Chemoinformatics approach to design and develop vanillin analogs as COX-1 inhibitor

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Abstract

Background: Coronary Heart Disease (CHD), commonly known as the silent killer, impacted the severity of COVID-19 patients during the pandemic era. Thrombosis or blood clots create the buildup of plaque on the coronary artery walls of the heart, which leads to coronary heart disease. Cyclooxygenase 1 (COX-1) is involved in the production of prostacyclin by systemic arteries; hence, inhibiting the COX-1 enzyme can prevent platelet reactivity mediated by prostacyclin. To obtain good health and well-being, the research of discovery of new drugs for anti-thrombotic still continue.

Objective: This study aims to predict the potential of 17 compounds owned by the vanillin analog to COX-1 receptor using *in silico*.

Methods: This research employed a molecular docking analysis using Toshiba hardware and AutoDock Tools version 1.5.7, ChemDraw Professional 16.0, Discovery Studio, UCSF Chimera software, SWISSADME and pKCSM, a native ligand from COX-1 (PDB ID: 1CQE) was validated.

Results: The validation result indicated that the RMSD was <2 Å. The 4-formyl-2-methoxyphenyl benzoate compound had the lowest binding energy in COX-1 inhibition with a value of -7.70 Å. All vanillin derivatives show good intestinal absorption, and the predicted toxicity indicated that they were non-hepatotoxic. All these compounds have the potential to be effective antithrombotic treatments when consumed orally.

Conclusion: In comparison to other vanillin derivative compounds, 4-formyl-2-methoxyphenyl benzoate has the lowest binding energy value; hence, this analog can continue to be synthesized and its potential as an antithrombotic agent might be confirmed by *in vivo* studies.

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Introduction

Coronary heart disease is the leading cause of death worldwide and is known as the silent killer. At the period of the COVID-19 pandemic, coronary heart disease had a greater impact on the severity of COVID-19 patients than on those without coronary heart disease.[1] This is due to inflammation in the myocardium and microvascular dysfunction carried on by SARS-CoV-2, which promotes coronary plaque formation and even mortality.[2] Coronary heart diseases are caused by the buildup of plaque on the coronary arteries and other arterial walls throughout the body. Plaque formation on the coronary artery walls of the heart is induced by two processes, namely atherosclerosis and thrombosis. Thrombosis refers to the formation of a blood clot in both veins and arteries. Blood flow in the passageways that exist between blood cells such as platelets, plasma proteins, coagulation factors, and the endothelial lining of arteries and veins can be restricted by obstructions.[3] Aspirin is a common antithrombotic treatment, which still has side effects in the form of gastrointestinal disturbances[4] and induce bleeding.[5] As a side effect, important to accomplish is the development of novel antithrombotic. To gain good health and

well-being, the efforts of discovery new compounds as antithrombotic agents continue developing. The utilization of technology, such as chemoinformatic remains a great assistance in drug discovery and drug development.

Chemoinformatic plays a crucial role in the characterization of natural materials with the physicochemical properties of a chemical compound.[6] Machine learning is essential for drug designers to collect chemical information from enormous databases of substances in order to build drugs with certain biological features.[7] Computer Aided Drugs Discovery is a computer method utilized for the discovery of novel drugs, such as molecular docking.[8] Computational approaches could offer a significant contribution to the development of natural ingredients-based drugs and support experimental research during the early discovery phase. An important aspect of chemoinformatic is the search for lead chemicals and target proteins that affect the targeted activity. Cyclooxygenase 1 is one of the target proteins for reducing the formation of thrombosis (COX-1). COX-1 is involved in the production of prostacyclin by the systemic arteries;[9] hence, inhibiting the COX-1 enzyme can prevent prostacyclin-mediated platelet reactivity.[10] An antithrombotic mechanism with a COX-1 target can block the actions of thromboxane A2 (TxA2) in enhancing platelet aggregation, which can lead to severe cardiovascular thrombosis and cerebrovascular illness. If COX-1 is suppressed, TxA2 synthesis is reduced and platelet aggregation along with plug formation are decreased as well.[11] Many substances have been shown to reduce thrombosis by inhibiting COX-1, including ferulic acid. Beside inhibit COX-1, ferulic acid may also interact with one of the $P2Y_{12}$ receptor.[12] Vanillin is precursor to synthesize ferulic acid.[13] It was reported that vanillin exhibited *in vitro* anti-platelet aggregation activity which is generated by arachidonic acid, although it was inferior to aspirin in the control group.[14] In the antiplatelet aggregation test with induction variation conducted, vanillin inhibits 100 % of the aggregation induced by arachidonic acid *in vivo*. However, vanillin displays only low inhibition effect in test animals induced by collagen, thrombin, and platelet activity factor.[15]

