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Insight on Estrogen Receptor Alpha Modulator from Indonesian Herbal Database: An *in-silico* analysis

Wawasan terhadap Modulator Estrogen Reseptor Alfa dari Database Herbal Indonesia: Analisis *In-Silico*

Muhammad Arba[1,2]*, Nadhifatul Aslikah[1], Arfan[1], Ruslin[1], Arry Yanuar[2]

[1]Faculty of Pharmacy, Universitas Halu Oleo
Jl. HEA Mokodompit, Kendari 93232, Indonesia.
[2]Faculty of Pharmacy, Universitas Indonesia
Kampus UI Depok, Pondok Cina, Beji, Depok 16424, Indonesia

*Corresponding author email: muh.arba@uho.ac.id

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ABSTRACT

Estrogen receptor α (ERα) is liable for regulating transcription factors which are an important part of hormonal signaling in breast cancer. This study intends to find hit compounds that are considered capable of inhibiting ERα by utilizing structure-based pharmacophores and molecular docking. Pharmacophore of the original ERα ligand (E4D600) has one hydrogen bond acceptor and three hydrogen bond donors which are used to select compounds from the Indonesian herbal database. This pharmacophore model had an Area under Curve of the Receiver Operating Characteristics (AUC-ROC) value is 0.80 and the Goodness of Hits (GH) value is 0.81. The selection process generated 330 compounds which proceed to the molecular docking stage to analyze their binding energy and interactions to ERα. The results indicated potential hit compounds seen from their binding energies in the range -5.42 to -10.01 kcal/mol. four of the best compounds including Lig57/(-)-Bidwillon A, Lig47/Quercetin 3-(6''-galloylgalactoside), Lig197/Multifloroside and Lig83/Erythrabyssin II provide promising information for their detailed analysis as ERα inhibitors.

Kata kunci: Estrogen receptor α, Indonesian herbal database, molecular docking, pharmacophore.

ABSTRACT

Estrogen receptor α (ERα) bertanggung jawab dalam mengatur faktor transkripsi yang berperan penting pada jalur pensinyalan hormonal kanker payudara. Penelitian ini bermaksud untuk menemukan senyawa yang dianggap mampu menghambat ERα dengan memanfaatkan pemodelan farmakofor berbasis struktur dan penambatan

molekuler. Farmakofor Ligan alami ERα (E4D600) memiliki satu akseptor ikatan hidrogen dan tiga donor ikatan hidrogen yang digunakan untuk memilih senyawa dari database herbal Indonesia. Model farmakofor ini memiliki nilai Area under Curve of Receiver Operating Characteristics *(AUC-ROC) sebesar 0,8 dan nilai* Goodness of Hits *(GH) sebesar 0,81. Penambatan molekuler terhadap 330 senyawa hit bertujuan untuk menganalisis energi ikatan dan interaksinya dengan ERα. Hasil penambatan molekuler menunjukkan potensi dari senyawa hit berdasarkan energi ikatannya dengan rentang -5,42 hingga -10,01 kkal/mol. Empat senyawa terbaik di antaranya Lig57/*(-)-Bidwillon A, *Lig47/*Quercetin 3-(6''-galloylgalactoside, *Lig197/*Multifloroside, dan Lig83/Erythrabyssin II *memberikan informasi yang menjanjikan untuk analisis lebih terperinci senyawa hit tersebut sebagai inhibitor ERα.*

***Keywords**: Database Herbal Indonesia, estrogen reseptor α, farmakofor penambatan molekuler.*

Introduction

Indonesia has a variety of plants and compounds that have long been used until now to treat various types of diseases (Sholikhah, 2016). Cancer is one of the causes of death in Indonesia (Tjindarbumi and Mangunkusumo, 2002). Cancer, one of which can be caused by abnormalities of estrogen receptor expression(Hua *et al*., 2018). The expression of the estrogen receptor can be a parameter for the diagnosis of breast cancer (Haque and Desai, 2019).

Estrogen receptors (ER) are mediators of intracellular signaling pathways and transcription factors involved in many parts of the biological system, including cell expansion and regulating the central nervous system, mammary glands, and reproduction. Estrogen receptors consist of 2 types encoded by different genes and activated by estrogens, namely ERα and ERβ (Souza *et al*., 2017). ERα can be found in the breast, bone, and reproductive tissue (uterus, ovary). ERα plays a role in tumorigenesis and the expansion of breast cancer. ERβ is found in the prostate, large intestine, immune system, cardiovascular system, and central nervous system. ERβ can directly inhibit the activity and expression of ERα (Jia *et al*., 2015). The most frequent and effective treatment given to breast cancer sufferers is with a selective estrogen receptor modulator (SERM) (Grande *et al*., 2018).

