

PAPER • OPEN ACCESS

Antibacterial based on IPBCC.08.610 glucose oxidase against UDP-N-acetyl glucosamine enolpyruvyl transferase and elongation factor G enzymes *in silico*

To cite this article: M R Hakim *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **299** 012035

View the [article online](#) for updates and enhancements.

Antibacterial based on IPBCC.08.610 glucose oxidase against UDP-N-acetyl glucosamine enolpyruvyl transferase and elongation factor G enzymes *in silico*

M R Hakim¹, T Sumaryada² and L Ambarsari^{1*}

¹Department of Biochemistry, IPB University, Bogor 16680, Indonesia

²Department of Physics, IPB University, Bogor 16680, Indonesia

*E-mail: ami_icha@yahoo.com

Abstract. The use of a synthetic antibacterial agent as food preservatives could develop several health problems thus the user should be reduced. Enzyme-based antibacterial is a natural antibacterial that could substitute the latter antibacterials. Enzyme-based antibacterials have several ways that can be used to inactivate microbes, one of them utilize enzyme catalysis products. The aim of this research is to study the interaction between IPBCC.08.610 glucose oxidase (NCBI accession number MH593586) catalysis product against MurA and EFG enzymes using computational investigation. We confirmed that the gene sequence from previous research is truly encoding the IPBCC.08.610 glucose oxidase. Gluconolactone and gluconic acid are less effectively absorbed by microbes. Gluconolactone and gluconic acid are capable to interact with Cys115 and Asp305 of MurA, meanwhile, it does not interact with Phe90 and Thr84 of EFG. Gluconolactone and gluconic acid can inhibit MurA effectively but not on EFG.

1. Introduction

Glucose oxidase is an oxidoreductase enzyme that catalyzes β -D-glucose to hydrogen peroxide and gluconolactone which then will be hydrolyzed non-enzymatically to gluconic acid when reacting with water. The enzyme has been used in the food industry as a preservative industry and has been registered as Generally Regarded as Safe (GRAS) [1]. Generally, the use of the enzyme glucose oxidase as a preservative is followed by adding catalase enzyme to control hydrogen peroxide content in food [2]. Glucose oxidase is widely produced by a certain mold such as *Aspergillus* and *Penicillium*. Indonesia has local isolates that can produce glucose oxidase, one of which is *Aspergillus niger* IPBCC.08.610. *A.niger* IPBCC.08.610 is one of Indonesia isolates which able to produce glucose oxidase [3]. The enzyme has been successfully characterized, immobilization [4], cloned and sequenced and can be used as biosensor [5]. The IPBCC.08.610 glucose oxidase sequence has been published from NCBI with accession number MH593586.

One of antibacterial mechanism inhibits key enzymes which needed in bacterial cell wall synthesis. UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) the main enzyme which catalyzes the formation of peptidoglycan walls from bacteria. MurA catalyzes the condensation reaction between UDP-N-acetylglucosamine and phosphoenolpyruvate to produce UDP-N-acetylglucosamine-enolpyruvate which is the first reaction in bacterial wall synthesis [6]. Furthermore, this study also focuses on inhibiting mechanism of cellular protein synthesis. Protein synthesis carried out in ribosomes is regulated by several transcriptional factors [7]. Elongation Factor G (EF-G) is one of transcriptions



factor that regulates amino acid elongation. EFG acts to move the peptidyl-tRNA molecule from ribosomal A site to P site when translating with the help of GTP hydrolysis [8].

The advantage of using glucose oxidase as a preservative is a controllable number of antibacterial agents desired. Moreover, without adding antibacterials periodically, glucose oxidase can produce hydrogen peroxide continuously when glucose is available therefore it is more efficient than convention preservatives as an antibacterial agent. Indeed, the use of IPBCC.08.610 glucose oxidase as an antibacterial agent has never been done. On the other hand, homology and active site of MH593586 have never been analyzed. IPBCC.08.610 glucose oxidase potential can be studied by interacting between glucose oxidase products and antibacterial targets such as MurA and EFG. This research aim is to study the interaction between IPBCC.08.610 glucose oxidase products (gluconolactone and gluconic acid) against UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) and Elongation factor G (EF-G) enzymes as antibacterials. Some parameters used in this study were homology and similarity active site IPBCC.08.610 Glucose Oxidase, free energy value, and inhibition constant value of the test ligands.

