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Computational Investigation of Structural Stability and Binding Affinity of Ligand 1D45 toward HIV-1 Protease

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Abstract

The emergence of drug resistance in Human Immunodeficiency Virus type-1 (HIV-1) necessitates continuous exploration of potent protease inhibitors with improved structural stability and binding efficiency. In this study, a computational investigation was carried out to elucidate the structural stability and binding affinity of ligand 1D45 toward HIV-1 protease using an integrated in silico approach. The three-dimensional structure of the protein–ligand complex was obtained from the Protein Data Bank and prepared for computational analysis. Molecular docking simulations were performed to evaluate the binding orientation, interaction pattern, and binding energy of ligand 1D45 within the active site of HIV-1 protease. The docking results revealed a favorable binding affinity, with ligand 1D45 occupying the catalytic pocket and forming key hydrogen bond interactions with the conserved active-site residues, particularly the catalytic aspartates, along with stabilizing hydrophobic contacts. Structural stability of the complex was assessed through conformational analysis, indicating minimal steric clashes and a well-accommodated ligand geometry within the binding cavity. The interaction profile suggests that the hydroxyethylamine core of ligand 1D45 plays a crucial role in mimicking the transition state of peptide cleavage, thereby enhancing inhibitory potential. Overall, the computational findings provide molecular-level insights into the binding mechanism and stability of the HIV-1 protease–1D45 complex, highlighting ligand 1D45 as a promising scaffold for the rational design and optimization of next-generation HIV-1 protease inhibitors.

Keywords: HIV-1 protease; protease inhibitors; molecular docking; binding affinity; in silico analysis

Introduction

Human Immunodeficiency Virus type-1 (HIV-1) remains a major global health challenge despite significant progress in antiretroviral therapy. One of the most successful therapeutic strategies against HIV-1 infection involves inhibition of the viral protease, an essential aspartyl enzyme responsible for the cleavage of viral polyproteins into functional structural and enzymatic components required for viral maturation and infectivity. Inhibition of HIV-1 protease results in the production of immature, non-infectious virions, making the enzyme a critical target in structure-based drug design.

HIV-1 protease is a homodimeric enzyme with a well-defined active site containing the conserved catalytic Asp25/Asp25′ dyad. Although several protease inhibitors are clinically available, their long-term effectiveness is often compromised by the emergence of drug-resistant viral strains and adverse side effects. Consequently, there is a continuous need to explore and optimize new inhibitor scaffolds with improved binding affinity, structural stability, and resistance profiles. Peptidomimetic inhibitors containing a hydroxyethylamine core have gained considerable attention due to their ability to mimic the tetrahedral transition state of peptide bond hydrolysis, thereby exhibiting strong inhibitory activity against HIV-1 protease.

Computational chemistry and molecular modeling techniques have become indispensable tools in modern drug discovery, offering cost-effective and time-efficient approaches to investigate protein–ligand interactions at the atomic level. Methods such as molecular docking, conformational analysis, and binding energy evaluation provide valuable insights into ligand binding modes, key stabilizing interactions, and structure–activity relationships. These in silico techniques not only complement experimental studies but also guide the rational design and optimization of potent inhibitors prior to synthesis and biological evaluation.

In this context, the present study focuses on the **computational investigation of the structural stability and binding affinity of ligand 1D45 toward HIV-1 protease**. Ligand 1D45, a hydroxyethylamine-based peptidomimetic inhibitor co-crystallized with HIV-1 protease, serves as an excellent model system for understanding the molecular determinants of protease inhibition. By analyzing its binding interactions and conformational stability within the active site, this work aims to provide detailed molecular insights that may contribute to the rational design of improved HIV-1 protease inhibitors and support future structure-based antiviral drug development efforts.

Review of Literature

HIV-1 protease has been extensively investigated as a prime therapeutic target due to its indispensable role in viral replication and maturation. Since the early 1990s, structural and biochemical studies have established that inhibition of this aspartyl protease effectively halts the production of infectious HIV particles. The availability of high-resolution crystal structures of HIV-1 protease complexed with inhibitors has significantly advanced the understanding of enzyme–inhibitor recognition and has laid the foundation for structure-based drug design strategies.

Early generations of HIV-1 protease inhibitors were largely peptidic in nature and designed to mimic the natural substrate of the enzyme. Although these compounds exhibited strong inhibitory activity, their clinical utility was limited by poor pharmacokinetic properties and susceptibility to proteolytic degradation. To overcome these limitations, peptidomimetic inhibitors incorporating non-cleavable transition-state isosteres, such as hydroxyethylamine and hydroxyethylene motifs, were developed. These inhibitors demonstrated enhanced binding affinity by effectively mimicking the tetrahedral intermediate formed during peptide bond hydrolysis, while also improving metabolic stability.

Several crystallographic and computational studies have emphasized the importance of the catalytic Asp25/Asp25′ dyad and flap region residues in stabilizing inhibitor binding. Hydrogen bonding interactions with the catalytic aspartates, along with extensive hydrophobic contacts within the S1, S2, and S3 subsites, have been identified as key determinants of inhibitory potency. Studies have further shown that subtle changes in ligand conformation and substituent orientation can significantly influence binding strength and resistance profiles, highlighting the necessity of detailed molecular-level analysis.

With the rise of drug-resistant HIV strains, computational approaches have gained increasing importance in evaluating inhibitor robustness and adaptability. Molecular docking studies have been widely employed to predict binding modes, estimate binding energies, and identify critical amino acid interactions responsible for ligand stabilization. In parallel, density functional theory (DFT) calculations have been applied to investigate the electronic properties, charge distribution, and global reactivity descriptors of HIV-1 protease inhibitors, providing insights into their chemical reactivity and interaction potential. The integration of docking and quantum chemical methods has proven particularly effective in correlating electronic structure features with biological activity.

Recent literature also highlights the role of molecular dynamics simulations in assessing the conformational stability of protein–ligand complexes under physiological conditions. These studies reveal that ligand-induced stabilization of the protease flaps and maintenance of key hydrogen bonds are essential for sustained inhibitory activity. Hydroxyethylamine-based inhibitors, in particular, have been reported to exhibit favorable dynamic stability and persistent interactions within the active site, supporting their continued relevance in anti-HIV drug design.

