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Research Article

Molecular Dynamics Simulation of Serotonin Transport Protein Complex with 6-Hydroxy-1-Methyl-1,2,3,4-Tetrahydro-β-Carboline Ligand from Chocolate (*Theobroma cacao L.*) Isolate

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ABSTRACT

6-hydroxy-1-methyl1,2,3,4-tetrahydro-β-carboline (6OHMTHβC) is a chocolate derivate that has antidepressant potency. It can increase dopamine and serotonin secretion, which leads to mood improvement. This method was carried out using computational molecular docking simulations (in silico). The research design was computational-based exploratory descriptive. The results of molecular docking showed the lowest energy score and the backbone RMSD value ≤2Å. The procedure performed was 6AWP receptor docking without ligand, with native ligand (6OHMTHβC), and with reference ligand (fluvoxamine). This study also performed molecular docking simulations of 6OHMTHβC towards 6AWP to find compounds in the receptor binding pocket. This study also performed dynamics simulations and identified the molecular determinants using PyPLIF-HIPPOS and YASARA Structure software 20.1.24.10 with the Windows 10 operating system. This study succeeded in determining the stability of the dynamics simulation of the serotonin transport protein complex with the reference ligand 6OHMTHβC for 50 ns, and this result corresponds to the RMSD value and binding energy. The determination of binding energy (BE) was calculated from the BE calculation available at YASARA and Ubuntu. The binding energy value of the original ligand was -11.6590 kJ/mol, and the reference ligand was -83880 kJ/mol. The highest RMSD value of the original ligand was 1.39292Å, while the RMSD value of the reference ligand was 1.71072Å. The essential amino acid carried out was 438Ser with hydrogen bond interactions, so 6OHMTHβC was considered a competent antidepressant candidate.

INTRODUCTION

Chocolate is known as a potential antidepressant because it has the capability to increase dopamine and serotonin levels. Dopamine and serotonin are hormones that can induce a positive attitude toward consumers (Scholey and Owen, 2013). 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline or 6OHMTHβC (Figure 1) is a compound that has been successfully isolated from chocolate and induces a greater serotonin amount (Herraiz, 2000). The anti-depressant role is also bridged by the 6AWP receptor as a serotonin (5-HT) transporter. The use of antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) has side effects and can lead to drug dependence. Nearly 30% of depressed patients do not respond to drug therapy, and 70% of patients fail to achieve complete recovery (Kulkarni and Kulkarni, 2013). Most Indonesian people habitually use

herbal medicine to cope with their diseases; therefore, it is important to screen and select natural ingredients that have active compounds mainly with antidepressant properties and also their formological mechanisms empirically, so the availability of the natural ingredients can be maximized for their use as medicines (Khanifah et al., 2021).

Molecular dynamics (MD) simulations were carried out to determine the interaction of the protein with the ligand atoms within a specified time. In addition, MD provides information on GG calculations and protein structure sampling as an implementation of the induced-fit theory in structure-based virtual screening (SBVS) Protein conformational stability in the molecular dynamics simulation process will be calculated with the root mean square deviation (RMSD) RMSD is the value obtained from calculating the square root of the average sum of the squares of the distances or displacements of atoms (only heavy atoms are selected) of a pose or snapshot result by atoms of reference poses (Isyastono, 2021). The docking parameter is valid when the RMSD backbone value is ≤2Å.A smallerr RMSD backbone value indicates that the position of the ligand is higher andclosere to the overview of the original molecule conformation. The interaction between the residues on the protein and the ligand isimportant,t so the proteins are tending to retain their structure. This will happen when the RMSD backbone value begins tostabilize. PyPLIFF-HIPPOS is a toolthat ist used in this research and aims to identifyproteins and ligandsd from molecular dynamics (MD) and combine lock theory and induced fit (Nugraha and Istyastono, 2021).

This research has the benefit and prospect of providing new additional information related to 6AWB bonding amino acid essentials from chocolate with antidepressant potency. Further, this finding will be studied in vitro and in vivo, respectively, using 6OHMTHβC chocolate isolate as an antidepressant drug candidate.

METHODS

This research method is descriptive, exploratory, and used in silico with a molecular simulation approach. Screening molecular cell data would form a model of the target compounds and could be used as an effective tool in designing drugs. Silico is one of the crucial steps in medicinal chemistry and is often done using several devices to support drug discovery and development.