2. Materials

The tools that used were laptops with specs Intel core i3 - 3217 specifications 1.80 GHz, 4GB RAM, Windows 10 64-bit operating system and software in the form of the Discovery Studio 2016 Client, AutoDock Vina Tools (The Scripps Research Institute, USA), MarvinView and LigPlot + 1.4.5.

The materials used in molecular tethering include 2D structures of gluconolactone, gluconic acid, comparative ligands (Fosfomycin and Fusidic acid). The three-dimensional structure of the UDP-N-acetylglucosamine enolpyruvyl transferase receptor (MurA) with the code 1UAE and Elongation factor G (EF-G) with code 4WQF downloaded from <https://www.rcsb.org/>.

3. Methods

The homology of IPBCC.08.610 glucose oxidase MH593586 was performed with BLASTn. Five best homologs sequence were translated, then the motives and the active site was analyzed using ClustalW. Ligand solubility was examined using Discovery Studio and AutoDock tools based on the five rules of Lipinski. Next, receptors stability of MurA and EFG were confirmed with Ramachandran diagram. Ligands were prepared by Discovery studio and Autodock tools. Finally, the interaction of ligand-receptor was visualized using LigPlot.

Grid box validation was done by redocking the natural fosfomycin (MurA) ligand and fusidic acid (EFG) ligands ten times using AutoDock vina. The coordinate of gluconolactone and gluconic acid into MurA are center_x = 37.591, center_y = 24.496, center_z = 43.464 with size_x = 20, size_y = 18, size_z = 16 and grid box using the coordinates EFG center_x = 166.741, center_y = -177.162, center_z = -173.451 with size_x = 30, size_y = 34, size_z = 34. The chemical binding (visualization) were analyzed using Ligplot and the affinity with Ki calculation methods [9].

4. Results and Discussion

4.1. Homology and active site analysis of GOX IPBCC.08.610 enzyme

Homology of HM593586 was analyzed using the BLAST program provided by NCBI. The BLASTn analysis produced five nucleotides with the best parameter values. Sequence alignment of MH593586 sequence has a high value of ident percentage up to 96%, with e-value of 0.0. Smaller the e-value and the higher the bit-score value, result in the higher homology level [10]. Based on Table 1, MH593586 gene homologs to glucose oxidase A. niger st Z-25, QYW3221, B1, BT18, and A9.

Every protein, have a specific region called "motive". The motive can be made as a marker to know their families or superfamilies. The motive also contains the active site in the protein. GMC (Glucose-Methanol-Choline) Oxidoreductase also have their own motives. In this project, active site analysis using the ClustalW program. ClustalW is the most familiar version of the Clustal Programs. Clustal W using the pairwise progressive algorithm. Although Clustal W is an old program, it still has respectable

performance in both speed and accuracy [11]. The result of Clustal W analysis produced similarities in conserved areas (motive) owned by the GMC (Glucose-Methanol-Choline) Oxidoreductase family. The conserved area owned by MH593586 even results same catalytic area as homologous residues, especially at H514 residue. The H514 residue is a highly conserved area in the glucose oxidase enzyme which acts as a proton acceptor from the substrate [12]. It is confirmed that MH593586 homologs with glucose oxidase.

Table 1. Homology of nucleotide sequences of MH593586 using BLASTn.

