Indonesian Journal of Global Health Research
Volume 7 Number 1, February 2025
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

DOCKING AND STRUCTURAL MODIFICATION OF FLAVONOID DERIVATIVE COMPOUNDS AS CYCLOXYGENAS-2 ENZYME INHIBITORS

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ABSTRACT
Seven flavonoid compounds have the activity of inhibiting the cyclooxygenase-2 (COX-2) enzyme, thus providing an anti-inflammatory effect. Molecular docking analysis is needed to determine the binding interaction between flavonoid compounds and the cyclooxygenase-2 (COX-2) enzyme. Objective: This study aims to determine the interaction of flavonoid compounds with the cyclooxygenase-2 (COX-2) enzyme along with the modification of the flavonoid compound structure to increase the binding energy to the cyclooxygenase-2 (COX-2) enzyme. Method: Flavonoid derivative compounds were geometry optimized using VegaZZ software, then target preparation, ligand preparation, docking method validation, and docking analysis were carried out to obtain the interaction between flavonoid compounds and the cyclooxygenase-2 (COX-2) enzyme which is expressed as ΔG and interaction patterns, which include hydrogen bonds, amino acids and functional groups involved, using the program using PyRx-Python 0.8 - AutoDock Vina. Results: The interaction pattern of seven flavonoid derivative compounds with COX-2 enzyme showed hydrogen bonds with amino acids ARG 121, ILE 113, LEU 93, VAL 117. The interaction is similar to the interaction of protoporphrin ix containing co which is the original ligand of the target protein. There is no relationship between the inhibitory activity of flavonoid derivatives and the free energy value of binding (ΔG). Modification of the new compound luteolin 7 glucosidase has a better amino acid interaction pattern, namely PRO 155, 154, ARG 44, TYR 131, LEU 153. Conclusions: The binding profile of flavonoid derivatives has the potential to be a candidate for oral cyclooxygenase-2 (COX-2) inhibitor compounds as an anti-inflammatory.

Keywords: adme; cyclooxygenase-2; docking molekuler; flavonoid; toxtree

How to cite (in APA style)
Listyani, T. A., Addawiyyah, M., Raharjo, D., & Chyang, P. (2024). Docking and Structural Modification of Flavonoid Derivative Compounds as Cycloxygenas-2 Enzyme Inhibitors. *Indonesian Journal of Global Health Research*, *7*(1), 311-322. https://doi.org/10.37287/ijghr.v7i1.4041.

INTRODUCTION

Pain is an evolutionarily protective perception of nonciceptive stimuli that indicate real or potential danger to the body. Pain is a global problem that is a major cause of disability, but long-term clinically available drugs do not provide analgesia in many pain patients and cause undesirable effects such as sedation and constipation, rapid tolerance, high drug potency and risk death. The side effects of using anti-inflammatory drugs that have been described previously have encouraged research related to the discovery of potential compounds and recruitment as drug candidates using natural ingredients because they have low side effects (Ifora et al., 2021). Drug development using the in silico method has advantages compared to testing using in vivo and in vitro, namely faster testing with lower costs to find more potent compounds and apply as drug candidates (Al-Khayri, 2022). The research was carried out with the aim of knowing the binding profile of flavonoid derivatives which have the potential to be candidates for cyclooxygenase-2 (COX-2) inhibitor compounds as anti-inflammatories with plans for new compounds to have better affinity. By finding information regarding the interaction of flavonoid derivative compounds with the cyclooxygenase-2 (COX-2) enzyme, along with the relationship between the free energy of binding on the binding site using

molecular docking analysis.

METHOD

The hardware consists of a Lenovo laptop model V14-IGL type 82C2 with N4020 processor specifications, dual-core CPU 1.10GHz, 4 Giga Byte RAM, 931.51 Giga Byte hard disk.The software used to process the data is the Windows 10 program, ChemDraw Ultra 22.0.00, Chem 3D Ultra 22.0.00, Discovery Studio Visualizer V21.1.0.20298, VegaZZ 3.2.3.28, PyMOL 1.3, PyRx-Python 0.8 – AutoDock VinaThe material used is the cyclooxygenase-2 3D structure receptor which can be downloaded from the Protein Data Bank with the identity 6OFY originating from the organism Spodoptera frugiperda, obtained from the 2.20 A resolution X-ray method (Dong et al., 2019). Ligand 3D structure test of seven compounds from NMR results of flavonoid derivative compounds using ChemOffice 2022; Afzelichin (Ondua et al, 2019), Epicathechin 3 gallat, Epicatechin, Luteolin, Luteolin 7 glukosidase, Quersetin, Rutin (Al-khayri et al, 2022). The original ligand or native ligand used is a protoporphyrin ix containing co structure which has bound to the cyclooxygenase-2 (COX-2) enzyme as a receptor (Dong et al., 2019). The positive control used was the structure of the celebrex drug compound as an anti-inflammatory which is already circulating on the market. Can be downloaded at https://pubchem.ncbi.nlm.nih.gov/. The structure of the drug compound used as a negative control, namely paracetamol, can be downloaded https://pubchem.ncbi.nlm.nih.gov/.

