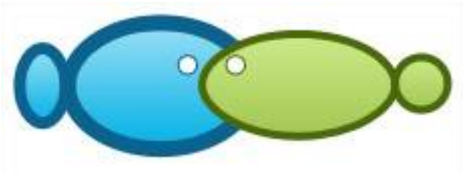

Goldband goatfish fatty acids as potential cyclooxygenase-2 blocking agents: a molecular docking approach

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Abstract. Fatty acids are essential for human health and well-being. The goldband goatfish (*Upeneus moluccensis* (Bleeker, 1855)) is a valuable food fish with high protein and fatty acid content. This in silico study identified potential anti-inflammatory activity of goldband goatfish fatty acids (palmitic acid, docosahexaenoic acid and eicosatetraenoic acid) through cyclooxygenase-2 (COX-2) protein inhibition. The fatty acid structure was redocked with cyclooxygenase protein, using naproxen as a control COX-2 inhibitor. Docking on specific cyclooxygenase-2 protein grids was implemented in Molegro virtual Docker version 5.0. Data were visualized and analyzed in Discovery Studio version 21.1.1. The ligand-protein complex interactions showed goldband goatfish fatty acids binding to cyclooxygenase-2 inhibitor residue sites with active residue performance similar to naproxen as the control inhibitor. This study concluded that goldband goatfish fatty acids have potential as cyclooxygenase-2 blocking agents to prevent inflammation. Further research is recommended on molecular dynamics and through *in vitro* studies.
Key Words: cyclooxygenase-2, goldband goatfish, inflammation, molecular docking.

Introduction. The goldband goatfish (*Upeneus moluccensis* (Bleeker, 1855)) is a valuable food fish with high protein and fatty acid content (Doğan & Ertan 2017). Known as „ikan biji nangka" in Indonesia, the goldband goatfish is associated with mangrove ecosystems which serve as feeding, nursery, and spawning grounds, such as the coastal waters and mangrove forests of Maumere Bay, Flores, Indonesia where it is typically caught using net-based gears, including gillnets and trammel nets (Vincentius 2020). Goldband goatfish production from Maumere Bay is quite high, ranging from 68 to 83 tons year^{-1} over the period 2016 to 2021 (Dinas Perikanan Kabupaten Sikka 2021).

The nutritional content of goldband goatfish has been reported as 78-80% water, 1.5-1.9% crude ash, 1.4-3.7% crude fat, and 20-22% crude protein, based on proximate analysis (Bilgin et al 2018). The main amino-acids composing the relatively high goldband goatfish crude protein fraction are lysine, leucine, aspartic acid, and glutamic acid (Bilgin et al 2018). Goldband goatfish mineral content is dominated by K and P, while other minerals include Ca, Na, Mg, Cd, Cr, Cu, Mn, Zn, and Fe (Öksüz et al 2011).

The fatty acid content of goldband goatfish is also relatively high and varied, including palmitic acid, stearic acid, palmitoleic acid, oleic acid, eicosatetraenoic acid (EPA), arachidonic acid, and docosahexaenoic acid (DHA) (Bilgin et al 2018). Essential fatty acids, especially polyunsaturated fatty acids, can promote human health and well-being. The consumption of fatty acids from fish can reduce the risk of cardiovascular disease, strokes, diabetes mellitus type-2, and cancer (Awuchi et al 2022). Docosahexaenoic acid and eicosatetraenoic acid can influence brain function, antitumor activity, lipid and glucose metabolism (Lane et al 2014; Zhang et al 2019). Omega-6 and omega-3 polyunsaturated fatty acid (PUFA) compounds are lipid precursors for

inflammation regulating compounds (Bilgin et al 2018). Arachidonic acid acts as a substrate for cyclooxygenase-1/2 to promote the synthesis of prostaglandin(PG)H_2 which can lead to inflammation. Similarities in the structure of PUFAs and arachidonic acid means that PUFAs can have anti-inflammatory properties (Wall et al 2010).

