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Biological Activity of BO-1236, a New Antipseudomonal Cephalosporin

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BO-1236, a new cephalosporin having an *N*-methyl-5,6-dihydroxyisoindolinium moiety on the 3-methylene of the cephem, showed potent activity against gram-negative organisms, including *Pseudomonas aeruginosa*. The in vitro activity of BO-1236 was superior or comparable to that of ceftazidime, cefotaxime, and cefoperazone in susceptibility tests with clinical isolates. BO-1236 was significantly more active than ceftazidime against *P. aeruginosa* strains susceptible or resistant to ceftazidime or gentamicin or both. MBCs were usually close to MICs, both of which were influenced by inoculum size to about the same degree as those of the other β-lactams. BO-1236 was stable to all types of β-lactamases except type I oxyiminocephalosporin-hydrolyzing enzyme, by which BO-1236 was slightly hydrolyzed. BO-1236 showed protective activity superior to that of ceftazidime and cefotaxime in experimental infections in mice caused by two strains of *P. aeruginosa* and showed activity comparable to that of ceftazidime and cefotaxime against other gram-negative bacterial infections.

Some new cephalosporins possess potent and extended antibacterial spectra and have made an important contribution to the treatment of bacterial infections (7). However, the search for new semisynthetic cephalosporins with improved spectra has intensified, since few of the cephalosporins are sufficiently active against glucose nonfermentative, gram-negative bacilli, including *Pseudomonas aeruginosa*. Furthermore, strains resistant to β-lactam antibiotics have been rapidly increasing in recent years (8).

We synthesized a new semisynthetic cephalosporin, BO-1236 (7-[(*z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(5,6-dihydroxy-2-methyl-2-isoindolium)methyl-3-cephem-4-carboxylate), the structure of which is shown in Fig. 1. BO-1236 exerts excellent activity against gram-negative bacteria, especially against nonfermenters, and that the two adjacent hydroxy groups on the isoindoline ring are requisite for the potent activity (S. Nakagawa, R. Ushijima, F. Nakano, N. Ban, K. Yamada, and A. Asai, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 363, 1985; M. Sanada, N. Hazumi, K. Matuda, and S. Nakagawa, 25th ICAAC, abstr. 364, 1985).

This paper describes the details of the biological activity of BO-1236 compared with those of ceftazidime (1, 11), cefoperazone (2, 6), and cefotaxime (4, 5).

MATERIALS AND METHODS

Antibiotics. BO-1236 and ceftazidime were synthesized at Okazaki Research Laboratories, Banyu Pharmaceutical Co., Ltd., Okazaki, Japan. Cefotaxime (Hoechst Japan, Tokyo), cefoperazone (Toyama Chemical Co., Ltd., Tokyo), penicillin G (Banyu Pharmaceutical Co., Ltd., Tokyo), and cephaloridine (Nippon Glaxo, Tokyo) were commercial products.

Organisms. The strains of gram-negative and gram-positive bacteria used in this study were recent clinical isolates. β-Lactamase-producing strains were kindly supplied by S. Mitsuhashi, Episome Institute, Gunma, Japan.

Determination of MICs. The MICs were determined by the agar dilution method. Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) was used for all assays except the following. Mueller-Hinton agar supplemented with 5% defibrinated horse blood was used for *Streptococcus* species, and Gifu anaerobic medium agar (Nissui Pharmaceutical Co., Ltd., Tokyo) was used for *Bacteroides fragilis*. Overnight cultures of test organisms were diluted to final concentrations of approximately 10^6 CFU/ml with buffered saline gelatin containing (grams per liter): NaCl, 8.5; NaH_2PO_4, 0.6; KH_2PO_4, 0.3; gelatin, 0.1. One loopful of diluted culture containing ca. 10^4 CFU was spotted with an inoculator (Microplanter; Sakuma Seisakusho, Tokyo) onto agar plates containing serial twofold dilutions of each antibiotic. These plates were incubated at 37°C for 18 h. For *Bacteroides fragilis* the incubation was done in a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.). The MIC was defined as the lowest concentration of antibiotic which prevented visible growth on agar.

