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In Silico Analysis of Flavonol Compound Against Mpro COVID-19

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Abstract. Mpro (main protease) is a protein that mediates the replication and transcription processes in viruses. Flavonols are flavonoid-derived compounds derived from Indonesian natural ingredients with potent antibacterial, anticancer, and antioxidant properties. This study aims to use molecular docking to investigate flavonol's potential as an inhibitor of SARS-CoV-2 main protease. The ligand structure was drawn using Avogadro, while the protein structure was retrieved from www.rcsb.org using the protein data bank (PDB) ID 6LU7. The docking method is validated by redocking the protein and native ligands to get an RMSD value of less than 2 Å. Bond energy for re-docking of native N3 ligands was -9.34 kcal/mol, and bond energy for molecular docking of flavonol compounds ranged from -8.51 to -8.47 kcal/mol. Based on bond energy value, compounds with Mpro inhibitor potential were 4',7-dihydroxy 3-ethoxy flavonol compounds.

INTRODUCTION

COVID-19 is a disease caused by infection with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is genomically similar to SARS-CoV and MERS, which appeared several years ago [1]. The main protease (Mpro) in SARS-CoV-2 is an enzyme that mediates viruses replication and transcription processes [2]. This Mpro generates new viral proteins by cutting polyproteins previously translated by RNA. Inhibiting the action of the Mpro enzyme is one technique to stop it from operating. Because no enzyme in the human body is equivalent to Mpro, this inhibitory process will not interfere with the functioning of other enzymes. The virus cannot multiply in the human body due to this inhibitory process [3].

Many investigations have now been conducted to discover chemical substances that can inhibit Mpro. Jin [2] found that molecular docking could reduce Mpro SARS-CoV-2 activity and that N-[(5-Methylisoxazol-3-Yl)Carbonyl]Alanyl-L-Valyl-N~1~-((1r,2z)-4-(Benzyloxy)-4-Oxo-1-{[(3r)-2-Oxopyrrolidin-3-Yl]Methyl}But-2-Enyl)-L-Leucinamide (N3) synthesis compounds could inhibit Mpro SARS-CoV-2 activity. In addition, Arora [4] has conducted further molecular docking experiments. This study discovered that numerous flavonoid derivative compounds and 21 flavonoid compounds were suspected to be potential inhibitors of Mpro SARS-CoV-2.

Flavonol compounds can be used as antiviral agents that target the SARS-CoV-2 enzyme [5]. Flavonoid molecules are secondary metabolites found in many plants and play a key role in their growth. Flavonoids are potent antioxidants, anti-inflammatory agents, anticancer agents, antibacterial, antifungal, and antiviral agents. Flavonoid compounds have been explored in vivo and in vitro for their antiviral properties [6,7].

The research that uses the in-silico method to predict the affinity and binding activity between drug candidates and proteins is molecular docking [8]. Researchers can use docking to examine compounds or ligands and their interactions with receptors or proteins by identifying the best possible active sites to obtain the optimum ligand-receptor (protein) complex geometry [9]. This method offers various advantages, including conserving

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research funds and eliminating the need for extra tools and supplies [10]. In this study, the binding of flavonols with the Mpro SARS-CoV-2 was carried out by docking molecules.

RESEARCH METHOD

Ligand and Protein Preparation

The SARS-CoV-2 main protease model was obtained from the protein database (www.rscb.org). A protein with the PDB code ID 6LU7 was chosen as a model, and the inhibitor N3 was removed. The N3 ligand was used as a control for method validation. Flavonol compounds as test ligands were prepared by visualization using Avogadro 2.4.1 [11] and geometric optimization using a quantum chemistry program called ORCA [12].

Molecular Docking

Autodock software [13] was utilized in all the docking experiments, with the optimized model as the docking target. Flavonols are known as compounds that have antibacterial and antiviral activity, so they are used in this study for further studies of SARS-CoV-2. Before docking the ligand test against the SARS-CoV-2 target protein, the structures of small molecules were optimized using the quantum mechanics B3LYP method.

The grid box (60 Å x 60 Å x 60 Å) centered at (-29.059; 9.486; 61.528) Å [14] of the protein were used in docking and re-docking calculation by utilizing Autodock tools and Autodock 4.2. The validation of the docking method was carried out to show that the test ligand could inhibit the protein. Hydrogen bonding and atomic interaction were determined using Ligplot programs [15].

RESULTS AND DISCUSSION

Re-docking Protein with Native N3 Ligand

A re-docking procedure was used to reconnect N3 natural ligand and Mpro protein, which were previously detached during protein synthesis. The act of re-docking validates the docking method [16]. The docking validation parameter is based on the bond energy value with the lowest RMSD value < 2 Å [4].

