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N-thiolated 2-oxazolidinones: A new family of antibacterial agents for methicillin-resistant *Staphylococcus aureus* and *Bacillus anthracis*

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Abstract—In this report, we describe a new family of N-thiolated 2-oxazolidinones having antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Bacillus anthracis*. The effect of ring substituents and stereochemistry on antibacterial activity of these oxazolidinones closely parallels that previously reported for N-thiolated β-lactam antibiotics.

The problem of bacterial drug resistance has reached a crisis level such that successful treatment of antibiotic-resistant infections in hospitals and health care centers can no longer be taken for granted. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming particularly difficult to treat with conventional antibiotics such as penicillin, leading to a sharp rise in clinical complications and deaths. The need for new antibacterial agents and protocols for treating MRSA infections is becoming extremely serious.

Recently, we reported a novel family of lipophilic N-thiolated β-lactams (Fig. 1) that are effective growth inhibitors of MRSA[1–7] and *Bacillus* species,[8] and whose mode of action and structure–activity profiles differ dramatically from those of traditional β-lactams.[9] Investigations in our laboratory[10]have shown that these β-lactam compounds can carry a wide range of substituents at the C_3 and C_4 centers; however, the *N*-organothio substituent is necessary for microbiological activity. The mechanism of action is under investigation but appears to depend on the ability of the compounds to transfer the organothio moiety onto a cellular thiol. This suggests that the role of the lactam ring is to provide a structural framework for the delivery of the thiol moiety and may not be absolutely required for the activity. To probe this possibility, and to expand on the structural diversity of anti-MRSA compounds available for clinical development, we decided to examine oxazolidinones as potential antibacterially active organothio carriers. Oxazolidinones are already recognized for their favorable pharmacological properties and are the only new class of antibacterial drugs introduced into clinical use in the last three decades.[11–13]

In the present study, a representative selection of differentially substituted N-thiolated 2-oxazolidinones **1–9** was prepared for antimicrobial screening by N-thiolation of the corresponding 2-oxazolidinones using protocols that have been previously reported by Miller and co-workers[14] for β-lactams. The structures of the compounds were confirmed by ^1H and ^{13}C NMR spectroscopy, and antibacterial assays were performed by Kirby–Bauer disk diffusion on agar plates according to NCCLS guidelines.[15] For these assays, we tested both

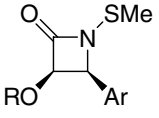

Figure 1. N-thiolated β-lactam.

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Table 1. Compound susceptibility measurements obtained from agar disk diffusion of oxazolidinones **1**–**9** against a methicillin-susceptible strain of *Staphylococcus aureus* (*S. aureus* ATCC 25923) and 10 strains of methicillin-resistant *S. aureus* (MRSA)

Bacterial strains	Lac	1	2	3	4	5	6	7	8	9	Pen G
S. aureus 849 (ATCC 25923)	25	31	16	17	21	19	30	29	20	21	33
MRSA USF919 (ATCC 43300)	—	32	11	19	20	19	30	28	21	13	—
MRSA USF920 (ATCC 33591)	—	32	0	19	16	19	28	28	18	15	—
MRSA USF652	30	30	12	17	16	19	30	30	19	12	8
MRSA USF653	30	32	19	20	20	20	31	30	27	25	15
MRSA USF654	26	30	14	18	20	22	29	26	23	20	10
MRSA USF655	25	30	14	17	20	22	29	28	22	18	14
MRSA USF656	28	31	15	19	22	20	31	28	22	20	12
MRSA USF657	27	30	12	19	21	22	29	27	22	19	12
MRSA USF658	26	31	14	17	18	21	29	27	16	19	19
MRSA USF659	24	28	16	19	19	20	26	26	22	22	16