In this study, vanillin was selected as the lead compound whose structure would be modified to generating some vanillin derivatives to enhance their bioactivity. Biological activity of a compound may be enhanced by modifying the molecular structure of the lead compound. The Topliss method is one of the molecular modification techniques that employs the various substituents that are predicted to increase the biological activity of compounds based on the physicochemical properties of substituents according to Hansch's concept of structure-activity relationships.

Attaching a substituent possessing lipophilic, electronic, and steric properties at specific position on the benzene ring, could modified the lead structure producing some molecules with greater activity.[16] According to previous report, modifying the structure of ferulic acid by changing the phenolic –OH group at the para position into an ester could produce additional hydrophobic interactions of alkyl groups (–R) and aromatic rings with amino acids at binding site of the receptor. Besides that, the presence of the additional carbonyl group in the ester formed increased the number of hydrogen bonds, which is needed for the increase in antiplatelet activity of the modified compound. In this study, the structure of the vanillin were modified by replacing the phenolic OH group with a various of esters.[12]

Then, modification of the benzene ring of the compound was a variation of the acyl group of the ester resulting in 17 designed compounds. The Topliss approach was used to generate 17 vanillin analogs that would be evaluated by *in silico* methods utilizing COX-1 enzyme to determine the compounds that would be developed as anti-thrombotic candidates. In addition to the inhibitory activity against COX-1, the ADME and toxicity (ADMET) profiles of the compound were important considerations in the selection of compounds to be developed as drug candidates. Prediction of ADMET profile could provide an important information of the type of compounds that would be present in the human body.[17] Therefore, the pkCSM tool will be used to evaluate the ADMET properties of the 17 designed compounds. On the basis of the *in silico* analysis of molecular docking, ADMET predictions, and the Lipinski Rules of Five, one compound having the best profiles in these study would be selected to be continued in research for antithrombotic agents.

Materials and Methods

Receptor preparation

The protein used as receptor was COX-1 (PDB ID: 1CQE) containing the native ligand FLP in chain A, which was downloaded from http://www.rcsb.org/pdb/ in a pdb format. Then the receptor was separated from its natural ligands and the water molecules using the Discovery Studio 2017 program. The docking validation was done by re-docking the natural ligand flurbiprofen to the receptor using the AutoDock Tools version 1.5.7 program. The validation parameter used was the RMSD (Root Mean Square of Deviation) and the validation successful criterion was the resulting RMSD value <2.0 Å.[18]

Ligands preparation

The structure of vanillin analogs as ligand was drawn in two-dimension (2D) using the ChemDraw version 20.1.1 program, then it was changed to a three dimension (3D) structure and saved in pdb format. The energy minimization of the 3D molecular structure was performed using the Avogadro program with the MMFF94 calculation which aims to obtain the most stable conformation before the simulation of ligand-receptor, then the optimized structures were save the file in pdb format. The classification of ligands in this study can be seen in Table 1.

Molecular docking analysis

After the validation met the requirements, the research was continued by performing molecular docking simulations for vanillin derivatives in the same way with two replications. The data obtained from the docking simulation were the binding energy (kcal/mol) that obtained from the best pose of ligand-receptor complex and the inhibition constant (Ki). The prepared ligands were set on 100 independent genetic algorithms (GA Runs). The parameter in docking simulation were set as: the population size 150, the maximum number of energy evaluations 2.500.000 (medium) and the maximum 27.000 generations, the maximum number of active sites 1, the gene mutation rate 0.02 and the crossover rate 0.8 used in the genetic algorithm method (4.2).[19]

Visualization of ligand-receptor interactions

In molecular docking studies, the visualization process plays an important role in determining the amino acid residue interactions that occur between the ligand and the target protein (receptor). All visualizations were evaluated using Discovery Studio program.[20]

Lipinski rules of five & ADMET prediction

In discovering the potential for new medicinal compounds, there were some rules that could be used to assess the effectiveness

of a compound whether pharmacologically active compounds could be promoted as a drug that would be given orally to humans, including the Lipinski Rule's of Five. To complete, it was also carried out an ADMET prediction to determine the pharmacokinetics of drug candidates including absorption, distribution, metabolism and excretion in the human body. Both predictions were done by processing the molecular structure of each compound in SMILES code at the pkCSM on line sites[21] and SWISSADME sites.[22]

Results

The results of molecular docking were shown in Table 1, which contained data of binding energy, inhibition constants, and hydrogen bond interactions with amino acid residues in COX-1.

