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# A Molecular Docking Study: Targetting HIV-1 Integrase Protein Against Selected Phytocompounds from *Calophyllum Lanigerum*

Rishabh Kulkarni[1,*], Shubham Wanarase[1]

***Abstract***

***Objectives:*** *The most common form of HIV that causes AIDS is HIV-1. The World Health Organization has estimated roughly 75 million plus HIV-1 infections till date and roughly 40 million deaths (as of 2021). Thus, this study was done to identify natural compounds from Calophyllum lanigerum, a medicinal plant largely endemic to South-East Asia, to inhibit the spread of this disease. It did so by using molecular docking methods, Lipinski drug-likeness prediction, and the ADME analysis.* ***Methods:*** *The Protein Data Bank database was used to retrieve the HIV-1 Integrase protein (core domain with catalytic activity). The ligands were obtained from PubChem. PyRx was used for the docking analysis, and Biovia Discovery Studio Visualizer was used to display the receptor ligand interactions. Swiss-ADME, an online tool, was used to conduct the ADMET and Lipinski drug-likeliness study.* ***Results:*** *Five compounds from C. lanigerum have been identified by molecular docking studies as having potential binding affinity to prevent the integration of HIV-1 dsDNA (reverse transcribed) into the genome of human immunocytes, such as CD4+ T cells, which would have an impact on viral replication. According to the ADMET profile and drug likeness prediction, each of the top 5 compounds is safe and possesses drug-like qualities.* ***Conclusion:*** *The current investigation identifies 5 phytocompounds in total as having the strongest binding affinities and potential inhibitory effects on HIV-1 Integrase activity.*

**Keywords:** HIV-1, AIDS, *Calophyllum lanigerum*, HIV-1 Integrase, CD4+ T cell, Viral replication

## INTRODUCTION

When numerous homosexual men, with an average age of around 34, (calculated from a report of the first 1000 documented HIV cases in the US) expired due to unusual opportunistic infections and uncommon cancers in 1981, Acquired Immune Deficiency Syndrome (AIDS) was first recognised as a unique disease [1]. Human immunodeficiency virus type 1 (HIV-1) was determined to be caused by a retrovirus, one of the most devastating viral infections in the recent past. According to the WHO, about 75 million individuals have been infected with HIV worldwide as of today, with around 37 million still alive and living with the infection [2, 3]. Africa has 26 million people, the Americas have 3.3 million, Southeast Asia has 3.5 million, Europe has 2.4 million, the eastern Mediterranean has 360,000, and the western Pacific has 1.5 million according to the population distribution of these patients [2]. The immune system gradually fails as a result of AIDS, which then promotes the growth of malignancies and opportunistic infections that can be fatal [4]. The usual survival period after infection ranges between 9 and 11 years [5], depending on the viral subtype.

HIV is typically a sexually transmitted disease that is spread through contact or transfer of blood,

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pre-ejaculate, semen, and vaginal secretions [6, 7]. Important immune system components such as dendritic cells, helper T cells (particularly CD4+ T cells), and macrophages are all susceptible to infection [8]. Through varying pathways, infection lowers levels of CD4+ T cells. These include direct killing of infected cells, [9] apoptosis of uninfected bystander cells, [10] pyroptosis (triggered by proinflammatory signals associated with inflammation) of abortively infected T cells, [10] and killing of infected by CD8+ cytotoxic lymphocytes that recognise them [11]. The body becomes more susceptible to opportunistic infections and the cell-mediated defence systems become ineffective when CD4+ T cell counts fall below a crucial threshold, which results in the development of AIDS.

Neither a treatment for HIV/AIDS nor a reliable vaccine exist today. The prevention, control, and treatment of it, however, have made great strides [12]. Since the advent of HAART in 1996, the rates of morbidity and mortality have been successfully lowered [13]. Research on vaccines and the creation of antimicrobial drugs are both continuing. Antiretroviral medications used to prevent mother-to-child transmission, [14–17] and voluntary male medical circumcision [18, 19] are two advancements in HIV prevention.

Even if HAART is beneficial, there are some serious side effects, which are particularly noticeable in patients receiving prolonged treatment. Additionally, the majority of currently used medicines are constrained by the development of multidrug resistance [20], and a novel approach is now necessary to address the problem of the body's HIV reservoirs for complete eradication of HIV and AIDS. Since they may prove to be more cost-effective therapeutic agents, the WHO has recommended that ethnomedicines be systematically evaluated against HIV [21, 22]. The study of natural compounds with activity against HIV-1enzymes like reverse transcriptase, proteases, and integrases accounted for most of the contribution during the 1990’s in this field [23]. The Calanolides (derivatives of coumarin), Ursolic and Betulinic acids (triterpenes), Polycitone A (alkaloid) etc are examples of such natural products. They have been suggested as viable options for anti-HIV medications [24].

