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INTERACTION OF FURANODIENONE, PROCURCUMENOL, CURDIONE, AND DEHYDROCURDIONEFOUND IN *CURCUMA ZEDOARIA* (CHRISTM.) ROSCOE) WITH HUMAN ESTROGEN RECEPTOR ALFA (ERα)

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ABSTRACT

ERα, induced by estradiol, which is its agonist, activating genes that play a role in the growth of breast cancer cells, thus making it an attractive target for anti-breast cancer drug discovery. Essential oils of white tumerics (*Curcuma zedoaria*), which consists of several compounds, for example, furanodienon, procurcumenol, curdion, and dehidrocurdion, could be expected to occupy the active ERα bag because it has inhibitory activity against MCF-7 cells in vitro testing. Molecular docking performed on compound furanodienon, procurcumenol, curdion, and dehidrocurdion to visualize the interaction between these ligands with ERα compared with estradiol and tamoxifen, using the software AutoDock Vina. Molecular docking results showed that the compound furanodienon, procurcumenol, curdion, dehidrocurdion, and tamoxifen interact with ERα whereas hydrophobic estradiol forming hydrogen bonds and hydrophobic interactions. It was found that all tested ligands could be located in the active pocket of ERα, based on the Ki values, which are 1,154µM, for furanodienone, procurcumenol, and dehydrocurdione, and 1,365µM, forcurdione.

Keywords : ERα, furanodienone, procurcumenol, curdione, dehydrocurdione, docking

INTRODUCTION

Natural estrogens, especially estradiol, ERα regulated by transcription factors, activating genes that play a role in cancer growth. Estradiol binding to ERα to activate the complex by binding polypeptide further role in DNA transcription. ERαi sweighing 68k Daprotein with

an amino acid constituent active pocket consisting of Met343, Leu346, Leu349, Ala350, Glu353, Leu384, Leu387, Met388, Leu391, Arg394, Phe404, Met421, Ile424, Phe425, Leu428, Gly521, His524, and Leu525 (Atkinson *et al*., 1999; Denger *et al*., 2001; Tanenbaum *et al*., 1998). Estradiol forming hydrophobic interactions inactive ERα pocket and form hydrogen bonds with glutamate 353 (Glu353), arginine394 (Arg394) and histidine524 (His524) (Blizzard *etal*., 2007). ERαis associated with breast cancer cause excessive over expressed in cancer tissue (Ikeda and Inoue, 2004).

According to Indonesia Health Profile, there are 8277 cases of breast cancer in 2007, which was the highest of all cancer cases in Indonesia (Departemen Kesehatan Republik Indonesia, 2009).

The in vivo study Hamid (2008) showed that the ethanol extract of the rhizomes of white tumerics with a dose of 300 mg/kg chemopreventive effects on mammary gland cells. Components of essential oils from white tumerics consists of several sesquiterpenoid compounds such as, furanodienone, procurcumenol, curdione, and dehydrocurdione (Carvalho *et al*., 2010; Jang *et al*., 2001). White tumerics essential oils can inhibit proliferation and induce apoptosis of breast cancer cells MCF-7 at a concentration of 150-400 mg/L (Pu and Zhao, 2009). According to in vitro studies, furanodienone can inhibit the growth of breast cancer cells MCF-7, T47D, and MDA-MB-231 at a concentration of 10-160 µM. Research shows furanodienone stimulation inhibits estrogen response resulting in decreased proliferative activity of cancer cells and subsequently induce apoptosis (Li *et al*., 2011).

This study reports on the study of the interaction and activity of anticancer compounds furanodienone, procurcumenol, curdione, and dehydrocurdione through methods based and docking base dapproach. Reported data will be very useful to supplement and verify data having been reported by above previous researchers.

METHODS

Tools

The hard ware used for the calculations, molecular modeling and molecular docking include personal computer with the technical specifications of AsusIntelPentium4 CPU2.4GHz, operating system WindowsXP Professional32-bit, capacity 250 Gb harddisk and 1 GB of RAM memory.

