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In Vitro Activity and β-Lactamase Stability of a New Carbapenem, SM-7338

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SM-7338, a new carbapenem, inhibited most members of the family *Enterobacteriaceae* at MICs of 0.015 to 0.25 μg/ml, including *Klebsiella oxytoca*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Proteus vulgaris* isolates resistant to cefotaxime, ceftazidime, piperacillin, and gentamicin. It was two- to eightfold more active than imipenem, but it inhibited *Pseudomonas aeruginosa* at 1 to 8 μg/ml, which was comparable to the activity of imipenem. *Haemophilus*, *Neisseria*, and *Branhamella* species were inhibited by ≤0.25 μg/ml, which was superior to the activity of imipenem. SM-7338 inhibited *Staphylococcus aureus* and coagulase-negative staphylococci at 0.25 μg/ml, but for methicillin-resistant isolates MICs were 4 to 16 μg/ml. Group A, B, and C streptococci and *Streptococcus pneumoniae* were inhibited by ≤0.03 μg/ml. *Bacteriodes* species, including clindamycin-resistant isolates, were inhibited by 0.25 μg/ml. There was no major inoculum size effect, and the MBCs were within a dilution of the MICs. SM-7338 was more active than imipenem at an acid pH under anaerobic conditions. Plasmid β-lactamases of TEM-1, TEM-2, TEM-3, TEM-5, SHV-1, SHV-2, PSE-1, PSE-2, PSE-3, OXA-2, OXA-3, OXA-4, OXA-5, and OXA-7; *Staphylococcus aureus* enzymes; and the chromosomal β-lactamases P-99 and K-1; *Morganella* species; and *Proteus vulgaris* did not hydrolyze SM-7338. The repeated transfer of organisms increased the MICs of SM-7338, as it did the MICs of imipenem.

The carbapenem imipenem has proved to be an extremely useful antibacterial agent because of its great β-lactamase stability and high intrinsic activity against a broad range of bacteria (3, 5, 11). Although carbapenems have been extremely stable to attack by β-lactamases, they are hydrolyzed by the zinc-activated β-lactamase that is present in *Bacillus* species, by *Xanthomonas maltophilia*, by some *Pseudomonas cepacia* isolates, and by occasional *Bacteroides* species (6, 10, 13). Most carbapenems are hydrolyzed by dihydropeptidases, which are present in renal tissue (3, 12, 13). We investigated the activity of a new carbapenem, SM-7338, that has been preliminarily reported to possess excellent antimicrobial activity (M. Sunagawa, H. Matsumura, T. Inoue, M. Fukasawa, and M. Kato, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 752, 1987). SM-7338 is of great interest since it has been reported to have excellent pharmacokinetic properties and to resist destruction by renal dehydropeptidase-1 (T. Tanio, H. Nouda, E. Tada, T. Kohzuki, M. Kato, M. Fukasawa, T. Okuda, and S. Kamidono, 27th ICAAC, abstr. no. 758, 1987).

MATERIALS AND METHODS

SM-7338 was supplied by Summitomo Pharmaceutical Company, Ltd., Osaka, Japan, and Stuart Pharmaceuticals, Wilmington, Del. The other antimicrobial agents were provided as gifts from their manufacturers. The organisms used were isolates from patients in hospitals in the Columbia University system and included isolates that were selected because of known β-lactamase activity or resistance to β-lactam antibiotics on the basis of β-lactamase or permeability changes, as determined by analysis of outer membrane proteins. Some isolates with the new cefotaxime-ceftazidime-hydrolyzing β-lactamases were gifts from workers in France (4).

Antimicrobial susceptibility tests. Antimicrobial activity was measured by an agar dilution method with Mueller-Hinton agar, unless otherwise specified. A final inoculum of 10^4 CFU was applied with a replicating device. Broth dilution tests were performed with 5×10^5 CFU in tubes containing 1 ml of broth. Incubation of test tubes containing agar and broth was done at 35°C for 18 h. The susceptibilities of *Neisseria* and *Haemophilus* species were determined with chocolate Mueller-Hinton agar in the presence of 5% CO_2. The susceptibilities of streptococci were determined by using Mueller-Hinton agar supplemented with 5% sheep blood, and the susceptibility of anaerobic species was determined with brucella agar supplemented with 5% sheep blood, hemin, and vitamin K. Incubation of anaerobic cultures was done for 48 h in jars (GasPak; BBL Microbiological Systems, Cockeysville, Md.). The susceptibilities of methicillin-resistant staphylococci were determined on Mueller-Hinton agar or in broth supplemented with 3% NaCl; isolates for which the MIC of oxacillin was >4 μg/ml were considered resistant. The MIC was defined as the lowest concentration of antibiotic that inhibited the development of visible growth on agar or in broth. The MBC was determined by subculturing 0.01 ml of culture from clear tubes onto antibiotic-free agar plates; the MBC was the concentration at which there was a 99.9% reduction in CFU, considering the pipetting error for the system (9). All assays were run with the following control strains (from the American Type Culture Collection, Rockville, Md.): *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 22913, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 29853.

β-Lactamase assays in inhibition studies. The presence of β-lactamase in the clinical isolates was determined by the nitrocefin assay (7). Enzymes used to analyze the stability of SM-7338 and other agents were prepared as described pre-

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viously (7). The β-lactamase stability was determined by a spectrophotometric assay as described previously (7). Inhibition assays were performed with either nitrocefin or cephaloridine as substrate, and various concentrations of SM-7338 were used to calculate 50% inhibitory concentrations. K_m and K_i values were calculated by using a standard Lineweaver-Burke plot.

