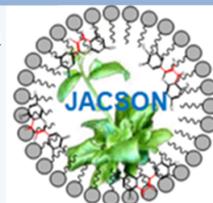




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### Inhibition Docking Simulation of Zerumbone, Gingerglycolipid B, and Curzerenone Compound of Zingiber zerumbet from Timor Island against MurA Enzyme

Origenes Boy Kapitan<sup>\*1,2</sup>, Laksmi Ambarsari<sup>3</sup>, and Syamsul Falah<sup>1</sup>

<sup>1</sup>Departement of Biochemistry, FMIPA, Institut Pertanian Bogor, 16680, INDONESIA, <sup>2</sup>Program Studi Agroteknologi, Faperta, Universitas Timor Jl. Km.09 Kelurahan Sasi, Kefamenanu, NTT, INDONESIA, <sup>3</sup>Pusat Penelitian Biofarmaka, Institut Pertanian Bogor, Jl. Taman Kencana No.3, Bogor 16128, INDONESIA

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#### ABSTRACT

Zingiber zerumbet is a medicinal plant which is traditionally used to treat ulcerative lesions. In this research, it has been done an in vitro studies of its antibacterial activity test from crude ethyl acetate, ethanol, and aqueous extracts. The result of LC-MS analysis showed that the most active antibacterial compounds in the ethyl acetate, ethanol, and aqueous extract were zerumbone, gingerglycolipid B, and curzerenone. Further molecular docking simulation was performed using Autodock Vina to filter based on the value of affinity zerumbone, gingerglycolipid B, and curzerenone in the active site of MurA enzymes ( $\Delta G_{\text{binding}}$ ). The result indicated that the affinity energy ( $\Delta G_{\text{binding}}$ ) molecular docking of the natural ligand (substrat), drug ligand (fosfomycin), zerumbone ligand, gingerglycolipid B ligand, and the curzerenone ligand against the MurA enzyme were respectively -10.1, -4.7, -8.3, -8.4 and -7.4 kcal mol<sup>-1</sup>. Further docking simulations indicated that there was a competitive reaction mechanism between zerumbone (test ligand) with an uridine-diphosphate-N-asetylglucosamine substrate in the enzyme as a test ligand to occupy the same spot as the substrate therefore inhibiting the formation of peptidoglycan, a major constituent of bacterial cell wall. Analysis of  $\Delta G_{\text{binding}}$  value and the result of docking simulations interaction finally indicated that the zerumbone ligand had the highest potential to replace fosfomycin drug in inhibiting the synthesis of bacterial peptidoglycan.

**Keywords:** cell wall, peptidoglycan, Lipinsky rule, phosphomycin, Autodock Vina

**\*Corresponding author:** [olivianusrkapitan@gmail.com](mailto:olivianusrkapitan@gmail.com), Tel/Fax:(+62)388-2433012/2433013

#### 1. Introduction

Computer aided drug design (CADD) is a method of drug development which is based on computational science (Meng et al., 2011). This method helps in understanding the structure of molecular biology and contributes to the advance research of drug candidates based on their structures. In this method, the prediction of the three-dimensional structure of the complex between protein targets and drug candidates plays a very important role particularly in structure-based drug design. The design can subsequently be observed from the interaction between the protein and the ligand (Wandzik, 2006). Among various CADD methods, molecular docking simulation is one of the most commonly CADD used methods. This method has been frequently used in studying the interaction

between drugs and receptors where the binding orientation between ligand candidate and target protein can be determined to find out the performance and affinity of the ligand (Meng et al., 2011; Kumar et al., 2012; Yanuar, 2012). Docking simulations can therefore be used to obtain the binding mechanism of a chemical compound or macromolecule in molecular scale which can also allow the possibility of designing the desired ligand structure (Vijesh et al., 2013).

Peptidoglycan is commonly known as a very essential and the largest spesific component of the bacterial wall cells which is found outside the cytoplasmic membrane (Vollmer et al., 2008). Peptidoglycan is a typical of heteropolimer glycan which is cross-linked with amino acids. This component plays an important role in restraining the

turgor pressure (Vollmer and Bertsche, 2008, de Pedro and Cava, 2015) and contributes to the shape and stiffness of bacteria wall cells (Margolin, 2009) that can protect the bacteria from the damage caused by the osmotic pressure. The survival of the bacteria was generally determined by the activity of the UDP N-acetylglucosamine transferase Enolpyruvyl enzyme, EC 2.5.1.7 (MurA) (Skarzynki et al., 1996; Eschenburg et al., 2005). This enzyme catalyzes the first step of peptidoglycan biosynthesis by condensing phosphoenolpyruvate (PEP) and UDP-N-acetylglucosamine (UDP-Glc-NAC) into UDP-GlcNAc-enolpyruvate. This step, however, does not occur in mammals hence the MurA becomes a target in antibacterial drug development (Eschenburg et al., 2005).

