Molecular docking of 5-o-benzoylpinostrobin derivatives from *Boesenbergia pandurata* roxb. as anti-inflammatory

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Abstract

Background: The use of NSAIDs, also known as non-steroidal anti-inflammatory drugs, has numerous adverse effects and consequences. For this reason, it is necessary to develop rational drugs as safer anti-inflammatory drugs with fewer side effects. Temu Kunci rhizome contains Pinostrobin (5-hydroxy-7-methoxyflavanone), which is believed to have anti-inflammatory properties.

Objective: This study aims to determine the strongest anti-inflammatory activity at the cyclooxygenase-2 (COX-2) receptor through the 5-O-Benzoylpinostrobin derivative design.

Methods: AutoDockTools on the COX-2 receptor (PDB code: 5IKR) were used in molecular docking in this study. The metrics employed were binding afinity (ΔG), inhibition constant (Ki), which serve as indicators of affinities, and amino acid residue similarity, which serves as a measure of the similarity of interactions. Predictive scores were confirmed by Molecular Docking Simulation.

Results: The top five 5-O-Benzoylpinostrobin derivatives show a high affinity for the COX-2 receptor compared to Pinostrobin as a marker compound of Boesenbergia pandurata Roxb and furthermore give the lowest inhibition constant (Ki) and the highest negative binding free energy (ΔG), 35.40, 45.21, 54.75, 64.43, 76.97 nM and -10.16, -10.02, -9.91, -9.81, -9.7 kcal/mol. Interestingly, the five 5-O-Benzoylpinostrobin derivatives also have higher affinity than the native ligand Mefenamic acid, which is known to be a non-selective COX-2 inhibitor. The highest predicted affinity was shown by 4-Nitro-5-O-benzoylpinostrobin for the COX-2 receptor (PDP ID: 5IKR), with a higher predicted affinity for Mefenamic acid.

Conclusion: The five selected 5-O-Benzoylpinostrobin derivatives were potent modifications of pinostrobin as an anti-inflammatory because they showed a higher affinity than Pinostrobin and Mefenamic acid. This study demonstrated that it is highly feasible to produce and test the novel 5-O-Benzoylpinostrobin derivative *in vivo*, specifically 4-Nitro-5-O-benzoylpinostrobin.

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Introduction

NSAIDs, also known as non-steroidal anti-inflammatory drugs, are among the most widely used over-the-counter medications in the world, making up 5% of all prescription medications globally.[1] According to the research results of Mehuys *et al.* (2018),[2] analgesics that are often used in America are paracetamol (68.6%) and NSAIDs (46.8%). The majority of NSAIDs are used to treat pain and inflammatory diseases include menstrual cramps, osteoarthritis, rheumatoid arthritis, surgical conditions, and chronic pain.[1] However, it is necessary to monitor the use of NSAIDs. There are many side effects and complications of using NSAIDs. Side effects and complications due to the use of these NSAIDs include impaired kidney function, edema, hypertension, and bleeding in the gastrointestinal tract.[1,3,4]

For this reason, it is necessary to develop rational drugs as safer anti-inflammatory drugs with fewer side effects. The use of traditional medicine in the community to overcome and reduce symptoms caused by inflammation is widely found. Bioactive substances with anti-inflammatory properties include flavonoids and diterpenoids. Boesenbergia pandurata Roxb., Andrographis panic-

ulata, Kaempferia galanga L., Hibiscus sabdariffa L., and Tripterygium wilfordii Hook, f. (TWHF) are a few examples of plants with anti-inflammatory properties.[5-7] This study raised one of the traditional medicinal plants that are believed to have efficacy as an alternative anti-inflammatory, namely the temu kunci plant (Boesenbergia pandurata Roxb.). Temu Kunci rhizome contains Pinostrobin (5-hydroxy-7-methoxyflavanone), which is believed to have anti-inflammatory properties.[8] The development of Pinostrobin can be done by modifying the structure. Beginning with the determination of 20 analogs of 5-O-Benzoylpinostrobin using the modified Topliss method based on binding interactions, the better or higher the activity, the more negative the binding affinity (ΔG). The energy required for drug-receptor interactions decreases with decreasing binding affinity (ΔG) values, meaning the drug-receptor connection is more stable and the biological activity of a chemical can be anticipated by this.[9]

Materials and Methods

Materials

A HP brand laptop with an AMD Ryzen 5 5500U processor, Radeon Graphics 2,10 GHz RAM, and Windows 10-64-bit operating system was used as the research equipment. The cyclooxygenase-2 (COX-2) receptor's molecular structure with its natural ligand, mefenamic acid (ID8 601), using the Protein Data Bank website (https://www.rcsb.org/structure/5IKR), download PDB ID: 5IKR.[10] ChemDraw (PerkinElmer ChemOffice Suite) was used to create the 2D structure of the 5-O-Benzoylpinostrobin analog, which was then copied into Chem3D, AutoDock (The Scripps Research Institute, Inc).

