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Molecular Docking Study of Bioactive Compounds from *Curcuma aeruginosa* Roxb. as Antioxidant and Anticancer Activities

Kajian Molecular Docking Senyawa Bioaktif dari *Curcuma aeruginosa* Roxb. sebagai Aktivitas Antioksidan dan Antikanker

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ABSTRACT

Cancer is one type of dangerous disease that is increasing every year. Free radicals are the cause of gene mutations (cancer). Cancer will develop uncontrollably due to the occurrence of the cell cycle and the presence of compounds that trigger cell proliferation and inhibit the process of apoptosis. This cancer treatment is carried out by giving cyclin-dependent kinase2 (CDK2) and cyclooxygenase-2 (COX-2) inhibitor drugs to inhibit cancer development, as well as lipoxygenase (LOX) inhibitor drugs for the formation of free radicals. *Curcuma aeruginosa* RoxB., the plant, is known to have the potential for antioxidant and anticancer properties. This study aims to determine the molecular interaction between the dominant compound in the ethanol extract of *C. aeruginosa* with CDK2, COX-2, and LOX receptors. The type of ligand interaction with the receptor was determined through the parameters of affinity energy (ΔG), inhibition constant (Ki), type of interaction, and percentage of binding site similarity (%BSS). The results showed that the gajutsulacton A had the best potential in inhibiting CDK2. The cucumenol may be a COX-2 inhibitor, and there are no compounds that can inhibit LOX as well as an antioxidant. Thus, our findings demonstrate the potential for *C. aeruginosa* bioactive to serve as anticancer candidate molecules against CDK2 and COX-2 receptors.

Keywords: Anticancer, antioxidant, CDK2, COX-2, LOX

ABSTRAK

Kanker merupakan salah satu penyakit berbahaya yang semakin meningkat setiap tahunnya. Radikal bebas merupakan penyebab terjadinya mutasi gen (kanker). Kanker akan berkembang tidak terkendali akibat terjadinya siklus sel dan adanya senyawa yang memicu proliferasi sel, serta menghambat proses apoptosis. Pada penelitian ini digunakan obat inhibitor cyclin-dependent kinase2 (CDK2) dan cyclooxygenase-2 (COX-2) untuk menghambat perkembangan kanker, serta obat inhibitor lipoxygenase (LOX) untuk pembentukan radikal bebas. *Curcuma aeruginosa* RoxB. merupakan tanaman yang diketahui memiliki potensi sebagai antioksidan dan antikanker. Penelitian ini bertujuan untuk mengetahui interaksi molekuler antara senyawa dominan pada ekstrak etanol *C. aeruginosa* dengan reseptor CDK2, COX-2, dan LOX. Interaksi ligan dengan reseptor ditentukan melalui parameter energi afinitas (ΔG), konstanta inhibisi (Ki), jenis interaksi, dan persentase *binding site similarity* (%BSS). Hasil penelitian menunjukkan bahwa gajutsulacton A mempunyai potensi terbaik dalam menghambat CDK2. Cucumenol dapat menghambat COX-2 dan tidak ada senyawa yang dapat menghambat LOX dengan baik sebagai antioksidan. Dengan demikian, temuan kami menunjukkan potensi bioaktif *C. aeruginosa* sebagai kandidat antikanker terhadap reseptor CDK2 dan COX-2.

Kata Kunci: Antikanker, antioksidan, CDK2, COX-2, LOX

INTRODUCTION

Cancer is one of the deadliest diseases in the world. The World Health Organization (WHO), on September 12, 2018, reported that cancer is the second leading cause of death in the world. A total of 9.6 million people in 2018 are known to have died from cancer. Cases of cancer sufferers in Indonesia, according to Health Research data, in 2018 also increased, recorded in 2013 as many as 374,780 people (1.4 prevalence by 1000 population) and in 2018 reaching 479183 people (1.79 prevalence by 1000 population) (Badan Penelitian dan Pengembangan Kesehatan, 2019; World Health Organization (WHO), n.d.).

Cancer can be caused by many factors, one of which is free radicals, often referred to as Reactive Oxygen Species (ROS). Free radicals are molecules or atoms that have unpaired electrons and short half-lives, so they are highly reactive. One of the enzymes that mediate the formation of endogenous free radicals is the lipoxygenase enzyme. The lipoxygenase enzyme is one of the enzymes in the arachidonic acid metabolic pathway that synthesizes the formation of leukotrienes and produces intermediates in the form of $O_2^{\bullet-}$ which is further processed into H_2O_2 (Wisastra & Dekker, 2014). The reaction of free radicals with DNA (Deoxyribonucleic acid) causes gene mutations, which downregulate the cell cycle, causing cancer cells to proliferate uncontrollably.

Cancer is a disease characterized by uncontrolled abnormal cell growth and can attack other body tissues and organs (Yu et al., 2015). Meanwhile, the rapid spread of cancer is partly due to cell cycle dysregulation leading to abnormal cell growth. Cyclin-dependent kinase-2 (CDK2), the protein responsible for mediating the cell cycle process in Gap 1 (G1) phase to the synthesis (S) phase, once inhibited, will cause cell cycle arrest and apoptosis (Ding et al., 2020).

Some cancers overexpress cyclooxygenase-2 (COX-2). However, overexpression of this enzyme increases the synthesis of prostanoids, a protein that upregulates cellular proliferation and prevents cell death in cancer cells (Fishbein et al., 2021). The COX-2 enzyme is an isoform of the cyclooxygenase enzyme, which converts arachidonic acid to prostanoids (prostaglandins and thromboxane). Hence, inhibition of this enzyme will reduce cell proliferation and trigger apoptosis (Nurrochmad, 2004).

Generally, cancer treatment includes surgery, radiotherapy, or hormonal therapy (Y. S. Sun et al., 2017) with known side effects on normal cells. Efforts to minimize the side effects incurred from cancer therapies encourage a variety of drug-related research that prevent the spread and establishment of cancer and have no side effects to a minimum. Recently, the prevention and control of cancer cells with antioxidants due to their ability to interact and neutralize free radicals has taken a toll.

Indonesia is second only to Brazil in biodiversity; Indonesians take advantage of this diversity by making it an alternative medicine. *Curcuma aeruginosa* Roxb., called *C. aeruginosa* or temu hitam in Indonesia, is a traditional medicinal plant widely found in Indonesia and some parts of Southeast Asia. *C. aeruginosa* rhizome is used as a traditional medicinal plant because of its bioactive compounds: saponins, flavonoids, polyphenols, terpenoids, and glucans (Jose & Thomas, 2014). Extracts of *C. aeruginosa* compounds based on research results are reported to have several bioactivities, including antimicrobial (Angel et al., 2012), anticancer (Jantan et al., 2005; Septaningsih et al., 2018), antiandrogenic (Suphrom et al., 2012), and antioxidant (Choudhury et al., 2013; Qiao et al., 2015; Wang et al., 2015; Waras et al., 2015).

