane (1:1); S1: N2-benzoyl-A-octahydroisoquinolin-2(1H)-yl]-3-hydroxy-1-phenylbutan-2-yl}-L-aspartamide; S2: A-octahydroisoquinolin-2(1H)-yl]-3-hydroxy-1-phenylbutan-2-yl}-N2-(3-oxo-3 phenylpropanoyl)-L-aspartamide

Methodology:

Designing and evaluation of ligands (structural analogs of Saquinavir)

The approved drug molecule Saquinavir for HIV-1 PR was retrieved as *.mol, .sdf, SMILES* and *InChi* formats at DrugBank online **[12].** Using Saquinavir as template, various modifications were made in Saquinavir side chains at Chemsketch version 12 **[13]** for Windows, manually. Two structures analogous to Saquinavir were designed with a possibility that multiple contacts with atoms or amino acid residues of HIV-1 PR may occur. Molecules with high hydrophobicity or charges or containing disulfide bonds were avoided. Strategies included replacing double rings with single rings, substituting separate rings with fused rings, or substitution of H-bond acceptor atoms with other electronegative atoms. Idea was to prune and cure the established drug to design its analogs in a manner which least interferes with its Lipinski profile. The new molecules, structural analogs of Saquinavir designed as above were named S1 to S2 and have been illustrated as **(Figure 1).**

Molecular docking and prediction of binding energies

The Lamarckian genetic algorithm in AutoDock 4.2 was used to perform docking experiments **[14]** using the *.mol* format of ligands and converted into *.pdb* at OpenBabel **[15].** The *.pdb* file for the crystal structure of native HIV-1 PR protein subtype A (PDB ID: 3ixo) **[10]** was obtained from the protein data bank (PDB) **[16].** The designed analogs S1 and S2 were obtained as 3D models and flexible docking with HIV-PR protein subtype-A (PDB ID: 3ixo) were performed. Binding energies of the different dockings with Saquinavir, S1 and S2 were listed. Molecular graphics and analyses were performed with the UCSF Chimera package **[17].**

Binding study analysis of docked ligands with HIV-1 PR-subtype A (PDB id: 3ixo) for resistance mutation

The unbound form of HIV-1 PR-subtype-A protein (PDB id: 3ixo) retrieved above was compared for those amino acids reported to undergo mutations for developing resistant protease variants **[11]** and are involved in binding with Saquinavir, S1 or S2 as ligands.

Prediction of pharmacokinetic properties

Saquinavir and the two designed analogs S1 with minimum and S2 with equal binding energy as that of Saquinavir were selected for pharmacokinetic studies as earlier **[18]** at ACD/I-lab **[13]** and the profile of the reference drug was collated to establish the practically desirable benchmark. The pharmacokinetic profile of the S1 and S2 molecules included absorption, distribution, metabolism, excretion (ADME) and toxicity, drug-like characteristics *viz.* including Lipinski profile, logP, pKa, solubility and toxicity tests for mutagenicity (AMES test), genotoxicity, $LD_{50,}$ endocrine disruption and health effects.

Results & discussion:

The two designed structural analogs of HIV-1 PR inhibitor Saquinavir with IUPAC names are illustrated as **(Figure 1).** Both the analogs showed binding with HIV-1 PR involving the residues in the enzyme catalytic site and the flap region. **Table 1 (see supplementary material)** lists the binding energies of Saquinavir, S1 and S2 analogs obtained after docking with HIV-PR at AutoDock-4.2. Saquinavir (reference drug, R) has a docking energy of -3.07 kcal/mol and S1 has least binding energy of -4.08 kcal/mol. Analog S2 had binding energy similar to the reference drug -3.07 kcal/mol. From the IUPAC names and corresponding structures of analogs it can be noted that in S2 the 3-oxo 3-phenyl propanoyl group replaces quinolin-2-yl carbonyl of Saquinavir at N2. In S1 however, there is an introduction of a N2-benzoyl group at N1 of Saquinavir.

