

## Antibacterial Activity of BMS-180680, a New Catechol-Containing Monobactam

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The *in vitro* activities of a new catechol-containing monobactam, BMS-180680 (SQ 84,100), were compared to those of aztreonam, ceftazidime, imipenem, piperacillin-tazobactam, ciprofloxacin, amikacin, and trimethoprim-sulfamethoxazole. BMS-180680 was often the most active compound against many species of the family *Enterobacteriaceae*, with MICs at which 90% of the isolates were inhibited (MIC<sub>90</sub>s) of  $\leq 0.5$   $\mu\text{g/ml}$  for *Escherichia coli*, *Klebsiella* spp., *Citrobacter diversus*, *Enterobacter aerogenes*, *Serratia marcescens*, *Proteus* spp., and *Providencia* spp. BMS-180680 had moderate activities (MIC<sub>90</sub>s of 2 to 8  $\mu\text{g/ml}$ ) against *Citrobacter freundii*, *Morganella morganii*, *Shigella* spp., and non-*E. aerogenes* *Enterobacter* spp. BMS-180680 was the only antibiotic evaluated that was active against >90% of the *Pseudomonas aeruginosa* (MIC<sub>90</sub>, 0.25  $\mu\text{g/ml}$ ), *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* (MIC<sub>90</sub>s, 1  $\mu\text{g/ml}$ ) strains tested. BMS-180680 was inactive against most strains of *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Pseudomonas diminuta*, and *Burkholderia pickettii*. BMS-180680 was moderately active (MIC<sub>90</sub>s of 4 to 8  $\mu\text{g/ml}$ ) against *Alcaligenes* spp. and *Acinetobacter lwoffii* and less active (MIC<sub>90</sub>, 16  $\mu\text{g/ml}$ ) against *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex. BMS-180680 lacked activity against gram-positive bacteria and anaerobic bacteria. Both *tonB* and *cir* *fiu* double mutants of *E. coli* had greatly decreased susceptibility to BMS-180680. Of the TEM, PSE, and chromosomal-encoded  $\beta$ -lactamases tested, only the K1 enzyme hydrolyzed BMS-180680 to any measurable extent. Like aztreonam, BMS-180680 bound preferentially to penicillin-binding protein 3. The MICs of BMS-180680 were not influenced by the presence of hematin or 5% sheep blood in the test medium or with incubation in an atmosphere containing 5% CO<sub>2</sub>. BMS-180680 MICs obtained under strict anaerobic conditions were significantly higher than those obtained in ambient air.

*Pseudomonas aeruginosa* continues to be a frequent opportunistic pathogen, capable of causing a wide variety of infections in the immunocompromised patient. These infections are often associated with significant morbidity and are difficult to treat. In a recent survey of 43 U.S. medical centers (10), approximately 11 to 12% of the 1,003 *P. aeruginosa* strains tested were resistant to ceftazidime and imipenem, with resistance rates as high as 24 to 28% in some institutions.

In *P. aeruginosa*, the outer membrane is a major contributing factor in its resistance to many antibiotics.  $\beta$ -Lactam antibiotics cross the outer membrane of *P. aeruginosa* two orders of magnitude more slowly than they cross the outer membrane of *Escherichia coli* (16). For most  $\beta$ -lactams, this diffusion is through nonspecific or general porins (17, 26). In recent years, a number of  $\beta$ -lactams containing an iron-chelating catecholic substituent have been reported (1, 9, 11, 13, 14, 18, 21, 24). These catecholic  $\beta$ -lactams have excellent antibacterial activity against gram-negative bacteria, including potent activity against *P. aeruginosa*. The potent activity of these compounds is due to their utilization of the TonB-dependent iron transport systems for transport across the bacterial outer membrane.

In this communication we describe the *in vitro* activity of a new  $\beta$ -lactam, BMS-180680 (SQ 84,100), that contains a cate-

chol analog (a quinoxaline) directly attached to the oxime side chain of the monobactam nucleus (Fig. 1). The antibacterial spectrum of BMS-180680 was compared with those of aztreonam, ceftazidime, imipenem, piperacillin-tazobactam (PIP-TAZO), ciprofloxacin, amikacin, and trimethoprim-sulfamethoxazole (TMP-SMX).

### MATERIALS AND METHODS

**Bacterial strains.** The >1,500 bacterial strains used were clinical isolates obtained from numerous sources with a broad geographical distribution and primarily from 1987 to 1992. Properties of the *E. coli* K-12 strains used are listed in Table 1. All isolates were maintained frozen in liquid nitrogen.

**Antibiotics.** BMS-180680 and aztreonam, amikacin and benzylpenicillin, imipenem, and ciprofloxacin were synthesized at the Bristol-Myers Squibb Pharmaceutical Research Institute in New Brunswick, N.J., Syracuse, N.Y., Regensburg, Germany, and Lognes, France, respectively. PIP-TAZO was obtained from Lederle Laboratories, Wayne, N.J.; ceftazidime was from Glaxo Pharmaceuticals, Research Triangle Park, N.C.; TMP-SMX was from Hoffmann-La Roche Inc., Nutley, N.J.; and cephaloridine was from Eli Lilly & Co., Indianapolis, Ind.

**Growth-inhibitory activity.** MICs were determined by the agar dilution method in accordance with the procedures outlined by the National Committee for Clinical Laboratory Standards (15) except that inocula were adjusted to yield approximately  $5 \times 10^4$  CFU per spot. The MIC was considered to be the lowest concentration that prevented visible growth or yielded fewer than six discrete colonies. Mueller-Hinton agar was used for all but the following: streptococci (Mueller-Hinton agar with 5% defibrinated sheep blood), *Haemophilus influenzae* (*Haemophilus* test medium [HTM]), *Neisseria* spp. (GC medium base with 1% supplement C), and anaerobes (Wilkins-Chalgren agar medium supplemented with 5% defibrinated sheep blood). All agar plates used for determining the MIC of TMP-SMX (1:19) contained 0.2 IU of thymidine phosphorylase per ml. Culture plates were incubated aerobically at 35°C for 18 h except for *Haemophilus* sp. and *Neisseria* spp. (5% CO<sub>2</sub> for 24 h) as well as anaerobes (48 h in an anaerobic atmosphere).