Anti-inflammatory properties have been reported in some previous research. Several bioactive compounds from ginger rhizomes can promote anti-inflammatory mechanisms through cyclooxygenase-1 and cyclooxygenase-2 inhibition (Choi et al 2017; Mahboubi 2019; Mao et al 2019; Sapkota et al 2019; Bare et al 2020; dos Reis Nunes et al 2020; Spisni et al 2020; Zhang et al 2021). Coffee compounds can also reduce the pain by inhibiting cyclooxygenase synthesis (Bare et al 2019a, b). Anthocyanins from black rice can also prevent or reduce inflammation through pro-inflammatory cytokine inhibition (Sari et al 2019). This study performed in-silico simulation of cyclooxygenase-2 inhibition by goldband goatfish fatty acids through a molecular docking approach.

Material and Method

Fatty acid structure retrieval. Fatty acid structures, including palmitic acid (CID 985), docosahexaenoic acid (CID 445580), eicosatetraenoic acid (CID 446284), docosapentaenoic acid (CID 5497182) were obtained from the PubChem NCBI database in sdf file format (Kim et al 2016). The ligand structures were imported to Molegro virtual docker 5.0 for molecular docking analysis (Bitencourt-Ferreira & de Azevedo 2019a).

Cyclooxygenase-2 protein retrieval and optimization. Cyclooxygenase-2 protein structure with accession code 3NTB was retrieved from the Protein Data Bank in pdb format (Duggan et al 2010). The protein structure was cleaned to remove undesirable ligands and molecules. The cleaned structure file was imported into Molegro virtual Docker version 5.0 (Bitencourt-Ferreira & de Azevedo 2019b). Cyclooxygenase protein binding cavities were identified using the van der Waals expansion maximum 5 as a molecular surface parameter. A cyclooxygenase-2 binding cavity was placed at X = -19.67; Y = 52.07; Z = -7.12; radius 12 on the protein grid was used to determine the docking position.

Molecular docking simulations. Goldband goatfish fatty acid compounds that interacted with cyclooxygenase protein were re-docked at the cyclooxygenase-2 binding site. The in silico docking was conducted in Molegro Virtual Docker version 5.0 (Bitencourt-Ferreira & de Azevedo 2019b). The docking settings were: score function, MolDock Score [Grid]; grid resolution, 0.30; algorithm, MolDock simplex evolution (SE); maximum SE iterations, 1500; maximum population size, 50; docking creation energy threshold, 100; maximum pose generator attempts, 10-30; simplex steps, 300; neighbour distance factor, 1.00; multiple pose (pose clustering), 5; energy threshold 0.00; Cartesian root-mean-square deviation (RMSD) threshold (cluster similar poses).

Ligands-protein complex visualization and analysis. Ligand-protein interaction energy was calculated from MolDock Score, Moldock Grid Score, and Rerank score (Bitencourt-Ferreira & de Azevedo 2019b). Bond energies were expressed in kJ mol^{-1} and represent the average of five ligand-protein complex binding models. Docking results were visualized in 3D and 2D in Discovery Studio program ver. 21.1.1 (Bare et al 2019a; Bare et al 2020; Sari et al 2022).

Results and Discussion. The palmitic acid-COX-2 complex has a binding energy of approximately -190.4 kJ mol^{-1}. We found seven amino acid residues which could be divided into three groups, as follows: VAL116, VAL349, VAL523, ALA527, LEU531 (Hydrophobic type Alkyl), TYR355, (Hydrophobic type Pi-Alkyl), and LEU531 (Unfavorable docking bump). Docosahexaenoic acid-COX-2 interaction binding energy was 299.6 kJ mol^{-1}. Several amino acid residues interacted with the ligand-like TYR385 (conventional hydrogen bond), VAL116, VAL349, VAL523, ALA527, LEU352, LEU359, LEU351, ILE345, VAL359, LEU531, ILE345, VAL349 and LEU351 (hydrophobic alkyl type bond), and with

TYR355, TRP387 and PHE518 (hydrophobic pi-alkyl type bond) (Table 1). The eicosapentaenoic acid-COX-2 interaction binding energy was -347 kJ mol^{-1} and showed interaction ligand-amino acid residue interactions in the following order: TYR385 (conventional hydrogen bond), SER530 (carbon hydrogen bond), VAL116, VAL349, VAL523, ALA527, LEU352, MET522, LEU352, LEU531, and ARG120 (hydrophobic alkyl type bond), TYR355 and PHE518 (hydrophobic pi-alkyl type bond) (Figure 1).