Description	Accession Number	Total Score	<i>E-value</i>	%ident (%)
GOX. <i>A. niger</i> st Z-25	FJ979866.1	3025	0.0	96%
GOX. <i>A. niger</i> st QYW3221	KC333175.1	3003	0.0	96%
GOX. <i>A. niger</i> st B1	AY803992.1	2881	0.0	96%
GOX. <i>A. niger</i> st BT18	DQ661005.1	2868	0.0	96%
GOX. <i>A. niger</i> st A9	DO836361.1	2832	0.0	96%

```

DQ661005.1 -----GIEASLLTDPKEVAGRTVDYIIAGGGGLGLTTAARL
DQ836361.1 -----GIEASLLTDPKEIAGCTVDYIIAGGGGLGLTTAARL
AY803992.1 -----GIEASLLTDPKEVAGRTVDYIIAGGGGLGLTTAARL
KC333175.1 MQTLFVSSLVSLAAALPHYIRSNGIEASLLTDPKEVAGRTVDYIIAGGGGLGLTTAARL
FJ979866.1 MQTLLVSSLVSLAAALPHYIRSNGIEASLLTDPKEVAGRTVDYIIAGGGGLGLTTAARL
GGOX      MQTLLVSSLVSLAAALPHYIRSNGIEASLLTDPKDVTRGTVDYIIAGGGGLGLTTAARL
          *****:*****
DQ661005.1 TENPDITVLVIESGSYESDRGPIIEDLNAYGDIGSSVDHAYETVELATNNQTALIRSGN
DQ836361.1 TENPDITVLVIESGSYESDRGPIIEDLNAYGDIGSSVDHAYETVELATNNQTALIRSGN
AY803992.1 TENPDITVLVIESGSYESDRGPIIEDLNAYGDIGSSVDHAYETVELATNNQTALIRSGN
KC333175.1 TENPDITVLVIESGSYESDRGPIIEDLNAYGDIGSSVDHAYETVELATNNQTALIRSGN
FJ979866.1 TENPDITVLVIESGSYESDRGPIIEDLNAYGDIGSSVDHAYETVELATNNQTALIRSGN
GGOX      TENPNITVLVIESGSYESDRGPIIEDLNAYGDIGSSVDHAYETVELATNNQTALIRSGN
          *****:*****
DQ661005.1 GLGGSTLVNGGTWTRPHKAQVDSWETVFGNEGWNWDSVAAYSLQAEARAPNAKQIAAGH
DQ836361.1 GLGGSTLVNGGTWTRPHKAQVDSWETVFGNEGWNWDSVAAYSLQAEARAPNAKQIAAGH
AY803992.1 GLGGSTLVNGGTWTRPHKAQVDSWETVFGNEGWNWDSVAAYSLQAEARAPNAKQIAAGH
KC333175.1 GLGGSTLVNGGTWTRPHKAQVDSWETVFGNEGWNWDSVAAYSLQAEARAPNAKQIAAGH
FJ979866.1 GLGGSTLVNGGTWTRPHKAQVDSWETVFGNEGWNWDSVAAYSLQAEARAPNAKQIAAGH
GGOX      GLGGSTLVNGGTWTRPHKAQVDSWETVFGNEGWNWDSVAAYSLQAEARAPNAKQIAAGH
          *****:*****
DQ661005.1 DQTTSTVRSRITSAGAGQGQAAWFATFNETF GDYAEKAHELLNTKLEQWAAEEAVARGGFH
DQ836361.1 DQTTSTVRSRITSAGAGQGQAAWFATFNETF GDYAEKAHELLNTKLEQWAAEEAVARGGFH
AY803992.1 DQTTSTVRSRITSAGAGQGQAAWFATFNETF GDYAEKAHELLNTKLEQWAAEEAVARGGFH
KC333175.1 DQTTSTVRSRITSAGAGQGQAAWFATFNETF GDYAEKAHELLNTKLEQWAAEEAVARGGFH
FJ979866.1 DQTTSTVRSRITSAGAGQGQAAWFATFNETF GDYAEKAHELLNTKLEQWAAEEAVARGGFH
GGOX      TRPPLPSAHALPPFPVDRDRPLGLSLPSTRPLATTPKRPTSCSTPSWSSGPKAEAVARGGFH
          *****:*****
DQ661005.1 NNTALLIQYENYRDWIVKDNVAYS-ELFLDTAGVASFDVWDLPLPFRGYVHILDKDPYLR
DQ836361.1 NNTALLIQYENYRDWIVKDNVAYS-ELFLDTAGVASFDVWDLPLPFRGYVHILDKDPYLR
AY803992.1 NNTALLIQYENYRDWIVKDNVAYS-ELFLDTAGVASFDVWDLPLPFRGYVHILDKDPYLR
KC333175.1 NNTALLIQYENYRDWIVKDNVAYS-ELFLDTAGVASFDVWDLPLPFRGYVHILDKDPYLR
FJ979866.1 NNTALLIQYENYRDWIVKDNVAYS-ELFLDTAGVASFDVWDLPLPFRGYVHILDKDPYLR
GGOX      NNTAFAHFPVRELPRDLDRQGQCRILGTLPLSTRPEWASFDVWDLPLPFRGYVHILDKDPYLR
          *****:*****
DQ661005.1 HFAYDPQYFLNELDLLGQAATQLARNISNSGAMQTYFAGETIPGDNLAYDADLSAWVEY
DQ836361.1 HFAYDPQYFLNELDLLGQAATQLARNISNSGAMQTYFAGETIPGDNLAYDADLSAWVEY
AY803992.1 HFAYDPQYFLNELDLLGQAATQLARNISNSGAMQTYFAGETIPGDNLAYDADLSAWVEY
KC333175.1 HFAYDPQYFLNELDLLGQAATQLARNISNSGAMQTYFAGETIPGDNLAYDADLSAWVEY
FJ979866.1 HFAYDPQYFLNELDLLGQAATQLARNISNSGAMQTYFAGETIPGDNLAYDADLSAWVEY
GGOX      HFAYDPQYFLNELDLLGQAATQLARNISNSGAMQTYFAGETIPGDNLAYDADLSAWVEY
          *****:*****