Cyclooxygenase-2 (COX-2) receptor macromolecule download

The structures of protein macromolecules that have an anti-inflammatory role are downloaded from the Protein Data Bank (PDB) with the identity 6OFY (Dong et al., 2019) through https://www.rcsb.org/structure/6OFY in .pdb format

Creation of 3D structure of test ligand

The two-dimensional structures of the seven test ligand compounds were created using ChemDraw ultra22.0 software, then converted to a three-dimensional model using Chem3D ultra22.0. The three-dimensional structure is saved using the PDB file format. The three-dimensional structure was optimized using VegaZZ software with the steps calculate, select ammp, click minimization, and save, click save as with the ligand name in .mol format for prediction and toxicity tests and in .pdb format for optimization in VegaZZ (Listyani et al., 2018)

Preparation of macromolecules

The Discovery Studio Visualizer application is used to separate macromolecular structures from test ligands. By opening the Discovery Studio Visualizer icon, clicking on the macromolecule file to be separated, the three-dimensional structure of the target protein appears. Unused molecular chains, ligands and residues will be removed by clicking the script selection select water molecules / ligands / protein chains edit delete. Macromolecules that are ready to be saved are in .pdb format (Listyani et al., 2018)

Preparation of test ligands

Optimization is carried out with the aim of producing the lowest molecular energy which shows the stability of the chemical structure of the ligand which has been optimized to produce a folded structure that is different from the initial structure. The three-dimensional structure of the compound to be used is opened using VegaZZ software and hydrogen atoms are added. The charge of the compound was corrected by adding partial gasteiger charges then adding the AutoDock forcefield and the compound was minimized by 3,000 steps to obtain

the most stable conformation. Each optimized compound is saved with the ligand name using the.pdb format for docking tests and the.mol format for toxicity tests (Listyani et al., 2018).

Molecular docking process

The molecular docking process uses PyRx 0.8 software with the AutoDock Vina system using gridbox. Macromolecules and ligands that have been optimized separately in the same folder. The parameters used in the molecular docking process are energy minimization which is in accordance with the validation results. Validation of the Root Mean Square Deviation or RMSD value is carried out using PyMOL software by selecting the file then open (search for ligands as a comparison) and open the file resulting from the docking of the PyRx application, then change the display from lines to sticks. Type in the command column, align the name of the ligand storage file, then enter, then the Root Mean Square Deviation value will be displayed after the calculation has been completed. Molecular docking uses PyRx 0.8 with the steps of opening the PyRx 0.8 application then loading the molecule in the storage folder. Right-click the available file, select AutoDock, then select the ligand and protein macromolecule to be tested. Vina Wizard click start here select local then start. The ligand and macromolecule in the select molecule are guaranteed to be selected because if the ligand and macromolecule have not been selected, the result is that the next step cannot be forwarded. The gridbox can be set in the table view, then directed to the binding site as the center point, there are x, y and z coordinates then recorded. Click Run, the docking process will start and wait until 100% of the results will appear in the form of an analyze result table. Click on the out ligand with the smallest binding affinity in the navigator table, click on molecule then select the out ligand that is already in the analyze result table. save in .PDB format (Listyani et al., 2018).

Visualization of molecular docking results

The molecular docking results that have been saved in .PDB format are then visualized using Discovery Studio Visualizer by hovering the cursor on the file then clicking open then selecting the macromolecule used for docking. The ligand that has been docked is inserted by hovering the cursor on the file then clicking open then selecting the ligand then copying the ligand then pasting it on the macromolecule that has been displayed. Interact with the ligand and target molecule by clicking structure then labels then add select the AminoAcid object then set the text color to black and change the background of the Discovery Studio Visualizer display to white by viewing then Display Style then selecting Graphics Background Color select Select Color click OK. Visualization can be done in 2D to find out the bonds that occur by clicking show 2D Diagram then the 2D interactions and types of bonds that occur appear (Listyani et al., 2018).

RESULTS

Creation of 2D and 3D structures of test ligands

Making the test ligand structure in 2D format using the ChemDraw Ultra 22.0 application. 2D structure creation is used to create a 3D structure of the test ligand. The depiction of 2D structures is guaranteed to be appropriate because it affects the 3D shape that will be separated from the structure (Dewantoro & Wilapangga, 2023). Making the structure in 2D format was continued in 3D using ChemDraw Ultra 3D 22.0 because in the docking stage everything was done on a 3D structure. When the structure is converted into 3D using ChemDraw Ultra 3D 22.0, it is ensured that the 3D structure will not come loose (Dewantoro & Wilapangga, 2023). An example of the 3D structure of the test ligand in the ChemDraw 3D Ultra 22.0 application can be seen in Figure 4.2 and Appendix 2.