It has been reported that fosfomycin is one of commercial drugs that can be used to inhibit the growth of the MurA enzyme (Skarzynki et al., 1996). However, recent research has prompted its deficiency due to further increase in the level of bacterial resistance to this drug (Eschenburg et al., 2005). Therefore, it is necessary to find an alternative replacement candidate derived from medicinal plants. One of medicinal plant candidate which has antibacterial activity and is very potential to be further explored is Zingiber zerumbet (Kader et al., 2011, Singh et al., 2012). Our previous research through an in vitro study indicated that crude extract of ethyl acetate, ethanol, and aqueous of *Z. zerumbet* has shown a potential antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*. Further analysis using LC-MS indicated that there were three specific and bioactive compounds of these three extracts which were responsible for their antibacterial activities including zerumbone, ginglycolipid B, and curzerenon (Kapitan et al., 2015).

## 2. Materials and Methods

### 2.1. Materials

Laptop (Intel Core i3, 2GB of DDR2 RAM, 250 Seagate HDD and VGA ati radeon x1200), Fast File extension, PDB, PDBQT, the 3D chemical structure of the MurA enzyme, the 2D chemical structure of uridinediphosphate-N-acetylglucosamine; fosfomycin; zerumbone; ginglycolipid B; and curzerenone, ADT 1.5.6, 1.9.2 VMD, Marvin Sketch, Discovery Studio v3.5, LigPlot + 4.5.3, and Autodock Vina.

### 2.2. Methods

#### 2.2.1. Determination of the MurA receptor structure

The initial step before performing a docking simulations is a determination of the receptor and the ligand which will be tested.

#### 2.2.2. Ligand preparation

The ligand structures including the 2-dimensional (2D) and the three dimensional (3D) were drawn by using Marvin Sketch software.

#### 2.2.3. Optimization of three-dimensional MurA geometry structure

The optimization of three-dimensional MurA structure was carried out by using an Autodock Tools (ADT) 1.5.6 software. It was initially done through the removal of water molecules (H<sub>2</sub>O) around proteins, hetero atoms and substrat or natural ligands. Next step is the addition of the Gasteiger charge and hydrogen. The resulting file is then stored in a PDBQT format and the stability of the structure can be pictured on Ramachandran plot of the VMD 1.9.2.

#### 2.2.4. Optimization of three dimensional ligands geometry structure

The structures of the ligands used were designed using a Marvin sketch software with pdb. storage format. The optimization of the structures were done using an ADT 1.5.6 software. The initial step was to perform nonpolar hydrogen merged and Gasteiger charge addition. All files were stored in a pdbqt. Format and all the resulting ligand compounds were further filtered according to the Lipinski rule by using an online tools (Web-1)

#### 2.2.5. Molecular docking simulation

Grid process and parameter validation were carried out using ADT 1.5.6. The following molecular docking was done by using Autodock Vina (Scripps Research Institute, USA) where all the rotatable bonds of the ligands can flexibly rotate and the macromolecules are assumed to be rigid. The following parameters are parameters that have been validated. The size of grid box was selected and validated as 80 x 80 x 80 Å with 0,375 Å spacing centered on the active site, while the x\_center, y\_center and z\_center were chosen and validated respectively as 36.021, 19.91, and 44.388. The area of the box included the surrounding of the entire MurA structure. The exhaustiveness was set at approximately 250. Subsequently, the resulting Vina folder was placed in C:\Vina where all the CONF.TXT files were saved with all the above parameters including its center and size values.

The execution of the molecular docking command was done on the CMD window as the following procedures. Initially, Vina.exe was called and the specific command of "C: \ vina --config conf.txt --log log.txt" was typed. The following step was done by pressing the enter command and waiting until the process is fully completed where the num-20 mode has to be removed. The ligands in the MurA complex with the smallest resulting Gibbs free energy

( $\Delta G_{\text{binding}}$ ) from the list were then stored in a PDBQT format (ADT 1.5.6) and further converted into PDB format with DSV 3.5.

### 2.2.6. Analysis

The results of molecular docking were analysed their Gibbs free energy ( $\Delta G_{\text{binding}}$ ), inhibition constant, hydrogen bonds, RMSD, binding residues, van der Waals forces and the functional group ligands using Ligplot + 4.5.3 with .pdb format for 2D visualization. DSV 3.5 was used for the analysis of van der Waals forces and the DNA binding mode while VMD was used for the visualization of the resulting 3D structures.

## 3. Results and Discussion

### 3.1. Characteristics of MurA enzyme

The three-dimensional MurA enzyme structure data used in this research was downloaded from the Protein Data Bank (PDB) (Web-2) with PDB ID: 1UAE (Skarzynski et al., 1996). These data are the result of analysis using several biophysical techniques such as X-ray crystallography or NMR spectroscopy which cover the structure, the active sites and the sequences. Fig. 1 shows the ternary complex of a protein, a substrate or a natural ligand (uridine-diphosphate-N-acetylglucosamine) and fosfomycin or drug ligand. As a commercial drug, fosfomycin can inhibit the action of this enzyme by acting as an analogue substrate which can react with Cys<sup>115</sup> residues.

