JPPIPA 9(8) (2023)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

In Silico Study of Derivative Compounds of Galangal Plants as Anti-Inflammatory

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Received: January 31, 2023
Revised: May 12, 2023
Accepted: August 25, 2023
Published: August 31, 2023

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DOI: 10.29303/jppipa.v9i8.3042

Abstract: A Inflammation is the basis of pathogenesis of several diseases, both degenerative and non-degenerative diseases. Galangal plants which are commonly found in Indonesia are commonly used as traditional medicines for several diseases and also have secondary metabolite compounds that are useful as anti-inflammatory. In this study, an in silico approach in the form of molecular docking has been applied to 5 compounds derived from the galangal plant to important inflammatory molecular targets such as the cyclooxygenase-2 (COX-2) receptor. Analysis of the biological activity of compounds derived from the galangal plant using the WAY2DRUG PASS prediction server. Prediction results of physicochemical properties of compounds derived from galangal plant using the SWISS-ADME server. This study aims to predict the ability of 5 compounds derived from the galangal plant to inhibit the COX-2 enzyme. Detailed information has been obtained using a molecular docking approach. Docking simulations for 5 compounds derived from the galangal plant have been carried out through the Autodock 4.2 application which is embedded in the MGL Tools 1.5.6 application. The molecular interactions of compounds derived from galangal against COX-2 receptors were visualized using Discovery Studio (Biova) software. Based on the results of the research that has been carried out, it can be concluded that the test compound Galanganal has the best affinity when compared to the compounds Galanganol A, Galanganol B, Galanganol C and Galangin. This can be seen from the bond free energy value of -8.98 kcal/mol and the inhibition constant of 261.59 nM. These results indicate that the Galanganal test compound has potential as an anti-inflammatory agent. However, further research is needed to study more compounds derived from the galangal plant to isolate the best conformation.

Keywords: Anti-inflammatory; galangal; cyclooxygenase-2; molecular docking; in silico study.

Introduction

Inflammation is a biological response by the immune system to various harmful stimuli such as pathogens, damaged cells, toxic compounds and radiation. The inflammatory process causes tissue damage which is characterized by redness, swelling, heat, pain, and loss of tissue function (Chen et al., 2018). Tissue damage is caused by stimuli that cause mast cell rupture and subsequently Nitrogen Oxide (NO), Prostaglandin (PG), Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α) (Utami et al., 2020) .

Non-steroidal anti-inflammatory drugs (NSAIDs) which are now widely used to treat inflammation are usually such as ibuprofen, aspirin, diclofenac and colecoxib. This drug functions to suppress the inflammatory process by inhibiting the activity of pro-inflammatory enzymes that synthesize prostaglandins.

How to Cite:
Aryasa, I. W. T., & Sugianta, I. K. A. (2023). In Silico Study of Derivative Compounds of Galangal Plants as Anti-Inflammatory. *Jurnal Penelitian Pendidikan IPA*, *9*(8), 6531–6539. https://doi.org/10.29303/jppipa.v9i8.3042

However, long-term use of NSAIDs can cause side effects such as stomach irritation, bleeding, kidney disease, bronchial disease, cardiovascular system, and perforation (Utami et al., 2020). Traditional medicine using herbal compounds is now an alternative for society. Medicinal plants contain phytochemical compounds such as steroids, glycosides, phenols, flavonoids, alkaloids, polysaccharides, terpenoids and cannabinoids, which have the same strength as the molecular mechanisms of synthetic drugs (Yatoo et al., 2018). The discovery of herbal medicines is expected to provide alternative treatments with fewer side effects. One of the plants that is usually used as raw material for herbal medicine is galangal.

