

## INTERACTION STUDY OF CURCULIGOSIDE A AND ITS AGLYCONES AS CYCLOOXYGENASE INHIBITORS USING COMPUTATIONAL MODELLING

NURSAMSIAR<sup>a,b\*</sup>, IDA MUSFIROH<sup>a,c</sup>, MUCHTARIDI<sup>c</sup>, SLAMET IBRAHIM<sup>a</sup>, DARYONO H. TJAHJONO<sup>a</sup>

<sup>a</sup>School of Pharmacy Bandung Institute of Technology, Jalan Ganesha 10, Bandung 40132, <sup>b</sup>Sekolah Tinggi Ilmu Farmasi Makassar, Jalan P. Kemerdekaan Km 13,7 Daya, Makassar 90423, <sup>c</sup>Faculty of Pharmacy, Padjadjaran University, Jalan Raya Jatinangor Km 21,5 Sumedang. \*Email: n.siar@yahoo.co.id

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## ABSTRACT

The aim of the present study was to determine the interaction of curculigoside A and its aglycone with COX-1 (3N8Z) and COX-2 (6COX) in terms of hydrogen bonds and binding free energy. Docking simulations were performed by AutoDock 4.2 package. Molecular docking showed that curculigoside A and its aglycone are able to compete with the natural ligand and bind to the COX-1 (3N8Z) and COX-2 (6COX) binding pocket. The binding free energy to COX-1 of curculigoside A and its aglycone was -5.16 kcal/mol and -7.72 kcal/mol, respectively, while the binding free energy to COX-2 was -7.43 kcal/mol and -7.59 kcal/mol, respectively. The interaction with COX-1(3N8Z) was mediated by a hydrogen bond with Arg120, while the interaction with the active site of the COX-2 (6COX) was mediated by a hydrogen bond with Gln192.

**Keywords:** Curculigoside A, Cyclooxygenase, Computational modeling.

## INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. Inflammation is a basic way in which the body reacts to infection, irritation or other injury. The key features are redness, warmth, swelling and pain. Inflammation is now recognized as a type of non-specific immune response [2].

Cyclooxygenase (COX) is an enzyme involved in the synthesis of arachidonic acid into prostaglandin, a substance that plays a role in the inflammation process. COX has two isoforms, i.e. COX-1 and COX-2. Despite similar structures, these enzymes have different amino acid sequences in their active site. COX-1 has isoleucine, while COX-2 has valine at position 523, which influences its lipophilicity [3]. COX-1 is a constitutively expressed enzyme with a role in producing prostaglandin, protecting the mucous and catalyzing thromboxane

A2 formation, which leads to platelet aggregation and vasoconstriction. Conversely, COX-2 is inducibly expressed in response to particular stimuli. In addition to catalyzing prostaglandin formation, COX-2 also catalyzes the formation of prostacyclin, which plays a role in inhibiting platelet aggregation [4].

Curculigoside A is a phenolic glycoside compound isolated from the rhizome *Curculigo orchoides* [5]. It is the major bioactive compound present in *Curculigo orchoides*. It stimulates the secretion of estradiol by cultured primary granulosa cells and exhibits potent inhibitory activity against matrix metalloproteinase-1 in cultured human skin fibroblasts [6,7]. It also attenuates human umbilical vein endothelial cell injury induced by H<sub>2</sub>O<sub>2</sub> [8], up-regulates VEGF in MC3T3-E1 cells [9], and decreases infarct volume, alleviates cerebral damage, and reduces the expression of HMGB1 and phosphorylation of I $\kappa$ B- $\alpha$  and NF- $\kappa$ B in ischemic brain tissue [10]. These effects of curculigoside A are correlated with inhibition of the inflammatory response.

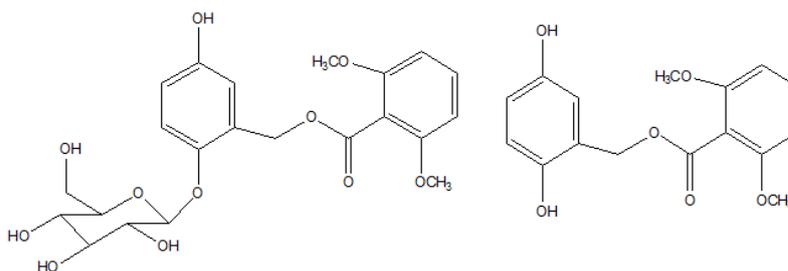


Fig. 1: Chemical structure of curculigoside A (a) and its aglycone (b)

## MATERIALS AND METHODS

## Materials

The ligand binding domain 3D (LBD 3D) structure of the cyclooxygenase-1 crystal complex with flurbiprofen (PDB: 3N8Z) with 2.90 Å resolution and the LBD 3D structure of the cyclooxygenase-2 complex with SC558 (PDB:6COX) with 2.80 Å resolution were obtained from the Protein Data Bank (www.pdb.org). The structures of curculigoside A and its aglycone were constructed using HyperChem v8.0.1, and then optimized using Austin Model 1 (AM1).

