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In Vitro Activity against Aerobic and Anaerobic Gram-Positive and Gram-Negative Bacteria and β-Lactamase Stability of RS-533, a Novel Carbapenem

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RS-533 is a novel carbapenem antibiotic. Its activity was compared with that of imipenem and the new cephalosporins, aztreonam, piperacillin, and tobramycin. RS-533 had activity comparable to that of imipenem, inhibiting the majority of the *Enterobacteriaceae*, streptococci, staphylococci, and *Bacteroides* species at concentrations of ≤2 μg/ml. RS-533 inhibited *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* resistant to ceftazidime, aztreonam, and cefoperazone, but RS-533 did not inhibit all methicillin-resistant *Staphylococcus aureus* or *Pseudomonas maltophilia*. It inhibited tobramycin-resistant members of the *Enterobacteriaceae* and *Pseudomonas aeruginosa*. RS-533 was stable against attack by common chromosomal and plasmid-mediated beta-lactamases and was an effective inhibitor of many beta-lactamases.

Many new beta-lactam antimicrobial agents have been introduced into clinical practice in the past decade. One class of compounds which has been of particular interest is the carbapenems. Imipenem, a thienamycin, has been extensively studied and is extremely useful in the treatment of a variety of infections (1, 2, 6). RS-533 (Fig. 1) is a new carbapenem. This agent differs significantly from imipenem, which has a basic alkythio side chain attached to the five-membered ring. We evaluated the antibacterial activity and beta-lactamase stability of RS-533 in comparison with those of other antimicrobial agents with particular reference to its activity against organisms resistant to other beta-lactam agents.

MATERIALS AND METHODS

Microorganisms. Gram-positive and gram-negative bacteria used in this study were clinical isolates from three hospitals in the Columbia University system. The isolates were specifically collected because of resistance to beta-lactam agents and aminoglycosides.

Antimicrobial agents. Standard antimicrobial powders were provided as follows: RS-533 from Sankyo International Corp., Tokyo, Japan; ceftazidime from Glaxo, Inc., Research Triangle Park, N.C.; cefoperazone from Pfizer Inc., New York, N.Y.; cefoxitin and imipenem from Merck Sharp & Dohme, Rahway, N.J.; piperacillin from Lederle Laboratories, Pearl River, N.Y.; aztreonam from E. R. Squibb & Sons, Princeton, N.J.; tobramycin from Eli Lilly & Co., Indianapolis, Ind.; and cefotaxime from Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. Antimicrobial solutions were freshly prepared on the day of use according to the directions of the manufacturers.

Susceptibility studies. Susceptibility testing was performed by a standard agar dilution technique by using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) which was supplemented with 5% defibrinated sheep blood for testing streptococci and with 5% chocolate-containing blood for testing *Haemophilus* spp., *Branhamella* spp., or *Neisseria* spp. Brucella agar supplemented with hemin and vitamin K was used for anaerobic species. Overnight cultures of test organisms in Mueller-Hinton broth (BBL), Todd-Hewitt broth (BBL) for streptococci, Schaedler broth for *Haemophilus* spp. and *Neisseria* spp., or chopped-meat glucose (Scott Laboratories, Inc. Providence, R.I.) for anaerobic species were diluted in Mueller-Hinton broth. Final inocula of approximately 10^5 CFU were applied by a multipoint spot inoculator. Plates were examined after 18 h of incubation at 35°C. Anaerobic organisms were incubated in GasPak jars (BBL) for 48 h at 35°C. All compounds were analyzed at the same time.

Susceptibility to RS-533 of five isolates of each of several bacterial species was determined by broth dilution technique. Tubes (1 ml) containing serial twofold dilutions of the compound in Mueller-Hinton broth were inoculated with log-phase organisms to yield a final inoculum of 5×10^5 CFU/ml. Tubes were incubated for 18 h at 35°C and inspected for lack of turbidity. Samples of 0.01 ml were removed to antibiotic-free plates which were incubated for 24 h at 35°C. The MBC, defined as a 99.9% reduction in the initial inoculum, was determined by the method of Pearson et al. (7), assuming a 5% pipetting error.

