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Molecular Docking on Kokosanolide A and C for Anticancer Activity Against Human Breast Cancer Cell MCF-7

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Abstract

Kokosanolide A (**1**), from the seeds of *Lansium domesticum* Corr. cv *Kokossan*, has been shown strong cytotoxic activities (IC_{50} = 8.62 µg/mL) against MCF-7 breast cancer cells. The aim of this work was to study the molecular interactions of kokosanolid A and kokosanolid C with the Estrogen Receptor α (ERα) using computer aided drug design approaches. Molecular docking using Autodock Vina (open-source software PyRx 0.8) was employed to explore the modes of binding of kokosanolid A (**1**) and kokosanolid C (**2**) with ERα. Compound **1** and **2** showed strong bond-free energy (-8.8 kcal/mol and -8.7 kcal/mol) to ERα. These two compounds have molecular mechanism to inhibit ERα in breast cancer cells.

Keywords: estrogen receptor alpha, *Lansium domesticum*, Meliaceae, kokosanolide A.

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1. INTRODUCTION

Breast cancer is a disease in which cell growth is abnormal cell growth or uncontrolled. (Novianti & Purnami, 2012). There are various risk factors of breast cancer. Some of them are regarded as non-modifiable risk factors and the others are regarded as modifiable ones. The former includes, such as age, mutations in the BRCA1 and BRCA2 genes, family history, reproductive history, and being exposed to high radiation to the chest. The latter are contributed by having, such as high endogenous estrogen, hormone therapy, obesity, and alcohol consumption. However, until now the effect of estrogen on breast cancer has attracted a lot of attention. This is due to its important role in causing breast cancer (Samavat & Kurzer, 2015). About 70% of breast cancers express estrogen receptor (ER) (Qi *et al.*, 2011). Inhibiting the receptor activities can be a strategy in the treatment of estrogen receptor-positive breast cancer, either by inhibiting estrogen production using aromatase inhibitors or by interfering with the binding site of estrogen using selective estrogen receptor modulators (SERMs) (Belev & Vrbanec, 2012). Estrogen receptors belong to the nuclear receptor family and have two subtypes, namely estrogen receptor alpha (ERα) and beta (ERβ). From these two subtypes, 78% of breast cancers express ERα receptors making it the most important predictor for breast cancer prognosis (Burns & Korach, 2012).

Current cancer research trends focus on developing anticancer drugs using natural compounds. Several methods such as in vitro, in vivo, and computation are used to see the potential of the drug. Molecular Docking has been used extensively in the prediction and design of drugs for cancer (Tabassum *et al.*, 2014; Zahra *et al.*, 2013). Investigating bioactive compounds derived from medicinal plants lies in their affordability and convenience to increase efficacy and minimize serious side effects (Sudha *et al.*, 2018). Two tetranortriterpenoids, kokosanolid A and C have been reported found in the

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seeds of L. domesticum Corr. Cv kokossan (Mayanti *et al.*, 2011). Kokosanolid A , have been shown to have strong cytotoxic activity (IC_{50} = 8.62 μg/mL) against MCF-7 breast cancer cells (Mayanti *et al.*, 2016). L. domesticum is a tropical plant that is widespread in Asian regions such as Malaysia, Thailand, Indonesia, and the Philippines (Lim, 2012). Several previous reports have described the interesting bioactivity of triterpenoids came from L. domesticum, such as antimalarial (Saewan *et al.*, 2006), insecticide (Leatemia & Isman, 2004), antifeedant (Mayanti *et al.*, 2011), antibacterial (Ragasa *et al.*, 2006), antimutagenic (Matsumoto *et al.*, 2018; Matsumoto *et al.*, 2019), and anticancer activity (Fadhilah *et al.*, 2020). In this paper, we report a study about interactions and potential activity of two tetranortriterpenoids, kokosanolide A (1) and kokosanolide C (2) (Figure 1) to inhibit ERα in breast cancer cells.