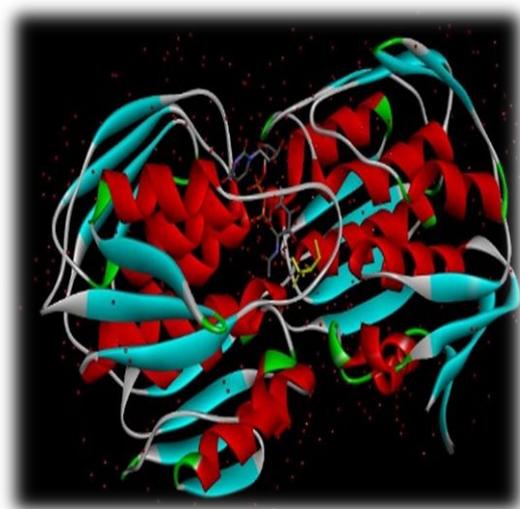


Fig. 1. The structure of MurA enzyme with PDB ID: 1UAE (Skarzynski et al. 1996)

A MurA enzyme consists of 418 residues with 34% of its helix composition consisting of 18 helix with 143 amino acid residues. Its beta sheet, on the other hand, consists of 34 strands with 113 residues. Secondary structure of the MurA enzyme can be seen in Fig. 2.

### 3.2. The stability of the receptor structure

The stability of the MurA enzyme structure is shown by the Ramachandran plot. The visualization of the Ramachandran plot was downloaded from the PDB. This diagram, as shown in Fig. 3, has four quadrants which are the most favored regions in quadrant I, additional allowed regions in quadrant II, generously allowed regions in quadrant III, and disallowed regions in quadrant IV. Phi ( $\Phi$ ) symbol indicates the x-axis, whereas psi ( $\psi$ ) expressed the y-axis of the amino acids in the protein structure (Ramachandran et al., 1963; Bosco and Brasseur, 2005).

The diagram indicates that the MurA enzyme is stable. This can be seen from the amino acids data where 97.8% of them are in the most favored region (407/406) and 100% are in the permitted (allowed) area (416/416). In addition, there are less than 1% of amino acid found in disallowed area. The more the percentage of amino acids residue in the most favored region and the lower the percentage of residues on disallowed region, the better the quality and the stability of receptor structure (Bosco and Brasseur, 2005).

### 3.3. Potential ligands according to the Lipinski rules

The test ligands used in this research was obtained from the crude extract of ethyl acetate, ethanol and aqueous from *Z. Zerumbet* which have been previously analysed by using LC-MS. These ligands have also been determined to have antibacterial activity (Kapitan et al., 2015). They are including zerumbone, gingerglycolipid B, and curzerenone. In total, there are five ligand used in this simulation including a natural ligand of the MurA enzyme, a fosfomycin ligand as a commercial drug MurA enzyme inhibitor and three test ligands. The ligands structure showed in Table 1.

It is generally accepted that the potential drug candidates have to meet some criteria according to Lipinski rules to be optimally absorbed in the body. The potential of these candidate ligands to penetrate through membrane cells and be absorbed by gastrointestinal were validated using Lipinski software. This software validates several parameters of these potential ligands including their molecular weight, the amount of group donor hydrogen bond, the number of hydrogen bond receptors, log P value and their molar refractivity (Lipinski et al., 2001). According to Leeson and Springthorpe (2007), a compound with molecular weight less than 500 g mol<sup>-1</sup> is able to pass through the

