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

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**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  $\beta$ -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



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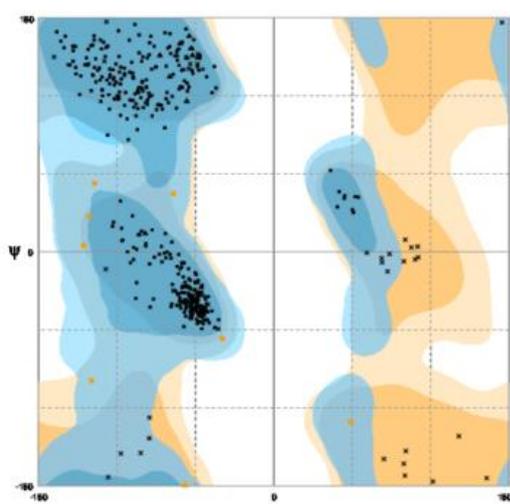
DQ661005.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSHVMTVFYA
DQ836361.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGRVIDGSIPPTQMSHVMTVFYA
AY803992.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSHVMTVFYA
KC333175.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSHVMTVFYA
FJ979866.1    IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSHVMTVFYA
GGOX          IPYNFRPNYHGVGTCSMPKEMGVVDNAARVYGQGLRVIDGSIPPTQMSHVMTVFYA
*****
DQ661005.1    MALKIADAILADYAS--
DQ836361.1    MALKIADAILADYASMQ
AY803992.1    MALKIADAVLADYASMQ
KC333175.1    MALKIADAILADYASMQ
FJ979866.1    MALKIADAILADYASMQ
GGOX          MALKIADAVLADYASMQ
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**Figure 1.** Results of Multiple Alignment of glucose oxidase using ClustalW Molecular Docking.

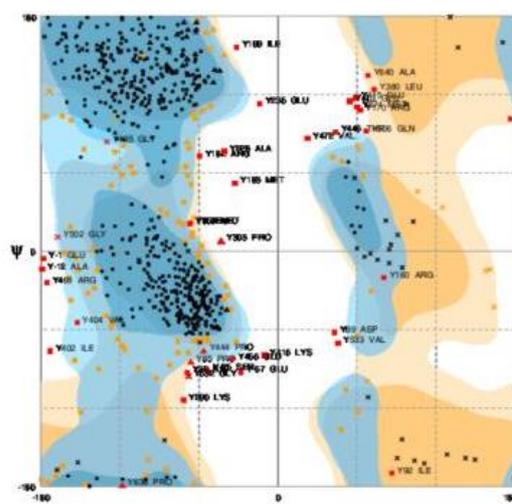
There are several parameters in the established Lipinski rules, namely molecular weight (BM) not more than 500 g / mL, partition coefficient (LogP) not more than 5, less than five hydrogen bond donors, less than ten hydrogen bond acceptors and molar refractivity between 40-130 [13]. Based on Table 2, 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
<b>Fosfomycin</b>	132	1	4	-1.948730
<b>Fusidic acid</b>	516	3	6	5.768120
<b>Gluconolactone</b>	178	4	6	-3.013201
<b>Gluconic acid</b>	196	6	7	-3.493100



