Communication

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Antiretroviral Promise: Amentoflavone and Nilotinib Strong Binding and Low Toxicity Against HIV-1 Reverse Transcriptase

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Abstract: In this preliminary investigation, a concise molecular docking study using the Pyrx program screened thousands of compounds against the active site of HIV-1 reverse transcriptase. The Virtual Screening library was employed to assess binding energetics scores, focusing on identifying candidates with high Vina Scores. Eight compounds, including Tetrophan, Imatinib, Amentoflavone, Alprazolam, Bilobetin, Bafetinib, Nilotinib, and Ginketin, emerged as promising candidates with significant binding energies.Recognizing compounds that strongly bind to HIV-1 reverse transcriptase holds potential for antiretroviral drug development in the fight against HIV infection. Subsequently, the study evaluated the toxicity properties of these candidates using pKCSM. Amentoflavone, a natural biflavonoid found in Ginkgo biloba and Nilotinib, a tyrosine kinase inhibitor, demonstrated lower overall toxicity profiles compared to other candidates. Further toxicity analysis using Pro-Tox-II revealed Amentoflavone's higher predictive LD50, lower toxicity class, and reduced probabilities of hepatotoxicity, carcinogenicity, mutagenicity, and moderate immunotoxicity compared to Nilotinib. While Nilotinib, a pharmaceutical drug, exhibited higher probabilities of adverse effects in these categories. In summary, Amentoflavone and Nilotinib present as promising candidates with lower toxicity concerns. Amentoflavone, as a natural substance, showcases a more favorable toxicity profile than Nilotinib, offering valuable insights for potential therapeutic applications.

Keywords: HIV-1 reverse transcriptase,Pyrx program; Docking approach; Pro-Tox-II; pkCSM

1. Introduction

It's important to note that while antiretroviral therapy (ART) can effectively control HIV infection and slow down disease progression, there is currently no cure for HIV. Ongoing research and development efforts aim to improve treatment options and potentially find a cure for this persistent viral infection [1].Understanding the structure and function of HIV-1 reverse transcriptase is crucial for the development of new antiretroviral drugs. Researchers continue to study the enzyme to identify novel targets for intervention and to improve existing therapies[2–4].

Reverse transcriptase is an essential enzyme in the retroviral life cycle. It catalyzes the conversion of the viral RNA genome into DNA, a process known as reverse transcription. This newly formed viral DNA is then integrated into the host cell's genome[5]. The HIV-1 virus contains RNA as its genetic material. Upon entering a host cell, reverse transcriptase synthesizes a complementary DNA (cDNA) strand from the viral RNA template.

This cDNA is then used as a template to synthesize the second DNA strand, resulting in a double-stranded DNA molecule. Due to its crucial role in viral replication, HIV-1 reverse transcriptase is a target for antiretroviral drugs[1–5].

There are two main classes of drugs that target reverse transcriptase:

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-Nucleoside Reverse Transcriptase Inhibitors (NRTIs): These drugs are analogs of nucleosides and become incorporated into the growing DNA chain, causing premature termination of DNA synthesis[6].

-Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs): These drugs bind to a specific site on the reverse transcriptase enzyme, inhibiting its activity[7].

The study considered a diverse set of compounds, including both synthetic drugs and natural substances. Natural substances can be of interest due to their potential pharmacological properties and may serve as sources for the development of new drugs. The method applied withj this goal was Molecular Docking, his computational technique involves predicting the preferred orientation of one molecule to a second when bound to each other to form a stable complex. In the context of drug discovery, it helps identify potential drug candidates that can bind to a target protein with high affinity[8,9].

One of the parameters most important of this computational tecnique is binding energy score reflects the strength of the interaction between the ligand and the target protein. A lower (more negative) binding energy score usually indicates a stronger binding affinity[8–10].

2. Material and Methods

HIV-1 Reverse Tracriptase was taken by Protein Data Bank, prepared accurately and performed by Pyrx program [11] by Autodock Vina [10]. Drugs and natural compounds were taked by PUBCHEM Database. Docking analysis was performed in Ligand Binding Site of HIV-1 Reverse Tracriptase in complex with Crystal ligand TB9 (4-CHLORO-8-METHYL-7-(3-METHYL-BUT-2-ENYL)-6,7,8,9-TETRAHYDRO-2H-2,7,9A-TRIAZA-BENZO[CD]AZULENE-1-THIONE).

HIV-1 Reverse Tracriptase (PDB Code: 1REV, 2.60 Å Resolution); Grid box Coordinates of binding Center X (1.17683606281), Y(-38.5113755271), Z(22.5823878823) ; Size_X = 19.5935839859 Å,Size_Y = 15.3113078957Å, Size_Z = 19.5935839859Å.

3. Results and Discussion

This molecular docking study has investigate by Pyrx program [11] to thousands compoundsin in the active site of HIV-1 reverse transcriptase. General Speaking Pyrx is a molecular graphics software and a user-friendly interface for the AutoDock suite of molecular docking tools. Virtual screening involves computationally screening a large number of compounds to identify potential drug candidates[11] .