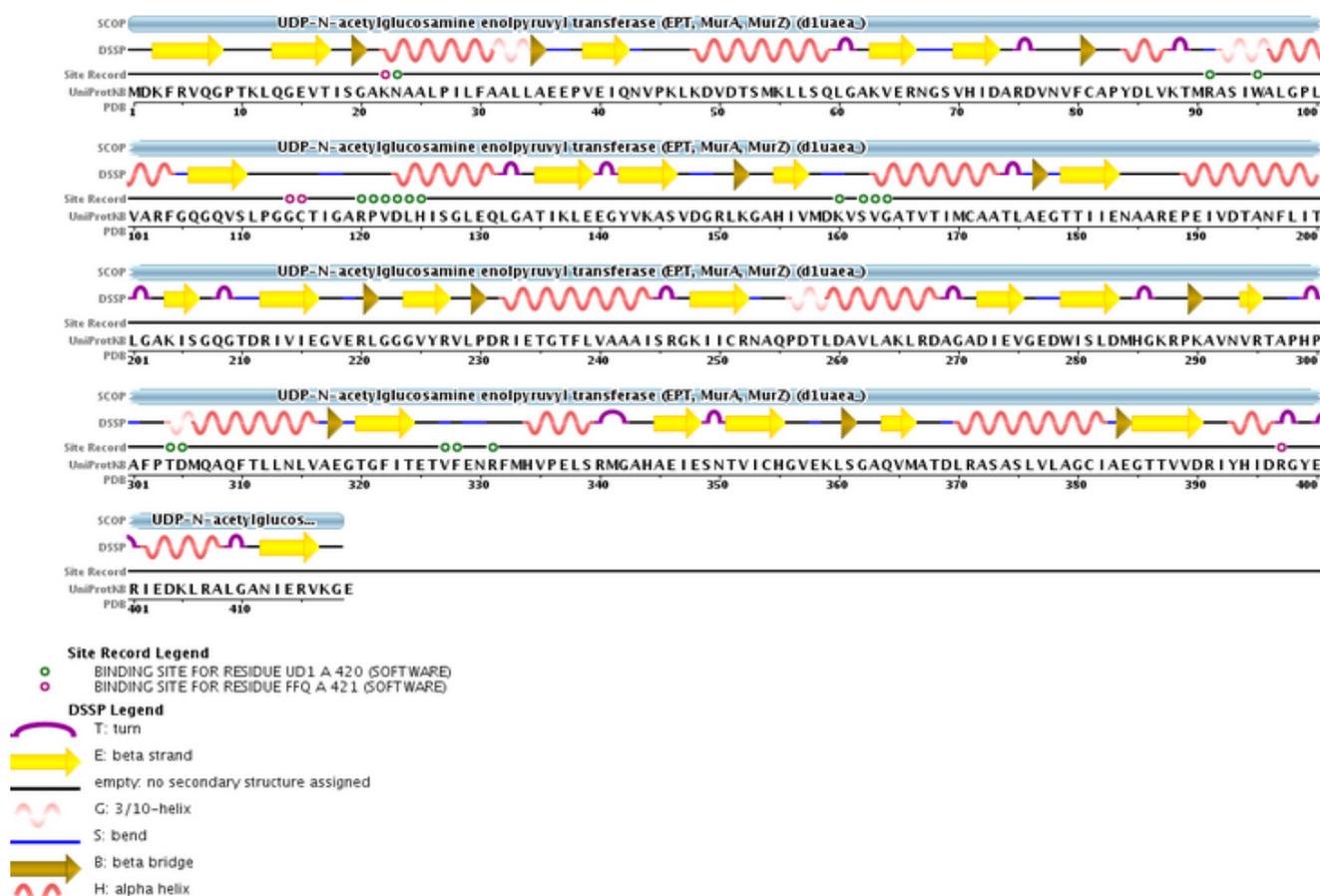


Fig. 2. Secondary structure of MurA receptors

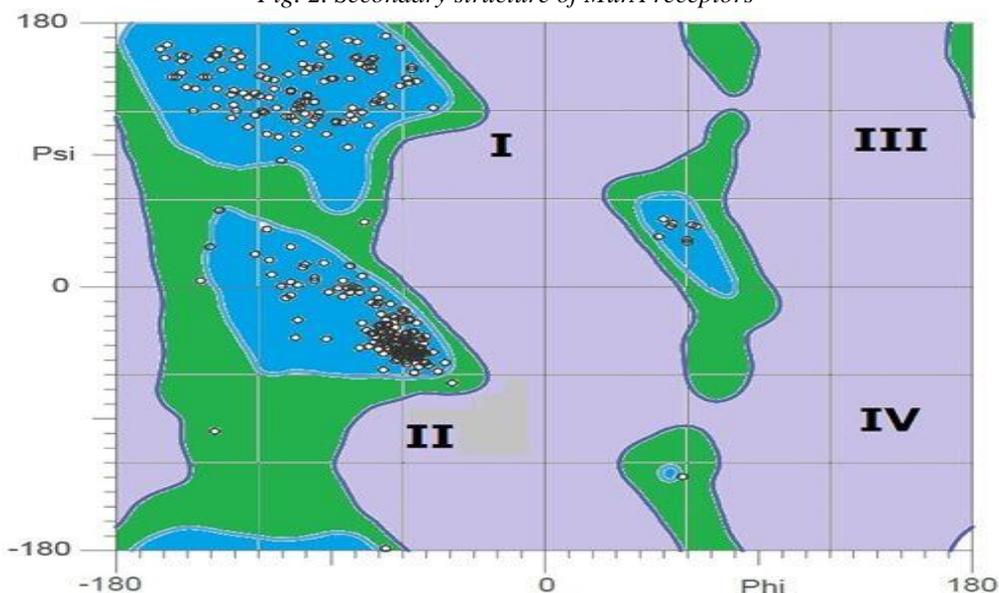
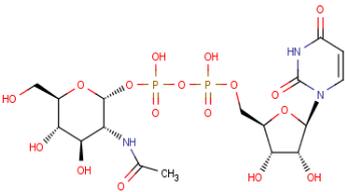
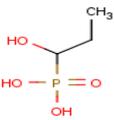
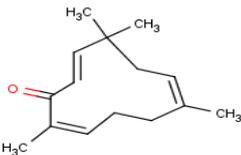
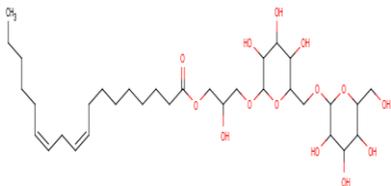
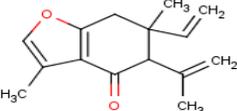


Fig. 3. The Ramachandran plot of MurA enzyme

walls of the gastrointestinal tract into the blood vessel by means of penetrating the wall cells of the gastrointestinal tract. This rule further require a potential ligand to have log P value less than 5 in order to be well absorbed by the intestinal. At this condition, the ligand candidate will undergo a passive diffusion

where it will spontaneously moves from higher concentration region to the lower one and this process will spontaneously and non selectively occurs. This transport will only stop once the concentration of the drug in the initial and target areas has reached equilibrium. This transport plays an important role

Tabel 1. 2D structure of ligands (substrate or UDP-*N*-GluAc, fosfomycin, zerumbone, ginglycolipid B, and curzerenone)

