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MOLECULAR DOCKING OF BRAZILIN FROM SECANG WOOD PLANT (*Caesalpinia sappan* L.) AS AN ANTI-BREAST CANCER

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Abstract

Breast cancer is a disease characterized by abnormal cell growth, which can invade adjacent tissues or metastasize to other organs. Secang (*Caesalpinia sappan* L.) is a plant that has been used as an alternative medicine for a variety of health conditions, including some types of cancer. This study aims to determine whether the brazilin compound found in secang wood can interact with the target receptors estrogen alpha, 17-β-HSD-1, and NUDT5, potentially serving as an anticancer candidate. Molecular docking simulations were employed to identify the molecular interactions of brazilin against estrogen receptor alpha (ERα) (PDB ID: 3ERT), 17β-HSD-1 (PDB ID: 3HB5), and NUDT5 (PDB ID: 5NQR) using AutoDockTools software. The results showed that the best free-binding energy (ΔG) value obtained for brazilin against the 17-β-HSD-1 receptor was -9.16 kcal/mol, with an inhibition constant of 192.45 nM. The ΔG value of brazilin with estrogen alpha was -6.68 kcal/mol, and the ΔG value for brazilin against NUDT5 was -4.8 kcal/mol. Brazilin has a higher potency compared to innate ligands based on the docking result of estrogen alpha receptor and NUDT5. Some structural similarities and interactions occurred between the amino acids GLY186 and TYR155 in brazilin with the binding of amino acids formed in the innate ligand against the 17-β-HSD-1 receptor, thus showing similar affinity to the 17-β-HSD1 receptor. In silico approaches provided valuable insights into the potential of brazilin as an anti-breast cancer agent.

INTRODUCTION

Cancer is a disease characterized by the abnormal growth of cells beyond normal boundaries that can then attack adjacent parts of the body and spread to other organs. Other terms used for cancer are malignant tumours and neoplasms. A malignant tumor, or cancer, enters the surrounding tissue and can go to a more distant area of other organs (metastasis) [1]. Breast cancer is one of the types of cancers that are most affected and contributes to the prevalence of cancer in women around the world, including in Indonesia. This type of cancer has a high mortality rate due to late, early detection. Breast cancer crossovers are generally detected in advanced stages. Early detection of breast cancer in Indonesia is carried out through the SADANIS program, a clinical examination of the breast performed by trained health personnel. This early detection can suppress mortality and health financing [2].

Secang (*Caesalpinia sappan* L.) is a plant that has been used as an alternative medicine for various health conditions, including some types of cancer [3, 4]. Several studies have suggested that secang wood extract exhibits anticancer activity through various mechanisms. It is known that the brazilin compound contained in the secang plant has an anticancer activity value of -57.7 kcal/mol and also has an IC_{50} value of 3.7 μM [5].

The chemical compounds found in secang wood include lactic acid, tannin, resin, resorcin, brazilin, brazilein, d-a-phellandrene, ocimene, and essential oils [6]. Phytochemical tests showed that secang wood contains a group of alkaloids, flavonoids, and saponins. Flavonoids and brazilin act as anticancer agents, whereas flavonoid is a natural phenolic compound that has antioxidant properties and potentially inhibits the growth of cancer cells [7]. Alpha-estrogen, also known as estradiol, is a type of estrogen hormone that is found at a higher rate in women during the reproductive stages. High levels of estradiol have

been linked to an increased risk of breast cancer. Some studies show that long-term exposure to high estradiol levels can lead to the development of breast cancer, promote cell proliferation, and inhibit apoptosis [4]. In addition, 17-β-hydroxysteroid dehydrogenase type 1 (17β-HSD-1) catalyzes the last step in the synthesis of estradiol and androstenediol in breast tumor tissue [8, 9, 10]. Another study says that NUDIX hydrolase 5 (NUDT5) is involved in nucleotide metabolism and cancer. NUDT5 has recently been identified as a factor in the production of ATP in the nucleus of breast cancer cells, and its thinning impedes the regulation of genes that depend on progesterone and estrogen [11, 12].