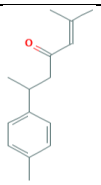
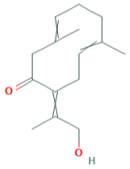
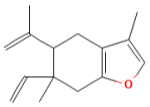
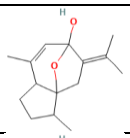
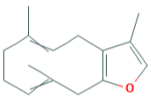
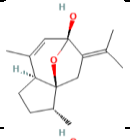
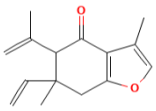
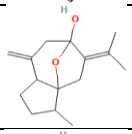
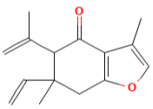
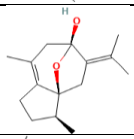
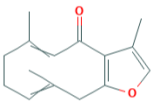
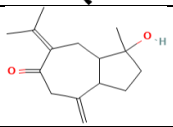
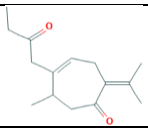
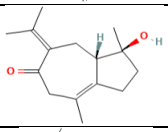
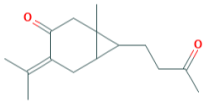
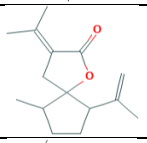
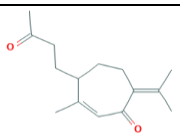
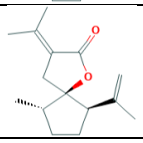
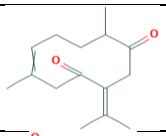
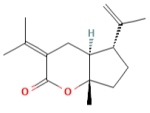
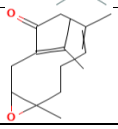
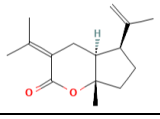
Recent findings from Septaningsih et al. (2018) show that the ethanol extract of C. aeruginosa inhibits free radicals as an antioxidant and has the potential for anticancer. The study showed 22 dominant active compounds in the form of a sesquiterpene group identified using Liquid Chromatography-Mass Spectrometry (LC-MS). This study aims to determine the potential of the dominant active compound of *C. aeruginosa* as an antioxidant and anticancer.

METHODS

Molecular anchoring simulation using Autodock Vina 1.5.6 software, MarvinView, Ligplot+ 4.3.5, and Discovery Studio Visualizer to visualize the interaction between ligands and receptors. The materials used in this study are a three-dimensional structure of Lipoxygenase (LOX-3) with code 1N8Q, Cyclooxygenase (COX-2) with code 4COX, and Cyclin-Dependent Kinase (CDK2) with code 1DI8 as a receptor downloaded from the RSCB Protein Data Bank. Three-dimensional structures of the test ligands and natural ligands were obtained from the PubChem database. The test ligands used were 22

dominant active compounds from the ethanol extract of *the C. aeruginosa* rhizome, these ligands belong to the terpenoids/sesquiterpen class (**Table 1**). The natural ligand used is an inhibitor compound that has been bound to the receptor.

Table 1. The Dominant Compunds of *Curcuma Aeruginosa* Selected for Molecular Docking

No.	Name compounds	Structure	No	Name compounds	Structure
1	Ar-Tumerone		12	13-hydroxygermacrone	
2	Curzerene		13	Curcumenol	
3	Furanodiene		14	4-epicurcumenol	
4	Curzerenone		15	Isocurcumenol	
5	5-epi-curzerenone		16	Neocurcumenol	
6	Furanodienone		17	Isoprocurcumenol	
7	Curmadione		18	Neoprocurcumenol	
8	Curcumenone		19	Curcumanolide A	
9	Isocurcumadione		20	Curcumanolide B	
10	Dehydrocurdione		21	Gajutsulactone A	
11	Germacrone		22	Gajutsulactone B	

Note: The structure of the compounds was obtained from Septaningsih et al. 2018

1. Receptor structure preparation

The receptor structure preparation was carried out according to the method developed by Dermawan et al. (2019) and Pingaew et al. (2018). The receptor used for the antioxidant test was LOX, while for the anticancer test, COX-2 and CDK2 were used. The downloaded three-dimensional receptor was prepared using the Discovery Studio Visualizer application; initial preparation included the removal of water molecules and ligands that were still

attached to the structure, the addition of hydrogen ions, and the Gasteiger-Marsili charge, after which the results were saved in PDB format. The prepared structure is formatted into PDBQT using the AutoDock 1.5.6 application.

2. Ligand structure preparation

Ligand structure preparation was carried out as previously described by Sumaryada et al. (2021). *C. aeruginosa* rhizome ethanol extract contains 22 dominant compounds available at www.pubchem.ncbi.nih.gov in pdf format. Marvin-view was used to convert Ligands with .sdf format to PDB format, followed by optimization by the addition of hydrogen ions using Autodock Tools 1.5.6.

3. Analysis of ligand bioavailability and toxicity

Analysis of test and natural ligands for their solubility and permeability based on Lipinski's rules was done by accessing the http://swissadme.ch/index.php page. Ligands that met the Lipinski rule are then measured for their toxicity by accessing the page http://lmmd.ecust.edu.cn/admetsar1/predict/.

4. Molecular docking simulation

Molecular docking simulations were carried out according to the method developed by Dermawan et al. (2019) and Pingaew et al. (2018). Molecular docking simulations were carried out using Autodock Vina using the 'targeted docking' technique. The ligand attachment zone is defined by a grid box around the active site of the receptor using AutoDock Tools with coordinates and a grid box corresponding to each receptor. The vina folder is placed on the C:\Vina drive and then filled with the CONF.TXT file with the above parameters according to the center number and its size. Execute the embedding command using commands via the Command Prompt window. Documents in PDBQT format can be converted to PDB using the Discovery Studio Visualizer software. In addition, a "log" document in txt format will also be formed containing data on the change in the value of Gibbs free energy (ΔG).

5. Ligand-receptor interaction analysis:

Analysis of the interaction of ligands with receptors was carried out according to the method developed by Sumaryada et al. (2021). Molecular docking produces a model of the interaction of the test ligand and the receptor. The ligand anchoring model with the selected receptor is the model that has the lowest G and three-dimensional (3D) visualization that is closest to the receptor region. The selected ligand model was combined with the receptor using the Discovery Studio Visualizer software. The merging of the two molecules is done by copying the selected model on the ligand tab screen, pasting it on the receptor tab screen, and then pulling it to the top to be attached to the receptor. The combined results are stored in the form of PDB and then analyzed for energy and chemical bonds. Molecular interaction analysis, including hydrogen bonding and hydrophobic interactions in two dimensions (2D), can be performed using Ligplot+ 4.3.5 software. The analysis was carried out by comparing the visualization of the ligand anchoring area on the receptor to that of the native ligand.

RESULTS & DISCUSSION

1. Receptor Structure and Molecular Docking Method Validation

The receptors used in this study were cyclin-dependent kinase2 (CDK2), cyclooxygenase-2 (COX-2), and lipoxygenase receptor (LOX). The prepared receptor was then validated by re-docking the native ligand to the receptor. Preparation of protein structure is needed to separate or remove the attached molecules in the form of water molecules and natural ligands. The omitted molecule aims to avoid the occurrence of a long duration of molecular docking and to ensure that the receptor will only interact with the test compound (Rachmania, Hariyanti, et al., 2018). This preparation process is also carried out by adding hydrogen atoms which aim to restore hydrogen atoms that may be lost during the crystallization process (Seeliger & De Groot, 2010).