Tamoxifen, toremifene, raloxifene, and arzoxifene are SERM groups that block estrogen from occupying with its receptor. the group has many problems including side effects such as stroke, heart disease, coronary events, bone fractures, low bioavailability, mutations that cause a high risk of breast cancer recurrence, and even death (Kucinska *et al*., 2016; Piperigkou and Karamanos, 2020; Dai and Wu, 2011; Zheng *et al*., 2020). This study aims to identify Indonesian natural compounds that have the potential to inhibit estrogen receptors by utilizing

virtual screening techniques along with the development of science and technology.

Virtual screening is a computer-aided drug design technique (CADD) in drug discovery that utilizes pharmacophores ligand information to obtain active compounds (ligand-based drug design) (Makrynitsa *et al*., 2018). The structure-based drug design included in CADD namely molecular docking (Batool, Ahmad, and Choi 2019) was also applied in this study to explore and analyze the binding energy and orientation of the hit compound on the estrogen receptor.

Material and Methods

Pharmacophore modeling and data base screening

LigandScout Advanced 4.3 (Wolber and Langer, 2005) was used to build a pharmacophore model of the E4D600 structure which is a native ligand from ERA (PDB ID: 1SJ0). The pharmacophore model generated was validated against 626 active compounds and 20773 decoys obtained from the Directory of Useful Decoys: Enhanced (DUD-E) (http://www.dude.docking.org/) (Mysinger *et al*., 2012). The best pharmacophore model chosen had features with one hydrogen bond acceptor and three hydrogen bond donors (Figure 1). These features are then used for screening compounds in the Indonesian Herbal database (http://herbaldb.farmasi.ui.ac.id/).

Molecular docking simulation

All hit compounds were subjected to molecular docking simulation using iDock software. iDock is a software under the apache 2.0 license which is available free of charge and open source (Li, *et al*., 2012) to ERα. ERα protein was downloaded from protein data bank (PDB ID: 1SJ0) (http://www.rcsb.org/pdb/). The protein was prepared by removing water molecules, added polar hydrogen, and the Kollman charge using AutoDockTools 1.5.6 software (Morris *et al*., 2009; Arba *et al*., 2018; Arba *et al*., 2018). The active site of the protein was regulated by copying the position of native ligands with coordinates center x = 30.660, center y = -1.067 dan center z = 23,464. The grid box size used is 40 x 40 x 40 Å with point spacing 0.375 Å. The results of molecular docking are then visualized using Discovery Studio Visualizer.

Result and Discussion

The pharmacophore model was chosen must fulfill the AUC-ROC and GH score criteria greater than or equal to 0.5. The best pharmacophore model was generated had features with one hydrogen bond acceptor and three hydrogen bond donors (Figure 1). The pharmacophore model fulfills the validation requirements with a Goodness of Hits (GH) value is 0.81 and an Area Under Curve value of the Receiver Operating Characteristic (AUC-ROC) is 0.80 (Figure 2). AUC-ROC values close to 1 indicate the good pharmacophore model and could be distinguishing between active and decoy compounds.

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Further, screening against the Indonesian Herbal database (1379 molecules) employing the validated pharmacophore model retrieved 330 hit molecules.

The interactions of 330 hit compounds were analyzed using molecular docking results based on their binding energy and conformation to ERA. Validation of the molecular docking process was achieved by obtaining a root mean square deviation (RMSD) value from the overlay between the crystallographic conformation of the native ligand (E4D600) and the docking result are 0.6252 Å (Figure 3).

Molecular docking on all hits to ERα resulted in conformations and binding energies in the interval of -5.42 to -10.01 kcal/mol. The binding energies of hit molecules were comparable to that of the ligand antagonist 4-D (-11.81 kcal/mol). Some interactions observed from the conformation of E4D600 include interactions with hydrogen bonds with residues Gly521, His524, Leu387, Glu353, and Arg394. The conformation also has interactions with Leu384, Leu349, Met421, and Leu354. It is known that His524, Leu348, and Met421 are important residues on the ERα active site (Kim *et al*., 2004).

Based on the binding energies and conformations, four best-docked hit molecules were selected. They were Lig57 or (-)-Bidwillon A (E = -10.01 kcal/mol), Lig47 or Quercetin 3-(6''-galloylgalactoside (E = -10.00 kcal/mol), Lig197 or Multifloroside (E = -9.87 kcal/mol), and Lig83 or Erythrabyssin II (E = -9.69 kcal/mol). Figure 4 shows the chemical structures of the four best docked hit molecules.

