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Interaction Mechanism of Inhibition of Palmitic Acid and α-Selinene Targeting FabH and FabI Enzymes in *Escherichia coli*: In Silico Study

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Abstract

Investigation studies of the interaction mechanism of palmitic acid and α-selinene in inhibiting FabH and FabI enzymes have been studied using an in silico approach. FabH (Beta-Ketoacyl-ACP Synthase III) and FabI (Enoyl-acyl carrier protein reductase) enzymes are two enzymes that are targets for the inhibition of candidate antibacterial compounds. This study aimed to determine the strongest candidate between palmitic acid and α-selinene as an antibacterial agent for *Escherichia coli*. The method used in this study is a random and directed molecular docking method using the Autodock Vina program, which is integrated into PyRx 0.8 software. The results of the molecular docking simulation include the pattern and strength of interaction between the ligand and the FabI and FabH enzymes. The interaction pattern includes the cluster pattern, the ligand poses on the protein surface, and the interaction strength based on the binding affinity value. Based on the results of random docking simulation data analysis, it was shown that the majority of α-selinene occupied the position of cluster 1 of the FabI enzyme and palmitic acid in cluster 2 of the FabH enzyme. Based on the binding affinity value, palmitic acid has a weaker interaction strength on the FabH enzyme (-5.7 kcal/mol) than on the FabI enzyme (-7.1 kcal/mol). The interaction strength of α-selinene on the FabI enzyme (-7.3 kcal/mol) was stronger than that of the FabH enzyme (-6.9 kcal/mol). The interaction strength of α-selinene in both FabI and FabH enzymes was greater than that of palmitic acid. α-selinene is projected to have a better potential as an antibacterial agent against *Escherichia coli* than palmitic acid based on its greater interaction strength.

1. Introduction

A severe condition that frequently results in fatalities is gastrointestinal illness. The digestive system digests every food and beverage that enters the human body [1]. One of the causes of diarrhea is infection with *Escherichia coli* in the digestive tract [2]. *Escherichia coli* is a pathogenic bacteria with a high level of pathogenicity and generally lives in the intestinal tract of humans and animals [3]. The bacterial infection by *Escherichia coli* can spread throughout the body, infecting the urinary tract, meningitis and gastrointestinal infections [4].

Escherichia coli possesses a bilayer membrane made of phospholipids and lipopolysaccharides, which allows it to defend itself despite having a 20% cell wall that is relatively thin [5, 6]. In addition, *Escherichia coli* has a thin peptidoglycan layer located in the periplasm and a lipopolysaccharide layer [1]. The formation of a lipid bilayer occurs due to the process of fatty acid biosynthesis produced from the enzymes FabH (Beta-Keto-Acyl Carrier Protein Synthase III) and FabI (enoyl-acyl carrier protein reductase), then forms phospholipids and lipopolysaccharides which will process in the formation of the cell membrane of *Escherichia coli* bacteria [7]. High growth rates of

pathogenic bacteria can result in the development of antibiotic resistance. As a result, it may be more challenging for the medication to inhibit or kill pathogenic microorganisms. Hence, killing harmful germs requires a potent compound.

Zheng *et al.* [8] proved that fatty acids have antibacterial activity and can inhibit the FabI enzyme, which is essential in bacterial growth. The ability of fatty acids to inhibit the FabI enzyme is predicted to be one of the mechanisms of killing bacteria. Palmitic acid is one of the fatty acids proven in vitro to have antibacterial activity [9]. Another bioactive compound with strong antibacterial activity against *Escherichia coli* is α-selinene [10]. The interaction mechanism between fatty acids, α- selinene, with components in bacteria has not been widely reported. The study was performed by determining the pattern and strength of the interaction between the ligand and protein using the molecular docking method. Previous research has studied inhibition mechanisms on FabI enzymes by palmitic acid inhibitors with BA -7.1 kcal/mol through the in silico method, which shows antibacterial activity against *Escherichia coli* [11].

Moreover, the FabH protein is frequently utilized as a target molecule in in-silico analysis to study molecular interactions related to antibacterial properties [12]. Khasanah [13] reported that inhibiting the FabH enzyme by the α-selinene inhibitor compound produced a binding affinity of -6.9 kcal/mol, which showed antibacterial activity against *Escherichia coli*, supported by hydrophobic interactions between α-selinene molecules and amino acid residues in pocket sites protein. The results of previous in silico studies showed that the bioactive α- selinene and palmitic acid could inhibit the FabH and FabI enzymes. However, the bioactive component of α- selinene and palmitic acid that can inhibit both FabH and FabI concurrently has not yet been identified. This study conducted tests through a cross-sectional process by interacting with the bioactive α-selinene with the FabI enzyme and palmitic acid with the FabH enzyme, which was studied in silico. The cross-section aimed to obtain the best bioactive prediction results that can simultaneously inhibit both FabH and FabI enzymes and become drug candidates for antibacterial against *Escherichia coli*.