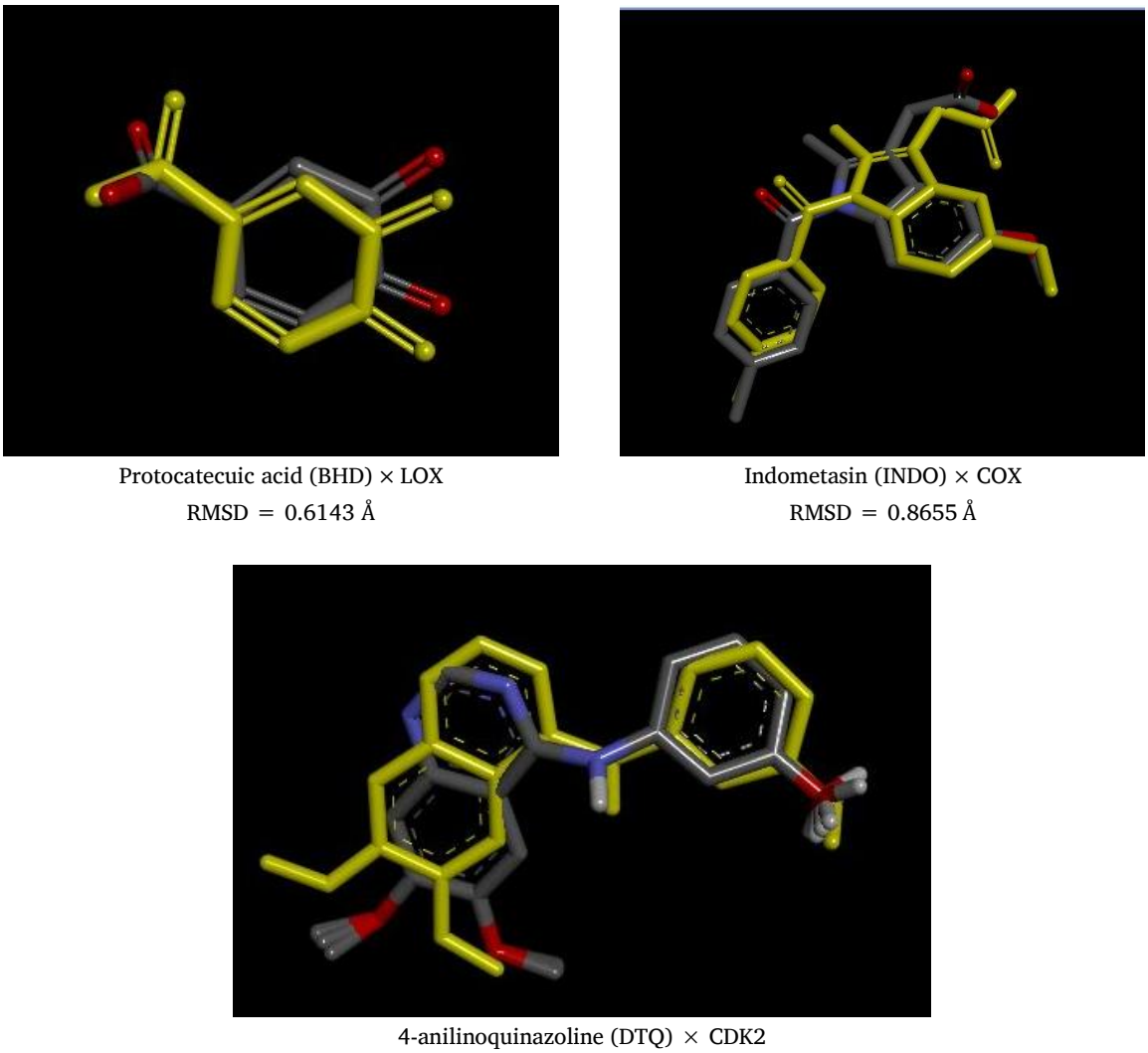
The native ligand used in this study was tethered 10 times to each receptor using Discovery Studio. Validation of the method is done by determining the grid box using the Autodock Vina program so that the coordinates of the binding site of a receptor are known. The determination of the grid-box is carried out in the area known as the active site of the receptor and will be used as the attachment site for the molecule.

Determining the grid box includes determining the size of the grid box using spacing (angstrom) and setting the location of the grid box parameters. The grid box on the lipoxygenase receptor (code:1N8Q) is

x = 21,287; y = 1.743; and z = 19,171, cyclooxygenase receptors (code: 4COX) namely x = 23,941; y = 21,867; and z = 13,892, and CDK2 receptors (code:1DI8), namely x = -8,615; y = 49,545; and z = 12,689 with each receptor using several points 40×40×40. Spacing (angstrom) for each receptor in this study was determined to be 0.375 Å.

The receptor structure that has been prepared through the Autodock Vina program is then saved in .pdbqt format. The .pdbqt format on the receptor indicates the presence of a partial charge on each atom (Syahputra et al., 2014). The prepared receptors then validated the method. Method validation was carried out to obtain a good method (Mardianingrum et al., 2015).

The validation of the molecular docking method was done by calculating the Root Mean Square Deviation (RMSD) value. Determination of the RMSD value was done using the Command Prompt (CMD) program and Discovery Studio. The RMSD value was obtained by measuring the two poses and comparing the atomic positions between the experimental structure and the structure to be predicted. The average RMSD value of the lipoxygenase receptor with protocatechuic acid was 0.6143 Å while the average RMSD value of the cyclooxygenase receptor with indomethacin was 0.8655 Å. The cyclin-dependent kinase-2 (CDK2) receptor with its ligand 4-anilinoquinazoline had an average RMSD value of 1.1322 Å (**Figure 1**).



Protocatecuic acid (BHD) × LOX
RMSD = 0.6143 Å

Indometasin (INDO) × COX
RMSD = 0.8655 Å

4-anilinoquinazoline (DTQ) × CDK2
RMSD = 1.1322 Å

Figure 1. Molecular docking validation of the native ligand (yellow) and the validated ligands (gray)

The validation of the docking method is considered successful if the value obtained is < 2.0 (Rachmania, Hariyanti, et al., 2018). The closer the RMSD value is to zero, the more similar the native ligand copy poses to the copy ligand (Purnomo et al., 2013). The results of the validation of the molecular docking method on each native ligand with its receptor obtained an RMSD value of less than 2, so it

can be stated that the procedure used is close to the native condition, and the natural ligand is attached to the active site of each receptor.

The COX-2 protein selected was an enzyme complex with the non-selective inhibitor indomethacin (a native ligand) obtained from fractionation by X-ray crystallography of mice (Mus musculus). Indomethacin is an indole-derived compound that can inhibit prostaglandin production by competitively blocking cyclooxygenase activity. The CDK2 protein used in this study is a protein bound to the selective inhibitor 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline (a natural ligand). CDK2 activity is ATP-dependent, and 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline can competitively inhibit CDK2 activity by occupying the ATP-binding pocket. LOX enzyme is a complex enzyme with Protocatechuic acid (native ligand) obtained from fractionation by X-ray crystallography of soybean seeds (Resnick cultivar). Inhibition of protocatechuic acid on lipoxygenase enzymes occurs competitively. The COX-2 protein selected was an enzyme complex with the non-selective inhibitor indomethacin (a native ligand) obtained from fractionation by X-ray crystallography of mice (Mus musculus). Indomethacin is an indole-derived compound that can inhibit prostaglandin production by competitively blocking cyclooxygenase activity. The CDK2 protein used in this study is a protein bound to the selective inhibitor 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline (a natural ligand). CDK2 activity is ATP-dependent, and 4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline can competitively inhibit CDK2 activity by occupying the ATP-binding pocket. LOX enzyme is a complex enzyme with Protocatechuic acid (native ligand) obtained from fractionation by X-ray crystallography of soybean seeds (Resnick cultivar). Inhibition of protocatechuic acid on lipoxygenase enzymes occurs competitively.

2. Ligand Bioavailability and Toxicity

A drug has good bioavailability if it meets Lipinski's Rule of Five. Lipinski criteria consist of five rules, namely molecular weight less than 500 Da, LogP value less than 5, hydrogen bond donor less than 5, hydrogen bond acceptor less than 10, and molar refractivity between 40-130. All the ligands used were in accordance with Lipinski's rules and can be used as oral drugs except for the natural ligand 3,4-dihydroxybenzoic acid, which has a molar refractivity value outside the range of 40-130, which is 37.45 (**Table 2**). Molar refractivity values that are outside the required range can cause a drug to be less than optimally absorbed in the body (Fakhruri et al., 2021).