Alpinia galangal (Zingiberaceae), often called galangal, is a herbal plant that is widespread in Asia. Many developing countries, including Indonesia, cultivate this plant. This plant has many benefits, from using it as a cooking spice to creating a distinctive aroma when cooked. Galangal is generally used in traditional medicine in many countries in the world to treat diabetes mellitus, bronchitis, heart disease, stomach ache, colic, diarrhea, emesis, indigestion, stomach ache, vomiting, respiratory disease, rheumatism, and inflammatory disorders (Basri et al., 2017; Das et al., 2020; Khairullah et al., 2020). Galangal is also effective in treating fever, menstrual irregularities, and increasing male fertility (Abubakar et al., 2018). Galangal rhizomes are used in several formulations to prevent cancer and tumors, and are also used to treat other diseases such as rheumatism, inflammation, diabetes, and neuropathy (Mundugaru et al, 2018; Basri et al., 2017). The compounds in galangal are compounds that can be used to treat several diseases in society (Khairullah et al., 2020). Galangal rhizome contains various phenolic compounds, polyphenols, flavonoids, saponins, phenylpropanoids, glycosides, diarylheptanoids, sesquiterpenoids, and diterpenoids (Das et al., 2020; Zhou et al., 2018).

The main active compounds in galangal rhizomes are galangal acetate, kaempferol and 1.8-cineole (Basri et al., 2017; Upadhye et al., 2018). Although the use of galangal rhizomes has been widely used and research on other parts of the galangal plant, namely the galangal flowers, can also provide additional benefits such as having antimicrobial and antioxidant properties, although the chemical composition of the parts of the galangal rhizome is different (Tang et al. , 2018). Therefore, this study aims to describe the use of galangal rhizomes in terms of their phytochemical properties, botanical content, and future prospects for the development of effective therapeutic compounds.

Flavonoid compounds can inhibit enzymes involved in prostaglandin synthesis. Triterpenoids as anti-inflammatory agents can be reduced by reducing cells that express inducible nitrate synthase (iNOS) such as lupeol or by reducing iNOS expression and inhibiting nitric oxide production (Owolabi et al., 2018). Several studies have shown that several active compounds from ficus religiosa have shown anti-inflammatory potential using a molecular docking approach (Utami et al., 2020).

Recent studies using bioinformatics-based approaches have the potential to reveal complex relationships between drugs, targets, and the diseases they target (He et al., 2021). The bioinformatics approach in drug development can now be done computer-based, which is called in silico. This method aims to target through molecular docking and identify the relationship between phytochemicals in plants and the therapeutic effects of medicinal plants (Gul et al., 2022). Therefore, this research was conducted to determine the levels of bioactive compounds in galangal (Alpinia galanga) which may have anti-inflammatory effects in silico.

Method

The macromolecule (receptor) used in this study is the Cyclooxygenase-II complex chain (PDB ID: 4PH9) taken from the site www.rcsb.org. Macromolecules were then prepared using the Biovia Discovery Studio Visualizer. The ligands used were selected from a list of compounds found in a previous study conducted by Prasetiawati et al. (2022), and also taken from USDA Dr. Duke Phytochemical (https://phytochem.nal.usda.gov/) then searched for canonical or isomeric SMILE (simplified molecular-input line-entry system) in the PubChem database (https:/ /pubchem.ncbi.nlm.nih.gov/). The first step taken was validation of docking between macromolecules and natural ligands found in macromolecules in Autodock software (Shah et al., 2019; Hassan et al., 2019). The next step is to carry out the docking process using the previously validated parameters between the receptor and galangal derivative compounds. The results of the docking process were then analyzed using Autodock Tools software and interactions were visualized using Biovia Discovery Studio Visualizer (Haque et al., 2022; Levita et al., 2017). The next step is that all galangal derivative compounds along with comparison compounds in the form of anti-inflammatory drugs are generally subjected to pre-ADME tests and toxicity tests. Furthermore, to observe its biological activity using the Server Prediction of Activity Spectra for Substances (PASS) prediction. The final stage is to carry out an analysis to determine the best compound for anti-inflammation.

Results and Discussion

The prediction of each galangal-derived compound used the Prediction Server of Activity Spectra for Substances (PASS) prediction and observed its biological activity. Prediction by the server is carried out to identify the biological activity associated with the biological activity being investigated, along with its probability spectrum. Table 1 shows the results of the bioactivity tests performed on celecoxib, Galanganal, Galanganol A, Galanganol B, Galanganol C and Galangin. The type of bioactivity that is relevant to the molecular analysis of this compound is anti-inflammatory activity. All compounds in this study showed a probability of activity as an anti-inflammatory agent, as indicated by the probability of activity (Pa) being greater than the probability of non-activity (Pi). The galangal derivatives analyzed showed Pa values ranging from 0.382 to 0.689. It can be seen that Galangin has the highest Pa value, followed by Celecoxib, Galanganol C, Galanganal, Galanganol A and Galanganol B.

