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In Vitro and In Vivo Activities of DN-9550, a New Broad-Spectrum Cephalosporin

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DN-9550 [(6R, 7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(1*H*-imidazol-4-yl)methoxyiminoacetamido]-3-[(1-pyridinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride] is a new semisynthetic cephalosporin with a broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria. The activity of DN-9550 against most species of the family *Enterobacteriaceae* was roughly comparable to that of ceftazidime, slightly lower than that of cefotaxime, and far exceeded that of cefoperazone. Against *Citrobacter freundii*, *Enterobacter cloacae*, and *Serratia marcescens*, DN-9550 was more active than ceftazidime and cefotaxime. DN-9550 and ceftazidime were significantly more active than cefotaxime against *Pseudomonas aeruginosa*, but DN-9550 and cefotaxime were clearly more active than ceftazidime against staphylococci and streptococci. *Haemophilus influenzae* and *Neisseria gonorrhoeae* were also highly susceptible to DN-9550, but *Bacteroides fragilis* was generally not susceptible to the compound. DN-9550 was stable to various types of β-lactamases and had high affinities for penicillin-binding protein 3 of both *Escherichia coli* and *P. aeruginosa*. When DN-9550 was administered subcutaneously to mice experimentally infected with *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, or *Pseudomonas aeruginosa*, its efficacy well reflected its in vitro potency.

The types of infection usually treated with cephalosporins and cephamycins are those in which several bacterial species are present and those caused by *Serratia marcescens*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* in patients whose host defenses have been compromised by a long hospitalization. Recently, progress has been made in the development of new cephalosporins with antibacterial activity against gram-negative bacteria, including the above organisms, although these drugs are less potent than older cephalosporins against gram-positive bacteria. For example, cefoperazone (4, 6) and cefotaxime (3, 5) have high intrinsic activities against gram-negative bacteria, but further improvement in activity against *Pseudomonas* and *Serratia* spp. is desirable. Moreover, cefotaxime has the disadvantage of metabolic degradation, which reduces its activity in the human body (9). Ceftazidime (8, 12) is highly active against these organisms but has poor activity against staphylococci. These circumstances have stimulated the search for newer antibiotics. One such compound is DN-9550 [(6R, 7R)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1*H*-imidazol-4-yl)methoxyiminoacetamido]-3-[(1-pyridinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride], a semisynthetic cephalosporin which has a broad antibacterial spectrum and high activity against *P. aeruginosa*, *S. marcescens*, and staphylococci. This paper summarizes our observations on the in vitro and in vivo activities of this new cephalosporin, its stability to β-lactamases, and its affinity for target proteins.

MATERIALS AND METHODS

Compounds. DN-9550 (Fig. 1) and ceftazidime were synthesized at the Research Institute, Daiichi Seiyaku Co., Ltd., Tokyo, Japan. Cefotaxime (Chugai Pharmaceuticals, Tokyo), cefoperazone (Toyama Chemical, Tokyo), cefsulodin (Takeda Chemical Industries, Osaka), ampicillin (Meiji Seika Kaisha, Tokyo), cephaloridine (Nippon Glaxo, Tokyo), and penicillin G (Banyu Pharmaceuticals, Tokyo) were commercial products.

Organisms. A total of 1,467 strains were used, of which 1,449 were recent clinical isolates (see Table 2). All strains were from the culture collections of the Research Institutes of Daiichi Seiyaku Co., Tokyo, and the Episome Institute, Gunma, Japan.

Determination of MICs. MICs were determined by the twofold agar dilution method. The media used are listed in Table 1. An overnight culture or gonococcal suspension in Difco Proteose Peptone no. 3 (Difco Laboratories, Detroit, Mich.) was adjusted to the density of a 0.5 McFarland Standard (about 10^8 CFU/ml) and diluted to 10^{-2}. One loopful (5 μl) of the diluted culture, corresponding to about 10^4 CFU, was inoculated onto 10 ml of compound-containing agar layers in petri dishes. Bacterial growth was observed after 18 h of incubation at 37°C, except for *Bacteroides fragilis*, which was incubated for 48 h. *Haemophilus influenzae* and *Neisseria gonorrhoeae* were incubated in a candle jar, and *B. fragilis* was cultured in an anaerobic glove box. The MIC was defined as the lowest concentration which prevented visible growth of bacteria.