Beta-lactamase assays and inhibition studies. The presence of beta-lactamases in clinical isolates was determined by the nitrocefin assay (5). The beta-lactamases used for the evaluation of the stability of the compound were either purified enzymes or partially purified enzymes as previously described (5). The stability of the compounds to beta-lactamase was determined by a spectrophotometric assay by using the change in absorption at the absorption maximum of each substrate. The λ_{max} used for RS-533 was 285 nm. Inhibition assays, with nitrocefin as the substrate, were performed with

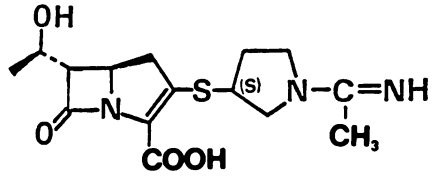

FIG. 1. Structure of RS-533.

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TABLE 1. Comparative activities of RS-533 and other agents against gram-negative isolates

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Escherichia coli[b] (30)	RS-533	≤0.06–0.25	0.06	0.12
	Imipenem	0.12–0.5	0.25	0.5
	Ceftazidime	0.12–1	0.12	0.5
	Cefoperazone	≤0.06–32	≤0.12	4
	Cefoxitin	1–>128	4	8
	Piperacillin	0.5–>128	>128	>128
	Aztreonam	≤0.06–0.12	0.12	0.12
	Tobramycin	0.25–8	0.5	1
Klebsiella pneumoniae[b] (30)	RS-533	≤0.06–2	0.12	0.5
	Imipenem	0.12–2	0.12	0.5
	Ceftazidime	0.12–2	0.25	0.5
	Cefoperazone	0.06–32	0.5	32
	Cefoxitin	4–32	4	16
	Piperacillin	4–>128	>128	>128
	Aztreonam	0.12–1	0.25	1
	Tobramycin	0.25–>8	0.5	>8
Klebsiella oxytoca[b] (15)	RS-533	≤0.06–0.12	0.06	0.12
	Imipenem	0.12–0.25	0.12	0.12
	Ceftazidime	≤0.06–0.25	0.06	0.12
	Cefoperazone	0.12–>128	0.5	4
	Cefoxitin	4–32	4	16
	Piperacillin	4–>128	>128	>128
	Aztreonam	≤0.06–8	0.06	0.5
	Tobramycin	0.25–>8	0.5	1
Enterobacter aerogenes[b,c] (30)	RS-533	0.12–8	0.5	1
	Imipenem	0.25–8	2	4
	Ceftazidime	0.5–32	0.5	16
	Cefoperazone	0.12–>128	0.5	32
	Cefoxitin	>128	>128	>128
	Piperacillin	1–>128	8	>128
	Aztreonam	≤0.06–16	0.12	16
	Tobramycin	0.25–>16	0.5	2
Enterobacter agglomerans[b,c] (10)	RS-533	0.12–2	0.25	0.5
	Imipenem	0.5–1	0.25	1
	Ceftazidime	0.12–1	0.25	0.5
	Aztreonam	≤0.06–0.5	0.12	0.5
	Tobramycin	0.5–8	0.5	1
Enterobacter cloacae[b,c] (35)	RS-533	0.25–2	0.25	1
	Imipenem	≤0.06–16	0.25	8
	Ceftazidime	≤0.06–>128	16	32
	Cefoperazone	≤0.06–128	2	32
	Cefoxitin	>128	>128	>128
	Piperacillin	1–>128	16	>128
	Aztreonam	≤0.06–>128	4	32
	Tobramycin	0.25–2	0.25	1
Salmonella spp. (30)	RS-533	≤0.06–1	0.12	0.12
	Imipenem	0.06–0.25	0.06	0.25
	Ceftazidime	≤0.06–1	0.1	0.5
	Cefoperazone	0.12–64	0.2	0.8
	Cefoxitin	1–8	1	8
	Piperacillin	0.5–>128	1	2
	Aztreonam	0.06–1	0.06	0.12
Shigella spp. (30)	RS-533	0.06–0.1	0.12	0.25
	Imipenem	0.06–0.5	0.06	0.25
	Ceftazidime	0.06–1	0.12	0.5
	Cefoperazone	0.06–128	0.25	0.5
	Cefoxitin	1–8	1	8
	Piperacillin	0.5–>128	1	2
	Aztreonam	0.06–1	0.06	0.12

