European Journal of Medicinal Chemistry 199 (2020) 112312

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Review article

Penicillin binding protein 2a: An overview and a medicinal chemistry perspective

Menna-Allah W. Shalaby [a, 1], Eman M.E. Dokla [a, **, 1], Rabah.A.T. Serya [a], Khaled A.M. Abouzid [a, b, *]

[a] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, 11566, Cairo, Egypt
[b] Department of Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Sadat City, Sadat City, Egypt

ARTICLE INFO

Article history:
Received 19 December 2019
Received in revised form
28 March 2020
Accepted 5 April 2020
Available online 18 April 2020

Keywords:
Antimicrobial resistance
Anti-MRSA
Penicillin binding protein 2a
β-lactams
Enzyme inhibitor
Allosteric control

ABSTRACT

Antimicrobial resistance is an imminent threat worldwide. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the "superbug" family, manifesting resistance through the production of a penicillin binding protein, PBP2a, an enzyme that provides its transpeptidase activity to allow cell wall biosynthesis. PBP2a's low affinity to most β-lactams, confers resistance to MRSA against numerous members of this class of antibiotics. An Achilles' heel of MRSA, PBP2a represents a substantial target to design novel antibiotics to tackle MRSA threat via inhibition of the bacterial cell wall biosynthesis.

In this review we bring into focus the PBP2a enzyme and examine the various aspects related to its role in conferring resistance to MRSA strains. Moreover, we discuss several antibiotics and antimicrobial agents designed to target PBP2a and their therapeutic potential to meet such a grave threat. In conclusion, we consider future perspectives for targeting MRSA infections.

Contents

* Corresponding author. Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, 11566, Cairo, Egypt.
** Corresponding author.
E-mail addresses: emanelawady@pharma.asu.edu.eg (E.M.E. Dokla), khaled.abouzid@pharma.asu.edu.eg (K.A.M. Abouzid).
[1] Both authors have equal contributions.

https://doi.org/10.1016/j.ejmech.2020.112312
0223-5234/

1. Introduction

Antimicrobial resistance is currently considered one of the major health threats worldwide with an alarming increase in infection-related morbidity and mortality rates [1]. One of the major causes of hospital- and community-acquired infections is the methicillin-resistant *Staphylococcus aureus* (MRSA) [2–4]. According to the Centers for Disease Control and Prevention (CDC), MRSA is ranked as a serious threat with more than 80,461 infections and 11,285 deaths per year in USA alone as stated in the antibiotic resistance threats report in 2013 [5]. Several β-lactams – *S. aureus* standard treatment option – have limited use as the microorganism developed various resistance mechanisms rendering these agents ineffective against many *S. aureus* strains including MRSA [6–9]. Production of penicillin binding protein 2a is the major mechanism developed by MRSA to exhibit a broad clinical resistance to the β-lactam antibiotics [10]. MRSA's resistance is mediated through the acquisition of a gene cassette containing *mec*A, which encodes the altered, low-affinity transpeptidase, PBP2a [11]. Consequently, there is an urgent need to develop effective antibiotics to meet the emerging threats of MRSA resistance.

S. aureus is a Gram-positive organism, considered as a serious threat to human health, since it is capable of causing a wide spectrum of infections [12,13]. Patients with severe staphylococcal infections usually had very poor outcomes until the introduction of penicillin in the early 1940s. However, few years later, the first penicillin-resistant strain of *S. aureus* was reported [14]. The first discovered resistance mechanism of *S. aureus* to β-lactams was due to the production of a β-lactamase (penicillinase), an inducible extracellular enzyme released in response to β-lactams exposure, which hydrolyzes the β-lactam ring, yielding inactive derivatives [15]. β-lactamases are encoded by the structural gene *blaZ*, which is controlled by two regulatory genes, *blaI* and *blaR1*. In the presence of penicillin, the sensor protein BlaR1 triggers a signaling cascade leading to the cleavage of the

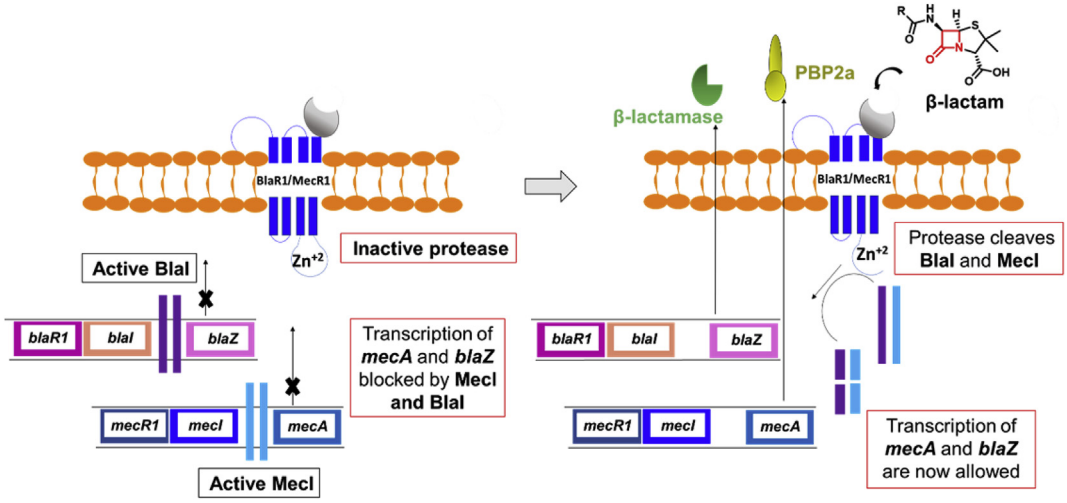


Fig. 1. β-lactamase and PBP2a expression regulation. In absence of β-lactams (left), the transcriptional repressors BlaI and MecI exist as dimers and block the transcription of *blaz* and *mecA*. In the presence of β-lactams (right), BlaR1 and MecR1 are acylated by the antibiotics, leading to the activation of a zinc protease that cleaves BlaI and MecI, leading to the transcription of *blaz* and *mecA* and the production of β-lactamase (encoded by *blaZ*) and PBP2a (encoded by *mecA*).

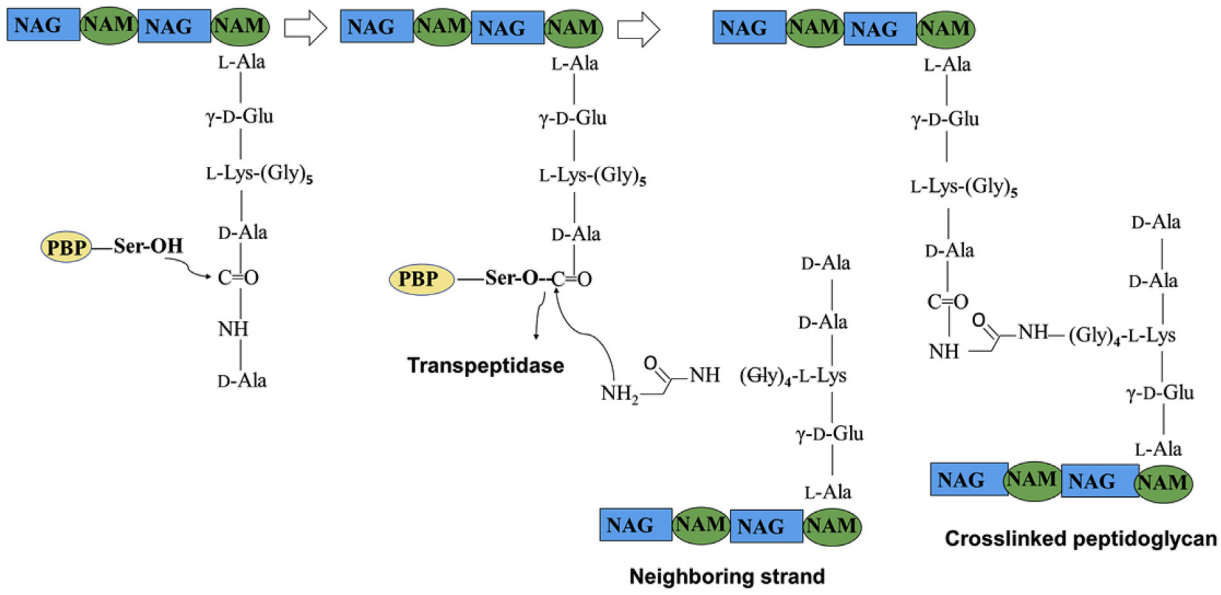


Fig. 2. Crosslinking of peptidoglycan strands stage of cell-wall synthesis.

transcriptional repressor BlaI and allows the production of β-lactamase at a high level [16]. To address this resistance mechanism, semisynthetic β-lactamase-resistant penicillins, such as methicillin and oxacillin, were developed during the early 1960s [17].

Soon after, several resistant strains were isolated and through the following years, there has been an increasing occurrence of various *S. aureus* strains that are resistant to multiple antibiotics, notably the methicillin-resistant *S. aureus* (MRSA) [13,18]. Methicillin resistance was conferred by the acquisition of a mobile genetic element called staphylococcal chromosomal cassette *mec* (SCC*mec*) [19–21]. This genetic element carries the *mecA* gene, which encodes a surrogate penicillin-binding protein (PBP2a), and the regulatory genes, *mecR1* and *mecI*. Upon exposure to β-lactam antibiotics, MecR1 cleaves MecI, thereby, disrupting its binding to the *mecA* promoter and allowing the

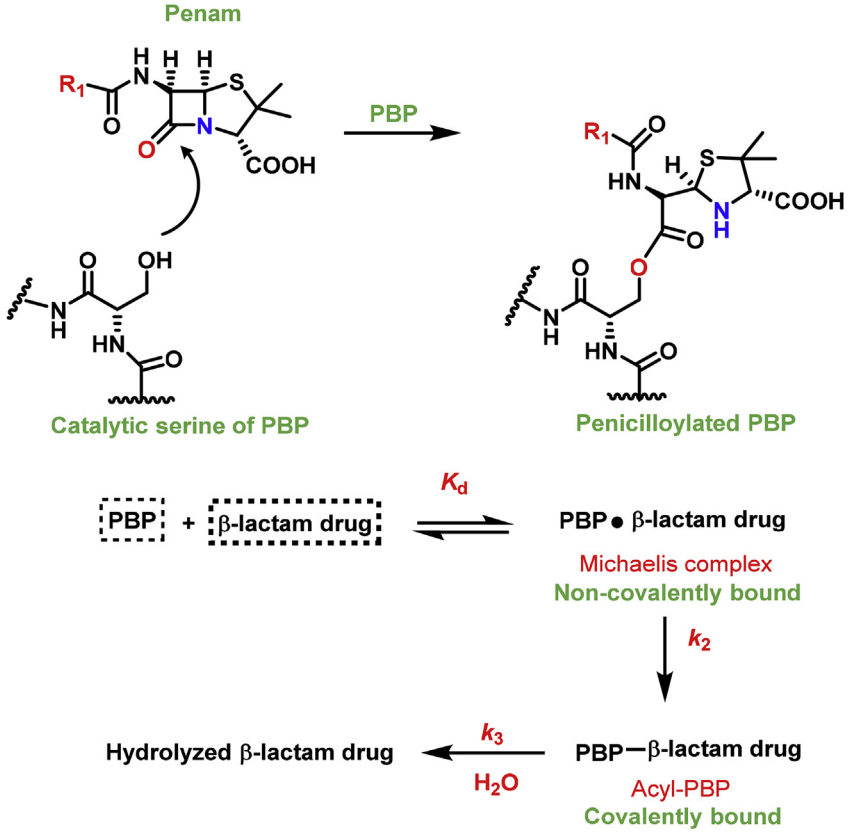


Fig. 3. PBPs inhibition by β-lactam antibiotics. K_d: dissociation constant of the Michaelis complex. k_2: rate constant for the formation of the acyl-PBP complex. k_3: rate constant for the hydrolysis of the acyl-PBP complex.

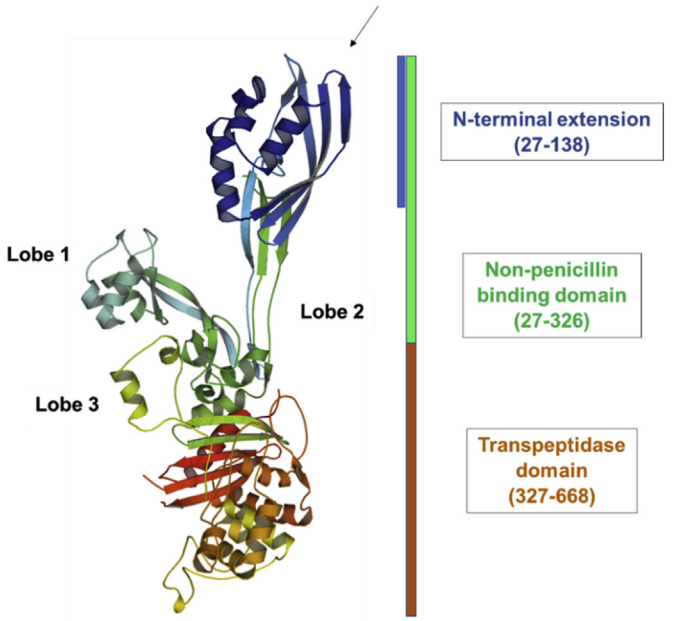


Fig. 4. Ribbon representation of the x-ray crystal structure of PBP2a (PDB 1VQQ). Displayed are the nPB domain (green), the N-terminal lobe (blue), and the transpeptidase domain (brown). The arrow indicates the point of attachment of the membrane anchor. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

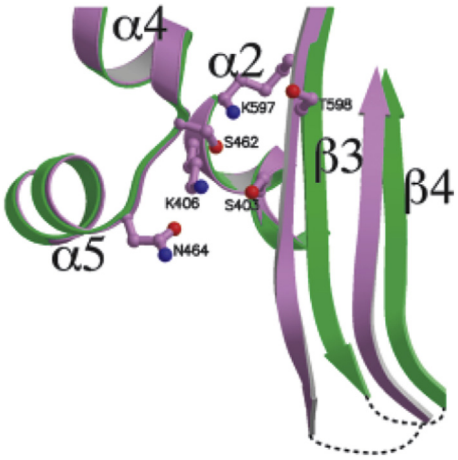


Fig. 5. The active site of the apo form of *S. aureus* PBP2a (colored in purple) superimposed on the complexed form of PBP2a (colored in green). The active site distortion and the conformational change of strand β3 are the main contributors to its reduced susceptibility toward traditional β-lactam antibiotics [46]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

production of PBP2a [13]. This penicillin-binding protein maintains the transpeptidase activity as the intrinsic set of PBPs (PBP 1 to 4) of *S. aureus*, but it differs by having a low affinity to many β-lactam antibiotics. Therefore, cell wall synthesis proceeds despite the presence of otherwise inhibitory concentrations of β-lactam antibiotics, thus evading cell death and lysis [22].

In summary, *S. aureus* has been found to resist β-lactams by two main mechanisms, using β-lactamases to inactivate the antibiotic and more critically through the employment of a low affinity PBP2a (Fig. 1).

2. Bacterial cell-wall biosynthesis and the role of penicillin-binding proteins

Cell wall biosynthesis pathway is one of the oldest bacterial targets exploited for antibiotic treatment, yet it remains an attractive aim for developing new antimicrobial agents. Bacterial cell wall synthesis is a multi-component process that comprises mainly the peptidoglycan biosynthesis, which is responsible for cell shape and integrity [23].

2.1. Cell wall biosynthesis

The peptidoglycan is composed of glycan chains of alternating N-acetylglucosamine and N-acetylmuramic acid cross-linked by short stem peptides attached to the N-acetylmuramic acid [24]. Peptidoglycan biosynthesis take place in three stages as follows: [25]

1 **Cytoplasmic stage**: Precursors as UDP-*N*-acetylmuramyl-pentapeptide (UDP-NAM) and UDP-*N*-acetylglucosamine (UDP-NAG) are synthesized in the cytoplasm of *S. aureus.*
2 **Membrane stage (transfer and elongation)**: Those precursors are transferred from the cytosol to the membrane and incorporated into the growing peptidoglycan.
3 **Extracellular stage (crosslinking)**: Synthesis of the peptidoglycan backbone is carried out by bifunctional PBPs, where the transglycosylase domain produce glycan strands, while the transpeptidase domain perform the crosslinking reaction. As illustrated in Fig. 2, NAM of murein monomers is connected to NAG by PBP transglycosylase (chain extension) and the terminal glycine is connected to the second D-alanine of another chain, with the consequent release of the terminal D-alanine by PBP transpeptidase (chain crosslinking) [26].

2.2. Penicillin-binding proteins: the key players in bacterial cell wall synthesis

2.2.1. Types of PBPs

PBPs comprise two classes, the high molecular mass (HMM) PBPs and the low molecular mass (LMM) PBPs [27]. HMM PBPs are subdivided into classes A and B, according to the number of reactions they can catalyze. Class A PBPs are bifunctional enzymes, as they have a C-terminal domain that catalyzes the transpeptidation (TP) reactions and an N-terminal domain that catalyzes transglycosylation (TG) reactions. As for class B PBPs, they are monofunctional enzymes responsible for transpeptidation reactions only, as they possess a catalytic C-terminal transpeptidation domain with an N-terminal domain that usually contributes to other non-penicillin-binding functions [28]. LMM PBPs are implicated in the regulation of the level of peptidoglycan crosslinking as they catalyze D,D-carboxypeptidation trimming reactions preventing further peptidoglycan crosslinking [29].

