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Antimicrobial Activity of DU-6681a, a Parent Compound of Novel Oral Carbapenem DZ-2640

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The in vitro antibacterial activity of DU-6681a, a parent compound of DZ-2640, against gram-positive and -negative bacteria was compared with those of penems and cephalosporins currently available. MICs at which 90% of the isolates are inhibited (MIC_{90}s) of the compound for clinical isolates of methicillin-susceptible and -resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, including methicillin-susceptible and -resistant strains, were 0.10, 25, and 12.5 μg/ml, respectively. DU-6681a inhibited the growth of all strains of *Streptococcus pyogenes* and of penicillin-susceptible and -insusceptible *Streptococcus pneumoniae* at 0.006, 0.025, and 0.20 μg/ml, respectively, and MIC_{90}s of the compound were 6.25 and >100 μg/ml for *Enterococcus faecalis* and *Enterococcus faecium*, respectively. MIC_{90}s of DU-6681a were 0.20, 0.10, and 0.025 μg/ml for *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Neisseria gonorrhoeae*, respectively. For *Pseudomonas aeruginosa*, the MIC_{50} and MIC_{90} of DU-6681a were 25 and 50 μg/ml, respectively. DU-6681a activity was not affected by different media, varied inoculum size (10^4 to 10^7 CFU), or the addition of human serum but was decreased under acidic conditions against gram-negative bacteria, under alkaline conditions against gram-positive bacteria, and in human urine, as was the activity of the other antibiotics tested. The frequency of spontaneous resistance to DU-6681a was less than or equal to those of the reference compounds. Time-kill curve studies demonstrated the bactericidal action of DU-6681a against *S. aureus*, *S. pneumoniae*, *Escherichia coli*, and *H. influenzae*.

DZ-2640 is a new oral carbapenem having a bicyclic imidazole ring as a side chain (Fig. 1). The bicyclic imidazole ring system influenced both in vitro activity and pharmacokinetics after oral administration as pivaloyloxymethyl (POM) ester prodrug in rats (10); orally administrated DZ-2640 was reported to be rapidly absorbed in mice, rats, monkeys, and dogs as potent drug (1). DU-6681 is a parent compound of DZ-2640 and DU-6681a is a sodium salt of DU-6681. In this study, we compared the antimicrobial activity of DU-6681a with those of oral penems and cephalosporins such as furopenem (3, 4), cefpodoxime (5, 15), and cefdinir (9) against freshly isolated bacteria and estimated the effect of growth conditions on the activity of DU-6681a and its β-lactamase stability. Cefpodoxime and cefdinir were brought to market in 1989 and 1993 in Japan, respectively, and there are no oral carbapenems on the market yet. The standard MIC method for determining antibacterial activity provides no information on initial killing kinetics. This study therefore includes the killing curve method to obtain additional information on the antibacterial properties of DU-6681a in vitro, particularly with regard to its initial bactericidal activity against selected strains. The morphological study using differential interference microscopy also provides additional information on its bactericidal activity.

MATERIALS AND METHODS

Antibacterial agents. DU-6681a, a parent compound of DZ-2640, and furopenem (3, 4), cefpodoxime (5, 15), cefdinir (9), GV-104326, imipenem, and cefaclor were synthesized at the New Product Research Laboratories I, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan. Methicillin sodium (Sigma Chemical Co., St. Louis, Mo.) and ampicillin (Sigma) were obtained commercially.

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Organisms. Thirty-one aerobic and 26 anaerobic standard strains stocked in the New Product Research Laboratories I, Daiichi Pharmaceutical Co., Ltd., were used. A total of 643 strains, isolated between 1990 and 1994 from patients at six medical centers located in the Tokyo and Tohoku areas in Japan were randomly used (one isolate per patient). The two reference strains *Escherichia coli* NIH JC-2 and *Staphylococcus aureus* FDA 209-P were included as internal controls throughout the study.

Determination of MICs. The MICs for standard strains were determined by the broth microdilution method with Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.) for aerobic strains or GAM broth (Nissui Seiyaku Co., Ltd., Tokyo, Japan) for anaerobic strains. MICs for clinical strains were determined by the agar dilution method (8) with Mueller-Hinton agar (MHA; Difco). MHA supplemented with 5% horse blood (chocolate agar) was used for streptococci, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Neisseria gonorrhoeae*, while GAM agar (Nissui) was used for *Bacteroides fragilis* (11). One loopful (5 μl) of an inoculum corresponding to 10^4 CFU per spot was inoculated on drug-containing agar plates, and the plates were incubated for 18 h at 37°C, except those with *B. fragilis*, which were incubated for 24 h. *H. influenzae* and *N. gonorrhoeae* were incubated under conditions of 10% CO_2, and *B. fragilis* was incubated in an anaerobic cabinet. The MIC was defined as the lowest drug concentration which prevented visible growth of bacteria. Tests to examine the effects of varied inoculum size and medium pH, as well as of the addition of human serum on DU-6681a activity, were also performed by the MHA dilution method. The effect of the type of medium was determined by the agar dilution method using nutrient agar (NA; Eiken Chemical Co., Ltd., Tokyo, Japan), tryptone soy agar (TSA; Eiken), heart infusion agar (HIA; Eiken), brain heart infusion agar (BHIA; Difco), and MHA. The effect of human urine was examined by the macrodilution tube method (15) using MHB or 100% human urine. The final inoculum size was 5×10^5 CFU/ml.