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TABLE 1—*Continued*

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Serratia marcescens[b,c] (25)	RS-533	0.25–2	0.25	1
	Imipenem	0.06–4	0.12	1
	Ceftazidime	≤0.06–8	≤0.12	2
	Cefoperazone	0.5–>128	2	16
	Cefoxitin	2–>128	16	>128
	Piperacillin	1–>128	>128	>128
	Aztreonam	0.06–64	0.12	4
	Tobramycin	0.5–>8	1	8
Citrobacter freundii[b,c] (30)	RS-533	0.12–1	0.25	0.5
	Imipenem	≤0.06–2	0.5	2
	Ceftazidime	≤0.06–>128	0.5	8
	Cefoperazone	≤0.06–>128	0.5	32
	Cefoxitin	16–>128	>128	>128
	Piperacillin	0.5–>128	8	128
	Aztreonam	0.12–>128	≤0.25	8
	Tobramycin	0.12–>8	0.5	2
Citrobacter diversus[c] (20)	RS-533	≤0.06–0.5	0.06	0.12
	Imipenem	0.12–2	0.12	0.25
	Ceftazidime	≤0.06–0.5	0.06	0.25
	Cefoperazone	≤0.06–0.5	0.12	0.5
	Cefoxitin	2–32	2	16
	Piperacillin	1–>64	8	64
	Aztreonam	≤0.06–0.12	0.06	0.12
	Tobramycin	0.25–2	0.5	1
Proteus mirabilis (20)	RS-533	≤0.06–2	0.12	0.5
	Imipenem	≤0.06–4	0.5	2
	Ceftazidime	0.12–2	0.12	0.25
	Cefoperazone	0.12–>128	0.5	0.5
	Cefoxitin	2–16	2	4
	Piperacillin	0.5–>128	1	2
	Aztreonam	≤0.06–0.12	0.06	0.06
	Tobramycin	0.12–2	1	2
Proteus vulgaris[b,c] (25)	RS-533	0.25–4	1	2
	Imipenem	0.12–2	0.5	2
	Ceftazidime	≤0.06–4	≤0.06	0.25
	Cefoperazone	1–>128	2	32
	Cefoxitin	4–>128	8	32
	Piperacillin	1–>128	8	32
	Aztreonam	≤0.06–4	0.5	4
	Tobramycin	0.25–1	0.25	0.25
Morganella morganii[b,c] (20)	RS-533	0.5–2	1	2
	Imipenem	≤0.06–4	1	4
	Ceftazidime	≤0.06–8	0.06	1
	Aztreonam	≤0.06–2	0.06	0.5
	Tobramycin	0.25–8	0.5	1
Providencia rettgeri[b,c] (25)	RS-533	≤0.06–2	0.5	1
	Imipenem	0.25–2	0.5	2
	Ceftazidime	≤0.06–4	0.12	1
	Cefoperazone	0.06–>128	1	32
	Cefoxitin	2–32	4	32
	Piperacillin	1–>128	8	32
	Aztreonam	≤0.06–>8	≤0.06	≤0.06
	Tobramycin	0.25–1	0.5	1
Providencia stuartii[b,c] (10)	RS-533	0.12–1	0.5	1
	Imipenem	0.5–2	1	2
	Ceftazidime	≤0.06–1	0.25	1
	Cefoperazone	≤0.06–16	1	8
	Cefoxitin	1–>128	8	32
	Piperacillin	1–>128	8	64