2.2.2. PBPs physiological function

The TP domain of PBPs comprises a five stranded β-sheet bounded by three α-helices subdomain and an all-helical subdomain, where the active site is positioned at their interface. The TP active site comprises three specific motifs: SXXK (catalytic serine and lysine), (S/Y)XN and (K/H)(S/T)G [27,30].

Different PBPs catalyze three main reactions: D-alanine carboxypeptidation, peptidoglycan transpeptidation, and peptidoglycan endopeptidation. Carboxypeptidation and transpeptidation reactions occur in three consecutive steps, first, a reversible non-covalent Michaelis complex between the muramyl peptide (donor

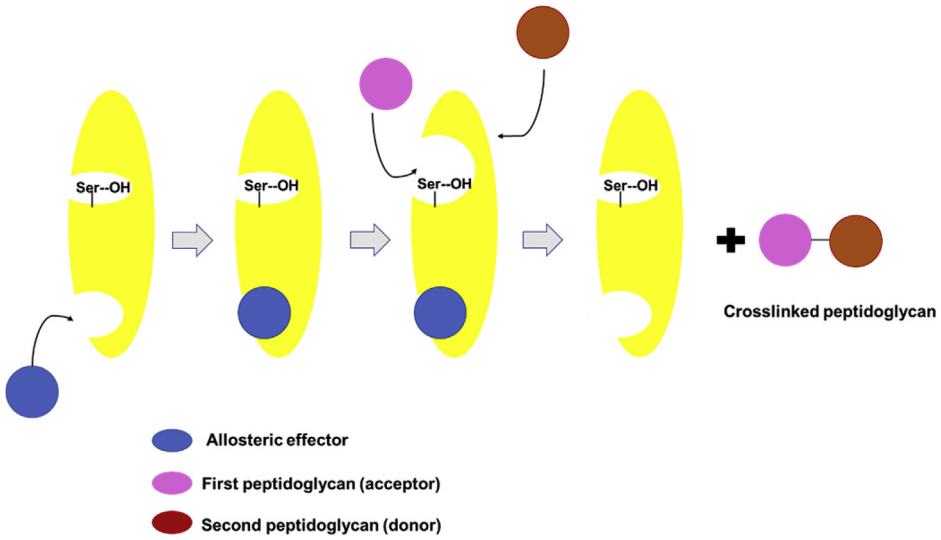


Fig. 6. Diagrammatic representation of the allosteric activation of PBP2a.

strand) and the enzyme is formed. Next, the catalytic serine attacks the carbonyl of the second D-alanine of the stem pentapeptide, forming a covalent acyl–enzyme complex with concomitant release of the terminal D-alanine residue. Finally, this complex can be deacylated to give a shortened peptidoglycan strand (carboxypeptidation) or it can be cross-linked with an amino group of a second strand to form a new peptide bond (transpeptidation) (Fig. 2) [31]. Endopeptidation reaction of PBPs results in the hydrolysis of the cross-bridge between glycan strands in a reverse activity to transpeptidation [30].

Penicillin-binding proteins represent the molecular targets for the β-lactam antibiotics [27]. Inhibition of the PBPs TP enzymatic activity is attributed to the structural mimicry between penicillins and the D-Ala-D-Ala dipeptide substrate, as proposed by the Tipper-Strominger hypothesis [32]. The catalytic serine attacks the β-lactam ring carbonyl group, resulting in ring opening and the formation of a stable covalent acyl-enzyme complex [33]. This complex is hydrolyzed very slowly, thus the enzyme is no longer available for its normal function (Fig. 3). As a result, transpeptidation is impaired leading to peptidoglycan cross-linking failure and subsequent bacterial death [34,35].

3. Understanding PBP2a resistance

The expression of PBP2a is the basis for the high-level resistance to the β-lactam antibiotics by MRSA. However, this resistance can be exhibited only in the presence of other PBPs as PBP2a catalyzes the transpeptidation reactions only in the cell wall biosynthesis process. Therefore, the co-functioning of the native class A PBP2 transglycosylase domain is required for successful peptidoglycan synthesis [36]. Additionally, the presence of the native class B PBP1 is also needed, even though the transpeptidation can be carried out by PBP2a, which suggests a different role for PBP1 in cell division apart from its involvement in the cross-linking of peptidoglycan [37]. Furthermore, *fem* (factor essential for methicillin resistance) and *aux* (auxiliary) factors contribute to the methicillin resistance of PBP2a [38,39]. For example, it is postulated that PBP2a can only cross-link peptidoglycan strands bearing penta- and triglycine, but not monoglycine. The presence of FemA enzyme that extends the monoglycine branch to triglycine on Lipid II using glycyl-charged tRNA molecules, is very important for PBP2a in order to crosslink the cell wall and exhibit resistance to β-lactam antibiotics [40,41].

3.1. Structural information on PBP2a

The first crystal structure of a soluble form of PBP2a (PDB ID 1VQQ) was published by Lim and Strynadka in 2002. The enzyme is displayed (Fig. 4) as an elongated protein comprising a C-terminal transpeptidase domain (residues 327–668) and a non-penicillin-binding domain (nPB) (residues 27–326), which includes an N-terminal extension subdomain (residues 27–138) [42]. The full-length protein also contains a transmembrane anchor segment (residues 1–23), which can be removed without affecting the β-lactams binding kinetics [43,44].

It was proposed that the nPB domain allows access to the transpeptidase domain from the cell membrane [42]. Subsequent analysis of a PBP2a complex with ceftaroline identified an allosteric site at the intersection of Lobe 1 (residues 166–240), Lobe 2 (residues 258–277), Lobe 3 (residues 364–390), and the top of the N-terminal extension domain. Binding to this allosteric site leads to several conformational changes that end up in the opening of the active site to permit the substrate entry and subsequent activation (cross-linking of the cell wall) or inhibition. Apart from PBP2a, most PBPs X-ray structures lack an allosteric domain indicating for the absence of an allosteric control [45].

The transpeptidase active site of PBP2a shares similar structural folds with the other transpeptidases, however its affinity to β-lactams differs owing to a closed active site conformation that requires unfavorable movement of β3 sheet to access the catalytic Ser403 (Fig. 5) [42].

3.2. Structural and kinetic resistance to β-lactam antibiotics by PBP2a

The interaction of a PBP with a β-lactam antibiotic starts by the rapid reversible formation of a noncovalent Michaelis complex (dissociation constant K_d). Next, Ser403 attacks the β-lactam ring to give a covalent acyl-PBP intermediate (rate constant k_2). Finally, deacylation by water attack on the acyl-PBP bond allow release of the product (rate constant k_3) (Fig. 3) [47]. Kinetically speaking, effective β-lactams should exhibit high acylation efficiency (k_2/K_d ratio), and low deacylation rate constants (k_3 values), hence the acyl-PBP complex is irreversibly inhibited [48]. In contrast, β-lactams interacts with PBP2a very slowly, primarily due to the decreased formation of the acyl-PBP intermediates (slow k_2), and

increased reversible pre-acylation complexes formation with the antibiotics (high K_d), indicating the low affinity of the enzyme for β-lactams in general [45,49].

Low rates of acyl-PBP formation is attributed to the inability of β-lactams to interact with the buried active-site Ser403, where PBP2a must undergo energetically-costing conformational changes at both strand β_3 and helix α_2 N-terminus to allow β-lactams access to Ser403, as illustrated in (Fig. 5) [42,45,50,51]. Additionally, the increased dissociation constant (K_d) for Michaelis complex is ascribed to the closed nature of the PBP2a active site pocket, compelled by the protein loops (α_2-α_3 and β_3-β_4 loops) position and the juxtaposed side chains of Met641 and Tyr446 residues that act as gatekeepers, hence β-lactams can't access the active site [51]. On the other hand, β-lactam-susceptible PBPs experience no such differences between the apo and the acyl-PBP structures, allowing smooth transition from the reversible to the irreversible acyl complex resulting in high affinity [52,53].

3.3. Allosteric control of PBP2a physiological function

The crystal structure of the apo PBP2a shows a closed active site conformation that enables the enzyme to resist the β-lactam antibiotics, while maintaining its transpeptidase activity (Fig. 5). This tight active site prevents access of the β-lactams to the catalytic Ser403 however it allows the binding of peptidoglycan substrate to achieve the crosslinking reaction [42]. The ability of PBP2a to accommodate

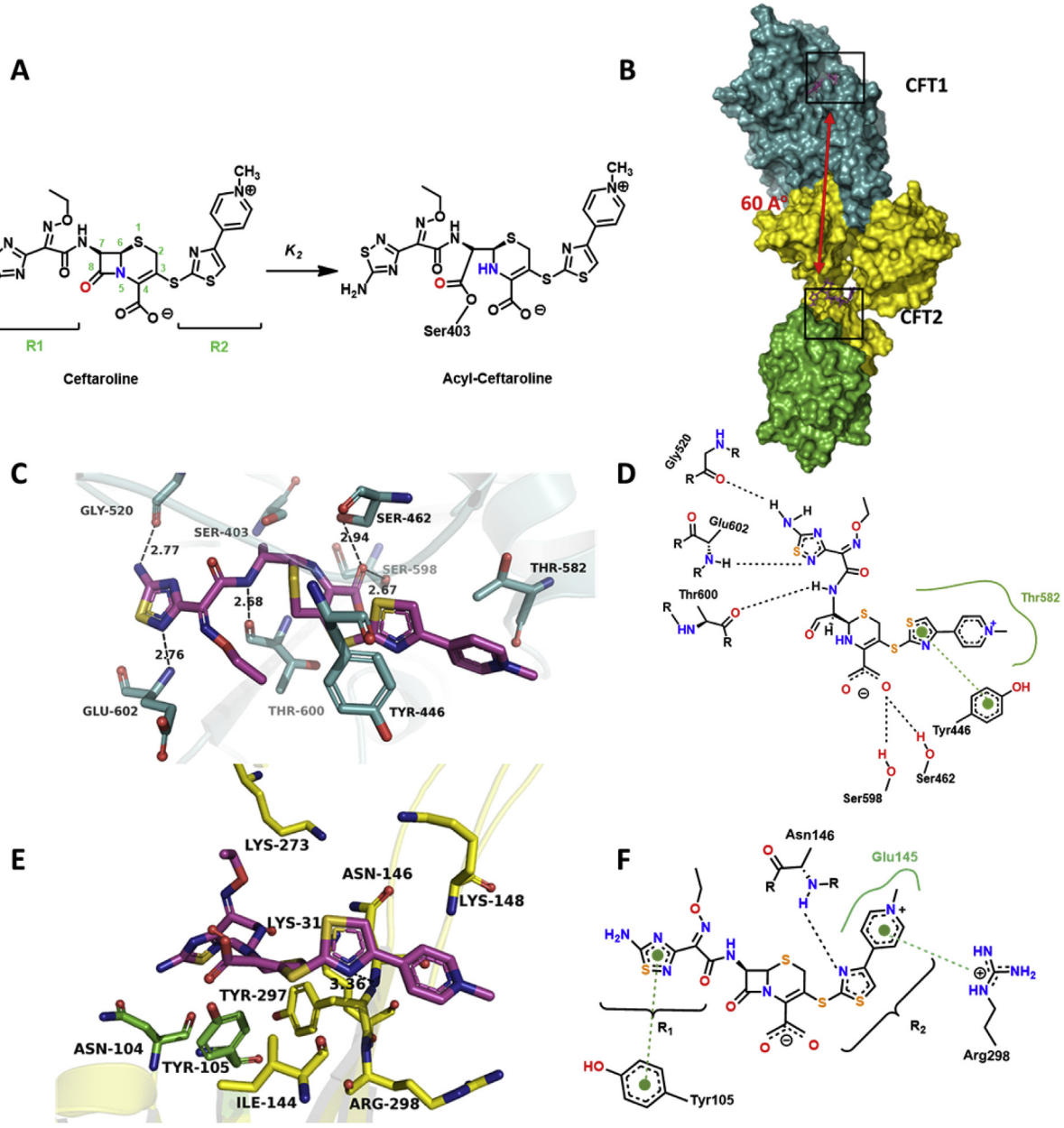


Fig. 7. Inhibition of the *S. aureus* PBP2a by ceftaroline. (A) Ceftaroline acylation by the exposed Ser403 at PBP2a active site. **(B)** X-ray crystal structure (PDB ID: 3ZFZ) of PBP2a bound to two ceftaroline molecules at the active site (CFT1) and the allosteric site (CFT2), the N-terminal extension, allosteric domain, and TPase domain are colored green, yellow, and cyan. **(C)**, **(D)** 3D and 2D views of ceftaroline (CFT1) respectively within the active site of PBP2a displaying the key amino acids involved in the interaction. Ceftaroline backbone is colored in magenta, while the hydrogen-bonding interactions are depicted as black dashes. **(E)**, **(F)** 3D and 2D views of ceftaroline (CFT2) respectively within the allosteric site of PBP2a displaying the key amino acids involved in the interaction. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

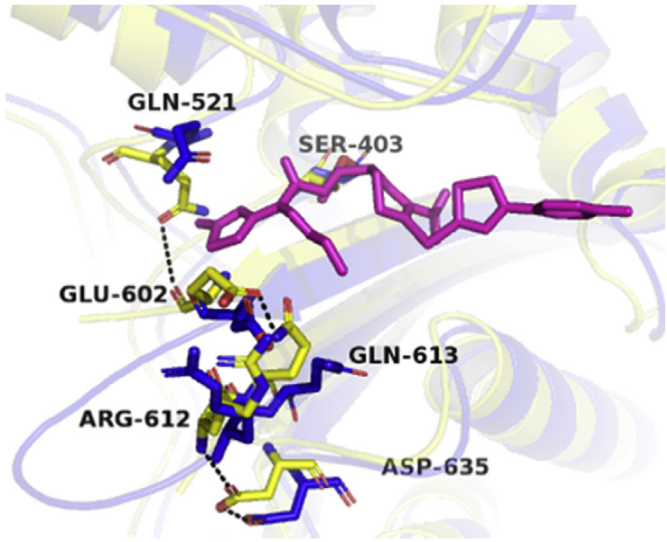


Fig. 8. Superimposition of the active sites of the unbound (PDB ID: 1VQQ**) and ceftaroline bound *S. aureus* PBP2a (PDB:** 3ZG0**).** The bound and unbound protein backbones are displayed in blue and yellow respectively. Ceftaroline is shown in magenta sticks and salt-bridge interactions are shown as black dashes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

two peptidoglycan strands using an open active site conformation (requires a volume larger than 1000 $Å^3$) [54] and the bias of the open versus closed conformations toward the peptidoglycan substrate was soon explained by allostery. Binding of a peptidoglycan chain (or allosteric ligand) to the allosteric binding site leads to several conformational changes resulting in expanding the active site volume to 1300 $Å^3$, allowing the accommodation of the other peptidoglycan chain [55]. While the allosteric site is located ~60 A° from the active site, it still can affect its conformation through several salt-bridge interactions that propagate sequentially to the catalytic site. Finally, these conformational changes expose the catalytic Ser403 residue and allow catalysis [45,51].

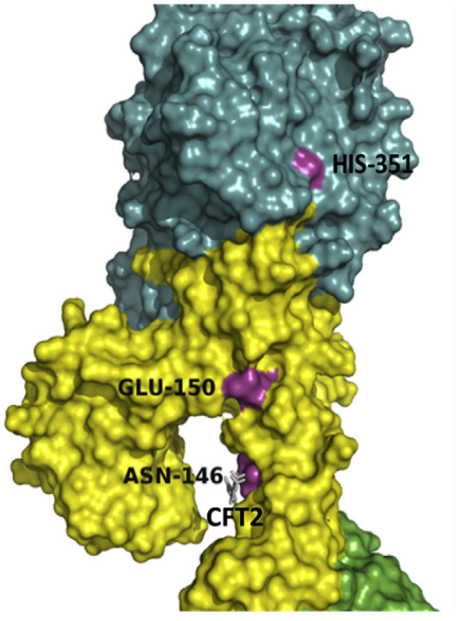


Fig. 9. Allosteric site mutations confer a low-level of ceftaroline resistance to the mutant MRSA strains. The locations of the point mutations are indicated in magenta and the allosteric ceftaroline (CFT2) is depicted as grey-capped sticks. The N-terminal extension is colored in green, the allosteric domain is colored in gold, and the transpeptidase domain is colored in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The allosteric control of PBP2a was proved by various experiments using different allosteric effectors such as synthetic peptidoglycan and β-lactam antibiotics (e.g. ceftaroline) as mimetics of the peptide stem of the peptidoglycan [45,51,56,57].