No.	Structure	IUPAC Name	Common name
1.		[({[2R,3S,4R,5R]-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}(hydroxyphosphoryl)oxy)({[2R,3R,4R,5S,6R]-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}phosphinic acid	Uridine-diphosphate- <i>N</i> -acetyl-glucosamine
2.		[(1R)-1-hydroxypropyl]phosphonic acid	Fosfomycin
3.		( <i>E,E</i> )-2,6,9,9-tetramethyl-2,6,10-cycloundecatrien-1-one	Zerumbone
4.		2-hydroxy-3-({[3,4,5-trihydroxy-6-({3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}methyl)oxan-2-yl]oxy}propyl (9 <i>Z</i> ,12 <i>Z</i> )-octadeca-9,12-dienoate	Ginglykolipid B
5.		6-ethenyl-3,6-dimethyl-5-(prop-1-en-2-yl)-4,5,6,7-tetrahydro-1-benzofuran-4-one	Curzerenone

proton acceptor is less than ten in order to positively increase the stability of the binding force between the potential compound and the protein target. The protein will have a stable conformational change in case large number of hydrogen bonds formed between the potential ligand (inhibitor) and the protein target (Lipinski et al., 2001; Arwansyah et al., 2014).

The tests were carried out using online tools (Web-2). The results of a Lipinski filter of the natural ligand, fosfomycin, zerumbone, ginglycolipid B, and the curzerenone can be seen in Table 2. The Lipinski filter result shows that the fosfomycin ligand and ginglycolipid B indicate does not fulfill the Lipinski rule, while zerumbone and curzerenone ligand meet Lipinski rules. It is shown on the table that the molar refractivities of fosfomycin ligand is less than 40, hence it is considered as a not optimal compound to be absorbed in the body. Meanwhile, the ginger-glycolipid B ligand have relative atomic mass and molar refractivity values larger than those that required. Therefore, it is clear that this ligand can not

become a substitute for the fosfomycin drug candidate because it will not also be optimally absorbed in the body.

### 3.4. Affinity Energy ( $\Delta G_{\text{binding}}$ ) molecular docking to a *MurA* enzyme)

Molecular docking predicted the orientation of a molecule to another molecule when they bind each other to form a new stable complex (Kroemer, 2003; Funkhouser, 2007). The result of analysis shows that the affinity energy ( $\Delta G_{\text{binding}}$ ) molecular docking of natural ligand, drug ligand, zerumbone ligand, ginglycolipid B ligand, and the curzerenone ligand were -10.1, -4.7, -8.3, -8.4 and -7.4 kcal Mol<sup>-1</sup> respectively (Fig. 4). The lower the Gibbs free energy value, the more stable the conformation form ed. Conversely, the higher the the Gibbs free energy value the more unstable the complexes formed (Kroemer, 2003). The differences in the interaction energy are also affected by the number of atoms, types of atoms, and ligand orientation.

Table 2. Results of Lipinski filter between the natural ligand, a ligand and a ligand drug test

Lipinski's rule	Ligand				
	Natural (Substrate)	Drugs	Zerumbone	Gingerglycolipid B	Curzerenone
A	-	138	226	684	240
B	-	1	1	9	1
C	-	4	1	14	2
D	-	-1.3715	4.2973	0.5845	3.0906
E	-	25.2772	70.4057	169.8644	69.7437

Ket: A: Molecular mass <500, B: Hydrogen bond donors <5, C: Hydrogen bond acceptors <10, D: Lipophilicity as Log P <5, E: Molar refractivity 40-130, -: Can not be analyzed by online tools

### 3.5. Ligand inhibition constant value

$\Delta G_{\text{binding}}$  value is used to determine the inhibition constants which represents the inhibition concentration of active compound to a half-life of enzyme reactions ( $K_i$ ). Theoretically, the smaller the  $K_i$  value the smaller of the inhibitor concentration required to inhibit the substrate.  $K_i$  value (inhibition constant) can be calculated by the equation:

$$\Delta G = RT \ln K_i$$

The equation clearly shows that the  $K_i$  values are in a linear correlation with  $\Delta G_{\text{binding}}$  value. In other words, it can be stated that the better the  $\Delta G$  value of a ligand the smaller the concentration of the ligand concentration needed as enzyme inhibitors. Table 3 shows the results of calculation of  $K_i$  values for each ligand.

### 3.6. Molecular docking interaction of ligand to the MurA enzymes

Results tethering interaction between ligand molecules and MurA enzymes were bond types, bond length, amino acid residues that interact with the enzyme, and the interacting functional group of the ligands (Kumar et al., 2012; Yanuar, 2012). The molecular docking results showed that there is an interaction between drug ligand, test ligand, and the natural ligand with the MurA enzyme as shown in Table 3.

The hydrogen bonds formed between amino acids and ligands indicated that there is a specific molecular interactions (Hubbard, 2010). These bonding occur between the non-covalent hydrogen atom with electronegative atom. It is important to notice that the distance between the ligand and the amino acid residues which interact also influence the resulting bonding in which the closer the distance

between the ligand and the target amino acid residue, the stronger and stabler the interactions that occur. The distance between donor and acceptor bond also determine the strength of hydrogen bonds (Table 3) (Jeffery, 1997). The closer the hydrogen bond the stronger the bond energy formed.

