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Study of Molecular Docking of Chalcone Analogue Compounds as Inhibitors for Liver Cancer Cells HEPG2

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ABSTRACT

Molecular docking study using chalcone analogue compounds with proteins target from modeling crystallographic structure of Tyrosine kinase enzymes with code 1T46 was carried out with the aid of a computer using the AutoDock Vina program. The aim this study to determine the activity of 5 chalcone analogue compounds obtained from previous studies and 3 chalcone analogues which were modified as inhibitors of liver cancer using 5-fluorouracil as a positive control. Based on the docking results, it has been carried out and shown those compounds 1, 2, and 3 have the potential as the active inhibitors againts HepG2 liver cancer with a successive affinity of -10.1 kcal/mol, -9.7 kcal/mol, and - 9.6 kcal/mol, respectively. For the modified chalcone analogue compounds, compound 8 has the best results with an affinity value of -8.3 kcal/mol and this compound also has six amino acid residues which are the same as 5-flourouracyl (i.e. positive control).

Keywords: chalcone, hepatocellular carcinoma, molecular docking, tyrosine kinase.

1. INTRODUCTION

Cancer is a disease characterized by uncontrolled growth and the spread of abnormal cells, these uncontrolled spread can cause death [1]. Lung, breast, stomach, colorectal, and liver cancers are the biggest cause of death every year [2].

Statistical data in 2018 on the incidence of the liver cancer in USA based on sex, it is showed that for new cases ranked 10th and for cases of cancer death ranked 5th [3]. While the incidence of liver cancer in Indonesia by sex shows that for new cases it ranks 7th and for cases of death ranks 3rd [2].

There are several types of liver cancer treatment, namely chemotherapy, radiotherapy and surgery [4]. The side effects of cancer treatment using chemotherapy methods cause hair loss, nausea, vomiting, diarrhea, susceptibility to infection, thrombocytopenia, neuropathy, and myalgia while radiation causes the effects of nausea and vomiting, and surgery is not entirely able to remove damaged tissue due to cancer [5]. Base on these conditions, it is necessary to conduct a search for cytotoxic substances that have a potential to treat liver cancer [6]. One of the compounds used as liver cancer inhibitors is chalcone [7].

Chalcone compounds are flavonoid secondary metabolites which have a base skeleton of 1,3-diarylpropanoid which can be found in plants [8]. Chalcone has attracted a lot of attention because of the diversity of biological activities such as antiviral [9]. anti-inflammatory[10]. antibacterial [11]. antioxidants [12], antimalarial [13], and anticancer [14].

Research to find active compounds for then these compounds can be used as liver cancer inhibitors is increasingly being carried out and the most widely used method is isolation and synthesis. But these two methods use complex equipment with relatively expensive reagents and to ensure the activity of a compound must be tested through invitro or invivo assay. Therefore, the insilico method with molecular docking technique was chosen to predict the activity of chalcone analogue compounds before laboratory experiments [15].

Molecular docking has many advantages, such as it certainly reduce the use of solvents and chemicals that can pollute the environment, and also save costs. This method can also be used to predict the activity of compounds such as chalcone by calculating the bonding or energy affinity involved in the interaction with the protein active site. The category of active compounds are if there are interaction of chalcone with the protein, and it is only requires low energy and the interation have to bind with the protein active site [15].

Study of molecular docking chalcone analogue compounds was carried out base on Mai et al (2014) [16]. However, so far there have not been many studies that have conducted insilico studies by modifying the structure of compounds. The purpose of this study was to determine the activity of 8 chalcone analogue compounds as inhibitors for liver cancer against enzyme residues on receptors and to compare the activity of some chalcone analogues with 5-fluorouracil drugs as liver anticancer. So, it can be used as an alternative medicine ingredient.

