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Interaction Study Between Alpha-mangostin, Beta-mangostin, and Gamma-mangostin with Cyclooxygenase Compared to Acetosal and SC-558 as Oral Antiinflammation Drugs.

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ABSTRACT

Cyclooxygenase (COX) plays role in the biosynthesis of prostaglandin, a mediator of inflammation. Cyclooxygenase presents in two isoforms, COX-1 and COX-2. The objective of this study is to analyze the interaction between alpha-mangostin, beta-mangostin and gamma-mangostin with COX-1 and COX-2. The analysis method was comparing the interactions of ligands in the ligand binding domain of COX-1 and COX-2. Acetosal and SC-558 were used as the references. Alpha-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 interact with cyclooxygenase receptor via hydrogen bonds, hydrophobic interaction, and van der waals interaction. Gamma-mangostin gives the best results based on the interaction energy. Beta-mangostin provides the best affinity based on the inhibition constant to COX-1, and has inhibition constant to COX-2 that half time weaker than acetosal. Alpha-mangostin, beta-mangostin, gamma-mangostin can be used as non selective COX-2 oral antiinflamatic drugs.

Keywords: Alpha-mangostin, Beta-mangostin, Cyclooxygenase, Docking simulation, Gamma-mangostin.

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INTRODUCTION

Inflammation is a response of the body due to mechanical injury, wounds, microbial infections, and other stimuli that injure the body. This process may include changes in blood flow, tissue damage, and formation of inflammatory mediators, ie prostaglandin, leukotrien, and platelet activating factors catalyzed by phospholipase A2 (PLA2), cyclooxygenase, and lipooxygenase [1]. One of the pathways of the inflammatory process is the expression of cyclooxygenase enzyme. Cyclooxygenase enzyme plays role in the biosynthesis of prostaglandin [2].

Cyclooxygenase (COX) enzymes are present in two isoforms, COX-1 and COX-2. The first type of isoform (COX-1) is present in most tissues, including blood, kidney, and gastrointestinal tracts. This enzyme protects the stomach by forming bicarbonate and mucilage and inhibits acid production. The second isoform (COX-2) under normal circumstances is not present in the tissues, but is formed during the inflammatory process [3].

COX-2 is expressed after induction by cytokines, chemokines, oxidative stress, and carcinogens. In chronic inflammation, COX-2 levels increase in proportion with excess production of prostaglandin in cells and tissues [4].

This enzyme attaches to the endoplasmic reticulum membrane through its hydrophobic amino acids. The active site of cyclooxygenase is present in the center of each enzyme monomer. To achieve the active site, the cyclooxygenase enzyme's substrate must pass through a hydrophobic pathway. After the first reaction occurs, the product of the reaction moves to the peroxidase site, resulting second reaction. The final product is then excreted into the cytoplasm [4].

Bioactive compounds from natural ingredients, especially flavonoids, generally have anti-inflammatory activity. Mangostin is a xanton compound contained in the skin of the mangosteen fruit (*Garcinia mangostana* L). which was in vivo performed by Chen (2006), showed antiinflammatory activity, but it is doubtful whether the inhibition was related to the COX enzyme or not [5-7].

Acetosal and SC-558 are used as reference compunds in silico related to their effectiveness as anti-inflammatory. Acetosal was used as a non-selective drug of the COX-1 and COX-2 enzymes [8]. While sc-558 was used as a selective drug of COX-2 enzyme [9].

In this study we examined the interaction between alpha-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 on cyclooxygenase enzyme through docking simulation and its interaction using Ligand Explorer Viewer. The principle of docking is the function of algorithm and scoring [10]. Docking information regarding the relative positions of ligand in receptors and the involved cluster bonds and the role of ligand subtituents in ligand-receptor interactions can be used to predict the strength of alpha-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 bonds to cyclooxygenase. The affinity of alpha-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 to cyclooxygenase can be predicted by docking simulation using a ligand based approach. In a ligand based approach, ligands that in vivo have biological activity and certain physicochemical properties are collected and then tested on a receptor. Analysis on Ligand Explorer Viewer is to know the type of amino acids and bonds that may form between the ligand with the binding site of the receptor, so as to predict the functional groups of the ligand that can bind to the active site of the receptor and then produce pharmacological effects.