Iron-depleted Mueller-Hinton broth (MHB) was prepared by the addition of 150  $\mu\text{M}$   $\alpha, \alpha'$ -dipyridyl. Sodium citrate (1 mM) was also used for induction of the citrate-dependent iron transport system.

**Effects of test conditions on BMS-180680 MICs.** The effect of atmospheric incubation condition on BMS-180680 was determined by using Mueller-Hinton

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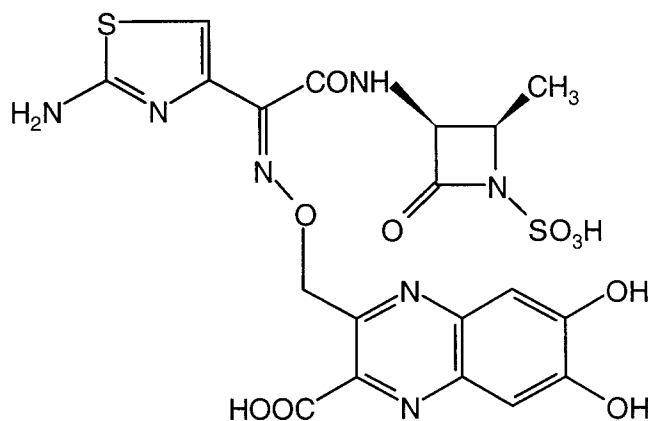


FIG. 1. Structure of BMS-180680.

agar. The atmospheric conditions examined were ambient air, 5% CO<sub>2</sub>, and anaerobic conditions. The effects of 5% sheep blood and hematin-containing media (HTM and GC medium containing 1% supplement C) were also examined.

**β-Lactamase assays.** All β-lactamases were purified from sonic extracts by Sephadex G-75 chromatography, followed by further purification steps using DE-52 chromatography for TEM-2 (23), a type B aminophenylboronic acid-agarose column for TEM-3 and TEM-5 (3), and QAE-Sephadex for P99 (20).

β-Lactamase hydrolysis studies were performed spectrophotometrically on a Gilford 250 or 2600 spectrophotometer. Spectral parameters used in these studies were as follows: cephaloridine, 295 nm, Δε = 889; ceftazidime, 260 nm, Δε = 8,660; imipenem, 295 nm, Δε = 12,600; aztreonam, 318 nm, Δε = 660; and BMS-180680, 310 nm, Δε = 681. V<sub>max</sub> values were calculated by the program ENZPACK (Elsevier). Relative V<sub>max</sub> values were normalized with respect to cephaloridine for the cephalosporinases and broad-spectrum β-lactamases and with respect to benzylpenicillin for the PSE enzymes.

**Penicillin-binding protein (PBP) assays.** Cells from *E. coli* SC 8294 and *P. aeruginosa* SC 8329 were harvested and sonicated. Solubilized membranes, prepared from the centrifuged pellet by homogenization with 2% Triton X-100, were incubated (145 μg of protein) at 30°C with β-lactam for 10 min in a total volume of 50 μl. Ten nmol of <sup>14</sup>C-penicillin G (54 μCi/μmol; Amersham, Arlington Heights, Ill.) was added, and the incubation was continued for another 10 min. Proteins were precipitated with six volumes of cold acetone and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography (22). The concentration of β-lactam that prevented <sup>14</sup>C-penicillin binding to PBPs by 50% was determined by densitometry.

TABLE 1. *E. coli* K-12 strains used

Strain	Relevant genotype	Reference or source
AB2847	<i>aroB malT thi tsx</i>	6
H873	Same as AB2847 but <i>fepA</i>	K. Hantke
BR158	Same as AB2847 but <i>tonB</i>	2
WA380	Same as AB2847 but <i>fecA12</i>	25
H455	Same as AB2847 but Δ( <i>pro lac</i> )	7
H1196	Same as H455 but <i>fhuA::Mu d1</i>	7
H1300	Same as H455 but <i>cir::Mu d1</i>	7
H1187	Same as H455 but <i>fepA::Mu d1</i>	7
C1076	Same as H455 but <i>tonB</i>	5
H1443	<i>araD Δ(lac) aroB deoC flbB ptsF rbsR reLA rpsL</i>	8
H1619	Same as H1443 but <i>fhuE::Mu d1X</i>	8
H1594	Same as H1443 but <i>fhu::Mu d1X</i>	8
C1001	Same as H1594 but <i>cir</i>	5
H1728	Same as H1443 but <i>cir fhu::Mu d1X</i>	K. Hantke
MS172	Same as H1443 but <i>fhuE::λ plac Mu</i>	K. Hantke
C600	<i>fhuA21 lacY1 leuB6 supE44 thi-1 thr-1</i>	22
GUC6	<i>fhuA21 lacY1 leuB6 supE44 thi-1 thr-1 tonB50</i>	B. Bachman

## RESULTS AND DISCUSSION

The antibacterial activity of BMS-180680 was determined for >1,500 recently obtained clinical isolates. BMS-180680 was compared to aztreonam, ceftazidime, imipenem, PIP-TAZO, ciprofloxacin, amikacin, and TMP-SMX. Among members of the family *Enterobacteriaceae*, BMS-180680 was extremely active (MICs at which 90% of the isolates were inhibited [MIC<sub>90s</sub>] of ≤0.5 μg/ml) against *Citrobacter diversus*, *Enterobacter aerogenes*, *E. coli*, *Klebsiella* spp. (*oxytoca* and *pneumoniae*), *Proteus* spp. (*mirabilis* and *vulgaris*), *Providencia* spp. (*rettgeri* and *stuartii*), *Salmonella enteritidis*, *Serratia marcescens*, and *Yersinia enterocolitica* (Table 2). Good activities (MIC<sub>90s</sub> of >0.5 but <8 μg/ml) were also observed with BMS-180680 against *Citrobacter freundii* (MIC<sub>90</sub>, 2 μg/ml), *Enterobacter cloacae* and other non-*E. aerogenes* *Enterobacter* spp. (MIC<sub>90s</sub>, 4 to 8 μg/ml), and *Morganella morganii* (MIC<sub>90</sub>, 2 μg/ml). Good activity was also observed against *Shigella* spp. (MIC<sub>90</sub>, 2 μg/ml). Assuming that the susceptible MIC breakpoint for BMS-180680 is the same as it is for aztreonam (i.e., at ≤8 μg/ml), BMS-180680 was active against all members of the family *Enterobacteriaceae*.

