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Pharmacophore-Based Virtual Screening and Docking of Approved Drugs and Philippine Natural Products Targeting HIV-1 Protease

Roxangeluv Costiniano, Junie Billones*

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Abstract

The human immunodeficiency virus (HIV) is the cause of acquired immunodeficiency syndrome, or AIDS, which has been a serious global health concern since its discovery in 1981. Finding novel treatment leads is crucial, as seen by the lack of effective antiretroviral drugs. To identify potential HIV-1 proteins, this study employed a technique known as pharmacophore-based virtual screening and molecular docking. Using a pharmacophore model that was created, compounds from DrugBank and an internal library of Philippine natural products were screened before being docked onto the crystal structure of HIV-1 protease. The top 10 hits that were selected for further analysis included seven-methyl-GpppA, remikiren, clomocycline, metrizamide, SC-74020, and 6-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-yl]amino}phenyl). ~(4Z) 5-methyl-4,5-dihydropyridazin-3(2H) -one -2-[(1R,2R)-1-amino-2-hydroxypropyl] -4-[(4-amino-1H-indol-3-yl)methylene] 5-oxo-4,5-dihydro-1H-imidazol-1-ylacetic acid, bevantolol, and two natural chemicals called quercetin 3-O-β-D-(2-O-galloyl-β-D-glucopyranosyl) 4-O-vinylpropionate with vitamin C together. Predicted ADMET characteristics and docking data showed that SC-74020 was the most promising inhibitor. 6-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-yl]amino~phenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one was the second-place pyridazinone derivative. These results identify potential lead compounds for further in vitro and in vivo testing in the development of novel HIV-1 protease inhibitors.

Keywords: HIV-1, Protease inhibitors, Natural products, Structure-based pharmacophore screening, Molecular docking, ADMET analysis

Introduction

Acquired immunodeficiency syndrome (AIDS), a chronic and sometimes lethal illness (Makhoahle *et al*., 2023; Tabassum *et al*., 2023; Karatas, 2024; Samyuktha & Syam, 2024), is caused by an HIV infection. HIV has remained a major global health concern since it was first discovered in 1981 (Malcangi *et al*., 2023; Botelho *et al*., 2024). According to UNAIDS (2022a), 38.4 million individuals worldwide are predicted to be HIV+ by 2023, and 40.1 million people have died from AIDS-related causes since the pandemic began. According to the Epidemiology Bureau–Department of Health (2022) and UNAIDS (2022b), the Philippines has one of the fastest-growing HIV epidemics in Asia and the Pacific, with an average daily increase of 356% in new infections and a 327% increase in HIV incidence between 2010 and 2021. This rapid escalation highlights the urgent need for continued research into effective treatments.

HIV belongs to the type of retroviruses known as lentiviruses, which are known to induce long-lasting infections with lengthy incubation periods (Kyei & Powderly, 2017). The two primary types are HIV-1, which accounts for roughly 95 percent of infections globally, and HIV-2, which is less virulent and less transmissible (Marlink *et al*., 1994; Ingole *et al*., 2013). There are four other HIV-1 classifications identified: P, O (outlier), N, and M (major). Group M accounts for more than 90% of infections globally (Maurez *et al*., 2013; Ndjoyi-Mbiguino *et al*., 2020). Retrovirus recombination has produced over 100 circulating recombinant forms (CRFs), such as CRF01_AE and CRF02_AG (Bbosa *et al*., 2019). CRF01_AE, an African recombinant form that has supplanted subtype B as the predominant circulating strain, is of special concern in Southeast Asia and the Philippines (Hemelaar *et al*., 2006; Junqueira *et al*., 2020).

An important enzyme in the HIV life cycle, the viral protease is a C2-symmetric homodimer comprising two 99-amino-acid subunits. The dimer interface is where its active site is located. Glycine-rich flaps that alter shape when the substrate binds partially cover this location (Lv *et al*., 2015). HIV protease degrades the Gag and Gag-Pol polyproteins, releasing structural proteins and vital enzymes that are required for the formation of infectious virions, such as reverse transcriptase, integrase, and protease itself (Dunn, 2013; Ghosh *et al*., 2016). Without a functional protease, viral particles remain immature and noninfectious, making this enzyme an attractive target for therapeutic intervention. Although HIV infection was successfully managed with highly active antiretroviral therapy (HAART), protease inhibitors (PIs) have not been used for very long due to side effects like hepatotoxicity, metabolic disorders, and cardiovascular complications (Noor, 2007; Gupta *et al*., 2012; Soontornniyomkij *et al*., 2014; Alnemer *et al*., 2022; Broers *et al*., 2023). Saquinavir, indinavir, and nelfinavir are among the early PIs that are no longer utilized in clinical practice because of their toxicity and undesirable pharmacological characteristic. (Guides for Adults and Adolescents on Antiretroviral Practices, Panel on, 2022).

