Journal of Bio-Molecule Research and Engineering
Vol. 1 | No. 2 | 66 – 72 | 2022
e-ISSN: 2962-343X
https://www.e-journal.unair.ac.id/biome

The comparative of free energy binding between Pyrimethamine-pDHFR double mutant and Pyrimethamine-pDHFR quadruple mutant: Structure-based approach

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ARTICLE INFO

Article history
Received 20th Sep 2022
Accepted 31st Nov 2022

Keywords:
Plasmodium falciparum
Pyrimethamine
malaria
antimalaria
molecular docking
md simulation

ABSTRACT

Structure-based was performed to understand the mechanism inhibition of pyrimethamine (CP6) against *Plasmodium Falciparum* mutants at the molecular level. A molecular docking process was carried out to obtain the initial conformation of each system. The data is shown in the form of RMSD values, hydrogen bonds, and grid scores. The results show that the RMSD value in the redocking process meets the criteria of the CP6-1J3J and WRA-1J3K complexes with a value of ≤ 2.00 Å. Several hydrogen bonds bind to receptor-active sites, including Ile14, Asp50, Ile164, and Asn108. Additionally, the grid score (kcal/mol) binds well on the active site: CP6-1J3J (-46.86), WRA-1J3K (-65.40), and CP6-1J3k (-44.71). Furthermore, molecular dynamics simulation was carried out to determine the free energy (ΔG_{bind}) of each complex using the MM/GBSA approach. The results show ΔG_{bind} (kcal/mol) in each complex, namely CP6-1J3J (-28.24), WRA-1J3K (-36.62), and CP6-1J3k (-24.23). Information on this research was expected to provide molecular insight into pyrimethamine as a *Plasmodium Falciparum* mutant inhibitor.

1. Introduction

Plasmodium falciparum parasite is the main target in the development of antimalarial drugs [1,2]. Nowadays, antimalarial drugs such as chloroquine experience resistance due to mutations in enzymes that play a role in the development of the *P. falciparum* parasite [3]. The main target of this enzyme is a mutant of the oxidoreductase transferase. As noted, it is the main target in the occurrence of malaria diseases. The parasites will do a lot of replication and translation to survive their host cells [4].

Molecular docking is a powerful method for determining or predicting the orientation of a ligand when it binds to a receptor through an empirical free energy function approach [5]. Molecular docking can determine some crucial variables in ligand and protein interaction, such as hydrogen bonds and van der Waals interactions [6]. The advantage of this method is that it can calculate the ligand coordinate that binds very well on the active site of the targeted protein. Moreover, box grid preparation plays a crucial role in the successful utilization of this method [5,6].

Research developments in computational drug design through predicting the biological activity of a compound against target proteins have been carried out by many studies on the combination of molecular docking and molecular dynamics (MD) simulations in predicting binding energy [7,8]. In particular, MD simulation is known as one of the reliable in silico methods for predicting the free energy binding of ligand-protein [9,10]. MD simulation can reduce costs and time in predicting drug biological activity to be used as trial samples in drug development.

The MM/PBSA (Molecular Mechanics Poisson-Boltzmann) and MM/GBSA (Generalized Born Surface Area) approaches based on MD simulations have been widely used to predict free energy binding [10,11]. The MM/GBSA approach is useful for evaluating ligand-protein interactions, especially for pharmaceutical research and development. This approach detailed the calculations of free energy binding in ligands, receptors, and complexes based on some parameters considered in MD simulations [12].

2. Materials and methods

2.1. Ligand and receptor preparation

The selected proteins obtained from the Protein Data Bank (PDB) are 1J3J (Double mutant: Arg59 and Asn108) and 1J3K (Quadruple mutant: Ile51, Arg59, Asn108, and Leu164) [13], each protein consisting of non-standard residues and standard amino acids (Figure 1). Native ligand preparation was extracted from both proteins, namely pyrimethamine (CP6) and WRA, by using the Chimera version 1.13 package. Meanwhile, the standard amino acid residues for each protein are used for receptors. Moreover, ligands and receptors were calculated for the missing parameters, such as bond and non-bonded parameters, through AMBER ff14SB. Additionally, the AM1-BCC method was applied to calculate the ligand and receptor charges. Finally, all files were saved in the form of the MOL2 file type.