EXPERIMENTAL

Materials

The hardware used for molecular calculation, molecular modeling and docking simulation was personal computer with Intel CoreTM 2 Duo 2.0, GHz 800 MHz FSB 2 MB L2 cache processor, Windows 7 Ultimate 32- bits, 250 GB hard disk space, and 1 GB of RAM memory .

The softwares used are as follows:

1. ChemOffice 2004 program package (by Cambridge Soft Corporation 2003 downloaded from www.cambridgesoft.com).
2. Portable HyperChem Release 8.0.7 (by Hypercube Incorporation 2007 downloaded from http://www.hyper.com).
3. SwissPDBViewer v.4.01 program package (by GlaxoSmithKline R & D downloaded from http: //www.expasy. Org)
4. AutoDockTools program in MGLTools v1.5.2 program package (Molecular Graphics Laboratory, The Scripps Research Institute 2009 downloaded from http://mgltools.scripps.edu).
5. ArgusLab v.4.0.1 (by Mark Thompson and Planaria Software 2004 downloaded from http://www.arguslab.com).
6. Ligand Explorer Viewer v.3.8 (by Research Collaboratory for Structural Bioinformatics, which is on-line data from http://www.pdb.org/pdb/explore)

The materials used contain of the three-dimensional structure of the COX-1 enzyme crystallized with flurbiprofen with a resolution of 2.7 Å (PDB code: 1EQH), COX-2 enzyme crystallized with flurbiprofen with a resolution of 2.5 Å (PDB code: 3PGH), and COX-2 enzyme crystallized with SC-558 with a resolution of 2.8 Å (PDB code: 1CX2). These three structures are derived from the data base on Protein Data Bank (www.pdb.org). Two- and three-dimensional structures of alpha-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 were drawn using the ChemOffice 2004 program package.

Preparation of Alfa-mangostin, Beta-mangostin, Gamma-mangostin, Acetosal, and SC-558

Two and three dimensional structures of all ligands were made using ChemOffice 2004. Geometric optimization was done using Portable Hyperchem Release 8.0.7 using AM1 Method. Then, the analysis of the chemical properties of alfa-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 were done.

Preparation of COX-1 Enzyme (PDB Code: 1EQH) and COX-2 (PDB Code: 3PGH)

COX-1 and COX-2 enzymes were downloaded from Protein Data Bank (www.pdb.org), then the chains were reduced into monomer form using SwissPDBViewer v4.01. Analysis of the active site was done using Ligand Explorer Viewer and Q-SiteFinder. Interactions of Ligand-Enzyme were analyzed using Ligand Explorer Viewer in Protein Data Bank (www.pdb.org).

Software Validation

Flurbiprofen crystalized was isolated using SwissPDBViewer v.4.01. Then, two and three dimensional structure were made and geometric optimization of flurbiprofen were done using AM1 and PM3 methods. These structures were overlaid using Portable HyperChem Release 8.0.7. Re-docking of flurbiprofen to COX-1 and COX-2 were done using AutoDockTools. Then we also done the docking of flurbiprofen that was made to COX-1 and COX-2 using AutoDockTools. Then, all data were analyzed.

Docking Simulation of Alfa-mangostin, Beta-mangostin, Gamma-mangostin, Asetosal, and SC-558

Docking simulation of Alfa-mangostin, Beta-mangostin, Gamma-mangostin, Asetosal, and SC-558 were done using AutodockTools. All the bonds formed were interpretated.

RESULT AND DISCUSSION

Two-dimensional structure of ligands were transformed into three-dimensional structures with Chemoffice 2004. The three-dimensional structures were then geometrically optimized to obtain the most stable conformation. Conformation changes before and after optimized shown in Figure 1.

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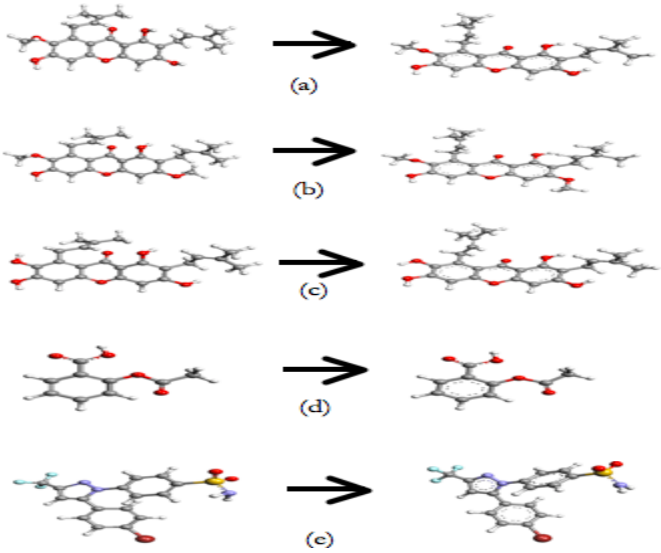