The work has evaluated the binding energetics scores, specifically using the Vina Scores, by Autodock Vina Algorithm [10], as a parameter to assess the strength of interaction between the compounds and the target protein. The focus was on selecting molecules with high binding energies. Generally, the negative values of binding energy indicate favorable interactions between the compounds and the target protein. Lower (more negative) binding energies generally suggest stronger binding.The study identified eight compounds with notable Vina Scores, suggesting strong binding affinity to HIV-1 reverse transcriptase. Here are the details of the selected compounds along with their corresponding binding energies:

-Tetrophan
Binding Energy: -11.2 kcal/mol
-Imatinib
Binding Energy: -9.8 kcal/mol
-Amentoflavone
Binding Energy: -12.5 kcal/mol
-Alprazolam
Binding Energy: -11.1 kcal/mol
-Bilobetin
Binding Energy: -12.0 kcal/mol
-Bafetinib
Binding Energy: -10.4 kcal/mol

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-Nilotinib
Binding Energy: -11.8 kcal/mol
-Ginketin
Binding Energy: -11.8 kcal/mol

The next step involved assessing the toxicity properties of the selected candidates using two prediction servers, namely pKCSM and Pro-Tox-II. The focus was on key toxicity parameters, and pKCSM, a machine learning platform, was utilized firstly for this evaluation.

Three crucial parameters were considered: Max.tolerated dose (human) (log mg/kg/day), Oral Rat Acute Toxicity (LD50) (mol/kg), and Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day).

The results revealed that among the eight candidates, only two demonstrated a lower overall toxicity profile when considering all the predicted toxicity properties analyzed by pKCSM[12]. These candidates are Amentoflavone, a biflavonoid found in Ginkgo biloba and Hypericum perforatum [13], and Nilotinib, a tyrosine kinase inhibitor known as AMN107[14]. Here are the main toxicity results for these two candidates:

-Amentoflavone:
Max.tolerated dose (human) (log mg/kg/day): 0.438 log mg/kg/day
Oral Rat Acute Toxicity (LD50) (mol/kg): 2.527 mol/kg
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day): 3.572 log mg/kg_bw/day
-Nilotinib:
Max.tolerated dose (human) (log mg/kg/day): 0.96 log mg/kg/day
Oral Rat Acute Toxicity (LD50) (mol/kg): 2.149 mol/kg
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day): 2.81 log mg/kg_bw/day

Both Amentoflavone and Nilotinib exhibited high values in the parameters of Max.tolerated dose (human), LD50, and LOAEL, suggesting potential as candidates with lower toxicity concerns. Amentoflavone, being a natural substance, and Nilotinib, a pharmaceutical drug, demonstrated promising toxicity profiles in this analysis.

In the conclusive phase, an in-depth examination of the toxicity properties of Amentoflavone and Nilotinib was conducted using Pro-Tox-II [15], a virtual lab designed for predicting toxicities of small molecules. Pro-Tox-II is proficient in forecasting various toxicological aspects, including acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity. Upon comparison, several noteworthy aspects emerge, which can be succinctly summarized as follows:

-Acute Toxicity (LD50):
Amentoflavone: Predictive LD50 of 3919 mg/kg.
Nilotinib: Predictive LD50 of 800 mg/kg.
-Toxicity Class:
Amentoflavone: Classified as toxicity class 5.
Nilotinib: Classified as toxicity class 4.

Specific Toxicity Predictions for Amentoflavone:
Hepatotoxicity: Probability of approximately 70%, indicating a lower likelihood.
Carcinogenicity: Probability of approximately 70%, suggesting a lower likelihood.
Mutagenicity: Probability of approximately 70%, implying a lower likelihood.
Immunotoxicity: Probability of approximately 50%, indicating a moderate likelihood.

Specific Toxicity Predictions for Nilotinib:
Hepatotoxicity: Probability of over 70%, suggesting a higher likelihood.
Carcinogenicity: Probability of over 70%, indicating a higher likelihood.
Mutagenicity: Probability of less than 70%, suggesting a lower likelihood.
Immunotoxicity: Probability of over 70%, indicating a higher likelihood.
Cytotoxicity: Probability of over 70%, suggesting a higher likelihood.

In summary, Amentoflavone, as a natural substance, exhibits a predictive LD50 that is higher than Nilotinib, and it is classified in a lower toxicity class. Additionally, Amentoflavone shows a lower likelihood of being hepatotoxic, carcinogenic, mutagenic, and moderately immunotoxic compared to Nilotinib, which demonstrates higher probabilities of adverse effects in these categories.

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These findings provide valuable insights into the comparative toxicity profiles of Amentoflavone and Nilotinib, informing further considerations for their potential use in therapeutic applications.