Naproxen interacted with the COX-2 protein and twelve amino acid residues interacted with the ligand-like VAL523 (carbon hydrogen bond), VAL523, ALA527 (hydrophobic pi-sigma bond), VAL116, LEU359, LEU352, VAL523 (hydrophobic alkyl bond) and TYR355, PHE518, VAL349, LEU531, ALA527 (hydrophobic pi-alkyl bond) with a binding energy of -208.4 kJ mol^{-1}.

Some residues, including VAL523, VAL116, ALA527, TYR355, VAL349, and LEU531, were identified at all fatty acid–cyclooxygenase–2 and Naproxen-cyclooxygenase-2 active interaction sites. A residue of docosahexaenoic acid, LEU359, also occurred with naproxen. Two cyclooxygenase–2 residues, LEU352 and PHE518, were identified in docosahexaenoic acid and eicosapentaenoic acid. The similarity of residues from fatty acid and naproxen is indicative of palmitic acid, docosahexaenoic acid and eicosapentaenoic acid.

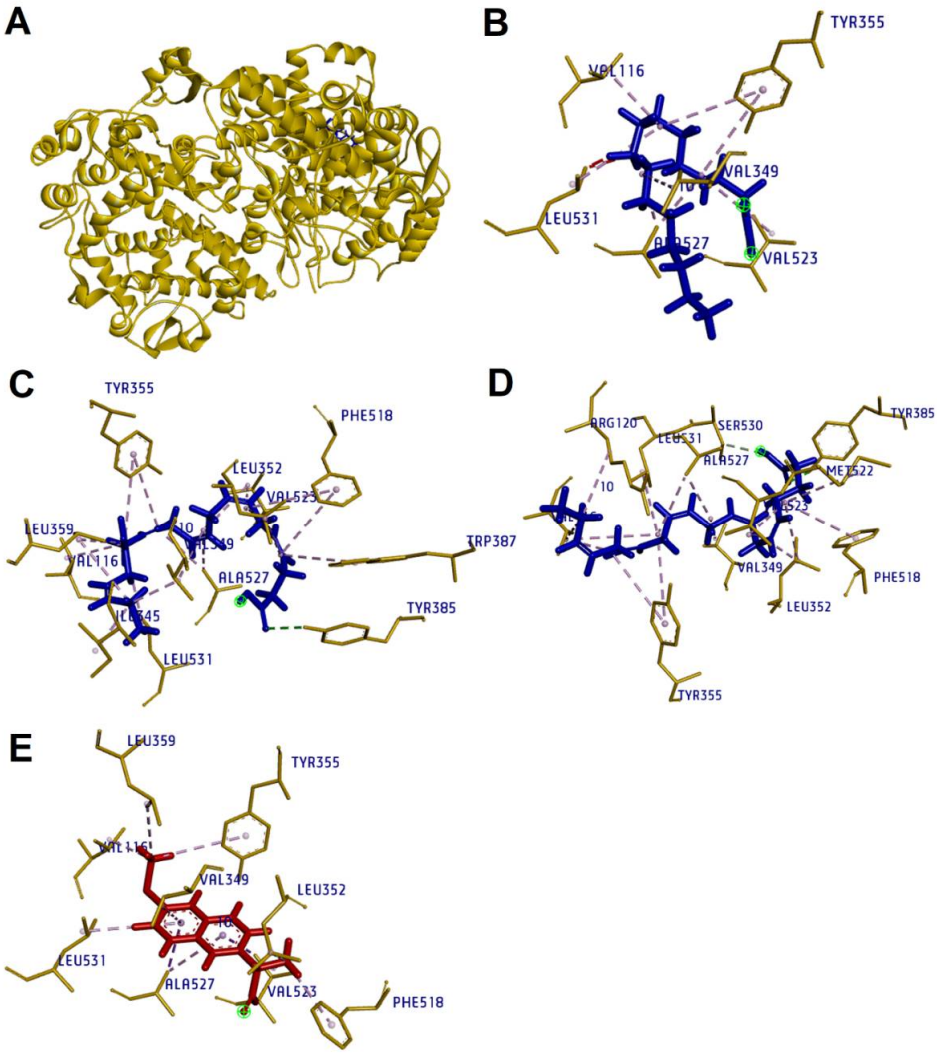

Figure 1. 3D structure of fatty acid-cyclooxygenase-2 binding interactions: A. overview of the ligand-protein complex structure; B. palmitic acid; C. docosahexaenoic acid; D. eicosatetraenoic acid; E. naproxen.

