

In Vitro Antibacterial Activity of DU-6859a, a New Fluoroquinolone

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The in vitro antibacterial activity of DU-6859a, a new fluoroquinolone, against a wide variety of clinical isolates was evaluated and compared with those of tosylfloxacin, ofloxacin, ciprofloxacin, and sparfloxacin. DU-6859a showed potent broad-spectrum activity against gram-positive, gram-negative, and anaerobic bacteria, and its activity was greater than those of the control quinolones. By comparison of MICs at which 90% of strains are inhibited, DU-6859a had potent activity against bacteria resistant to the control quinolones. The time-killing curves of quinolones showed that the number of viable cells decreased rapidly during 2 to 4 h of incubation, and regrowth was not seen even after 8 h of incubation. At a concentration of four times the MIC, the frequencies of appearance of spontaneous mutants of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* resistant to DU-6859a were $\leq 4.0 \times 10^{-9}$ to 1.9×10^{-8} . The 50% inhibitory concentrations of DU-6859a were 0.86 and 1.05 $\mu\text{g/ml}$ for the supercoiling activities of DNA gyrases isolated from *E. coli* and *P. aeruginosa*, respectively. The rank order of the 50% inhibitory concentrations observed for both DNA gyrases roughly paralleled the MICs.

A number of new quinolone antibacterial agents such as tosylfloxacin (1), ofloxacin (8), ciprofloxacin (9), and sparfloxacin (5) have been developed and introduced into the market. These drugs have broad spectra of activity and potent activities against gram-positive and gram-negative bacteria.

DU-6859a, (-)-7[(7*S*)-amino-5-azaspiro[2,4]heptan-5-yl]-8-chlore-6-fluoro-1-[(1*R*,2*S*)-*cis*-2-fluoro-1-cyclopropyl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid sesquihydrate, a new fluoroquinolone antimicrobial agent, has a broad antibacterial spectrum (6). In the study described here, the activity of DU-6859a was compared with those of other fluoroquinolones.

Determination of DU-6859a MIC. The bacterial strains used in the study were reference strains and clinical isolates collected from several hospitals and laboratories in Japan between 1985 and 1992. All isolates were maintained at the Episome Institute.

The following antimicrobial agents were provided by the indicated manufacturers: DU-6859a, Daiichi Seiyaku Co., Ltd., Tokyo, Japan; tosylfloxacin, Toyama Chemical Co., Ltd., Tokyo, Japan; ofloxacin, Daiichi Seiyaku Co., Ltd., Tokyo, Japan; ciprofloxacin, Bayer Yakuin, Ltd., Osaka, Japan; sparfloxacin, Dainiphon Seiyaku, Ltd., Osaka, Japan; and vancomycin, Shionogi Seiyaku, Ltd., Osaka, Japan.

MICs were determined by the twofold serial agar dilution method with Sensitivity Disk Agar-N (SDA; Nissui Pharmaceutical, Tokyo, Japan), which was supplemented with 5% defibrinated horse blood for streptococci, 5% Fildes enrichment (Difco Laboratories, Detroit, Mich.) for *Haemophilus influenzae*, and 10% defibrinated horse blood with heating (chocolate agar) for *Neisseria gonorrhoeae*. For anaerobic bacteria, GAM agar (Nissui) was used.

Overnight broth cultures of the bacterial strains were diluted with corresponding fresh broth to result in a final concentration of approximately 10^6 CFU/ml, and an inoculum of 10^4 CFU per spot was applied with an inoculating apparatus (Microplanter; Sakuma Seisakusho, Tokyo, Japan) to agar plates containing graded concentrations of drug. The plates were

incubated at 37°C for 18 h except for those containing *N. gonorrhoeae*, which were incubated in a candle jar for 24 h, and anaerobes, which were incubated in an anaerobic chamber for 18 h. The MIC was defined as the lowest concentration of drug that inhibited visible growth on the plate.

The in vitro activities of DU-6859a, tosylfloxacin, ofloxacin, ciprofloxacin, and sparfloxacin against a variety of clinical isolates are given in Table 1. DU-6859a showed potent antibacterial activity against gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, and *Enterococcus* spp. in comparison with the control quinolones tested.

The DU-6859a MICs at which 90% of strains are inhibited ($\text{MIC}_{90\text{s}}$) for *Staphylococcus aureus* (methicillin susceptible), *Staphylococcus epidermidis* (methicillin susceptible), *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* were 0.025, 0.05, 0.10, 0.05, and 0.20 $\mu\text{g/ml}$, respectively, indicating the potent activity of DU-6859a (Table 1).