The software used was YASARA-Structure 20.1.24 and ran on a computer with Windows 10 as the operating system. The hardware that was needed was an Intel® Celeron® N4000 CPU (1.1GHz) and an LED monitor. The research material was ligand 6OHMTHβC, derived from the isolation of the natural compound, and the structural formula was made using ChemDraw. The crystal structure of the antidepressant receptor was obtained from https://www.rscb.org/structure/6AWP (3.80Å, homo sapiens, PDB ID code 6AWP). Accessed on September 22, 2022.

The data were collected as follows: (1) preparation: The receptor database was downloaded from the Protein Data Bank. Protein macromolecule receptors were separated from other molecules and their ligands. This study was carried out in three stages: (i) 6AWP receptor binding without ligand; (ii) binding of the fluvoxamine reference ligand to the 6AWP receptor; (iii) binding of the 6OHMTHβC candidate ligand, which is expected to be the potential antidepressant candidate for the 6AWP receptor. Each molecular anchorage space was determined by 5Å based on the reference ligand fluvoxamine and 6OHMTHβC with the amino acids in the bond pocket. The molecular dynamics were carried out for 50 ns using the macro md_run_50 ns_ss10.mcr. pH 7.4, temperature 298K, pressure 1 bar, simulation snapshots every 250000 fs, save interval = 250000, density = 0.997, duration = 5000 picoseconds.

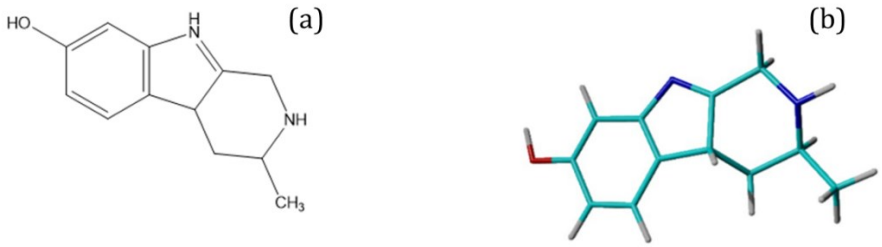

Figure 1. Structure visualization of 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6OHMTHβC) using Chemdraw (a) and 3D Structure visualization of 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6OHMTHβC) using YASARA (b)

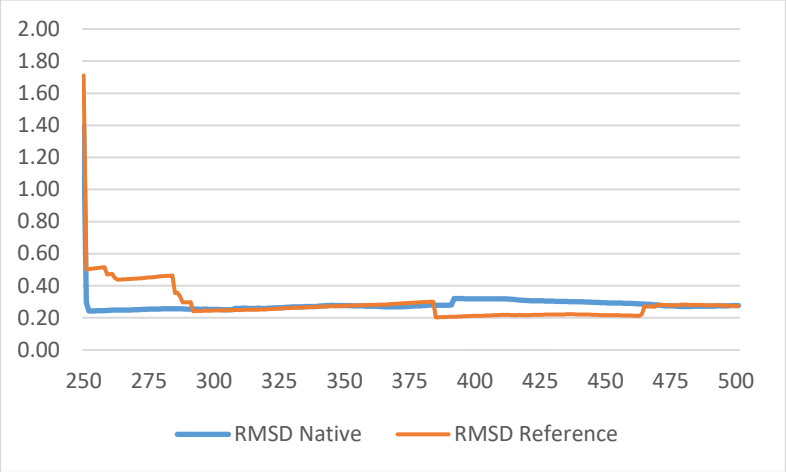

Figure 2. RMSD value of MD results on native and reference ligands every 5ns

The molecular dynamics simulation results were analyzed using the default YASARA structure md_analyze.mcr as a standard to observe the backbone RMSD value. When the results of the dynamic reached equilibrium, the free energy (ΔG) was analyzed, and the lowest energy that was found was used as a target for binding the ligand model 1000 times using the macro dock_run_1000.mcr. Quantitative formal analysis was carried out to find the results of these parameters, respectively: (1) protein stability with RMSD values; (2) results analysis; and (3) molecular determinant identification.

The results of molecular dynamics simulations on the ligand and protein complexes are files formed with the extension *.sim and then converted into files with the extension *.pdb. Identification of moleculaeterminants was performed on *pdb files using the PyPLIF-HIPPOS software.

RESULTS AND DISCUSSION

This research was conducted to observe the stability of the serotonin transport protein complex with the 6OHMTHβC ligand based on (i) RMSD values and (ii) molecular determinants that play an important role in the stability of the protein complex.