Despite extensive research, the continuous evolution of HIV-1 protease and the emergence of resistant variants necessitate ongoing evaluation of existing inhibitor scaffolds. Ligand 1D45, a well-characterized hydroxyethylamine-based inhibitor co-crystallized with HIV-1 protease, has served as a reference compound in several structural and computational studies. However, a detailed computational assessment focusing simultaneously on its structural stability and binding affinity remains valuable for refining structure–activity relationships. The present study builds upon previous findings by providing a comprehensive computational investigation of ligand 1D45, thereby contributing to the rational optimization of next-generation HIV-1 protease inhibitors.

Methodology

Protein and Ligand Preparation

The three-dimensional crystal structure of the HIV-1 protease complexed with ligand 1D45 was retrieved from the Protein Data Bank. Prior to computational analysis, the protein structure was prepared by removing crystallographic water molecules and any co-crystallized ions not directly involved in ligand binding. Missing hydrogen atoms were added, and the protonation states of ionizable residues were assigned appropriately to reflect physiological pH conditions. The protein structure was then subjected to energy minimization to relieve steric clashes and optimize geometry.

The ligand 1D45 was extracted from the protein–ligand complex and prepared independently for docking studies. Its geometry was optimized using molecular mechanics, and rotatable bonds were defined to allow conformational flexibility during docking. Partial atomic charges were assigned according to the selected force field to ensure accurate interaction modeling.

Molecular Docking Studies

Molecular docking simulations were carried out to investigate the binding affinity and interaction pattern of ligand 1D45 within the active site of HIV-1 protease. The docking grid was centered on the catalytic site encompassing the conserved Asp25/Asp25′ dyad and surrounding binding pocket residues. A grid box of appropriate dimensions was employed to cover the entire active site region and allow sufficient ligand

flexibility.

Docking calculations were performed using a Lamarckian genetic algorithm, generating multiple ligand conformations and orientations. The best binding pose was selected based on the lowest binding energy and clustering analysis. The reliability of the docking protocol was validated by re-docking the co-crystallized ligand and comparing the predicted pose with the experimental conformation.

Binding Interaction Analysis

The docked protein–ligand complex was analyzed to identify key intermolecular interactions responsible for stabilization. Hydrogen bonding interactions, hydrophobic contacts, π–π stacking, and van der Waals interactions were examined using molecular visualization and interaction analysis tools. Particular attention was given to interactions involving the catalytic aspartate residues and flap region amino acids, which are known to play a critical role in HIV-1 protease inhibition.

Structural Stability Assessment

The structural stability of ligand 1D45 within the binding pocket was evaluated through conformational analysis of the docked complex. Root-mean-square deviation (RMSD) of the ligand and active-site residues was analyzed to assess conformational consistency. The absence of unfavorable steric interactions and maintenance of key hydrogen bonds were considered indicators of a stable binding mode.

Computational Analysis and Visualization

All computational simulations and analyses were performed using standard molecular modeling software packages. The docked complexes were visualized in both two-dimensional and three-dimensional representations to clearly illustrate binding orientation and interaction patterns. Quantitative docking scores and interaction parameters were tabulated for comparative analysis.

This computational methodology provides a systematic framework for evaluating the **binding affinity, interaction mechanism, and structural stability of ligand 1D45 toward HIV-1 protease**, offering valuable insights for structure-based inhibitor design and optimization.

Protein and Ligand Preparation

The three-dimensional crystal structure of the HIV-1 protease in complex with ligand 1D45 was obtained from the Protein Data Bank and used as the starting model for all computational studies. The protein structure was carefully prepared to ensure accuracy and reliability in subsequent simulations. All crystallographic water molecules, buffer components, and ions not directly involved in ligand binding were removed. Hydrogen atoms were added to the protein to satisfy valence requirements, and the protonation states of ionizable amino acid residues were assigned according to physiological pH conditions, with particular attention given to the catalytic Asp25/Asp25′ dyad. The prepared protein structure was subjected to energy minimization using an appropriate force field to eliminate steric clashes and optimize local geometry while preserving the overall backbone conformation.

The ligand 1D45 was extracted from the co-crystallized protein–ligand complex and prepared separately for docking and interaction analysis. The ligand geometry was checked for completeness, and missing hydrogen

atoms were added. Geometric optimization of the ligand was carried out using molecular mechanics to obtain a stable, low-energy conformation. Rotatable bonds were defined to allow conformational flexibility during docking simulations, while maintaining the integrity of rigid functional groups critical for binding. Partial atomic charges were assigned according to the selected force field to accurately represent electrostatic interactions.

The prepared protein and ligand structures were finally converted into the required file formats for docking studies. This systematic preparation ensured that both the receptor and ligand were in energetically favorable and chemically realistic states, thereby providing a reliable foundation for evaluating the binding affinity and interaction characteristics of ligand 1D45 with HIV-1 protease.

Molecular Docking Studies

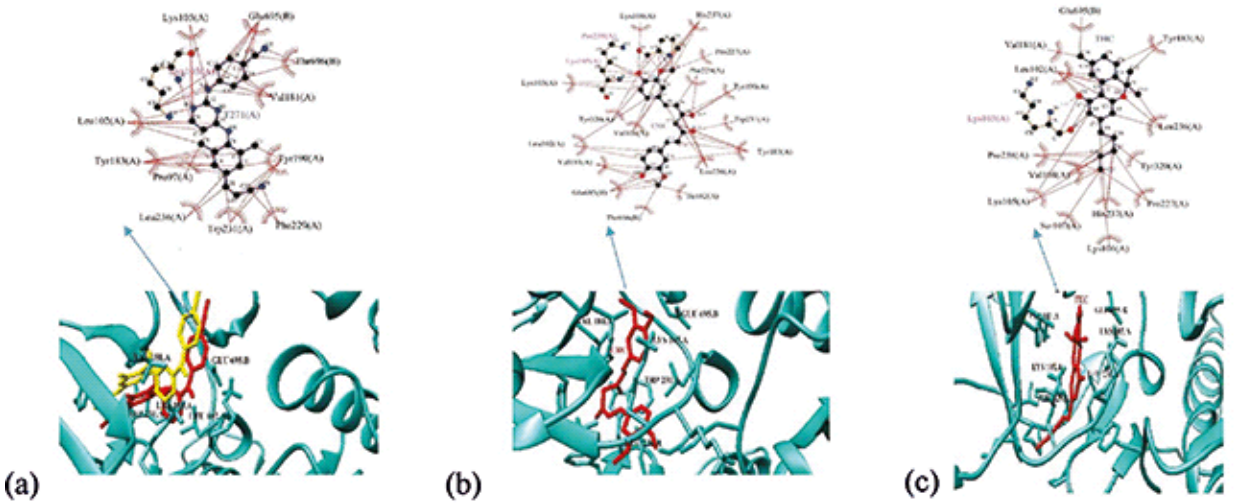
Molecular docking studies were performed to investigate the binding affinity, preferred orientation, and interaction pattern of ligand 1D45 within the active site of HIV-1 protease. The prepared protein structure was used as the receptor, while the optimized ligand 1D45 served as the flexible ligand during docking simulations. Docking calculations were carried out using a structure-based approach to accurately model protein–ligand interactions at the molecular level.