Effect of inoculum size on MICs and MBCs. Test strains were cultured overnight in Mueller-Hinton broth. Cultures diluted 10-fold (10^5 to 10^8 CFU/ml) were inoculated into broth containing twofold serial dilutions of each antibiotic. The MBCs (the lowest concentrations which yielded >99.9% reduction in CFU after 18 h of incubation at 37°C) were determined by transferring 0.05 ml from each tube without visible growth for the MIC assay onto antibiotic-free Mueller-Hinton agar plates.

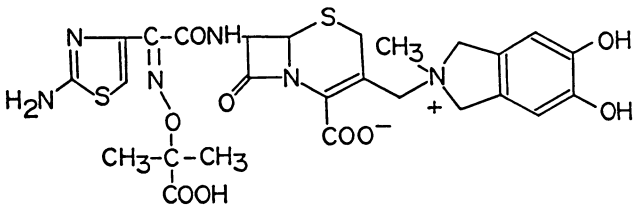

FIG. 1. Chemical structure of BO-1236.

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Stability to β-lactamases. The various β-lactamases used in this study were partially purified by the methods of Mitsuhashi and Inoue (3). Short-term β-lactamase hydrolysis analysis was performed with a UV spectrophotometer at the appropriate wavelength for each β-lactam antibiotic (10). Reaction mixtures consisted of 3 ml of a 100 μM substrate solution in 50 mM phosphate buffer (pH 7.0) and 50 μl of enzyme solution. The enzymatic reaction was carried out at 30°C in a water-jacketed spectrophotometer (model UV-240; Shimadzu Seisakusho, Kyoto, Japan), and the Lineweaver-Burk plot was determined.

In vivo tests. In vivo antibacterial activity was determined against experimental infections in mice. Test organisms were cultured with shaking overnight at 37°C in brain heart infusion broth (Difco). The cultures were diluted with 5% gastric mucin to provide 5- to 100-fold of the 50% lethal dose as determined in each study. Mice in six groups, each group containing 10 male, 4-week-old ddY mice (18 to 20 g), were intraperitoneally inoculated with 0.5 ml of the bacterial suspension. One hour after infection, the test compound was administered subcutaneously in aseptic water solution (0.1 ml/10 g of body weight). All the untreated mice died within 3 days at the inoculum used. The 50% effective dose was calculated by the probit method from the survival rate recorded on day 5 after infection.

Male ddY mice weighing 19 to 21 g were used for acute toxicity studies of BO-1236. Mice in groups of 10 were injected via the tail vein with 0.4 ml of BO-1236 in water. The concentrations of BO-1236 used were serial 1.15-fold dilutions in water from 150 to 74.6 mg/ml (six dose levels). The 50% lethal dose was calculated by the probit method from the lethal rate recorded on day 5 after dosing.

RESULTS

In vitro antibacterial activity. The results of comparative susceptibility tests with 1,172 clinical isolates are summarized in Table 1. BO-1236 was highly active against gram-negative bacteria, especially against glucose nonfermentative bacilli, including *Pseudomonas aeruginosa*. BO-1236 was active against *Streptococcus pyogenes* and *Streptococcus pneumoniae* with MICs for 90% of strains tested (MIC_{90}s) of 0.78 μg/ml, but the agent was less active against *Staphylococcus aureus* and was inactive against *Streptococcus faecalis*.

BO-1236 tested at 1.56 μg/ml or less inhibited 90% of all members of the family *Enterobacteriaceae*. The activity was superior or comparable to that of ceftazidime, cefotaxime, and cefoperazone. In particular, BO-1236 showed greater than eightfold-higher activity than the other antibiotics against *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Morganella morganii*. Geometric mean MICs for 36 strains resistant to ceftazidime (MIC ≧ 12.5 μg/ml) of four genera selected from the *Enterobactericeae* isolates are shown in Table 2 in comparison with those for ceftazidime-susceptible strains (MIC ≦ 6.25 μg/ml) of the same genera. It is interesting to note that the resistant strains were similarly resistant to cefotaxime and cefoperazone, but susceptible to BO-1236. The ceftazidime-susceptible strains were all susceptible to BO-1236 and other comparative β-lactams with the exception of cefoperazone, which showed a higher MIC for *Serratia marcescens* (MIC, 7.33 μg/ml). The resistant strains produced various levels of β-lactamase ranging from 9.8 to 0.7 U/mg of protein (mean, 2.83 U/mg), although the production of β-lactamase may not be the sole mechanism of resistance.