```

```

DQ661005.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSSHVMTVFYA
DQ836361.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSSHVMTVFYA
AY803992.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSSHVMTVFYA
KC333175.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSSHVMTVFYA
FJ979866.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSSHVMTVFYA
GGOX          IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSSHVMTVFYA
*****
DQ661005.1    MALKIADAILADYAS--
DQ836361.1    MALKIADAILADYASMQ
AY803992.1    MALKIADAVLADYASMQ
KC333175.1    MALKIADAILADYASMQ
FJ979866.1    MALKIADAILADYASMQ
GGOX          MALKIADAVLADYASMQ
*****

```

Figure 1. Results of Multiple Alignment of glucose oxidase using ClustalW Molecular Docking.

There are several parameters in the established Lipinski rules, namely molecular weight (BM) not more than 500 g / mL, partition coefficient (LogP) not more than 5, less than five hydrogen bond donors, less than ten hydrogen bond acceptors and molar refractivity between 40-130 [13]. Based on Table 2, gluconolactone and gluconic acid ligands are antibacterial compounds that are less effective for bacteria to absorb. The comparative ligand used was fosfomycin for MurA and fusidic acid for EFG. The two comparative ligands used were commercial antibiotics or inhibitors that have been used to inhibit the target enzyme. Predictions using the Lipinski rule state that fosfomycin and fusidic acid are less effective compounds used as an antibacterial agent. However, the compounds have been widely used as inhibitors that targeted each of these enzymes.

Table 2. Lipinski rule of five results.

Ligand	Relatives Mass (Da)	Donor H	Acceptor H	Log P
Fosfomycin	132	1	4	-1.948730
Fusidic acid	516	3	6	5.768120
Gluconolactone	178	4	6	-3.013201
Gluconic acid	196	6	7	-3.493100

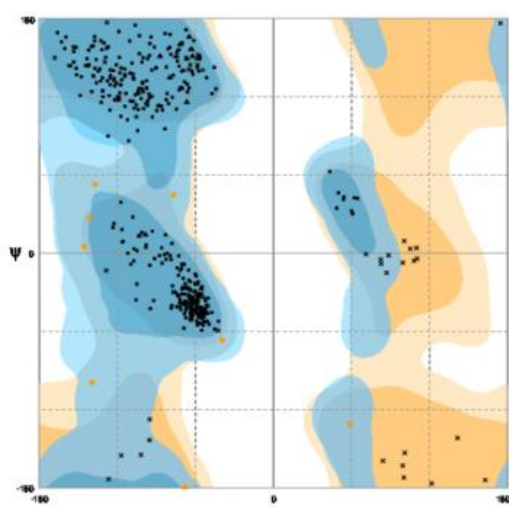


Figure 2. Ramachandran Plot result of MurA.

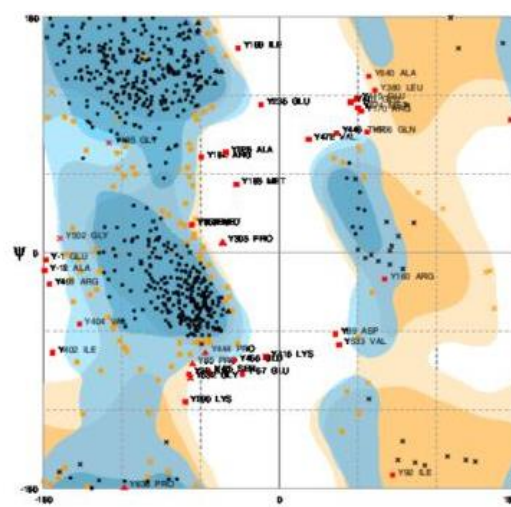


Figure 3. Ramachandran Plot result of EFG.

The MurA enzyme with code 1UAE has 1.8 Å resolution, EFG Receptor with code 4WQF has 2.8 Å resolution. Ramachandran plot showed that MurA has 98.1% (408/418) amino acid residues located

in the most favorable region and 1.9% (8/418) amino acid residues were located in the allowed region (Figure 2). EFG has 79.9% (580/826) amino acid residues in the most favorable region, 14.6% (106/826) in the allowed region and 5.5% (40/826) in the disallowed region (Figure 3). It shows that both receptors have a good and stable structure that can provide accurate result in the next step.

