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Molecular docking study of sappan wood extract to inhibit PBP2A enzyme on methicillin-resistant *Staphylococcus aureus* (MRSA)

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Abstract:
Background: PBP2a is a type of penicillin-binding proteins (PBPs) that cause resistivity in methicillin-resistant *Staphylococcus aureus* (MRSA) from β-lactam antibiotics. MRSA susceptible with cefttobiprole (fifth generation of cephalosporin as an anti-MRSA agent) which inhibits PBP2a and stops its growth. Contrary to its efficacy, ceftobiprole causes taste disturbance more than any other cephalosporins; furthermore, its mechanism is unknown. This study aims to explore an *in silico* study of a natural compound, which serves as a potential alternative to overcome MRSA with minimum adverse side effects.
Methods: A molecular docking study was performed using Molegro Virtual Docker version 5.5. Brazilin and proto-sappanins A–E are phytochemical compounds contained in sappan wood extract and are docked into the binding site of PBP2a (Protein Data Bank: ID 4DKI).
Results: Brazilin and proto-sappanins A–E have some interaction with Ser 403 amino acid residue which is an important interaction to inhibit PBP2a protein. The result of the molecular docking study showed that the MolDock score of proto-sappanins D and E is lower than that of methicillin but higher than that of its native ligand (ceftobiprole).
Conclusions: The results of this study suggest that proto-sappanins D and E have an excellent potential activity as an alternative to ceftobiprole in limiting MRSA growth through PBP2A enzyme inhibition.

Keywords: antimicrobial, molecular docking, MRSA, PBP2a, sappan wood

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of gram-positive bacteria that is genetically different from the other *S. aureus* [1]. MRSA causes life-threatening bloodstream infections, pneumonia, and surgical site infections in humans at health care facilities. Hospital-acquired infections caused by MRSA are often systemic infections, whereas community-acquired MRSA infections are skin and soft tissue infections [2]. These MRSA bacteria are developed by horizontal transfer gene or multiple drug resistance to β-lactam antibiotics, including penicillin derivate (methicillin or oxacillin), cephalosporin, or carbapenem [3]. MRSA resistance is caused by structural modification of penicillin-binding proteins (PBPs) [4], [5]. The mecA gene is a biomarker responsible gene found in bacteria, which causes resistance to methicillin and other β-lactam antibiotics. The mecA gene encodes is PBP2a, which differs from other PBPs that do not allow methicillin and other β-lactam antibiotics bind to their active site [1], [6], [7].

Ceftobiprole is a broad-spectrum antibiotic which shows activity against MRSA [8]. Ceftobiprole is the frontrunner of the fifth cephalosporin generation, which is used as a comparator drug to an alternative natural compound with potential anti-MRSA activity. Although some β-lactam antibiotics in the fifth-generation broad spectrum show their activity against MRSA, their adverse side effects cause limitation to their usage as comparator drugs [3], [9]. Natural compound with anti-microbial effects in search for alternative drugs against MRSA. This study aims to explore natural compounds which serve as a potential alternative to overcome MRSA with minimum adverse side effects.

Sappan wood extract is known to have anti-microbial properties. It has been tested against different microorganisms for its potential anti-microbial activity, including *S. aureus* [10]. The investigation of the chemical

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constituents of sappan wood extract shows that it has various types of phenolic compounds including brazilin and proto-sappanin [11], [12]. Hence, in this study a natural compound from sappan wood extract was analyzed for its effectiveness with respect to the existing drugs (ceftobiprole) against MRSA through *in silico* studies (molecular docking) using Molegro Virtual Docker (MVD) version 5.5.

