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Discovery of GPX4 inhibitor by molecular docking simulation as a potential ferroptosis inducer

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ABSTRACT

As one of the most complex diseases in the world, cancer continues as one of the significant public health problems. It was recorded by 2014 that cancer caused 1,551,000 death in Indonesia. One type of programmed cell death (PCD) that played a role in cancer cell treatment is Ferroptosis. Ferroptosis is PCD on iron and characterized by the inactivation of glutathione-dependent peroxidase (GPx4). In this research, a new therapeutic strategy for cancer was developed through the computational approach on synthetic compounds to discover its potential as an inhibitor of GPx4. About 688 compounds derivative from mercaptosuccinic acid acquired from the Zinc15 database. These compounds screened through the Lipinski's Rule of Three and pharmacological prediction to eliminate ligands with undesired molecular properties. After that, the ligands underwent both rigid and flexible molecular docking simulations to predict their inhibition activity toward GPx4. From molecular docking simulation, (2S)-2-[(Z)-3-phenylprop-2-enyl]sulfanylbutanedioic acid show favorable characteristics as a drug candidate.

Keywords: *Cancer; Ferroptosis; GPx4; Mercaptosuccinic Acid; Molecular Docking Simulation.*

1. INTRODUCTION

Cancer is a complicated disease that remains one of the major public health problems. It was recorded in the year 2018 that cancer caused 207,210 death in Indonesia [1]. Cell death or apoptosis is an event in the life of a biological cell ceasing to carry out its function. Cell death can be classified as 'regulated' or 'accidental' routines. Regulated cell death is activated by a stimulus or encoded through the genome, while physical or mechanical stresses induce accidental cell death. Ferroptosis is a type of regulated cell death. Functionally, ferroptosis is defined as cell death driven by the accumulation of iron dependent-lipid peroxides that result in cell death. This specific profile is unique to ferroptosis, not only among regulated cell death but also within the oxidative forms of cell death.

Ferroptosis is initiated by the inactivation of the glutathione peroxidases (GPX), resulting in unchecked lipid peroxidation [2]. The lipid peroxidation generates reactive oxygen species (ROS), leading to several undesired redox reactions and, ultimately, cell death. GPX is an enzyme family that catalyzes the peroxidase activity using monomeric glutathione and other reductants as a substrate [3]. There are several GPX encoded by different genes in the human body. Among those GPX, glutathione peroxidases 4 (GPX4) is the enzyme that directly associated with ferroptosis [4]. GPX4 is an enzyme in the human body that is encoded by the GPX4 gene. In a way, this enzyme is unique in its function to catalyze the reduction of hydroperoxide groups (-OOH) such as hydrogen peroxide and fatty acid esterified in membrane phospholipids [5]. As a result, this enzyme could inhibit the ferroptosis through the production of glutathione disulfide and lipid-alcohol using the glutathione and lipid-hydroxide [3]. Thus, the inhibition of GPX4 has been established as a possible approach to reactivate cell death by ferroptosis in cancer treatment [6].

Mercaptosuccinic acid or thiomalic acid is derivate of dicarboxylic acid that has been employed widely in the synthesis of various antimicrobial and antitubercular [7]. Furthermore, due to the strong reducibility properties of sulfhydryl in mercaptosuccinic acid, it's also commonly used in cancer treatment. Through the salt or ester form of mercaptosuccinic acid, it acts as cancer therapy via inhibition of enzyme glutathione peroxidases [8]. The inhibition activity of mercaptosuccinic acid is performed by competing with glutathione (GSH) to bind to the active site of enzyme GPX [6]. This inhibition accumulates the reactive oxygen species (ROS) from lipid peroxidation, such as polyunsaturated fatty acids (PUFAs) and arachidonic acid (AA) [9]. The reduction of PUFAs and AA resulted in the inactivation of GPX, and ultimately ferroptosis [4,10].