The RMSD is the atomic distance from one conformation to the nearest atom of the same type as another conformation. The smaller the RMSD value, the closer the ligand position to the initial position [17].

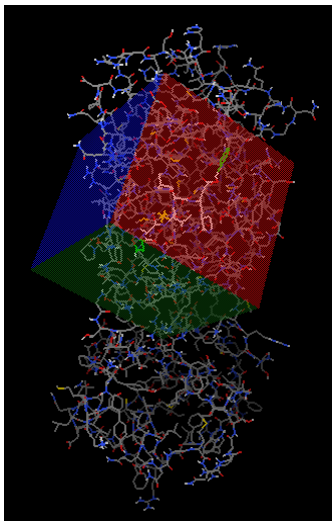

FIGURE 1. Grid box Mpro

The purpose of re-docking is to locate the protein's grid box or active site to make the protein docking process with the test ligand easier. This grid box includes setting the location and spacing box (Å) [18]. The grid box also serves to limit the movement of the ligands so that it will be easier for the ligands to find the active site of the protein.

TABLE 1. RMSD value from re-docking protein 6LU7 with N3 ligand

Sub Rank	Binding Energy (kcal/mol)	Reference Energy (kcal/mol)
1	-9.34	1.03
2	-9.29	1.0
3	-9.29	0.99
4	-9.29	1.0
5	-9.27	1.02
6	-9.15	0.98
7	-9.09	1.05
8	-9.06	1.07
9	-9.00	1.11
10	-8.98	1.09

Based on the RMSD table above, it is found that the bond energy produced is low up to -9.34, and the resulting RMSD value in all conformations is < 2. In addition, the number of hydrogen bonds formed and the type of residue can be seen from the following visualization.

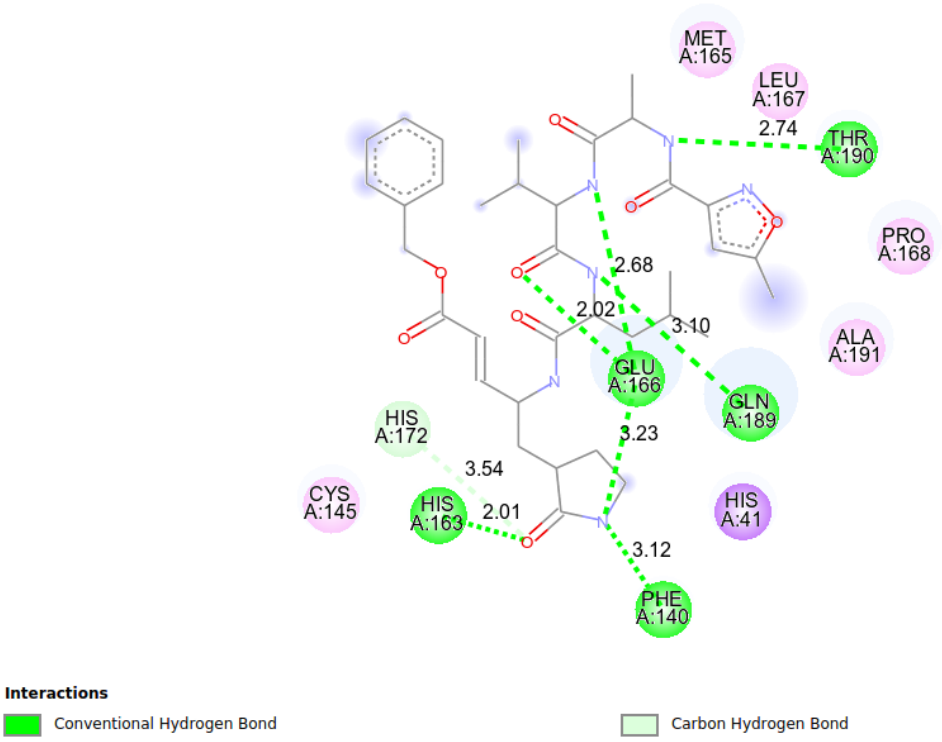

FIGURE 2. Structure re-docking of N3 ligand with 6LU7 protein

In the study of Jin [2], the amino acid residues present in the active group of proteins and hydrogen bonds with native N3 ligands (Fig. 2) are PHE140, GLU166, THR190, GLN189, HIS163, and HIS172. In addition, this ligand has a disulfide covalent bond with the CYS145 residue.

Based on the re-docking done, two amino acid residues have hydrogen bonds with N3 ligands, namely PHE140 and GLN189. The amino acid residue PHE140 interacts with the amine group with a bond distance of 3.12 Å. The residue GLN189 interacts with the amine group with a bond distance of 3.10 Å. Meanwhile, there is 1 type of disulfide bond formed, namely between the native N3 ligand and CYS145. This bond is formed because the C atom in the native N3 ligand is thought to be covalently bonded to the S atom in CYS145.