Induction of β-lactamases. One strain each of *Pseudomonas aeruginosa* and *Serratia marcescens* was grown overnight in broth and diluted 100-fold to achieve exponential growth. During incubation at 35°C, SM-7338 was added at concentrations that were one and four times the MIC, and incubation was continued for 2 h. Bacteria were harvested by centrifugation, washed in 0.05 mM potassium phosphate buffer (pH 7), and subsequently disrupted by sonication. Cellular debris was removed by centrifugation, and the samples were dialyzed at 4°C for 24 h against phosphate buffer. β-Lactamase activity was determined by using nitrocefin as the substrate, with the activity based on the micromoles of substrate hydrolyzed per milliliter of protein. The two organisms used were previously shown to be inducible with cefoxitin, CGP 31608, and other β-lactams (8).

Development of progressive resistance. The development of progressive resistance to SM-7338 and imipenem was determined for single isolates of *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Staphylococcus aureus*, and *Escherichia coli*. An inoculum of 5×10^5 CFU was placed in tubes of 1 ml containing twofold increasing concentrations of either SM-7338 or imipenem. After 24 h of incubation, samples were removed from the tubes with the highest dilution showing visible growth and were reinoculated into tubes containing an increasing concentration of either of the two antibiotics. This process was repeated daily for a period of 10 days. Organisms selected in this manner were then repeatedly passed for 1 week on antibiotic-free medium to determine the stability of the resistance selected by this technique.

To determine the frequency of spontaneous point mutational resistance to SM-7338, 10^{10} organisms obtained by centrifugation of cultures of different species were exposed to the drug in agar at four times the MIC. Plates were incubated for 48 h at 35°C. Organisms growing on these plates were subsequently tested to establish the MIC for the organism.

Postantibiotic suppression effect. Exponentially growing bacteria at 10^6 CFU were exposed to a concentration of antibiotic at twice the MBC for 2 h. Samples were removed, diluted 10,000-fold in fresh antibiotic-free medium to yield 10^6 CFU/ml, and placed in a 37°C shaking water bath. Samples were removed every 2 h and plated onto agar to determine the CFU remaining and the rate of regrowth. Control organisms not exposed to antibiotic were processed in an identical fashion. The postantibiotic effect was calculated as described previously (2).

Synergy studies. Synergy was determined by using a checkerboard agar method, with concentrations of drugs varying twofold. Synergy was defined as a fractional inhibitory concentration of ≤0.5, and antagonism was defined as a fractional inhibitory concentration of ≥4.

RESULTS

The comparative in vitro activities of SM-7338 and other agents against gram-negative aerobic species are shown in Table 1. Overall, SM-7338 had an extremely narrow range of inhibitory values for most species. The MIC of SM-7338 for 90% of the strains (MIC_{90}) of members of the family *Enterobacteriaceae*, with the exception of *Serratia marcescens*, was ≤0.25 μg/ml. The MIC_{90} for *S. marcescens* was 0.5 μg/ml. SM-7338 was more active than imipenem against the majority of the *Enterobacteriaceae*. For example, the SM-7338 MIC_{90} for *Escherichia coli* was 0.03 μg/ml compared with an imipenem MIC_{90} of 0.5 μg/ml; the SM-7338 MIC_{90} for *Enterobacter cloacae* was 0.25 μg/ml compared with an imipenem MIC_{90} of 2 μg/ml; and the SM-7338 MIC_{90} for *Citrobacter freundii* was 0.12 μg/ml compared with an imipenem MIC_{90} of 2 μg/ml. The largest difference in MIC was seen for *Providencia stuartii*, with an MIC_{90} of SM-7338 of 0.12 μg/ml compared with an MIC_{90} of imipenem of 4 μg/ml. SM-7338 inhibited isolates of *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Citrobacter freundii* which were resistant to cefotaxime and ceftazidime at MICs of >128 μg/ml for some of these isolates. It also inhibited *Klebsiella pneumoniae*, *Citrobacter freundii*, *Proteus rettgeri*, *Providencia stuartii*, and *Serratia marcescens*, which were resistant to gentamicin.

SM-7338 was twofold more active than imipenem against *Pseudomonas aeruginosa* isolates, inhibiting isolates that were resistant to ceftazidime, piperacillin, and gentamicin. In general, the SM-7338 MICs for imipenem-resistant isolates were twofold lower; that is, for isolates with imipenem MICs for 16 μg/ml the SM-7338 MICs were 4 or 8 μg/ml. SM-7338 did not inhibit most isolates of *Pseudomonas cepacia*, and all of the *Xanthomonas maltophilia* isolates were resistant. Of the other *Pseudomonas* species tested, *P. putida*, *P. stutzeri*, *P. fluorescens*, and *P. diminuta* were inhibited by concentrations of 0.02 to 4 μg/ml. SM-7338 and imipenem showed equal activity against the *Acinetobacter* species, inhibiting isolates that were resistant to cefotaxime, ceftazidime, piperacillin, and gentamicin. SM-7338 was more active than imipenem against *Haemophilus influenzae* isolates, but was less active than cefotaxime. SM-7338 inhibited *Neisseria gonorrhoeae* at lower concentrations than those of imipenem, similar to the concentrations of cefotaxime and ceftazidime. It also inhibited β-lactamase-producing *N. gonorrhoeae* (four isolates; data not shown) and two isolates of *N. gonorrhoeae* (data not shown) that were resistant to spectinomycin and tetracycline. The MIC of SM-7338 for eight isolates of *Branhamella catarrhalis* (0.015 to 0.25 μg/ml) was eightfold lower than that of imipenem and was equivalent to those of cefotaxime and ceftazidime. Three isolates of *Neisseria meningitidis* (data not shown) were inhibited by concentrations of 0.12 μg/ml.