## Methods

The MGL tools 1.5.4 program package (*Molecular Graphics Laboratory, The Scripps Research Institute*) was used to prepare protein structures, ligand structures, grid parameters and docking

parameters, whereas the AutoGrid v4.2 program package (*The Scripps Research Institute*) was used to prepare the grid. The AutoDock v4.2 program package (<http://autodock.scripps.edu>) was used to stimulate the docking process with the Cygwin program package ([www.cygwin.com](http://www.cygwin.com)). In the docking of curculigoside A and its aglycone to COX-1 (PDB: 3N8Z), the grid box was 40x40x40 with a volume of 0.375 Å, while the grid center of *x,y,z* were -20.975, 50.155, 10.484 respectively. The running number was 10, with a total population of 150 and evaluation energy of 2500000. The docking parameters of curculigoside A and its aglycone to COX-2 (PDB: 6COX) applied a grid box of 50x50x50 with a volume = of 0.375Å and grid center *x,y,z* of 22.541, 23.526, 46.984, respectively. In addition, the running number was 10, with a total population of 150 and evaluation energy of 2500000. Evaluation of the interaction was based on their binding free energy ( $\Delta G$ ), inhibition constant ( $K_i$ ) and hydrogen bonds between ligands and the enzymes.

## RESULTS AND DISCUSSION

## Geometric Optimization of Curculigoside A and Its Aglycone

Table 1: Geometric parameters of optimized curculigoside A and its aglycone

Compounds	Log P	Mass (amu)	Volume (Å <sup>3</sup> )	Energy (kcal/mol)
Curculigoside	-3.47	466.44	1189.31	-6190.22
Aglycone	-2.01	304.30	847.97	-4115.46

## Validation of the Docking Method of Autodock 4.2 Software

Validation of the docking method was performed by redocking flurbiprofen into COX-1 (PDB:3N8Z) and SC-558 into COX-2 (PDB:6COX). Table 2 shows the validation results.

Redocking flurbiprofen into the COX-1 binding site and SC-558 into the COX-2 binding site showed that these two compounds were capable of interacting with the active sites of these two enzymes. When flurbiprofen interacted with COX-1, it formed hydrogen bonds

with the Arg120 and Tyr355 residues. The interaction of SC558 with COX-2 showed that SC558 formed hydrogen bonds with the Arg120, Gln192, Leu352 and Tyr355 residues. The root mean square deviation (RMSD) of redocking flurbiprofen to COX-1 was 0.578 Å, while that of redocking SC558 to COX-2 was 1.204 Å. The model is considered valid if the RMSD is equal to or less than 2 Å [11].

According to the validation results, the AutoDock 4.2 software can be applied to simulate the docking of curculigoside A and its aglycone into the COX-1 and COX-2 binding site.

Table 2: Data of re-docking of the natural ligand into the binding site of COX-1 and COX-2

Crystal structure	ΔG (kcal/mol)	K <sub>i</sub> (nM)	Σ hydrogen bond	RMSD (Å)
Flurbiprofen	-9.0	251.93	4	0.578
SC-588	-10.29	28.43	4	1.204

## Computational Modeling for the Interaction of Curculigoside a and its Aglycone with COX-1 and COX-2

Table 3: Parameters of the interaction between curculigoside A and its aglycone with COX-1

Compounds	ΔG (kcal/mol)	K <sub>i</sub> (M)	Σ Hydrogen bond	Amino Acid residues
Curculigoside A	-5.16	1.66. 10 <sup>-8</sup>	4	His90, Leu93, Val116, Arg120, Val349, Leu352, Ser353, Tyr355, Leu357, Leu359, Phe381, Trp387, Phe518, Met522, Ile523, Gly526, Ala527, Ser530, Leu531
Aglycone	-7.72	2.18. 10 <sup>-6</sup>	4	Met113, Val116, Arg120, Val349, Leu352, Ser353, Tyr355, Leu359, Phe381, Tyr385, Phe518, Met522, Ile523, Gly526, Ala527, Ser530, Leu531