The introduction of this Benzoyl group in S1 perhaps lowers the number of contacts between the HIV-1 PR and ligand as reflected in the lowered binding energy of the corresponding complex **(Table 1).** The binding results of the amino acid residues involved at catalytic or flap region of protein in forming bonds between Saquinavir/S1/S2 with HIV-PR subtype-A (PDB id: 3ixo) have also been listed in **Table 2 (see supplementary material).**

Resistance to protease inhibition occurs largely due to mutations within the active site and non-catalytic distal sites of the protein that results in lowered affinity of protease inhibitors with respect to substrate, the biological activity of the enzyme protein in parallel **[11].** The number of contacts between the ligands Saquinavir, S1 and S2 with HIV-1 PR subtype-A protein

are 211, 112 and 182 respectively. All the three ligands bind to the active site of the HIV-1 PR involving nine amino acid residues at positions 8, 23, 25, 26, 27, 28, 29, 30, 32 and 48, 49, 50, 82, 84 of the flap region. Unlike that in Saquinavir, one additional position 47 of the flap region is seen involved in binding with S1 and S2.

According to a resistance mutation update using HIV patients' data **[11]** the mutations in the protease gene associated with protease inhibitor Saquinavir, take place at 08 minor and 02 major positions where amino acid substitution occurs conferring resistance to HIV-1 PR towards Saquinavir. In the 99 amino acid residues long HIV-1-PR backbone, Gly 48 and Leu 90 are sites for major resistance mutations whereas, Leu 10, Leu 24, Iso 54, Iso 62, Arg 71, Gly 73, Val 77, Val 82 and Iso 84 are documented as sites for minor resistance mutations **[11].** Interestingly of these 10 possible mutation sites, only 03 (01 major and 02 minor) sites are noted to be involved in bound forms of 3ixo with Saquinavir, or analogs S1 and S2 **(Table 2).** More surprisingly all these three mutational positions lay in the flap region of HIV-1 PR subtype-A **(Table 2).** From the results it can be inferred that both S1 and S2 have similar chances of resistance to HIV-1 PR mutants as that of Saquinavir. Therefore, owing to the involvement of position 47 in the former there is a probability that S1/S2 would prove a better inhibitor for HAART therapy in patients with HIV-1 PR subtype-A than Saquinavir. On the other hand using conformational dynamics studies common polymorphic changes in HIV-1 non-subtype B clades as reported by Mao **[6]** suggest 21 most common resistance-related mutation patterns against all HIV-PR inhibitors. The bound form of HIV-1 PR subtype-B (PDB id: 1hxb) **[19]** using conformational dynamics studies with Saquinavir is reported to have resistant-mutation positions (10, 24 and 32) in the enzyme active site and positions (46, 47, 48, 50, 53, 54, 82 and 84) in the flap-region of the same protein.

Drug resistance not only influences protease binding but also generates differential binding affinity between the substrate and inhibitors. The protein backbone of HIV-1 subtype-A and B are similar except that in subtype B the scaffold has amino acid substitutions I13V, K20R, M36I, R41K, H69K and L89M. Though the crystal structure of HIV-1 PR subtype-A (3ixo) is reported to differ from subtype B in having closed flap region as compared to the open structure observed in subtype B, their crystal contacts are similar to the unbound or bound forms of PR crystallized. These workers opined that as there appeared differing RMSD for HIV-1 PR crystal structures from subtypes across the clades, the conformation of the flaps in the different subtypes, bound and unbound may be a 'cause-and-effect' observation only. In our study no amino acid residues that confer structural differences in binding with ligand between subtype A and B are involved therefore both the novel Saquinavir analogs (S1 and S2) are expected to work with similar potential as Saquinavir in blocking HIV-1 PR across the clades.

The results of the physico-chemical parameters studied at ACD/ilab for Saquinavir, and its two designed structural analogs S1 and S2 are listed as **Table 3 (see supplementary material).** The Lipinski profile for S2 was the best among the three molecules studied. Both S1 and S2 have a lower molecular weight of 619.79 and 661.83 respectively, than Saquinavir (mol wt. 670.84). Number of H-bond donors was six for all the 3 molecules. Number of H-bond acceptors was 11 for Saquinavir and S2 however, the number decreased to 10 for S1. Number of rotatable bonds was 13 for both reference drug and S1 whereas it was more i.e. 15 for analog S2 **(Table 3).** Of the three molecules the topological polar surface area (TPSA) was comparatively larger for S2, followed by Saquinavir and S1. LogP values decreased as Saquinavir>S2>S1 respectively. Predicted pKa values for the strongest base were similar for the three molecules whereas pKa for strongest acid was lower for S2 than Saquinavir or S1. Solubility calculated twice as logSw (AB/logSw 2.0) and Sw was almost similar for all three molecules tested **(Table 3).**