Drug resistance further compromises HAART efficacy, as viral mutations in or near the protease active site can reduce inhibitor

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binding and lower competitive inhibition (Zdanowicz, 2006). Despite the fact that non-B subtypes of HIV-1 are increasingly more common worldwide, the majority of medication development research has traditionally concentrated on subtype B (Kantor, 2006). These difficulties highlight the necessity of novel therapeutic approaches (Dipalma *et al*., 2022; Harmouche *et al*., 2022; Sugimori *et al*., 2022; Son & Lee, 2024). From target identification to clinical approval, however, traditional drug discovery is expensive and time-consuming, frequently requiring more than 10 years (Al Qaraghuli *et al*., 2017; Parvathaneni *et al*., 2019). Computational techniques like virtual screening and pharmacophore modeling have become more popular in response, allowing for the quick identification of possible inhibitors as well as the early assessment of ADMET (absorption, distribution, metabolism, excretion, and toxicity) characteristics (Vrbanac & Slauter, 2017). By concentrating on substances with proven safety profiles, drug repurposing significantly speeds up research. In light of this, the current study used molecular docking of authorized medications and Philippine natural products as well as pharmacophore-based virtual screening to find prospective inhibitors of the HIV-1 protease CRF01_AE variant.

Materials and Methods

Experimentation in this study was carried out in four major stages: (1) 3D pharmacophore generation, (2) virtual screening, (3) molecular docking, and (4) in-silico ADMET analysis. All computational procedures were performed using Accelrys (now BIOVIA) Discovery Studio (DS) Client v2.5 () on a Windows 7 Professional 64-bit system (Intel® Core™ i7-3770 CPU @ 3.40 GHz, 8 GB RAM). Graphical outputs were generated with DS Visualizer 2021 on an ASUS M509D notebook (Windows 10 64-bit, AMD Ryzen 3 3200U with Radeon Vega Mobile Graphics @ 2.60 GHz, 4 GB RAM).

Pharmacophore Model Generation

Before pharmacophore-based screening, the HIV-1 CRF01_AE protease structure complexed with darunavir (PDB ID: 3LZS; www.rcsb.org) was prepared using the *Clean Protein and Prepare Protein* tools of DS 2.5. The optimized protein underwent energy minimization with the *Smart Minimizer* algorithm and was superimposed with the original structure to compute the root-mean-square deviation (RMSD), ideally 0–1.2 Å. A pharmacophore, defined as the spatial arrangement of essential interaction features, was then generated from the bound ligand using *Define Selected Molecule as Receptor* and *Feature Mapping*. Features were refined with *Cluster Current Feature* and *Keep Only Cluster Centers*, and the resulting model was used to screen the compound databases.

Virtual Screening

Approximately 10,330 compounds—approved and experimental drugs from DrugBank (https://go.drugbank.com) and natural products from an in-house Philippine collection (Castro & Billones 2024)—were screened. Ligands were first prepared with the *Prepare Ligands* protocol and compiled into 3D databases using *Build 3D Database*. Screening was performed via the *Screen Library* protocol; compounds with fit values ≥ 3.0 in both rigid and flexible fitting modes advanced to molecular docking.

Molecular Docking

Docking predicts the binding orientation and affinity of ligands to the target protein. The Dock Ligands (CDOCKER) protocol was applied to compounds that passed the pharmacophore screen, and binding energies were calculated using *Calculate Binding Energies*. The ten top-scoring ligands were ranked and visualized using Show 2D Diagram in DS Visualizer 2021.

In-silico ADMET Analysis

Top-scoring natural products were further evaluated for pharmacokinetic and toxicity profiles. ADME properties (absorption, distribution, metabolism, excretion) were predicted with SwissADME (www.swissadme.ch), which provides key tools such as the bioavailability radar (assessing lipophilicity, molecular size, polarity, solubility, saturation, and flexibility), iLOGP (lipophilicity), and BOILED-Egg (gastrointestinal absorption and blood–brain barrier permeation). Toxicity was assessed using the TOPKAT module of DS 2.5, which estimates rodent carcinogenicity, Ames mutagenicity, developmental toxicity potential (DTP), and aerobic biodegradability by statistically comparing unknown compounds to reference datasets (Duraimurugan *et al*., 2022; Basher *et al*., 2023). Probabilities ≤ 0.3 indicate absence of the property, ≥ 0.7 indicate presence, and 0.3–0.7 are indeterminate (Dassault Systèmes BIOVIA, 2014).