The next step is docking between each receptor (protein) and ligands (gluconolactone and gluconic acid). Docking uses the unique mathematical algorithm to solve the “best” matching between two molecules. The complexity of the docking algorithm is huge. It involves the efficient search (and matching) algorithm, which cover relevant conformational space, and selective scoring function [14]. AutoDock Vina uses a semiempirical scoring function which combines the molecular force field and function optimization with regression method on empirical data.

Cys115 and Asp305 contribute to condensation of phosphoenolpyruvate (PEP) and UDP-N-Acetylglucosamine [15]. Cys115 residue acts as an acid in the protonation process of PEP during the reaction, while Asp305 acts as a base in the deprotonation process of UDP-N-acetylglucosamine [16]. The interaction of gluconolactone and Cys115 residues relatively weak but successfully bound Asp305 residues strongly with hydrogen interactions (Figure 4). Gluconic acid Interaction with Cys115 was stronger than gluconolactone because it has two interactions of hydrogen with Cys115 and one bond is less than 3.2 Å and it could interact with Asp305 hydrophobically (Table 3).

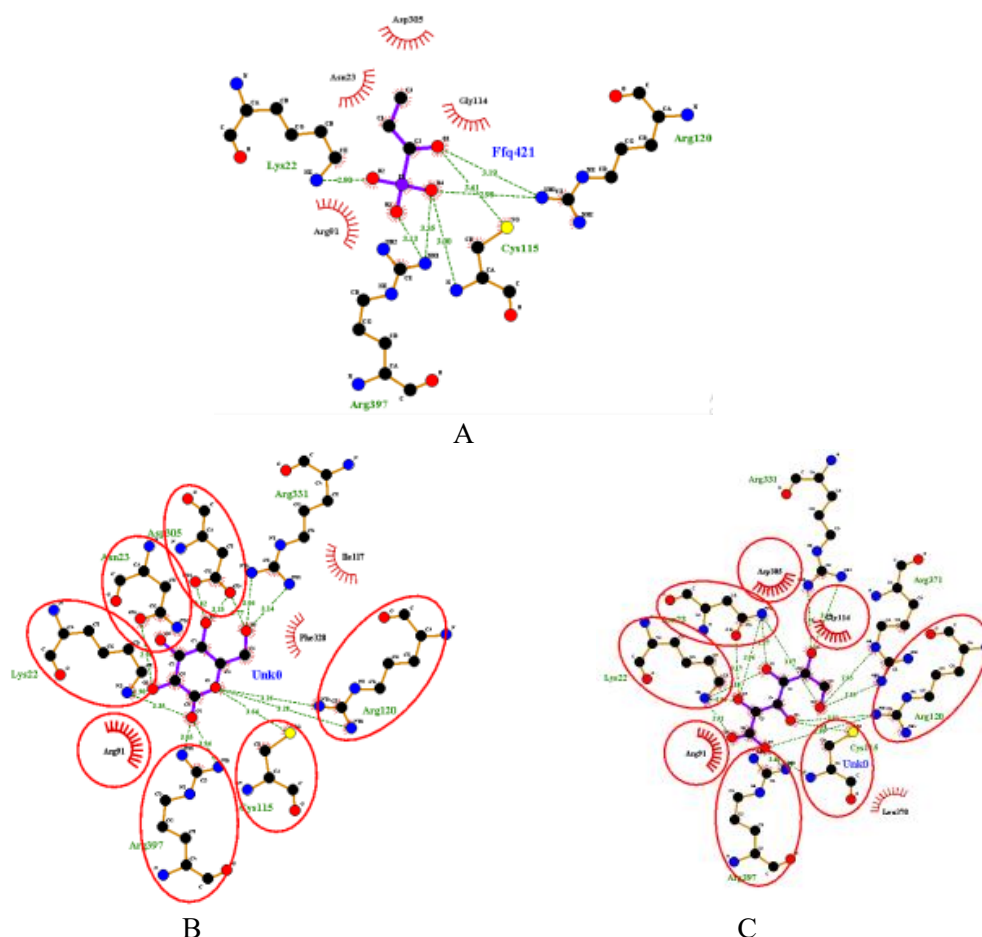


Figure 4. 2D visualization docking result in MurA; A. Fosfomycin B. Gluconolactone C. Gluconic Acid.

Table 3. Molecular docking result on MurA.