Materials and methods

Receptor and ligand preparation

PBP2a receptors obtained from Protein Data Bank (PDB) were prepared using the MVD version 5.5 software, as shown in Figure 1. PBP is an enzyme that catalyzes processes of the building cell wall process of some bacteria. PBP2a is a type of PBP that causes resistivity in MRSA from β-lactam antibiotics. PBP2a was downloaded with PDB ID code 4DKI from www.rcsb.org [13]. The comparative ligand from PBP2a receptor is ceftobiprole (its native ligand) from the x-ray crystallography was downloaded and stored in the form of mol.files for re-docking in order to validate the MVD program, used to docked the brazilin and proto-sappanins A–E (they are phytochemical compounds contained in sappan wood extract). Before the docking process, brazilin and proto-sappanin A–E structures were built with ChemBioDraw Ultra 11.0 and their geometry optimizations were performed using MMFF94 [14]. The phytochemical compounds from sappan wood extract were docked into the active site of PBP2a receptors that have the active site occupied by their native ligand ceftobiprole, as shown in Figure 1.

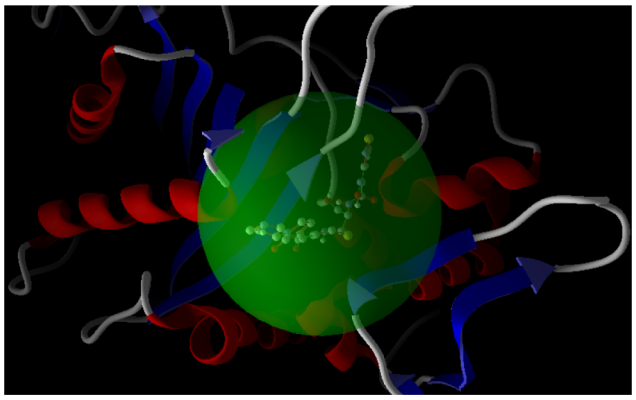
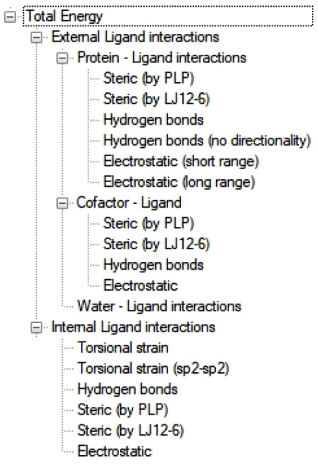


Figure 1: Active site of the PBP2a receptor.

Ligand receptor docking study and visualization of docking results

The docking is carried out with the native ligand, brazilin, and proto-sappanins A–E using MVD version 5.5. The result of Molecular docking study was evaluated using the MolDock score as scores interpreted as predictions of bond interactions between ligand and receptors.

The MolDock score is the total energy from external ligand interaction plus internal ligand interaction. External ligand interaction is the sum of energy consisting of protein-ligand interaction and cofactor-ligand interaction. Internal energy interaction is the sum of energy dependent on the chemical structure of the ligand such as torsional strain, torsional strain sp^2-sp^2, steric, and electrostatic. The MolDock score formula is as shown in Figure 2.



$$E_{score} = E_{inter} + E_{intra}$$

$$E_{inter} = \sum_{i \in ligand} \sum_{j \in protein} \left[E_{PLP}(r_{ij}) + 332.0 \frac{q_i q_j}{4 r_{ij}^2} \right]$$

E_{intra} is the internal energy of the ligand:

$$E_{intra} = \sum_{i \in ligand} \sum_{j \in ligand} E_{PLP}(r_{ij}) + \sum_{flexible\ bonds} A[1 - \cos(m \cdot \theta - \theta_0)] + E_{clash}$$

Figure 2: Formula of the MolDock score.

The lower MolDock score indicates that smaller amount of energy is required for forming the ligand-receptor interaction. The MolDock score interprets all accumulation energy between the ligand-receptor interaction, which includes hydrogen bond, steric interaction, and electronic interaction.

Validation of the docking process

Molecular docking of the drug to be tested was done in order to analyze the stability of the receptor protein. The *in silico* validation was carried out by re-docking the native ligand (ceftobiprole) into its active site. The criterion of acceptance is set with the value of root mean square deviation (RMSD) calculated for the protein's backbone atoms of less than 2.0 Å, as shown in Figure 3.