According to the allosteric regulation concept, ceftaroline was successful in tackling MRSA by binding noncovalently to the PBP2a allosteric site and covalently to the active site (Fig. 6) [45]. Based on these premises, PBP2a allosteric site targeting by novel antibiotics or small molecule can circumvent the resistance of MRSA.

4. Targeting PBP2a

As previously discussed, PBP2a constitutes the major mechanism by which MRSA exhibits its resistance to most β-lactam antibiotics. PBP2a can be targeted at either its active site, allosteric site or both, hereby, proposing an attractive approach to design novel antibacterials to target MRSA infections. In this section, we will provide a thorough discussion of the numerous reported inhibitors, their activity scope, mode of action, and developed resistance, to demonstrate the validity of this target and aid future design of new antimicrobials.

4.1. β-lactam antibiotics active against PBP2a

4.1.1. Ceftaroline

Ceftaroline is a fifth-generation cephalosporin that was approved by FDA in 2010, with a wide-spectrum activity against Gram-positive bacteria, particularly, several multidrug resistant strains [58–61].

4.1.1.1. Mechanism of inhibition of PBP2a. Ceftaroline exerts its action through the covalent binding to the active site of penicillin-binding proteins, thus inhibiting cell wall crosslinking (Fig. 7A). It has a high affinity for the native PBPs of *S. aureus* (PBP1-3) with an IC_{50} values below 0.1 μg/ml and for the mutant PBP2a with an IC_{50} value of 0.16 μg/ml, however, it shows lower affinity for PBP4 (IC_{50} > 1 μg/ml) [62,63]. This affinity translates into favorable low MICs (0.25–1 μg/mL) against several MRSA strains [58,63,64].

As discussed before, PBP2a is resistant to most β-lactam antibiotics, due to its closed active site conformation that places the catalytic Ser403 in a tight narrow channel inaccessible by these antibiotics. Nevertheless, ceftaroline could overcome this resistance owing to its ability to bind to the allosteric site in a non-covalent manner. Ceftaroline interactions with the allosteric site lead to several conformational changes that culminate into the opening of the active site. Consequently, a second molecule of ceftaroline can access the active site and acylate the now-exposed Ser403 leading to PBP2a inhibition and cell wall synthesis blocking [45].

This unique ability of ceftaroline is a result of its side chain that mimics the muropeptide chain of peptidoglycans, thus enabling it to interact with the allosteric site and conformationally open the active site in a similar manner to the native peptidoglycan substrate [58]. This mechanism was confirmed by analyzing the crystal structure of PBP2a in complex with ceftaroline (PDB 3ZFZ, Fig. 7B). This structure reveals two ceftaroline molecules crystallized with PBP2a, where the first molecule is non-covalently bound in the allosteric site, while the second one acylates the active site serine [45].

4.1.1.2. Ceftaroline binding mode within the active and the allosteric sites of PBP2a. Within the active site, the ceftaroline acyl-PBP2a complex displays the following interaction features responsible for the high affinity of PBP2a for the ceftaroline antibiotic in

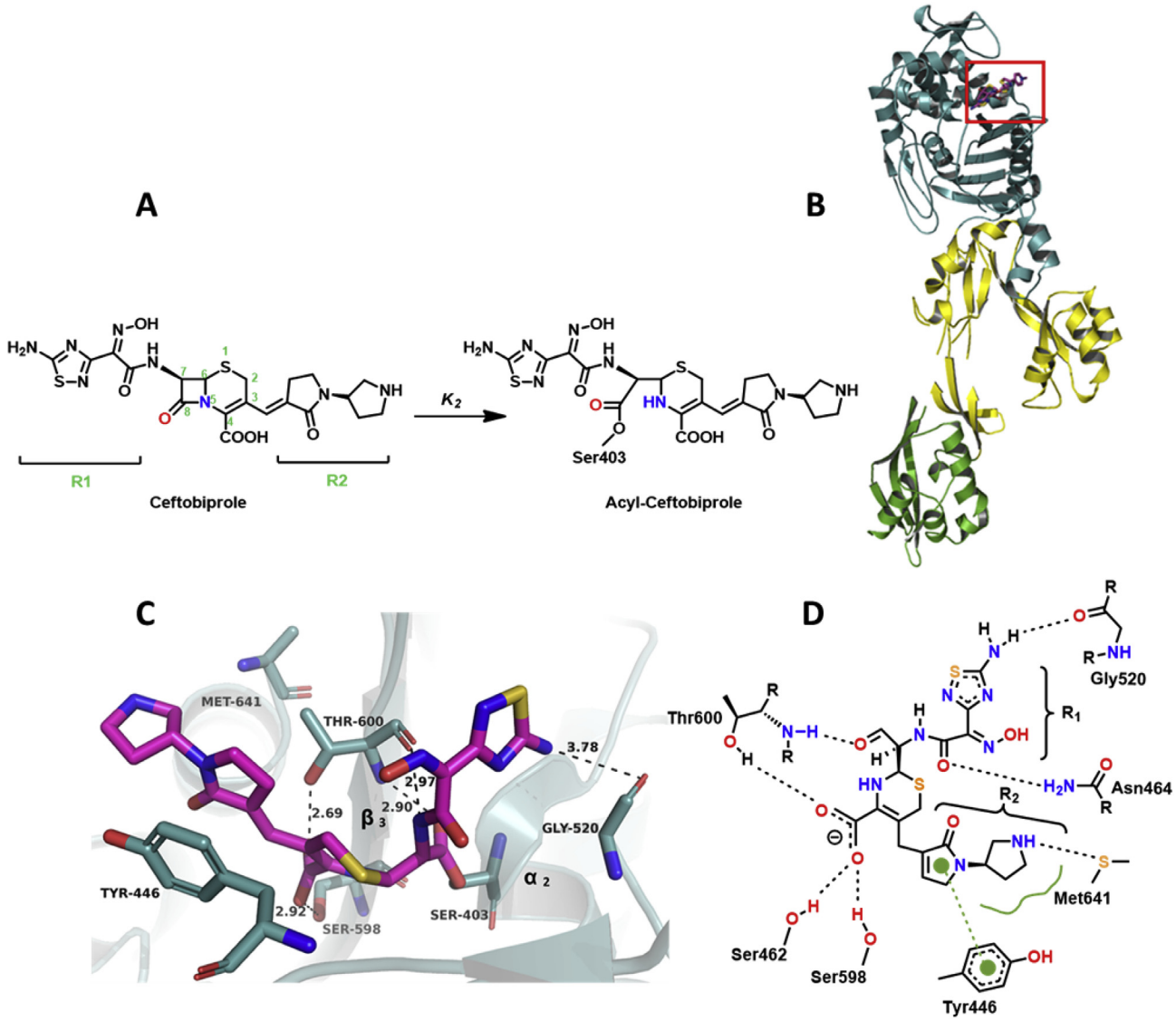


Fig. 10. Inhibition of the *S. aureus* PBP2a by ceftobiprole. **(A)** Ceftobiprole acylation by Ser403 at PBP2a active site. **(B)** X-ray crystal structure (PDB ID: 4DKI) of PBP2a bound to a ceftobiprole molecule at the active site, the N-terminal extension, allosteric domain, and TPase domain are colored green, yellow, and cyan. **(C)**, **(D)** 3D and 2D views of ceftobiprole respectively within the active site of PBP2a displaying the key amino acids involved in the interaction. Ceftobiprole backbone is colored in magenta, while the hydrogen-bonding interactions are depicted as black dashes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

addition to the covalent bond formed with the catalytic Ser403 (Fig. 7C, **D**).

[1] Ceftaroline's R2 group is sandwiched between Tyr446 on one side and Thr582 on the other side. A pi-pi interaction is formed between Tyr446 and the 1,3-thiazole ring.
[2] The carboxylate group forms two hydrogen bonds with both Ser462 and Ser598 hydroxyl groups.
[3] The amide nitrogen at 7-position of the cephem scaffold forms a hydrogen bond with the backbone carbonyl of Thr600.
[4] R1 group resides in a pocket formed by Gly520, Gln521, and Glu602 amino acids, where the N2 of the 1,2,4-thiadiazole ring is hydrogen bonded to Glu602, whereas, the amino group attached to the 3rd position of the 1,2,4-thiadiazole ring is hydrogen bonded to Gly520.

At the allosteric site, ceftaroline exhibits a non-covalent binding mode and demonstrates the following interaction features (Fig. 7E, **F**).

[1] R2 group is positioned between Arg298, Glu145, and Asn146 amino acids, where the N-methyl pyridine ring forms a pi-cation interaction with Arg298 and the N3 of the 1,3-thiazole ring forms a hydrogen bond with the backbone NH of Asn146.
[2] 1,2,4-thiadiazole ring exhibits a pi-pi interaction with Tyr105.

The additional interactions imparted by the R2 group of ceftaroline (while lacking in other β-lactams), are predicted to contribute to the increased binding affinity of ceftaroline to the allosteric binding site of PBP2a (Fig. 8) [63]. Other β-lactams (e.g. oxacillin, cefepime, ceftazidime) have weak affinity for PBP2a as they partially occupy the allosteric site. So, they are incapable of inducing the required conformational changes for the complete opening of the active site. This observation was supported by the findings of molecular dynamics (MD) simulation of the closed conformation of apo-PBP2a that showed only partial β3-β4 motion, and thus the active site remained closed hindering the acylation reaction [51]. In conclusion, optimum occupancy of the allosteric site in a proper way is required to provide the needed protein reorganization to open the active site.

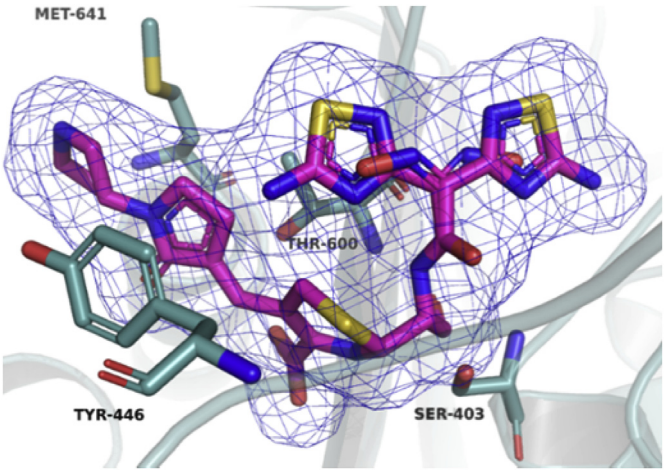


Fig. 11. View of ceftobiprole within the active site of PBP2a showing the R1 group in both conformations (PDB ID: 4DKI).

4.1.1.3. Resistance. Shortly, after the introduction of ceftaroline to the market in 2010, several cases of MRSA strains with various levels of resistance were reported [65–69]. Mutations in the transpeptidase active site lead to high-level resistance while mutations remote from the active site (e.g. allosteric site) lead to low-level resistance to ceftaroline. Three clinically isolated ceftaroline-resistant MRSA strains from a patient with high-level resistance (MIC > 32 μg/ml) demonstrated mutations in two amino acids (Y446N and E447K) in the transpeptidase active site. These mutations replace tyrosine (Y446) with asparagine (bulky phenol in place of a small amide group), and glutamic acid (E447) with lysine (switching a negatively charged amino acid with a positively charged one) [66]. Considering that these two amino acids are positioned in the penicillin-binding pocket [42], such replacement can affect ceftaroline binding to the active site drastically, leading to a high-level of resistance.

A low-level of ceftaroline resistance results from an uncommon mechanism, where point mutations of certain amino acids disrupt the allosteric control of the active site, thus diminish its responsiveness to ceftaroline (MIC = 2–4 μg/ml) [65,67]. Moreover, such resistance can result from a single point mutation of the active site (Y446N or E447K alone) [66]. Two clinical variants, ST228 (N146K and E150K mutations) and ST247 (N146K, E150K, and H351N mutations), manifested low-level resistance to ceftaroline [70]. Two of these mutations (N146K and E150K) are situated in the allosteric site, while the third one (H351N) is positioned outside both sites (Fig. 9). Allosteric site mutations impart resistance to ceftaroline through two possible mechanisms, first, they can impede the ability of ceftaroline to bind to the allosteric binding site (indicated by an increased dissociation constant K_d), second, they alter the conformational changes resulting from binding to the allosteric site, hence suppressing the active site opening [56].

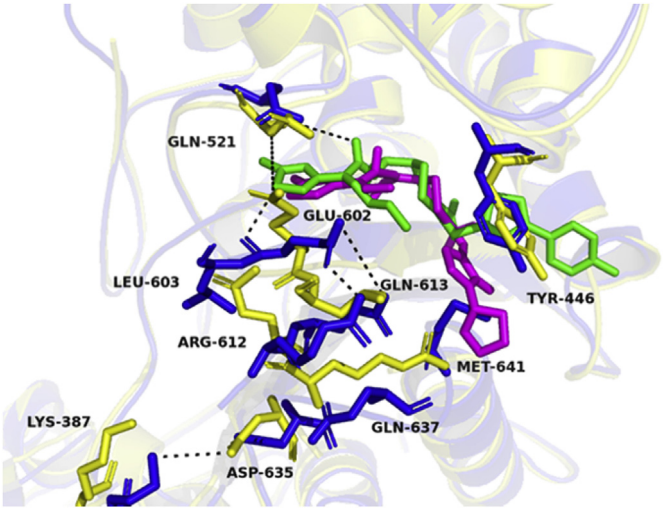


Fig. 12. Comparison of the binding mode and interactions of ceftaroline (green, PDB: 3ZG0**) and ceftobiprole (magenta, PDB:** 4DKI**) within the PBP2a active site.** Amino acids involved in the interactions are displayed in blue (ceftaroline) and yellow (ceftobiprole) and polar interactions are shown as dashed lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.1.2. Ceftobiprole

Ceftobiprole is the second fifth-generation cephalosporin, first approved in Canada in 2008 for the treatment of complicated skin and soft tissue infections. Although it was approved in several European countries, it is not approved by the FDA for use in the United States. It exhibits a very broad spectrum of activity against Gram-positive and Gram-negative pathogens, particularly, against MRSA strains [71–77].

4.1.2.1. Mechanism of action. Ceftobiprole is a β-lactam antibiotic (Fig. 10A, **B**) that exhibits a potent bactericidal activity by binding to PBPs, thus inhibiting transpeptidation and suppressing the cell wall synthesis. It is a good inhibitor for all native PBPs of *S. aureus* (IC_{50} < 1 μg/ml) and for the mutant PBP2a ($IC_{50} \leq$ 0.47 μg/ml) [78]. Such activity is reflected in low MIC values (0.5–4 μg/ml) against MRSA [71,78,79]. Ceftobiprole activity is attributed to the increased hydrophobic interaction of the R2 substituent with the PBP2a active site, which results in an improvement in the binding affinity (decreased K_d) and a subsequent greater overall acylation efficiency (k_2/K_d). In contrast, neither penicillin nor methicillin demonstrate such interactions. The increased stabilization and prevalence of the Michaelis complex compensate for the energetic cost of conformational rearrangement needed to reach the buried active-site Ser403, thus enabling ceftobiprole to irreversibly inhibit PBP2a [42,50].

Ceftobiprole is a cephalosporin with an oxyimino aminothiadiazolyl substituent (R1 group) connected to the 7-amino group of the cephem nucleus, which protects the molecule from hydrolysis by many lactamases. The 1,3′-bipyrrolidin-2-one moiety (R2 group) located at the 3-position of the cephem nucleus sits deep in the narrow cavity of the PBP2a binding site making it closer to Tyr-446, Met-641, and Thr-600 and favoring the acylation of PBP2a (Fig. 10C, **D**). In addition, the planarity of R2 makes it more similar to the pentaglycine cross-bridge [50]. In conclusion, the higher acylation rate, the increased intrinsic affinity, and the lower deacylation rate are the three main contributors that work in concert to make the ceftobiprole a potent inhibitor of the active site of the PBP2a [80].

4.1.2.2. Ceftobiprole binding mode within the active site of PBP2a. Ceftobiprole acyl-PBP2a complex displays the following interaction features, in addition to the covalent bond formed with the catalytic Ser403 (Fig. 10C, **D**).

[1] Ceftobiprole R2 group is located between Tyr446, Met641, and Thr600, where the pyrrolidine ring is in hydrophobic contact with Met641 and the pyrrolidine NH forms a hydrogen bond with the side chain sulfur of Met641. The pyrrolidinone ring forms a pi-pi interaction with Tyr446 and has hydrophobic contact with Thr600. Both the planarity and the hydrophobicity of R2 group are essential to allow access

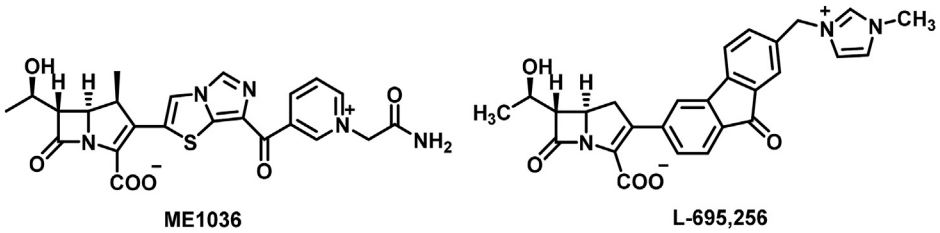


Fig. 13. Chemical structures of two investigational β-lactam antibiotics, ME1036 and L-695,256.