In each case, 20 μg of the test compound in CH_2Cl_2 was applied to 6 mm cellulose disks prior to inoculation and incubation. The value corresponds to average diameter in mm (triplicate experiments) for the zone of growth inhibitions observed after 24 h of incubation at 37 °C. *S. aureus* (ATCC 25923) and methicillin-resistant *S. aureus* (labeled MRSA USF652-659 and USF919-920) were obtained from Lakeland Regional Medical Center, Lakeland, FL. Lac is the N-thiolated β-lactam shown in Figure 2. Pen G is penicillin G (potassium salt). Error values are within ±1 mm.

an ATCC strain of methicillin-susceptible *S. aureus* as well as 10 strains of methicillin-resistant *S. aureus* obtained either from ATCC sources or as clinical isolates from a local hospital. The zones of growth inhibition produced by the compounds against each of these microbes after 24 h of incubation are presented in Table 1. Our first goal was to determine the effect of substitution at the C_4 and C_5 centers of the oxazolidinone ring on anti-*Staphylococcus* activity. Accordingly, N-thiolated oxazolidinones **1**–**5** (as racemates) were examined and compared to two reference compounds, *N*-methylthio lactam **Lac** (Fig. 2) and penicillin G.

In almost every case, the five oxazolidinones displayed about equal activity against both *S. aureus* and MRSA, as did the corresponding β-lactam (Lac), and were uniformly much more effective than penicillin G (Pen G) against the MRSA strains. The most potent of these five oxazolidinones, compound **1**, produced zones of similar dimensions against *S. aureus* to that of penicillin G. Oxazolidinone **2**, on the other hand, showed much more moderate activity against both *S. aureus* and MRSA. Monosubstituted oxazolidinones **3**–**5** also possessed strong anti-MRSA activity, surpassing disubstituted derivative **2**, indicating that substituents can be placed at either the C_4 or C_5 centers, or at both, without significantly affecting bioactivity. This stands in contrast to previous observations from studies of mono- versus disubstituted N-thiolated β-lactams, in which disubstitution on the ring provides for the best anti-MRSA properties.[10] Additionally, replacement of the *N*-methylthio moiety of compound **4** for *N*-*sec*-butylthio (compound **5**[16]) leads to no significant improvement in anti-MRSA activity.[17]

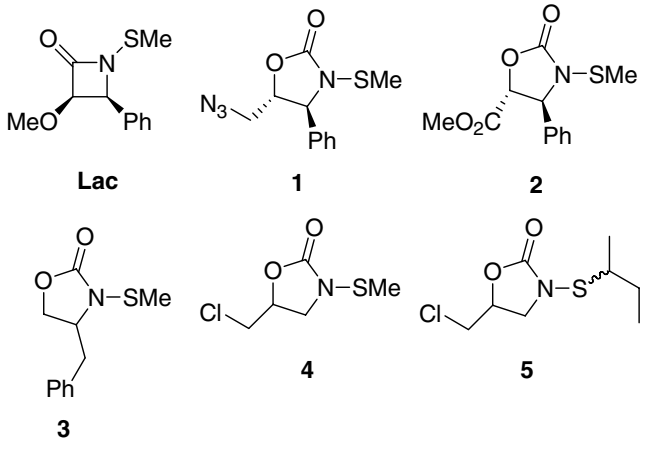


Figure 2. A reference N-thiolated β-lactam (**Lac**) and *N*-alkylthio 2-oxazolidinones **1**–**5.**

Enantiomerically paired oxazolidinones **6**, **7** and **8**, **9** were then evaluated for anti-MRSA properties to probe whether absolute stereochemistry was a determinant of activity (Fig. 3). These four compounds were individually prepared from their commercially available *N*-protio precursors and subjected to Kirby–Bauer testing. First, from these assays, we noted that the phenyl-substituted oxazolidinones **6** and **7** afforded somewhat larger inhibition zones than the isopropyl-bearing oxazolidinones **8** and **9**, indicating stronger anti-MRSA activity. Indeed, oxazolidinone **7** exhibited a lower broth MIC value (8 μg/mL) against both *S. aureus* and MRSA than that of oxazolidinone **8** (16 μg/mL). Second, the *S* enantiomer in each case was found, on average, to be slightly more active than the *R*-isomer. Indeed, the growth inhibition zones for *R*-configured compound **9** were visibly not as clear as they were for the *S*-stereoisomer **8**, indicative of incomplete growth inhibition. Thus, there may be a small but discernible difference in bioactivities of the two enantiomeric forms, which should be further evaluated.

