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VIRTUAL INHIBITION ANALYSIS OF BIOACTIVE COMPOUND BRAZILIN (*Caesalpinia sappan* L.) TOWARDS PROGESTERON RECEPTOR OR LONAPRISAN IN BREAST CANCER PROLIFERATION

ANALISIS PENGHAMBATAN VIRTUAL ANTARA SENYAWA BIOAKTIF BRAZILIN SECANG (Caesalpinia sappan L.*) DENGAN RESEPTOR PROGESTERON ATAU LONAPRISAN PADA PROLIFERASI SEL KANKER PAYUDARA*

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ABSTRACT

Breast cancer is one of the leading causes of death worldwide. The pathway of breast cancer in KEGG shows that the most effective pathway is through the progesterone receptor (PR). Brazilin is a bioactive compound of secang (Caesalpinia sappan L.) used to inhibit breast cancer through survivin and Bcl-2 pathway but the interaction with PR route is unknown. This research was conducted to determine the virtual interaction between brazilin and PR and its comparison with lonaprisan, so the potential of breast cancer drugs that can overcome through three targets at once with minimal side effects is expected to be known. There are five docking interactions, including the interaction of PR-progesterone, PR-brazilin, PR-brazilin-progesterone, PR-lonaprisan, and PR-lonaprisan-progesterone. Protein and ligand preparation was performed by using Discovery Studio Client 2019 and PyRx 0.8, molecular docking was performed by using Hex 8.0.0 and visualization used Discovery Studio Client 2019. Virtual interaction results shows that lonaprisan has the most stable bond (lowest binding energy), -333.8kJ/mol but when progesterone was docked afterwards the result shows the opposite. Brazilin has a more stable bond compared to lonaprisan with a difference of 2.1kJ/mol and supported by hydrophobic bonds also capable of changing the position of progesterone in binding to PR so that it is estimated that brazilin has the potential as SPRMs, an alternative breast cancer drug to replace lonaprisan. Herbal medicine with brazilin can be estimated to fight breast cancer through 3 targets at once (survivin, Bcl-2, PR).

Keywords: *Brazilin, breast cancer, lonaprisan, PR, progesterone*

ABSTRAK

Kanker payudara adalah salah satu penyebab utama kematian di seluruh dunia. Pathway penyakit ini pada KEGG menunjukkan bahwa jalur paling efektif melalui reseptor progesteron (PR). Brazilin merupakan senyawa bioaktif secang (Caesalpinia sappan L.) yang telah terbukti mampu menghambat kanker payudara melalui jalur protein survivin dan Bcl-2, namun interaksi dengan target PR belum diketahui. Penelitian ini bertujuan untuk mengetahui interaksi virtual brazilin terhadap PR dan perbandingannya dengan lonaprisan sehingga diharapkan dapat diketahui potensi obat kanker payudara yang mampu mengatasi tiga jalur sekaligus dengan efek samping minimal. Terdapat lima perlakuan docking dalam penelitian ini, yaitu interaksi antara PR-progesteron, PR-brazilin, PR-brazilin-progesteron, PR-lonaprisan, dan PR-lonaprisan-progesteron. Preparasi protein dan ligan dilakukan dengan software Discovery Studio Client 2019 dan PyRx 0.8, docking menggunakan Hex 8.0.0 serta visualisasi menggunakan Discovery Studio Client 2019. Hasil interaksi virtual menunjukkan bahwa lonaprisan memiliki ikatan paling stabil (energi binding

terendah), yaitu -333,8kJ/mol namun hasil interaksi ketika didocking bersamaan progesteron menunjukkan hal yang sebaliknya. Brazilin memiliki ikatan yang lebih stabil (energi binding terendah) dibandingkan lonaprisan dengan selisih sebesar 2,1kJ/mol dan didukung oleh ikatan hidrofobik serta mampu mengubah posisi progesterone berikatan dengan PR sehingga diperkirakan brazilin berpotensi sebagai SPRMs, alterfnatif obat kanker payudara menggantikan lonaprisan. Obat herbal dengan senyawa brazilin berpotensi melawan kanker payudara melalui 3 target sekaligus (survivin, Bcl-2, PR).