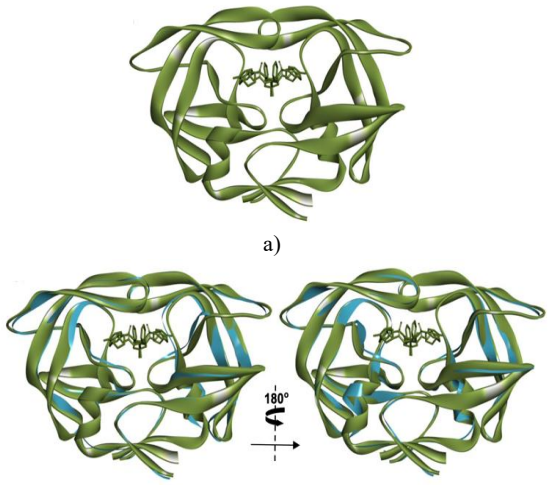
Results and Discussion

Protein Structure Preparation

After being obtained from the RCSB PDB, the crystal structure of HIV-1 CRF01_AE protease in association with darunavir (PDB ID: 3LZS, 1.95 Å resolution) was created in DS using the Prepare Protein procedure. According to Dassault Systèmes BIOVIA (2014), this stage cleans the protein, protonates residues using expected pK values, eliminates superfluous water molecules, deletes alternate conformers, adds missing atoms to incomplete residues, and standardizes atom names. The retention of a crucial water molecule in the active site.

Energy minimization was then performed with the *Minimization* protocol, applying the CHARMm force field and the *Smart* algorithm for 500 steps. The initial potential energy of the HIV-1 protease crystal was –3,896.56 kcal mol^{-1}; after minimization it decreased to –4,255.65 kcal mol^{-1}, indicating a more stable conformation (Mackay *et al*., 1989).

To assess structural changes, the minimized protein was superimposed on the original crystal structure using the *Superimpose Proteins* tool. According to Kufareva and Abagyan (2012), alignment based on Cα atoms produced a root-mean-square deviation (RMSD) of 0.735 Å, indicating that the reduced structure kept its original fold and shape **(Figure 1)**.



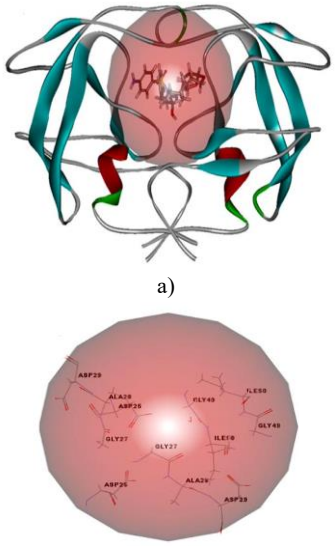
a)

b)

Figure 1. Ribbon diagram and structural alignment of HIV-1 protease CRF01_AE: a) Original crystal structure complexed with darunavir (PDB ID: 3LZS); b) Molecular overlay of the raw structure (olive) and the prepared, energy-minimized structure (cyan) showing an RMSD of 0.735 Å.

Active Site Definition and Validation

The *Define Selected Molecule as Receptor* tool was used to specify the HIV-1 protease, with the co-crystallized darunavir removed. The active site was defined around the darunavir binding pocket using *Define Sphere from Selection*, generating a sphere centered at (–10.0062, 15.4609, 0.9561) Å with a final radius of 10.014 Å to encompass key catalytic residues Asp25, Gly27, Ala28, Asp29, Gly49 and flap residue Ile50 (Yadav *et al*., 2012; Deb *et al*., 2014) **(Figure 2)**.



