Journal of TROPICAL BIODIVERSITY E-ISSN : 2774-4116

Therapeutic Potential of Quercetin Derivatives: In Silico Investigation of HIV-1 Protease Inhibition

Husna Abdul Aziz[1], Yeremiah Rubin Camin[1], Vivitri Dewi Prasasty[1]*

Department of Biology, Faculty of Biology and Agriculture, Universitas Nasional, Jakarta

Correspondence Author: vivitri.prasasty@unas.ac.id

Submission	:	January, 09th 2024
Revision	:	March 08th 2024
Publication	:	April 30th 2024

Abstract

Human Immunodeficiency Virus (HIV) type 1 is the predominant strain known for its impact on the immune system and its propensity for mutation. According to the World Health Organization (WHO), the global infection count reached 37.9 million in 2018, with alarming rates of mortality and morbidity. Ongoing drug discovery endeavors encompass various facets, including investigations into HIV protease—a key enzyme in the cleavage process of gag and gag-pol polyprotein chains essential for the genesis of new virions. While numerous studies suggest the inhibitory potential of quercetin against HIV-1, comprehensive exploration regarding its interaction with the HIV-1 protease receptor remains limited. This study aimed to elucidate the therapeutic potential of quercetin derivative compounds as viable candidates for HIV protease inhibition. Employing in silico analysis, molecular docking of 36 quercetin derivative compounds with the HIV-1 protease receptor (code 3SO9) using the Pyrx-Autodock Vina-Open Babel platform was conducted. Prior to docking, ligand preparation was meticulously performed using Autodock Tools 1.5.6, with geometry optimization utilizing Avogadro software. The interaction was assessed through Gibbs free energy (ΔG) scoring, where a more negative ΔG value indicated a stronger binding propensity between the ligand and receptor. The docking results revealed that 22 quercetin derivative compounds exhibited Gibbs energy (ΔG) values lower than the original ligand, darunavir. However, 5 compounds deviated from Lipinski's rule, while 17 compounds adhered to Lipinski's criteria. Consequently, these 17 quercetin derivative compounds exhibit promising potential as candidate drugs for HIV-1 protease inhibition.

Keyword: Quercetin, HIV-1 protease, inhibitor, drug candidate

INTRODUCTION

The Human Immunodeficiency Virus (HIV) belongs to the genus Lentivirus of the Retroviridae family and targets the immune system (Balasubramaniam, Pandhare, & Dash, 2019). It is categorized into two main types, HIV type 1 and HIV type 2, based on genetic characteristics and differences in viral antigens. Among these, HIV type 1 predominantly affects humans and exhibits a high mutation rate, rendering it a dynamic target for therapeutic intervention. Consequently, extensive research efforts, particularly focusing on HIV-1 inhibitors, persist in the pursuit of treatment.

According to the World Health Organization (WHO), as of 2018, there were 37.9 million people globally infected with HIV, with 23.3 million receiving antiretroviral treatment (Eriksen et al., 2020). However, despite treatment efforts, the number of new infections remains substantial, particularly in countries like Indonesia, where HIV cases have been on the rise annually, reaching 48,300 cases in 2017 (Mahadewi, Hilmy, Heryana, & Wiharto, 2021). Such prevalence underscores the significant morbidity associated with HIV.

Current treatments for HIV aim to suppress viral replication by targeting key enzymes involved in the replication process, such as reverse transcriptase, integrase, and protease enzymes (Gill, Hassan, & Ahemad, 2019). Protease inhibitors, introduced in 1995 due to the high mutation rate of reverse transcriptase, play a crucial role in inhibiting the cleavage of polyproteins during viral replication, thereby preventing the formation of new virions. Notably, the combination of reverse transcriptase and protease inhibitors has proven effective in reducing viral load, with darunavir being one of the protease inhibitor drugs commonly used.

With advancements in technology, drug screening processes have evolved, with virtual screening methods, also known as in silico methods, offering significant advantages in terms of time and cost efficiency. Virtual screening for HIV-1 protease inhibitors has gained prominence due to its ability to expedite drug discovery processes and reduce costs (Chuntakaruk et al., 2024). Research has identified quercetin, a natural product compound, as a potential HIV-1 protease inhibitor. However, the interaction of quercetin and its derivatives with the HIV-1 protease receptor remains relatively unexplored.

This study aimed to investigate the binding energy affinity of quercetin derivative compounds as ligands for the HIV-1 protease receptor through molecular docking, utilizing in silico methods. The hypothesis posits that quercetin derivative compounds hold promise as inhibitors of the HIV-1 protease enzyme. By elucidating the conformational energy of interactions between quercetin derivatives and the HIV-1 protease receptor, this research seeks to contribute to the development of HIV-1 protease inhibitor drug candidates derived from natural sources, with the ultimate goal of advancing HIV treatment and patient care.

METHOD

Materials

The materials used in this study consist of two components. Firstly, the three-dimensional structure of HIV-1 protease was retrieved from the Protein Data Bank (www.rcsb.org) with the identity code 3SO9, which is bound to its original ligand, darunavir in .pdb format. Secondly, thirty-six ligands comprising quercetin derivative compound structures retrieved from ZINC docking (https://zinc.docking.org) with the following codes: ZINC100824387, ZINC14952519, ZINC85815592, ZINC95620386, ZINC85815508, ZINC13479087, ZINC3973253, ZINC4096846, ZINC13515662, ZINC59764611, ZINC95620863, ZINC100825220, ZINC100825216, ZINC14644527, ZINC100825198, ZINC4175638, ZINC4349592, ZINC100825193, ZINC14684664, ZINC14644557, ZINC4349687, ZINC59764324, ZINC38428701, ZINC13740559, ZINC14644472, ZINC4096845, ZINC3869685, ZINC517261, ZINC84428502, ZINC14684644, ZINC4654812, ZINC84858038, ZINC6484697, ZINC6484693, ZINC84422547, and ZINC100772239.