SM-7338 inhibited methicillin-susceptible *Staphylococcus aureus* strains at concentrations of 0.06 to 1 μg/ml (Table 2). The MIC_{90} of SM-7338 was twofold greater than that of imipenem, but was manyfold lower than the MIC_{90}s of comparable agents that were tested. SM-7338 had MIC_{90}s of 8 μg/ml for methicillin-resistant staphylococci; these concentrations were similar to the concentrations found for imipenem. The majority of these isolates were also resistant to gentamicin. The activity of SM-7338 against coagulase-negative staphylococci which were methicillin susceptible was similar to its activity against methicillin-susceptible *Staphylococcus aureus* and similar to the activity of imipenem. It did not inhibit all of the methicillin-resistant, coagulase-negative staphylococci, which included both *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, but it was more active than imipenem. SM-7338 inhibited five isolates of *Staphylococcus saprophyticus* at 0.12 μg/ml (data not shown). It also inhibited *Streptococcus pyogenes*, *Streptococcus agalactiae*, and hemolytic strepto-

TABLE 1. Comparative in vitro activities of SM-7338 and other antimicrobial agents against gram-negative organisms

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (30)	SM-7338	≤0.015–0.06	≤0.015	0.03
	Imipenem	0.03–0.5	0.25	0.5
	Cefotaxime	0.015–0.25	0.06	0.12
	Ceftazidime	0.03–1	0.25	0.5
	Piperacillin	0.25–>128	32	>128
	Gentamicin	0.06–2	1	2
Klebsiella pneumoniae (29)	SM-7338	≤0.015–0.06	0.03	0.06
	Imipenem	0.12–2	0.25	1
	Cefotaxime	0.015–0.5	0.06	0.12
	Ceftazidime	0.06–2	0.25	1
	Piperacillin	2–>128	16	>128
	Gentamicin	0.25–>128	0.5	32
Klebsiella oxytoca (20)	SM-7338	0.03–0.06	0.03	0.06
	Imipenem	0.12–1	0.25	0.5
	Cefotaxime	≤0.015–0.25	0.03	0.12
	Ceftazidime	0.06–1	0.25	0.5
	Piperacillin	2–>128	8	128
	Gentamicin	0.25–2	0.5	1
Enterobacter aerogenes (14)	SM-7338	0.03–0.25	0.06	0.12
	Imipenem	0.12–2	1	1
	Cefotaxime	0.06–64	0.12	32
	Ceftazidime	0.06–64	0.25	32
	Piperacillin	2–128	4	64
	Gentamicin	0.25–1	0.5	1
Enterobacter cloacae (29)	SM-7338	0.03–0.5	0.12	0.25
	Imipenem	0.03–2	0.5	2
	Cefotaxime	0.06–>128	1	64
	Ceftazidime	0.06–>128	2	>128
	Piperacillin	0.5–>128	4	>128
	Gentamicin	0.12–64	0.5	32
Citrobacter freundii (20)	SM-7338	0.03–0.25	0.6	0.12
	Imipenem	0.25–2	1	2
	Cefotaxime	0.12–>128	2	128
	Ceftazidime	0.25–>128	1	>128
	Piperacillin	2–>128	4	>128
	Gentamicin	0.5–>128	1	32
Citrobacter diversus (12)	SM-7338	0.03–0.06	0.03	0.06
	Imipenem	0.25–4	0.5	1
	Cefotaxime	0.06–0.25	0.06	0.12
	Ceftazidime	0.06–0.25	0.12	0.25
	Piperacillin	4–>128	8	32
	Gentamicin	0.25–2	1	1
Proteus mirabilis (15)	SM-7338	0.06–0.12	0.06	0.12
	Imipenem	1–8	2	8
	Cefotaxime	0.015–0.12	0.015	0.03
	Ceftazidime	0.06–0.25	0.06	0.12
	Piperacillin	0.25–1	0.5	1
	Gentamicin	0.5–8	1	2
Morganella morganii (15)	SM-7338	0.03–0.25	0.12	0.25
	Imipenem	1–8	4	4
	Cefotaxime	0.015–4	0.03	1
	Ceftazidime	0.015–8	0.12	4
	Piperacillin	0.12–32	2	8
	Gentamicin	0.25–1	0.5	1
Proteus vulgaris (15)	SM-7338	0.03–0.25	0.06	0.12
	Imipenem	0.25–8	1	2
	Cefotaxime	≤0.06–16	0.25	2
	Ceftazidime	≤0.06–4	≤0.06	0.25
	Piperacillin	0.25–>128	4	>128
	Gentamicin	0.25–16	0.5	4