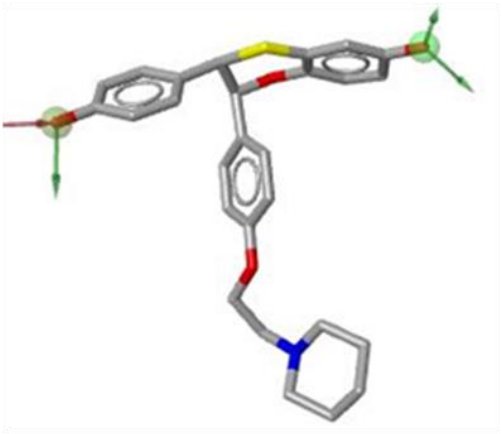

Figure 1. 3D pharmacophore model composed of one hydrogen bond acceptor (red line) and three hydrogen bond donor (green lines) features

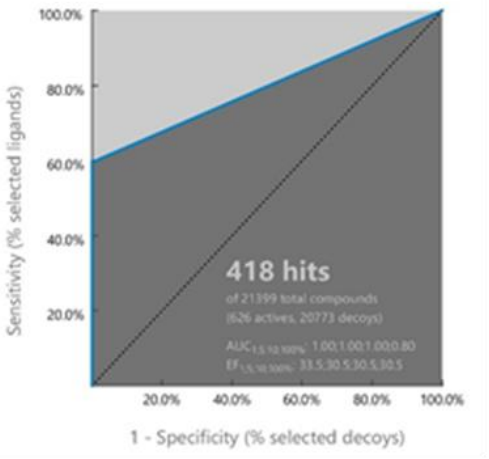

Figure 2. The Area under Curve (AUC) of Receiver Operating Characteristic (ROC) curve

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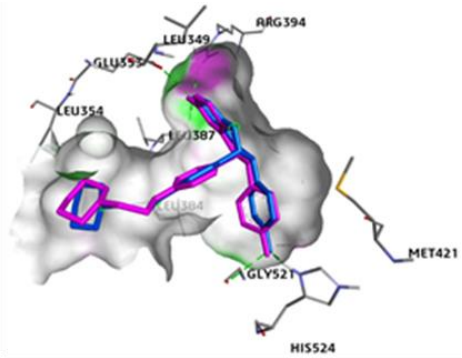

Figure 3. The superimposed 3D conformations of both experimental (purple) and docked (blue) experiments with green dash line represent the hydrogen bond

The interaction of Lig47 or Quercetin 3-(6''-galloylgalactoside) with ERA was supported by hydrogen bonds with Glu353 and Gly521 residues, while hydrophobic interactions were observed with Leu384, Leu387, His524, Leu525. Leu349. In Lig57 or (-)-Bidwillon A, the interaction with ERα was dominated by hydrophobic interactions with residues Glu353, His524, Leu384, Leu387, Leu525, Leu349.

Hydrogen bonds in Lig197 or Multifloroside interact with Gly420, Gly521, Ile424 residues, as well as in Lig83 or Erythrabyssin II interact with Glu353, Arg394, Ile424, Leu387 residues on the active site of ERα. Hydrophobic Interactions with residues His524, Leu525, Leu384, and Met421 were observed in both ligands. All hit compounds can interact with important residues on the active site of ERA. Figure 5 shows the conformation and interactions of the four best docked hit molecules.