Preparation of three-dimensional structure optimization of test compounds

The test ligands used in this research are seven flavonoid derivative compounds in 3D form using ChemDraw 3D Ultra, the structure of protoporphyrin ix containing co which binds to the COX-2 enzyme as a receptor used to compare amino acid residues because it is the original ligand, the positive control is a compound the celebrex drug which is already circulating on the market and the structure of the paracetamol drug compound which is used as a negative control which has been geometrically optimized using VegaZZ. The aim of geometric optimization is to produce a compound conformation with the lowest energy that shows the best stability in the optimized chemical structure of the ligand, which will produce a structure with folds that are different from the initial structure (Pratama et al., 2021). Previous research conducted (Pratama et al., 2021) showed that the test ligand was optimized using gasteiger charges plus forcefield autodock, the compound was minimized by 10,000 steps after being tested. previous research conducted by (Listyani et al., 2018) and the test ligand compound was more stable.

Preparation of three-dimensional structures of macromolecules

Preparation of the macromolecular structure used will determine the predicted interactions that will occur, so selecting the target macromolecule before molecular docking is a very important step. The method chosen for determining macromolecular structures is X-Ray Difraction because it can be applied to macromolecular structures that are large in size (>100 KDa) and complexed with native ligands whose activity can be estimated to be the same as the test ligand and with greater precision. The organisms used are human macromolecules (homo sapiens) or animals that are close to human macromolecules, but the macromolecular code does not contain information about human macromolecules so the macromolecules used are organisms other than humans (Pratama et al., 2021). The macromolecular structure used was downloaded from the Protein Data Bank (PDB) via the site https://www.rcsb.org/structure/ with the identity 6OFY. Information on the target macromolecules used can be seen in table 1.

Table 1.
Crystallographic macromolecular and ligand information (Dong et al., 2019)

Parameter	Antiinflamasi
PDB ID	6OFY
Organisme	*Spodoptera frugiperda*
Metode	*X-Ray Difraction*
Resolusi	2.20 A
Ligan	Protoporphyrin ix containing co

The macromolecular structure used is bound to ligands and water molecules, so it must be removed because it can interfere with the docking process. Water molecules will mediate the interaction of the ligand with the receptor, so that the docking results are better. The presence of a ligand bound to the active site of the macromolecule will hinder the interaction of the ligand attached to the docking process (Listyani et al., 2018). Macromolecules that have been separated from water and ligand residues are ready for docking, so the results of molecular docking are better because there is nothing blocking the molecular docking process when they are docked with the test ligand (Listyani et al., 2018).Protoporphyrin ix containing co in macromolecules was dissolved in V3491 murine COX-2 (muCOX-2). V3491 muCOX-2 is used to substitute for trapping arachidonic acid in the conformation leading to 15R-HETE (Dong et al., 2019).

Validation of the molecular docking method

After the macromolecules were optimized, the method was validated using PyRx software. The general principle of docking validation is by redocking the original ligand to the target protein, the ligand and protein are prepared using docking methods and parameters that will

be used for docking studies on the test ligand. Validation of the docking method was carried out on original ligands extracted from macromolecules and optimized by VegaZZ software (Pratama et al., 2021) The gridbox setting is carried out before validation by means of the macromolecules being arranged in such a way as to determine the gridbox. The purpose of setting the gridbox is to determine the binding space for the ligand to be docked. Ligands that have bonds will have a space referring to the ligands that are already bound to the macromolecule at the time it is downloaded. Gridbox settings including center_x, center_y and center_z are used to set the location of box parameters on macromolecules. Size_x, size_y and size_z are used to determine the size of the gridbox for the binding space for the ligand (Listyani et al., 2018). Information regarding the gridbox settings used can be seen in table 2.

Table 2.
Macromolecular gridbox arrangement.

	Gridbox					
	Center			Dimensi		
Macromolecules	X	Y	Z	X	Y	Z
Protoporphyrin ix containing co	-24.856	-39.366	-28.8358	75.0098	93.9729	75.4800

The parameter used for method validation is the Root Mean Square Deviation or RMSD value. The Root Mean Square Deviation or RMSD value is the value used to determine whether the bond mode prediction is successful and is important for validating the docking program. The RMSD value is the conformation resulting from the docking obtained which is aligned to the conformation of the native ligand resulting from crystallographic measurements which is expressed in the macromolecule value whose gridbox has been set and then tested with the original ligand (crystallographic results) which is called method validation with the RMSD value using PyMOL software to obtain the RMSD value smallest. The results of calculating the RMSD validation value between the original ligand and the docking results using PyMOL software produced a value of 2.1, while the RMSD value resulting from docking using PyRx software produced a value of 2.2. These results indicate that method validation can be used for molecular docking testing because the RMSD value is said to be good if the value is ≤ 2 (Listyani et al., 2018). Information regarding RMSD values and binding affinity values can be seen in table 4 and figure 1.