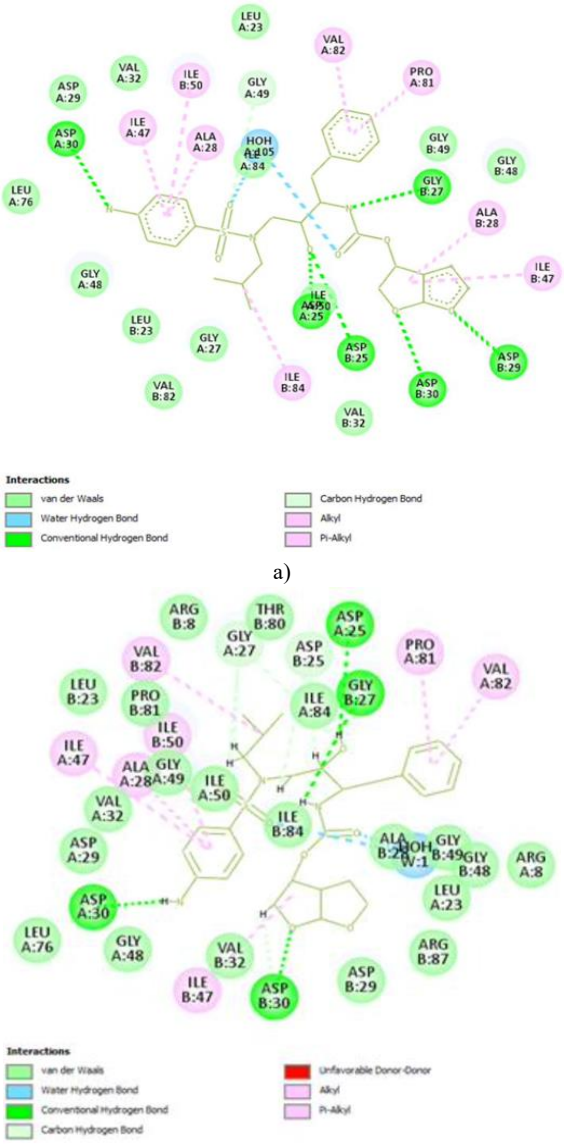
a)

b)

Figure 2. Binding site characterization of HIV-1 protease: a) Binding site sphere on the crystal structure complexed with darunavir; b) Key active-site amino acid residues reported in the literature as crucial for inhibitor binding.

For validation, darunavir was re-docked into the prepared protease using the docking protocol. The three best poses showed RMSD values of 0.80–0.97 Å relative to the co-crystallized ligand, demonstrating that the docking procedure reliably reproduced the experimental binding mode.

Interaction diagrams of the re-docked ligand and the reference complex revealed that five of the seven original hydrogen-bond interactions—those involving Gly27′, Asp25/25′ and Asp30/30′—were retained, including the water-mediated bridge with Ile50/50′. Most hydrophobic (π-alkyl) contacts with Ala28/28′, Ile47/47′, Ile50′, Pro81 and Val82 were also preserved **(Figure 3)**. Slight differences in van der Waals contacts likely reflect minor structural adjustments during preparation.



a)

b)

Figure 3. a) 2D interaction diagram of the original 3LZS complex; b) 2D interaction diagram of darunavir re-docked onto the prepared and energy-minimized HIV-1 protease crystal.

Pharmacophore Generation

A structure-based pharmacophore model was developed using the synthesized HIV-1 protease. The spatial configurations of interactions necessary for ligand binding are represented by pharmacophoric characteristics, which include hydrogen donors and acceptors, ionizable groups, aromatic rings, and hydrophobic moieties (Akram *et al*., 2016; Cortes-Cabrera *et al*., 2016).

Using the *Feature Mapping* protocol, 31 features were initially identified and visualized as colored spheres (green = acceptor, magenta = donor, sky-blue = hydrophobic, red = positive ionizable, orange = aromatic). Hierarchical clustering with *Cluster Current Feature* followed by *Keep Only Cluster Centers* reduced these to 13 key features: five hydrogen acceptors, three hydrogen donors, two hydrophobic features, one positive ionizable group and two aromatic rings **(Figure 4)**. The virtual screening template was based on this pharmacophore.

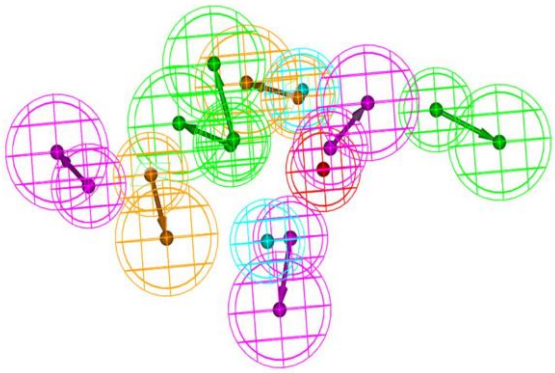


Figure 4. A thirteen-feature pharmacophore model that shows hydrogen bond donors (magenta), hydrogen bond acceptors (green), aromatic rings (orange), hydrophobic regions (sky blue), and positively charged ionizable features (red).