The HIV genome instructs a small number of viral proteins that work together with host proteins to form cooperative partnerships, penetrate host cells, and take over their internal mechanisms [25]. The virion has a diameter of about 100 nm. The cone-shaped core that makes up its innermost portion contains two copies of the (positive sense) ssRNA genome, as well as the primary core protein, some smaller proteins, and the enzymes reverse transcriptase, integrase, and protease [26].

An essential step in the replication cycle of retroviruses is the integration of a nucleic acid copy of the virus into the host genome [27]. Although integration can happen almost everywhere in the genome, some areas are favoured [28]. Through cycles of cell division, the integrated viral DNA is kept stable and duplicated alongside the cellular DNA. This makes it difficult to treat retroviral infections. Despite significant advancements in antiviral development, the integrated virus nevertheless exists in cells with a lengthy lifespan [29]. The Integrase protein, which is encoded by a virus, is the main enzyme involved in this process [30]. Through a sequence of DNA cutting and joining events, the HIV-1 Integrase protein catalyses the integration of viral DNA into the host genome.

Following virion entrance into a cell and reverse transcription of the RNA genome in dsDNA, this enzyme activity occurs. It has three domains: the core domain has the catalytic DDE (transposase) motif, which is necessary for its enzymatic activity; the C-terminal domain has an SH3-like (beta barrel) fold, which binds DNA non-specifically [31]. The N-terminal domain has a His2Cys2 (histidine and cysteine amino acids) motif, which chelates zinc (metal-protein interactions).

A genus of tropical flowering plants called *Calophyllum* belongs to the *Calophyllaceae* family. Although some species can be found in Africa, the Americas, Australasia, and the Pacific Islands,

 

they are primarily found in Asia [32]. Numerous secondary metabolites, including coumarins, xanthones, chromanones, triterpenes, steroids, and glycosides, have been discovered by phytochemical investigations [33–35].

The genus *Calophyllum* frequently contains coumarins, which are primarily biosynthesized in the leaves. They have a wide range of pharmacological functions and can be applied as biomarkers. In the past 30 years, they have drawn attention as potential orally accessible non-peptidic antiviral medicines. Numerous lead compounds with coumarin scaffolds, whether natural, semi-natural, or synthetic, have been found in the past and are currently in various stages of pharmacological development [36]. Despite the fact that certain older assessments have already been released, the majority of them have emphasised the anti-HIV potential of coumarin derivatives [37].

In the current work, a total of 52 distinct active phytoconstituents from *C. lanigerum* were used in an effort to find new active and stable inhibitors against HIV-1 Integrase.

## METHODS

### Protein Selection

The Protein Data Bank (PDB), an online protein structure database maintained by the Research Collaboratory for Structural Bioinformatics (RCSB), was used to find the 3D protein structure of HIV-1 Integrase [38]. The crystal structure of the catalytic core domain of HIV-1 integrase in association with 2-(tert-butoxy)-2-(2-(3-cyclohexylureido)-3,6-dimethyl-5-(5-methylchroman-6-yl)pyridin-4-yl)acetic acid (PDB ID: 7D83) was the structure that was recovered. The structure has a crystal resolution of 2.43. The protein contains 166 residues (based on the deposited residue count), and there is just one unique protein chain.

### Protein Preparation

Utilising the Biovia Discovery Studio programme, the protein purification was completed. The purification involved the removal of water molecules, the ligand molecules associated with the protein as well as other heteroatoms. This was followed by the addition of polar hydrogen atoms to the structure for charge stabilization and optimization purposes. The active sites for binding of ligands were then identified (a total of 5 active binding sites are present). The file was saved in PDB format.

### Ligand Selection

For the screening of potential inhibitory phytochemicals, the three-dimensional structures of a total of 52 phytocompounds present in *C. lanigerum* were obtained from PubChem database in the form of a Structure Data File (SDF) [39].

### Ligand Preparation

The three processes of ligand preparation were completed. The ligand structures were all first optimised. The next step was to reduce their energies, and the last step was converting the ligands to the 3D PDB format using the Open Babel tool found in the PyRx software [40].

### Molecular Docking

Small molecule behaviour in the binding site of target proteins can be studied using the molecular docking techniques. By mimicking the atomic-level interaction between tiny molecules and a protein, they also aid in the analysis of fundamental biological processes. For the current study, the PyRx virtual screening tool was utilised [41].