Ligands preparation

The test ligands used are Pinostrobin, 5-O-Benzoylpinostrobin parent, and substituted (Table 1). ChemDraw was used to create the 2-dimensional structure of the analog of 5-O-benzoylpinostrobin (PerkinElmer ChemOffice Suite) and then copied into Chem3D, MMFF94 is used to measure the minimum energy, then saved as .mol2. The docking software used was AutoDock from The Scripps Research Institute. One of the advantages of AutoDock is that it can provide predictive value for the inhibition constant (K_i), which can give predictions for the *in vitro* analysis process later. All ligands were loaded and torque-adjusted using AutoDock.

Receptors preparation

Using AutoDockTools, receptors were downloaded in .pdb format. Unused components, such as water molecules, were eliminated, non-polar hydrogen was inserted, charged, and the size and coordinate grid were organized. By making the location of the ligand and the grid box's center, the size and coordinates of the grid box are automatically adjusted to the position of the ligand of each receptor.[11]

By docking pinostrobin and its analogs to the cyclooxygenase-2 (COX-2) receptor, PDB ID: 5IKR, anti-inflammatory action was predicted. Mefenamic acid, an over-the-counter anti-inflammatory medication, was utilized as a benchmark. The findings take the form of the binding affinity (ΔG) and the inhibition constant (Ki), which represent the interaction energy between the test substance and the 5IKR receptor.

Validation of docking protocol

The validation process is done through re-docking cocrystal ligands that have been extracted from receptors in the active site. When validating a ligand cocrystal at a chosen binding site, the Root Mean Square Deviation (RMSD) metric is used. Docking

Table 1. The 5-O-benzoylpinostrobin derivatives test compound.

No	Code	Compounds name	Functional Group		
			R1	R2	R3
1	P5O	5-O-benzoylpinostrobin	H	H	H
2	P2Cl	2-Chloro-5-O-benzoylpinostrobin	Cl	H	H
3	P3Cl	3-Chloro-5-O-benzoylpinostrobin	H	Cl	H
4	P4Cl	4-Chloro-5-O-benzoylpinostrobin	H	H	Cl
5	P2,4Cl2	2,4-Dichloro-5-O-benzoylpinostrobin	Cl	H	Cl
6	P3,4Cl	3,4-Dichloro-5-O-benzoylpinostrobin	H	Cl	Cl
7	P4Cl,3CF3	4-Chloro-3-trifluoromethyl-5-O-benzoylpinostrobin	H	CF_3	Cl
8	PNO2,3CF3	4-Nitro-3-trifluoromethyl-5-O-benzoylpinostrobin	H	CF_3	NO_2
9	P4F	4-Fluoro-5-O-benzoylpinostrobin	H	H	F
10	P4I	4-Iodo-5-O-benzoylpinostrobin	H	H	I
11	P3N(CH3)2	3-Dimethylamine-5-O-benzoylpinostrobin	H	$N(CH_3)_2$	H
12	P4Br	4-Bromo-5-O-benzoylpinostrobin	H	H	Br
13	P3CF3	3-Trifluoromethyl-5-O-benzoylpinostrobin	H	CF3	H
14	P4CF3	4-Trifluoromethyl-5-O-benzoylpinostrobin	H	H	CF_3
15	P4NO2	4-Nitro-5-O-benzoylpinostrobin	H	H	NO_2
16	P2CH3	2-Methyl-5-O-benzoylpinostrobin	CH_3	H	H
17	P4CH3	4-Methyl-5-O-benzoylpinostrobin	H	H	CH_3
18	P2OCH3	2-Methoxy-5-O-benzoylpinostrobin	OCH_3	H	H
19	P4C(CH3)3	4-t-Butyl-5-O-benzoylpinostrobin	H	H	$(CH_3)_3$
20	P3CH3	3-t-Butyl-5-O-benzoylpinostrobin	H	$(CH_3)_3$	H

software was used to predict the results from experimental positions with an RMSD of not more than 2.0 Å.[12,13]

Molecular docking

The goal of molecular docking is to determine the test ligand's most energy-efficient form of binding to the target receptor. The validation procedure was followed for docking both test ligands, using identical grid box dimensions and positions for each cocrystal ligand.