Molecular docking is a computational method that aims to mimic the interaction event of a ligan molecule with a protein targeted in an in vitro test [13, 14, 15]. Although brazilin is often used as a traditional medicine, the precise mechanisms by which it exerts its anticancer effects remain unclear, particularly concerning breast cancer. By using molecular docking, we can predict the binding affinity and interactions of brazilin against important receptor proteins that contribute to the development of breast cancer. Based on this background, the research was conducted to identify the potential of the brazilin compound contained in the secang plant as an anticancer by inhibiting the signals of the estrogen receptors alpha, 17β-HSD-1, and NUDT5 with molecular docking methods [16].

EXPERIMENT

Receptor and Ligan Preparations

This study was carried out on a computer equipped with a processor AMD Ryzen 5 3500U with Radeon Vega Mobile Gfx 2.10 GHz, and RAM memory 8 GB. The receptors structure was prepared from the Protein Data Bank (PDB) and then separated from the original ligands, non-standard residues, water, and added charges using the BIOVIA Discovery Studio Visualizer 2017 software [17]. The design was intended to provide space (pocket/cavity) so that the pocket shape and pocket coordinates are known as the docking material [18]. The receptors used were estrogen alpha, 17-β-HSD-1, and NUDT5, downloaded through the Protein Data Bank (PDB) website with codes 3ERT, 3HB5, and 5NQR, respectively. The downloaded files were then opened using AutoDockTools-1.5.6 [19], and the water molecules were removed, after which the hydrogen atom was added. If the receptor used was simmered, then remove one of the atomic chains and then separate between the ligand and the protein, naming the file with the receptors and ligands. The test ligands are then prepared by matching the coordinate of the test ligand to the standard ligand using BIOVIA Discovery Studio. Select the downloaded ligand and brazilin files, and then the ligand structure was copied into the brazilin structure document and saved under the name of the ligand. To optimize the geometric 3D structure of ligands, clean geometry option, Dreiding-like forcefield [20], were applied using BIOVIA Discovery Studio. Open AutoDockTools to set up the receptor, and select ligand.pdb, and save in pdbqt format. After that, the parameter file for GridBox was prepared to determine the coordinates and size of the binding site of the receptor. The file was stored under the name grid.gpf. The docking parameter was prepared by selecting receptor.pdbqt.

Validation of Methods

A ligand re-attached to a protein receptor was prepared using the AutoDockTools program. The result of this molecular docking was based on the root mean square deviation (RMSD) value [17, 18].

Molecular docking of brazilin against target receptors

A series of molecular docking experiments were carried out using the AutoDockTools software to ensure binding efficiency through the interaction of the brazilin molecule with the selected target. The brazilin structure and target protein prepared in PDB format were translated into PDBQT format using AutoDockTools software, after which a 40×40×40 points with 0.375Å space grid box was created to know the binding site specific to each targeted protein to obtain a reliable docking result. The conformation of molecular restraint simulation results was clustered using RMSD 2.0Å tolerance. Ligand conformation with a low binding energy (ΔG) of the cluster was the best used for analysis stages using BIOVIA Discovery Studio 2017 software. Analysis of free-binding energy as a docking result was seen in the output in AutoDockTools. Conformation of the selected ligand protein complex that has the smallest free bonding energy value was chosen for further analysis [18]. The

visualization of the docking results was employed using the BIOVIA Discovery Studio program.

RESULT AND DISCUSSION

The receptor and ligand preparation was done using the BIOVIA Discovery Studio program. The purpose of this preparation was to provide space as a test compound binding to the protein. This process was very important in the docking process because it can affect the quality of the docking results. In the docking process, receptor preparation takes place by preparing the 3D structure of the target protein or its receptor. The preparation of receptors includes the cleaning of residues and the removal of water molecules and metal ions. Water molecules must be removed so as not to interfere with docking and the interaction formed so that only receptors and ligands are interacting [22].

The preparation of the receptors was carried out by separating the alpha estrogen receptor (3ERT) from the OHT600 ligand (hydroxytamoxifen); 17β-hydroxysteroid dehydrogenase-1 (3HB5) from the E2B ligand (ethinyl estradiol); and NUDT5 (5NQR) from the 958302 ligand (TH5427), respectively (**Figure 1**).