Table 2 contains the results of the parameters in Lipinski Rule's of Five (RO5) were analysis using SwissADME.

The result of ADMET profiles of vanillin analogs can be seen

Table 1. Moleculer docking results of vanillin analogs with COX-1 enzyme.

Compound	COX-1 (ΔG) (kcal/mol)	Inhibition constant (uM)
Vanillin	-4.94	239.24
Native Ligand	-7.99	1.38
Aspirin	-4.70	356.01
4-formyl-2-methoxyphenyl 4-acetat	-5.39	112.34
4-formyl-2-methoxyphenyl 4-propionate	-5.49	94.42
4-formyl-2-methoxyphenyl butyrate	-5.78	58.32
4-formyl-2-methoxyphenyl pentanoat	-6.14	31.53
4-formyl-2-methoxyphenyl hexanoate	-6.55	15.86
4-formyl-2-methoxyphenyl heptanoate	-6.22	27.36
4-formyl-2-methoxyphenyl octanoate	-6.30	24.24
4-formyl-2-methoxyphenyl nonanoate	-7.00	7.39
R= substituted aromatic ring with donating subtituents		
4-formyl-2-methoxyphenyl benzoate	-7.70	2.28
4-formyl-2-methoxyphenyl 4-methoxybenzoate	-6.52	16.51
4-formyl-2-methoxyphenyl 4-methylbenzoate	-6.68	12.76
4-formyl-2-methoxyphenyl 4-hydroxybenzoate	-6.51	16.86
R= substituted aromatic ring with withdrawing subtituents		
4-formyl-2-methoxyphenyl 4-(trifluoromethyl)benzoate	-7.13	5.89
4-formyl-2-methoxyphenyl 4-fluorobenzoate	-7.40	3.77
4-formyl-2-methoxyphenyl 4-chlorobenzoat	-6.68	12.64
4-4((4-formyl-2-methoxyphenoxy)carbonyl)benzoic acid	-5.86	50.71
4-formyl-2-methoxyphenyl 4-bromobenzoate	-6.70	12.29

Table 2. The physicochemical properties of vanillin analogs according to Lipinski rule's of five obtained using SWISSADME.

Compounds	Molecule weight (<500) g/mol	Log P (<5)	H-bond donor (<5)	H-acceptor (<10)	Molar refractivity (40-130)
4-formyl-2-methoxyphenyl 4-acetat	194.18	1.43	4	4	49.82
4-formyl-2-methoxyphenyl 4-propionate	208.21	1.82	0	4	54.63
4-formyl-2-methoxyphenyl butyrate	222.24	2.21	0	4	59.43
4-formyl-2-methoxyphenyl pentanoat	236.26	2.60	0	4	64.24
4-formyl-2-methoxyphenyl hexanoate	250.29	2.99	0	4	69.05
4-formyl-2-methoxyphenyl heptanoate	264.32	3.38	0	4	73.86
4-formyl-2-methoxyphenyl octanoate	278.34	3.77	0	4	78.66
4-formyl-2-methoxyphenyl nonanoate	292.37	4.16	0	4	83.47
4-formyl-2-methoxyphenyl benzoate	256.25	2.73	0	4	69.72
4-formyl-2-methoxyphenyl 4-methoxybenzoate	286.28	2.74	0	5	76.21
4-formyl-2-methoxyphenyl 4-methylbenzoate	270.28	3.04	0	4	74.69
4-formyl-2-methoxyphenyl 4-hydroxybenzoate	272.25	2.43	1	5	71.75
4-formyl-2-methoxyphenyl 4-(trifluoromethyl)benzoate	324.25	4.90	0	7	74.72
4-formyl-2-methoxyphenyl 4-fluorobenzoate	274.24	3.29	0	5	69.68
4-formyl-2-methoxyphenyl 4-chlorobenzoat	290.70	3.38	0	4	74.73
4-4((4-formyl-2-methoxyphenoxy)carbonyl)benzoic acid	300.26	2.43	1	6	76.68
4-formyl-2-methoxyphenyl 4-bromobenzoate	335.15	3.49	0	4	77.42

in Table 3.