Kata kunci: Brazilin, kanker payudara, lonaprisan, PR, progesteron

INTRODUCTION

Breast cancer is one of the diseases that cause the biggest death in the world, including Indonesia. Based on the data report in 2008, there were 1/3 women per 1000 people suffering from breast cancer [1]. According to Data and Information Center, Indonesian Ministry of Health, in 2013, the number of new breast cancer cases was 819 cases with 217 death cases. This number continues to increase every year [2].

The pathway of breast cancer in KEGG shows that the most effective pathway is through the progesterone receptor (PR) [3]. PR plays an important role in the cell proliferation in breast cancer. Progesterone is the natural ligand of PR. When progesterone binds to PR, cell proliferation will be induced and spur cancer cells. The inhibition of PR by compounds, known as Selective Progesterone Receptor Modulators (SPRMs) that can compete with the hormone progesterone, can inhibit the proliferation of cancer cells [4].

Some chemical drugs such as lonaprisan and mifepristone are used to inhibit cancer cell proliferation. Lonaprisan and mifepriston belong to the SPRMs group. SPRMs have a comparable effect with PR antagonists or PR (progestin) agonists that bind to PR in the nucleus. Changes in the configuration of the receptor structure can change due to the presence of these bonds and then DNA binds to the receptors. Agonists act as inhibitors of gene transcription by being co-activators, whereas antagonists inhibit gene transcription as co-repressors [5][6]. However, chemotherapy with lonaprisan can cause various undesirable side effects, so alternative natural compounds are needed to suppress safer cancer cell proliferation.

Secang (*Caesalpinia sappan* L.) is a Leguminosae group that has been widely known and favoured by the public because it can increase the freshness of drinks [7]. Secang's brazilin compound can inhibit the survivin protein involved in the activation of caspase-3 and caspase-9, related to the mechanism of apoptosis that has the potential to treat cancer [8]. This compound also acts as an anticancer agent through the Bcl-2 pathway [9]. The interaction of brazilin compound with the PR route is unknown [10].

Therefore, in this research focus on studying the potential of brazilin compounds of secang to treat breast cancer through the PR pathway. It is expected that the study of virtual interaction of brazilin with PR and its comparison with lonaprisan can predict the potential of breast cancer drugs that can overcome three targets at once (survivin, Bcl-2, PR) with minimal side effects compared to lonaprisan.

METHODS

Protein and ligand preparation. Protein structure of progesterone receptor was obtained from RCSB PDB (ID: 1E3K) in PDB format and was prepared using Discovery Studio 2019 to remove ligands and water molecules [11]. Ligands structures were obtained from PubChem (CID: 5994, progesterone), (CID: 73384, brazilin), (CID: 6918548, lonaprisan) in SDF format. Biological activity prediction of ligands were done by using molinspiration [12]. The ligands were prepared using PyRx 0.8 to minimize their energy and then converted to PDB format [13].

Protein-ligand docking and visualization. Protein and ligand interactions were performed by docking using Hex 8.0.0 (blind docking) to determine the position of brazilin that can bind which PR region, without limiting the possibility to bind outside the binding site [14][15]. It has an efficiency algorithm to calculate the amount of intermolecular energy from atomic and electrostatic desolvation energy as a correlation function for all

configurations and calculate protein-ligand docking by utilizing the Spherical Polar Fourier correlation (SPF) to speed up the calculation and one of the few docking programs that have been built in the graph to see its effect [16][17].