Fig 1: Three-dimensional structures before and after geometric optimization of (a) alpha-mangostin (b) beta-mangostin, (c) gamma-mangostin, (d) acetosal, and (e) SC-558.

The energy required by alpha-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 to obtain stable conformation shown in Table 1. From the results, it indicate that all ligands require large energy to obtain stable conformation.

From Table 1, we know that all ligands having mass below 500 amu. The ligands can be made into oral dosage form because it fulfill the requirement of Lipinski's Rule of Five.

Volume of all ligands are larger than the volume of cyclooxygenase active site which is 329 $Å^3$ (Table 1). It indicates that the ligands structures could not fully enter into the cyclooxygenase active site.

Table 1: Chemical properties of alfa-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558

Ligands	Energy (Kkal.mol^{-1})	cLog P	Volume ($Å^3$)	Mass (amu)
Alfa-mangostin	-5988,6876	-1,02	1166,22	410,47
Beta-mangostin	-6257,4770	-0,99	1228,26	424,49
Gamma-mangostin	-5720,6215	-1,05	1113,00	396,44
Acetosal	-2335,5697	-0,26	537,04	180,16
SC-558	-4000,8105	0,92	969,97	446,24

Coefficient of partition (Log P) must fulfill the Lipinski's Rule of Five, it has to be under five. The Log P value indicates the solubility of the drug. The Log P value of compounds that will be developed as an oral drug should be in the range that required by Lipinski's Rule of Five, so the compound can be absorbed and distributed in the body in order to reach drug's target. In this study, the Log P value is calculated using software, so the notation is written as cLog P (calculated Log P). Table 1 shows that all ligands fulfill the Lipinski's Rule of Five. All ligands have moderate hydrophilicity so it will easily transport through cell fluid. Gamma-mangostin is the most hydrophilic compound, while the SC-558 is the most lipophilic. High lipophilicity of SC-558 allows this compound to interact with hydrophobic cyclooxygenase active site.

Another property used to assess the feasibility of compounds that will be formulated into oral dosage form based on Lipinski's Rule of Five are the number of hydrogen bond donor and acceptor. Based on Lipinski's Rule of Five, a compound can be an oral drugs if it has maximum of five hydrogen bond donor and maximum of ten hydrogen bond acceptor.

Hydrogen bond donor and acceptor from the compounds can interact with amino acids at the active site of COX-1 and COX-2. The number of hydrogen bond donor and acceptor can be seen in Table 2. These five compounds meet oral drug criteria based on Lipinski's Rule of Five.

Table 2: Hydrogen bond donor and acceptor of ligands

Ligands	Number of hydrogen bond donor	Number of hydrogen bond acceptor
Alfa-mangostin	3	6
Beta-mangostin	2	6
Gamma-mangostin	3	6
Acetosal	1	4
SC-558	8	2

The enzymes selected for this study were COX-1 and COX-2 crystallized with flurbiprofen (non-selective antiinflammatory drugs) and with SC-558 (COX-2 selective antiinflammatory agent). The resolution values of COX-1 (code PDB: 1EQH) and COX-2 with flurbiprofen (code PDB: 3PGH) were 2.7Å and 2.5Å, respectively, while COX-2 with SC-558 (PDB code: 1CX2) was 2.8 Å.

The chains were then reduced to produce a monomer chain A. Ligand Explorer Viewer and Q-Site Finder software were used to analyze the amino acids that at the active site of COX-1 and COX-2.

