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SUSCEPTIBILITY

# Susceptibility of Methicillin-Resistant *Staphylococcus aureus* to Five Quinazolinone Antibacterials

**Sara Ceballos,[a] Choon Kim,[b] Yuanyuan Qian,[b] Shahriar Mobashery,[b] Mayland Chang,[b] Carmen Torres[a]**

[a]Area of Biochemistry and Molecular Biology, University of La Rioja, Logroño, Spain
[b]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana, USA

**ABSTRACT** The *in vitro* activities of five quinazolinone antibacterials, compounds Q1 to Q5, were tested against 210 strains of methicillin-resistant *Staphylococcus aureus* (MRSA). The $MIC_{50}$/$MIC_{90}$ values (in $\mu$g/ml) were as follows: Q1, 0.5/2; Q2, 1/4; Q3, 2/4; Q4, 0.06/0.25; and Q5, 0.125/0.5. Several strains with high MIC values (from 8 to >32 $\mu$g/ml) for some of these compounds exhibited amino acid changes in the penicillin-binding proteins, which are targeted by these antibacterials.

**KEYWORDS** methicillin-resistant *Staphylococcus aureus*, MRSA, penicillin-binding proteins, quinazolinones, staphylococci

Nosocomial and community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections remain major clinical problems. Patients with MRSA infections have a 64% higher risk of mortality than those infected with nonresistant bacteria (1). According to the Centers for Disease Control and Prevention, MRSA accounts for >80,000 severe infections and kills >11,000 individuals annually in the United States alone (2). Historically, $\beta$-lactam antibiotics have been agents of choice in the treatment of *S. aureus* infections (3). $\beta$-Lactams target inhibition of penicillin-binding proteins (PBPs), which are enzymes of cell wall biosynthesis. *S. aureus* has four native PBPs, but MRSA has acquired an additional PBP, designated PBP 2a, which affords broad resistance to $\beta$-lactam antibiotics in the face of their challenge (4–6).

We have described the discovery of the quinazolinones as cell wall-active antibacterials with anti-*S. aureus* activity, including MRSA strains (7). We documented that the quinazolinones target PBP 1 and PBP 2a for inhibition (7). Preliminary structure-activity relationships and descriptions of *in vitro* and *in vivo* activities for the class have been described (7–14). In this report, we investigated the antibacterial activity profile of five quinazolinones of our design, compounds Q1 to Q5 (Fig. 1), against a collection of 210 MRSA strains (108 strains from the United States and 102 from Spain). The collection encompasses distinct clonal complexes and 54 MRSA strains with additional mechanisms of antimicrobial resistance, including resistance to the second-generation penicillin methicillin through the *mecC* gene and resistance to vancomycin or linezolid (Table 1). The 108 strains from the United States were obtained through BEI Resources. The 102 Spanish strains were obtained from four hospitals in three different regions (Aragón, La Rioja, and Madrid) ($n = 94$) and from animal, food, and water origins ($n = 8$, mostly with *mec*C mechanism).

The microdilution method supplemented with 2% NaCl was used to determine the MIC values of quinazolinones Q1 to Q5 (15), using *S. aureus* NCTC8325 as a reference and a quality-control strain (MIC ranges for Q1 to Q5, 0.125 to 1 $\mu$g/ml) (Table 1). Two clinically used anti-MRSA agents, ceftaroline (a $\beta$-lactam) and linezolid (an oxazolidinone), were included for the purpose of comparison. Vancomycin (a glycopeptide antibiotic) was included for vancomycin-resistant *S. aureus* (VRSA), vancomycin-intermediate *S. aureus* (VISA), and heterogeneous vancomycin-intermediate *S. aureus*

**Citation** Ceballos S, Kim C, Qian Y, Mobashery S, Chang M, Torres C. 2020. Susceptibility of methicillin-resistant *Staphylococcus aureus* to five quinazolinone antibacterials. Antimicrob Agents Chemother 64:e01344-19. https://doi.org/10.1128/AAC.01344-19.



Address correspondence to Mayland Chang, mchang@nd.edu, or Carmen Torres, carmen.torres@unirioja.es.