Ligands that have met the Lipinski criteria are then predicted for their toxicity. Ligand toxicity describes the effects that occur due to the involvement of drugs on body metabolism that will cause cell or organ damage in an organism. The parameters used in this study consisted of three things: inhibition of Human Ether-A-Go-Go Related Gene (herG), carcinogenicity, and acute oral toxicity. Prediction results on herG parameters indicate that all ligands were categorically weak inhibitors, while in carcinogenicity prediction, it is understood that all ligands included in the non-carcinogenic category cannot cause cancer except for ar-tumerone compounds with a value of 0.5168. The prediction results for acute oral toxicity showed that indomethacin was included in category I with a value of 0.7774, while curmadione and dehydrocurdione compounds were included in category II with values of 0.4856 and 0.4719, respectively. The value of acute oral toxicity on other compounds shows that these compounds fall into category III.

The bioavailability of the ligands in this study was analyzed to see if a drug had good criteria. Oral bioavailability is the ability of a drug or substance to be available to target tissues after administration of the drug or substance to the body. A drug is said to have good bioavailability if it can comply with Lipinski's Rule of Five. Lipinski's rule is used to determine the physicochemical properties and the level of absorption or permeability of a compound to the lipid bilayer so that it can be absorbed by the body (Rachmania, Supandi, et al., 2018). Lipinski's rules are complied with to design a drug compound so that it can be administered orally. Compounds that are used as drugs orally must have the ability to penetrate the intestinal wall and work actively on the desired target. The criteria that must be met for a compound in Lipinski's rule include a molecular weight of less than 500 Da, a LogP value of less than 5, a hydrogen bond donor of less than 5, a hydrogen bond acceptor of less than 10, and a molar refractivity between 40-130 (Lipinski, 2016).

Table 2. Results of Lipinski rules analysis of ligands

Ligand	Molecular Weight (Da)	Hydrogen Donors	Hydrogen Acceptors	LogP	Molar Refractivity
3,4-dihydroxybenzoic acid	154.12	3	4	0.65	37.45*
4-anilinoquinazoline (4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline)	297.31	2	5	2.40	84.09
Indometasin	357.79	1	4	3.63	96.12
Ar-Tumerone	216.32	0	1	4.48	69.75
Curzerene	216.32	0	1	4.54	68.74
Furanodiene	216.32	0	1	4.63	69.00
Curzerenone	230.31	0	2	3.88	69.16
5-epi-curzerenone	230.31	0	2	3.88	69.16
Furanodienone	230.30	0	2	3.97	69.42
Curmadione	234.33	0	2	3.02	71.56
Curcumenone	234.33	0	2	2.88	69.66
Isocurcumadione	234.33	0	2	3.02	71.56
Dehydrocurdione	234.33	0	2	3.26	71.56
Germacrone	234.33	0	2	3.61	70.37
13-hydroxygermacrone	234.33	1	2	3.17	72.04
Curcumenol	234.33	1	2	3.96	69.25
4-epicurcumenol	234.33	1	2	3.96	69.25
Isocurcumenol	234.33	1	2	3.75	69.25
Neocurcumenol	234.33	1	2	2.88	69.25
Isoprocurcumenol	234.33	1	2	3.03	70.44
Neoprocurcumenol	234.33	1	2	3.08	70.44
Curcumanolide A	234.33	0	2	3.01	70.37
Curcumanolide B	234.33	0	2	3.57	70.37
Gajutsulactone A	234.33	0	2	4.69	70.37
Gajutsulactone B	234.33	0	2	3.54	70.37

* = not according to the Lipinski rules

This study showed that all ligands had a molecular weight below 500 Da (**Table 2**). The molecular weight of a drug compound will affect the absorption of the drug in the body, the greater the molecular weight, the more difficult it will be for the compound to be absorbed into the body (Fakhruri et al., 2021). Another criterion that must be met is the Log P-value. This log P value is related to the absorption process or the permeability of a compound to the lipid bilayer. The log P value shows the coefficient of solubility in fat/water, which has a range of -0.4 – 5 (Syahputra et al., 2014). A log P value of more than five indicates that the compound has a high level of toxicity because it is retained in the lipid bilayer and causes the sensitivity of ligand binding to the receptor to decrease (Fakhruri et al., 2021), besides that, the log P value should not be too negative, negativity will cause these compounds to be unable to pass through the lipid bilayer membrane (Rachmania, Supandi, et al., 2018). All ligands used in this study have log P values according to Lipinski's rule.

The number of hydrogen bond donors and acceptors in the docking molecule was carried out to determine the hydrogen bonding capacity. Hydrogen bonding with a high capacity will require a large amount of energy for the absorption process to occur (Syahputra et al., 2014). The number of hydrogen bond donors and acceptors in the ligands in this study complied with Lipinski's rules. The Lipinski rule states that a drug can be administered orally if it does not violate more than two of its rules (Kartasasmita et al., 2015). All ligands showed that the compound had high bioavailability because there was only one natural ligand that violated one of Lipinski's 5 rules, 3,4-dihydroxybenzoic acid. This shows that the test ligand has good permeability to cross cell membranes and the ligand can easily bind to the receptor (Tumilaar et al., 2021).

Ligands that have met the Lipinski criteria are then predicted for their toxicity. The results of this prediction aim to see the degree of damage that will be caused by a drug molecule and determine the dose limit for the administration of a drug. Prediction of herG inhibition showed that the results of all the ligands used in this study were weak inhibitors of herG. HerG is one of the parameters which is a protein-coding gene derived from the K ion channel. HerG channel is associated with heart rate control so that if the work of the channel is inhibited by drug compounds, it will cause loss of consciousness and an increased risk of sudden death (Pratama et al., 2017).

Carcinogenicity parameters are parameters that describe the ability of compounds to cause tumors and cancer in humans. Classification of carcinogenicity according to the International Agency of Research on Cancer (IARC) is divided into four groups, namely group one indicates the compound is carcinogenic, group two has carcinogenic properties, group three indicates the compound cannot be classified as carcinogenic, and group four is a group whose compounds are carcinogenic. non-carcinogenic (Fakhruri et al., 2021). Prediction of carcinogenicity of the ligands used showed all ligands were non-carcinogenic except for ar-tumerone which was carcinogenic with a value of 0.5168.

Acute oral toxicity was performed to measure the degree of toxic effect within 24 hours. This toxicity indicates the acute toxic nature of a drug compound that enters orally. The LD50 value determines the level of toxicity of a ligand. These levels are classified into 4 categories: category one has an LD50 of 50 mg/kg, category two LD50 values have a range between 50-500 mg/kg, category three LD50 values are in the range of 500-5000 mg/kg, and category 4 has an LD50 value of greater than 5000 mg/kg (Fakhruri et al., 2021). In the prediction of acute oral toxicity, all the ligands used were in the third category except for indomethacin which was included in category one, and curmadione and dehydrocurdione were included in the second category (**Table 3**).