Table 1

Fatty acid-cyclooxygenase–2 interactions

Ligand (binding energy (kJ mol^{-1}))	*Residues (distance (A))*		
	Hydrogen bond	*Hydrophobic*	*Unfavorable*
Palmitic acid (-190.4)		Alkyl: VAL116 (4.1); VAL349 (4.7); VAL523 (5.02); ALA527 (3.9); ALA527 (3.5); LEU531 (5.4) Pi-Alkyl: TYR355 (5.3); TYR355 (5.1)	Unfavorable Bump: LEU531 (1.3), LEU531 (1.4)
Docosahexaenoic acid (-299.6)	Conventional Hydrogen Bond: TYR385 (3.84)	Alkyl: VAL116 (2.8), VAL349 (5.1), VAL523 (3.8), VAL523 (4.9), ALA527 (4.2), ALA527 (3.9), LEU352 (4.9), LEU352 (4.2), LEU359 (4.6), LEU531 (5.2), ILE345 (3.4), VAL349 (3.0), LEU359 (4.5), LEU531 (4.8) Pi-Alkyl: TYR355 (5.0), TYR355 (5.2), TRP387 (5.2), PHE518 (5.1), PHE518 (5.3)	
Eicosapentaenoic acid (-347)	Carbon Hydrogen Bond: TYR385 (3.0), SER530 (3.2)	Alkyl: VAL116 (5.4), VAL116 (3.6), VAL349 (4.2), VAL523 (4.8), VAL523 (4.7), ALA527 (3.7), ALA527 (4.2), LEU352 (4.9), MET522 (5.1), LEU352 (3.8), LEU531 (5.0), VAL116 (4.6), ARG120 (4.7) Pi-Alkyl: TYR355 (5.4), TYR355 (5.1), PHE518 (4.6)	
Naproxen (-208.4)	Carbon Hydrogen Bond: VAL523 (2.8)	Pi-Sigma: VAL523 (3.8), ALA527 (3.4) Alkyl: VAL116 (3.6), LEU359 (4.5), LEU352 (3.8), VAL523 (4.3). Pi-Alkyl: TYR355 (5.3), PHE518 (4.1), VAL349 (5.0), LEU531 (5.2), ALA527 (4.4)	

Cyclooxygenase-2 enzyme is an enzyme that regulates inflammatory response through catalyzing the synthesis of PGH2. This study revealed that the goldband goatfish fatty acid inhibited the cyclooxygenase-2 protein at inhibitor sites; as described by Duggan et al (2010), VAL523, VAL116, ALA527, TYR355, VAL349, and LEU531 are cyclooxygenase-2 protein inhibitor sites (Figure 2).

Several other compounds are also reported as having anti-inflammatory properties, preventing inflammation as cyclooxygenase-2 inhibitors. These include malic acid, xylose, benzoic acid, succinic acid, fumaric acid, rhamnose, and an ethyl butyrate extract from *Elaeocarpus sphaericus* fruit (Primiani et al 2022). Walnut oil compounds have also shown inhibitory effects on cyclooxygenase-2 (Sundari et al 2022), while chlorogenic acid and quinic acid can inhibit cyclooxygenase activity by blocking the COX-2 substrate sites (Bare et al 2019a, b).

