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RESEARCH ARTICLE

Docking study and molecular dynamic approach to predicting the activity of 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid against COX-1 enzyme

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Keywords
4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid
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Abstract

Background: Ferulic acid is a phenolic acid compound that has anti-inflammatory and anti-thrombosis activity. However, ferulic acid has the disadvantage of poor absorption. Structural modifications can be made to increase the biological activity of the compound. In this research, the structure of ferulic acid was modified into 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid. **Objective:** The purpose of this research is to predict the activity of 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid. **Method:** It was carried out using the Pass Online Prediction, Autodock 1.5.7, Discovery Studio and Maestro Schrödinger 2020-1 software. **Result:** The activity prediction results showed that 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid had anti-inflammatory and anti-thrombotic activity. The molecular docking results showed that 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid had lower free bond energy values than ferulic acid, hence, predicted to have greater activity. Molecular dynamics also reveal better stability interactions of the 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid with COX-1 protein than the ferulic acid. **Conclusion:** The compound, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid is feasible to be synthesised.

Introduction

COVID-19 is an infectious disease and a significant challenge to global health. WHO declared the COVID-19 outbreak a global pandemic on March 11, 2020 (Cucinotta & Vanelli, 2020). Based on data from WHO, COVID-19 has reached 254 million cases globally and four million cases in Indonesia as of November 2021 (WHO, 2021). The severity of this disease is varied. Some people are asymptomatic and appear mild, such as flu, fever, dry cough, fatigue, or muscle aches. Coronaviruses have a functional receptor, namely angiotensin-converting enzyme 2 (ACE2). The binding of spike protein to ACE2 can cause infection due to its expression in type II pneumocytes in the respiratory system. There are cardiovascular implications of COVID-19 because ACE2 receptors are also widely expressed in the cardiovascular system. Patients with COVID-19 may have thrombolytic and coagulation disorders, induce a hypercoagulable state, and result in increased rates of thrombolytic and thromboembolic events (Ortega-Paz *et al*., 2021). COX-1 is the main form present in mature platelets in the blood, where it converts arachidonic acid to the intermediate PG-G/H, which is then converted to thromboxane A2 (Alegbeleye *et al*., 2020).

Ferulic acid or 3-methoxy-4-hydroxycinnamic acid belongs to the phenolic acid group, the sub-group of hydroxycinnamic acid (Contardi *et al*., 2021). Based on in vivo studies, ferulic acid had antithrombotic activity by decreasing the expression of αIIbβ3/FIB and

phosphorylation of AKT in THR-stimulated platelet activation (Choi *et al*., 2017). However, ferulic acid has the disadvantage that is difficult to penetrate the biological lipid bilayer membrane (Zhang *et al*., 2015). Structural modifications can be done to increase the biological activity of compounds (Siswandono, 2016).

Ferulic acid consists of an aromatic ring, a double bond (-C-C-), a carboxylic group (-COOH), a phenol hydroxy group (-OH), and a methoxy group (-OCH3). Modification of the phenolic -OH group of ferulic acid into carbonyl ester by binding methoxy at the position of the aromatic ring so that ferulic acid derivatives are formed, namely 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid. Based on research by Ekowati and authors (Ekowati *et al*., 2018), it can be seen that the MDS value of ferulic derivatives against the P2Y12 receptor is smaller than ferulic acid, so it predicted that ferulic acid derivatives have stronger anti-thrombotic activity than ferulic acid. Therefore, researchers will modify the structure of ferulic acid in the hope that 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid can increase its activity.

Methods

Activity prediction

The structure of the compound was drawn using the ChemDraw version 19.0 and then copied into the Chem3D version 19.0 to form a 3D structure and measure the minimum energy using MMF94 then saved as mol file {MDL Molfile (*.mol)}. Activity prediction is done using the Pass Online Prediction.