**Figure 2.** Ramachandran Plot result of MurA.



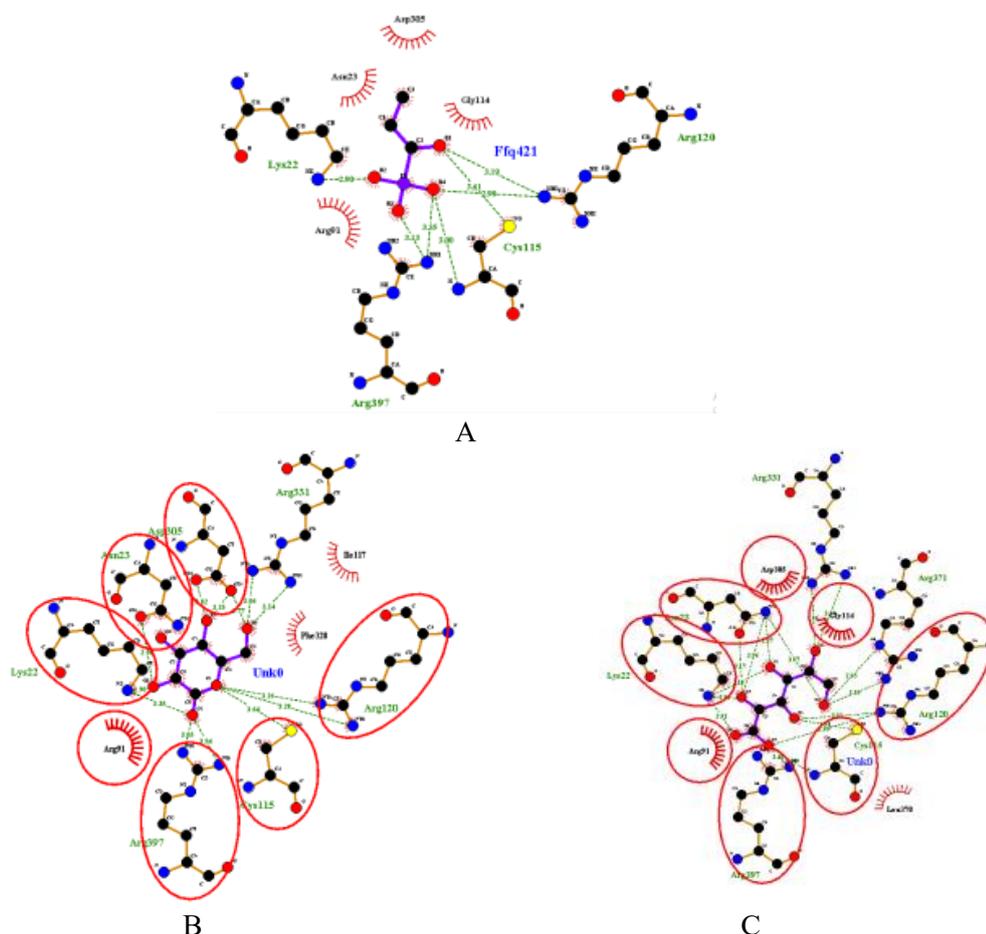
**Figure 3.** Ramachandran Plot result of EFG.

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).