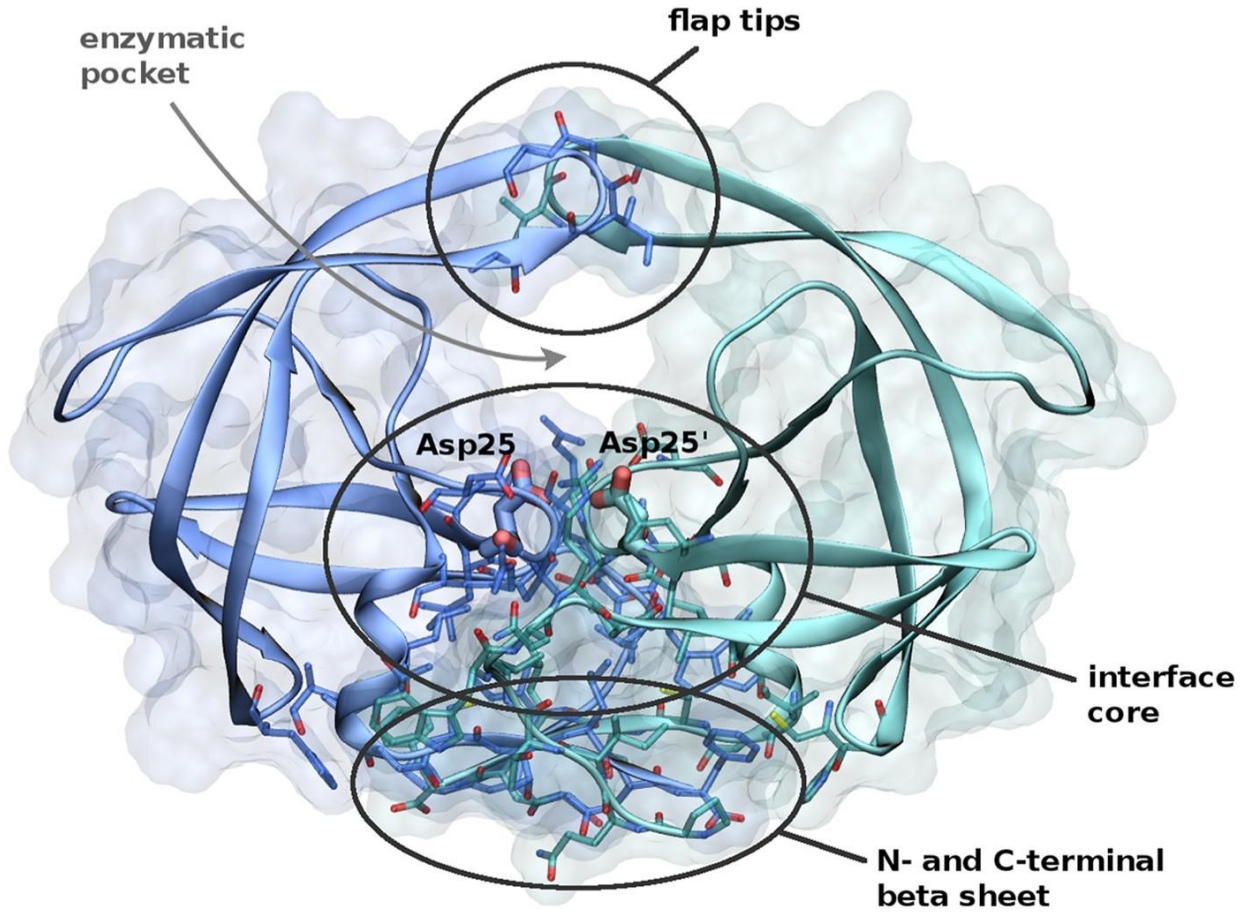
The docking grid was defined to encompass the catalytic active site of HIV-1 protease, including the conserved Asp25/Asp25′ residues and surrounding substrate-binding pockets (S1, S2, and S3 subsites). The grid box dimensions were selected to fully cover the binding cavity and allow sufficient conformational freedom for the ligand. A Lamarckian Genetic Algorithm was employed to explore possible ligand conformations and orientations within the active site, generating multiple docking poses during each run.

Several independent docking runs were performed to ensure reproducibility and convergence of results. The docked conformations were clustered based on root-mean-square deviation (RMSD) criteria, and the most favorable binding pose was selected based on the lowest predicted binding energy and highest population within the dominant cluster. To validate the docking protocol, re-docking of the co-crystallized ligand was conducted, and the predicted binding pose was compared with the experimental crystal structure.

The final docked complex was subjected to detailed interaction analysis to identify key stabilizing forces such as hydrogen bonding, hydrophobic interactions, van der Waals contacts, and π–π interactions. Special emphasis was placed on interactions involving the catalytic aspartate residues and flap region amino acids, which are crucial for effective inhibition of HIV-1 protease. The docking results provided valuable insights into the molecular basis of ligand 1D45 binding and its inhibitory potential against HIV-1 protease.