Based on Ligand Explorer Viewer software, there were 12 amino acids at the active site of COX-1, such as Val116, Arg120, Val349, Leu352, Tyr355, Leu359, Met522, Ile523, Gly526, Ala527, Ser530, Leu531 and there were 14 amino acids at the active site of COX-2, such as Val116 , Arg120, Val349, Leu352, Tyr355, Leu359, Met522, Ile523, Gly526, Ala527, Ser530, Leu531 Tyr385 and Trp387. While based Q-SiteFinder, the amino acids at the active site of COX-1 were Met113, Val116, Arg120, Tyr348, Val349, Leu352, Ser353, Tyr355, Leu359, Phe381, Leu384, Tyr385, Trp387, Phe518, Met522, Ile523, Gly526, and the amino acids at the active site of COX-2 were Met113, Val116, Arg120, Tyr348, Val349, Leu352, Ser353, Tyr355, Leu359, Phe381, Leu384, Tyr385, Trp387.

Validation was done to make sure that all procedures valid for this study,. Two- and three-dimensional flurbiprofen structures were made, then were optimized using AM1 and PM3 methods. These structures were overlaid with isolated fluobiprofen from the enzymes. The overlay Root Mean Square Deviation (RMSD) value for AM1 method was 0.39Å, while for PM3 method was 0.58Å. This value indicates that the optimization method fulfill the validation requirement, which is must less than 2Å.

The average Root Mean Square Deviation (RMSD) value for three replication of re-docking flurbiprofen on the COX-1 and COX-2 enzymes was 0.4Å. It indicates that docking procedures have proven valid.

The interaction energy of alpha-mangostin, beta-mangostin, gamma-mangostin, and acetosal with COX-1 and COX-2 are negative. It suggests that the four compounds are capable to interact spontaneously with COX-1 and COX-2. When the value of interaction energy is smaller, ligands will be easier to bind with enzymes. Gamma-mangostin is the compound that most easily enter or interact spontaneously with COX-1 and COX-2 because it has the smallest interaction energy (Table 3 and Table 4).

Inhibition constant (KI) shows the affinity of the ligand to bind with receptor. The compound with smaller KI value will be easier to bind with receptor. Beta-mangostin is a compound that has the smallest inhibition constant to COX-1, and has KI value to COX-2 half times weaker than acetosal (Table 3 and Table 4).

Besides value of interaction energy and inhibition constant, the evaluation of the docking results can also seen from the hydrogen bonds and the interactions formed. Comparison of alpha-mangostin, beta-

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mangostin, gamma-mangostin, and acetosal interactions with COX-1 and COX-2 receptors can be seen in Table 3 dan Table 4.

Table 3: Docking of alpha-mangostin, beta-mangostin, gamma-mangostin, and acetosal in COX-1

Ligands	IE[a]	KI[b]	Hydrogen bond	AAR[c]
Alfa-mangostin	-7,56	0,204	O-AM→H-Ile523 O-AM→H-Met522	Met113, Val116, Leu117, Arg120, Tyr348, Val349, Leu352, Tyr355, Leu359, Leu384, Tyr385, Trp387, Met522, Ile523, Gly526, Ala527, Ser530, Leu531
Beta-mangostin	-7,25	0,189	-	Met113, Val116, Leu117, Arg120, Tyr348, Val349, Leu352, Tyr355, Leu359, Leu384, Tyr385, Trp387, Phe518, Met522, Ile523, Ala527, Ser530, Leu531
Gamma-mangostin	-7,61	7,5	O-GM →NH2-Arg120 O-GM→HE-Arg120	Met113, Arg120, Tyr348, Val349, Leu352, Ser353, Tyr355, Leu359 , Tyr385, Trp387, Met522, Ile523, Gly526, Ala527, Ser530, Leu531
Acetosal	-7,44	0,203	O-ASP→H Ser530	Val349, Leu352, Tyr348, Phe381, Leu384, Tyr385, Trp387, Met522, Ile523, Gly526, Ser530

Note: [a] Interaction Energy (Kcal/mol) [b] Inhibition constant (μM) [c]Amino acid residues

Table 4: Docking of alpha-mangostin, beta-mangostin, gamma-mangostin, and acetosal in COX-2

