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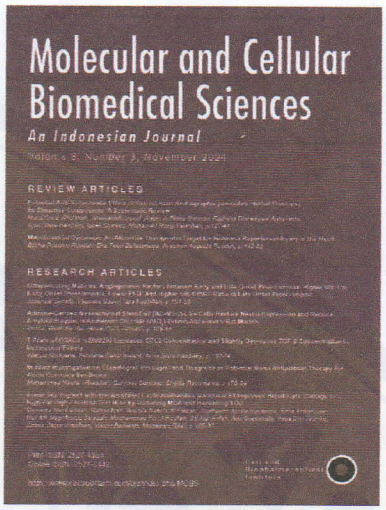

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RESEARCH ARTICLE

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In silico Investigation on Clopidogrel, Prasugrel and Ticagrelor as Potential Mono Antiplatelet Therapy for Acute Coronary Syndrome

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Background: In acute coronary syndrome (ACS), antiplatelet therapy is crucial for inhibiting platelet aggregation. Dual antiplatelet therapy (DAPT) commonly employs aspirin along with clopidogrel, prasugrel, or ticagrelor. This is well known that aspirin acts as a cyclooxygenase (COX)-1 inhibitor, while clopidogrel, prasugrel, and ticagrelor act as P2Y12 inhibitors. Despite DAPT's proven efficacy in more effectively reducing cardiovascular events in ACS patients, this is associated with an increased risk of bleeding compared to mono antiplatelet therapy (MAPT). To minimize the cost and side effect that might arise from the use of DAPT, this is necessary to assess the potential of MAPT using a P2Y12 inhibitor drug, to understand whether they are capable of binding to both COX-1 and P2Y12. Hence, this study was conducted to identify P2Y12 inhibitor drugs that have the ability to bind to COX-1, allowing them to be proposed as MAPT.
Materials and methods: Molecular docking was employed to assess binding affinity, interaction types, amino acid residues, binding distances, and visualizations in both 3D and 2D formats. The applications utilized were BIOVIA Discovery Studio, AutoDock and PyMol, while the websites utilized were research collaboratory for structural bioinformatics protein data bank (RCSB PDB) and PubChem.
Results: *In silico* findings reveal differences in binding strength among clopidogrel, prasugrel, and ticagrelor to COX-1 and P2Y12, with ticagrelor emerging as the stronger ligand due to a higher number of bindings and/or closer binding distances. Notably, only prasugrel and ticagrelor demonstrate the ability to bind to the active site of COX-1.
Conclusion: Therefore, prasugrel and ticagrelor emerge as potential MAPT agents for ACS patients.

Keywords: *clopidogrel, prasugrel, ticagrelor, antiplatelet, ACS, in silico, molecular docking*

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Introduction

Coronary artery disease (CAD) is an imbalance in the supply and consumption of blood and oxygen to the myocardium, caused by a blockage in the coronary artery and it can be initiated by vascular calcification.[1,2] CAD has become the third leading cause of mortality worldwide, with 17.8 million deaths annually.[3] There are two types of CAD, namely acute coronary syndrome (ACS) and stable ischemic heart disease (SIHD). The mortality ratio for ACS is higher than SIHD, at 12% and 1-2% respectively every year.[4,5] ACS is initiated when an atherosclerotic plaque ruptures, causing the exposure of tissue factor to blood flow. This event activates the coagulation cascade and circulates platelets, which play a crucial role in forming a thrombus. The platelets adhere to the damaged endothelium, releasing adenosine diphosphate (ADP) and thromboxane (TXA-2), contributing to thrombus formation. Consequently, blood flow may be completely or partially blocked.[1,6]

In ACS, antiplatelet therapy is currently employed to inhibit platelet aggregation in the thrombotic process and prevent complications during percutaneous coronary intervention (PCI) therapy. Antiplatelet agents comprise several groups, including cyclooxygenase (COX)-1 inhibitors, P2Y12 inhibitors, glycoprotein IIb/IIIa inhibitors, phosphodiesterase (PDE)-3 inhibitors, and protease-activated receptor (PAR-1) inhibitors.[7] The European Society of Cardiology recommended dual antiplatelet therapy (DAPT) using aspirin and clopidogrel as platelet aggregation inhibitors for ACS patients undergoing planned PCI.[8]

Although DAPT has been proven to be more effective in reducing cardiovascular events in ACS patients by inhibiting two important target proteins, namely COX-1 and P2Y12, a systematic review study shows that DAPT also comes with an increased risk of bleeding compared to mono antiplatelet therapy (MAPT). Although clopidogrel exhibits better efficacy compared to aspirin in reducing thrombotic events, while aspirin is more prone to cause gastrointestinal side effects[9,10]; however, clopidogrel is currently also falling out of favor due to research evidence suggesting a prolonged pharmacological requirement and an association with genetic variability. Based on above reasons, adopting MAPT might offer more benefits in terms of minimizing costs and side effects.[11,12]

