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In Vitro Evaluation of Tigemonam, a Novel Oral Monobactam

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Tigemonam, a novel, orally administered monobactam, exhibited potent and specific activity in vitro against members of the family *Enterobacteriaceae*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. Its activity was variable to poor against gram-positive bacteria, *Acinetobacter* spp., *Pseudomonas aeruginosa*, and anaerobes. Within its spectrum of activity, tigemonam was far superior to oral antibiotics currently available, including amoxicillin-clavulanic acid, cefaclor, and trimethoprim-sulfamethoxazole. In addition, tigemonam was superior to cefuroxime, which is under development as an oral pro-drug, and more active than cefixime against several genera of the *Enterobacteriaceae*. The activity of tigemonam against the enteric bacteria, *Haemophilus* species, and *Neisseria* species was, in general, comparable to that of the quinolone norfloxacin. The excellent activity of tigemonam against β-lactamase-producing bacteria reflected its marked stability to hydrolysis by isolated enzymes. The expanded spectrum of activity against gram-negative bacteria observed with tigemonam thus extends oral β-lactam coverage to include members of the *Enterobacteriaceae* that are intrinsically or enzymatically resistant to broad-spectrum penicillins and cephalosporins.

The use of oral antibiotics in the therapy of infectious diseases is generally limited to mild, community-acquired infections. Although community-acquired pathogens have historically been susceptible to current oral agents, resistant and multiply resistant isolates are becoming increasingly prevalent. The clinical utility of the oral β-lactam antibiotics is being rapidly eroded by the increasing isolation of β-lactamase-producing strains of *Escherichia coli*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*, due to the acquisition and transfer of plasmid-mediated determinants (6, 8). Plasmid-mediated trimethoprim and tetracycline resistance is relatively common and may occur concomitantly with β-lactamase production (4, 6).

The limitations on the use of oral antibiotics in nosocomial infections are even greater. The most common pathogens, particularly among members of the family *Enterobacteriaceae*, may not only carry β-lactamase or other acquired resistance determinants, but also are often intrinsically resistant to many of the oral agents (10). The potential impact of oral therapy on reducing hospital-associated infection risks and costs is therefore limited.

Herein we describe a new oral monobactam, tigemonam (Fig. 1), that exhibits an expanded gram-negative spectrum, high-level activity, and β-lactamase stability similar to those of several newer parenteral cephalosporins and the first monobactam, aztreonam. With its excellent pharmacokinetic properties and efficacy in laboratory animals (5), tigemonam should prove to be a significant advancement in oral antibacterial therapy.

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MATERIALS AND METHODS

Bacteria. All strains were clinical isolates maintained as frozen (−70°C) stocks by the Squibb Culture Collection.

Antibiotics. Tigemonam, ampicillin, and amoxicillin were prepared by E. R. Squibb and Sons, Princeton, N.J. Clavulanic acid was obtained from Beecham Laboratories, Bristol, Tenn.; cephalexin and cefuroxime were from Glaxo Inc., Research Triangle Park, N.C.; cefaclor was from Eli Lilly Co., Indianapolis, Ind.; cefixime was from Fujisawa Pharmaceutical Co, Ltd., Osaka, Japan; norfloxacin was from Merck Sharp and Dohme, Inc., Rahway, N.J.; oxytetracycline was from Lab Pro-Ter, Milan, Italy; and trimethoprim and sulfamethoxazole were from Hoffmann-LaRoche, Nutley, N.J. All compounds were prepared fresh as directed by their respective manufacturers.

Susceptibility testing. Comparative MICs were determined by the agar dilution method. Diagnostic Sensitivity Test agar (Oxoid Ltd., Columbia, Md.) was used for all testing except for *N. gonorrhoeae* and *H. influenzae*, for which Proteose Agar no. 3 (Difco Laboratories, Detroit, Mich.) was used.