The catalytic core domain of HIV-1 Integrase was sequentially docked with each of the 52 active phytocompounds of C. lanigerum using the PyRx programme. The synthesised receptor molecule and ligand files were chosen for the current study. For docking, the receptor protein was loaded and converted into a macromolecule (PDBQT file). The Open Babel programme was then used to import

 

the ligands [42]. After that, a grid box was defined by pressing the "maximise" button in order to carry out "blind docking" and examine every potential protein-ligand interaction. A table containing the binding affinities of each ligand was generated once all changes were made, and docking was initiated by hitting the forward button. In PDB file format, the top 5 ligands with the highest negative binding affinities (measured in kcal/mol) were saved after being chosen for additional screening study. Utilising Discovery studio visualizer 21.1 [43], the protein-ligand interactions in 2D and 3D were seen.

**ADMET Analysis**

The terms "absorption, distribution, metabolism, excretion, and toxicity" or "ADMET" are used in pharmacokinetics and pharmacology. These five factors all have a major impact on drug levels in the system as well as the kinetics of drug exposure to tissues, which affects a compound's efficacy and pharmacological activity as a medicine [44]. For the drug likeliness and ADMET analyses in this investigation, the top 5 phytocompounds with the highest negative binding affinity values were selected. Swiss-ADME was used to conduct this analysis [45]. The same platform was also used for boiled-egg analysis. For ADME analysis, the Lipinski rule of five was considered. It predicts whether a chemical will behave in a similar way as a medicine. Any molecule must meet two or more of the requirements listed below:

a. The molecular mass of the compound should be less than 500 Dalton,
b. The value of MLog(P) should be less than 4.15 (refers to degree of lipophilicity of phytocompound)
c. There should not be more than five H-bond donors.
d. There should not be more than ten H-bond acceptors.
e. Molar refractivity of the compound should lie between 40 and 130.

ChemAGG platform was used to check the aggregation characteristic of the phytocompounds while ProTox-II tool was used to predict the toxicity of different phytocompounds [46, 47].

**RESULTS**

**Molecular Docking**

According to the study, a number of phytochemicals from *C. lanigerum* have a high affinity for the catalytic core domain of HIV-1 Integrase protein. 52 phytocompounds found in *C. lanigerum* were examined for their ability to dock with the core domain of the HIV-1 Integrase protein using PyRx software. The top five phytochemicals with the highest affinity for the receptor protein are listed in the Table 1.

**Table 1.** Top 5 phytocompounds with highest binding affinities.

| S.N. | PubChem compound ID | Name of the phytocompound | Shortened name (synonym) | Binding energy (kcal/mol) |
|---|---|---|---|---|
| 1 | CID_5352087 | Calanone | CHEMBL518283 | -8.4 |
| 2 | CID_467236 | (+)-Pseudocordatolide C | CHEMBL507953 | -8.2 |
| 3 | CID_468350 | (10R)-11,12-Dihydro-12alpha-hydroxy-4-propyl-10alpha-ethyl-6,6,11alpha-trimethyl-2H,6H,10H-benzo[1,2-b:3,4-b':5,6-b"]tripyran-2-one | CHEMBL267208 | -8.1 |
| 4 | CID_454258 | Calanolide D | CHEMBL267693 | -7.9 |
| 5 | CID_468343 | (+/-)-10,11-Dihydro-6,6,10,11,11-pentamethyl-4-propyl-2H,6H,12H-benzo[1,2-b:3,4-b':5,6-b""] tripyran-2,12-dione | CHEMBL7270 | -7.9 |

**Molecular Visualization**

Using Biovia Discovery Studio Visualizer 21.1, the interactions between the top 5 phytocompounds and their receptor ligands were portrayed. The phytocompounds were saved in PDB file format using

 

PyRx and then opened with the purified protein in DS visualizer (Figures 1–4). The phytocompounds with the highest negative binding affinity and the lowest RMSD value (represented by 1 out of the 9 binding modes for each) were chosen. Numerous interactions between phytocompounds and the catalytic core domain of HIV-1 Integrase were seen in 2D and 3D. Van der Waal forces, conventional and carbon hydrogen bonds, pi-sigma interactions, alkyl and pi-alkyl contacts and pi-cation interactions can all be seen in the diagram of 2D interactions.

*Calanone* (CHEMBL518283)

Multiple interactions between calanone and the core domain of HIV-1 Integrase occur. Leu 101, Val 113, Lys 127, and Ile 135 are involved in pi-alkyl interactions, while Phe 121 and Trp 131 form typical hydrogen bonds (Figure 5).

