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In Vitro Activity of a New Carbapenem Antibiotic, BO-2727, with Potent Antipseudomonal Activity

SUSUMU NAKAGAWA, TERUTAKA HASHIZUME,* KOUJI MATSUDA, MINORU SANADA, OSAMU OKAMOTO, HIROSHI FUKATSU, AND NOBUO TANAKA

Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., 3 Okubo, Tsukuba 300-33, Japan

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BO-2727, a new 1-β-methyl-carbapenem, was active at concentrations of 6.25 μg/ml or less against gram-positive and gram-negative bacteria, including some imipenem- and/or meropenem-resistant (MICs, ≥12.5 μg/ml) *Pseudomonas aeruginosa* strains, against which it proved generally fourfold more active than imipenem and meropenem. BO-2727's antipseudomonal activity and its broad spectrum merit further investigation for clinical use by itself, since it was stable in the presence of renal dehydropeptidase I.

Among a variety of β-lactam antibiotics, imipenem was the first carbapenem antibiotic with a broad spectrum including *Enterococcus faecalis* and *Pseudomonas aeruginosa* (3, 4, 6). Imipenem is, however, not used alone clinically because of its instability in the presence of dehydropeptidase I (DHP-I). Carbapenem antibiotics such as panipenem (RS-533), meropenem (SM-7338), and biapenem (LJC10,627) have been developed since the introduction of imipenem (8, 9, 13, 14). Meropenem and biapenem, both of which have a β-methyl group at the 1 position of the nucleus, were reported to overcome instability in the presence of DHP-I without the help of a DHP-I inhibitor (5, 12) and to have increased potency against gram-negative bacteria, including *P. aeruginosa*. However, the emergence of carbapenem-resistant *P. aeruginosa* has continued to be a concern (7, 10). In the course of modification of carbapenem 2-side chains, we synthesized a new parenteral 1β-methyl carbapenem, BO-2727 (1*R*,5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-{(3*S*,5*S*)-5-[(*R*)-1-hydroxy-3-*N*-methylaminopropyl]pyrrolidin-3-ylthio}-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride hydrate (Fig. 1). The introduction of the methyl group at the 1β position and the pyrrolidinylthio side chain carrying a 1-hydroxy-3-*N*-methylamino-propyl group into the 2 position improved both stability in the presence of DHP-I and activity against *Staphylococcus aureus* and *P. aeruginosa*. In this report, we describe the in vitro antibacterial activities of BO-2727 against clinical isolates and its stability in the presence of DHP-I, and we compare the results with those obtained with reference antibiotics.

BO-2727 and meropenem were synthesized at the Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Tsukuba, Japan. Imipenem and amikacin were products of Banyu Pharmaceutical Co., Ltd., Tokyo, Japan. Ceftazidime was purchased from Nippon Glaxo Co., Ltd., Tokyo, Japan. The antibiotics were dissolved in a 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.0) on the day of use.

The clinical isolates used in this study were our stock cultures which have been collected since 1983 in Japan. Susceptibility testing was performed by a standard agar dilution technique with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) based on the standards of the Japan Society of Chemotherapy (2). The medium was supplemented with 5% horse blood for streptococci, and chocolate agar was used for *Haemophilus influenzae*. For *Bacteroides fragilis*, GAM agar (Nissui Seiyaku Co., Ltd., Tokyo, Japan) was used. The culture grown overnight at 37°C was diluted to 10^6 CFU/ml, and about 2×10^3 CFU of the culture per spot was inoculated onto agar plates containing serial twofold dilutions of antibiotics with a replicating device (Microplanter; Sakuma Seisakusyo, Tokyo, Japan). The plates were incubated at 37°C for 20 h, except for methicillin-resistant *S. aureus* and *B. fragilis*, which were incubated at 35°C for 20 h in Mueller-Hinton agar supplemented with 4% sodium chloride and at 37°C for 24 h in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.), respectively. The MIC was defined as the lowest concentration of antibiotic which prevented visible growth.

