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Binding Modes of Doxorubicin Compared to Estratetrol and Tamoxifen

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Abstract

Doxorubicin, a compound isolated from S*treptomyces peucetius* var Caesius, is commonly used in the treatment of breast cancer. This drug works by interacting on human nucleic acids. This work was aimed to study the binding modes of doxorubicin with estrogen receptor alpha (ERα). Estratetrol and tamoxifen were used as natural ligand and standard drug, respectively. Molecular docking simulations was performed by AutoDock v.3.05 using minimum coordinates -34, -6, -15 (x, y, z) and the maximum coordinates -13, 13, 3 (x, y, z). Tamoxifen formed one hydrogen bond with Glu353 (Ki=3.78 µM); estratetrol binds to Glu-353, Arg394, Gly521, and His524 (Ki=0.01 µM). Doxorubicin only formed one hydrogen bond with Ser317 (Ki=N/A). In conclusion, doxorubicin could not interact appropriately with ERα due to its voluminioues structure which hinder its entrance to binding pocket of the macromolecule.

Keywords: doxorubicin, estrogen receptor alpha.

Introduction

World Health Organization (WHO) states 8-9% of women in the world are at risk of breast cancer. This makes breast cancer the most common type of cancer in women. Every year more than 250,000 new cases of breast cancer are diagnosed in Europe and about 17000 in the United States. Data collected in hospitals showed that breast cancer was ranked first among other cancers in women in Indonesia.[1,2]

Estrogen hormone is one of the sex hormone steroids, because it has a steroid-like chemical structure that is physiologically produced mostly by the endocrine glands of the female production system. Men also produce estrogen but in much smaller amounts. Its primary function is closely related to the function of the primary and female genitalia[3] Estrogen has two types of receptors, namely ERα and ERβ derived from different genes and located in the cell nucleus. The estrogen receptor (ER) is a ligand activated transcription factor that can increase or decrease the mRNA synthesis of the target gene depending on the compound it occupies. ERα plays a major role in breast cancer because of its large number in breast compared to ERβ. . ERα is a heterotetramer

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with 4 different types of monomer [4]

Doxorubicin is an anthracycline antibiotic. It is widely used for the treatment of various types of cancer due to its interaction with nucleic acids. Doxorubicin is also known as adriamicin. However, the information regarding its effectiveness in breast cancer treatment was limited. [5]

This study was aimed to compare the compare the binding mode of doxorubicin with estratetrol to discover anti-breast cancer drug.

Methods

Tools and materials

The hardware used for molecular calculation, molecular modeling and docking included a personal computer with Windows XPTM Home Basic (2009) operating with an Intel Dual Core Genuine CPU CPU E5300 2.60 GHZ, Windows XPTM Home Basic operating system (2010), 80 GB hard disk capacity, and 1 GB RAM memory.

1. ChemOffice 2004.
2. HyperChem Release 8.0.7.
3. SwissPDBViewer v.4.0.1
4. AutoDock v.3.05 in MGLTools v.1.5.4
5. ArgusLab v.4.0.1
6. Ligand Explorer Viewer v.3.8

3D structure of ERα was downloaded from www.pdb.org (code: 3LO3). 2D and 3D structure of doxorubicin was built using the ChemOffice 2004.

Method

1. Geometry optimization and analysis of molecular properties of doxorubicin performed using Hyperchem Release 8.0.7
2. Reduction of ERα receptor chain into its monomer form using SwissPDBViewer v4.01.
3. Analysis of a binding pocket was performed using Ligand Explorer Viewer v.3.8.
4. Validation of software.
5. Molecular docking simulation of doxorubicin was performed by using Autodock v3.05.

Results and Discussion

Preparation of ligands is an early stage in the docking process. Preparation of ligand serves to prepare ligands used in the docking process in order to have a conformation in accordance with the actual situation in nature. Preparation of ligands consists of several stages, including the making of two- and three-dimensional structures, geometric optimization, and molecular properties analysis. Conformational changes before and after gemoetry optimization was shown in Figure 1.

The cLog P of doxorubicin compounds was smallest compared to the other ligands, indicating lipophilicity property. Doxorubicin is more polar, hence it will be easier to be distributed in the circulation. Based on this character, doxorubicin was predicted could not interact with the non-polar character of hydrophobic ERα binding pocket.

Table 2 provides the number of HBD and HBA of the ligands. According to the Lipinski Rule of five principle, a compound can be used as a drug with peroral route if it has a maximum of 5 donors and ten acceptor hydrogen bonds.

The number of HBD and HBA also determines the ability of a compound to bind with the amino acid residue via hydrogen bonding. The more hydrogen bonds are formed, the stronger the bond between the receptor ligand.[6]

Preparation of ERα showed that this macromolecule is a heterotetramer structure,

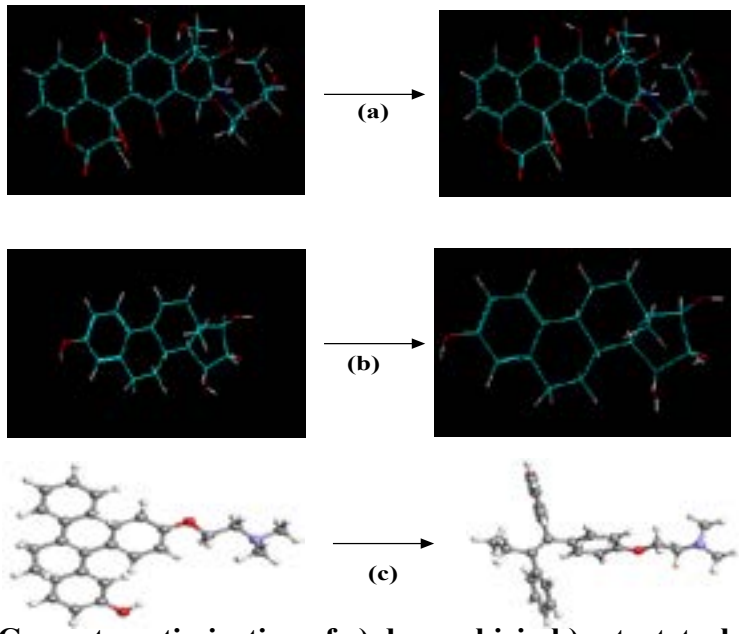

Figure 1. Geomety optimization of a) doxorubicin b) estratetrol c) tamoxifen

consist of four different monomers (chan A, chan B, chan C, chain D). Estratetrol was observed bound to chain A.