One of the approaches to investigate the potential of the ligands as an inhibitor of a receptor is by Computer-aided drug design (CADD) [11]. CADD estimates the interaction between ligand-receptor through the binding energy and dynamic stability of intermolecular interaction properties. Aside from that, we can also push the cost of drug development in the wet lab by using this method. One example of CADD is molecular docking simulation [12]. Studies describe molecular docking simulation as a study simulation to predict the conformation and affinity of ligands with a receptor [13,14]. Through various parameters such as the binding energy of ligand-receptor and root mean square deviation (RMSD), molecular docking simulation is employed [15].

From this research, we found a suitable compound as an inhibitor of GPX4 to triggers ferroptosis through the molecular docking simulation. We used mercaptosuccinic acid derivatives as

ligands in molecular docking simulation. Finally, we conducted the ADME-Tox test to predict the drug candidate effect on the human body.

2. MATERIALS AND METHODS

This research has been done through the molecular docking simulation as a form of computational drug delivery by employing the Molecular Operating Environment (MOE) 2014.09 software, OsirisDataWarrior, and ToxTree. The 3D structure of GPX4 was acquired from the National Center of Biological Information Protein Data Bank (NCBI PDB). The database of the ligand was obtained from the Zinc15 database.

2.1. The Optimization of Structure GPx4 Protein.

The 3D structure protein of GPX4 enzyme was obtained from the National Center of Biological Information Protein Data Bank (NCBI PDB) with PDB ID: 2OBI. Then, the Molecular Operating Environment (MOE) 2014.09 was used to optimize the structure of protein GRPx4 in PDB format using the LigX feature. This optimization was performed to eliminate the undesired molecules such as water and unwanted ligands bonded with the protein. After that, we saved the enzyme in .moe format.

2.2. The Construction of Potential Ligand Database.

We obtained the standard RLH3 from the PubChem database while we acquired the ligands, mercaptosuccinic acid derivatives, from the Zinc15 database. The ligands were screened through the Lipinski's Rule of Three (RO3) and toxicity test by the OsirisDataWarrior software. After that, the ligands were optimized through MOE 2014.09. In MOE 2014.09, we set the force field to MMF94x, and the value of root mean square deviation (RMSD) to 0.001 as optimization parameters. Lastly, we saved the ligand database in .mdb format.

2.3. Molecular Docking Simulations of Ligand.

The selected ligands that we prepared from the previous step were docked into GPX4 using the MOE 2014.09 software twice. The rigid receptor protocol was performed first, and after that, we performed the flexible-receptor protocol or induced fit protocol. As the parameters, we set the standard protocol to AMBER:10EHT, and during the molecular docking simulation either with rigid or induced-fit, we keep the retain pose at 30 and 10012. The ligands with the most favorable RMSD, Gibbs binding energy, and molecular interaction were selected for the next step.

2.4. Mutagenicity and Carcinogenicity Prediction.

The potential ligands from molecular docking simulation underwent mutagenicity and carcinogenicity properties prediction. Toxtree software was used to screen those ligands.

3. RESULTS

3.1. The optimization of structure GPx4 protein

Glutathione peroxidase 4 is an enzyme responsible for the inhibition of ferroptosis [2]. GPx4 is an enzyme that is considered as a potential candidate for cancer therapy. For this research, we acquired the 3D structure of this enzyme from the National Center of Biological Information Protein Data Bank (NCBI PDB) with PDB ID: 2OBI [16]. The structure was shown in Figure 1.

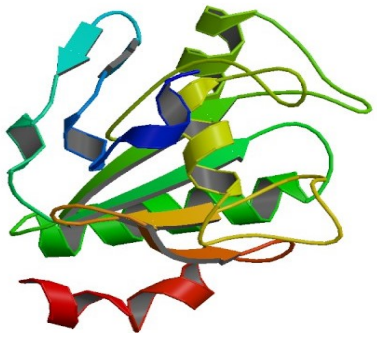


Figure 1. 3 D Structure of GPX4.

As the protein structure was visualized through the diffraction of x-rays, we need to optimize this protein first. To optimize this enzyme, we used the Molecular Operating Environment (MOE) 2014.09 software. This optimization is performed to eliminate the undesired molecules such as water and unwanted ligands bonded with the protein. The elimination of these undesired molecules was achieved, so when we docked this protein, these undesired molecules will not affect the molecular mechanism and electrostatic force calculated in molecular docking simulation. Moreover, to optimize the protein in the most suitable condition for protein, macromolecules, and nucleic acid, we optimized and minimized the structure in AMBER:10EHT forcefield.