It was found that the interaction of the test ligand, zerumbone with amino acid residues in the active site of enzyme is similar to the interaction between drug and natural ligands in the enzyme. This can be attributed to the similarity of the amino acid residues on both zerumbone and drug ligands which is Arg<sup>91</sup> and that of the natural ligand which is His<sup>125</sup> residue. More importantly, natural ligand is bound to similar amino acid residue spot on both zerumbone and drug ligand which are in the amino acids residues of Arg<sup>120</sup> and His<sup>125</sup>. These results indicate that there is a competitive reaction mechanisms between zerumbone and the substrate (the natural ligand) in the enzyme which was due to the competitive occupation of similar spot on enzyme by zerumbone test ligand and the substrate. The drug ligand bind to the amino acid residues of Arg<sup>120</sup> therefore preventing the substrate from binding amino acid residues at the same spot. This reveals that the drug ligands can act not only as an analogue for the substrate through the reveralkylation thiol groups of the Cys<sup>115</sup> amino acids but also as reactant in the competitive reaction between the ligand drug and the substrate.

Gingerglycolipid B ligand is found to have a similar binding site to the common drug ligands at the active site of enzymes, which are characterized by the similarity binding spot of amino acid residues Arg<sup>91</sup>. This ligand might also occupy the same site as the natural ligand of the MurA enzyme, which is attributed to the similarity of hydrophobic residue interactions, i.e, Asn<sup>23</sup>, Arg<sup>91</sup> and Phe<sup>328</sup>.

Table 3. The results of belay molecular interactions between ligands and MurA enzyme

Ligand types	natural/substrate	Drugs/fosfomycin	gingerglycolipid B ligand test	Zerumbone ligand test	Curzerenone ligand test
$\Delta G$ (Kkal mol <sup>-1</sup> )	-10.1	-4.7	-8.4	-8.3	-7.4
Ki value (mol <sup>-1</sup> )	0.012	0.126	0.025	0.026	0.038
RMSD	1.570	1.484	1.693	0.986	1.274
The number of H-bonds	4	4	3	2	1
Hydrogen interaction residue	NH from Arg <sup>120</sup> N from His <sup>125</sup> N from Val <sup>163</sup> N from Gly <sup>164</sup>	N from Arg <sup>91</sup> NH from Arg <sup>120</sup> N from Arg <sup>120</sup> N from Gly <sup>164</sup>	NH from Arg <sup>91</sup> N from Val <sup>161</sup> NH from Arg <sup>232</sup>	NH from Arg <sup>91</sup> N from His <sup>125</sup>	N from Asn <sup>23</sup>
Atom ligands that interacts	C <sub>1B</sub> -O <sub>4B</sub> P <sub>A</sub> -O <sub>2A</sub> C <sub>6'</sub> -O <sub>6'</sub> C <sub>5B</sub> -O <sub>5B</sub>	P <sub>1</sub> -O <sub>4</sub> C <sub>2</sub> -O <sub>1</sub> C <sub>2</sub> -O <sub>1</sub> P <sub>1</sub> -O <sub>3</sub>	C <sub>32</sub> -O <sub>13</sub> C <sub>4</sub> -O <sub>3</sub> C <sub>29</sub> -O <sub>10</sub>	C <sub>9</sub> -O C <sub>9</sub> -O	C <sub>4</sub> -O <sub>1</sub>
Hydrogen bond distance	2.80 3.13 2.98 2.87	3.28 2.92 3.11 2.81	2.79 3.29 2.74	3.02 3.19	3.28
Bond strength	medium medium medium medium	weak medium medium medium	medium weak medium	medium medium	weak
Hydrophobic interaction residue	Lys <sup>22</sup> , Asn <sup>23</sup> , Leu <sup>24</sup> , Leu <sup>26</sup> , Trp <sup>95</sup> , Arg <sup>91</sup> , Ala <sup>92</sup> , Pro <sup>121</sup> , Ser <sup>162</sup> , Val <sup>317</sup> , Phe <sup>328</sup>	Ser <sup>162</sup> , Val <sup>163</sup> , Phe <sup>32</sup>	Asn <sup>23</sup> , Val <sup>157</sup> , Lys <sup>160</sup> , Val <sup>163</sup> , Glu <sup>183</sup> , Asn <sup>184</sup> , Glu <sup>188</sup> , Asp <sup>211</sup> , Pro <sup>298</sup> , Thr <sup>304</sup> , Asp <sup>305</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Trp <sup>95</sup> , Ser <sup>162</sup> , Val <sup>163</sup> , Gly <sup>164</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Arg <sup>120</sup> , Val <sup>163</sup> , Glu <sup>188</sup> , Arg <sup>232</sup> , Pro <sup>298</sup> , Pro <sup>303</sup> , Thr <sup>304</sup> , Asp <sup>305</sup> , Val <sup>327</sup> , Phe <sup>328</sup>
Uncharged polar amino acids	Asn <sup>23</sup> , Trp <sup>95</sup> , His <sup>125</sup> , Ser <sup>162</sup>	Ser <sup>162</sup>	Asn <sup>23</sup> , Glu <sup>183</sup> , Asn <sup>184</sup> , Glu <sup>188</sup> , Thr <sup>304</sup>	Trp <sup>95</sup> , His <sup>125</sup> , Ser <sup>162</sup>	Asn <sup>23</sup> , Glu <sup>188</sup> , Thr <sup>304</sup>
Charged polar amino acids	Lys <sup>22</sup> , Arg <sup>91</sup> , Arg <sup>120</sup>	Arg <sup>91</sup> , Arg <sup>120</sup>	Arg <sup>91</sup> , Lys <sup>160</sup> , Asp <sup>211</sup> , Asp <sup>305</sup> , Arg <sup>232</sup>	Arg <sup>91</sup>	Arg <sup>120</sup> , Arg <sup>232</sup> , Asp <sup>305</sup>
Non-polar amino acids	Leu <sup>24</sup> , Leu <sup>26</sup> , Ala <sup>92</sup> , Pro <sup>121</sup> , Val <sup>161</sup> , Val <sup>163</sup> , Gly <sup>164</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Val <sup>163</sup> , Gly <sup>164</sup> , Phe <sup>328</sup>	Val <sup>157</sup> , Val <sup>161</sup> , Val <sup>163</sup> , Pro <sup>298</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Val <sup>163</sup> , Gly <sup>164</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Val <sup>163</sup> , Pro <sup>298</sup> , Val <sup>327</sup> , Phe <sup>328</sup>
Similarity interaction amino acids against substrates	Lys <sup>22</sup> , Asn <sup>23</sup> , Leu <sup>26</sup> , Arg <sup>91</sup> , Ala <sup>92</sup> , Trp <sup>95</sup> , Arg <sup>120</sup> , Pro <sup>121</sup> , Leu <sup>124</sup> , His <sup>125</sup> , Val <sup>161</sup> , Ser <sup>162</sup> , Val <sup>163</sup> , Gly <sup>164</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Arg <sup>91</sup> , Arg <sup>120</sup> , Ser <sup>162</sup> , Val <sup>163</sup> , Gly <sup>164</sup> , Phe <sup>328</sup>	Asn <sup>23</sup> , Arg <sup>91</sup> , Val <sup>157</sup> , Lys <sup>160</sup> , Val <sup>161</sup> , Val <sup>163</sup> , Glu <sup>183</sup> , Asn <sup>184</sup> , Glu <sup>188</sup> , Asp <sup>211</sup> , Arg <sup>232</sup> , Pro <sup>298</sup> , Thr <sup>304</sup> , Asp <sup>305</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Arg <sup>91</sup> , Trp <sup>95</sup> , His <sup>125</sup> , Ser <sup>162</sup> , Val <sup>163</sup> , Gly <sup>164</sup> , Val <sup>327</sup> , Phe <sup>328</sup>	Asn <sup>23</sup> , Arg <sup>120</sup> , Val <sup>163</sup> , Glu <sup>188</sup> , Arg <sup>232</sup> , Pro <sup>298</sup> , Pro <sup>303</sup> , Thr <sup>304</sup> , Asp <sup>305</sup> , Val <sup>327</sup> , Phe <sup>328</sup>
The percentage of amino acid similarity to the substrate interactions	16 (100)	6 (37,50)	6 (37,50)	8 (50)	5 (31,25)