Determination of MBCs. The MBCs of DN-9550 against each of 50 isolates randomly selected from those of *Escherichia coli*, *S. marcescens*, *P. aeruginosa*, and *Staphylococcus aureus* were determined by the twofold broth microdilution method with the Dynatech MIC 2000 system. Volumes (1.5 μl each) of the undiluted cultures described above, each containing 10^5 CFU, were inoculated into 0.1 ml of compound-containing Mueller-Hinton broth in microtiter wells. The inoculum size was 10^6 CFU/ml (final concentration). After 18 h of incubation at 37°C, the lowest compound concentration that allowed no visible growth was defined as the MIC. The content of each well was stirred on a Vortex mixer before sampling. Samples (10 μl each) were inoculated onto compound-free Mueller-Hinton agar plates. The MBC

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was defined as the lowest concentration that yielded no colony formation after 24 h of incubation at 37°C and after 18 h of exposure to DN-9550.

Stability to β-lactamases. The β-lactamases used were purified enzymes (2). Stability to various β-lactamases was determined by spectrophotometric assay by measuring the absorbance at the absorption maximum of each compound. The maximum rate of hydrolysis (relative V_{max}) was determined by use of a Lineweaver-Burk plot.

Assay of affinity for target proteins. The affinities of DN-9550 for target proteins, i.e., penicillin-binding proteins (PBPs), were examined by the procedure of Noguchi et al. (7). Various concentrations of DN-9550 (5 μl), together with [^{14}C]penicillin G (0.25 μCi in 5 μl), were added to the membrane proteins (0.7 mg in 30 μl) prepared from *Escherichia coli* K-12 L3 and *P. aeruginosa* 2084 L3, respectively, both of which were β-lactamase-less mutants, and incubated for 10 min at 30°C. The reaction was terminated by adding Sarkosyl and unlabeled penicillin G. ^{14}C-labeled PBP complexes were visualized after sodium dodecyl sulfate-slab gel electrophoresis and fluorography. The extent of competitive inhibition of [^{14}C]penicillin G binding to PBPs by DN-9550 was determined with a densitometer.

In vivo test. In vivo activities were determined against systemic infections in mice. Ten male, 5-week-old STD:ddY mice, each weighing 20 to 30 g, were used for each treatment dose and as untreated controls. An overnight culture on slants of Tryptose blood agar base (Difco) at 37°C was suspended in 0.033 M potassium phosphate buffer (pH 7.0) or in 3% gastric mucin. A 0.2-ml volume of bacterial suspension, equal to 4 to 64 times higher than the 50% lethal dose, was inoculated intraperitoneally. Immediately and 4 h after infection, the mice were weighed and treated subcutaneously with each compound solution in aseptic distilled water in a fixed volume of 0.1 ml/10 g of body weight. The mice infected with *P. aeruginosa* were treated three times, i.e., immediately and at 3 and 6 h after infection. Control groups were also weighed and received distilled water; all the control mice died within 3 days after challenge. The total number of mice surviving at each dose level was recorded 1 week after infection, and the 50% effective dose was calculated by the Litchfield-Wilcoxon probit method (1).

RESULTS

Antibacterial activity. The in vitro antibacterial activity of DN-9550 was compared with those of ceftazidime, cefotaxime, and cefoperazone (Table 2). The MICs of DN-9550 at which 90% of the isolates were inhibited (MIC_{90}s) were 1.56 and 6.25 μg/ml for *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively. At this level, DN-9550 was 2 to 4 times more active than cefoperazone, 4 to 16 times more active than ceftazidime, and comparable in activity to cefotaxime against these organisms and 4 times more active than cefotaxime against *Staphylococcus saprophyticus*.

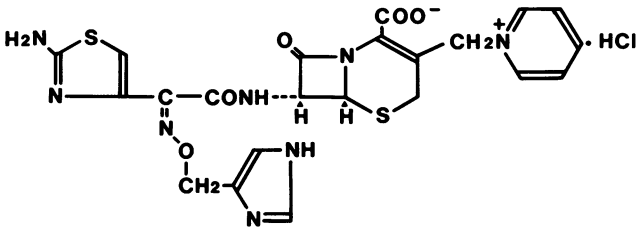

FIG. 1. Chemical structure of DN-9550.