Frequency of spontaneous resistant mutants. The test strains were precultured in MHB for 20 h at 37°C. The bacterial suspension was plated onto MHA plates containing 2, 4, or 8 times the MIC of each compound. After 48 h at 37°C, colonies were counted and the frequency of spontaneous resistance was calculated as the ratio of the number of colonies that grew on drug-containing plates to those on drug-free plates.

Determination of bactericidal activity. The organisms incubated in MHB for 18 h at 37°C were diluted with fresh broth to about 10^5 CFU/ml, and the diluted cultures were then incubated with agitation for 2 h at 37°C. For *H. influenzae*, MHB supplemented with 5% Fildes enrichment was used. After this preincubation, cultures were incubated in the absence of the indicated drug or in its presence at levels equivalent to one-quarter, one-half, one, two, and four times the MIC, and samples were removed at intervals, serially diluted, and plated for viable cells. The colonies were counted after 24 h of incubation at 37°C. Samples

TABLE 1. Antimicrobial spectra of DU-6681a and reference antibiotics against aerobic bacteria[a]

Organism	MIC (μg/ml)					
	DU-6681a	FRPM	CPDX	CFDN	CCL	IPM
Staphylococcus aureus FDA 209-P	0.013	0.05	0.78	0.05	0.78	0.006
Staphylococcus aureus Smith	0.025	0.10	1.56	0.10	0.78	0.013
Staphylococcus epidermidis 56556	0.025	0.05	0.78	0.05	1.56	0.013
Streptococcus pyogenes G-36	0.006	0.025	0.025	0.025	0.20	0.006
Streptococcus mitis IID 685	0.05	0.05	0.10	0.20	6.25	0.025
Enterococcus faecalis ATCC 19433	0.78	0.78	3.13	0.78	50	0.78
Bacillus subtilis ATCC 6633	0.025	0.025	6.25	1.56	0.39	0.025
Escherichia coli NIHJ	0.006	0.20	0.025	0.05	0.78	0.20
Escherichia coli KL-16	0.013	0.39	0.39	0.20	NT[b]	0.20
Shigella flexneri 2a 5503	0.013	0.39	0.20	0.20	1.56	0.20
Salmonella enteritidis IID 604	0.013	0.39	0.20	0.20	0.78	0.39
Hafnia alvei IID 978	0.013	0.78	0.39	0.39	6.25	0.20
Citrobacter freundii IID 976	0.006	0.39	1.56	0.78	3.13	0.20
Proteus vulgaris 08601	0.025	0.39	0.025	0.20	100	0.39
Proteus vulgaris 08602	0.013	0.20	0.05	0.20	>100	0.39
Morganella morganii IID 602	0.05	1.56	0.39	1.56	100	3.13
Providencia rettgeri 08500	0.39	3.13	0.39	1.56	>100	0.78
Proteus mirabilis IFO 3849	0.013	0.20	0.05	0.10	1.56	0.20
Klebsiella pneumoniae subsp. *pneumoniae* type 1	0.013	0.20	0.10	0.10	1.56	0.10
Klebsiella oxytoca 07600	0.013	0.39	3.13	1.56	>100	0.20
Enterobacter cloacae 03400	0.025	1.56	0.78	0.78	25	1.56
Enterobacter aerogenes ATCC 8329	0.025	1.56	0.20	0.39	100	1.56
Serratia marcescens 10100	0.025	1.56	0.39	0.78	50	0.39
Yersinia enterocolitica TE 591	0.013	1.56	6.25	1.56	50	0.20
Pseudomonas aeruginosa PAO1	6.25	>100	>100	>100	>100	0.78
Pseudomonas putida IID 5121	12.5	>100	>100	>100	>100	0.78
Burkholderia cepacia IID 1340	25	>100	>100	>100	>100	6.25
Stenotrophomonas maltophilia IID 1275	>100	>100	>100	>100	>100	>100
Flavobacterium meningosepticum ATCC 13253	3.13	12.5	100	100	>100	6.25
Alcaligenes faecalis ATCC 19108	0.025	0.39	1.56	0.39	0.39	0.20
Alcaligenes denitrificans subsp. *xylosoxydans* ATCC 27061	0.78	3.13	>100	>100	100	0.78

[a] The broth microdilution method (10^4 CFU of each strain/ml) was used. Abbreviations for antibiotics: FRPM, furopenem; CPDX, cefpodoxime; CFDN, cefdinir; CCL, cefaclor; IPM, imipenem.

[b] NT, not tested.

of bacterial suspensions used in the above-mentioned experiment were observed by differential interference microscopy.

β-Lactamase stability. Cell sonic extracts which were prepared from late-exponential cultures in MHB were used as enzyme solutions. Stability to β-lactamases from *Stenotrophomonas maltophilia* was measured with purified group 3 (L-1) and 2e (L-2) β-lactamases (2, 12). Hydrolytic rates of β-lactams were measured by a modified spectrophotometric method with 100 μM substrate at 30°C (6, 14).

Kinetic analysis of β-lactamases. K_m and maximum rate of metabolism (V_{max}) values were determined from hydrolytic rates of various concentration of substrates by using a Lineweaver-Burk plot with an enzyme preparation partially purified by DEAE-Cephacel and Sephadex G-50. The inhibitory constant (K_i), with cephaloridine or penicillin G as the substrate, was determined by using a Dixon plot.