Separation of HIV-1 Protease Receptor from Original Ligand

The downloaded three-dimensional structure of the HIV-1 protease macromolecule from the Protein Data Bank is initially bound to the ligand, darunavir, necessitating separation. Using Discovery Studio 2019 client software, this process unfolds as follows: (a) Open the structure within the software. (b) Select Ligands from the Scripts menu, followed by deletion of the ligand via keyboard commands. (c) Save the isolated HIV-1 protease receptor in .pdb format. (d) Reopen the downloaded structure from the protein data bank. (e) Use the Select Protein function to remove the receptor. (f) Save the separated original ligand in .pdb format.

Optimization of HIV-1 Protease Receptor and the Original Ligand (Darunavir)

Optimizing the HIV-1 protease receptor was achieved with Discovery Studio 2019 client software, involving the removal of non-standard residues. These residues typically consisted of water molecules dispersed around the protein structure, which could disrupt binding interactions during docking by potentially forming hydrogen bonds with the ligand. Similarly, optimization of the ligand structure entailed removing water molecules using the same software to eliminate any residual interference.

Preparation of HIV-1 Protease Macromolecule and the Ligand

The preparation of the HIV-1 protease macromolecule, separated from its original ligand, involved treatment using AutoDockTools-1.5.6 software. The preparation includes adding hydrogen molecules to the HIV-1 protease receptor, which is then saved in .pdbq format. Meanwhile, ligand preparation involves adding Gasteiger charges using AutoDockTools-1.5.6 software. After adding Gasteiger charges, torsion adjustment was performed, and then the ligand is saved in .pdbqt format.

Molecular Docking

Thirty-six quercetin derivative compounds undergo molecular docking with the HIV-1 protease macromolecule using Pyrx-Autodock Vina (Dallakyan & Olson, 2015), employing identical grid settings as the docking validation with the original ligand, darunavir. This ensures consistent treatment across validation and test ligand docking processes.

Visualization of Docking Results

Chemical interaction analysis between the quercetin derivatives and HIV-1 protease is conducted using Discovery Studio 2019 client software (18287, 2019) and LigPlot++ (Laskowski & Swindells, 2011)are employed to visualize docking results in two and three dimensions. This includes both the original ligand (for validation) and the docking outcomes for quercetin derivative compounds, facilitating the observation of optimal ligand conformations binding to the HIV-1 protease receptor. Additionally, these tools enable the identification of surrounding amino acid residues and interaction types, providing insight into the revealing potential interactions such as hydrogen bonds and hydrophobic interactions formed between the tested ligands and the HIV-1 receptor and the factors influencing them.

RESULT

Molecular Docking

The results of the molecular docking were first validated by the original ligand (darunavir), the interactions that occurred and the amino acid residues involved around the interactions are presented in Table 2.

Table 2. Results of docking validation against the original ligand (Darunavir)

Ligand	RMSD Value	Gibbs Energy (kcal/mol)	Inhibition Constant (μM)	Hydrogen Bond	Hydrophobic Interaction
Darunavir	0	-8.9	0.29	Asn25(A), Gly48(B)	Ile50B, Gly49B, Gly27A, Gly49A, Val84A, Leu23B, Ala28A, Pro81B, Asp29A, Thr82B, Gly48A, Val32B,

					Val84B, Ile50A, Ile47B, Ala28B, Gly27B, Pro81A

From Table 2, it can be seen that the Root Mean Square Deviation (RMSD) value resulting from the docking between darunavir, and the HIV-1 protease receptor is zero, which indicates the stability of the interaction of the ligand with the receptor as well as the similarity of the structure of the overlapping ligand. The interaction that occurs between the original ligand (darunavir) and the HIV-1 receptor was depicted in Figure 1.

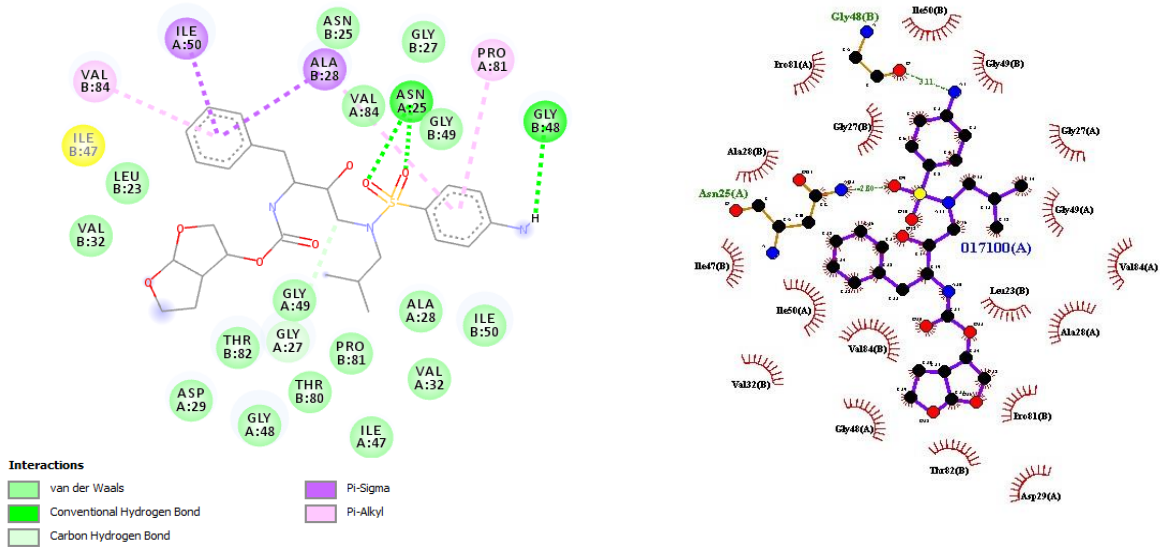

Figure 1. Interaction of Darunavir with HIV-1 Protease

Based on the validation test results, it was found that the best RMSD was 0 Å and the Gibbs energy was -8.9 kcal/mol. The interactions obtained when docking the original ligand (darunavir) with the HIV-1 protease are hydrogen interactions with residues Asn25 on chain A and Gly48 on chain B. Apart from hydrogen interactions, there are also van der wall interactions found on chain A (Gly27, Gly48, Gly49, Val84 , Ala28, Asp29, Ile50, Pro81) and chain B (Ile50, Gly49, Leu23, Pro81, Thr82, Val32, Val84, Ile47, Ala28, Gly27).