In the visualization above, it is possible to conclude that the re-docking of the resulting N3-protein native ligand complex is close to the ligand's initial position before separation. Furthermore, the hydrogen bonds formed are consistent with previous studies, allowing docking to continue with the test ligand.

Result of Protein Interaction with Test Ligand

Ligand affinity parameters such as energy, inhibitor constant, ligand efficiency, and the amount of hydrogen formed are used to determine the best position of the molecular docking result between protein and ligand. Bond energy is the energy needed to bind the protein to ligands. Ligand efficiency is used to measure the bond energy ratio for the number of non-hydrogen atoms of the compound. While the inhibitor constant (Ki) indicates how much potential the ligand has as an inhibitor, including the concentration required to produce half the maximum inhibition. Bond energy is closely related to the efficiency of the ligand and the inhibitor constant, where the value of the bond energy is directly proportional to the efficiency of the ligand and the inhibitor constant. The smaller the value of bond energy, inhibitor constant, and ligand efficiency, the higher the affinity of the ligand [7]. In addition, the more hydrogen bonds formed, the greater the affinity of the ligand [13]. The docking results between the protein and the test ligands resulted in several conformations. Conformation is the arrangement of a compound with different positions due to the group's rotation around the bond. In this study, ten conformations were used to obtain various docking positions between the protein and the ligand so that there were more opportunities to get a better conformation.

TABLE 2. Result of docking molecular 6LU7 with ligand test

Ligand	Bond energy (kcal/mol)	Ligand Efficiency	Inhibitor Constant (μM)	Interact formed
N3 (Native)	-9.34	-0.19	0.2	PHE140, GLU166, THR190, GLN189, HIS163, HIS172
4',7-dihydroxy-3-methoxy flavonol	-8.51	-0.41	0.58	GLU166, ASP187, THR190
4',7-dihydroxy-3-ethoxy flavonol	-8.51	-0.39	0.58	GLU166, ASP187, THR190, TYR54

In Table 2, the bond energy value obtained in conformation 1 for complex 4',7-dihydroxy-3-methoxy flavonol is -8.51 kcal/mol, ligand efficiency of -0.41, inhibitor constant of 0.58 μM with the number of interactions formed is five. These two types of interactions can be seen in Fig. 3 that are three hydrogen bonds formed between the ligands and residues GLU166, ASP187, THR190 and the others is van der waals interaction (light green). The bond distance of amino acid residues ASP187, THR190, and GLU166 with the hydroxyl groups on the ligands were 1.99 Å, 1.98 Å, and 2.06 Å.