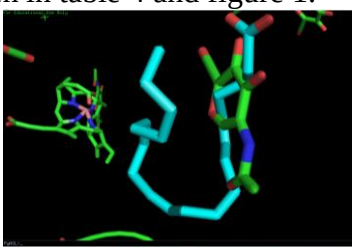

Figure 1. Validation results of the docking method with PyMOL (PyMOL 1.3).

Table 3.
RMSD and Binding affinity values

Makromolekul	Binding affinity (kcal/mol)	RMSD
Protoporphyrin ix containing co	-4.7	2.221
Validasi PyMOL		2.1

The results of the molecular docking validation of the native ligand in the form of protoporphyrin ix containing co with the macromolecular target were visualized using BIOVIA to determine the interaction of protoporphyrin ix containing co which was then used for comparison between test ligands to determine their anti-inflammatory activity. The visualization results of molecular docking of protoporphyrin ix containing co are SER 120, VAL 117, VAL 89, PRO 84, TYR 116, ARG 121, LEU 93, ILE 113, TRP 100. The results of molecular docking of protoporphyrin ix containing co are in accordance with the results of the native ligand protoporphyrin ix containing co which binds to the COX-2 enzyme namely

arachidonic acid binds in a C-shaped pose, rotating the side chain of LEU 531. ILE 349 is positioned to protect the addition of carbon 15 oxygen in a manner similar to that proposed for the side chain of SER-530 (Dong et al., 2019).

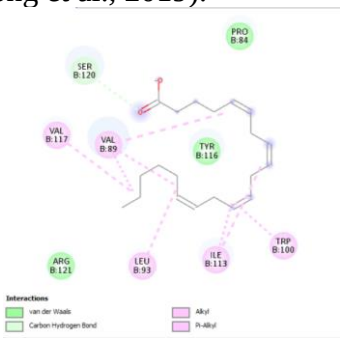

Figure 2. Visualization results of protoporphyrin ix containing co (BIOVIA V21.1.0.20298)

Molecular docking results

Seven ligands were docked to target macromolecules to see potential interactions using the PyRx application. The gridbox used for docking is the gridbox during method validation, so the results will be more accurate. Analysis of docking results in this study includes RMSD values, ΔG binding and interactions between ligands and protein residues. The conformations resulting from docking of each ligand are ranked based on the RMSD and ΔG binding values. Small RMSD and ΔG binding values indicate that the conformation of the complex formed is stable, if large RMSD and ΔG binding values indicate that the complex formed is less stable (Pratama et al., 2021). The molecular docking results can be seen in table 4.

Table 4.
Results of Molecular Docking of Test Ligands

Senyawa Flavonoid	Nilai ΔGbind (kkal/mol)	Nilai RMSD	Senyawa Flavonoid	Nilai ΔGbind (kkal/mol)	Nilai RMSD
Protoporphyrin ix containing co	-4,5	2,2	Epicatechin 3 gallat	-7,8	1,6
Kontrol positif (celebrex)	-8,3	1,5	Luteolin	-8,0	1,9
Kontrol negatif (paracetamol)	-5,4	3,2	Luteolin 7 glukosidase	-9,3	1,8
Afzelechin	-5,9	2,1	Quersetin	-7,9	1,8
Epicatechin	-6,0	1,7	Rutin	-8,1	1,8

Data from the molecular docking process of test ligands against 6OFY macromolecules shows that of the 10 docked ligands, 7 test ligands and 3 comparison ligands produced the best (lowest) RMSD and ΔG binding values. Data from docking obtained bond energy values ranging from -4.7 kcal/mol to -9.3 kcal/mol and RMSD values ranging from 1.5 to 2.2. The results of the RMSD and ΔG binding values are in accordance with the literature, namely an RMSD value of less than 3 for structural conformation alignment is still acceptable but the most optimal value is less than 2. The smaller the ΔG binding value, the better the interaction with the reference compound (Listyani et al., 2018) . The molecular docking results of the 7 test ligands on the ΔG binding value when compared with protoporphyrin ix containing co as a comparison, the best positive control (Celebrex) and negative control (paracetamol), namely the compound luteolin 7 glucosidase because it has the lowest ΔG binding value, so it is better and more stable when compared with other test ligands