Interaction between Quercetin derivative compounds with HIV-1 Protease

The docking process was carried out using the same grid box as the original ligand validation process for 36 quercetin derivative compounds downloaded from the ZINC Docking website (https://zinc.docking.org/substances/search/?q=quercetin) using the Pyrx program -autodock vina and gridbox settings X: 13.6319, Y: -3.2468, Z: -8.7628.

The file format of the quercetin derivative compound will be automatically changed by the Open Babel software or program, then with the same gridbox settings as the docking validation, the docking process for all the test ligands is carried out. The results of the docking are in the form of RMSD values, Gibbs free energy values (ΔG) and ligand conformations in the .pdbqt file format.

From the molecular docking carried out on 36 quercetin derivative compounds, the RMSD and Gibbs energy values were obtained which are presented in Table 3.

Table 3. Molecular docking results for 36 quercetin derivative compounds (RMSD values and Gibbs energy)

No	Quercetin Derivative	RMSD Value	Gibbs Energy (kcal/mol)	Ki (µM)	No	Quercetin Derivative	RMSD Value	Gibbs Energy (kcal/mol)	Ki (µM)
1	ZINC100824387	0	-11.5	0,00	19	ZINC14684664	0	-9.3	0,15
2	ZINC14952519	0	-10.9	0,01	20	ZINC14644557	0	-9.2	0,18
3	ZINC85815592	0	-10.6	0,02	21	ZINC4349687	0	-9.1	0,21
4	ZINC95620386	0	-10.5	0,02	22	ZINC59764324	0	-8.9	0,29
5	ZINC85815508	0	-10.3	0,03	23	ZINC38428701	0	-8.8	0,35
6	ZINC13479087	0	-10.3	0,03	24	ZINC13740559	0	-8.8	0,35
7	ZINC3973253	0	-10.3	0,03	25	ZINC14644472	0	-8.6	0,49
8	ZINC4096846	0	-10.2	0,03	26	ZINC4096845	0	-8.4	0,69
9	ZINC13515662	0	-10.1	0,04	27	ZINC3869685	0	-8.3	0,81
10	ZINC59764611	0	-10.1	0,04	28	ZINC517261	0	-8.3	0,81
11	ZINC95620863	0	-9.9	0,05	29	ZINC84428502	0	-8.3	0,81
12	ZINC100825220	0	-9.9	0,05	30	ZINC14684644	0	-8.0	1,35
13	ZINC100825216	0	-9.9	0,05	31	ZINC4654812	0	-7.8	1,89
14	ZINC14644527	0	-9.7	0,08	32	ZINC84858038	0	-7.8	1,89
15	ZINC100825198	0	-9.5	0,11	33	ZINC6484697	0	-7.6	2,65
16	ZINC4175638	0	-9.5	0,11	34	ZINC6484693	0	-7.4	3,71
17	ZINC4349592	0	-9.5	0,11	35	ZINC84422547	0	-7.3	4,40
18	ZINC100825193	0	-9.4	0,13	36	ZINC100772239	0	-6.0	39,54

The docking results for 36 quercetin derivative compounds as presented in table 3 above show that the quercetin derivative with the code ZINC100824387 has the smallest Gibbs free energy (ΔG), namely -11.5 kcal/mol with the RMSD value was 0 (zero). Gibbs energy (ΔG) showed the strength of the bond between the ligand and the macromolecule or protein with darunavir with the results of docking quercetin derivative compounds are presented in Table 4.

Table 4. Comparison of Darunavir Interactions with Quercetin Derivative Compounds

No	Quercetin Derivative	Gibbs Energy	Hydrogen Bond	Van der waals Interaction	Pi-Sigma/ Pi-anion	Pi-Alkyl
1	Darunavir	-8,9	Asn25A, Gly48B	Leu23B, Asn25B, Gly27A, Gly27B, Ala28A, Asp29A, Val32A, Val32B, Ile47A, Ile47B, Gly48A, Gly49A, Gly49B, Ile50B, Thr80B, Pro81B, Thr82B, Val84A	Ala28B, Ile50A	Pro81A, Val84B
2	ZINC100824387	-11.5	Asn25A, Asn25B, Gly27A, Asp30A, Thr82B	Gly49A, Leu23B, Asn83B, Thr80B, Gly27B, Thr80A, Val32A, Thr31A, Leu76A, Ile47A	Ala28A	Ile50A, Ile50B, Pro81B, Val84A, Val84B
3	ZINC14952519	-10.9	Asn25A, Asp29A	Asp30A, Val32A, Gly49A, Gly49B, Val32B, Val84B, Ala28B, Asn25B, Gly27B, Val84A, Gly27A	-	Ala28A, Ile47A, Ile50A, Ile50B
4	ZINC858155592	-10.6	-	Thr82A, Val84A, Thr80A, Ala28A, Asn25B, Asn25A, Pro81B, Gly27A, Thr80B, Val84B, Ile50A, Thr82B, Gly48B, Val32B, Ile47B, Ala28B, Ile50	-	-
5	ZINC956203866	-10.5	Asp30A, Gly48A	Pro81B, Thr82B, Gly49A, Leu23B, Thr80B, Val84B, Ile50A, Gly27A, Ile50B, Val32A, Asp29A, Arg8B	Ala28A	Ile47A
6	ZINC858155088	-10.3	Ile50A, Arg8B, Thr82A, Gly48A	Pro81B, Val84B, Gly27A, Gly49A, Ile47A, Asn25A, Thr80A, Leu23A, Gly49B, Asp29A, Gly27B, Ala28B, Thr82B, Asn25B	-	Ile50B, Ala28A, Val84A, Pro81A, Leu23B
7	ZINC134790887	-10.3	Ile50A, Asn25A, Gly27B, Gly48A	Gly48B, Pro81A, Thr82A, Leu23A, Asp30A, Leu76A, Ile47A, Gly49A, Ile50A, Asn25B, Ala28B, Val84B, Pro81B, Thr82B, Gly27A	-	Ala28A, Val32A, Val84A