The most outstanding characteristic of BO-1236 was its superior activity against *Pseudomonas aeruginosa*, including strains resistant to ceftazidime or gentamicin or both (Table 1). The MIC_{90} of BO-1236 for strains susceptible to ceftazidime and gentamicin was 0.2 μg/ml, 8- and 32-fold more active than ceftazidime and cefoperazone, respectively. The MIC_{90} of BO-1236 for ceftazidime-resistant strains was 0.78 μg/ml, whereas the MIC_{90} of ceftazidime was 50 μg/ml, and the MIC_{90} for gentamicin-resistant strains was 1.56 μg/ml. BO-1236 was significantly more active in terms of MIC_{50} against *Pseudomonas cepacia* and *Pseudomonas maltophilia* than were the reference drugs, although MIC_{90}s were substantially higher. BO-1236 was highly active against *Acinetobacter calcoaceticus* with an MIC_{50} and MIC_{90} of 0.1 and 0.39 μg/ml, respectively, while the MIC_{50} and MIC_{90} of the reference drugs were 3.12 to 50 μg/ml and 12.5 to >100 μg/ml, respectively.

Haemophilus influenzae and *Branhamella catarrhalis* were susceptible to BO-1236, with MIC_{90}s of 0.78 and 0.39 μg/ml, respectively. *Bacteroides fragilis* was resistant to all the drugs tested, including BO-1236.

Effect of inoculum size on MICs and MBCs. The effect of inoculum size was determined with concentrations of 10^5, 10^6, 10^7, and 10^8 CFU of six strains of organisms (Table 3). In general, for BO-1236 MBCs were close to MICs. The MBCs were equal to the MICs at each concentration of *Escherichia coli* Juhl and *Klebsiella pneumoniae* BB5710. The MBC-to-MIC ratios for *Serratia marcescens* BB5713 and *Pseudomonas aeruginosa* BB5701 and BB5722 were less than 4 at most of the concentrations, which were comparable to those of ceftazidime. However, the MBC-to-MIC ratios were larger for *Pseudomonas aeruginosa* AKR17 resistant to ceftazidime. The MICs and MBCs of BO-1236, ceftazidime, and cefotaxime increased with increasing inoculum levels. In particular, a strong effect was observed with BO-1236 and the comparative β-lactams with *Serratia marcescens* BB5713 and *Pseudomonas aeruginosa* BB5722 and AKR17. The MICs and MBCs of BO-1236 for *Pseudomonas aeruginosa* BB5701 were the least affected by the increase in inoculum level.

Stability to β-lactamases. The stability of BO-1236 against enzymatic hydrolysis was tested with various types of β-lactamases. BO-1236 was stable to plasmid-mediated penicillinases from four strains of *E. coli*, W3630 (Rms212), W3630 (Rms 213), W3630 (Rte16), and ML4901 (PSE-1), and to chromosome-mediated cephalosporinases from *Enterobacter cloacae* GN7471, *Citrobacter freundii* GN346, *P. aeruginosa* GN10362, and *E. coli* GN5482. Furthermore, BO-1236 was also stable to a new type of β-lactamase, oxyiminocephalosporin-hydrolyzing enzyme (CXase) type II from *Pseudomonas maltophilia* GN12873 (L-1), but it was slightly hydrolyzed by type I CXase produced by GN12873 (L-2) (9), with a relative rate of 0.6 when the rate (V_{max}) of cephaloridine is set as 100. Ceftazidime was, in general, stable to β-lactamases, although it was slightly hydrolyzed by type II penicillinase from *E. coli* W3630 (Rms213) and type I and II CXase produced by *Pseudomonas maltophilia* GN12873, with relative rates of 3.8, 0.5, and 0.8, respectively.