Pharmacophore-based Virtual Screening

To find new inhibitors from chemical libraries, pharmacophore-based virtual screening was used (Rani *et al*., 2021; Reshma & Prakasan, 2021; Giordano *et al*., 2022). Ligand structures (approved and experimental DrugBank compounds and an in-house natural products collection) were prepared using *Prepare Ligands*, which removes duplicates, enumerates tautomers and generates 3D conformations. The Build 3D Database protocol compiled the ligands into searchable databases.

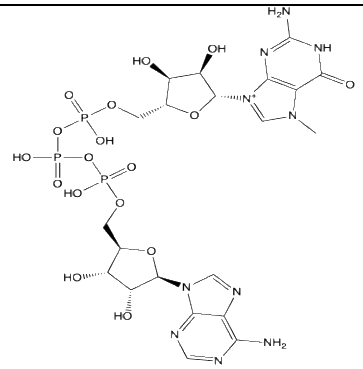
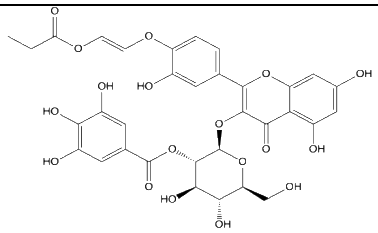
Screening with the *Screen Library* protocol employed rigid and flexible fitting in succession. From 10,330 compounds, those matching the 13-feature pharmacophore were shortlisted for docking.

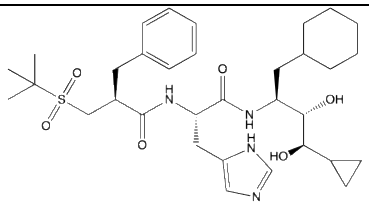
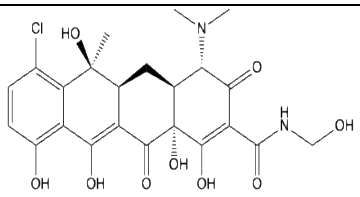
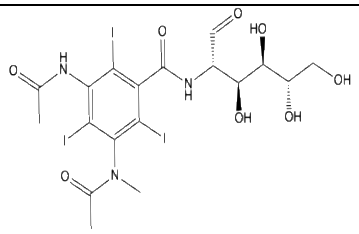
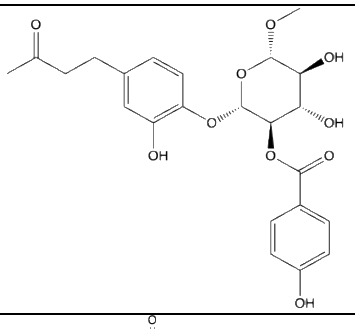
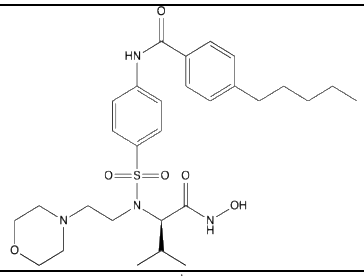
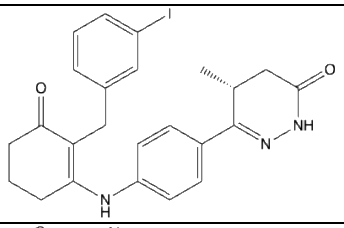
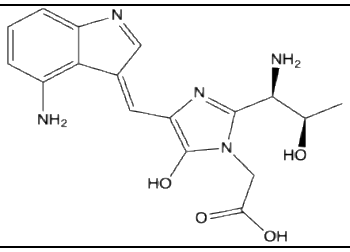
Molecular Docking

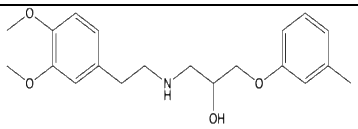
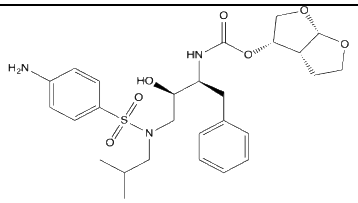
According to Pinzi and Rastelli (2019), molecular docking, which is carried out with Dock Ligands (CDOCKER), predicts the binding mode and calculates the binding energy of ligands with HIV-1 protease. A more negative value indicates stronger, spontaneous binding. Darunavir, re-docked as a control, gave an energy of –4,719.54 kcal mol⁻¹ (Yoong *et al*., 2022; Attenborough *et al*., 2023; Ghati *et al*., 2023; Wolderslund *et al*., 2024).

