AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

SUSCEPTIBILITY

# Activities of Oxadiazole Antibacterials against *Staphylococcus aureus* and Other Gram-Positive Bacteria

**Sara Ceballos,[a] Choon Kim,[b] Derong Ding,[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 activities of four oxadiazoles were investigated with 210 methicillin-resistant *Staphylococcus aureus* (MRSA) strains. $MIC_{50}$ and $MIC_{90}$ values of 1 to 2 and 4 $\mu$g/ml, respectively, were observed. We also evaluated the activity of oxadiazole ND-421 against other staphylococci and enterococci and in the presence of oxacillin for selected MRSA strains. The MIC for ND-421 is lowered severalfold in combination with oxacillin, as they synergize. The $MIC_{90}$ of ND-421 against vancomycin-resistant enterococci is $\leq$1 $\mu$g/ml.

**KEYWORDS** *Enterococcus*, MIC, MRSA, PBPs, *Staphylococcus* spp., oxadiazole

*Staphylococcus aureus* is a leading cause of health care-related infections, with more than 80,000 severe methicillin-resistant *S. aureus* (MRSA) infections per year in the United States alone (1). The oxadiazoles are a new class of antibacterials with activity against Gram-positive bacteria; they are bactericidal and target cell-wall biosynthesis (2, 3). The oxadiazoles emerged from *in silico* screening against the structure of penicillin-binding protein (PBP) 2a. PBPs are involved in bacterial cell-wall biosynthesis. There are four PBPs (PBP1 to PBP4) in *S. aureus*, with MRSA having acquired the additional PBP2a, which confers resistance to $\beta$-lactam antibiotics (4, 5). The oxadiazoles bind to PBP2a, and likely to other PBPs, as activity is observed against *S. aureus* strains without PBP2a (2). The structure-activity relationship of the oxadiazoles revealed that phenol, indole, or pyrazole rings on the left-hand side of the molecule were tolerated (2, 6). The lead oxadiazole, ND-421, shows efficacy in murine models of MRSA infection, similar or superior to that of linezolid (3, 7). In the present report, we selected four oxadiazoles (Fig. 1), including ND-421 (compound 3), and investigated their activities with 210 MRSA strains (108 strains from the United States and 102 from Spain). This collection included 54 MRSA strains with relevant mechanisms of resistance (vancomycin-, linezolid-, and *mecC*-related) (Table 1). The Spanish collection of 102 strains was obtained from different institutions and origins, including different clonal complexes widely distributed in Spanish hospitals. The remaining 108 strains in the US were obtained from BEI resources, including different clones and staphylococcal cassette chromosome *mec* element (SCC*mec*) types.

The MIC values of oxadiazoles 1 to 4 were determined by the microdilution method (8). *S. aureus* ATCC 29213 was used as the reference and quality control strain. Ceftaroline (9) and linezolid were included for comparison of antimicrobial activities with oxadiazoles. Table 1 shows the range of MIC values determined for the 210 MRSA strains (including the 54 strains with specific mechanisms of resistance), as well as the $MIC_{50}$ and $MIC_{90}$ values of these antibacterials. For oxadiazoles 1, 2, and 3, the $MIC_{50}$ and $MIC_{90}$ values were 2 and 4 $\mu$g/ml, respectively. Oxadiazole 1 contains a phenol ring, while 2 and 3 have the phenol group replaced by an indole ring (Fig. 1). Oxadiazole 4, containing a pyrazole ring, had $MIC_{50}$ and $MIC_{90}$ values of 1 and 4 $\mu$g/ml, respectively. A few strains showed higher MIC values for some oxadiazoles, as follows: 16 $\mu$g/ml (four

**Received** 7 March 2018 **Returned for modification** 28 March 2018 **Accepted** 26 May 2018

**Accepted manuscript posted online** 4 June 2018

**Citation** Ceballos S, Kim C, Ding D, Mobashery S, Chang M, Torres C. 2018. Activities of oxadiazole antibacterials against *Staphylococcus aureus* and other Gram-positive bacteria. Antimicrob Agents Chemother 62:e00453-18. https://doi.org/10.1128/AAC.00453-18.