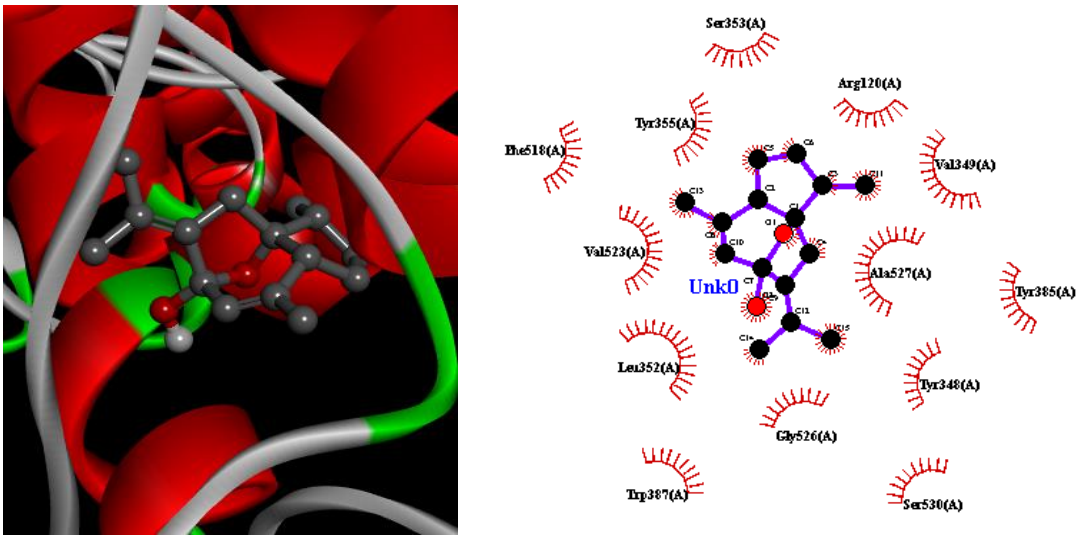
Table 3. Prediction of ligand toxicity

Ligand	Human *Ether-A-Go-Go Related Gene* (herG) Inhibition		Carcinogenity		Oral acute toxicity level	
	Category	Score	Category	Score	Category	Score
3,4-dihydroxybenzoic acid	Weak Inhibitor	0.9818	Non-carcinogen	0.9154	III	0.5059
4-anilinoquinazoline (4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline)	Weak Inhibitor	0.9684	Non-carcinogen	0.9428	III	0.5446
Indometasin	Weak Inhibitor	0.9818	Non-carcinogen	0.8728	I	0.7774
Ar-Tumerone	Weak Inhibitor	0.8356	Carcinogen	0.5168	III	0.7877
Curzerene	Weak Inhibitor	0.8277	Non-carcinogen	0.8102	III	0.6049
Furanodiene	Weak Inhibitor	0.5304	Non-carcinogen	0.8288	III	0.7044
Curzerenone	Weak Inhibitor	0.8922	Non-carcinogen	0.8836	III	0.5212
5-epi-curzerenone	Weak Inhibitor	0.8922	Non-carcinogen	0.8836	III	0.5212
Furanodienone	Weak Inhibitor	0.6449	Non-carcinogen	0.9037	III	0.5738
Curmadione	Weak Inhibitor	0.8461	Non-carcinogen	0.6753	II	0.4856
Curcumenone	Weak Inhibitor	0.8266	Non-carcinogen	0.8551	III	0.5665
Isocurcumadione	Weak Inhibitor	0.6807	Non-carcinogen	0.7937	III	0.4857
Dehydrocurdione	Weak Inhibitor	0.6613	Non-carcinogen	0.8223	II	0.4719
Germacrone	Weak Inhibitor	0.8810	Non-carcinogen	0.8563	III	0.7023
13-hydroxygermacrone	Weak Inhibitor	0.6357	Non-carcinogen	0.8398	III	0.7966
Curcumenol	Weak Inhibitor	0.8603	Non-carcinogen	0.9526	III	0.6221
4-epicurcumenol	Weak Inhibitor	0.8603	Non-carcinogen	0.9526	III	0.6221
Isocurcumenol	Weak Inhibitor	0.8803	Non-carcinogen	0.9504	III	0.6501
Neocurcumenol	Weak Inhibitor	0.8803	Non-carcinogen	0.9566	III	0.6331
Isoprocurcumenol	Weak Inhibitor	0.9538	Non-carcinogen	0.8989	III	0.6836
Neoprocurcumenol	Weak Inhibitor	0.9538	Non-carcinogen	0.9120	III	0.7132
Curcumanolide A	Weak Inhibitor	0.8776	Non-carcinogen	0.8602	III	0.7308

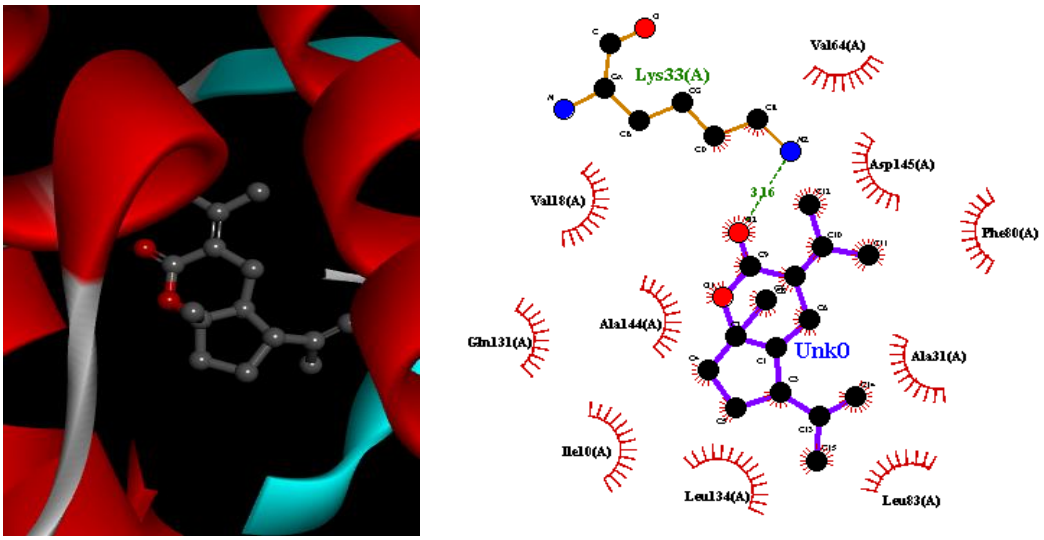
Ligand	Human *Ether-A-Go-Go Related Gene* (herG) Inhibition		Carcinogenity		Oral acute toxicity level	
	Category	Score	Category	Score	Category	Score
Curcumanolide B	Weak Inhibitor	0.8776	Non-carcinogen	0.8602	III	0.7308
Gajutsulactone A	Weak Inhibitor	0.8603	Non-carcinogen	0.9237	III	0.7159
Gajutsulactone B	Weak Inhibitor	0.8603	Non-carcinogen	0.9237	III	0.7159

3. Molecular Docking and Binding Site Similarity Ligand

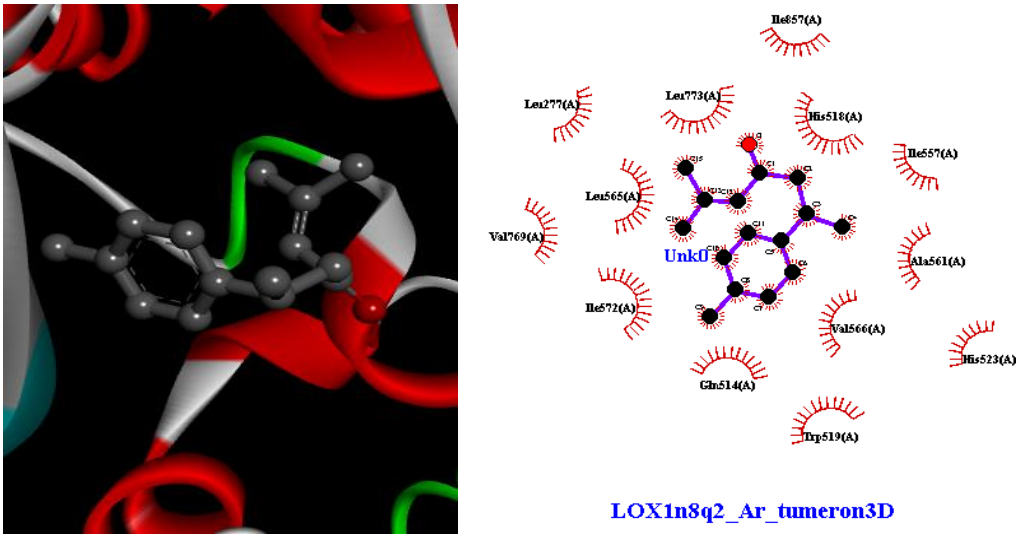
Ligand and receptor binding results will be visualized using the LigPlot+ program. Example binding models and Ligplot+ results for COX-2_Curcumenol, CDK2_gajutsulactoneA, and LOX_artumeron are presented in **Figure 2**. The percent value of Binding Site Similarity (%BSS) was used to calculate the similarity of the amino acid residues from the binding between the native ligand and the test ligand tethered to the receptor. The results showed that 4-anilinoquinazoline as a native ligand at the CDK-2 receptor had a total of 14 interactions, one hydrogen bonding interaction in the form of Lys33 (2.80). Ala144, Asp145, Val18, Leu134, Ile10, Gln131, Asp86, Gln85, Ala31, Phe82, Glu81, Leu83, Phe80 are thirteen hydrophobically bonded residues.