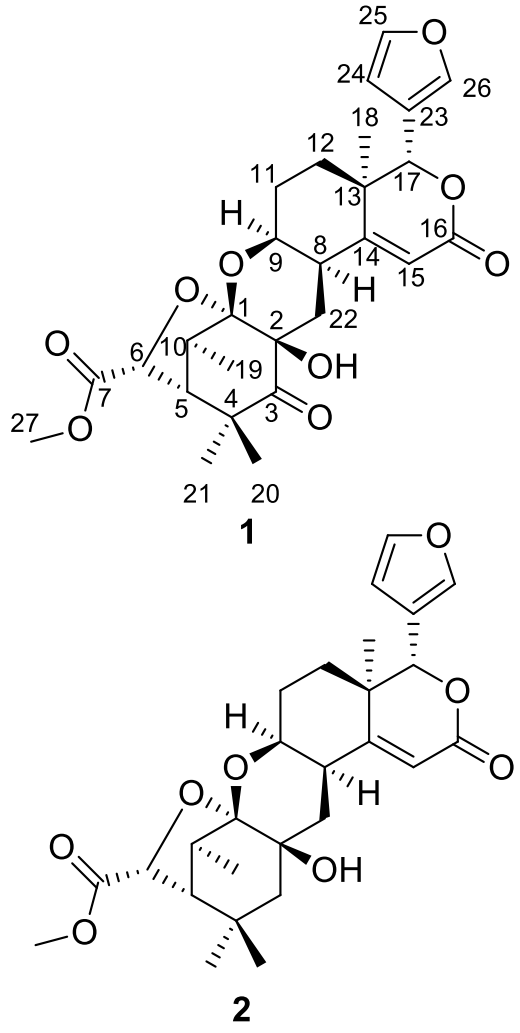


Figure 1. Structures of Compounds **1** and **2**

2. MATERIAL AND METHODS

Molecular Docking Assay

ER-α (protein data bank ID: 3ERT) was used in this research for screening compound mechanism against breast cancer cells activity. The 3-dimensional structures were retrieved from RSCB Protein Data Bank (https://www.rcsb.org/) obtained in PDB format. Afimoxifene (4-OHT) was used as a positive control ligand and estradiol ligand for comparison. The 3D structures of the compounds were modeled by using Biovia Discovery Studio program (Mukund *et al.*, 2019).

Autodock Vina (open-source software PyRx 0.8) was used for the ligand-protein docking and virtual screening for anticancer activity of the compounds. Compound 1, compound 2, 4-OHT, and estradiol were subjects for binding to ER-α as protein target, the ligand was free for blind docking. Before the ligand is thetered to the receptor, it is necessary to identify the active pocked that binds to the receptor through grid parameters. The grid box was produced by redocking tamoxifen against ERα is x=30.282; y=1.1913; z=24.207 with a space volume of 40x40x40 points. The conformation was selected based on binding energy, the one with the lowest binding affinity score. The docking simulation was performed at the center of the active side of the receptor with coordinates (x = 30.010, y = -1.913, and z = 24.207) selected.

The docking results were visualized by using Biovia Discovery Studio program. Ligand-residue interaction and docking poses in the 3-dimension molecular picture were showed by the program. The docking pose of each protein-ligand complex was compared by looking at the side of the amino acid residue that binds the ligand. The similarity from ligation poses of compound 1 and 2, 4-OHT, and estradiol that bound on amino acid residues was determined and the relation of the docking pose of the ligands and protein targets being studied was selected. Prediction of pharmacokinetics was carried out with a SMILE structure analyzed using swiss ADME http://www.swissadme.ch/index.php) (Daina *et al.*, 2017).

3. RESULTS AND DISCUSSIONS

The molecular mechanism prediction of the compounds binding the ERα was performed by molecular docking simulations.

Estrogen receptors are a group of proteins that are activated by estrogen hormones (17β-estradiol) (Dahlman *et al.*, 2006). The MCF-7 cell lines have characterized as expressing ERα or proliferate in response to estradiol (Gross & Yee, 2002).