In vivo tests. The in vivo antibacterial activity of BO-1236 in experimental infections in mice was compared with that of ceftazidime and cefotaxime (Table 4). BO-1236 showed excellent activity in infections caused by gram-negative bacteria, but it was less active in infection caused by *Staphylococcus aureus* Smith. BO-1236 was as efficacious as ceftazidime and cefotaxime against *E. coli* Juhl and *Klebsi-*

TABLE 1. Comparative in vitro activity of BO-1236 and other β-lactam antibiotics

Organism (no. of strains)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (104)	BO-1236	0.39–>100	25	50
	Ceftazidime	3.12–>100	3.12	50
	Cefotaxime	1.56–>100	1.56	12.5
	Cefoperazone	0.78–>100	1.56	>100
Staphylococcus epidermidis (50)	BO-1236	25–>100	25	50
	Ceftazidime	3.12–>100	3.12	12.5
	Cefotaxime	0.2–>100	0.78	3.12
	Cefoperazone	0.39–>100	0.78	1.56
Streptococcus pyogenes (30)	BO-1236	0.78–6.25	0.78	0.78
	Ceftazidime	0.05–0.39	0.05	0.05
	Cefotaxime	≦0.006–0.05	0.006	0.006
	Cefoperazone	0.025–0.78	0.05	0.2
Streptococcus pneumoniae (21)	BO-1236	0.1–0.78	0.39	0.78
	Ceftazidime	0.0125–0.2	0.1	0.1
	Cefotaxime	≦0.006–0.025	≦0.006	0.0125
	Cefoperazone	≦0.006–0.05	0.025	0.05
Streptococcus faecalis (54)	BO-1236	50–>100	>100	>100
	Ceftazidime	25–>100	>100	>100
	Cefotaxime	1.56–>100	50	>100
	Cefoperazone	12.5–>100	12.5	25
Escherichia coli (157)	BO-1236	≦0.006–1.56	0.0125	0.1
	Ceftazidime	0.025–3.12	0.05	0.1
	Cefotaxime	≦0.006–0.78	0.025	0.05
	Cefoperazone	≦0.006–12.5	0.1	1.56
Klebsiella pneumoniae (116)	BO-1236	0.0125–3.12	0.025	0.2
	Ceftazidime	0.025–3.12	0.05	0.1
	Cefotaxime	≦0.006–1.56	0.025	0.025
	Cefoperazone	0.025–>100	0.1	0.39
Serratia marcescens (50)	BO-1236	0.2–12.5	0.39	1.56
	Ceftazidime	0.1–50	0.39	3.12
	Cefotaxime	0.1–100	0.78	25
	Cefoperazone	0.39–>100	6.25	>100
Enterobacter aerogenes (50)	BO-1236	0.05–12.5	0.2	0.78
	Ceftazidime	0.1–>100	0.1	12.5
	Cefotaxime	0.05–>100	0.1	12.5
	Cefoperazone	0.1–>100	0.4	>100
Enterobacter cloacae (50)	BO-1236	0.1–>100	0.2	0.78
	Ceftazidime	0.05–>100	0.2	25
	Cefotaxime	0.0125–>100	0.1	50
	Cefoperazone	0.0125–>100	0.39	25
Citrobacter freundii (50)	BO-1236	0.0125–12.5	0.1	1.56
	Ceftazidime	0.05–>100	0.2	25
	Cefotaxime	0.025–50	0.1	12.5
	Cefoperazone	0.1–50	0.39	25
Providencia rettgeri (25)	BO-1236	0.1–6.25	0.1	0.78
	Ceftazidime	0.05–25	0.05	0.78
	Cefotaxime	≦0.006–12.5	0.025	0.78
	Cefoperazone	0.2–>100	1.56	12.5
Morganella morganii (25)	BO-1236	0.025–6.25	0.2	0.39
	Ceftazidime	0.05–>100	0.05	6.25
	Cefotaxime	0.0125–50	0.05	3.12
	Cefoperazone	0.39–100	0.78	12.5
Proteus vulgaris (25)	BO-1236	0.1–>100	0.1	0.78
	Ceftazidime	0.025–>100	0.05	0.78
	Cefotaxime	0.0125–>100	0.025	12.5
	Cefoperazone	0.39–50	0.78	12.5
Proteus inconstans (26)	BO-1236	0.05–1.56	0.05	0.2
	Ceftazidime	0.0125–1.56	0.05	0.39
	Cefotaxime	≦0.006–3.12	0.0125	0.39
	Cefoperazone	0.1–>100	1.56	12.5
Proteus mirabilis (25)	BO-1236	0.1–0.78	0.1	0.39
	Ceftazidime	0.025–0.1	0.025	0.025
	Cefotaxime	0.025–0.05	0.025	0.025
	Cefoperazone	0.39–3.12	0.39	1.56

