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Estimation of Hydrazone Derivative Compounds as Anti-Bacterial Agents Against *Staphylococcus aureous* Through Molecular Docking and Self Consisted Field

Neni Frimayanti[1,*], Melzi Octaviani[1], Gety Wulandari[1] and Adel Zamri[2]

[1]*Sekolah Tinggi Ilmu Farmasi Riau, Pekanbaru 28293, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematic and Natural of Mathematics and Natural Sciences, Universitas Riau, Riau 28293, Indonesia*

([*]Corresponding author's e-mail: nenifrimayanti@stifar-riau.ac.id)

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Abstract

Molecular docking is an important computational method for drug design. It can be used to predict the binding interaction of receptor with the ligand. Hydrazone were reported as chemical compounds with =CH-N-NH group. In addition, hydrazone derivative compounds were also reported have various activities, such as antibacterial agents against *Staphylococcus aureus*. *S. aureus* is a very serious problem because this bacteria is increasingly resistant to various types of antibiotics (multidrug resistance). *S. aureus* is also having extraordinary adaptability thus, it can be resistant to many antibiotics. The purpose of this study is to predict whether the hydrazone compounds were active as anti-bacterial agents and also to ensure that the binding interaction is stable before and after docking calculation. Molecular docking research was conducted using the protein target 1N67 (PDB ID), which was derived from the crystallographic structure of hydrazone derivative chemicals. Four hydrazone derivative substances were docked to the protein in this investigation with grid boxes along x, y, and z radii of 67, 86 and 66, respectively. The positive control was chloramphenicol. Self-Consisted Field (SCF) was calculated using AM1 as basis set and molecular dynamic simulation was performed using CHARMM27 force field. The results showed that from 4 tested compounds, only compound 3 with the binding free energy of −6.535 kcal/mol was estimated as active agent against *S. aureus.* This compound showed very good potential to be an anti-bacterial with the lowest binding free energy value with the highest stability level.

Keywords: Anti-bacterial, Hydrazone, Docking, SCF, *Staphylococcus aureus*, Anti-biotic, Molecular dynamic

Introduction

Antibacterial is a substance that can interfere with the growth or even kill bacteria by interfering the metabolism of harmful microbes [1]. Several microorganisms have been become resistance, one of them is *Staphylococcus aureus* (*S. aureus*). Currently, *S. aureus* is a very serious problem because this bacteria is increasingly resistant to various types of antibiotics (multidrug resistance). *S. aureus* is also having extraordinary adaptability thus, it can be resistant to many antibiotics [2].

The rise, dissemination, and persistence of multidrug-resistant (MDR) bacteria, colloquially known as "superbugs" which cause infections that do not respond to traditional therapies, has led to antibiotic resistance being one of the most important public health challenges on this century [3,4]. One of the main factors that contributing to the emergence and spread of antimicrobial resistance is the rising use and abuse of antibiotics in both humans and animals, as well as the lack of innovation in antibiotic research (reduction in the number of new antibiotic classes) [5]. Both the creation of novel chemical entities as antibacterial agents and policies to regulate the unwarranted and irrational use of antibiotics are urgently required [6].

Hydrazone compounds are reported as chemical compounds with an =CH-N-NH group that have various activities such as antibacterial, antitumor, anticancer, analgesic, and anti-HIV [7]. Hydrazone has 2 nitrogen atoms with different properties and linked together, besides =C=NH conjugated with nitrogen free electrons. To date, several studies have been performed to discover new agents for antibacterial [8] but unfortunately, thus far, hydrazone compounds have not been extensively studied for their antibacterial activity.

Due to *in silico* study such as molecular docking, molecular dynamic simulation is an important computational method for drug design. It can be used to predict the binding orientation of small molecule drug candidates to their protein targets. In addition, docking is also can be applied to predict small molecule affinity and activity. Thus, molecular docking has an important role in drug discovery and rational design [9,10]. Self-Consisted Field (SCF) is also applied in this research, it can create the usage of fixed in the difference combination of algorithms: At the certain time, atoms force is measured and pooled with the recent position to produce a new position and also the velocities to generate new velocities in a very short time for each step ahead.