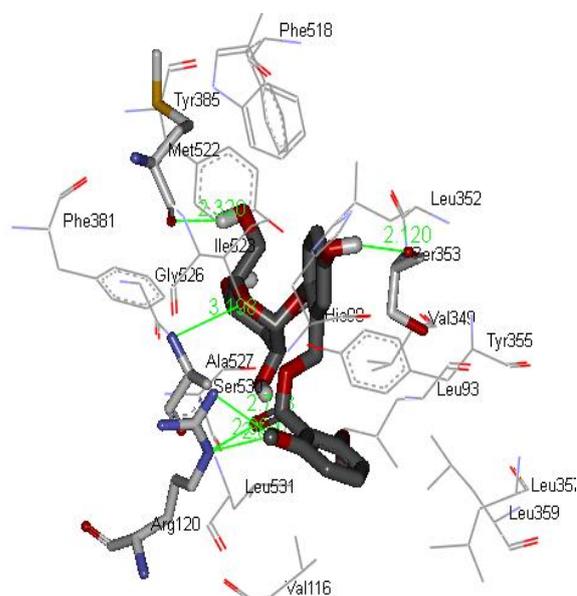


Fig. 2: Interaction of Curculigoside A with the binding pocket of COX-1

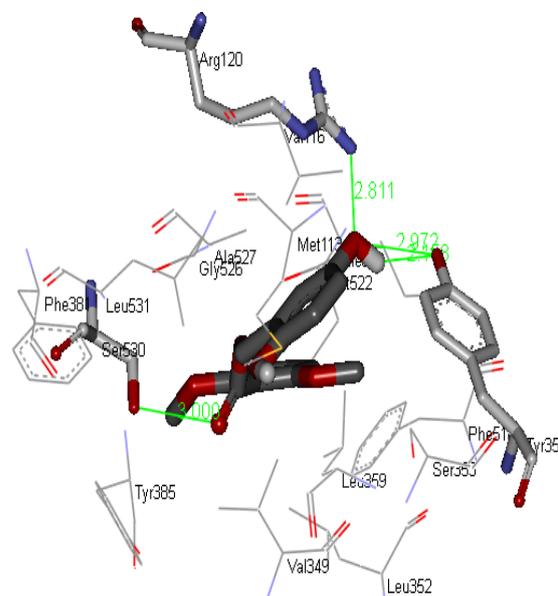


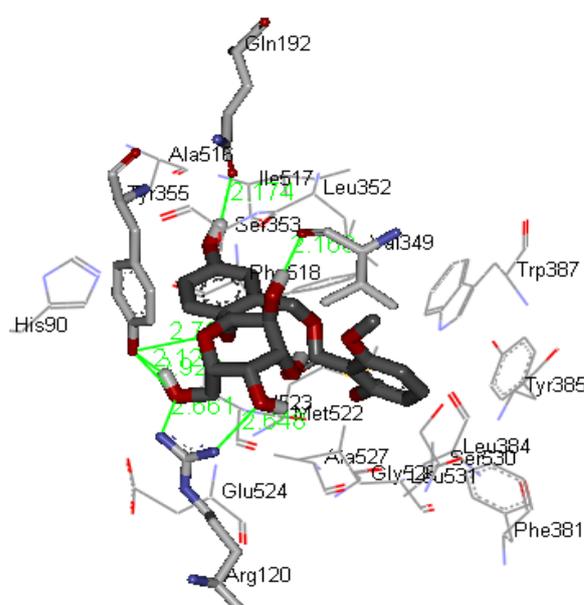
Fig. 3: Interaction of Aglycone with the binding pocket of COX-1

Upon interaction with COX-1, the binding free energy ( $\Delta G$ ) and inhibition constant ( $K_i$ ) for curculigoside A and its aglycone were  $-5.16$  kcal/mol,  $1.66 \times 10^{-4}$  M and  $-7.72$  kcal/mol,  $2.18 \times 10^{-6}$  M, respectively. Curculigoside A showed an interaction with the active site of COX-1 (Fig. 2) through the formation of hydrogen bonds between the methoxy oxygen of the aromatic group (HB donor) and Arg120 (HB acceptor), the hydrogen of the hydroxyl aromatic group (HB donor) and Ser353, the oxygen of the glucose group (HB acceptor) and Ala527 (HB donor), the hydrogen of the

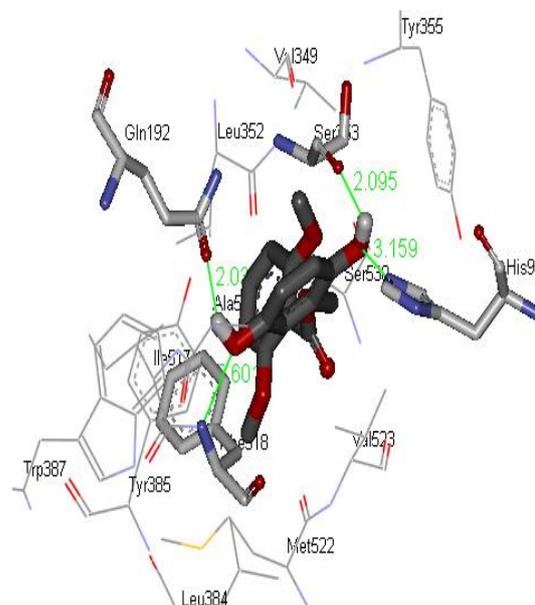
glucose group (HB donor) and Met522, as well as hydrophobic bonds with other amino acid residues. The aglycone showed interactions with the active site of COX-1 (Fig. 3) through hydrogen bonds between the oxygen atom of the aromatic group (HB acceptor) and Arg120 (HB donor), the hydrogen atom of the aromatic group (HB donor) and Tyr353 (HB acceptor), the oxygen atom of the carbonyl group (HB acceptor) and Ser353 (HB donor), and the hydrogen of the aromatic group (HB acceptor) and Tyr355 (HB donor).