8	ZINC3973253	-10.3	Asn25A, Ile50B, Thr82B, Gly48A	Asp30A, Asn25B, Val32A, Gly48B, Ile47B, Ala28B, Gly49B, Val32B, Pro81A, Gly27B, Val84A, Val84B, Thr80B, Gly49A, Gly27A	Asp29A	Ile47A, Ala28A, Pro81B, Ile50A
9	ZINC4096846	-10.2	Asn25A, Asn25B, Gly27B	Arg8B, Leu23B, Thr82B, Gly48B, Ala28B, Gly49B, Leu23A, Pro81A, Ile50B, Thr82A, Val84A, Ala28A, Gly27A, Gly49A, Ile47A	Asp29A	Ile50A, Val84B
10	ZINC13515662	-10.1	Asp29A, Ile50B	Leu46A, Thr82, Pro81, Ile50A, Gly49A, Val32A, Ala28A, Ile47A, Asp30A, Gly48A	-	-
11	ZINC59764611	-10.1	Ile50A	Gly49A, Asn25B, Gly27A, Val84B, Ala28B, Asp29B, Gly48B, Asp30B, Gly49B, Asn25A, Gly27B	-	-
12	ZINC95620863	-9.9	Thr80A	Val84, Ile50B, Pro81B, Thr82B, Val84B, Ile50A, Gly48A, Gly49A, Ala28A, Asp30A. Val32A	-	-
13	ZINC100825220	-9.9	-	Asp30A, Gly48A, Val32A, Val84A, Ile50B, Thr80A, Pro81A, Thr82A, Asn25A, Thr82B, Val84B, Pro81B, Gly27A, Ile50A, Gly49A, Ala28A, Ile47A	-	-
14	ZINC100825216	-9.9	Asp29A, Asp30A, Thr82A	Ile47A, Gly49B, Ile50B, Val32A, Gly27B, Leu23A, Pro81A, Thr80A, Val84A, Asn25A, Asn25B, Val32B, Thr80B, Pro81B, Thr82B, Gly27A, Gly49A	Ala28A	Ile50A, Ala28B, Val84B

15	ZINC14644527	-9.7	Gly48B	Ile47B, Asn25B, Val32B, Val84B, Thr80B, Pro81B, Leu23B, Thr82B, Gly48A, Gly27A, Gly49A, Asp29A, Ile47A, Asp30A, Val32A, Val84A, Asn25A, Gly49B	Ile50A	Ala28A, Ile50B
16	ZINC100825198	-9.5	Asn25B, Asp29A, Asp30A	Gly27A, Gly27B, Val84B, Val32B, Gly48B, Ile47B, Gly49B, Gly49A, Gly48A, Ala28A, Ile47A, Ala28A, Val32A, Val84A	Ile50A	Ala28B, Ile50B
17	ZINC4175638	-9.5	Ile50B, Asp29A, Asp30A, Gly48A	Gly48B, Pro81A, Gly49B, Asn25B, Ile50B, Asn25A, Gly27B, Gly27A, Ala28A, Ile47A, Val32A, Val84A, Gly49A, Val32B, Asp30B, Leu76B	Ala28B	Ile47B, Ile50A
18	ZINC4349592	-9.5	Ile50A	Val84A, Asn25A, Gly49A, Pro81B, Arg8B, Leu23B, Arg87A, Gly48A, Gly27A, Ile47A, Ala28A, Asp30A, Val32A, Ile50B, Pro81A, Gly49B, Gly27B, Ala28B	Thr82B, Asp29	-
19	ZINC100825193	-9.4	Gln58B, Thr74B, Gln92B, Asp30B, Asp29B, Arg87B, Leu5A	Lys45B, Thr91B, Trp6A, Asn88B, Gln61B, Gly73B, Ile72B, Leu89B, Asp60B	-	-
20	ZINC14684664	-9.3	Asn25A, Asn25B, Gly27A, Thr82A	Leu23B, Gly49A, Ala28A, Val84A, Ile50B, Leu23, Arg8A, Asp29B, Gly48B,	-	Val84B, Ile50A, Pro81B, Ala28B

				Pro81A, Gly49B, Thr80B		
21	ZINC14644557	-9.2	Asp29A, Asp30A, Thr82A, Ile50B	Gly49B, Gly48B, Thr82B, Pro81B, Val84B, Asn25B, Leu23B, Gly49A, Asn25A, Val32A, Thr31A, Leu76A, Ile47A, Thr80A		Ala28A, Pro81A, Val84A
22	ZINC4349687	-9.1	Gly27A, Asn25B, Asp29A, Gly48A	Leu23B, Thr82B, Ala28A, Pro81B, Gly49A, Ile47A, Asp30A, Val84A, Thr80A, Asn25A, Pro81A, Thr82A	Ile50B	Val84B, Ile50A
23	ZINC59764324	-8.9	Thr82A, Asn25B, Gly48B, Asn25A Arg8A, Gly27A Ile50A	Gly27B, Pro81A, Val84A, Gly49B, Ile50B, Asp29B, Leu76B, Asp30B, Val84B, Gly49A, Ala28A, Leu23B, Arg87B	-	Ala28B, Ile47B

