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Vitual screening and binding mode elucidation of curcumin analogues on Cyclooxygenase-2 using AYO_COX2_V1.1 protocol

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Abstract. Curcumin is yellow colored phenolic compounds contained in Curcuma longa. Curcumin is known to have biological activities as anti-inflammatory, antiviral, antioxidant, and anti-infective agent [1]. Synthesis of curcumin analogue compounds has been done and some of them had biological activity like curcumin. In this research, the virtual screening of curcumin analogue compounds has been conducted. The purpose of this research was to determine the activity of these compounds as selective Cyclooxygenase-2inhibitors in in-silico. Binding mode elucidation was made by active and inactive representative compounds to see the interaction of the amino acids in the binding site of the compounds. This research used AYO_COX2_V.1.1, a structure-based virtual screening protocol (SBVS) that has been validated by Mumpuni E et al, 2014 [2]. AYO_COX2_V.1.1 protocol using a variety of integrated applications such as SPORES, PLANTS, BKchem, OpenBabel and PyMOL. The results of virtual screening conducted on 49 curcumin analogue compounds obtained 8 compounds with 4 active amino acid residues (GLY340, ILE503, PHE343, and PHE367) that were considered active as COX-2 inhibitor.

1. Introduction

Curcumin is known to have biological activities as anti-inflammatory, antiviral, antioxidant, and anti-infective agents [1]. However, curcumin is an unstable compound, because it is easily degraded through hydrolysis at neutral and alkaline pH [3]. Therefore, A range of research has been done to modify the curcumin compound such as works conducted by Nurfina et al [4], Sardjiman et al [5] and Supardjan et al [6]. Several of synthesized curcumin analogues compounds, through the in vivo and in vitro test, showed biological activity as a curcumin. One of the biological activities is the ability to inhibit the cyclooxygenase-2 enzyme (COX-2).

COX-2 enzyme is responsible for inflammatory and cancer mediator. Overexpression of COX-2 in cancer cells resulting in overproduction of prostanoid leading to increase of cell proliferation and prevention of apoptosis. In colon cancer cells, the overproduction of the COX-2 enzyme is also accelerated by the process of angiogenesis. It is caused by the catalysis product of COX-2 that will stimulate the activity of angiogenic factors [7, 8]. This shows the importance of research to find compounds that can inhibit the action of the COX-2 enzyme as the design of drug discovery. Utilization of computers in the study of drug discovery, *in silico* methode, is an analogue with *in vitro*

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and *in vivo*. The approach of virtual screening was used to identify active compounds based on structure of certain enzymes, so called the Structure-Based Virtual Screening (SBVS), which simulated docking molecules often used as a basis for the development of SBVS protocols. Molecular docking, used in the study SBVS is regarded as an efficient way in drug discovery. SBVS protocol in this study is the protocol built for identifying COX-2 inhibitors compounds.

The protocol is called AYO_COX2_V.1.1 that has been validated by E. Mumpuni. et al, 2014 [2]. AYO_COX2_V.1.1 protocol is validated retrospectively to see the value of Enrichment Factor (Comparative between the percent of True Positive and False Positive percent.) as the objective function by using the default settings and modified settings. In the default setting obtained value of EF20% = 3.016 and the value of EFmax = infinity (~), had the quality of structure-based screening protocol as "excellent" by reference [9]. In addition to using EF20% and EFmax, the protocol used an assessment of the value of Matthews correlation coefficient (MCC) and the EF value at the time the highest MCC value (MCCmax). The MCCmax value was used to find ligands which were used as comparison ligand that capable to distinguish active and inactive compounds for virtual screening. MCCmax value = 0.188 and EF values when MCCmax = 4,105 which was the value of active ligand on the DUD data set with the code ZINC03814604. The ligand can be used as comparison ligand. Furthermore, This study used a protocol that has been validated namely the default protocol with reference compounds ZINC03814604 to find COX-2 inhibitors. The results are expected to obtain curcumin analogues compounds which have activity as selective COX-2 inhibitors in *in silico*, so that it can be analyzed as more potential of curcumin analogues compounds that can be used as candidate drugs known as coxib (COX-2 inhibitor).