Molecular docking

The receptor used was COX-1 (PDB ID: 1CQE). The molecular structure of the receptor can be downloaded via the Protein Data Bank website. COX-1 has a native ligand, Flubriprofen code FLP_1650 [A]. The ligand used is ferulic acid and 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid. Ligand structures were drawn using the ChemDraw version 19.0 and then copied into the Chem3D version 19.0 to form a 3D structure and perform MMF94 minimisation then saved as mol2 file {SYBYL2 (*.mol2)}. The validation method was done by re-docking between the receptor and the native ligand. Validation is accepted if the root mean square deviation (RMSD) < 2.0. The molecular docking study of the compound was carried out using AutoDock 1.5.7. The result of molecular docking is shown in the form of free energy of binding. Visualisation of molecular docking was performed using Discovery Studio.

Molecular dynamics

Molecular dynamics (MD) study was carried out using Desmond integrated with Maestro Schrödinger 2020-1 software (Bower *et al.,* 2006; Zubair *et al.,* 2021). The MD system was generated using an SPC (simple point charge) water box to immerse protein-ligand complexes at 10 Å; then, charge neutralisation of the system was carried out to simulate physiological conditions by adding counter ions and salts containing sodium and chloride and set at 0.15 M. The MD process was carried out under OPLS_2005 forcefield and NPT conditions (pressure 1.63 bar and temperature 300 K) which were carried out for 120 ns with recording set at intervals of 1.2 ps for energy and 20 ps for trajectory.

Results

Activity prediction

Activity prediction is done using the Pass Online Prediction. The results showed that 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid was predicted to have anti-inflammatory activity (Pa = 0.683 ; Pi = 0.018) and anti-thrombotic activity (Pa = 0.589 ; Pi = 0.015).

Molecular docking

The first step that needs to be done is the validation method by re-docking the receptor with the native ligand three times. The RMSD value at the COX-1 receptor (PDB ID: 1CQE) was 1.322 ± 0.006. The molecular docking method has been valid so that further molecular docking can be carried out on ferulic acid and 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid ligands. Molecular docking results using AutoDock are shown as the free energy of binding (kcal/mol). The result of binding of the COX-1 receptor to ferulic acid was -6.27 ± 0.07 kcal/mol and to 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid was -8.28 ± 0.25 kcal/mol. The interaction between the ligands and the amino acids of the receptor can be seen in Table I.

Table I: Interaction between the ligands and the amino acid of the receptors

Compound Name	COX-1 (PDB ID: 1CQE)		
	Functional groups binding	Amino acid residue binding	Bound distance*
Flurbiprofen (Ligand native of COX-1)	$-CH_3$	116-Val	4.88
	-OH carboxyl	120-Arg	2.71
	-CO carboxyl	120-Arg	3.28
	$-CH_3$	349-Val	4.91
	Aromatic	349-Val	5.12
	Aromatic	352-Leu	5.30
	-OH carboxyl	355-Tyr	1.93
	$-CH_3$	359-Leu	4.50
	Aromatic	522-Met	5.82
	Aromatic	523-Ile	5.04
	Aromatic	527-Ala	3.42
	Aromatic	527-Ala	5.11
	-F	530-Ser	3.27
	$-CH_3$	531-Leu	4.83
Ferulic Acid	-CO carboxyl	120-Arg	2.85
	$-OCH_3$	348-Tyr	4.67
	$-OCH_3$	349-Val	4.25
	Aromatic	352-Leu	3.90
	$-OCH_3$	352-Leu	4.45
	-OH carboxyl	353-Ser	3.31
	-CO carboxyl	355-Tyr	2.76
	Aromatic	518-Phe	5.89
	Aromatic	526-Gly	3.87
	Aromatic	527-Ala	4.99
	$-OCH_3$	530-Ser	1.80
4-(4-methoxy) benzoyloxy-3 methoxycinnamic acid	$-OCH_3$	116-Val	5.35
	Aromatic	352-Leu	3.56
	$-OCH_3$	349-Val	4.27
	$-OCH_3$	359-Leu	4.06
	$-OCH_3$	381-Phe	5.16
	$-OCH_3$	384-Leu	4.47
	$-OCH_3$	385-Tyr	3.65
	$-OCH_3$	387-Trp	5.14
	$-OCH_3$	526-Gly	3.58
	Aromatic	526-Gly	4.16
	Aromatic	527-Ala	4.09
	Aromatic	531-Leu	5.33
	$-OCH_3$	531-Leu	4.04

* Conventional hydrogen bond; Alkyl/Pi-alkyl; Amide-Pi stacked/Pi-Pi stacked; Attractive charge; Pi-Sulfur; Pi-Sigma; Carbon hydrogen bond

The RMSD plot shows stable interactions among ligand-protein complexes (Figure 1A). In contrast with RMSD, the RMSF plot (Figure 1B) exhibits stable interactions between ligands and specific amino acids. The flurbiprofen, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid, and ferulic acid showed good

RMSF values (<2 Å) even for ARG120 (0.818 Å, 0.955 Å, 0.651 Å) and TYR355 (0.824 Å, 0.998 Å, 0.541 Å) respectively.