The software used was as follows:

1. 3D ChemBio Program v12.0.2 Free Trial (Serial number: 186-410320-7811)
2. Hyper Chem Program v8.0 Professional Edition (Verification Code: 0-28331).
3. SwissPDBViewer program package v.4.01 (by GlaxoSmithKline R & D downloaded from http://www.expasy. Org)
4. Auto Dock Vina program (Molecular Graphics Laboratory, The Scripps Research Institute in 2011 downloaded from http://vina.scripps.edu/download.html)
5. Program Open Babel v.2.1.1 (software downloaded from http://openbabel.org in 2007).
6. Ligand Explorer Viewer v.3.8 program (by the Research Collaboratory for Structural Bioinformatics, which was the data on line from http://www.pdb.org/pdb/explore)
7. Q-SiteFinder program, which was a freeware online that are accessed from www.bioinformatics.leeds.ac.uk/qsitefinder/
8. Program Tools v1.5.6 MGL (Molecular Graphics Laboratory, The Scripps Research Institute in 2012 downloaded from http://mgltools.scripps.edu)
9. The program PyMOL (The PyMOL Molecular Graphics System 2002 by Delano, WL downloaded from http://www.pymol.org).

Material

Three-dimensional structure of the estrogen receptor alpha (ERα), which had been crystallized with estradiol at 2.8 Å resolution (PDB code: 1A52), obtained from the online database of Protein Data Bank (www.pdb.org). Two and three-dimensional structure of furanodienone, procurcumenol, curdione, dehydrocurdione, estradiol, and tamoxifen were drawn using the program package ChemBio 3D v12.0.2 Free Trial (Serial number: 186-410320-7811).

Method

1. Preparation ERα (PDB code: 1A52) obtained from the Protein Data Bank (www.pdb.org).

a. Download data from the Protein Data Bank ERα.

b. Reduction ERα chain to form monomer (chain A) using SwissPdbViewer software v.4.01.

c. Analysis bag Ligand Explorer Viewer bond with the Protein Data Bank (www.pdb.org).

2. Analysis of ligand-receptor interactions with ligands Explorer Viewer in the Protein Data Bank (www.pdb.org).

a. Preparation of ligand furanodienon, procurcumenol, curdione, dehydrocurdione, estradiol, and tamoxifen with 3D ChemBio software v12.0.2 Free Trial (Serial number: 186-410320-7811).
b. Making two-and three-dimensional structures with a 3D ChemBio program v12.0.2 Free Trial (Serial number: 186-410320-7811).
c. Optimization of geometry with HyperChem software v8.0 Professional Edition (Verification Code: 0-28331) using appropriate methods.
c. Analysis of molecular properties by HyperChem software v8.0 Professional Edition (Verification Code: 0-28331).

3. AutoDock Vina program validation by crystallized redocking inhibitors to ERα binding pockets. Stacking structure model with inhibitor that has been crystallized using HyperChem software v8.0 Professional Edition (Verification Code: 0-28331).

a. Overlay model with ligand crystal structures that have been crystallized using HyperChem software v8.0 Professional Edition (Verification Code: 0-28331).
b. Redocking inhibitors which have crystallized into ERα binding pockets using the program AutoDock Vina.
c. Analysis of data validation results.

4. Furanodienon molecular docking, procurcumenol, curdione, dehydrocurdione, estradiol, and tamoxifen using AutoDock Vina program.
5. Interpretation of data from molecular docking.

RESULTS AND DISCUSSION

1. Results Preparation ERα (PDB code: 1A52)

ERα obtained from the Protein Data Bankwas the result of crystallization of protein crystals with ligands, estradiol, which was isolated from Homosapiens, was a homodimermolecule, which was a polymer consisting of two monomers of the same type (chain A and B). However, to focus on the dockingarea, carried out the reduction of one of the chain, which in this study was the Bchain, using v.4.01Swiss PDBV that achieved more accurate results. The selection was based on a chain leash to which a ligand binds. As ligand binding to both chains (homodimer), then both chains could be selected.

Pocket active receptor was then analyzed using the program Ligand Explorer Viewer and Q-Site Finder.

