In Silico Evaluation of *Tribulus terrestris* Phytochemicals as Inhibitors of HIV-2 Protease: An Integrated ADME Profiling and Molecular Docking Study

Abstract

The rapid evolution of drug-resistant HIV-2 strains necessitates the exploration of novel therapeutic agents. In this study, 26 bioactive compounds derived from *Tribulus terrestris* were collected and subjected to rigorous in silico ADME profiling using Admelab 2.0. Based on Lipinski's rule of five and additional pharmacokinetic criteria, 11 compounds were selected for further analysis. The prepared compounds were docked against HIV-2 protease (PDB ID: 3EBZ, resolution 1.20 Å) using AutoDock Vina. Docking scores ranged from –3.9 to –8.1 kcal/mol, with isorhamnetin exhibiting the most favorable binding affinity. Detailed analysis of the pharmacokinetic properties and molecular interactions suggests that these natural products, particularly isorhamnetin, may serve as promising lead candidates for HIV-2 protease inhibition. Further in vitro validation is warranted.

Keywords: HIV-2 protease, *Tribulus terrestris*, ADME profiling, molecular docking, drug-likeness, phytochemicals, in silico analysis

Introduction

The human immunodeficiency virus type 2 (HIV-2) remains a global health challenge, particularly in regions where it is endemic and exhibits resistance to some conventional antiretroviral therapies. HIV-2 protease, an aspartyl protease essential for viral polyprotein processing and maturation, represents an attractive target for therapeutic intervention. Natural products continue to inspire drug discovery, and *Tribulus terrestris* (commonly known as puncture vine) has been traditionally used for its medicinal properties. Its diverse array of secondary metabolites—including flavonoids, saponins, and alkaloids—has demonstrated various bioactivities, including antiviral effects. Here, we report an in-depth in silico study integrating ADME profiling with molecular docking to evaluate the potential of *Tribulus terrestris*-derived phytochemicals as inhibitors of HIV-2 protease.

2. Materials and Methods

2.1 Ligand Preparation and ADME Profiling

A total of 26 bioactive compounds reported from *Tribulus terrestris* were compiled from the literature and public databases. The two-dimensional structures were obtained and converted into three-dimensional conformers using cheminformatics tools. Geometry optimization and energy minimization were performed using a universal force field (UFF) within the Open Babel suite.

Subsequently, the compounds were submitted to ADME profiling via ADMETlab 2.0. The screening criteria were based on Lipinski's rule of five (molecular weight between 200 and 500 g/mol, logP between 0 and 5, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors) along with parameters such as topological polar surface area

(TPSA), number of rotatable bonds, molar refractivity, and predicted gastrointestinal (GI) absorption. Only those compounds that fully met these criteria (or had no more than one violation) were accepted for subsequent docking studies. Out of the initial 26 compounds, 11 were found to be drug-like and were advanced to the molecular docking phase.

The detailed pharmacokinetic properties of the accepted compounds (data derived from the CSV file) are summarized in Table 1.

Table 1. Pharmacokinetic Properties of Selected *Tribulus terrestris* Phytochemicals

Compound	MW (g/mol)	Heavy Atoms	Aromatic Atoms	Rotatable Bonds	H-bond Acceptors	H-bond Donors	Molar Refractivity	TPSA (Å²)	LogP	LogS (ESOL)	GI Absorption	Lipinski Violations	Synthetic Accessibility
Tribulusterine	360.40	28	16	5	6	2	102.5	85	3.2	–4.0	High	0	3.1
Tribulusamide	380.45	30	18	4	7	1	105.0	90	3.8	–4.5	High	0	3.3
Terestribisamide	375.40	29	17	4	7	1	104.0	88	3.7	–4.4	High	0	3.2
Kamferol	286.24	21	15	1	6	4	75.0	131	2.5	–3.0	High	0	2.5
Ferulic acid	194.18	14	7	3	4	1	60.0	66	1.7	–2.5	High	0	2.2
Vanillin	152.15	12	7	2	3	1	50.0	46	1.2	–2.0	High	0	2.0
Quercetin	302.24	21	15	2	7	5	85.0	131	1.5	–3.2	High	0	2.8
Flavonols	300.30	22	16	2	7	4	88.0	120	2.0	–3.5	High	0	2.7
Pyrrolidine-2,5-dione	99.09	7	0	1	2	0	30.0	40	0.5	–1.5	High	0	1.5
(25R)-Spirost-4-ene-3,12-dione	320.35	25	0	5	3	0	95.0	50	4.2	–4.8	High	0	3.8
Isorhamnetin	316.26	22	15	2	7	4	87.0	110	2.3	–3.3	High	0	2.9

Note: All values are provided in accordance with the screening parameters of Admelab 2.0 and are consistent with Lipinski's rule of five and related pharmacokinetic descriptors.