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TABLE 1—*Continued*

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
	Aztreonam	≤0.06–1	≤0.03	≤0.03
	Tobramycin	1–>8	2	2
Pseudomonas aeruginosa[b,c] (40)	RS-533	1–64	2	8
	Imipenem	1–64	4	8
	Ceftazidime	1–64	2	16
	Cefoperazone	1–>128	16	128
	Aztreonam	0.25–128	4	16
	Piperacillin	1–>128	16	>128
	Tobramycin	0.5–>8	2	>8
Pseudomonas spp.[b,c,d] (10)	RS-533	0.1–>128	0.25	2
	Imipenem	≤0.06–>128	0.25	8
	Ceftazidime	1–>128	2	8
Acinetobacter spp.[b,c] (30)	RS-533	0.06–4	0.12	1
	Imipenem	0.12–4	1	2
	Ceftazidime	1–>64	8	64
	Cefoperazone	>128	>128	>128
	Cefoxitin	8–>128	>128	>128
	Piperacillin	8–>128	32	>128
	Aztreonam	4–>64	16	64
	Tobramycin	1–>8	1	>8
Aeromonas hydrophila[b] (10)	RS-533	0.12–2	0.25	1
	Imipenem	0.12–4	0.5	2
	Ceftazidime	0.12–1	0.25	0.5
	Aztreonam	≤0.06–0.5	≤0.06	0.5
Yersinia enterocolitica[b] (10)	RS-533	≤0.06–0.25	0.12	0.25
	Imipenem	0.03–0.25	0.12	0.25
	Ceftazidime	≤0.06–4	≤0.06	2
	Aztreonam	0.12–1	0.25	0.5

[a] 50% and 90%, MIC for 50 and 90% of isolates tested, respectively.
[b] Ampicillin resistant, beta-lactamase positive.
[c] Cefazolin resistant.
[d] *P. maltophilia* (3 isolates) and *P. cepacia* (6 isolates).

a final volume of 3 ml. The enzyme and inhibitor were incubated at various concentrations at 35°C for 10 min, and subsequently, nitrocefin was added. The change in absorption with nitrocefin was followed over 10 min in a temperature-controlled recording spectrophotometer.

Induction of beta-lactamases. *Enterobacter cloacae* 10469 and *Pseudomonas aeruginosa* 10524 were grown overnight in Mueller-Hinton broth and diluted 10^{-2} in fresh broth. After resumption of exponential growth, beta-lactam agents were added at concentrations equal to, twice, and four times the MIC, and incubation continued for 2 h at 35°C. Bacterial cells were harvested by centrifugation, washed in phosphate buffer, and disrupted by sonication. Cell debris was removed by centrifugation, and the material was dialyzed at 4°C for 24 h. Beta-lactamase in uninduced cells and in induced cells was determined with the nitrocefin assay with a correction made for protein contents of the preparations. Assays were run with duplicate organisms with a reproducibility between assays of 95%. One unit of activity equalled 1 nmol of substrate hydrolyzed.

Selection of resistant mutants. Single isolates of *E. cloacae*, *Serratia marcescens*, *Citrobacter freundii*, and *P. aeruginosa* were concentrated by centrifugation to yield 10^9 CFU. Organisms were incorporated into agar containing antibiotic at eight times the MIC. Organisms were isolated, and resistance was confirmed by agar dilution.

RESULTS

The results of agar susceptibility studies against gram-negative aerobic bacteria are shown in Table 1. RS-533 had broad antibacterial activity inhibiting the majority of members of the *Enterobacteriaceae* and *Pseudomonas* spp., with the exception of *Pseudomonas maltophilia*, at concentrations of ≤2 μg/ml. RS-533 overall had activity similar to that of imipenem with minor differences in MICs noted for individual isolates. In general, RS-533 had activity similar to that of ceftazidime, except against *Proteus* spp., where ceftazidime was more active, and against *Providencia* spp., where a number of agents had equal activity, with aztreonam the most active. RS-533 inhibited at 2 μg/ml all the *Klebsiella* spp., *Escherichia coli*, and *Proteus* spp. which were resistant to cefoperazone and to piperacillin (MIC ≥ 128 μg/ml). It also inhibited the majority of the tobramycin-resistant *Enterobacteriaceae* and *P. aeruginosa* at ≤8 μg/ml.