Ten compounds exhibited more negative CHARMM energies than darunavir **(Table 1)**. Interaction analyses using Show 2D Diagram and Ligand Interaction revealed that darunavir formed seven hydrogen bonds, four electrostatic contacts, five hydrophobic (alkyl) interactions and 21 van der Waals (VDW) contacts. The top hit, 7-methyl-GpppA, bound with energy of –5,109.75 kcal mol⁻¹ through nine hydrogen bonds, 23 VDW and four hydrophobic interactions—exceeding darunavir's hydrogen-bond count. The best-scoring natural product, quercetin 3-O-β-D-(2-O-galloyl-β-D-glucopyranosyl)-4-O-vinyl-propionate, with nine hydrogen bonds and 18 VDW interactions.

Table 1. Top ten hit compounds with their calculated binding energies and fit values

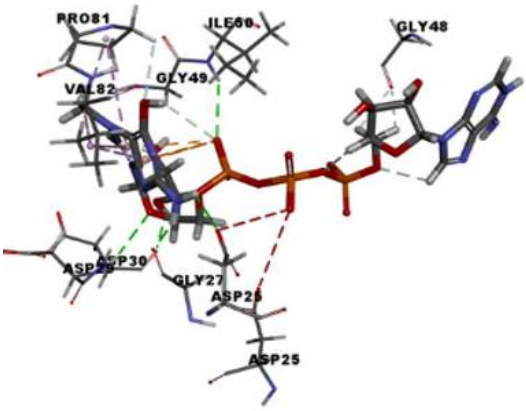
#	Compound	Structure	DrugBank ID	CHARMM Energy (kcal/mol)
1	7-methyl-GpppA		DB01649	–5109.75
2	Quercetin 3-O-β-D-(2-O-galloyl-β-D-glucopyranosyl)-4-O-vinyl-propionate (NP)		–	–4855.43

3	Remikiren		DB00212	–4842.90
4	Clomocycline		DB00453	–4826.70
5	Metrizamide		DB01578	–4811.42
6	Vitexfolin C (NP)		–	–4807.27
7	SC-74020		DB01630	–4806.98
8	6-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-yl]amino}phenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one		DB01640	–4772.83
9	{(4Z)-2-[(1R,2R)-1-amino-2-hydroxypropyl]-4-[(4-amino-1H-indol-3-yl)methylene]-5-oxo-4,5-dihydro-1H-imidazol-1-yl}acetic acid		DB01641	–4737.54

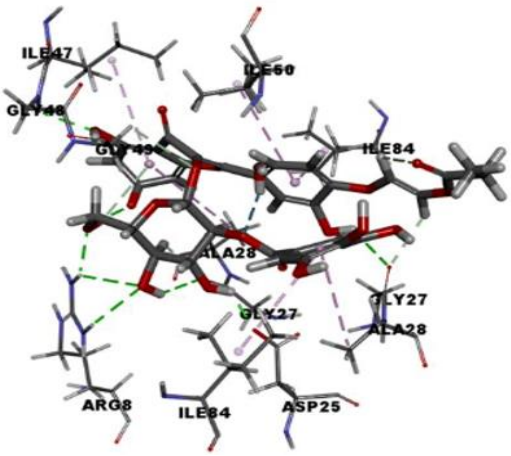
10	Bevantolol		DB01295	–4736.54
	Darunavir (Reference)		–	–4719.54

Legend: DB = DrugBank; NP = natural product.