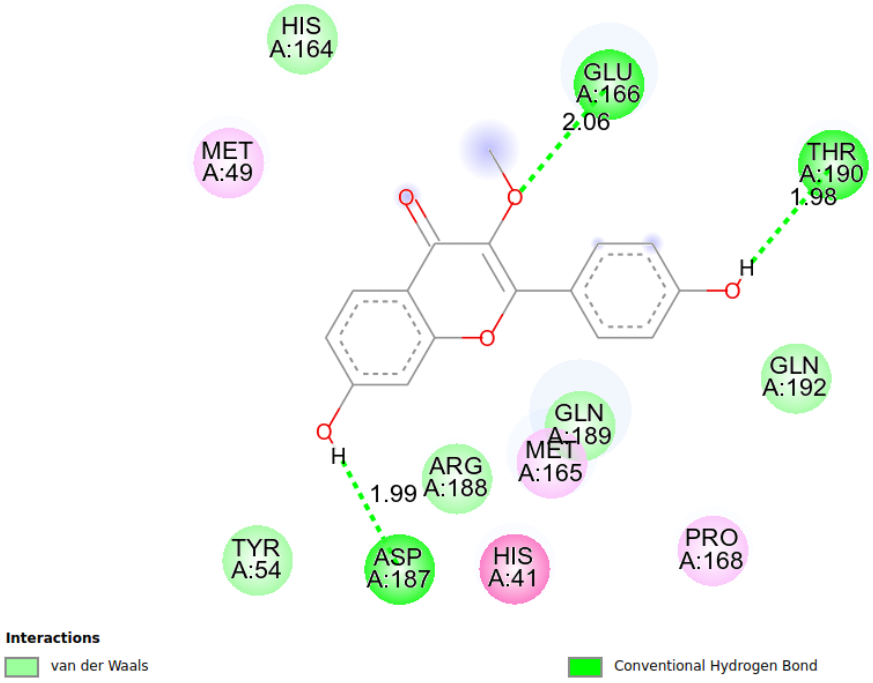

FIGURE 3. Structure 2D complex 4',7-dihydroxy-3-methoxy flavonol

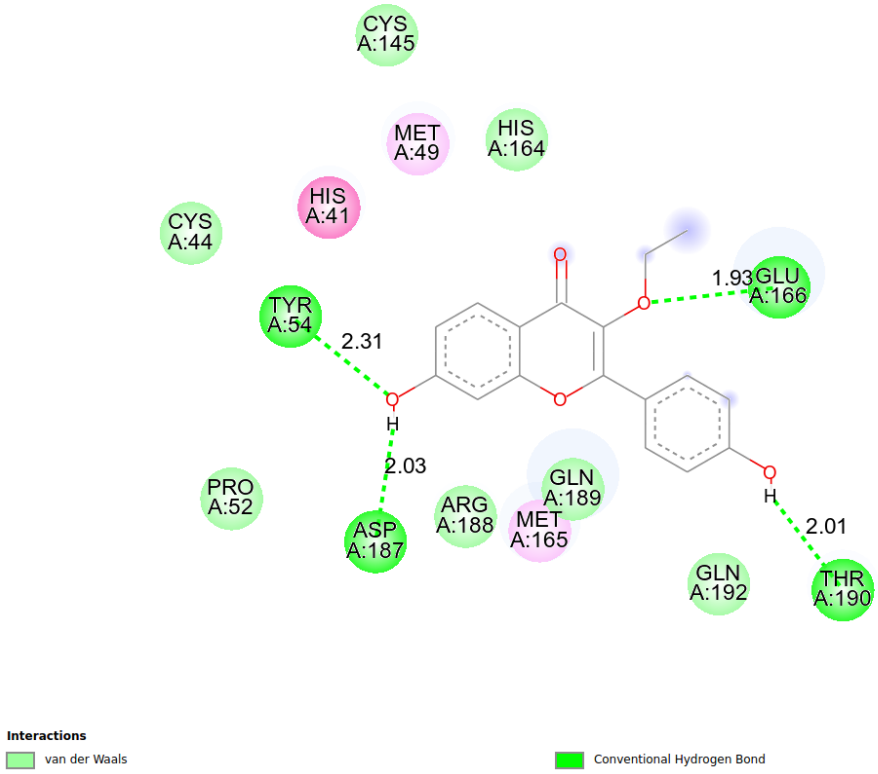

FIGURE 4. Structure 2D complex 4',7-dihydroxy-3-ethoxy flavonol

The bond energy value obtained in complex 4',7-dihydroxy-3-ethoxy flavonol is -8.51 kcal/mol, ligand efficiency of -0.39, inhibitor constant of 0.58 M with the number of interactions formed 4. Of the four interactions produced, it can be seen in Fig. 4 that the hydrogen bonds formed between the ligands and residues are GLU166, ASP187, THR190, and TYR54, respectively. The amino acid residues GLU166, ASP187, THR190, and TYR54 interact with the ligands' hydroxyl groups were 1.93 Å, 2.03 Å, 2.01 Å and 2.31Å.

The N3 native ligand in the table above is used as a comparison against other test ligands or as a control test, where the N3 native ligand has the lowest bond energy, ligand efficiency, and inhibitor constant even though it produces fewer hydrogen bonds. This is presumably because, during the re-docking of the native N3 protein-ligand complex, other types of interactions cannot be detected by docking and visualization, such as van Der Waals and phi-cation interactions occur between the benzene ring on the ligand and other amino acid residues.

Furthermore, the test ligand with the best position can be seen by several parameters such as bond energy, ligand efficiency, inhibitor constant, and hydrogen bonds formed. The test ligand data above found that the ligand with the lowest bond energy, ligand efficiency, and inhibitor constant is 4',7-dihydroxy 3-ethoxy flavonol. If the affinity of the resulting ligand tends to be small, then the possibility for that ligand to inhibit is also slighter. So it is feared that the ligand cannot act as a suitable inhibitor. While 4',7-dihydroxy 3-ethoxy flavonol has bond energy, ligand efficiency, and inhibitor constant is very small and close to the value of the test ligand, so it tends to be more stable and robust when compared to other test ligands.

From the results of this molecular docking, it can be concluded that the 4',7-dihydroxy 3-ethoxy flavonol ligand is a promising ligand compared to the other three test ligands and is thought to be an excellent ligand to be used as a Mpro SARS-CoV-2 inhibitor.

CONCLUSION

Based on the research, it can be concluded that the 4',7-dihydroxy 3-ethoxy flavonol ligand is a promising ligand and is thought to be used as a Mpro SARS-CoV-2 inhibitor with constant binding energy, ligand efficiency, and inhibitor value for the compound -8.51 kcal/mol, -0.41 and 0.58 M, respectively.

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