Consequently, new antiplatelet agents, such as prasugrel and ticagrelor, have been proposed for their improved efficacy and reduced side effects.[8] Numerous randomized controlled trials (RCTs) study that enrolled 13,608 ACS patients treated prasugrel showed a mortality hazard ratio of 0.81 compared to clopidogrel, meanwhile 18,624 patients were treated ticagrelor exhibit a mortality hazard ratio of 0.84 compared to clopidogrel.[13,14] Generally, aspirin has been discovered to acts as a COX-1 inhibitor, while clopidogrel, prasugrel, and ticagrelor act as P2Y12 inhibitors.[7] They all share the same effect, *i.e.*, antiplatelet activity, but the binding potency of clopidogrel, prasugrel and ticagrelor to COX-1 is still unknown.

Up to date, there is no existing research comparing the binding strengths of clopidogrel, prasugrel and ticagrelor to both COX-1 and P2Y12. DAPT used in ACS could be reconsidered and might be potentially replaced with MAPT using clopidogrel, prasugrel, and ticagrelor if they are capable of binding to COX-1. Additionally, it is also interesting to investigate whether prasugrel and ticagrelor are still superior to clopidogrel in terms of binding strength to COX-1. Therefore, the objective of this research is to compare the binding strengths of clopidogrel, prasugrel, and ticagrelor to the COX-1 enzyme and P2Y12 receptor in ACS therapy.

Materials and Methods

Protein Preparation

The COX-1 enzyme (PDB ID:6Y3C) and P2Y12 receptor (PDB ID: 4NTJ) were used as target proteins in this research with the .pdb format, downloaded from research collaboratory for structural bioinformatics protein data bank (RCSB PDB). To prepare the protein file, AutoDock tools 1.5.6 (https://autodocksuite.scripps.edu/adt/) and BIOVIA Discovery Studio v21.1.0.20298 (https://discover.3ds.com/discovery-studio-visualizer-download) applications were used. To prepare the protein file, the H_2O group and native ligands on the protein were removed. This was because H_2O on the target protein can impair the molecular docking process and lower accuracy. Hydrogen atoms on polar groups also need to be added in protein preparation to account for hydrogen atoms in the protein so that hydrogen bonds can be formed and Kollman charges added. Subsequently, the protein file was saved in .pdbqt format.

Ligand Preparation

The 3D structures of the test and comparison ligands were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.

gov/) in .sdf format. The ligands used in this research include arachidonic acid, ADP, aspirin, clopidogrel, prasugrel, and ticagrelor. For COX-1 docking, arachidonic acid served as the native ligand; aspirin as the positive control; while clopidogrel, prasugrel, and ticagrelor as the test ligands. For P2Y12 docking, ADP acted as the native ligand; clopidogrel, prasugrel, and ticagrelor served as positive control; while aspirin as the test ligands. The term "native ligand" refers to a ligand binding with the protein in physiological processes, "positive control" indicates a ligand that has been established or proven to bind with the protein, and "test ligand" denotes a ligand used for comparing docking results with the native ligand and positive control. The ligands' format was changed to .pdb using BIOVIA Discovery Studio to align with the molecular docking process. Subsequently, ligands were prepared using AutoDock tools 1.5.6 to add hydrogen atoms, Gasteiger charge and assess rotation. The prepared ligand files were then saved in .pdbqt format.

Grid Box

The grid box was arranged to generate precise binding locations between proteins and ligands. Additionally, the grid box arrangement was performed to identify the binding site coordinates within a protein. The grid box size was adjusted on the protein using the x, y, z axis, and angstrom (Å) units until encompassing the entire active surface of the protein. The active sites of Cox-1 include Leu-117, Arg-120, Phe-205, Phe-209, Val-344, Ile-345, Tyr-348, Val-349, Leu-352, Ser-353, Tyr-355, Leu-359, Phe-381, Leu-384, Tyr-385, Trp-387, Phe-518, Ile/Val-523, Gly-526, Ala-527, Ser-530, Leu-531, Gly-533, and Leu-534.[15,16] Meanwhile, the active sites of P2Y12 consist of Cys-97, Val-102, Tyr-105, Phe-106, Tyr-109, Met-152, Leu-155, Ser-156, Asn-159, His-187, Val-190, Asn-191, Cys-194, Phe-252, Ala-255, Arg-256, Tyr-259, Leu-276, and Val-279.[17] The grid center and grid size coordinates of the proteins were saved in a notepad file in .txt format for use in molecular docking simulations.