The molecular interactions with TYR355 were reported at 66%, slightly higher than flurbiprofen with identical interaction types. However, the interactions with ARG120 and the carbonyl group were mediated by a water molecule and lower than flurbiprofen. The weakest interactions were found on ferulic acid with 10% for TYR355 and ARG120 (Figure 2).

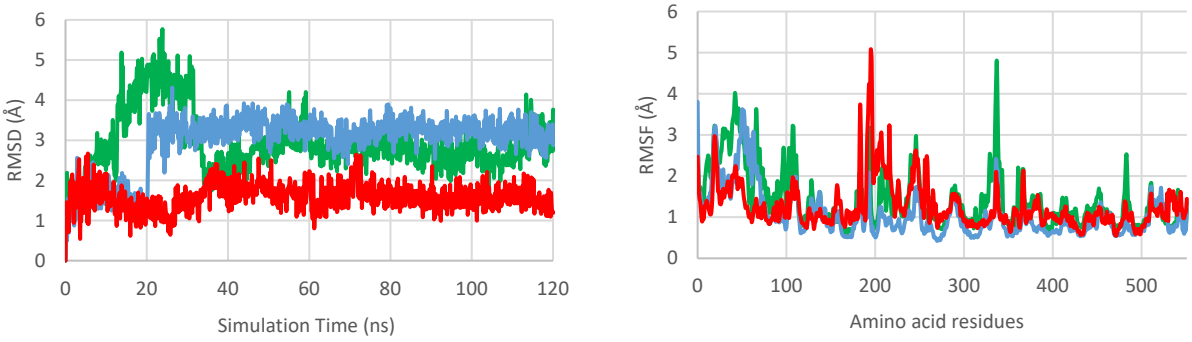

Figure 1: Root Mean Square Deviation (RMSD) (A; Left) and Root Mean Square Fluctuation (RMSF) (B; Right) analysis of ligand-protein complexes trajectories (red = Fluorbiprofen; green = 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid; blue = ferulic acid)

