Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2229–2231

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

N-Thiolated β-Lactams: Novel Antibacterial Agents for Methicillin-Resistant *Staphylococcus aureus*

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Received 10 January 2002; accepted 2 April 2002

Abstract—In this report we describe a new family of *N*-thiolated β-lactams that have antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The compounds show unprecedented structure–activity features and an unusual mode of action for a β-lactam antibiotic. © 2002 Elsevier Science Ltd. All rights reserved.

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming extremely difficult to treat with conventional antibiotics, leading to a sharp rise in clinical complications.[1,2] The need for new antibacterial agents and protocols for treating MRSA infections is serious. In this report we describe a novel family of lipophilic β-lactam antibacterials that are effective against MRSA, and whose mode of action and structure–activity profiles differ dramatically from those of traditional β-lactam drugs.[3] In addition, their selectivity for *Staphylococcus* bacteria over most other common microorganisms, and their stability to β-lactamase proteins, make these β-lactam molecules interesting leads for further investigation.

In this study, a selection of *N*-methylthio-substituted β-lactams (**1**–**9**, Fig. 1) were examined for antimicrobial activity by the Kirby–Bauer disk diffusion method on agar plates. A variety of common Gram-positive and Gram-negative bacteria were tested, including clinical isolates of methicillin-resistant *S. aureus* (MRSA). Table 1 gives the zones of growth inhibition that were observed after 24 h. The data indicate that lactams **1**–**9** are most active against *Staphylococcus* and *Micrococcus* bacteria, but act only weakly against *Neisseria gonorrhoeae*, *Bacteroides fragalis,* and *Haemophilus influenzae*. The compounds have no activity against other common microorganisms we examined, including *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Vibrio cholorae*, *Streptococcus pyrogenes (GAS)*, *Streptococcus*

Table 1. Compound susceptibility measurements obtained from agar disk diffusion experiments using 6-mm air-dried disks impregnated with 20 μg of the test compound

Bacterial strain	1	2	3	4	5	6	7	8	9	PenG
S. aureus (ATCC 25923)	27	18	25	26	15	12	14	23	27	34
MRSA USF652	31	19	30	30	17	12	14	23	28	8
MRSA USF653	30	23	30	30	22	14	16	27	28	16
MRSA USF654	28	16	26	26	16	12	12	23	27	10
MRSA USF655	29	17	25	26	14	14	12	23	29	14
MRSA USF656	30	19	28	28	18	11	13	25	28	12
MRSA USF657	30	17	27	28	18	12	10	23	26	12
MRSA USF658	27	16	26	27	17	12	12	22	26	19
MRSA USF659	25	15	24	24	11	13	12	20	24	15
S. epidermidis	30	23	31	29	20	12	20	25	28	50
S. simulans (ATCC 11631)	21	11	14	16	13	0	0	14	0	13
S. saprophyticus (ATCC 3552)	22	12	22	20	15	8	14	15	20	30
M. luteus	23	20	21	24	22	20	21	21	15	40
N. gonorrhoeae	13	11	14	19	12	11	12	12	11	0

The values correspond to the average diameters in mm (triplicate experiments) for the zone of growth inhibition observed after 24 h. *S. aureus* (ATCC 25923) and β-lactamase-producing strains of methicillin-resistant *S. aureus* (labeled as *MRSA* USF652–659) were obtained from Lakeland Regional Medical Center, Lakeland, FL. *S. epidermidis*, *S. simulans*, *S. saprophyticus,* and *M. luteus* are clinical isolates from University of South Florida Medical Clinic. *N. gonnorhoeae* (β-lactamase positive) was obtained from the Tampa Branch State Laboratory. PenG is penicillin G (potassium salt).
Small zones (<15 mm) were observed against *B. fragalis* and *H. influenzae 561*. No zones were observed against *K. pneumoniae 512*, *L. monocytogenes*, *V. cholerae 1018 (CDC E5906, toxin +)*, *V. cholerae 1019 (CDC 1074–78, toxin-)*, *S. pyrogenes (GAS)*, *S. agalactiae (GBS)*, *S. marcessens 519* (ATCC 29634), *S. typhimurium 515*, *P. aeruginosa* (ATCC 15442), *P. mirabilis*, *M. smegmatis*, *E. cloacae*, *or E. coli* (ATCC 23590).