After the 3D structure of GPx4 was optimized, the 'site finder' feature of MOE 2014.09 was utilized to predict the binding site of GPx4. According to research done by Cozza et al (2017), we could see that the binding site was compromised of 25 amino acid residues (Gly 131, Lys 135, Arg 152, Asn 52, Trp 36, Cys 46, Gly 47, Lys 48, Phe 138, Asp 21, Ile 22, Asp 23, Lys 31, Lys 90, Ala 93, Lys 121, Lys 125, Lys 127, Gly 128, Ile, 129, Leu 130, Ala 94, Val 98, Lys 99) [5]. Lastly, we saved the optimized protein in the .moe format.

3.2. The Construction of the potential ligand database.

Around 688 compounds of mercaptosuccinic acid derivatives were obtained from Zinc15 database. After that, the ligands were screened by Lipinski's Rule of Three (RO3). This rule stated that compounds with molecular weight bigger than 300 Da, logP value less than 3, the number of hydrogen bonds less than 3, and the number of hydrogen acceptor lower than three should be eliminated. To employ this rule, we used the Osiris Data Warrior Software [17]. Finally, we also removed ligands with drug-likeness lower than 0, mutagenic, tumorigenic, and reproductive effect and irritant. From these steps, only 49 compounds of mercaptosuccinic acid derivatives were used for molecular docking analysis, and we saved them in the .mdb format as the ligand for the next step.

3.3. Molecular docking simulations of ligand.

The molecular docking simulation is a study that conjures the orientation of ligand in the receptor (enzyme or protein) [18]. Aside from that, the simulation is employed to predict the conformation of small-molecule ligands through the Gibbs binding energy when the ligand interacted with the receptor's binding site — ultimately creating a complex of the ligand with the receptor. Its potential also can be determined through the score computed by software. As a result, through years, molecular docking

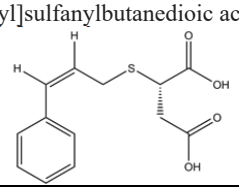
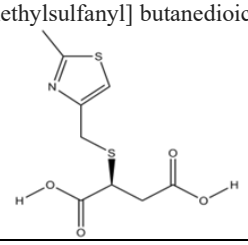
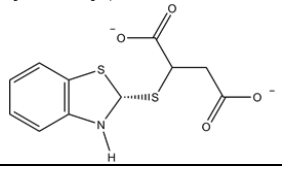
simulation as part of computational drug delivery has developed significantly and become a crucial part of a new compound as a potential drug [13,19]. However, the molecular docking simulation may lead to false results as the rigid receptor docking employed in molecular docking simulation is not resembling the real characteristic of the receptor. To overcome that, scientists develop a method called flexible receptor docking or what commonly known as induced-fit.

In this research, the selected ligands from previous steps were docked into the binding site of the enzyme glutathione peroxidase 4 (GPx4) using the molecular operating environment (MOE) 2014.09 four times. The first docking simulation was the rigid-receptor protocol and, after that, the induced-fit which is commonly known as flexible receptor protocol. During the docking simulation either with rigid or flexible, we kept the retain of 30 and 100 repetitions respectively.

From Table 1, (2S)-2-[(Z)-3-phenylprop-2-enyl] sulfanyl butane dioic acid, (2S)-2-[(2-methyl-1,3-thiazol-4-yl) methylsulfanyl] butanedioic acid and 2-(1H-benzimidazole-2-ylsulfanyl) butanedioic acid had $\Delta G_{binding}$ lower than $\Delta G_{binding}$ of RSL3 as the standard molecule with GPx4. However, we shouldn't take the $\Delta G_{binding}$ as the sole factor in determining the inhibitor potential of the ligands. Root mean square deviation (RMSD) should be considered as the factor for determining the inhibitory potential of the ligand [15]. RMSD value determines the ligand conformation in a binding site, which is visualized through a value lower or equal than 2Å. Based on Table 1, compound (2S)-2-[(Z)-3-phenylprop-2-enyl]sulfanylbutanedioic acid is the best ligand because it has the lowest RMSD than the other ligands.