Drug design essentially focuses on optimizing the binding interactions of ligands with their targets. However a compound with the best binding interactions is not necessarily the best drug because there are other critical issues downstream. A clinically used drug has to travel through the body to reach its target. There isn't wisdom in perfecting a compound with good drug-target interactions if it has no chance of reaching its target. These issues are grouped as pharmacokinetics and broadly qualified as ADME properties (Absorption, Distribution, Metabolism and Excretion). **Table 3** also lists the absorption, distribution, metabolism and excretion (ADME) properties of Saquinavir, S1 and S2 predicted *in silico* in terms of Absorption, Bioavailability and logBB (blood-brain barrier) at ACD/ilab. Absorption min^{-1} was in the order Saquinavir>S1>S2. All 3 molecules studied had 100% passive absorption and 30% oral bioavailability. Potential to cross blood brain barrier as suggested by logBB values for the three was the least for S2 **(Table 3).**

Toxicity Tests

Getting the drug to the market invariably involves toxicity tests. This phase is significantly more expensive in terms of time and money than either lead discovery or drug design. Table 3 lists the important toxicity parameters for which Saquinavir and its analogs S1 and S2 were tested. Potential mutagenicity of a molecule predicted by AMES test suggests that all of the three molecules have a probability of positive AMES test below 1 indicating almost no potential for mutagenicity and can be used as drug **(Table 3).** Moreover, none of the three molecules have any genotoxicity hazard. *In silico* test to measure whether Saquinavir and its analogs may (not) block the HERG K^+ ion channels of the heart suggest S2 to be a better molecule with minimum HERG inhibition value of 0.12 followed by S1 (0.13) and Saquinavir drug (0.14). The LD_{50} value which measures the dosage in mg/kg which is fatal for an organism was least for Saquinavir but increased for S1 and S2 implying thereby that even at higher doses the two analogs would not be fatal. Toxicity increases numerically. This study places all the three molecules in similar toxicity category of 3 or 4. No endocrine disruption was noted for all the three ligands studied herein. Note that the HIV which causes immune deficiency creates a situation where the lesser the health effects of an administered molecule on the housekeeping organs the better it is for long term use upon infection. The various health effects listed in Table 3 suggest S2 to be the safest among the three ligands with its significantly lowered effect on cardio-vascular system and lungs that suggest important health implications. The maximum recommended daily dose (MRDD)

in correspondence with LD_{50} above assigns the largest value to S2 (13.79 mg/kg/day) > S1 (8.50 mg/kg/day) which is better than the marketed drug **(Table 3).** Results suggest that analog S2 has more potential to evade the problem of drug resistance mutation against HIV-1 PR subtype-A. S2 can be further developed and tested clinically as a real alternative drug for HIV-1 PR across the clades in future.

Conclusion:

Computational lead discovery and lead design can be attempted using chemoinformatics tools and resources. Analogs S1 and S2 of Saquinavir, the approved drug were computationally designed and tested for binding to the drug target, and also with respect to toxicity profile. The position N2 of Saquinavir inhibitor can perhaps be potentially targeted for designing new but potential HIV-PR drugs. Among the two analogs studied the designed molecule S2 appears to be the best. S2 has a better pharmacodynamic profile and all desirable physico-chemical properties required as a drug. It can therefore be concluded that for a realistic understanding of the identified lead analog S2, *in vitro* and/or *in vivo* tests in animal models may be carried out prior to its recommendation as a drug.

Conflict of interest:

There is no conflict of interest and all the authors have contributed equally to this work.

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References:

[1] http://www.unaids.org/en/resources/documents/2012/name,76120,en.asp
[2] http://www.unaids.org/en/resources/documents/2013/
[3] http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm
[4] Montaner JS *et al. PLoS One*. 2014 **9:** e87872 [PMID: 24533061]
[5] Jayaraman S & Shah K, *In Silico Biol.* 2008 **8:** 427 [PMID: 19374129]
[6] Mao Yi, *BMC Structural Biology*. 2011 **11:** 31 [PMID: 21740562]
[7] http://www.who.int/world-health-day/2011/world-health-day2011-brochure.pdf.
[8] http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf.
[9] Wainberg MA *et al. Curr Opin Virol.* 2012 **2:** 569 [PMID: 23025913]
[10] Robbins AH *et al. Acta Crystallogr D Biol Crystallogr.* 2010 **66**: 233 [PMID: 20179334]
[11] Johnson VA *et al. Top HIV Med.* 2009 **17**: 138 [PMID: 20068260]
[12] Knox C *et al. Nucleic Acids Res.* 2011 39: D1035 [PMID: 21059682]
[13] www.acdlabs.com.
[14] Morris GM *et al. J Comput Chem* 2009 **16:** 2785 [PMID: 19399780]
[15] Noel M O'Boyle *et al. J Cheminform*. 2011 **3:** 33 [PMID: 21982300]
[16] Berman HM *et al. Nucleic Acids Research*. 2000 **28:** 235 [PMID: 10592235]
[17] Pettersen EF *et al. J Comput Chem*. 2004 **25**: 1605 [PMID: 15264254]
[18] Shah K *et al. Arch Virol*. 2014 [Epub ahead of print] [PMID: 24643333]
[19] Krohn A *et al. J Med Chem* 1991 **34**: 3340 [PMID 1956054]