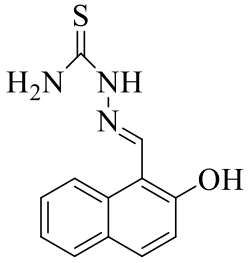
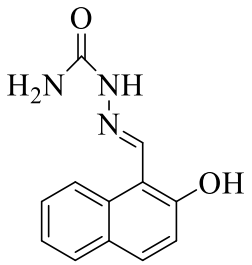
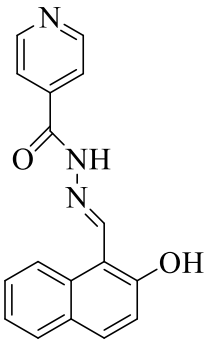
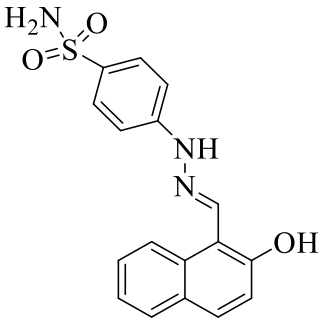
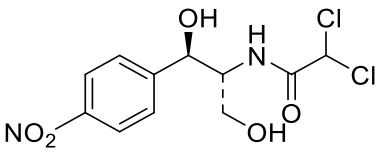
Materials and methods

Molecular docking

Ligand preparation

Molecular structure of hydrazone compounds and positive control are depicted in **Table 1**, they were sketched using ChemDraw Professional 15.0 application and saved in ".cdx" format. Furthermore, the energies of all the ligands were minimized using MMFF94x force field with gradient of 0.0001 and saved as a ligand database.

Table 1 Molecular structure of hydrazone compounds.

No	Structure	No	Structure
1		2	
3		4	
Positive control			

Protein preparation

The protein structure with PDB ID 1N67 was downloaded from the website www.rcsb.org. in PDB format. The protein crystal structure was then prepared using BIOVIA Discovery Studio Visualizer (DSV) 2020 and Molecular operating environment, MOE 2021.0901 (Chemical Computing Group) software package. The protein consisted of 1 chain (A), then the water molecule that bounded with protein was removed for then the native ligand that attached in the protein was also removed. Furthermore, the energy

of protein molecular structure was minimized using selected force field CHARMM 27 with coordinates x, y and z of 5.44, 46.42 and 66.20, respectively. RMS gradient was set up to 0.001 (kcal/mol/ Å). QuickPrep tools in MOE 2021.0901 was used for the protein preparation. Finally, this protein was saved in PDB format for then it was ready used as receptor [11].

Molecular docking

MOE 2021.0901 (Chemical Computing Group) software package was used for docking. The potential was first set up using a force field with a grid box among radius x, y, and z of 67, 86 and 66, respectively, using CHARMM27. Refinement was set into 100. Based on certain metrics such as binding free energy, root mean square deviation (RMSD), and binding factor, the ideal docking positions were chosen (the same binding interaction with positive control).

Self-consisted field (SCF)

Molecular structures of hydrazone compounds and positive control were drawn using ChemDraw Professional 15.0. All of the molecular structures were exported to MOE 2021.0901 (Chemical computing group) for SCF preparation. On the Compute menu, click SCF calculation, followed by energy minimization using MOPAC. Before running the analysis, the following parameters have to be set up such as AM1 is selected as the basis set, AM1 is a semi-empirical approach to computing the quantum structure of molecules in computational chemistry. AM1 usually use as a basis set and in some cases, forcefield parameterizations in molecular modelling are started using the results of AM1 computations. MOPAC is selected as engine, and singlet/closed is selected as multiplicity. Furthermore, once the parameters are set up, the analysis can start running and be presented.