Docking Method Validation

Before conducting molecular docking, the validation of the docking location method was necessary for reliability verification in docking simulations. This validation was performed separately using the protein and native ligand in BIOVIA Discovery Studio and then conducting redocking with AutoDock Vina. The redocking results were compared with the native ligand in the initial file using PyMOL 2.5.4 (https://pymol.org/) to assess conformational alignment, which was quantified by root mean square deviation (RMSD). The docking method was considered valid if the RMSD value is <2Å.[15,18] In this research, COX-1 (PDB ID: 6Y3C) exhibited an RMSD value of 0.989 Å, whereas P2Y12 (PDB ID: 4NTJ) showed RMSD value of 0.258 Å.

Molecular Docking Simulation

After preparing the protein and ligand structures in.pdbqt format, molecular docking was conducted using AutoDock Vina. Before performing the docking, a configuration file needed to be created, containing the receptor and ligand file names, grid center and size coordinates, and the docking result file name. Molecular docking was executed using the command prompt.

The result of this process includes binding affinity and structure prediction from the docking results. Binding affinity characterizes the efficiency of protein-ligand, protein-peptide, and protein-protein docking.[19] The more negative the score, the less energy was needed for the ligand to form a complex with the protein. Consequently, the ligand binds to the protein more easily and stably.[20] If the binding affinity of the test ligand was more negative than that of the comparison ligand, it can be concluded that the test ligand has the potency to inhibit the target protein.[21] The conformation with the lowest binding affinity complex was chosen for further analysis.

Analysis and Visualization

Visualization of the complex structure was conducted using BIOVIA Discovery Studio v21.1.0.20298. This visualization aimed to identify the amino acid residues of the target protein and ligand. Subsequently, the data obtained can be used to analyze the potency of the ligand as an antiplatelet. Various interactions, such as hydrogen bonds and van der Waals bonds were examined during this visualization process.

Results

The results of this research include binding affinity scores, interaction types, amino acid residue types, binding distances, and visualizations in both 3D and 2D formats.

Arachidonic Acid and Aspirin Binding to COX-1

Each ligand exhibited nine interaction models, with the lowest binding affinity score for arachidonic acid being -5.2

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kcal/mol, while aspirin had a score of -6.5 kcal/mol (Table 1). Arachidonic acid and aspirin could bind to the COX-1 active site . Arachidonic acid formed a hydrogen bond with Arg-120 and a van der Waals bond with Tyr-355, whereas aspirin formed van der Waals bonds with Tyr-348, Tyr-385, and Trp-387 (Figure 1).

Clopidogrel, Prasugrel, and Ticagrelor Binding to COX-1

Each ligand exhibited nine interaction models, with the lowest binding affinity scores for clopidogrel, prasugrel, and ticagrelor were -6.3 kcal/mol, -7.1 kcal/mol, and -7.9 kcal/mol, respectively (Table 1). Only prasugrel and ticagrelor could bind to the active site of COX-1. Prasugrel formed a hydrogen bond with Arg-120, whereas ticagrelor formed a van der Waals bond with Arg-120 (Figure 1).

The docking of clopidogrel, prasugrel, and ticagrelor to COX-1 showed lower binding affinity than arachidonic acid, the native ligand (Table 1). When compared to aspirin, used as a positive control, only prasugrel and ticagrelor exhibit lower binding affinity. Among the test ligands, ticagrelor demonstrated the lowest binding affinity, followed by prasugrel and then clopidogrel.

ADP Binding to P2Y12

ADP had nine interaction models, with the lowest binding affinity score being -6.7 kcal/mol (Table 2). ADP could bind to the active site of P2Y12, forming a hydrogen bond with Tyr-109, Arg-256, and Asn-191. Additionally, van der Waals interactions occurred with Val-102, Tyr-105, Asn-159, Val-190, Cyst 194, Leu-276, and Phe-252 (Figure 2).