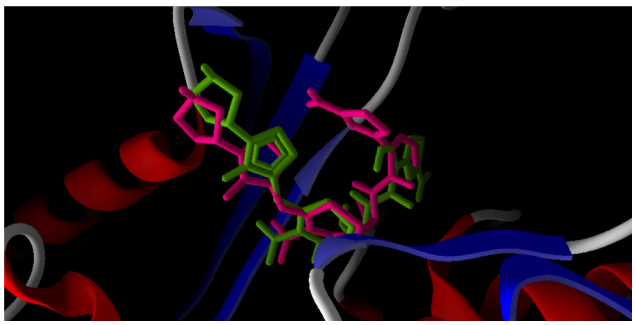


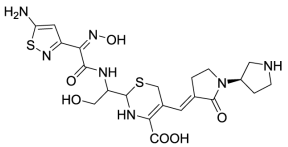
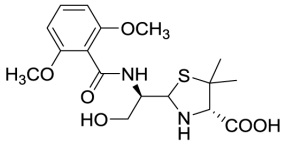
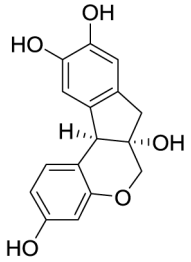
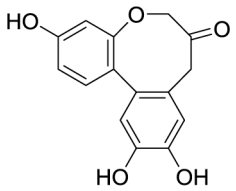
Figure 3: Comparison of native ligand (green) with docking result simulation (pink) by the Molegro Virtual Docker (MVD) software, version 5.5. The RMSD is 1.98 Å.

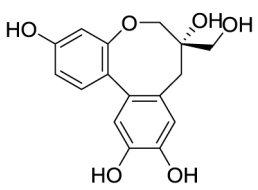
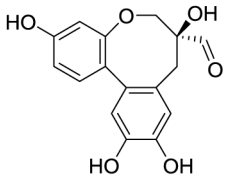
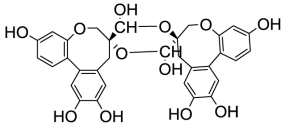
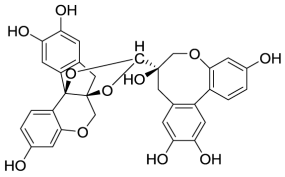
After the re-docking process, the brazilin and proto-sappanins A–E were docked into the active site of the PBP2a receptor. The binding affinity between the ligand and receptor (docking score) was evaluated using the MolDock score, and we also compared the scores of ceftobiprole as its native ligand and methicillin (as a standard microbiological activity against MRSA) with brazilin and proto-sappanins A–E.