Ligand	ΔG (kcal/mol)	H Bond Distance (Å)	H Bond (AA)	Hydrophobic bond (AA)	%BSS
Fosfomycin	-4.5	2.90 3.61, 3.00 3.19, 2.98 3.35, 3.13	Lys ²² Cys ¹¹⁵ Arg ¹²⁰ Arg ³⁹⁷	Gly ¹¹⁴ Arg ⁹¹ Asn ²³ Asp ³⁰⁵	0
Gluconolactone	-5.8	2.90, 3.35 2.96 3.25, 3.02, 2.77 3.26, 3.28 3.64 3.94, 3.05 3.06 3.14	Lys ²² Asn ²³ Asp ³⁰⁵ Arg ¹²⁰ Cys ¹¹⁵ Arg ³⁹⁷ Arg ³³¹	Arg ⁹¹ Phe ³²⁸ Ile ¹¹⁷	87.5%
Gluconic Acid	-5.4	2.97, 3.96, 3.17, 3.87 3.03, 2.99 3.91, 3.06 3.40 3.10, 2.80 2.92 3.59, 2.86 2.85, 2.89	Asn ²³ Arg ¹²⁰ Cys ¹¹⁵ Arg ³⁹⁷ Lys ²² Arg ³³¹ Arg ³⁷¹	Arg ⁹¹ Asp ³⁰⁵ Gly ¹¹⁴ Leu ³⁷⁰	100%

Gluconolactone not interacted with Phe90 but could interact with Thr84 (Figure 5). The interaction with Thr84 was not strong enough to inhibit EFG because the bond with Thr84 is only conformational stabilizing. Gluconic acid could not produce interactions with both target residues (Table 4). The interaction between ligand and Phe90 causes no EFG conformational changes, whereas the interaction between fusidic acid and Thr84 results in a more stable conformation [17]. Gluconolactone and gluconic acid not able to inhibit EFG.

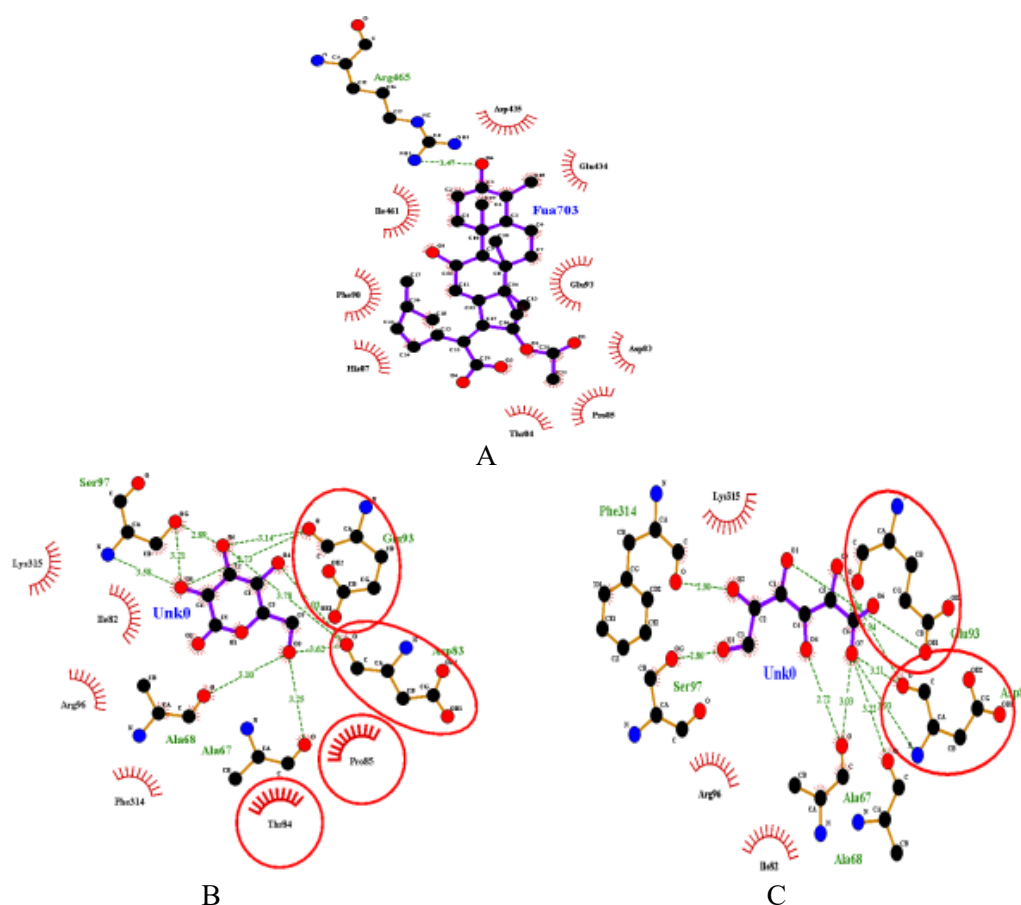


Figure 5. 2D visualization docking result in EFG; A. Fusidic acid B. Gluconolactone C. Gluconic Acid.