2. Methods

2.1. Materials

The protein used were FabI (enoyl-ACP reductase) with a PDB code of 1C14 and FabH (β-ketoacyl-ACP synthase III) with a PDB code of 1HNH. The ligands were Palmitic acid, α-selinene, acetyl CoA, and triclosan. The software was Autodock vina (PyRx 0.8 Tools), Autodock Tool 1.5.6, Chimera 1.11.12, Open Babel GUI 2.3.1, and LigPlot++1.4.5.

2.2. Preparation and Validation of Protein 3- dimensional Structures

The 3-dimensional structure of the FabH and FabI proteins were downloaded in .pdb format via www.rcsb.org/pdb. The FabH protein used was the FabH protein (open) with the PDB code 1HNH (1 chain A bound to the CoA ligand), while the FabI protein used the (open) protein with the PDB code 1C14 (1 chain A bound to triclosan and NAD ligands). Ligand separation from protein was carried out using the Chimera 1.11.2 program and saved in .pdb format. FabH and FabI proteins were minimized using Chimera 1.11.2 to obtain a 3-dimensional structure with the lowest energy.

The 3-dimensional structure of the protein was validated by uploading the structure before and after minimization to the open-source website MolProbity via the site http://molprobity.biochem.duke.edu. The validation results were seen through the clashscore and MolProbity, which were then compared between the proteins before and after minimization. The structure can be valid when the clashscore and MolProbity produce a smaller value and a larger percentile value than before being minimized.

2.3. Preparation of 3-dimensional Structures of Ligand

Three-dimensional standard and sample ligands structures were downloaded from https://pubchem.ncbi.nlm.nih.gov/ and http://www.chemspider.com/ as SDfile (.sdf) or Molfile (.mol). Files in .sdf and .mol format were converted to .pdb using the Open Babel GUI program. The 3-dimensional ligand structure validation was done by uploading the 3-dimensional ligand structure validation results to the page http://www.scfbio- iitd.res.in/software/drugdesign/lipinski.jsp.

2.4. Molecular Docking

Molecular docking was simulated using PyRex 0.8, integrated with the Autodock vina program. Confirmation was conducted by carrying out directional docking at the protein binding site. The size of the directional docking grid box was matched to the size of the protein binding site. The data generated from the docking process was the binding affinity value.

Docking was done ten times which produced nine .pdb files in one docking. Each docking result contains each ligand's conformation, sorted based on the binding affinity value. The best molecular structure of the ligand was determined based on the lowest binding affinity, indicating the most stable interaction. Docking results were analyzed using Chimera 1.11.2 and LigPlot++ with attention to binding affinity and orientation position. Random docking was performed on all ligands with the maximum grid box size.

2.5. Analysis and Visualization

The position and orientation of ligands in the enzyme were predicted based on an analysis of docking results. Analysis of cluster patterns resulting from docking on the surface or focusing on protein binding sites, binding affinity values, and the amount and types of amino acid residues around docking ligands were performed. Data comparing the number of docked ligands in each cluster were analyzed using the Microsoft

Excel. The results were visualized in 2D using LigPlot++ 1.4.5 to determine the number and types of amino acid residues around the docked ligands. Meanwhile, the docking results were visualized in 3D using Chimera 1.11.2 to describe the distribution of ligands in the enzyme.

3. Results and Discussion

3.1. Preparation and Validation of 3-dimensional Structures of Proteins and Ligands

The preparation and validation of 3-dimensional structures of proteins and ligands aimed to obtain valid 3- dimensional structures of proteins and ligands at their lowest energy state. Docking simulation of valid protein and ligand structures would produce more accurate data and be closer to its natural state.

3.1.1. Preparation and Validation of Protein 3- dimensional Structures

The 3-dimensional structures of the FabH and FabI proteins in the form of the PDB code for each protein were downloaded in .pdb file format via the website www.rcsb.org/pdb. The FabH protein used was the FabH protein (open) with PDB code 1HNH (1 chain A bound to CoA ligand), while the FabI protein uses a protein (open) with PDB code 1C14 (1 chain A bound to triclosan and NAD ligands) which shown in Figure 1. The ligand was separated from the protein using the Chimera 1.11.2 software. The FabH and FabI proteins were minimized using the minimize structure program integrated on Chimera 1.11.2 to obtain a 3-dimensional structure with the lowest energy.