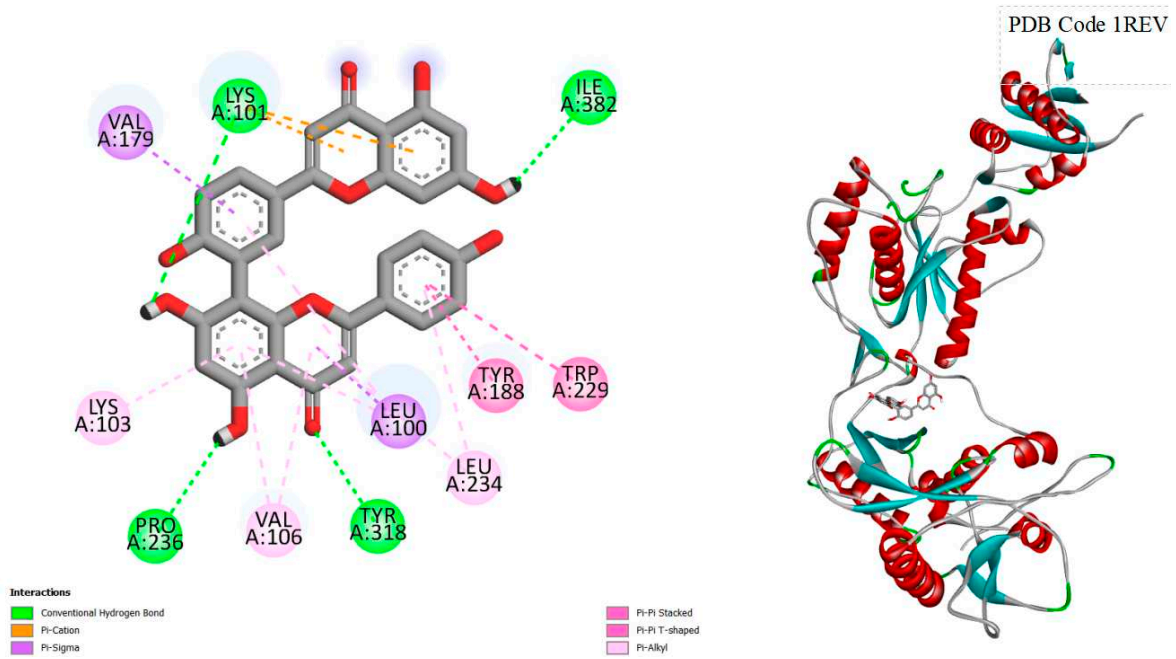

Figure 1. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Amentoflavone within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Amentoflavone. Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Amentoflavone.

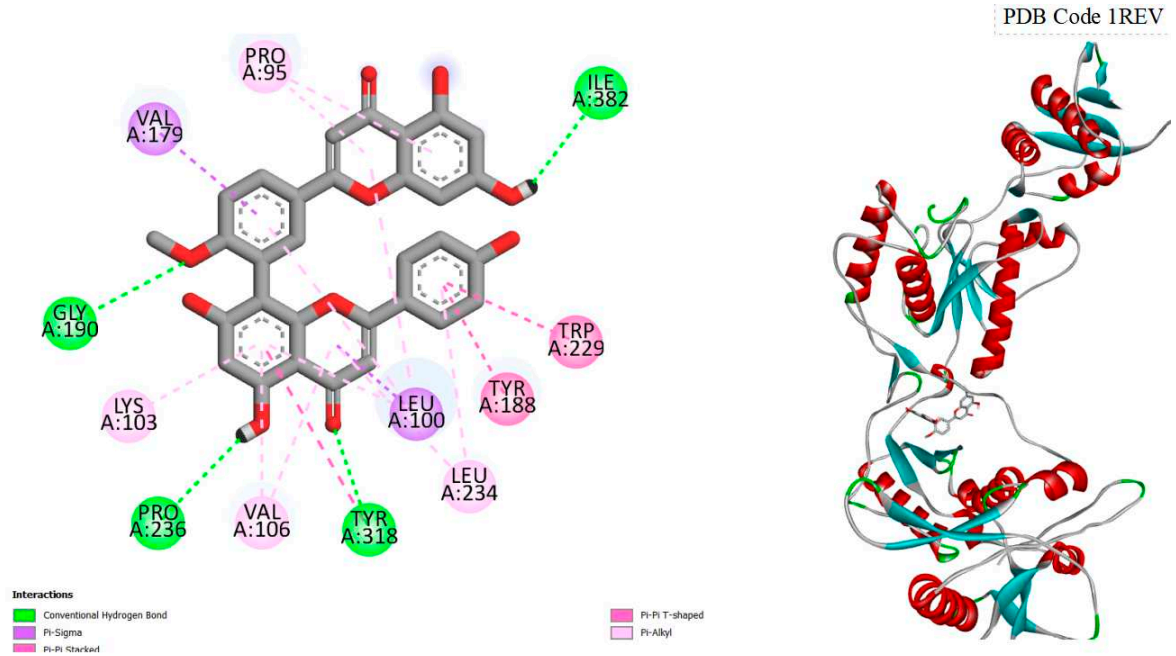

Figure 2. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Bilobetin within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Bilobetin. Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Bilobetin.

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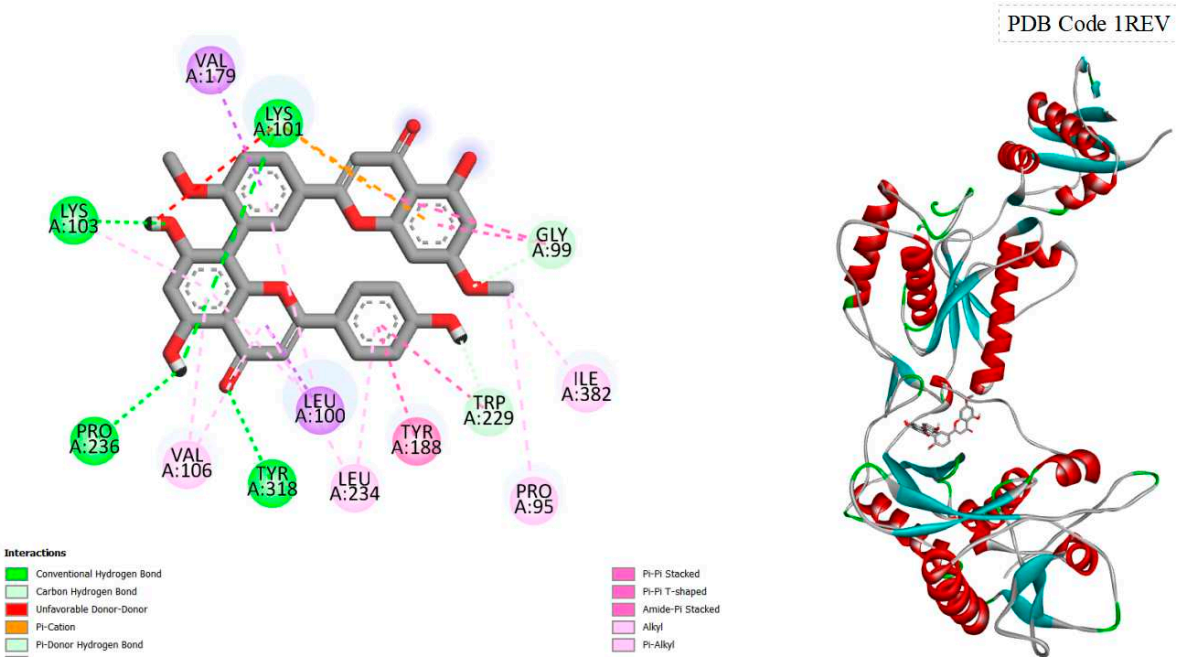