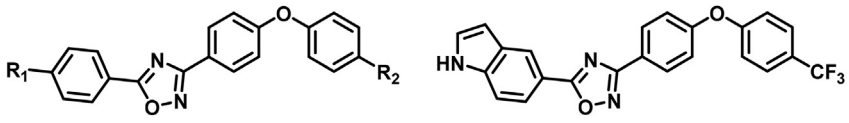


Fig. 14. 1,2,4-oxadiazole derivatives that exhibit activity against MRSA.

to the narrow active site pocket and achieve the required van der Waals interactions within the active site in contrast to other β-lactams lacking such functionality [81].

[2] The cephem carboxylate group forms three hydrogen bonds with Ser462, Ser598, and Thr600 hydroxyl groups.

[3] The carbonyl of the opened β-lactam ring forms a hydrogen bond with the backbone NH of Thr600.

[4] The amide carbonyl at 7-position of the cephem ring forms a hydrogen bond with the side chain NH_2 of Asn464.

[5] R1 group exists in two distinct conformations within the active site (Fig. 11) [50], one of them resides in a pocket formed by Gly520, Gln521, and Glu602 amino acids, where the amino group attached to the 3rd position of the 1,2,4-thiadiazole ring forms a weak hydrogen bond with Gly520 (Fig. 10C, **D**). The second conformation points away from that pocket toward the R2 group, thus the whole structure acquires a U-shaped conformation and the hydrogen bond formed by the other conformation with Gly520 is lost. These conformations probably result in an entropic gain for the bound ceftobiprole molecule [82].

4.1.2.3. Resistance. The analysis of MRSA strains resistant to ceftobiprole (MIC, $\geq$ 4 μg/ml) [76,83,84] reveals several amino acid mutations within the allosteric site. These mutations include E150K, E237K, and E239K [85,86]. Such mutations result in the disruption of the communication between the allosteric and the active site, thus, affecting the binding of ceftobiprole to the active site cavity [85].

4.1.2.4. Comparison of PBP2a in complex with ceftaroline and ceftobiprole. While both ceftaroline and ceftobiprole PBP2a acyl-enzyme complexes demonstrated similar structural changes at the strand β3 and the N terminus of helix α2, there are notable differences between these structures (Fig. 12). First, the Glu602-Arg612 salt bridge observed in the ceftaroline complex as a consequence of the interaction with its R1 group is absent in the ceftobiprole complex. Second, the R2 group in the ceftobiprole complex is stacked between Tyr446 and Met641, whereas in the ceftaroline complex, Tyr446 on the α2–α3 loop interacts with Met641 to close the active site and keep ceftaroline within a narrow cavity. Third, the salt-bridge interaction between Lys387 and Asp635 is not observed in the ceftobiprole acyl-enzyme structure due to a different positioning of Asp635 that prevents the formation of this salt-bridge interaction [45,50].

4.1.3. ME1036

ME1036 [87,88] (formerly CP5609) is a carbapenem undergoing clinical trials (Fig. 13). It exhibits broad-spectrum activity against MRSA, *H. influenzae*, *S. pneumoniae*, and *Enterococcus faecalis* with potency even greater than ceftaroline (MIC, 0.06–0.5 μg/ml against 148 CA-MRSA strains) [89]. ME1036 activity against MRSA is related to its high affinity for the mutant PBP2a [90].

4.1.4. L-695,256

L-695,256 [91] is an investigational synthetic carbapenem β-lactam antibiotic (Fig. 13) exhibiting broad spectrum activity against resistant *S. aureus*, *S. epidermidis*, *E. faecalis*, and *S. pneumoniae* strains [92] (MIC, 0.016–2 mg/ml against MRSA isolates [91]). L-695,256 binds with a high degree of affinity to PBP2a with a mean IC_{50} of 1.2 μg/ml [93]. L-695,256 forms insoluble crystals, thus it can't be used in humans [94].

4.2. Oxadiazoles derivatives

1,2,4-oxadiazoles are a recent class of non-β-lactam antibacterial agents. They are active against vancomycin- and linezolid-resistant MRSA and several other Gram-positive bacterial strains, with a good *in vivo* efficacy and oral bioavailability. Their activity against MRSA is owed to their ability to inhibit PBP2a with the subsequent suppression of peptidoglycan synthesis [95].

4.2.1. Development of oxadiazoles and their antimicrobial activity

Through an *in-silico* docking study of 1.2 million compounds from the ZINC database using the x-ray structure of PBP2a of MRSA (PDB code 1VQQ), several compounds were selected and examined for their antibacterial activity, which lead to the identification of a 1,2,4-oxadiazole compound (**1**) as an initial hit (Fig. 14). Compound **1** manifested a poor but consistent MIC values against *S. aureus* and *E. faecium* bacteria. A 370-derivatives library was assembled and tested, where three active derivatives (**2**–**4**) were identified with excellent MIC values (1–2 μg/ml) against various MRSA and VRSA strains [95]. Two following SAR studies of this series reported additional 120 and 59 analogs. Compound **5** (**ND-421**) was

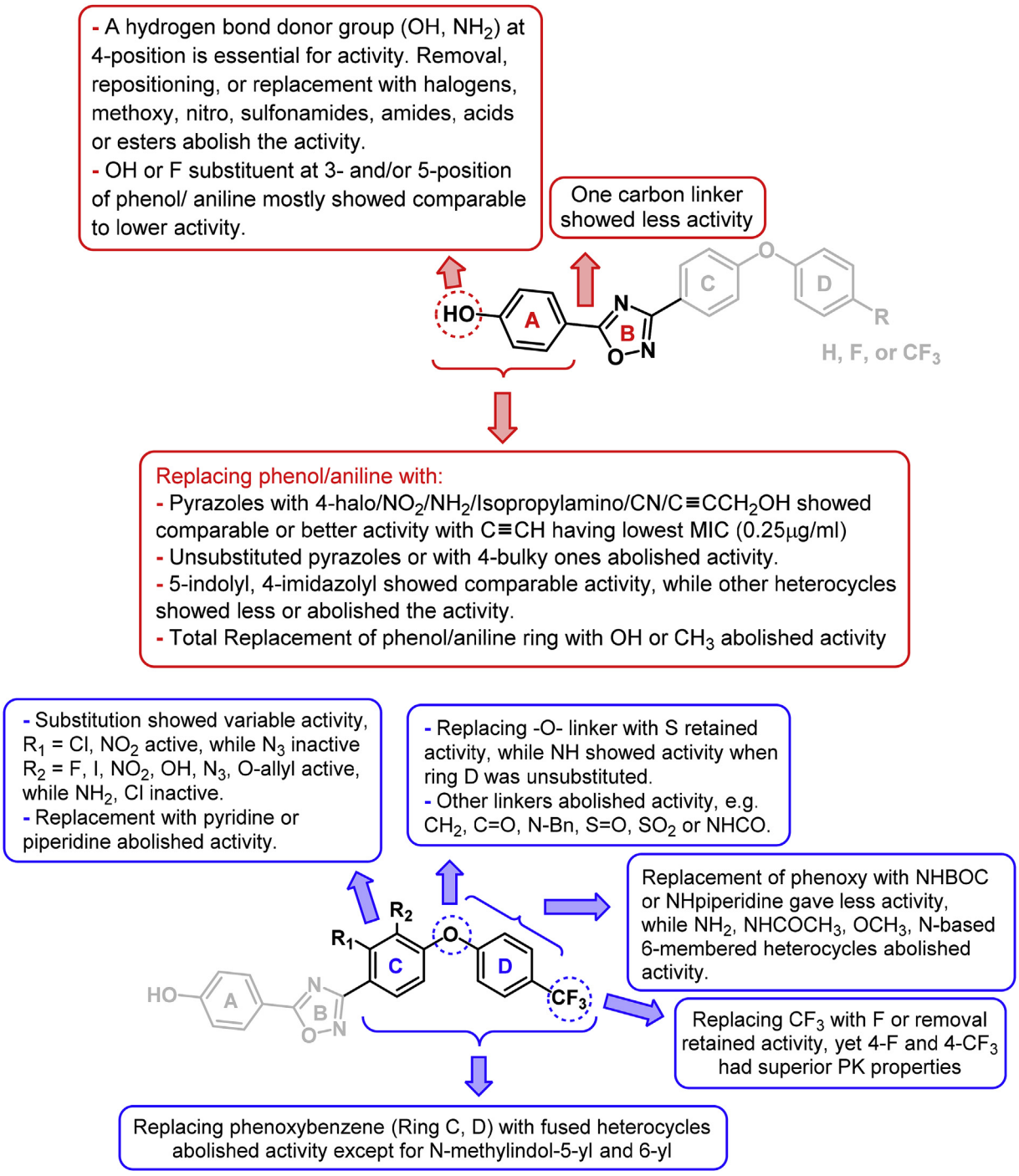


Fig. 15. General structure–activity relationship for the oxadiazole class of antibiotics. Variation of ring A yielded 120 derivatives, where their SAR is summarized (upper panel). Ring C and D variation yielded an additional 59 derivatives, where their SAR is shown (lower panel).

identified as the best candidate with optimal pharmacokinetic properties as demonstrated in animal models [96,97]. Further investigation of **ND-421** revealed its comparable efficacy to Linezolid and synergistic effect with β-lactam antibiotics [98,99]. However, the mechanism by which these compounds inhibit PBP2a and whether they bind to the active or allosteric site is not reported.

4.2.2. SAR

The reported 1,2,4-oxadiazoles comprise four rings, A, B, C, and D, as depicted in Fig. 15. To define a clear structure–activity relationship (SAR) for this novel class of antibacterials, two studies were reported [96,97]. First, diversification of ring A yielded 120 derivatives with varied antimicrobial activities. A general SAR for this series is summarized in Fig. 15, upper panel. Next, variation of both ring C and D generated an additional 59 derivatives, where their SAR is outlined in Fig. 15, lower panel.

Moreover, a three-dimensional quantitative structure–activity relationship (3D-QSAR) model was developed using 77 oxadiazole derivatives as the training set (containing active and inactive compounds) and 25 compounds as a test set to validate the *in-silico* model [100]. This model identified certain important antibacterial structure–activity features of the oxadiazole compounds. At the 4-

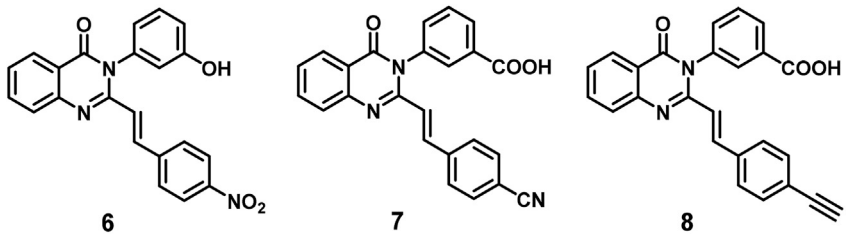


Fig. 16. Initial hit and optimized 4(3*H*)-quinazolinone antibacterials.

position of ring A, electropositive functional groups are preferred, whereas the presence of negatively charged substituent may abolish the antibacterial activity. On the other hand, presence of electronegative groups is preferred at both 3- and 5-positions of ring A (corresponding to the nitrogen and 4-substituents in pyrazole derivatives). Moreover, large substituents on ring A is disfavored leading to inactive compounds. On the other side, the presence of a bulky steric group represented by the substituted ring D is essential for the oxadiazoles to exert their antibacterial activity.

4.2.3. Resistance

An *in vitro* study using serial passages of a MRSA strain (*S. aureus* COL) generated two oxadiazole-resistant strains. Such resistance was attributed to selected mutations in some of the genes implicating the cell wall stress stimulon [101].

4.3. Quinazolinone derivatives

4.3.1. Development and antimicrobial activity

Through the same *in-silico* docking and screening program that led to the discovery of the oxadiazole antibacterials, a second hit, (*E*)-3-(3-hydroxyphenyl)-2-(4-nitrostyryl) quinazolin-4(3*H*)-one (**6**) was identified (Fig. 16), with an MIC value of 2 μg/ml against *S. aureus* ATCC 29213 (a MSSA strain) [102]. Screening of a library of

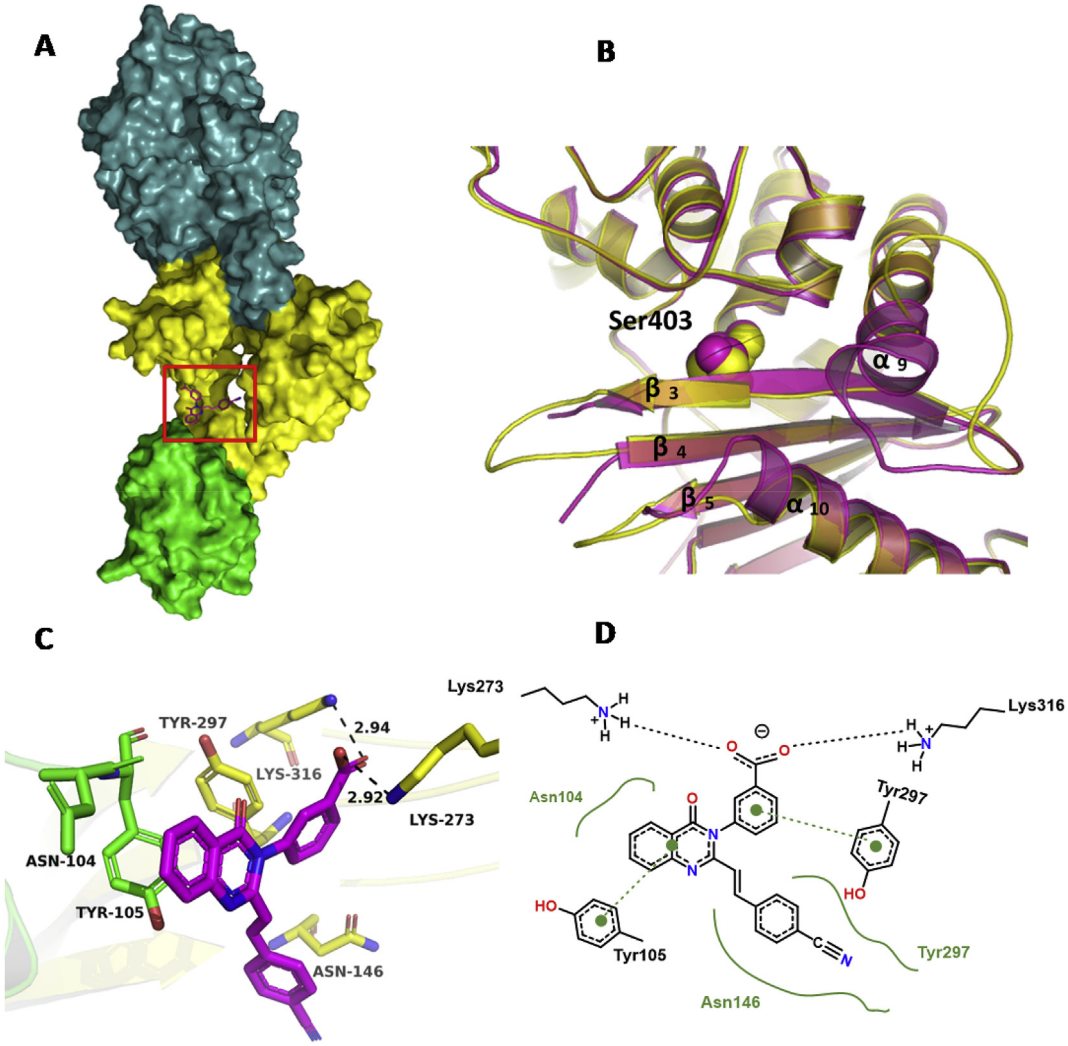


Fig. 17. Inhibition of *S. aureus* PBP2a by quinazolinone derivative, compound 7. (A) X-ray crystal structure (PDB ID: 4CJN) of PBP2a bound to compound **7** at the allosteric site, the N-terminal extension, allosteric domain, and TPase domain are colored green, yellow, and cyan. **(B)** Superimposition of the active site of apo PBP2a (PDB ID: 1VQQ) colored in purple and PBP2a-compound **7** complex (PDB ID: 4CJN) colored in yellow. Structural changes occur at loops α9-β3, β3-β4, and β5-α10. **(C)**, **(D)** 3D and 2D views of compound **7** respectively within the active site of PBP2a displaying the key amino acids involved in the interaction. Compound **7** backbone is colored in magenta, while the hydrogen-bonding interactions are depicted as black dashes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

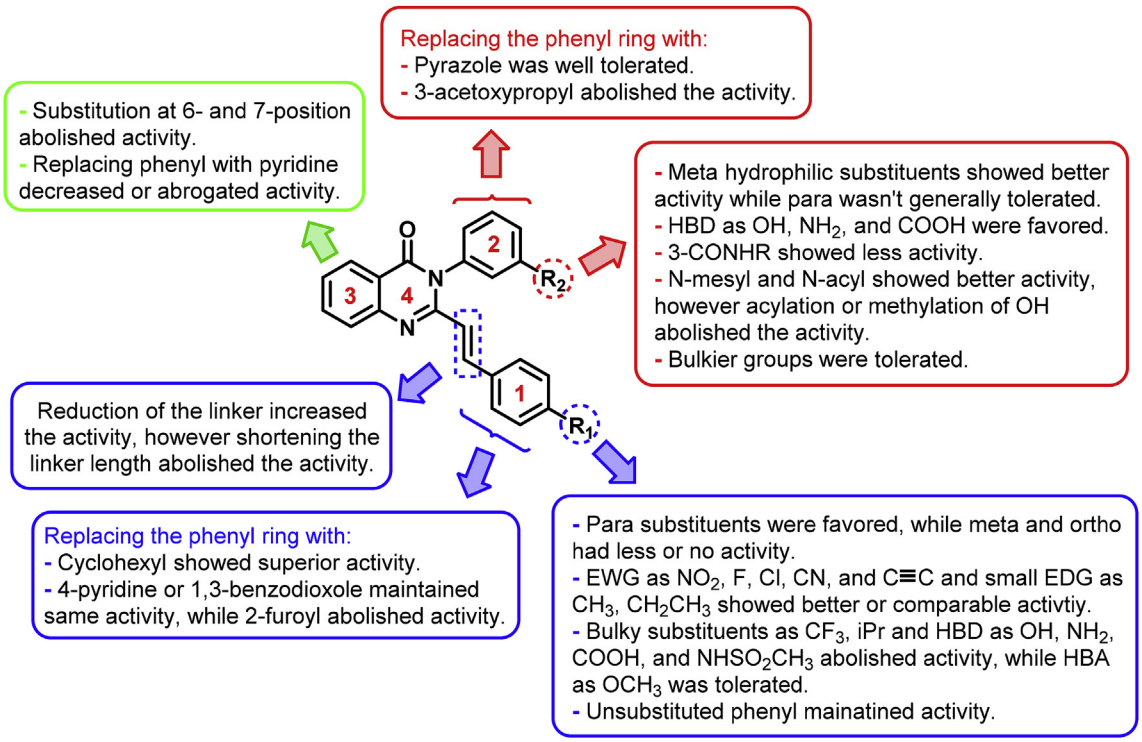


Fig. 18. General structure–activity relationship for the quinazolinone class of antibiotics.