Validation of the 3-dimensional structure of the protein was carried out by uploading the 3-dimensional structure of the protein before and after minimization on the MolProbity open-source website via the site http://molprobity.biochem.duke.edu. Validation of the 3- dimensional structure of the protein can be seen through the clashscore and MolProbity scores with a smaller value and a larger percentile than before minimization, indicating a more valid structure [14]. The results of validating the MolProbity 3-dimensional structure of the FabI and FabH enzyme proteins before and after minimization are presented in Table 1.

Table 1. Results of the validation of the 3-dimensional structure of enzymes

Validation parameters			Before minimization	After minimization
FabI Enzyme (1C14)	Clashscore*	Score value	9.49	4.22
		Percentile***	84th	99th
	MolProbity score*	Score value	2.27	2.01
		Percentile***	52th	73th
Enzyme FabH (1HNH)	Clashscore*	Score value	9.08	1.49
		Percentile***	82th	100th
	MolProbity score*	Score value	1.99	1.16
		Percentile***	69th	100th

*_Clashscore_: number of overlapping steric atoms (> 0.4 Å) per 1000 atoms

**_MolProbity score_: combining the overall evaluation values into one score comparable to the X-ray diffraction resolution value used when determining the 3-dimensional structure of a protein. The smaller the value, the better

***Percentile: 100th shows the best value and 0th shows the worst value

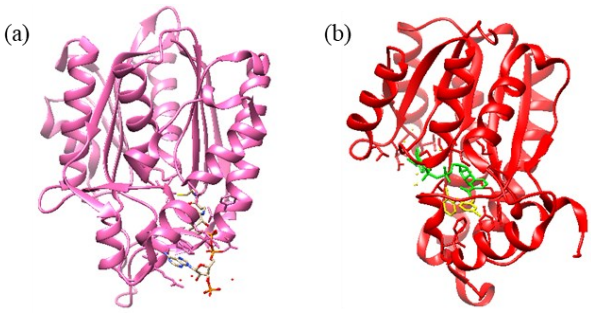

Figure 1. (a) FabH (1HNH) and (b) FabI (1C14) enzymes

3.1.2. Preparation and Validation of 3-dimensional Structures of Ligands

The ligands used in this study consisted of palmitic acid, acetyl CoA as an inhibitor of the FabH enzyme, α-selinene, and triclosan as a ligand to inhibit the FabI enzyme. The use of α-selinene and palmitic acid ligands has a pharmacological effect as an antibacterial against *Escherichia coli*. The FabI and FabH enzymes are inhibited by the α-selinene and palmitic acid, which also prevents the development of cell membranes and ends the growth or death of Escherichia coli bacteria. In this study, the standard ligands used were triclosan and acetyl CoA, which are native ligands that bind to the crystal structures of the enzymes FabI and FabH. The preparation stage for the 3-dimensional structure of the ligand was done by downloading the 3-dimensional structure of the ligand from an online database via the https://pubchem.ncbi.nlm.nih.gov website. The 3-dimensional structure of the ligand was saved in .pdb format using the Open Babel GUI program. The complete structure of all ligands is presented in Figure 2.

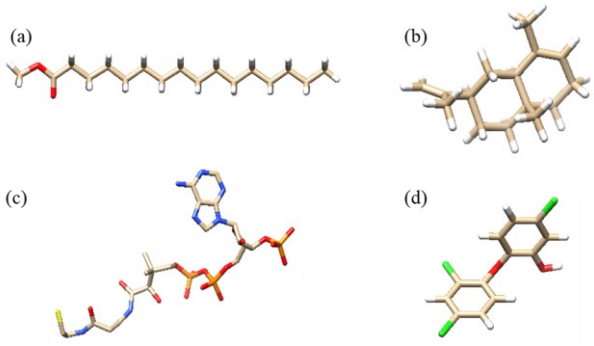

Figure 2. 3-dimensional structures of the ligands (a) palmitic acid, (b) α-selinene, (c) acetyl CoA, (d) triclosan

The data in Table 2 shows the characteristics of the ligands that meet Lipinski's Rule of Five (RO5) parameters. This antibacterial drug candidate must satisfy the RO5 parameters for the ligand to reach the intended target successfully. The criteria for RO5 are molecular weight ≤ 500, log P ≤ 5, hydrogen bond donors ≤ 5, and hydrogen bond acceptors ≤ 10. The greater the

log P value of a molecule, the more hydrophobic it is. A molecule can not penetrate the lipid bilayer membrane if the log P value is too negatively polar (negative). The number of hydrogen bond donors and acceptors describes that the higher the hydrogen bond capacity, the higher the energy required for absorption. The Lipinski rule generally describes the solubility of certain compounds to penetrate the cell membrane by passive diffusion [15].