The 6AWP receptor was downloaded from the protein data bank as a target receptor that has a reference ligand and is crucial in the construction of SBVS (Isyastono, 2021). After the target receptor was found, the docking process was performed. The docking was done in three ways: (i) without ligand-receptor docking, (ii) with native receptor docking, and (iii). 6OHMTHβC is a brown isolate used as a reference ligand. Reference ligands are very useful for defining binding pockets. The MD simulation was carried out by setting the AMBER14 force field, pH 7.4, temperature 298K, pressure 1 bar, and running with the command md_runmembrane.mcr.

According to Istyastono and Riswanto's research, analysis of the protein stability complex should be carried out every 50 ns to produce 500 snapshots. This snapshot succeeded in producing good results. The best snapshot is obtained from the calculation of the lowest bond-free energy. MD Stability is also seen from the RMSD value, and if the RMSD is ≤2Å for 2 ns, the protein is considered stable (Nugraha and Istyastono, 2021). RMSD calculations are performed every 5 seconds to produce 250 RMSD. MD was performed on the native ligand, fluvoxamine, and the reference ligand, 6OHMTHβC, which was isolated from chocolate. Sample preparation was carried out at the RMSD stage without ligand, with native ligand, and with reference ligand. The RMSD values for native and reference ligands have good stability, as can be seen in Figure 2. There is no significant spike before 2Å, meaning that the results of the dynamic have reached equilibrium. The highest RMSD value is at native MD1.39292Å, and the highest RMSD value is at reference MD 1. A comparison of the RMSD Natives and reference values can be seen in Figure 2.

Binding energy is determined from the binding energy (BE) calculation available on YASARA and uses Ubuntu by BE_native grep 001 *.log > grep_log. The best snapshot can be seen from the lowest energy binding using the

command BE_native grep 11.6590 *.log. The lowest binding energy of the native ligand (fluvoxamine) was -11.6590 kJ/mol at 346.pdb snapshot, and the lowest binding energy of the reference ligand (6OHMTHβC) was -8.3880 kJ/mol at 383.pdb snapshot. The best snapshot of the reference ligand is in pose 383.pdb because it has the lowest energy among the other poses in the reference ligand. The lowest-energy system is selected for further internal validation tests to observe the quality of the resulting protocol. Validation was performed by separating the selected receptor structures from their ligands; the receptor structures were stored as 346_receptor.sce, and the ligands were stored as 346_ligandref.yob. This system was selected and further analyzed as a validated virtual target. In terms of thermodynamics, the best receptor-ligand interaction occurs when the resulting complex has a lower potential energy (Schneider, 2014). This supports the results of research that the bond energies of -11.6590 kJ/mol and -83880 kJ/mol are the best energy at certain snapshots.

Ligan reference (6OHMTHβC) stability can be seen in Figure 3, and it shows that the hydrogen bond space that formed between ser438 and the reference ligand is 1,952 Å in 383.pdb pose. As can be seen in Figure 4, the distance of hydrogen bonding between ser336 and the native ligand is 1.91 Å. The distance between hydrogen bonding and receptor in every ligand, namely reference ligand and native ligand, is almost similar. Thus, it expects there to be such bioactivity in the reference ligand as if it were a native ligand.

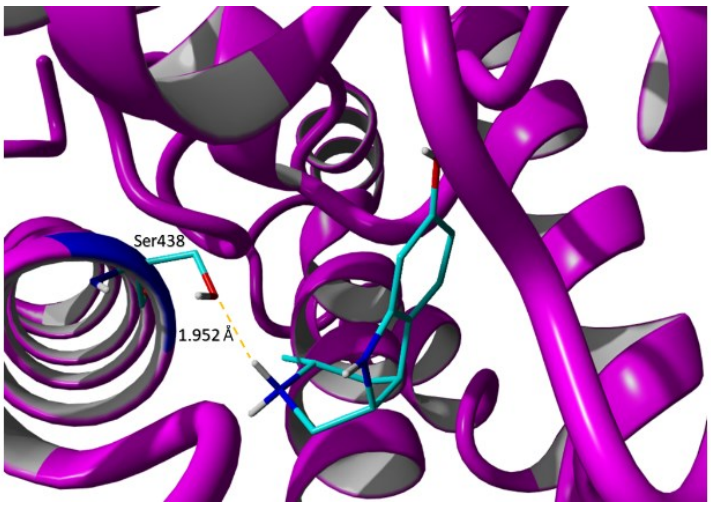

Figure 3. The best snapshot of the reference ligand that binds to the Ser438 amino acid