BMS-180680 and aztreonam were often the most active β-lactams against many species of *Enterobacteriaceae*. Compared to aztreonam, BMS-180680 was more active against *E. aerogenes*, *S. marcescens*, *S. enteritidis*, and *Y. enterocolitica* but was less active against non-*E. aerogenes* *Enterobacter* spp., *Shigella* spp., *P. mirabilis*, and *M. morganii* (Table 2). Of the strains tested, the majority of *E. aerogenes* were resistant (as judged by the MIC<sub>90</sub> being higher than the susceptible MIC breakpoint) to ceftazidime and PIP-TAZO; *P. rettgeri* was resistant to PIP-TAZO, amikacin, and TMP-SMX; *P. stuartii* was resistant to TMP-SMX; and *S. enteritidis* was resistant to PIP-TAZO.

BMS-180680 was moderately active (MIC<sub>90s</sub> of 8 μg/ml) against members of the family *Vibrionaceae* (*Vibrio cholerae* and *Aeromonas hydrophila*) but was less active than aztreonam against these organisms (Table 2). BMS-180680 was also less active than aztreonam against members of the family *Neisseriaceae*. The MIC<sub>90s</sub> of BMS-180680 were 0.25 and 2 to 8 μg/ml against *Neisseria meningitidis* and *Neisseria gonorrhoeae*, respectively (Table 2). BMS-180680 had good activity against *H. influenzae* (MIC<sub>90s</sub> of 0.13 to 0.25 μg/ml).

BMS-180680 was extremely active against certain *Pseudomonas* spp. and related bacteria (Table 2). MIC<sub>90s</sub> of BMS-180680 were 0.25 μg/ml for *P. aeruginosa* and *Pseudomonas putida*; 0.5 μg/ml for *Comamonas testosteroni*; and 1 μg/ml for *Comamonas acidovorans* and *Burkholderia cepacia*. Most impressive was the potent activity of BMS-180680 (MIC<sub>90</sub> of 1 μg/ml) against *Stenotrophomonas maltophilia*. BMS-180680 was inactive (MIC<sub>90s</sub> of ≥16 μg/ml) against most strains of *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Pseudomonas diminuta*, and *Burkholderia pickettii*.

BMS-180680 was more active than aztreonam against pseudomonads and related organisms. In fact, the MIC<sub>90</sub> of aztreonam was 16 μg/ml against *P. aeruginosa* and >32 μg/ml against the other species of *Pseudomonas* and related organisms. For the other comparative agents, ceftazidime was active (MIC<sub>90s</sub> of 2 to 8 μg/ml) against *P. aeruginosa*, *P. fluorescens*, *P. putida*, *B. cepacia*, and *C. acidovorans*; imipenem was active (MIC<sub>90s</sub> of 0.5 to 4 μg/ml) against *P. aeruginosa*, *P. putida*, *P. stutzeri*, *P. diminuta*, *B. cepacia*, *B. pickettii*, and *C. acidovorans*; ciprofloxacin was active (MIC<sub>90s</sub> of 0.13 to 1 μg/ml) against *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *B. pickettii*, *C. acidovorans*, and *C. testosteroni*; PIP-TAZO was active (MIC<sub>90s</sub> of 4 to 64 μg/ml) against *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. diminuta*, *B. cepacia*, *B. pickettii*, *C. acidovorans*, and *C.*

TABLE 2. Expanded evaluation of monobactam BMS-180680 against clinical isolates

Organism tested (no. tested) <sup>a</sup>	Compound tested	MIC (µg/ml)		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Citrobacter diversus</i> (32)	BMS-180680	0.008–0.5	0.13	0.5
	Aztreonam	0.015–1	0.03	0.13
	Ceftazidime	0.06–4	0.13	0.5
	Imipenem	0.06–2	0.13	0.25
	PIP-TAZO <sup>b</sup>	0.05–32	4	8
	Ciprofloxacin	0.008–0.5	0.015	0.06
	Amikacin	0.5–64	2	4
<i>Citrobacter freundii</i> (32)	TMP-SMX	0.03–>128	0.06	0.5
	BMS-180680	≤0.001–8	0.015	2
	Aztreonam	0.03–16	0.13	4
	Ceftazidime	0.06–>128	0.25	4
	Imipenem	0.13–1	0.25	1
	PIP-TAZO	0.25–64	2	8
	Ciprofloxacin	0.008–2	0.015	0.06
<i>Enterobacter aerogenes</i> (32)	Amikacin	1–32	2	2
	TMP-SMX	0.06–>128	0.13	0.5
	BMS-180680	0.004–2	0.015	0.25
	Aztreonam	0.015–16	0.06	16
	Ceftazidime	0.06–64	0.13	32
	Imipenem	0.13–2	0.5	1
	PIP-TAZO	0.25–64	2	32
<i>Enterobacter cloacae</i> (32)	Ciprofloxacin	0.008–0.06	0.03	0.06
	Amikacin	0.5–4	2	4
	TMP-SMX	0.03–2	0.25	0.25
	BMS-180680	0.004–16	0.13	4
	Aztreonam	0.015–64	0.06	2
	Ceftazidime	0.06–>128	0.13	4
	Imipenem	0.13–2	0.25	0.5
<i>Enterobacter spp.</i> (28)	PIP-TAZO	0.5–>128	1	8
	Ciprofloxacin	0.008–1	0.015	0.03
	Amikacin	1–4	2	2
	TMP-SMX	0.06–64	0.13	0.5
	BMS-180680	0.002–8	0.06	8
	Aztreonam	0.008–0.5	0.03	0.13
	Ceftazidime	0.015–0.5	0.06	0.5
<i>Escherichia coli</i> (32)	Imipenem	0.13–1	0.13	0.5
	PIP-TAZO	0.015–8	1	4
	Ciprofloxacin	0.008–1	0.015	0.06
	Amikacin	0.25–8	1	2
	TMP-SMX	0.03–2	0.25	1
	BMS-180680	0.015–0.5	≤0.015	0.06
	Aztreonam	0.015–0.25	0.03	0.13
<i>Klebsiella oxytoca</i> (32)	Ceftazidime	0.03–1	0.13	0.25
	Imipenem	0.06–0.5	0.13	0.25
	PIP-TAZO	0.25–4	1	2
	Ciprofloxacin	0.002–0.03	0.015	0.03
	Amikacin	1–8	2	4
	TMP-SMX	0.03–64	0.06	1
	BMS-180680	0.002–0.25	0.015	0.06
<i>Klebsiella pneumoniae</i> (32)	Aztreonam	0.008–16	0.03	0.13
	Ceftazidime	0.03–0.25	0.06	0.13
	Imipenem	0.06–0.5	0.13	0.25
	PIP-TAZO	0.5–>128	1	4
	Ciprofloxacin	0.008–0.06	0.03	0.03
	Amikacin	1–2	2	2
	TMP-SMX	0.03–0.25	0.06	0.13
<i>Morganella morganii</i> (31)	BMS-180680	0.001–8	0.015	0.5
	Aztreonam	0.008–32	0.015	0.13
	Ceftazidime	0.06–>128	0.13	0.5
	Imipenem	0.06–16	0.13	0.13
	PIP-TAZO	0.5–32	2	8
	Ciprofloxacin	0.015–1	0.03	0.25
	Amikacin	1–32	2	2
	TMP-SMX	0.06–>128	0.06	2
	BMS-180680	0.004–8	0.13	2