Table 4. Molecular docking result on EFG.

Ligand	ΔG (kcal/mol)	H Bond Distance (Å)	H Bond (AA)	Hydrophobic bond (AA)	%BSS
Fosfomycin	-7.0	3.47	Arg ⁴⁶⁵	Asp ⁴³⁵ , Glu ⁴³⁴ Glu ⁹³ , Asp ⁸³ Pro ⁸⁵ , Thr ⁸⁴ His ⁸⁷ , Phe ⁹⁰ Ile ⁴⁶¹	0
Gluconolactone	-5.3	2.73, 3.14 3.03, 3.78, 3.62 3.25 3.10 3.21, 3.58, 2.89	Glu ⁹³ Asp ⁸³ Ala ⁶⁷ Ala ⁶⁸ Ser ⁹⁷	Pro ⁸⁵ , Thr ⁸⁴ Phe ³¹⁴ , Arg ⁹⁶ Ile ⁸² , Lys ³¹⁵	40%
Gluconic Acid	-4.6	2.92 3.21, 3.93 2.94, 3.22 3.03 2.72 2.86, 2.90	Glu ⁹³ Asp ⁸³ Ala ⁶⁷ Ala ⁶⁸ Ser ⁹⁷ Phe ³¹⁴	Lys ³¹⁵ , Arg ⁹⁶ Ile ⁸²	20%

4.2. Affinity Energy and Inhibition of Constant

The results show that the interaction ability of gluconolactone and gluconic acid to MurA is more stable than fosfomycin. Meanwhile, ligand gluconolactone and gluconic acid have a smaller affinity than fusidic acid so that it has a far unstable interaction compared to fusidic acid. Aside from being a stability parameter of the ligand-receptor interaction, the affinity energy generated from molecular tethering can also be used to determine the inhibition constant (K_i) value. K_i value itself describes the strength of affinity binding between ligand and receptor. K_i can be calculated used the formula :

$$\Delta G = RT \ln\left(\frac{K_i}{c_0}\right) \quad (1)$$

The R value used was 1,986 cal/mol K and the T value of 298 K and c_0 was the concentration on the standard thermodynamic state (1 mol/L). Based on these results, gluconolactone and gluconic acid had great potential as MurA inhibitors but not on EFG (Figure 4).

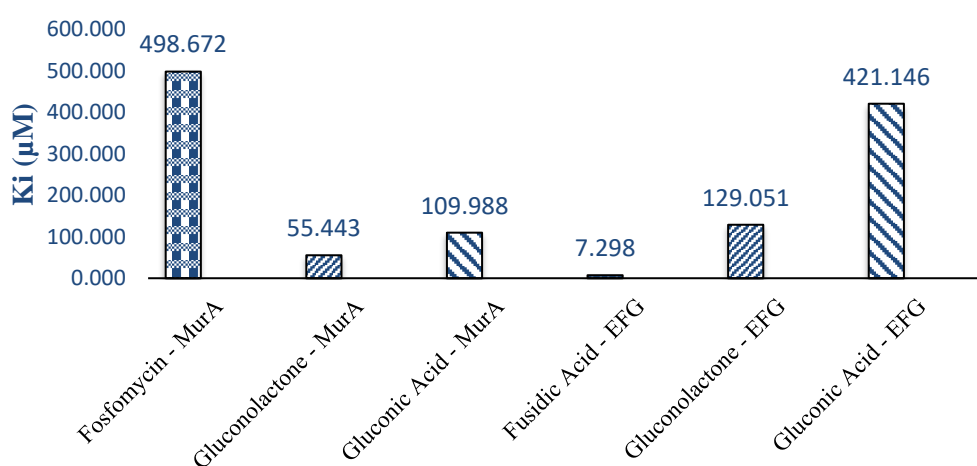


Figure 6. Inhibition Constant result.