Clopidogrel, Prasugrel, Ticagrelor, and Aspirin Binding to P2Y12

Each ligand had 9 interaction models, with the lowest binding affinity scores being -6.7 kcal/mol for clopidogrel, -7.5 kcal/mol for prasugrel, -8.7 kcal/mol for ticagrelor, and -6.7 kcal/mol for aspirin (Table 2). All ligands could bind to the P2Y12 active site. Clopidogrel formed a hydrogen bond with Asn-191 and Arg-256, van der Waals bonds with Tyr-109, Ala-255, and Tyr-259, and other bonds with Tyr-105 and Phe-252. Prasugrel formed van der Waals bonds with Tyr-109, Val-190, Cys-194, Phe-252, Ala-255, Arg-256, and Val-279, and other bonds with Tyr-105, Asn-191, Tyr-259, and Leu-276. Ticagrelor formed hydrogen bonds with Tyr-109, Asn-159, and Asn-191, van der Waals bonds with His 187, Val-190, Phe-252, Ala-255, and Val-279, and other bonds with Tyr-105, Arg-256, Tyr-259, and Leu-276. Aspirin formed van der Waals bonds with Cys-97 and Tyr-105.

The docking of prasugrel and ticagrelor to P2Y12 had lower binding affinity than ADP as the native ligand, while clopidogrel and aspirin exhibit the same binding affinity as ADP (Table 2). Among the test ligands, ticagrelor demonstrated the lowest binding affinity, followed by prasugrel, then clopidogrel and aspirin.

Discussion

Several factors can affect binding affinity, including the number and distance of bindings. The more bindings formed with the fewest distances, the lower the binding affinity score.[22-26] The docking results of this research reveal that the binding affinities of clopidogrel, prasugrel, and ticagrelor to COX-1 are -6.3 kcal/mol, -7.1 kcal/mol, and -7.9 kcal/mol, respectively.

Ticagrelor has a lower binding affinity to COX-1 than prasugrel and clopidogrel, indicating that forming a complex with COX-1 requires less energy and is easier for ticagrelor compared to prasugrel and clopidogrel. However, the number of bonds formed by ticagrelor to COX-1 is slightly less than prasugrel, *i.e.*, 15 and 16, respectively. Therefore, the lower binding affinity of ticagrelor compared to prasugrel may be caused by the fact that the binding distance of ticagrelor is closer to COX-1 than prasugrel. Additionally, the number of bonds formed by ticagrelor to COX-1 is more than that of clopidogrel, *i.e.*, 15 and 14, respectively, resulting in a lower binding affinity score.

All these drugs exhibited lower binding affinity than arachidonic acid, suggesting a stronger potential to bind to COX-1 than arachidonic acid. However, only prasugrel and ticagrelor can bind to the active site of COX-1, thereby influencing COX-1. *In silico* studies demonstrated the superiority of prasugrel and ticagrelor over clopidogrel in binding to COX-1. Prasugrel forms a hydrogen bond with Arg-120, while ticagrelor forms a van der Waals bond with Arg-120. Arg-120 is a crucial amino acid known for its primary role in COX-1 catalysis.[27] This study also revealed that arachidonic acid binds to Arg-120, supporting previous research indicating arachidonic acid's binding to Arg-120, Tyr-355, and Ser-530.[28] There is a similarity in the amino acid residues bound by arachidonic acid, prasugrel, and ticagrelor to COX-1, suggesting that these drugs may prevent arachidonic acid from binding to COX-1, inhibiting the production of TXA-2 and preventing platelet

Table 1. The comparison involves evaluating the binding affinity, interaction type, and amino acids that have bound between clopidogrel, prasugrel, and ticagrelor to COX-1.

Ligand	Binding Affinity (kkal/mol)	Interaction Type between Ligand and COX-1			Number of Binding
		Hydrogen (distance)	Van der Waals	Others	
Arachidonic Acid	-5,2	Arg-120 (2.46 Å)[1], Glu-524 (1.86 Å)	Thr-89, Leu-112, Tyr-355[1], Leu-357	Arg-83, Pro-84, Pro-86, Leu-93, Leu-115, Val-116, Val-119	Total: 13 (H: 2; V: 4; O: 7), In active site: 2 (H: 1; V: 1; O: 0)
Aspirin	-6,5	Ala-202 (2.30 Å), Thr-206 (2.58 Å)	Ala-199, His-207, Tyr-348[1], Tyr-385[1], Trp-387[1], Leu-390	Gln-203, His-388	Total: 11 (H: 2; V: 8; O: 1), In active site: 3 (H: 0; V: 3; O: 0)
Clopidogrel	-6,3	Arg-83 (2.86 Å)	His-43, Gln-44, Thr-62, Tyr-64, Thr-76, Arg-79, Asn-80, Asn-122, Lys-468, Gly-471		Total: 11 (H: 1; V: 10; O: 0), In active site: 0 (H: 0; V: 0; O: 0)
Prasugrel	-7,1	Gln-44 (2.85 Å), Arg-120 (2.38 Å)[1]	His-43, Tyr-64, Arg-79, Thr-76, Val-119, Lys-468, Arg-469, Phe-470, Gly-471, Glu-524, Pro-528	Asn-80, Arg-83, Leu-123	Total: 16 (H: 2; V: 11; O: 3), In active site: 1 (H: 1; V: 0; O: 0)
Ticagrelor	-7,9	Glu-524 (1.89 Å)	Arg-83, Pro-84, Pro-86, Phe-99, Leu-112, Arg-120[1], Leu-357	Thr-89, Leu-92, Leu-93, Trp-100, Leu-115, Val-116, Val-119	Total: 15 (H: 1; V: 7; O: 7), In active site: 1 (H: 0; V: 1; O: 0)