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

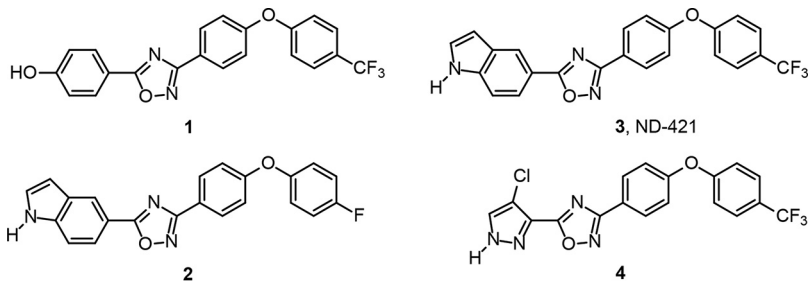


**FIG 1** Structures of the four oxadiazoles used in this study.

strains), 32 (one strain), or >32 μg/ml (six strains). The $MIC_{90}$ values of the oxadiazoles 1 to 4 were within 2-fold of those of linezolid and within 2- to 4-fold of that of ceftaroline (Table 1). Ceftaroline and the oxadiazoles are bactericidal, while linezolid is bacteriostatic. All of the *mecC*-mediated MRSA strains (which lack PBP2a but harbor PBP2c) (10), exhibited low MIC values for the oxadiazoles tested (MIC ≤ 4 μg/ml). For linezolid-resistant MRSA strains (LRSA), the MIC values for the oxadiazoles (2 to 8 μg/ml) were lower than those for linezolid (8 to >16 μg/ml). The MIC range for compound 3 (ND-421) in vancomycin-resistant MRSA isolates was 1 to 4 μg/ml, whereas vancomycin had an MIC of >32 μg/ml (Table 1).

The sequences of *pbp1*, *pbp2*, *mecA*, *pbp3*, and *pbp4* genes in four selected MRSA isolates that showed high MIC values (>32 μg/ml) for oxadiazoles 2 and 4 were analyzed by PCR and sequencing (Table 2) (11, 12). The nucleotide sequences and their translation into amino-acid sequences were aligned and compared with reference sequences available from the NCBI database, namely, methicillin-susceptible *Staphylococcus aureus* (MSSA) strain ATCC 25923 for *pbp1*, *pbp2*, *pbp3*, and *pbp4* (GenBank accession number CP009361.1) and MRSA strain N315 for *mecA* (GenBank accession number BA000018.3). The MRSA clonal complex 398 (CC398) strain (with an MIC of >32 μg/ml for compound 4) had four amino-acid changes in PBP1 (F465L, D480E, D662N, and S664T), four in PBP2 (D270E, T439V, D489E, and T691A), one in PBP2a (G246E), and one in PBP4 (E398A), but none in PBP3 (Table 2). No amino-acid changes in PBP1 and PBP2a were detected in the three MRSA CC5 strains (one strain with an MIC of >32 μg/ml for oxadiazole 4, and two strains for oxadiazole 2), although changes were identified in PBP2 (A285P ± A58V), PBP3 (K504R and D563E), and PBP4 (T189S ± A25T). The relationship between the mutations detected in *S. aureus* PBPs and oxadiazole resistance is not known; however, increased MIC is not seen for ND-421, indicating that replacement of the indole in ND-421 with the pyrazole ring in oxadiazole 4 and replacement of trifluoromethyl with fluorine in oxadiazole 2 increases resistance.

ND-421 synergized *in vitro* with β-lactam antibiotics, as well as with oxacillin, in a

**TABLE 1** MIC values (in μg/ml) for antibacterial agents against 210 MRSA strains and *S. aureus* ATCC 29213 and for selected MRSA strains with different mechanisms of resistance

| Antibacterial agent | MIC data for all MRSA strains (*n* = 210) | | | MIC range for: MRSA strains with specific mechanisms of resistance[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ | VRSA (*n* = 15) | VISA (*n* = 20) | hVISA (*n* = 6) | *mecC* MRSA (*n* = 7) | LRSA (*n* = 6) | *S. aureus* ATCC 29213 |
| Ceftaroline | 0.125–2 | 0.5 | 1 | 0.25–2 | 0.25–2 | 0.5–1 | 0.5–1 | 0.5–2 | 0.125 |
| Linezolid | 0.5–>16 | 2 | 2 | 1–2 | 0.5–2 | 0.5–2 | 1–2 | 8–>16 | 2 |
| Vancomycin | | | | >32 | 4–8 | 2–8 | ≤2 | 1–4 | 1 |
| Oxadiazole 1 | 1–32 | 2 | 4 | 2–4 | 1–4 | 4–32 | 1–2 | 4–8 | 1–2 |
| Oxadiazole 2 | 0.5–>32 | 2 | 4 | 1–4 | 1–8 | 2–8 | 1–2 | 2–8 | 0.5–1 |
| Oxadiazole 3[a] | 0.5–8 | 2 | 4 | 1–4 | 1–4 | 2–4 | 1–4 | 2–8 | 1–2 |
| Oxadiazole 4 | 0.25–>32 | 1 | 4 | 1–16 | 1–16 | 1–4 | 0.5–2 | 2–8 | 0.5–1 |

[a]Oxadiazole 3 = ND-421.