Table 1. Analysis of the biological activity of galangal derivative compounds and medicinal compounds using the server using the WAY2DRUG PASS prediction

Compound Name	Bioactive Activity	Active Probability (Pa)	Inactive Probability (Pi)
Celecoxib	Anti-inflammatory	0.663	0.021
Galanganal	Anti-inflammatory	0.415	0.088
Galanganol A	Anti-inflammatory	0.382	0.105
Galanganol B	Anti-inflammatory	0.382	0.105
Galanganol C	Anti-inflammatory	0.418	0.087
Galangin	Anti-inflammatory	0.689	0.017

The physicochemical properties of galangal derivatives based on Lipinski's rule 5 show that all compounds fulfill Lipinski's rule 5. Therefore, the six test compounds, including celecoxib as a comparison compound, are expected to be well absorbed for oral use. The physicochemical properties of galangal derivative compounds based on Lipinski's rule 5 show that all compounds fulfill Lipinski's rule 5. Therefore, the five test compounds, including the reference compound celecoxib, can be absorbed well and are expected to be used as oral formulations.

Table 2. ADME prediction results for galangal derivatives and medicinal compounds using the SWISS-ADME server

Compound Name	Parameter				
	Molecular weight < 500 g/mol	Proton donors < 5	Proton acceptor < 10	Log P < 5	Refractory Molar 40-130
Celecoxib	381.37	1	7	3.40	89.96
Galanganal	280.32	2	3	3.22	84.81
Galanganol A	300.35	4	4	2.48	86.62
Galanganol B	300.35	4	4	2.48	86.62
Galanganol C	432.51	4	5	3.93	125.36
Galangin	270.24	3	5	1.99	73.99

According to Lipinski's rule 5, in drug development and discovery of drug candidates for oral use, five conditions must be met, known as the "rule of five", namely a drug candidate that has a molecular weight of no more than 500, hydrogen bond donors of no more than 5 , hydrogen bond acceptors no more than 10, log P no more than 5, and molar refractivity between 40-130 (Sahu et al., 2022; Ruswanto et al., 2022; Shaikh et al., 2022).

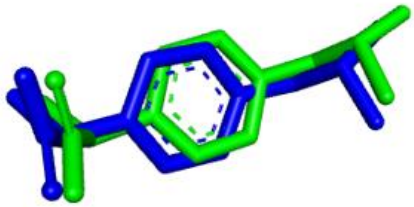


Figure 1. Visualization of the overlap of the natural ligand 4PH9 (Green) with the redocked ligand (Blue)

This research was carried out to look at the three-dimensional structure of Cyclooxygenase-II which was downloaded from the PDB site with the code 4PH9. Before the process of docking macromolecular molecules (receptors) with the test compound, a validation process is first carried out by separating the natural ligand from the receptor using Discovery Studio Visualizer and then docking it again using AutoDock Tools software to obtain the appropriate RMSD value.

Table 3. Parameters Validation of the docking process using MGL Tools software version 1.5.6 which is equipped with Autodock Tools version 4.2

Validation Parameters	Validation Results
Grid Box Size	X: 40; Y: 40; Z: 40
Spacing	0.375
Grid Center	X: 13.578; Y: 23.024; Z: 25.205
RMSD	0.749 Å
Bond Free Energy	-8.33 kcal/mol

The RMSD value obtained from the receptor with code 4PH9 after the results of redocking is 0.749 Å, this value is good because the docking method is said to be valid if it has RMSD (<) 2Å. RMSD (Root Mean Square Deviation) is a parameter used to evaluate the similarity of two structures. This similarity is measured based on the difference in distance between similar atoms. A smaller RMSD value indicates that the position of the redocking ligand is closer to the position of the crystallographic ligand (Parks et al., 2020; Su et al., 2018; Yang et al., 2022; Sagitasa et al., 2021; Aarthy & Singh, 2018; Aziz et al., 2022).