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Supplementary material:

Table 1: Docking energies of Saquinavir drug-reference and its 2 designed analogs with HIV Protease (PDB ID: 3ixo)

S. No.	Ligand	Binding Energy with HIV Protease (kcal/mol)
1	Saquinavir-reference	-3.07
2	S-1	-4.08
3	S-2	-3.07

Table 2: Clustering results of the three bound forms of non-mutated HIV-PR subtype A (PDB ID: 3ixo) with Saquinavir and structural analogs S1 and S5 to detect residues that may influence the protein-ligand binding

Ligand	No. of Contacts	Coupled with Active site	Coupled with flap region
Saquinavir	211	8, 23, 25, 26, 27, 28, 29, 30, 32	48, 49, 50, 82, 84
S1	112	8, 23, 25, 26, 27, 28, 29, 30, 32	47, 48, 49, 50, 82, 84
S2	182	8, 23, 25, 26, 27, 28, 29, 30, 32	47, 48, 49, 50, 82, 84

* Only the clusters containing the active sites and flaps are shown here.
(1) Residues shared by all the bound forms are in italics
(2) Residues belonging to the 21 most common resistant mutations for HIV-PR subtype B (i.e. mutation position shared by at least two inhibitors) include positions 10, 24, 32, 46, 47, 48, 50, 53, 54, 82 and 84 and are underlined.
(3) Major and minor [19] resistance mutations in Protease gene.

Table 3: The Physico-chemical, ADME and Toxicity properties predicted for Saquinavir-Reference and its structural analogs S1 and S2 at ACD/ilab

S No	Properties	Reference	S-1	S-2
A	**PHYSICO-CHEMICAL**			
	Lipinski-Type Properties			
(i)	Molecular Weight	670.84	619.79	661.83
(ii)	No. of H-Bond Donors	6	6	6
(ii)	No. of H-Bond Acceptors	11	10	11
(iv)	TPSA (Topological Polar Surface Area)	166.75	153.86	170.93
(v)	Number of Rotatable Bonds	13	13	15
2	**LogP** (ACD/Labs)	4.44 ± 0.85	4.17±0.85	4.38 ± 0.83
3	**pKa** Strongest pKa(Acid)	11.7 ± 0.6	11.7 ± 0.6	7.3 ± 0.8
4	**Solubility**			
	LogSw (AB/LogSw 2.0)	-3.85	-3.27	-3.70
B	**ADME**			
1	**Absorption rate (Ka) (min^{-1})**	0.037	0.034	0.029
2	**Bioavailability**			
	Oral bioavailability	< 30%	< 30%	< 30%
	Solubility, Stability & Passive Absorption	Acceptable	Acceptable	Acceptable
3	**logBB (Blood Brain Barrier)**	0.18	0.30	-0.02
	Fraction unbound I n plasma	0.02	0.03	0.06
C	**TOXICITY**			
1	Probability of positive AMES test	0.08	0.03	0.04
2	Genotoxicity Hazards	Nil	Nil	Nil
3	hERG In (Ki < 10 uM, patch-clamp) probability	0.14	0.13	0.12
	Reliability	Moderate (RI = 0.51)	Borderline(RI = 0.49)	Borderline (RI = 0.39)
4	LD_{50} (mg/kg) :Intraperitoneal in mouse	1300	1400	1500
5	Toxicity Categories	3 or 4	3 or 4	3 or 4
6	Endocrine Disruption	Nil	Nil	Nil
7	Health Effects on: Blood	0.98	0.97	0.97
	Cardiovascular system	0.69	0.63	0.15

	Gastrointestinal system	0.99	0.98	0.98
	Kidney	0.71	0.67	0.56
	Liver	0.93	0.80	0.80
	Lungs	0.87	0.54	0.75
8	MRDD (Max Recommended Daily Dose) (mg/kg/day)	4.56	8.50	13.79