In this paper, the in silico study of isolated compounds is carried out to determine the stereoselective and regioselective factors on the target receptor. The parameters observed are the binding affinity and the residues binding of ERα. The bond energy or binding affinity (ΔG) used to measure as to rank order hits binding to the target and help design drugs that bind their targets selectively and specifically . The smaller or more negative ΔG value represents, the stronger stability and strength of the bond are (Gupta *et al.*, 2015).

The analysis of result as shown in Table 1 presented the interaction of isolated compounds with the ligand-binding domain (LBD) of the estrogen receptor alpha (ERα). This reveals the strength of an interaction between each compounds and ERα that is able to be observed from the relative binding affinity (RBA).

Molecular docking was carried out on the ERα and 4-OHT structures to validate the molecular docking method used. The best molecular docking results were obtained with an RMSD (root mean square deviation) value of 0.987 Å from the previous position obtained from the X-ray conformation (Figure 2).

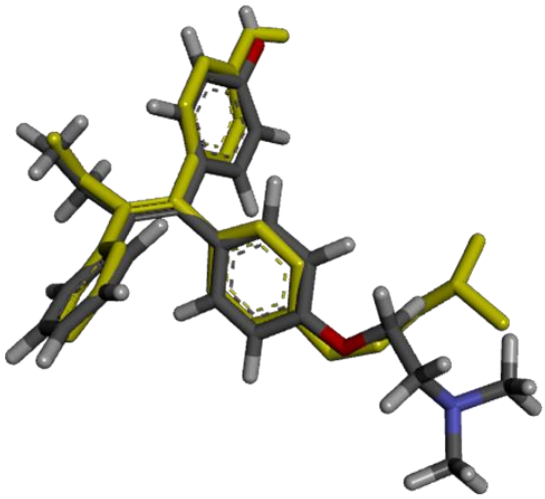


Figure 2. The result of molecular docking on the 4-OHT ligand with an RMSD value of 0.987 Å. Yellow color represents after redocking.