TABLE 1. Media used for preculture and susceptibility tests

Media[a]	Organism
For preculture	
BHIB	*Streptococcus* spp.
BHIA + hemin (10 μg/ml) + β-NAD (2 μg/ml)	*H. influenzae*
GC agar + 1% hemoglobin + 1% IsoVitalex	*N. gonorrhoeae*
GAM broth	*B. fragilis*
MHA	Other organisms
For MIC determination	
Chocolate agar	*Streptococcus* spp. *H. influenzae*
GC agar + 1% hemoglobin + 1% IsoVitalex	*N. gonorrhoeae*
GAM agar	*B. fragilis*
MHA	Other organisms

[a] Abbreviations and sources: BHIB, brain heart infusion broth (Difco); β-NAD, Sigma Chemical Co.; GC agar, Difco; GAM broth, Nissui; MHB, Mueller-Hinton broth (Difco); chocolate agar, Mueller-Hinton agar (Difco) plus 10% defibrinated horse blood with heating; GAM agar, Nissui; MHA, Mueller-Hinton agar (Difco).

Against *Streptococcus pneumoniae* and *Streptococcus pyogenes*, DN-9550 exhibited excellent activity, as did cefotaxime: all the isolates were inhibited by ≤0.05 μg/ml, whereas 0.19 to 0.39 μg of ceftazidime or cefoperazone per ml was required for corresponding activity. DN-9550 and these reference cephalosporins showed poor activity against *Streptococcus faecalis*.

DN-9550 was highly active against various species of the family *Enterobacteriaceae*, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis*, with MIC_{90}s of ≤0.39 μg/ml for each species. Its activity was roughly comparable to that of ceftazidime, slightly inferior to that of cefotaxime, and much greater than that of cefoperazone. Against indole-positive *Proteus* spp., DN-9550, with an MIC_{90} of 1.56 μg/ml, was less active than ceftazidime and cefotaxime but more active than cefoperazone. The MIC_{90} of DN-9550 for *Serratia marcescens* was less than 1.56 μg/ml. The activity of DN-9550 was two to eight times those of ceftazidime and cefotaxime and far exceeded that of cefoperazone. *Citrobacter freundii* and *Enterobacter* spp. were less susceptible to DN-9550 than were the other species of *Enterobacteriaceae*, but DN-9550 inhibited these organisms at concentrations lower than that of any other compound tested.

The activity of DN-9550 against *Pseudomonas aeruginosa* was an important feature of its antibacterial spectrum. DN-9550, ceftazidime, and cefsulodin inhibited 90% of the isolates at concentrations below 6.25 μg/ml. Cefotaxime was the least active compound tested, having an MIC_{90} of 100 μg/ml. DN-9550, cefotaxime, and cefoperazone were less active than ceftazidime against *Pseudomonas maltophilia* and *Alcaligenes faecalis*. *H. influenzae* and *N. gonorrhoeae* was highly susceptible to DN-9550, with MIC_{90}s of 0.19 and 0.78 μg/ml, respectively.

DN-9550 displayed low activity against *B. fragilis*, as did the other cephalosporins tested, and many of the isolates were resistant to these compounds.

Bactericidal activity. The bactericidal activity of DN-9550 is shown in Table 3. The MBC_{90}s of DN-9550 against

TABLE 2. Antibacterial activities of DN-9550, ceftazidime, cefotaxime, cefoperazone, cefsulodin, and ampicillin against fresh clinical isolates[a]