The susceptibility of BO-2727 to hydrolysis by renal DHP-I was compared with those of imipenem and meropenem by using partially purified swine renal DHP-I (specific activity, 0.3 U/mg of protein). One unit of activity was defined as the amount of enzyme hydrolyzing 1 μmol of glycyldehydrophenylalanine per min when the substrate (50 μM) was incubated at 35°C in 50 mM MOPS buffer, pH 7.0. The reaction mixture consisted of 50 μM substrate and 0.04 U of DHP-I per ml in 50 mM MOPS buffer (pH 7.0). Hydrolysis was monitored spectrophotometrically at 298, 299, and 298 nm for BO-2727, imipenem, and meropenem, respectively. The relative hydrolysis rate was determined, taking the hydrolysis rate of imipenem as 1.0.

The in vitro antibacterial activity of BO-2727 against the clinical isolates of 21 species was compared with those of meropenem, imipenem, and ceftazidime (Table 1). In tests with *P. aeruginosa*, amikacin was also examined. BO-2727 was active against gram-positive and gram-negative bacteria. It inhibited all strains of methicillin-susceptible *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Strep-*

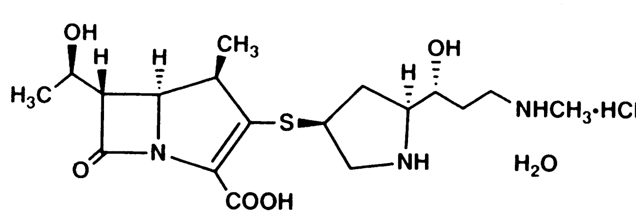

FIG. 1. Chemical structure of BO-2727.

* Corresponding author.

TABLE 1. Comparative in vitro antibacterial activities of BO-2727 and reference antibiotics

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (27)	BO-2727	0.05–0.39	0.1	0.39
	Meropenem	0.1–0.78	0.1	0.78
	Imipenem	0.025–0.1	0.025	0.1
	Ceftazidime	6.25–100	6.25	100
Staphylococcus aureus, methicillin resistant (50)	BO-2727	6.25–25	12.5	25
	Meropenem	6.25–50	25	50
	Imipenem	3.13–100	25	100
	Ceftazidime	>100–>100	>100	>100
Staphylococcus epidermidis (22)	BO-2727	0.012–0.78	0.05	0.78
	Meropenem	0.05–1.56	0.1	1.56
	Imipenem	≤0.006–0.2	0.012	0.2
	Ceftazidime	1.56–12.5	6.25	12.5
Streptococcus pyogenes (13)	BO-2727	≤0.006–0.78	≤0.006	0.05
	Meropenem	≤0.006–0.78	0.012	0.1
	Imipenem	≤0.006–0.39	≤0.006	0.1
	Ceftazidime	0.1–1.56	0.1	0.78
Streptococcus pneumoniae (13)	BO-2727	0.012–0.78	0.025	0.025
	Meropenem	0.012–0.78	0.025	0.025
	Imipenem	≤0.006–0.2	≤0.006	0.012
	Ceftazidime	0.2–3.13	0.2	1.56
Enterococcus faecalis (25)	BO-2727	0.39–3.13	1.56	3.13
	Meropenem	0.78–6.25	6.25	6.25
	Imipenem	0.39–1.56	0.78	1.56
	Ceftazidime	100–>100	>100	>100
Escherichia coli (13)	BO-2727	0.05–0.1	0.05	0.05
	Meropenem	0.012–0.05	0.025	0.025
	Imipenem	0.1–0.2	0.2	0.2
	Ceftazidime	0.05–0.2	0.2	0.2
Klebsiella pneumoniae (14)	BO-2727	0.05–0.1	0.05	0.1
	Meropenem	0.025–0.05	0.025	0.05
	Imipenem	0.1–0.2	0.2	0.2
	Ceftazidime	0.05–3.13	0.1	0.2
Serratia marcescens (33)	BO-2727	0.1–0.78	0.2	0.78
	Meropenem	0.025–0.78	0.05	0.39
	Imipenem	0.2–1.56	0.39	0.78
	Ceftazidime	0.1–50	0.39	6.25
Enterobacter cloacae (12)	BO-2727	0.05–0.2	0.05	0.1
	Meropenem	0.025–0.39	0.05	0.1
	Imipenem	0.2–0.39	0.2	0.39
	Ceftazidime	0.1–25	0.2	12.5
Citrobacter freundii (13)	BO-2727	0.05–1.56	0.1	0.78
	Meropenem	0.025–1.56	0.025	0.78
	Imipenem	0.2–3.13	0.39	3.13
	Ceftazidime	0.1–>100	0.39	100
Proteus mirabilis (13)	BO-2727	0.1–0.39	0.2	0.39
	Meropenem	0.05–0.1	0.05	0.05
	Imipenem	0.2–1.56	0.78	1.56
	Ceftazidime	0.05–0.1	0.05	0.1
Proteus inconstans (24)	BO-2727	0.025–0.78	0.78	0.78
	Meropenem	≤0.006–0.2	0.05	0.1
	Imipenem	0.05–1.56	0.78	0.78
	Ceftazidime	0.05–6.25	0.1	1.56