Constituent amino acids activated ERα pocket can be seen in Table1.

Tabel 1.Amino Acid Residues in the Pocket Active Compiler of Estrogen Receptor Alpha (ERα)

Asam Amino Residue	***Ligand Explorer Viewer***	***Q-SiteFinder***
Met343	√	√
Leu346	√	√
Thr347	-	√
Leu349	-	√
Ala350	-	√
Asp351	√	√
Glu353	√	√
Trp383	-	√
Leu384	-	√
Leu387	√	√
Met388	√	√
Leu391	√	√
Arg394	√	√
Phe404	√	√
Met421	-	√
Ile424	√	√
Phe425	-	√
Leu428	-	√
His524	√	-
Leu525	√	-

Constituent amino acid residues in the active pocket Ligand Explorer Viewer was less than the Q-SiteFinder due to the distance of observation on Ligand Explorer Viewer was narrower, only based on the interaction between the receptor (ERα) with the crystal ligand (estradiol), whereas observations with Q- SiteFinder more widely, based on a calculation using the interaction energy between macromolecules (ERα) with the van der Waals loop to look for bags that are considered the most preferred binding by the ligand from an energy standpoint (pocket bond interactions with the lowest energy). In addition, through Q-SiteFinder also known coordinates of the pocket active receptor. According to Q-SiteFinder, minimum

coordinates found in 94, 4, 87 (x, y, z) and the maximum coordinates at 115, 25, 107 (x, y, z) with an active pocket volume of 561 A^3. The area in between the minimum and maximum coordinates is called an active enclave receptor and can be used in determining the grid on the docking process.

Based on the analysis done by Ligand Explorer Viewer, estradiol was known to interact with ERα through, hydrogen bonds with amino acid residues Glu353, Arg394, and His524, as well as hydrophobic interactions with amino acid residues Ala350, Leu387, Met388, Leu391, Phe404, Ile424, and Leu525 .

2. Results Preparation of Ligand Furanodienone, Procurcumenol, Curdione, Dehydrocurdione, Estradiol, and Tamoxifen

Preparation of ligands serve to prepare the ligand used in the docking process that has the conformation corresponding to the actual situation in nature. Preparation of ligands consists of several stages, making the structure of two-dimensional (2D) and three-dimensional structures (3D) geometry optimization, and analysis of molecular properties.

Two-dimensional structure made using 3D ChemBio program v12.0.2 Free Trial (Serial number: 186-410320-7811). Once created in 2D, the compound was in the form of 3D geometry to be optimized using the program HyperChem v8.0 Professional Edition (Verification Code: 0-28331). Geometry optimization was the process of formation of the most stable conformation of a compound with the lowest energy. This process aims to make the compound has the conformation corresponding to the actual situation in nature. The next step was the analysis of the properties of molecules using HyperChem software v8.0 Professional Edition (Verification Code: 0-28331). The results of the analysis are presented in Table 2.

Table 2. Molecular Nature Analysis Results

Compounds	Energy (Kcal.mol^{-1})	^{c}Log P	Volume (Å)	Mass (amu)
Furanodienone	-3646,7946	2,71	726,71	234,34
Procurcumenol	-3906,8198	2,71	726,71	234,34
Curdione	28, 189754	4,01	758,51	236,35
Dehydrocurdione	31,771456	3,63	735,80	234,34

Estradiol	-4549,3567	4,01	812,11	272,39
Tamoxifen	-6191,2810	5,63	1210,84	387,52

Note: Tamoxifen used was its metabolite (4-Hydroxitamoxifen)