(a)



(b)



(c)

Figure 2. Binding modes and Ligplot+ results of best docked molecules with their target receptors (a) COX-2_Curcumenol (b) CDK2_gajutsulactoneA (c) LOX_artumeron

The results of the test ligand docking at the CDK2 receptor showed that all the test ligands had %BSS ≥ 50%, and three test ligands had the most residues in common, namely isoprocurcumenol, curzerene, and neoprocurcumenol compounds with a large %BSS of 78.57%. The interactions of these ligands include isoprocurcumenol, which lacks hydrogen bonding, and hydrophobic interactions with Asp145, Ala144, Phe80, Val18, Glu81, Phe82, Ala31, Leu83, Leu134, Gln131, Ile10. Curzerene exhibited interactions of twelve bonds without any hydrogen bond interactions and hydrogen interactions with Lys33, Val18, Asp145, Ile10, Ala31, Glu81, Leu83, Leu134, Phe82, Val64, Ala144, Phe80. Neoprocurcumenol has 13 hydrophobic interactions with residues Leu83, Leu134, Ala31, Ile10, Glu81, Val64, Asp86, Lys33, Asn132, Ala144, Val18, Asp145, Gln131, without any hydrogen bonding interactions.

Indomethacin as a natural ligand with the COX receptor has a total of 15 interactions, two hydrogen bond interactions in the form of Ser530 (3.27) and Arg120 (2.93) and hydrophobic interactions with Leu352, Tyr355, Val532, Ser353, Leu359, Val349, Leu531, Ala527, Met522, Gly526, Leu384, Trp387, Tyr385. The test ligand tethered to the COX-2 receptor had the largest %BSS value in the curcumenone and curmadione test compounds of 80.00%. Each of these compounds has 14 bonds of amino acid residues. The curmadione compound had two interactions with amino acids through hydrogen bonding to the amino acids Ser530 (2.85) and Tyr355 (2.98) and hydrophobic interactions with Leu352, Met522, Phe518, Trp387, Phe381, Gly526, Ala527, Arg120, Tyr355, Ser535, Val523, Val349. The curcumenone compound has a hydrophobic amino acid interaction with Phe518, Met522, Tyr385, Ser530, Gly526, Trp387, Leu352, Val523, Phe381, Ser353, Tyr355, Ala527, Arg120, Val349. Other test ligands, as many as 14 ligands, have similarities above 50%.

Protocatechuic acid is a natural ligand that is tethered to the LOX receptor and has no hydrogen bonding interaction but hydrophobic interactions with Ile557, Ala561, Ile857, Leu773, Leu565, His523, Trp519, His518, Val566, Gln514, Ile572. All the test compounds tethered to LOX had %BSS values above 50%. Most of the tested ligands had a percentage value of 90.90%-100% except for curcumenone, curmadione, curzerene, and gajutsulactone A compounds.

Molecular docking is a useful bioinformatics technique used to show drug targets, target structures, and active sites in determining new drug candidates (Pujiastuti & Sanjaya, 2017). Molecular docking results were determined from the value of binding energy and the inhibition constant of the ligand to the protein, as well as the interactions that occurred between the ligand and the target protein. The interaction of ligands with proteins involves amino acid residues and the type of binding, both hydrogen bonds and hydrophobic bonds (Pujiastuti & Sanjaya, 2017).

The bond that is important in biological activity is hydrogen bonding because hydrogen bonds can stabilize the bond between the ligand and the

receptor (Rachmania, Hariyanti, et al., 2018). Hydrogen bonding is a bond that involves the interaction of a hydrogen atom covalently bonded with electronegative atoms such as flour (F), nitrogen (N), and oxygen (O) (Głowacki et al., 2013). Other interactions that can also increase conformational stability are electrostatic interactions and Van der Waals interactions (Rachmania, Hariyanti, et al., 2018). Hydrophobic interactions are interactions that avoid the liquid environment and tend to group in the inner globular structure of the protein so that the interaction of nonpolar residues with water can be minimized (Sumaryada et al., 2021).

The results of docking visualization are the appearance of hydrogen bonds and hydrophobic bonds of amino acid residues that occur between the ligand and the receptor. The percentage value of Binding Site Similarity (%BSS) will also be calculated by comparing the number of similarities of amino acid residues between natural and test ligands (Tu & Shi, 2013). The percentage of BSS shows the binding mechanism of the test ligand to the receptor. The test ligand will have the same binding mechanism as the natural ligand, a competitive inhibitor if it has a %BSS above 50%, whereas if the test ligand is below 50% then there is a possibility that another mechanism will occur in its binding with the natural ligand (Salahudeen & Nishtala, 2017).

CDK is a protein kinase known to be inactive and can be activated by phosphorylation of serine or threonine and binding to cyclin proteins. CDK2 activity is ATP-dependent, and the presence of an ATP-binding pocket makes it a target site for CDK2 inhibition because it is deposited in all types of kinases. CDK2 protein has two lobes, namely a small lobe of the amino-terminal domain, which is dominated by -sheets, and a large lobe of the carboxy-terminal domain, which consists mostly of helices. Between the two lobes, there is a gap that is hydrophobic and there is an ATP binding site as the active site (Arba et al., 2017). Compounds or small molecules that can occupy the ATP-binding pocket will be able to competitively inhibit CDK2 activity. The ATP site region involves several amino acid residues, including Lys33, Glu51, and Asp145.

Shewchuk et al. (2000) explained that 4-anilinoquinazoline (DTQ) as a CDK2 inhibitor was found to inhibit CDK2 at the ATP site where the quinazoline ring interacts with the -strand of CDK2 (residues 81-83) linking the two protein domains. This study demonstrated that all test ligands could interact with the CDK2 ATP binding site. This can be seen from the interaction formed with one or more amino acid residues in the ATP region. The interaction of DTQ with CDK2 also showed that Leu83 and Asp145 residues were key residues in CDK2 inhibition. 4-anilinoquinazoline (DTQ), as a natural ligand at the CDK2 receptor, has a total of 14 interactions; one hydrogen bond interaction and 13 hydrophobic bonds. All the tested ligands in this study produced a %BSS value of more than 50%, so it can be stated that all ligands have a mechanism similar to natural ligands (competitive inhibitors). The three compounds that had the highest %BSS were isoprocurcumenol, curzerene, and neoprocurcumenol.