The ligand used in this research is a compound derived from the galangal plant (*Alpinia galanga (L.) Willd.*). The three-dimensional structure of the ligand was downloaded from the Pubchem site. Molecular docking in the test compound is carried out in the same way as the validation process by using grid box size determination and parameters, then autogrid calculations will produce mapping parameters. Before docking, the parameters needed to dock are first prepared, namely grid parameters (Grid Parameter File) and docking parameters (Docking Parameter File). Grid settings include determining coordinates and volume.

The coordinates used in the molecular docking are the center coordinates (X, Y, Z) respectively, namely 13.578; 23,024; and 25,205. The volume of the mooring grid used in this study is 40 x 40 x 40 Å with a spacing of 0.375. In the mooring parameters, changes were made to the Number of GA Runs to 100. For each Number of GA Runs the Maximum Number of Evaluation Medium is used, namely 2,500,000.

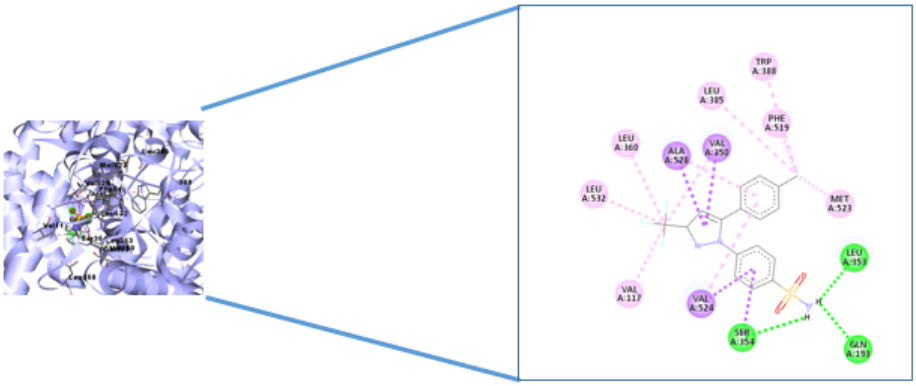
In Table 2 it can be seen that the parameters observed to determine the affinity of the test compound for the receptor are the ΔG (binding free energy) and (KI) inhibition constant values. The affinity of the test compound for the receptor is determined by the binding free energy and inhibition constant values. The more negative the binding energy value and the smaller the inhibition constant value, the higher the ligand affinity (Terefe & Ghosh, 2022). Apart from that, the results of the visualization of the tethering and the interactions that occurred were also observed. Based on the results of the docking between the test compound and the target receptor, the conformation of the test compound with the lowest energy is obtained. The binding free energy is a measure of the drug's ability to bind to the receptor as it decreases (Sukmawaty et al., 2022).

Table 4. Affinity calculation of medicinal compounds and compounds derived from galangal uses MGL Tools version 1.5.6 software equipped with Autodock Tools version 4.2

Compound name	Bond Free Energy	Inhibition constant
Celecoxib	-9.78 kcal/mol	68.13 nM
Galanganal	-8.98 kcal/mol	261.59 nM
Galanganol A	-8.37 kcal/mol	736.50 nM
Galanganol B	-8.24 kcal/mol	916.44 nM
Galanganol C	-7.80 kcal/mol	1.93 µM
Galangin	-8.25 kcal/mol	893.31 nM

The smaller the bond free energy value, the higher the affinity between the receptor and the test compound. Vice versa, if the value of the bond free energy is greater, the affinity between the test compound and the receptor is lower (Sukmawaty et al., 2022). The lower the value of the bond free energy released during the interaction of the compound and the receptor, the stronger the bond between the compound and the receptor complex due to the stability and strength of the non-covalent bond interactions between the compound and the receptor, so that it will easily enter the cell and disrupt DNA replication or other processes metabolism, resulting in cell death (Adeboye et al., 2022). In table 2 it can be seen that the value of the lowest bond free energy (ΔG) and inhibition constant (KI) is the Celecoxib comparator compound and the test compound that is close to this value is the Galanganal compound with a bond free value of -8.98 kcal/mol, followed by by other test compounds sequentially namely Galanganol A compound with a bond-free value of -8.37 kcal/mol, Galangin compound with a bond-free value of -8.25 kcal/mol, Galanganol B compound with a bond-free value of -8, 24 kcal/mol and the Galanganol C compound with a bond-free value of -7.80 kcal/mol. So, of the 5 test compounds that have the potential to be anti-inflammatory, it is the Galanganal compound.