The interactions of aspirin and 4-formyl-2-methoxyphenyl benzoate visualized using the Discovery Studio (DSV) 2017 program, which can be seen in Figure 1, denoted dashed-line showing the interaction between the ligand and amino acids in the receptor.

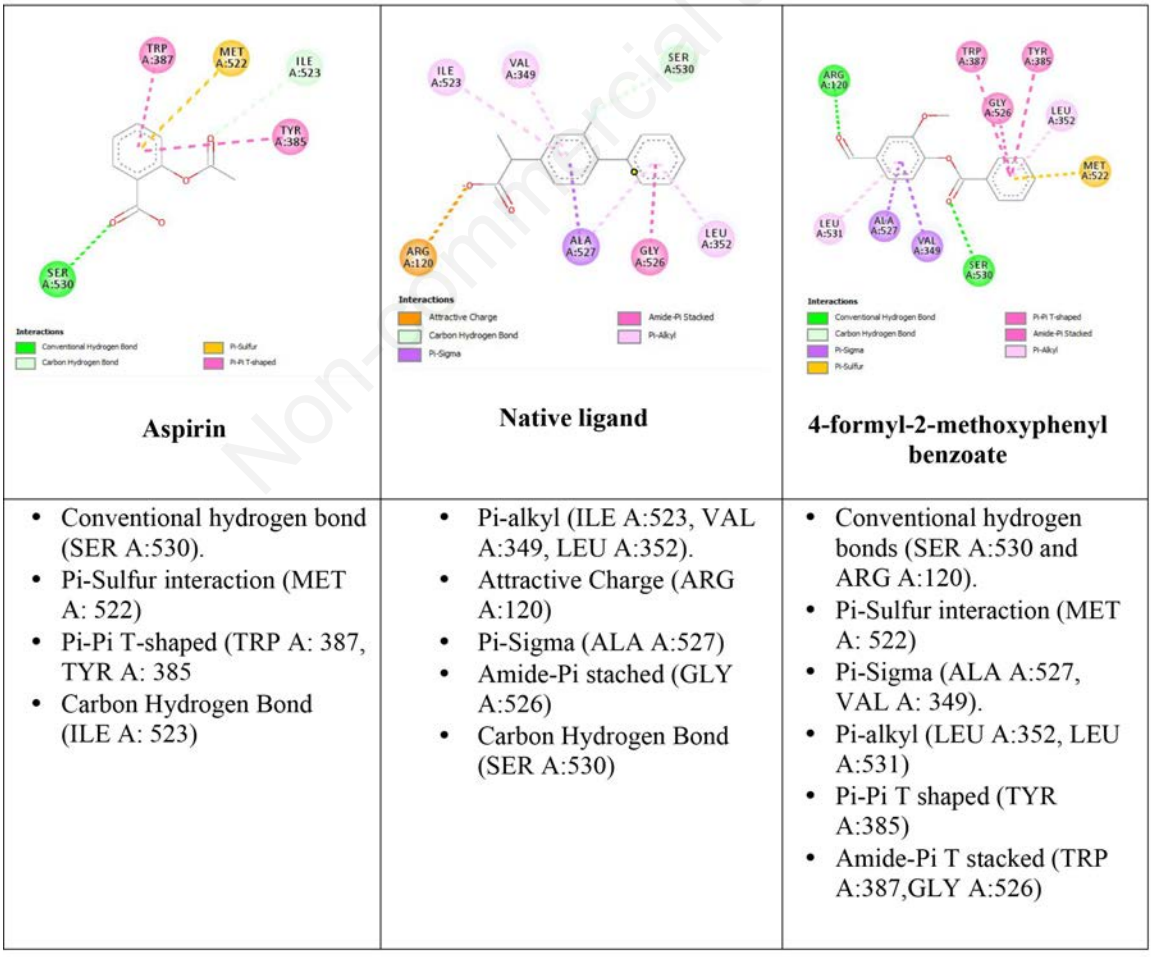
Figure 2 showed visualizations of the type of amino acids involved in ligand-receptor interaction and hydrophobic interactions of the selected ligands.