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TABLE 1—*Continued*

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Proteus vulgaris (26)	BO-2727	0.05–3.13	0.78	1.56
	Meropenem	0.025–0.39	0.05	0.2
	Imipenem	0.2–6.25	1.56	3.13
	Ceftazidime	0.025–3.13	0.1	3.13
Providencia rettgeri (22)	BO-2727	0.025–0.78	0.2	0.78
	Meropenem	0.012–0.39	0.05	0.05
	Imipenem	0.05–1.56	0.39	0.78
	Ceftazidime	0.025–12.5	0.05	0.39
Morganella morganii (13)	BO-2727	0.39–1.56	0.78	1.56
	Meropenem	0.05–0.2	0.1	0.2
	Imipenem	1.56–3.13	1.56	3.13
	Ceftazidime	0.05–>100	0.1	25
Haemophilus influenzae (20)	BO-2727	0.1–0.78	0.39	0.78
	Meropenem	0.012–0.1	0.05	0.05
	Imipenem	0.1–3.13	0.78	1.56
	Ceftazidime	0.05–3.13	0.1	0.1
Branhamella catarrhalis (15)	BO-2727	≤0.006–0.025	0.012	0.025
	Meropenem	≤0.006–≤0.006	≤0.006	≤0.006
	Imipenem	≤0.006–0.025	0.012	0.025
	Ceftazidime	0.012–0.05	0.025	0.05
Pseudomonas aeruginosa, imipenem susceptible (80)	BO-2727	0.1–3.13	0.39	1.56
	Meropenem	0.05–12.5	0.39	3.13
	Imipenem	0.2–6.25	0.78	3.13
	Ceftazidime	0.39–>100	3.13	50
	Amikacin	0.39–50	3.13	12.5
Pseudomonas aeruginosa, imipenem resistant (14)	BO-2727	3.13–12.5	3.13	6.25
	Meropenem	1.56–25	12.5	25
	Imipenem	12.5–50	25	25
	Ceftazidime	1.56–100	12.5	50
	Amikacin	0.78–25	6.25	25
Pseudomonas aeruginosa meropenem resistant (10)	BO-2727	0.78–12.5	3.13	6.25
	Meropenem	12.5–25	12.5	25
	Imipenem	1.56–25	25	25
	Ceftazidime	6.25–>100	12.5	>100
	Amikacin	3.13–25	12.5	25
Acinetobacter calcoaceticus (12)	BO-2727	0.1–1.56	0.2	0.39
	Meropenem	0.2–0.78	0.39	0.78
	Imipenem	0.2–0.39	0.2	0.39
	Ceftazidime	1.56–12.5	3.13	6.25
Pseudomonas cepacia (12)	BO-2727	0.39–12.5	3.13	12.5
	Meropenem	0.39–3.13	0.39	3.13
	Imipenem	0.39–6.25	3.13	6.25
	Ceftazidime	0.78–50	0.78	50
Bacteroides fragilis (27)	BO-2727	0.39–1.56	0.39	0.78
	Meropenem	0.1–1.56	0.1	0.39
	Imipenem	0.05–0.78	0.05	0.2
	Ceftazidime	3.13–>100	12.5	>100

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

tococcus pneumoniae, and *E. faecalis* at concentrations of 3.13 μg/ml or less. Imipenem was, however, fourfold more active than BO-2727 against methicillin-susceptible *S. aureus* and *S. epidermidis*. BO-2727 was also active against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Providencia rettgeri*, *Morganella morganii*, *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, *H. influenzae*, *Branhamella catarrhalis*, *Acinetobacter calcoaceticus*, and *B. fragilis*, with MICs for 90% of the strains tested ranging from 0.025 to 1.56 μg/ml. BO-2727 was 4-fold more active than imipenem against *E. coli*, *E. cloacae*, *C. freundii*, and *Proteus mirabilis*, but it was 4-fold to 16-fold less active