2. METHODS

2.1 TOOLS

The tools used in this research is the Intel laptop Core i3-6006U, 2.0 GHz, dengan RAM 4 GB. *Chemdraw Ultra* 12.0 (CambridgeSoft), *Discovery Studio Visualizer* (BIOVIA), MGL *Tools* 1.5.6, *AutoDock Tools* 1.5.6, *AutoDock Vina*, dan *PyMOL*.

2.2 MATERIAL

The material used in this study is protein 1T46 which was downloaded from www.pdb.org in the GDP format. The ligand used is the 5-Fluorouracil structure, 5 chalcone analogues that have been studied by Mai et al (2014) and 3 modified chalcone analogues shown in Table 1.

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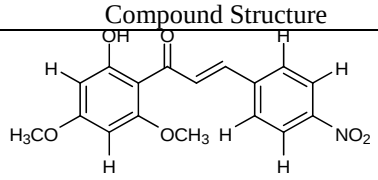
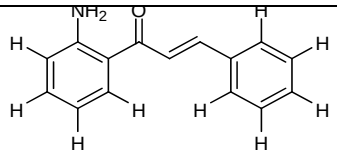
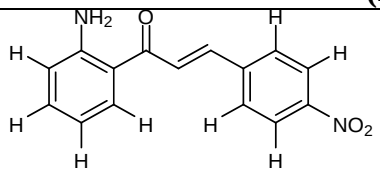
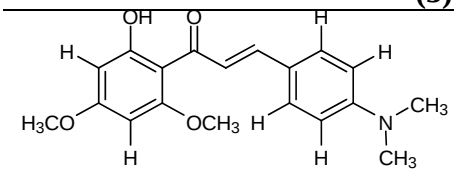
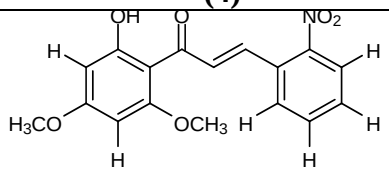
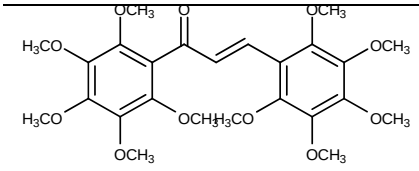
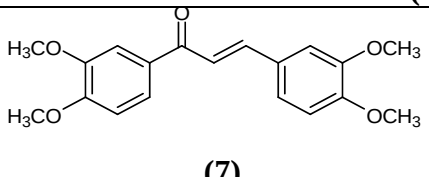
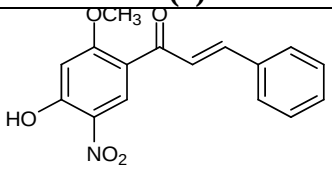
TABLE 1.
Structure of Analogous Chalcone Compounds as Ligands

Compound Structure	IC_{50}(µM)
 **(1)**	8,60
 **(2)**	11,43
 **(3)**	13,08
 **(4)**	>100
 **(5)**	>100
 **(6)**	modification compound
 **(7)**	modification compound
 **(8)**	modification compound

2.3 PROTEIN PREPARATION

The water molecules are removed using DSV. Furthermore, hydrogen atoms are added using AutoDock Tools 1.5.6 and stored in PDBQT format.

2.4 LIGAND PREPARATION

The molecular structure of chalcone is drawn using Chemdraw Ultra 12.0 (Cambridge), then save it in PDB format using DSV. Ligands in PDB format are 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 save in the PDBQT format.

2.5 GRID BOX SETTINGS

In AutoDock Tools 1.5.6, the grid menu is selected to open proteins that have been prepared in PDBQT format, then the grid box is created to determine the space in which the docking simulation will run. The size of the distance 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 side of the protein. In this study, the grid box is located at coordinates x: 31,925, y: 25,893, and z: 35,098. Grid box data will be needed to create configuration data.

2.6 MAKING CONFIGURATION DATA

Configuration data is created in the work folder in the form of a Text Document with the name ‘conf.txt 'and filled in according to the name of the protein that is the receptor and ligand in PDBQT format, followed by the dimensions and the position of the angles x, y and z in the grid box settings.