Figure 3. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Ginkgetin within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Ginkgetin. Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Ginkgetin.

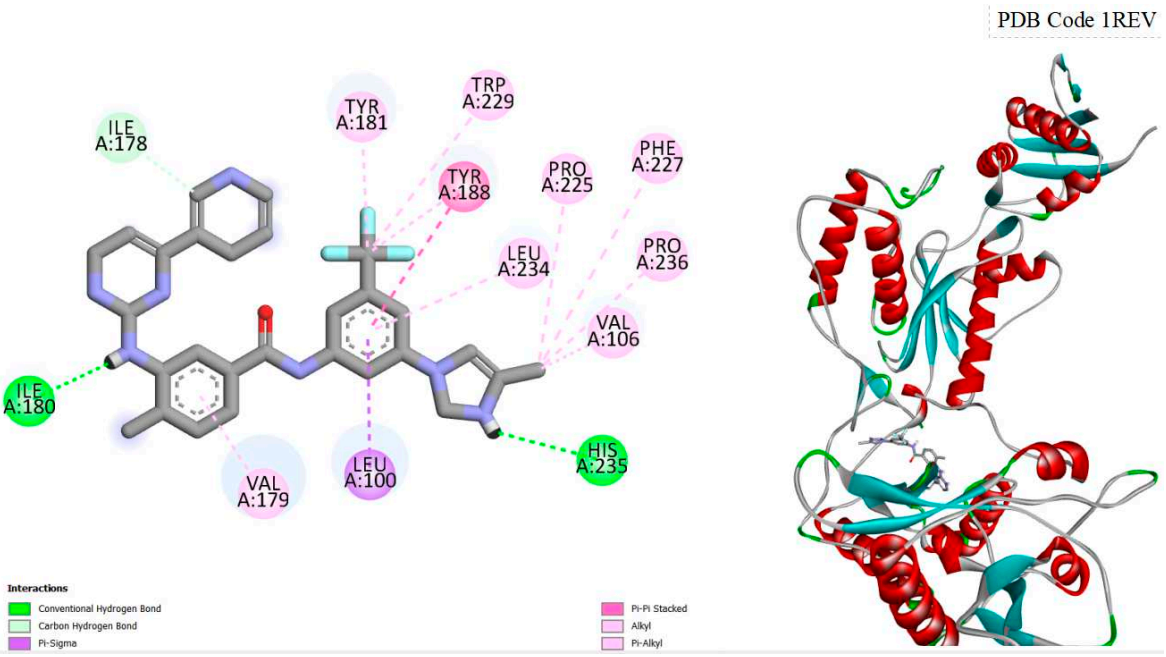

Figure 4. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Nilotinib within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Nilotinib. Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Nilotinib.

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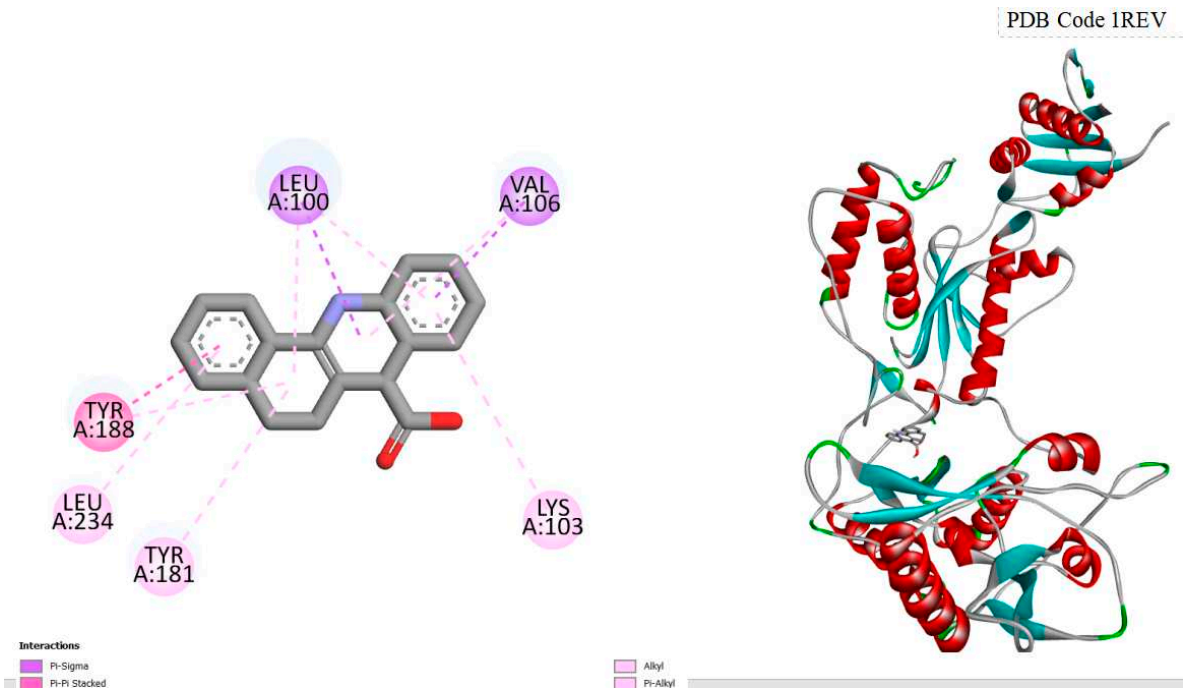

Figure 5. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Tetrophan within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Tetrophan . Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Tetrophan .