Continued

TABLE 2—Continued

Organism tested (no. tested) <sup>a</sup>	Compound tested	MIC (µg/ml)			
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>	
<i>Proteus mirabilis</i> (32)	Aztreonam	0.008–16	0.015	0.13	
	Ceftazidime	0.06–32	0.13	2	
	Imipenem	1–4	2	4	
	PIP-TAZO	0.13–64	0.25	0.5	
	Ciprofloxacin	0.008–0.25	0.015	0.03	
	Amikacin	0.5–128	1	8	
	TMP-SMX	0.06–>128	0.13	1	
	BMS-180680	0.004–0.5	0.008	0.06	
	Aztreonam	0.004–0.015	0.008	0.008	
	Ceftazidime	0.03–0.13	0.06	0.13	
<i>Proteus vulgaris</i> (32)	Imipenem	0.5–4	1	1	
	PIP-TAZO	0.25–1	0.5	0.5	
	Ciprofloxacin	0.015–0.13	0.03	0.06	
	Amikacin	1–8	2	4	
	TMP-SMX	0.03–0.06	0.06	0.06	
	BMS-180680	0.004–0.25	0.008	0.03	
	Aztreonam	0.008–0.5	0.015	0.03	
	Ceftazidime	0.03–0.13	0.06	0.13	
	Imipenem	0.25–4	1	4	
	PIP-TAZO	0.13–1	0.5	1	
<i>Providencia rettgeri</i> (32)	Ciprofloxacin	0.015–0.13	0.03	0.06	
	Amikacin	0.5–8	1	4	
	TMP-SMX	0.06–>128	0.25	0.5	
	BMS-180680	0.008–0.5	0.015	0.13	
	Aztreonam	0.002–0.25	0.008	0.06	
	Ceftazidime	0.03–4	0.25	1	
	Imipenem	0.5–2	1	2	
	PIP-TAZO	0.25–128	1	64	
	Ciprofloxacin	0.015–2	0.13	1	
	Amikacin	0.5–128	2	32	
<i>Providencia stuartii</i> (32)	TMP-SMX	0.06–>128	4	128	
	BMS-180680	0.002–2	0.002	0.03	
	Aztreonam	0.008–16	0.015	0.03	
	Ceftazidime	0.13–2	0.25	1	
	Imipenem	0.13–2	1	2	
	PIP-TAZO	1–8	2	4	
	Ciprofloxacin	0.03–16	0.13	2	
	Amikacin	0.5–4	2	4	
	TMP-SMX	0.13–>128	2	>128	
	BMS-180680	0.001–8	0.004	0.008	
<i>Salmonella enteritidis</i> (30)	Aztreonam	0.008–0.25	0.06	0.13	
	Ceftazidime	0.06–4	0.25	1	
	Imipenem	0.06–0.5	0.25	0.25	
	PIP-TAZO	0.25–128	2	32	
	Ciprofloxacin	0.008–0.06	0.015	0.03	
	Amikacin	0.5–2	1	2	
	TMP-SMX	0.015–2	0.03	0.13	
	BMS-180680	0.004–0.13	0.015	0.06	
	Aztreonam	0.06–8	0.13	0.25	
	Ceftazidime	0.13–8	0.25	1	
<i>Serratia marcescens</i> (32)	Imipenem	0.25–2	0.5	1	
	PIP-TAZO	0.5–128	2	8	
	Ciprofloxacin	0.015–16	0.13	1	
	Amikacin	2–>128	4	8	
	TMP-SMX	0.03–>128	0.25	1	
	BMS-180680	0.004–2	0.06	2	
	Aztreonam	0.008–4	0.03	0.06	
	Ceftazidime	0.03–1	0.13	0.13	
	Imipenem	0.13–1	0.13	0.25	
	PIP-TAZO	≤0.008–2	0.5	1	
<i>Shigella spp.</i> (32)	Ciprofloxacin	0.004–0.03	0.015	0.03	
	Amikacin	2–8	4	8	
	TMP-SMX	≤0.008–>128	0.03	0.25	
	BMS-180680	0.002–0.03	<0.002	0.03	
	Aztreonam	0.002–2	0.13	0.5	
	Ceftazidime	0.03–4	0.13	1	
	<i>Yersinia enterocolitica</i> (16)	BMS-180680	0.002–0.03	<0.002	0.03
		Aztreonam	0.002–2	0.13	0.5
		Ceftazidime	0.03–4	0.13	1