In evaluating the effectiveness of a pharmacological compound when used via oral route, Lipinski's Rule of Five was used as a reference theory. This rule describes the important properties of a molecule, so it has significant value when viewed from theside of the pharmacokinetic. Sixth compounds had a mass below 500 amu, in accordance with the principle of Lipinski's Rule of Five. The compound has a mass that was too large, it can be experienced first-pass effect, compounds immediately eliminated without a chance to be absorbed. ^{C}log P suggested a compound lipophilicity. According to Lipinski, orally active compounds that have a ^{C}log P value terms, ie -2 <Clog P <5. The larger the Clog P values, the compounds are more easily penetrate cell membranes, which means having good absorption ability. It can be concluded, furanodienone, procurcumenol, and dehydrocurdione, have poorer absorption capability compared with its natural ligand, estradiol. Meanwhile, curdione have the same absorption capability with estradiol. Tamoxifen has a ^{C}log P value above five indicating extremely hydrophobic nature that tamoxifen predicted soluble in blood. Compounds that are too hydrophobic to bind strongly to the cell membrane and therefore reduces its bioavailability. If viewed from the active ERα bag that has a volume of 561Å , it can beconcluded, furanodienone, procurcumenol, curdione, dehydrocurdione, estradiol, and tamoxifen could only go partially in the active ERα bag because it has a larger volume than 561Å.

The next requirement that must be met for the oral route of drug compounds is to have a maximum of five and a maximum of ten donor hydrogen bond acceptor. According to Lipinski *et al*. (2001), a compound that has more than five donor and ten acceptor hydrogen bonds have poor permeability abilities in passing the membrane bilayer.

Table 3. Number of hydrogen bond donors and acceptors

Compounds	**Donor**	**Acceptor**
Furanodienone	-	2
Procurcumenol	1	2
Curdione	-	2

Dehydrocurdione	-	2
Estradiol	2	2
Tamoxifen	1	3

Which acts as a hydrogen bond donor to compound procurcumenol, estradiol, and tamoxifen is the polar hydrogen atoms while acting as a hydrogen bond acceptor is charged electronegative atom, the atom O and N. The number of hydrogen bond donors and acceptors also determines the ability of a compound to bind to amino acid residues pocket active receptors via hydrogen bonds. Hydrogen bond is formed between the polar H atoms with atoms that have lone pairs (PEB). Judging from the number of hydrogen bond donors and acceptors, the six compounds met the criteria of oral drugs based on Lipinski's Rule of Five.

3. Validation Software AutoDock Vina Results

The first step in the validation process was the manufacture of two-and three-dimensional structure of estradiol. Three-dimensional structure and then optimized and stacked with crystal estradiol. Overlay repetitions performed three times with an average RMSD value of 0.34 Å. A model compound considered to have the same crystal structure of the ligand RMSD if the value was less than two.

The next step was the process redocking. In the process redocking, estradiol had been isolated in the previous phase was fed back into the bag ties and then compared the results with the results of the Ligand Explorer Viewer. Through this process it is known that the feasibility of a software installed in a computer to be used in molecular docking.

Table 4. Comparison of Amino Acid Residues Implementing Active Pouch ERα Redocking

Results with Ligand Explorer Viewer

Asam Amino Residues	***Ligand Explorer Viewer***	***Redocking results***
Met343	√	-
Leu346	√	-
Asp351	√	-
Glu353	√	√
Leu387	√	√
Met388	√	√

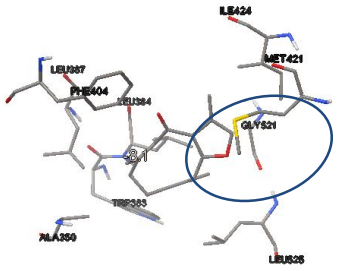
Leu391	√	√
Arg394	√	√
Phe404	√	-
Ile424	√	√
His524	√	√
Leu525	√	√

Table 4 showed little ligand shift position, seen from a different configuration of constituent amino acid residues in the active pockets. Docking results were able to reproduce the type of binding but show little ligand shift position was classified as a category close (Jones *et al.*, 1997). Results docking using AutoDock Vina software in this study were grouped under the category of "near".

4. Molecular Docking results of Furanodienone, Procurcumenol, Curdione, Dehydrocurdione, Estradiol, and Tamoxifen Using AutoDock Vina Program

Furanodienone, procurcumenol, curdione, and dehydrocurdione a test ligands, derived from *Curcuma zedoaria*, which were dockied against ERα. Meanwhile, as the natural ligand estradiol and tamoxifen as a synthetic ligand. The purpose was to predict the docking conformation and binding affinity of each ligand with the receptor. Prediction of the conformation and the active site of a ligand and its receptor is very important because it can be used to inform the development of further drug.