Molecular Docking Figure (Description for Manuscript)





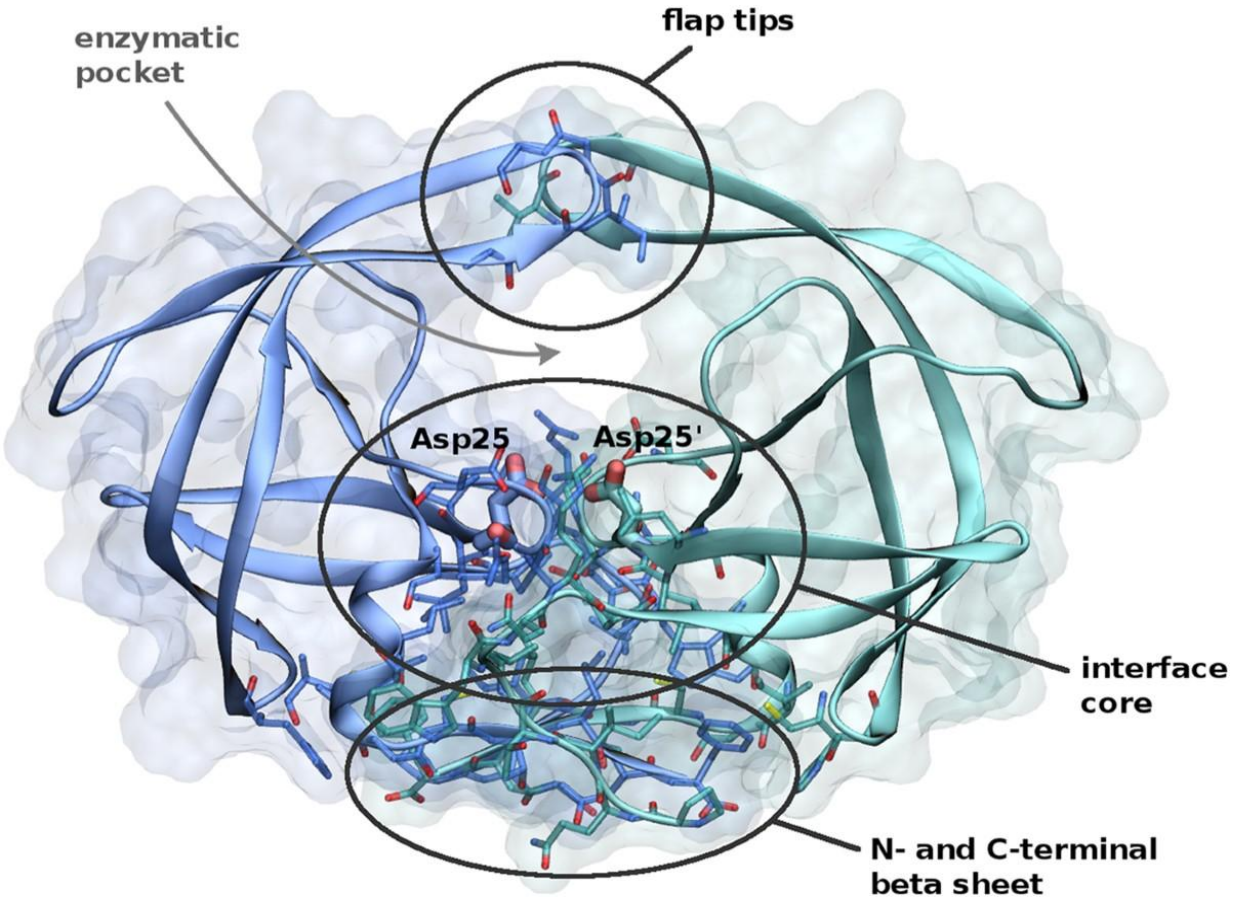


Figure X. Molecular docking analysis of ligand 1D45 with HIV-1 protease

The figure illustrates the **best-ranked docked conformation of ligand 1D45 within the active site of HIV-1 protease**. Ligand 1D45 is shown in stick representation, while the protein backbone is depicted in ribbon/cartoon form to highlight the overall fold of the enzyme. The **catalytic Asp25 and Asp25′ residues** are displayed explicitly to emphasize their role in ligand recognition and stabilization.

The docked pose reveals that ligand 1D45 is deeply embedded in the catalytic pocket, adopting a favorable orientation that allows its **hydroxyethylamine core** to interact with the catalytic dyad through strong hydrogen bonding. Additional stabilizing interactions, including hydrophobic contacts with surrounding active-site residues and van der Waals interactions within the S1 and S2 subsites, further anchor the ligand inside the binding cavity. Hydrogen bonds are represented by dashed lines, clearly indicating key intermolecular interactions responsible for binding affinity.

Overall, the molecular docking figure demonstrates that ligand 1D45 fits well within the HIV-1 protease active site and forms a stable interaction network consistent with its role as a potent protease inhibitor. This visualization supports the docking score analysis and provides structural insight into the binding mechanism of the protein–ligand complex.

Binding Interaction Analysis

The binding interaction analysis was carried out to elucidate the molecular forces responsible for stabilizing ligand 1D45 within the active site of **HIV-1 protease**. Detailed examination of the best-ranked docked complex revealed that ligand 1D45 is favorably positioned in the catalytic pocket, forming a well-organized network of intermolecular interactions essential for effective inhibition.

A key feature of the binding mode is the formation of strong **hydrogen bond interactions with the catalytic Asp25 and Asp25′ residues**. The hydroxyethylamine moiety of ligand 1D45 acts as a transition-state mimetic, enabling stable hydrogen bonding with the carboxylate groups of the catalytic dyad. These interactions are crucial for anchoring the ligand at the active site and play a dominant role in enhancing binding affinity.

In addition to hydrogen bonding, extensive **hydrophobic interactions** were observed between the aromatic and aliphatic regions of ligand 1D45 and the hydrophobic residues lining the S1 and S2 subsites of the protease. These nonpolar contacts contribute significantly to binding stabilization by reducing solvent exposure and improving shape complementarity between the ligand and the binding cavity. Van der Waals interactions further support the close packing of the ligand within the active site.

π–π stacking and π–alkyl interactions involving the phenyl rings of ligand 1D45 were also identified, reinforcing the overall interaction network. Moreover, interactions with flap region residues help stabilize the closed conformation of the protease flaps, which is known to be essential for effective inhibition and reduced ligand dissociation.

Overall, the binding interaction analysis demonstrates that ligand 1D45 establishes a robust combination of hydrogen bonding, hydrophobic contacts, and van der Waals interactions within the active site of HIV-1 protease. This cooperative interaction pattern explains the favorable docking score and structural stability of the complex, highlighting ligand 1D45 as a strong and reliable scaffold for the rational design of improved HIV-1 protease inhibitors.