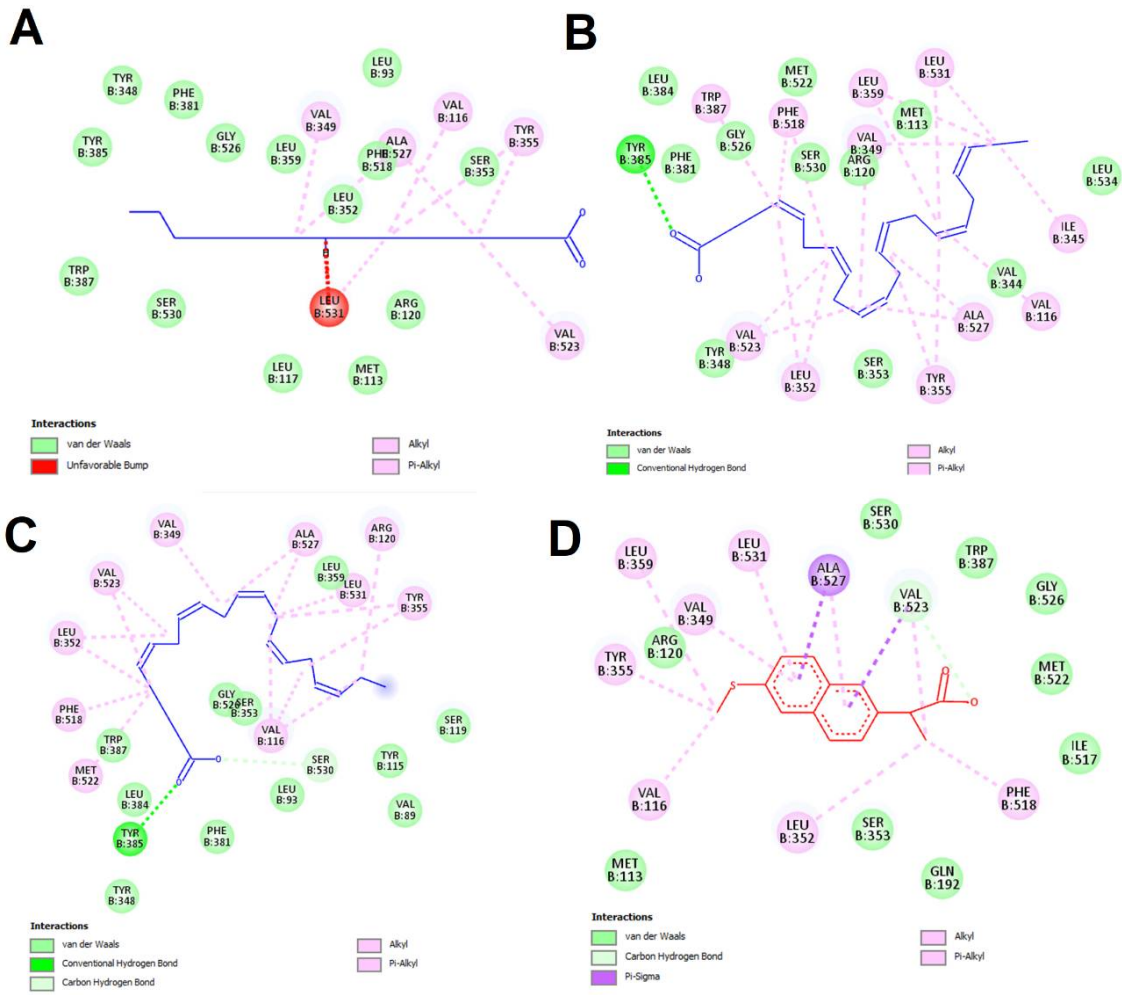

Figure 2. 2D structure of interaction fatty acid-cyclooxygenase-2: A. palmitic acid; B. docosahexaenoic acid; C. eicosatetraenoic acid; D. naproxen.

Conclusions. The ligand-protein complex interactions showed goldband goatfish fatty acids binding to cyclooxygenase-2 inhibitor residue sites with active residue performance similar to naproxen as the control inhibitor. In-silico molecular docking analysis of goldband goatfish fatty acid compounds, palmitic acid, docosahexaenoic acid and eicosapentaenoic acid, showed anti-inflammatory activity of these compounds through cyclooxygenase-2 inhibition.

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Conflict of interest. The authors declare that there is no conflict of interest.

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