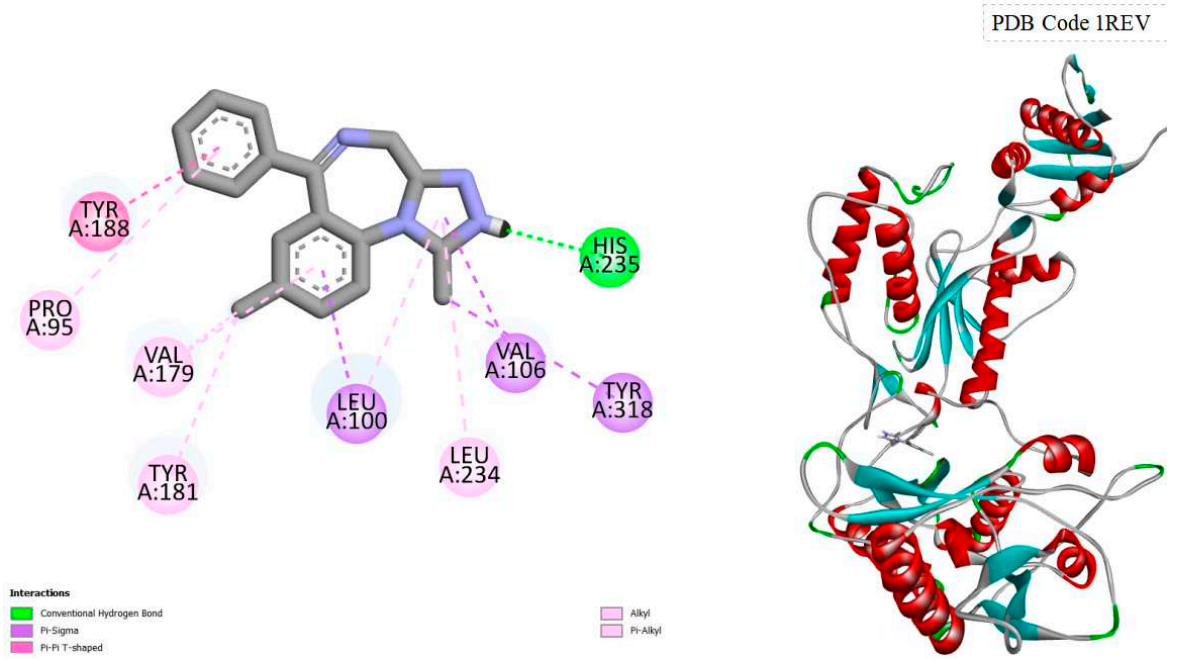

Figure 6. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Alprazolam within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Alprazolam. Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Alprazolam.

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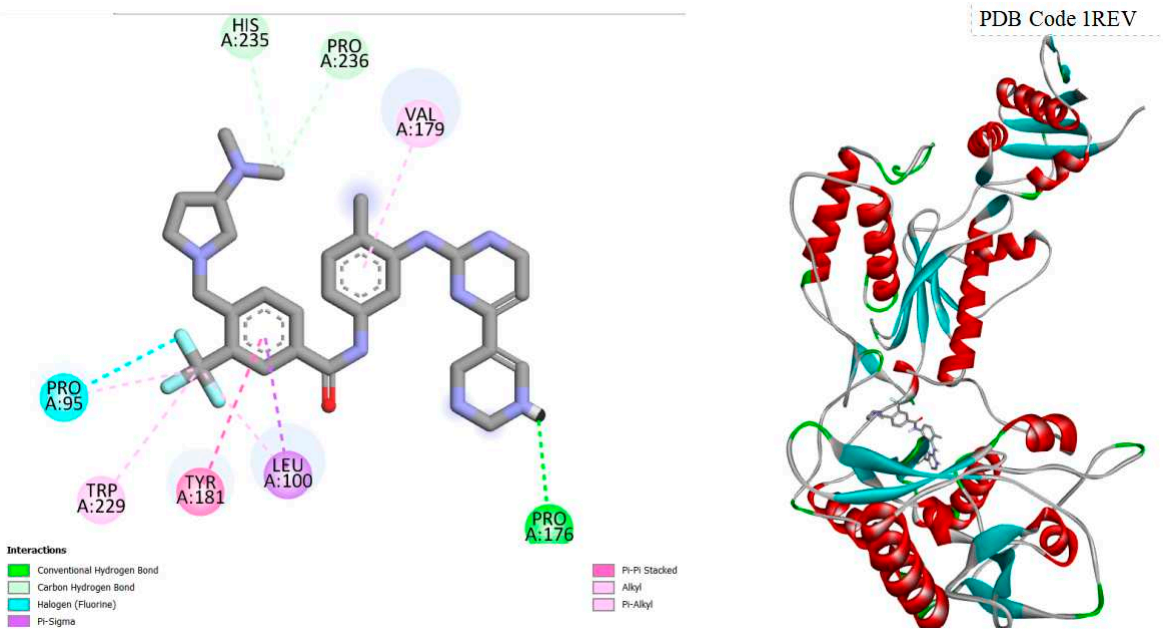

Figure 7. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Bafetinib within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Bafetinib. Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Bafetinib.