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TABLE 1—*Continued*

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Proteus rettgeri (10)	SM-7338	0.03–0.06	0.06	0.06
	Imipenem	0.25–1	0.5	1
	Cefotaxime	≤0.015–64	0.06	16
	Ceftazidime	0.03–32	0.25	4
	Piperacillin	0.25–>128	0.5	>128
	Gentamicin	0.25–>16	2	>16
Providencia stuartii (20)	SM-7338	0.03–2	0.06	0.12
	Imipenem	0.5–4	2	4
	Cefotaxime	≤0.015–2	0.06	1
	Ceftazidime	0.03–32	0.5	1
	Piperacillin	0.25–>128	8	>128
	Gentamicin	0.5–128	8	32
Serratia marcescens (19)	SM-7338	≤0.015–0.5	0.06	0.5
	Imipenem	0.06–16	1	4
	Cefotaxime	0.12–16	1	4
	Ceftazidime	0.06–2	0.25	1
	Piperacillin	1–>128	16	>128
	Gentamicin	0.25–32	1	16
Pseudomonas aeruginosa (28)	SM-7338	0.25–8	2	8
	Imipene	0.5–32	4	16
	Cefotaxime	16–>128	128	>128
	Ceftazidime	0.25–128	4	32
	Piperacillin	8–>128	16	64
	Gentamicin	0.5–>128	4	64
Pseudomonas cepacia (11)	SM-7338	0.25–>64	2	>64
	Imipenem	2–>128	16	128
	Cefotaxime	4–>128	16	128
	Ceftazidime	2–>128	2	64
	Piperacillin	2–>128	8	>128
	Gentamicin	0.5–>128	128	>128
Xanthomonas maltophilia (10)	SM-7338	16–>64	>64	>64
	Imipenem	>128	>128	>128
	Cefotaxime	64–>128	128	>128
	Ceftazidime	4–128	32	128
	Piperacillin	16–>128	128	>128
	Gentamicin	1–>128	8	64
Other *Pseudomonas* species[a] (10)	SM-7338	0.12–4	0.25	0.5
	Imipenem	0.25–2	1	2
	Cefotaxime	0.5–64	4	64
	Ceftazidime	0.25–4	0.5	4
	Piperacillin	1–32	2	16
	Gentamicin	0.5–>128	4	64
Acinetobacter anitratus (15)	SM-7338	0.12–4	0.5	1
	Imipenem	0.06–2	0.25	1
	Cefotaxime	1–64	16	32
	Ceftazidime	1–>128	8	32
	Piperacillin	0.5–>128	8	>128
	Gentamicin	0.25–>128	2	8
Salmonella species (15)	SM-7338	0.06–0.12	0.06	0.12
	Imipenem	0.5–1	0.5	1
	Cefotaxime	0.06–0.5	0.12	0.5
	Ceftazidime	0.25–4	0.5	4
	Piperacillin	2–>128	4	>128
	Gentamicin	0.25–2	0.5	2
Shigella species (15)	SM-7338	0.03–0.06	0.03	0.06
	Imipenem	0.25–2	0.25	1
	Cefotaxime	0.015–0.12	0.03	0.12

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TABLE 1—*Continued*

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
	Ceftazidime	0.12–4	0.25	2
	Piperacillin	0.25–>128	2	>128
	Gentamicin	0.25–2	1	1
Aeromonas hydrophila (15)	SM-7338	≤0.015–0.5	0.03	0.12
	Imipenem	0.12–2	0.25	2
	Cefotaxime	0.03–32	0.06	2
	Ceftazidime	0.12–32	0.25	16
	Piperacillin	1–>128	4	64
	Gentamicin	0.25–4	1	1
Yersinia enterocolitica (9)	SM-7338	0.03	0.03	
	Imipenem	0.12–0.5	0.25	
	Cefotaxime	0.03–1	0.06	
	Ceftazidime	0.06–1	0.12	
	Piperacillin	0.5–16	2	
	Gentamicin	0.25–1	0.5	
Haemophilus influenzae (15)	SM-7338	0.015–0.5	0.12	0.5
	Imipenem	0.5–8	1	4
	Cefotaxime	≤0.008–0.12	0.015	0.12
	Ceftazidime	≤0.015–0.25	0.12	0.25
	Piperacillin	0.06–4	0.06	4
	Gentamicin	2–8	4	4
Branhamella catarrhalis (8)	SM-7338	0.015–0.25		
	Imipenem	0.5–4		
	Cefotaxime	0.015–0.25		
	Ceftazidime	0.015–0.25		
Neisseria gonorrhoeae (11)	SM-7338	≤0.008–0.12	0.015	0.03
	Imipenem	0.12–4	0.5	1
	Cefotaxime	0.008–0.12	0.008	0.03
	Ceftazidime	≤0.015–2	0.03	0.06
	Piperacillin	≤0.015–2	0.015	0.12
	Gentamicin	0.5–8	4	8

[a] The other *Pseudomonas* species were *P. fluorescens*, *P. putida*, *P. stutzeri*, and *P. diminuta*.

cocci belonging to groups C and G; but it was less active than imipenem. SM-7338 was severalfold less active against viridans group streptococci, which included isolates of *Streptococcus mutans*, *Streptococcus sanguis*, and *Streptococcus salivarius*. In contrast, SM-7338 had activity equal to that of imipenem against *Streptococcus pneumoniae*. None of the pneumococci were penicillin resistant. SM-7338 was eightfold less active than imipenem against *Enterococcus faecalis* (MIC_{90}, 8 μg/ml). For isolates of *Enterococcus faecium*, which had imipenem MICs of >8 μg/ml, the MICs of SM-7338 were >8 μg/ml. Imipenem also was more active against *Listeria monocytogenes* isolates, with an MIC_{90} of 0.5 μg/ml compared with 4 μg/ml for SM-7338.

SM-7338 had activity comparable or twofold better than that of imipenem against *Bacteroides fragilis*, inhibiting isolates that were resistant to piperacillin and clindamycin. SM-7338 inhibited *Bacteroides thetaiotaomicron* which were clindamycin and piperacillin resistant at concentrations similar to that of imipenem, and it inhibited *Bacteroides vulgatus* isolates that were resistant to piperacillin. SM-7338 inhibited peptostreptococci, *Propionibacterium acnes*, and *Clostridium perfringens* isolates at concentrations similar to those of imipenem. It was more active against *Clostridium perfringens* than was imipenem, inhibiting 90% of the isolates at 0.12 μg/ml. SM-7338 inhibited peptococci, fusobacteria, and eubacteria (three isolates of each; data not shown) at concentrations of ≤0.03 μg/ml.