To ensure that the test ligand binds to the ideal position for each ligand, the binding site orientation is carried out by the blind docking method, and the results of all test ligands show a cavity with the highest affinity equal to the comparative ligand.[14] For this reason, the same grid box size is used for the docking process as the validation process. With a total of 100 genetic algorithm runs, a population size of 150, a moderate maximum energy evaluation number of 2,500,000, and a maximum number of generations of 27,000, this study used the Lamarckian genetic algorithm docking search settings. The main variables employed in the docking method were binding affinity (ΔG), inhibition constant (Ki), amino acid residues, and the amount of hydrogen bonds.[15] Binding affinity (ΔG) and inhibition constant (Ki) values are used to determine the strength of drug-receptor binding. The stronger drug-receptor connection is indicated by a greater negative binding affinity (ΔG) value and a lower inhibition constant (Ki) value. To determine the potential of the tested ligands as receptor blockers, all of the tested ligands were compared with the results of cocrystal ligand validation. To demonstrate the similarity of interactions, the amino acid residues of all the tested ligands were compared to the amino acid residues of the cocrystal ligands. The likelihood of the test ligand having similar activity to the cocrystal ligand increases with the degree of similarity between its amino acid residues.[16] The ligand-receptor interaction was seen using the BIOVIA Discovery Studio Visualizer.

Results

The validation process for the docking method

The validation process for the docking method uses a grid box size of XYZ (40, 40, 40) and the coordinates, and Table 2 shows the RMSD values obtained.

The mefenamic acid native ligand overlap

The Mefenamic acid native ligand overlap before and after redocking results can be seen in Figure 1.

The molecular docking parameters

Table 3 below shows the outcomes of molecular docking parameters.

Table 2. Grid center size and RMSD value validation results of 5IKR cyclooxygenase docking.

PDB ID	Grid center			Grid box			RMSD
	X	Y	Z	X	Y	Z	
5IKR	38.042	2.131	61.28	40	40	40	0.596 Å

RMSD, Root Mean Square Deviation.

Table 3. Molecular docking results parameters.

No	Compound name	G (kcal/mol)	Inhibition constant/K_i (nM)
1	4-Nitro-5-O-benzoylpinostrobin	-10.16	35.40
2	4-Chloro-5-O-benzoylpinostrobin	-10.02	45.21
3	3-t-Butyl-5-O-benzoylpinostrobin	-9.91	54.75
4	4-Bromo-5-O-benzoylpinostrobin	-9.81	64.43
5	4-Iodo-5-O-benzoylpinostrobin	-9.70	76.97
6	4-Fluoro-5-O-benzoylpinostrobin	-9.67	81.30
7	3-Chloro-5-O-benzoylpinostrobin	-9.60	92.29
8	2,4-Dichloro-5-O-benzoylpinostrobin	-9.58	94.44
9	4-Trifluoromethyl-5-O-benzoylpinostrobin	-9.47	115.00
10	4-Methyl-5-O-benzoylpinostrobin	-9.46	116.01
11	2-Chloro-5-O-benzoylpinostrobin	-9.43	122.61
12	2-Methyl-5-O-benzoylpinostrobin	-9.31	149.13
13	2-Methoxy-5-O-benzoylpinostrobin	-9.26	162.00
14	3,4-Dichloro-5-O-benzoylpinostrobin	-9.24	170.04
15	4-t-Butyl-5-O-benzoylpinostrobin	-9.20	181.01
16	3-Dimethylamine-5-O-benzoylpinostrobin	-8.82	344.43
17	5-O-Benzoylpinostrobin	-8.73	400.32
18	3-Trifluoromethyl-5-O-benzoylpinostrobin	-8.60	500.84
19	4-Chloro-3-trifluoromethyl-5-O-benzoylpinostrobin	-8.35	751.95
20	4-Nitro-3-trifluoromethyl-5-O-benzoylpinostrobin	-8.01	1.35
21	Native ligand: Mefenamic acid (ID8_601)	-7.67	2.38
22	Pinostrobin	-7.06	6.72

The visualization of the docking

Discovery Studio Visualizer was employed to obtain a visualization of the two-dimensional ligand-receptor interaction. Figure 2 presented a visualization of the docking results of 4-Nitro-5-O-benzoylpinostrobin compared to Mefenamic acid, Pinostrobin, and 5-O-Benzoylpinostrobin.