Analysis of test compounds that have been tethered to target receptors can be seen through visualization by looking at amino acid residues and the number of hydrogen bonds formed as a result of interactions with each test compound and target receptor. This can be seen in Figure 1.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LEU A:385
PHE A:519
LEU A:360
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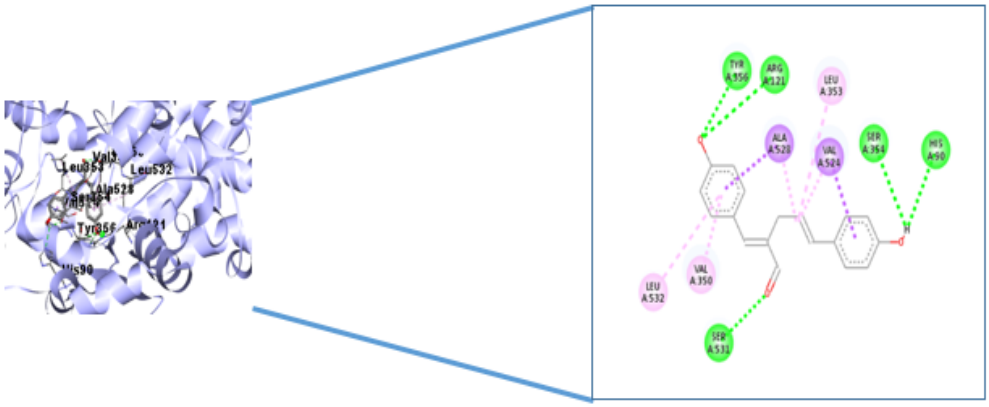
VAL A:350
LEU A:532
MET A:523
LEU A:353
VAL A:117
VAL A:524
SER A:354
GLN A:193

a


TYR A:356
ARG A:121
LEU A:353
ALA A:528
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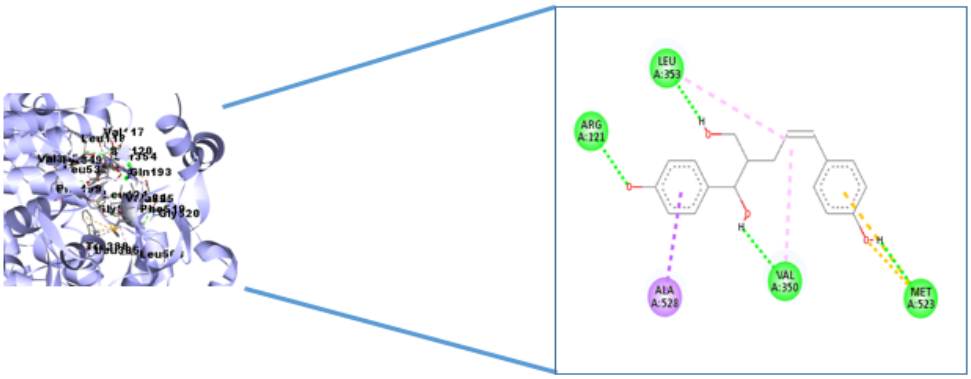
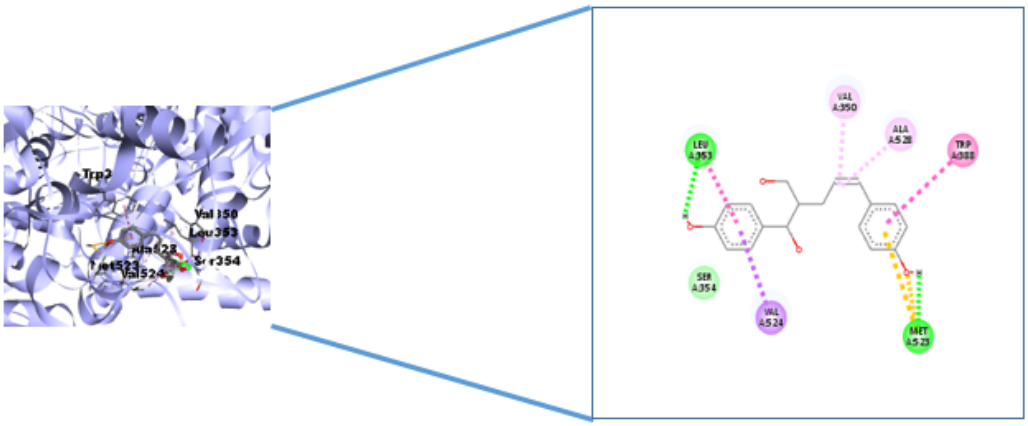
SER A:354
HIS A:90
VAL A:350
LEU A:532
SER A:531