2.2. Molecular docking

The molecular docking step was performed by the DOCK6 package with a Linux-based operating system [5]. Molecular docking preparation was carried out to create a box grid of each complex by selecting cluster spheres contained in the receptor surface. In detail, the size and center of each selected protein is 1J3J (center (X: 28.16, Y: 5.31, Z: 58.26) and size (X: 26.60, Y: 27.80, and Z: 29.00)) and 1J3K (center (X: 28.69, Y: 7.00, Z: 59.28) and size (X: 26.65, Y: 26.91, and Z: 29.04)). Meanwhile, the redocking step between ligand and receptor aimed to obtain the initial coordinates, such as grid score, hydrogen bond, and root-mean-square deviation (RMSD) [6].

2.3. Molecular dynamics simulation

The MD simulation was performed to calculate the free energy binding (ΔGbind) between the CP6 and the targeted receptors (1J3J and 1J3K) [8]. The results of molecular docking are used as an initial coordinate to generate the topology for each system. In processing, MD simulation consists of several stages, such as minimization, heating, equilibrium, production, and trajectory analysis [14],[15]. All simulations were carried out for ten ns using the sander and pmemd.cuda tools. The ΔG_{bind} calculation was performed by the MM/GBSA approach available in AMBER18 [10].

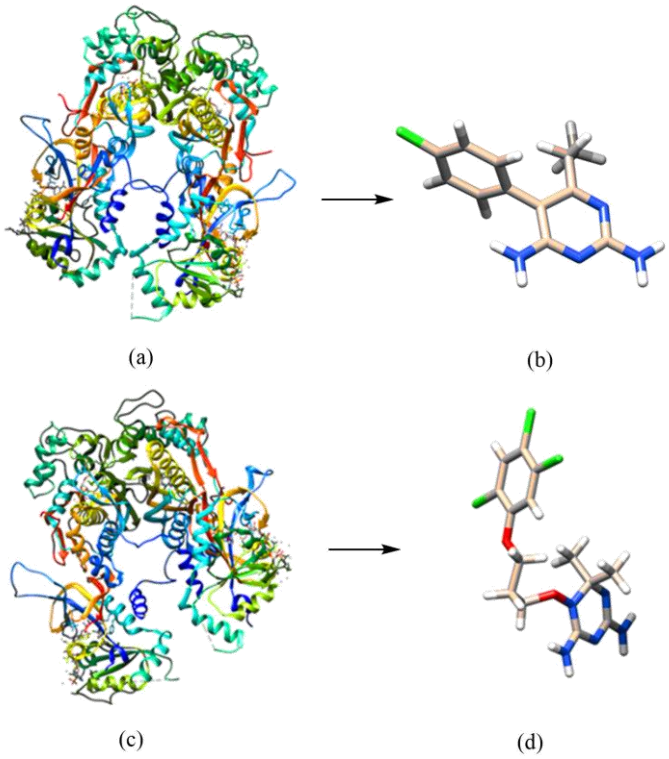

Figure 1. Molecular structures: (a) 1J3J, (b) CP6, (c) 1J3K, and (d) WRA

3. Results and discussion

3.1. Redocking: Active site determination

The process of molecular docking is carried out through several stages, including creating a box grid that aims to have a cluster sphere or area in the receptor part that the ligand wants to occupy (Figure 2). As mentioned in the molecular docking methodology, the center and size of the grid box are crucial in determining the cost and time needed to dock the ligand into the receptor. Therefore, the box size must follow the calculation needed to determine the best initial coordinate [16].