Media were supplemented with 1% hemoglobin (BBL Microbiology Systems, Cockeysville, Md.) and 1% IsoVitaleX (BBL) for the testing of *Haemophilus* and *Neisseria* species, 5% sheep blood for streptococci, and 5% lysed sheep blood plus 0.5 μg of vitamin K per ml for anaerobes.

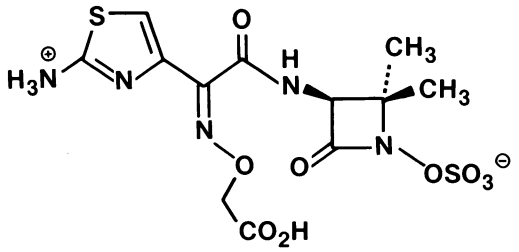

FIG. 1. Structure of tigemonam.

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TABLE 1. Comparative activity of tigemonam against members of the *Enterobacteriaceae*

Organism (no. of strains)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
E. coli (26)	Tigemonam	<0.1–1.6	0.3	0.7
	Ampicillin	0.8–>100	18.8	>100
	Amoxicillin	1.6–>100	10.5	>100
	Amoxicillin-clavulinic acid	0.8–12.5	4.3	11.0
	Cephalexin	6.3–>100	12.0	39.9
	Cefaclor	6.3–>100	31.2	>100
	Cefuroxime	0.2–50	3.1	8.8
	Cefixime	<0.1–1.6	0.4	1.2
	SXT	0.4–25	1.6	7.5
	Oxytetracycline	1.6–>100	>100	>100
	Norfloxacin	<0.1–0.4	<0.1	0.2
Enterobacter spp. (25)	Tigemonam	0.2–25	0.8	3.9
	Ampicillin	12.5–>100	>100	>100
	Amoxicillin	100–>100	>100	>100
	Amoxicillin-clavulinic acid	12.5–100	31.9	79.2
	Cephalexin	100–>100	>100	>100
	Cefaclor	100–>100	>100	>100
	Cefuroxime (21)[a]	3.1–>100	23.4	>100
	Cefixime	0.8–>100	7.4	>100
	SXT	0.8–>100	2.5	5.6
	Oxytetracycline	3.1–>100	3.0	7.9
	Norfloxacin	0.1–0.4	0.1	0.2
Proteus mirabilis (24)	Tigemonam	<0.1–0.1	<0.1	<0.1
	Ampicillin	0.8–>100	1.2	>100
	Amoxicillin	0.8–>100	0.9	>100
	Amoxicillin-clavulinic acid	0.4–12.5	0.4	1.3
	Cephalexin	6.3–100	9.4	12.5
	Cefaclor	0.8–>100	1.9	4.9
	Cefuroxime (22)	0.8–3.1	1.4	2.8
	Cefixime	<0.1	<0.1	<0.1
	SXT	1.6–50	2.6	35.0
	Oxytetracycline	100–>100	>100	>100
	Norfloxacin	<0.1–0.2	<0.1	0.2
Proteus spp. (indole positive) and *Providencia* spp. (26)	Tigemonam	<0.1–0.2	<0.1	<0.1
	Ampicillin	0.8–>100	>100	>100
	Amoxicillin	0.8–>100	>100	>100
	Amoxicillin-clavulinic acid	0.4–>100	60.7	97.9
	Cephalexin	3.1–>100	>100	>100
	Cefaclor	0.8–>100	>100	>100
	Cefuroxime (12)	6.3–>100	>100	>100
	Cefixime	<0.1–3.1	0.2	1.5
	SXT	1.6–100	6.3	42.0
	Oxytetracycline	1.6–>100	>100	>100
	Norfloxacin	<0.1–12.5	<0.1	0.7
Citrobacter spp. (25)	Tigemonam	0.2–6.3	0.4	1.2
	Ampicillin	6.3–>100	52.5	>100
	Amoxicillin	25–>100	>100	>100
	Amoxicillin-clavulinic acid	1.6–100	17.7	45.3
	Cephalexin	3.1–>100	45.8	>100
	Cefaclor	1.6–>100	75.0	>100
	Cefuroxime (23)	1.6–>100	6.9	>100
	Cefixime	0.1–>100	1.0	5.7
	SXT	0.8–12.5	1.3	3.1
	Oxytetracycline	1.6–>100	2.2	3.1
	Norfloxacin	<0.1–0.4	<0.1	0.2
Klebsiella pneumoniae (24)	Tigemonam	<0.1–0.8	0.3	0.6
	Ampicillin	25–>100	>100	>100
	Amoxicillin	25–>100	>100	>100
	Amoxicillin-clavulinic acid	1.6–25	1.4	10.8
	Cephalexin	3.1–50	5.0	12.1
	Cefaclor	1.6–>100	3.0	>100
	Cefuroxime	0.4–>100	2.5	19.9
	Cefixime	<0.1–0.4	<0.1	<0.1
	SXT	3.1–>100	3.6	>100
	Oxytetracycline	1.6–>100	3.1	>100
	Norfloxacin	<0.1–1.6	0.1	0.2