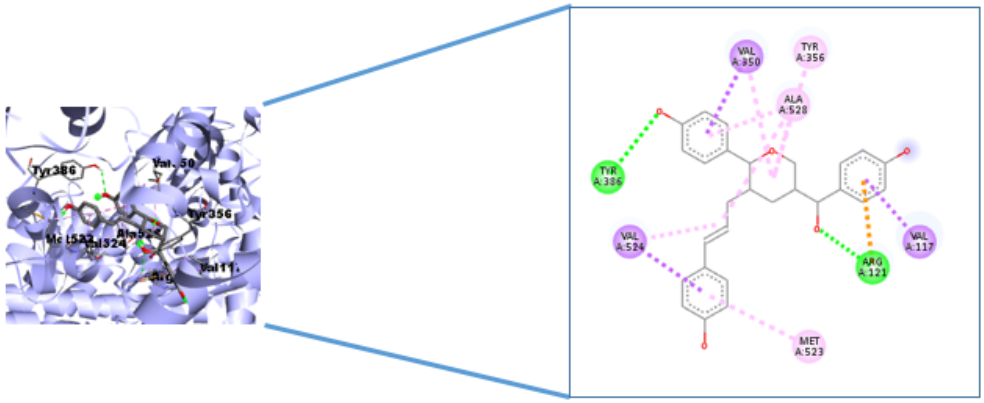
b


LEU A:353
ARG A:121
ALA A:528
VAL A:350
MET A:523

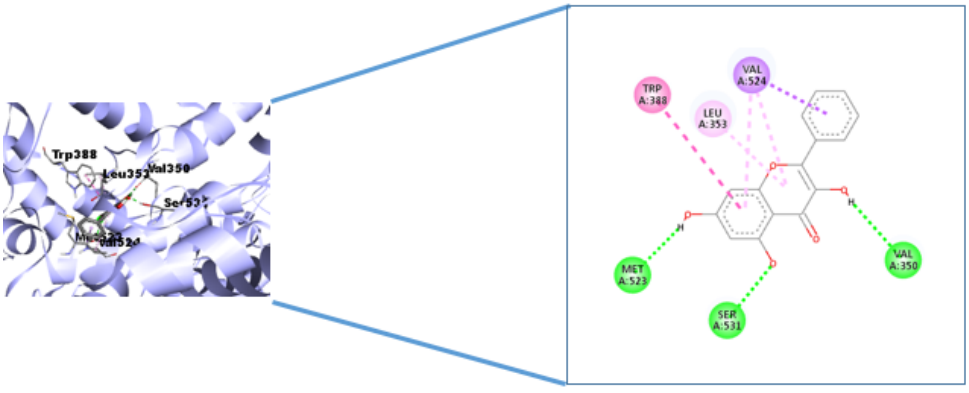
c



d



e



f

Figure 2. Molecular interactions of compounds (A) Celecoxib, (B) Galanganal, (C) Galanganol A, (D) Galanganol B, (E) Galanganol C, and (F) Galangin against the Cyclooxygenase-II (COX2) receptor