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PII: S0960-894X(02)00343-8

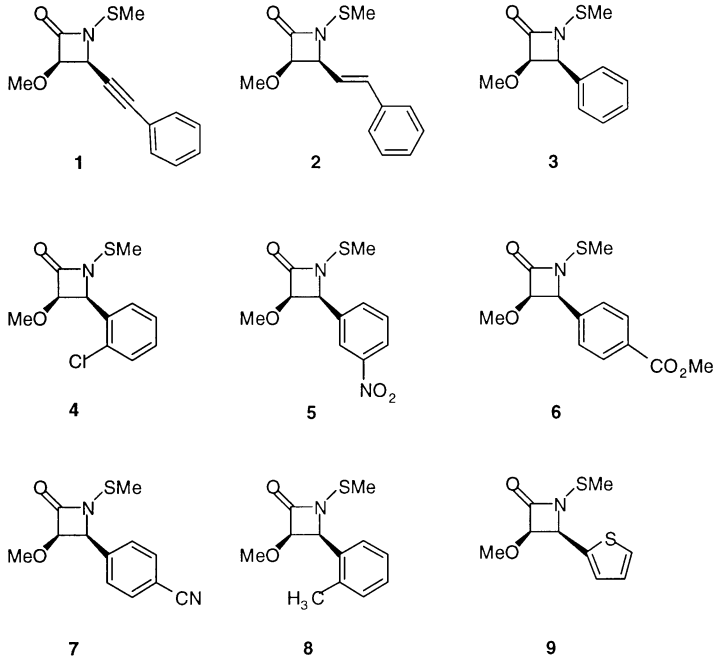

Figure 1. *N*-Methylthio β-lactam antibacterials **1**–**9**.

agalactiae (GBS), *Serratia marcessens*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Mycobacterium smegmatis*, *Enterobactor cloacae*, or *Escherichia coli*. Thus, the lactams provide a narrow-spectrum of antibacterial activity, with high selectivity for *Staphylococcus* species. Most intriguing is the observation that the lactams retain their full effectiveness against the β-lactamase producing MRSA mutants.

Preliminary structure–activity profiling of these active analogues suggests that the β-lactam ring can carry a wide variety of unsaturated substituents at the C_4 center, including alkynyl, alkenyl, aryl, or heteroaryl moieties (Table 2). However, the *N*-alkylthio substituent is required, since oxidation to the *N*-SO_2Me derivative or reduction to the *N*-H analogue completely destroys the biological activity. Minimum inhibitory concentration (MIC) values for lactam **4**, the most active analogue among the nine studied, were determined from agar dilution experiments[4] to be 15 μg/mL for *S. aureus* and 5–10 μg/mL for the MRSA strains. Lactam **4** is considerably more active in controlling MRSA than penicillin, whose MIC's were >64 μg/mL.

The structure–bioactivity patterns observed for these *N*-thiolated lactams are highly atypical for a β-lactam antibiotic. The compounds are lipophilic and devoid of the typical acidic ring functionality required for recognition by the penicillin binding proteins.[3] The implication that lactams **1**–**9** are not acting in the same manner or on the same enzymes as the traditional β-lactam drugs is supported by electron microscopy experiments, which confirm that cell morphology is unaffected by lactam **4**.[5] Whereas cells grown in the presence of penicillin G or vancomycin (inhibitors of cell wall biosynthesis) appear wrinkled, deformed, or even sheared upon high magnification, and take on a pink coloration upon Gram staining,[6] those treated with lactam **4** display no visible damage or morphological deformities. In fact, only small isolated colonies of surviving cells can be found, all of which appear perfectly normal in size, shape, smoothness, and clustering behavior. Gram staining of these cluster of colonies produces a uniform purple coloration indicative of cells having a fully formed and intact cell wall (identical to that of untreated cells). The complete absence of any deformed, fused, folded or pink-stained cells (after staining) within these colonies indicates that lactam **4** is not altering cell wall crosslinking. Pulse radiolabeling experiments[7,8] indicate that lactam **4** blocks the bacterial uptake of ^{3}H-uridine and ^{3}H-leucine at low (MIC) levels.