1: Reference to amino acids is included in the protein active site . H: Hydrogen bond. V: van der Waals bond. O: Other.

aggregation. Additionally, other studies have shown that P2Y12 inhibitors can reduce TXA-2 levels, despite TXA-2 being a major product of COX-1 activity.[29,30] This suggests the possibility that P2Y12 inhibitors may bind to COX-1 and inhibit its activity, as P2Y12 receptors are involved in other cellular processes such as the activation of glycoprotein IIb/IIIa.[31] These findings support the argument for transitioning from using DAPT to MAPT in ACS patients, as prasugrel and ticagrelor can bind to two target proteins, COX-1 and P2Y12. It may provide a benefit by avoiding the increased bleeding risk associated with using DAPT.

When compared with aspirin, a drug established to inhibit COX-1, prasugrel and ticagrelor exhibit lower binding affinity, while clopidogrel shows higher binding affinity. However, there are differences in amino acid residues bound by prasugrel and ticagrelor to COX-1 compared to aspirin. Aspirin binds to Tyr-348, Tyr-385, and Trp-387, whereas prasugrel and ticagrelor do not bind to

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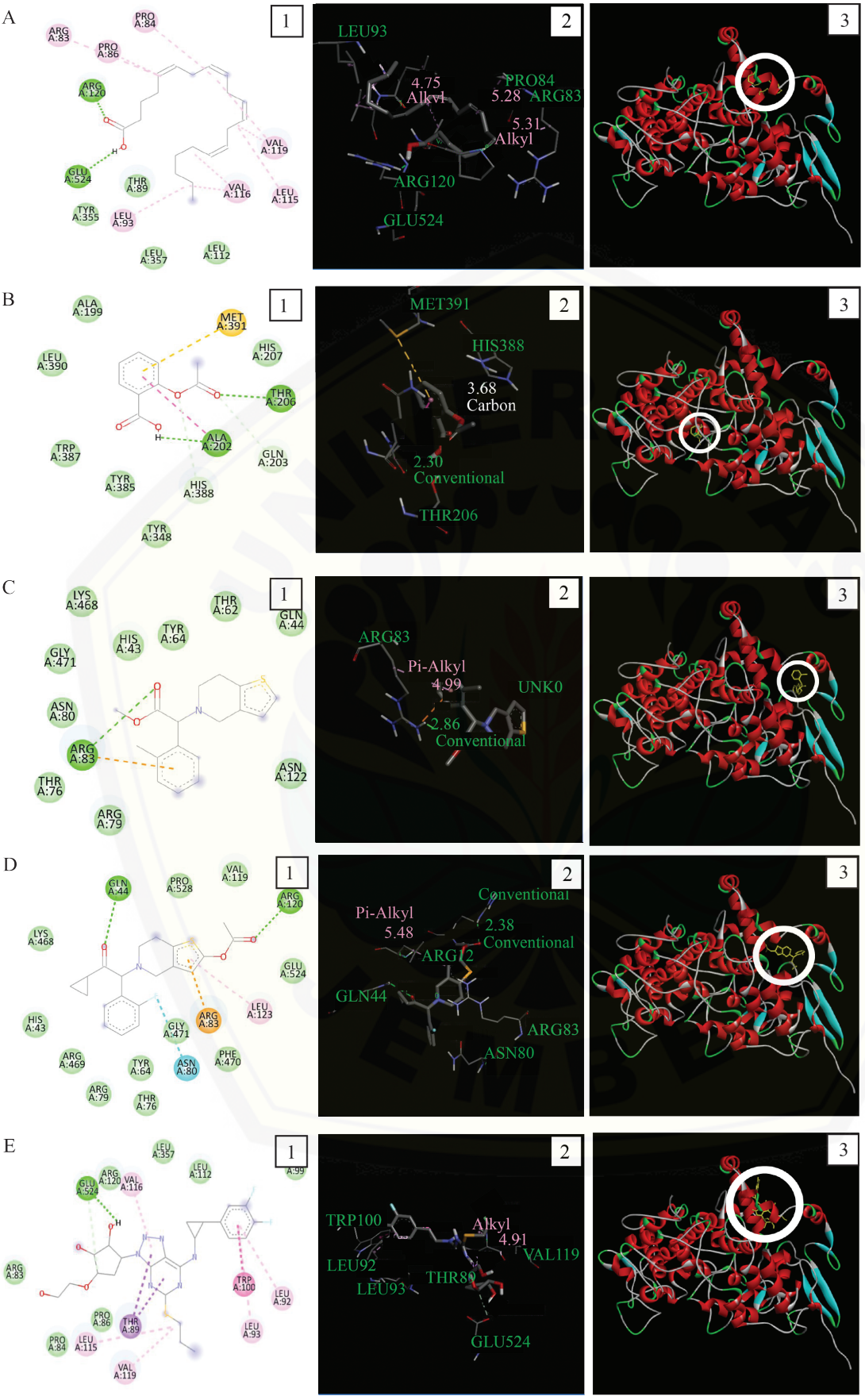