2.7 DOCKING SIMULATION USING AUTODOCK VINA

Docking simulation using AutoDock Vina is done by entering commands in the command promt (cmd) according to the installation location of the AutoDock Vina program and the work folder on the computer. After the running process is complete, in the folder there will be two files, namely 'result.txt' which is the result of a docking, it contained the value of affinity and 'out.pdbqt' which contained the ligand conformations after docking. The conformation can be separated by entering the split to cmd command.

2.8 VISUALIZATION OF DOCKING RESULTS WITH PyMOL AND DSV

PyMol is used to combine proteins that have been prepared with ligands from docking into a complex and stored in PDB format. The interaction formed between enzymes and ligands can be seen using DSV in 2D and 3D.

2.9 DATA ANALYSIS

Data that has been obtained from the docking process of chalcone analogue compounds using AutoDock Vina, obtained 9 affinity results for each compound, then each affinity with the smallest value was selected and then visualized using

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PyMOL to combine enzymes that had been prepared with ligands. The docking results can be seen in the table, where the interaction between the ligand and its receptors will then be read using the DSV program both 2D and 3D.

3. RESULTS AND DISCUSSION

TABLE 2.
Docking results of Chalcone and 5-Fluorouracil Analogue Compounds

Compound	Parameter		
	Affinity (Bond Free Energy) (kcal/mol)	Amount of Amino Acid Compatibility with 5-fluorouracil	Hidrogen Bond
1	-10,1	5	Not Formed
2	-9,7	6	Formed
3	-9,6	9	Formed
4	-7,0	4	Formed
5	-7,2	2	Not Formed
6	-5,6	1	Not Formed
7	-7,0	3	Not Formed
8	-8,3	6	Not Formed
5-fluorouracyl	-5,1	-	Formed