Discussion

Cyclooxygenase 1 (COX-1) plays a role in producing prostacyclin by systemic arteries,[9] so that when the COX-1 enzyme was inhibited, it could block the reactivity of platelets which mediated by prostacyclin.[10] One of the COX inhibitor drugs is Aspirin, belonging to the NSAID class, whose side effects often occur was a gastrointestinal disturbance which could lead to gastric ulcers.[4] So that there is a need for the discovery of new drugs from natural ingredients that are relatively safer and more nutritious. In this study, the receptor used was protein COX-1 (PDB:1CQE), where it still bound to its natural ligand, flurbiprofen.[23] The separation of the receptor from its natural ligand was carried out using the Discovery Studio 2021 program, and firstly the water molecule had to be removed so that the docking simulation could run effectively.[24]

The validation process was carried out to determine the suitability of the method we were working on and the RMSD value which described the stability of distance of the docked ligands.[25] RMSD of the validation process must be worth <2 Å so that it could be interpreted that the method used was appropriate and correct. In the validation process, the result was 1.42 Å by setting the grid box in the dimensions of x, y, z as 40, 40, 40 and the coordinates of the center grid box as x (31.082), y (37.894), and z (205.665) which aims to determine the binding site where the interaction between the ligand and the target protein take placed.[26]

Further studies were carried out on fifteen vanillin compounds classified in 3 groups, namely chain extension, aromatic substitution with electron-donating groups, and aromatic substitution with electron-withdrawing groups, for COX-1 as protein target. The results of the binding of binding energy were arranged according to the ranking from the best binding energy (Table 1). The lower the binding energy value, the more stable the conformation of the ligand-target protein complex.[27] The compound 4-formyl-2-methoxyphenyl benzoate had the best binding energy against COX-1 with a value of -7.70 Å among the other compounds. Aspirin has a binding energy of -4.70 Å, while all compounds of the modified vanillin showed better potency than Aspirin in inhibiting COX-1. The inhibition constant indicates the strength of the inhibitory action of a compound with the receptor, the smaller value of Ki, the stronger the inhibition.[28] The compound 4-formyl-2-methoxyphenyl benzoate also has the best Ki value among the



Aspirin	Native ligand	4-formyl-2-methoxyphenyl benzoate
• Conventional hydrogen bond (SER A:530). • Pi-Sulfur interaction (MET A: 522) • Pi-Pi T-shaped (TRP A: 387, TYR A: 385 • Carbon Hydrogen Bond (ILE A: 523)	• Pi-alkyl (ILE A:523, VAL A:349, LEU A:352). • Attractive Charge (ARG A:120) • Pi-Sigma (ALA A:527) • Amide-Pi stached (GLY A:526) • Carbon Hydrogen Bond (SER A:530)	• Conventional hydrogen bonds (SER A:530 and ARG A:120). • Pi-Sulfur interaction (MET A: 522) • Pi-Sigma (ALA A:527, VAL A: 349). • Pi-alkyl (LEU A:352, LEU A:531) • Pi-Pi T shaped (TYR A:385) • Amide-Pi T stacked (TRP A:387,GLY A:526)

Figure 1. Visualization of interactions of amino acid with aspirin, native ligand, 4-formyl-2-methoxyphenyl benzoate.

other compounds, which was 2.28 μM, while Aspirin has Ki of 356.01 μM.

In molecular docking studies, the visualization plays an important role. One of them is to determine the amino acid involved in interaction that occurs between the ligand and the target protein (receptor). Visualization of amino acid residues involved in interactions aims to see the type of interactions including van der Walls interactions, electrostatic interactions, hydrogen bonds, hydrophobic interactions (pi-alkyl, alkyl-alkyl, pi-sigma pi-pi interactions), and halogens.[29] The type of binding that was important in the molecular interactions was the hydrogen bond; in the DSV program, this bond might be divided into two conventional hydrogen and carbon hydrogen. Conventional hydrogen bonds are stronger than carbon hydrogen bonds,[30] besides supported interactions such as hydrophobic interactions, they also showed that compounds were increasing in their hydrophilicity, thereby increasing in the stability of ligand-receptor interactions.[31]

Compared to binding energy of the native ligand (-7.99) and 4-formyl-2-methoxyphenyl benzoate (-7.70), the binding energy of aspirin was the lowest (-4.70) (Figure 1). The number of hydropho-

Table 3. The results of ADMET profiles of vanillin analogs predicted using pkCSM.