Table 2. Ligand characteristics based on Lipinski RO5 parameters

Ligand	Log P	BM	Hydrogen donor	Hydrogen receptors	Molar refractory
Triclosan	2.828	289.5	1	2	60.859
Acetyl CoA	-3.837	731	0	23	139.089
Palmitic Acid	5.64	270	0	2	82.327
α-selinene	4.725	204	0	0	66.742

3.2. Ligand Cluster Pattern on FabI (1C14) Enzyme Surface

The ligand cluster pattern on the enzyme surface was obtained by random docking simulation of the triclosan standard ligand and α-selinene target ligand on the FabI enzyme surface in the open chain A state (.pdb code: 1C14). The 3-dimensional structure of the FabI enzyme used was derived from *Escherichia coli*. This docking simulation used the Autodock Vina program integrated into the Pyrx. The random docking method allows the ligands to interact freely to find the best position and orientation on the entire surface of the FabI enzyme. The docked ligand-protein interaction position data were grouped using the visualized clustering method using Chimera 1.11.12. The depiction of the scattering pattern of ligands resulting from random docking on the surface of the FabI enzyme is presented in Figure 3.

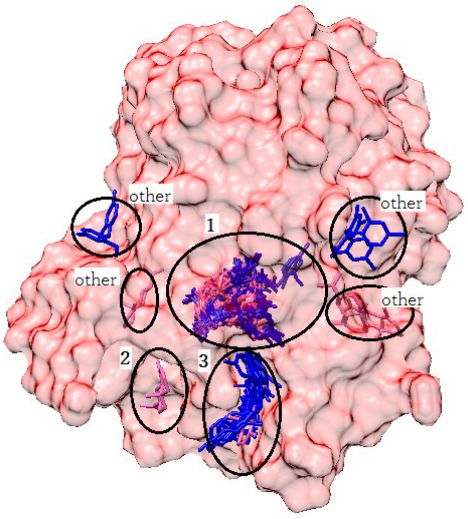

Figure 3. Ligand cluster patterns on the surface of the FabI 1C14 enzyme

The clustering analysis results showed that the ligand interaction pattern on the surface of the FabI 1C14 enzyme formed clusters 1, 2, and 3 and other clusters. Other clusters are clusters that are only occupied by one to three ligands. α-selinene ligands are depicted in pink, while triclosan ligands are shown in blue. The results of calculating the percentage of ligands present in each cluster and the strength of the interaction (binding affinity) are presented in Table 3.

Table 3. Percentage of ligand interactions in each cluster on the surface of the FabI enzyme

Enzyme	Ligand		Cluster 1	Cluster 2	Cluster 3	Other clusters
FabI (1C14)	α-selinene	Amount	88.88%	5.55%	1.11%	4.44%
		Best BA (kcal/mol)	-7.4	-7.2	-7.2	-7.2
	Triclosan	Amount	74.44%	-	22.22%	3.33%
		Best BA (kcal/mol)	-7.5	-	-7.1	-7

The docking simulation results performed ten times resulted in ten docking poses with their respective bond energies. α-selinene ligand in cluster 1 has a percent interaction value of 88.88%, with the best BA value of -7.4 kcal/mol. This shows that the tendency of the majority of α-selinene ligands has the greatest chance of interacting in cluster 1. The standard triclosan ligand interaction pattern also has the greatest chance of interacting in cluster 1 at 74.44%, with the best BA value of -7.5 kcal/mol. α-selinene is projected to have a substantial inhibitory effect on the FabI enzyme because the results indicate that it has similar opportunities and strong interactions with triclosan.

3.3. Ligand Cluster Patterns on the Enzyme FabH (1HNH) surface

The ligand cluster pattern on the enzyme surface was obtained by random docking simulation of the acetyl CoA standard ligand and palmitic acid target ligand on the FabH enzyme surface in the open chain A state (.pdb code: 1HNH). The 3-dimensional structure of the FabH enzyme used was derived from *Escherichia coli*. This docking simulation used Autodock Vina integrated into the Pyrx software. The random docking method allows ligands to interact freely to find the best position and orientation on the entire surface of the FabH enzyme. The docked ligand-protein interaction position data were grouped using the visualized clustering method using Chimera 1.11.12. The depiction of the scattering pattern of ligands resulting from random docking on the surface of the FabH enzyme is presented in Figure 4.