*(+)-Pseudocordatolide C* (CHEMBL507953)

(+)-Pseudocordatolide C forms multiple interactions with HIV-1 Integrase core domain. It includes conventional hydrogen bonding with residues Trp 131; Carbon hydrogen bond with residue Ile 135; pi-cation and pi-sigma interactions with residue Lys 127 (Figure 6).

*(10R)-11,12-Dihydro-12alpha-hydroxy-4-propyl-10alpha-ethyl-6,6,11alpha-trimethyl-2H,6H,10H-benzo[1,2-b:3,4-b':5,6-b"]tripyran-2-one* (CHEMBL267208)

Multiple interactions between CHEMBL267208 and the HIV-1 Integrase core domain are formed. It also includes pi-sigma interaction with residue Phe 121, pi-alkyl interaction with residue Lys 127, alkyl interaction with residues Leu 101, Val 113, Val 126, and Lys 127, as well as conventional hydrogen bonding with residues Val 126 and Trp 131 (Figure 7).

*Calanolide D* (CHEMBL267693)

HIV-1 Integrase core domain and Calanolide D interact in many ways. Pi-alkyl, alkyl, and pi-cation interactions with residue Lys 127 are among them, as are interactions with residue Phe 121 and Lys 127 through the pi-sigma, pi-alkyl, and alkyl interactions (Figure 8).

*(+/-)-10,11-Dihydro-6,6,10,11,11-pentamethyl-4-propyl-2H,6H,12H-benzo[1,2-b:3,4-b':5,6-b""] tripyran-2,12-dione* (CHEMBL7270)

Multiple interactions between CHEMBL7270 and the core domain of HIV-1 Integrase are formed. With residue Val 126, carbon hydrogen bonds are formed, as are pi-alkyl interactions, alkyl interactions, and pi-cation interactions with residue Lys 127 (Figure 9).

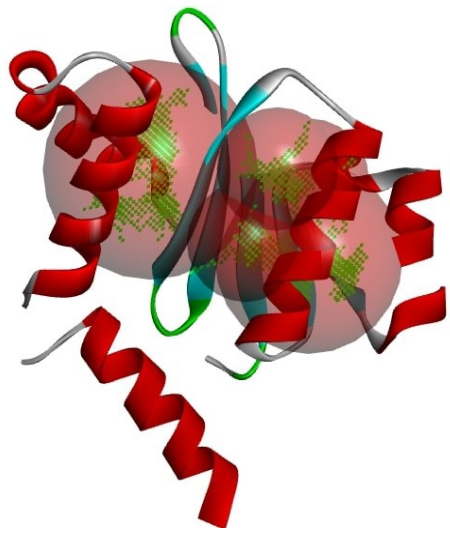

**Figure 1.** HIV-1 - Purified catalytic core domain (DS Visualizer) of Integrase with the five active binding sites depicted as spheres.

 

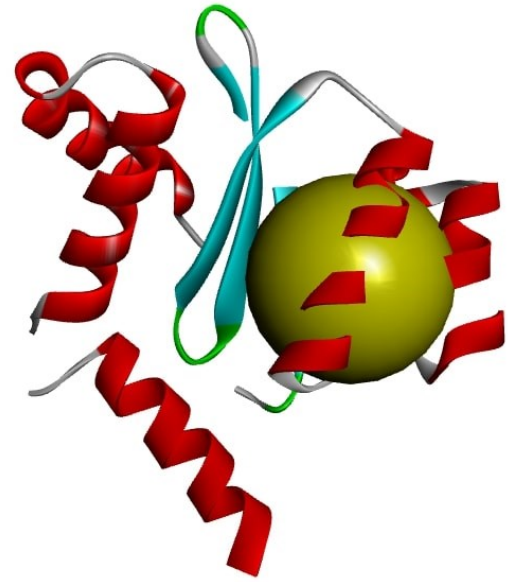

**Figure 2.** Active binding site number 2 highlighted (out of the total 5 present in the protein).

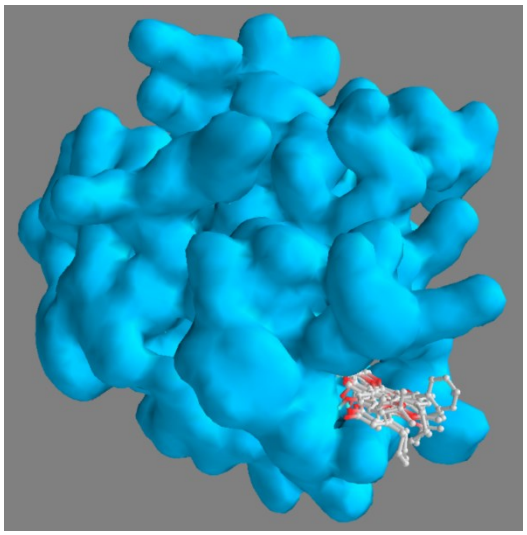

**Figure 3.** Ligand receptor visualization using PyRx. Note that all the five selected phytocompounds dock to the protein in the same pocket (binding site) which is seen in Figure 2 of DS Visualizer (Active site 2 out of 5).