To evaluate the drug-likeness of these ligands, we screened them with Lipinski's rule. The Lipinski's rule states that molecules with molecular weight less than 500 dal, H-donor and H-acceptor less than 5 and logP less than 5 is a molecule that most suitable as a drug candidate [17]. From tests showed in Table 1, we could see that (2S)-2-[(Z)-3-phenylprop-2-enyl] sulfanylbutanedioic acid is the most suitable as a drug candidate.

Table 1. Molecular Properties of the Selected Results from Molecular Docking Simulation.

No	Molecules Name & Structures	$\Delta G_{binding}$ (kcal/mol)	RMSD (Å)	logP (o/w)	MW	H-Don	H-Acc
1	(2S)-2-[(Z)-3-phenylprop-2-enyl]sulfanylbutanedioic acid 	-6.3263	1.2083	-2.4474	266.311	4	0
2	(2S)-2-[(2-methyl-1,3-thiazol-4-yl)methylsulfanyl] butanedioic acid 	-6.0020	1.9881	-4.0052	261.013	5	0
3	2-(1H-benzimidazol-2-ylsulfanyl)butanedioic acid 	-5.9475	1.1602	-3.7493	283.327	3	6

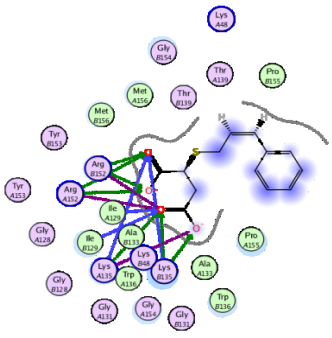


Figure 2. Interactions compound (2S)-2-[(Z)-3-phenylprop-2-enyl] sulfanylbutanedioic acid with GPx4 protein.

As shown in Fig. 2 (2S)-2-[(Z)-3-phenylprop-2-enyl]sulfanylbutanedioic acid has 25 interactions with the amino acids residue in the binding site. Six interactions in the form of hydrogen bonds are binding the ligand in the binding pocket such as Arg152 and Lys135.

3.4. Mutagenicity and carcinogenicity prediction.

To predict the mutagenicity and carcinogenicity of ligand, we used a software called Toxtree [20]. This software is capable of making a prediction based on the module encoded by Romualdo Benigni and Cecilia Bossa. The module stated that the mutagenic and carcinogenicity properties of molecules/compounds could be predicted from the functional group of the molecules/compounds. The function group regarded as mutagenic or carcinogenic is no less than acyl halide, epoxide, aliphatic halogen, aldehyde, isocyanate, aromatic nitro, polyaromatic hydrocarbon, and thiocarbonyl [21]. The result from Toxtree software could be seen in Table 2.

Table 2. The result of carcinogenicity and mutagenicity based on the rule of Benigni-Bossa at ToxTree.

Carcinogenicity and Mutagenicity Test	(2S)-2-[(Z-3-phenylprop-2-enyl] sulfanyl butane dioic acid	(2S)-2-[(2-methyl-1-3-thiazol-4-yl) methylsulfanyl] butanedioic acid	2-(1H-benzimidazole-2-ylsulfanyl) butanedioic acid)
Potential carcinogen based on QSAR	No	No	No
Potential *S.typhimurium* TA100 mutagen based on QSAR	No	No	No
Non-genotoxic carcinogenicity	No	No	No
Genotoxic Carcinogenicity	No	No	No

4. CONCLUSIONS

The result shows that 2-[(Z)-3-phenylprop-2-enyl]sulfanylbutanedioic acid has excellent potential as a drug candidate for inhibiting GPx4 protein. The ligand has a lower $\Delta G_{binding}$ and RMSD with the protein than the ligand standard, RSL3. Finally, our result must be examined through in vivo and in vitro methods to investigate its potential in biological conditions.

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