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TABLE 1—*Continued*

Organism (no. of strains)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Pseudomonas aeruginosa, gentamicin and ceftazidime susceptible (110)	BO-1236	0.0125–12.5	0.025	0.2
	Ceftazidime	0.05–6.25	0.78	1.56
	Cefotaxime	0.1–100	6.25	25
	Cefoperazone	0.2–25	1.56	6.25
Pseudomonas aeruginosa, ceftazidime resistant (12)	BO-1236	0.1–3.12	0.78	0.78
	Ceftazidime	25–>100	25	50
	Cefotaxime	12.5–>100	>100	>100
	Cefoperazone	12.5–>100	50	>100
Pseudomonas aeruginosa, gentamicin resistant (28)	BO-1236	0.025–3.12	0.1	1.56
	Ceftazidime	0.39–100	1.56	25
	Cefotaxime	1.56–>100	25	50
	Cefoperazone	1.56–>100	12.5	50
Pseudomonas cepacia (14)	BO-1236	0.003–>100	0.006	12.5
	Ceftazidime	1.56–>100	0.78	>100
	Cefotaxime	3.12–>100	3.12	>100
	Cefoperazone	3.12–>100	3.12	>100
Pseudomonas maltophilia (25)	BO-1236	0.05–>100	3.12	>100
	Ceftazidime	1.56–>100	>100	>100
	Cefotaxime	3.12–>100	>100	>100
	Cefoperazone	3.12–>100	>100	>100
Acinetobacter calcoaceticus (35)	BO-1236	0.1–25	0.1	0.39
	Ceftazidime	0.78–>100	3.12	12.5
	Cefotaxime	1.56–100	6.25	25
	Cefoperazone	6.25–>100	50	>100
Alcaligenes faecalis (25)	BO-1236	0.025–12.5	0.1	6.25
	Ceftazidime	0.2–12.5	0.78	6.25
	Cefotaxime	0.39–50	1.56	25
	Cefoperazone	0.2–12.5	1.56	6.25
Haemophilus influenzae (40)	BO-1236	0.05–0.78	0.39	0.78
	Ceftazidime	0.025–0.78	0.1	0.2
	Cefotaxime	≦0.006–0.1	0.025	0.05
	Cefoperazone	≦0.006–0.78	0.0125	0.05
Branhamella catarrhalis (14)	BO-1236	0.2–0.39	0.2	0.39
	Ceftazidime	0.025–0.05	0.025	0.05
	Cefotaxime	0.025–0.39	0.2	0.39
	Cefoperazone	0.2–1.56	0.78	1.56
Bacteroides fragilis (25)	BO-1236	3.12–>100	12.5	>100
	Ceftazidime	3.12–>100	6.25	>100
	Cefotaxime	0.78–>100	1.56	>100
	Cefoperazone	3.12–>100	6.25	>100

ella pneumoniae BB5710. BO-1236 was as active as ceftazidime against *Serratia marcescens* BB5713 and was about 20-fold more active than cefotaxime. BO-1236 exhibited in vivo activity which was superior to ceftazidime against two strains of *Pseudomonas aeruginosa*. BO-1236 was comparable to ceftazidime against *Pseudomonas aeruginosa* BB5701 and considerably more active than ceftazidime against *Pseudomonas aeruginosa* BB5722 and BB5746.