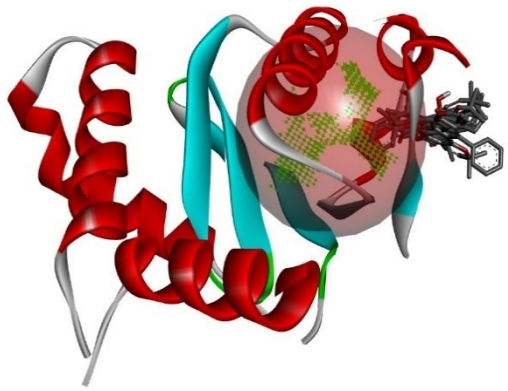

**Figure 4.** Zoomed-up view of active binding site 2 along with the 5 docked phytocompounds (note that all of them bind to the protein in the same receptor cavity).

 

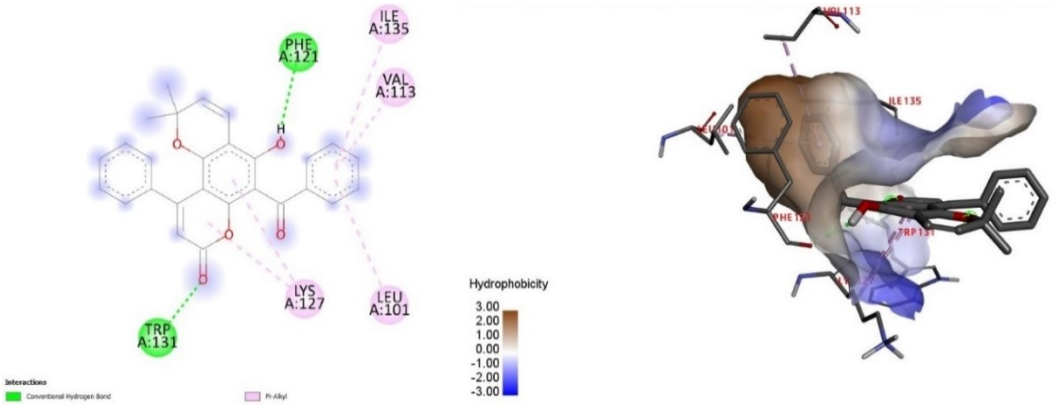


**Figure 5.** Calanone - receptor 2D and 3D interactions.

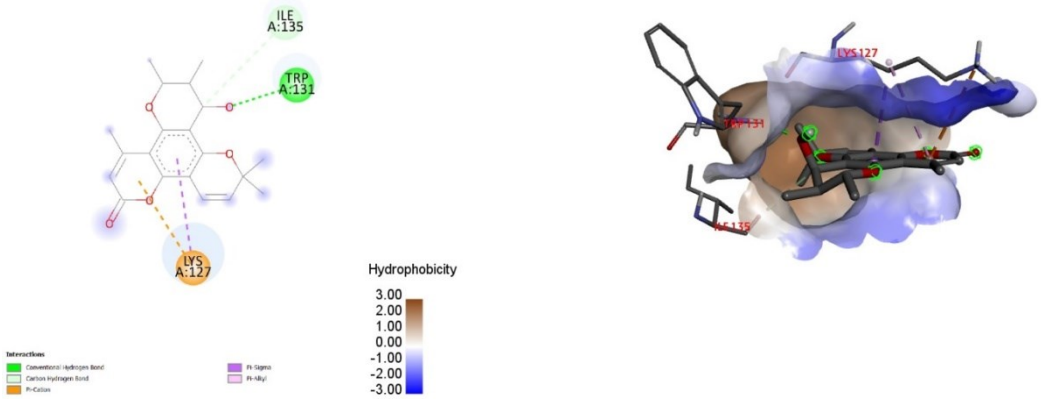


**Figure 6.** (+)-Pseudocordatolide - receptor 2D and 3D interactions.

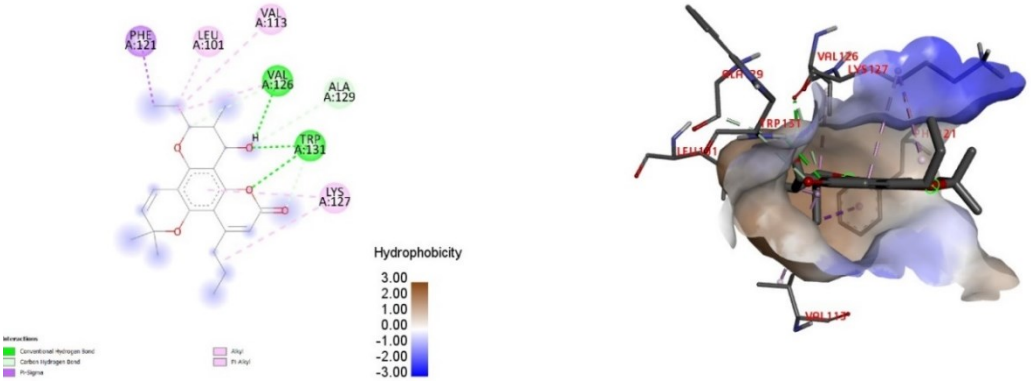


**Figure 7.** CHEMBL267208 – receptor 2D and 3D interactions.

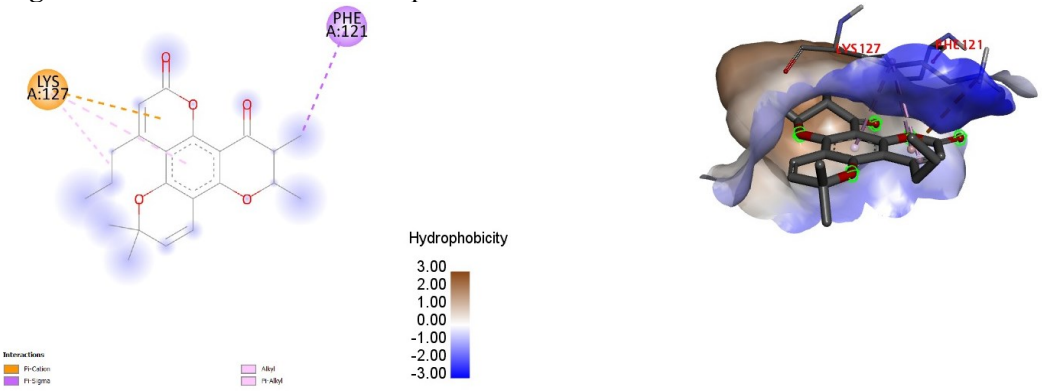


**Figure 8.** Calanolide D - receptor 2D and 3D interactions.

 

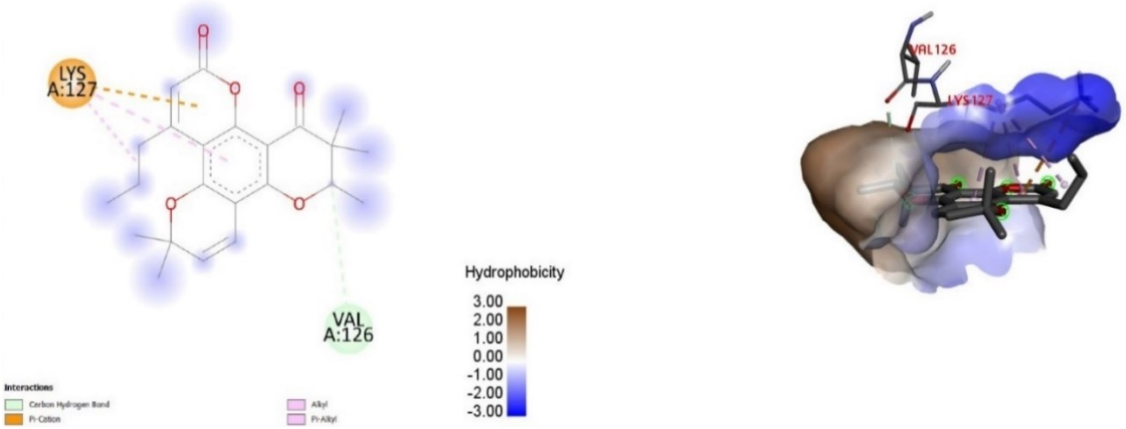


**Figure 9.** CHEMBL7270 - receptor 2D and 3D interactions.

**Drug-likeliness prediction and ADMET analysis**

To discriminate between substances that are more likely to be included in therapeutic formulations and those that are not, Lipinski's rule of five is required. The Lipinski's rule of five was taken into account (Table 2) while predicting the drug-likeliness of the best docking phytocompounds. Swiss-ADME web server was used to execute the ADME (Table 3) and Boiled-Egg analysis (Figure 10). This was done to forecast the likelihood that the molecule would pass through the Blood-Brain Barrier (BBB) and the selected phytocompounds' GI absorption. Additionally, the effects of best-docked substances on the mutagenic Pgp-sub (Permeability Glycoprotein substrate) were evaluated on the standard scale. Table 4 shows Toxicity and Aggregation analysis of the top 5 phytocompounds.