Interaction of the test ligand with the receptor

The amino acids that bind to the active site of the COX-2 enzyme as an anti-inflammatory are branched-chain amino acids or BCAAs (leucine or LEU, valine or VAL and isoleucine or ILE) which are specific amino acids in the COX-2 binding pocket. Branched-chain amino

acids or BCAAs are essential amino acids that play an important role in protein structure, metabolism and regulation (He et al., 2018).The interactions resulting from the ligand were seen using Discovery Studio Visualizer software and the results were compared with protoporphyrin ix containing co, which is a ligand that binds to the target macromolecule as the original ligand and celebrex as a positive control, which is an anti-inflammatory drug on the market which has more potential capabilities.Ligand-protein interactions are noncovalent interactions such as hydrogen bonds, Vander Waals bonds, π-π interactions, hydrophobic and donor interactions. Computational methods yield predictions of interactions and binding affinities as well as conformations of ligand-macromolecule complexes. Hydrogen bonds are described as a form of electrostatic interaction between a hydrogen atom bonded to an electronegative atom and another electronegative atom. Hydrogen bonds contribute to the structure and are a characteristic property of molecules.

Table 5.
Ligand Interactions With Receptors

Senyawa Flavonoid	Ikatan Hidrogen	Ikatan Vanderwaals	Interaksi π- π	Donor
Protoporphyrin ix containing co	SER 120	ARG 121, PRO 84, TYR 116	VAL 117, 89, LEU 93, ILE 113, TRP 100	-
Kontrol positif (celebrex)	ARG 514, HIS 90, SER 531,354, TYR 386, LEU 353	-	TYR 356, 349, VAL 117, 524, ALA 528, ILE 350, LEU 360, TRP 388	-
Kontrol negatif (paracetamol)	THR 207, TYR 386	-	ALA 203	-
Afzelechin	-	-	ILE 346,350, LEU 532, ALA 528	-
Epicatechin	LEU 172	-	LYS 460, PRO 103	ARG457
Epicatechin 3 gallat	ALA 157, PRO 155, GLY 136	-	HIS 34, TYR 137, CYS 36,47, PRO 154	-
Luteolin	ASN 383, ALA 200	GLN 204	HIS 208, ALA 203	-
Luteolin 7 glukosidase	SER 531, ARG 121	-	VAL 524, LEU 353, ILE 350, ALA	PHE 519
Quersetin	TYR 356	-	VAL 117, 524, LEU 353, ILE 350, ALA 528	-
Rutin	HIS 352, 357, LEU 360, SER 580	LYR 359	-	PHE 581

Note: bold print indicates the similarity of the amino acid residues of the test ligand to the native ligand.

Vander Waals bonds are the force of attraction between molecules or atoms that have no charge and are located close together or 4-6 meters apart. Vander Waals bonds occur due to the polarization of molecules or atoms (Listyani et al., 2018).The results of the interaction of the test ligand with the receptors of the 7 test ligands when compared with protoporphyrin ix containing co as the original ligand used for comparison and the drug celebrex as a positive control which is already on the market as an anti-inflammatory shows that the best ligand is luteolin 7 glucosidase because it has the same amino acid similarity. namely SER 531, ARG 121, VAL 524, LEU 353, and ILE 350. The results of the interaction of the test ligand with the receptor can be seen in table 5.

Afzelechin

Afzelechin is a metabolite and monomer compound that can interact with COX-1 and COX-2 by forming hydrogen bonds. Afzelechin is included in the flavonoid compound flavan-3-ol with other names 3,5,7,4-tetrahydroxyflavan and 3,4,5,7-flaventretro (Winata et al., 2023).

The results of molecular docking research on the afzelechin compound show an RMSD value of 2.19 and a ΔG binding value of -5.9 which is more stable compared to protoporphyrin ix containing co as the original ligand, a ΔG binding value of -4.5, but the afzelechin compound is unstable when compared to the positive control (celebrex) ΔG binding value is -8.3. Analysis of the interaction of the afzelechin ligand with the COX-2 receptor has π-π interactions including ILE 346, 350, LEU 532 and ALA 528 which interact on the benzene aromatic ring when targeted with the target macromolecule. Bond. The π-π interaction is an amino acid bond that is compatible with the original ligand, namely ILE 350, 346, LEU 532.