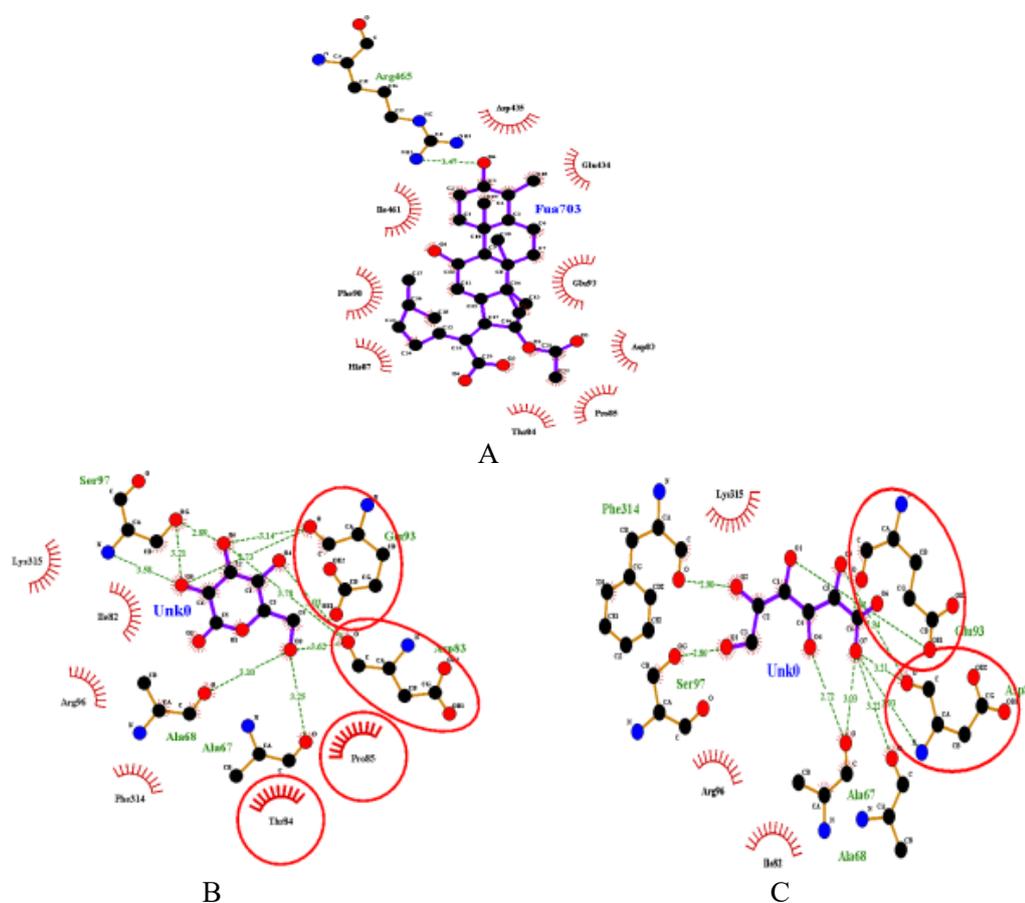


**Figure 4.** 2D visualization docking result in MurA; A. Fosfomycin B. Gluconolactone C. Gluconic Acid.

**Table 3.** Molecular docking result on MurA.

Ligand	$\Delta G$ (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 <sup>22</sup> Cys <sup>115</sup>  Arg <sup>120</sup>  Arg <sup>397</sup>	Gly <sup>114</sup> Arg <sup>91</sup>  Asn <sup>23</sup> Asp <sup>305</sup>	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 <sup>22</sup>  Asn <sup>23</sup> Asp <sup>305</sup>  Arg <sup>120</sup>  Cys <sup>115</sup> Arg <sup>397</sup>  Arg <sup>331</sup>	Arg <sup>91</sup>  Phe <sup>328</sup> Ile <sup>117</sup>	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 <sup>23</sup>    Arg <sup>120</sup>  Cys <sup>115</sup>  Arg <sup>397</sup> Lys <sup>22</sup>  Arg <sup>331</sup>  Arg <sup>371</sup>	Arg <sup>91</sup>  Asp <sup>305</sup>  Gly <sup>114</sup> Leu <sup>370</sup>	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.

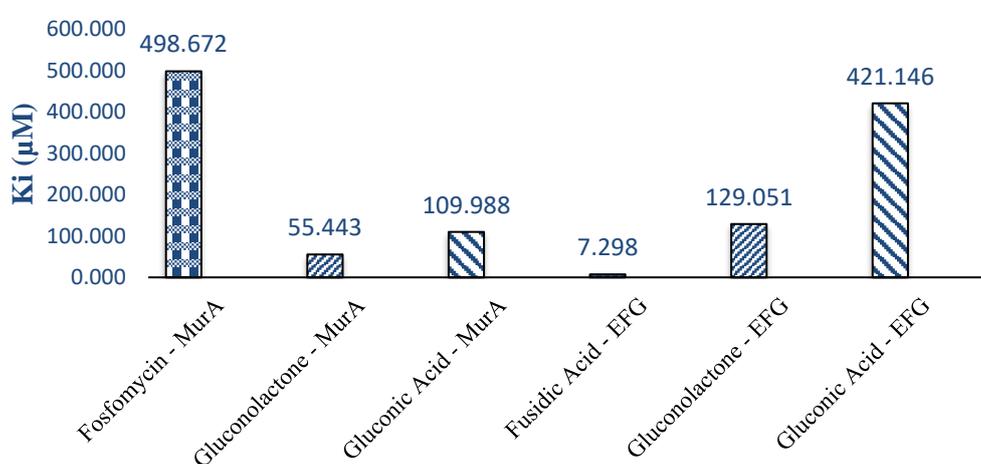
Ligand	$\Delta G$ (kcal/mol)	H Bond Distance (Å)	H Bond (AA)	Hydrophobic bond (AA)	%BSS
Fosfomycin	-7.0	3.47	Arg <sup>465</sup>	Asp <sup>435</sup> , Glu <sup>434</sup> Glu <sup>93</sup> , Asp <sup>83</sup> Pro <sup>85</sup> , Thr <sup>84</sup> His <sup>87</sup> , Phe <sup>90</sup> Ile <sup>461</sup>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

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