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Staphyloccus aureus (150)	DN-9550	0.39–12.5	1.56	1.56
	Ceftazidime	1.56–100	6.25	12.5
	Cefotaxime	0.39–50	0.78	1.56
	Cefoperazone	0.19–100	1.56	3.13
Staphylococcus epidermidis (150)	DN-9550	≤0.05–50	0.78	6.25
	Ceftazidime	≤0.05–>100	12.5	25
	Cefotaxime	≤0.05–>100	1.56	6.25
	Cefoperazone	≤0.05–>100	1.56	12.5
Staphylococcus saprophyticus (15)	DN-9550	0.78–6.25	3.13	3.13
	Ceftazidime	12.5–100	50	50
	Cefotaxime	1.56–12.5	6.25	12.5
	Cefoperazone	3.13–12.5	6.25	12.5
Streptococcus pneumoniae (25)	DN-9550	≤0.05	≤0.05	≤0.05
	Ceftazidime	0.19–0.39	0.39	0.39
	Cefotaxime	≤0.05	≤0.05	≤0.05
	Cefoperazone	≤0.05–0.19	≤0.05	0.10
Streptococcus pyogenes (40)	DN-9550	≤0.05	≤0.05	≤0.05
	Ceftazidime	≤0.05–0.39	0.10	0.19
	Cefotaxime	≤0.05	≤0.05	≤0.05
	Cefoperazone	≤0.05–0.39	≤0.05	0.19
Streptococcus faecalis (104)	DN-9550	0.39–>100	25	>100
	Ceftazidime	6.25–>100	100	>100
	Cefotaxime	0.19–>100	6.25	>100
	Cefoperazone	3.13–>100	12.5	50
Escherichia coli (100)	DN-9550	≤0.05–1.56	0.10	0.19
	Ceftazidime	≤0.05–0.78	0.19	0.39
	Cefotaxime	≤0.05–0.78	≤0.05	0.10
	Cefoperazone	≤0.05–>100	0.39	6.25
Klebsiella pneumoniae (100)	DN-9550	≤0.05–6.25	0.10	0.39
	Ceftazidime	≤0.05–6.25	0.19	1.56
	Cefotaxime	≤0.05–3.13	≤0.05	0.19
	Cefoperazone	0.19–12.5	0.19	12.5
Klebsiella oxytoca (70)	DN-9550	≤0.05–6.25	0.10	0.39
	Ceftazidime	≤0.05–1.56	0.10	0.19
	Cefotaxime	≤0.05–3.13	≤0.05	0.19
	Cefoperazone	≤0.05–>100	1.56	100
Proteus mirabilis (89)	DN-9550	≤0.05–0.78	0.19	0.39
	Ceftazidime	≤0.05–0.39	0.10	0.19
	Cefotaxime	≤0.05	≤0.05	≤0.05
	Cefoperazone	0.10–25	0.78	3.13
Indole-positive *Proteus* spp. (90)[b]	DN-9550	≤0.05–25	0.39	1.56
	Ceftazidime	≤0.05–3.13	0.19	0.39
	Cefotaxime	≤0.05–50	≤0.05	0.78
	Cefoperazone	≤0.05–100	1.56	6.25
Serratia marcescens (100)	DN-9550	≤0.05–25	0.78	1.56
	Ceftazidime	≤0.05–100	0.78	3.13
	Cefotaxime	0.10–>100	1.56	25
	Cefoperazone	0.39–>100	12.5	>100
Citrobacter freundii (70)	DN-9550	≤0.05–100	0.39	25
	Ceftazidime	≤0.05–>100	0.39	100
	Cefotaxime	≤0.05–100	0.19	50
	Cefoperazone	≤0.05–>100	1.56	100

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TABLE 2—*Continued*

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Enterobacter spp. (75)[c]	DN-9550	≤0.05–50	0.39	12.5
	Ceftazidime	≤0.05–100	0.78	25
	Cefotaxime	≤0.05–>100	0.78	50
	Cefoperazone	≤0.05–>100	0.78	50
Pseudomonas aeruginosa (150)	DN-9550	0.19–50	1.56	6.25
	Ceftazidime	0.19–25	1.56	6.25
	Cefotaxime	0.39–>100	12.5	100
	Cefoperazone	0.19–>100	3.13	25
	Cefsulodin	≤0.05–>100	1.56	6.25
Pseudomonas maltophilia (25)	DN-9550	1.56–>100	100	>100
	Ceftazidime	0.19–>100	6.25	50
	Cedotaxime	3.13–>100	100	>100
	Cefoperazone	3.13–>100	50	>100
Alcaligenes faecalis (25)	DN-9550	0.10–100	3.13	25
	Ceftazidime	≤0.05–50	0.78	6.25
	Cefotaxime	≤0.05–100	0.78	50
	Cefoperazone	0.19–>100	1.56	12.5
Haemophilus influenzae (25)	DN-9550	≤0.05–0.19	0.19	0.19
	Ceftazidime	0.10–0.19	0.19	0.19
	Cefotaxime	≤0.05	≤0.05	≤0.05
	Cefoperazone	≤0.05–0.19	≤0.05	≤0.05
Neisseria gonorrhoeae (22)	DN-9550	≤0.05–0.78	0.10	0.78
	Ceftazidime	≤0.05–1.56	0.10	0.78
	Cefotaxime	≤0.05–0.19	≤0.05	0.19
	Cefoperazone	≤0.05–0.78	0.10	0.78
	Ampicillin	≤0.05–3.13	0.39	1.56
Bacteroides fragilis (25)	DN-9550	6.25–>100	>100	>100
	Ceftazidime	12.5–>100	>100	>100
	Cefotaxime	1.56–>100	50	>100
	Cefoperazone	3.13–>100	50	>100

