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² Interaction of mutant PBP2a and bioactive compounds from Streptomyces with anti-MRSA activities

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³ **Abstract.** Methicillin-Resistant *Staphylococcus aureus* (MRSA) is the leading cause of nosocomial infections in hospitals. Treatment of MRSA infection using ceftaroline has been reported to be resistant due to mutations in the Penicillin Binding Protein (PBP)2a. In silico's approach through virtual screening can analyze the bioactive compounds that can bind effectively to mutant PBP2a. The potential source of bioactive compounds with anti-MRSA activities is Streptomyces, which is the main antibiotic-producing bacteria. Thus, the study aimed to analyze the interactions of PBP2a/mutant PBP2a against ceftaroline and the interactions between mutant PBP2a against bioactive compounds from Streptomyces. The protein receptors were PBP2a (PDB 3ZG0) and mutant PBP2a (PDB 4CPK). The ligands used were ceftaroline (CID 9852981) as control and nine bioactive compounds produced by Streptomyces. Protein preparation and visualization used Discovery Studio, ligand preparation used Marvin, and molecular docking used Autodock4. The alignment results showed that mutant PBP2a has a more extended amino acid sequence (643 amino acids) than PBP2a (641 amino acids). The mutations that occurred in mutant PBP2a caused conformation changes in the active site of mutant PBP2a so that the interaction between ceftaroline and mutant PBP2a decreased. The virtual screening results indicated that 1-acetyl- β -carboline was the most potent compound as anti-MRSA with the lowest binding energy (-7.12 Kcal/mol) compared to ceftaroline (-6.32 Kcal/mol). The amino acids involved in the binding of 1-acetyl- β -carboline with PBP2a mutant were Ser403, Ser461, Asn464, Thr600, Ser462, Tyr446, and Ala642. This result suggests that 1-acetyl- β -carboline has better interaction with mutant PBP2a, hence might serve as a potential anti-MRSA compound.

⁷ Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is *Staphylococcus aureus* bacteria resistant to β -lactam antibiotics such as penicillin and its derivatives, namely methicillin, oxacillin, dicloxacillin, cefaclor, and cephalosporin. Resistance to methicillin is caused by the *MecA* gene that produces Penicillin Binding Protein (PBP) 2a, which is insensitive to β -lactam antibiotics [1]. PBP has a significant role in the peptidoglycan biosynthetic reaction in the formation of bacterial cell walls [2]. There are four types of PBP, namely PBP1, PBP2, PBP3, and PBP4, which are complex proteins with glycosylase and transpeptidase activities. In MRSA, there is a fifth type of PBP, namely PBP2a, coded by the *MecA* gene [3]. This PBP2a causes MRSA to be insensitive to β -lactam class antibiotics and causes multiresistant strains.

One of the options for treating MRSA infection is the use of ceftaroline antibiotic, which serves as transpeptidase inhibitor in PBP2a [4]. However, Fishovitz et al. [5] have reported a mutation in PBP2a



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so that MRSA⁵ becomes resistant to ceftaroline. Resistance occurs due to the mutation of two amino acids on the allosteric site of PBP2a, which causes changes in the conformation of the active site. These changes cause a decrease in the effectiveness of ceftaroline antibiotic binding and result in the ineffectiveness of ceftaroline for MRSA therapy [6]. The incidence of MRSA resistance to ceftaroline encourages the development of new bioactive compounds as anti-MRSA. WHO has even classified MRSA in the High-Risk category on the Priority Pathogen List for research and development of new antibiotics [7].

Exploring bioactive compounds that are antagonistic can be done through virtual screening of potentially bioactive compounds [8]. Molecular docking can be performed between the mutant PBP2a receptor and bioactive compounds as ligands to obtain bioactive compounds capable of interacting with mutant PBP2a. Molecular docking is an in-silico approach to pairing a protein molecule on the active side with other molecules with a specific purpose, for example, looking for potential new drugs [9]. It is known that ceftaroline antibiotic binds PBP2a on the active side at the Serin 403 position [10]. Therefore, the active site of PBP2a can be used as a reference for molecular docking to determine the interaction of PBP2a with other bioactive compounds to test the compound's potential as anti-MRSA. Lavanya et al. [11] conducted molecular docking to screen potential compounds from the ZINC database capable of interacting with MRSA that is resistant to ceftaroline. Mohamed et al. [12] reported that indigenous Sudanese natural ingredients, namely isoflavonoids-17, chalcon-21, diphyllin, and coumarin-29, were able to bind effectively with PBP2a in Ser404 using the molecular docking method.