Stability to DHP-I. The susceptibility of DU-6681a to renal dehydropeptidase-I (DHP-I) was compared with that of imipenem by using partially purified swine renal DHP-I. Hydrolytic rates of compounds were measured by a modified spectrophotometric method with 100 μM substrate at 30°C (7). The relative hydrolysis rate was determined, taking the hydrolysis rate of imipenem as 100.

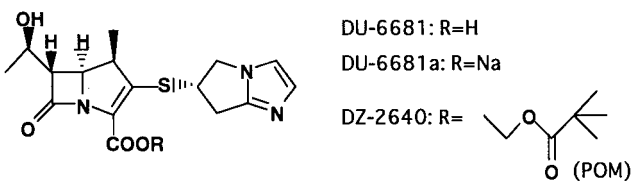

FIG. 1. Chemical structure of DU-6681a.

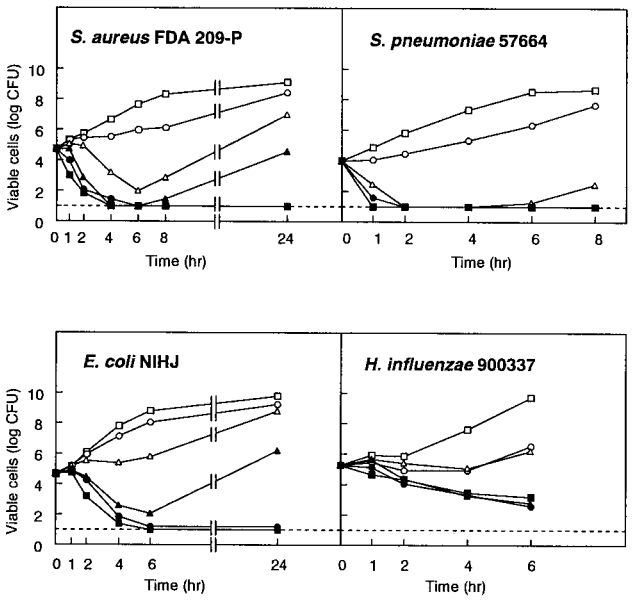

FIG. 2. Bactericidal kinetics of DU-6681a. MICs (▲) were 0.025 μg/ml for *S. aureus*, 0.013 μg/ml for *S. pneumoniae*, 0.013 μg/ml for *E. coli*, and 0.10 μg/ml for *H. influenzae*. Symbols: □, control; ○, one-quarter the MIC; △, one-half the MIC; ●, twice the MIC; ■, four times the MIC.

TABLE 2. Antimicrobial spectra of DU-6681a and reference antibiotics against anaerobic bacteria[a]

Organism	MIC (μg/ml)				
	DU-6681a	FRPM	CPDX	CFDN	IPM
Clostridium perfringens 22	0.10	0.78	12.5	1.56	1.56
Clostridium oroticum ATCC 13619	0.39	0.78	50	12.5	3.13
Clostridium sordellii ATCC 9714	≤0.05	≤0.05	0.20	0.10	≤0.05
Clostridium indolis ATCC 25771	1.56	1.56	>100	50	3.13
Clostridium difficile ATCC 9689	1.56	3.13	>100	25	25
Eubacterium moniliforme VPI 5518	≤0.05	≤0.05	0.78	0.39	0.10
Eubacterium aerofaciens ATCC 25986	0.10	0.20	1.56	0.39	1.56
Eubacterium aerofaciens X-3	0.10	0.39	1.56	0.39	1.56
Eubacterium limosum ATCC 8486	0.20	0.20	3.13	0.78	1.56
Propionibacterium acnes X-18	0.10	≤0.05	≤0.05	≤0.05	≤0.05
Peptostreptococcus asaccharolyticus VPI 5045	≤0.05	0.10	1.56	0.78	0.78
Peptostreptococcus prevotii ATCC 9321	0.10	0.39	1.56	0.78	0.20
Peptostreptococcus magnus X-36	0.20	0.20	50	6.25	3.13
Peptostreptococcus anaerobius ATCC 27337	0.20	0.39	0.78	0.78	0.20
Streptococcus intermedius VPI 3372	≤0.05	≤0.05	0.39	0.39	0.20
Bacteroides fragilis PA-2-II	0.10	≤0.05	100	25	0.78
Bacteroides vulgatus F-92	1.56	≤0.05	6.25	6.25	0.39
Bacteroides distasonis E-32	0.20	≤0.05	25	12.5	1.56
Bacteroides ovatus ATCC 8483	0.39	0.20	100	50	1.56
Bacteroides uniformis ATCC 8492	0.20	0.20	50	25	0.78
Fusobacterium varium ATCC 8501	0.20	0.39	50	6.25	6.25
Fusobacterium nucleatum subsp. *nucleatum* IPP 143	0.39	0.78	25	100	3.13
Fusobacterium perfoetens CCI	0.39	0.78	50	3.13	12.5
Fusobacterium mortiferum ATCC 9817	0.10	0.20	1.56	0.78	3.13
Fusobacterium gonidiaformans X-52	≤0.05	≤0.05	≤0.05	≤0.05	0.10
Veillonella parvula ATCC 10790	≤0.05	0.20	0.39	0.20	0.39

[a] See footnote *a* to Table 1 for details.