Effect of assay conditions. The in vitro activity of SM-7338 against 30 members of the family *Enterobacteriaceae*, 30 *Pseudomonas aeruginosa* isolates, 30 *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates, and 10 *Enterococcus faecalis* isolates did not differ by more than twofold when determined in Mueller-Hinton agar, Columbia agar, or tryptic soy digest medium with different conductivities and osmolalities. The MICs for *Xanthomonas maltophilia*, when determined on nutrient agar, were 8 μg/ml, in contrast to the MICs determined on Mueller-Hinton agar, which were >64 μg/ml. Imipenem MICs remained >64 μg/ml. Broth dilution MICs for *Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Pseudomonas aeruginosa* (five isolates of each species) were within a factor of 2 of the agar MICs.

The MICs and MBCs of SM-7338 were determined against a number of members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*, all of which contained β-lactamases. The MBCs were within a factor of 2 of the MICs for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Staphylococcus aureus*. The MBCs of *Serratia*

TABLE 2. Comparative in vitro activities of SM-7338 and other antimicrobial agents against gram-positive and anaerobic organisms

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (19)	SM-7338	0.06–1	0.25	0.25
	Imipenem	0.03–2	0.06	0.12
	Cefotaxime	1–16	2	8
	Ceftazidime	2–32	8	16
	Piperacillin	0.5–128	8	64
	Gentamicin	4–16	4	8
Staphylococcus aureus, methicillin resistant (13)	SM-7338	≤0.015–16	2	8
	Imipenem	0.03–>128	2	>128
	Cefotaxime	1–128	4	64
	Ceftazidime	2–>128	16	>128
	Piperacillin	0.12–>128	32	>128
	Gentamicin	2–>128	8	>128
Staphylococcus epidermidis, methicillin susceptible (13)	SM-7338	0.06–0.5	0.12	0.25
	Imipenem	0.03–2	0.06	0.5
	Cefotaxime	0.12–32	0.5	2
	Ceftazidime	2–16	4	16
	Piperacillin	0.12–64	1	32
	Gentamicin	0.5–>128	2	8
Staphylococcus epidermidis, methicillin resistant (15)	SM-7338	0.06–16	0.12	8
	Imipenem	0.03–128	0.12	32
	Cefotaxime	0.25–128	1	128
	Ceftazidime	2–128	4	64
	Piperacillin	0.5–64	1	64
	Gentamicin	2–>128	>128	>128
Streptococcus pyogenes (20)	SM-7338	0.008–0.015	0.008	0.008
	Imipenem	0.008–0.015	0.008	0.008
	Cefotaxime	0.008–0.015	0.008	0.008
	Ceftazidime	0.03–0.25	0.12	0.012
	Piperacillin	0.12	0.12	0.12
	Gentamicin	0.25–8	1	2
Streptococcus group B (21)	SM-7338	0.008–0.03	0.015	0.03
	Imipenem	0.008–0.03	0.008	0.008
	Cefotaxime	0.008–0.06	0.015	0.06
	Ceftazidime	0.12–0.5	0.25	0.5
	Piperacillin	≤0.12	≤0.12	≤0.12
	Gentamicin	0.5–16	8	16
Streptococcus groups C and G (20)	SM-7338	0.008–0.03	0.008	0.015
	Imipenem	0.008–0.03	0.015	0.03
	Cefotaxime	0.015–0.06	0.015	0.03
	Ceftazidime	0.12–0.25	0.25	0.25
	Piperacillin	≤0.12	≤0.12	≤0.12
	Gentamicin	1–4	2	4
Viridans group streptococci (27)	SM-7338	0.12–4	0.25	4
	Imipenem	0.015–02	0.06	1
	Cefotaxime	0.06–64	0.25	1
	Ceftazidime	0.06–32	4	16
	Piperacillin	≤0.12–8	2	8
	Gentamicin	1–16	4	16
Streptococcus pneumoniae (15)	SM-7338	0.008–0.03	0.015	0.015
	Imipenem	0.008–0.03	0.008	0.015
	Cefotaxime	0.008–0.06	0.015	0.03
	Ceftazidime	0.12–1	0.25	0.5
	Piperacillin	0.06–0.12	0.06	0.06
	Gentamicin	2–16	8	16
Enterococcus faecalis (20)	SM-7338	2–8	4	8
	Imipenem	1–2	1	1
	Cefotaxime	2–>128	128	>128
	Ceftazidime	32–>128	>128	>128
	Piperacillin	2–8	2	4
	Gentamicin	2–16	2	8