There are five interactions in this study (Table 1). The first interaction aims to determine how strong the binding between PR and its native ligand (progesterone). The second interaction aims to determine the interaction of PR with brazilin. The third interaction aims to know the ratio of binding power of brazilin to PR compared to its native ligand. The fourth interaction aims to know the interaction of PR with the common drug (lonaprisan). The fifth interaction aims to know the ratio of binding power of common drug compared to its native ligand. The interaction with the common drug was used as a control group. Docking results were visualized using Discovery Studio 2019 [11].

Table 1. Research design: molecular docking interaction

		Molecules	
Interaction	Protein	Ligand 1	Ligand 2
1	PR	Progesterone	-
2	PR	Brazilin	-
3	PR	Brazilin	Progesterone
4	PR	Lonaprisan	-
5	PR	Lonaprisan	Progesterone

RESULTS AND DISCUSSION

Biological activity prediction of ligands. Among the three compounds predicted by Molinspiration for their biological activity, progesterone and brazilin obeyed the Lipinski's Rules and showed good drug-likeness score. Mi log P values of those compounds are <5 (3.81 and 1.29) that showed that progesterone and brazilin have good permeability to across the cell membrane. TPSA scores are <160Å, molecular weight (MW) <500, number of hydrogen bond donors (nON) <5, hydrogen bond acceptors (nOHNH) <4, number of rotatable flexible bonds (nrotb) <5, and n-violations 0. Bioactivity score of progesterone, brazilin, and lonaprisan showed the highest activity as nuclear receptor ligand (>0). These compounds are potentially to become ligand for PR that belong to nuclear receptors [12][18].

Virtual analysis of progesterone receptor interacted with progesterone, brazilin or lonaprisan. Virtual interactions between progesterone receptors and all tested ligands (progesterone, brazilin, and lonaprisan) bind in almost the same position and at amino acid residues PHE895, ILE896, ARG899 all interactions involve it (Figure 1). This result shows that all three ligands (progesterone, brazilin, or lonaprisan) are predicted to have the same role and it is possible to inhibit the bond of the progesterone receptor with its native ligand. The strongest binding strength occurs in the interaction of progesterone receptors with lonaprisan, which is E: -333.8kJ/mol. The binding energy value is stronger even when compared to the native ligand. The binding energy plays an important role in determining the strength of bonds between molecules. Interactions that have a minimum binding energy value have a stronger bond, and the more negative, the stronger the bond. The small ΔGbind value indicates that the conformation formed is stable, while the large ΔGbind value indicates that the complex formed is less stable [19].

Virtual analysis of progesterone receptor interacted with brazilin or lonaprisan and progesterone. The combination of native ligands (progesterone) with brazilin (bioactive compound of *Caesalpinia sappan* L.) and lonaprisan (chemical drugs for breast cancer as a control) causes native ligands to change position in binding to the receptor (Figure 2). The binding position of native ligand (progesteron) to PR is changed significantly after lonaprisan or brazilin is docked to the receptor. The binding position of progesteron on PR changed to LYS885, HIS888, ILE920, PRO927 amino acid residues. The binding position of progesterone on PR changed to LYS885, HIS888, ILE920, PRO927 amino acid residues. In this region, it has acted as AF2; mediates transcriptional activation [20]. AF-2 is needed for recruitment of hormone-dependent, dimerized coactivators and interactions with companion proteins in an inactive state then transcription modulation and proliferation of breast cancer cells are disrupted [21][22]. Additionally, all bonds that occur are not on the binding site PR-Progesterone ARG766 [23]. These results indicate that brazilin and lonaprisan can inhibit the binding of native ligand to its receptor as an allosteric inhibitor.