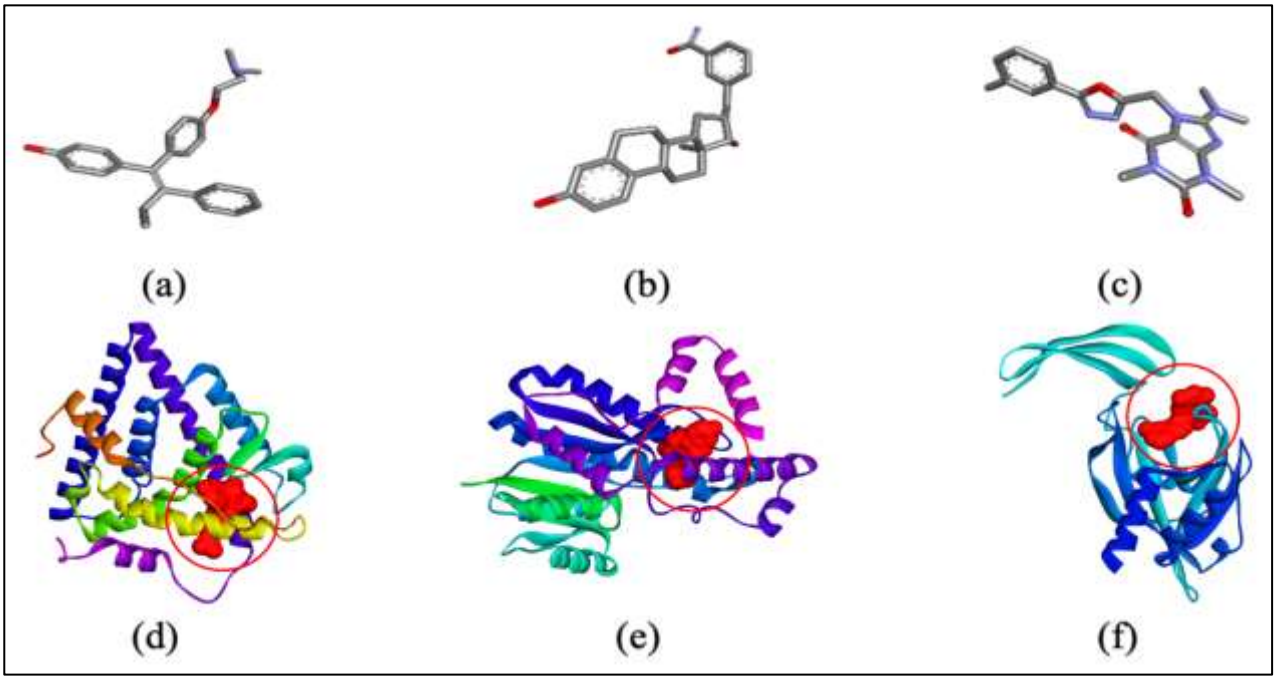

Figure 1. The structure of (a) OHT600 (hydroxy tamoxifen); (b) E2B (ethynyl estradiol); (c) 958302 (TH5427); (d) 3ERT receptor; (e) 3HB5 receptor; and (f) 5NQR receptor.

Method validation was carried out using the AutoDockTools program by redocking the respective ligands with the prepared receptors. Each of the ligands of these three receptors: estrogen alpha (3ERT), 17-β-HSD-1 (3HB5), and NUDT5 (5NQR) were redocked. The important thing when validating the molecular constriction method was the setting of the grid box. The grid box was designed to show the space where the interaction between the ligand and the amino acids in the target compound will result in a pharmacological effect.

In this study, for the alpha estrogen receptor, the size of the grid box used was 40x40x40 with a space of 0.375 Å. While for the 17-β-HSD-1 receptor, a grid box size was 30x38x30 with 0.375Å space. Whereas for the NUDT5 receptor, the grade box size used was 30x30x30. RMSD (Root Mean Square Deviation) is a parameter in the validation of molecular restraint. The RMSD value shows a comparison of the conformation of the ligand of the receptor before validation with the redocking ligand. RMSD of a validation result less than 2.0 Å was said to be good and validated. The method validation results of the three receptors can be seen in **Table 1**.

Table 1. The results of method validation obtained from the receptors.