Epicatechin 3 gallate

Epicatechin 3 gallate is a flavonoid compound that has anti-inflammatory activity because it is selective for the COX-2 enzyme and has high bond strength and stability (Ahsana et al., 2021). The results of molecular docking of the compound epicatechin 3 gallate in this study showed an RMSD value of 1.6 and ΔG binding -7.8 which is more stable compared to protoporphyrin ix containing co as the original ligand with a ΔG binding value of -4.5, but the compound epicatechin 3 gallate is unstable if compared to the positive control (celebrex) the ΔG binding value was -8.3. The results of this research are in line with previous research conducted by Ahsana et al., 2021, the results of molecular docking of the epicatechin 3 gallate compound against the COX-2 enzyme with a ΔG binding value of -9.3. Analysis of the interaction of the epicatechin 3 gallate ligand against the COX-2 receptor produces π-π interactions including HIS 34, TYR 137, CYS 36, 47, and PRO 154. The hydrogen bonds formed are ALA 157, PRO 155 and GLY 136. Through the interaction π-π and the hydrogen bonds formed are compatible with the original ligands, namely PRO 155 and 154, which are formed on the O-H-O side chain and on the benzene ring when targeted at the target macromolecule.The results of this study are not in line with previous research regarding the molecular docking of the epicatechin 3 gallate compound with the COX-2 receptor code 6COX. The positive control celebrex has great potential as a selective anti-inflammatory COX-2 inhibitor (Abdillah, 2023).

Epicatechin

The results of molecular docking of the epicatechin compound in this study show an RMSD value of 1.7 and ΔG binding -6.0, which is more stable compared to protoporphyrin ix containing co as the original ligand, a ΔG binding value of -4.5, but the epicatechin compound is unstable when compared to the positive control (celebrex) ΔG binding value is -8.3. The results of this research are in line with previous research conducted (Ahsana in 2021), the results of molecular docking of the epicatechin compound against the COX-2 enzyme produced a ΔG binding value of -8.24.Analysis of the interaction of the epicatechin ligand with the COX-2 receptor produces π-π interactions, namely LYS 460, PRO 103 and the hydrogen bond formed is LEU 172. Through the π-π interaction and the hydrogen bonds formed, there is a compatibility of the amino acids formed with the original ligand, namely LEU 172, PRO 103 and ARG 457 which are formed on the O-H-O side chain and on the benzene ring when targeted to the target macromolecule.

Luteolin

Luteolin is a flavonoid compound in the flavone group which has tetrahydroxyflavone with four hydroxy groups. Luteolin has antioxidant, anti-inflammatory and chemopreventive activity. Luteolin is often found in medicinal plants used to treat hypertension, inflammation and cancer (Abdillah, 2023). The results of molecular docking in this study, the luteolin compound produced an RMSD value of 1.9 and a ΔG binding value of -8.0, which was more stable compared to protoporphyrin ix containing co as the original ligand, a ΔG binding value of -4.5, but the luteolin compound was unstable when compared with the control positive

(celebrex) ΔG binding value of -8.3. The results of the research are in line with previous research conducted by Abdillah in 2023. The results of molecular docking of the luteolin compound against the COX-2 enzyme have an anti-inflammatory affinity and the resulting ΔG binding value is -10.3 kcal/mol. Analysis of the interaction of the luteolin ligand with the COX-2 receptor produces π-π interactions including HIS 208 and ALA 203, the hydrogen bonds formed are ASN 383 and ALA 200, the Vander Walls bonds formed are GLN 204. Through π-π interactions, hydrogen bonds and the Vander Walls bond formed in this study is a weak bond because the amino acid interactions formed have nothing in common with the original ligand. These results are not in line with previous research on molecular docking of the compound luteolin with the COX-2 receptor code 1CX2 as an anti-inflammatory, having a higher affinity for COX-2 compared to the control celecoxib (Abdillah, 2023) Luteolin 7 glucosidase is a flavonoid compound in the flavon subclass which has the main active component found in the compound luteolin. Luteoin 7 glucosidase has bioactive activities such as anti-tumor, antioxidant, anti-inflammatory and nervous system protection (Gunarti & Hidayah, 2022).