RESULTS

Antibacterial activity. The antimicrobial spectra of DU-6681a and reference antibiotics against individual aerobic and anaerobic strains are shown in Tables 1 and 2. DU-6681a was found to possess antibacterial activity against a wide range of bacteria. Tables 3 and 4 compare the activity of DU-6681a against groups of clinical isolates of gram-positive and -negative bacteria with those of the reference compounds. The MIC_{90}s of DU-6681a for methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) (MIC of methicillin, ≥12.5 μg/ml), and *S. epidermidis* were 0.10, 25, and 12.5 μg/ml, respectively. The activity against MSSA was 2- to 64-fold higher than those of the other reference compounds at MIC_{90}s. DU-6681a showed the highest activity against *S. aureus* and *S. epidermidis* among the antibiotics tested. MIC_{90}s for *Streptococcus pyogenes* and penicillin-susceptible and -insusceptible *S. pneumoniae* were 0.006, 0.025, and 0.20 μg/ml, respectively. For *Enterococcus faecalis* and *Enterococcus faecium*, the MIC_{90}s of DU-6681a were 6.25 and >100 μg/ml, respectively.

Against various species of *Enterobacteriaceae*, DU-6681a was at least fourfold more active than the reference compounds. DU-6681a inhibited 90% of isolates of *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Morganella morganii* at ≤0.20 μg/ml and inhibited 90% of isolates of *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, and *Providencia stuartii* at 6.25, 0.10, 0.39, 1.56, and 0.78 μg/ml, respectively. DU-6681a showed potent activity against cephalosporin- and/or penem-resistant *E. cloacae*. For *Pseudomonas aeruginosa*, MIC_{90}s of all compounds showed were greater than 50 μg/ml. Other glucose-nonfermentable gram-negative bacteria, including *Alcaligenes faecalis*, *Alcaligenes denitrificans*, and *Acinetobacter calcoaceticus*, were mostly susceptible to DU-6681a (MIC_{90}, 6.25 μg/ml).

H. influenzae, including β-lactamase-producing strains, *M. catarrhalis*, and *N. gonorrhoeae* were susceptible to DU-6681a, with MIC_{90}s for these strains being 0.20, 0.10, and 0.025 μg/ml, respectively. The MIC_{50} and MIC_{90} of DU-6681a for *B. fragilis* were 0.20 and 1.56 μg/ml, respectively.

The reference MICs of DU-6681a for *E. coli* NIH JC-2 and *S. aureus* FDA 209-P control strains were 0.006 and 0.013 μg/ml, respectively.

Factors affecting activity. The activities of DU-6681a against *S. aureus* FDA 209-P, *S. epidermidis* 56556, *E. coli* NIH JC-2, *K. pneumoniae* type I, and *P. aeruginosa* PAO1 were closely similar in all five different media tested, namely, MHA, NA, HIA, BHIA, and TSA (data not shown). Varying the pH of MHA between 7 and 8.5, adding human serum of 10, 25, and 50% to the medium, increasing the inoculum size from 10^4 to 10^7 CFU, or the presence of inoculum in human urine had no significant effect on the activity of DU-6681a against each strain, except for a fourfold increase in the MIC for *S. epidermidis* at pH 8.5. However, activity in the pH 5.5 MHA was 4-fold higher than that in the pH 7.0 MHA against staphylococci and 8- and 16-fold lower against *E. coli* and *K. pneumoniae*, respectively, than were activities of the other drugs tested.

Frequency of spontaneous resistant mutants. The frequencies of spontaneous resistance at two, four, and eight times the MIC of each drug are shown in Table 5. The frequencies of spontaneous resistance to DU-6681a were lower than those to the comparator compounds except for *S. aureus* FDA 209-P. Cefdinir (1.1×10^{-6}) and cefpodoxime (7.0×10^{-6}) against *S. aureus* ATCC 25923, cefdinir (1.4×10^{-6}) against *E. coli*

TABLE 3. Antibacterial activities of DU-6681a and reference antibiotics against clinical isolates of gram-positive bacteria

Organism (no. of strains)	Agent	MIC (μg/ml) range	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
MSSA (27)	DU-6681a	0.025–0.20	0.05	0.10
	Furopenem	0.10–0.39	0.20	0.20
	GV104326	0.05–0.20	0.10	0.10
	Cefpodoxime	1.56–12.5	3.13	6.25
	Cefdinir	0.10–1.56	0.39	1.56
	Methicillin	0.39–6.25	3.13	3.13
MRSA (19)	DU-6681a	0.10–25	12.5	25
	Furopenem	0.39–>100	>100	>100
	GV104326	0.10–>100	50	100
	Cefpodoxime	25–>100	>100	>100
	Cefdinir	1.56–>100	>100	>100
	Methicillin	12.5–>100	>100	>100
S. epidermidis (22)	DU-6681a	0.025–12.5	0.10	12.5
	Furopenem	0.10–>100	0.20	50
	GV104326	0.05–100	0.20	50
	Cefpodoxime	0.39–>100	3.13	>100
	Cefdinir	0.05–>100	0.39	>100
	Methicillin	0.78–>100	3.13	>100
S. pyogenes (25)	DU-6681a	≤0.003–0.006	0.006	0.006
	Furopenem	0.013–0.025	0.025	0.025
	GV104326	0.006–0.025	0.013	0.025
	Cefpodoxime	0.013–0.025	0.025	0.025
	Cefdinir	0.013–0.025	0.025	0.025
	Ampicillin	0.006–0.025	0.013	0.025
S. pneumoniae (penicillin susceptible)[a] (18)	DU-6681a	0.006–0.05	0.013	0.025
	Furopenem	0.013–0.025	0.013	0.013
	GV104326	0.013–0.025	0.013	0.025
	Cefpodoxime	0.025–1.56	0.025	1.56
	Cefdinir	0.05–3.13	0.10	1.56
	Ampicillin	0.003–0.05	0.025	0.05
S. pneumoniae (penicillin insusceptible)[b] (21)	DU-6681a	0.05–0.78	0.20	0.20
	Furopenem	0.10–1.56	0.39	0.78
	GV104326	0.10–1.56	0.39	0.78
	Cefpodoxime	0.39–6.25	1.56	3.13
	Cefdinir	0.39–25	6.25	12.5
	Ampicillin	0.10–6.25	1.56	6.25
E. faecalis (25)	DU-6681a	1.56–6.25	3.13	6.25
	Furopenem	0.78–3.13	1.56	3.13
	Cefpodoxime	>100–>100	>100	>100
	Cefdinir	3.13–>100	25	100
	Ampicillin	0.78–1.56	1.56	1.56
E. faecium (21)	DU-6681a	1.56–>100	100	>100
	Furopenem	6.25–>100	>100	>100
	Cefpodoxime	>100	>100	>100
	Cefdinir	12.5–>100	>100	>100
	Ampicillin	0.39–>100	100	>100