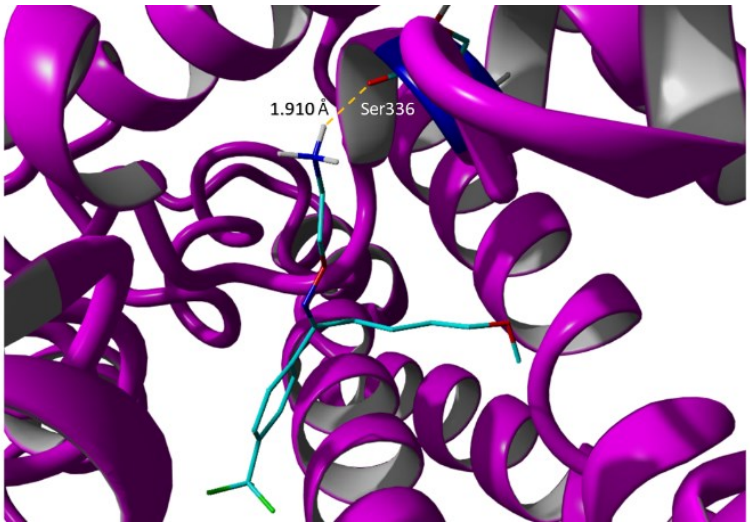

Figure 4. Best snapshot of native ligands bound to amino acids Ser336

The best snapshot with the lowest energy was then used as a target for attaching the ligand molecule 1000 times using the macro dock_run_1000.mcr. This protocol aims to determine the stability of the ligand at the receptor. Previous researchers reported that the binding of the molecule is stochastic (Istyastono, 2021). Re-attaching more than once is highly recommended to reduce the instability of the ligand to the receptor, and it can be done 1000 times. The stability of the ligand in the receptor can be seen if 95% of the best poses resulting from re-patching have RMSD ≤2Å (Istyastono, 2021). The results of the optimization of the molecular dynamics of the mooring of the native ligand (fluvoxamine) and reference ligand (6OHMTHβC) molecules obtained a maximum RMSD value of each 1.953Å, meaning below 2Å. Re-docking and dynamics simulations have a RMSD≤2Å value. It supported the stable form of the binding since the smaller RMSD value indicates the minimum error deviation in docking since the RMSD value describes the value of the deviation from or the error possibility at the process (Cole et al., 2005).

Molecular determinant identification plays an important role in the stability of complexes. It is carried out with the Discovery Studio tool and PyPLIFF-HIPPOS software and results in molecular dynamics simulations (Riandono and Istyastono, 2022). Comparisons between vital amino acids with different ligands can be seen in Table 1.

The relationship between structure and activity can be seen from the types of bonds that are formed. Hydrogen bonds are stronger than Van der Waals bonds. This is because hydrogen bonds will be formed even though the distance between the ligand and the receptor is quite far (Bulusu and Desiraju, 2022). Hydrophobic interactions also play a role in determining the stability of the ligand at the receptor. The formation of hydrophobic bonds minimizes the interaction of nonpolar residues and water (Escobar and Ballester, 2021). The higher hydrogen capacity will require more energy. Based on Table 1, the results show off the essential amino acid reference ligand SER438, which is the type of H-bond acceptor with a bond distance of 1.952 Å; meanwhile, the native ligands are amino acids SER336 and the type of H-bond acceptor bond with an atomic bond distance of 1.910 Å. This is relevant to previous research, which reported that the essential amino acid in serotonin transport protein (6AWP) is SER336 (Coleman et al., 2016). Other research mentions that the essential amino acids for serotonin transport are Ser438/Thr439 or Ala169/Ile172/Ala173 (Chan et al., 2022). The results of this study reported that chocolate allegedly had activity against receptors. It can be seen from the formation of several bonds with vital or essential amino acids.

CONCLUSIONS

Dynamics simulation of the serotonin transporter protein complex with their reference ligand 6OHMTHβC for 50 ns results shows that the backbone RMSD value is ≤2Å. This results in a low bond energy between each ligand and the 6awp receptor, caused by the balance of ligands in the binding pocket. The limitation of this research was carried out using laptop hardware at a temperature of 298K. In further research, an in vitro-in vivo test will be conducted using 6OHMTHβC chocolate isolate as an antidepressant candidate.

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