**Table 2.** Lipinski analysis.

| S.N. | Ligand Name | Molecular weight (g/mol) | H-bond donors | H-bond acceptors | MLog(P) | Molar refractivity | Drug likeliness |
|---|---|---|---|---|---|---|---|
| 1 | CHEMBL518283 | 424.44 | 1 | 5 | 3.38 | 123.94 | Yes (0 violations) |
| 2 | CHEMBL507953 | 342.39 | 1 | 5 | 2.4 | 96.5 | Yes (0 violations) |
| 3 | CHEMBL267208 | 384.47 | 1 | 5 | 3.06 | 110.92 | Yes (0 violations) |
| 4 | CHEMBL267693 | 368.42 | 0 | 5 | 2.77 | 105.37 | Yes (0 violations) |
| 5 | CHEMBL7270 | 382.45 | 0 | 5 | 2.98 | 109.92 | Yes (0 violations) |

**Table 3.** ADME analysis.

| S.N. | Ligand name | GI absorption | Pgp-sub | BBB permeant | Carcinogenicity (Probability score) |
|---|---|---|---|---|---|
| 1 | CHEMBL518283 | High | No | No | Active (0.54) |
| 2 | CHEMBL507953 | High | Yes | Yes | Inactive (0.50) |
| 3 | CHEMBL267208 | High | Yes | Yes | Inactive (0.59) |
| 4 | CHEMBL267693 | High | No | Yes | Inactive (0.56) |
| 5 | CHEMBL7270 | High | No | Yes | Inactive (0.56) |

**Table 4.** Toxicity and Aggregation Analysis.

| S.N. | Ligand name | LD50 value (mg/kg) | Toxicity class | Aggregator type* | Probability associated with aggregator type |
|---|---|---|---|---|---|
| 1 | CHEMBL518283 | 2500 | 5 | 0 | 0.425 |
| 2 | CHEMBL507953 | 832 | 4 | 0 | 0.25 |
| 3 | CHEMBL267208 | 1213 | 4 | 0 | 0.061 |
| 4 | CHEMBL267693 | 1679 | 4 | 0 | 0.133 |
| 5 | CHEMBL7270 | 1679 | 4 | 0 | 0.188 |

**NOTE: Class 0 refers to a non-aggregator type of molecule while class 1 refers to an aggregator type of molecule.*

 