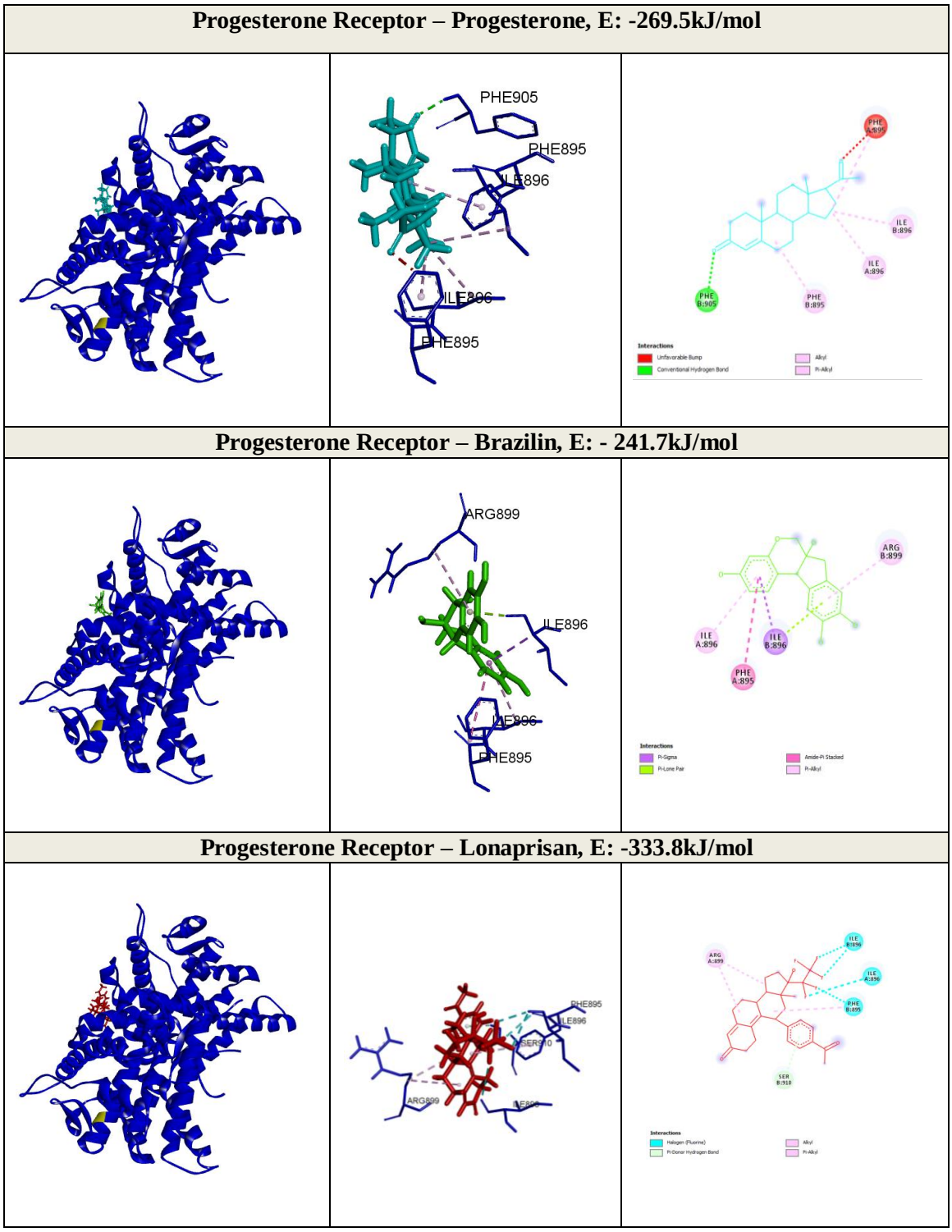

Figure 1. Virtual analysis of progesterone receptor interacted with progesterone, brazilin or lonaprisan. Difference of colors: progesterone receptor-dark blue, progesterone-light blue, brazilin-green, lonaprisan-red, binding site-yellow.