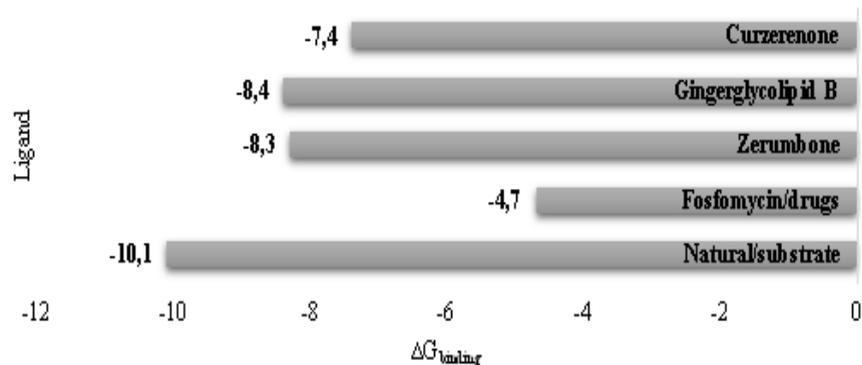


Fig. 4. Gibbs free energy of the natural ligand (substrate), a drug ligand (fosfomicin) and the test ligand (zerumbone, gingerglykolipid B, and curzerenone) against enzyme MurA