Figure 1. In silico analysis of the interactions of arachidonic acid (A), aspirin (B), clopidogrel (C), prasugrel (D), and ticagrelor (E) to COX-1. 1: The number of interactions, types of interactions, and amino acid types bound to COX-1 in 2D visualization. 2: The binding distance to COX-1. 3: The binding to COX-1 in 3D visualization was depicted with white circle.

Table 2. The comparison involves evaluating the binding affinity, interaction type, and amino acids that have bound between clopidogrel, prasugrel, and ticagrelor to P2Y12.

Ligand	Binding Affinity (kkal/mol)	Interaction Type between Ligand and P2Y12			Number of Binding
		Hydrogen (distance)	Van der Waals	Others	
ADP	-6,7	Tyr-109 (2.19Å) [1], Asn- 191 (2.31Å) [1], Arg-256 (2.56Å) [1], Lys-280 (3.02Å)	Ser-101, Val-102 [1], Tyr-105 [1], Met-108, Asn-159 [1], Val-190 [1], Cyst-194 [1], Gln-195, Phe-252 [1], Leu-276 [1]		Total: 14 (H: 4; V: 10; O: 0), In active site: 10 (H: 3; V: 7; O: 0
Aspirin	-6,7		Tyr-32, Thr-76, Phe-77, Phe-79, Cys-97 [1], Thr-100 Ser-101, Tyr-105 [1], Ser-288	Lys-80, Phe-104, Leu-284	Total: 12 (H: 0; V: 9; O: 3), In active site: 2 (H: 0; V: 2; O: 0)
Clopidogrel	-6,7	Asn-191 (2.45Å) [1], Arg-256 (2.10Å; 2.81Å) [1]	Tyr-109 [1], Gln-195, Ala-255 [1], Tyr-259 [1], Thr-283	Tyr-105 [1], Phe-252 [1], Lys-280	Total: 10 (H: 2; V: 5; O: 3), In active site: 7 (H: 2; V: 3; O: 2)
Prasugrel	-7,5		Tyr-109 [1], Val-190 [1], Cys-194 [1], Gln-195, Phe-252 [1], Ala-255 [1], Arg-256 [1], Thr-260, Val-279 [1]	Tyr-105, Asn-191, Tyr-259, Leu-276, Lys-280	Total: 14 (H: 0; V: 9; O: 5), In active site: 7 (H: 0; V: 7; O: 0)
Ticagrelor	-8,7	Tyr-109 (2,19Å; 2.91Å) [1], Asn-159 (2.65Å) [1], Asn-191 (2.49Å; 2.89Å) [1], Gln-195 (1.79Å; 2.23Å)	His-187 [1], Val-190 [1], Cys-194, Phe-252 [1], His-253, Ala-255 [1], Gln-263, Val-279 [1], Thr-283	Tyr-105 [1], Arg-256 [1], Tyr-259 [1], Arg-265, Asp-266, Leu-276 [1], Lys-280	Total: 20 (H: 4; V: 9; O: 7), In active site: 12 (H: 3; V: 5; O: 4)

1: Reference to amino acids is included in the protein active site . H: Hydrogen bond. V: van der Waals bond. O: Other.

these amino acid residues, even though they still bind to other active sites of COX-1. In comparison with another study, aspirin forms a hydrogen bond to Tyr-385 and Ser-530.[32] In addition, prior studies reported that aspirin irreversibly inhibits COX or prostaglandin endoperoxide synthase (PGHS) by acetylating a serine residue at position 529 and acetylating Ser530 to inhibit catalysis by preventing access of arachidonic acid substrate in the COX-1 isoenzyme.[33,34] Although prasugrel and ticagrelor bind to different amino acid residues than aspirin, there are other drugs that also bind to Arg-120 in COX-1 and still have antiplatelet effects, such as Ibuprofen, although with less efficacy than Aspirin.[35-37] It may imply that prasugrel and ticagrelor also have an NSAID-like effect similar to Ibuprofen. Based on binding affinity, prasugrel and ticagrelor show potential to inhibit COX-1 more effectively than aspirin. However,

