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Docking Studies of Chalcone Analogue Compounds as Inhibitors for Breast Cancer MCF7 Cell Line

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ABSTRACT

*Molecular docking studies of chalcone analogue with the protein target from the crystal structure modeling of H-RAS P21 Triphospate enzyme (PDB ID: 5P21) was carried out using computational approach with Autodock Vina program. The aims of this study is to determine the activity of 15 chalcone analogue, these compounds were obtained from the previous studies as breast cancer inhibitors and also to compare the activity of several chalcone analogue with the doxorubicin. Doxorubicin is used as a positive control. Based on the docking results, it is showed that compound **4** has a greater potential to be used as an inhibitor of MCF-7 breast cancer cells than the other test compounds. Compound **4** has the binding affinity energy of -9.0 kcal/mol, it is indicated that ligand perform a bonding with the receptor through the eleven active sites of doxorubicin. This compound can be further analyzed especially in vivo test to prove its inhibitory activity.*

Keywords: Breast Cancer, Chalcone, MCF-7 cell line, Moleculer docking**.**

INTRODUCTION

Cancer is one of the leading causes of death in the worldwide. Breast cancer is the second most common cancer in the world and this cancer that occurs in women with an estimated 1.67 million (25%) new cancer cases diagnosed in 2012 [1]. In Indonesia, breast cancer is the number two cancer after cervical cancer and there is a tendency from year to year in the incidence increases. The number of breast cancers in Indonesia is approximately 23140 new cases every year [2].

Most likely the caused of breast are related to changes in the genetic material (DNA) in our cells. DNA changes are often related to our lifestyle, but some can be due to age and other factors [3]. The etiology of breast cancer can be explained as variety of interlated factors, such as hormones, the environmental sociobiology and physicology among others can influence breast cancer development [4]. The development of breast cancer is started in any part of breast such as in invasive or infiltrating ductal carcinoma (IDC) and invasive or infiltrating lobular carcinoma (ILC) [5].

There are some medication of cancer such as chemotherapy, radiotherapy and surgery. Chemotherapy is a potential choice for cancer treatment, currently, a cancer chemotherapy agent that is still widely used is doxorubicin. Doxorubicin is included in anthracycline antibiotics. However, the use of doxorubicin can cause side effects including cardiotoxicity which can end with heart failure [6], hepatotoxicity [7] and resistance [8].
MCF-7 is a breast cancer cell taken from the site of breast cancer pleural metastatic effusion in a 69-year-old Caucasian woman [9]. MCF-7 cells are cells that are commonly used to test the effect of breast cancer *in vitro* because of the best form of all types of human breast cancer cells [10].

Chalcone is a common structure that is widely found in flavonoids [11]. Chalcone or 1,3-diphenyl-2-propene-1-on, in the form of two aromatic rings that are mutually bound [12]. Chalcone is a bioactive compound that has various biological activities such as antibacterials [13], antitumor [14-16], anticancer [17,18] and antioxidants [14]. One new method used to predict the activity of compounds for anticancer is the molecular docking. Molecular docking is a computational procedure that can predict non-covalent bonds of macromolecules (receptors) with a small molecule (ligand), the efficiently using its structure through molecular docking simulations is also can measured [19].
Research on molecular docking was conducted to determine the activity of 15 chalcone analogue compounds which are obtained from Mai et al. [20] with the IC_{50} value as an inhibitor of breast cancer. Interaction between amino acid residues on receptors and the ligand (i.e. 15 chalcones) is very important to know the reason that compound is active or not. The aim of this study is to determine the activity of 15 chalcone analogue compounds with doxorubicin was used as positive control. The best activity of the chalcone analogues compound was then can be used as new inhibitors for breast cancer and also it can be applied as a reference for alternative medicine.