[a] Agar dilution method; 10^4 CFU.
[b] Includes 59 *P. vulgaris*, 10 *P. rettgeri*, 10 *P. inconstans*, and 11 *P. morganii* strains.
[c] Includes 63 *E. cloacae* and 12 *E. aerogenes* strains.

Escherichia coli, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were identical to or, at most, twice as high as the MIC_{90}s.

Resistance to β-lactamases. Hydrolysis of DN-9550 by various types of β-lactamases was expressed as the relative rate of hydrolysis, taking the absolute rate of cephaloridine or penicillin G as 100. DN-9550 was highly stable to all the cephalosporinases tested, as were ceftazidime and cefotaxime, whereas cefoperazone was less stable than the other compounds to cephalosporinases from *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa* (Table 4). DN-9550 and cefoperazone were hydrolyzed to some extent by the oxyimino cephalosporin-hydrolyzing β-lactamase cefuroximase (2) from *Pseudomonas cepacia*, against which ceftazidime was stable, but DN-9550 was quite stable to the cefuroximase from *Proteus vulgaris*, as was ceftazidime. Cefotaxime was not stable to either of the cefuroximases. All the compounds, including DN-9550, were hydrolyzed to some extent by the cefuroximase from *B. fragilis*. DN-9550 was also highly stable to penicillinase types I, II, III, and IV mediated by R plasmids. DN-9550 was considered to be a cephalosporin resistant to β-lactamases, and this stability probably contributed to its broad spectrum of activity.

Affinity for PBPs. The affinities of DN-9550 to PBPs of *Escherichia coli* and *Pseudomonas aeruginosa* were examined by measuring the competition of unlabeled DN-9550 with [^{14}C]penicillin G for binding to PBPs. Figure 2 shows the competition patterns quantitatively. DN-9550 had a remarkably high affinity for *Escherichia coli* and *Pseudomonas aeruginosa* PBP-3, which participates in septum formation (11). DN-9550 also had high affinities for PBP-1Bs of *Escherichia coli* and PBP-1A of *Pseudomonas aeruginosa*. The affinities of DN-9550 for PBP-1A, -2, and -4 of

TABLE 3. Comparison of inhibitory and killing activities of DN-9550 against fresh clinical isolates[a]

Organism[b]	MIC (μg/ml)		MBC (μg/ml)	
	Range	90%	Range	90%
Escherichia coli	≤0.05–0.78	0.19	≤0.05–1.56	0.19
Serratia marcescens	≤0.05–50	6.25	0.10–50	6.25
Pseudomonas aeruginosa	0.78–50	12.5	1.56–50	25
Staphylococcus aureus	0.39–3.13	1.56	0.39–3.13	1.56

[a] Broth dilution; 10^6 CFU/ml.
[b] A total of 50 strains of each species were used.

TABLE 4. Stability of DN-9550, ceftazidime, cefotaxime, and cefoperazone to various β-lactamases