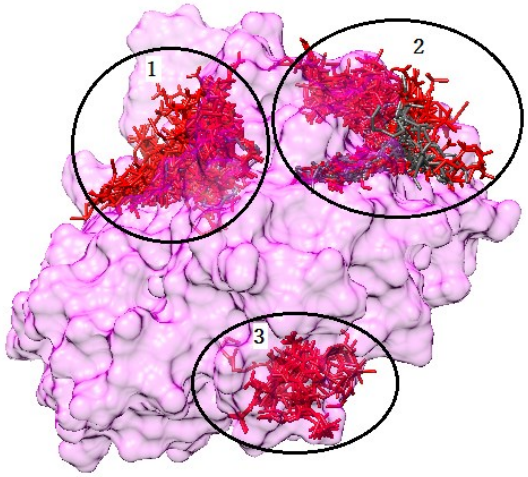

Figure 4. Ligand cluster patterns on the surface of the FabH 1HNH enzyme

The clustering analysis results showed that the ligand interaction patterns on the surface of the FabH 1HNH enzyme formed three clusters, namely clusters 1, 2, and 3. The palmitic acid ligands are depicted in gray, while the Acetyl CoA ligands are shown in red. The results of calculating the percentage of ligands present in each cluster and the strength of the interaction (binding affinity) are presented in Table 4.

Table 4. Percentage of ligand interactions in each cluster on the surface of the FabH enzyme

Enzyme	Ligand		Cluster 1	Cluster 2	Cluster 3
FabH (1HNH)	Palmitic acid	Amount	6.66%	93.33%	-
		Best BA (kcal/mol)	-4.6	-5.8	-
	Acetyl *CoA*	Amount	37.77%	51.11%	11.11%
		Best BA (kcal/mol)	-6.8	-7	-6.7

The docking simulation results done ten times resulted in 10 poses with their respective bond energies. Palmitic acid ligands in cluster 1 had a percentage interaction value of only 6.66%, with the best BA value of -4.6 kcal/mol. In comparison, cluster 2 had more interaction percentage values of 93.33% with a BA value of -5.8 kcal/mol. This shows that the tendency of the majority of palmitic acid ligands has the greatest chance of interacting in cluster 2. The standard ligand interaction pattern acetyl CoA also has the greatest chance of interacting in cluster 2 at 51.11%, with the best BA value of -7 kcal/mol. It is projected that palmitic acid will be more challenging to inhibit the FabH enzyme because the analysis revealed that the acetyl CoA ligand interacts more strongly than the palmitic acid ligand.

3.4. Position, Orientation, and Binding Affinity of Ligand-Protein Interactions at the Active Site of FabI Enzymes

The position, orientation, and binding affinity value of the ligand interaction were assessed based on the results of directional docking on the active site of the FabI enzyme. Position and orientation were determined by comparing them to the positions and orientations of the typical triclosan ligands in the FabI enzyme. The directional docking process was performed by directing the ligand to the active site of the FabI enzyme by arranging the grid box at the coordinates according to the position of the standard triclosan ligand. The size of the enzyme grid box was set at space center coordinates of 5.7834, 20.9500, 134.8107, and dimensions of 12.2669 × 12.2303 × 12.2082 Å. The best ligand position and orientation prediction were determined based on the most negative binding affinity value and the highest number of perfectly overlapping ligands. The best position and orientation can represent the greatest opportunity for ligands to interact at the proper position with strong interaction. The best position and orientation of the ligands on the active site of the FabI enzyme can be seen in Figure 5, which shows the best positions and orientations: best ligands (bold-colored), non-best ligands (thin-colored), and standard triclosan ligands (bold-black).

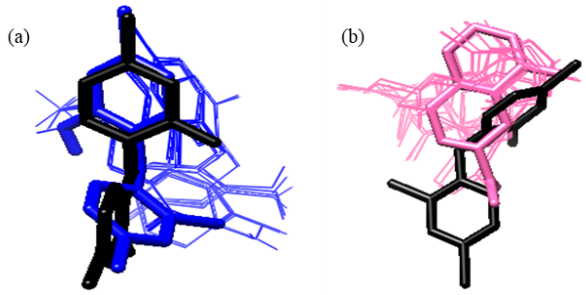

Figure 5. The best position and orientation of the docked ligands is directed towards the active site of the FabI enzyme (a) triclosan (20) BA -7, (b) α-selinene (29) BA - 7.3

The best ligand position and orientation prediction in FabI enzymes was determined based on the most negative binding affinity value and the highest number of perfectly overlapping ligands. The more ligands that overlap in a particular position and orientation indicate that the orientation position is the most preferred by the docking ligands. There are 20 ligands overlapped with the original triclosan ligands from the FabI enzyme; these ligands have significant contact with amino acid residues at the orientation position of BA -7 kcal/mol, which can be said to be a highly negative value. In the α-selinene ligand, 29 ligands overlap with the original triclosan ligand at BA -7.3 kcal/mol. The results of this study were better than those of the Khasanah [13], which only managed to obtain a BA value of -6.9 kcal/mol from the ligand α-selinene when it interacted with the FabH enzyme. As a result, the α-selinene ligand utilized as an inhibitor is anticipated to interact with the FabI enzyme more favorably, increasing its effectiveness in killing *Escherichia coli*.