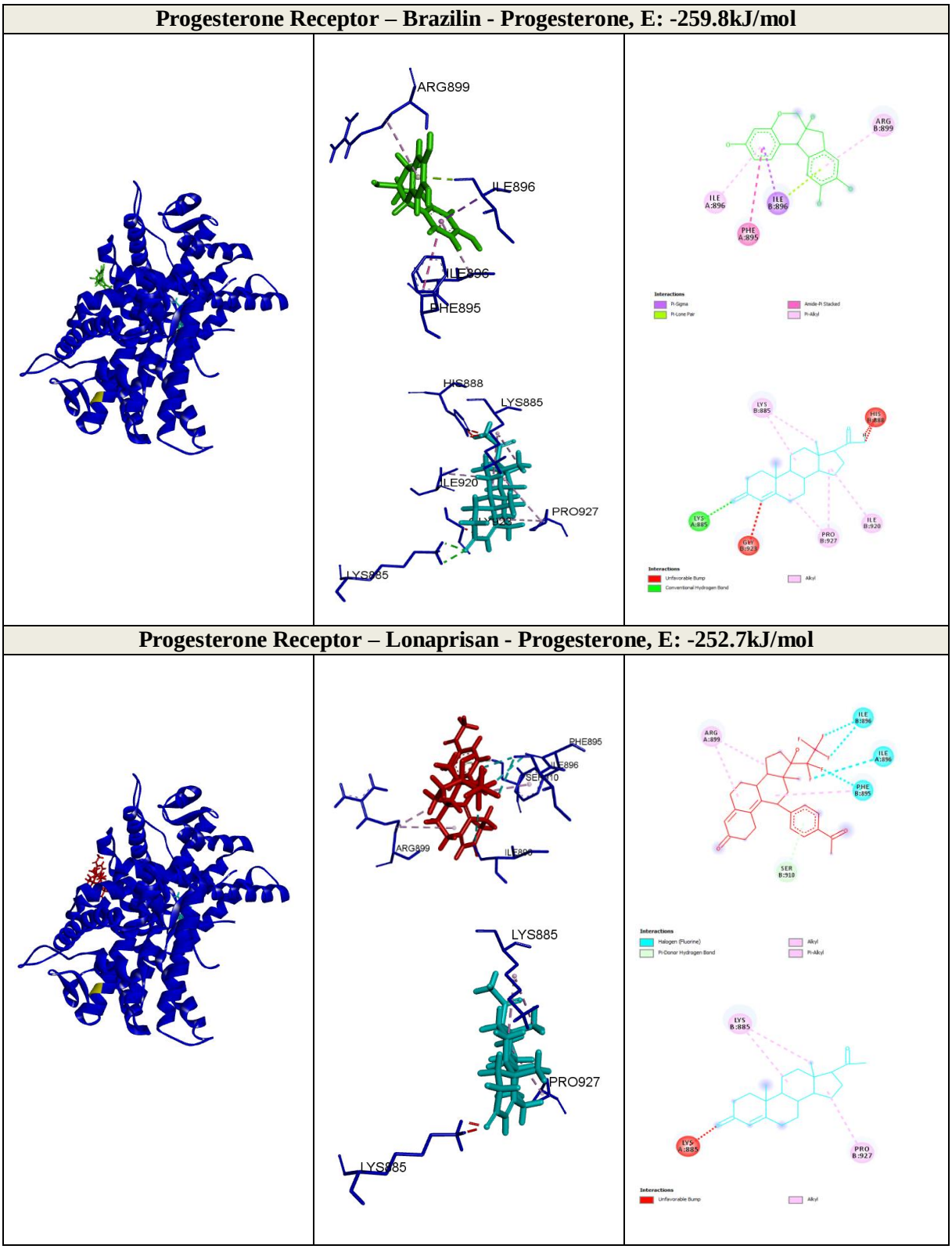

Figure 2. Virtual interaction between native ligands and brazilin or lonaprisan against PR. Difference of colors: progesterone receptors-dark blue, progesterone-light blue, brazilin-green, lonaprisan-red, binding site-yellow.