The best active compound is luteolin 7 glucosidase because only luteolin 7 glucosidase has the smallest binding energy value and occupies the same binding pocket as the original ligand, showing an RMSD value of 1.8 and ΔG binding -9.3 so it is more stable and better than protoporphyrin ix containing co as the original ligand, the ΔG binding value is -4.5 and the positive control (celebrex) ΔG binding value is -8.3, luteolin 7 glucosidase can produce a stable conformation in the complex formed, by having an anti-inflammatory affinity that can inhibit the COX- 2 with several amino acid bonds identical to the original ligand (protoporphyrin ix containing co) and positive control (celebrex), but the luteolin 7 glucosidase compound is toxic to genotoxic carcinogenicity and there is a structural warning for S. typhimurium mutagenicity. Amino acid residues in the test ligand for the COX-2 receptor form π-π interactions namely VAL 524, LEU 353, ILE 350 and ALA 528 on the benzene aromatic ring, hydrogen bonds namely SER 531, ARG 121 and HIS 90 on the O-H-O side chain, bonds donor PHE 519. Has similarities with the original ligand in the form of hydrogen bonds on amino acids SER 531 and ARG, has similarities with the Pi interaction bonds namely VAL 524, LEU 353, ILE 350. The amino acid residue luteolin 7 glucosidase has similarities with the positive control (celebrex) in the form of hydrogen bonds in the amino acids SER 531 and ARG 121 which have similarities with the Pi interaction bonds namely VAL 524, LEU 353, ILE 350, the original ALA is located in the amino acids PRO 155,154, ARG 44, TYR 131 and LEU 153, better than luteolin 7 glucosidase as the parent compound. The modified ligand also has the same amino acids as the positive control (celebrex) as an anti-inflammatory drug already circulating on the market, namely ARG 44, TYR 131 and LEU 153, so that the modified ligand can be developed into an oral dosage as an anti-inflammatory drug with good stability and safe to use. . Information regarding the results of modifications528. The interaction of luteolin 7 glucosidase with the original ligand and positive control (celebrex) can identify similarities in its mechanism of action on proteins, namely competing competitively with celebrex and the original ligand.

Quercetin

Quercetin is a flavonol compound from the polyphenolic flavonoid group which has several pharmacological activities such as anti-inflammatory, antioxidant, anticancer and antipyretic. Quercetin can inhibit COX-2 which works by inhibiting prostaglandin biosynthesis. Quercetin can inhibit eicosanoids so that the cyclooxygenase pathway as an inflammatory mediator is blocked (Nurfitriah et al., 2021). The results of molecular docking of the Quersetin compound in this study showed an RMSD value of 1.87 and ΔG binding -7.9, which was more stable compared to protoporphyrin ix containing co as the original ligand, a ΔG binding value of -

4.5, but the Quersetin compound was unstable when compared to the positive control (celebrex) ΔG binding value is -8.3. The results of this research are in line with previous research conducted by Abdillah in 2023, the results of molecular docking of the Quersetin compound against the COX-2 enzyme have an anti-inflammatory affinity and the resulting ΔG binding value is -9.5 kcal/mol. Analysis of the interaction of the Quercetin ligand against the COX-2 receptor produces π-π interactions namely VAL 117, 524, LEU 353, ILE 350 and ALA 528. The hydrogen bond formed is TYR 356. Through the π-π interaction and the hydrogen bond formed has the same acidity. amino in the π-π interaction which corresponds to the original ligand, namely VAL 117,524, LEU 353 and ILE 350 on the benzene aromatic ring, but in this study luteolin 7 glucosidase has a high affinity for COX-2 in terms of free energy and amino acid residue interactions. The results of this research are in line with previous research regarding the molecular docking of the compound quercetin with the COX-2 receptor code 1CX2 as an anti-inflammatory which has a higher affinity for COX-2 compared to the control celecoxib (Abdillah, 2023).

Routine

Rutin is a glycoside compound of the flavonoid group resulting from the condensation of quercetin aglycone withrutinose. Rutin has activity as a natural antioxidant and anti-inflammatory (Satari et al., 2021). The results of molecular docking research on the routine compound show an RMSD value of 1.8 and ΔG binding -8.1 which is more stable compared to protoporphyrin ix containing co as the original ligand, a ΔG binding value of -4.5, but the routine compound is unstable when compared with the positive control (celebrex) ΔG binding value of -8.3. The results of this research are in line with previous research conducted by (Hakim in 2018), the results of molecular docking of routine compounds against the COX-2 receptor produced a ΔG binding value of -10.Analysis of routine ligand interactions with the COX-2 receptor has hydrogen bond interactions, namely HIS 352, 357, LEU 360, SER 580. The van der Waals bond is LYR 359 and the donor bond is PHE 581. Through hydrogen bonds, Van der Waals bonds and donor bonds. The amino acid bond is compatible with the original ligand, namely the amino acids LEU 360 and SER 580 formed in the O-H-O side chain. The results of this study are not in line with previous research regarding molecular docking of the compound routine with the COX-2 receptor, which showed that the inhibition stability between the routine and the COX-2 receptor was more stable than dexamethasone as a positive control (Hakim et al., 2018).