Indomethacin is a non-selective inhibitor compound that binds to the cyclooxygenase active site. The presence of indomethacin at the cyclooxygenase active site inhibits arachidonic acid from binding to cyclooxygenase, so the formation of prostaglandins is also inhibited. Cyclooxygenase has a structure consisting of three different domains, namely the N-terminal epidermal growth factor (EGF) domain, the membrane-binding motif domain, and the catalytic C-terminal domain, which has the active sites of cyclooxygenase and peroxidase. Indomethacin on COX-2 is known to penetrate deep into the hydrophobic channel that is part of the cyclooxygenase active site. The opening to the active site is limited by the constriction formed by Arg120, Tyr-355, and Glu-524. The COX-2 and indomethacin complexes show inhibitory binding at the cyclooxygenase active site above the constriction (Xu et al., 2019).

The results of the study (Appendix 4) on the COX receptor showed that the natural ligand indomethacin had a total of 15 interactions: two hydrogen bonds and 13 hydrophobic residues. According to Das & Samanta (2015), indomethacin inhibits COX-2 through key interactions with amino acid residues Arg120 and Tyr355. Most of the test compounds used had similar residue interactions with natural ligands except isocurcumenol, neocurcumenol, furanodienone, and neoprocurcumenol which did not have any residue in common with natural ligands. The test compounds that have a mechanism as competitive inhibitors, such as natural ligands, are as many as 14 compounds with %BSS above 50%. The two compounds with the most similarities were found in curmadion and curcumenone with 80% BSS.

LOX is an enzyme with a single iron cofactor. LOX plays a role in the metabolism of unsaturated

fatty acids. Protocatechuic acid is a LOX inhibitor that prevents the formation of free radicals, which are by-products of leukotrienes. The natural protocatechuic acid (3,4-dihydroxybenzoic acid, DHB) ligand bound to the LOX receptor has a total of 11 interactions. Protocatechuic acid has no hydrogen bonding interactions and has 11 hydrophobic bonded residues. The test ligand compounds tethered to the LOX receptor have bound interactions like natural ligands, so they can be said to have competitive inhibitory mechanisms. Monika et al. (2013) stated that protocatechuic acid interacts with lipoxygenase enzymes. The interaction occurs through binding to the amino acid residues His523, Gln514, His518, and Leu565.

Several test ligands showed interactions with the same amino acid residues but with different bonds. Amino acid residues that interact hydrogenic on one ligand can bind hydrophobic to the other ligand. This can happen because the hydrogen bonds in each test ligand have different strengths and can be seen based on the bond distance. Weak hydrogen bonds will cause these bonds to break easily and turn into other bonds, such as hydrophobic bonds. The good hydrogen bonding range is at 2.5-3.5Å (Syahputra et al., 2014).

4. Ligand Affinity Energy

The bond energy of the ligand-receptor complex is an important parameter in the analysis of molecular anchoring. Gibbs free energy (ΔG) is the bond energy referred to in this study. The results of molecular good belay can be observed by looking at the value of ΔG and inhibiting constants (Ki). Experimentally, the Gibbs free energy is directly related to the inhibition constant by the equation:

$$\Delta G = -RT \ln Ki$$

Where R is the ideal gas constant of 1,987 cal/mol.k and T is the absolute gas temperature with a value of 298.15 K. The constant of inhibition is an indication of the inhibitory strength of the substrate and the inhibitor in binding certain proteins.

This study showed that all ligands tethered to the COX-2 enzyme had negative ΔG values. Indomethacin, as a native ligand, has the most negative Gibbs free energy value of -10.6 kcal/mol, and the lowest Ki value is 0.0167 μM. The test ligand that has the most negative Gibbs free energy value is Furanodiene with a value of -9.2 kcal/mol and a Ki value of 0.1774 μM followed by Curcumanolide A and Curcumenol with the same Gibbs free energy value of -9.1 kcal/mol and a Ki value of 0.2100 μM. The test ligands with the highest Gibbs free energy and Ki values were found in dehydrocurdione and 13-hydroxygermacrone, namely -7.1 kcal/mol and 6.1643 μM.

The molecular binding to the CDK2 receptor resulted in a negative affinity energy value for all test ligands. Gajutsulactone A is the test ligand that has the most negative Gibbs free energy value of -9.0 kcal/mol and the lowest Ki value of 0.2487 μM. The ligands with the highest Gibbs free energy and Ki values were found in neoprocurcumenol, -6.7 kcal/mol and 12,1175 μM. The native ligand tethered to the CDK2 receptor has a Gibbs free energy value of -8.8 kcal/mol and a Ki value of 0.3487 μM comparable to the Furanodienone test ligand. The test ligand compound gajutsulactone A has a lower free energy value than the native ligand compound. Other test ligands that have lower Gibbs free energy and Ki values than natural ligands are Curcumenol and Furanodiene compounds, which are -8.9 kcal/mol and 0.2945 μM. All test ligands can also be classified as strong inhibitors because they have a Ki value below 100 μM.

The most negative Gibbs free energy value at the lipoxygenase receptor was -5.5 kcal/mol and the lowest Ki value was 92.04 μM through natural ligand binding. The test ligand with the Gibbs free energy value and the lowest Ki value of -4.5 kcal/mol, and the Ki value of 498.67 μM was ar-tumerone. Subsequent test ligands with the lowest G values were 4-epicurcumenol and curcumenone -2.8 kcal/mol and -2.4 kcal/mol, respectively. The test ligands that had the highest Gibbs free energy and Ki values were found in germacron, 4.0 kcal/mol and 861539098.41 μM. Test ligands that had negative G values were only found in 9 test ligands.

Gibbs free energy is the thermodynamic potential that is minimized when the system reaches equilibrium at constant pressure and temperature (Adelina, 2018). A strong bond will be characterized by a low ΔG value. The low ΔG value also indicates that the conformation formed is stable and takes place spontaneously, and the activity of the ligand-receptor complex is also getting better. In addition, ΔG describes the affinity energy that occurs in the ligand and receptor complex. Binding affinity is a measure of a drug's ability to bind to a receptor (Ruswanto et al., 2015). This ability is the result of the sum of the total intermolecular energy, total internal energy, and free torsional energy in the absence of system energy (Anitha et al., 2013).

The value of ΔG will be proportional to the value of Ki (inhibition constant). The smaller the Ki value of a ligand, the reaction equilibrium will tend towards the formation of a complex between the compound (ligand) and the receptor or the greater the binding affinity to inhibit the activity of an enzyme (Pujiastuti & Sanjaya, 2017). Research by Zheng & Polli (2010) states that an inhibitor with a Ki value below 100 μM can be said to be a strong inhibitor, whereas if a Ki value is above 100 μM it can be said to be a less powerful inhibitor.

The results of binding to COX-2 protein showed that indomethacin, as a native ligand, had the best potential as an inhibitor, as evidenced by the lowest ΔG value and Ki value compared to all test ligands. Test ligands that have the best potential after native ligands are furanodiene compounds (ΔG -9.2 kcal/mol), curcumanolide A (ΔG -9.1 kcal/mol), and curcumenol (ΔG -9.1 kcal/mol). The test compound curcumenol had the largest %BSS value of 73.33% compared to the two compounds furanodiene and curcumenolide A. The compound curcumenol had a Ki value of 0.2100 μM, so it was included in the category of strong inhibitor. This shows that curcumenol is the most potential test ligand because it has a similar mechanism to native ligands and fulfills all Lipinski rules and toxicity tests. Antiinflammation has been identified in curcumenol from various medicinal plants (Makabe et al., 2006; Pintatum et al., 2020; D. X. Sun et al., 2010). Several mechanisms have been reported as anti-inflammatory in curcumenol compounds including downregulation of Akt and p38 MAPKs signaling and inhibited activation of Akt-dependent NF-κB (Lo et al., 2015), inhibited NF-κB and MAPK pathways (Yang et al., 2021).