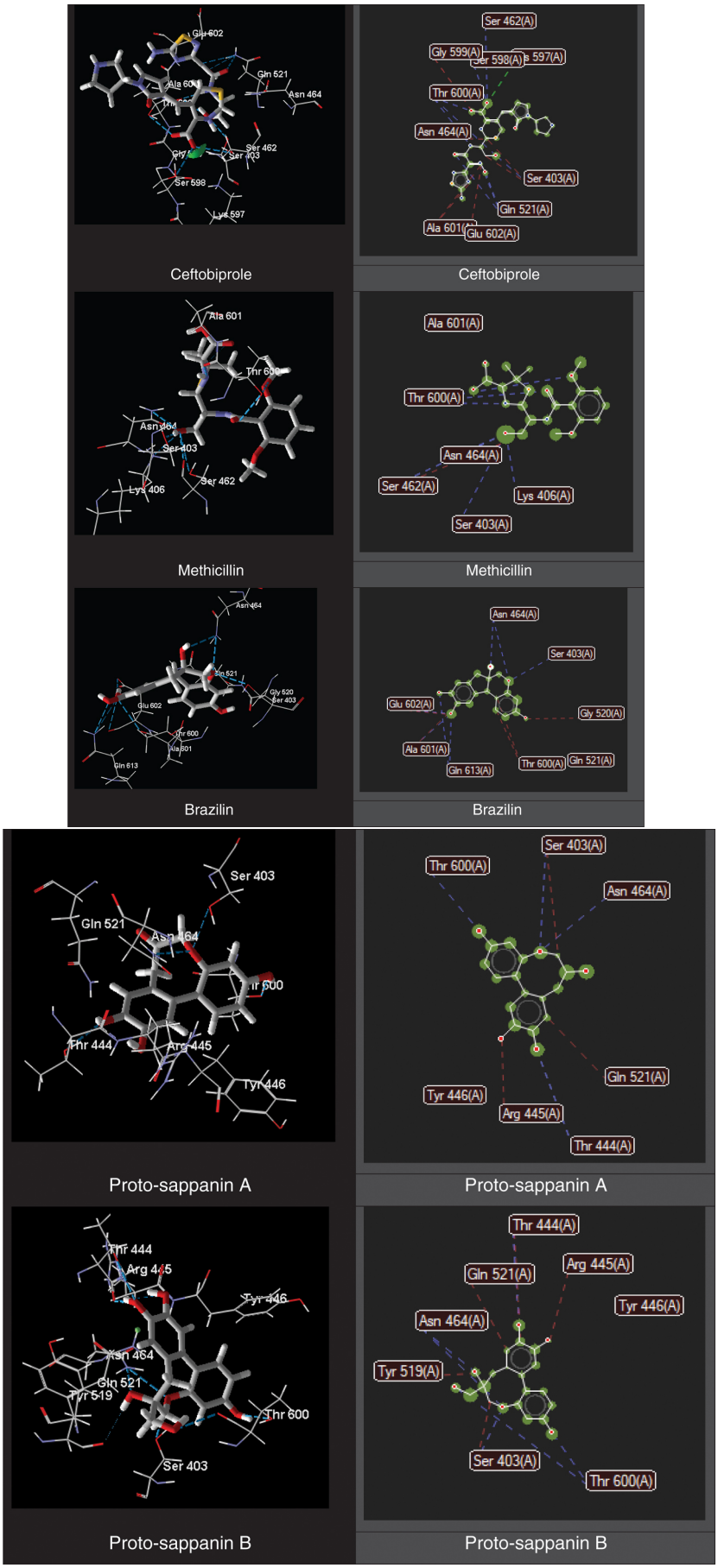
Results

In this research for the molecular docking study, we docked brazilin and proto-sappanins A–E (phytochemical compounds from sappan wood extract) compared with methicillin and ceftobiprole (native ligand from PBP2a). The docking result is shown in Table 1 and Figure 4. The MolDock score of proto-sappanin D (−144.21 kcal/mol) and E (−146.48 kcal/mol) is lower than that of methicillin (−119.50 kcal/mol), but higher than that of ceftobiprole (−171.14 kcal/mol), an anti-MRSA agent; moreover, the MolDock score of proto-sappanins A–C and brazilin is higher than that of methicillin and ceftobiprole. The comparison of the native ligand is shown in Figure 3.

Table 1: Molecular docking result on PBP2a (penicillin-binding proteins)

Compound	MolDock score, kcal/mol	Docked pose	∑ Hydrogen bond, kcal/mol	Amino acids involved	∑ Steric interaction, kcal/mol	Amino acids involved	∑ Electronic interaction, kcal/mol
 Native ligand (ceftobiprole)	−171.14 ± 0.37	√	6	Ser 403 Gln 521 Asn 464 Thr 600 Ser 598 Ser 462	4	Ser 403 Ala 601 Glu 602 Gly 599	Lys 587
 Methicillin	−119.50 ± 1.08	√	5	Ser 403 Lys 406 Ser 462 Asn 464 Thr 600	5	Ser 403 Lys 406 Ser 462 Asn 464 Thr 600 Ala 601	–
 Brazilin	−98.60 ± 0.36	√	5	Ser 403 Asn 464 Ala 601 Glu 602 Gln 613	5	Gly 520 Gln 521 Thr 600 Ala 601 Glu 602	–
 Proto-sappanin A	−92.46 ± 0.37	√	4	Ser 403 Thr 444 Asn 464 Thr 600	4	Ser 403 Arg 445 Try 446 Gln 521	–