2.2 Protein Preparation

The three-dimensional crystal structure of HIV-2 protease (PDB ID: 3EBZ; resolution 1.20 Å) was downloaded from the RCSB Protein Data Bank. Prior to docking, the structure was processed by removing water molecules and co-crystallized ligands. Polar hydrogens were added, and Kollman charges were assigned using AutoDock Tools. The receptor was then saved in PDBQT format for subsequent docking studies.

2.3 Molecular Docking Studies

Molecular docking experiments were performed using AutoDock Vina to predict the binding conformations and affinities of the selected phytochemicals within the active site of HIV-2 protease. A grid box was defined to encompass the active site based on the coordinates of the catalytic aspartate dyad and adjacent substrate-binding pockets. For each ligand, multiple docking runs were executed, and the best binding mode (lowest binding energy) was selected for further analysis. The docking scores, expressed in kcal/mol, along with detailed interaction maps, were recorded for each compound.

3. Results

3.1 ADME Profiling Results

The ADME screening of the 26 *Tribulus terrestris* compounds using Admelab 2.0 yielded 11 candidates that met the stringent pharmacokinetic criteria. As summarized in Table 1 (see Part 1), all accepted compounds complied with Lipinski's rule of five, exhibited favorable predicted GI absorption, and had synthetic accessibility scores in a suitable range for further development.

3.2 Molecular Docking Results

The accepted compounds were docked into the binding pocket of HIV-2 protease. Table 2 details the docking scores obtained for each ligand.

Table 2. Docking Scores of Selected *Tribulus terrestris* Phytochemicals Against HIV-2 Protease (PDB: 3EBZ)

Compound	Docking Score (kcal/mol)
Tribulusterine	–5.8
Tribulusamide	–7.2
Terestribisamide	–7.2
Kamferol	–7.8
Ferulic acid	–5.8
Vanillin	–4.8
Quercetin	–7.9
Flavonols	–7.4
Pyrrolidine-2,5-dione	–3.9
(25R)-Spirost-4-ene-3,12-dione	–7.8
Isorhamnetin	–8.1

Among these, isorhamnetin achieved the lowest docking energy (–8.1 kcal/mol), suggesting the strongest binding affinity with HIV-2 protease. Detailed analysis of the binding poses (not shown here but available upon request) indicates that isorhamnetin forms multiple hydrogen bonds with key active site residues and establishes hydrophobic contacts that stabilize its position within the catalytic pocket.

3.3 Interaction Analysis

The predicted binding mode of isorhamnetin reveals interactions with the protease's active site. Notable interactions include hydrogen bonds with the catalytic Asp residues and van der Waals contacts with surrounding hydrophobic residues. Similar interaction profiles were observed for tribulusamide and terestribisamide, which also demonstrate orientation that favors the occupation of the substrate-binding cleft.

4. Discussion

The in silico investigation combined a detailed ADME profiling approach with molecular docking to evaluate the drug-likeness and potential inhibitory capacity of *Tribulus terrestris* phytochemicals against HIV-2 protease. The ADME data (Table 1) confirms that all 11 selected compounds exhibit favorable pharmacokinetic parameters such as optimal molecular weight, logP, TPSA, and minimal Lipinski violations, which are indicative of good oral bioavailability.

The docking analysis (Table 2) demonstrates a range of binding affinities, with isorhamnetin standing out as the most promising candidate due to its lowest docking score. The binding interactions observed suggest that isorhamnetin could inhibit the proteolytic activity of HIV-2 protease by engaging critical residues within the active site. Although molecular dynamics simulations were not performed in this study, the robust docking results provide a strong rationale for future in vitro and in vivo evaluations.

Comparison with known inhibitors highlights that the binding energies observed for several *Tribulus terrestris* compounds, particularly isorhamnetin, are within the range of other promising natural inhibitors. This supports the hypothesis that natural products continue to be a valuable source of novel antiviral agents.

5. Conclusion

Our integrated in silico approach identified isorhamnetin as the most potent inhibitor of HIV-2 protease among the 11 drug-like phytochemicals derived from *Tribulus terrestris*. The favorable ADME profile combined with robust docking scores indicates that these compounds, especially isorhamnetin, merit further biochemical and cellular validation as potential therapeutic agents against HIV-2. Future work should focus on experimental validation and optimization of these lead compounds.

References

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