Most top hits formed key interactions with the catalytic Asp25/Asp25′ and maintained contacts similar to darunavir **(Figure 5)**. Some unfavorable donor–donor or negative–negative contacts were observed (e.g., in 7-methyl-GpppA, remikiren and metrizamide), likely due to the semi-flexible docking protocol, which models the receptor as rigid (Minovski, 2021).



a)



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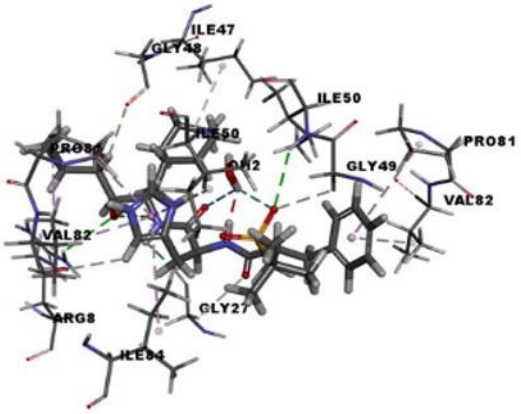
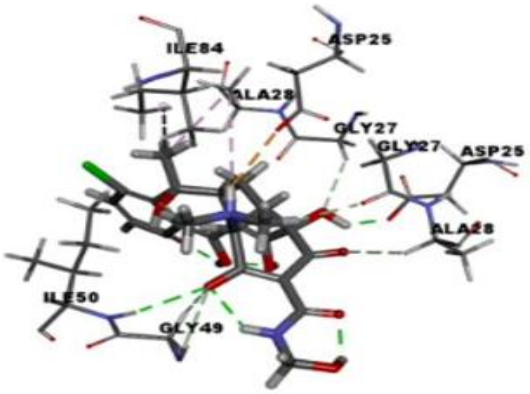
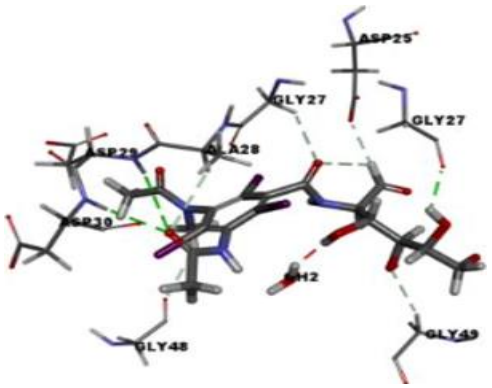


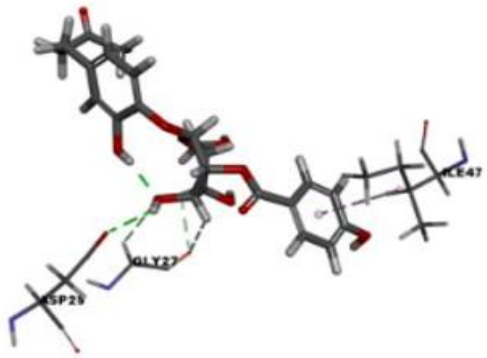
c)



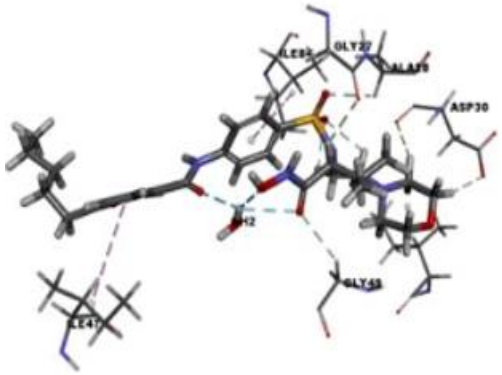
d)



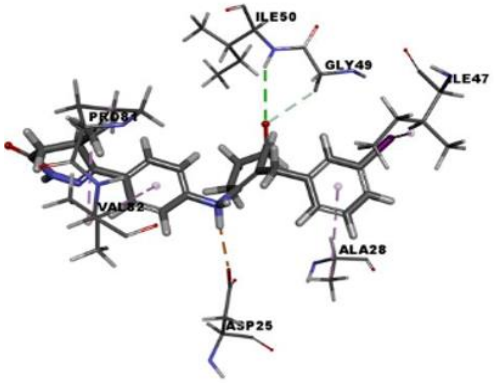
e)



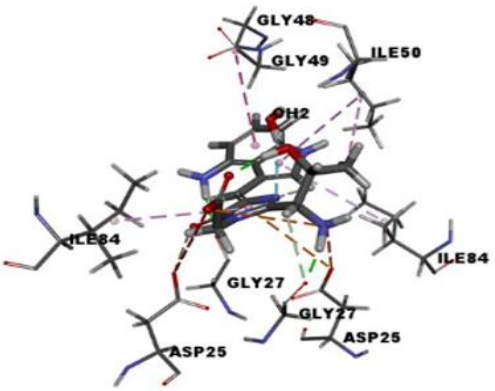
f)