One of the potential sources of bioactive compounds is actinomycetes, especially the genus Streptomyces which is the main antibiotic-producing bacteria. Streptomyces are known to produce antibiotic, anticancer, anti-inflammatory, anti-fungal, protein inhibitor, and other active compounds [13]. Indeed, the genus Streptomyces can produce potential compounds with anti-MRSA activity. Kemung et al. [14] have reviewed bioactive compounds with anti-MRSA activity produced by Streptomyces. Asnani et al. [15] have also reported that Streptomyces from mangrove sediment at Segara Anakan has potential as anti-MRSA. Hence, this study aimed to compare PBP2a protein interaction with mutant PBP2a against ceftaroline and screen bioactive compounds from Streptomyces as anti-MRSA candidates. The bioactive compounds with the most potential to bind to mutant PBP2a are expected to be the most potential anti-MRSA candidates.

2. Materials and methods

The PBP2a receptor protein was PDB 3ZGO, a PBP2a crystal structure that binds to ceftaroline [10]. The mutant PBP2a was PDB 4CPK, which is a crystal structure of PBP2a double clinical mutant N146K-E150K from MRSA [5]. The crystal structures of the two proteins were downloaded from the Protein Data Bank. The alignment of mutant PBP2a and PBP2a used Clustal Omega (1.2.4) multiple sequence alignment to analyze the differences caused by mutations. Protein preparation used Autodock4 [9] version 1.5.6.

The ligands were the antibiotic ceftaroline as control and nine bioactive compounds produced by Streptomyces, namely 1-acetyl- β -carboline, marinopyrrole A, cyslabdan, 4'-deacetyl griseucin A, griseucin A, polyketomycin, heliquinomycin, and chaxamycin D. All ligand structures were downloaded from PubChem and optimized with MarvinSketch 20.3.

Interaction of mutant PBP2a and bioactive compounds from Streptomyces was carried out based on the active side of mutant PBP2a, which is Serin 403 coordinates [10] using Autodock4 [9] version 1.5.6. Mutant PBP2a protein was not given a degree of freedom. In contrast, ligands were given a degree of freedom to predict the best position on the active site of the protein. The bond coordinates were x: -27.959; y: -19.845; and z: 6.711. The box's size to predict binding position was 30 on Autodock and docking parameters used LGA (Lamarckian Genetic Algorithm). Visualization of the amino acid bonds involved in the binding position and the interactions between the receptor protein and the ligand used the BIOVIA Discovery Studio Visualizer.

3. Results and discussion

3.1. Alignment of PBP2a and mutant PBP2a

Protein receptors alignment were performed by comparing the crystal structure of PBP2a (PDB 3ZG0), which binds to ceftaroline antibiotics with the crystal structure of mutant PBP2a (PDB 4CPK). Figure 1 shows the differences in the structures of PBP2a and mutant PBP2a. The mutations that occur in mutant PBP2a are on the allosteric site, which causes changes in the protein conformation so that ceftaroline cannot effectively bind to the active site [5]. This mutation causes MRSA to become resistant to ceftaroline.

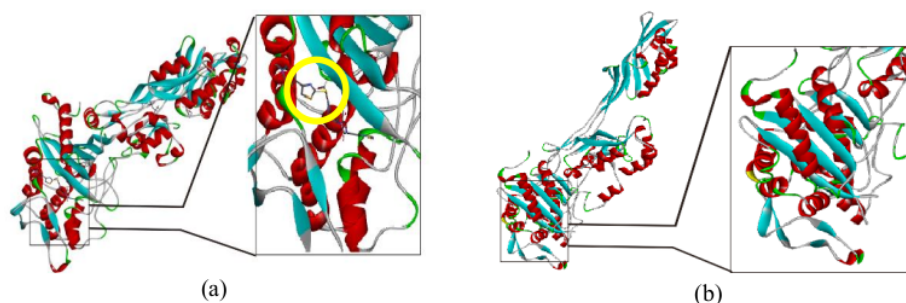


Figure 1. The crystal structure (a) PBP2a binds to the ceftaroline antibiotic on the active site of the A chain indicates by the yellow circle, (b) mutant PBP2a resistant to antibiotics does not bind to ligands on the active site of the A chain.

In addition to differences in the molecular structure of mutant PBP2a and PBP2a, there are also differences in the amino acid sequences of the two proteins. The results of the alignment analysis of the amino acid sequences of mutant PBP2a and PBP2a showed differences in the number of amino acids. The mutant PBP2a has a more extended amino acid sequence than PBP2a. The longer mutant PBP2a protein presumably is caused by a mutation on the allosteric site that changes the protein conformation. Table 1 shows the differences in amino acids of PBP2a (PDB 3ZG0) and mutant PBP2a (PDB 4CPK).

