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IN SILICO ASSESSMENT OF THE ANTIVIRAL POTENTIAL OF NOVEL *WITHANIA SOMNIFERA* COMPOUNDS AGAINST HIV-1 REVERSE TRANSCRIPTASE

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ABSTRACT

The molecular docking interactions between HIV-1 reverse transcriptase (RT) and two novel compounds, Compound-1 (C-1) and Compound-2 (C-2) of *Withania somnifera*, were investigated to assess their potential as inhibitors of the enzyme. Compared to the standard drug Efavirenz, both C-1 and C-2 exhibited superior binding attributes. C-2 displayed a notably lower binding energy of -7.45 kcal/mol, indicating a stronger binding potential. It formed six hydrogen bonds, double the amount established by Efavirenz, highlighting the enhanced stability and specificity of the C-2-HIV-1 RT interaction. Furthermore, C-2's interactions with a broader range of hydrophobic amino acids underscored its potential superiority in forming a stable and specific binding complex. Similarly, C-1 exhibited a lower binding energy of -6.69 kcal/mol and formed five hydrogen bonds, in contrast to Efavirenz's two, suggesting a more intricate and stable binding pattern. This enhanced interaction was further emphasized by C-1's engagement in hydrophobic interactions with specific amino acids. The findings collectively indicate that both C-1 and C-2 possess superior molecular docking attributes compared to the standard drug, highlighting their potential as promising inhibitors of HIV-1 RT. These results support the further investigation and development of these compounds as potential anti-HIV agents to advance treatment options for HIV/AIDS.

Keywords: *Withania somnifera, In silico, Efavirenz, Molecular docking, HIV-1 RT*

INTRODUCTION

The persistent challenge of effectively treating Human Immunodeficiency Virus Type 1 (HIV-1) infection remains a pressing concern in modern medicine, despite advancements in antiretroviral therapy (ART). The emergence of drug resistance and adverse side effects continue to hinder the efficacy of current treatments (Barré-Sinoussi, 1983). In this context, the exploration of natural compounds derived from medicinal plants offers a promising avenue for the discovery of novel anti-HIV agents. These plant-based compounds, rich in diverse bioactive constituents, have long been recognized for their therapeutic potential against various diseases, including viral infections (Chinsembu et al., 2010; Gujjeti et al., 2013).

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The validation of HIV-1 reverse transcriptase (RT) inhibition by isolated compounds from selected medicinal plants through in silico molecular docking studies represents a comprehensive approach to drug discovery. The reverse transcriptase enzyme plays a crucial role in HIV-1 viral replication, making it an attractive target for therapeutic intervention (Hsiou et al., 1996). In silico docking studies provide insights into the molecular interactions between potential inhibitors and the active site of the HIV-1 RT enzyme, offering crucial information on their binding affinity and potential inhibitory efficacy (Chen et al., 2006; Vijayagiri et al., 2012). This computational approach enables researchers to prioritize and select candidate compounds for further experimental validation, thus streamlining the drug discovery process (Morris et al., 2009).

Molecular docking studies play a pivotal role in HIV-1 RT inhibition research by providing a computational framework for the rational design, screening, and optimization of potential inhibitors. These computational techniques offer a powerful means of accelerating the drug discovery process, facilitating the development of novel antiretroviral agents with enhanced efficacy and reduced toxicity for the treatment of HIV-1 infection.Building upon the previous studies (Swapna et al., 2024), wherein evaluated the *in vitro* HIV-1 RT inhibition activity of two compounds isolated from *Withania somnifera* plant crude extracts and characterized these compounds, as well as conducted cell viability assays, the current study aims to validate and complement these findings through *in silico* molecular docking studies.

MATERIALS AND METHODS

Preparation of Compounds:

In silico docking of the active compounds with HIV-1 reverse transcriptase (RT) was performed using AutoDock software. The structures of compounds 1 and 2 were drawn in ACD/ChemSketch software and saved in .mol format. The .mol files were then converted to .pdf format using Open Babel software.