We next turned to examine the antibacterial capabilities of the oxazolidinones against *Bacillus anthracis*, the causative agent of anthrax infections, and six other species of

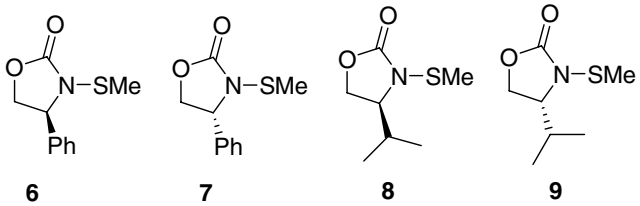


Figure 3. Enantiomerically pure *N*-methylthio 2-oxazolidinones **6**–**9.**

Table 2. Compound susceptibility measurements obtained from agar disk diffusion of oxazolidinones **6**–**9** against *Bacillus anthracis* (Sterne strain) and six other strains of *Bacillus*

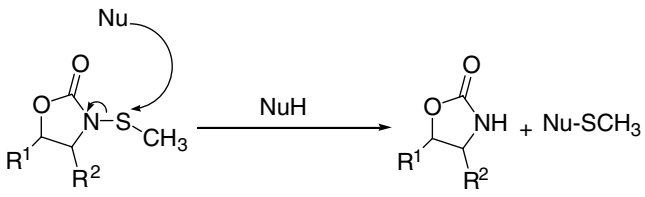
Bacillus species	6	7	8	9
B. anthracis	23	23	23	15
B. globigii	15	17	15	17
B. thurigensis	17	15	16	0
B. megaterium	18	20	18	10
B. subtilus	19	19	17	19
B. cereus	24	23	22	15
B. coagulans	17	17	17	0

In each case, 20 μg of the test compound in CH_2Cl_2 was applied to 6 mm cellulose disks prior to incoculation and incubation. The value corresponds to average diameter in mm (triplicate experiments) for the zone of growth inhibitions observed after 24 h of incubation at 37 °C. Error values of these measurements are ±1 mm.

Bacillus.[18] Concerns about the possible use of *B. anthracis* as a biological weapon have led to widespread efforts to develop antibiotics and vaccines for anthrax infections.[19] For this initial examination, we chose N-thiolated 2-oxazolidinones **6**–**9** for Kirby–Bauer testing. The data shown in Table 2 indicate that each of the *N*-methylthio 2-oxazolidinones inhibits the growth of all seven species of *Bacillus*. Of these four optically pure compounds, however, **6** and **7** had identical activity,while the *R* compound **9** possessed much weaker and more sporadic activity compared to that of the *S*-enantiomer **8**. The reasons for this seemingly anomalous, but reproducible, difference in bioactivity are still under investigation.

As previously described for N-thiolated β-lactams, the antibacterial activity of these agents shows only a small dependence on the ring substituents, but requires the *N*-alkylthio group.[10] In each case, N-thiolated 2-oxazolidinones exhibited antibacterial activity, whereas the corresponding *N*-protio oxazolidinones have no antibacterial activity. We therefore tentatively postulate that these N-thiolated oxazolidinones, like their β-lactam counterparts[10], react covalently with their biological target through transfer of the organothio side chain as shown in Scheme 1.

Further studies to assess the mode of action of these anti-MRSA, anti-*Bacillus* compounds, and to identify their cellular target(s), are currently underway in our laboratory and will be reported in due course.



Scheme 1.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.058.

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