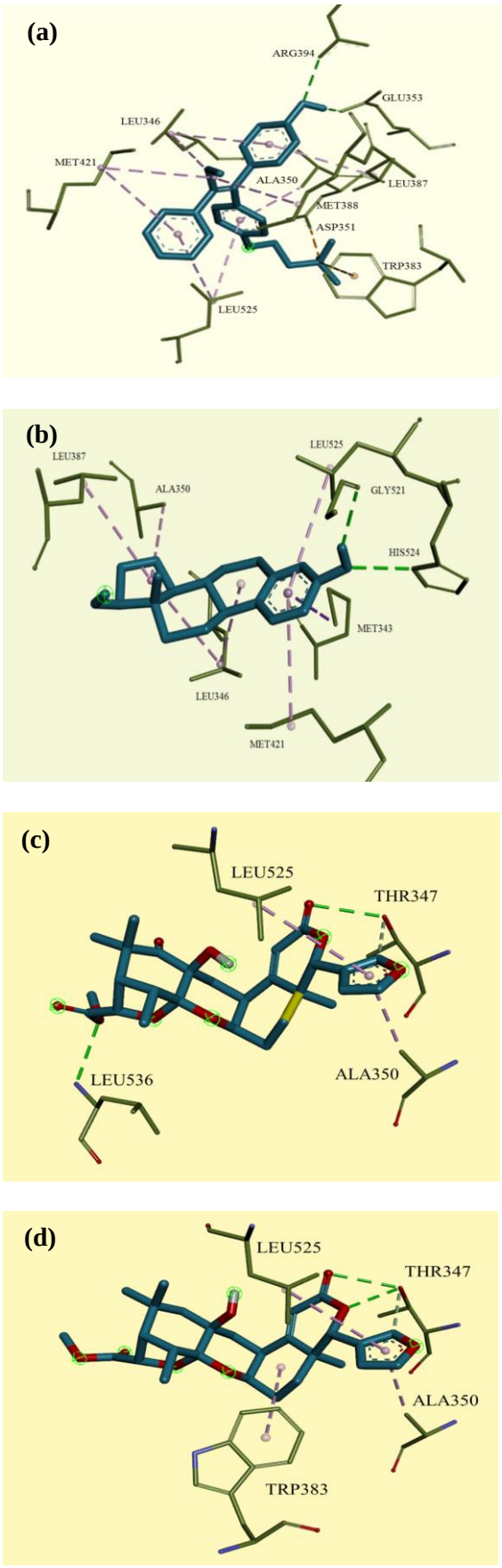


Figure 3. Binding site on ERα for: (**a**) Estradiol (**b**) 4-OHT (**c**) Compound **1** (**d**) Compound **2**

From 100 runs, it was obtained one cluster with an average binding energy value of -11.53 kcal/mol. Molecular docking shows the presence of four hydrogen bond interactions between 4-OHT and Arg394, Glu353, Asp351, TRP383, as well as hydrophobic interactions with ALA350, LEU346, MET388, LEU387, MET421, LEU525 (Figure 3 (3a) and Figure 4).

Molecular docking is also carried out using a natural ligand from ERα, namely estradiol. This ligand is obtained from a crystalline structure. Molecular tethering is carried out using the same protocol as before. The result of molecular docking showed ΔG value of -9.0 kcal/mol. From the best results, two hydrogen bonding interactions were obtained with HIS524 and GLY521 and 6 hydrophobic interactions with LEU346, LEU387, LEU525, MET343, MET421 and ALA350 (Figure S2 (2b)).

Molecular docking on the ERα with compound 1 and 2 showed free energy -8.8 kcal/mol and -8.7 kcal/mol respectively. Based on this data, compound 1 and 2 have molecular mechanism to inhibit ERα in breast cancer cells which is shown through the formation of hydrogen bonds with ERα. Moreover, compound 1 and 2 attaches the same residues with positive controls, 4-OHT, in ER-α (Ala350 and Leu525 residues) (Table 1 and Figure 3).

Figure 5 shows the bioavailability radar of molecules. The bioavailability radar gives a main scan at the drug-likeness of a compound. All the compounds passed the drug-likeliness test as shown in Table 2; they also passed the Lipinski rule of five, a criteria used as a guide in drug design (the molecules that adhere to three rules out of the four rules are said to obey to Lipinski rule (Daina et al., 2017)).