**Received** 2 July 2019
**Returned for modification** 17 July 2019
**Accepted** 8 October 2019
**Accepted manuscript posted online** 14 October 2019
**Published** 20 December 2019

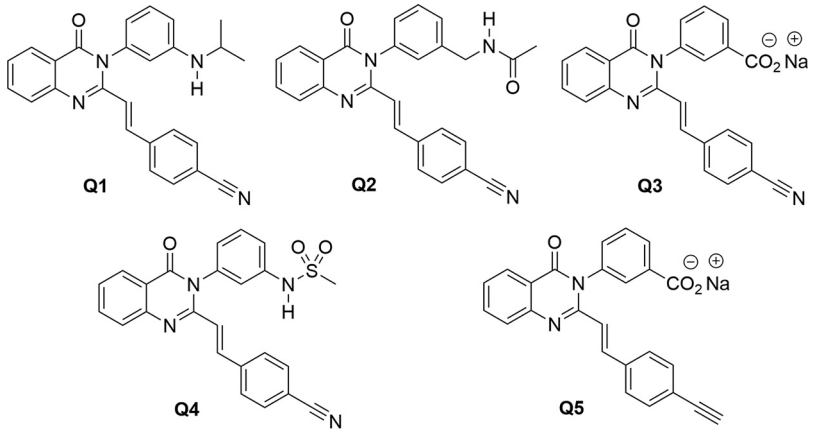


**FIG 1** Chemical structures of quinazolinones Q1 to Q5.

isolates. The MIC ranges for the five quinazolinones with the 210 MRSA strains and the values for $MIC_{50}$ and $MIC_{90}$ are shown in Table 1. The range of quinazolinone MIC values for the 210 MRSA strains was broad, from ≤0.03 to >32 μg/ml. High MICs for the quinazolinones of >32, 16, and 8 μg/ml were observed in 15, 2, and 18 strains, respectively. The $MIC_{50}$/$MIC_{90}$ values (in μg/ml) of the quinazolinones were as follows: Q1, 0.5/2; Q2, 1/4; Q3, 2/4; Q4, 0.06/0.25; and Q5, 0.125/0.5. Notwithstanding the broad MIC ranges for Q4 and Q5, note that compound Q4 $MIC_{50}$/$MIC_{90}$ values were 8- to 4-fold and 32- to 8-fold lower than those of ceftaroline and linezolid, respectively, and compound Q5 $MIC_{50}$/$MIC_{90}$ values were 4- to 2-fold and 16- to 4-fold lower than those of ceftaroline and linezolid, respectively (Table 1). For *mecC*-dependent MRSA and linezolid-resistant *S. aureus* (LRSA) strains, all quinazolinones had MIC values of ≤2 μg/ml (except for two LRSA strains with MICs of 4 μg/ml). Quinazolinones Q4 and Q5 showed excellent MICs of ≤1 μg/ml against the collected strains with few exceptions: for Q4, one VISA isolate with >32 μg/ml and one VRSA isolate with 4 μg/ml; and for Q5, two MRSA isolates with >32 μg/ml and one VRSA isolate with 2 μg/ml.

Four MRSA strains (C1657, C5287, K399, and C2878) that showed high MICs (>32 μg/ml) for the quinazolinones were selected for sequence analysis of *pbp1*, *pbp2*, *mecA*, *pbp3*, and *pbp4* genes by PCR and DNA sequencing (16, 17); these are the five PBP genes in MRSA. The corresponding amino acid sequences were compared with reference sequences available in the NCBI database: MRSA strain N315 for the *mecA* gene (GenBank accession number BA000018.3) and methicillin-susceptible *S. aureus* (MSSA) strain NCTC 8325 for the *pbp1*, *pbp2*, *pbp*3, and *pbp4* genes (GenBank accession

**TABLE 1** Susceptibility of 210 MRSA strains, including *S. aureus* NCTC8325, to quinazolinones 1 to 5, ceftaroline, linezolid, and vancomycin