Compound	Water solubility	Caco2 permeability	IA	VDss	CNS	BBB	CYP2D6	CYP3A4	Total clearance	Hepatoxity	LD50
Vanillin	-1.264	1.213	89.636	0.029	-2.221	-0.248	No	No	0.601	No	2.037
Aspirin	-1.868	0.09	76.938	-1.716	-2.489	-0.332	No	No	0.72	No	2.286
4-formyl-2-methoxyphenyl 4-acetat	-1.328	1.281	96.666	-0.379	-2.437	-0.203	No	No	0.727	No	2.032
4-formyl-2-methoxyphenyl 4-propionate	-2.689	1.281	97.723	-0.285	-2.862	0.095	No	No	0.442	No	2.112
4-formyl-2-methoxyphenyl butyrate	-3.111	1.305	97.348	-0.22	-2.858	0.048	No	No	0.456	No	2.086
4-formyl-2-methoxyphenyl pentanoat	-3.54	1.328	96.515	-0.161	-2.52	0.02	No	No	0.473	No	2.059
4-formyl-2-methoxyphenyl hexanoate	-3.977	1.525	95.919	-0.098	-2.523	0.01	No	No	0.49	No	2.038
4-formyl-2-methoxyphenyl heptanoate	-4.415	1.543	95.563	-0.035	-2.526	-0.015	No	No	0.508	No	2.025
4-formyl-2-methoxyphenyl octanoate	-4.837	1.561	95.213	0.025	-2.532	-0.056	No	No	1.506	No	2.018
4-formyl-2-methoxyphenyl nonanoate	-5.235	1.579	94.869	0.079	-2.54	-0.098	No	No	1.531	No	2.012
R= substituted aromatic ring with donating subtituents											
4-formyl-2-methoxyphenyl benzoate	-3.952	1.355	97.313	-0.463	-2.273	-0.2	No	Yes	0.595	No	2.042
4-formyl-2-methoxyphenyl 4-methoxybenzoate	-4.366	1.314	1.314	-0.414	-2.436	-0.248	No	No	0.752	No	2.35
4-formyl-2-methoxyphenyl 4-methylbenzoate	-4.236	1.354	1.354	-0.349	-2.207	-0.216	No	No	0.596	No	2.027
4-formyl-2-methoxyphenyl 4-hydroxybenzoate	-3.703	1.112	95.092	-0.442	-2.461	-0.394	No	No	0.71	No	2.107
R= substituted aromatic ring with withdrawing subtituents											
4-formyl-2-methoxyphenyl 4-(trifluoromethyl)benzoate	-5.067	1.447	94.04	-0.528	-2.116	-0.189	No	Yes	0.581	No	2.677
4-formyl-2-methoxyphenyl 4-fluorobenzoate	-4.342	1.352	97.049	-0.568	-2.318	-0.474	No	No	0.667	No	2.209
4-formyl-2-methoxyphenyl 4-chlorobenzoat	-4.576	1.38	96.084	-0.394	-2.166	-0.229	No	No	0.013	No	2.234
4-4((4-formyl-2-methoxyphenoxy)carbonyl)benzoic acid	-3.219	1.204	100	-2.141	-3.159	-0.657	No	No	0.752	No	2.301
4-formyl-2-methoxyphenyl 4-bromobenzoate	-4.678	1.385	96.017	-0.378	-2.144	-0.23	No	No	-0.008	No	2.249