Continued on following page

TABLE 2—*Continued*

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Listeria monocytogenes (15)	SM-7338	0.06–4	2	4
	Imipenem	0.03–1	0.5	0.5
	Cefotaxime	0.25–64	16	16
	Ceftazidime	4–>128	>128	>128
	Piperacillin	0.12–4	2	4
	Gentamicin	0.12–4	1	4
Corynebacterium jekeii (8)	SM-7338	>16		
	Imipenem	>16		
	Cefotaxime	>16		
	Ceftazidime	>16		
Bacteroides fragilis (21)	SM-7338	0.12–0.5	0.12	0.25
	Imipenem	0.06–1	0.12	0.5
	Cefoxitin	0.25–32	8	16
	Clindamycin	0.03–16	0.5	16
	Piperacillin	2–>128	8	128
	Metronidazole	0.5–128	1	2
Bacteroides thetaiotaomicron and *Bacteriodes vulgatus* (9)	SM-7338	0.25–2	0.25	
	Imipenem	0.12–2	0.5	
	Clindamycin	0.5–32	8	
	Piperacillin	16–>128	64	
	Metronidazole	0.5–1	0.5	
Peptostreptococcus species (7)	SM-7338	≤0.015–0.25	0.03	
	Imipenem	≤0.015–0.5	0.03	
	Cefoxitin	0.03–4	1	
	Clindamycin	0.06–0.5	0.12	
	Piperacillin	≤0.015–4	0.12	
	Metronidazole	0.25–>32	1	
Propionibacterium acnes (8)	SM-7338	0.03–0.06	0.03	
	Imipenem	<0.015–0.03	<0.015	
	Cefoxitin	0.12–2	0.25	
	Clindamycin	0.06–1	0.06	
	Piperacillin	0.06–0.5	0.25	
	Metronidazole	0.05–>32	>32	
Clostridium perfringens (17)	SM-7338	0.008–1	0.015	0.12
	Imipenem	0.06–2	0.12	1
	Cefoxitin	0.25–4	1	4
	Clindamycin	0.03–8	0.12	1
	Piperacillin	0.06–1	0.12	1
	Metronidazole	0.12–1	0.25	0.5

marcescens and *Pseudomonas aeruginosa* were 10- and 16-fold higher, respectively, than the MICs.

Table 3 shows the effect of pH and anaerobic incubation on the MICs of SM-7338 for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Serratia marcescens* compared with the MICs of imipenem under similar conditions. The increase in the MICs of SM-7338 was at most twofold compared with a two- to eightfold increase in the MICs of imipenem at pH 5.5 under anaerobic conditions similar to those that would be found in an abscess. MICs of SM-7338 for *Staphylococcus aureus* and *Enterococcus faecalis* were determined under anaerobic conditions and

TABLE 3. Effect of pH on MICs of SM-7338 and imipenem under anaerobic conditions

Organism (no. tested)	Geometric mean MIC (μg/ml [range])			
	SM-7338		Imipenem	
	pH 5.5	pH 7.5	pH 5.5	pH 7.5
Escherichia coli (6)	0.06	0.03 (0.015–0.03)	1.59 (1–4)	0.31 (0.25–0.5)
Klebsiella pneumoniae (6)	0.08 (0.06–0.12)	0.04 (0.03–0.06)	2.52 (1–4)	0.56 (0.5–1)
Enterobacter cloacae (6)	0.05 (0.03–0.06)	0.03 (0.03–0.06)	2.24 (1–8)	1.78 (0.5–8)
Serratia marcescens (5)	0.12 (0.06–0.5)	0.08 (0.03–0.5)	2.29 (1–8)	1 (0.5–2)

TABLE 4. Effect of inoculum on MICs of SM-7338 and imipenem

Organism (no. of isolates)	Geometric mean MIC (μg/ml [range]) at CFU of:					
	10^3		10^5		10^7	
	SM-7338	Imipenem	SM-7338	Imipenem	SM-7338	Imipenem
Escherichia coli (5)	0.02 (≤0.008–0.12)	0.14 (0.06–0.5)	0.03 (0.015–0.12)	0.29 (0.12–1)	0.07 (0.03–0.25)	0.57 (0.25–1)
Klebsiella pneumoniae (5)	0.03 (0.03–0.03)	0.19 (0.12–0.5)	0.03 (0.03–0.06)	0.44 (0.25–1)	0.07 (0.06–0.12)	1.15 (1–2)
Enterobacter cloacae (5)	0.03 (0.015–0.06)	0.25 (0.12–1)	0.07 (0.03–0.5)	0.76 (0.25–2)	0.99 (0.05–4)	6.96 (2–16)
Serratia marcescens (5)	0.04 (0.03–0.06)	0.5 (≤0.015–0.03)	0.05 (0.03–0.06)	0.87 (0.5–2)	0.19 (0.06–1)	6.96 (4–16)
Pseudomonas aeruginosa (5)	0.5 (0.25–2)	2 (1–4)	1 (0.25–4)	3.03 (1–4)	2.3 (1–4)	6.06 (2–16)
Staphylococcus aureus (5)	0.09 (0.06–0.12)	0.02 (≤0.015–0.03)	0.16 (0.12–0.25)	0.03 (≤0.015–0.03)	0.19 (0.12–0.25)	0.05 (0.03–0.12)

were within a factor of 2 of the MICs obtained aerobically (data not shown).

Table 4 shows the effect of inoculum size on SM-7338 and imipenem MICs for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Although there was an increase at 10^7 CFU in the MICs for *Enterobacter cloacae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. The MICs of SM-7338 remained lower than the MICs of imipenem, except for *Staphylococcus aureus*.

In 50% normal human serum, the MICs and MBCs for two isolates each of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* were identical or within a factor of 2 lower or higher than the MICs and MBCs determined in Mueller-Hinton broth. For the same strains, MICs determined in sterile filtered urine (pH 5.5) were within a factor of 2 of the MICs determined in broth.