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TABLE 1—*Continued*

Organism (no. of strains)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Klebsiella spp. (19)	Tigemonam	0.4–6.3	0.4	1.4
	Ampicillin	100–>100	>100	>100
	Amoxicillin	>100	>100	>100
	Amoxicillin-clavulinic acid	6.3–100	20.2	52.4
	Cephalexin	3.1–>100	12.2	>100
	Cefaclor	12.5–>100	>100	>100
	Cefuroxime (18)	3.1–100	5.6	70.1
	Cefixime	<0.1–>100	0.1	4.8
	SXT	1.6–>100	75.0	>100
	Oxytetracycline	3.1–>100	>100	>100
	Norfloxacin	<0.1–6.3	0.4	1.8
Serratia marcescens (33)	Tigemonam	0.2–3.1	0.5	1.5
	Ampicillin	25–>100	>100	>100
	Amoxicillin	50–>100	>100	>100
	Amoxicillin-clavulinic acid	12.5–>100	23.0	87.1
	Cephalexin	>100	>100	>100
	Cefaclor	>100	>100	>100
	Cefuroxime (28)	25–>100	45.8	>100
	Cefixime	0.2–25	0.5	2.7
	SXT	3.1–>100	5.7	42.5
	Oxytetracycline	12.5–>100	15.6	>100
	Norfloxacin	0.2–50	0.3	3.0
Salmonella spp. (24)	Tigemonam	0.4–1.6	0.7	1.4
	Ampicillin	1.6–>100	1.5	>100
	Amoxicillin	0.8–>100	1.3	>100
	Amoxicillin-clavulinic acid	0.8–12.5	0.7	8.8
	Cephalexin	6.3–50	5.4	12.2
	Cefaclor	3.1–>100	2.7	19.9
	Cefuroxime	6.3–12.5	7.2	11.5
	Cefixime	0.2–0.8	0.3	0.5
	SXT	1.6–12.5	1.5	5.8
	Oxytetracycline	1.6–>100	3.9	>100
	Norfloxacin	0.1–0.2	0.1	0.2
Shigella spp. (21)	Tigemonam	0.1–1.6	0.3	1.0
	Ampicillin	1.6–>100	>100	>100
	Amoxicillin	1.6–>100	>100	>100
	Amoxicillin-clavulinic acid	0.8–50	7.1	42.5
	Cephalexin	6.3–>100	18.8	>100
	Cefaclor	1.6–>100	42.5	>100
	Cefuroxime (19)	1.6–100	5.1	46.3
	Cefixime	<0.1–100	0.6	49.5
	SXT	0.8–12.5	1.8	6.1
	Oxytetracycline	0.8–>100	2.5	>100
	Norfloxacin	<0.1–0.1	<0.1	<0.1
Enterobacteriaceae (247)	Tigemonam	<0.1–25	0.3	1.2
	Ampicillin	0.8–>100	>100	>100
	Amoxicillin	0.8–>100	>100	>100
	Amoxicillin-clavulinic acid	0.4–>100	10.0	63.2
	Cephalexin	3.1–>100	22.4	>100
	Cefaclor	0.8–>100	>100	>100
	Cefuroxime (217)	0.2–>100	5.7	>100
	Cefixime	<0.1–>100	0.3	7.9
	SXT	0.4–>100	2.9	33.1
	Oxytetracycline	0.8–>100	8.0	>100
	Norfloxacin	<0.1–50	<0.1	0.4

[a] Numbers within parentheses after cefuroxime indicate the number of strains against which this antimicrobial agent was tested.