The minimum coordinates of ERα binding pocket at -34, -6, -15 (x, y, z) and the maximum coordinates are at -13, 13, 3 (x, y, z). The area between the minimum and the maximum is the active receptor side region with a volume of 460 Å3.

Geometry optimization results of both estratetrol molecules were overlayed and calculated its Root Mean Square Deviation (RMSD). A model compound is considered to have a structural similarity with its crystalline ligand if the RMSD value was less than two. The RMSD value of the overlay was 1.79Å, meaning that the RMSD value meets the requirements of less than two and it can be said that the estratetrol structure of the model is close to the crystalline estratetrol structure.

Table 3 shows the comparison between crystallized estratetrol and its model. The results showed that their interaction energy (EI), inhibition constants (Ki), and hydrogen bonds were relatively similar. Six amino acids were available in the re-docking of crystallized estratetrol *i.e.,* Glu353, Met388, Arg394, Phe404, Ile424, and His524. In its model, there were three additional amino acids: Leu384, Leu387, and Gly521. There was no significant difference between the crystallized estratetrol profile and its model. Therefore, it can be concluded that the program AutoDockTools v.3.05 is valid.

Table 1. Analysis of molecular characteristics

Ligand	Energy (kcal/mol)	cLog P	Volume (Å^3)	Mass (amu)
Doxorubicin	-7572.18	-0.08	1323.64	571.54
Estratetrol	-4761.69	2.54	846.58	304.39
Tamoxifen	-6181.30	1.85	1211.00	387.52

Table 2. Prediction of hydrogen bonds donor (HBD) and acceptor (HBA)

Ligand	HBD	HBA	Aromatic ring
Doxorubicin	5	6	4
Estratetrol	2	2	1
Tamoxifen	1	3	3

EI is the energy required by a ligand to enter and bind to its receptor. EI of tamoxifen and estradiol were negative, showing that they can bind to the receptors spontaneously without energy, while doxorubicin was positive. Doxorubicin needs a lot of energy to bind freely to the receptors. The amount of free energy influences the magnitude of the Ki. The lower the free energy, the lower the value of Ki.[7,8]

The number of hydrogen bonds on ligand-receptor interactions depends on the presence of H, O, N, or S atoms surrounding the ligand. Atom H acts as a hydrogen bonding donor, while the O, N, and S atoms act as hydrogen bond acceptor.[9] Amino acid residues are amino acids around the ligands at a certain distance. Amino acids that are about 4-6Å will form the interaction of van der Waals. Van der Waals interactions have an effect on the solubility of a ligand in lipids. The more van der Waals interactions formed, the higher the solubility in lipids. In addition, this interaction also plays a role in the strength of ligand-receptor interactions.[10-12] Table 5

Table 3. Docking and re-docking of estratetrol

Parameter	Replication	Crystallized Estratetrol	Estratetrol Model
EI (kkal/mol)	1	-4.22	-4.21
	2	-4.23	-4.23
	3	-4.25	-4.19
Ki (M)	1	0.000935	0.000933
	2	0.000963	0.000967
	3	0.000967	0.000955
Hydrogen bond distance	1	1.627 Å	1.928 Å
	2	1.995 Å	2.081 Å
	3	2.217 Å	2.035 Å
Amono acid residues	1	Glu353, Met388, Arg394, Phe404, Ile424, His524	Glu353, Leu384, Leu387, Met388, Leu391, Arg394, Ile424, Gly521, His524
	2	Glu353, Met388, Arg394, Phe404, Ile424, His524	Glu353, Leu384, Leu387, Met388, Leu391, Arg394, Ile424, Gly521, His524
	3	Glu353, Met388, Arg394, Phe404, Ile424, His524	Glu353, Leu384, Leu387, Met388, Leu391, Arg394, Ile424, Gly521, His524

Table 4. Results of Docking Simulation

Parameter	Doxorubicin	Tamoxifen	Estratetrol
EI	1104.254	-8.90	-10.74
Ki	0.00	3.78	0.0108
Hydrogen bond	H-dox.. O- Ser317	¬¬H-fen..O- Glu353	H-est..O- Glu353 O-est..H- Arg394 H-est..O- Gly521 O-tin..H- His524
Amino acid residues	Gly 442, Phe444, Gln443, Leu320, Ser317, Leu319, Asp313, Leu489, Lys492, Leu448, Cys447, Glu444, and Lys449	Leu346, Leu349, Ala350, Glu353, Leu384, Leu387, Met388, Phe404, Met421, Ile424, Phe425, Leu428, Gly521, His524, Leu525	Glu353, Leu384, Leu387, Met388, Leu387, Met388, Leu391, Arg394, Ile424, Leu428, Gly521, His524

shows that all ligands have several van der Waals interactions, indicating that the ligand-receptor bond is strong.

Conclusion

Doxorubicin could not interact appropriately with ERα due to its voluminious structure which hinder its entrance to binding pocket of the macromolecule.

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research authorship, and/or publication of this article

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