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TABLE 2—Continued

Organism tested (no. tested) <sup>a</sup>	Compound tested	MIC (µg/ml)		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Aeromonas hydrophila</i> (12)	Imipenem	0.13–0.5	0.25	0.5
	PIP-TAZO	0.06–4	0.25	1
	Ciprofloxacin	0.004–0.03	0.015	0.03
	Amikacin	1–8	4	8
	TMP-SMX	0.06–0.13	0.13	0.13
	BMS-180680	0.015–16	0.13	8
	Aztreonam	0.008–0.015	0.015	0.015
	Ceftazidime	0.06–0.25	0.13	0.25
	Imipenem	0.13–2	0.5	2
	PIP-TAZO	0.25–4	0.5	2
	Ciprofloxacin	0.004–0.06	0.008	0.015
	Amikacin	0.5–4	1	4
<i>Vibrio cholera</i> (12)	TMP-SMX	0.13–2	0.25	2
	BMS-180680	0.5–16	2	8
	Aztreonam	0.25–8	0.5	0.5
	Ceftazidime	0.06–0.5	0.13	0.25
	Imipenem	1–4	2	2
	PIP-TAZO	0.008–2	0.13	0.5
	Ciprofloxacin	0.002–0.03	0.002	0.008
	Amikacin	1–4	4	4
	TMP-SMX	0.03–0.13	0.06	0.13
	BMS-180680	0.015–16	1	8
	Aztreonam	0.004–2	0.13	0.5
	Ceftazidime	0.008–0.25	0.03	0.13
<i>Neisseria gonorrhoeae</i> P– (31)	Imipenem	0.03–0.5	0.06	0.25
	PIP-TAZO	0.002–0.5	0.03	0.25
	Ciprofloxacin	0.001–0.25	0.004	0.008
	Amikacin	8–64	32	64
	BMS-180680	0.25–8	0.5	2
	Aztreonam	0.06–0.5	0.13	0.13
	Ceftazidime	0.015–0.13	0.03	0.06
	Imipenem	0.03–0.25	0.06	0.06
	PIP-TAZO	0.25–>16	0.5	2
	Ciprofloxacin	0.002–0.015	0.004	0.008
	Amikacin	8–64	32	64
	<i>Neisseria meningitidis</i> (27)	BMS-180680	0.03–4	0.13
Aztreonam		0.008–0.13	0.03	0.03
Ceftazidime		0.008–0.06	0.03	0.03
Imipenem		0.015–0.06	0.03	0.06
PIP-TAZO		≤0.001	<0.001	<0.001
Ciprofloxacin		0.002–0.008	0.004	0.008
Amikacin		8–32	32	32
BMS-180680		0.25–2	0.5	2
Aztreonam		0.5–4	1	2
Ceftazidime		0.03–0.25	0.06	0.13
Imipenem		0.008–0.13	0.03	0.06
PIP-TAZO		0.03–1	0.25	0.25
<i>Moraxella catarrhalis</i> P+ (30)	Ciprofloxacin	0.015–0.06	0.03	0.06
	Amikacin	0.25–1	0.5	1
	TMP-SMX	0.13–0.5	0.25	0.25
	BMS-180680	0.015–0.25	0.13	0.25
	Aztreonam	0.008–0.06	0.03	0.06
	Ceftazidime	0.002–0.25	0.06	0.13
	Imipenem	0.004–2	1	1
	PIP-TAZO	0.001–0.13	0.015	0.06
	Ciprofloxacin	0.001–0.03	0.015	0.015
	Amikacin	4–64	16	32
	TMP-SMX	0.06–1	0.13	1
	BMS-180680	0.06–0.25	0.13	0.13
<i>Haemophilus influenzae</i> P– (32)	Aztreonam	0.015–0.13	0.06	0.13
	Ceftazidime	0.06–0.25	0.13	0.13
	Imipenem	0.06–8	1	2
	PIP-TAZO	0.004–0.13	0.03	0.06
	Ciprofloxacin	0.008–0.03	0.015	0.015
	Amikacin	8–32	16	32
	TMP-SMX	0.13–16	0.5	1

Continued

TABLE 2—Continued

Organism tested (no. tested) <sup>a</sup>	Compound tested	MIC (µg/ml)		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Burkholderia cepacia</i> (32)	BMS-180680	0.004–1	0.03	1
	Aztreonam	1–>128	16	64
	Ceftazidime	1–8	4	4
	Imipenem	1–32	8	16
	PIP-TAZO	0.25–32	4	32
	Ciprofloxacin	0.5–8	2	4
	Amikacin	2–>128	128	>128
	TMP-SMX	0.13–32	1	8
	BMS-180680	0.5–16	4	16
	Aztreonam	64–>128	128	>128
	Ceftazidime	4–16	16	16
	Imipenem	0.25–8	1	4
<i>Burkholderia pickettii</i> (10)	PIP-TAZO	0.5–8	1	8
	Ciprofloxacin	0.06–0.13	0.13	0.13
	Amikacin	4–>128	128	>128
	TMP-SMX	0.06–0.5	0.13	0.5
	BMS-180680	0.13–2	0.25	1
	Aztreonam	4–128	8	32
	Ceftazidime	0.13–2	0.5	2
	Imipenem	0.13–1	0.5	0.5
	PIP-TAZO	0.008–8	2	8
	Ciprofloxacin	0.06–4	0.13	0.25
	Amikacin	32–>128	128	>128
	TMP-SMX	0.03–8	0.06	0.25
<i>Comamonas acidovorans</i> (32)	BMS-180680	0.03–0.5	0.25	0.5
	Aztreonam	4–128	8	32
	Ceftazidime	0.13–2	0.5	2
	Imipenem	0.13–1	0.5	0.5
	PIP-TAZO	0.008–8	2	8
	Ciprofloxacin	0.06–4	0.13	0.25
	Amikacin	32–>128	128	>128
	TMP-SMX	0.03–8	0.06	0.25
	BMS-180680	0.03–0.5	0.25	0.5
	Aztreonam	4–32	8	32
	Ceftazidime	0.5–8	1	4
	Imipenem	0.03–0.13	0.06	0.13
<i>Comamonas testosteroni</i> (10)	PIP-TAZO	≤0.008–8	0.13	4
	Ciprofloxacin	0.015–0.25	0.08	0.13
	Amikacin	4–128	8	64
	TMP-SMX	0.06–32	0.5	32
	BMS-180680	0.001–1	0.06	0.25
	Aztreonam	0.06–64	4	16
	Ceftazidime	0.13–32	2	8
	Imipenem	0.13–16	1	2
	PIP-TAZO	0.5–128	4	16
	Ciprofloxacin	0.06–32	0.25	1
	Amikacin	1–32	4	8
	TMP-SMX	0.25–>128	8	>128
<i>Pseudomonas aeruginosa</i> (50)	BMS-180680	32–>64	>64	>64
	Aztreonam	32–>128	>128	>128
	Ceftazidime	16–>128	32	128
	Imipenem	0.13–2	1	1
	PIP-TAZO	0.5–32	2	16
	Ciprofloxacin	0.015–32	4	32
	Amikacin	0.5–>128	4	128
	TMP-SMX	0.06–16	2	16
	BMS-180680	0.13–16	2	16
	Aztreonam	16–64	32	64
	Ceftazidime	1–32	2	8
	Imipenem	1–8	2	8
PIP-TAZO	2–16	4	16	
Ciprofloxacin	0.06–1	0.06	1	
Amikacin	0.5–128	1	128	
TMP-SMX	0.25–32	1	16	
<i>Pseudomonas fluorescens</i> (18)	BMS-180680	0.008–1	0.03	0.25
	Aztreonam	16–>128	32	64
	Ceftazidime	2–32	4	8
	Imipenem	0.25–2	0.5	1
	PIP-TAZO	2–>128	16	64
	Ciprofloxacin	0.06–8	0.25	0.5
	Amikacin	0.25–128	1	4
	TMP-SMX	2–>128	16	128
	BMS-180680	0.06–128	128	128
	Aztreonam	0.06–>128	16	>128