Information about the types of residues interacting with standard and sample ligands was obtained from visualization results using the LigPlot++ 1.4.5 program. The description of the number and types of interactions of triclosan and α-selinene ligands in the best position and orientation with amino acid residues on the binding site of the FabI enzyme is presented in Figure 6.

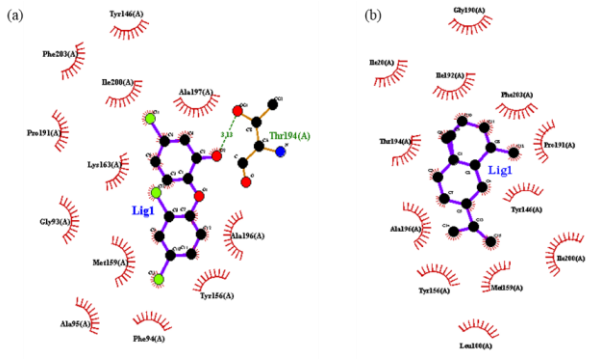

Figure 6. Hydrogen and hydrophobic ligand-protein interactions at the binding site of FabI (a) triclosan, (b) α-selinene

The analysis results of the LigPlot++ 1.4.5 program showed hydrogen and hydrophobic interactions between the ligands and the residues closest to the protein. The hydrogen interactions are represented by green dotted lines, while red semicircular lines represent the hydrophobic interactions.

The number and type of interactions between the ligand and the active residues of the FabI enzyme determined the strength of the interaction. The more active residues around the ligand, the stronger the interaction. In general, hydrogen interactions are stronger than hydrophobic interactions. The description of the number and types of residues on the active site of the FabI enzyme involved in interactions with the ligands is presented in Table 5.

Table 5. Results of interaction between ligand and active residues in FabI enzyme

Bond type	Amino acid residue			
	Triclosan	α-selinene	Triclosan*	Palmitic acid*
Binding affinity (kcal/mol)	-7	-7.3	-8.9	-7.1
Hydrogen Bonds	Thr194	-	Tyr156	-
Hydrophobic Bond	Ala095	Ala196	-	-
	Ala196	gly190	Ala196	Ala196
	Ala197	Ile020	Ala197	gly093
	gly093	Ile192	gly093	gln040
	Ile200	Ile200	Ile200	Ile200
	Lys163	Leu100	Leu100	Phe094
	Met159	Met159	Lys163	Phe293
	Phe094	Phe203	Met159	Tyr146
	Phe203	Pro191	Tyr146	Tyr156
	Pro191	Thr194	-	-
	Tyr146	Tyr146	-	-
	Tyr156	Tyr156	-	-
Total Active Residue	13	12	9	8
JRS Triclosan	-	8	-	5

* Ligands from previous research [11]

The results of this study indicate that the standard triclosan ligand only forms hydrogen interactions with the Thr194 amino acid residue. Triclosan also forms hydrophobic interactions with residues Ala095, Ala196, Ala197, Gly093, Ile200, Lys163, Met159, Phe094, Phe203, Pro191, Tyr146, Tyr156. Meanwhile, α-selinene does not form hydrogen interactions; it only forms hydrophobic interactions with residues Ala196, Gly190, Ile020, Ile192, Ile200, Leu100, Met159, Phe203, Pro191, Thr194, Tyr146, Tyr156. The results of the residual interactions between the triclosan ligand and α-selinene show many similarities in the interaction of the residues. Previous studies found that the results of the interaction of amino acid residues in the FabI enzyme were interacted with using palmitic acid ligands. Only five amino acids were the same between the residues of the triclosan ligand and palmitic acid. Amino acid residues that determine the active site of the FabI enzyme in terms of similarities between this study and previous studies are Ala196, Ile200, Tyr146, and Tyr156 [11].

In the FabI enzyme inhibition process, the Tyr156 residue is an important target to be blocked by sample ligands because it is a catalytic residue on the active site of the FabI enzyme. The hydride from NADH is transferred to C-3 from the C2-C3 double bond of the enoyl substrate to form the enolate at C-1. This enolate then accepts hydrogen from Tyr156 to form the enol, which tautomerizes it into the final product. Based on the data from Table 5, the ligand sample α-selinene interacts with the Tyr156 residue.

Triclosan is a standard ligand that binds to the crystal structure of the FabI enzyme. The triclosan compound has been shown in vitro as an inhibitor of the FabI enzyme through a competitive inhibition mechanism and as an antibacterial [8]. Triclosan will prevent the substrate from occupying its active site, inhibiting the FabI enzyme and causing the bacteria to die. The search for potential antibacterial sample ligands was based on the similarity of position and orientation with the standard triclosan ligands and the strength of the interaction based on their binding affinity values.