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

**TABLE 2** Mutations detected in PBPs in MRSA strains with high MIC values for oxadiazoles (>32 μg/ml)

| Strain | MRSA description[a] | | | | Compound (MIC [μg/ml]) | Location(s) of amino-acid substitution(s) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *spa* | MLST | CC | SCC*mec* | | PBP1 | PBP2 | PBP2a | PBP3 | PBP4 |
| C1992 | t011 | ST398 | CC398 | IVa | Oxadiazole 4 (>32)<br>ND-421 (2) | F465L<br>D480E<br>D662N<br>S664T | D270E<br>T439V<br>D489E<br>T691A | G246E[b] | None | E398A |
| C3203 | t067 | ST125 | CC5 | IVc | Oxadiazole 4 (>32)<br>ND-421 (4) | None | A285P | None | K504R<br>D563E | A25T<br>T189S |
| K370 | t067 | ST5 | CC5 | IVc | Oxadiazole 2 (>32)<br>ND-421 (4) | None | A285P | None | K504R<br>D563E | T189S |
| C2899 | t067 | ST125 | CC5 | IVc | Oxadiazole 2 (>32)<br>ND-421 (2) | None | A58V<br>A285P | None | K504R<br>D563E | A25T<br>T189S |

[a]MLST, multilocus sequence type; CC, clonal complex.
[b]The amino-acid change G246E is present in ceftaroline-susceptible *S. aureus* isolates and seems to be not related to resistance (15).

murine MRSA infection model (7). In this study, the MIC values of ND-421 in the absence and presence of oxacillin at 1/2, 1/4, and 1/8 MIC were determined with 11 selected MRSA strains that displayed high MIC values for this oxadiazole (five isolates with MICs of 8 μg/ml and six isolates with MICs of 4 μg/ml), using the microdilution method (8). The MICs of oxacillin ranged from 32 to >512 μg/ml. In three isolates with very high oxacillin MICs (≥512 μg/ml), we tested ND-421 in the presence of oxacillin at 1/4 and 1/8 MIC (but not at 1/2 MIC). The addition of oxacillin lowered the MICs for ND-421 to ≤0.03 to 2 μg/ml (with 1/2 and 1/4 MIC of oxacillin) or to ≤0.03 to 4 μg/ml (with 1/8 MIC of oxacillin). The ND-421 MIC values decreased from 8 μg/ml to 0.25 to 1 μg/ml in the presence of 1/4 MIC of oxacillin (Table 3). In the presence of β-lactam antibiotics, cooperation between the transpeptidase domain of PBP2a (inhibited by the oxadiazoles) and the transglycosylase domain of PBP2 (inhibited by oxacillin) is required for cell-wall synthesis (13). We hypothesize that this could be the basis of synergy between ND-421 and oxacillin.

The susceptibility of ND-421 to 55 additional Gram-positive isolates (32 non-*S. aureus* staphylococci and 23 vancomycin-resistant enterococci [VRE]) was evaluated (Table 4). VRE strains results in 20,000 annual drug-resistant enterococcal infections in the United States alone, resulting in 1,300 deaths (1). The MIC values in all cases were ≤2 μg/ml, with the exception of five methicillin-resistant (MR) coagulase-negative staphylococci strains, which showed MICs of >8 μg/ml (MR-*S. epidermidis* and MR-*S. haemolyticus*), and one *Enterococcus faecalis* strain carrying the *vanB2* gene, with an MIC of 4 μg/ml. The range of MICs for ND-421 exhibited by the 11 *S. pseudintermedius* strains was 0.25 to 2 μg/ml, and the range for most of the 15 *vanA/vanB1/vanB2*-carrying enterococci tested was ≤0.25 to 1 μg/ml (with only one exception, with an MIC of 4 μg/ml); the eight *Enterococcus gallinarum* and *Enterococcus casseliflavus* isolates with intrinsic vancomycin resistance (carrying *vanC1* and