Receptor	RMSD
Estrogen Alpha	1.240 Å
17-β-HSD-1	1.443 Å
NUDT5	1.225 Å

Based on the results of the validation method of the three receptors obtained RMSD results that are already less than 2.0 Å. The estrogen alpha receptor obtained a value of 1.24 Å, 17-β-HSD-1 obtained a value of 1,443 Å, and NUDT5 was 1.225 Å, which means that the validating results can be said to meet the requirements and have been validated. The receptors can be used for the next stage of the docking against the brazilin target compound (**Figure 2**).

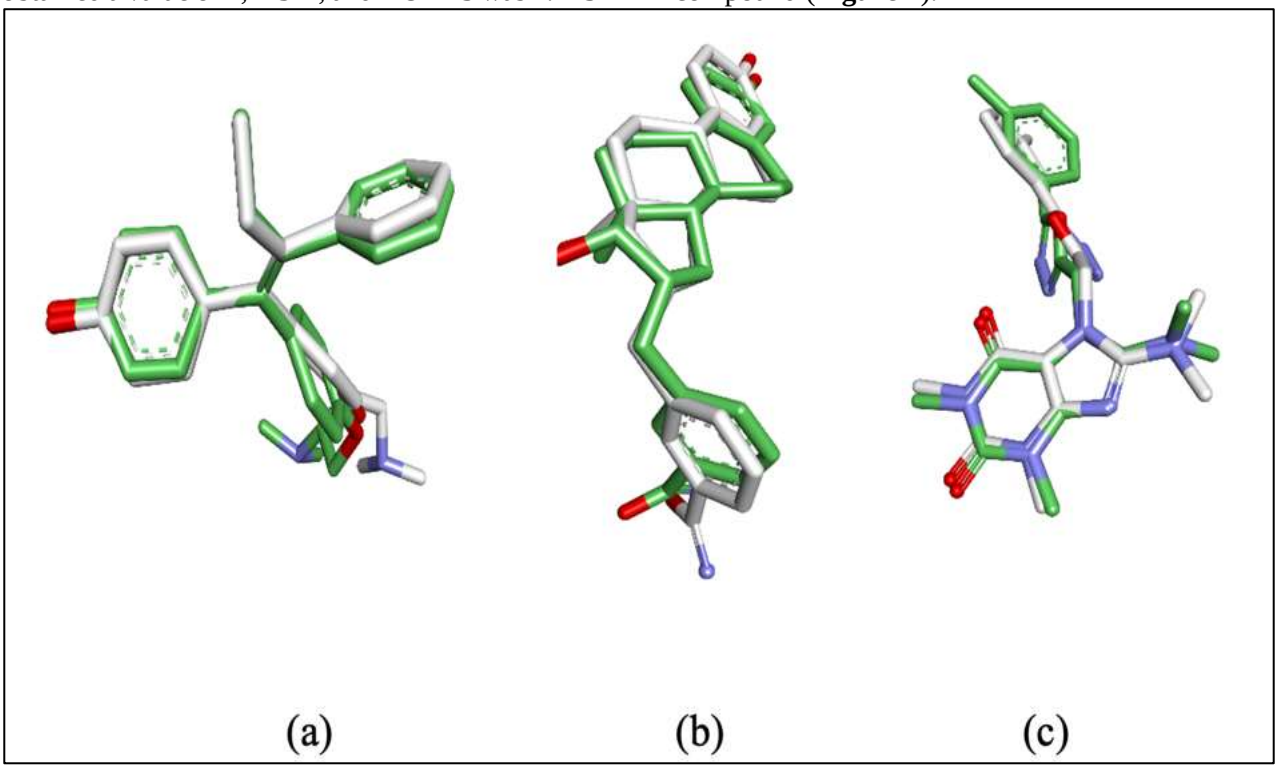

Figure 2. Comparison of the origin and validated ligand results in (a) alpha estrogen, (b) 17-β-HSD-1, and (c) NUDT5.

The brazilin compound was prepared first using the AutoDockTools application, after which the docking processes were performed using the receptors that had passed the validation process. The result of the molecular docking of brazilin and E2B as a reference ligand against the three receptors can be seen in **Table 2**.

Table 2. The results of molecular docking were obtained from the interaction between brazilin and the receptors.