In all cases, trimethoprim-sulfamethoxazole (SXT; 1:19, wt/wt) was tested in medium containing 5% lysed horse blood (9).

Inocula were prepared by dilution of overnight broth cultures (Antibiotic Assay Broth no. 3; BBL) to ca. 5×10^8 CFU/ml. Plates were inoculated with a multipoint inoculator (Denly Instruments, Sussex, U.K.) delivering 0.001 ml resulting in a test inoculum of 5×10^5 CFU. In the panel of β-lactamase-producing bacteria the test inocula were 10^4 and 10^6 CFU. Plates were incubated for 18 to 20 h at 37°C in air, in 5% CO_2 (*Neisseria* and *Haemophilus* species and streptococci), or anaerobically (Forma Scientific, Marietta, Ohio).

MICs of amoxicillin-clavulanic acid were expressed as the concentration of amoxicillin contained in the amoxicillin-clavulanic acid (2:1, wt/wt) mixture. Similarly, the MICs of

TABLE 2. Comparative activity of tigemonam against *H. influenzae* and *N. gonorrhoeae*

Organism (no. of strains)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
H. influenzae, Amp^s (14)	Tigemonam	0.1–0.2	0.1	0.2
	Ampicillin	0.2–0.4	0.2	0.3
	Amoxicillin	0.4–0.8	0.5	0.7
	Amoxicillin-clavulinic acid	0.2–0.4	0.2	0.3
	Cephalexin	6.3–100	6.3	86.0
	Cefaclor	3.1–50	12.5	32.5
	Cefuroxime	0.4–12.5	0.8	1.5
	Cefixime	<0.1–0.1	<0.1	<0.1
	SXT	0.1–1.6	0.6	0.8
	Oxytetracycline	0.8–3.1	0.8	1.5
	Norfloxacin	<0.1–0.1	<0.1	<0.1
H. influenzae, Amp^r (20)	Tigemonam	<0.1–0.2	<0.1	0.1
	Ampicillin	>100	>100	>100
	Amoxicillin	>100	>100	>100
	Amoxicillin-clavulinic acid	0.4–0.8	0.6	0.8
	Cephalexin	6.3–50	8.2	12.1
	Cefaclor	12.5–50	13.8	23.8
	Cefuroxime	0.8–1.6	0.6	1.1
	Cefixime	<0.1	<0.1	<0.1
	SXT (19 strains)	0.4–0.8	0.6	0.8
	Oxytetracycline	0.8–50	0.7	1.5
	Norfloxacin	<0.1–0.1	<0.1	<0.1
N. gonorrhoeae (23)	Tigemonam	<0.1–0.8	<0.1	0.2
	Ampicillin (22 strains)	<0.1–100	0.2	0.7
	Amoxicillin	<0.1–100	0.2	0.7
	Amoxicillin-clavulinic acid	<0.1–0.8	0.1	0.3
	Cephalexin	0.8–50	2.4	9.9
	Cefaclor	0.1–3.1	0.5	1.6
	Cefixime	<0.1–0.1	<0.1	<0.1
	SXT	3.1–25	5.0	12.3
	Oxytetracycline	0.4–6.3	0.8	3.8
	Norfloxacin	<0.1–0.1	<0.1	<0.1