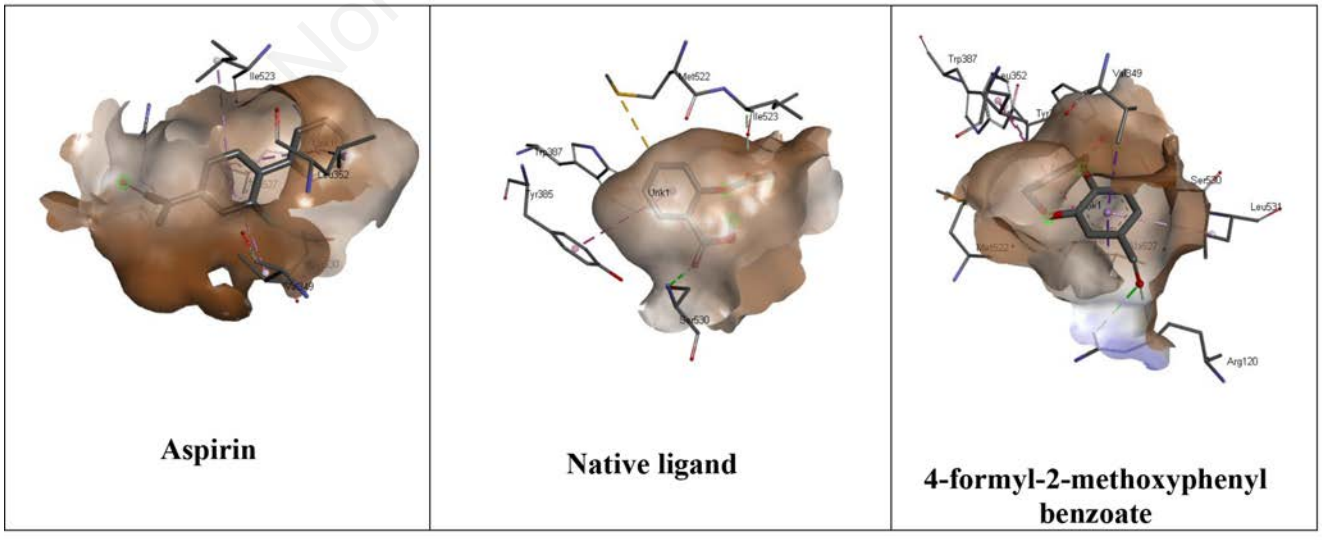


Figure 2. Visualization of hydrophobic environment of aspirin, native ligand, and 4-formyl-2-methoxyphenyl benzoate in binding interactions with COX-1.

bic interactions and the presence of hydrogen bonds determined the intensity of the binding between ligand and receptor (Figure 2). Aspirin formed one conventional hydrogen bond with SER A:530, whereas 4-formyl-2-methoxyphenyl benzoate has two conventional hydrogen bonds with SER A:530 and ARG A:120, giving it a lower binding energy so a better binding affinity. Furthermore, aspirin has 4 hydrophobic interactions, including Pi- Sulfur interaction, Pi-Pi T shaped interaction, Carbon Hydrogen Bond interaction with TRP A:387, MET A:522, ILE A:523, and TYR A:385, while the compound 4-formyl-2-methoxyphenyl benzoate has 8 hydrophobic interactions, namely Pi-Sulfur interaction, Pi-sigma interaction, Pi-alkyl interaction, Pi-pi T shaped interaction and Amide-Pi T stacked interaction with TRP A:387,GLY A:526,TYR A:385, LEU A:352, MET A:522, LEU A:531, ALA A:527, and VAL A:349, so this also offers binding affinity better than aspirin.

The compound 4-formyl-2-methoxyphenyl benzoate shared similarities with native ligand, specifically an amide-Pi Stacked interaction with GLY A:526 and Pi-Sigma interaction of benzene ring with ALA A:527. In the native ligand, there was an attractive charge-charge interaction between the negatively charged and deprotonated carboxylate group O with ARG A:120, which might strengthen the ligand-receptor binding. Therefore, the native ligand has slightly higher binding energy than 4-formyl-2-methoxyphenyl benzoate.