Molecular dynamic

Preliminary study of molecular dynamic was carried out using the Nanoscale Molecular Dynamics program version 2.9 and CHARMM27 as the best force field. CHARMM 27 is widely used as better force field in molecular dynamic simulation. Popular additive atomistic Force field model for Molecular dynamic is CHARMM especially CHARMM27. CHARMM Force Field is implemented in several cutting-edge molecular dynamic simulation packages due to its comprehensive and versatile functional form, which is perfectly equivalent to that of other well-established force fields and the availability of parameters for a vast, constantly expanding number of current biomolecular systems. The modeled protein was solvated in a TIP3P water molecule box that extended in all directions more than 2.5 Å from the coordinated structure.

The simulated gradual heating in the constant temperature, constant volume (number volume temperature) assembling from 0 to 300 K over 100 ps was applied to the modeled systems. Then, for each system in an isothermal isobaric ensemble (number pressure temperature) with periodic boundary conditions, 50-ns time scale molecular dynamic (MD) simulations were performed. The coupling parameters for temperature and pressure were adjusted to 1.0 ps. When sampling, the coordinates were recorded every 0.1 ps. The conformations were produced by the simulations, which were then applied to the subsequent calculations of the binding and decomposition free energies.

Results and discussion

Molecular docking

Table 2 is presented the docking results for these hydrazone compounds. Based on docking results, chloramphenicol was employed as a positive control and based on docking results, it has the lowest binding free energy of −6.06 kcal/mo. Chloramphenicol was interacted with amino acids residues Asn284, Asn343 and Glu342 through hydrogen bonding. Chloramphenicol was also able to perform the van der Waals interaction and hydrophobic interaction with amino acid Asp312, Asp 340 and Lys281, respectively. **Figure 1** is presented the spatial arrangement of chloramphenicol.

Table 2 Docking results.

Compounds	Binding free energy (kcal/mol)	RMSD	Hydrogen bond	Hydrophobic	VDW	*Binding factor*
Positive control (chloramphenicol)	−6.064	1.099	Asn284, Asn343, Glu342	Lys281	Asp312, Asp 340	11
1	−5.125	0.892	Asp340, Thr289	Lys281, Asp312, Ser290	Glu342	4
2	−5.168	1.153	Asn343, Glu342	Lys281, Asp312, Ser290	Asp340	9
3	−6.535	0.738	Ser290, Glu342	Lys281	Asp340, Asp312	9
4	−6.137	1.317	Asp312, Asn284, Lys281, Asn314	Val279, Lys281	Asp340, Glu342	5

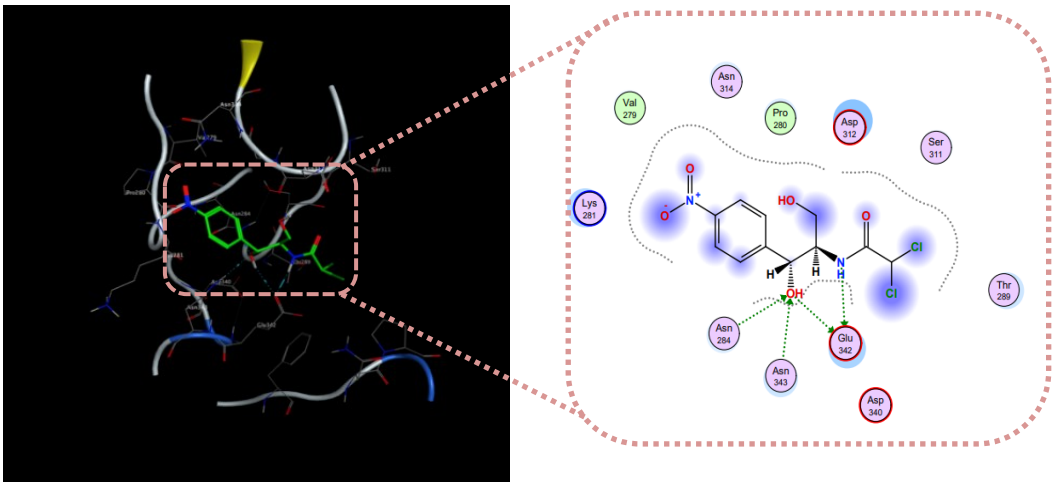


Figure 1 Spatial arrangement of chloramphenicol.