Organism	Type of β-lactamase[a]	Relative rate of hydrolysis (V_{max}) of[b]:					
		CER	DN-9550	CAZ	CTX	CPZ	PCG
Escherichia coli GN5842	CSase	100	<1	<1	<1	4	63
Enterobacter cloacae GN7471	CSase	100	<1	<1	<1	1	12
Citrobacter freundii GN7391	CSase	100	<1	<1	<1	<1	3
Serratia marcescens GN10857	CSase	100	<1	<1	<1	2	3
Proteus rettgeri GN4430	CSase	100	<1	<1	<1	<1	3
Proteus morganii GN5407	CSase	100	<1	<1	<1	<1	16
Pseudomonas aeruginosa GN10362	CSase	100	<1	<1	<1	5	29
Proteus vulgaris GN7919	CXase	100	<1	<1	84	15	20
Pseudomonas cepacia GN11164	CXase	100	12	<1	174	10	161
Bacteriodes fragilis GN11478	CXase	100	8	4	7	7	3
Escherichia coli W3630/Rms212	PCase type I	130	<1	<1	—[c]	—	100
Escherichia coli W3630/Rms213	PCase type II	263	<1	<1	—	—	100
Escherichia coli W3630/Rte16	PCase type III	23	<1	<1	—	—	100
Pseudomonas aeruginosa M1/Rms139	PCase type IV	20	<1	<1	—	—	100

[a] See reference 2. Abbreviations: CSase, cephalosporinase; CXase, cefuroximase; PCase, penicillinase.
[b] Hydrolysis of each substrate is expressed as the relative rate of hydrolysis, taking the absolute rate of cephaloridine or penicillin G as 100. V_{max}, Maximum rate of hydrolysis. Abbreviations: CER, cephaloridine; CAZ, ceftazidime; CTX, cefotaxime; CPZ, cefoperazone; PCG, penicillin G.
[c] —, Not tested.

Escherichia coli and PBP-1B, -2, and -4 of *Pseudomonas aeruginosa* were low, and affinities for PBP-5 and -6 of both organisms could not be detected.

In vivo efficacy. The protective effects of DN-9550 on systemic infections in mice are shown in Table 5. The 50% effective dose of DN-9550 against *Staphylococcus aureus* E46 infection was 0.6 mg/kg; DN-9550 was twice as active as cefotaxime and cefoperazone and 10 times as active as ceftazidime. DN-9550 and cefotaxime were 20 to 30 times more effective than ceftazidime and cefoperazone against *Streptococcus pyogenes* G36 infections. The activities of DN-9550 against *Escherichia coli* E77156, *K. pneumoniae* 3167, and *Serratia marcescens* 13001 infections, with 50% effective doses of 4.9, 3.7, and 4.6 mg/kg, respectively, were 2 to 6 times greater than those of ceftazidime, 3 to 13 times that of cefotaxime, and far exceeded that of cefoperazone. The 50% effective dose of DN-9550 against *Pseudomonas aeruginosa* PI-III infection was 10.3 mg/kg, indicating that DN-9550 is one-third as active as ceftazidime and cefsulodin; both cefotaxime and cefoperazone was ineffective against this infection.

DISCUSSION

DN-9550 showed excellent activity against members of the family *Enterobacteriaceae*, being roughly comparable to ceftazidime, although it was slightly less active than cefotaxime. DN-9550 was the most active cephalosporin tested against *Serratia marcescens*, *C. freundii*, and *Enterobacter cloacae*. DN-9550 also exhibited high activity against *Pseudomonas aeruginosa*, against which cefotaxime was much less active. Moreover, DN-9550 was highly active against staphylococci, against which ceftazidime was quite poor in activity. Thus, it could be said that DN-9550 has a well-balanced antibacterial spectrum with excellent potency against aerobic bacteria.

DN-9550 had extremely high affinities for PBP-3 of both *Escherichia coli* and *Pseudomonas aeruginosa*, and for PBP-1Bs of *Escherichia coli* and PBP-1A of *Pseudomonas aeruginosa*. PBP-1Bs of *Escherichia coli* are known to participate in cell elongation (10), and PBP-3 participates in septum formation (11); these proteins are supposed to correspond to PBP-1A and -3 of *Pseudomonas aeruginosa*,