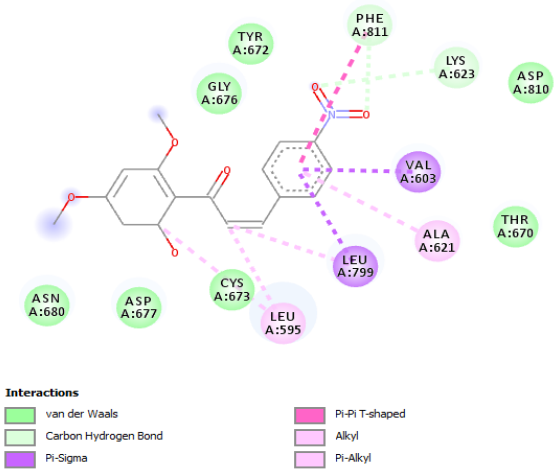

FIGURE 1. Interaction of Protein Residues with Compound **1**

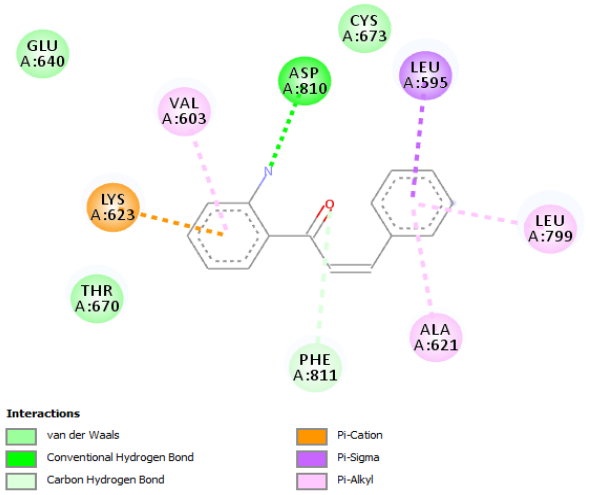

FIGURE 2. Interaction of Protein Residues with Compound **2**

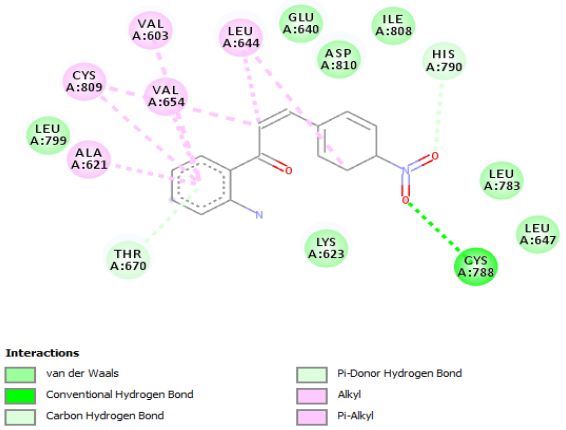

FIGURE 3. Interaction of Protein Residues with Compound **3**

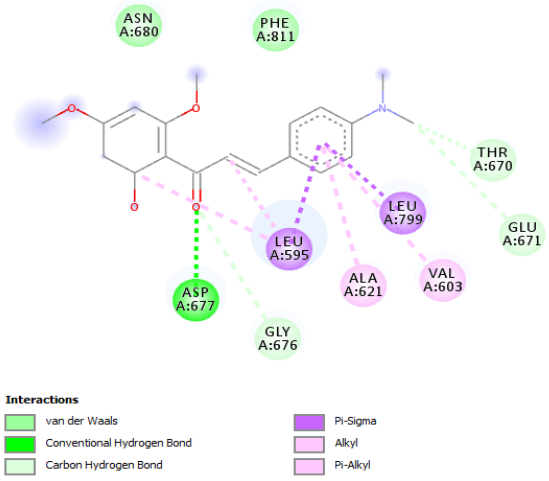

FIGURE 4. Interaction of Protein Residues with Compound **4**

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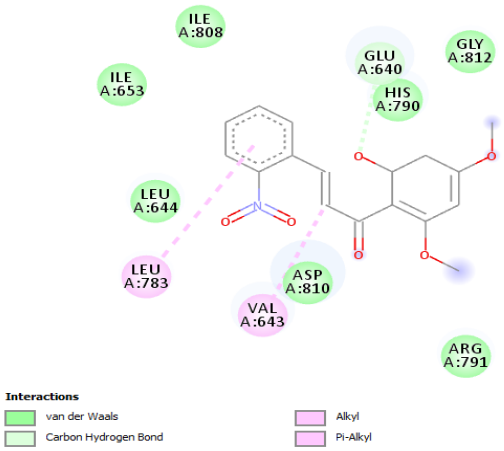