The conformations that have been formed from the results of the docking simulation were then analyzed using the BIOVIA Discovery Studio 2021 software. The interactions of the reference compound (Celecoxib) and the galangal derivative test compound against the Cyclooxygenase-II receptor were identified with the aim of knowing more about the characteristics of the binding area of the galangal derived compound. Based on Figure 1, the reference compound (Celecoxib and all galangal derivatives) have similar conformational binding results to the cyclooxygenase-II receptor binding site. The reference compound (Celecoxib) shows hydrogen bonds with amino acid residues in 3 interactions, namely SER354, LEU353, and GLN193. The docking results of the Galanganal test compound showed hydrogen bonds with amino acid residues of 3 interactions, namely SER354, HIS90, TYR356, AGR121, and SER531. The docking results of the Galanganol A test compound

showed hydrogen bonds with amino acid residues of 4 interactions, namely AGR121, LEU353, VAL350, and MET523. The docking results of the Galanganol B test compound showed hydrogen bonds with amino acid residues of 2 interactions, namely LEU353 and MET523. The docking results of the Galanganol C test compound showed hydrogen bonds with amino acid residues of 2 interactions, namely TYR38 and AGR121. The anchoring results of the Galangin test compound shows hydrogen bonds with 3 amino acid residues, namely MET523, SER53, and VAL350. Where several important amino acid residues in the interaction of COX-2 receptors with several anti-inflammatory compounds, namely ARG, SER, and TYR (Ahmadi et al., 2022). This is found in the comparison compound (Celecoxib) and the test compound Galanganal.

Table 5. Prediction of the toxicity of galangal derivative compounds and medicinal compounds using the Toxtree application

Compound name	Cramer Rules	Kroes TTC	Carcinogenicity and Mutagenicity
Celecoxib	High (Class III)	Substance would not be expected to be a safety concert	Nagative for genotoxic
Galanganal	High (Class III)	Substance would not be expected to be a safety concert	Nagative for genotoxic
Galanganol A	High (Class III)	Substance would not be expected to be a safety concert	Nagative for genotoxic
Galanganol B	High (Class III)	Substance would not be expected to be a safety concert	Nagative for genotoxic
Galanganol C	High (Class III)	Substance would not be expected to be a safety concert	Nagative for genotoxic
Galangin	High (Class III)	Substance would not be expected to be a safety concert	Nagative for genotoxic

The final step is to predict the toxicity of Zizyphine analog compounds with three parameters, namely Creamer rules, Kroes TTC, and Carcinogenicity and Mutagenicity using Toxtree software version 3.1.0. Table 3 shows the results of the prediction of the toxicity of the four galangal derivative compounds and based on the Cramer rules parameters, the results of the tested compounds belong to class III or have a high potential for toxicity. Then the Kroes TTC parameter states that galangal derivative compounds are still within the exposure limit with a low risk of exposure. Meanwhile, the Carcinogenicity and Mutagenicity parameters obtained negative results and did not cause carcinogenicity or mutagenicity.

Conclusion

Based on the research results above, it was concluded that of the 5 test compounds that had the potential to act as an anti-inflammatory, it was the Galanganal compound with a binding free energy value of -8.98 kcal/mol and an inhibition constant of 261.59 nM. Based on the Lipinski 5 rule, it shows that all compounds fulfill the Lipinski 5 rule. So it can be predicted that the 5 test compounds, including the reference drug, have good absorption for use as oral preparations. The five test compounds also have anti-inflammatory potential as seen using the WAY2DRUG PASS prediction server.

Acknowledgment
We would like to express our thanks to all parties who have provided support and assistance for this research. We also thank the Bali International University for their support in writing this article.

Authors Contribution
In this research, the first author contributed to determining research methods, processing research data and interpreting research data. The second author contributed, namely assisting in the data processing process and creating the manuscript for this article and carrying out the editing process for this article.

Funding
Funding for our research was obtained from a grant from the Bali International University.

Conflicts of Interest
The author has declared that there is no conflict of interest related to the publication of this article.

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