TABLE 3. Comparative activity of tigemonam against nonfermenters

Organism (no. of strains)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Pseudomonas aeruginosa (26)	Tigemonam	100–>100	>100	>100
	Ampicillin	>100	>100	>100
	Amoxicillin	100–>100	>100	>100
	Amoxicillin-clavulinic acid	50–>100	>100	>100
	Cephalexin	>100	>100	>100
	Cefaclor	>100	>100	>100
	Cefuroxime (21 strains)	>100	>100	>100
	Cefixime	50–>100	80.8	>100
	SXT	100–>100	>100	>100
	Oxytetracycline	25–50	26.8	45.4
	Norfloxacin	0.4–6.3	0.6	2.2
Acinetobacter spp. (25)	Tigemonam	3.1–>100	37.5	>100
	Ampicillin	0.8–>100	17.2	40.6
	Amoxicillin	0.8–>100	16.5	40.6
	Amoxicillin-clavulinic acid	0.8–>100	4.4	11.8
	Cephalexin	1.6–>100	>100	>100
	Cefaclor	0.4–>100	87.5	>100
	Cefixime	0.8–>100	9.8	22.3
	SXT	0.8–>100	2.4	6.0
	Oxytetracycline	1.6–25	4.8	11.4
	Norfloxacin	0.8–50	4.0	11.4

SXT were expressed as the concentration of sulfamethoxazole. The MICs for 50 and 90% of isolates (MIC_{50} and MIC_{90}, respectively) were calculated by extrapolation of the cumulative percent inhibition curves.

Bactericidal activity was determined by quantitative subculture from antibiotic-containing broths onto antibiotic-free agar. MBCs were determined by microbroth dilution testing in cation-supplemented Mueller-Hinton (BBL) broth (9) at 5×10^5 CFU/ml. After incubation at 37°C for 18 to 24 h, 10 μl was subcultured from each well. The MBC was defined as the concentration of drug killing 99.9% of the original inoculum. The kinetics of bactericidal activity (time-kill) was determined for *E. coli* SC 8294. An overnight broth culture was diluted into Antibiotic Assay Broth no. 3 containing the indicated concentrations of antibiotic, and cultures were incubated at 37°C with aeration. Samples of 0.1 ml were quantitatively subcultured onto 20 ml of antibiotic-free agar at the times indicated.

β-Lactamase. Partially purified preparations of K1 (*Klebsiella oxytoca* SC 10436), SHV-1 (*Klebsiella pneumoniae* SC 10999), OXA-1 (*E. coli* SC 10854), PSE-1 (*Pseudomonas aeruginosa* SC 12128), PSE-2 (*P. aeruginosa* SC 12138), PSE-3 (*P. aeruginosa* SC 12127), and PSE-4 (*P. aeruginosa* SC 12130) β-lactamases were prepared by using a freeze-thaw method followed by chromatography on Sephadex G75 with 0.05 M phosphate buffer (pH 7.0) as the eluent (3). TEM-2 (*E. coli* SC 11101) and P99 (*Enterobacter cloacae* SC 10435) β-lactamases were purified to greater than 95% homogeneity (11). All preparations contained a single β-lactamase as determined by isoelectric focusing (LKB Multiphor, prepared Ampholine PAGplates, pH 3.5 to 9.5; LKB Instruments, Inc., Gaithersburg, Md.). The identification of β-lactamases was based upon isoelectric points and substrate profiles (3).

Hydrolysis of tigemonam was determined spectrophotometrically (λ_{max} = 320 nm; ε = 416 per M per cm) (3). Assays were performed at 25°C by adding β-lactamase (0.05 to 600 μl, depending upon the activity of the preparation) to 0.20 to 1.00 ml of test solution and monitoring absorbance

TABLE 4. Activity of tigemonam against gram-positive and anaerobic bacteria

Organism (no. of strains)	Antimicrobial agent	MIC range (μg/ml)
Staphylococcus aureus (10)	Tigemonam	>100
	Aztreonam	>100
Streptococcus faecalis (10)	Tigemonam	>100
	Aztreonam	>100
Streptococcus group A (8)	Tigemonam	0.8–1.6
	Aztreonam	12.5
Streptococcus group B (7)	Tigemonam	50
	Aztreonam	>100
Streptococcus pneumoniae (3)	Tigemonam	3.1–>100
	Aztreonam	50–>100
Corynebacterium spp. (2)	Tigemonam	>100
	Aztreonam	>100
Bacillus fragilis (14)	Tigemonam	12.5–>100
	Cefoxitin	3.1–12.5
Clostridium spp. (5)	Tigemonam	12.5–>100
	Cefoxitin	0.4–25