Receptor	Result			
	ΔG		Ki	
	Reference	Brazilin	Reference	Brazilin
Estrogen Alpha	-11,69 kcal/mol	-6,68 kcal/mol	2,71 nM	12,71 µM
17-β-HSD-1	-8,73 kcal/mol	-9,16 kcal/mol	401,7 nM	192,45 nM
NUDT5	-6,99 kcal/mol	-4,8 kcal/mol	7,49 µM	301,65 µM

A good docking result can be analyzed by looking at the value of the binding-free energy (ΔG) and the constants of inhibition (Ki). The more negative or lower the value of binding-free energy value indicates a good level of stability of the ligand and receptor bond so that the bond formed is stronger and the inhibitory activity is maximum [23]. The binding-free energy value (ΔG) of the estrogen alpha receptor was -11.69 kcal/mol, and the docking output against brazilin was -6.68 kcal/mol. The binding-free energy value of the 17-β-HSD-1 receptor of the E2B as a reference ligand was -8.73 kcal/mmol and the docking outcome against the brazilin was -9.16 kcal/mmol. Whereas the binding free energy (ΔG) value of the NUDT5 receptor ligand was -6.99 kcal/mol and the docking result against brazilin was -4.8 kcal/mol.

The receptors tested in this study are the alpha estrogen, 17-β-HSD-1, and NUDT5, where these three receptors play an active role in breast cancer. Mechanisms of estrogen alpha receptors on breast cancer are that they affect the progression of breast cancer by stimulating the growth of cancer cells, i.e., stimulating breast cancer cell growth by binding the estrogen alpha receptors to the cancer cell, then inducing the proliferation of cancer cells and suppressing apoptosis. 17-β-HSD-1 plays a role because if there is an increase in the 17-β-HSD-1 then it will result in increased levels of estrogen in the breast tissue that can increase the risk of breast growth. It can allow cancer cells to continue to grow [8].

Based on the results of studies of the estrogen alpha receptor, the binding-free energy value of the standard ligand OHT600 showed more negative compared to the binding-free energy value of the brazilin; for the 17-β-HSD-1 receptor, the binding-free energy produced by the suspension with the brazilin compound was more negative than the E2B, whereas for the NUDT5 receptor, the binding-free energy values of the innate ligan are more negative. It is also influenced by the number of hydrogen bonds and their hydrophobic nature because the abundance of hydrogen and hydrophobic bonds plays a major role in the increasingly negative binding-free energy. Based on the results, brazilin has the best affinity value for inhibiting the 17-β-HSD-1 receptor at -9.16 kcal/mol. This is the best value because it has the most binding energy with 7 hydrogen bonds and 4 hydrophobic bonds. On the other hand, the NUDT5 and estrogen alpha receptors produce good binding-free energy values of -4.8 and -6.68 kcal/mol with the standard AutoDockTools error program ± 2-3 kcal/mol, so it can be said that NUDT5 and estrogen alpha still have potential as anti-breast cancer agents.

The Ki value becomes important in the docking process because Ki is a parameter used to evaluate the ability of a compound to inhibit the activity of a particular enzyme or receptor. Based on the Ki results, brazilin has the smallest Ki values for the 17-β-HSD-1 receptor of 192.45 nM. For the other two receptors, NUDT5 and estrogen alpha, brazilin showed a higher Ki value than the standard ligand. This implies that brazilin was predicted to be able to inhibit the 17-β-HSD-1 receptor as an anti-breast cancer agent. An intermolecular interaction is an interaction that occurs in the three ligands of the 17-β-HSD-1, NUDT5, and estrogen alpha receptors against the brazilin compound, and this interaction can be seen in the docking results using the AutoDockTools program. An interaction that occurs can increase the affinity of the bonding of the ligand with the brazilin, so it is necessary to know the intermolecular interactions that occur (**Table 3**). By default, AutoDockTools software will categorize hydrogen bonds into classical, non-classical, water, and salt bridges. It also includes the length, angle, and atom type. If the criterion is not fulfilled, the software will not categorize it as a hydrogen bond.