Similar to imipenem, RS-533 inhibited *E. cloacae* and *C. freundii* which were resistant to ceftazidime, cefoperazone, and aztreonam. RS-533 was more active than cefoperazone, cefoxitin, or aztreonam against *S. marcescens*. RS-533, imipenem, and ceftazidime were the most active agents tested against the highly resistant *P. aeruginosa* and the *Acinetobacter* spp., but ceftazidime inhibited some of the *P. maltophilia* isolates resistant to RS-533 and imipenem.

TABLE 2. Comparative activities of RS-533 against gram-positive and anaerobic species

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus (methicillin susceptible) (20)	RS-533	≤0.015–0.5	0.03	0.5
	Imipenem	≤0.015–0.5	≤0.015	0.5
	Nafcillin	0.12–8	0.5	2
	Cefotaxime	0.5–4	2	4
Staphylococcus aureus (methicillin resistant) (20)	RS-533	0.25–>16	0.12	16
	Imipenem	0.5–8	2	8
	Nafcillin	>16	>16	>16
	Cefotaxime	>16	>16	>16
Staphylococcus epidermidis (methicillin susceptible) (20)	RS-533	≤0.015–0.5	≤0.015	0.5
	Imipenem	0.03–0.5	0.12	0.5
	Nafcillin	1–8	1	8
	Cefotaxime	1–8	4	4
Staphylococcus epidermidis (methicillin resistant) (20)	RS-533	0.5–16	1	16
	Imipenem	0.03–16	1	8
	Nafcillin	>16	>16	>16
	Cefotaxime	>16	>16	>16
Streptococcus pyogenes (10)	RS-533	≤0.015–0.03	≤0.015	≤0.015
	Imipenem	≤0.015–0.25	≤0.015	≤0.015
	Cefotaxime	≤0.015–0.25	≤0.015	0.25
Streptococcus agalactiae (10)	RS-533	≤0.015–0.03	≤0.015	≤0.015
	Imipenem	≤0.015–1	≤0.015	0.015
	Cefotaxime	≤0.015–0.06	≤0.015	0.06
Streptococcus, groups C, G, and F (20)	RS-533	≤0.015–0.12	≤0.015	0.03
	Imipenem	≤0.015–0.06	0.015	0.06
	Cefotaxime	≤0.015–0.5	≤0.015	0.25
Streptococcus faecalis (10)	RS-533	0.5–2	1	1
	Imipenem	0.5–1	0.5	1
	Cefotaxime	>32	>32	>32
Streptococcus pneumoniae (10)	RS-533	≤0.015–1	≤0.015	0.25
	Imipenem	0.12–1	0.12	0.25
	Cefotaxime	≤0.015–1	≤0.015	0.12
Listeria monocytogenes (10)	RS-533	≤0.12	≤0.12	≤0.12
	Imipenem	≤0.12	≤0.12	≤0.12
	Cefotaxime	>32	>32	>32
Corynebacterium JK sp. (10)	RS-533	8–>32	>32	>32
	Imipenem	16–>32	>32	>32
	Cefotaxime	>32	>32	>32
Bacteroides spp.[b] (30)	RS-533	≤0.015–0.5	0.03	0.06
	Imipenem	≤0.015–0.5	0.03	0.03
	Cefotaxime	1–>32	>32	>32
Clostridium spp.[c] (15)	RS-533	≤0.015–8	1	8
	Imipenem	≤0.015–4	≤0.015	2
	Cefotaxime	≤0.015–32	0.5	32

[a] 50% and 90%, MIC for 50 and 90% of the strains tested, respectively.
[b] Includes *B. fragilis*, *B. vulgatus*, *B. bivius*, *B. thetaiotaomicron*, and *B. melaninogenicus*.
[c] Includes *C. perfringens*, *C. difficile*, *C. novyi*, *C. septicum*, *C. ramosum*, *C. subterminale*, *C. butyricum*, and *C. sphenoides*.