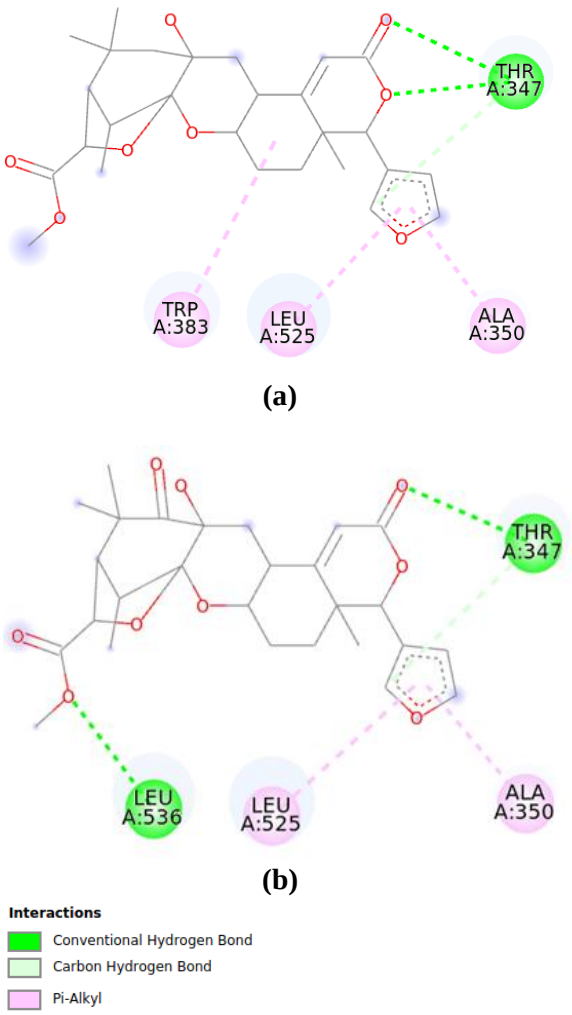


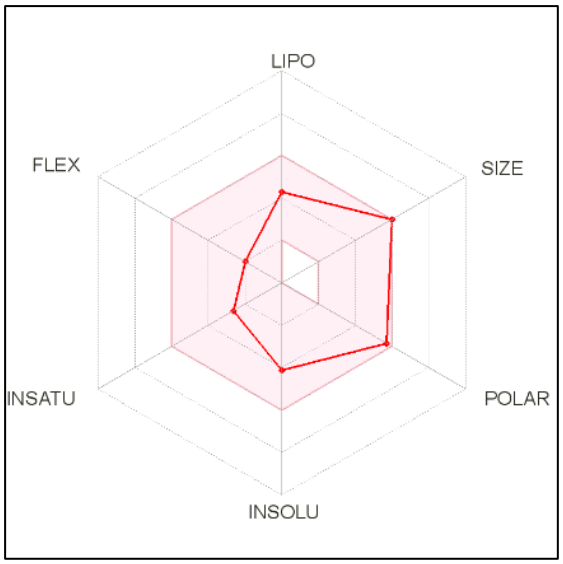
Figure 4. The 2D binding site on ERα for compound **1** (**a**) compound **2 (b)**

Tabel 1. Attached residues to ligands

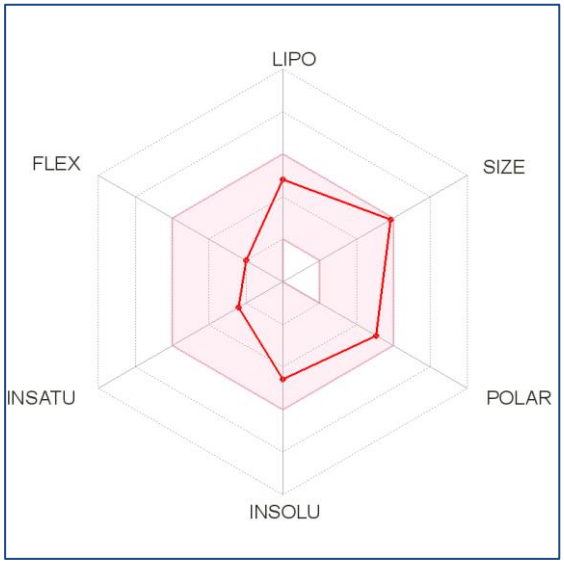
Residues binding at ligand-protein complex	
Ligands	ERα
4-OHT	Arg394, Glu353, Met421, Ala350, Leu387, Leu346, Leu525, Met388, Asp351, and Trp383
Estradiol	Gly521, His524, Met421, Leu346, Met343, Leu387, Leu525 and Ala350
1	Thr347, Ala350, Leu525, and Leu536
2	Thr347, Ala350, Leu525, and Trp383

Table 2. Pharmacokinetics properties

Compound	MW (g/mol)	Log P	TPSA (Å^2)	HBA	HBD	Rule of 5 violation
Kokosanolid A	500.54	2.41	121.50	9	1	1
Kokosanolid C	48.55	3.05	104.43	8	1	0



(a)



(b)

Figure 5. The bioavailibility radar for: compound Compound **1** (**a**) Compound **2 (b)**

4. CONCLUSIONS

Two tetranortriterpenoids, kokosanolide A (**1**) and kokosanolide C **(2)** showed strong bond-free energy (-8.8 kcal/mol and -8.7 kcal/mol) to ERα. These two compounds have a molecular mechanism to inhibit ERα in breast cancer cells.

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