FIGURE 5. Interaction of Protein Residues with Compound **5**

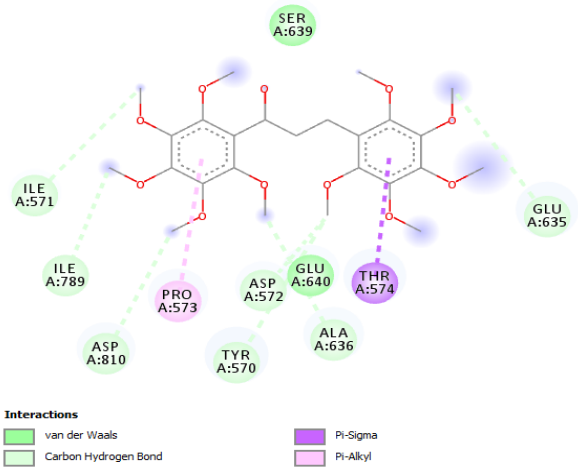

FIGURE 6. Interaction of Protein Residues with Compound **6**

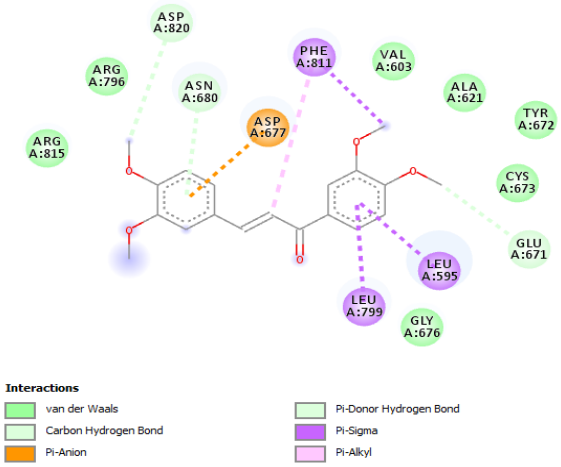

FIGURE 7. Interaction of Protein Residues with Compound **7**

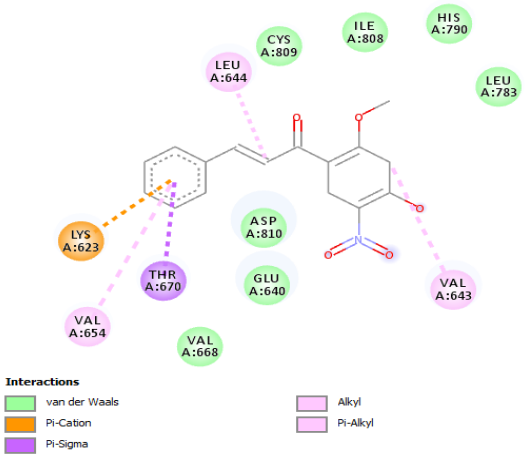

FIGURE 8. Interaction of Protein Residues with Compound **8**

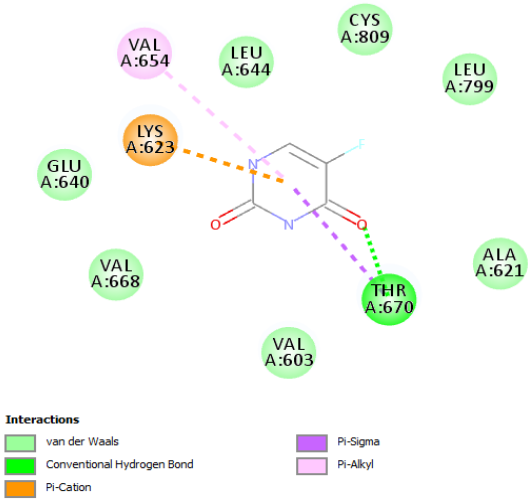

FIGURE 9. Interaction of protein residues with 5-fluorouracil

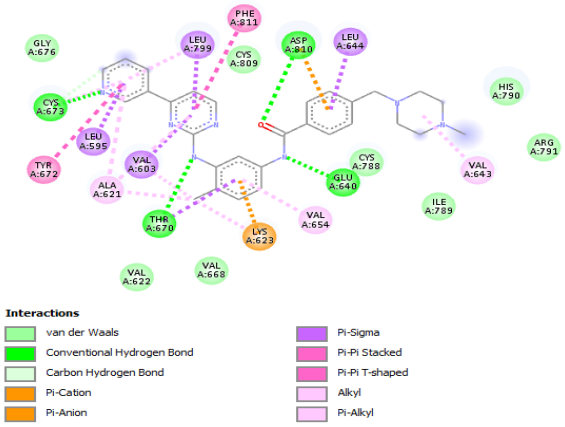

FIGURE 10. Active Side of Protein 1T46 2D

In this study, the steps taken were the selection of proteins used as receptors and the selection of ligands as compounds that would be tethered to the receptor. The crystallographic structure of a protein or 3D enzyme is provided on the website