The activity of DU-6859a against gram-negative bacteria was roughly comparable to those of tosylfloxacin and sparfloxacin and was usually fourfold greater than those of ofloxacin and ciprofloxacin. Its activity against *Pseudomonas* spp. and *Chryseobacterium meningosepticum* was similar to those of tosylfloxacin and was 4- to 32-fold greater than those of ofloxacin and ciprofloxacin. The activity of DU-6859a against streptococci and enterococci was equal to or 2- or 64-fold greater than those of tosylfloxacin, ofloxacin, ciprofloxacin, and sparfloxacin.

Among the strains tested, we chose strains resistant to fluorinated quinolones on the basis of the $\text{MIC}_{90\text{s}}$ that we obtained (Table 2). It was remarkable that, in a comparison of the $\text{MIC}_{90\text{s}}$, DU-6859a had potent activity against bacteria resistant to the control quinolones, ranging from 0.20 to 12.5 $\mu\text{g/ml}$ (*S. epidermidis*, *Clostridium difficile*).

Bactericidal activity of DU-6859a. The time-killing curves of DU-6859a, ofloxacin, and ciprofloxacin against *S. aureus* Smith and *Pseudomonas aeruginosa* GN11189 are shown in Fig. 1.

Mid-logarithmic-phase cells (approximately 10^6 CFU/ml) were exposed to the test drug at a concentration of one-quarter, one-half, one, two, or four times the MIC. A 0.1-ml sample was removed at fixed times, and serial 10-fold dilutions were prepared in saline and plated onto the drug-free SDA (Nissui). The number of colonies was counted after 24 h of incubation at 37°C.

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TABLE 1. Antibacterial activities of DU-6859a and the other compounds against clinical isolates

Organism (no. of isolates)	Compound	MIC ($\mu\text{g/ml}$)		
		Range	50%	90%
<i>Staphylococcus aureus</i> , methicillin susceptible (70)	DU-6859a	≤ 0.006 –3.13	0.025	0.025
	Tosufloxacin	0.013–12.5	0.025	0.05
	Ofloxacin	0.10–>100	0.39	0.39
	Ciprofloxacin	0.20–>100	0.39	0.78
	Sparfloxacin	0.025–50	0.05	0.10
	Vancomycin	0.78–1.56	0.78	0.78
<i>Staphylococcus epidermidis</i> , methicillin susceptible (32)	DU-6859a	≤ 0.006 –0.10	0.025	0.05
	Tosufloxacin	0.025–1.56	0.05	0.20
	Ofloxacin	0.10–6.25	0.20	0.78
	Ciprofloxacin	0.05–12.5	0.20	1.56
	Sparfloxacin	0.05–6.25	0.10	0.20
<i>Streptococcus pyogenes</i> (97)	DU-6859a	0.025–0.20	0.05	0.10
	Tosufloxacin	0.05–0.78	0.20	0.39
	Ofloxacin	0.20–6.25	0.78	3.13
	Ciprofloxacin	0.39–12.5	0.78	3.13
	Sparfloxacin	0.20–3.13	0.78	1.56
<i>Streptococcus pneumoniae</i> (25)	DU-6859a	0.013–0.10	0.025	0.05
	Tosufloxacin	0.025–3.13	0.10	0.78
	Ofloxacin	0.39–12.5	1.56	3.13
	Ciprofloxacin	0.30–50	1.56	6.25
	Sparfloxacin	0.10–6.25	0.39	1.56
<i>Enterococcus faecalis</i> (99)	DU-6859a	0.05–0.39	0.10	0.20
	Tosufloxacin	0.05–0.78	0.20	0.39
	Ofloxacin	0.78–3.13	1.56	3.13
	Ciprofloxacin	0.39–3.13	0.78	1.56
	Sparfloxacin	0.20–0.78	0.39	0.39
<i>Escherichia coli</i> (97)	DU-6859a	≤ 0.006 –0.10	0.013	0.025
	Tosufloxacin	≤ 0.006 –0.39	0.025	0.025
	Ofloxacin	0.05–1.56	0.05	0.10
	Ciprofloxacin	0.013–0.39	0.025	0.05
	Sparfloxacin	≤ 0.006 –0.39	0.025	0.025
<i>Shigella</i> spp. (100)	DU-6859a	≤ 0.006 –0.10	0.013	0.013
	Tosufloxacin	≤ 0.006 –0.20	0.013	0.013
	Ofloxacin	0.013–1.56	0.05	0.10
	Ciprofloxacin	≤ 0.006 –0.20	0.013	0.025
	Sparfloxacin	≤ 0.006 –0.39	0.013	0.025
<i>Salmonella</i> spp. (108)	DU-6859a	≤ 0.006 –0.025	0.013	0.025
	Tosufloxacin	0.013–0.39	0.025	0.05
	Ofloxacin	0.05–0.20	0.10	0.20
	Ciprofloxacin	0.013–0.39	0.025	0.025
	Sparfloxacin	0.013–0.39	0.05	0.05
<i>Klebsiella pneumoniae</i> (108)	DU-6859a	≤ 0.006 –0.39	0.025	0.05
	Tosufloxacin	≤ 0.006 –0.78	0.05	0.05
	Ofloxacin	0.05–1.56	0.20	0.20
	Ciprofloxacin	0.013–1.56	0.05	0.10
	Sparfloxacin	0.013–0.78	0.10	0.10
<i>Klebsiella oxytoca</i> (100)	DU-6859a	≤ 0.006 –0.05	0.013	0.025
	Tosufloxacin	≤ 0.006 –0.39	0.025	0.025
	Ofloxacin	0.10–0.78	0.20	0.20
	Ciprofloxacin	0.013–0.10	0.025	0.10
	Sparfloxacin	0.013–0.10	0.05	0.05
<i>Proteus mirabilis</i> (102)	DU-6859a	0.013–0.20	0.025	0.05
	Tosufloxacin	0.025–0.78	0.10	0.20
	Ofloxacin	0.05–0.78	0.10	0.20
	Ciprofloxacin	0.025–0.20	0.05	0.05
	Sparfloxacin	0.05–1.56	0.20	1.56
<i>Proteus vulgaris</i> (95)	DU-6859a	0.013–1.56	0.05	0.20
	Tosufloxacin	0.025–1.56	0.05	0.39