Table 4 showed a comparison of the docking results of darunavir as the original ligand with the docking results of quercetin derivative compounds. In docking darunavir against the HIV-1 protease, the Gibbs free energy (ΔG) result was -8.9 kcal/mol with an RMSD of 0, which means there was structural similarity as measured based on the distance of similar atoms. Meanwhile, in the docking results of quercetin derivative compounds, the results obtained for 21 compounds showed that the Gibbs energy was more negative (ΔG < -8.9 kcal/mol) compared to the Gibbs free energy (ΔG) found in darunavir as a control HIV-1 protease inhibitor, compounds- These compounds are quercetin with ZINC100824387, ZINC14952519, ZINC85815592, ZINC95620386, ZINC85815508, ZINC13479087, ZINC3973253, ZINC4096846, ZINC13515662, ZINC597646 11, ZINC95620863, ZINC100825220, ZINC100825216, ZINC14644527, ZINC100825198, ZINC4175638, ZINC4349592, ZINC100825193, ZINC14684664, ZINC14644557, ZINC4349687, ZINC59764324, ZINC38428701 , and there is one quercetin derivative compound which has a Gibbs energy whose Gibbs energy is the same as the energy found in darunavir (-8.9 kcal/mol), namely quercetin with the code ZINC59764324, while fourteen other quercetin derivative compounds have a higher binding affinity of Gibbs free energy.

Docking darunavir with the HIV-1 protease receptor, there are several interactions that occur, including two hydrogen bonds that occur in both polyprotein chains with the Asn25 amino acid residue in chain A and the Gly48 amino acid residue in chain B. Another bond that is formed is the hydrophobicity characterized by van der Waals interactions, pi-sigma bonds, and pi-alkyl. The interactions that occur in the docking of quercetin derivative compounds with the HIV-1 protease receptor are almost the same as the interactions that occur in docking the original ligand, mostly hydrogen bonds, van der Waals interactions, pi-sigma and pi-alkyl bonds are found in the interactions of quercetin derivative compounds with HIV-1 protease receptor.

A comparison of the interactions that occur between the docking results of the original ligand (darunavir) and one of the test ligands is shown in Figure 2.

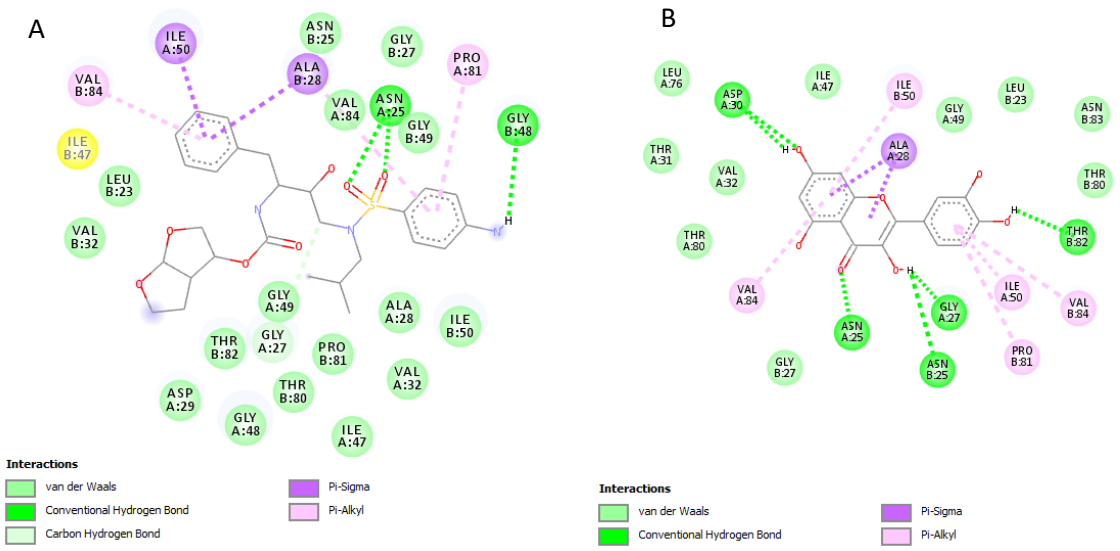

Figure 2. A: Interactions that occur with Darunavir; B: Interaction with one of the quercetin derivative compounds code ZINC100824387

The investigation of the physicochemical properties and drug likeness of the original ligand and the test ligand was carried out using the SwissADME webtools (http://www.swissadme.ch/index.php) and the results are presented in Table 5 and Table 6.

Table 5. Comparison of the physicochemical properties of darunavir with HIV-1 protease inhibitor compounds

No	Quercetin Derivative	Molecular Weight (g/mol)	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Log P	TPSA (Å²)
1	Darunavir	547.66	8	3	2.47	148.80
2	ZINC100824387	302.24	7	5	0	131.36
3	ZINC14952519	316.26	7	4	0	120.36
4	ZINC85815592	464.38	12	8	2	210.51
5	ZINC95620386	464.38	12	8	2	210.51
6	ZINC85815508	610.52	16	10	3	269.43
7	ZINC13479087	448.38	11	7	2	190.28
8	ZINC3973253	506.41	13	7	3	216.58
9	ZINC4096846	464.38	12	8	2	210.51
10	ZINC13515662	550.42	15	8	3	253.88
11	ZINC59764611	360.36	7	1	0	83.45
12	ZINC95620863	360.36	7	1	0	83.45