**Table 4: Parameters of the interaction between curculigoside A and its aglycone with COX-2**

Compounds	$\Delta G$ (kcal/mol)	$K_i$ (M)	$\Sigma$ Hydrogen bond	Amino acid residues
Curculigoside A	-7.43	$3.57 \cdot 10^{-6}$	7	His90, Arg120, Gln192, Val349, Leu352, Ser353, Tyr355, Phe381, Leu384, Tyr385, Trp387, Ala516, Ile517, Phe518, Met522, Val523, Glu524, Gly526, Ala527, Ser530, Leu531
Aglycone	-7.59	$2.72 \cdot 10^{-6}$	4	His90, Gln192, Val349, Leu352, Ser353, Tyr355, Leu384, Tyr385, Trp387, Ala516, Ile517, Phe518, Met522, Val523, Ser530



**Fig. 4: Interaction of curculigoside A with the binding pocket of COX-2**



**Fig. 5: Interaction of the aglycone with the binding pocket of COX-2**

Curculigoside A and its aglycone can interact with the active site of COX-2, as shown by the docking simulation. The binding free energy ( $\Delta G$ ) and  $K_i$  for curculigoside A and its aglycone were  $-7.43$  kcal/mol,  $3.57 \times 10^{-6}$  M and  $-7.59$  kcal/mol,  $2.72 \times 10^{-6}$  M, respectively. Curculigoside A was found to interact with the active site of COX-2 (Fig. 4) through the formation of hydrogen bonds between the hydrogen of the aromatic group (HB donor) and Gln192 (HB acceptor), the hydrogen of the glucose group (HB donor) and Val349 and Tyr355 (HB acceptor), the oxygen of the glucose group (HB acceptor) and Arg120 and Tyr355 (HB donor), as well as hydrophobic bonds with other amino acid residues. The aglycone interacted with the active site of COX-2 (Fig. 5) through hydrogen bonds between the hydrogen atom of the aromatic group (HB donor) and Gln192 and Ser353 (HB acceptor), the oxygen atom of the aromatic group (HB acceptor) and His90 and Phe518 (HB donor), as well as hydrophobic bonds with other amino acid residues.

Methoxy functional groups bound to aromatic and carbonyl groups of curculigoside A are features that can interact with the active site of COX-1, while in the aglycone, aromatic groups are features at the

active site of COX-1. Although the glycoside has more active features, its activity against COX-1 was lower compared to its aglycone, as indicated by smaller binding free energy (Table 3). In addition, aromatic functional groups on the two structures (the glycoside and its aglycone) were the feature capable of interacting with the active site of COX-2; the binding free energy values are shown in Table 4.

This study showed that the two compounds had activities against COX-1 and COX-2. The interaction between curculigoside A and COX-1 had higher activity compared to the interaction with the aglycone. The active site of COX-1 is different from that of COX-2 at Ile523 (Val523 in COX-2), which contributes to its lipophilic properties [3]. The active site of COX-1 possesses higher lipophilicity compared to that of COX-2, thus the aglycone (which is more lipophilic than the glycoside) can interact more strongly with active site of COX-1, as shown by the higher binding affinity ( $K_i$ ). Meanwhile, the affinity of the aglycone of curculigoside A for the active site of COX-2 was slightly lower than that of the glycoside. This was due to the more hydrophilic COX-2 site active, thus the glycoside can interact more strongly with COX-2.

## CONCLUSIONS

The docking simulation showed that curculigoside A and its aglycone can interact with the active sites of COX-1 (3N8Z) and COX-2 (6COX). Curculigoside A and its aglycone showed interactions with the active site of COX-1 through hydrogen bonds with Arg120, while binding to the active site of COX-2 occurred through hydrogen bonds with Gln192. The active feature of curculigoside A which interacts to the active site of COX-1 was methoxy group bound to aromatic and carbonyl group, whereas the active feature of its aglycone was aromatic group which bind hydroxyl groups. The active feature of both structures (the glycoside and its aglycone) binding to the active site of COX-2 was aromatic group binding to hydroxyl groups.

## ACKNOWLEDGEMENT

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