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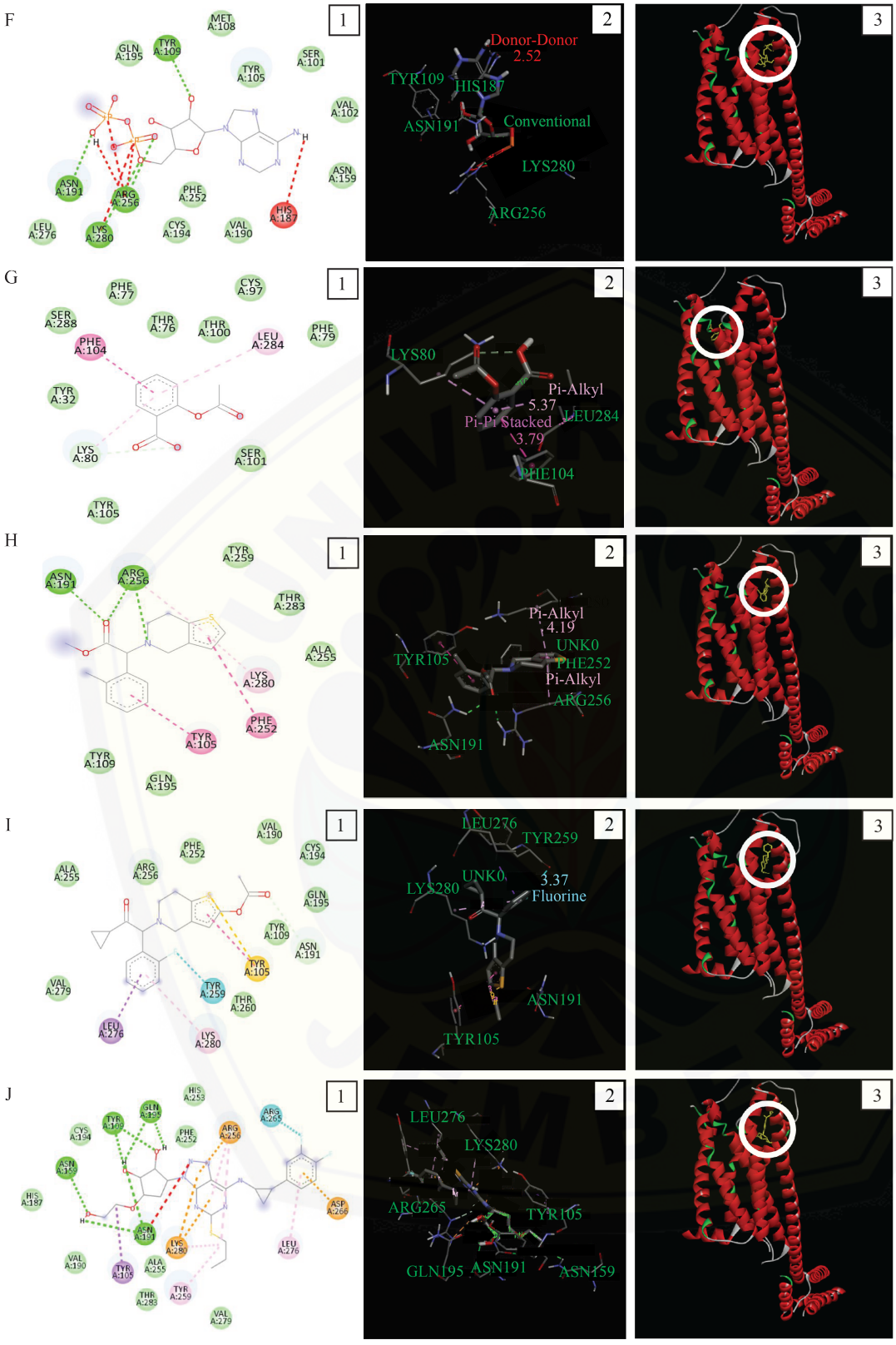

Figure 2. In silico analysis of the interactions of ADP (F), aspirin (G), clopidogrel (H), prasugrel (I), and ticagrelor(J) to P2Y12. 1: The number of interactions, types of interactions, and amino acid types bound to P2Y12 in 2D visualization. 2: The binding distance to P2Y12. 3: The binding to P2Y12 in 3D visualization. was depicted with white circle.

considering the different amino acid residues they bind to, it cannot be conclusively stated that they have a better antiplatelet effect than aspirin as a COX-1 inhibitor.