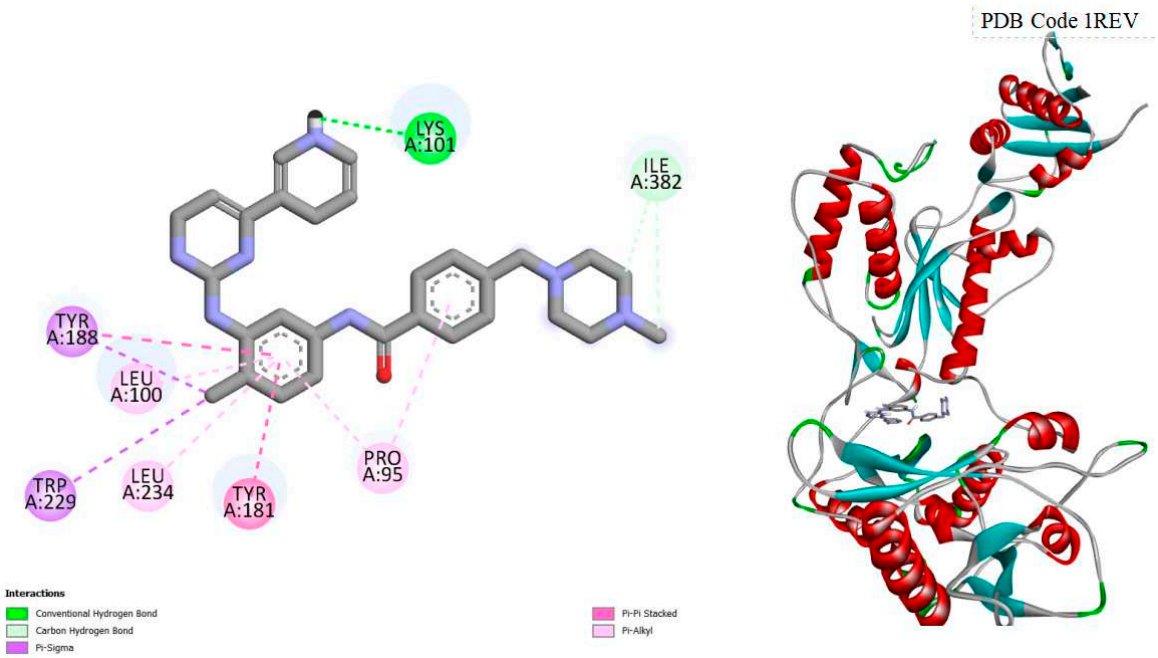

Figure 8. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Imatinib within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Imatinib . Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Imatinib.

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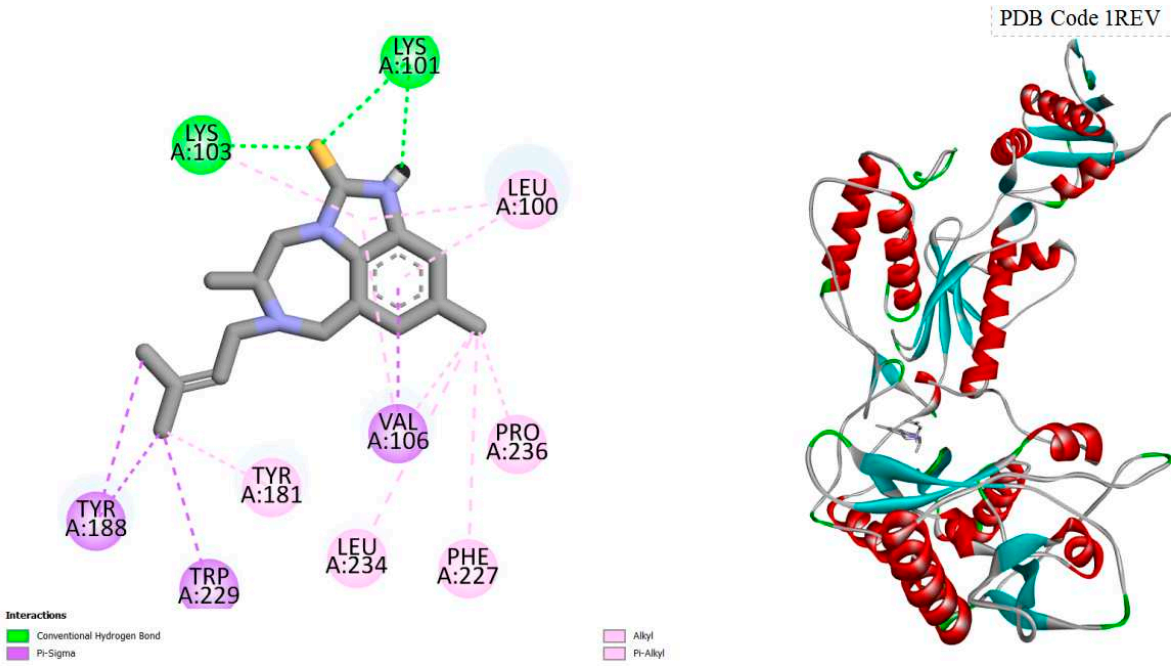

Figure 9. displays the docking outcomes of HIV-1 Reverse Transcriptase in conjunction with Crystal Ligand TB9 (4-CHLORO-8-METHYL-7-(3-METHYL-BUT-2-ENYL)-6,7,8,9-TETRAHYDRO-2H-2,7,9A-TRIAZA-BE ZO[CD]AZULENE-1-THIONE) within the Ligand Binding Site, as analyzed by Autodock Vina through the Pyrx program. On the left side, 2D diagrams illustrate the residue interactions between the target and Crystal Ligand TB9. Meanwhile, the right side exhibits the Ligand Binding Site of the protein, highlighting the specific location of Crystal Ligand TB9.