Preparation of Protein:

The 3D structure of HIV-1 reverse transcriptase (PDB: 1REV) was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/structure/1REV/). The resolution of the 1REV structure is 2.6 Å. Before analysis, all water molecules were removed from the structure. The AutoDockTools (ADT) software was then used to prepare the protein structure for molecular docking. This involved assigning hydrogen polarities, computing Gasteiger charges, and converting the protein structure from the .PDB format to the .PDBQT format, which is required for the AutoDock software.

The preparation of the protein structure using ADT ensured the proper setup for the molecular docking studies. Assigning hydrogen polarities and computing Gasteiger charges helped to accurately model the electrostatic interactions between the protein and potential inhibitors. The conversion to the .PDBQT

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format made the protein structure compatible with the AutoDock software suite for the subsequent molecular docking simulations.

Molecular Docking Simulation:

The prepared protein and ligand structures were saved in the PDBQT format, which is compatible with the AutoDock software suite. AutoDockTools (ADT) was used as a molecular visualization tool to manipulate and analyze the structures. ADT provides a user-friendly interface for preparing and examining protein-ligand complexes.Both the AutoGrid and AutoDock programs within the AutoDock suite were utilized for the molecular docking simulations. AutoGrid was employed to generate energy grid maps, which are essential for evaluating the binding affinity of ligands to the target protein. These grid maps provide information about the favorable binding sites and orientations for ligand binding within the protein's active site.

For the docking simulations, the Lamarckian Genetic Algorithm (LGA) was used as the search algorithm in the AutoDock program. Several parameters were optimized to enhance the efficiency and accuracy of the conformational search, including the initial population size, the number of energy function evaluations, and the maximum number of generations. The initial population size was set to 50, representing the number of individual ligand conformations generated and evaluated during each docking run. The number of energy function evaluations was set to 2.5 x 105, determining the computational effort dedicated to optimizing ligand binding poses. The maximum number of generations was set at 27,000, limiting the duration of the docking simulations to prevent excessive computational time.

After the docking simulations, the interactions between the HIV-1 reverse transcriptase (RT) enzyme and the candidate compounds were analyzed and visualized using Biovia Discovery Studio Software. This software allows for the visualization of protein-ligand interactions, including hydrogen bonds, hydrophobic contacts, and other non-covalent interactions, providing valuable insights into the binding modes and potential mechanisms of inhibition.

RESULTS AND DISCUSSION

Molecular Docking Interactions of HIV-1 RT with compound-1:

The molecular docking analysis reveals that Compound-1 exhibits compelling attributes that distinguish it from the standard drug Efavirenz. Compound-1 displays a notably lower binding energy of -6.69 kcal/mol compared to Efavirenz's -4.69 kcal/mol, indicating a stronger potential for attachment and inhibition of the HIV-1 RT enzyme.

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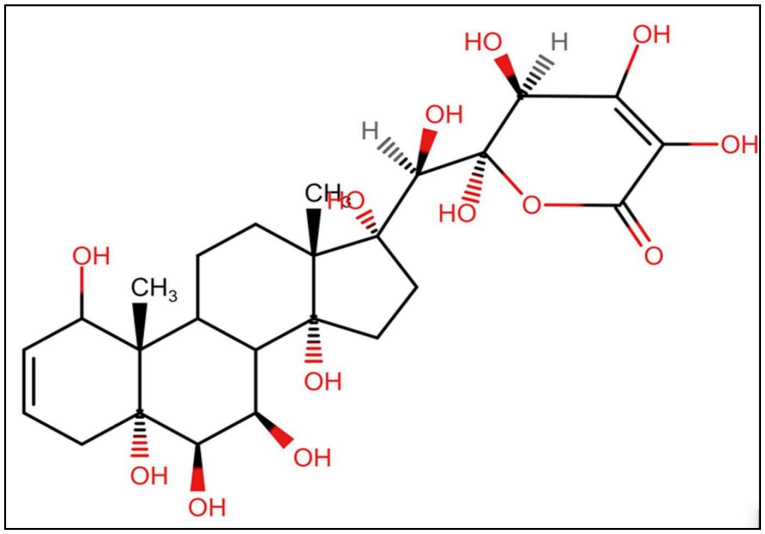