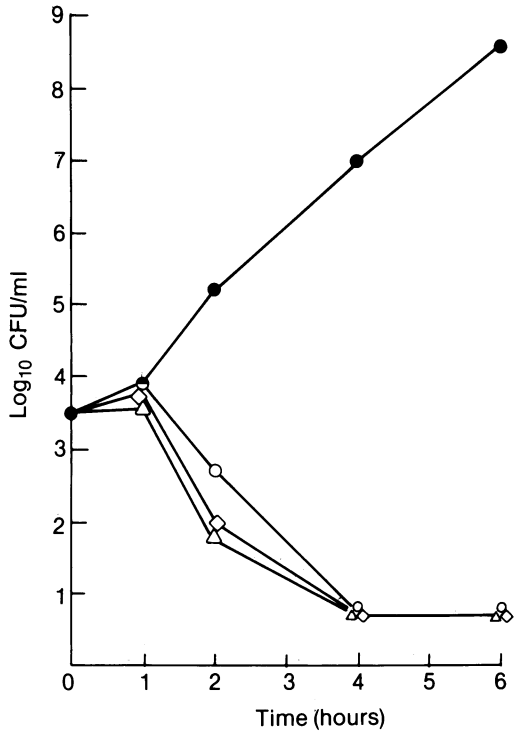

FIG. 2. Bactericidal kinetics of tigemonam against *E. coli* SC 8294. Symbols: (●) untreated; (◇) cephalexin, 100 μg/ml (MIC 12.5 μg/ml; (○) tigemonam, 1.0 μg/ml (MIC 0.4 μg/ml); (△) tigemonam, 5.0 μg/ml.

changes on a Gilford spectrophotometer (model 250 or 2600; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Rates of hydrolysis were calculated as micromoles of β-lactam hydrolyzed per minute per microgram of protein. Kinetic constants, K_m and V_{max} (micromoles of substrate hydrolyzed per minute per milligram of protein), were determined for each enzyme from Lineweaver-Burk plots of at least four different substrate concentrations. Values for V_{max} for cephaloridine (nanomoles per minute per microgram of protein) with each enzyme were as follows: TEM-2, 2,480; SHV-1, 24.9; OXA-1, 0.90; K1, 620; P99, 1,100. The V_{max}/K_m ratio (efficiency of hydrolysis [11]) was normalized with respect to cephaloridine (V_{max}/K_m = 100).

RESULTS

The inhibitory activity in vitro of tigemonam compared with other oral antibacterial agents is shown in Tables 1 through 4. Against members of the *Enterobacteriaceae* tigemonam was far more active than the oral penicillins (including amoxicillin-clavulanic acid), cephalosporins, SXT, and oxytetracycline (Table 1). Tigemonam was also generally more active than the aminothiazole cephalosporin cefixime, especially against *Enterobacter* and *Shigella* species. When compared with norfloxacin against members of the *Enterobacteriaceae*, similar activity was observed.

Tigemonam exhibited excellent activity against *N. gonorrhoeae* and *H. influenzae*. This activity was not affected by β-lactamase and was comparable to the activity of cefixime and norfloxacin (Table 2).

Tigemonam was not significantly active against *P. aeruginosa* and exhibited variable activity against *Acinetobacter* spp. (Table 3). Poor or variable activity was also seen against anaerobes and gram-positive cocci except Lancefield group A streptococci (Table 4).

Tigemonam exhibited bactericidal kinetics typical of β-lactam antibiotics (Fig. 2). The inhibitory activity of tigemonam was identical to its bactericidal activity (data not shown).