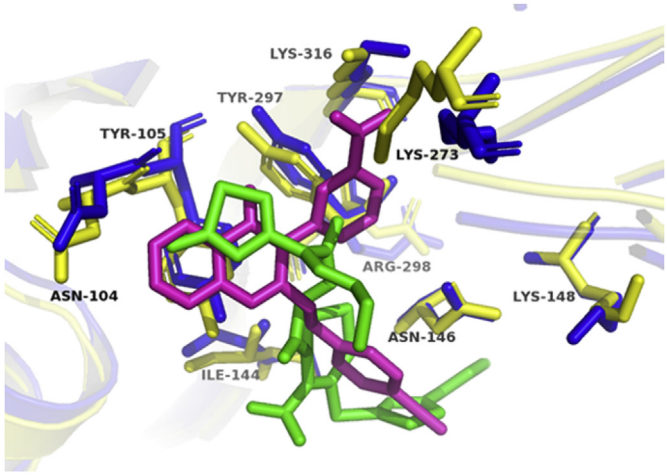


Fig. 19. Structural comparison of the ceftaroline and compound 7 interaction with the PBP2a allosteric site. Superposition of the allosteric site of ceftaroline-PBP2a complex (PDB: 3ZFZ) and quinazolinone-PBP2a complex (PDB ID code 4CJN), ceftaroline is depicted in green and quinazolinone in magenta. Side chains of relevant residues are shown as sticks for the ceftaroline-PBP2a (blue) and the quinazolinone-PBP2a (yellow) complexes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

80 quinazolinone derivatives generated an optimum agent, (*E*)-3-(3-carboxyphenyl)-2-(4-cyanostyryl)-quinazolin-4(3*H*)-one (**7**) (Fig. 16), which exhibits excellent *in vivo* efficacy, good oral bioavailability, and demonstrates a distinctive safety profile. Compound **7** inhibits several *S. aureus* strains at MIC value range of 2–16 μg/ml. It exerts its action by restricting the cell wall biosynthesis, through inhibition of PBP1 and PBP2a with average IC_{50} values of 78, 140 μg/ml respectively. X-ray crystal structure of PBP2a with compound **7** (PDB: 4CJN) reveals that compound **7** is bound to the allosteric site (Fig. 17A), resulting in the opening of the active site as indicated by the reorganization of the loops surrounding the active site and the repositioning of several residues within the active site (Lys406, Lys597, Ser598, Glu602, and Met641) (Fig. 17B). Pertinent to these findings, it was proposed that compound **7** inhibits PBP2a by either binding to the allosteric and active site (competitive inhibition) cognate to ceftaroline binding mode or it binds to the allosteric site only, prompting a negative allosteric control of the active site. Competitive inhibition premise is supported by the finding that compound **7** inhibits PBP1 at the active site as it lacks an allosteric one, yet a crystal structure of compound **7** within the PBP2a active site was never obtained [102].

A follow up SAR study of another 77 derivatives identified a more potent derivative, (*E*)-3-(3-carboxyphenyl)-2-(4-ethynylstyryl)-quinazolin-4(3*H*)-one (**8**) [103] (Fig. 16) with MIC value of 0.03 μg/ml against MRSA. Compound **8** surpassed its precedent, compound **7**, as it demonstrated better *in vivo* efficacy using a mouse neutropenic thigh infection model and excellent pharmacokinetic properties and safety profile.

A recent study reported the bactericidal efficacy of a triple combination of piperacillin (PBP inhibitor), tazobactam (β-lactamase inhibitor), and compound **8** sodium salt *in vitro* and *in vivo* using mouse infection model. Such combination demonstrated synergistic antibacterial activity at sub-MIC values of all three drugs. A proposed working hypothesis starts with the binding of compound **8** to the PBP2a allosteric site, culminating in the opening of the active site to allow piperacillin binding and subsequent inhibition of PBP2a. Tazobactam protects piperacillin from the actions of β-lactamases allowing it to bind to PBP2a to exert it effect. This postulate was confirmed by the x-ray crystal structure of PBP2a complexed with compound **8** and piperacillin which located compound **8** within the allosteric site and piperacillin covalently bound to Ser403 within the active site (PDB: 6H5O and unreleased 6Q9N) [57]. Based on these premises, ineffective antibiotics can be revived for use in synergistic combination with allosteric PBP2a inhibitors against resistant MRSA strains.

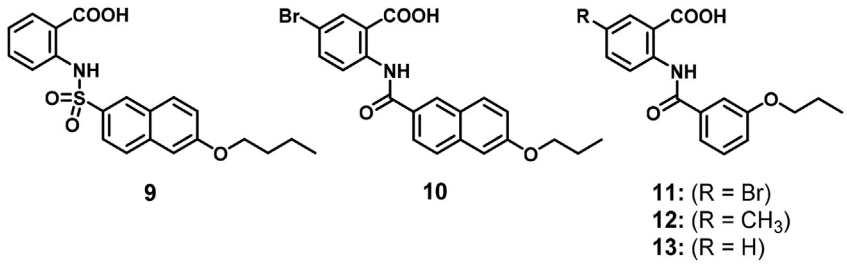


Fig. 20. Non-covalent inhibitors of PBP2a.

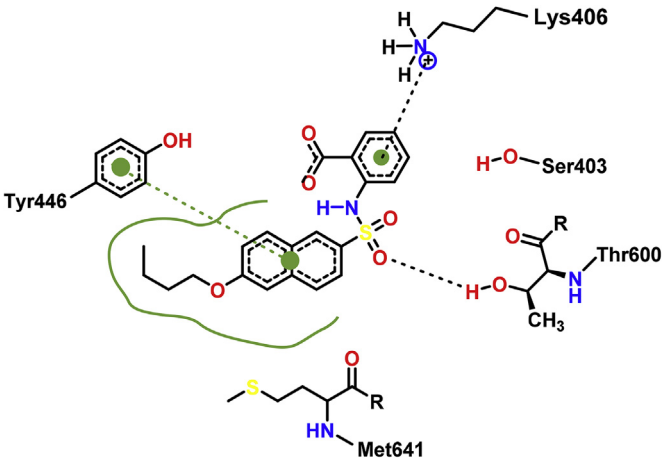


Figure 21. 2D interaction diagram for the postulated binding mode of a non-covalent inhibitor, compound 9, within the PBP2a active site.

4.3.2. SAR

The structure–activity relationship for the 4(3*H*)-quinazolinones antibacterial class was explored by introducing a systematic variation at rings 1, 2, and 3 (Fig. 18) [103]. Seventy-seven analogs were screened for antibacterial activity and a relevant SAR was deduced.

4.3.3. Quinazolinone binding mode within the allosteric site of PBP2a

At the allosteric site, compound **7** binds in a non-covalent mode (PDB: 4CJN) and demonstrates the following interaction features (Fig. 17C, **D**).

[1] The carboxylate group is positioned within a pocket formed by Lys316, Lys273, and Glu294 amino acids, where it is anchored by forming two hydrogen bonds with the side chain NH_2 of both Lys316 and Lys273.
[2] A pi-pi interaction is formed between Tyr297 and the phenyl ring at the 3-position of quinazolinone.
[3] The fused benzene ring of quinazolinone is in hydrophobic contact with Asn104 and Tyr105 side chains.
[4] The phenyl ring of the cyanostyryl substituent at the 2-position of quinazolinone is in hydrophobic contact with Asn146 side chain.

Comparison between ceftaroline and quinazolinone binding mode within the allosteric site. Ceftaroline and compound **7** share the same binding pocket of the PBP2a allosteric site, yet they show different binding modes (Fig. 19). Both ceftaroline R1 group and compound **7** quinazolinone core occupy the same hydrophobic pocket formed by Tyr297, Tyr105, and Asn104 and show similar hydrophobic interactions (Fig. 7E, **F** and Fig. 17C, **D**). On the other hand, ceftaroline R2 group extends deeper into the second pocket formed by Asn146, Arg298, and Ile144 and exhibits an additional hydrogen bond and a pi-cation interaction, while compound **7** forms weaker hydrophobic interactions with this pocket. Conversely, compound **7** extends into a pocket formed by Lys316, Lys273, and Glu294 amino acids establishing two strong hydrogen bonds with Lys316 and Lys273, while ceftaroline lacks such interactions.

4.4. Non-covalent inhibitors

4.4.1. Anthranilic acid derivatives

4.4.1.1. Development and antimicrobial activity. Screening of an in-house library of 250 compounds against the three PBPs, PBP2a, PBP2x (penicillin binding protein 2x of *Streptococcus pneumoniae*), and PBP5fm led to the identification of a small non-covalent inhibitor, compound **9** (Fig. 20), which inhibits PBP2a and PBP2x with IC_{50} values of 97, 391 μM respectively. Similarity search of the ChemBridge database using compound **9** structure as a template, retrieved eleven similar compounds, which were evaluated for their PBPs inhibitory activity. Among these derivatives, compounds, **10**–**13** (Fig. 20) exhibit modest activity against PBP2a with IC_{50} values of 210, 230, 680, and 910 μM respectively. Further evaluation of their antibacterial activity, compounds **9**–**13** showed moderate to weak MIC values of 32, 128, 32, 256, and 512 μg/ml respectively against two MRSA strains [104].

4.4.1.2. Binding interactions of compound 9 within the PBP2a active site. A hypothesized binding mode for compound **9** into the PBP2a active site was suggested based on a docking study using the unbound PBP2a x-ray crystal structure (PDB: 1VQQ) (Fig. 21) [104]. Compound **9** was postulated to form three major interactions with the key amino acids within the PBP2a active site comprising:

1] Hydrogen bond between the sulfonamide oxygen and the OH group of Thr600 amino acid.
2] Pi-cation interaction between anthranilic acid phenyl ring and the side chain NH_2 of Lys406.
3] Hydrophobic interaction between naphthalene ring and Tyr446 and Met641 side chains.

4.4.1.3. Miscellaneous PBP2a inhibitors. Several antibacterials and/or antibacterial potentiators (Fig. 22) were reported to target MRSA through putative inhibition of PBP2a, yet a clear mechanistic validation is lacking.

4.5. Aspermerodione

It is a fungal metabolite (**14**) with antimicrobial activity against MRSA (MIC = 32 μg/ml). Inverse docking study identified PBP2a as the possible target and this finding was supported by aspermerodione binding affinity assessment to PBP2a using a microscale thermophoresis (MST) method which showed a K_d value of 18.4 ± 1.29 μM [105].

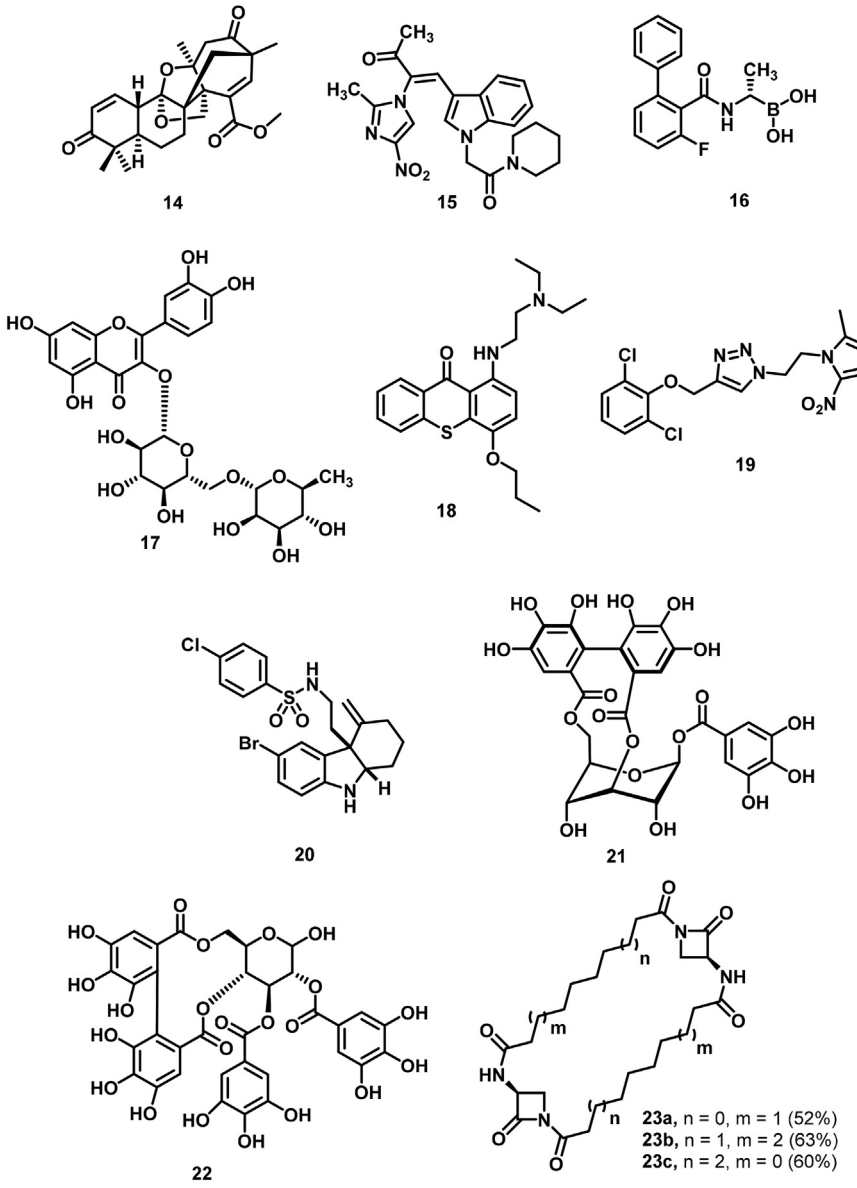


Fig. 22. Miscellaneous PBP2a inhibitors.

4.6. Indole-nitroimidazole conjugates

Eighteen indole-nitroimidazole hybrids were synthesized and evaluated for their antimicrobial activity where compound **15** demonstrated good inhibition of MRSA (MIC = 1 μg/ml). Based on a molecular modeling binding study, it was postulated that these derivatives can exert their action through binding to the PBP2a active site [106].

4.7. Alkyl boronic acid derivatives

Guided by the ability of boronic acid-based compounds to inhibit nucleophilic enzymes through covalent binding, a library of boronic acid derivatives was evaluated for its PBP1b inhibitory activity. Eleven alkyl boronic acid derivatives were identified and crystallized with PBP1b to evaluate their binding modes. An optimum derivative, compound **16** demonstrates modest activity against two MRSA strains with MIC value of 32 μg/ml and inhibits PBP2a at 4–10 times its MIC. In the PBP1b-compound **16** complex, the active site catalytic Ser460 forms a covalent bond with the boron atom, likewise, PBP2a inhibition is predicted to involve the same mechanism [107].