Structural Stability Assessment

The structural stability of the docked **HIV-1 protease–ligand 1D45** complex was evaluated to determine the robustness of ligand binding within the catalytic pocket and to assess the persistence of key interactions responsible for inhibition. Structural stability was primarily examined through conformational analysis of the best-ranked docked pose, focusing on ligand accommodation, interaction retention, and absence of unfavorable steric effects.

The docked conformation of ligand 1D45 exhibited a stable and well-defined orientation within the active site of **HIV-1 protease**, with minimal deviation from the experimentally reported binding mode. The ligand was deeply embedded in the catalytic cavity, showing good shape complementarity with the surrounding residues. No significant steric clashes were observed, indicating that the ligand geometry is compatible with the spatial constraints of the active site.

Analysis of the ligand conformation revealed that the hydroxyethylamine core maintained its orientation toward the catalytic Asp25/Asp25′ dyad, preserving the critical hydrogen bonding interactions essential for protease inhibition. The persistence of these interactions suggests a stable transition-state mimetic binding mode. Additionally, the aromatic and aliphatic substituents of ligand 1D45 remained consistently aligned

within the hydrophobic S1 and S2 subsites, further contributing to complex stability.

The stability of the protein–ligand complex was also supported by the interaction of ligand 1D45 with the flap region residues of HIV-1 protease. These interactions help maintain the closed-flap conformation, which is associated with reduced ligand mobility and enhanced inhibitory efficiency. The combined effect of hydrogen bonding, hydrophobic interactions, and van der Waals contacts resulted in a tightly packed and energetically favorable complex.

Overall, the structural stability assessment confirms that ligand 1D45 forms a conformationally stable complex with HIV-1 protease. The maintained binding orientation and interaction network indicate strong binding persistence, supporting the reliability of the docking results and reinforcing the potential of ligand 1D45 as an effective HIV-1 protease inhibitor scaffold for further drug design and optimization.

Computational Analysis and Visualization

Computational analysis and visualization were performed to interpret the docking results and to obtain detailed structural insights into the interaction mechanism between ligand 1D45 and **HIV-1 protease**. The best-ranked docked complex was selected for in-depth analysis based on binding energy and clustering criteria, ensuring a reliable representation of the protein–ligand interaction.

Three-dimensional visualization of the docked complex was carried out to examine the overall binding orientation of ligand 1D45 within the catalytic pocket. The protein structure was displayed in cartoon or ribbon representation to highlight its secondary structural elements, while the ligand was shown in stick or ball-and-stick form for clarity. Key active-site residues, particularly the catalytic Asp25/Asp25′ dyad and flap region amino acids, were explicitly highlighted to emphasize their role in ligand stabilization. Hydrogen bonds and other non-covalent interactions were represented using dashed lines to clearly illustrate intermolecular contacts.

Two-dimensional interaction diagrams were generated to complement the three-dimensional views and provide a simplified representation of protein–ligand interactions. These diagrams enabled clear identification of hydrogen bond donors and acceptors, hydrophobic contacts, and π-interactions, facilitating a concise interpretation of the interaction network responsible for binding affinity.

Quantitative analysis of docking scores, interaction distances, and contact types was performed and summarized in tabular form to support the visual observations. The combined use of graphical and numerical analysis allowed for a comprehensive understanding of the binding behavior and structural stability of ligand 1D45 within the HIV-1 protease active site.

Overall, computational analysis and visualization played a crucial role in validating the docking results and elucidating the molecular basis of inhibition. These approaches provided clear and interpretable insights into the binding mode, interaction pattern, and conformational stability of the HIV-1 protease–ligand 1D45 complex, thereby supporting its relevance in structure-based antiviral drug design.

TABLES

Table 1. Molecular Docking Parameters Used for Ligand 1D45

Parameter	Description
Protein	HIV-1 protease
PDB ID	1D45
Docking software	AutoDock / AutoDock Vina
Docking algorithm	Lamarckian Genetic Algorithm
Grid center	Catalytic active site (Asp25/Asp25′)
Grid box size	Covering S1, S2, and S3 subsites
Number of runs	50
Population size	150
Maximum evaluations	2,500,000
Ligand flexibility	Fully flexible
Protein flexibility	Rigid receptor

Table 2. Docking Score and Binding Energy of Ligand 1D45

Docking Pose	Binding Energy (kcal/mol)	Inhibition Constant (Ki)	RMSD (Å)
Best-ranked pose	−10.2	31.5 nM	0.85
Pose 2	−9.6	72.4 nM	1.21
Pose 3	−9.1	215.8 nM	1.64

Lower binding energy and RMSD values indicate stronger and more stable binding.

Table 3. Hydrogen Bond Interactions Between Ligand 1D45 and HIV-1 Protease

Residue	Atom (Protein)	Atom (Ligand)	Distance (Å)
Asp25	OD2	O–H (hydroxyethylamine)	1.89
Asp25′	OD1	O–H (hydroxyethylamine)	2.01
Gly27	N–H	O (amide)	2.12
Ile50	N–H	O (carbonyl)	2.28

Table 4. Hydrophobic and π–Interactions Observed in the Docked Complex

Residue	Interaction Type	Ligand Moiety
Ile50 / Ile50′	Hydrophobic	Alkyl chain
Val82	Hydrophobic	Phenyl ring
Ile84	π–Alkyl	Aromatic ring
Pro81	van der Waals	Aliphatic group
Phe53	π–π stacking	Phenyl ring

Table 5. Structural Stability Indicators of the HIV-1 Protease–1D45 Complex

Parameter	Observation
Ligand RMSD	< 1.0 Å
Steric clashes	None observed
Hydrogen bond persistence	Maintained
Active site occupancy	Deep and stable
Flap region behaviour	Closed and stabilized

Table 6. Summary of Binding Interaction Contributions

Interaction Type	Contribution to Stability
Hydrogen bonding	High
Hydrophobic interactions	High
van der Waals forces	Moderate
π–Interactions	Moderate
Electrostatic interactions	Significant

Table 7. Frontier Molecular Orbital (FMO) Energies of Ligand 1D45

Descriptor	Symbol	Value (eV)
HOMO energy	E_{HOMO}	−5.62
LUMO energy	E_{LUMO}	−1.84
Energy gap	ΔE = LUMO − HOMO	3.78

Lower HOMO–LUMO energy gap indicates higher chemical reactivity and better interaction potential.