2. Methodology

2.1. Construction of the test compounds

In terminal mode applications were used AYO_COX2_V.1.1 with command line interface with the command: ./AYO_COX2_v.1.1.sh. *BkChem* application window appeared and each test compounds in Table 2.1 was drawn in the form of two-dimensional and exported with the file name uji.mol. After that, the AYO_COX2_V.1.1 application returned ChemPLP score that stored with the file name hasil.txt, besides the scores of test compounds on the results file also found ChemPLP score of comparison compounds, this steps were done by replication at least 3 times. Binding mode elucidation has done by selected representative active and inactive compounds were used for visualizing using *PyMOL* to do elucidation modes of bonding in the receptor binding site and determining the amino acid residues that actively interacted with the ligand as a COX-2 inhibitor.Positive control test was also conducted using the compound of the drugs known as coxibs.

3. Results and Discussion

Virtual screening was done by preparing the test compounds and comparison compounds. Each compound was drawn using *BKchem* applications that opened automatically when running SBVS protocol. Screening was done at least three times replication to obtain the docking simulation results score called *chemPLP* score which was the total energy calculated from one docking process [9]. *ChemPLP* Score of the test compounds was compared with the comparison compound ZINC03814604. The test compounds were active in *in silico* on COX-2 binding pocket if it had a smaller score (more negative) than the score of comparison compound, meaning that the compound had good stability. The docking simulation results and in *in silico* compound activity are presented in Table 1.

Based ChemPLP score obtained 8 predicted active compounds i.e : 1,7-Diphenil-1,6-heptadien-3,5-dione, 1,7-bis(4-hydroxyphenyl)-1,6-heptadien-3,5-dione, 1,7-bis(3-methoxyphenyl)-1,6-heptadien-3,5-dione, 1,7-bis(4-hydroxy-3,5-dimethylphenyl)-1.6-heptadien-3,5-dion, 1,7-bis(3,5-diethyl-4-hydroxyphenyl)-1.6-heptadien-3,5-dione, 1,7-bis(2-methoxyphenyl)-1.6-heptadien-3,5-dione, 1,7-bis(4-chlorophenyl)-1.6-heptadien-3,5-dione, 1,7bis (4-methylphenyl) -1.6-heptadien-3,5-dione, with

the average of ChemPLP score respectively : -91.9147, -86.1098, -90.2596, -88.5337, -90.5843, -95.8059, -87.3040, and -88.0893. Based on the *ChemPLP* score, 1,7-bis (2-methoxyphenyl) -1.6-heptadien-3,5-dione had the highest affinity in the receptor COX-2 because it had the smallest *ChemPLP* score (most negative) than the another test compound.

Having obtained a score of each test compound and the comparison compound, then statistical analysis of one-tailed paired t-test was performed. In statistical testing, if p-value was less than 0.05 at the 95% confidence level, it can be stated that average score of test compound was significant or meaningful that can represent the entire population. The predicted active compounds as COX-2 inhibitors are 1,7-Diphenil 1,6-heptadien-3,5-dione, 1,7-bis (4-hydroxyphenyl) -1.6-heptadien-3, 5-dione, 1,7-bis (3-methoxyphenyl) -1.6-heptadien-3,5-dione, 1,7-bis (4-hydroxy-3,5-dimethylphenyl) -1.6-heptadien- 3,5-dione, 1,7-bis (3,5-diethyl-4-hydroxyphenyl) -1.6-heptadien-3,5-dione, 1,7-bis (2-methoxyphenyl) -1,6- heptadien-3,5-dione, 1,7-bis (4-chlorophenyl) -1.6-heptadien-3,5-dione, 1,7-bis (4-methylphenyl) -1.6-heptadien-3, 5-dione with a p-value respectively 0.0002, 0.0087, 0.0013, 0.0011, 0.0008, 0.0002, 0.0085, 0.0004. All of them had a p-value less than 0.05, this showed that all of these compounds had activity as active inhibitors of COX-2.