4.8. Quercetin 3-O-rutinoside

Quercetin, a natural product with weak antibacterial activity showed synergistic effect when combined with β-lactams against sensitive MRSA strain [108]. Docking and molecular dynamic simulation studies of quercetin derivatives postulated that quercetins, particularly, quercetin 3-O-rutinoside (**17**), exert their action through binding to the PBP2a allosteric and active sites [109].

4.9. Thioxanthones

Screening a library of 40 xanthone derivatives identified few compounds active against MRSA strain represented by compound **18**, which showed synergistic activity when combined with ampicillin and oxacillin against MRSA. Docking studies proposed that

compound **18** binds to the allosteric binding site of PBP2a, which may explain its synergistic effect when combined with oxacillin [110].

4.10. Metronidazole-triazole hybrids

Thirty-five metronidazole-triazole derivatives were evaluated for their anti-MRSA activity, where compound **19** was identified as a good candidate with good activity against several MRSA strains at MIC value as low as 4 μg/ml. Compound **19** exhibits synergistic effect with oxacillin with MIC value of 1 μg/ml. *In silico* studies suggested that these derivatives exert their action through binding to the PBP2a active site [111].

4.11. Polycyclic indole alkaloids

A library of 120 polycyclic indolines were synthesized and evaluated for their potential as resistance-modifying agents. Compound **20** was found to potentiate the activity of several β-lactams, notably, oxacillin (128-fold) against MRSA strain [112].

4.12. Peptide-penicillin conjugates

A synthetic peptide library was constructed by coupling selected peptides with a penicillin side chain and evaluated for their PBP2a inhibitory activity. An appropriate 11-residue oligopeptide conjugate potentiated the PBP2a IC_{50} of 6-aminopenicillanic acid from >500 mM to 7 mM (>100-fold). Such improvement might be contributed to the additional binding interactions conferred by the peptide chain contact with the active site cognate to those formed by the side chain of cephalosporins [113]. This study provides a novel strategy to enhance the efficacy of β-lactams against PBP2a and MRSA.

4.13. Chitosan-ferulic acid conjugate (CFA)

Combinations of chitosan-ferulic acid conjugate and β-lactam antibiotics demonstrated a synergistic activity, where penicillin, ampicillin, and oxacillin showed 3–4 folds reduction in MIC in association with CFA against two MRSA strains. CFA exhibits its activity through inhibition of the expression of *mecA* gene, resulting in PBP2a suppression [114].

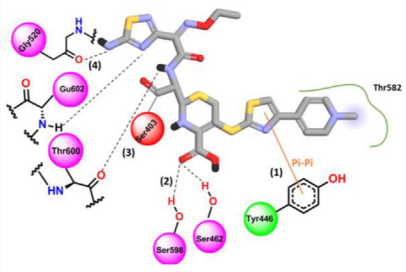
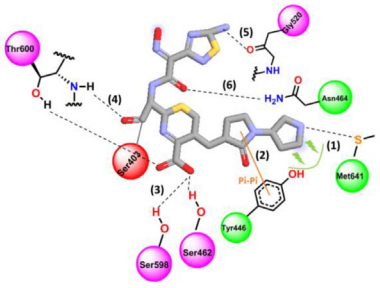
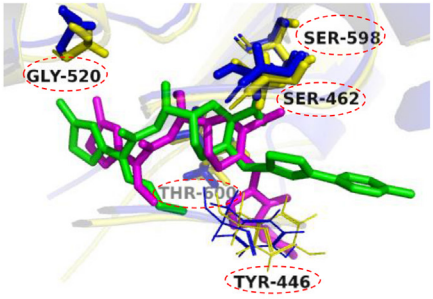
4.14. Polyphenols

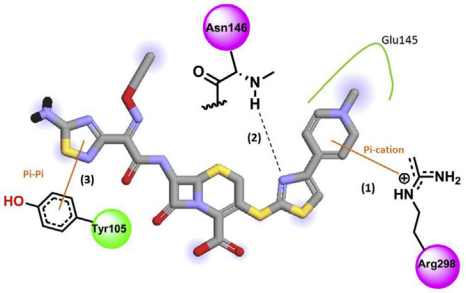
Two polyphenols, corilagin (**21**) and tellimagrandin I (**22**) were reported to potentiate the activity of oxacillin against four MRSA strains (100–1000-fold). Such effect is manifested through inhibition of PBP2a binding ability thus restoring β-lactams activity against MRSA [115].

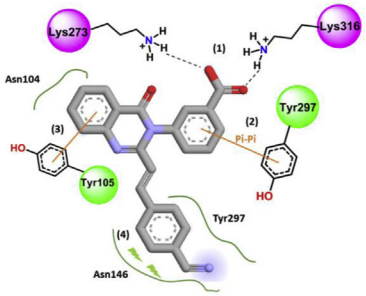
4.15. Bis-2-oxoazetidinyl macrocycles

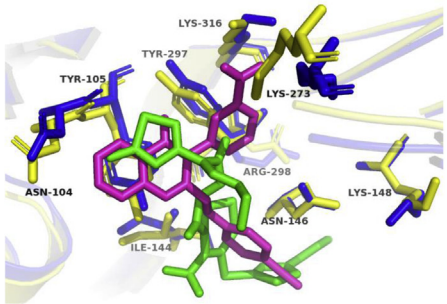
A library of bis-2-oxoazetidinyl macrocycles was synthesized and evaluated against R39 (PBP model), PBP2a of MRSA, and PBP5 of resistant *Enterococcus faecium*. Three cyclic dimers (**23a-c**) showed good activity with 22–33% PBP2a residual activity at 1 mM concentration of the dimers. Docking studies suggested that these dimers binds to the active site forming an acyl-enzyme complex and their flexibility would assist fitting to the closed conformation of PBP2a [116].

Summary

	Ceftaroline	Ceftobiprole	Quinazolinone
IC_{50} for the mutant PBP2a/MIC values	IC_{50} value of 0.16 μg/ml. MICs (0.25–1 μg/mL) against several MRSA strains.	IC_{50} ≤ 0.47 μg/ml. MIC values (0.5 – 4 μg/ml) against several MRSA strains.	IC_{50} value of 140 μg/ml. MIC values (2-16 μg/ml) against several MRSA strains.
X-ray PDB code	**3ZFZ, 3ZG0**	**4DKI**	**4CJN**
Binding mode at the active site	 **4 main interactions are required as illustrated in the diagram.**	 **6 main interactions are required as illustrated in the diagram.**	No molecule was reported at the active site.
Comparison of the binding mode between Ceftaroline and Ceftobiprole within PBP2a active site	 **Crystal structure of PBP2a complexed with ceftaroline (green, PDB: 3ZG0) is aligned to the crystal structure of PBP2a complexed with ceftobiprole (magenta, PDB: 4DKI) within the PBP2a active site.** Common amino acids involved in the interactions are displayed in blue (ceftaroline) and yellow (ceftobiprole).		

<table>
<tr><th></th><th>Ceftaroline</th><th>Ceftobiprole</th><th>Quinazolinone</th></tr>
<tr><td>Binding mode at the allosteric site</td><td> 

3 main interactions are required as illustrated in the diagram.</td><td>No molecule was reported at the allosteric site.</td><td> 

4 main interactions are required as illustrated in the diagram.</td></tr>
<tr><td>Comparison of the binding mode between Ceftaroline and Quinazolinone within PBP2a allosteric site</td><td colspan="3"> 

Crystal structure of ceftaroline-PBP2a complex (PDB ID code 3ZFZ) is aligned to the crystal structure of quinazolinone-PBP2a complex (PDB ID code 4CJN) within the allosteric site, ceftaroline is depicted in green and quinazolinone in magenta. Side chains of relevant residues are shown as sticks for the ceftaroline-PBP2a (blue) and the quinazolinone-PBP2a (yellow) complexes.</td></tr>
<tr><td>Notes</td><td colspan="3">Oxadiazoles: The mechanism by which these compounds inhibit PBP2a and whether they bind to the active or allosteric site is not reported.
Covalent inhibitors (Anthranilic acid derivatives):A hypothesized binding mode for one of these compounds into the PBP2a active site was suggested based on a docking study using the unbound PBP2a x-ray crystal structure (PDB: 1VQQ) (Figure 21).
Other miscellaneous compounds: Aspermerodione, Indole-nitroimidazole conjugates, Alkyl boronic acid derivatives, Quercetin 3-O-rutinoside, Thioxanthones, Metronidazole-triazole hybrids, Polycyclic indole alkaloids, Peptide-penicillin conjugates, Chitosan-ferulic acid conjugate (CFA), Polyphenols, and Bis-2-oxoazetidinyl macrocycles. All of these compounds work either by directly binding to PBP2a or by inhibiting its expression. None of them was co-crystallized with PBP2a.</td></tr>
</table>

5. Future perspectives

Current antibiotics targeting MRSA exert their action through three main mechanisms: cell wall biosynthesis inhibition, protein synthesis inhibition, and nucleotide synthesis suppression [117]. Nonetheless, MRSA competently developed resistance to all these mechanisms and nowadays, even the last-resort antibiotics as vancomycin [118], daptomycin [119], ceftaroline [120], and linezolid [121] have disclosed records of MRSA resistance [122]. Such observation suggests that MRSA will probably develop more resistance in the near future. Therefore, new strategies for antibiotic discovery are urgently needed to face the inexorable development of antibiotic-resistance mechanisms acquired by MRSA.

Considering recent literature signifying PBP2a-mediated MRSA resistance and the profound knowledge detailing the enzyme structure, mechanism of resistance, allosteric control, and successful attempts at inhibition, targeting PBP2a constitutes a promising approach for antimicrobial therapy. Three main approaches are suggested to effectively inhibit PBP2a:

a) The first approach is to design new compounds that can bind exclusively at the PBP2a active site with higher binding affinity. This can be achieved by increasing the number of noncovalent interactions between the inhibitor and the active site, as exemplified by ceftobiprole and non-covalent inhibitors.
b) The second approach is to tackle the allosteric control of PBP2a by targeting the allosteric site by non-covalent inhibitors, where upon binding, these inhibitors trigger a series of conformational changes and predispose the enzyme to be inactivated by a second molecule of the same compound or a combination of a β-lactam antibiotic (synergy). Ceftaroline and quinazolinone are good examples for this approach.
c) The third approach targets PBP2a indirectly by blocking a regulatory pathway or by inhibition of related enzymes. For example, FemA enzyme ability to extend the monoglycine branches to triglycine on lipid II is crucial for PBP2a to achieve cell wall crosslinking, as it can only crosslink peptidoglycan strands bearing penta- and triglycine, but not monoglycine [40]. Accordingly, design of FemA inhibitors can target PBP2a indirectly. Furthermore, a previous study reported the development of DNAzymes to knock down *mecR1* and *blaR1* genes thus suppressing the transcription of *mecA* and *blaZ* which encode the low affinity PBP2a and β-lactamases respectively, thereby, reinstating the susceptibility of MRSA to oxacillin antibiotic [123].

Alternatively, different steps of the cell wall biosynthesis can be targeted other than PBP2a [124], but this is beyond the scope of this review.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We are grateful to Salma Magdi for her kind help in preparing the graphical abstract. The authors declare no funding was received for this work.

References

[1] World Health Organization, Antimicrobial Resistance Global Report on Surveillance : 2014 Summary, World Health Organization, 2014. https://apps.who.int/iris/handle/10665/112647.

[2] M. Haque, M. Sartelli, J. McKimm, M. Abu Bakar, Health care-associated infections - an overview, Infect. Drug Resist. 11 (2018) 2321–2333, https://doi.org/10.2147/IDR.S177247.

[3] E.F. Kong, J.K. Johnson, M.A. Jabra-Rizk, Community-Associated methicillin-resistant Staphylococcus aureus: an enemy amidst us, PLoS Pathog. 12 (2016), https://doi.org/10.1371/journal.ppat.1005837 e1005837.

[4] N.A. Turner, B.K. Sharma-Kuinkel, S.A. Maskarinec, E.M. Eichenberger, P.P. Shah, M. Carugati, T.L. Holland, V.G. Fowler, Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research, Nat. Rev. Microbiol. 17 (2019) 203–218, https://doi.org/10.1038/s41579-018-0147-4.

[5] Biggest threats and data | antibiotic/antimicrobial resistance | CDC (n.d.), https://www.cdc.gov/drugresistance/biggest_threats.html#mrsa. (Accessed 23 July 2019).

[6] K. Poole, Resistance to β-lactam antibiotics, Cell. Mol. Life Sci. 61 (2004) 2200–2223, https://doi.org/10.1007/s00018-004-4060-9.

[7] C.C.S. Fuda, J.F. Fisher, S. Mobashery, β-Lactam resistance in Staphylococcus aureus: the adaptive resistance of a plastic genome, Cell. Mol. Life Sci. 62 (2005) 2617–2633, https://doi.org/10.1007/s00018-005-5148-6.

[8] T.J. Foster, Can β-lactam antibiotics Be resurrected to combat MRSA? Trends Microbiol. 27 (2019) 26–38, https://doi.org/10.1016/j.tim.2018.06.005.

[9] M. Gajdács, The continuing threat of methicillin-resistant Staphylococcus aureus, Antibiotics 8 (2019) 52, https://doi.org/10.3390/antibiotics8020052.

[10] S.J. Peacock, G.K. Paterson, Mechanisms of methicillin resistance in *Staphylococcus aureus*, Annu. Rev. Biochem. 84 (2015) 577–601, https://doi.org/10.1146/annurev-biochem-060614-034516.

[11] C. Fuda, M. Suvorov, S.B. Vakulenko, S. Mobashery, The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus, J. Biol. Chem. 279 (2004) 40802–40806, https://doi.org/10.1074/jbc.M403589200.

[12] I.A. Myles, S.K. Datta, Staphylococcus aureus: an introduction, Semin. Immunopathol. 34 (2012) 181–184, https://doi.org/10.1007/s00281-011-0301-9.

[13] S.O. Jensen, B.R. Lyon, Genetics of antimicrobial resistance in *Staphylococcus aureus*, Future Microbiol. 4 (2009) 565–582, https://doi.org/10.2217/fmb.09.30.

[14] M. Barber, M. Rozwadowska-Dowzenko, Infection by penicillin-resistant staphylococci, Lancet (London, England) 2 (1948) 641–644, https://doi.org/10.1016/s0140-6736(48)92166-7.

[15] F.D. Lowy, Antimicrobial resistance: the example of Staphylococcus aureus, J. Clin. Invest. 111 (2003) 1265–1273, https://doi.org/10.1172/JCI18535.

[16] H.Z. Zhang, C.J. Hackbarth, K.M. Chansky, H.F. Chambers, A proteolytic transmembrane signaling pathway and resistance to β-lactams in staphylococci. Sci. (Washington, DC, United States) 291 (2001) 1962–1965, https://doi.org/10.1126/science.1055144.

[17] R.P. Novick, D. Bouanchaud, Extrachromosomal nature of drug resistance in Staphylococcus aureus, Ann. N. Y. Acad. Sci. 182 (1971) 279–294, https://doi.org/10.1111/j.1749-6632.1971.tb30664.x.

[18] A.L. Panlilio, D.H. Culver, R.P. Gaynes, S. Banerjee, T.S. Henderson, J.S. Tolson, W.J. Martone, Methicillin-resistant Staphylococcus aureus in U.S. hospitals, 1975-1991, Infect. Control Hosp. Epidemiol. 13 (1992) 582–586, https://doi.org/10.1086/646432.

[19] M.C. Enright, D.A. Robinson, G. Randle, E.J. Feil, H. Grundmann, B.G. Spratt, The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA), Proc. Natl. Acad. Sci. 99 (2002) 7687–7692, https://doi.org/10.1073/pnas.122108599.

[20] Y. Katayama, T. Ito, K. Hiramatsu, A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus, Antimicrob. Agents Chemother. 44 (2000) 1549–1555, https://doi.org/10.1128/AAC.44.6.1549-1555.2000.

[21] R. Deurenberg, E. Stobberingh, The molecular evolution of hospital- and community-associated methicillin-resistant Staphylococcus aureus, Curr. Mol. Med. 9 (2009) 100–115, https://doi.org/10.2174/156652409787581637.

[22] M.G. Pinho, S.R. Filipe, H. de Lencastre, A. Tomasz, Complementation of the essential peptidoglycan transpeptidase function of penicillin-binding protein 2 (PBP2) by the drug resistance protein PBP2A in Staphylococcus aureus, J. Bacteriol. 183 (2001) 6525–6531, https://doi.org/10.1128/JB.183.22.6525-6531.2001.

[23] D.W. Green, The bacterial cell wall as a source of antibacterial targets, Expert Opin. Ther. Targets 6 (2002) 1–20, https://doi.org/10.1517/14728222.6.1.1.

[24] P. Giesbrecht, T. Kersten, H. Maidhof, J. Wecke, Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin, Microbiol. Mol. Biol. Rev. 62 (1998) 1371–1414.

[25] Y. Liu, E. Breukink, The membrane steps of bacterial cell wall synthesis as antibiotic targets, Antibiot. (Basel, Switzerland) 5 (2016) 28, https://doi.org/10.3390/antibiotics5030028.