The binding of compounds on the allosteric site induce change of native ligand binding position on its receptor. These results show that brazilin and lonaprisan have a potential to interrupt the binding of progesteron to its receptor and could possibly cancel the activation of PR. This is the reason why the targeting of PR on the allosteric site is more specific than that of the kinase enzyme inhibitor, which works on the active site of the protein, so that the allosteric inhibitor used clinically better [24].

The binding energy value plays an important role in determining the strength of bonds between molecules. Results of this study indicate that binding energy of PR-B-P (PR-Brazilin-Progesterone) interactions is higher than that of PR-L-P (PR-Lonaprisan-Progesterone) by a difference of 2.1kJ/mol so it can be concluded that brazilin has a higher ability than lonaprisan to inhibit breast cancer when combined with natural ligands.

Chemical bonds formed in protein-ligand virtual interactions. In addition to the binding position and binding energy, chemical bonds from ligand-protein interactions play an important role in determining the outcome of interactions. The molecular docking of progesterone receptor with ligands such as progesterone, brazilin, and lonaprisan formed several chemical bonds as shown in Table 2.

Each bond has different roles. Covalent bonds are formed when two atoms use one pair of electrons together. Drug compound interaction with receptor through covalent bonds produce quite stable complexes and these properties can be used for certain treatment purposes such as anticancer drugs. Van der Waals bonds are involved in the interaction of the benzene ring with the plane of the receptor and the interaction of the hydrocarbon chain with macromolecules or receptors. Hydrophobic bond has an important role in the process of combining non-polar regions of drug molecules with non-polar regions of biological receptors [25]. Results of this study show that the dominant chemical bond is a hydrophobic bond which is one of the important forces in the process of combining non-polar regions of drug molecules with non-polar regions of biological receptors.

Table 2. Chemical bonds formed in protein-ligand virtual interactions

Interaction	Name	Chemistry Bound	Type
PR-P (PR-Progesterone)	B:PHE905:HN - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond
	:LIG1 - A:ILE896	Hydrophobic	Alkyl
	:LIG1 - B:ILE896	Hydrophobic	Alkyl
	A:PHE895 - :LIG1	Hydrophobic	Pi-Alkyl
	B:PHE895 - :LIG1	Hydrophobic	Pi-Alkyl
PR-B (PR-Brazilin)	B:ILE896:CA - :LIG1	Hydrophobic	Pi-Sigma
	B:ILE896:O - :LIG1	Other	Pi-Lone Pair
	A:PHE895:C,O;ILE896:N - :LIG1	Hydrophobic	Amide-Pi Stacked
	:LIG1 - B:ARG899	Hydrophobic	Pi-Alkyl
	:LIG1 - A:ILE896	Hydrophobic	Pi-Alkyl
PR-L (PR-Lonaprisan)	A:ILE896:O - :LIG1:F	Halogen	Halogen (Fluorine)
	B:PHE895:O - :LIG1:F	Halogen	Halogen (Fluorine)
	B:ILE896:O - :LIG1:F	Halogen	Halogen (Fluorine)
	B:ILE896:O - :LIG1:F	Halogen	Halogen (Fluorine)
	B:SER910:HG - :LIG1	Hydrogen Bond	Pi-Donor Hydrogen Bond
	A:ARG899 - :LIG1	Hydrophobic	Alkyl
	A:ARG899 - :LIG1	Hydrophobic	Alkyl
	B:PHE895 - :LIG1	Hydrophobic	Pi-Alkyl
PR-B-P (PR-Brazilin-Progesterone)	A:PHE895:C,O;ILE896:N - B:LIG1	Hydrophobic	Amide-Pi Stacked
	B:ILE896:CA - B:LIG1	Hydrophobic	Pi-Sigma