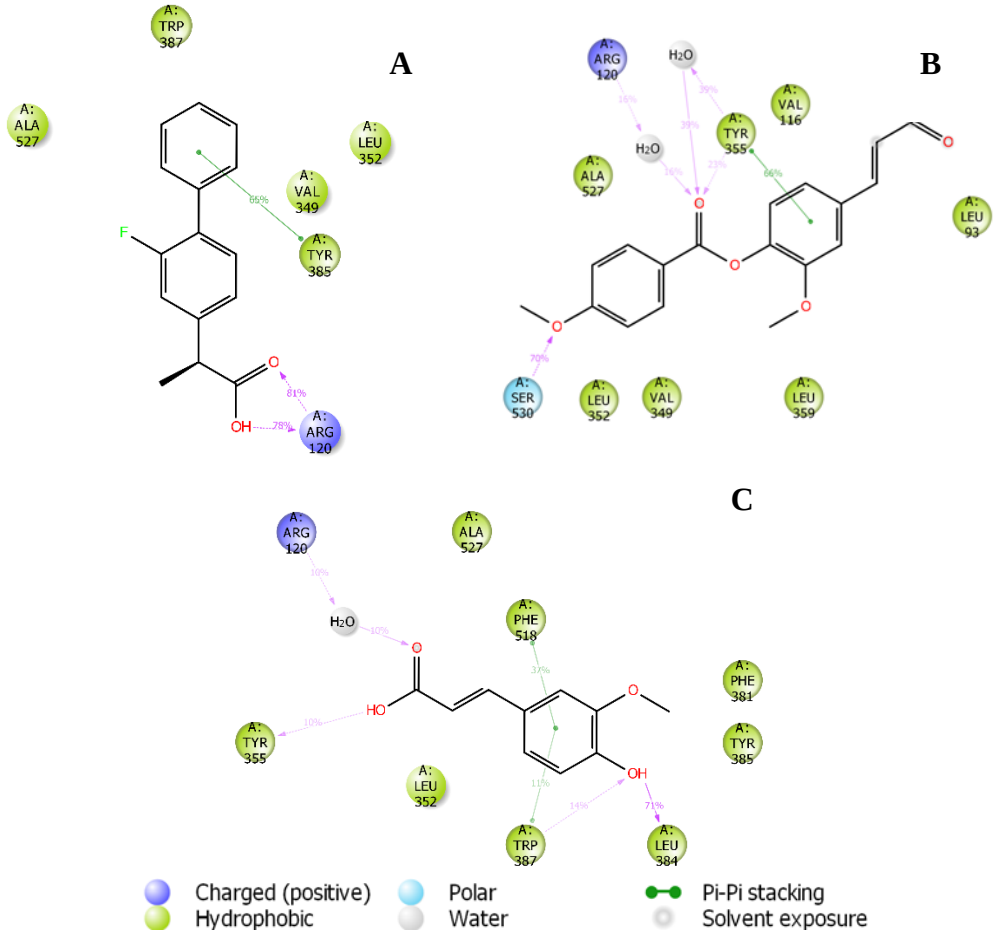

Figure 2: Molecular interactions analysis of ligand-protein after 120 ns simulation times (A= Flurbiprofen; B= 4-(4-methoxy) benzoyloxy-3-methoxycinnamic acid; C= ferulic acid)

According to the MMGBSA calculation result, the compounds with the highest to lowest stability interactions for 120 ns are FLurbiprofen>4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid > ferulic acid (Table II).

Table II: Molecular mechanics/generalised born surface area (MMGBSA) calculations after 120 ns simulation times

Compound	MMGBSA (kcal/mol)
Flurbiprofen	-56.90
Ferulic acid	-42.94
4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid	-49.49

Discussion

The results of activity prediction using the Pass Online Prediction are expressed by estimated either probabilities to be active (Pa) or probabilities to be inactive (Pi). If Pa > 0.7 has a relatively high probability of finding activity experimentally and may have a high degree of similarity with drug compounds in the same activity. If the compound has a value of 0.5 < Pa < 0.7 then it is less likely to find activity experimentally and may be less similar to a drug compound with the same activity. If a value of Pa < 0.5 has a lower probability of finding activity experimentally but if the activity is successful it is proven that the compounds found can be the parent compounds of a new group of chemical compounds on the biological activity (Filimonov *et al*., 2014). The compound, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid is predicted to have anti-inflammatory and anti-thrombotic activity with a value of 0.5 < Pa < 0.7 so it can find activity experimentally and may be similar to drug compounds with the same activity.

Molecular docking was performed using AutoDock 1.5.7 and method validation was performed. Theoretically, if the RMSD is less than 2.0, the molecular docking procedure is correct and the binding of the ligand to the receptor is stable. The smaller the RMSD value, the conformation of the native ligand of the re-docking result, and the closer it is to the native ligand result from crystallography so the deviation is smaller (Susanti *et al*., 2019). The result of the validation test shows that the RMSD value of the COX-1 receptor (PDB ID: 1CQE) is 1.322 ± 0.006. Therefore, it can be concluded that the molecular docking method has been valid so that further testing can be carried out on the binding of the receptor molecule, COX-1 to ferulic acid and 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid.

The result of molecular docking is shown as the free energy of binding. The lower the bond energy, the more stable the bond is formed and the stronger its activity (Ekowati *et al*., 2018). The sum of the intermolecular energy (ΔGintermolecular) and the free energy of torsion (ΔGtorsion) will give the value of the free energy of binding (ΔGbond) in Autodock. Energy intermolecular forces consist of van der Waals interaction, hydrogen bonds, and desolvation energies. Torsion energy is estimated to be obtained by multiplying the weight factor (Wtorsion) and the total ligand torsion (Ntorsion) (Sriramulu *et al*., 2019). Based on the test results, it can be seen that 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid, a ferulic acid derivative has a lower free energy of binding value than its parent compound, ferulic acid. The 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid showed more stable interaction because of the number of hydrophobic interactions, namely alkyl/Phi-alkyl increases with the same amino acid as flurbiprofen which is the native ligand of COX-1 and proving that the modified compound of ferulic acid interacted at the same active site as the native ligand., so it is predicted to have stronger activity against COX-1 receptor.