[26] S.O. Meroueh, K.Z. Bencze, D. Hesek, M. Lee, J.F. Fisher, T.L. Stemmler, S. Mobashery, Three-dimensional structure of the bacterial cell wall peptidoglycan, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 4404–4409, https://doi.org/10.1073/pnas.0510182103.

[27] C. Goffin, J.M. Ghuysen, Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs, Microbiol. Mol. Biol. Rev. 62 (1998) 1079–1093.

[28] C. Goffin, J.-M. Ghuysen, Biochemistry and comparative genomics of SxxK superfamily acyltransferases offer a clue to the mycobacterial paradox: presence of penicillin-susceptible target proteins versus lack of efficiency of penicillin as therapeutic agent, Microbiol. Mol. Biol. Rev. 66 (2002) 702–738, https://doi.org/10.1128/MMBR.66.4.702-738.2002.

[29] C. Morlot, M. Noirclerc-Savoye, A. Zapun, O. Dideberg, T. Vernet, The d,d-carboxypeptidase PBP3 organizes the division process of Streptococcus pneumoniae, Mol. Microbiol. 51 (2004) 1641–1648, https://doi.org/10.1046/j.1365-2958.2003.03953.x.

[30] E. Sauvage, F. Kerff, M. Terrak, J.A. Ayala, P. Charlier, The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis, FEMS Microbiol. Rev. 32 (2008) 234–258, https://doi.org/10.1111/j.1574-6976.2008.00105.x.

[31] M.A. McDonough, J.W. Anderson, N.R. Silvaggi, R.F. Pratt, J.R. Knox, J.A. Kelly, Structures of two kinetic intermediates reveal species specificity of penicillin-binding proteins, J. Mol. Biol. 322 (2002) 111–122, https://doi.org/10.1016/S0022-2836(02)00742-8.

[32] D.J. Tipper, J.L. Strominger, Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-d-alanyl-d-alanine, Proc. Natl. Acad. Sci. U.S.A. 54 (1965) 1133–1141, https://doi.org/10.1073/PNAS.54.4.1133.

[33] A.P. Kuzin, H. Liu, J.A. Kelly, J.R. Knox, Binding of cephalothin and cefotaxime to d-Ala-d-Ala-peptidase reveals a functional basis of a natural mutation in a low-affinity penicillin-binding protein and in extended-spectrum beta-lactamases, Biochemistry 34 (1995) 9532–9540, https://doi.org/10.1021/bi00029a030.

[34] J.M. Ghuysen, Molecular structures of penicillin-binding proteins and beta-lactamases, Trends Microbiol. 2 (1994) 372–380, https://doi.org/10.1016/0966-842x(94)90614-9.

[35] A.F. Kluge, R.C. Petter, Acylating drugs : redesigning natural covalent inhibitors, Curr. Opin. Chem. Biol. 14 (2010) 421–427, https://doi.org/10.1016/j.cbpa.2010.03.035.

[36] M.G. Pinho, H. de Lencastre, A. Tomasz, An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci, Proc. Natl. Acad. Sci. 98 (2001) 10886–10891, https://doi.org/10.1073/pnas.191260798.

[37] S.F.F. Pereira, A.O. Henriques, M.G. Pinho, H. de Lencastre, A. Tomasz, Role of PBP1 in cell division of Staphylococcus aureus, J. Bacteriol. 189 (2007) 3525–3531, https://doi.org/10.1128/JB.00044-07.

[38] B. Berger-Bächi, L. Barberis-Maino, A. Strässle, F.H. Kayser, FemA, a host-mediated factor essential for methicillin resistance in Staphylococcus aureus: molecular cloning and characterization, Mol. Gen. Genet. 219 (1989) 263–269, https://doi.org/10.1007/bf00261186.

[39] H. de Lencastre, A. Tomasz, Reassessment of the number of auxiliary genes essential for expression of high-level methicillin resistance in Staphylococcus aureus, Antimicrob. Agents Chemother. 38 (1994) 2590–2598, https://doi.org/10.1128/aac.38.11.2590.

[40] V. Srisuknimit, Y. Qiao, K. Schaefer, D. Kahne, S. Walker, Peptidoglycan cross-linking preferences of Staphylococcus aureus penicillin-binding proteins have implications for treating MRSA infections, J. Am. Chem. Soc. 139 (2017) 5–8, https://doi.org/10.1021/jacs.7b04881.

[41] J.M. Monteiro, G. Covas, D. Rausch, S.R. Filipe, T. Schneider, H.-G. Sahl, M.G. Pinho, The pentaglycine bridges of Staphylococcus aureus peptidoglycan are essential for cell integrity, Sci. Rep. 9 (2019) 5010, https://doi.org/10.1038/s41598-019-41461-1.

[42] D. Lim, N.C.J. Strynadka, Structural basis for the β lactam resistance of PBP2a from methicillin-resistant Staphylococcus aureus, Nat. Struct. Biol. 9 (2002) 870–876, https://doi.org/10.1038/nsb858.

[43] W.-P. Lu, Y. Sun, M.D. Bauer, S. Paule, P.M. Koenigs, W.G. Kraft, Penicillin-binding protein 2a from methicillin-resistant Staphylococcus aureus : kinetic characterization of its interactions with β-lactams using electrospray mass spectrometry, Biochemistry 38 (1999) 6537–6546, https://doi.org/10.1021/bi990025e.

[44] C.Y. Wu, J. Hoskins, L.C. Blaszczak, D.A. Preston, P.L. Skatrud, Construction of a water-soluble form of penicillin-binding protein 2a from a methicillin-resistant Staphylococcus aureus isolate, Antimicrob. Agents Chemother. 36 (1992) 533–539, https://doi.org/10.1128/aac.36.3.533.

[45] L.H. Otero, A. Rojas-Altuve, L.I. Llarrull, C. Carrasco-Lopez, M. Kumarasiri, E. Lastochkin, J. Fishovitz, M. Dawley, D. Hesek, M. Lee, J.W. Johnson, J.F. Fisher, M. Chang, S. Mobashery, J.A. Hermoso, How allosteric control of Staphylococcus aureus penicillin binding protein 2a enables methicillin resistance and physiological function, Proc. Natl. Acad. Sci. 110 (2013) 16808–16813, https://doi.org/10.1073/pnas.1300118110.

[46] P. Macheboeuf, C. Contreras-Martel, V. Job, O. Dideberg, A. Dessen, Penicillin binding proteins: key players in bacterial cell cycle and drug resistance processes, FEMS Microbiol. Rev. 30 (2006) 673–691, https://doi.org/10.1111/j.1574-6976.2006.00024.x.

[47] G. Nicola, J. Tomberg, R.F. Pratt, R.A. Nicholas, C. Davies, Crystal structures of covalent complexes of β-lactam antibiotics with Escherichia coli penicillin-binding protein 5: toward an understanding of antibiotic specificity, Biochemistry 49 (2010) 8094–8104, https://doi.org/10.1021/bi100879m.

[48] W.P. Lu, E. Kincaid, Y. Sun, M.D. Bauer, Kinetics of beta-lactam interactions

with penicillin-susceptible and -resistant penicillin-binding protein 2x proteins from Streptococcus pneumoniae. Involvement of acylation and deacylation in beta-lactam resistance, J. Biol. Chem. 276 (2001) 31494–31501, https://doi.org/10.1074/jbc.M102499200.
[49] C. Fuda, D. Hesek, M. Lee, K. Morio, T. Nowak, S. Mobashery, Activation for catalysis of penicillin-binding protein 2a from methicillin-resistant Staphylococcus aureus by bacterial cell wall, J. Am. Chem. Soc. 127 (2005) 2056–2057, https://doi.org/10.1021/ja0434376.
[50] A.L. Lovering, M.C. Gretes, S.S. Safadi, F. Danel, L. De Castro, M.G.P. Page, N.C.J. Strynadka, Structural insights into the anti-methicillin-resistant Staphylococcus aureus (MRSA) activity of ceftobiprole, J. Biol. Chem. 287 (2012) 32096–32102, https://doi.org/10.1074/jbc.M112.355644.
[51] K.V. Mahasenan, R. Molina, R. Bouley, M.T. Batuecas, J.F. Fisher, J.A. Hermoso, M. Chang, S. Mobashery, Conformational dynamics in penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus, allosteric communication network and enablement of catalysis, J. Am. Chem. Soc. 139 (2017) 2102–2110, https://doi.org/10.1021/jacs.6b12565.
[52] B. Thomas, Y. Wang, R.L. Stein, Kinetic and mechanistic studies of penicillin-binding protein 2x from *Streptococcus pneumoniae*, Biochemistry 40 (2001) 15811–15823, https://doi.org/10.1021/bi011368r.
[53] M.S. Wilke, T.L. Hills, H.-Z. Zhang, H.F. Chambers, N.C.J. Strynadka, Crystal structures of the Apo and penicillin-acylated forms of the BlaR1 beta-lactam sensor of Staphylococcus aureus, J. Biol. Chem. 279 (2004) 47278–47287, https://doi.org/10.1074/jbc.M407054200.
[54] Q. Shi, S.O. Meroueh, J.F. Fisher, S. Mobashery, A computational evaluation of the mechanism of penicillin-binding protein-catalyzed cross-linking of the bacterial cell wall, J. Am. Chem. Soc. 133 (2011) 5274–5283, https://doi.org/10.1021/ja1074739.
[55] J. Fishovitz, N. Taghizadeh, J.F. Fisher, M. Chang, S. Mobashery, The Tipper–Strominger hypothesis and triggering of allostery in penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus* (MRSA), J. Am. Chem. Soc. 137 (2015) 6500–6505, https://doi.org/10.1021/jacs.5b01374.
[56] J. Fishovitz, A. Rojas-Altuve, L.H. Otero, M. Dawley, C. Carrasco-López, M. Chang, J.A. Hermoso, S. Mobashery, C. Carrasco-lo, M. Chang, J.A. Hermoso, S. Mobashery, Disruption of allosteric response as an unprecedented mechanism of resistance to antibiotics, J. Am. Chem. Soc. 136 (2014) 9814–9817, https://doi.org/10.1021/ja5030657.
[57] J. Janardhanan, R. Bouley, S. Martínez-Caballero, Z. Peng, M. Batuecas-Mordillo, J.E. Meisel, D. Ding, V.A. Schroeder, W.R. Wolter, K.V. Mahasenan, J.A. Hermoso, S. Mobashery, M. Chang, The quinazolinone allosteric inhibitor of PBP 2a synergizes with piperacillin and tazobactam against methicillin-resistant Staphylococcus aureus, Antimicrob. Agents Chemother. 63 (2019), https://doi.org/10.1128/aac.02637-18 e02637-18.
[58] C. Duplessis, N.F. Crum-Cianflone, Ceftaroline: a new cephalosporin with activity against methicillin-resistant Staphylococcus aureus (MRSA), Clin. Med. Rev. Therapeut. 3 (2011) 1–17, https://doi.org/10.4137/CMRT.S1637.
[59] N. Lounsbury, M.G. Reeber, G. Mina, C. Chbib, A mini-review on ceftaroline in bacteremia patients with methicillin-resistant Staphylococcus aureus (MRSA) infections, Antibiot. (Basel, Switzerland) 8 (2019) 30, https://doi.org/10.3390/antibiotics8010030.
[60] S.-H. Lan, S.-P. Chang, C.-C. Lai, L.-C. Lu, C.-M. Chao, Ceftaroline efficacy and safety in treatment of complicated skin and soft tissue infection: a systemic review and meta-analysis of randomized controlled trials, J. Clin. Med. 8 (2019) 776, https://doi.org/10.3390/jcm8060776.
[61] K.-N. Tran, M.J. Rybak, β-Lactam combinations with vancomycin show synergistic activity against vancomycin-susceptible Staphylococcus aureus , vancomycin-intermediate S. aureus (VISA), and heterogeneous VISA, Antimicrob. Agents Chemother. 62 (2018), https://doi.org/10.1128/AAC.00157-18 e00157-18.
[62] H. Moisan, M. Pruneau, F. Malouin, Binding of ceftaroline to penicillin-binding proteins of Staphylococcus aureus and Streptococcus pneumoniae, J. Antimicrob. Chemother. 65 (2010) 713–716, https://doi.org/10.1093/jac/dkp503.
[63] K. Kosowska-Shick, P.L. McGhee, P.C. Appelbaum, Affinity of ceftaroline and other beta-lactams for penicillin-binding proteins from Staphylococcus aureus and Streptococcus pneumoniae, Antimicrob. Agents Chemother. 54 (2010) 1670–1677, https://doi.org/10.1128/AAC.00019-10.
[64] R.N. Jones, R.E. Mendes, H.S. Sader, Ceftaroline activity against pathogens associated with complicated skin and skin structure infections: results from an international surveillance study, J. Antimicrob. Chemother. 65 (2010), https://doi.org/10.1093/jac/dkq252 iv17–iv31.
[65] R.E. Mendes, A. Tsakris, H.S. Sader, R.N. Jones, D. Biek, P. McGhee, P.C. Appelbaum, K. Kosowska-Shick, Characterization of methicillin-resistant Staphylococcus aureus displaying increased MICs of ceftaroline, J. Antimicrob. Chemother. 67 (2012) 1321–1324, https://doi.org/10.1093/jac/dks069.
[66] S.W. Long, R.J. Olsen, S.C. Mehta, T. Palzkill, P.L. Cernoch, K.K. Perez, W.L. Musick, A.E. Rosato, J.M. Musser, PBP2a mutations causing high-level ceftaroline resistance in clinical methicillin-resistant Staphylococcus aureus isolates, Antimicrob. Agents Chemother. 58 (2014) 6668–6674, https://doi.org/10.1128/AAC.03622-14.
[67] D.O. Andrey, P. François, C. Manzano, E.J. Bonetti, S. Harbarth, J. Schrenzel, W.L. Kelley, A. Renzoni, Antimicrobial activity of ceftaroline against methicillin-resistant Staphylococcus aureus (MRSA) isolates collected in 2013–2014 at the Geneva University Hospitals, Eur. J. Clin. Microbiol. Infect. Dis. 36 (2017) 343–350, https://doi.org/10.1007/s10096-016-2807-5.
[68] M. Nigo, L. Diaz, L.P. Carvajal, T.T. Tran, R. Rios, D. Panesso, J.D. Garavito, W.R. Miller, A. Wanger, G. Weinstock, J.M. Munita, C.A. Arias, H.F. Chambers, Ceftaroline-resistant, daptomycin-tolerant, and heterogeneous vancomycin-intermediate methicillin-resistant Staphylococcus aureus causing infective endocarditis, Antimicrob. Agents Chemother. 61 (2017), https://doi.org/10.1128/AAC.01235-16 e01235–16.
[69] R. Ślusarczyk, A. Bielejewska, A. Bociek, M. Bociek, Resistance to ceftaroline - 2018 review, Eur. J. Biol. Res. 8 (2018) 112–120, https://doi.org/10.5281/zenodo.1304435.
[70] W.L. Kelley, A. Jousselin, C. Barras, E. Lelong, A. Renzoni, Missense mutations in PBP2A Affecting ceftaroline susceptibility detected in epidemic hospital-acquired methicillin-resistant Staphylococcus aureus clonotypes ST228 and ST247 in Western Switzerland archived since 1998, Antimicrob. Agents Chemother. 59 (2015) 1922–1930, https://doi.org/10.1128/AAC.04068-14.
[71] P. Hebeisen, I. Heinze-Krauss, P. Angehrn, P. Hohl, M.G.P. Page, R.L. Then, In vitro and in vivo properties of Ro 63-9141, a novel broad-spectrum cephalosporin with activity against methicillin-resistant staphylococci, Antimicrob. Agents Chemother. 45 (2001) 825–836, https://doi.org/10.1128/AAC.45.3.825-836.2001.
[72] R.A. Stein, R.M. Goetz, G.M. Ganea, Ceftobiprole: a new β-lactam antibiotic, Int. J. Clin. Pract. 63 (2009) 930–943, https://doi.org/10.1111/j.1742-1241.2009.02041.x.
[73] C. Vidaillac, M.J. Rybak, Ceftobiprole: first cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*, Pharmacotherapy 29 (2009) 511–525, https://doi.org/10.1592/phco.29.5.511.
[74] H.F. Chambers, Ceftobiprole: in-vivo profile of a bactericidal cephalosporin, Clin. Microbiol. Infect. 12 (2006) 17–22, https://doi.org/10.1111/j.1469-0691.2006.01404.x.
[75] M.Z. David, M. Dryden, T. Gottlieb, P. Tattevin, I.M. Gould, Recently approved antibacterials for methicillin-resistant Staphylococcus aureus (MRSA) and other Gram-positive pathogens: the shock of the new, Int. J. Antimicrob. Agents 50 (2017) 303–307, https://doi.org/10.1016/j.ijantimicag.2017.05.006.
[76] M.A. Pfaller, R.K. Flamm, L.R. Duncan, J.M. Streit, M. Castanheira, H.S. Sader, Antimicrobial activity of ceftobiprole and comparator agents when tested against contemporary Gram-positive and -negative organisms collected from Europe (2015), Diagn. Microbiol. Infect. Dis. 91 (2018) 77–84, https://doi.org/10.1016/J.DIAGMICROBIO.2017.12.020.
[77] M.I. Morosini, M. Díez-Aguilar, R. Cantón, Mechanisms of action and antimicrobial activity of ceftobiprole, Rev. Española Quimioter. 32 (Suppl 3) (2019) 3–10. http://www.ncbi.nlm.nih.gov/pubmed/31364335. (Accessed 28 November 2019).
[78] T.A. Davies, M.G.P. Page, W. Shang, T. Andrew, M. Kania, K. Bush, Binding of ceftobiprole and comparators to the penicillin-binding proteins of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae, Antimicrob. Agents Chemother. 51 (2007) 2621–2624, https://doi.org/10.1128/AAC.00029-07.
[79] D.J. Farrell, R.K. Flamm, H.S. Sader, R.N. Jones, Activity of ceftobiprole against methicillin-resistant Staphylococcus aureus strains with reduced susceptibility to daptomycin, linezolid or vancomycin, and strains with defined SCCmec types, Int. J. Antimicrob. Agents 43 (2014) 323–327, https://doi.org/10.1016/j.ijantimicag.2013.11.005.
[80] M.G. Page, Cephalosporins in clinical development, Expet Opin. Invest. Drugs 13 (2004) 973–985, https://doi.org/10.1517/13543784.13.8.973.
[81] D.T. King, S. Sobhanifar, N.C.J. Strynadka, One ring to rule them all: current trends in combating bacterial resistance to the β-lactams, Protein Sci. 25 (2016) 787–803, https://doi.org/10.1002/pro.2889.
[82] D.L. Mobley, K.A. Dill, Binding of small-molecule ligands to proteins: "what you see" is not always "what you get, Structure 17 (2009) 489–498, https://doi.org/10.1016/j.str.2009.02.010.
[83] G. Morroni, A. Brenciani, L. Brescini, S. Fioriti, S. Simoni, A. Pocognoli, M. Mingoia, E. Giovanetti, F. Barchiesi, A. Giacometti, O. Cirioni, High rate of ceftobiprole resistance among clinical methicillin-resistant Staphylococcus aureus isolates from a hospital in Central Italy, Antimicrob. Agents Chemother. 62 (2018), https://doi.org/10.1128/AAC.01663-18 e01663-18.
[84] L.C. Chan, L. Basuino, B. Diep, S. Hamilton, S.S. Chatterjee, H.F. Chambers, Ceftobiprole- and ceftaroline-resistant methicillin-resistant Staphylococcus aureus, Antimicrob. Agents Chemother. 59 (2015) 2960–2963, https://doi.org/10.1128/AAC.05004-14.
[85] R. Banerjee, M. Gretes, L. Basuino, N. Strynadka, H.F. Chambers, In vitro selection and characterization of ceftobiprole-resistant methicillin-resistant Staphylococcus aureus, Antimicrob. Agents Chemother. 52 (2008) 2089–2096, https://doi.org/10.1128/AAC.01403-07.
[86] Y. Katayama, H.-Z. Zhang, H.F. Chambers, PBP 2a mutations producing very-high-level resistance to beta-lactams, Antimicrob. Agents Chemother. 48 (2004) 453–459, https://doi.org/10.1128/aac.48.2.453-459.2004.
[87] M. Kurazono, T. Ida, K. Yamada, Y. Hirai, T. Maruyama, E. Shitara, M. Yonezawa, In vitro activities of ME1036 (CP5609), a novel parenteral carbapenem, against methicillin-resistant Staphylococci, Antimicrob. Agents Chemother. 48 (2004) 2831–2837, https://doi.org/10.1128/AAC.48.8.2831-2837.2004.
[88] A. Villegas-estrada, M. Lee, D. Hesek, S.B. Vakulenko, S. Mobashery, Co-opting the cell wall in fighting methicillin-resistant Staphylococcus aureus (MRSA):