**TABLE 3** The effect of oxacillin on MIC values for ND-421 in selected MRSA strains

| Strain | MRSA description | | | MIC (μg/ml) for: | | | | |
|---|---|---|---|---|---|---|---|---|
| | *spa* | MLST | CC | Oxacillin | ND-421 | ND-421 + 1/2 MIC oxacillin | ND-421 + 1/4 MIC oxacillin | ND-421 + 1/8 MIC oxacillin |
| K118 | t109 | ST228 | CC228 | >512 | 8 | ND[a] | 1 | 1 |
| C5282 | t067 | ST5 | CC5 | 512 | 8 | ND | 0.25 | 4 |
| C5269 | t067 | ST5 | CC5 | 512 | 8 | ND | 0.5 | 2 |
| C5382 | t067 | ST5 | CC5 | 128 | 4 | 0.25 | 2 | 4 |
| C5380 | t067 | ST5 | CC5 | 128 | 4 | ≤0.25 | 0.5 | 2 |
| C5171 | t067 | ST5 | CC5 | 128 | 4 | 2 | 1 | 1 |
| C3787 | t7892 | ST125 | CC5 | 128 | 4 | ≤0.25 | ≤0.25 | ≤0.25 |
| C3212 | t032 | ST22 | CC22 | 128 | 4 | ≤0.25 | 1 | 2 |
| C5294 | t008 | ST8 | CC8 | 64 | 8 | ≤0.03 | ≤0.03 | 0.125 |
| C4058 | t7892 | ST125 | CC5 | 64 | 4 | ≤0.25 | ≤0.25 | ≤0.25 |
| C5177 | t002 | ST5 | CC5 | 32 | 8 | ≤0.03 | ≤0.03 | ≤0.03 |

[a]ND, not determined (because of the very high MIC for oxacillin for these strains).

**TABLE 4** Range of MIC values for the oxadiazole ND-421 for *Staphylococcus* (non-*S. aureus*) and vancomycin-resistant *Enterococcus*[a] strains

| Species[b] | No. of strains tested | MIC range ($\mu$g/ml) |
|---|---|---|
| MR *Staphylococcus pseudintermedius* | 9 | 0.25–1 |
| MS *S. pseudintermedius* | 2 | 1–2 |
| MR *Staphylococcus epidermidis* | 10 | ≤0.25–>8 |
| MR *Staphylococcus haemolyticus* | 10 | ≤0.25–>8 |
| MS *Staphylococcus saprophyticus* | 1 | ≤0.25 |
| *Enterococcus faecium* carrying *vanA/vanB1/vanB2* | 6 | ≤0.125–0.25 |
| *Enterococcus faecalis* carrying *vanA* | 1 | 1 |
| *E. faecalis* carrying *vanB1* | 1 | 1 |
| *E. faecalis* carrying *vanB2* | 4 | 1–4 |
| *Enterococcus durans/Enterococcus hirae* carrying *van*A | 3 | ≤0.125 |
| *Enterococcus gallinarum/Enterococcus casseliflavus* | 8 | ≤0.125–1 |

[a]All *vanA/vanB*-carrying enterococci showed an MIC for vancomycin of ≥32 $\mu$g/ml, and *E. gallinarum/E. casseliflavus* showed an MIC of 8 to 16 $\mu$g/ml.
[b]MR, methicillin-resistant; MS, methicillin-susceptible.

*vanC2* genes, respectively) also showed an MIC range of ≤0.25 to 1 $\mu$g/ml (Table 4). In particular, the activity of ND-421 against VRE is of note. Linezolid has been reported to show $MIC_{90}$ values of 2 $\mu$g/ml for *E. faecalis* carrying *vanA*, *E. faecium* carrying *vanA*, and *E. faecium* carrying *vanB* (14). According to these results, ND-421, which was initially designed for PBP2a of MRSA, is also active in other Gram-positive bacteria, such as VRE, supporting the hypothesis that it targets other PBPs. In any case, regardless of the target, the result is the inhibition of cell-wall biosynthesis.

In summary, we show that the oxadiazoles have $MIC_{90}$ values of 4 $\mu$g/ml in 210 strains of MRSA. The MIC values for ND-421 are consistently lowered when used in combination with oxacillin. The compound ND-421 has shown an MIC of ≤1 $\mu$g/ml with most of VRE tested.

## ACKNOWLEDGMENTS

The work with 102 MRSA and other Gram-positive strains from Spain (S. Ceballos and C. Torres) was funded by project SAF2016-76571-R from the Agencia Estatal de Investigación (AEI) of Spain and the Fondo Europeo de Desarrollo Regional (FEDER). The work with 108 MRSA strains from the United States (C. Kim, D. Ding, S. Mobashery, and M. Chang) was supported by a grant from the National Institutes of Health (AI90818). S. Ceballos has a predoctoral fellowship from the University of La Rioja (Spain).