Continued on following page

TABLE 2—Continued

Organism tested (no. tested) <sup>a</sup>	Compound tested	MIC (μg/ml)		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Stenotrophomonas maltophilia</i> (32)	Ceftazidime	0.13->128	>128	>128
	Imipenem	0.25-2	0.5	1
	PIP-TAZO	0.03->128	32	>128
	Ciprofloxacin	0.008-0.13	0.03	0.13
	Amikacin	1-8	2	4
	TMP-SMX	0.13-4	0.5	1
	BMS-180680	0.03-8	0.25	1
	Aztreonam	32->128	>128	>128
	Ceftazidime	1-128	16	64
	Imipenem	64-128	>128	>128
	PIP-TAZO	16-128	128	>128
<i>Acinetobacter calcoaceticus-Acinetobacter baumannii</i> complex (35)	Ciprofloxacin	0.25-8	2	8
	Amikacin	4->128	128	>128
	TMP-SMX	0.25-32	0.5	1
	BMS-180680	0.5-32	1	16
	Aztreonam	4-128	16	32
	Ceftazidime	1-16	2	8
	Imipenem	0.06-0.5	0.25	0.25
	PIP-TAZO	≤0.008->128	<0.008	32
	Ciprofloxacin	0.06-8	0.25	1
	Amikacin	0.5-128	4	64
	TMP-SMX	0.015-32	0.06	8
<i>Acinetobacter lwoffii</i> (32)	BMS-180680	0.015-16	0.5	8
	Aztreonam	1->128	8	64
	Ceftazidime	0.25-128	2	8
	Imipenem	0.03-1	0.13	0.5
	PIP-TAZO	≤0.008-128	<0.008	2
	Ciprofloxacin	0.06-1	0.13	0.5
	Amikacin	0.25-32	2	4
	TMP-SMX	0.004-2	0.06	0.25
	BMS-180680	0.06-4	0.25	4
	Aztreonam	4-64	32	64
	Ceftazidime	0.5-8	2	4
<i>Alcaligenes</i> spp. (32)	Imipenem	0.13-0.5	0.25	0.5
	PIP-TAZO	0.06-2	0.5	0.5
	Ciprofloxacin	0.13-8	1	2
	Amikacin	4-32	8	32
	TMP-SMX	0.015-8	0.13	0.5
	BMS-180680	>128	>128	>128
	Aztreonam	>128	>128	>128
	Ceftazidime	4-16	8	16
	Imipenem	0.008-0.15	0.015	0.015
	PIP-TAZO	0.06-0.5	0.13	0.25
	Ciprofloxacin	0.13-0.25	0.25	0.25
<i>Staphylococcus aureus</i> MS, P+ (11)	Amikacin	1-4	2	4
	TMP-SMX	0.03-0.5	0.06	0.5
	BMS-180680	>128	>128	>128
	Aztreonam	>128	>128	>128
	Ceftazidime	2-8	4	4
	Imipenem	0.008-0.06	0.015	0.015
	PIP-TAZO	0.06-0.5	0.13	0.25
	Ciprofloxacin	0.13-0.25	0.25	0.25
	Amikacin	1-4	2	4
	TMP-SMX	0.03-0.25	0.13	0.13
	BMS-180680	64->128	128	128
<i>Streptococcus agalactiae</i> (11)	Aztreonam	64->128	>128	>128
	Ceftazidime	0.13-1	0.5	0.5
	Imipenem	0.008-0.06	0.015	0.015
	PIP-TAZO	0.06-0.5	0.25	0.25
	Ciprofloxacin	0.5-4	1	2
	Amikacin	≥128	>128	>128
	TMP-SMX	0.25-0.5	0.5	0.5
	BMS-180680	16-64	32	32
	Aztreonam	8-16	16	16
	Ceftazidime	0.13-0.25	0.25	0.25
	Imipenem	0.002-0.004	0.003	0.008