Excellent activity of tigemonam was also noted against β-lactamase-producing, gram-negative bacilli at low and high inocula (Table 5), reflecting its excellent stability to β-lactamases (Table 6). Tigemonam was markedly stable to the common, plasmid-mediated β-lactamases TEM-2, SHV-1, and OXA-1. This was in contrast to the instability exhibited by the penicillins and the broad-spectrum cephalosporins. Only cefixime was as stable as tigemonam to hydrolysis by the TEM-2 and SHV-1 β-lactamases, but cefixime was readily destroyed by the OXA-1 enzyme. As with the plasmid-mediated enzymes, tigemonam was stable to the chromosomally mediated β-lactamases K1, from *Klebsiella* sp. and P99, from *Enterobacter* sp. Once again, the early β-lactams were unstable. Cefixime was readily hydrolyzed by the P99 β-lactamase, consistent with its relatively poor activity against *Enterobacter* strains (Table 1).

Tigemonam was also stable to hydrolysis by the plasmid-mediated PSE β-lactamases. Efficiencies of hydrolysis of tigemonam were 0.67, 0.5, and 0.22 for PSE-1, PSE-3, and PSE-4 β-lactamases, respectively, relative to cephaloridine (V_{max}/K_m = 100). The K_m of tigemonam for the PSE-2

TABLE 5. Activity of tigemonam against β-lactamase-producing bacteria

Organism (β-lactamase)	SC strain no.	MIC (μg/ml) at the indicated inoculum (10^4 or 10^6 CFU)															
		Tigemonam		Ampicillin		Amoxicillin		Amoxicillin-clavulinic acid		Cephalexin		Cefaclor		Cefuroxime		Cefixime	
		10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6
E. coli (TEM-1)	10404	0.2	0.8	>100	>100	>100	>100	25	25	6.3	25	3.1	>100	3.1	6.3	0.2	0.8
E. coli (OXA-1)	10854	0.4	1.6	>100	>100	>100	>100	25	50	12.5	50	12.5	25	6.3	25	1.6	3.1
Citrobacter freundii	10204	0.2	0.8	50	>100	>100	>100	100	100	100	>100	50	>100	1.6	100	1.6	25
Shigella sonnei	10944	0.8	1.6	>100	>100	>100	>100	100	100	>100	>100	>100	>100	50	50	50	50
E. cloacae (P99)	10435	6.3	50	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
E. cloacae	9965	<0.1	0.2	100	>100	>100	>100	100	100	>100	>100	>100	>100	3.1	25	0.8	6.3
K. oxytoca (K1)	10436	1.6	12.5	>100	>100	>100	>100	25	50	100	>100	>100	>100	>100	>100	0.2	1.6
K. pneumoniae	11066	0.4	1.6	>100	>100	>100	>100	25	25	6.3	25	25	>100	3.1	6.3	0.2	0.4
Providencia rettgeri	8217	<0.1	<0.1	50	>100	>100	>100	100	>100	>100	>100	25	>100	3.1	100	<0.1	0.4
P. stuartii	11104	<0.1	<0.1	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	12.5	100	0.8	12.5
Proteus vulgaris	10950	<0.1	<0.1	>100	>100	>100	>100	6.3	12.5	>100	>100	100	>100	>100	>100	<0.1	0.1
S. marcescens	8247	0.4	0.8	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	12.5	>100

TABLE 6. Stabilities of β-lactam antibiotics to β-lactamases

Antibiotic	TEM-2		SHV-1		OXA-1		K1		P99	
	K_m (μM)	Rel V_{max}[a]/K_m	K_m (μM)	Rel V_{max}/K_m	K_m (μM)	Rel V_{max}/K_m	K_m (μM)	Rel V_{max}/K_m	K_m (μM)	Rel V_{max}/K_m
Cephaloridine	650	100	160	100	640	100	150	100	730	100
Tigemonam	350	0.014	740	0.015	ND[b]	≤0.5[c]	1,800	0.31	980	0.007
Ampicillin	53	970	48	1,000	17	15,000	170	180	20	0.60
Amoxicillin	56	790	62	1,200	61	5,800	140	150	13	3.2
Cephalexin	1,500	0.37	3,600	0.95	62	16	190	3.0	76	47
Cefaclor	190	19	2,800	6.2	590	150	220	35	300	48
Cefuroxime	2,000	0.29	650	0.17	78	29	160	19	16	0.13
Cefixime	2,100	0.004	ND[b]	≤0.01[c]	52	12	560	0.18	2.0	16