| Antibacterial agent | MIC for total MRSA population ($n$ = 210) (μg/ml) | | | MIC range for MRSA with following resistance mechanisms[a] (μg/ml): | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ | VRSA ($n$ = 15) | VISA ($n$ = 20) | hVISA ($n$ = 6) | *mecC*-MRSA ($n$ = 7) | LRSA ($n$ = 6) | MIC for MSSA[b] NCTC8325 (μg/ml) |
| Ceftaroline | 0.125 to 2 | 0.5 | 1 | 0.25 to 2 | 0.25 to 2 | 0.5 to 1 | 0.5 to 1 | 0.5 to 2 | 0.125 |
| Linezolid | 0.5 to >16 | 2 | 2 | 1 to 2 | 0.5 to 2 | 0.5 to 2 | 1 to 2 | 8 to >16 | 1 |
| Vancomycin | | | | >32 | 4 to 8 | 2 to 8 | 1 to 2 | 1 to 4 | 1 |
| 1 | 0.03 to >32 | 0.5 | 2 | 0.125 to 2 | 0.25 to 4 | 0.125 to 4 | 0.25 to 2 | 0.25 to 1 | 0.25 |
| 2 | 0.06 to >32 | 1 | 4 | 0.125 to 8 | 0.125 to 8 | 0.25 to 2 | 0.5 to 2 | 1 to 4 | 0.5 |
| 3 | 0.5 to 16 | 2 | 4 | 2 to 16 | 0.5 to 16 | 0.5 to 2 | 1 to 2 | 1 to 4 | 1 |
| 4 | ≤0.03 to >32 | 0.06 | 0.25 | 0.06 to 4 | 0.03 to >32 | ≤0.03 to 0.125 | ≤0.03 to 0.06 | 0.06 to 0.25 | 0.25 |
| 5 | ≤0.03 to >32 | 0.125 | 0.5 | 0.06 to 2 | ≤0.03 to 1 | ≤0.03 to 0.125 | 0.06 to 0.25 | 0.06 to 0.25 | 0.125 |

[a]VRSA, vancomycin-resistant *S. aureus*; VISA, vancomycin-intermediate *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; LRSA, linezolid-resistant *S. aureus*.

[b]MSSA, methicillin-susceptible *S. aureus*. MIC values for ATCC 29213 (quality control strain) were in the range of those shown by CLSI for ceftaroline, linezolid, and vancomycin.

**TABLE 2** Amino acid changes of PBPs in MRSA strains that showed high MIC values (>32 μg/ml) to quinazolinones

| Strain | *spa* | MLST | CC | MIC for β-lactams[a] (μg/ml) | Compound with MIC >32 μg/ml | Location of amino acid substitutions for: | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | PBP 1 | PBP 2 | PBP 2a | PBP 3 | PBP 4 |
| C1657 | t127 | ST1 | CC1 | CPT, 0.5; BPR, 1; TZP, >256; MEM, >32 | Q2 | V617M | None | G246E | H556L | T189S |
| C5287 | t008 | ST8 | CC8 | CPT, 0.5; BPR, 1; TZP, >256; MEM, >32 | Q1 | None | R30K | None | None | None |
| K399 | t051 | ST247 | CC247 | CPT, 1; BPR, 2; TZP, >256; MEM, >32 | Q1, Q2, Q5 | None | None | G246E | None | None |
| C2878 | t1451 | ST398 | CC398 | CPT, 1; BPR, 1; TZP, >256; MEM, 6 | Q1, Q2, Q5 | F465L, D480E, D662N, S664T | D270E, T439V, D489E, T691A | S225R | None | E398A |

[a]TZP, piperacillin-tazobactam; MEM, meropenem; CPT, ceftaroline; BPR, ceftobiprole.

number LS483365.1). The resultant variations in the amino acids of the proteins are listed in Table 2, in addition to the MIC values for the β-lactams ceftaroline, ceftobiprole, meropenem, and piperacillin-tazobactam in these MRSA strains. The clonal complexes (CC) of these four selected strains were known to belong to CC1, CC8, CC247, and CC398, respectively (18–20). Strain C1657, with an MIC for compound Q2 of >32 μg/ml, showed amino acid substitutions in all PBPs except PBP 2. Strain C5287 (MIC for Q1, >32 μg/ml) exhibited a single amino acid substitution in PBP 2. Strain K399 (MICs for Q1, Q2, and Q5, >32 μg/ml) showed one substitution in PBP 2a (G246E) and none in the other PBPs. Finally, strain C2878 (MICs for Q1, Q2, and Q5, >32 μg/ml) exhibited amino acid changes in all PBPs except PBP 3. The relationship between the amino acid substitutions and the quinazolinone reduced activity is not clear and should be further investigated. However, mutations in PBP 1 and PBP 2a are of special interest, in that these two PBPs are targets of quinazolinones (7, 14).