Table 1. The results of the alignment analysis of 3ZG0 and 4CPK.

PBP2a	Amino acids		Position	
	PDB 3ZG0	PDB 4CPK	PDB 3ZG0	PDB 4CPK
A chain	-	Lysine	-	1
	Asparagine	Lysine	120	121
	Glutamic acid	Lysine	124	125
	-	Glutamic acid	-	643
B chain	-	Lysine	-	1
	Asparagine	Lysine	120	121
	Glutamic acid	Lysine	124	125

3.2. Ligand optimization

The ligands used in this study were ceftaroline antibiotics as control and nine bioactive compounds with potency as anti-MRSA from Streptomyces. Structure optimization of ligands used MarvinSketch. Structure optimization aims to ensure the correct structure of the ligands. It minimizes the energy to

stabilize the structures before the ligands are used in molecular docking. The ligand optimization generated structure with energies ranges from 46.47 to 227.48 Kcal/mol (table 2). The compounds that have been minimized were then used as ligands in molecular docking.

Table 2. The Energy minimization from ligands optimization

No	Compounds	CID	Energy (Kcal/mol)
1	Ceftaroline	138756682	127.30
2	1-Acetyl- β -carboline	638667	46.47
3	Frigocyclinone	11476774	109.56
4	Marinopyrrol A	24797083	85.86
5	Cyslabdan	24778014	113.11
6	4'-deacetyl griseusin A	56834145	90.09
7	Griusisin A	16102131	89.14
8	Polyketomycin	10395823	227.48
9	Heliquinomycin	101714016	127.30
10	Chaxamycin D	53344649	164.45

3.3. Interaction of mutant PBP2a and ligands

Molecular docking was performed 20 times from the LGA calculation to produce the best position with the lowest binding energy. The smaller binding energy with the receptor protein indicates good results in which the binding is more stable than that with the higher binding energy. The results of the molecular docking of nine bioactive compounds from Streptomyces based on binding energy are presented in table 3. The 1-acetyl- β -carboline compound has the lowest binding energy (-7.12 Kcal/mol) compared to other bioactive compounds, including the control ceftaroline (-6.32 Kcal/mol). This result suggests that the interaction between 1-acetyl- β -carboline and mutant PBP2a is the most effective so that 1-acetyl- β -carboline has the potential as anti-MRSA. The best interactions between ligands and receptor proteins were further visualized using Discovery Studio to analyze the amino acid bonds.

Table 3. The binding energy from the interaction of protein and ligand.

No	Compounds	Binding Energy (Kcal/mol)
1	Ceftaroline	-6.32
2	1-acetyl- β -carboline	-7.12
3	Frigocyclinone	-6.66
4	Marinopyrrol A	-6.27
5	Cyslabdan	-5.67
6	4'-deacetyl-griseusin A	-5.10
7	Griusisin A	-4.55
8	Polyketomycin	-4.49
9	Heliquinomycin	-3.55
10	Chaxamycin D	-1.41

The docking result of ceftaroline on the active site of the mutant PBP2a has a binding energy of -6.32 Kcal/mol. The amino acid involved in the binding of ceftaroline with mutant PBP2a are lysine at a

position of 430 (Lys430) with hydrogen bonding (figure 2). Amino acids involved in the binding interactions of ceftaroline and mutant PBP2a through hydrogen bonds are Lys430, Tyr446, Asn464, Gln521, Thr600, Ala601, and Gln613 in the A chain.

Ceftaroline is known to have a high affinity for PBP2a. It binds to the active site of the PBP2a on the amino acid Ser403 [10]. The results of visualization of ceftaroline binding with mutant PBP2a showed different binding to PBP2a. This difference in binding is probably the cause of the different responses of MRSA containing mutant PBP2a to ceftaroline therapy. Allosteric site changes that occur in mutant PBP2a has changed the conformation on the active site of mutant PBP2a [5]. Apparently, the binding affinity of the receptor to ceftaroline decreases due to conformation changes of the active site of the protein, thus resulting in resistance to ceftaroline.