Ligands	IE[a]	KI[b]	Hydrogen bond	AAR[c]
Alfa-mangostin	-4,89	0,078	-	Met113, Val116,Leu117, Ile345, Tyr348, Val349, Leu352, Ser353, Tyr355 Leu359, Tyr385, Trp387, Phe518, Met522, Val523, Gly526, Ser530, Leu531
Beta-mangostin	-5,29	0,051	O-BM → H-Ser530	Met113, Val116, Arg120, Val349, Tyr355, Leu359, Phe381, Leu384, Tyr385, Trp387, Phe518, Met522, Val523, Gly526, Ala527, Ser530, Leu531
Gamma-mangostin	-9,11	7,61	-	Met113, Val116, Arg120, Val349, Tyr355, Leu359, Phe381, Leu384, Tyr385, Trp387, Phe518, Met522, Val523, Gly526, Ala527, Ser530, Leu531
Acetosal	-7,33	0,026	O-ASP→H Ser530	Leu352, Trp387, Leu384, Met522, Gly526, Ala527, Ser530
SC-558	-	-	O-SC →O His90 O-SC →NH2 Arg513 O-SC → O Phe518	His90, Arg120, Gln192, Val349, Leu352, Ser353, Tyr355, Leu 359, Trp387, Arg513, Ala516, Ile517, Phe518, Val523, Gly526, Ala527, dan Leu531.

Note: [a] Interaction Energy (Kcal/mol) [b] Inhibition constant (μM) [c] Amino acid residues

Pharmacophore which produces hydrogen bond interactions between mangostin and cyclooxygenase is hydroxyl group. Beta-mangostin is potential to inhibit COX-2, its strength was half-times weaker than acetosal. Hydrophobic interactions occur in Phe518, it indicates that this compound is non selective inhibitor.

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CONCLUSION

The docking simulationindicated that alpha-mangostin, beta-mangostin, gamma-mangostin, acetosal, and SC-558 interact with cyclooxygenase receptor via hydrogen bonds, hydrophobic, and van der waals interaction. Alpha-mangostin, beta-mangostin, gamma-mangostin can be used as non selective COX-2 oral antiinflamatic drugs, based on the hydrogen bonds and hydrophobic interaction formed.

REFERENCES

[1] Maslinska, D & M. Gajewski., Some aspects of the inflammatory process, Folia Neuropathol, 36(4), pp. 199-204, 1998.

[2] Ricciotti, E & Garret A.F., Prostaglandin and Inflammation, Arterioscler Thromb Vasc Biol, 31(5), pp. 986–1000, May, 2011.

[3] Fitzpatrick F.A. Cyclooxygenase enzymes: regulation and function, Curr Pharm Des, 10(6), pp. 577-588, 2004.

[4] Amasino, A., Y. Deng, S. Huang, I. Lee, A. Lesi, Y. Pu, and P. V. Velden. COX-1 and COX-2 enzymes synthesize prostaglandins and are inhibited by NSAIDS (Nonsteroidal Anti-inflammatory Drugs), University of Wisconsin-Madison, http://cbm.msoe.edu/images/contentImages/smartTeams/alumni/2004-05/2004MadisonWestfinalposter8x11.pdf (2005).

[5] Chen , L. G., Yang, L, L., Wang, C,C.Anti-inflammatory activity of mangostins from Garcinia mangostana. Food Chem Toxicol, 46(2008), pp. 688-693, Sept. 2007.

[6] Gutierrez-Orozco, F, C. Chitchumroonchokchai, G. B. Liseinski, S. Suksamrarn & M.L. Failla. α-Mangostin: Anti-Inflammatory Activity and Metabolism by Human Cells, J Agric Food Chem, 61(16), pp. 3891–3900, Apr. 2013.

[7] Atluri, N, R. Holur, V. Thirumnalanadhuni & U.M.D. Palempalli.Modulation of pro-inflammatory genes by α-mangostin from Garcinia mangostana, Int J Pharm Sci Invent, 3 (5), pp. 23-29, May. 2014.

[8] Gunawan S.G. Farmakologi dan Terapi. Universitas Indonesia Press, pp. 230-246, 2007.

[9] Vittorio, L, Massimiliano B, Luciana M, Matteo M, Francesco L, Andrea C, Ettore N, Michele P. Dual binding node of SC-558 in COX-2: a new frontier in COX inhibition, ETHzurich, https://www.ethz.ch/content/specialinterest/chab/physical-chemistry/parrinello-group/en/news-and-events/past-seminars/seminars-2008.html (8 September 2008).

[10] Halperin I, Ma B, Wolfson H, Nussinov R, Principles of docking: An overview of search algorithms and a guide to scoring functions, Proteins, 47(4), pp. 409-43, Jun. 2002.