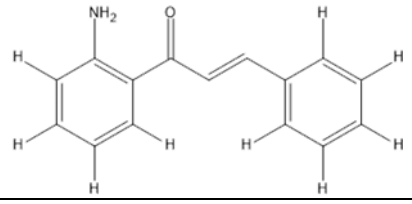
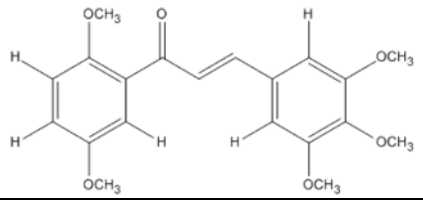
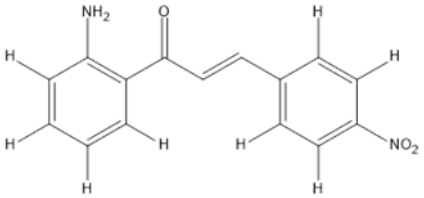
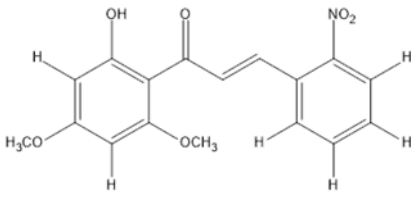
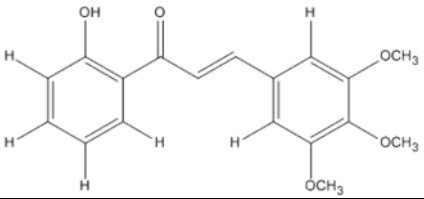
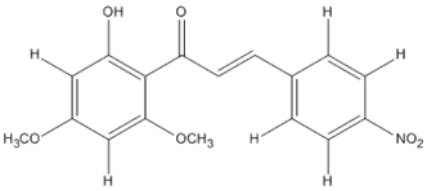
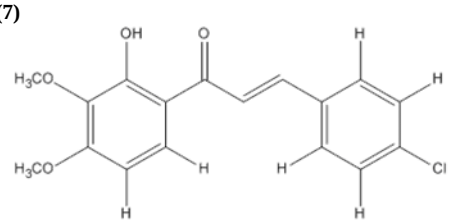
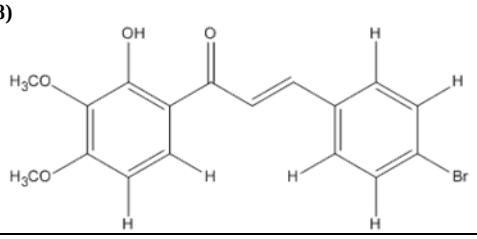
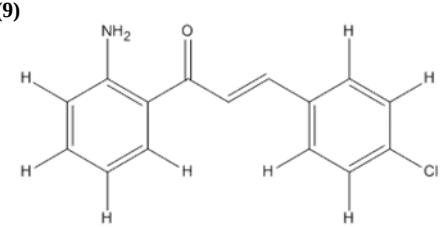
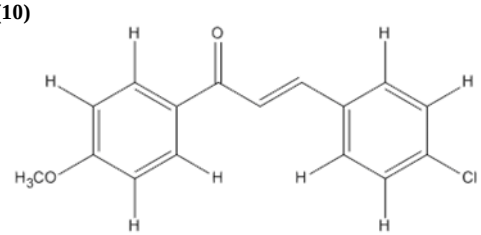
MATERIALS AND METHODS

Tools and material

A set of Aspire V5-471G 1.40 GHz computers, with 4096 MB RAM. The program used are Chemdraw Ultra 13.0 (CambridgeSoft), Discovery Studio Visualizer (DSV), MGL Tools 1.5.6 (including AutoDock Tools 1.5.6, AutoDock Vina), and pyMOL.

The materials used for molecular docking are target proteins obtained from the modeling of the crystal structure of the H-RAS P21 Triphospate enzyme in the format of PDB (Protein Data Bank) with 5P21 code with a resolution of 1.35 Å, doxorubicin as positive control and the ligand used is structure of chalcone analogue compounds are presented in Table 1.

Table 1: Molecular ligand structure

Molecular structure	Molecular structure
(1) 	**(2)** 
(3) 	**(4)** 
(5) 	**(6)** 
(7) 	**(8)** 
(9) 	**(10)** 

Protein preparation

The structure of crystallographic protein or enzyme with PDB-ID 5P21 is downloaded from the site www.pdb.org in PDB format, the water molecules were then removed using discovery studio visualizer (DSV). Preparation is done by adding the hydrogen using AutoDock Tools 1.5.6 and it was saved into PDBQT format.

Ligand preparation

The molecular structure of the chalcone analogues were skeched using ChemDraw Ultra 13.0, then saved in PDB format using DSV. Ligand in PDB format are then inputted to the AutoDock Tools 1.5.6 program for further preparation. Rotating bonds can be corrected in the torsion tree panel for ligand flexible properties. Then the ligand is saved in the form of PDBQT.

Grid box settings

In AutoDock Tools 1.5.6, the Grid menu is selected to open enzymes that have been prepared in the PDBQT format, then the grid box is created to determine the space for docking simulation will be running. The distance size in the grid box is set to 1 Å and the dimensions of the x, y, and z are arranged in such a way that the grid box can load the active site of the enzyme. The data grid box must be kept in mind because it will be needed for creating the configuration data.

Docking simulation using autodock vina

Docking simulation was run using AutoDock Vina. It has been done by entering the commands in the command prompt (cmd) base on the installation location of the AutoDock Vina program and also the work folder on the computer. After completed the running process, in the work folder there will be two files, they are "log.txt" and "out.pdbqt". "log.txt" is the docking results, it is consisted of the affinity value and "out.pdbqt", it is contained the conformation of ligand after docking. The conformation can be separated by entering the split to cmd command to perform one by one of the conformation.