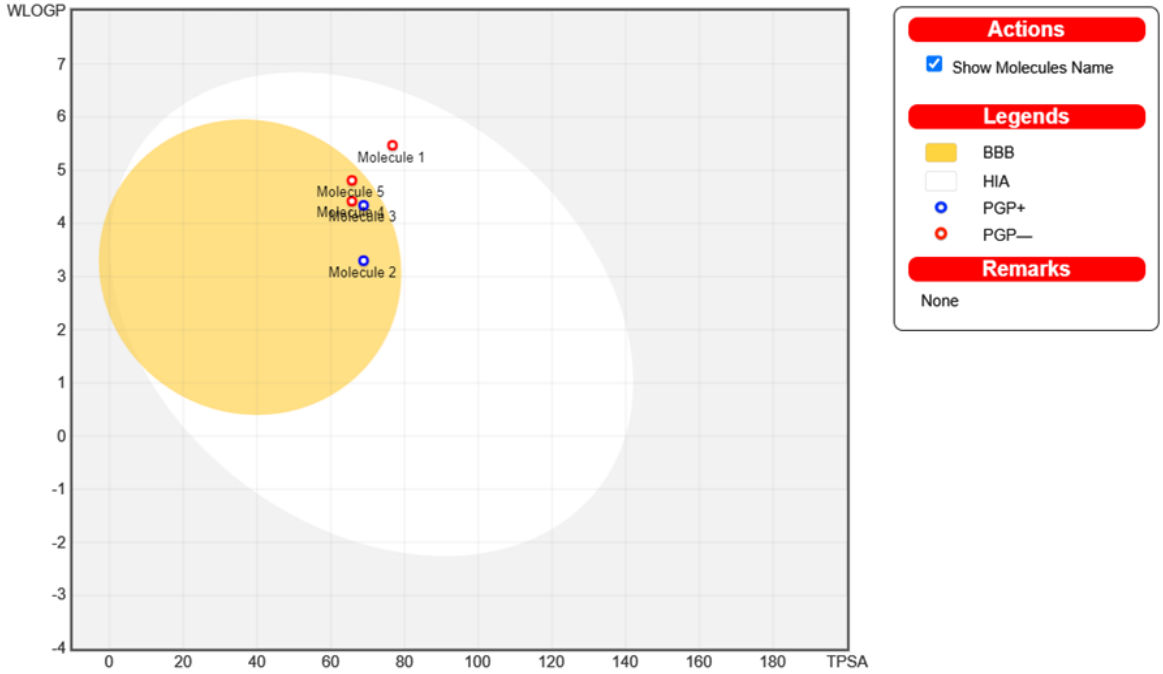


**Figure 10.** Boiled-Egg analysis: Calanone, (+)-Pseudocordatolide C, CHEMBL267208, Calanolide D, CHEMBL7270 (in order from molecule 1 to molecule 5).

## DISCUSSION

A number of in silico techniques, including molecular docking, ADMET analysis, and visualisation of receptor-ligand interactions, have proven to be helpful in analysing binding scores, receptor-ligand interactions, and the inherent stability of the receptor-ligand complex in *in vitro* and *in vivo* assays. To further investigate the interactions with the receptor protein, a total of 5 phytocompounds from C. lanigerum have been discovered. Being a crucial protein necessary for the integration of viral dsDNA (reverse transcribed from RNA) into the human genome, HIV-1 Integrase is a good candidate for the development of therapies.

The structure of HIV-1 Integrase (core domain) was acquired from PDB and purified using Discovery Studio Visualizer for the specific research topic. A total of 52 phytocompounds from *C. lanigerum* were tested against the purified protein for the in silico analysis. *Calanone, (+)-Pseudocordatolide, (10R)-11,12-Dihydro-12alpha-hydroxy-4-propyl-10alpha-ethyl-6,6,11alpha-trimethyl-2H,6H,10H-benzo[1,2-b:3,4-b':5,6-b"]tripyran-2-one, Calanolide D and (+/-)-10,11-Dihydro-6,6,10,11,11-pentamethyl-4-propyl-2H,6H,12H-benzo[1,2-b:3,4-b':5,6-b""] tripyran-2,12-dione* were among the top 5 phytocompounds with the strongest binding affinities.

The five compounds were subsequently examined for an ADMET analysis after the molecular docking investigation to confirm their drug-like and hazardous properties. These phytochemicals' molecular interactions with the receptor have the capacity to suppress Integrase's integration activity, obstruct viral infection in general, and impede viral replication cycles inside host human immunocytes (mainly CD 4+ T cells).

## CONCLUSION

The goal of the current investigation was to identify possible phytocompounds from *C. lanigerum* that could treat HIV-1 infection and limit viral replication that would otherwise cause AIDS. The epidemic of HIV/AIDS commenced in 1981, and it is till date a global public health issue [48]. The HIV-1 Integrase protein is a crucial component needed for the integration of viral DNA into the human genome to complete infection. Targeting this protein with phytocompounds from the medicinal plant, *C. lanigerum*, we can conclude that a total of 5 phytocompounds have the potential to

 

inhibit the activity of HIV-1 Integrase (as described previously). The top 5 phytocompounds with drug-like properties, and permissible ADMET properties may aid in the process of developing inhibitors of HIV-1 Integrase as well as anti-HIV therapeutics.

**Acknowledgement**

I thank the BioNome faculty for their assistance with training and research projects.

**Authors' Contributions**

There is a single author contribution to this paper.

**Conflicts of Interest**

The author declares no conflict of interest.

**Abbreviations**

| | |
|---|---|
| HIV-1 | Human Immunodeficiency Virus Type 1 |
| AIDS | Acquired Immunodeficiency Syndrome |
| HAART | Highly Active Antiretroviral Treatment |
| PDB | Protein Data Bank |
| PDBQT | Protein Data Bank, Partial Charge (Q), and Atom Type (T) format |
| ADMET | Absorption, Distribution, Metabolism, Excretion, Toxicity |
| BBB | Blood Brain Barrier |
| Pgp | P-glycoprotein |
| GI | Gastrointestinal |
| DS | Discovery Studio |
| RMSD | Root Mean Square Deviation |

**List of Amino acids**

| | |
|---|---|
| Trp | Tryptophan |
| Leu | Leucine |
| Ile | Isoleucine |
| Phe | Phenylalanine |
| Gln | Glutamine |
| Val | Valine |
| Lys | Lysine |

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