Quinazolinone Q5 has shown similar *in vivo* activity to the closely related Q3 in mouse models of infection by MRSA (9). Whereas the MIC for MRSA NRS70, the strain used in mouse neutropenic thigh infection, is 8-fold lower for Q5 than for Q3, the plasma protein binding of Q5 is very high (99.6% ± 0.04% for Q5 versus 96.5% ± 0.7% for Q3) (9), decreasing the *in vivo* efficacy of Q5. Q4 has slightly lower MICs (Table 1); however, its PK properties are poor, resulting in low systemic exposure and very high clearance in mice (9). As a result, Q4 shows poor efficacy in the mouse peritonitis MRSA model of infection (9). Thus, both compounds Q3 and Q5 were selected for susceptibility testing (microdilution method) with 32 additional methicillin-susceptible and methicillin-resistant non-*aureus* staphylococci isolates, including 4 linezolid-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* (Table 3). Linezolid MIC was tested by the microdilution method in this study. All staphylococci included in the study belonged to the strain collection of the University of La Rioja (Spain). The range of MICs for Q5 in all *Staphylococcus pseudintermedius* strains was 4 to 16 μg/ml, with only one methicillin-resistant strain showing a value of 16 μg/ml. Within the coagulase-negative staphylococci (CoNS), all *S. epidermidis* and *S. haemolyticus* strains showed MIC values for Q5 of ≤0.125 μg/ml, and the *Staphylococcus saprophyticus* strain exhibited an MIC of 2 μg/ml. The higher MIC of *S. saprophyticus* for the quinazolinones may be explained by the fact that methicillin-sensitive *S. saprophyticus* is resistant to most of

**TABLE 3** MIC for quinazolinones Q3, Q5, and linezolid against non-*aureus* staphylococci

| Species[a] | No. of strains tested | MIC range (μg/ml) for: | | |
|---|---|---|---|---|
| | | Q3 | Q5 | Linezolid |
| MR *S. pseudintermedius* | 9 | 16 to 32 | 4 to 16 | 0.5 to 1 |
| MS *S. pseudintermedius* | 2 | 8 | 4 to 8 | 0.5 |
| MR *S. epidermidis* | 10 | 0.06 to >32 | ≤0.03 to 0.06 | 0.25 to 32[b] |
| MR *S. haemolyticus* | 10 | 0.125 to >32 | ≤0.03 to 0.125 | 0.5 to 16[b] |
| MS *S. saprophyticus* | 1 | >32 | 2 | 1 |

[a]MR, methicillin resistant; MS, methicillin susceptible.
[b]Two MR *S. epidermidis* and two MR *S. haemolyticus* were linezolid resistant.

the antibiotics used for treatment of urinary tract infections, including ceftriaxone (21). Accordingly, compound Q5 appears to be highly effective against some CoNS, such as *S. epidermidis* and *S. haemolyticus*, including linezolid-resistant ones, which are important clinically as opportunistic pathogens causing catheter-associated urinary tract or bloodstream infections (22–24). The activity of Q3 in non-*aureus* staphylococci was lower (Table 3).

In summary, the quinazolinones have excellent *in vitro* activity against a broad range of MRSA strains. Quinazolinone Q5 stands out for its low $MIC_{50}$/$MIC_{90}$ values against *S. aureus* isolates and its high antibacterial activity against other staphylococcal species.

## ACKNOWLEDGMENTS

The 94 Spanish clinical isolates were provided by C. Aspiroz, Hospital Royo Villanova, and A. Rezusta, Hospital Universitario Miguel Servet, Zaragoza, Aragón, Spain; J.M. Azcona, Hospital San Pedro, La Rioja, Spain; and E. Cercenado, Hospital General Universitario Gregorio Marañon, Madrid, Spain. The 108 U.S. clinical isolates of *S. aureus* were provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources, NIAID, NIH.

The work in Spain was funded by project SAF2016-76571-R from the Agencia Estatal de Investigación (AEI) of Spain and the Fondo Europeo de Desarrollo Regional (FEDER) of EU (to C.T.). S.C. was funded by a predoctoral fellowship from the University of La Rioja. The work in the United States was supported by NIH grants AI116548 (to M.C.) and AI104987 (to S.M.). Y.Q. is a Ruth L. Kirschtein National Research Service Award Fellow of the Chemistry-Biochemistry-Biology Interface Program at the University of Notre Dame, supported by training grant T32GM075762 from NIH.

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