Continued

TABLE 2—Continued

Organism tested (no. tested) <sup>a</sup>	Compound tested	MIC (μg/ml)		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Streptococcus pneumoniae</i> (11)	PIP-TAZO	0.03-0.06	0.06	0.06
	Ciprofloxacin	0.5-1	1	1
	Amikacin	≥128	>128	>128
	TMP-SMX	0.25-0.5	0.5	0.5
	BMS-180680	16->128	32	64
	Aztreonam	32->128	128	>128
	Ceftazidime	0.06-4	0.25	0.5
	Imipenem	0.002-0.06	0.015	0.015
	PIP-TAZO	≤0.008-0.5	0.015	0.13
	Ciprofloxacin	0.5-4	2	2
	Amikacin	16-128	128	>128
<i>Enterococcus faecalis</i> (11)	TMP-SMX	0.06-1	0.25	0.5
	BMS-180680	>128	>128	>128
	Aztreonam	>128	>128	>128
	Ceftazidime	>128	>128	>128
	Imipenem	0.5-1	1	1
	PIP-TAZO	1-4	2	2
	Ciprofloxacin	0.25-1	1	1
	Amikacin	64-128	128	>128
	BMS-180680	64->128	>128	>128
	Aztreonam	≥128	>128	>128
	Ceftazidime	16->128	32	>128
<i>Bacteroides fragilis</i> (12)	Imipenem	0.25-0.5	0.5	0.5
	PIP-TAZO	0.25-16	4	8
	Ciprofloxacin	1-8	2	2
	Amikacin	>128	>128	>128
	TMP-SMX	1-4	2	4
	BMS-180680	8->128	32	>128
	Aztreonam	64->128	>128	>128
	Ceftazidime	0.13-4	1	2
	Imipenem	0.015-0.06	0.03	0.06
	PIP-TAZO	0.03-1	0.06	0.5
	Ciprofloxacin	0.13-0.25	0.25	0.25
<i>Clostridium perfringens</i> (12)	Amikacin	≥128	>128	>128
	TMP-SMX	2-128	8	32
	BMS-180680	>128	>128	>128
	Aztreonam	>128	>128	>128
	Ceftazidime	≥128	>128	>128
	Imipenem	2-8	4	4
	PIP-TAZO	2-16	8	8
	Ciprofloxacin	2-8	4	8
	Amikacin	≥128	>128	>128
	TMP-SMX	64->128	128	>128
	<i>Clostridium difficile</i> (12)	BMS-180680	>128	>128
Aztreonam		>128	>128	>128
Ceftazidime		≥128	>128	>128
Imipenem		2-8	4	4
PIP-TAZO		2-16	8	8
Ciprofloxacin		2-8	4	8
Amikacin		≥128	>128	>128
TMP-SMX		64->128	128	>128

<sup>a</sup> P-, penicillinase negative; P+, penicillinase positive; MS, methicillin susceptible.<sup>b</sup> The MIC reported is for PIP. The TAZO concentration is held constant (4 μg/ml).

*tetosteroni*; and TMP-SMX was active (MIC<sub>90</sub>s of 0.25 to 1 μg/ml) against *P. stutzeri*, *B. pickettii*, *C. acidovorans*, and *S. maltophilia*. Thus, BMS-180680 was the only antibiotic evaluated that was active against >90% of the *P. aeruginosa*, *B. cepacia*, and *S. maltophilia* strains tested, three important species that may cause nosocomial lower respiratory tract infections (12).

BMS-180680 was moderately active (MIC<sub>90</sub>s of 4 to 8 μg/ml) against *Alcaligenes* spp. and *Acinetobacter lwoffii*, but it was less active (MIC<sub>90</sub> of 16 μg/ml) against *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex (Table 2). Except for amikacin, TMP/SMX, and aztreonam, the other agents compared were active against these *Acinetobacter* and *Alcaligenes* spp.

As with most known monobactams, BMS-180680 was inactive against gram-positive bacteria (Table 2). In addition, BMS-180680 had no activity against anaerobic bacteria. The

TABLE 3. Susceptibilities of *E. coli* mutants to BMS-180680, aztreonam, and ceftazidime

Strain	Relevant genotype	MIC ( $\mu\text{g/ml}$ ) in untreated MHB			BMS-180680 MIC ( $\mu\text{g/ml}$ ) in MHB plus 150 $\mu\text{M}$ dipyriddy and 1 mM Na citrate
		BMS-180680	Az-treonam	Cefta-zidime	
AB2847	Wild type	0.015	0.06	0.13	0.005
H873	<i>fepA</i>	0.008	0.06	0.13	0.001
BR158	<i>tonB</i>	8	0.06	0.13	8
WA380	<i>fecA</i>	0.0005	0.13	0.25	0.00025
H455	Wild type	0.015	0.06	0.13	0.001
H1196	<i>fhuA</i>	0.015	0.06	0.13	0.001
H1300	<i>cir</i>	0.06	0.13	0.25	0.001
H1187	<i>fepA</i>	0.015	0.06	0.13	0.001
C1076	<i>tonB</i>	8	0.06	0.13	8
H1443	Wild type	0.008	0.06	0.13	0.002
H1619	<i>fhuE</i>	0.008	0.06	0.25	0.004
H1594	<i>fiu</i>	0.015	0.06	0.25	0.004
C1001	<i>cir fiu</i>	8	0.06	0.25	8
H1728	<i>cir fiu</i>	8	0.06	0.13	8
MS172	<i>fhuE</i>	0.008	0.06	0.25	0.002
C600	<i>fhuA</i>	0.03	0.06	0.13	0.004
GUC6	<i>tonB</i>	4	0.06	0.13	2

susceptibilities of *E. coli* iron transport mutants (Table 1) to BMS-180680, aztreonam, and ceftazidime are listed in Table 3. The highest (approximately 1,000-fold) increases in MICs to BMS-180680 were observed in *tonB* mutants and in the *cir fiu* double mutants. The TonB protein couples the cytoplasmic membrane energy needed to transport substances bound on the iron-regulated outer membrane proteins across the outer membrane (19); therefore, *tonB* mutants cannot transport BMS-180680 via the iron transport pathway. The Cir and Fiu proteins are believed to be the iron-regulated outer membrane receptors used for transport of monomeric catecholic cephalosporins (5, 18). With the exception of a 4-fold increase and a 32-fold decrease in the BMS-180680 MIC versus those for the *cir* and *fecA* mutants, respectively, there was essentially no change in the MIC of this catecholic monobactam for the other iron transport mutants tested. The aztreonam and ceftazidime MICs for the mutants and their respective parents were the same, consistent with their lack of uptake by the iron transport system.