13	ZINC100825220	478.36	13	8	2	227.58
14	ZINC100825216	478.36	13	8	2	227.58
15	ZINC14644527	434.35	11	7	2	190.28
16	ZINC100825198	382.30	10	5	0	183.11
17	ZINC4175638	478.36	13	8	2	227.58
18	ZINC4349592	492.43	12	6	2	188.51
19	ZINC100825193	506.41	13	7	3	216.58
20	ZINC14684664	434.35	11	7	2	190.28
21	ZINC14644557	448.38	11	7	2	190.28
22	ZINC4349687	434.35	11	7	2	190.28
23	ZINC59764324	550.42	15	8	3	253.88

Table 6. Drug likeness properties of quercetin derivative compounds

No	Quercetin Derivative	Solubility (Log S)	Lipinski's Rule Violation	Bioavailability Score
1	Darunavir	-4.46	1	0.55
2	ZINC100824387	-3.16	0	0.55
3	ZINC14952519	-3.36	0	0.55
4	ZINC85815592	-3.04	2	0.17
5	ZINC95620386	-3.04	2	0.17
6	ZINC85815508	-3.30	3	0.17
7	ZINC13479087	-3.33	2	0.17
8	ZINC3973253	-3.15	3	0.17
9	ZINC4096846	-3.64	2	0.17
10	ZINC13515662	-3.08	3	0.11
11	ZINC59764611	-3.64	0	0.55
12	ZINC95620863	-3.64	0	0.55
13	ZINC100825220	-3.27	2	0.11
14	ZINC100825216	-3.27	2	0.11
15	ZINC14644527	-3.27	2	0.17
16	ZINC100825198	-3.07	0	0.11
17	ZINC4175638	-3.27	2	0.11
18	ZINC4349592	-3.13	2	0.17
19	ZINC100825193	-3.15	3	0.17
20	ZINC14684664	-2.99	2	0.17
21	ZINC14644557	-3.33	2	0.17
22	ZINC4349687	-3.27	2	0.17
23	ZINC59764324	-3.08	3	0.11

The results of the physicochemical analysis of darunavir have a molecular weight of more than 500 Daltons, three hydrogen bond donors, eight hydrogen bond acceptors and an octanol/water partition coefficient of 2.47, which means there is 1 deviation from Lipinski's rule and this shows that darunavir has high permeability as a drug. The results of physicochemical analysis of 22 quercetin derivative compounds which have the most negative binding affinity compared to the original ligand (darunavir), it was found that five compounds had 3 deviations from the Lipinski rule, namely quercetin derivative compounds with codes ZINC85815508, quercetin ZINC3973253, quercetin ZINC13515662, quercetin ZINC100825193 and quercetin ZINC5976 4324, twelve quercetin derivative compounds with 2 deviations from Lipinski's law, namely ZINC85815592, ZINC95620386, ZINC13479087, ZINC4096846, ZINC100825220, ZINC100825216, ZINC14644527, ZINC4175638, ZINC4349592, Z INC14684664, ZINC14644557, ZINC4349687 and 5 quercetin derivative compounds that do not have deviations from Lipinski's law, namely quercetin with code ZINC100824387 , ZINC14952519, ZINC59764611, ZINC95620863, ZINC100825198.

DISCUSSION

HIV-1 protease is an enzyme in the HIV-1 virus which plays a role in the maturation of new viruses by cutting polyprotein chains. This enzyme is a homodimer and consists of two identical polyprotein chains connected noncovalently and consisting of 99 amino acids (Tambani, 2019). The active site of this enzyme is found between dimers containing aspartate catalytic residues where each residue contains subunits to form two motifs Asp-25-Thrs2 and Gly27. The active site of this enzyme contains a site where the inhibitor can bind tightly (Sousa, Tamames, Fernandes, & Ramos, 2011).

Protease inhibitors are one of the HIV drugs that work by binding to the protease enzyme which plays an important role in the development and replication of the virus in the body. This protease enzyme functions to cut the polyprotein chains on the gag and gag-pol sides during the virus maturation process. These protease inhibitor drugs work in the final phase of virus replication and their effect on HIV is stronger than reverse transcriptase inhibitors (Sluis-Cremer & Tachedjian, 2008).

In silico analysis is a computer-based method used to describe the interaction of a molecule as a ligand with a protein or receptor in drug discovery studies so that the length of time for the search process and the costs incurred can be reduced (Sarkar & Sen, 2020). The docking validation used as a reference is the docking between the original ligand, namely darunavir, and the HIV-1 protease receptor.

In this study, thirty-six quercetin derivative compounds were tested which were taken from the ZINC Docking database (https://zinc.docking.org) and these compounds were docked. Previous study regarding flavonoid activity against HIV-1 reported that quercetin has HIV-1 inhibitory activity of >64% (Pasetto, Pardi, & Murata, 2014).

Quercetin belongs to flavonoids, where flavonoids are phenolic compounds that have a basic carbon framework consisting of 15 carbon atoms with two benzene rings strongly connected to a propane chain forming a C6-C3-C6 arrangement. In general, flavonoids are divided into 4 large classes, namely flavones, flavonols, flavonones and flavanols. The most abundant flavonoid compounds in nature are the falvonol group. This compound occurs in various ways in nature depending on the position of the OH group on the phonol (Bose, Sarkar, Bose, & Mandal, 2018). Quercetin is the most common type of flavonoid found in nature and has antiviral capabilities, so it has the potential to fight the HIV-1 virus (Septembre-Malaterre et al., 2022)

Docking is carried out using the Pyrx, a virtual screening tools program which is a program for carrying out virtual screening in which there are several programs for the docking process such as the

Autodock Tools program which is used to prepare ligands and macromolecules before docking, the Autodock Vina program which is used to carry out docking, and the docking program. Open Babel is used to convert protein or ligand file formats, so that the existing format can be adapted to the needs of the Autodock Vina program when docking.

The more negative the energy value, the greater the value of the bond formed between the ligand and receptor on the macromolecule (Arefin et al., 2021) while the RMSD value shows the distance between an atom in one conformation and the nearest atom that has the same type as that atom in another conformation. The smaller the RMSD value indicates the better the position of the ligand formed because it is closer to the original conformation of the ligand (Liu, Watanabe, & Kokubo, 2017).