The activity of RS-533 was compared with that of cefotaxime, an aminothiazolyl cephalosporin more active against gram-positive species than many of the beta-lactamase-stable cephalosporins, and imipenem (Table 2). RS-533 was more active against methicillin-susceptible staphylococci than was cefotaxime and was comparable to imipenem. It did not inhibit all methicillin-resistant *Staphylococcus aureus* or *Staphylococcus epidermidis* but showed appreciable activity, inhibiting 50% at 0.12 μg/ml. RS-533 had activity equal to that of cefotaxime and imipenem against the hemolytic streptococci, but RS-533 had comparable activity to that of imipenem against *Streptococcus faecalis* and *Streptococcus faecium* (five isolates, data not shown). RS-533 also inhibited *Listeria monocytogenes*, but corynebacteria of

TABLE 3. Effect of inoculum size on MICs of RS-533

Organism[a]	Geometric mean MIC (μg/ml) at inoculum size (CFU):		
	10^3	10^5	10^7
Escherichia coli	0.06	0.29	0.66
Klebsiella pneumoniae	0.08	0.38	2.64
Enterobacter cloacae	0.76	2.64	6.06
Serratia marcescens	0.19	0.15	9.19
Morganella morganii	0.5	4	6.96
Staphylococcus aureus	0.02	0.04	0.1
Staphylococcus aureus (methicillin resistant)	0.06	0.12	1.1
Streptococcus faecalis	0.76	1.2	4
Pseudomonas aeruginosa	2.6	2.6	10.4

[a] Five isolates for each species; all beta-lactamase positive.

the JK group were resistant, as they were to imipenem and cefotaxime.

RS-533 had activity comparable to that of imipenem against *Bacteroides* species, and it inhibited *Clostridium perfringens* at ≤1 μg/ml, but the MICs for *Clostridium difficile* were 8 μg/ml. Overall, the activities of imipenem and RS-533 were similar against the various anaerobes tested. RS-533 inhibited the majority of *Fusobacterium* spp. (five isolates), *Eubacterium* spp. (six isolates), peptococci (five isolates), and peptostreptococci (five isolates) at ≤0.015 to 2 μg/ml. It inhibited *Bifidobacterium* sp. at 0.03 μg/ml (one isolate), *Lactobacillus acidophilus* at 0.03 μg/ml (two isolates), *Propionibacterium* sp. at ≤0.015 μg/ml (three isolates), and *Veillonella parulla* at <0.015 μg/ml (one isolate).

Broth dilution studies. MICs of RS-533 against *E. coli*, *Klebsiella pneumoniae*, *E. cloacae*, *S. marcescens*, *Morganella morganii*, *S. aureus*, and *P. aeruginosa* (five isolates of each) were comparable to those determined by agar dilution. RS-533 was bactericidal at concentrations identical or equal to fourfold above the MIC for 31 of the 35 strains, and the MBCs for *S. marcescens* (one isolate), *M. morganii*, (one isolate), and *P. aeruginosa* (two isolates) were more than fourfold above the MICs. *S. faecalis* (five isolates) required MBCs of 64 μg/ml compared with MICs of 1 μg/ml, but the susceptible methicillin-resistant *S. aureus* required MBCs of 2 μg/ml compared with MICs of 0.25 μg/ml, even when the assays were performed in the presence of 5% NaCl and plates were held for 30 h.

The effect of inoculum size is shown in Table 3. There was an increase in MICs at 10^7 CFU which was most marked for *S. marcescens* but was also found for *P. aeruginosa*, *M. morganii*, and *E. cloacae*. The MICs for the susceptible, methicillin-resistant *S. aureus* did not exceed 2 μg/ml at 10^7 CFU.

Beta-lactamase stability. RS-533 was extremely stable against attack by plasmid beta-lactamases of the TEM, SHV-1, OXA-1, -2, -3, and PSE-1, -2, -3, and -4 types. It also was not hydrolyzed by enzymes from organisms containing chromosomally mediated Richmond-Sykes type Ia beta-lactamases in *E. cloacae*, *C. freundii*, and *M. morganii*. Neither *S. aureus* nor *Bacteroides fragilis* beta-lactamases hydrolyzed RS-533, and there was no hydrolysis of RS-533 by *Proteus vulgaris* or *Klebsiella oxytoca* beta-lactamases, which hydrolyze cefotaxime and aztreonam, respectively.