The fact that compounds **1**–**9** retain their full antibacterial activity against β-lactamase-producing strains of MRSA suggests that these lactams are unaffected by bacterial penicillinases. In vitro experiments confirm that lactams **1**–**9** are indeed hydrolytically-resistant to penicillinases for prolonged periods, and that they do not inhibit β-lactamase-induced cleavage of penicillin G. Thus, the compounds appear to be transparent to β-lactamase proteins. Moreover, the resilience these β-lactams have toward β-lactamases and the unprecedented structure–activity patterns[9] they display supports the hypothesis that the compounds are operating through a unique mode of action. Preliminary experiments indicate that the lactams are not cytotoxic to healthy mammalian cells (human fibroblasts) at concentrations more than three times the bacterial MIC's.[10] Our current studies are aimed at defining the biological mechanism and the cellular target of these fascinating new β-lactam antibacterials.

Table 2. Selected characterization data for lactams **1–9**

Lactam	Mp (°C)	^{1}H NMR data
1	74–76	δ 7.42 (d, J = 8.8 Hz, 2H), 7.30 (m, 3H), 4.72 (d, J = 4.8 Hz, 1H), 4.63 (d, J = 4.8 Hz, 1H), 3.56 (s, 3H), 2.58 (s, 3H)
2	92–94	δ 7.31 (m, 5H), 6.80 (d, J = 15.8 Hz, 1H), 6.25 (dd, J = 15.8, 9.4 Hz, 1H), 4.70 (d, J = 4.7 Hz, 1H), 4.41 (dd, J = 9.4, 4.7 Hz, 1H), 3.48 (s, 3H), 2.44 (s, 3H)
3	51–54	δ 7.31 (m, 5H), 4.76 (d, J = 4.9 Hz, 1H), 4.72 (d, J = 4.9 Hz, 1H), 3.08 (s, 3H), 2.29 (s, 3H)
4	71–73	δ 7.35 (d, J = 7.4 Hz, 1H), 7.24 (m, 3H), 5.29 (d, J = 4.9 Hz, 1H), 4.80 (d, J = 4.9 Hz, 1H), 3.16 (s, 3H), 2.40 (s, 3H)
5	Oil	δ 8.25 (ABm, 2H), 7.71 (d, J = 7.3 Hz, 1H), 7.61 (t, J = 7.7 Hz, 1H), 4.96 (d, J = 5.0 Hz, 1H), 4.85 (d, J = 5.0 Hz, 1H), 3.25 (s, 3H), 2.42 (s, 3H)
6	106–107	δ 8.06 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 4.88 (d, J = 4.9 Hz, 1H), 4.81 (d, J = 4.9 Hz, 1H), 3.92 (s, 3H) 3.15 (s, 3H), 2.37 (s, 3H)
7	88–90	δ 7.64 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 4.79 (ABm, 2H), 3.14 (s, 3H), 2.37 (s, 3H)
8	80–81	δ 7.23 (m, 4H), 5.11 (d, J = 5.0 Hz, 1H), 4.85 (d, J = 5.0 Hz, 1H), 3.21 (s, 3H), 2.45 (s, 3H), 2.37 (s, 3H)
9	Oil	δ 7.43 (d, J = 5.0 Hz, 1H), 7.21 (d, J = 2.9 Hz, 1H), 7.06 (dd, J = 5.0, 2.9 Hz, 1H), 5.08 (d, J = 4.7 Hz, 1H), 4.82 (d, J = 4.8 Hz, 1H), 3.32 (s, 3H), 2.30 (s, 3H)

References and Notes

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10. Cytotoxicity experiments were carried out in the laboratories of Professor Q. Ping Dou at the H. Lee Moffitt Cancer Center and Research Institute at University of South Florida.