Interaction	Name	Chemistry Bound	Type
	B:ILE896:O - B:LIG1	Other	Pi-Lone Pair
	B:LIG1 - A:ILE896	Hydrophobic	Pi-Alkyl
	B:LIG1 - B:ARG899	Hydrophobic	Pi-Alkyl
PR-L-P (PR-Lonaprisan-Progesterone)	A:ILE896:O - B:LIG1:F	Halogen	Halogen (Fluorine)
	B:PHE895:O - B:LIG1:F	Halogen	Halogen (Fluorine)
	B:ILE896:O - B:LIG1:F	Halogen	Halogen (Fluorine)
	B:ILE896:O - B:LIG1:F	Halogen	Halogen (Fluorine)
	B:SER910:HG - B:LIG1	Hydrogen Bond	Pi-Donor Hydrogen Bond
	A:ARG899 - B:LIG1	Hydrophobic	Alkyl
	A:ARG899 - B:LIG1	Hydrophobic	Alkyl
	B:PHE895 - B:LIG1	Hydrophobic	Pi-Alkyl

The similarity of brazilin and progesterone structure. This research conducted a literature study on Selective Progesterone Receptor Modulators (SPRMs) besides discussing some of the results of these virtual interactions. SPRMs is the substance of synthetic steroids that have an agonist and/or antagonistic effect on PR. This compound has a structure similar to progesterone, so it can stick to PR [26]. Brazilin's structure is almost similar to progesterone (Figure 3) [27][28] therefore, it can bind to PR because both have amino acid residues ILE896 and PHE895.

Changes in conformation result from PR and SPRMs bonds are also caused by the accumulation of co-repressors or co-activators in the bond domain involved. Co-repressors and co-activators are correctors whose proportions influence the agonist and antagonistic effects of SPRMs. The SPRMs agonist's usually only binds with SHC-1 that is a co-activator. However, PR that binds with mifepristone (member of SPRMs) can also bind SHC-1 and silencing mediator co-repressors for retinoid and thyroid hormone receptors (SMRT) together so that they can have the right effect on different cells based on the corrector [6]. Therefore, further research involving co-regulators to explore the effects of agonists and antagonists in conformational change. Besides, in vitro and in vivo studies are more needed because the effect of the bonds of PR with SPRMs and co-regulators is different in each type of cell. In vitro and in vivo studies are needed to confirm the validity of the results in this study.

CONCLUSION

Brazilin is predicted as a competitor of progesterone binding with its receptors. Comparison of this interaction with lonaprisan to inhibit breast cancer proliferation shows that lonaprisan has better stability without progesterone (lowest binding energy), but brazilin is almost as stable as lonaprisan when docked with progesterone. These interaction also shows the two ligands can change bind to the receptor in region which has acted as AF2 mediates transcriptional activation, estimated to play a role as SPRMs Herbal medicine with brazilin can be estimated to fight breast cancer through three targets at once (survivin, Bcl-2, PR).

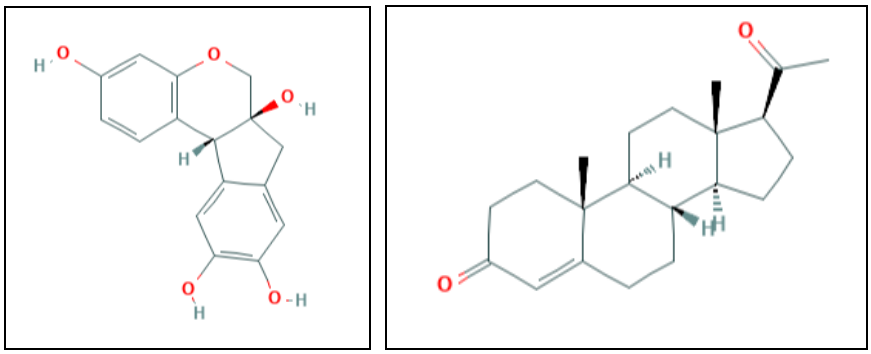

Figure 3. 2D Structure of Brazilin (left) and Progesterone (right)

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