Docking arrangements for furanodienone, procurcumenol, curdion, dehydrocurdione, estradiol, and tamoxifen treated equally. Grid box wass used to direct the ligand compound to interact to active enclave of a protein. Variations grid box used consists of six variations were 15x15x15 (3375 Å), 15x20x15 (4500 Å), 15x15x20 (4500 Å), 20x15x15 (4500 Å), 20x15x20 (6000 Å), and 20x20x15 (6000 Å) to coordinate 106.32 ; 15.01; 96.81 (x, y, z). Variations grid box was intended to obtain the best conditions of the interaction of the ligand with the receptor. Grid box size should be adjusted to the volume of the ligand. Size of the grid box that was too small resulting in a larger ligand can not interact completely (only partially) on the active enclave and grid box sizes that are too large could cause ligand interacts in some enclaves active so the results would not accurate. Further variations of the grid box, grid box size 20x20x15 (6000 Å) was selected because it produced the amino acid residue similarity best with constituent amino acid residues in the active pocket ERα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TRP383
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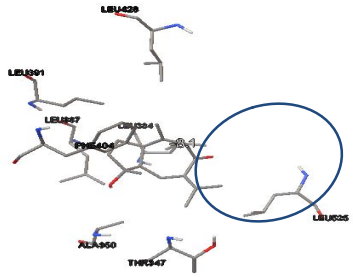
LEU525

(a)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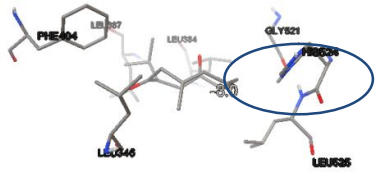
THR347

(b)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ARG524
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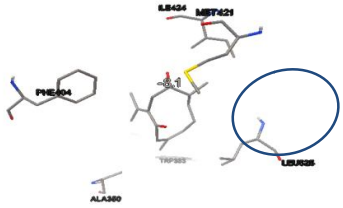
LEU525

(c)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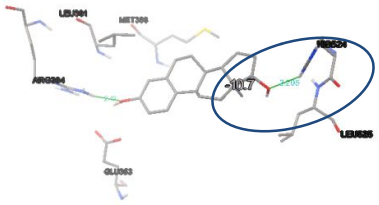
ALA380

(d)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ARG524
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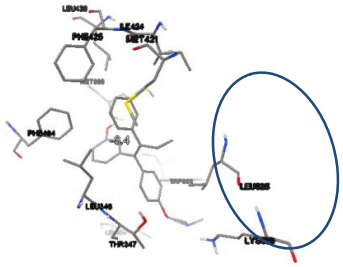
LEU525
GLU383

(e)



(f)

Figure1. Furanodienone docking results(a), procurcumenol(b), curdione(c), dehydrocurdione(d), estradiol(E), andtamoxifen(f) withERα

Note:

:hydrogenbonding region

: ligand region

Furanodienone, procurcumenol, curdione, dehydrocurdione, andtamoxifendid notform hydrogen bonds with the amino acid residues making up the active pocket ERα although when seeing its structure, these compounds could potentially form hydrogen bonds because it had a hydrogen bond acceptor and donor. This was due to the position and conformation of these compounds on the active ERαbag did not allow the formation of hydrogen bonds. Hydrogen bonding occurs in the bond distances from 1.72 to 2.85Å (Bohm and Schneider, 2003). A scan be seen in Figure1, the distance between acceptor and donorhydrogen bond test compounds with hydrogen bond acceptor and donor in the amino acid residues are too far away so it is not possible to form hydrogen bonds, where as estradiol form hydrogen bonds with the amino acids Arg394 with distance2, 10ÅandHis524 with a distance of 3.205Å for its atomic O and H atoms in the polarstructure.

5.Molecular *Docking*data interpretation

Toexamine the effectiveness of furanodienone, procurcumenol, curdione, dehydrocurdione, tamoxifen, and estradiol then analyzed by comparing the docking profile.