The 50% lethal dose of BO-1236 for male ddY mice was 2,201 mg/kg (95% confidence limit, 2,081 to 2,329 mg/kg). The maximum dose of BO-1236 which caused no death was 1,715 mg/kg.

TABLE 2. Comparison of activity of BO-1236 with that of β-lactams against ceftazidime-resistant and susceptible strains

Organism	Susceptibility[a] (no. of strains)	Geometric mean MIC (μg/ml)[b]			
		BO-1236	CAZ	CTX	CPZ
Serratia marcescens	R (4)	1.6	42.0	84.1	42.0
	S (46)	0.53	0.34	0.94	7.33
Enterobacter cloacae	R (14)	0.43	60.9	86.2	95.2
	S (36)	0.27	0.25	0.19	0.40
Enterobacter aerogenes	R (7)	0.53	55.2	60.9	55.2
	S (43)	0.28	0.20	0.18	0.77
Citrobacter freundii	R (11)	1.2	56.7	32.2	56.7
	S (39)	0.13	0.41	0.19	0.86

[a] R, Ceftazidime-resistant strains (MIC, ≧12.5 μg/ml); S, ceftazidime-susceptible strains (MIC, ≦6.25 μg/ml).
[b] CAZ, Ceftazidime; CTX, cefotaxime; CPZ, cefoperazone.

TABLE 3. Effect of inoculum size on MICs and MBCs of BO-1236

Organism	Inoculum size (CFU/ml)	BO-1236		CAZ[a]		CTX[a]	
		MIC[b]	MBC[b]	MIC	MBC	MIC	MBC
Escherichia coli Juhl	0.82×10^8	12.5	12.5	6.25	6.25	1.56	1.56
	0.82×10^7	3.12	3.12	1.56	1.56	0.39	0.39
	0.82×10^6	0.78	0.78	0.1	0.2	0.2	0.2
	0.82×10^5	0.39	0.39	0.1	0.2	0.05	0.05
Klebsiella pneumoniae BB5710	0.98×10^8	6.25	6.25	6.25	6.25	0.78	0.78
	0.98×10^7	3.12	3.12	1.56	3.12	0.39	0.39
	0.98×10^6	1.56	1.56	0.2	0.39	0.1	0.1
	0.98×10^5	0.39	0.39	0.39	0.39	0.1	0.1
Serratia marcescens BB5713	0.78×10^8	>100	>100	>100	>100	>100	>100
	0.78×10^7	50	>100	>100	>100	>100	>100
	0.78×10^6	3.12	3.12	0.39	0.78	25	100
	0.78×10^5	0.78	3.12	0.39	0.78	0.78	0.78
Pseudomonas aeruginosa BB5701	1.3×10^8	1.56	12.5	12.5	>100	>100	>100
	1.3×10^7	3.12	3.12	3.12	12.5	>100	>100
	1.3×10^6	1.56	3.12	1.56	12.5	50	>100
	1.3×10^5	0.78	3.12	0.78	12.5	25	>100
Pseudomonas aeruginosa BB5722	1.7×10^8	>100	>100	>100	>100	>100	>100
	1.7×10^7	0.39	1.56	1.56	1.56	12.5	>100
	1.7×10^6	0.39	0.39	1.56	1.56	12.5	50
	1.7×10^5	0.2	0.39	0.78	0.78	12.5	50
Pseudomonas aeruginosa AKR17	6.9×10^8	>100	>100	>100	>100	>100	>100
	6.9×10^7	25	>100	>100	>100	>100	>100
	6.9×10^6	3.12	50	>100	>100	>100	>100
	6.9×10^5	1.56	25	>100	>100	>100	>100

[a] CAZ, Ceftazidime; CTX, cefotaxime.
[b] In micrograms per milliliter. The MBC was defined as the lowest concentration which yielded >99.9% reduction in CFU after 18 h of incubation at 37°C.