[a] V_{max} values for cephaloridine are cited in the text for each enzyme. Rel, Relative.
[b] Rates were too slow for a reliable determination of K_m.
[c] Relative V_{max}, using the V_{max} for cephaloridine as 100.

enzyme could not be reliably determined because of the slow rate of hydrolysis. However, the relative V_{max} of ≤0.4 (V_{max} of cephaloridine = 100) indicated that tigemonam was stable to hydrolysis by the PSE-2 β-lactamase.

DISCUSSION

Tigemonam is a novel oral synthetic monobactam. The activity in vitro of tigemonam was not unlike that of the first parenteral monobactam, aztreonam (2). Although it lacks the notable activity of aztreonam against *P. aeruginosa*, tigemonam was highly active against members of the *Enterobacteriaceae*, *H. influenzae*, and *N. gonorrhoeae* and was stable to the common plasmid-mediated and chromosomally mediated β-lactamases.

The activity in vitro of tigemonam was superior to that of ampicillin, amoxicillin, amoxicillin-clavulanic acid, cephalexin, and cefaclor due to the β-lactamase stability and intrinsic activity of tigemonam. In addition, the spectrum of activity of tigemonam extends to members of the *Enterobacteriaceae* that are intrinsically resistant to penicillins and cephalosporins (10). Tigemonam inhibited >90% of the *Enterobacteriaceae* tested at ≤1.6 μg/ml. At this concentration ampicillin, amoxicillin, amoxicillin-clavulanic acid, cephalexin, cefaclor, and cefuroxime inhibited <30% of the strains.

Tigemonam was also superior to the non-β-lactam oral agents SXT and oxytetracycline (a representative tetracycline) with the added advantage of being bactericidal. At 1.6 μg/ml, SXT and oxytetracycline inhibited 27.1 and 6.5% of the *Enterobacteriaceae* tested, respectively.

Tigemonam had activity generally similar to that of cefixime, a new oral cephalosporin (7). However, tigemonam was much more active against *Enterobacter* spp. (tigemonam MIC_{90}, 3.9 μg/ml versus >100 μg/ml for cefixime), *Proteus* spp. and *Providencia* spp. (tigemonam MIC_{90}, ≤0.1 μg/ml versus 1.5 μg/ml for cefixime), and *Shigella* spp. (tigemonam MIC_{90}, 1.0 μg/ml versus 49.5 μg/ml for cefixime). This may reflect the excellent stability of tigemonam compared with cefixime to chromosomal β-lactamases.

Of the oral antibiotics tested, tigemonam was most similar to norfloxacin in activity (1). Greater than 95% of the *Enterobacteriaceae*, *H. influenzae*, and *N. gonorrhoeae* were inhibited by either tigemonam or norfloxacin at ≤1.6 μg/ml (294 of 304 isolates).

The in vitro and in vivo antibacterial activity of tigemonam indicates a potential for orally administered treatment of infectious diseases caused by gram-negative bacteria. Alone or in combination with complementary agents against gram-positive bacteria, tigemonam extends outpatient antibiotic coverage to include the β-lactamase-producing gram-negative bacteria that are increasingly prevalent in community-acquired infections. With the risk of acquiring nosocomial infections and the increasing pressure to decrease hospital-associated costs, a role for tigemonam as an outpatient adjunct to primary parenteral therapy can be envisaged (12).

Thus, tigemonam is an oral antibiotic with potential for the treatment of many infectious diseases that are either less predictably treatable or untreatable with the currently available oral agents.

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