potent inhibition of penicillin-binding protein 2a (PBP 2a) by ceftaroline, J. Am. Chem. Soc. 130 (2008) 9212–9213, https://doi.org/10.1021/ja8029448.
[89] H.S. Sader, T.R. Fritsche, R.N. Jones, Antimicrobial activities of ceftaroline and ME1036 tested against clinical strains of community-acquired methicillin-resistant Staphylococcus aureus, Antimicrob. Agents Chemother. 52 (2008) 1153–1155, https://doi.org/10.1128/AAC.01351-07.
[90] Y. Hirai, S. Takahata, K. Yamada, T. Ida, K. Maebashi, Correlation of the antimicrobial activity of ME1036 with its binding affinities to the penicillin-binding proteins from Streptococcus pneumoniae strains, J. Antibiot. (Tokyo). 64 (2011) 741–746, https://doi.org/10.1038/ja.2011.76.
[91] M. Rylander, J. Rollof, K. Jacobsson, S.R. Norrby, Comparative antibacterial activity of L-695,256, a carbapenem active against methicillin-resistant staphylococci, Antimicrob. Agents Chemother. 39 (1995) 1178–1181, https://doi.org/10.1128/AAC.39.5.1178.
[92] G. Malanoski, L. Collins, C.T. Eliopoulos, R.C. Moellering, G.M. Eliopoulos, Comparative in vitro activities of L-695,256, a novel carbapenem, against gram-positive bacteria, Antimicrob. Agents Chemother. 39 (1995) 990–995, https://doi.org/10.1128/aac.39.4.990.
[93] H.F. Chambers, In vitro and in vivo antistaphylococcal activities of L-695,256, a carbapenem with high affinity for the penicillin-binding protein PBP 2a, Antimicrob. Agents Chemother. 39 (1995) 462–466, https://doi.org/10.1128/aac.39.2.462.
[94] D.M. Livermore, Can β-lactams be re-engineered to beat MRSA? Clin. Microbiol. Infect. 12 (2006) 11–16, https://doi.org/10.1111/j.1469-0691.2006.01403.x.
[95] P.I. O'Daniel, Z. Peng, H. Pi, S.A. Testero, D. Ding, E. Spink, E. Leemans, M.A. Boudreau, T. Yamaguchi, V.A. Schroeder, W.R. Wolter, L.I. Llarrull, W. Song, E. Lastochkin, M. Kumarasiri, N.T. Antunes, M. Espahbodi, K. Lichtenwalter, M.A. Suckow, S. Vakulenko, S. Mobashery, M. Chang, Discovery of a new class of non-β-lactam inhibitors of penicillin-binding proteins with gram-positive antibacterial activity, J. Am. Chem. Soc. 136 (2014) 3664–3672, https://doi.org/10.1021/ja500053x.
[96] E. Spink, D. Ding, Z. Peng, M.A. Boudreau, E. Leemans, E. Lastochkin, W. Song, K. Lichtenwalter, P.I. O'Daniel, S.A. Testero, H. Pi, V.A. Schroeder, W.R. Wolter, N.T. Antunes, M.A. Suckow, S. Vakulenko, M. Chang, S. Mobashery, Structure–activity relationship for the oxadiazole class of antibiotics, J. Med. Chem. 58 (2015) 1380–1389, https://doi.org/10.1021/jm501661f.
[97] D. Ding, M.A. Boudreau, E. Leemans, E. Spink, T. Yamaguchi, S.A. Testero, P.I. O'Daniel, E. Lastochkin, M. Chang, S. Mobashery, Exploration of the structure-activity relationship of 1,2,4-oxadiazole antibiotics, Bioorg. Med. Chem. Lett 25 (2015) 4854–4857, https://doi.org/10.1016/j.bmcl.2015.06.044.
[98] J. Janardhanan, J.E. Meisel, D. Ding, V.A. Schroeder, W.R. Wolter, S. Mobashery, M. Chang, In vitro and in vivo synergy of the oxadiazole class of antibacterials with β-lactams, Antimicrob. Agents Chemother. 60 (2016) 5581–5588, https://doi.org/10.1128/AAC.00787-16.
[99] S. Ceballos, C. Kim, D. Ding, S. Mobashery, M. Chang, C. Torres, Activities of oxadiazole antibacterials against Staphylococcus aureus and other gram-positive bacteria, Antimicrob. Agents Chemother. 62 (2018), https://doi.org/10.1128/AAC.00453-18 e00453-18.
[100] E. Leemans, K.V. Mahasenan, M. Kumarasiri, E. Spink, D. Ding, P.I. O'Daniel, M.A. Boudreau, E. Lastochkin, S.A. Testero, T. Yamaguchi, M. Lee, D. Hesek, J.F. Fisher, M. Chang, S. Mobashery, Three-dimensional QSAR analysis and design of new 1,2,4-oxadiazole antibacterials, Bioorg. Med. Chem. Lett 26 (2016) 1011–1015, https://doi.org/10.1016/j.bmcl.2015.12.041.
[101] Q. Xiao, S. Vakulenko, M. Chang, S. Mobashery, Mutations in mmpL and in the cell wall stress stimulon contribute to resistance to oxadiazole antibiotics in methicillin-resistant Staphylococcus aureus, Antimicrob. Agents Chemother. 58 (2014) 5841–5847, https://doi.org/10.1128/AAC.03501-14.
[102] R. Bouley, M. Kumarasiri, Z. Peng, L.H. Otero, W. Song, M.A. Suckow, V.A. Schroeder, W.R. Wolter, E. Lastochkin, N.T. Antunes, H. Pi, S. Vakulenko, J.A. Hermoso, M. Chang, S. Mobashery, Discovery of antibiotic (E)-3-(3-carboxyphenyl)-2-(4-cyanostyryl)quinazolin-4(3H)-one, J. Am. Chem. Soc. 137 (2015) 1738–1741, https://doi.org/10.1021/jacs.5b00056.
[103] R. Bouley, D. Ding, Z. Peng, M. Bastian, E. Lastochkin, W. Song, M.A. Suckow, V.A. Schroeder, W.R. Wolter, S. Mobashery, M. Chang, Structure-activity relationship for the 4(3H)-Quinazolinone antibacterials, J. Med. Chem. 59 (2016) 5011–5021, https://doi.org/10.1021/acs.jmedchem.6b00372.
[104] S. Turk, O. Verlaine, T. Gerards, M. Živec, J. Humljan, I. Sosič, A. Amoroso, A. Zervosen, A. Luxen, B. Joris, S. Gobec, New noncovalent inhibitors of penicillin-binding proteins from penicillin-resistant bacteria, PloS One 6 (2011), https://doi.org/10.1371/journal.pone.0019418 e19418.
[105] Y. Qiao, X. Zhang, Y. He, W. Sun, W. Feng, J. Liu, Z. Hu, Q. Xu, H. Zhu, J. Zhang, Z. Luo, J. Wang, Y. Xue, Y. Zhang, Aspermerodione, a novel fungal metabolite with an unusual 2,6-dioxabicyclo[2.2.1]heptane skeleton, as an inhibitor of penicillin-binding protein 2a, Sci. Rep. 8 (2018) 5454, https://doi.org/10.1038/s41598-018-23817-1.
[106] Z.-Z. Li, V.K.R. Tangadanchu, N. Battini, R.R.Y. Bheemanaboina, Z.-L. Zang, S.-L. Zhang, C.-H. Zhou, Indole-nitroimidazole conjugates as efficient manipulators to decrease the genes expression of methicillin-resistant Staphylococcus aureus, Eur. J. Med. Chem. 179 (2019) 723–735, https://doi.org/10.1016/j.ejmech.2019.06.093.
[107] C. Contreras-Martel, A. Amoroso, E.C.Y. Woon, A. Zervosen, S. Inglis, A. Martins, O. Verlaine, A.M. Rydzik, V. Job, A. Luxen, B. Joris, C.J. Schofield, A. Dessen, Structure-guided design of cell wall biosynthesis inhibitors that overcome β-lactam resistance in *Staphylococcus aureus* (MRSA), ACS Chem. Biol. 6 (2011) 943–951, https://doi.org/10.1021/cb2001846.
[108] I. Hirai, M. Okuno, R. Katsuma, N. Arita, M. Tachibana, Y. Yamamoto, Characterisation of anti-Staphylococcus aureus activity of quercetin, Int. J. Food Sci. Technol. 45 (2010) 1250–1254, https://doi.org/10.1111/j.1365-2621.2010.02267.x.
[109] N. Rani, S. Vijayakumar, L. PTV, A. Arunachalam, Allosteric site-mediated active site inhibition of PBP2a using Quercetin 3-O-rutinoside and its combination, J. Biomol. Struct. Dyn. 34 (2016) 1778–1796, https://doi.org/10.1080/07391102.2015.1092096.
[110] L.J. Bessa, A. Palmeira, A.S. Gomes, V. Vasconcelos, E. Sousa, M. Pinto, P. Martins da Costa, Synergistic effects between thioxanthones and oxacillin against methicillin-resistant *Staphylococcus aureus*, Microb. Drug Resist. 21 (2015) 404–415, https://doi.org/10.1089/mdr.2014.0162.
[111] B. Negi, D. Kumar, W. Kumbukgolla, S. Jayaweera, P. Ponnan, R. Singh, S. Agarwal, D.S. Rawat, Anti-methicillin resistant Staphylococcus aureus activity, synergism with oxacillin and molecular docking studies of metronidazole-triazole hybrids, Eur. J. Med. Chem. 115 (2016) 426–437, https://doi.org/10.1016/j.ejmech.2016.03.041.
[112] J.D. Podoll, Y. Liu, L. Chang, S. Walls, W. Wang, X. Wang, Bio-inspired synthesis yields a tricyclic indoline that selectively resensitizes methicillin-resistant Staphylococcus aureus (MRSA) to β-lactam antibiotics, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 15573–15578, https://doi.org/10.1073/pnas.1310459110.
[113] S. Li, R.W. Roberts, A novel strategy for in vitro selection of peptide-drug conjugates, Chem. Biol. 10 (2003) 233–239, https://doi.org/10.1016/s1074-5521(03)00047-4.
[114] S.-H. Eom, S.-K. Kang, D.-S. Lee, J.-I. Myeong, J. Lee, H.-W. Kim, K.-H. Kim, J.-Y. Je, W.-K. Jung, Y.-M. Kim, Synergistic antibacterial effect and antibacterial action mode of chitosan-ferulic acid conjugate against methicillin-resistant Staphylococcus aureus, J. Microbiol. Biotechnol. 26 (2016) 784–789, https://doi.org/10.4014/jmb.1511.11046.
[115] S. Shiota, M. Shimizu, J. Sugiyama, Y. Morita, T. Mizushima, T. Tsuchiya, Mechanisms of action of corilagin and tellimagrandin I that remarkably potentiate the activity of β-lactams against methicillin-resistant *Staphylococcus aureus*, Microbiol. Immunol. 48 (2004) 67–73, https://doi.org/10.1111/j.1348-0421.2004.tb03489.x.
[116] A. Sliwa, G. Dive, A. Zervosen, O. Verlaine, E. Sauvage, J. Marchand-Brynaert, Unprecedented inhibition of resistant penicillin binding proteins by bis-2-oxoazetidinyl macrocycles, Med. Chem. Commun. 3 (2012) 344–351, https://doi.org/10.1039/C2MD00251E.
[117] I.P. Thomsen, G.Y. Liu, Targeting fundamental pathways to disrupt Staphylococcus aureus survival: clinical implications of recent discoveries, JCI Insight 3 (2018), https://doi.org/10.1172/jci.insight.98216 e98216.
[118] C. Walsh, Molecular mechanisms that confer antibacterial drug resistance, Nature 406 (2000) 775–781, https://doi.org/10.1038/35021219.
[119] G.L. French, Bactericidal agents in the treatment of MRSA infections–the potential role of daptomycin, J. Antimicrob. Chemother. 58 (2006) 1107–1117, https://doi.org/10.1093/jac/dkl393.
[120] N. Mendoza, S. Hernandez, S.K. Tyring, S. Lema, Ceftaroline in complicated skin and skin-structure infections, Infect. Drug Res. 5 (2012) 23–35, https://doi.org/10.2147/IDR.S17432.
[121] M.H. Wilcox, Update on linezolid: the first oxazolidinone antibiotic, Expet Opin. Pharmacother. 6 (2005) 2315–2326, https://doi.org/10.1517/14656566.6.13.2315.
[122] P.C. Appelbaum, The emergence of vancomycin-intermediate and vancomycin-resistant Staphylococcus aureus, Clin. Microbiol. Infect. 12 (2006) 16–23, https://doi.org/10.1111/j.1469-0691.2006.01344.x.
[123] Z. Hou, Y. Zhou, H. Wang, H. Bai, J. Meng, X. Xue, X. Luo, Co-blockade of mecR1/blaR1 signal pathway to restore antibiotic susceptibility in clinical isolates of methicillin-resistant Staphylococcus aureus, Arch. Med. Sci. 7 (2011) 414–422, https://doi.org/10.5114/aoms.2011.23404.
[124] H. Hao, G. Cheng, M. Dai, Q. Wu, Z. Yuan, Inhibitors targeting on cell wall biosynthesis pathway of MRSA, Mol. Biosyst. 8 (2012) 2828–2838, https://doi.org/10.1039/c2mb25188d.