[a] MIC of ampicillin ≦ 0.05.
[b] MIC of ampicillin ≧ 0.10.

TABLE 4. Antibacterial activities of DU-6681a and reference antibiotics against clinical isolates of gram-negative bacteria

Organism (no. of strains)	Agent	MIC (μg/ml) range	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
E. coli (25)	DU-6681a	0.006–0.05	0.013	0.025
	Furopenem	0.39–3.13	0.78	1.56
	GV104326	0.20–3.13	0.39	0.78
	Cefpodoxime	0.10–100	0.39	25
	Cefdinir	0.10–100	0.20	25
C. freundii (25)	DU-6681a	0.013–12.5	0.10	6.25
	Furopenem	0.78–50	3.13	25
	GV104326	0.39–25	3.13	25
	Cefpodoxime	1.56–>100	>100	>100
	Cefdinir	0.39–>100	>100	>100
E. cloacae (25)	DU-6681a	0.013–0.20	0.05	0.20
	Furopenem	0.20–12.5	3.13	6.25
	GV104326	0.10–12.5	3.13	6.25
	Cefpodoxime	0.025–>100	25	>100
	Cefdinir	0.05–>100	100	>100
K. pneumoniae (25)	DU-6681a	0.013–0.05	0.025	0.05
	Furopenem	0.39–6.25	0.78	6.25
	GV104326	0.20–6.25	0.78	3.13
	Cefpodoxime	0.05–1.56	0.20	1.56
	Cefdinir	0.10–3.13	0.20	1.56
P. mirabilis (25)	DU-6681a	0.05–0.10	0.05	0.10
	Furopenem	0.78–1.56	1.56	1.56
	GV104326	0.10–0.39	0.20	0.39
	Cefpodoxime	0.05–0.20	0.10	0.10
	Cefdinir	0.10–0.20	0.10	0.20
P. vulgaris (25)	DU-6681a	0.025–0.39	0.10	0.39
	Furopenem	0.39–12.5	1.56	12.5
	GV104326	0.10–25	0.39	25
	Cefpodoxime	0.10–>100	0.39	>100
	Cefdinir	0.20–>100	3.13	>100
P. rettgeri (25)	DU-6681a	0.05–3.13	0.39	1.56
	Furopenem	0.20–50	3.13	25
	Cefpodoxime	0.013–12.5	0.78	6.25
	Cefdinir	0.013–50	1.56	12.5
P. stuartii (24)	DU-6681a	0.025–0.78	0.20	0.78
	Furopenem	0.39–12.5	3.13	6.25
	Cefpodoxime	0.025–6.25	0.78	3.13
	Cefdinir	0.025–6.25	0.39	3.13
M. morganii (25)	DU-6681a	0.05–0.39	0.20	0.20
	Furopenem	1.56–12.5	3.13	3.13
	Cefpodoxime	0.10–>100	0.78	25
	Cefdinir	0.78–100	12.5	25
S. marcescens (25)	DU-6681a	0.05–25	0.20	25
	Furopenem	3.13–>100	12.5	100
	GV104326	1.56–50	6.25	50
	Cefpodoxime	0.78–>100	3.13	>100
	Cefdinir	1.56–>100	25	>100
P. aeruginosa (50)	DU-6681a	3.13–>100	25	50
	Furopenem	25–>100	>100	>100
	GV104326	12.5–>100	100	>100
	Cefpodoxime	>100	>100	>100
	Cefdinir	>100	>100	>100
S. maltophilia (23)	DU-6681a	>100	>100	>100
	Furopenem	>100	>100	>100
	Cefpodoxime	>100	>100	>100
	Cefdinir	>100	>100	>100