Molecular docking is a useful approach for predicting and matching the required binding locations, comprehending potential compound conformations, and further elucidating the binding interactions between ligand and receptor. In this study, 4 compounds of hydrazone derivative were docked into protein (PDB ID: 1N67) to interpret the interaction between ligand and active site. The lowest binding free energy is the main parameter to determine the binding stability between ligand and protein. This also showed that the ligand interaction with the receptor was more stable due to the decreased binding free energy value [12]. In addition, selection of the best poses for docking results was also carried out using root mean square deviation (RMSD). This stage is considered valid if resulting RMSD value is less than 2 [13]. The RMSD value is used to evaluate the error rate belong the docking process, smaller RMSD value was considered less error rate.

Based on the docking results (**Table 2**) indicated that compound 3 was estimated as active antibacterial against *S. aureus*. Compound 3 with binding free energy of −6.535 kcal/mol, it has lower binding free energy than positive control. Compound 3 was also able to construct the hydrogen bond with Ser290 and Glu342. Hydrophobic interaction was constructed between compound 3 with amino acid residue Lys281. It also observed the van der Waals interaction with amino acid residue Asp340 and Asn312. Thus, compound 3 predicted to be active compounds. The lowest binding free energy and lowest RMSD value of this molecule, which may presumably cause compound 3 to has better binding affinity compare to other compounds [14]. Superimposition is binding method that showed several residues play an important role in determine binding interactions for all ligands. Superimposition was performed to check the

orientation of compound 3. It seems that this compound has the same orientation to bind with the protein. The spatial arrangement of compound 3 is depicted in **Figure 2**.