Visualization of docking results with PyMOL and DSV

PyMOL is used to combine the enzymes with ligands from docking into a complex and saved it in PDB format. The interaction between enzymes and ligands can be displayed using the DSV program in 2D and 3D dimension.

Data analysis

The obtained data from the docking process of chalcone analogue compounds will come out the binding free energy with nine ligands conformations. The best pose will selected base on the smallest value of the binding free energy. The interaction between the active site of the protein with the ligands are performed in 2D and 3D using DSV.

RESULTS AND DISCUSSION

The data from docking results consisted of binding affinity (bond-free energy). Docking results is also performed**.** Protein 5P21 has active site Phe28, Lys147, Ser145, Ala146, Leu120, Asp119, Val14, Gly15, Ala18, Gly13, Gly12, Ala11, Lys16, Gly60, Ala59, Pro34, Thr35, Mg168, Ser17, Asp33, Glu31, Val29, Asp30, Asn11, and Lys117, and it is located at coordinates x: 6.811, y: 23.969, z: 9.121 in the grid box settings. In addition, the higher number of hydrogen bond, may account for ligand is more active [21]. The docking results of these ligands and also doxorubicin are presented in Table 2.

Based on the docking results, it was found that doxorubicin has the best binding affinity compared than the other 15 chalcone analogue compounds. Doxorubicnin has binding affinity value of -10.2 kcal / mol. 15 active sites was performed for doxorubicin after the docking process with receptors, these amino acimo acids are mentioned below one by one Ser145, Asp119, Phe28, Lys147, Lys117, Gly15, Lys16, Ser17, Thr35, Pro34, Gly13, Asp30, Leu120, Ala146 and Tyr32. Doxorubicin is displayed the same active site with the protein 5P21, doxorubicin are able to perform van der Waals interaction with Lys117, pi-sigma bond with Asp119, pi-sigma bond, pi-alkyl bond, alkyl bond, and 3 hydrogen bonds with Lys117, Asp119, Asp30.

Table 2: Results of docking of chalcone analog compounds

Compound	Affinity (Bond free energy) (kcal /mol)	Total Match Amino Acids to Doxorubicin	Hydrogen Bond
1	-8.4	7	Not formed
2	-8.4	10	Not formed
3	-8.9	10	Not formed
4	-9	11	Formed
5	-9.1	11	Not formed
6	-8.4	10	Not formed
7	-9	9	Not formed
8	-8.9	3	Not formed
9	-8.4	8	Not formed
10	-8.6	11	Not formed
Doxorubicin	-10.2		Formed

In this study, doxorubicin was used as a positive control against chalcone analogue compounds, chalcone analogue compounds have been docking on the active site of the protein 5P21 from the receptor to determine the bonding and affinity value of each compound. Based on the docking results, it is found that compounds 4, 5 and 7 are better activity, it was seen from their respective affinity of -9.0 kcal/mol, -9.1 kcal/mol, -9.0 kcal/mol, respectively. The smallest value of the binding affinity, it means that the energy needed for drug-receptor interaction is also small, so that the drug-receptor bond is more stable and also will be increasing the predicted activity [22]. The spatial arrangement of docking compounds 4, 5 and 7, is depicted in Figures 1-3. Compound 4 has better activity since it have the same active site like doxorubicin.