Table 1. ChemPLP score and inhibitors activity of test compounds and comparison compound

Test Compounds	ChemPLP score of test compounds	ChemPLP score of comparison compound	COX-2 inhibitor Activity
Natural analogue curcumin as reference compound			
Curcumin (Enol)	-86.2946	-84.7546	active
Curcumin (Keto)	-87.4828	-84.6900	active
Desmethoxycurcumin	-85.5663	-84.7107	active
Bidesmethoxycurcumin	-86.1965	-84.7078	active
Analogue Curcumin CompoundsSynthesized by Nurfina et al 1997 [4]			
1,7-Diphenyl-1,6-heptadien-3,5-dion	-91.9147	-84.7398	**active**
1,7-bis(4-hydroxyphenyl)-1,6-heptadien-3,5-dion	-86.1098	-84.5319	**active**
1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dion	-82.5414	-84.7290	inactive
1,7-bis(3-methoxyphenyl)-1,6-heptadien-3,5-dion	-90.2596	-84.7467	**active**
1,7-bis(3,4,5-trimethoxyphenyl)-1,6-heptadien-3,5-dion	-70.7774	-84.7197	inactive
1,7-bis(4-hydroxy-3,5-dimethylphenyl)-1,6-heptadien-3,5-dion	-88.5337	-84.6948	**active**
1,7-bis(3,5-diethyll-4-hydroxyphenyl)-1,6-heptadien-3,5-dion	-90.5843	-84.7361	**active**
1,7-bis(4-hydroxy-3,5-diisopropylphenyl)-1,6-heptadien-3,5-dion	-78.2153	-84.7174	inactive
1,7-bis(2-methoxyphenyl)-1,6-heptadien-3,5-dion	-95.8059	-84.7337	**active**
1,7-bis(4-chlorophenyl)-1,6-heptadien-3,5-dion	-87.3040	-84.7251	**active**
1,7-bis(4-methoxyphenyl)-1,6-heptadien-3,5-dion	-84.1965	-84.7284	inactive
1,7-bis(4-methylphenyl)-1,6-heptadien-3,5-dion	-88.0893	-84.7441	**active**
1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadien-3,5-dion	-81.4540	-84.7291	inactive
1,7-bis(3,5-Di-tert-buthyl-4-hydroxyphenyl)-1,6-heptadien-3,5-dion	-37.2678	-84.7282	inactive
1,7-bis(3,4-dibenzyloksphenyl)-1,6-heptadien-3,5-dion	-33.4720	-84.7070	inactive
1,7-bis(4-hydroxy-3,5-dimethoxyphenyl)-1,6-heptadien-3,5-dion	-75.4518	-84.7206	inactive

Table 1. (Continue)