Based on the analysis results the compound brazilin can interact with the 17-β-HSD-1 receptor by forming hydrogen bonds in the GLY186 and TYR155 amino acids, where the hydrogen binding is the same as that formed in the 17-β-HSD-1 receptor ligand. This suggests that the active site of the bond between the ligand and brazilin in the 17-β-H SD-1 receptor was corresponding so that it will produce the same affinity with the standard ligand in inhibiting the 17-β-HSD-1. The two dimensions of the three receptors' interaction can be seen in **Figure 3.**

The estrogen alpha receptor forms four identical amino acid bonds: ARG 394, GLU 353, LEU 387, and THR 347, but although it has the same hydrogen bond with the same amino acids, its binding-free energy value and the inhibition constant value of the alpha inherent estrogen ligan, OHT600 were greater than the docking result against the brazilin. This could happen because the structure of the brazilin and the shape of the structural ligand is different as well as NUDT5, and these differences can affect the binding strength and stability. Meanwhile, the interaction between brazilin and the 17-β-HSD-1 receptor may have lower binding-free energy values and inhibition constants due to similar structures. The hydrogen bond formed by GLY 186 was a flexible neutral amino acid that has flexibility in forming hydrogenic bonds; for TYR155, it was an aromatic amino acid that has a hydroxyl group that can form hydrogen binding and hydrophobic interaction, thus inhibiting the 17-β-HSD-1 target receptor. The best binding to the 17-β-HSD-1 receptor was seen from the affinity value of -9.16 kcal/mol, and the brazilin has activity as anti-breast cancer that was demonstrated by the way that the brazilin works by inhibiting the 17-β-H SD-1 receptors, so there was no increase in estrogen levels in the breast tissue and does not increase the risk of breast cancer growth. Thus the cancer cells cannot proliferate and apoptosis may occur [11].

The in silico studies on brazilin`s anticancer activity have encouraging breast cancer therapeutic implications. Whereas identifying specific

molecular targets of brazilin could lead to the development of targeted therapies. Molecular docking simulations might not accurately capture the complexities of real-world interactions. To confirm these results and evaluate the safety and effectiveness of brazilin, in vivo, studies are essential to provide a valuable starting point for further research and offer hope for developing novel and effective breast cancer treatments.

Table 3. Molecular docking results of the interaction between brazilin and receptors.

Receptor	Innate ligand				Brazilin			
	Number of bond		Amino acid		Number of bond		Amino acid	
	Hydrogen	Hydrophobic	Hydrogen	Hydrophobic	Hydrogen	Hydrophobic	Hydrogen	Hydrophobic
Estrogen Alpha	4	10	ARG 394 GLU 353 LEU 387 THR 347	LEU 346 MET 421 PHE 404 ALA 350 LEU 387 LEU 391 ALA 350 LEU 525 MET 421 LEU 525	4	8	ARG 394 GLU 353 LEU 387 THR 347	LEU 346 ALA 350 MET 388 ALA 350 LEU 387 LEU 391 ALA 350 LEU 525
17-β-HSD-1	7	11	LEU 95 TYR 155 LEU 95 ASN 152 LEU 95 GLY 186 TYR 155	PHE 259 TYR 155 PHE 192 VAL 143 LEU 149 PRO 187 VAL 225 LEU 149 VAL 225 MET 193	7	5	ILE 14 GLY 15 ASN 90 TYR 155 LYS 159 GLY 186 SER 142	ILE 14 CYS 185 ILE 14 PHE 192 CYS 185
NUDT5	3	13	ARG 51 ARG 51 GLU 166	TRP 28 TRP 28 TRP 28 TRP 28 ILE 141 TRP 28 TRP 28 TRP 28 TRP 28 TRP 28 LEU 98 ALA 96 LEU 98	5	5	LEU 98 GLY 97 TRP 28 TRP 28 TRP 28	TRP 28 TRP 28 LEU 98 TRP 28 LEU 98