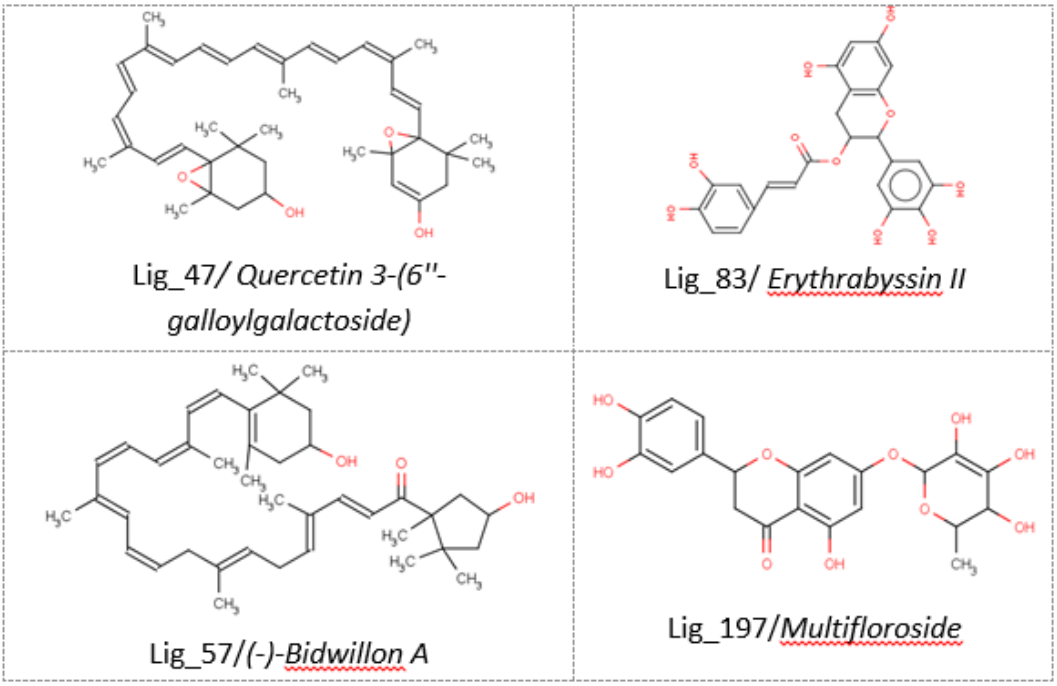

Figure 4. The chemical structures of the four best docked hit molecules

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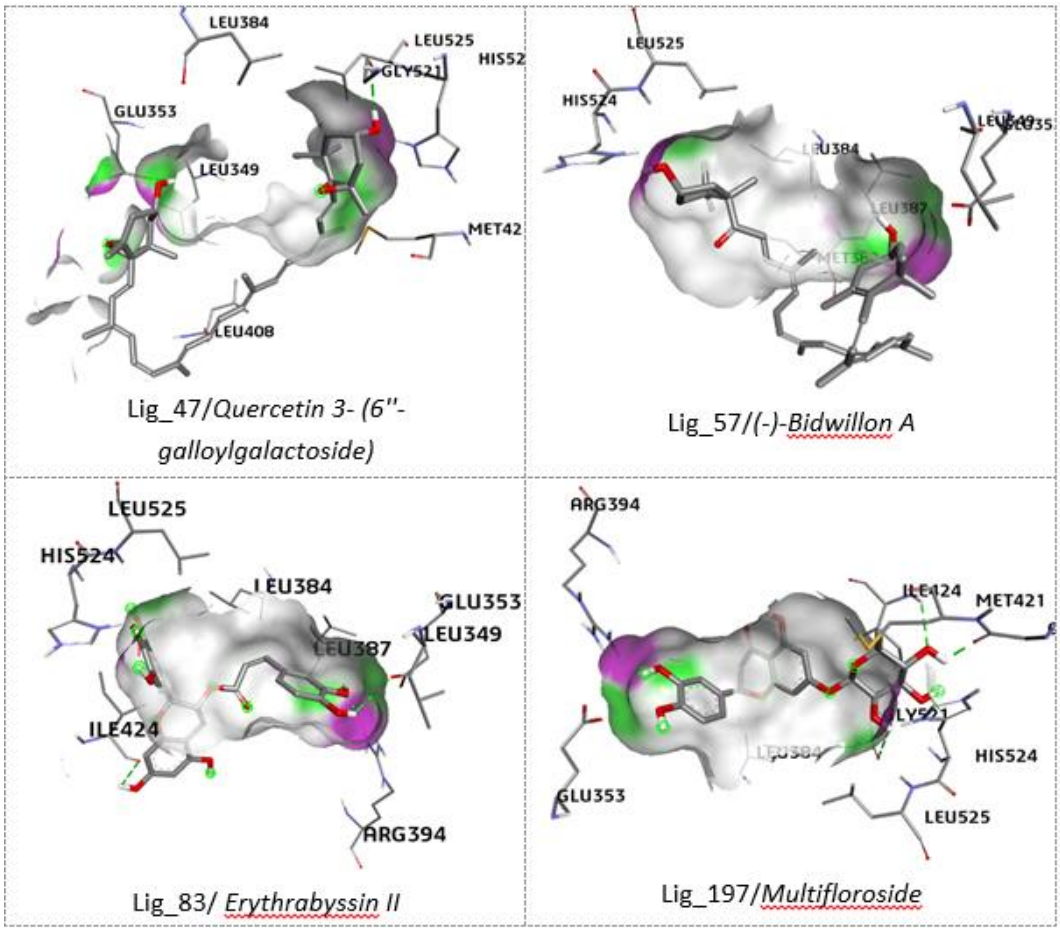

Figure 5. The binding orientation of Quercetin 3- (6''-galloylgalactoside), (-)-Bidwillon A, Erythrabyssin II, and Multifloroside in the active site of ERα

Conclusion

This study was effectively able to identify potential estrogen receptor inhibitors from the Indonesian herbal database which contained 1379 molecules. This illustrates that all the best compounds were able to occupy the active site of ERα as showed by molecular docking simulations. The binding energy of four best hits (Lig57/(-)-Bidwillon A, Lig47/Quercetin 3-(6''-galloylgalactoside), Lig197/Multifloroside and Lig83/Erythrabyssin II), were comparable with native ligand E4D600. It represents their potential to inhibit ERα and to be considered in the further experimental study.

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