The position and orientation similarity is based on the similarity of the active residues that interact between the sample ligands and the standard ligands. The more similar the number of residues, the more similar the positions and orientations are with the standard triclosan ligands. The similarity of positions and orientations will enable the sample ligands to block the active site of triclosan properly. The residues belonging to α-selinene exhibit the same number of 8 due to its resemblance to the triclosan ligand. The α-selinene ligand (BA -7.3 kcal/mol) is projected to be a potent inhibitor of the FabI enzyme of *Escherichia coli* because it interacts with the enzyme more strongly than the triclosan ligand, which has a BA of -7 kcal/mol.

3.5. Position, Orientation, and Binding Affinity of Ligand-Protein Interactions on Active Sites of FabH Enzymes

In the FabH enzyme to determine the position, orientation, and binding affinity value of the ligand interaction was performed based on the results of directional docking on the active site of the FabH

enzyme. The determination of position and orientation was based on its similarity to the position and orientation of the standard ligand, namely acetyl CoA. The directional docking process was carried out by directing the ligand to the active site of the FabH enzyme by arranging the grid box at the coordinates according to the position of the acetyl CoA standard ligand. Gridbox size was set for the FabH enzyme at space center coordinates of 30.1031, 10.1542, 35.0747, and dimensions 22.5037 × 26.1176 × 15.1086 Å. The best ligand position and orientation prediction was determined based on the most negative binding affinity value and the highest number of perfectly overlapping ligands. The best position and orientation can represent the greatest opportunity for ligands to interact at the proper position with strong interaction. The best position and orientation of the ligands on the active site of the FabH enzyme can be seen in Figure 7. It shows that the variations in the positions and orientations of the docked ligands (thin colored) are around the best ligand positions (bold-colored) and standard acetyl CoA (bold-black).

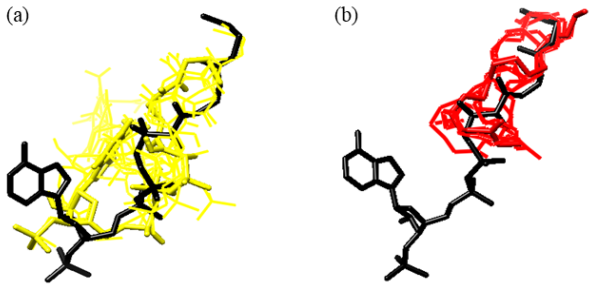

Figure 7. The best position and orientation of the docked ligands is directed towards the active site of the FabH enzyme(a) acetyl CoA (6) BA -7.1, (b) palmitic acid (8) BA -5.7

Based on the most negative binding affinity value and the highest number of perfectly overlapping ligands, this result is used as the best ligand position and orientation of the FabH enzyme. The more ligands overlap perfectly in a particular position and orientation, and the more docking ligands prefer the orientation position. Six ligands in the standard acetyl CoA ligand overlapped with the original from the FabH enzyme. The orientation position at BA -7.1 kcal/mol is quite negative and has strong interactions with amino acid residues. In contrast, eight ligands overlapped with the original acetyl CoA ligand in the palmitic acid ligand with a BA value of - 5.7 kcal/mol. This is an unfavorable result compared to previous research, which obtained a BA value of -7.1 kcal/mol [11]. The prediction results showed that the palmitic acid ligand as an inhibitor is not optimal when interacting with the FabH enzyme. However, the palmitic acid ligand would be better when it interacts with the FabI enzyme, which is antibacterial against *Escherichia coli*.

Information about the types of residues interacting with standard and sample ligands was obtained from visualization results using the LigPlot++ 1.4.5 program. The description of the number and type of interaction of acetyl CoA and palmitic acid ligands in the best position and orientation with amino acid residues on the binding site of the FabH enzyme is presented in Figure 8.

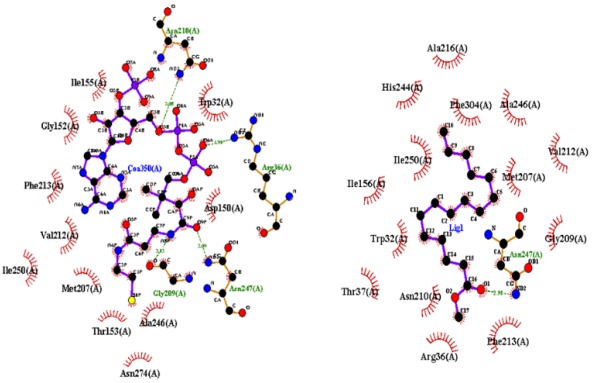

Figure 8. Hydrogen and hydrophobic ligand-protein interactions at the binding site of the FabH enzyme

The analysis results of the LigPlot++ 1.4.5 program show hydrogen interactions depicted by the green dotted line and hydrophobic interactions depicted by the red half-circle line. The number and type of interactions between the ligand and the active residues of the FabH enzyme determine the strength of the interaction. The more active residues around the ligand, the stronger the interaction. The description of the number and types of residues on the active site of the FabH enzyme involved in interactions with the ligands is presented in Table 6.