Analogue Curcumin CompoundsSynthesized by Sardjiman et al 1997 [5]			
2,6-bis(4-hidroxybenzyl)cyclohexanone	-68.8778	-84.7441	inactive
2,6-bis(4-hydroxy-3-methoxybenzyl)cyclohexanone	-72.8071	-84.7243	inactive
2,6-bis(4-hydroxy-3,5-dimethylbenzyl)cyclohexanone	-69.0245	-84.7297	inactive
2,6-bis(3,5-diethyl-4-hydroxybenzyl)cyclohexanone	-71.1657	-84.7287	inactive
2,6-bis(4-hydroxy-3,5-diisopropylbenzyl)cyclohexanone	-60.1065	-84.7187	inactive
2,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)cyclohexanone	-22.7815	-84.7077	inactive
2,6-bis(4-hydroxy-3,5-dimethoxybenzyl)cyclohexanone	-64.6337	-84.7222	inactive
2,6-bis(3,5-dichloro-4-hydroxybenzyl)cyclohexanone	-69.9008	-84.7135	inactive
2,5-bis(4-hydroxybenzyl)cyclopentanone	-77.0433	-84.7305	inactive
2,5-bis(4-hydroxy-3-methoxybenzyl)cyclopentanone	-72.5252	-84.7192	inactive
2,5-bis(4-hydroxy-3,5-dimethylbenzyl)cyclopentanone	-84.4206	-84.7253	inactive
2,5-bis(3,5-diethyll-4-hydroxybenzyl)cyclopentanone	-84.0432	-84.6667	inactive
2,5-bis(4-hydroxy-3,5-diisopropylbenzene)cyclopentanone	-72.8438	-84.7381	inactive
2,5-bis(3,5-di-tert-butyl-4-hydroxybenzyl)cyclopentanone	-33.4951	-84.7132	inactive
2,5-bis(4-hydroxy-3,5-dimethoxybenzyl)cyclopentanone	-72.4623	-84.7088	inactive
2,5-bis(3,5-dichloro-4-hydroxybenzyl)cyclopentanone	-79.8052	-84.7561	inactive
1.5-bis(4-hydroxyphenyl)1.4-pentadien-3-on	-76.6006	-84.6985	inactive
1.5-bis(4-hydroxy-3-methoxyphenyl)1.4-pentadien-3-on	-76.0701	-84.7191	inactive
1.5-bis(4-hydroxy-3.5-dimethylphenyl)1.4-pentadien-3-on	-78.469	-84.7457	inactive
1.5-bis(4-hydroxy-3.5-dimethoxyphenyl)1.4-pentadien-3-on	-69.9995	-84.7023	inactive
1.5-bis(3.5-dichloro-4-hydroxyphenyl)1.4-pentadien-3-on	-74.8726	-84.7396	inactive
Analogue Curcumin CompoundsSynthesizedby Supardjan *et al* 1999[6]			
4-methyl-curcumin	-69.3172	-84.7261	inactive
4-ethyllcurcumin	-71.5290	-84.7385	inactive
4-n-propylcurcumin	-70.7039	-84.7020	inactive
4-isopropylcurcumin	-67.5033	-84.7318	inactive
4-n-buthylcurcumin	-72.1470	-84.7258	inactive
4-benzylcurcumin	-55.4535	-84.7254	inactive
4-phenylcurcumin	-56.2741	-84.6900	inactive
4-(p-fluorophenyl)curcumin	-76.8823	-84.7336	inactive
4-(p-methoxyphenyl) curcumin	-57.5191	-84.7146	inactive
4-(p-methylphenyl)curcumin	-59.1340	-84.7254	inactive
4-(m-trifluoromethylphenyl) curcumin	-60.4516	-84.6687	inactive
4-(o,p-dinitrophenyl) curcumin	-44.3679	-84.7177	inactive
Compounds of Coxib group as reference compounds			
Celecoxib	-88.5473	-84.7317	active
Etoricoxib	-85.2023	-84.7160	active
1.5-bis(3-etoksi-4-hydroxyphenyl)1.4-pentadien-3-on (Deetoksi EHP)	-85.2539	-84.7298	active

3.1. Binding Mode Elucidation of Representative Compounds

Binding mode elucidation was depicted with *PyMOL* application. In the processes, desmethoxycurcumin, bisdesmethoxycurcumin, 1,7-bis (4-hydroxyphenyl) -1.6-heptadien-3,5-dion, 1,7-bis (3-methoxyphenyl) -1.6-heptadien-3,5-dione, 1,7-bis (4-hydroxy-3,5-dimethylphenyl)-1.6-heptadien-3,5-dion, 1,7-bis(3,5-diethyl-4-hydroxyphenyl)-1.6-heptadien-3,5-dione, 1,7-bis (2-methoxyphenyl) -1.6-heptadien-3.5 -dion, 1,7-bis (4-chlorophenyl) -1.6-heptadien-3,5-dione, 1,7-bis (4-methylphenyl) -1.6-heptadien-3,5-dion were used to identify amino acid residues that actively

interacted with the active representative compounds. The number of amino acid residues that interact with all of them respectively 28, 20, 22, 24, 26, 26, 26, 24 and 25 amino acid residues. Binding mode elucidation of inactive representative compounds was done on 1,7-bis (3,4-dibenzyloksphenyl)-1.6-heptadien-3,5-dione and obtained 37 amino acids residues interaction. Amino acid residues that interacted with active and inactive representative compounds are shown in Table 2.