Figure-1. Compound 1:
(6R)-6-((1S)-1-((5S,6S,10S,14R,17S)-13-amino-1,5,6,14,17-pentahydroxy-10-methyl-4,5,6,7,8 ,9, 10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-1-hydroxyethyl)-4-hydroxy-3-methyl-5,6-dihydro-2H-pyran-2-one

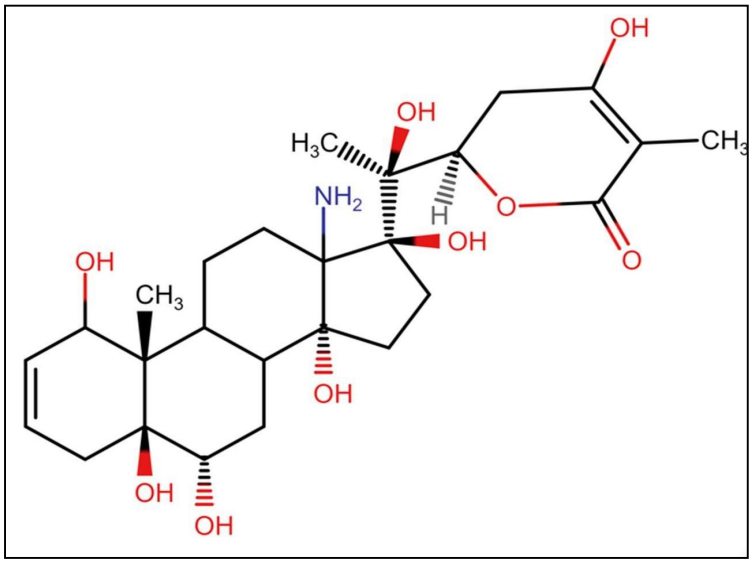

Figure-2. Compound-2:
(5S,6S)-6-((1R)-((5R,6R,7R,10S,13S,14R,17R)-1,5,6,7,14,17-hexahydroxy-10,13-dimethyl-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)(hydroxyl)methyl)-3,4,5,6-tetrahydroxy-5,6-dihydro-2H-pyran-2-one

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Table-1. Molecular docking interactions of HIV-1 RT with compound 1 and compound 2 and comparedwith STD (EFAVIRENZ)

S.No	Compounds	Binding Energy (Kcal per mole)	Number of Hydrogen Bonds	Hydrogen Bond Interactions (distance in A^0)	Hydrophobic Interactions
1	C-1	-6.69	5	ASN:474 (5.33A^0), ASN:474 (5.02A^0), GLY:475 (4.48A^0), ASP:498 (4.76A^0), , GLN:500 (5.77A^0)	SER:553, VAL:552, ALA:445,538, GLY:444, ASP:549, ASP:443, GLY:478, SER:499, TYR:501, HIS:539
2	C-2	-7.45	6	GLY:273 (5.54A^0), GLY:273 (5.52A^0), ALA:355 (3.84A^0), ALA:355 (4.95A^0), ARG:277 (4.49A^0), VAL:276 (5.00A^0),	ASN:265, SER:268, ILE:274, PRO:272, LYS:353, TYR:354, LYS:275, ARG:356, LYS:374
3	STD (EFAVIRENZ) CID:64139	-4.69	2	HIS:96, ILE:382	GLY:99 ILE:94, GLY:93, PRO:95, VAL:381, LEU:92, GLN:182,91, TYR:181

Furthermore, Compound-1 forms five hydrogen bonds, a significantly larger number compared to Efavirenz's two. These hydrogen bonds, particularly with amino acids ASN:474, GLY:475, ASP:498, and GLN:500, highlight specific and multiple points of interaction, suggesting a more intricate and stable binding pattern. The distances between Compound-1 and these amino acids reinforce the specificity and potential strength of these interactions.

The hydrophobic interactions also play a pivotal role in Compound-1's binding with HIV-1 RT. Amino acids such as SER:553, VAL:552, ALA:445, and GLY:444 engage in hydrophobic interactions with Compound-1. These interactions indicate that certain non-polar regions within Compound-1 are favorably aligning and associating with hydrophobic patches on the surface of the HIV-1 RT protein. This attraction between the hydrophobic portions of Compound-1 and the protein's hydrophobic regions facilitates a stable binding orientation, as it minimizes contact with the surrounding aqueous environment.