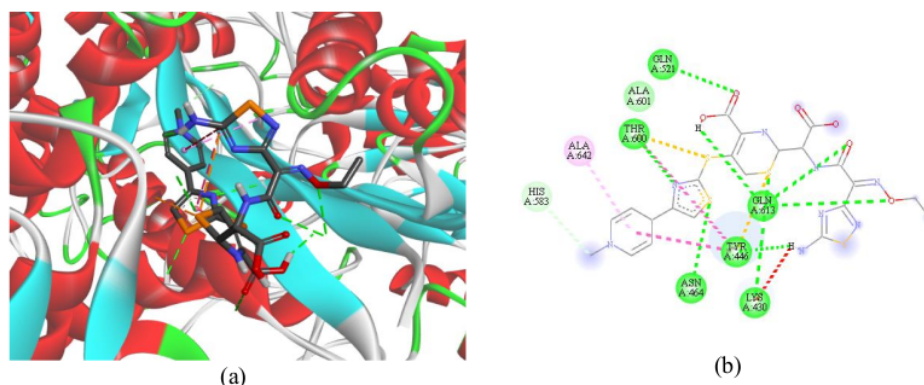


Figure 2. (a) 3D visualization of ceftaroline-protein receptor binding interactions; (b) 2D visualization of the amino acids involved.

Nine bioactive compounds from *Streptomyces* were successfully docking on the active site of mutant PBP2a with the same active site as PBP2a. Based on the docking results, 1-acetyl- β -carboline and frigocyclinone compounds are potential anti-MRSA compounds with bond energies of -7.12 and 6.62 Kcal/mol. The affinity binding of the 1-acetyl- β -carboline and frigocyclinone to mutant PBP2a are stronger than that of ceftaroline.

1-Acetyl- β -carboline is a compound of the alkaloid indole group isolated from *Streptomyces sp.* 04DH52 [16]. This molecule could enter and bind to the active site of mutant PBP2a with the least bond energy compared to other ligands. The small binding energy shows the bond's strength in which the smaller the value, the stronger the bonds formed. Docking of the 1-acetyl- β -carboline with the mutant PBP2a receptor protein gave binding energy of -7.12 Kcal/mol. Amino acids involved in the binding of 1-acetyl- β -carboline with receptor proteins through hydrogen bonding are Ser403, Ser461, Asn464, Thr600; and through the pi-sigma bonds are Ser462, Tyr446, and Ala642 in the A chain (figure 3A). Frigocyclinone is a bioactive compound isolated from *Streptomyces griseus* strain NTK 97 [17]. Frigocyclinone has good inhibitory activity against Gram-positive bacteria *Bacillus subtilis* DSM 10 and *S. aureus* DSM 20231. Docking of the frigocyclinone molecule with the mutant PBP2a receptor protein gave binding energy of -6.66 Kcal/mol. Amino acids involved in the binding of frigocyclinone with receptor through hydrogen bonds are Lys430, Gln521, Gln613, Glu602; and through phi-sigma bonds is Met641 on the A chain (figure 3B).

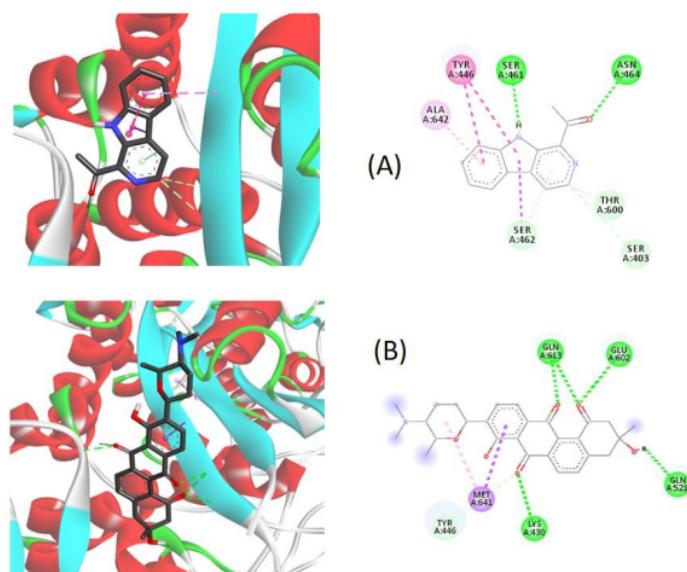


Figure 3. 3D visualization and 2D visualization of the amino acids involved in the interaction of (a) 1-acetyl- β -carboline and mutant PBP2a protein receptor, and (b) frigocyclinone and mutant PBP2a.

4. Conclusion

A virtual screening approach can be used to analyze the potential of bioactive compounds as anti-MRSA. Virtual screening of nine bioactive compounds from *Streptomyces* using molecular docking showed that 1-acetyl- β -carboline and frigocyclinone are potential compounds as anti-MRSA compared to ceftaroline control based on binding energy. This approach is expected to save time, effort, and cost before drug design and development is carried out for production.

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