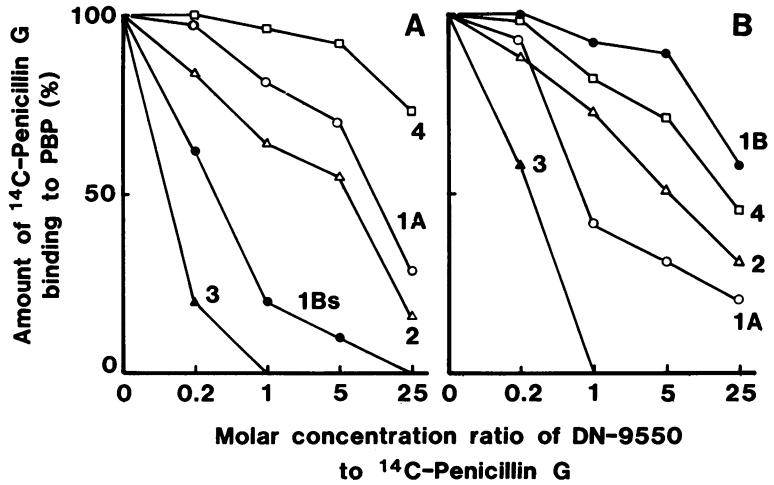

FIG. 2. Affinity of DN-9550 for PBPs of *Escherichia coli* (A) and *Pseudomonas aeruginosa* (B). Relative amounts of [^{14}C]penicillin G were measured with a densitometer, taking the amount of [^{14}C]penicillin G binding without DN-9550 as 100. ^{14}C-labeled PBP complexes were designated 1A, 1Bs, 2, 3, and 4, in order of increasing electrophoretic mobility.

TABLE 5. Protective effect of DN-9550, ceftazidime, cefotaxime, cefoperazone, and cefsulodin on systemic infections in mice

Organism	Inoculum size (CFU)[a]	Compound[b]	MIC (μg/ml)	ED_{50} (mg/kg)[c]	95% Confidence limit
Staphylococcus aureus E46	6.7×10^7 (8×)	DN-9550	0.78	0.6	0.4–0.8
		Ceftazidime	3.13	5.6	4.6–7.2
		Cefotaxime	0.78	1.4	1.1–1.7
		Cefoperazone	0.78	1.4	1.1–1.7
Streptococcus pyogenes G36	3.4×10^6 (32×)	DN-9550	0.05	0.1	0.1–0.2
		Ceftazidime	0.78	4.1	3.1–5.4
		Cefotaxime	0.05	0.2	0.1–0.3
		Cefoperazone	0.10	2.9	2.2–3.9
Escherichia coli E77156	2.6×10^7 (8×)	DN-9550	0.19	4.9	3.2–9.4
		Ceftazidime	0.19	16.8	12.8–20.9
		Cefotaxime	0.05	16.3	11.9–21.1
		Cefoperazone	0.19	>100	
Klebsiella pneumoniae 3167	4.0×10^3 (64×)	DN-9550	0.19	3.7	2.1–6.1
		Ceftazidime	0.19	5.4	3.0–9.6
		Cefotaxime	0.05	11.2	7.7–16.1
		Cefoperazone	0.39	>100	
Serratia marcescens 13001	2.9×10^7 (16×)	DN-9550	0.10	4.6	1.3–11.3
		Ceftazidime	0.19	28.3	12.8–86.8
		Cefotaxime	0.19	59.9	45.8–76.7
		Cefoperazone	0.78	>100	
Pseudomonas aeruginosa PI-III	5.1×10^5 (4×)	DN-9550	3.13	10.3	6.4–15.9
		Ceftazidime	1.56	3.0	2.0–4.4
		Cefotaxime	25	>100	
		Cefoperazone	6.25	>100	
		Cefsulodin	1.56	3.0	2.3–3.8

[a] Administered intraperitoneally with gastric mucin, except *E. coli* and *K. pneumoniae*, which were administered without gastric mucin. Numbers in parentheses represent 50% lethal doses.

[b] Subcutaneous regimen immediately and 4 h after infection with each organism, except the infection with *Pseudomonas aeruginosa*, which was treated with the subcutaneous regimen immediately and 3 and 6 h after infection.

[c] ED_{50}, 50% Effective dose.

respectively (7). The blocking of these proteins causes inhibition of cell division and, eventually, cell death. The excellent activity of DN-9550 may be attributed in part to its high affinities for these target proteins.

After intravenous administration in rats and monkeys, DN-9550 was well distributed to the kidneys, trachea, lungs, liver, and muscles and was excreted efficiently in unchanged form in urine (H. Tachizawa, K. Matsubayashi, T. Kurata, S. Shintani, and O. Okazaki, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 24th, Washington, D.C., abstr. no. 730, 1984). The broad spectrum of activity and the pharmacokinetic characteristics of DN-9550 warrant further evaluations of its clinical usefulness.

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