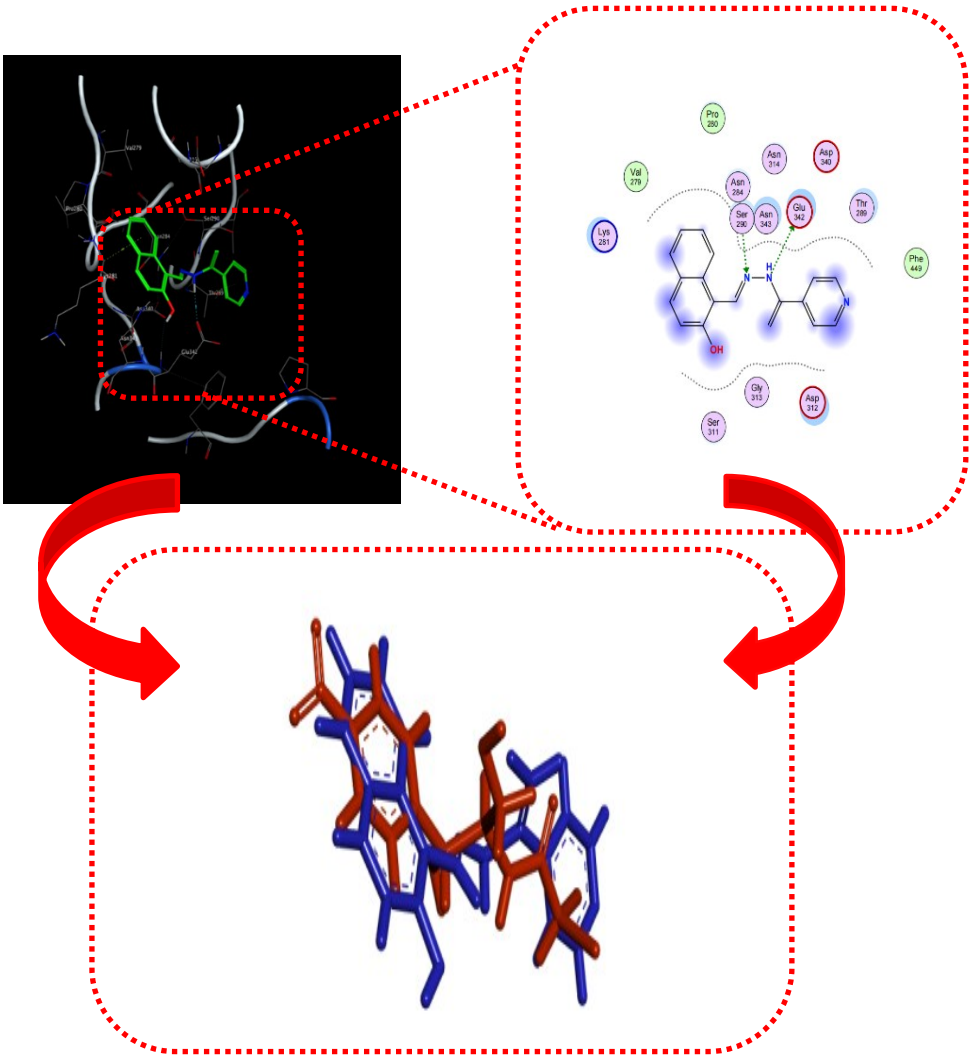


Figure 2 Spatial arrangement of compound 3 with superimposition of compound 3 (blue) and chloramphenicol (red).

Based on docking results for compound 1 and 2 were observed the binding free energy value of −5.125, −5,168 kcal/mol, respectively. although both of this compounds have hydrogen bonding, yet, based on their binding free energy value are higher than positive control (i.e. chloramphenicol) and its cannot be considered as potentially active compounds.

Different case for compound 4, although this compound has lower binding free energy than positive control and also displayed hydrogen bonds with Asp312, Asn284, Lys281, Asn314. However, these binds diverge from the positive control's amino acid sequence. In addition, the size of the molecule made compound 4 unable to enter deep into the cavity on the active site of the receptor, it probably caused the formation of complex receptor-compound 4 become difficult to form and it make several different interactions with positive control. Hence, this compound cannot be thought of as potential antibacterial.

Self-consisted field

SCF analysis was carried out to determine the stability of compound after docking simulation [15]. In this study, there some parameters for SCF calculation such as electron, SCF energy, Dipole, Delta H, Delta E HOMO and LUMO. Based on SCF calculation as depicted in **Table 3**, the results for SCF calculation for positive control (i.e. chloramphenicol) have 110 electrons, SCF Energy −159.297, Dipole

value 6.880D, Delta H value 124.768 kcal/mol, Delta E value 0.339au, HOMO value −0.041 and LUMO value −0.381.

Table 3 Results for SCF calculation.

Senyawa	*Electron*	SCF *Energy*	*Dipole* (D)	*Delta* H (kcal/mol)	*Delta* E(au)	*Homo* (au)	*Lumo* (au)
chloramphenicol	110	−159.297	68.80	124.768	0.339	−0.042	−0.381
1	86	−108.340	2.370	3.109	0.301	−0.019	−0.320
2	86	−108.339	2.400	3.261	0.300	−0.019	−0.320
3	108	−126.757	1.920	28.521	0.291	−0.020	−0.311
4	122	−152.777	3.890	23.285	0.289	−0.033	−0.313

One hundred and 8 electrons in compound 3 are atomic sub-particles that have a negative charge [16]. The difference of 24 electrons between chloramphenicol and compound 3 probably caused the same amount of electrons to be shifted, thus, it allowing this compound to bind with the same orientation with chloramphenicol. Delta value is the amount of energy that the system has taken in from its environment. The impact of the orbitals and the unit on the SCF results on the value of Delta H. The difference values of Delta for compound 3 is therefore not very great, and it can be said that this compound has a steady energy.