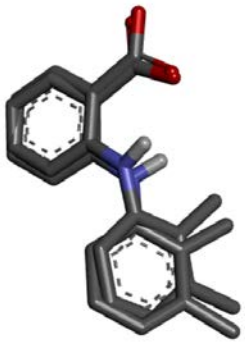

Figure 1. Mefenamic acid native ligand overlap before and after redocking.

Discussion

The validation process for the docking method

Docking parameter validation is a step that needs to be done before docking the test ligand. Re-docking the native ligand on the target protein's active site after it has been separated from its native ligand is one of the docking characteristics. The docking parameter is said to be valid if the design can anchor the native ligand or ligand complex to its original position with an RMSD value of less than 2Å also depending on the size of the ligand.[17]

The mefenamic acid native ligand overlap

The redocking process shows differences in the conformational structure of the ligand before and after the docking results in Figure 1 happens because the RMSD value obtained in the validation process is 0.596 Å. The smaller or closer to zero the RMSD value, the less the conformational change in the native ligand structure.

The parameters of molecular docking

Molecular docking validate with parameters in the form of RMSD value. The RMSD number can be used to compare the native ligand's conformation after docking to the original ligand's

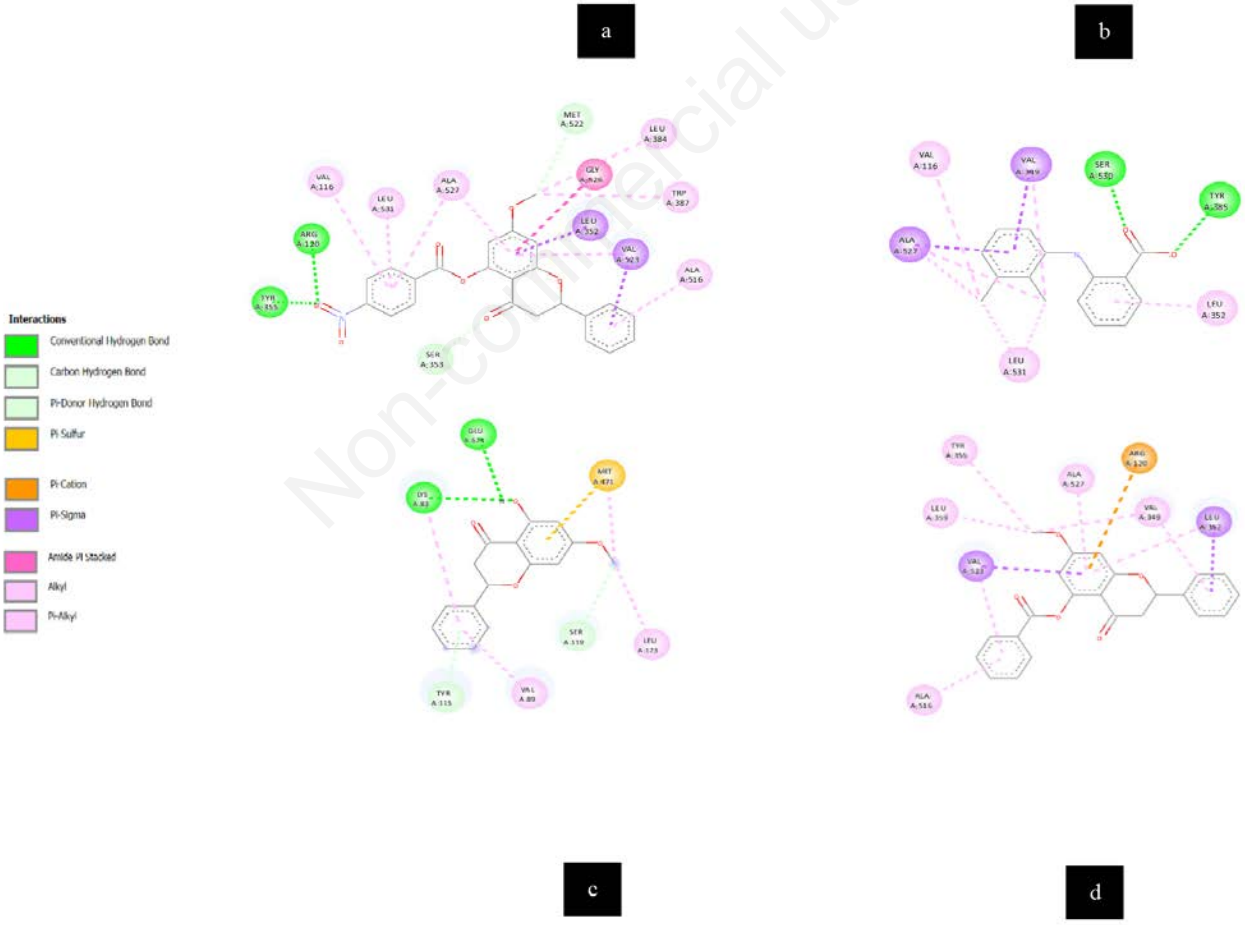

Figure 2. 2D visualization of the docking results of 4-Nitro-5-O-benzoylpinostrobin (a), Mefenamic acid (b), Pinostrobin (c), and 5-O-Benzoylpinostrobin (d).

conformation after crystallographic measurements.[18] The validation results show an RMSD of 0.596 Å. The results of self-docking of native ligands with cyclooxygenase-2 enzymes prove that the parameters are valid because the RMSD value is less than 2Å and is ready to be used for molecular docking that the test ligands.

When comparing the values of binding affinity (Δ G), inhibition constant (Ki), and hydrogen bond interactions between ligands and enzymes, good docking values may be seen. The energy of the binding affinity between the target and the ligand shows the stability and spontaneity of the binding. The bond is said to be more spontaneous and stable if the value of binding affinity (ΔG) is lower.[19] Comparing the 5-O-Benzoylpinostrobin analog compound to the native ligand of cyclooxygenase-2 and the comparative compound of anti-inflammatory medications, namely Mefenamic acid, the docking findings in Table 3 demonstrate the value of binding affinity (ΔG) and the inhibition constant (Ki) of the molecule, so it can be said that 5-O-Benzoylpinostrobin analog compounds are much more stable than native ligand compounds and Mefenamic acid comparison compounds.