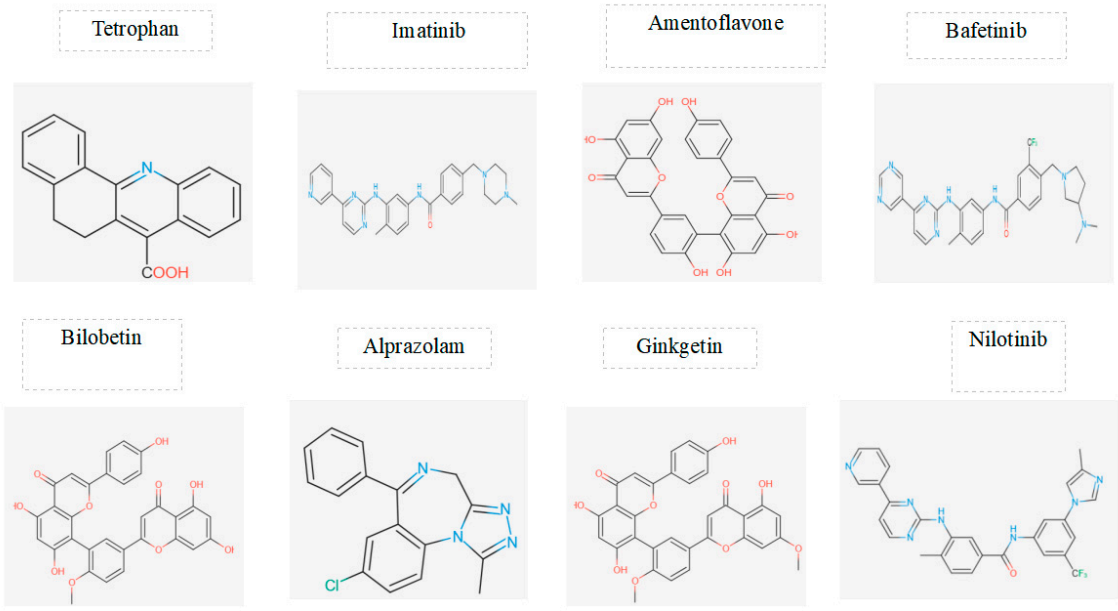

Figure 10. Comparison 3D Chemical Structures of Best potential candidates investigated for HIV-1 Reverse Transcriptase.

Table 1. Comparison of Best Binding Energies scores of potential candidates investigated for HIV-1 Reverse Transcriptase, by Autodock Vina through Pyrx program in the Ligand Binding Site pocket.

Compounds	Binding Energies scores (kcal/mol)
Crystal Ligand TB9	-10.7

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Tetrophan	-11.2
Imatinib	-9.8
Amentoflavone	-12.5
Alprazolam	-11.1
Bilobetin	-12.0
Bafetinib	-10.4
Nilotinib	-11.8
Ginketin	-11.8

Table 2. Comparison of predicted toxicity parameters of potential candidates investigated for HIV-1 Reverse Transcriptase, by pkCSM Server.

Compounds	AMES toxicity	Max. tolerated dose (human) (log mg/kg/day)	Oral Rat Acute Toxicity (LD50) (mol/kg)	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	Hepatotoxicity	Skin Sensitisation	T. Pyriformis toxicity (log ug/L)	Minnow toxicity (log mM)
Tetrophan	No	0.544	2.057	1.789	yes	no	0.285	-0.631
Imatinib	No	0.317	2.9	1.409	yes	no	0.285	2.089
Amentoflavone	**No**	**0.438**	**2.527**	**3.572**	**yes**	**no**	**0.285**	**2.685**
Bafetinib	No	0.383	2.85	1.321	yes	no	0.285	2.28
Nilotinib	**No**	**0.96**	**2.149**	**2.81**	**yes**	**no**	**0.285**	**5.136**
Bilobetin	No	0.437	2.56	2.217	yes	no	0.273	-0.003
Alprazolan	No	0.373	2.347	0.729	yes	no	0.359	-0.448
Ginkgetin	No	0.427	2.733	2.475	no	no	0.285	-2.351