The redocking step is validated through the native ligand superimposed between the co-crystal and docked pose to obtain the RMSD value. The results showed each native ligand RMSD value fulfills the criteria with the RMSD ≤ 2 Å [6]. In detail, the value of each native ligand superimposed is CP6: 0.27 Å and WRA: 0.29 Å. This finding indicated the obtained coordinate can be used for further analysis.

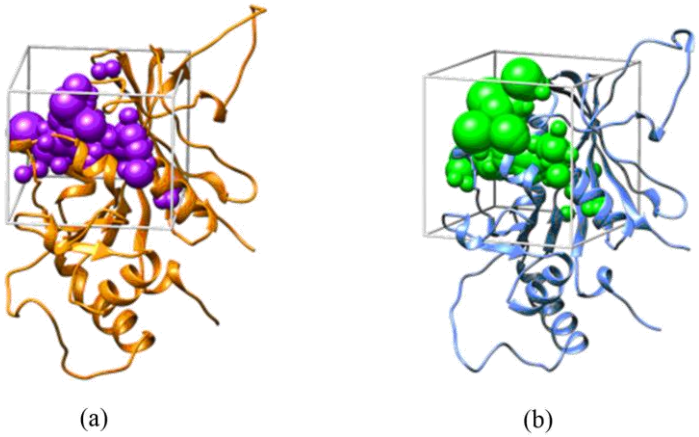

Figure 2. Box-grid created by dock6 package: (a) 1J3J and (b) 1J3K.

3.2. Molecular docking analysis: Hydrogen bond and energy contribution

The obtained coordinate from the redocking step was used to analyze several variables, such as hydrogen bond and grid score. In particular, the hydrogen bond is formed in each complex. The interaction was identified between the ligand and specific amino acids with different distances (Figure 3). The hydrogen bond is a stronger interaction that plays a crucial role in the ligand-receptor interaction [17],[18]. However, bond strength is generally considered weak. Furthermore, molecular docking calculates the energy interaction in the gas phase. The parameters measured at this step are the grid score, van der Waals (EvdW), electrostatic (Eele), and internal repulsive energy (Eint) [5]. In detail, the energy interaction of each complex is provided in Table 1.

Table 1. Energy contribution at the gas phase of each complex

parameters	CP6-1J3J	WRA-1J3K	CP6-1J3K
Grid Score (kcal/mol)	-46.86	-65.40	-44.71
E_{vdw} (kcal/mol)	-43.26	-64.45	-44.06
E_{ele} (kcal/mol)	-3.60	-0.95	-0.65
E_{int} (kcal/mol)	4.39	8.89	5.30

Following the analysis, the CP6 ligand was docked into the 1J3K receptor to produce an initial coordinate using the previous parameters. The results show that only one hydrogen bond was formed with Asn108, with a distance of 1.99 Å (Figure 3). However, the grid score of the CP6-1J3K complex is higher than the native ligand (WRA-1J3K) (Table 1). This indicates that WRA has a better binding pose compared to CP6. In particular, the CP6 ligand poses through two different enzymes showed the complex grid score with the trend CP6-1J3J < CP6-1J3K. It indicates that the binding pose of CP6-1J3K is weaker than CP6-1J3J. Thermodynamically, this finding describes that CP6 requires less energy to bind with 1J3J than 1J3K. However, this result needs further evaluation through free energy binding calculation following MD simulation for accurate results [8],[14].