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www.pdb.org. In this study, the protein was used the crystallographic structure of the tyrosine kinase enzyme, which is from the Spodoptera frugiperda (GDP-ID 1T46) with the resolution of 1.6 Å and it was released in 2004 by Mol et al. It is presented in Figure 1. Crystallography of 1T46 protein has water molecules, the water have to remove so as not to interfere with analysis and docking calculations because it is feared that there will be interaction between ligands and water molecules rather than receptors. In addition, the release of test ligands from proteins was carried out so that the test compounds used could be tethered to liver cancer cell receptors and replace test ligands. Most of the proteins provided by the site, unfortunately, it does not have hydrogen atoms, because of that hydrogen have to be added. Generally, hydrogen bonding was occured between ligands and receptors. Protein 1T46 has the active site Cys673, Cyc788, Cys809, Gly676, His790, Arg791, Ile789, Tyr672, Leu595, Leu644, Leu799, Val603, Val654, Val643, Val622, Val668, Ala621, Thr670, Lys623, Glu640, Asp810, and Phe811 shown in Appendix 3 and located in coordinates x : 31,925, y : 25,893, and z : 35,098 in *grid box* settings.

In this study 5 chalcone analogues were compounds that have been shown to have cytotoxic effects on HepG2 in vitro by Mai et al (2014), 3 compounds were taken from the lowest IC_{50} and 2 more compounds were selected from the highest IC_{50}. Compound **1** has the lowest IC_{50} value of 8.60 µM, compound **2** with IC_{50} value of 11.43 µM, compound **3** with IC_{50} 13.08 µM and compound **4** and **5** has IC_{50} value> 100 µM. 3 other compounds are modified compounds from compound 1 because they have the best cytotoxic activity.

Based on the docking results, it was found that 5-fluorouracil (compound **9**) has high affinity value compared to the 8 chalcone analog compounds tested which were -5.1 kcal / mol. 5-fluorouracil has 9 active sides formed after the docking process with receptors including Glu640, Lys623, Val654, Leu644, Cys809, Leu799, Ala621, Thr670, and Val603. 5-fluorouracil generally binds to the same amino acid with the active site of the 1T46 protein except Val668, with each bond in the form of van der Walls bond, hydrogen bond, pi-cation bond, pi-sigma bond, and pi-alkyl bond which can be seen in Figure 9.

The docking results of all 8 compounds showed different affinity values. Compounds **1, 2**, and **3** have the smallest affinity in a row, namely -10.1 kcal / mol, -9.7 kcal / mol, and -9.6 kcal / mol, from all 8 chalcone analog compounds tested, so it can be said that the activity is better than the other 5 compounds, but it is still has to be seen by its interaction with proteins or receptors. The results of the docking of all 8 compounds showed different affinity values. Compounds **1**, **2**, and **3** have the smallest affinity of -10.1 kcal / mol, -9.7 kcal / mol, and -9.6 kcal / mol, respectively. From all 8 chalcone analogue compounds tested, so it can be said that the activity is better than the other 5 compounds, but it is still active since they have the interaction with proteins Tyr570, Tyr672, Asn680, Asp677, Asp572, Asp810, Asp820, Cys673, Cys788, Cys809, Leu595, Leu783, Leu799, Leu644, Aala621, Ala636, Thr670, Thr574, Val603, Val643, Val654, Val668, Lys623, Phe811, Gly676, Gly812, Ile808, Ile653, Ile571, Ile789, His790, Glu640, Glu635, Glu671, Ser639, Pro573, Arg791, Arg815, dan Arg796. However, not all of these protein residues become the active side of 1T46.

CONCLUSION

Chalcone compounds 1, 2, and 3 showed affinity of -10,1 kcal / mol, -9,7 kcal / mol, and -9,6 kcal / mol, respectively. After 2D visualization there were 10 residues that interacted with the sites active protein 1T46, according to Mai et al (2014) this compound has the potential as an inhibitor against liver cancer (HepG2). While compound 8 which is a modified compound can be used as a reference if you want to be directed as an inhibitor for liver cancer (HepG2), because it has an affinity of -8.3 kcal / mol after 2D visualization There are 7 residues that interact with the active side of 1T46 protein and 6 acid residues amino has similarities with 5-fluorouracil.

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substituted chalcone analogs in vitro. *Medicinal Chemistry Research*. 18 : 407-420.

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