Table 6. Result of ligand interaction with active residues in the FabH enzyme

Bond Type	Amino acid residue			
	Acetyl CoA	Palmitic Acid	Acetyl CoA*	α-selinene*
Binding Affinity (kcal/mol)	-7.1	-5.7	-6.6	-6.9
Hydrogen Bonds	Arg036	Asn247	Arg036	-
	Asn210	-	Arg151	-
	Asn247	-	Asn210	-
	gly209	-	Asn247	-
		-	Thr028	-
Hydrophobic Bond	Ala246	Arg036	Ala246	Ala246
	Asn274	Asn210	gly209	Asn247
	Asp150	Ala216	Ile156	Asn274
	glly152	Ala246	Phe213	gly209
	Ile155	gly209	Trp032	His244
	Ile250	Ile156	Thr037	Ile156
	Met207	Ile250	-	Ile250
	Phe213	His244	-	Met207
	Thr153	Met207	-	Phe157
	Trp032	Phe213	-	Phe213
	Val212	Phe304	-	Phe304
	-	Thr037	-	Val212
	-	Trp032	-	-
	-	Val212	-	-
Total Active Residue	15	15	11	12
JRS Acetyl CoA	-	10	-	5

* Ligands from previous research [13]

The results of this study indicate that the standard Acetyl CoA ligand forms hydrogen interactions with the amino acid residues Arg036, Asn210, Asn247, and Gly209. Acetyl CoA also forms hydrophobic interactions with residues Ala246, Asn274, Asp150, Gly152, Ile155, Ille250, Met207, Phe213, Thr153, Trp032, Val212. Meanwhile onpalmitic acid only forms one hydrogen interaction, namely Asn247, palmitic acid also forms hydrophobic interactions with residues Arg036, Asn210, Ala216, Ala246, Gly209, Ille156, Ille250, His244, Met207, Phe213, Phe304, Thr037, Trp032, Val212. From the residual interaction between the Acetyl CoA and palmitic acid ligands, there are 10 of the same amino acid residues. Acetyl CoA ligands are the original ligands in *Escherichia coli,* which will interact with the FabH enzyme during forming of fatty acids. From the similarity of the residue results, it can be interpreted that the acid palmitate will later be able to inhibit the FabH enzyme and block the position of Acetyl CoA during the fatty acid synthesis process so that it will result in the inhibition of the formation of bacterial cell membranes.

In previous studies, the results of the interaction of amino acid residues in the FabH enzyme were interacted with using ligandsα-selinene only has five amino acids that are the same between the residues of Acetyl CoA and α-selinene. Amino acid residues that determine the active site of the FabH enzyme in terms of similarities between this study and previous studies are Asn247, Gly209, Ala246. and Phe213 [13]. Enzyme FabH has a catalytic residue side Asn 274 which is the target of inhibition on the active site of the enzyme, this catalytic residue is a residue that contributes directly to the enzyme catalytic reaction, but only the α-selinene sample ligand from previous studies contained Asn274 residue.

This indicates that the ligandα-selinene is predicted to be the best ligand to be used as a drug candidate that can inhibit FabI and FabH enzymes due to the inhibition mechanism on α-selinene with FabI and FabH enzymes which have catalytic residues which prove that α-selinene will inhibit better than palmitic acid ligands, and reviewed from the binding affinity value produced by the α-selinene ligand (-7.3 kcal/mol) which is more negative than the palmitic acid ligand (-5.7 kcal/mol), so it will be able to interact more strongly with the target protein.

From the results of this study, it is predicted that the best antibacterial mechanism for *Escherichia coli* is through the FabI enzyme because when it interacts with palmitic acid and α-selinene ligands, it produces BA which is more negative and more which overlaps with the original ligands of each enzyme.

4. Conclusion

The results of the in silico study showed that α-selinene (BA -7.3) was the best ligand for inhibiting *Escherichia coli*. The interaction between the FabI enzyme and the α-selinene and palmitic acid ligands showed a more optimal inhibition of *Escherichia coli*. The amino acid residues determining the FabI enzyme's active site were Ala196, Ille200, Tyr146, and Tyr156. Meanwhile, the FabH enzymes were Asn247, Gly209, Ala246. and Phe213.

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