RS-533 inhibited all of the organisms possessing the aforementioned various plasmid and chromosomal beta-lactamases at ≤8 μg/ml. Comparative MICs of other agents were as follows: cefoperazone for *P. aeruginosa* containing PSE-4, 128 μg/ml; aztreonam for *K. oxytoca*, 16 μg/ml; and cefotaxime for *E. cloacae* P99, 128 μg/ml. RS-533 inhibited at 4 μg/ml six isolates of *P. aeruginosa* derepressed for beta-lactamase production and at 0.5 μg/ml isolates of *E. cloacae* (six isolates) and *C. freundii* (nine isolates), which were derepressed for production of beta-lactamase. The MICs for cefotaxime and ceftazidime were 8 to >32 μg/ml for these strains.

RS-533 was an effective inhibitor of both plasmid and chromosomal beta-lactamases (Table 4), particularly of the TEM-1 enzyme and the type Ic enzymes. Inhibition of K-1 and the Sabath-Abraham *P. aeruginosa* beta-lactamases was less efficient than that of the plasmid enzymes.

RS-533 induced beta-lactamase activity in *E. cloacae* 10469 and *P. aeruginosa* 10524. The beta-lactamase activity induced in *P. aeruginosa* 10524 by RS-533 was 1,860 U/mg per min compared with 176 U/mg per min for cefoxitin and 168 U/mg per min for moxalactam. Uninduced activity was 9 U/mg per ml. For *E. cloacae* 10469 RS-533 produced 20,930 U/mg per min, cefoxitin produced 153 U/mg per min, and moxalactam produced 293 U/mg per min. Uninduced activity was 38 U/mg per ml. RS-533 was 10-fold more active as an inducer than was cefoxitin or moxalactam for *P. aeruginosa* 10524 and 100-fold more active for *E. cloacae* 10469. However, RS-533 remained active against strains in which it induced beta-lactamase with MICs of ≤2 μg/ml. Induction was similar with two and four times the MIC. Spontaneous resistance to RS-533 at eight times the MIC could not be selected by plating 10^9 CFU, whereas it was possible to select strains resistant to cefoperazone, aztreonam, and ceftazidime. RS-533 inhibited these strains selected for resistance (Table 5).

TABLE 4. Inhibition of RS-533 and the hydrolysis of nitrocefin by beta-lactamases[a]

Organism	Beta-lactamase	Richmond-Sykes classification	% Inhibition at concn (μM):		
			100	10	1
Escherichia coli	TEM-1	III	97.6	95.7	93.3
Klebsiella pneumoniae	SHV-1	III	97	94.3	93.2
Enterobacter cloacae	P99	Ia	98.4	96.2	84.2
Morganella morganii		Ia	95.3	86.5	58.8
Proteus vulgaris		Ic	100	98.8	93.8
Pseudomonas sp.	Sabath-Abraham	Id	80.6	33.8	
Klebsiella oxytoca	K-1	IV	63	51.1	
Pseudomonas sp.	PSE-4	V	61	56.5	
Pseudomonas sp.	OXA-2	V	99.2	98.3	96.6

[a] Enzymes were preincubated with RS-533 at the indicated concentrations for 10 min, and nitrocefin was added at a concentration of 100 μM.

DISCUSSION

Thienamycin, a product of *Streptomyces cattleya*, was discovered some 8 years ago, but it was an unstable sub-

TABLE 5. Activity of RS-533 against bacteria resistant to other agents

Organism	MIC (μg/ml)			
	RS-533	Ceftazidime	Cefoperazone	Aztreonam
Enterobacter cloacae 10469	0.5	16	64	16
Serratia marcescens 9146	0.5	8	32	8
Citrobacter freundii 8875	0.5	16	128	32
Pseudomonas aeruginosa 10524	4	32	128	64

stance (1). Imipenem is a useful amidine derivative of thienamycin that inhibits many bacteria and is beta-lactamase stable (1, 3, 6, 8, 9).