According to Zia *et al.* (2013) [18], hydrogen peroxide production from glucose oxidase is a major cause of inhibited bacterial growth. The use of hydrogen peroxide as a test ligand cannot be done because of the very small molecular size. In addition, gluconolactone and gluconic acid contribute to the pH deposition of the environment [19]. Based on this study, gluconolactone and gluconic acid contributed more to the mechanism of bacterial growth inhibition by inhibited MurA.

References

- [1] Wong CM, Wong KH, Chen XD. 2008. Glucose oxidase: natural occurrence, function, properties, and industrial applications. *Appl Microbiol Biotechnol.* **78**: 927-938.
- [2] Eissa AA. 2012. *Structure and Function of Food Engineering*. Vienna (AT): InTech. DOI: 10.5772/1615.
- [3] Kurniatin PA, Ambarsari L, Putri RP. 2012. Produksi dan pemurnian enzim glukosa oksidase dari *Aspergillus niger* isolat lokal (IPBCC.08.610). *Prosiding seminar nasional kimia 2012*; 2012 Februari 25; Surabaya, Indonesia. ID: Unesa University Press. D26 – D34.
- [4] Rohmayanti T, Ambarsari L, Maddu A. 2017. Enzymatic activity of glucose oxidase from *Aspergillus niger* IPBCC.08.610 on modified carbon paste electrode as glucose biosensor. *IOP Conf. Series: Earth and Environmental Science.* **58**. 1-6.
- [5] Ambarsari L, Maddu A, Rohmayanti T, Nurcholis W. 2018. An optimized glucose biosensor as a potential micro-fuel cell. *Rasayan J Chem.* **11**: 32-36.

- [6] Hrast M, Sosic I, Sink R, Gobec S. 2014. Inhibitors of the peptidoglycan biosynthesis enzymes MurA-F. *Bioorganic Chem.* **55**: 2-15.
- [7] Steitz TA. 2008. A structural understanding of the dynamic ribosome machine. *Nat Rev Mol Cell Biol.* **9**: 242–253.
- [8] Schmeing TM, Ramakrishnan V. 2009. What recent ribosome structures have revealed about the mechanism of translation. *NATURE.* **461**: 1234-1242.
- [9] Fikrika H, Ambarsari L, Sumaryada T. 2016. Molecular docking studies of catechin and its derivatives as an anti-bacterial inhibitor for glucosamine-6-phosphate synthase. *IOP Conf. Series: Earth and Environmental Science.* **31**: 1-6.
- [10] Khadeja N, Amin F, Rashid S, Munir I, Nasim Z. 2015. Study of multiple sequence alignment and phylogenetic analysis of CFA8 protein causing hemophilia A. *Int J Pure Appl Sci Technol.* **28**: 50-62.
- [11] Baxevanis AD, Brady JE. 2003. *Current Protocols in Bioinformatics*. New York (US): John Wiley & Sons.
- [12] Hille R, Miller SM, Palfey B. 2013. *Handbook of Flavoprotein*. Gottingen (GR): Hubert & Co.
- [13] Benet LZ, Hosey CM, Ursu O, Oprea TI. 2016. BDDCS, the rule of 5 and drugability. *Adv Drug Deliv Rev.* **101**: 89-98.
- [14] Halperin I, Ma B, Wolfson H, Nussinov R. 2002. Principles of Docking: An Overview of Search Algorithms and a Guide to Scoring Functions. *PROTEINS: Structure, Function, and Genetics.* **47**: 409-443.
- [15] Ahad A, Ahmad A, Din S, Rao AQ, Shahid AA, Husnain T. 2015. In silico study for diversifying the molecule pathway pigment formation: an alternative to manual coloring in cotton fibers. *Frontiers in Plant Sci.* **6**: 1-10.
- [16] Barreateau H, Kovac A, Boniface A, Sova M, Gobec S, Blanot D. 2008. Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiol Rev.* **32**: 168-207.
- [17] Gao YG, Selmer M, Dunham CM, Weixlbaumer A, Kelley AC, Ramakrishnan V. 2009. The structure of the ribosome with elongation factor g trapped in the posttranslocational state. *Science.* **326**: 694-699.
- [18] Zia MA, Riaz A, Rasul S, Abbas RZ. 2013. Evaluation of antimicrobial activity of glucose oxidase from *Aspergillus niger* EBL-A and *Penicillium notatum*. *Braz Arch Biol Technol.* **56**: 956-961.
- [19] Bradshaw CE. 2011. An in vitro comparison of the antimicrobial activity of honey, iodine and silver wound dressings. *Biosci Horizons.* **4**: 61-70.