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TABLE 4—*Continued*

Organism (no. of strains)	Agent	MIC (μg/ml) range	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
A. faecalis (24)	DU-6681a	0.05–>100	0.10	6.25
	Furopenem	0.39–>100	0.78	25
	Cefpodoxime	25–>100	100	>100
	Cefdinir	1.56–>100	3.13	>100
A. denitrificans subsp. *xylosoxydans* (23)	DU-6681a	0.20–100	0.78	6.25
	Furopenem	3.13–>100	3.13	12.5
	Cefpodoxime	>100	>100	>100
	Cefdinir	100–>100	>100	>100
A. calcoaceticus (25)	DU-6681a	0.78–25	3.13	6.25
	Furopenem	1.56–25	12.5	25
	Cefpodoxime	3.13–200	12.5	100
	Cefdinir	0.78–100	6.25	50
H. influenzae (24)	DU-6681a	0.05–0.78	0.10	0.20
	Furopenem	0.78–12.5	1.56	6.25
	GV104326	0.10–1.56	0.20	0.78
	Cefpodoxime	0.05–0.39	0.10	0.20
	Cefdinir	0.39–1.56	0.39	0.78
H. influenzae (β-lactamase producing) (14)	DU-6681a	0.025–0.10	0.05	0.10
	Furopenem	0.20–0.78	0.39	0.78
	GV104326	0.10–0.39	0.20	0.20
	Cefpodoxime	0.025–0.10	0.10	0.10
	Cefdinir	0.20–0.78	0.39	0.39
M. catarrhalis (25)	DU-6681a	0.025–0.10	0.05	0.10
	Furopenem	0.025–0.78	0.39	0.78
	GV104326	0.006–0.10	0.05	0.05
	Cefpodoxime	0.10–1.56	0.78	0.78
	Cefdinir	0.05–0.39	0.20	0.39
N. gonorrhoeae (24)	DU-6681a	≤0.003–0.025	0.013	0.025
	Furopenem	0.006–0.10	0.05	0.10
	Cefpodoxime	≤0.003–0.10	0.013	0.05
	Cefdinir	≤0.003–0.025	0.013	0.025
B. fragilis (22)	DU-6681a	0.10–>100	0.20	1.56
	Furopenem	0.025–>100	0.78	3.13
	GV104326	0.05–>100	0.20	0.78
	Cefpodoxime	6.25–>100	>100	>100
	Cefdinir	6.25–>100	>100	>100

KL-16, and furopenem (1.0×10^{-6}) and cefdinir (1.3×10^{-6}) against *K. pneumoniae* type I resulted in higher frequencies of resistance than did the other compounds. Except for the five cases mentioned above, there were no significant differences in the frequencies of resistance to the antibiotics tested.

Bactericidal activity. Killing curve studies showed rapid bactericidal activity of DU-6681a against *S. aureus* FDA 209-P, *S. pneumoniae* 57664, and *E. coli* KL-16 at the concentrations higher than the MIC (Fig. 2). However, in the case of *H. influenzae*, viable cell counts decreased only 1/10 of initial counts after a 6-h exposure to DU-6681a at concentrations one, two, and four times the MIC. Morphological alteration of *E. coli* after exposure to DU-6681a is shown in Fig. 3 and 4. *E. coli* cells became ovoid at concentrations one-eighth to eight times the MIC, while filamentous cells were observed after exposure to cefdinir.

Stability to β-lactamase and DHP-I. The β-lactamase stabilities of the antibiotics tested are shown in Table 6. DU-6681a was stable to various types of β-lactamases except *S. maltophilia* type 3 enzyme and showed potent antimicrobial activity against β-lactamase-producing strains except *S. maltophilia* and *P. aeruginosa*. The relative hydrolysis rate of DU-6681a by renal DHP-I was 6, while the hydrolysis rate of imipenem was defined as 100.

Kinetic analysis of β-lactamases. Table 6 also shows K_m, V_{max}, and apparent K_i values of DU-6681a and reference com-

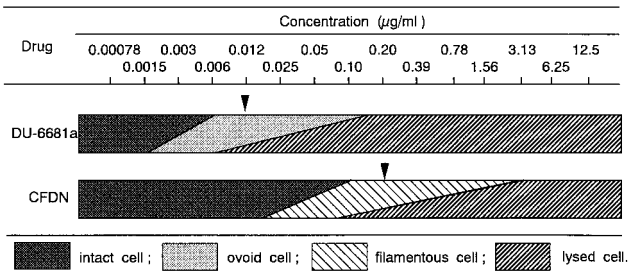

FIG. 3. Summary of morphological alterations of *E. coli* KL-16 exposed to DU-6681a and cefdinir (CFDN) for 4 h. Arrowheads show the MICs of the compounds.

TABLE 5. Frequency of spontaneous resistance to DU-6681a and reference antibiotics[a]