Table 2. Amino Acid Resiudes of Active and Inactive Representative Compounds

Group	Representatif Compounds	Amino Acid Residues
Natural analogues curcumin	Demethoxycurcumin (active)	ARG106, ARG499,ALA513, GLY340, GLY512, GLY519, ILE98, LEU78, LEU338, LEU345,LEU370,LEU511, LEU517, MET508, PHE367, PHE504, SER339, SER516, TYR101, TYR334,TYR341, TYR371, TRP373, VAL74, VAL102, VAL330, VAL335, VAL509
	Bidemethoxycurcumin (active)	ARG106,ALA513,GLY512, GLY519, HIS75, ILE503, LEU338, LEU345, LEU511, LEU517, MET99, PHE504, SER339, SER516, TYR334,TYR341, TRP373, VAL330, VAL335, VAL509
Derivatife compounds of 1,7-Diphenyl-1,6-heptadien-3,5-dione	1,7-bis(3-methoxyphenyl)-1,6-heptadien-3,5-dione (active)	ALA513, GLY512, GLY519, ILE331, LEU103, LEU109, LEU338, LEU345, LEU370, LEU517, LEU511, MET99, MET508, PHE367, PHE504, SER339, SER516, TYR341, TYR371, TRP373, VAL102, VAL330, VAL335, VAL509
	1,7-bis(4-hydroxyphenyl)-1,6-heptadien-3,5-dione (active)	ARG106, ARG499, ALA513, GLY512, GLY519, HIS75, ILE503, LEU338, LEU345, LEU511, LEU517, MET99, PHE504, SER339, SER516, TYR334, TYR341, TRP373, VAL102, VAL330, VAL335, VAL509
	1,7-bis(4-hydroxy-3,5-dimethylphenyl)-1,6-heptadien-3,5-dione(active)	ARG106, ALA513, GLY512, GLY519, ILE98, LEU78, LEU338, LEU345, LEU370, LEU511, LEU517, MET508, PHE343, PHE367, PHE504, SER339, SER516, TYR101, TYR334, TYR341, TYR371, VAL74, VAL102, VAL330, VAL335, VAL509
	1,7-bis(3,5-diethyll-4-hydroxyphenyl)-1,6-heptadien-3,5-dione(active)	ARG106, ALA513, GLY512, GLY519, ILE98, LEU78, LEU338, LEU345, LEU370, LEU511, LEU517, MET508, PHE343, PHE367, PHE504, SER339, SER516, TYR101, TYR334, TYR341, TYR371, VAL74, VAL102, VAL330, VAL335, VAL509
	1,7-bis(2-methoxyphenyl)-1,6-heptadien-3,5-dione(active)	ARG106, ALA513, GLY512, GLY519, HIS75, ILE331, LEU103, LEU109, LEU338, LEU345, LEU370, LEU511, LEU517, MET99, MET508, PHE367, PHE504, SER339, SER516, TYR334, TYR341, TYR371, TRP373, VAL330, VAL335, VAL509
	1,7-bis(4-chlorophenyl)-1,6-heptadien-3,5-dione(active)	ARG106, ALA513, GLY512, GLY519, LEU78, LEU338, LEU345, LEU370, LEU511, LEU517, MET508, PHE367, PHE504, SER339, SER516, TYR101, TYR341, TYR371, TRP373, VAL74, VAL102, VAL330, VAL335, VAL509
	1,7-bis(4-methylphenyl)-1,6-heptadien-3,5-dione(active)	ARG106, ALA513, GLY512, GLY519, ILE98, LEU78, LEU338, LEU345, LEU370, LEU511, LEU517, MET508, PHE367, PHE504, SER339, SER516, TYR101, TYR341, TYR371, TRP373, VAL74, VAL102, VAL330, VAL335, VAL509
	1,7-bis(3,4-dibenzyloksphenyl)-1,6-heptadien-3,5-dione(inactive)	ARG106, ARG499, ALA513, GLU506, GLU510, GLY512, GLY519, HIS75, ILE98, ILE331, LEU78, LEU103, LEU109, LEU338, LEU345, LYS344, LYS346, LEU370, LEU511, LEU517, LEU520, MET99, MET508, PRO71, PHE504, SER339, SER516, TYR101,TYR334, TYR341, TYR371, TRP373, VAL74, VAL102, VAL330, VAL335, VAL509