Compound 1, 2 and 4 has big difference value of electron and delta H value with positive control. It can be estimated that compound 1, 2 and 4 has a high level of instability. The HOMO (highest occupied molecular orbital) and LUMO can be used to approximate a molecule's ionization potential (lowest occupied molecular orbital). The valence orbital that most recently gained valence electrons is called HOMO. The vacant orbital just above the HOMO is the LUMO. An electron and a proton make up a molecule. Molecule can establish bonds by sharing electrons among themselves, and it's likely that 2 atoms sharing 2 electrons can also form a chemical connection. Atoms can work together to produce single bonds, double bonds, or triple bonds using 1 electron, 2 electrons, or 3 electrons. The 2 most crucial features for an atom or molecule are the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO) [17,18]. When the HOMO and LUMO are maintained together, a frontier orbital can be created. If an electron is placed in the outermost orbital, HOMO can be detected, and LUMO can be detected if there are no electrons in the first orbital [19]. It may cause that these compounds have different binding orientation with positive control.

Molecular dynamic

MD simulations are frequently used in modern drug design. In order to forecast the binding pose during the docking process and also to understand the drug-receptor interaction. By treating both the protein and the ligand as flexible entities and computing the movements of the atoms over time, MD replicates the dynamic behaviour of molecular systems as a function of time. As a result, MD trajectories can be used as sampling engines, allowing for the usage of the protein conformations produced by MD in molecular docking. In this approach, MD is seen as a docking-coupled technique that may be used to improve/rescore docking poses in addition to evaluating their stability.

The fulfilment of the docking prediction can also be usefully revealed by MD simulation [20]. The protein-ligand system fluctuates around the original conformation at the beginning of the MD simulation, while the ligand maintains its initial binding mode [21]. The effectiveness of hydrogen binding and the binding sites in the most active compounds, compound 3 was evaluated before and after a 50 ns and 300K MD simulation to check the results. The conformation was often preserved in the case of compound 3 to bind well with the identical residues before and after operation, and the hydrogen bond distance is less than 2.9 Å. The other examined hydrazone appeared to lose their activity due to the presence of some connections between ligands and receptors that were not sustained and also the distance of hydrogen bond is larger than 2.9 Å. **Table 4** is listed the interactions with amino acids. According to MD simulation, compound 3 appeared to keep up its connections with the same amino acids following simulation, suggesting that it might be employed as a possible antibacterial against *S. aureus*. **Figure 3** is depicted visualization of MD simulation.

Table 4 Molecular dynamic.

Compound	After docking	MD simulation	Distance of H bond
1	Asp340, Thr289	-	-
2	Asn343, Glu342	-	-
3	Ser290, Glu342	Ser290, Glu342	2.9 Å
4	Asp312, Asn284, Lys281, Asn314	-	-

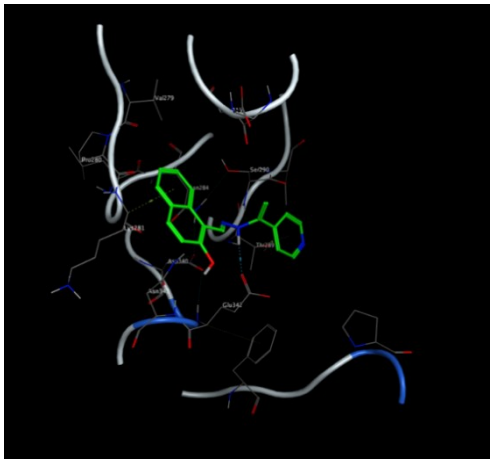


Figure 3 MD visualization of compound 3.

Conclusions

Molecular docking, molecular dynamic and SCF analysis have been successfully estimated the potentiality of 4 hydrazone derivative compounds as antibacterial against *S. aureus.* Based on molecular docking, compound 3 can binding well with the active site of 1N67 protein. Compound 3 has binding free energy of −6.535 kcal/mol, this compound also able to construct the hydrogen bond with Ser290 and Glu342. Hydrophobic interaction was constructed between compound 3 with amino acid residue Lys281 and van der Waals interaction was obtained with amino acid residue Asp340 and Asn312. Compound 3 is also showed the highest stability in SCF analysis. Hence, it make compound 3 can be used as potential candidate for antibacterial and also as the reference for the next stage in drug design.

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