Since molecular docking studies were able to analyse the interactions of ligand-protein in rigid conditions, the authors conducted a molecular dynamic study to analyse the interactions in the dynamic behavior of both protein and ligand to evaluate the stability of binding mode within the binding cavities. To identify the complex stability of the selected compounds against the COX-1 receptor, the authors simulated the complex system up to 120 ns which is fairly enough to evaluate the ligand and Cα proteins stability interactions. The Root Mean Square Deviation (RMSD) measures the average change in displacement or conformation of a selection of atoms for a particular frame to a reference frame. The RMSD value interprets coordinates of differences in position from rigid to dynamic conditions on the atomic scale in macromolecular structures (Sargsyan *et al*., 2017). The RMSD plot shows stable interactions among ligand-protein complexes (Figure 1A). Flurbiprofen as the native ligand showed good stability interactions with COX-1 binding sites based on RMSD value <3 Å for 120 ns simulation times. Similar to flurbiprofen, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid also exhibits suitable interactions stability; unfortunately, these ligands seek the optimal binding mode for the first 30 ns simulation times and make the stable interactions until simulations are over. Meanwhile, ferulic acid bound stably only for the first 20 ns; however, the RMSD value was increased up to 4 Å,

suggesting these ligands were diffused away from the binding sites. In contrast with RMSD, the RMSF plot (Figure 1B) exhibits stable interactions between ligands and specific amino acids. The protein of COX-1 has two catalytic sites i.e., TYR355 and ARG120. These amino acids contribute to COX-1 activity, therefore binding in these essential amino acids could inhibit the activity of the enzyme (Barry *et al.,* 2001). Refer to the RMSF plot (Figure 1B), the flurbiprofen, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid, and ferulic acid showed good RMSF value (<2 Å) even for ARG120 (0.818 Å, 0.955 Å, 0.651 Å) and TYR355 (0.824 Å, 0.998 Å, 0.541 Å) respectively.

Despite the RMSD and RMSF criteria, the percentage of molecular interaction between the complex of ligands and specific amino acids of COX-1 influences the stabilisation profiles. The highest binding proportion occurred on flurbiprofen with the catalytic sites of the enzyme up to 65% on TYR385 by hydrophobic interactions (π-π), while ARG120 by Involving positive charge interaction for 78% as well as 81% among in the hydroxyl and carbonyl group respectively. These interactions are also supported by hydrophobic interactions in the enzyme cavity (VAL349, LEU352, TRP387, and ALA527). Similar to flurbiprofen, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid could interact with both catalytic sites. The molecular interactions with TYR355 were reported at 66%, slightly higher than flurbiprofen with identical interaction types. Although, the interactions with ARG120 and the carbonyl group were mediated by a water molecule and lower than flurbiprofen. The weakest interactions were found on ferulic acid with 10% for TYR355 and ARG120 (Figure 2). These results were suitable for the MMGBSA value, which is widely used to calculate the free energy of the binding of ligands to proteins (Godschalk *et al.,* 2013). As the MM-GBSA binding energies are approximate free energies of binding, the more negative value implicates the better (i.e. stronger) binding (Bathini *et al.,* 2016). According to the MMGBSA calculation result, the compounds with the highest to lowest stability interactions for 120 ns are FLurbiprofen>4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid > ferulic acid (Table II).

Based on the ADMET profile in our previous article (Ekowati *et al*., 2018), docking studies, and their molecular dynamics results, it can be seen that 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid is more active against COX-1 protein and prospect as an anti-thrombotic agent.

Conclusion

The compound, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid is predicted to have greater activity than ferulic acid. So, 4-(4-methoxy)benzoyloxy-3-methoxycinnamic acid is feasible to synthesise.

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