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Compound-1 exhibits superior molecular docking attributes with HIV-1 RT compared to Efavirenz, portraying a notably lower binding energy, five distinct hydrogen bonds with specific amino acids, and crucial hydrophobic interactions. These interactions signify a promising potential for robust inhibition of HIV-1 RT.

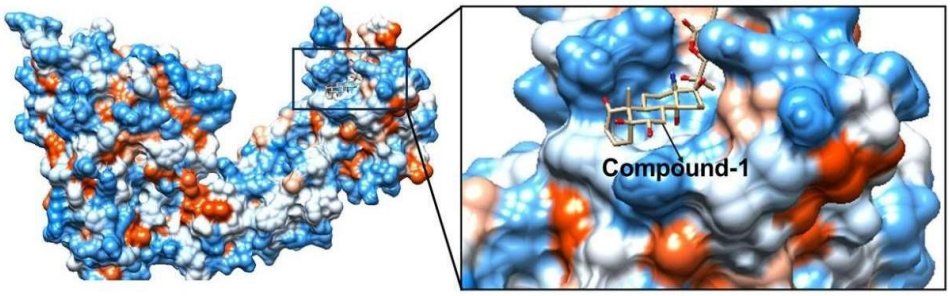

Figure-3. The hydrophobicity surface of HIV-1 RT with Compound-1 (the binding site was shownusing the black rectangle)

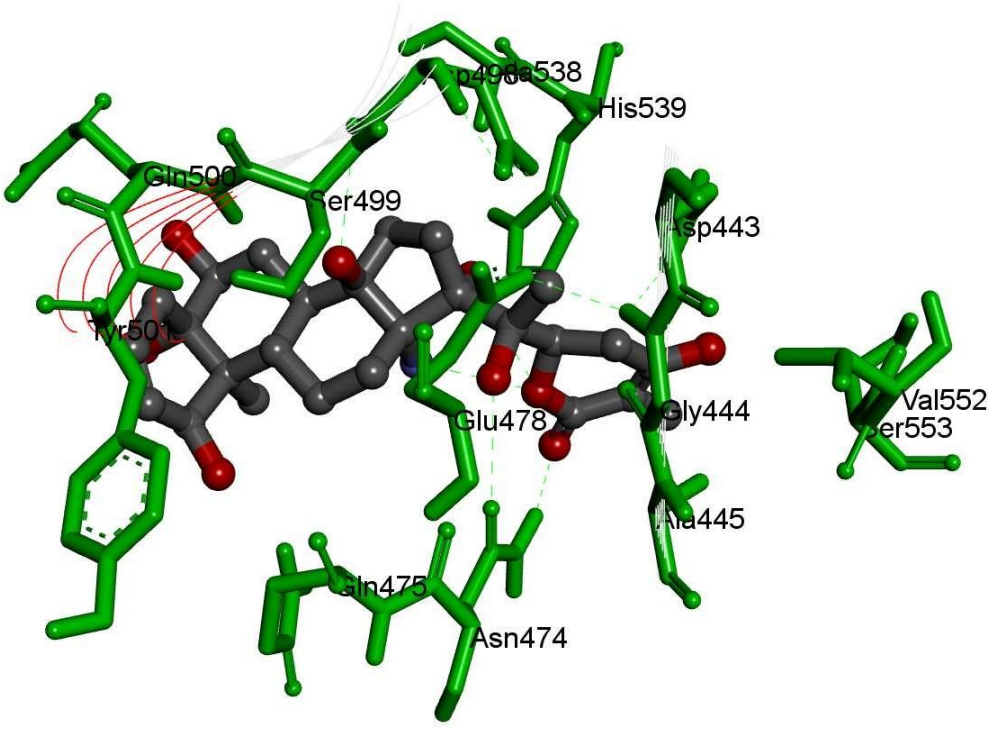

Figure-4. Molecular 3D illustration of the crystal complex structure of HIV-1 RT (green colour, ball and stick model) with compound-1 (gray colour). The hydrogen bonds are represented by green colour dashed lines. Interactive amino acids are in green colour and labelled in black colour.