Organism	Amt of agent[b]	Frequency of spontaneous resistance to:			
		DU-6681a	FRPM	CPDX	CFDN
S. aureus FDA 209-P	2MIC	3.2×10^{-7}	$<3.2 \times 10^{-8}$	$<3.2 \times 10^{-8}$	3.2×10^{-8}
	4MIC	$<3.2 \times 10^{-8}$	$<3.2 \times 10^{-8}$	$<3.2 \times 10^{-8}$	$<3.2 \times 10^{-8}$
	8MIC	$<3.2 \times 10^{-8}$	$<3.2 \times 10^{-8}$	$<3.2 \times 10^{-8}$	$<3.2 \times 10^{-8}$
S. aureus ATCC 25923	2MIC	$<3.7 \times 10^{-7}$	$<3.7 \times 10^{-7}$	7.0×10^{-6}	1.1×10^{-6}
	4MIC	$<3.7 \times 10^{-7}$	$<3.7 \times 10^{-7}$	$<3.7 \times 10^{-7}$	$<3.7 \times 10^{-7}$
	8MIC	$<3.7 \times 10^{-7}$	$<3.7 \times 10^{-7}$	$<3.7 \times 10^{-7}$	$<3.7 \times 10^{-7}$
S. epidermidis 56556	2MIC	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$
	4MIC	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$
	8MIC	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$	$<4.5 \times 10^{-8}$
E. coli KL-16	2MIC	$<1.7 \times 10^{-8}$	$<1.7 \times 10^{-8}$	$<1.7 \times 10^{-8}$	1.4×10^{-6}
	4MIC	$<1.7 \times 10^{-8}$	$<1.7 \times 10^{-8}$	$<1.7 \times 10^{-8}$	1.7×10^{-8}
	8MIC	$<1.7 \times 10^{-8}$	$<1.7 \times 10^{-8}$	$<1.7 \times 10^{-8}$	$<1.7 \times 10^{-8}$
K. pneumoniae type 1	2MIC	4.3×10^{-8}	1.0×10^{-6}	4.2×10^{-7}	1.3×10^{-6}
	4MIC	$<1.1 \times 10^{-8}$	1.1×10^{-8}	1.1×10^{-8}	1.8×10^{-7}
	8MIC	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$
P. aeruginosa PAO1	2MIC	2.4×10^{-8}	NT[c]	NT	NT
	4MIC	$<4.0 \times 10^{-9}$	NT	NT	NT
	8MIC	$<4.0 \times 10^{-9}$	NT	NT	NT

[a] See footnote *a* to Table 1 for explanation of abbreviations.
[b] DU-6681a and the reference antibiotics were used at two, four, and eight times the MIC (2MIC, 4MIC, and 8MIC).
[c] NT, not tested.

pounds for various types of β-lactamase. Cephalosporinase-type enzymes from *E. cloacae* GN7471, *E. coli* 1154, and *P. aeruginosa* 2006-1 showed high affinities (low K_m values) for cefpodoxime and cefdinir and hydrolyzed those compounds at low rates. For the enzymes from *P. vulgaris* GN76, *E. coli* ML1410/RGN238, and *E. coli* J53/TEM3, cefpodoxime and cefdinir displayed higher V_{max} values than did type 1 enzymes. In contrast, DU-6681a and furopenem were not hydrolyzed by all enzymes tested (low V_{max} values) except for *S. maltophilia* L-1 enzyme and inhibited the enzymes at low concentrations (low K_i values).

DISCUSSION

This study showed that DU-6681a, a recently discovered oral carbapenem, possesses antibacterial activities against staphylococci and streptococci more potent than those of furopenem, cefpodoxime, and cefdinir. The highly antibacterial activity of furopenem against penicillin-susceptible and -resistant *S. pneumoniae* was reported by Spangler et al. (13), whose MIC_{50}s and MIC_{90}s of furopenem were similar to ours. Moreover, DU-6681a showed a potent activity against *Enterobacteriaceae*, *H. influenzae*, *M. catarrhalis*, and *N. gonorrhoeae*, which are known as significant pathogens in clinical fields.

Time-kill curve studies showed the rapid bactericidal action of DU-6681a against *S. aureus*, *S. pneumoniae*, and *E. coli*. Its bactericidal activity against *H. influenzae* is noteworthy. The bactericidal activity of DU-6681a was higher than that of the other compound tested (data not shown). Morphological alterations of *E. coli* exposed to DU-6681a were different from those of *E. coli* exposed to cefdinir. Ovoid cells were observed, suggesting that DU-6681a might have a high affinity for penicillin-binding protein 2 of *E. coli.*

DU-6681a was not hydrolyzed by various types of β-lactamases, including the extended-spectrum β-lactamases TEM-3 and -7, some of which hydrolyzed oxyiminocephalosporin antibiotics. This high stability of the compound to β-lactamases contributed to its potent antibacterial activity against the β-lac-