## REFERENCES

1. Centers for Disease Control and Prevention (CDC). 2013. Antibiotic resistance threats in the United States, 2013. Atlanta, GA.
2. O'Daniel PI, Peng Z, Pi H, Testero SA, Ding D, Spink E, Leemans E, Boudreau MA, Yamaguchi T, Schroeder VA, Wolter WR, Llarrull LI, Song W, Lastochkin E, Kumarasiri M, Antunes NT, Espahbodi M, Lichtenwalter K, Suckow MA, Vakulenko S, Mobashery S, Chang M. 2014. Discovery of a new class of non-$\beta$-lactam inhibitors of penicillin-binding proteins with gram-positive antibacterial activity. J Am Chem Soc 136:3664–3672. https://doi.org/10.1021/ja500053x.
3. Spink E, Ding D, Peng Z, Boudreau MA, Leemans E, Lastochkin E, Song W, Lichtenwalter K, O'Daniel PI, Testero SA, Pi H, Schroeder VA, Wolter WR, Antunes NT, Suckow MA, Vakulenko S, Chang M, Mobashery S. 2015. Structure-activity relationship for the oxadiazole class of antibiotics. J Med Chem 58:1380–1389. https://doi.org/10.1021/jm501661f.
4. Macheboeuf P, Contreras-Martel C, Job V, Dideberg O, Dessen A. 2006. Penicillin binding proteins: key players in bacterial cell cycle and drug resistance processes. FEMS Microbiol Rev 30:673–691. https://doi.org/10.1111/j.1574-6976.2006.00024.x.
5. Zapun A, Contreras-Martel C, Vernet T. 2008. Penicillin-binding proteins and $\beta$-lactam resistance. FEMS Microbiol Rev 32:361–385. https://doi.org/10.1111/j.1574-6976.2007.00095.x.
6. Ding D, Boudreau MA, Leemans E, Spink E, Yamaguchi T, Testero SA, O'Daniel PI, Lastochkin E, Chang M, Mobashery S. 2015. Exploration of the structure-activity relationship of 1,2,4-oxadiazole antibiotics. Bioorganic Med Chem Lett 25:4854–4857. https://doi.org/10.1016/j.bmcl.2015.06.044.
7. Janardhanan J, Meisel JE, Ding D, Schroeder VA, Wolter WR, Mobashery S, Chang M. 2016. *In vitro* and *in vivo* synergy of the oxadiazole class of antibacterials with $\beta$-lactams. Antimicrob Agents Chemother 60:5581–5588. https://doi.org/10.1128/AAC.00787-16.
8. Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—tenth edition. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA.
9. Otero LH, Rojas-Altuve A, Llarrull LI, Carrasco-López C, Kumarasiri M, Lastochkin E, Fishovitz J, Dawley M, Hesek D, Lee M, Johnson JW, Fisher JF, Chang M, Mobashery S, Hermoso JA. 2013. How allosteric control of *Staphylococcus aureus* penicillin binding protein 2a enables methicillin resistance and physiological function. Proc Natl Acad Sci U S A 110:16808–16813. https://doi.org/10.1073/pnas.1300118110.
10. Paterson GK, Harrison EM, Holmes MA. 2014. The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*. Trends Microbiol 22:42–47. https://doi.org/10.1016/j.tim.2013.11.003.
11. Argudín MA, Dodémont M, Taguemount M, Roisin S, de Mendonça R, Deplano A, Nonhoff C, Denis O. 2017. *In vitro* activity of ceftaroline against clinical *Staphylococcus aureus* isolates collected during a national

survey conducted in Belgian hospitals. J Antimicrob Chemother 72:56–59. https://doi.org/10.1093/jac/dkw380.
12. Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC. 2011. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 55:3765–3773. https://doi.org/10.1128/AAC.00187-11.
13. Pinho MG, de Lencastre H, Tomasz A. 2001. An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. Proc Natl Acad Sci U S A 98:10886–10891. https://doi.org/10.1073/pnas.191260798.
14. Krause KM, Renelli M, Difuntorum S, Wu TX, Debabov DV, Benton BM. 2008. *In vitro* activity of telavancin against resistant gram-positive bacteria. Antimicrob Agents Chemother 52:2647–2652. https://doi.org/10.1128/AAC.01398-07.
15. Kelley WL, Jousselin A, Barras C, Lelong E, Renzoni A. 2015. 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 59:1922–1930. https://doi.org/10.1128/AAC.04068-14.