Amino acid residues that interacted with both of active and inactive representative compounds means amino acid residues had no effect or even a negative effect on the activity of active representative compounds. Amino acid residues that interactedwith the active representative compounds and did not interact with inactive representative compounds meant that the amino acid residues influenced the activity of COX-2 enzyme inhibition of active representativecompounds. Amino acid residues exclusively bound to the active compounds but not present in inactive compounds were as follows :GLY340 (Glycine 340), ILE503 (Isoleucine 503), PHE343 (Phenylalanine 343), and PHE367 (Phenylalanine 367). Ligands had a binding site with specific amino acids in the receptor. Ligand-receptor interaction occured because there were hydrogen bonds, Van Deer Waals bonding, or electrostatic interactions. Using PyMOL, bond distance between the ligand and amino acids can be known. Distance bond with the amino acid receptor ligands will affect the bond strength (affinity) of receptor-ligands. The smaller the distance of the bond are the greater affinity of the ligand-receptor. 3D visualization of active compounds were done on 1,7-bis(2-methoxyphenyl)-1,6-heptadien-3,5-dion, using PyMOL application, the bond between the ligand and amino acids can be known. The bond between the ligand and amino acids can be seen in Figure 1.

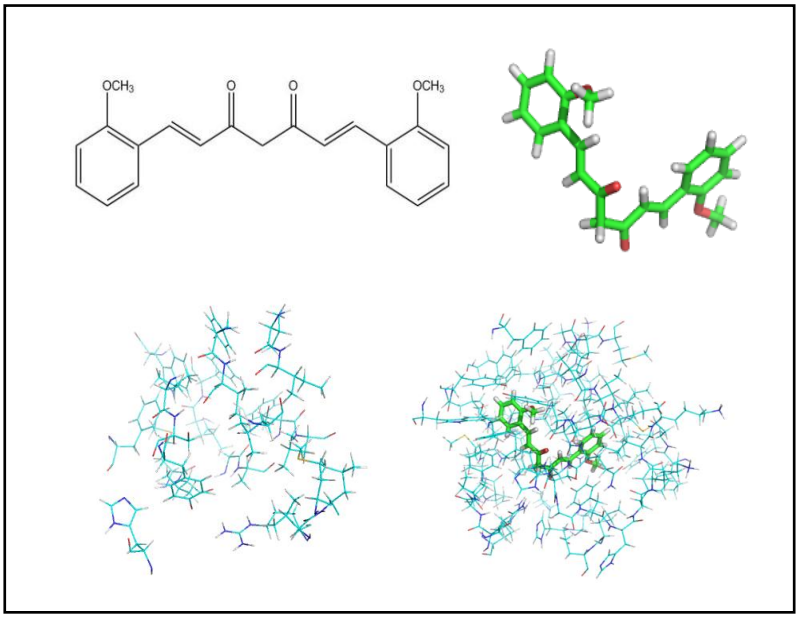

Figure 1. active representative compound 1,7-bis(2-methoxyphenyl)-1,6-heptadien-3,5-dion a) 2D model, b) 3D model c) bonding of compound on binding pocket COX-2

4. Conclusions

Of the 49 test compounds which were synthesized curcumin analogues compounds, there were eight compounds predicted active as COX-2 inhibitors using AYO_COX2_V .1.1 protocol i.e : 1,7-bis(3-methoxyphenyl)-1.6-heptadien-3,5-dione, 1,7-bis(4-hydroxyphenyl)-1.6-heptadien-3,5-dion, 1,7-bis (3-methoxyphenyl) -1.6-heptadien-3,5-dione, 1,7-bis(4-hydroxy-3,5-dimethylphenyl)-1.6-heptadien-3,5-dion, 1,7-bis(3,5-diethyl-4-hydroxyphenyl)-1.6-heptadien-3,5-dione, 1,7-bis (2-methoxyphenyl)-1.6-heptadien-3,5-dione, 1,7-bis(4-chlorophenyl)-1.6-heptadien-3,5-dione, and 1,7-bis (4-methylphenyl) -1,6-heptadien-3,5-dion and the active amino acids residues that increased the affinity of a COX-2 inhibitor i.e : GLY340 (Glycine 340), ILE503 (Isoleucine 503), PHE343 (phenylalanine 343), and PHE367 (phenylalanine 367).

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