RS-533 is a novel carbapenem which differs chemically from imipenem, which contains a basic alkythio side chain attached to the five-membered ring. In this study, it had in vitro activity comparable to that of imipenem against members of the *Enterobacteriaceae*, *P. aeruginosa*, *B. fragilis*, staphylococci, and hemolytic streptococci. RS-533 did not inhibit all methicillin-resistant staphylococci, but it actually was more active than imipenem against some isolates. RS-533, like imipenem, inhibited organisms such as *E. cloacae* and *P. aeruginosa* possessing derepressed chromosomal beta-lactamases which are resistant to ceftazidime, cefoperazone, and aztreonam. This may be related to easy entry through the cell wall due to small size or its probable binding to PBP2 and Ib in such species (1, 4). RS-533 also inhibited *Acinetobacter* species which are resistant to most beta-lactam agents except imipenem. Furthermore, RS-533 inhibited *Enterobacteriaceae* and *P. aeruginosa* resistant to tobramycin as does imipenem. The sole species which were resistant to RS-533, similar to imipenem, were *P. maltophilia* and some methicillin-resistant staphylococci.

RS-533 was bactericidal near the MICs for *E. coli*, *K. pneumoniae*, *E. cloacae*, *S. marcescens*, *M. morganii*, and methicillin-susceptible *S. aureus*. Increasing the inoculum size to 10^7 CFU did not increase the mean MICs above 10 μg/ml.

RS-533 was stable against attack by most beta-lactamases and was an effective inhibitor of a number of plasmid and chromosomal beta-lactamases. Like imipenem and the penem FCE 22101, RS-533 was an effective inhibitor of TEM and SHV-1 beta-lactamases (6, 10). Unlike many of the aminothiazolyl cephalosporins, RS-533 was a poor inhibitor of *K. oxytoca* K-1, *P. aeruginosa* PSE-4, and the Sabath-Abraham enzyme of *P. aeruginosa*.

The properties of RS-533 shown in this study demonstrate that variation in the carbapenem structure can yield highly active agents (3). Studies of the activity of RS-533 against the rare isolates of *Bacteroides* spp. and of *Bacillus cereus* which hydrolyze imipenem should be performed to determine whether this agent overcomes these defects of imipenem. Further studies of the stability of RS-533 to renal dipeptidases and of its potential for nephrotoxicity are required since imipenem in the absence of cilastatin has these two flaws (1). Overall, the results of this study of RS-533 show that its in vitro activity is comparable to that of other carbapenems and penems (1, 10).

LITERATURE CITED

1. **Birnbaum, J., F. H. Kahan, H. Kropp, and J. S. MacDonald.** 1985. Carbapenems, a new class of beta-lactam antibiotics. Am. J. Med. **78**(Suppl. 6A):3–21.
2. **Jones, R. N.** 1985. Review of the in vitro spectrum of activity of imipenem. Am. J. Med. **78**(Suppl. 6A):22–32.
3. **Neu, H. C.** 1983. Structure activity relation of new beta-lactam antibiotics and in vitro activity against common bacteria. Rev. Infect. Dis. **5**(Suppl.):9–20.
4. **Neu, H. C.** 1985. Summary of imipenem/cilastatin symposium. Am. J. Med. **78**(Suppl. 6A):165–167.
5. **Neu, H. C.** 1986. Antibiotic inactivating enzymes and bacterial resistance, p. 454–473. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
6. **Neu, H. C., and P. Labthavikul.** 1982. Comparative in vitro activity of *N*-formimidoyl thienamycin against gram-positive and gram-negative aerobic and anaerobic species and its β-lactamase stability. Antimicrob. Agents Chemother. **21**:180–187.
7. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18**:699–708.
8. **Richmond, M. H.** 1981. The semisynthetic thienamycin derivative MK 0787 and its properties with respect to a range of beta-lactamases from clinically relevant bacterial species. J. Antimicrob. Chemother. **7**:217–285.
9. **Tally, F. P., and N. V. Jacobus.** 1983. Susceptibility of anaerobic bacteria to imipenem. J. Antimicrob. Chemother. **12**:47–51.
10. **Wise, R., J. M. Andrews, and G. Danks.** 1983. Comparison of in vitro activity of FCE 22101, a new penem, with those of other β-lactam antibiotics. Antimicrob. Agents Chemother. **24**:909–914.