In general, the inhibition constant (Ki) value indicates the concentration required for a ligand to inhibit a macromolecule or protein. The smaller the inhibition constant (Ki) value, the better the ligand (Costa et al., 2018). The inhibition constant value is calculated using the formula (Morris et al., 1998):

$$Ki = Kd = \exp^{(\Delta G/RT)}$$

Where:

ΔG = Gibbs free energy (kcal/mol)
R = Gas Constant (1.986 kcal/mol)
T = Temperature (25°C = 298 K)

By using the equation above, the results of the inhibition constant values for each quercetin derivative compound are obtained and are presented in Table 3 and it can be said that the majority of quercetin derivative compounds have a conformation close to the original ligand.

In previous research conducted by Pasetto et al. (2014) showed that quercetin has inhibitory activity against HIV-1. Inhibitory activity is proven by the increasingly negative Gibbs energy, which indicates the stronger the bond between the ligand and the HIV-1 protease receptor. Based on the data in Table 4 above, there are 22 quercetin derivative compounds that have potential as HIV-1 protease inhibitors.

Gibbs free energy is influenced by changes in enthalpy and entrophy (Morris et al., 1998), which means that the binding affinity that occurs cannot be separated from the influence of the interaction of molecules on the surface of the ligand and the HIV-1 protease receptor, the bonds formed can be hydrogen or other bonds that influence each other, such as van der Waals interactions and other interactions. The more interactions that occur, the stronger the bond affinity formed between the ligand and the receptor (Bernaldez, Billones, & Magpantay, 2018).

The interactions that occurred between the HIV-1 protease receptor and the ligand were analyzed using the Discovery Studio 2019 software. Apart from being free to use, this software was chosen to also be able to visualize interactions both in 2D and 3D. The interactions formed between the ligand and the receptor contribute to the increase in binding affinity that occurs (Bernaldez et al., 2018).

In relation to being a drug candidate, we do not only pay attention to the energy affinity and binding that occurs between the ligand and the receptor, but we also need to pay attention to the physicochemical properties to assess and estimate the absorption, distribution, metabolism, excretion (ADME) process of the drug candidate (Lombardo et al., 2017).

The physicochemical properties of a compound are very important because they can quickly estimate the pharmacokinetic process (ADME), especially in the absorption of drug compounds by the body (Lombardo et al., 2017). ADME includes the process of drug absorption by the body (absorption), distribution of drugs in the body (distribution), drug metabolism in the body

(metabolism) and excretion of drugs from the body (excretion). Apart from that, a compound is said to have high permeability if it meets 2 or more criteria of Lipinski's rule, namely first, the molecular weight is not more than 500 daltons, the octanol/water partition coefficient (Log P) is less than 5, the hydrogen bond donor is not more than 5, and hydrogen bond acceptors no more than 10 (Lipinski; Roskoski Jr, 2023).

Molecules that have good solubility are an advantage in drug development because these physicochemical properties are very important in the pharmacokinetic process, especially for drugs administered orally and parenteral drugs that must be very soluble in water. Solubility assessment is carried out computationally using the SwissADME webtools with the Estimated SOLubility (ESOL) method developed by Delaney with assessment criteria not exceeding 6 (Daina, Michielin, & Zoete, 2017). The partition coefficient between n-octanol and water is a lipophilicity parameter which is a very important compound design because it is related to the absorption and protein binding processes (Bahmani, Saaidpour, & Rostami, 2017) with a value ranging from −0.7 to 6.0. In this study, both the original ligand and the test ligand (quercetin derivative compound) had a score of no more than 6, which means that 22 of these compounds were classified as compounds that had good solubility.

The molecular weight of a compound greatly influences the permeability of a drug. Apart from being influenced by molecular weight, permeability is also influenced by hydrogen bond acceptors and hydrogen bond donors. The larger the hydrogen bond acceptor and donor, the worse the ligand permeability will be (Lipinski). So, it will affect the process of absorption and distribution of the drug itself. Topological polar surface area (TPSA) is the area of all polar surfaces of a drug compound. The TPSA value can be used to optimize the ability of drugs to penetrate cell membranes (de Oliveira, Santana, Josino, Lima e Lima, & de Souza de Sales Júnior, 2021).

After absorption, the drug will of course be metabolized and distributed throughout the body so that the effect of administering the drug can be achieved and of course this process is influenced by the physicochemical properties of a drug such as molecular weight, hydrogen bond acceptor and donor, TPSA as well as the drug's solubility properties and score. This can be shown by the bioavailability value. In general, the bioavailability value shows a measurement of the rate at which the active ingredient is absorbed from a drug at the site of action with a score of at least 10% with oral administration of the drug (Daina et al., 2017). Seen from Table 4, both the original ligand (darunavir) and the test ligand (quercetin derivative compound) using the computational method using SwissADME have a bioavailability value of > 10%.

High permeability affects the excretion process, the higher the permeability of a substance, the easier it will be excreted. Meanwhile, permeability itself is influenced by the physicochemical properties of a substance (Zhao, Ukidve, Krishnan, & Mitragotri, 2019). Judging from the physicochemical properties presented in Table 3 and the drug likeness properties presented in Table 4, it can be seen that of the 22 quercetin derivative compounds that have the smallest Gibbs free energy, there are 17 quercetin derivative compounds that have good permeability, seen from the number of deviations from Lipinski's law. This shows that the 17 quercetin derivative compounds fulfill Lipinski's rules and have high binding energy affinities, making them potential candidates for HIV-1 protease inhibitors.