The docking results of this research demonstrate that the binding affinity of clopidogrel, prasugrel, and ticagrelor to P2Y12 is -6.7 kcal/mol, -7.5 kcal/mol, and -8.7 kcal/

mol, respectively. Ticagrelor has a lower binding affinity than prasugrel and clopidogrel to P2Y12, indicating that ticagrelor binds more strongly to P2Y12 compared to prasugrel and clopidogrel. One factor contributing to this difference is the higher number of bindings of ticagrelor to P2Y12, *i.e.*, 20, compared to prasugrel[14] and clopidogrel.[10]

Ticagrelor and prasugrel exhibit lower binding affinity than ADP, indicating a stronger binding potential than ADP, the native ligand, to P2Y12. In contrast, clopidogrel shows the same binding affinity as ADP to P2Y12, suggesting a competitive or equal chance of binding to P2Y12. Moreover, all these drugs can bind to the active site of P2Y12. Clopidogrel forms a hydrogen bond to Asn-191 and Arg-256, van der Waals bond to Tyr-109, Ala-255, and Tyr-259, and other bonds to Tyr-105 and Phe-252. Prasugrel forms van der Waals bonds to Tyr-109, Val-190, Cys-194, Phe-252, Ala 255, Arg-256, and Val-279, and other bonds to Tyr-105, Asn-191, Tyr-259, and Leu-276. Ticagrelor forms hydrogen bonds to Tyr-109, Asn-159, and Asn-191, van der Waals bonds to His 187, Val-190, Phe-252, Ala-255, and Val-279, and other bonds to Tyr-105, Arg-256, Tyr-259, and Leu-276. This aligns with real-world facts or clinical trials, confirming that all these drugs can be used as antiplatelet therapy by inhibiting P2Y12 in ACS treatment.[7] Additionally, prasugrel and ticagrelor, which have lower binding affinity than clopidogrel, align with clinical trials indicating the superiority of prasugrel and ticagrelor in terms of higher efficacy and lower side effects compared to clopidogrel.[13,14]

Aspirin, an established drug inhibiting COX-1, has a binding affinity of -6.7 kcal/mol when docked with P2Y12. This score is consistent with the docking results of clopidogrel and ADP to P2Y12. It suggests that aspirin may competitively or equally bind to P2Y12, inhibiting glycoprotein IIb/IIIa activation responsible for platelet aggregation. Aspirin forms two bonds with amino acid residues in the active site of P2Y12. However, compared with clopidogrel, prasugrel, and ticagrelor, which are established drugs inhibiting P2Y12, there is only one amino acid residue binding that is the same, namely Tyr-105, similar to clopidogrel. Based on binding affinity, aspirin has similar potency to clopidogrel and ADP to bind to P2Y12. However, concerning amino acid residues, aspirin has only one common amino acid residue binding with clopidogrel, indicating weaker binding potency and effectiveness against P2Y12 compared to clopidogrel, prasugrel, and ticagrelor. These results support the argument that transitioning from DAPT to MAPT in ACS patients may not include aspirin due to its weaker potency. Additionally, no other research to date suggests the potency of aspirin in inhibiting P2Y12, and based on other study it has lower efficacy with higher side effects compared to P2Y12 inhibitors as antiplatelet agents.[9,10]

This is crucial to note that all results from this research were obtained using *in silico* methods. Consequently, further research involving *in vitro*, *in vivo*, and clinical trials is necessary to obtain more accurate and clinically relevant results. *In silico* methods have limitations as they may not effectively replicate the molecular-to-physiological transition with complex biological phenomena in the field of medicine.[40] Further validation in future studies is needed to explain the interaction of prasugrel and ticagrelor via Arg120, and aspirin via Tyr-348, Tyr-385, and Trp-387, regarding the possible type of interaction (acetylation or deacetylation). Additionally, incorporating methods such as molecular dynamics could enhance the dataset, providing more comprehensive insights. Nevertheless, the molecular docking performed in this study still yields valid results, as it employs verified websites and applications.

Conclusion

Based on an *in silico* study, prasugrel and ticagrelor are the P2Y12 inhibitor drugs that have the ability to bind to COX-1, allowing them to be proposed as MAPT in ACS patients.

Authors Contributions

MNH was involved in conducting the research and drafting the manuscript, while SS and SR contributed by providing critical revisions to the study.

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