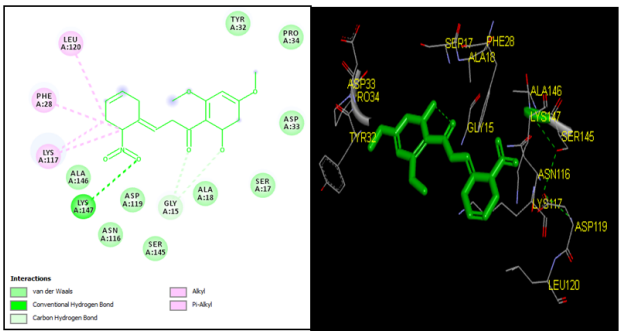


Figure 1: Interaction of enzyme residues with compound 4 in 2D and 3D

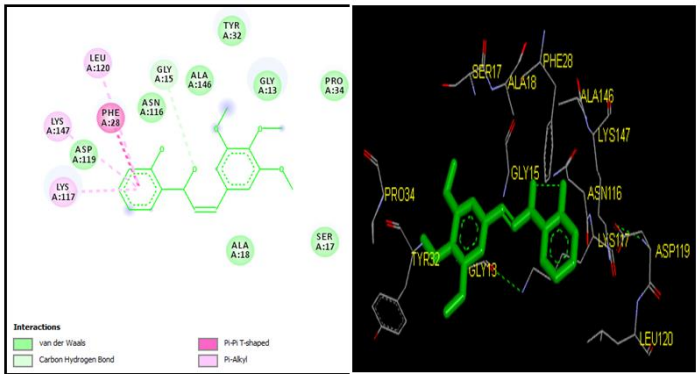


Figure 2: Interaction of enzyme residues with compound 5 in 2D and 3D

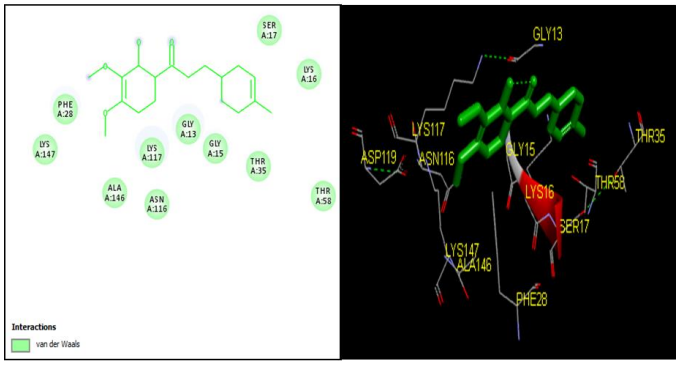


Figure 3: Interaction of enzyme residues with compound 7 in 2D and 3D

Compound 4 was able to build five type of bonding such as van der Waals, hydrogen bond, carbon-hydrogen bond, alkyl bond, and pi-alkyl bond with the same residues like doxorubicin, they are: Leu120, Phe28, Lys117, Ala146, Asp119, Ser145, Gly15, Lys147, Ser17, Pro34,, and Tyr32.

Side chain (substituent) will influence the difference number of bonds. Compound 4 has one nitro (NO_2) group, one hydroxy (OH) group, two methoxy groups (OCH_3), compound 5 has one hydroxy (OH) group, three methoxy groups (OCH_3) and compound 7 has one chloride group (Cl), one hydroxy (OH) group, two methoxy groups (OCH_3). All these three compounds have the same chain, one hydroxy (OH) group, but the other side chain is different. Compound 4 has good results are obtained because this compound contains one NO_2 group. Based on the research by Suhud et al. [23], the presence of NO_2 groups can improve the electronic properties (affect the process of drug-receptor interaction and also affect the penetration of biological membranes) because they contain electronegative atoms, due to the insertion of these substituents will affected to the biological activity of the compound. Compound 4 has only one hydrogen bond in the amino acid Lys117, compounds 5 and 7 do not have hydrogen bonds. The higher number of hydrogen bonds indicated that the compound is more active [21,24].

For compound 5, hydroxy (OH) and methoxy groups (OCH_3) have small electronegativity properties of nitro (NO_2) groups so that the bonds in compound 5 is less than compounds 4. Compounds 7 only has very small amount of bond with Cl side chain, it is probably because of the low activity of compounds substituted by Cl have not been theoretically explained, so that further studies are needed to find the answer.

In this study compounds 1, 2, and 3 should become the active compounds since these compounds have the lower IC_{50} value than compound 4. Unfortunately, base on the docking results compounds 1, 2, and 3 are not active because in these three compounds have a greater affinity, in addition they are also have less amino acid binding with the doxorubicin active site compare than compound 4. Hydrogen bonds are important roles in a ligand bond with a receptor, if hydrogen bonds are formed, the compound is more active but compounds 1, 2 and 3 do not form hydrogen bonds. In the theory the docking of these three compounds is not active, whereas according to research conducted by Mai et al (2014) the compound is active. Therefore these compounds need to be tested further. Based on the analysis and visualization of molecular docking results data of compound 4 has greater potential to be used as an inhibitor of MCF-7 breast cancer cells compared than other test compounds. So that it can be conclude that this ligand can inhibit the growth of cancer cells from a receptor. Based on docking results, it is showed that compound 4 has a greater potential to be used as an inhibitor of MCF-7 breast cancer cells than other test compounds, with a low energy (affinity) of -9.0 kcal / mol.

CONCLUSION

Based on docking results, it is showed that compound 4 has a greater potential and can be used as an inhibitor of MCF-7 breast cancer cells. Compound 4 has low energy (affinity) of -9.0 kcal / mol and also this ligand has a stable bond with the receptor and it also has eleven interaction with amino acid like interaction of doxorubicin as a positive control. The important amino acids involved are Leu120, Phe28, Lys117, Ala146, Asp119, Ser145, Gly15, Lys147, Ser17, Pro34, and Tyr32, with 5 types of bonds include van der Waals bonds, hydrogen bonds, carbon-hydrogen bonds, alkyl bonds, and pi-alkyl bonds.

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