Activity against strains possessing characterized β-lactamases. SM-7338 inhibited TEM-1-containing *Escherichia coli*, *Salmonella* species, *Haemophilus influenzae*, and *Neisseria gonorrhoeae* at 0.015 μg/ml. It inhibited strains of *Pseudomonas aeruginosa* which contained PSE-1, PSE-2, PSE-3, PSE-4, OXA-2, OXA-3, OXA-4, and OXA-7 β-lactamases at a concentration of ≤4 μg/ml. It inhibited isolates of *Enterobacter cloacae* containing the P-99 β-lactamase, for which the cefotaxime MIC was >64 μg/ml, and *Klebsiella oxytoca* isolates containing the K-1 β-lactamase, for which the aztreonam MIC was 32 μg/ml at a concentration of 0.25 μg/ml. It also inhibited *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Serratia marcescens* isolates containing TEM-3 at ≤0.5 μg/ml; and it inhibited *K. pneumoniae* isolates containing TEM-4 and TEM-5. It inhibited *Klebsiella pneumoniae* isolates containing SHV-1, SHV-2, and SHV-3 at ≤0.12 μg/ml. The SHV-2, SHV-3 isolates had cefotaxime and ceftazidime MICs of ≥32 μg/ml. It inhibited at a concentration of 0.25 μg/ml *Staphylococcus aureus* which hydrolyzed cefazolin and *Proteus vulgaris* at a concentration of 0.12 μg/ml. This *Proteus* isolate hydrolyzed cefotaxime.

β-Lactamase stability and inhibitory activity. SM-7338 was not hydrolyzed by the majority of plasmid or chromosomal β-lactamases (Table 5). V_{max} values for cephaloridine for these enzymes ranged from 15 to 8,560 μM/mg of protein. SM-7338 was an effective inhibitor of both plasmid and chromosomal enzymes, including the recently described TEM-3 (CTX-1) enzyme (Table 6). It also inhibited *Staphylococcus aureus* β-lactamase. The K_m for TEM-1 β-lactamase was 0.936 mM and the K_i was <0.01 μM. The K_m for the *Enterobacter cloacae* P-99 β-lactamase was 0.533 mM, and the K_i was <0.001 μM.

β-Lactamase induction. Placement of a 10-μg disk of SM-7338 opposite a disk of piperacillin on agar seeded with *Pseudomonas aeruginosa* caused a reduction in the zone of inhibition for 9 of 10 strains. Similarly, there was a reduction

TABLE 5. β-Lactamase stability of SM-7338 compared with that of imipenem

β-Lactamase	Source organism	Richmond-Sykes classification	Relative rate of hydrolysis[a]	
			SM-7338	Imipenem
TEM-1	*Escherichia coli*	III	<0.1	<0.1
TEM-2	*Escherichia coli*	III	<0.1	<0.1
SHV-1	*Klebsiella pneumoniae*	III	<0.1	<0.1
P-99	*Enterobacter cloacae*	Ia	<0.1	<0.1
	Morganella morganii	Ia	<0.1	<0.1
	Proteus vulgaris	Ic	<0.1	<0.1
Sabath-Abraham	*Pseudomonas aeruginosa*	Id	<0.1	<0.1
K-1	*Klebsiella oxytoca*	IV	<0.1	<0.1
PSE-1	*Pseudomonas aeruginosa*	V	<0.1	<0.1
PSE-2	*Pseudomonas aeruginosa*	V	<0.1	<0.1
PSE-3	*Pseudomonas aeruginosa*	V	<0.1	<0.1
PSE-4	*Pseudomonas aeruginosa*	V	<0.1	<0.1
OXA-2	*Pseudomonas aeruginosa*	V	<0.1	<0.1
PC-1	*Staphylococcus aureus*		<0.1	<0.1
TEM-3	*Escherichia coli*		<0.1	<0.1
TEM-3	*Enterobacter aerogenes*		<0.1	<0.1
TEM-3	*Serratia marcescens*		<0.1	<0.1
TEM-3	*Klebsiella pneumoniae*		<0.1	<0.1
TEM-5	*Klebsiella pneumoniae*		<0.1	<0.1
	Xanthomonas maltophilia		9.1	12.7
	Bacteroides fragilis		<0.1	<0.1
Richmond, I	*Staphylococcus aureus*		<0.1	<0.1
Richmond, II	*Staphylococcus aureus*		<0.1	<0.1
	Branhamella catarrhalis		<0.1	<0.1

[a] Based on cephaloridine as 100.

TABLE 6. Inhibition of β-lactamase by SM-7338

β-Lactamase	Source organism	Richmond-Sykes classification	% Inhibition of hydrolysis
TEM-1	*Escherichia coli*	IIIa	97.8[a]
P-99	*Enterobacter cloacae*	Ia	99.2[a]
	Morganella morganii	Ia	88.9[a]
Sabath-Abraham	*Pseudomonas aeruginosa*	Id	91.4[a]
K-1	*Klebsiella oxytoca*	IV	98.7
TEM-3 (CTX-1)	*Klebsiella pneumoniae*	III	96
PSE-2	*Pseudomonas aeruginosa*	V	98.5[b]
OXA-2	*Pseudomonas aeruginosa*	V	99.6[b]
PC-1	*Staphylococcus aureus*		82.7[b]

[a] Nitrocefin (100 μM) was used as the substrate.
[b] Cephaloridine (100 μM) was used as the substrate.

in the zone of inhibition of piperacillin against piperacillin-susceptible *Enterobacter cloacae* and *Serratia marcescens* isolates. One isolate each of *Pseudomonas aeruginosa* and *Serratia marcescens* was tested for the induction of β-lactamase. There was a 22-fold increase in the production of the β-lactamase of a *Pseudomonas aeruginosa* strain and a 1.1-fold increase in the β-lactamase of a *Serratia marcescens* strain. Both of these strains showed comparable inductions with cefoxitin, imipenem, and CGP 31608 (8).

Selection of resistant mutants. The MICs of SM-7338 showed a progressive increase for *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Serratia marcescens* but a minimal increase for *Staphylococcus aureus* and *Escherichia coli* (Table 7). Increases in MICs were noted for imipenem with the same strains. The MICs for the strains remained stable after multiple passages in the absence of antibiotics, and there was an increase in the MICs of both antimicrobial agents. The final MICs of SM-7338 were one dilution higher than the MICs of imipenem for *Enterobacter cloacae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*.