Modification of new compounds

The design of new compounds was carried out using the structure of the flavonoid derivative as the parent structure, then the parent structure of the functional groups in the flavonoid derivative was modified with the aim of increasing the bond energy and reducing the toxicity effect, based on the results of molecular docking and toxicity tests of the flavonoid derivatives (Listyani et al., 2018). The compound luteolin 7 glucosidase was chosen as the parent compound because it has similar amino acid interactions with the original ligand (protoporphyrin ix containing co) and the positive control (celebrex), but luteolin 7 glucosidase is a mutagen and carcinogen and is included in the hight or class 3 category which is not safe if consumed too much. excessive and has significant toxicity activity, so modifications need to be made to reduce the toxicity effect without reducing the ligand interaction with the target protein. Modification of the luteolin 7 glucosidase compound produces a new compound that does not cause carcinogens or mutagens, so the compound does not cause cancer or tumors, mutations in chromosomes or DNA and does not cause changes in genetic information (Indrasari et al., 2022). The change occurs due to eliminating the α, β unsaturated carbonyl structure in the luteolin 7 glucosidase compound which causes

carcinogens and mutagens, so that it can produce new compounds that are not carcinogens and mutagens (Toxtree, 2023).

Table 7.
Comparison of molecular docking of new compound modifications

Senyawa flavonoid	Nilai ΔGbind (kkal/mol)	Nilai RMSD
Protoporphyrin ix containing co	-4,5	2,2
Kontrol positif (celebrex)	-8,3	1,5
Luteolin 7 glukosidase	-9,3	1,8
7-(3,4-dihydroxycyclohexyl)-4,5-dihydroxy-5,8dihydronaphthalen-2-yl) oxy)-6-(hydroxymethyl) cyclohexane1,2,3-triol	-7,6	2,0

The results of molecular docking on the Δgbind and RMSD values for the compound luteolin 7 glucosidase 1.8 (-9.3 kcal/mol) and the positive control in the form of celebrex 1.5 (-8.3 kcal/mol) are more stable compared to the results of the modification of the new compound 2.0 (-7.6 kcal/mol) can be seen in table 4.6, but the Δgbind and RMSD values have no effect on the bonds formed, because the modified compound has better bond similarities compared to luteolin 7 glucosidase towards the original ligand and positive control (celebrex) as an anti-inflammatory drug on the market.

Table 8.
Comparison of ligand interactions with modified receptors of new compounds

Senyawa Flavonoid	Ikatan Hidrogen	Ikatan Vanderwaals	Interaksi π- π	Donor
Protoporphyrin ix containing co	SER 120	ARG 121, PRO 84, TYR 116	VAL 117, 89, LEU 93, ILE 113, TRP 100	-
Kontrol positif (celebrex)	ARG 514, HIS 90, SER 531,354, TYR 386, LEU 353	-	TYR 356, 349, VAL 117, 524, ALA 528, ILE 350, LEU 360, TRP 388	-
Luteolin 7 glukosidase	SER 531, ARG 121, HIS 90	-	VAL 524, LEU 353, ILE 350, ALA 528	PHE 519
7-(3,4-dihydroxycyclohexyl)-4,5-dihydroxy-5,8dihydronaphthalen-2-yl) oxy)-6-(hydroxymethyl) cyclohexane1,2,3-triol	CYS 47, GLU 46, PRO 155	-	ARG 44, TYR 131, LEU 153, PRO 154	ASN 39

Note: Bold print indicates the similarity of the amino acid residues of the test ligand to the native ligand**.**

Luteolin 7 glucosidase and the modified ligand have almost the same structure, but have bonds with residues that are slightly different from each other due to the difference in conformation and the single ethylene bridge of the compound can rotate freely, making the conformation formed allowing the ligand to have the most suitable conformation. stable on target proteins (Pratama et al., 2021). The results of the modification of the new compound produce binding energy values that are quite small, close to the scores of the original ligand and positive control (celebrex), forming amino acid residues which play a role in the anti-inflammatory process, which can be seen in table 4.7. Hydrogen bonds and Pi interactions are one of the residues in the binding pocket that play a role in inhibiting the target protein of the original ligand. The similarity of the modification results with the original ligand lies in the amino acids PRO 155,154, ARG 44, TYR 131 and LEU 153 which are better than luteolin 7 glucosidase as the parent compound. The modified ligand also has the same amino acids as the positive control (celebrex) as an anti-inflammatory drug already circulating on the market, namely ARG 44, TYR 131 and LEU 153, so that the modified ligand can be developed into an oral dosage as an anti-inflammatory drug with good stability and safe to use.

CONCLUSION

Flavonoid derivative compounds interact with protoporphyrin ix containing co as the target macromolecule for the COX-2 enzyme, having an interaction pattern in the form of amino acid residues, namely ARG 121, VAL 524, LEU 353 and ILE 350. The binding free energy in the form of the binding affinity value influences the binding side of the 6OFY protein as a

COX-2 inhibitor, if the binding affinity value is the smallest (more stable) so it has a good interaction pattern. The design of the luteolin 7 glucosidase compound produces a new compound that has a better interaction pattern in the form of amino acid residues and is similar to protoporphyrin ix containing co as the original ligand, namely PRO 155, 154, ARG 44, TYR 131, LEU 153.

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