The BMS-180680 MICs determined in iron-depleted MHB medium were often 4- to 10-fold lower than those obtained when MHB was used (Table 3). The decreased MICs were presumably due to induction of the iron transport proteins, including the Cir and Fiu receptors for increased BMS-180680 uptake, under iron-limiting conditions. Since *tonB* mutants cannot actively transport BMS-180680 across the outer membrane via the iron transport pathway, these mutants showed no

TABLE 4. Effect of incubation atmosphere on BMS-180680 MICs

Organism	BMS-180680 MIC ( $\mu\text{g/ml}$ ) in:		
	Air	5% CO <sub>2</sub>	Anaerobic conditions
<i>E. coli</i> A27538	0.03	0.016	0.5
<i>K. pneumoniae</i> A27464	0.016	0.016	0.5
<i>E. cloacae</i> A27451	0.5	1	2
<i>P. aeruginosa</i> A27471	0.13	0.06	Poor growth
<i>P. fluorescens</i> A24234	0.13	0.5	Poor growth
<i>B. cepacia</i> A27491	0.016	0.06	No growth

TABLE 5. Stability of BMS-180680 in the presence of  $\beta$ -lactamases

$\beta$ -Lactamase	Relative $V_{\text{max}}$ for $\beta$ -lactam				
	Cephalo-ridine	Benzyl-penicillin	Cefta-zidime	Az-treonam	BMS-180680
Plasmid-mediated					
TEM-2	100	86	0.001	0.23	0.01
TEM-3	100	104	8.5	0.36	3.2
TEM-5	100	33	25	15	0.61
PSE-1	9.1	100	0.005	0.01	$\leq 0.04$
PSE-2	16	100	$\leq 0.02$	0.97	$\leq 0.4$
PSE-3	4.7	100	0.043	0.19	$\leq 0.1$
PSE-4	12	100	$\leq 0.01$	0.01	$\leq 0.01$
Chromosomal <sup>a</sup>					
K1	100	280	0.03	40	1.5
P99	100	1.5	0.002	$< 0.001$	$< 0.01$
SC 8329	100	ND <sup>b</sup>	$\leq 0.1$	$\leq 0.2$	$\leq 0.05$
A26145	100	ND	0.013	0.16	$\leq 0.03$
A26363	100	ND	0.004	0.017	$\leq 0.01$

<sup>a</sup> The K1  $\beta$ -lactamase was isolated from *K. oxytoca*, and the P99 enzyme was isolated from *E. cloacae*. SC 8329, A26145, and A26363 are three chromosomal enzymes with  $pI$ s of  $>9.0$ , 8.2, and 8.8, respectively, from three different strains of *P. aeruginosa*.

<sup>b</sup> ND, not determined.

difference in susceptibility to BMS-180680 in iron-sufficient versus iron-deficient medium (Table 3). Since uptake of aztreonam and ceftazidime is not dependent on the iron transport system, their MICs were the same for the two test media (data not shown).

The effects of in vitro testing conditions on the activities of BMS-180680 were assessed. The MICs of BMS-180680 determined by incubation in ambient air and in the presence of 5% CO<sub>2</sub> were within fourfold of each other (Table 4). However, BMS-180680 was less active under anaerobic conditions, presumably because of iron depletion due to the increased solubility of iron under strict anaerobiosis (4). Because the iron transport system is regulated by iron in the growth medium, we examined whether supplementation of the growth medium with 5% sheep blood or with hematin (a supplement in HTM and GC media) would interfere with the inhibitory effects of BMS-180680. Hematin supplementation and the addition of 5% sheep blood did not alter the BMS-180680 MICs for the strains listed in Table 4 (data not shown).

BMS-180680 was quite stable in the presence of the TEM-2 and PSE  $\beta$ -lactamases (Table 5). Hydrolysis of BMS-180680 was much slower than that observed with aztreonam. The extended-spectrum  $\beta$ -lactamase TEM-3 was capable of hydrolyzing BMS-180680 but at a rate that appeared less than that observed with ceftazidime. BMS-180680 was the poorest substrate for TEM-5, with hydrolysis of this compound being at least an order of magnitude slower than that for aztreonam or ceftazidime. Of the chromosomal  $\beta$ -lactamases, only the broad-spectrum K1 enzyme hydrolyzed BMS-180680 to any measurable extent (Table 5). This monobactam was extremely stable in the presence of all the cephalosporinases tested.

BMS-180680 bound specifically to PBP 3 of both *E. coli* and *P. aeruginosa*. PBP 3 also has good affinity for aztreonam (Table 6). Comparison of the PBP profiles for BMS-180680 and aztreonam indicates that their IC<sub>50</sub>s for the *E. coli* and *P. aeruginosa* PBP 3 were similar but that MICs for aztreonam were higher. These results emphasize that an enhanced ability to penetrate the outer membrane may contribute to the excellent in vitro activity of BMS-180680 against gram-negative bacteria.

TABLE 6. Binding of BMS-180680 to PBP

Organism	Antibiotic	Concn ( $\mu\text{g/ml}$ ) of antibiotic required to inhibit binding of $^{14}\text{C}$ -penicillin G by 50%						MIC ( $\mu\text{g/ml}$ )
		PBP 1a	PBP 1b	PBP 2	PBP 3	PBP 4	PBP 5/6	
<i>E. coli</i> SC 8294	BMS-180680	3	100	>200	0.01	>100	>200	0.004
	Aztreonam	1.6	50	>200	0.005	46	>200	0.13
<i>P. aeruginosa</i> SC 8329	BMS-180680	16	4.7	>200	$\leq 0.2$	23	>200	0.015
	Aztreonam	22	6.5	>200	0.004	12	>200	2

In conclusion, BMS-180680 is a monocatecholic monobactam which appears to use the Cir and Fiu iron-regulated outer membrane receptor proteins and the TonB-dependent iron transport system for enhanced uptake across the bacterial outer membrane. As a result, BMS-180680 has excellent activity against many gram-negative bacteria, including many species of *Enterobacteriaceae* and *P. aeruginosa*, *B. cepacia*, and *S. maltophilia*.

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