In contrast to the progressive development of resistance, attempts to select spontaneously resistant mutants of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Serratia marcescens* (five isolates each) with MICs that were fourfold above the original MICs were not successful, whereas exposure of the same organisms to cefotaxime, ceftazidime, and aztreonam caused the selection of mutant isolates that constitutively produced higher levels of β-lactamase.The organisms that were resistant to the aforementioned agents with MICs of >32 μg/ml were tested against both SM-7338 and imipenem and showed no increase in MICs compared with the original organisms.

TABLE 7. Effect of repeated subculture of organisms in the presence of SM-7338

Organism	Antimicrobial agent	MIC (μg/ml) on:			
		Day 1	Day 3	Day 7	Day 10
Staphylococcus aureus	SM-7338	0.25	0.25	0.5	0.5
	Imipenem	0.06	0.06	0.12	0.12
Escherichia coli	SM-7338	0.03	0.03	0.06	0.06
	Imipenem	0.12	0.25	0.5	0.5
Klebsiella pneumoniae	SM-7338	0.06	0.25	4	4
	Imipenem	1	2	4	4
Enterobacter cloacae	SM-7338	0.5	2	8	16
	Imipenem	1	2	4	8
Serratia marcescens	SM-7338	0.06	0.25	4	8
	Imipenem	1	2	4	4
Pseudomonas aeruginosa	SM-7338	2	8	32	64
	Imipenem	4	16	32	32

Killing data and postantibiotic suppression. The killing activity of SM-7338 was studied with a β-lactamase-producing *Escherichia coli* strain that was resistant to ampicillin, cefazolin, cefoperazone, and trimethoprim and with a *Pseudomonas aeruginosa* strain that was resistant to piperacillin and gentamicin. At four times the MICs, SM-7338 and imipenem produced a 3-log-unit decrease in the CFU after 2 h, and by 8 h there was a 5-log-unit decrease. Regrowth was not seen at 24 h. Ceftazidime produced only a 1.5-log-unit decrease in 2 h and a 4-log-unit decrease in 8 h. At concentrations that were four times the MICs, all three agents produced a 3-log-unit decrease in the CFU of *Escherichia coli*, and regrowth was not encountered in 24 h. SM-7338 produced a 3.4-h PAE for the *Pseudomonas aeruginosa* strain after a 2-h exposure and a 3.1-h postantibiotic effect for the *Escherichia coli* strain.

Synergy studies. The effect of the combination of SM-7338 with gentamicin was determined against 10 isolates each of *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Serratia marcescens*. Antagonism was not encountered; however, synergy was seen for nine of the *Pseudomonas aeruginosa* isolates, seven of the *Escherichia coli* isolates, and six of the *Serratia marcescens* isolates.

DISCUSSION

The knowledge gained from the analysis of the naturally occurring carbapenem thienamycin showed that introduction of a hydroxy ethyl substituent at C-6 yielded a chemically stable, β-lactamase-resistant agent (3). SM-7338 shares the basic carbapenem nucleus with imipenem. It differs, however, in that it has a 5-dimethyl carbamoylpyrrolidin group which is not present in imipenem. It has been reported that a beta-methyl substitution at C-1 on the carbapenem nucleus reduces the ability of dihydropeptidase-1 to attack the β-lactam molecule (H. Kropp, J. C. Sundelof, J. S. Kahan, J. Huber, D. Dohn, L. Gerckens, F. M. Kahan, and J. Birnbaum, 23rd ICAAC, abstr. no. 331, 1983). Results of this study illustrate that the in vitro activity of SM-7338 is slightly superior to that of imipenem against the majority of the members of the family *Enterobacteriaceae*, particularly some of the *Proteus* and *Providencia* species, and is comparable or slightly less than the activity of imipenem against the majority of gram-positive species such as *Streptococcus pneumoniae*, hemolytic streptococci, and staphylococci. Imipenem is more active against enterococci, and neither agent inhibits some *E. faecium* isolates. SM-7338, in general, was a factor of two- to fourfold more active than imipenem against ceftazidime-resistant *Pseudomonas aeruginosa* isolates. SM-7338, at 8 μg/ml, inhibited isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis or other respiratory infections; imipenem MICs were ≥8 μg/ml for these isolates.

SM-7338 was not destroyed by chromosomal or plasmid β-lactamases, including enzymes which inactive broad-spectrum cephalosporins (4). Although it induced β-lactamase activity, as do cephamycins, other carbapenems, and clavams, the induced strains remained susceptible to the compound; it did not select mutants which constitutively produced high levels of β-lactamase, as do a number of the cephalosporins and cephamycins. The single β-lactamase

which destroyed SM-7338 was that present in *Xanthomonas maltophilia*, which also destroyed imipenem and agents such as ceftazidime and cefotaxime.

Similar to imipenem and other penems (1, 8), SM-7338 caused rapid killing of *Escherichia coli* and *Pseudomonas aeruginosa* and produced a postantibiotic effect on these two organisms.

Overall, these studies demonstrate the excellent in vitro properties of the novel carbapenem SM-7338. Pharmacological and clinical studies will ultimately establish the clinical utility of this agent in comparison with those of other β-lactams and imipenem. The high activity of this agent against *Pseudomonas aeruginosa* makes it of particular interest in an era with organisms that are resistant to extended-spectrum cephalosporins, monobactams, and the available carbapenem, imipenem.

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