The Lipinski Rule's of Five (RO5) analysis (Table 2) aims to explore the pharmacokinetic properties of a drug molecule including absorption, distribution, metabolism and excretion in the human body[32,33] and predict the similarity of the molecular properties of compounds with an existing drug (Drug Likeness). The results obtained were that the molecular weight of 17 compound of vanillin analogs were less than 500 Da, meaning that the compounds abled to diffuse and penetrate cell membranes. The log P values was related to the ability of the compound to dissolve in a non-polar solvent, lipids, fats, and oils. All compounds showed log P values< 5 meaning that the compound was hydrophobic and could penetrate the lipid bilayer. Hydrophobicity plays a role in determining the drug's distribution in the body after the absorption process and the rates for metabolism and excretion.[34]

The number of hydrogen bond donor (HBD) has the requirement that it must be<5 and the number of hydrogen bond acceptor (HBA), which must be<10 and all compounds met the criteria. This number HBD and HBA showed that the more the number of H-bonds, the more energy was needed in the absorption process.[35] Molar refractivity (MR) indicated the polarity of a compound and has a requirement that it must be in the range of 40-130 and the MR of all compounds falls within that range. All tested compounds of vanillin analogs met the requirement of RO5, which meant these compounds have good absorption and were potentially effective for oral consumption by humans.

The permeability of caco-2 is used to see the process of drug absorption through the intestinal epithelial cell barrier in humans.[36] Only aspirin had a low permeability, while all vanillin derivatives possess a good value. Then, intestinal absorption (IA) plays an important role in maintaining the bioavailability of a drug to reach its target site. The ability of the compound to be absorbed is poor if the IA percentage<30%,[21] and all vanillin derivatives have a better value than aspirin and the parent vanillin. Furthermore, volume of distribution at steady state (VDss) is the theoretical volume that the dose of total drug needs to be distributed evenly to deliver the same concentration as in blood plasma. The higher the value volume of distribution, the more drugs are distributed in the tissue than plasma. Log VDss value>0.45 indicates a high distribution volume, while the Log VDss value<-0.15 indicates a low distribution volume.[21] Based on the results obtained, only compounds 4-formyl-2-methoxyphenyl 4-acetate, 4-formyl-2-methoxyphenyl 4-propionate, 4-formyl-2-methoxyphenyl butyrate, 4-formyl-2-methoxyphenyl pentanoate, and aspirin which were used has a low VDss value. Then, regarding the Central Nervous System (CNS), these parameters play an important role in the targeted drug assessment on CNS. Compounds with a CN log value >-2 means that they can penetrate CNS (good), whereas a compound which has a log value of CN <-3 is considered unable to penetrate the CNS[21], and all vanillin analogs meet good criteria. The results indicate that 17 vanillin derivatives have the potential to be used to treat CNS diseases (Table 3).

The Blood Brain Barrier (BBB) or the blood brain barrier is a system, a protective barrier consisting of tightly packed endothelial cells to filter certain substances to enter, from the blood into the brain.[37] The BBB permeability (log BB) can also be used as a parameter which can help to predict the process of reducing side effects and toxicity or increasing the pharmacological efficacy of a drug designed to target the receptor in the brain. Compounds having log BB value >0.3 considered to be well distributed or able to cross the blood barrier brain, whereas compounds with Log BB<-1 were not well distributed into the blood brain barrier.[21] According to the results, all compounds have log BB less than -2, so it can be interpreted that the compounds were predicted to be less potential to be used as drug candidates that work to penetrate the blood brain barrier.

There are two main isoenzymes responsible for drug metabolism, namely cytochrome P2D6 (CYP2D6) and cytochrome P3A4 (CYP3A4). Almost all vanillin derivatives do not affect or inhibit CYP2D6 and CYP3A4 enzymes, except the compounds 4-formyl-2-methoxyphenyl benzoate and 4-formyl-2-methoxyphenyl 4-(trifluoromethyl) benzoate, which can affect CYP3A4 enzymes, so it can be predicted that their derivatives tend to be metabolized by P450 enzymes in the body.

Total clearance shows the rate of excretion of a drug and plays a role in determining the drug dose in achieving steady-state concentrations and drug bioavailability.[21] High clearance rate will cause fast drug excretion, while a low clearance rate will cause the drug to be slowly excreted and can cause toxicity. All the test compounds have a range of amounts and excretion rates between -0.008 to 1.531. Lastly, in the prediction of toxicity, all vanillin derivative compounds do not have hepatotoxic properties, and the LD50 value is in the range of 2,012–2,267, included in category 5, which is non-toxic.

Conclusions

From all results of this study, it was concluded that all designed vanillin derivatives had good drug likeness and ADMET profiles. The compound 4-formyl-2-methoxyphenyl benzoate has the greatest potential activity as an anti-thrombotic agent based on its *in silico* interaction with COX-1 enzyme. These results must be proven by further research with synthesis and *in vivo* study.

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