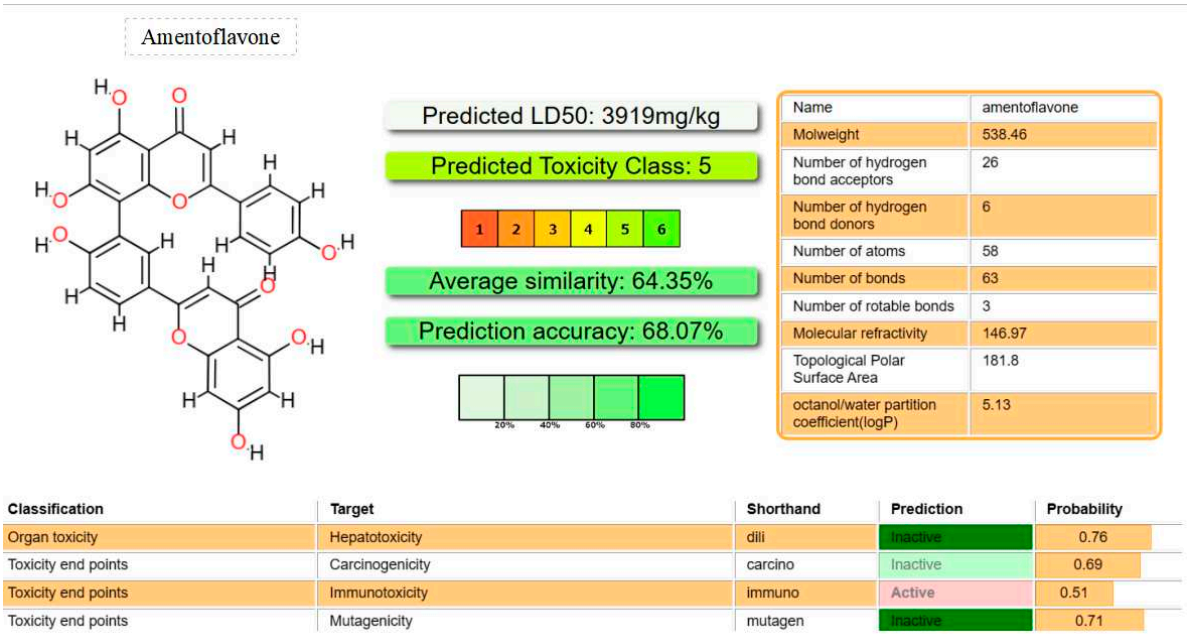

Figure 11. Comparison of predicted toxicity parameters of Amentoflavone investigated for HIV-1 Reverse.

Transcriptase, using Pro-Tox-II.

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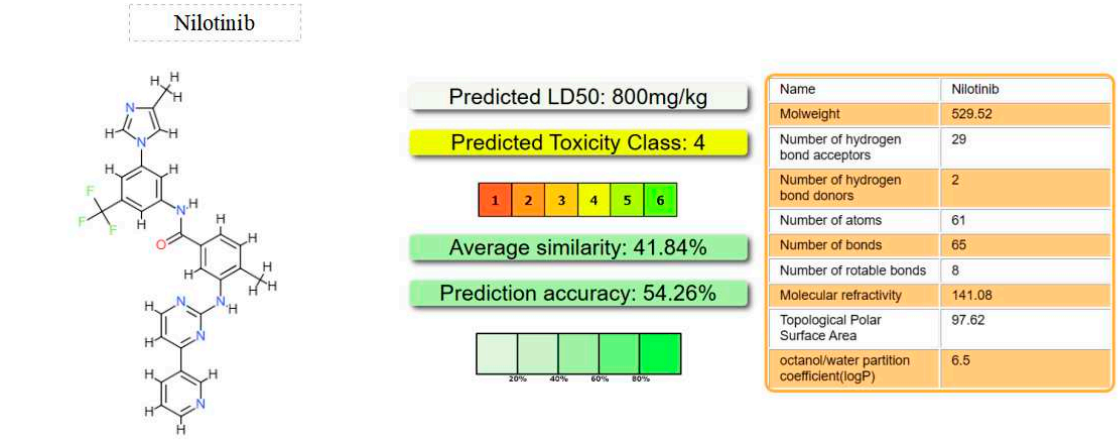

Name	Nilotinib
Molweight	529.52
Number of hydrogen bond acceptors	29
Number of hydrogen bond donors	2
Number of atoms	61
Number of bonds	65
Number of rotable bonds	8
Molecular refractivity	141.08
Topological Polar Surface Area	97.62
octanol/water partition coefficient(logP)	6.5

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.82
Toxicity end points	Carcinogenicity	carcino	Active	0.53
Toxicity end points	Immunotoxicity	immuno	Active	0.98
Toxicity end points	Mutagenicity	mutagen	Inactive	0.59
Toxicity end points	Cytotoxicity	cyto	Inactive	0.72

Figure 12. Comparison of predicted toxicity parameters of Nilotinib investigated for HIV-1 Reverse.

Transcriptase, using Pro-Tox-II.

4. Conclusion

In this preliminary study, a succinct molecular docking investigation was conducted using the Pyrx program, screening a multitude of compounds against the active site of HIV-1 reverse transcriptase. Employing the Virtual Screening library, the focus was on evaluating binding energetics scores, with an emphasis on identifying candidates possessing substantial Vina Scores. Eight compounds, namely Tetrophan, Imatinib, Amentoflavone, Alprazolam, Bilobetin, Bafetinib, Nilotinib, and Ginketin, emerged as promising candidates with noteworthy binding energies.

The identification of compounds that exhibit strong binding to HIV-1 reverse transcriptase holds potential for the development of antiretroviral drugs in the battle against HIV infection. Subsequently, the study delved into assessing the toxicity properties of these candidates using pKCSM.

Amentoflavone, a natural biflavonoid found in Ginkgo biloba and Hypericum perforatum, and Nilotinib, a tyrosine kinase inhibitor, displayed lower overall toxicity profiles compared to their counterparts.

Further scrutiny through Pro-Tox-II unveiled Amentoflavone's superior predictive LD50, lower toxicity classification, and diminished probabilities of hepatotoxicity, carcinogenicity, mutagenicity, and moderate immunotoxicity in contrast to Nilotinib. Conversely, Nilotinib, a pharmaceutical drug, exhibited higher probabilities of adverse effects in these categories. To summarize, Amentoflavone and Nilotinib emerge as promising candidates with reduced toxicity concerns.

Amentoflavone, being a natural substance, demonstrates a more favorable toxicity profile than Nilotinib, providing valuable insights for potential therapeutic applications. In vitro and in vivo studies would be necessary to assess their potential as antiretroviral agents.

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