 Proto-sappanin B	−99.30 ± 1.15	√	4	Ser 403 Thr 444 Asn 464 Thr 600	5	Ser 403 Arg 445 Try 446 Tyr 519 Gln 521	–
 Proto-sappanin C	−100.55 ± 0.01	√	4	Ser 403 Thr 444 Asn 464 Thr 600	5	Ser 403 Arg 445 Try 446 Tyr 519 Gln 521	–
 Proto-sappanin D	−144.21 ± 0.01	√	8	Ser 403 Ser 462 Lys 587 Ser 598 Asn 464 Glu 447 Ser 461	12	Gly 520 Gln 521 Thr 600 Ala 601 Glu 602 Etc	–
 Proto-sappanin E	−146.48 ± 0.01	√	5	Ser 403 Ser 462 Asn 464 Tyr 519 His 583	9	Ser 403 Thr 600 Asn 464 Ser 462 Tyr 519 Gln 521 Etc	–



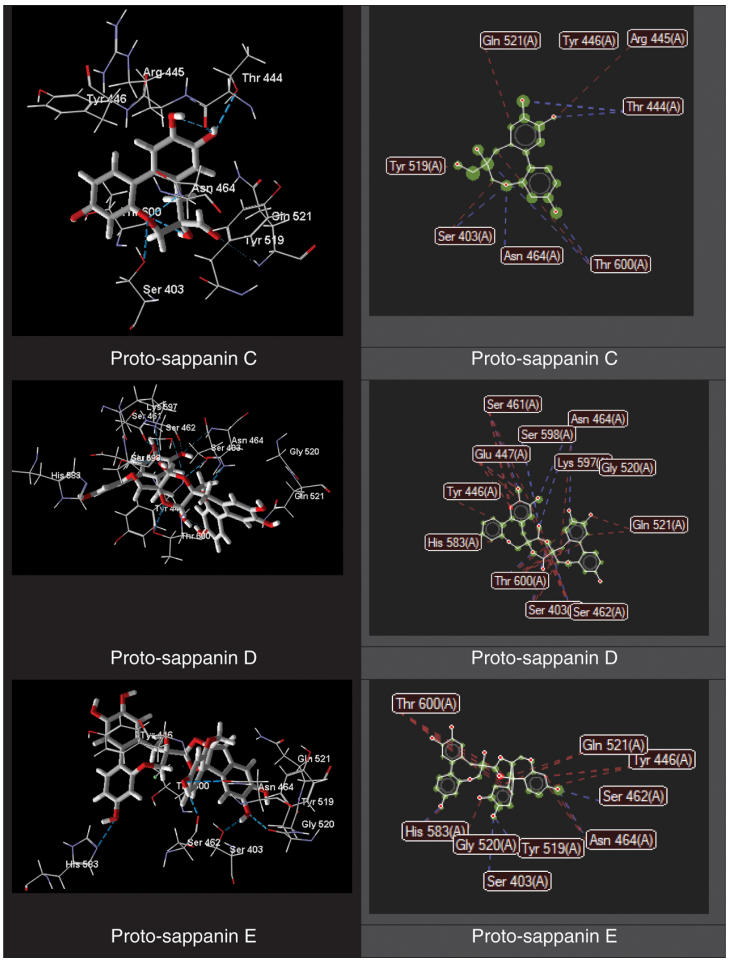


Figure 4: The interaction between its native ligand ceftobiprole, methicillin, brazilin, and proto-sappanins A–E into the active site of PBP2a.

Discussion

Brazilin and proto-sappanins A–E were docked into the active site of PBP2a. This enzyme was chosen because it catalyzes the cell wall of bacteria that had been resistant to methicillin, which we called MRSA. PBP2a is an enzyme transpeptidase that catalyzes bacterial wall processes as shown in Figure 5, especially in gram-positive bacteria, which generally contain peptidoglycan.