As a flag chemical of Boesenbergia pandurata Roxb, the top five 5-O-Benzoylpinostrobin derivatives in Table 3 exhibit a high affinity for the COX-2 receptor and also offer the strongest negative binding affinity (G) and the lowest inhibition constant (Ki), respectively, -10.16, -10.02, -9.91, -9.81, -9.7 kcal/mol and 35.40, 45.21, 54.75, 64.43, 76.97 nM. Interestingly, the five 5-O-Benzoylpinostrobin derivatives also have a higher affinity than the native ligand Mefenamic acid, which is known to be a non-selective COX-2 inhibitor.

The highest predicted affinity was shown by 4-Nitro-5-O-benzoylpinostrobin for the COX-2 receptor (PDP ID: 5IKR), with a higher predicted affinity for Mefenamic acid. The main difference in the position of the ligand from the docking results is mainly the 4-nitrobenzoyl group, which interacts at a different position from the position of the entire functional group of Mefenamic acid.

The visualization of the docking

According to the study and visualization in Figure 2, the natural ligand Mefenamic acid and the test ligand 4-Nitro-5-O-benzoylpinostrobin share the identical two hydrogen bonds, the presence of hydrogen bonds provides conformational stability to the COX-2 receptor. Hydrophobic and electrostatic interactions are two additional influencing elements that affect the Gibbs free energy value between the ligand and receptors in addition to the relationship between hydrogen bonds.

To learn the outcomes of the docking between the test ligand and the reference ligand, visualization, and analysis of drug-receptor interactions were carried out. The visualization outcomes demonstrate how ligands and amino acid residues interact. It appears from the interaction that the ligand might attach to the COX-2 receptor and exert inhibitory function. The ligand's binding site, which is a protein-binding site, will have an impact on the protein's conformation and functionality. The amino acid residues known as binding sites are crucial in the formation of interactions including hydrogen bonds, hydrophobic bonds, and electrostatic bonds between ligands and macromolecules.[20] The encouraging results were shown in all the test ligands which had a higher affinity as anti-inflammatory compared to the native ligand Mefenamic acid, only Pinostrobin had a lower affinity.

Conclusions

This research has succeeded in designing the highest potential 5-O-Benzoylpinostrobin derivative as an anti-inflammatory, the five selected 5-O-Benzoylpinostrobin derivatives were potent modifications of Pinostrobin as an anti-inflammatory because they showed a higher affinity than Pinostrobin and Mefenamic acid. This study demonstrated that it is highly feasible to synthesis and test the novel 5-O-Benzoylpinostrobin derivatives *in vivo*, in particular 4-Nitro-5-O-benzoylpinostrobin.

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