The binding of the ligand to the CDK2 receptor showed that the gajutsulactone A test ligand had the lowest ΔG value and the lowest Ki value among all the ligands used, even better than the native ligand. The ΔG value of gajutsulactone A was -9.0 kcal/mol, while 4-anilinoquinazoline (a native ligand) had a ΔG value of -8.8 kcal/mol. Besides gajutsulactone A, two test ligands have a lower ΔG value than native ligands, namely curcumenol and furanodiene with a value of -8.9 kcal/mol. The gajutsulactone A compound has the potential to be better because it has the largest % value compared to curcumenol and furanodiene and is a strong inhibitor because it has a Ki value of 0.2487 μM. Research related to the anticancer mechanism of gajutsulactone A has never been reported, so the potential for further development is needed through preclinical and clinical studies. However, the anticancer mechanism of curcumenol and furanodiene has been reported. Zhang *et al.* reported that curcumenol is efficacious as an anticancer by inducing ferroptosis through a ceRNA network (Zhang et al., 2022). Meanwhile, furanodiene is productive as an anticancer by suppressing the occurrence of metastases in cancer cells (Zhong et al., 2014).

The results of the binding to the LOX receptor showed that protocatechuic acid, as a native ligand, had the lowest G value and Ki values of -5.5 kcal/mol and 92.04 M. The test compound that had the best G value after the native ligand was ar-tumerone of -4.5 kcal/mol. and with a very good %BSS value of 100%. The low G value of the compound ar-tumerone does not make the compound potential as a LOX inhibitor because it is a carcinogen based on toxicity tests.

The affinity energies of several test ligands have low values, but the number of similarity residues is small or vice versa, high affinity energies but the number of similarity residues is large (**Table 4**). This could be due to differences in the type and number of residue interactions of each ligand with the receptor. Hydrogen bonding can stabilize and increase the bond strength between the ligand and the receptor (Rachmania, Hariyanti, et al., 2018). The interactions that occur between ligands and receptors are not only hydrogen bonds but also hydrophobic bonds. Another binding can occur due to flexible ligands interacting with receptors, including non-covalent interactions or non-binding interactions that can increase the affinity of the ligand to the receptor. Electrostatic interactions and van der Waals bonds are the most common interactions (Syahputra et al., 2014).

Table 4. Energy afinity and inhibition constants

Receptors	Ligand	ΔG (kcal/mol)	Inhibition constant (μM)	Binding hydrogen	Binding hidrofobic
Cyclooxygenase	Indometachin	-10.6	0.0167	2	13
	4-epicurcumenol	-8.1	1.1378	2	7
	5-epicurzerenone	-8.2	0.9609	0	12
	13-hydroxygermacrone	-7.1	6.1643	0	13

Receptors	Ligand	ΔG (kcal/mol)	Inhibition constant (μM)	Binding hydrogen	Binding hidrofobic
	Ar-tumerone	-8.6	0.4888	1	10
	Curcumanolide A	-9.1	0.2100	0	11
	Curcumanolide B	-7.9	1.5953	0	11
	Curcumenol	-9.1	0.2100	0	13
	Curcumenone	-8.3	0.8115	0	14
	Curmadione	-8.6	0.4888	2	12
	Curzerene	-7.3	4.3967	0	9
	Curzerenone	-7.2	5.2060	1	8
	Dehydrocurdione	-7.1	6.1643	1	10
	Furanodiene	-9.2	0.1774	1	10
	Furanodienone	-7.5	3.1359	0	8
	Gajutsulactone A	-8.5	0.5788	1	11
	Gajutsulactone B	-8.1	1.1378	0	11
	Gemacrone	-7.3	4.3967	2	5
	Isocurcumadione	-7.3	4.3967	1	7
	Isocurcumenol	-7.8	1.8889	3	6
	Isoprocurcumenol	-9.0	0.2487	1	11
	Neocurcumenol	-7.7	2.2366	3	7
	Neoprocurcumenol	-7.5	3.1359	1	8
Cyclin dependent	4-anilinoquinazoline	-8.8	0.3487	1	13
Kinase -2 (CDK2)	4-epi-curcumenol	-8.7	0.4128	1	8
	5-epi-curzerenone	-7.9	1.5953	1	10
	13-hydroxygermacrone	-6.9	8.6427	1	9
	Ar-tumerone	-7.6	2.6484	0	10
	Curcumanolide A	-8.5	0.5788	0	12
	Curcumanolide B	-8.2	0.9609	0	12
	Curcumenol	-8.9	0.2945	1	9
	Curcumenone	-7.6	2.6484	1	11
	Curmadione	-7.2	5.2060	0	9
	Curzerene	-7.7	2.2366	0	12
	Curzerenone	-7.9	1.5953	1	10
	Dehydrocurdione	-7.8	1.8889	0	10
	Furanodiene	-8.9	0.2945	0	10
	Furanodienone	-8.8	0.3487	1	8
	Gajutsulactone A	-9.0	0.2487	1	10
	Gajutsulactone B	-8.0	1.3473	1	10
	Germacrone	-7.1	6.1643	0	11
	Isocurcumadione	-7.4	3.7131	3	10
	Isocurcumenol	-8.4	0.6854	2	10
	Isoprocurcumenol	-8.5	0.5788	0	11
	Neocurcumenol	-8.0	1.3473	2	9
	Neoprocurcumenol	-6.7	12.1175	0	13
Lipoxygenase	Protocatechuic acid (3,4-dihydroxybenzoic acid, DHB)	-5.5	92.04	0	11
	4-epi-curcumenol	-2.8	8816.73	3	10
	5-epi-curzerenone	-0.4	508712.61	0	11
	13-hydroxygermacrone	1.7	17680391.25	2	10
	Ar-tumerone	-4.5	498.67	0	13
	Curcumanolide A	-0.1	844535.92	0	13
	Curcumanolide B	-0.4	508712.61	0	15

Receptors	Ligand	ΔG (kcal/mol)	Inhibition constant (μM)	Binding hydrogen	Binding hidrofobic
	Curcumenol	0.2	1402050.80	0	12
	Curcumenone	-2.4	17331.45	0	14
	Curmadione	-1.4	93897.44	0	15
	Curzerene	-0.1	844535.92	0	12
	Curzerenone	-0.3	602357.57	0	11
	Dehydrocurdione	2.7	95787892.74	0	14
	Furanodiene	2.3	48728508.67	0	13
	Furanodienone	2.0	29351986.28	0	13
	Gajutsulactone A	3.2	222956425.55	1	10
	Gajutsulactone B	2.4	57698562.71	0	14
	Gemacrone	4.0	861539098.41	0	12
	Isocurcumadione	-1.3	111182.29	0	15
	Isocurcumenol	-0.9	218556.20	2	11
	Isoprocurcumenol	1.2	7595957.00	1	11
	Neocurcumenol	0.7	3263421.15	0	15
	Neoprocurcumenol	2.4	57698562.71	1	12

CONCLUSION

In conclusion, all the active compounds used in this study met the Lipinski test parameters and had low toxicity, so they were potential candidates for oral drugs. Several active compounds have the potential as competitive inhibitors on the activity of CDK2 protein, COX-2 enzymes, and LOX enzymes. Gajutsulactone A, curcumenol, and furanodiene have better affinity energy and Ki values than natural ligands in inhibiting CDK2 protein activity. Furanodiene, curcumonolide A, and curcumenol compounds have the potential to inhibit COX-2 enzyme activity but are not better than indomethacin as a natural ligand. Furthermore, the tethering of the lipoxygenase enzyme showed that no compound had the potential to inhibit LOX, so it could not be used as an antioxidant compound. The active compounds identified in this study as having potential in silico must be further investigated in preclinical and clinical investigations. Further evidence of the possibility of active compounds can also be carried out on other receptors in their activity to inhibit certain types of cancer.

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