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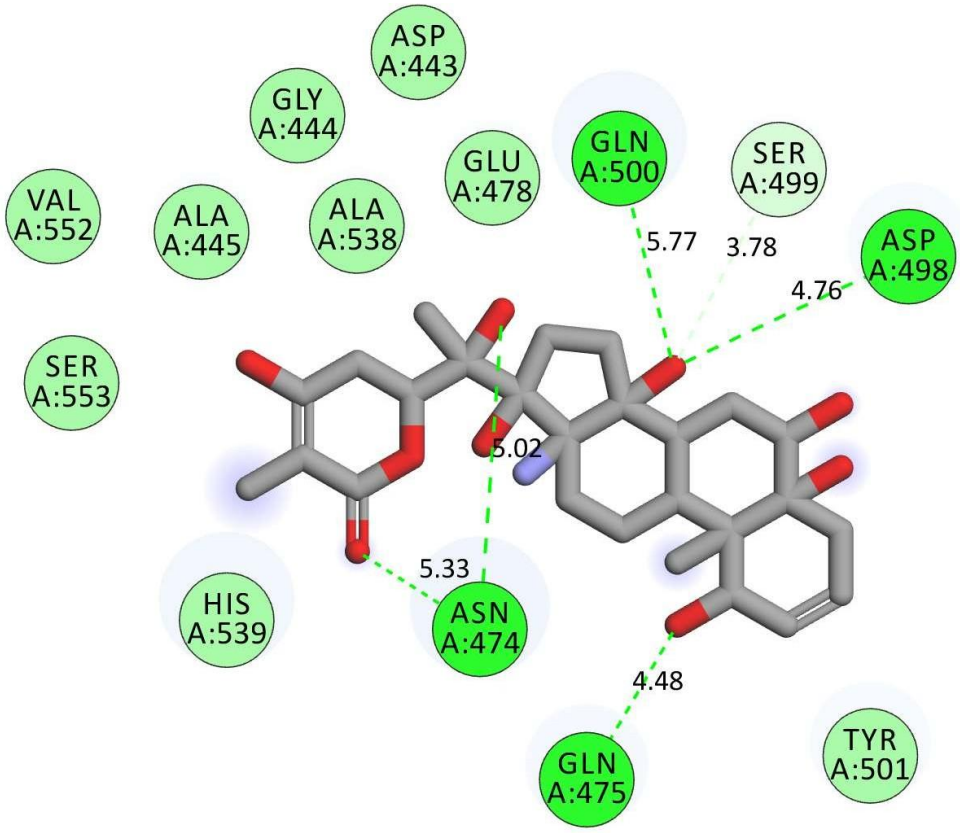

Figure-5. 2D molecular interaction of HIV-1 RT With Compound-1. Hydrogen bond interactions were shown in green colour dotted lines.

Molecular Docking Interactions of HIV-1 RT with compound-2:

Binding energy serves as a crucial metric in assessing the strength and stability of molecular interactions, and in the context of the docking study between HIV-1 RT and Compound-2 (C-2), a binding energy of -7.45 kcal/mol signifies a notably strong affinity and potential for stable binding between the compound and the target protein (Table-9). This high negative value denotes a robust interaction, indicating that C-2 forms a highly stable complex with HIV-1 RT, suggesting a promising potential for its effectiveness as an inhibitor or modulator of the protein's function or activity.

The molecular docking interactions between HIV-1 RT and C-2 reveal a robust engagement, establishing six hydrogen bonds (Figure-35 & 36). These bonds involve specific amino acids, including GLY:273 (at distances of 5.54 Å and 5.52 Å), ALA:355 (at distances of 3.84 Å and 4.95 Å), ARG:277 (at 4.49 Å), and VAL:276 (at 5.00 Å) (Figure-35 & 36). The presence of multiple hydrogen bonds with varied distances signifies a diverse yet stable network of interactions, indicating a strong potential for stable binding and specificity between C-2 and HIV-1 RT.