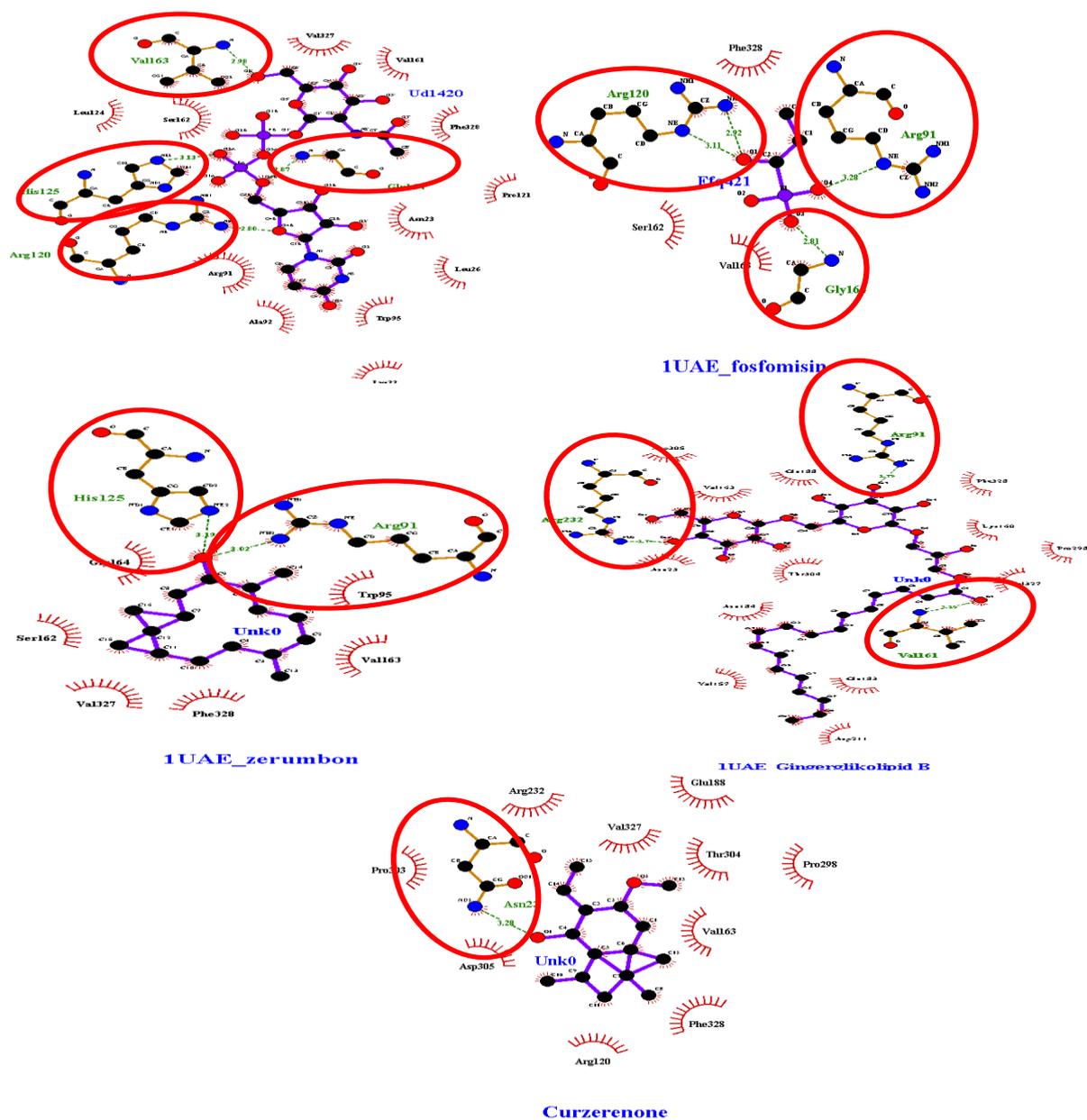


Fig. 5. 2D visualization of complex of MurA enzyme with natural ligand (substrate), a drug ligand (fosfomicin) and the test ligand (zerumbone, gingerglykolipid B, and curzerenone)

Curzerenone test ligand, is similarly suspected to occupy the same active side as that of the natural ligand that can be explained from the similarity of position and the hydrophobic residue interaction with those that bound by curzerenone ligand such as Asn<sup>23</sup>, Arg<sup>120</sup>, Val<sup>163</sup> and Phe<sup>328</sup>.

As shown on Fig. 4, the affinity energy and the  $\Delta G_{\text{binding}}$  of the Gingerglycolipids B is better than those of the zerumbone. However, ginger-glycolipids B still need to be reconsidered as a replacement fosfomycin drug because it does not meet several of the Lipinski rules. Lipinski rules set the drug distribution in the body. It can sub-sequently be assumed that the zerumbone ligand is more capable of being distributed in the body, therefore it is better considered to be a substitute for the fosfomycin drug candidates medicine. Zerumbone is preferably chosen and considered as a new candidate drug to inhibit bacterial cell wall synthesis as seen from the results of the interaction between zerumbone with MurA, where zerumbone occupy or bind to the same amino acid residues as the substrate or drugs fosfomycin.

#### 4. Conclusion

It has been shown that the result of Energy affinity ( $\Delta G_{\text{binding}}$ ) molecular docking of the substrat, drug ligand (fosfomycin), zerumbone, gingerglycolipid B, and the curzerenone against the MurA enzyme were -10.1, -4.7, -8.3, -8,4 and -7.4 kcal mol<sup>-1</sup> respectively. The results of the docking simulation indicate that there is a competitive reaction mechanisms between zerumbone with a substrate of MurA enzyme (uridine-diphosphate-N-acetylglucosamine) in enzyme to occupy the same site as the substrate therefore preventing the formation of peptidoglycan which composes bacterial cell wall. Subsequently, the result of  $\Delta G_{\text{binding}}$  value analysis and interaction docking simulations suggest that zerumbone ligand has the largest potential to replace fosfomycin drug in inhibiting the synthesis of bacterial peptidoglycan.

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