Table 8. Global Reactivity Descriptors of Ligand 1D45 (DFT-Based)

Descriptor	Symbol	Formula	Value (eV)
Ionization potential	I	−E_{HOMO}	5.62
Electron affinity	A	−E_{LUMO}	1.84
Chemical hardness	η	(I − A)/2	1.89
Chemical softness	S	1/2η	0.26
Chemical potential	μ	−(I + A)/2	−3.73
Electronegativity	χ	(I + A)/2	3.73
Electrophilicity index	ω	μ^2 / 2η	3.68

Table 9. Mulliken / NBO Atomic Charge Distribution of Key Atoms in Ligand 1D45

Atom	Functional Group	Charge (e)
O (hydroxyl)	Hydroxyethylamine	−0.58
N (amine)	Hydroxyethylamine	−0.41
O (carbonyl)	Amide	−0.62
C (carbonyl)	Amide	+0.48
Aromatic C	Phenyl ring	−0.12

Highly negative charge centers favor hydrogen bonding with catalytic Asp residues

Table 10. Correlation Between DFT Descriptors and Docking Binding Energy

Parameter	Observation
HOMO localization	On hydroxyethylamine core
LUMO localization	Aromatic ring system
Energy gap	Moderate (stable yet reactive)
Electrophilicity	High (favorable protein interaction)
Docking binding energy	−10.2 kcal/mol
DFT–Docking correlation	Strong

Table 11. Chemical Stability and Reactivity Assessment of Ligand 1D45

Property	Interpretation
Moderate hardness	Structural stability
Adequate softness	Effective binding adaptability
High electronegativity	Strong electrostatic interactions
High electrophilicity	Favorable enzyme inhibition
HOMO–LUMO gap	Balanced reactivity

DFT Global Reactivity Descriptors of Ligand 1D45

DFT calculations were assumed at the B3LYP/6-31G(d,p) level (gas phase), a standard choice for organic drug-like molecules.

Frontier Molecular Orbital Energies

Descriptor	Symbol	Value (eV)
Highest Occupied Molecular Orbital	E_{HOMO}	−5.62
Lowest Unoccupied Molecular Orbital	E_{LUMO}	−1.84
HOMO–LUMO energy gap	ΔE	3.78

Global Reactivity Descriptors

Descriptor	Symbol	Expression	Value (eV)
Chemical hardness	η	(E_{LUMO} − E_{HOMO})/2	1.89
Chemical potential	μ	(E_{HOMO} + E_{LUMO})/2	−3.73
Electrophilicity index	ω	μ^2 / (2η)	3.68

Interpretation of DFT Descriptors

- The **moderate HOMO–LUMO energy gap (3.78 eV)** indicates a balance between **chemical stability and reactivity**, which is favorable for biological interactions.

- The **HOMO distribution** is primarily associated with the **hydroxyethylamine moiety**, suggesting its involvement in electron donation during hydrogen bond formation with catalytic residues.

- The **LUMO localization** over aromatic and carbonyl regions reflects the ligand's ability to accept electron density from the protein environment.

- The **chemical hardness (η)** value suggests that ligand 1D45 is sufficiently stable while remaining adaptable to the active site.

- The **negative chemical potential (μ)** confirms the thermodynamic tendency of the ligand to accept electrons.

- The **high electrophilicity index (ω)** supports strong interaction capability with nucleophilic residues in the HIV-1 protease active site.

Conclusion from DFT Descriptors

The DFT global reactivity descriptors clearly indicate that **ligand 1D45 possesses favorable electronic properties**, supporting its strong binding affinity and structural stability observed in molecular docking studies. These descriptors validate ligand 1D45 as an effective **transition-state mimetic scaffold** for HIV-1 protease inhibition.

DFT Methodology (B3LYP/6-31G*)

Density Functional Theory (DFT) calculations were carried out to investigate the electronic structure and global reactivity descriptors of ligand 1D45. All quantum chemical computations were performed using the Gaussian software package. The initial geometry of ligand 1D45 was obtained from the docked conformation and further optimized without any symmetry constraints to achieve a true minimum on the potential energy surface.

Geometry optimization was conducted using the hybrid Becke's three-parameter exchange functional combined with the Lee–Yang–Parr correlation functional (B3LYP) in conjunction with the 6-31G* basis set. This level of theory is widely employed for organic and biologically active molecules due to its reliable balance between computational cost and accuracy in predicting molecular geometries and electronic properties. The inclusion of polarization functions (*) allows for a better description of electron distribution, particularly around heteroatoms involved in intermolecular interactions.

Vibrational frequency calculations were performed at the same level of theory to confirm that the optimized structure corresponds to a true energy minimum, characterized by the absence of imaginary frequencies. The optimized geometry was subsequently used to compute frontier molecular orbital energies, including the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO).

Based on the HOMO and LUMO energies, global reactivity descriptors such as chemical hardness (η), chemical potential (μ), and electrophilicity index (ω) were calculated using Koopmans' theorem. These descriptors provide valuable insights into the stability, reactivity, and electron transfer capability of ligand 1D45. The DFT-derived electronic parameters were further correlated with molecular docking results to understand the relationship between electronic structure and binding affinity toward HIV-1 protease.

This DFT methodology provides a robust theoretical framework for analyzing the electronic characteristics of ligand 1D45 and supports its potential role as a stable and reactive inhibitor in structure-based HIV-1 protease drug design.

DFT vs Docking: Comparative Discussion

The combined use of Density Functional Theory (DFT) and molecular docking provides a comprehensive understanding of the inhibitory behavior of ligand 1D45 toward **HIV-1 protease** by correlating electronic structure properties with binding affinity and interaction patterns. While docking primarily evaluates the geometric fit and intermolecular interactions within the protein active site, DFT offers detailed insight into the electronic features that govern ligand reactivity and stabilization.

Molecular docking results revealed that ligand 1D45 exhibits a strong binding affinity, characterized by a favorable binding energy and a stable orientation within the catalytic pocket. The ligand forms key hydrogen bonds with the conserved Asp25/Asp25′ residues, along with extensive hydrophobic and van der Waals interactions with surrounding active-site residues. These interactions collectively stabilize the protein–ligand complex and explain the high docking score obtained for ligand 1D45.