CONCLUSION

The in silico study on 36 quercetin derivative compounds with the HIV-1 protease receptor resulted significant findings, where 22 of these compounds exhibited higher binding energy affinity than the original ligand, darunavir, while 17 compounds showed potential as HIV-1 protease inhibitors based on Gibbs free energy (ΔG) and adherence to Lipinski rules. Future investigations could entail in vitro tests to assess the inhibitory activity of these compounds identified through in silico analysis against the HIV-1 protease.

REFERENCES

18287, B. D. S. C. v. (2019). Accelrys Discovery Studio. In: Accelrys Software Inc. San Diego, CA, USA.

Arefin, A., Ema, T. I., Islam, T., Hossen, M. S., Islam, T., Al Azad, S., . . . Alam, N. U. (2021). Target specificity of selective bioactive compounds in blocking α-dystroglycan receptor to suppress Lassa virus infection: an in silico approach. Journal of biomedical research, 35(6), 459.

Bahmani, A., Saaidpour, S., & Rostami, A. (2017). A simple, robust and efficient computational method for n-octanol/water partition coefficients of substituted aromatic drugs. Scientific reports, 7(1), 5760.

Balasubramaniam, M., Pandhare, J., & Dash, C. (2019). Immune control of HIV. Journal of Life Sciences (Westlake Village, Calif.), 1(1), 4.

Bernaldez, M., Billones, J., & Magpantay, A. (2018). In silico analysis of binding interactions between GSK983 and human DHODH through docking and molecular dynamics. Paper presented at the AIP Conference Proceedings.

Bose, S., Sarkar, D., Bose, A., & Mandal, S. C. (2018). Natural flavonoids and its pharmaceutical importance. The pharma review, 94, 61-75.

Chuntakaruk, H., Boonpalit, K., Kinchagawat, J., Nakarin, F., Khotavivattana, T., Aonbangkhen, C., . . . Rungrotmongkol, T. (2024). Machine learning-guided design of potent darunavir analogs targeting HIV-1 proteases: A computational approach for antiretroviral drug discovery. Journal of Computational Chemistry.

Costa, J. d. S., Ramos, R. d. S., Costa, K. d. S. L., Brasil, D. d. S. B., Silva, C. H. T. d. P. d., Ferreira, E. F. B., . . . Santos, C. B. R. d. (2018). An in silico study of the antioxidant ability for two caffeine analogs using molecular docking and quantum chemical methods. Molecules, 23(11), 2801.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports, 7(1), 42717.

Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. Chemical biology: methods and protocols, 243-250.

de Oliveira, E. C. L., Santana, K., Josino, L., Lima e Lima, A. H., & de Souza de Sales Júnior, C. (2021). Predicting cell-penetrating peptides using machine learning algorithms and navigating in their chemical space. Scientific reports, 11(1), 7628.

Eriksen, J., Carlander, C., Albert, J., Flamholc, L., Gisslén, M., Navér, L., . . . Sönnerborg, A. (2020). Antiretroviral treatment for HIV infection: Swedish recommendations 2019. Infectious Diseases, 52(5), 295-329.

Gill, M. S. A., Hassan, S. S., & Ahemad, N. (2019). Evolution of HIV-1 reverse transcriptase and integrase dual inhibitors: Recent advances and developments. European journal of medicinal chemistry, 179, 423-448.

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. In: ACS Publications.

Lipinski, C. A. Lipinski's rule of five.

Liu, K., Watanabe, E., & Kokubo, H. (2017). Exploring the stability of ligand binding modes to proteins by molecular dynamics simulations. Journal of computer-aided molecular design, 31, 201-211.

Lombardo, F., Desai, P. V., Arimoto, R., Desino, K. E., Fischer, H., Keefer, C. E., . . . Broccatelli, F. (2017). In Silico absorption, distribution, metabolism, excretion, and pharmacokinetics (ADME-PK): utility and best practices. an industry perspective from the international

consortium for innovation through quality in pharmaceutical development: miniperspective. Journal of Medicinal Chemistry, 60(22), 9097-9113.

Mahadewi, E. P., Hilmy, M. R., Heryana, A., & Wiharto, M. (2021). Learning Assistance And Capacity Building For Outreach To Volunteers And People With Hiv-Aids Ngo Jakarta Plus Center In Cirebon Indonesia. International Journal of Educational Research and Social Sciences (IJERSC), 2(5), 1246-1254.

Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., & Olson, A. J. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry, 19(14), 1639-1662.

Pasetto, S., Pardi, V., & Murata, R. M. (2014). Anti-HIV-1 activity of flavonoid myricetin on HIV-1 infection in a dual-chamber in vitro model. PLoS One, 9(12), e115323.

Roskoski Jr, R. (2023). Rule of five violations among the FDA-approved small molecule protein kinase inhibitors. Pharmacological Research, 106774.

Sarkar, A., & Sen, S. (2020). A comparative analysis of the molecular interaction techniques for in silico drug design. International Journal of Peptide Research and Therapeutics, 26, 209-223.

Septembre-Malaterre, A., Boumendjel, A., Seteyen, A.-L. S., Boina, C., Gasque, P., Guiraud, P., & Sélambarom, J. (2022). Focus on the high therapeutic potentials of quercetin and its derivatives. Phytomedicine Plus, 2(1), 100220.

Sluis-Cremer, N., & Tachedjian, G. (2008). Mechanisms of inhibition of HIV replication by non-nucleoside reverse transcriptase inhibitors. Virus research, 134(1-2), 147-156.

Sousa, S. r. F., Tamames, B., Fernandes, P. A., & Ramos, M. J. (2011). Detailed atomistic analysis of the HIV-1 protease interface. The Journal of Physical Chemistry B, 115(21), 7045-7057.

Tambani, T. (2019). Overexpression and structure-function characterization of HIV-1 Subtype C. reverse transcriptase and protease.

Zhao, Z., Ukidve, A., Krishnan, V., & Mitragotri, S. (2019). Effect of physicochemical and surface properties on in vivo fate of drug nanocarriers. Advanced drug delivery reviews, 143, 3-21.