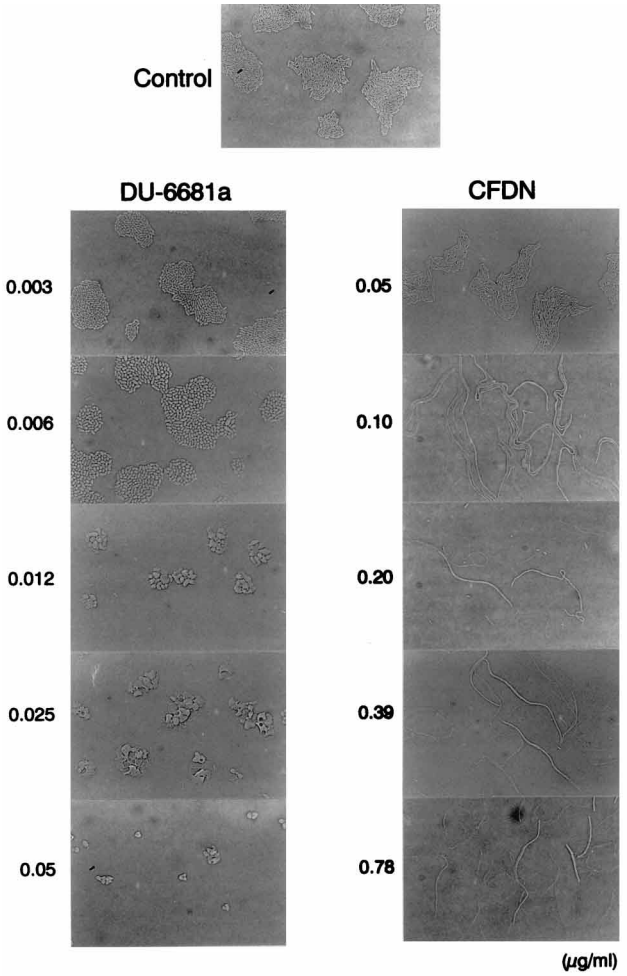

FIG. 4. Differential interference micrographs of *E. coli* KL-16 exposed to DU-6681a and cefdinir (CFDN) for 4 h. MICs of DU-6681a and cefdinir were 0.012 and 0.20 μg/ml, respectively.

TABLE 6. Hydrolysis and inhibition kinetic parameters for DU-6681a and reference antibiotics

Organism (type of β-lactamase[a])	Agent	MIC[b]	Stability[c]	K_m (μM)	Relative V_{max}[d]	K_i (μM)[e]
E. cloacae GN7471 (1)	DU-6681a	0.10	<0.1	—[f]	—	0.53
	Furopenem	3.13	<0.1	—	—	0.69
	Cefpodoxime	>100	0.5	27	0.068	NT[g]
	Cefdinir	>100	0.2	59	0.059	NT
E. coli 1154 (1)	DU-6681a	0.013	0.8	—	—	3.5
	Furopenem	0.78	1.7	—	—	0.19
	Cefpodoxime	25	2.1	28	0.70	NT
	Cefdinir	25	0.7	34	0.36	NT
P. vulgaris GN76 (2e)	DU-6681a	0.10	<0.1	—	—	0.061
	Furopenem	1.56	<0.1	—	—	1.8
	Cefpodoxime	0.20	15.2	370	26	NT
	Cefdinir	0.39	5.8	130	10	NT
P. aeruginosa 2006-1 (1)	DU-6681a	12.5	0.7	—	—	0.12
	Furopenem	>100	0.9	—	—	0.35
	Cefpodoxime	>100	15.1	15	1.4	NT
	Cefdinir	>100	2.1	17	0.52	NT
S. maltophilia IID 1275 (2e)	DU-6681a	>100	<0.1	NT	NT	NT
	Furopenem	>100	<0.1	NT	NT	NT
	Cefpodoxime	>100	59.8	NT	NT	NT
	Cefdinir	>100	2.7	NT	NT	NT
E. coli ML1410/RGN823 (2b)	DU-6681a	0.025	<0.1	—	—	0.042
	Furopenem	0.78	<0.1	—	—	52
	Cefpodoxime	0.20	<0.1	—	—	NT
	Cefdinir	0.39	<0.1	—	—	NT
K. pneumoniae GN5574 (2b)	DU-6681a	0.013	1.1	—	—	2.1
	Furopenem	0.39	1.2	—	—	28
	Cefpodoxime	0.10	2.9	—	—	NT
	Cefdinir	0.20	0.5	—	—	NT
E. coli ML1410/RGN238 (2b)	DU-6681a	0.025	<0.1	—	—	1.4
	Furopenem	0.78	<0.1	360	4.5	2.7
	Cefpodoxime	0.39	8.4	110	7.6	NT
	Cefdinir	0.39	2.3	210	11	NT
E. coli J53/TEM3 (2be)	DU-6681a	0.025	<0.1	—	—	0.091
	Furopenem	1.56	<0.1	—	—	1.4
	Cefpodoxime	25	111	120	150	NT
	Cefdinir	1.56	8.1	39	9.5	NT
E. coli J53/TEM7 (2be)	DU-6681a	0.025	<0.1	—	—	0.19
	Furopenem	0.78	<0.1	—	—	5.8
	Cefpodoxime	1.56	0.9	310	0.41	NT
	Cefdinir	0.39	<0.1	72	0.020	NT
S. maltophilia IID 1275 (3)	DU-6681a	>100	36	55	15	NT
	Furopenem	>100	28	67	9.6	NT
	Cefpodoxime	>100	8.2	80	3.4	NT
	Cefdinir	>100	3.9	750	9.1	NT

[a] Bush-Jacoby-Medeiros classification (2).
[b] Agar dilution method; results in micrograms per milliliter.
[c] Relative rate of hydrolysis (penicillinase, penicillin G = 100; cephalosporinase, cephaloridine = 100).
[d] Based on a value of 100 for cephaloridine (types 1 and 2e) or penicillin G (types 2b, 2be, and 3).
[e] Apparent K_i.
[f] —, could not be determined because of high stability to the β-lactamase.
[g] NT, not tested.

tamase-producing strains tested. However, *S. maltophilia* L-1 enzyme, which is a metalloenzyme, hydrolyzed DU-6681a as well as other β-lactams, including furopenem (4). K_i values of DU-6681a for β-lactamases were lower than those of furopenem except for the enzyme from *E. coli* 1154. This indicates that DU-6681a has a high affinity for and a high stability to β-lactamases.

Final decisions on the apparently greater efficacy of DU-6681a compared with other β-lactams must await further studies, such as studies of affinity for penicillin-binding proteins,

studies of efficacy for animal models, pharmacokinetic and toxicity studies, and clinical evaluations.

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