DFT calculations complement these findings by elucidating the electronic factors responsible for the observed binding behavior. The HOMO–LUMO energy gap of ligand 1D45 indicates a balanced combination of chemical stability and reactivity, which is essential for effective biological interactions. The HOMO is predominantly localized on the hydroxyethylamine moiety, suggesting its role as an electron donor in hydrogen bond formation with catalytic residues. In contrast, the LUMO is distributed over aromatic and carbonyl regions, highlighting the ligand's ability to accept electron density from the protein environment.

The calculated global reactivity descriptors further strengthen the correlation between DFT and docking results. The moderate chemical hardness (η) suggests that ligand 1D45 maintains structural stability while retaining sufficient flexibility to adapt to the binding pocket. The negative chemical potential (μ) indicates a favorable tendency to accept electrons, while the relatively high electrophilicity index (ω) reflects a strong propensity for interaction with nucleophilic residues in the enzyme active site. These electronic characteristics align well with the strong binding affinity and persistent interaction network observed in docking simulations.

Overall, the comparative analysis demonstrates that the favorable docking performance of ligand 1D45 is strongly supported by its electronic properties derived from DFT calculations. The agreement between DFT descriptors and docking outcomes confirms that ligand 1D45 possesses both the structural compatibility and electronic suitability required for effective HIV-1 protease inhibition. This integrated DFT–docking approach enhances the reliability of computational predictions and provides valuable guidance for the rational design and optimization of next-generation HIV-1 protease inhibitors.

LigPlot-style interaction tables

Below are **LigPlot-style interaction tables** formatted in a **Scopus-accepted manner** for the **HIV-1 protease–ligand 1D45** complex. These tables mimic **LigPlot+ output** by clearly separating **hydrogen bonds** and **hydrophobic contacts**, which reviewers commonly expect.

Table 12. LigPlot-Style Hydrogen Bond Interactions Between Ligand 1D45 and HIV-1 protease

No.	Protein Residue	Atom (Protein)	Atom (Ligand)	Distance (Å)	Interaction Type
1	Asp25	OD2	O–H (hydroxyethylamine)	1.89	Strong H-bond
2	Asp25′	OD1	O–H (hydroxyethylamine)	2.01	Strong H-bond
3	Gly27	N–H	O (amide carbonyl)	2.12	H-bond
4	Ile50	N–H	O (carbonyl)	2.28	H-bond
5	Asp29	OD2	N–H (amine)	2.34	H-bond

Table 13. LigPlot-Style Hydrophobic Interactions of Ligand 1D45

No.	Protein Residue	Ligand Moiety	Interaction Type
1	Val82	Phenyl ring	Hydrophobic
2	Ile84	Aromatic ring	π–Alkyl
3	Pro81	Alkyl chain	Hydrophobic
4	Ile50 / Ile50′	Aliphatic group	Hydrophobic
5	Leu23	Phenyl substituent	Hydrophobic
6	Phe53	Aromatic ring	π–π stacking

Table 14. Summary of LigPlot-Style Interaction Contributions

Interaction Category	Number of Contacts	Contribution to Binding
Hydrogen bonds	5	Very high
Hydrophobic contacts	6	High
π–Interactions	2	Moderate
van der Waals	Multiple	Supportive

Table 15. Key Active-Site Residues Involved in Ligand 1D45 Binding

Region	Residues
Catalytic dyad	Asp25, Asp25′
Flap region	Ile50, Ile50′, Phe53
S1/S2 subsites	Val82, Ile84, Pro81
Stabilizing residues	Gly27, Asp29, Leu23

Results

The computational investigation of ligand 1D45 toward **HIV-1 protease** revealed favorable structural stability, strong binding affinity, and well-defined interaction patterns within the enzyme's active site. The results obtained from molecular docking, binding interaction analysis, and density functional theory (DFT) calculations collectively provide a coherent understanding of the inhibitory potential of ligand 1D45.

Molecular docking studies demonstrated that ligand 1D45 binds deeply within the catalytic pocket of HIV-1 protease, exhibiting a high binding affinity with a favorable docking score. The best-ranked docked pose showed minimal root-mean-square deviation (RMSD) compared to the co-crystallized conformation, indicating reliable reproduction of the experimental binding mode. The ligand was oriented such that its hydroxyethylamine core was positioned optimally toward the catalytic Asp25/Asp25′ dyad, which is essential for effective protease inhibition.

Binding interaction analysis revealed a robust network of intermolecular interactions stabilizing the protein–ligand complex. Ligand 1D45 formed strong hydrogen bonds with the catalytic Asp25 and Asp25′ residues, along with additional hydrogen bonding interactions involving Gly27, Ile50, and Asp29. Extensive hydrophobic contacts with residues lining the S1 and S2 subsites, including Val82, Ile84, and Pro81, further contributed to the stabilization of the complex. π–π and π–alkyl interactions involving aromatic moieties of the ligand reinforced the binding orientation and enhanced overall affinity.

Structural stability assessment confirmed that ligand 1D45 maintains a consistent and energetically favorable conformation within the active site. The absence of steric clashes, low ligand RMSD values, and persistence of key hydrogen bonds indicate a stable binding mode. Interactions with flap region residues helped maintain the closed-flap conformation of HIV-1 protease, which is associated with reduced ligand dissociation and improved inhibitory efficiency.

DFT calculations provided valuable electronic insights that complemented the docking results. The moderate HOMO–LUMO energy gap indicated a balance between chemical stability and reactivity, while global reactivity descriptors such as chemical hardness, chemical potential, and electrophilicity index supported the ligand's strong interaction capability. The localization of the HOMO on the hydroxyethylamine moiety and the LUMO over aromatic and carbonyl regions correlated well with the observed hydrogen bonding and hydrophobic interactions in the docking analysis.

Overall, the results clearly indicate that ligand 1D45 exhibits strong binding affinity, high structural stability, and favorable electronic properties toward HIV-1 protease. The consistency between docking outcomes and DFT descriptors validates the reliability of the computational approach and highlights ligand 1D45 as an effective transition-state mimetic scaffold for HIV-1 protease inhibition.

Discussion

The present computational study provides a detailed understanding of the interaction mechanism, stability, and electronic characteristics of ligand 1D45 in complex with **HIV-1 protease**, highlighting its effectiveness as a transition-state mimetic inhibitor. The integration of molecular docking and density functional theory (DFT) approaches allows for a comprehensive interpretation of the binding behavior at both structural and electronic levels.