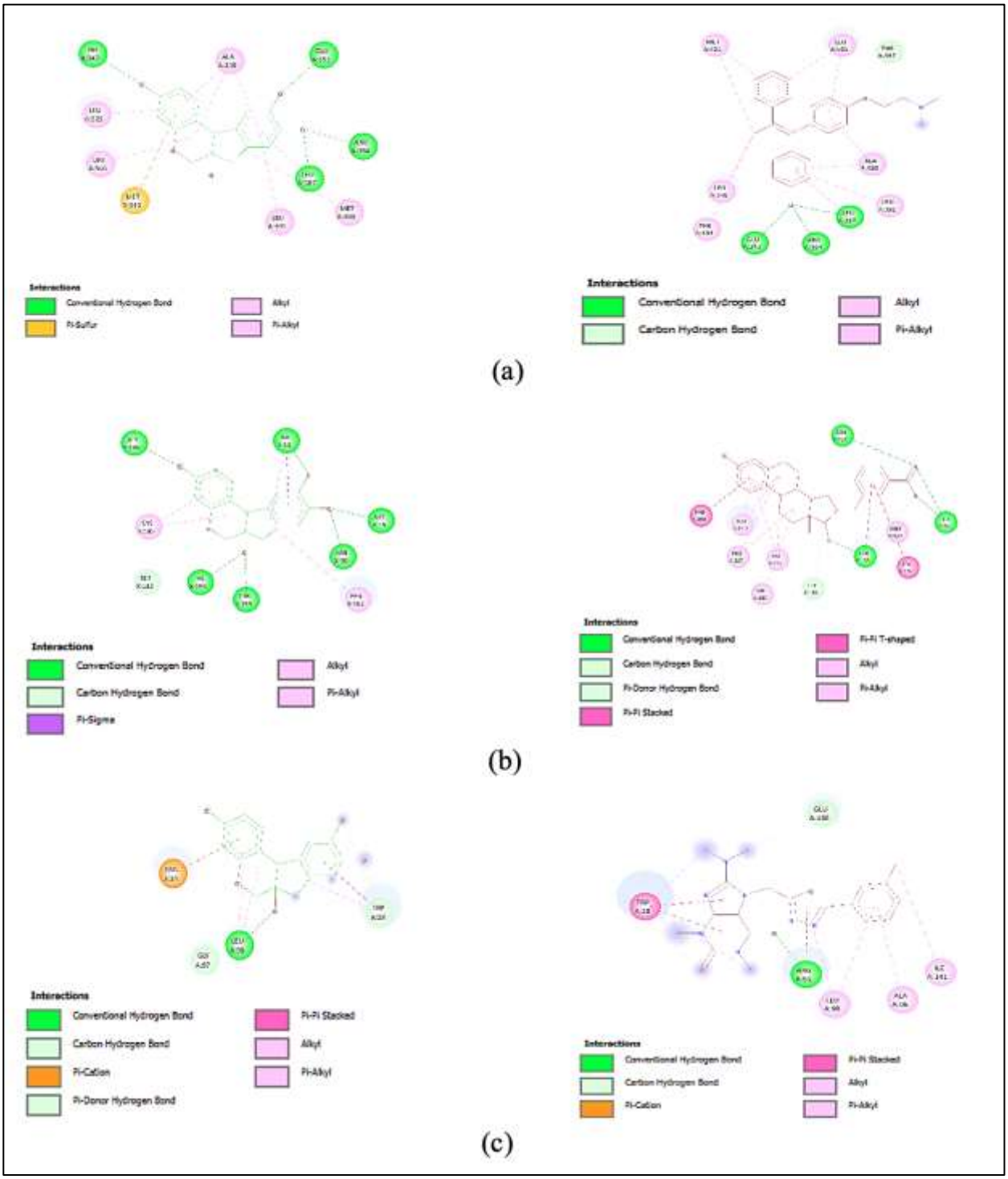

Figure 3. Visualization of the brazilin against ligands (a) OHT600, (b) E2B, and (c) 958302.

CONCLUSION

The molecular docking study of the brazilin compound from the secang plant against three targets: estrogen alpha, 17-β-HSD-1, and NUDT5. From the results of the study obtained, the docking of brazilin against the 17-β- HSD-1 receptor has the best results achieved with an affinity value of -9.16 kcal/mol. The hydrogen bonding in the amino acids GLY186 and TYR155 was the same as the bonding that occurs in the standard ligand E2B. The mechanism of brazilin inhibition activity to the receptor 17-β-HSD-1 was suppressing increased levels of estrogen in the breast tissue so that it does

not increase the risk of breast cancer growth and proliferation. The interaction of brazilin and the target receptor works and can be identified that the brazilin has a potential as an anti-breast cancer candidate.

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