The ligands comparisoncan be seen in Table5.

Table5. Comparison of furanodienone, procurcumenol, curdion, dehidrocurdione, Estradiol, dan Tamoxfen docking results

Ligand	EI (kcal/mol)	Ki (µM)	Hidrogen bonds	RAA	Hydrofobikc Interaction
Furanodienone	-8,1	1,154	-	Ala350, Trp383, Leu384, Leu387, Phe404, Met421, Ile424, Gly521, Leu525	Ala350, Trp383, Leu384, Leu387, Met421, Ile424, Leu525
Procurcumenol	-8,1	1,154	-	Thr347, Ala350, Leu384, Leu387, Leu391, Phe404, Leu428, Leu525	Thr347, Leu384, Leu387, Leu391, Phe404, Leu428, Leu525
Curdione	-8,0	1,365	-	Leu346, Leu384, Leu387, Phe404, Gly521, His524, Leu525	Leu346, Leu384, Leu387, Phe404, Gly521, His524, Leu525
Dehydrocurdione	-8,1	1,154	-	Ala350, Trp383, Phe404,	Ala350, Trp383, Phe404,

				Met421, Ile424, Leu525	Met421, Ile424, Leu525
Estradiol	-10,7	0,014	Arg394 His524	Glu353, Met388, Leu391, Arg394, His524, Leu525	Met388, Leu391, Leu525
Tamoxifen	-8,4	0,695	-	Leu346, Thr347, Leu354, Trp383, Met388, Phe404, Met421, Ile424, Phe425, Leu428, Leu525, Lys529	Leu346, Thr347, Leu354, Trp383, Met388, Phe404, Ile424, Phe425, Leu525

Note: Tamoxifen used was its metabolite (4-OH Tamoxifen)

Interaction energy (EI) in the sixth compound was negative, meaning that the six compounds were easily interact with ERα. The smaller the value of EI, the ease of ligand binding to the receptor will increase. EI values sequentially from the smallest of the estradiol < tamoxifen < furanodienone, procurcumenol, dehydrocurdione < curdione.

Inhibition constants (Ki) showed the smallest concentration required by the ligand to interact with the receptors. Experimental Ki values were in the ranga of 10^{-12}-10^{-2}M (Bohmand Schneider, 2003). From the research that has been done, it can be concluded all test compounds within the same range. Ki values sequentially from the smallest of the estradiol <tamoxifen <furanodienone, procurcumenol, dehydrocurdione< curdione.

Although the test compounds did not form hydrogen bonds with the amino acid residues making up the active ERα bag, test compounds forming hydrophobic interactions although individually weak, but the sum of these binding factor interactions was quite significant. Hydrophobic interactions (distance 3to 4Å) plays an important role in the process of merging the area of drug nonpolar molecules with nonpolar regions of biological receptors. As ligand-receptor might form many hydrophobic interactions building a stronger bond, it could be concluded that the bond between the test compound to the receptor was strong enough. Best Potential owned by estradiol with Ki value of 0.014 µM. While the test compound, the best potential possessed by furanodienone, procurcumenol, and dehydrocurdione the value of Ki 1.154 µM. Potential curdione slightly weaker with Ki value of 1.365 µM.

CONCLUSION AND RECOMMENDATIONS

Based on the this study of interaction, it can be concluded all test compounds, namely furanodienone, procurcumenol, curdione, and dehydrocurdione, derived from the plant *Curcuma zedoaria* (Christm.) Roscoe and comparator compounds, namely estradiol and tamoxifen, predicted to occupy active ERα bag. Furanodienone, procurcumenol, curdione, dehydrocurdione, and tamoxifen interact with ERα whereas hydrophobic estradiol forming hydrogen bonds and hydrophobic interactions. Successive Ki values of the best test compound, 1.1154 µM for furanodienone, procurcumenol, dehydrocurdione, and 1.365 µM for curdione.

It is recommended that further research need to be done to get the test compound toxicity data as a breast-cancer drug discovery efforts.

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