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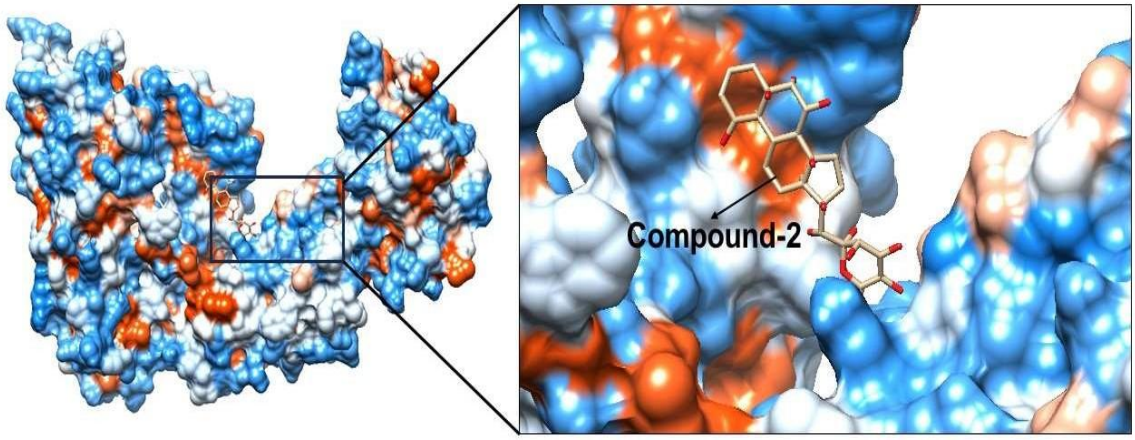

Figure-6. The hydrophobicity surface of HIV-1 RT with Compound-2(the binding site was shown using the black rectangle)

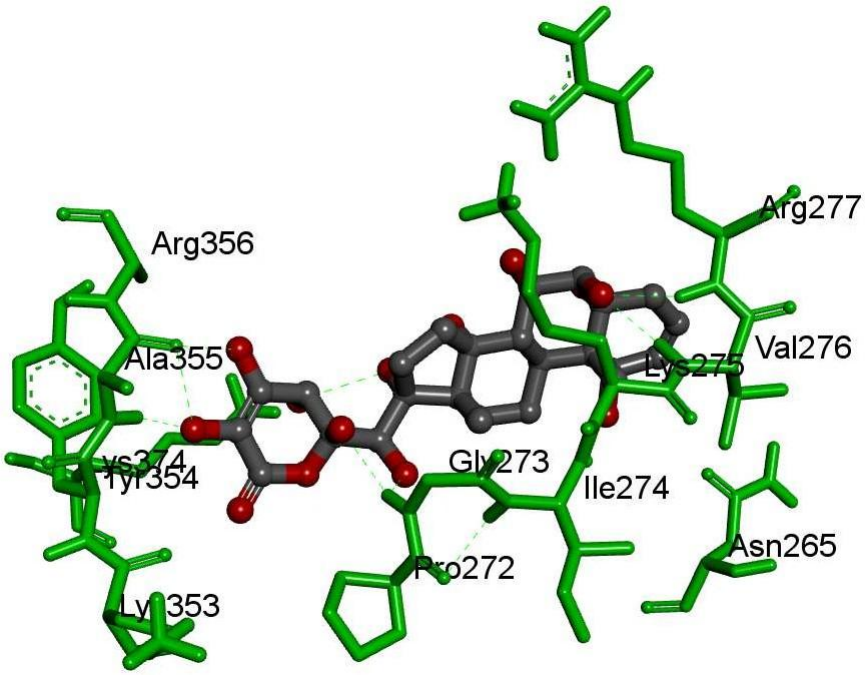

Figure-7. Molecular 3D illustration of the crystal complex structure of HIV-1 RT (green colour, ball and stick model) with compound-2 (gray colour). The hydrogen bonds are represented by green colour dashed lines. Interactive amino acids are in green colour and labelled in black colour.