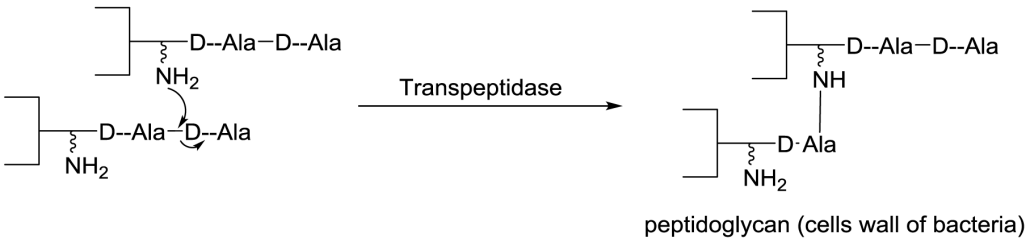


Figure 5: Transpeptidase that catalyzes bacterial wall processes by cross-linking.

PBP2a is highly resistant to methicillin. Methicillin is a standard antibiotic which indicates that bacteria are still sensitive or resistant to the β-lactam antibiotic groups.

Methicillin is not used in medical treatment because of its nephrotoxic side effects, while ceftobiprole is a fifth-generation cephalosporin antibiotic that can be an anti-MRSA agent.

Based on molecular docking result shown in Table 1, we find that ceftobiprole has the lowest MolDock score because of its accumulation energy from hydrogen bond (in amino acid residues such as Ser 403, Gln 521, Asn 464, Thr 600, Ser 598, Ser 462), steric interaction (on amino acid residues Ser 403, Ala 601, Glu 602, Gly 599),

and electronic interaction on Lys 587 residue, which shows that the least energy is needed by ceftobiprole to interact with PBP2a.

Based on the molecular docking data, the most important amino acid residue on the process of opened β-lactam ring is Ser 403. PBP2a inactivation directly depends on covalent bond with serin residue on the process of opened β-lactam ring. Oxocan-3-one ring of proto-sappanins A–C and pyran ring of brazilin have some interactions with Ser 403 residue. The interaction of Proto-sappanins A–C on active site PBP2a enzyme are weaker than methicillin and ceftobiprole because β-lactam ring is strongly reactive and in the molecular level, the mechanism of β-lactam ring is nucleophile attack from hydroxyl functional group from serin (transpeptidase) to carbonyl β-lactam ring followed by SN-acyl reaction and therefore inactivated biosynthesis of peptidoglycan, as shown in Figure 6.

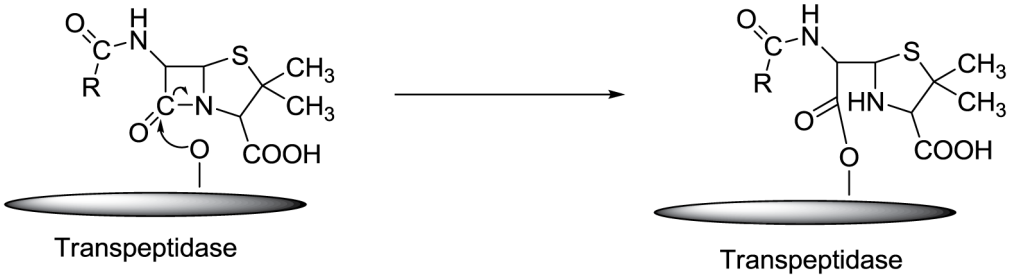


Figure 6: Inactivated transpeptidase by β-lactam ring.

The interaction of proto-sappanins A–C different with proto-sappanins D and E, both have condensated ring between another proto-sappanin or with brazilin. The condensated ring is easier to be the opened ring and interaction with Ser 403 residue than oxocan-3-one and pyran ring; therefore, the MolDock score of proto-sappanins D and E is lower than that of methicillin but higher than that of ceftobiprole. Finally, to prove the proto-sappanin D and E activity against MRSA, it is necessary to perform isolation and *in vitro* antibacterial test against MRSA strains.

Conclusions

Proto-sappanins D and E are predicted to have an activity that inhibits the growth of MRSA better than methicillin but not as well as ceftobiprole.

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Competing interests: The authors state no conflict of interest.

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