DISCUSSION

BO-1236 possesses potent antibacterial activity against gram-negative bacteria, including *P. aeruginosa*. BO-1236 showed considerably lower MIC_{90}s and MIC_{50}s relatively closer to MIC_{90}s for *Serratia marcescens*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Morganella morganii* than did ceftazidime, cefotaxime, and cefoperazone. It is noteworthy that strains resistant to ceftazidime selected from the members of the *Enterobacteriaceae* tested were simlarly resistant to cefotaxime and cefoperazone, whereas they were susceptible to BO-1236. BO-1236 was distinguished from ceftazidime and two other

TABLE 4. Protective effect of BO-1236 on experimental infections in mice[a]

Strain	Inoculum size (CFU/mouse)	Compound[b]	MIC (μg/ml)[c]	ED_{50} (mg/kg)[d] (95% confidence limit)
Staphylococcus aureus Smith	3.3×10^6	BO-1236	100	24.2 (13.7–32.5)
		CAZ	100	8.4 (6.3–10.9)
		CTX	12.5	3.0 (2.6–3.6)
Escherichia coli Juhl	1.8×10^6	BO-1236	0.025	0.05 (0.03–0.08)
		CAZ	0.1	0.02(0.005–0.04)
		CTX	0.1	0.09 (0.06–0.12)
Klebsiella pneumoniae BB5710	3.7×10^6	BO-1236	0.025	0.04 (0.02–0.06)
		CAZ	0.05	0.11 (0.07–0.19)
		CTX	0.05	0.09(0.07–0.13)
Serratia marcescens BB5713	1.9×10^5	BO-1236	0.39	0.66 (0.14–1.35)
		CAZ	0.39	0.50 (0.3–1.13)
		CTX	1.56	15.2 (8.4–61.4)
Pseudomonas aeruginosa BB5701	1.9×10^5	BO-1236	3.12	2.2 (1.1–3.4)
		CAZ	3.12	4.1 (0.17–8.1)
		CTX	25	>227
Pseudomonas aeruginosa BB5722	3.5×10^5	BO-1236	0.78	1.4 (0.5–2.4)
		CAZ	1.56	13.0 (7.5–23.2)
		CTX	50	>137
Pseudomonas aeruginosa BB5746	1.2×10^5	BO-1236	0.2	3.9 (2.3–6.6)
		CAZ	0.78	18.7 (10.5–38.4)
		CTX	12.5	>114

[a] Four-week-old male ddY strain mice in groups of 10 were infected intraperitoneally with the strains in 5% gastric mucin. Therapy was given subcutaneously 1 h after challenge.
[b] CAZ, Ceftazidime; CTX, cefotaxime.
[c] Agar dilution method with Mueller-Hinton agar at 37°C for 18 h.
[d] ED_{50} (50% effective dose) was determined as described in the text.

β-lactams by its potent activity against *Pseudomonas aeruginosa*. BO-1236 was significantly more active than ceftazidime against *Pseudomonas aeruginosa* strains susceptible to ceftazidime and, in addition, inhibited completely the strains resistant to ceftazidime or gentamicin or both at concentrations of 3.12 μg/mg or less.

The MBC-to-MIC ratios of BO-1236 were, in general, less than 4, except those for a strain of *Pseudomonas aeruginosa* resistant to ceftazidime. Inoculum size effects were generally similar to those of ceftazidime and cefotaxime. BO-1236 was stable to most of the β-lactamases under the conditions of the assay utilized, but further kinetic study is needed to better understand the interactions with β-lactamases. The potent antibiotic activity of BO-1236 against gram-negative bacteria including *Pseudomonas aeruginosa* appears to be largely attributable to its stability to β-lactamases.

The in vivo activity of BO-1236 in experimental infections in mice reflected the in vitro activity. BO-1236 was less active against infection caused by *Staphylococcus aureus*, but showed strong activity against gram-negative bacterial infections. BO-1236 was significantly more efficacious than ceftazidime against infections caused by *Pseudomonas aeruginosa* BB5722 and BB5746. The excellent antipseudomonal activity including strains resistant to ceftazidime, cefotaxime, cefoperazone, and gentamicin offers a great advantage, considering the increased incidence of refractory pseudomonal infections, especially in immunocompromised patients. These results and the low acute toxicity warrant further studies of BO-1236 as a useful antibacterial agent.

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