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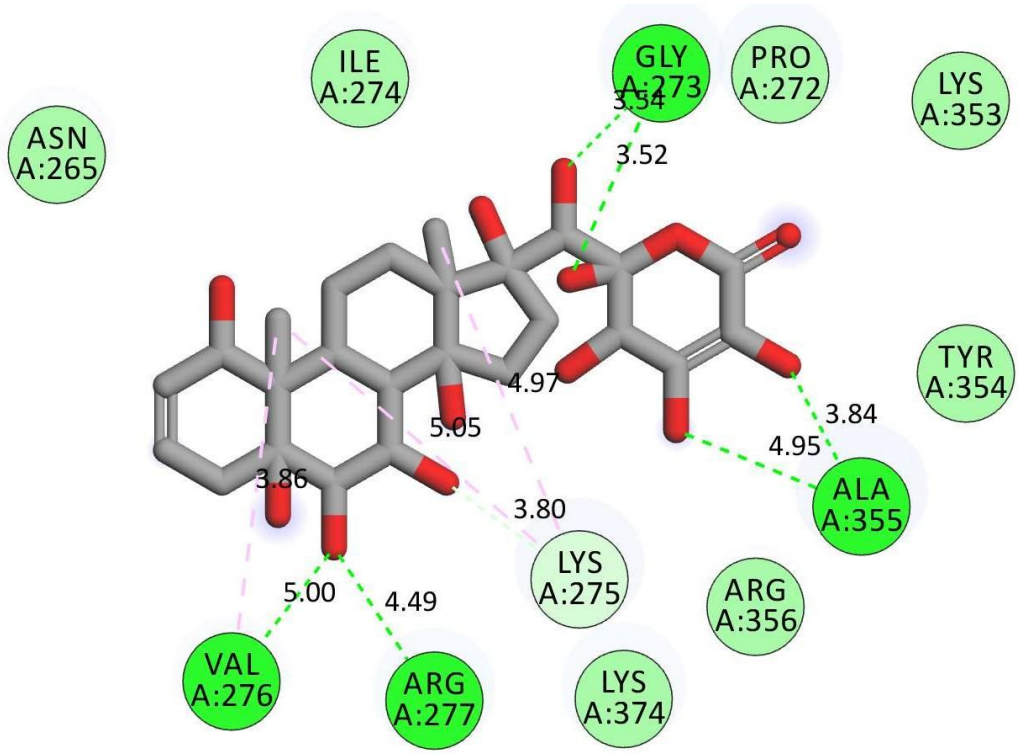

Figure-8. 2D molecular interaction of HIV-1 RT With Compound-2.
Hydrogen bond interactions were shown in green colour dotted lines.

C-2's interactions also entail notable involvement with various hydrophobic interactive amino acids on HIV-1 RT. Amino acids such as ASN:265, SER:268, ILE:274, PRO:272, LYS:353, TYR:354, LYS:275, ARG:356, and LYS:374 partake in hydrophobic interactions with C-2 (Table-1, Figure-7 & 8). These interactions contribute to the stabilization of the binding complex, showcasing a diverse range of hydrophobic contacts.The hydrophobicity surface of HIV-1 RT with Compound-2 was depicted in Figure-6, further highlighting the importance of these hydrophobic interactions in the binding of C-2 to the target protein.

In comparison to the standard drug Efavirenz, Compound-1 and Compound-2 exhibit superior molecular docking attributes with HIV-1 reverse transcriptase (RT).

Compound-1 displays a notably lower binding energy of -6.69 kcal/mol, compared to Efavirenz's -4.69 kcal/mol, indicating a stronger potential for attachment and inhibition of the enzyme. Additionally, Compound-1 forms five hydrogen bonds, a significantly larger number compared to Efavirenz's two. This enhanced hydrogen bonding, particularly with specific amino acids, suggests a more intricate and stable binding pattern between Compound-1 and HIV-1 RT. Furthermore, Compound-1's engagement in hydrophobic interactions with various amino acids contributes to a stable binding orientation, further underscoring its potential superiority over Efavirenz.

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Similarly, Compound-2 demonstrates a strong affinity and stable binding potential, with a binding energy of -7.45 kcal/mol. It forms six hydrogen bonds, double the number established by Efavirenz, and engages in multiple hydrophobic interactions with amino acids on HIV-1 RT. This diverse network of interactions, including both hydrogen bonds and hydrophobic contacts, highlights the enhanced stability and specificity of the Compound-2-HIV-1 RT complex compared to the Efavirenz-HIV-1 RT interaction. These findings collectively indicate that both Compound-1 and Compound-2 possess superior molecular docking attributes compared to Efavirenz, highlighting their potential as promising inhibitors of HIV-1 RT (Al-Masri et al., 2023; Serna-Arbeláez et al., 2023).