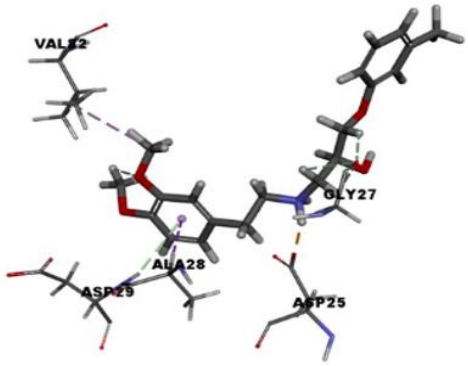
g)



h)



i)



j)

Figure 5. Three-dimensional interaction graphs show how the top docked compounds from DrugBank and the in-house natural product library (PDB ID: 3LZS) attach to the HIV-1 protease active site, along with the initial ligand, darunavir: 3-O-β-D-quercetin (2-O-galloyl-β-D-glucopyranosyl) and 6-methyl-GpppA a) and b) Remikiren, clomocycline, metrizamide, vitexfolin C, 4-O-vinylpropionate, SC-74020, Five-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-yl]amino}phenyl) h) One i) \(4Z) three(2H) (5)-methyl-4,5-dihydropyridazin [(1R,2R)-1-amino-2-hydroxypropyl- (3-yl)methylene [(4-amino-1H-indol] -2- 4-Acetetic acid OneH-imidazol-1-yl, -5-oxo-4,5-dihydro, and j) bevantolol.

ADMET Analysis

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) of darunavir and the top 10 hits were assessed using the SwissADME web server (www.swissadme.ch) to ascertain drug-likeness (Patatou *et al*., 2022; Seoane-Viaño *et al*., 2024). To produce information on physicochemical characteristics, lipophilicity, water solubility, pharmacokinetics, drug-likeness (Lipinski's rule), and medicinal chemistry filters, compounds were transformed into SMILES strings and submitted. All ten top-hit compounds from DrugBank and the in-house natural product library, along with the reference drug darunavir, were evaluated for their ADMET profiles using SwissADME and TOPKAT. Apart from vitexfolin C, the majority of the hits, "(1R,2R)-1-amino-2-hydroxypropyl" ((4-amino-1H-indol-3-yl)methylene) Bevantolol and -5-oxo-4,5-dihydro-1H-imidazol-1-ylacetic acid, like darunavir, have molecular weights over 500 Da and break one to three of Lipinski's rules, albeit substances that are transporter substrates can withstand such infractions. Only quercetin 3-O-β-D-(2-O-galloyl-β-D-glucopyranosyl)-4-O-vinyl-propionate and clomocycline showed logP values indicative of good oral absorption, while 7-methyl-GpppA had a negative logP, reflecting high aqueous affinity. Two hits, 6-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-yl]aminophenyl)-5-methyl-4,5-dihydropyridazin-3(2H), were found using BOILED-Egg analysis. -one and bevantolol—possessed significant blood–brain barrier penetration capability, a beneficial characteristic for treating neurological diseases linked to HIV. TOPKAT toxicity predictions showed that only SC-74020 met all criteria for a good drug—non-carcinogenic, non-mutagenic, non-toxic, and non-degradable—followed by 6-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-

yl]amino}phenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one, which met three of the four. Taken together, SC-74020 emerged as the most promising HIV-1 protease inhibitor, displaying the strongest binding affinity surpassing that of re-docked darunavir, along with a favorable overall ADMET profile.

Conclusion

Using 10,330 natural items from an internal collection and licensed and experimental medications from DrugBank, this study employed virtual screening, structure-based pharmacophore modeling, and molecular docking to find compounds with a high affinity for the HIV-1 protease. The CRF01_AE. The compound list was reduced by the pharmacophore model that was produced, and the protein–ligand complexes' binding energies were computed. Ten compounds, including two natural products (quercetin 3-O-β-D-(2-O-galloyl-β-D-glucopyranosyl)-4-O-vinyl-propionate and vitamin C) and eight experimental DrugBank entries (DB01649, DB00212, DB00453, DB01578, DB01630, DB01640, DB01641, and DB01295), demonstrated stronger binding than the re-docked reference ligand darunavir. SC-74020 [6-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-yl]amino~phenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one] was the most probable candidate based on binding energies and ADMET predictions, with favorable interactions and a pharmacokinetic profile. Further database screening, de novo design, and follow-up in vitro and in vivo research are advised to validate these computational findings and move possible HIV-1 protease inhibitors closer to drug development, given the ongoing increase in HIV diagnoses.

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