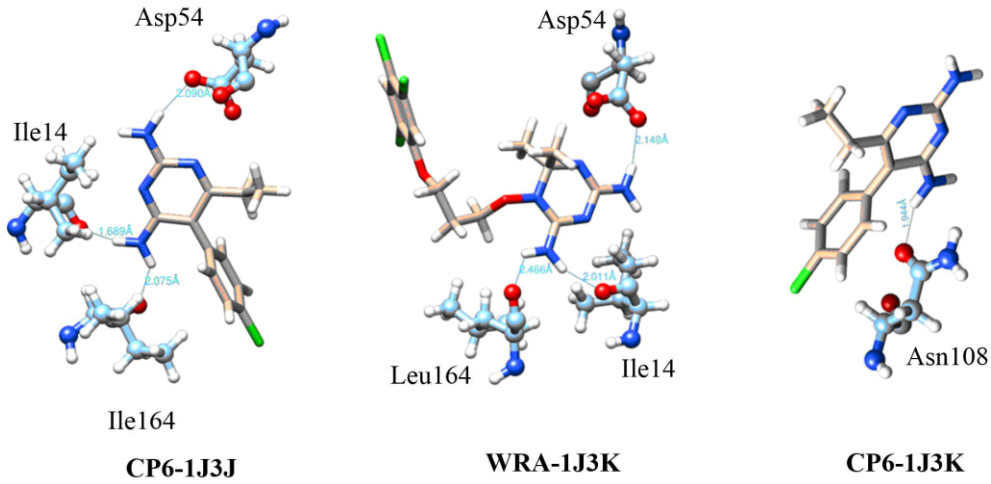

Figure 3. The hydrogen bonds analysis of each complex is shown by distance (Å).

3.3. MD simulation: Free energy binding

MD simulation was performed using the AMBER18 package based on the Linux operating system. The data shows that several steps must be passed to produce free energy binding (ΔGbind). The topology system was figured out by the tleap tool available in the AMBER18 package. It aims to produce a complexes-solvated topology of each system for the MD simulations calculation needed [15].

Table 2. Free energy binding (ΔG_{bind}) of each complex. Data are shown as mean ± standard deviation.

System	ΔG_{bind} (kcal/mol)	±SD (kcal/mol)
CP6-1J3J	-28.24	2.47
CP6-1J3K	-24.23	1.93
WRA-1J3K	-36.62	1.74

The ΔGbind analysis was performed using the MM/GBSA approach based on the AMBER force field ff14SB to analyze the results at the production stage [19],[20]. Overall, the standard deviation was calculated to see the ΔGbind deviation or different values during the simulation of 10 ns quantitatively. The lower ΔGbind value from the simulated system is the WRA-1J3K complex (Table 2). This finding is supported by previous data showing that WRA inhibitory activity is considered more sensitive to quadruple mutants than pyrimethamine and cycloguanil. In detail, all activities shown in Ki (WRA: 0.037 ± 0.005 nm, PC6: 283 ± 22 nm, and Cyc: 254 ± 33 nm) and IC50 (WRA: 0.018 ± 0.01 nm, PC6: > 100 nm, and Cyc: > 100 nm) [13]. Therefore, WRA has a stronger binding affinity compared to CP6. Meanwhile, CP6 binding poses to the two targeted receptors showed the trend as CP6-1J3J < CP6-1J3K. This finding led to CP6 binding strongly with the double mutant compared to the quadruple mutant. It should be noted that the ligand with a lower ΔGbind value will be able to bind more effectively to the active site of the targeted receptor [12],[19]. It aims to inhibit the regulatory process of the enzyme in the life cycle of the P. falciparum parasite.

4. Conclusions

In this work, the interaction of pyrimethamine (CP6) with double mutant (1J3J) and quadruple mutant (1J3K) was performed through molecular docking and MD simulations. Through the redocking step, the validation initial coordinates are determined with the RMSD value below 2.0 Å. The results suggest the binding pose of CP6 has a better energy interaction with 1J3J compared to 1J3K. This is because CP6 is the original ligand from the non-standard residue found in the double mutation, and it requires less energy to bind with 1J3J than the 1J3K. Thus, our observation suggests that CP6 has the potential as an inhibitor double mutation compared to quadruple mutant.

Acknowledgments

Not Available

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