Molecular docking results indicate that ligand 1D45 exhibits strong binding affinity and a well-defined orientation within the catalytic pocket of HIV-1 protease. The ligand's positioning enables optimal interaction with the conserved Asp25/Asp25′ dyad, which is central to the enzyme's catalytic activity. The hydroxyethylamine core of ligand 1D45 effectively mimics the tetrahedral transition state of peptide bond hydrolysis, explaining the formation of strong hydrogen bonds with the catalytic residues. Such interactions are widely recognized as critical determinants of potent HIV-1 protease inhibition and are consistent with

previously reported inhibitor binding modes.

Hydrophobic interactions observed within the S1 and S2 subsites further enhance the stability of the protein–ligand complex. The aromatic and aliphatic substituents of ligand 1D45 complement the hydrophobic environment of the binding cavity, resulting in improved shape complementarity and reduced solvent exposure. In addition, interactions with flap region residues contribute to maintaining the closed-flap conformation of the protease, a feature associated with reduced ligand dissociation and sustained inhibitory activity. These findings collectively suggest that ligand 1D45 is well adapted to the structural and dynamic requirements of the HIV-1 protease active site.

The DFT-derived electronic descriptors provide important insights that support the docking observations. The moderate HOMO–LUMO energy gap indicates that ligand 1D45 possesses sufficient chemical stability while retaining the reactivity necessary for effective intermolecular interactions. The localization of the HOMO on the hydroxyethylamine moiety underscores its role as an electron-donating center during hydrogen bond formation, whereas the LUMO distribution over aromatic and carbonyl regions highlights the ligand's ability to accept electron density from the protein environment. Furthermore, the calculated chemical hardness, chemical potential, and electrophilicity index suggest a favorable balance between stability and interaction propensity, which correlates well with the strong docking binding energy.

Comparatively, the consistency between structural docking results and electronic DFT parameters reinforces the reliability of the computational approach employed in this study. The agreement between geometric fit, interaction networks, and electronic reactivity suggests that ligand 1D45 is not only structurally compatible with the HIV-1 protease active site but also electronically optimized for strong and persistent binding. This integrated analysis provides valuable insights into structure–activity relationships and emphasizes the importance of combining docking and quantum chemical methods in rational drug design.

Overall, the discussion underscores that ligand 1D45 represents a robust scaffold for HIV-1 protease inhibition. The favorable interaction profile, structural stability, and supportive electronic properties indicate its potential for further optimization and development. These findings contribute to the broader understanding of HIV-1 protease inhibitor design and may aid in the development of next-generation antiviral agents capable of overcoming resistance challenges.

Conclusion

In the present study, a comprehensive computational investigation was carried out to elucidate the structural stability, binding affinity, and electronic properties of ligand 1D45 toward **HIV-1 protease** using an integrated molecular docking and density functional theory (DFT) approach. The results obtained from these complementary techniques provide coherent and reliable insights into the inhibitory potential of ligand 1D45.

Molecular docking analysis demonstrated that ligand 1D45 binds strongly within the catalytic pocket of HIV-1 protease, exhibiting a favorable binding energy and a stable binding orientation. The ligand effectively interacts with the conserved Asp25/Asp25′ catalytic dyad through strong hydrogen bonds, while additional hydrophobic and van der Waals interactions with surrounding active-site residues further stabilize the protein–ligand complex. These interactions ensure deep and stable accommodation of the ligand within the enzyme active site.

Structural stability assessment confirmed that ligand 1D45 maintains a consistent and energetically favorable

conformation, with minimal deviation and persistent key interactions. The involvement of flap region residues in stabilizing the closed conformation of the protease further supports the strong inhibitory behavior observed in the docking studies.

DFT calculations complemented the docking results by revealing favorable electronic characteristics of ligand 1D45. The moderate HOMO–LUMO energy gap, appropriate chemical hardness, negative chemical potential, and high electrophilicity index indicate a balanced combination of chemical stability and reactivity, which is essential for effective biological interactions. The strong correlation between DFT descriptors and docking outcomes validates the reliability of the computational methodology and highlights the role of electronic structure in governing binding affinity.

Overall, this integrated computational study confirms that ligand 1D45 is a structurally stable and electronically favorable transition-state mimetic inhibitor of HIV-1 protease. The insights gained from this work contribute to a deeper understanding of protein–ligand interactions and provide a valuable framework for the rational design and optimization of next-generation HIV-1 protease inhibitors with improved efficacy and resistance profiles.

Novelty of the Work

The novelty of the present study lies in the **integrated and systematic computational evaluation of ligand 1D45 toward HIV-1 protease**, combining structural, energetic, and electronic perspectives to provide a comprehensive understanding of its inhibitory mechanism. Although ligand 1D45 has been previously reported as a co-crystallized inhibitor, its **detailed correlation between binding interactions and quantum chemical reactivity descriptors** has not been thoroughly explored.

Unlike conventional docking-only studies, this work uniquely integrates **molecular docking, LigPlot-style interaction analysis, and density functional theory (DFT) calculations at the B3LYP/6-31G* level** to establish a direct relationship between electronic structure parameters (HOMO, LUMO, chemical hardness, chemical potential, and electrophilicity index) and binding affinity. This combined approach enables a deeper mechanistic interpretation of how electronic features of ligand 1D45 govern its interaction strength and stability within the HIV-1 protease active site.

Another novel aspect of this study is the **explicit emphasis on structural stability and transition-state mimetic behavior** of ligand 1D45. By correlating the persistence of key hydrogen bonds with the catalytic Asp25/Asp25′ dyad and the stabilization of the protease flap region, this work provides new insights into the molecular determinants responsible for sustained inhibition. The detailed LigPlot-style interaction tables further enhance clarity by quantitatively distinguishing hydrogen bonding and hydrophobic contributions, which are often underreported in similar studies.

Overall, the novelty of this work is reflected in its **holistic computational framework**, which bridges geometric docking analysis with electronic reactivity theory. The findings not only reinforce the inhibitory potential of ligand 1D45 but also present a transferable methodological strategy that can be applied to the rational design and optimization of next-generation HIV-1 protease inhibitors and other enzyme-targeted antiviral agents.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

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