TABLE 2. Comparative antibacterial activities of BO-2727 and reference antibiotics against β-lactamase-producing strains

Strain	β-Lactamase(s)[a]	Group(s)[b]	MIC (μg/ml) of[c]:			
			BO-2727	Meropenem	Imipenem	Ceftazidime
Escherichia coli ML4901	TEM-1	2b	0.05	0.025	0.2	0.39
Escherichia coli ML4901	TEM-2	2b	0.05	0.025	0.2	0.39
Escherichia coli ML4901	OXA-1	2d	0.05	0.025	0.2	0.2
Escherichia coli ML4901	PSE-1	2c	0.05	0.012	0.1	0.2
Escherichia coli GN5482	CSase	1	0.1	0.025	0.1	0.2
Klebsiella oxytoca GN10650	CXase	2b′	0.05	0.025	0.1	0.2
Morganella morganii GN5407	CSase	1	0.2	0.05	1.56	0.2
Proteus vulgaris GN7919	CXase	2e	0.05	0.025	0.2	3.13
Citrobacter freundii GN346	CSase	1	0.05	0.05	0.2	50
Enterobacter cloacae GN7471	CSase	1	0.05	0.025	0.1	3.13
Pseudomonas aeruginosa GN10362	CSase	1	0.39	0.2	0.78	1.56
Pseudomonas cepacia GN11164	CXase	2b	6.25	1.56	6.25	0.78
Xanthomonas maltophilia GN12873	L2, L1	2e, 3	>100	>100	>100	50
Bacteroides fragilis BB6065	CXase	2e	0.39	0.2	0.2	>100

[a] Abbreviations: CSase, cephalosporinase; CXase, oxyiminocephalosporinase.
[b] Based on Bush classification (1).
[c] Agar dilution method with Mueller-Hinton agar (Difco) and an inoculum size of about 2×10^3 CFU per spot.

than meropenem against other *Proteus* spp., *P. rettgeri*, *M. morganii*, *H. influenzae*, *B. catarrhalis*, and *Pseudomonas cepacia*. *A. calcoaceticus* and *P. cepacia* were as susceptible to BO-2727 as to imipenem, while *B. fragilis* was fourfold less susceptible to BO-2727 than to imipenem.

The antipseudomonal activity of BO-2727 was examined in detail. The MICs of BO-2727, meropenem, imipenem, ceftazidime, and amikacin for 90% of the imipenem-susceptible *P. aeruginosa* strains tested were 1.56, 3.13, 3.13, 50, and 12.5 μg/ml, respectively. It was noted that BO-2727 was active at 12.5 μg/ml or less against the isolates resistant to imipenem (MIC ≥ 12.5 μg/ml) and those resistant to meropenem (MIC ≥ 12.5 μg/ml). Of the 10 meropenem-resistant *P. aeruginosa* isolates, 2 were paradoxically susceptible to imipenem (MIC ≤ 3.13 μg/ml), and 9 were inhibited by BO-2727 at 6.25 μg/ml or less.

The various strains producing well-characterized plasmid- or chromosome-mediated β-lactamases, except for *P. cepacia* and *Xanthomonas maltophilia*, were inhibited by BO-2727 at concentrations of ≤0.39 μg/ml (Table 2). The strain of *X. maltophilia* that we tested is known to produce a carbapenem-hydrolyzing metalloenzyme (11), by which all the carbapenems were hydrolyzed.

The comparative study of the hydrolysis of the carbapenems by DHP-I showed that BO-2727 was quite stable in the presence of swine renal DHP-I showed that BO-2727 was quite stable in the presence of swine renal DHP-I. The relative hydrolysis rates were 0.11, 0.18 and 1.0 for BO-2727, meropenem, and imipenem, respectively.

In conclusion, BO-2727 is active against gram-positive and gram-negative bacteria, especially *P. aeruginosa* (including some imipenem- and/or meropenem-resistant strains) and is more stable in the presence of DHP-I than meropenem. Therefore, BO-2727 merits further investigation for clinical use by itself.

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