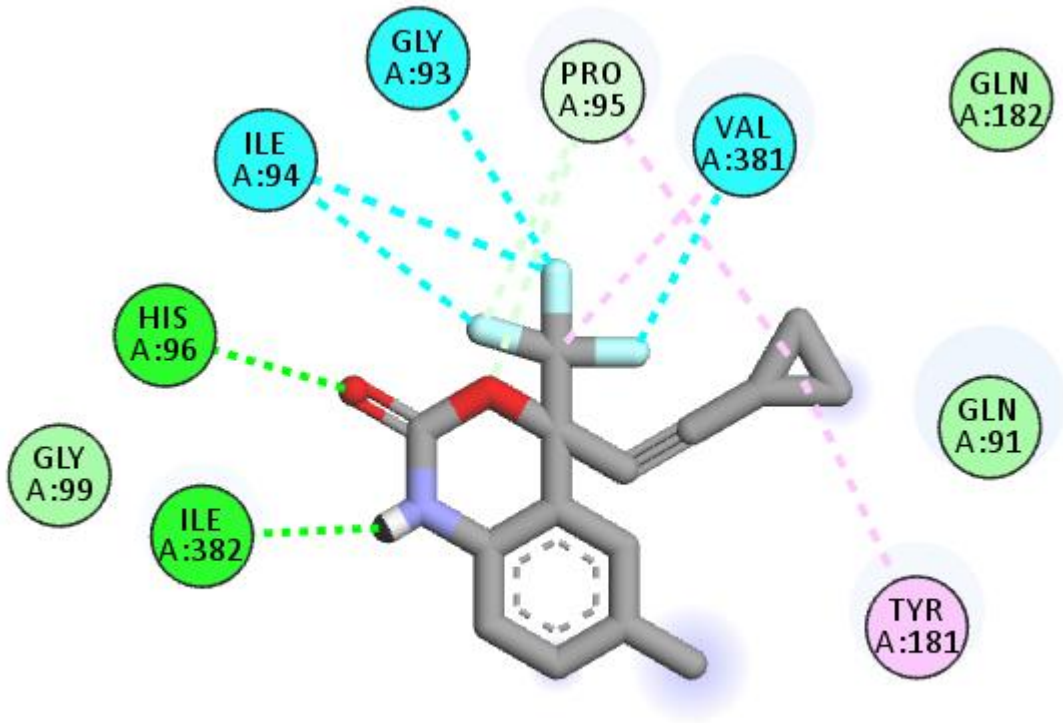

Figure-9. 2D molecular interaction of HIV-1 RT With standard drug Efavirenz. Hydrogen bond interactions were shown in green colour dotted lines.

These findings collectively indicate that both Compound-1 and Compound-2 possess superior molecular docking attributes compared to the standard drug Efavirenz, suggesting their potential as promising inhibitors of HIV-1 RT (Figure-9). The lower binding energies, increased hydrogen bonding, and extensive hydrophobic interactions observed for these compounds underscore their promising inhibitory properties and their potential as lead candidates for the development of novel antiretroviral therapies targeting HIV-1 RT.

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CONCLUSION

The molecular docking studies of HIV-1 reverse transcriptase (RT) with Compound-1 and Compound-2 have revealed their promising potential as inhibitors of the enzyme. Compared to the standard drug Efavirenz, both compounds exhibit superior binding energies and form a higher number of hydrogen bonds, indicating enhanced stability and specificity in their interactions with HIV-1 RT. Compound-1 displays a notably lower binding energy and five hydrogen bonds, while Compound-2 demonstrates a robust binding affinity with six hydrogen bonds and extensive hydrophobic interactions. These findings support the further investigation and development of Compound-1 and Compound-2 as potential anti-HIV agents, with the aim of advancing the treatment options for HIV/AIDS.

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