Continued on following page

TABLE 1—Continued

Organism (no. of isolates)	Compound	MIC ($\mu\text{g/ml}$)		
		Range	50%	90%
	Ofloxacin	0.05–1.56	0.10	1.56
	Ciprofloxacin	0.025–0.20	0.05	0.39
	Sparfloxacin	0.05–3.13	0.10	0.39
<i>Morganella morganii</i> (70)	DU-6859a	≤ 0.006 –3.13	0.013	0.025
	Tosufloxacin	0.025–25	0.05	0.20
	Ofloxacin	0.05–50	0.10	0.10
	Ciprofloxacin	0.013–25	0.013	0.025
	Sparfloxacin	0.025–25	0.10	0.20
<i>Providencia rettgeri</i> (50)	DU-6859a	≤ 0.006 –0.20	0.05	0.78
	Tosufloxacin	0.013–1.56	0.10	0.78
	Ofloxacin	0.10–25	0.39	6.25
	Ciprofloxacin	0.013–3.13	0.39	6.25
	Sparfloxacin	0.013–12.5	0.20	3.13
<i>Providencia stuartii</i> (74)	DU-6859a	≤ 0.006 –0.20	0.05	0.10
	Tosufloxacin	≤ 0.006 –0.39	0.10	0.20
	Ofloxacin	0.05–1.56	0.39	0.78
	Ciprofloxacin	0.025–0.78	0.20	0.39
	Sparfloxacin	0.013–0.10	0.10	0.20
<i>Citrobacter freundii</i> (96)	DU-6859a	≤ 0.006 –6.25	0.05	0.39
	Tosufloxacin	0.025–100	0.10	0.78
	Ofloxacin	0.10–100	0.20	1.56
	Ciprofloxacin	0.013–25	0.05	0.78
	Sparfloxacin	0.025–100	0.20	1.56
<i>Enterobacter cloacae</i> (100)	DU-6859a	≤ 0.006 –0.78	0.025	0.10
	Tosufloxacin	≤ 0.006 –3.13	0.05	0.20
	Ofloxacin	0.025–12.5	0.10	0.78
	Ciprofloxacin	0.013–6.25	0.025	0.20
	Sparfloxacin	≤ 0.006 –3.13	0.05	0.20
<i>Yersinia enterocolitica</i> (43)	DU-6859a	≤ 0.006 –0.025	0.025	0.025
	Tosufloxacin	≤ 0.006 –0.025	0.025	0.025
	Ofloxacin	0.05–0.39	0.20	0.20
	Ciprofloxacin	0.013–0.10	0.025	0.05
	Sparfloxacin	0.013–0.10	0.025	0.05
<i>Chryseobacterium meningosepticum</i> (38)	DU-6859a	0.05–12.5	1.56	3.13
	Tosufloxacin	0.10–3.13	0.78	1.56
	Ofloxacin	0.78–12.5	3.13	6.25
	Ciprofloxacin	0.78–50	6.25	12.5
	Sparfloxacin	0.05–0.78	0.39	0.78
<i>Acinetobacter</i> spp. (35)	DU-6859a	0.013–0.20	0.05	0.10
	Tosufloxacin	0.013–0.20	0.025	0.10
	Ofloxacin	0.10–1.56	0.20	0.78
	Ciprofloxacin	0.10–3.13	0.20	0.78
	Sparfloxacin	0.013–0.10	0.025	0.05
<i>Haemophilus influenzae</i> (38)	DU-6859a	≤ 0.006 –0.05	≤ 0.006	0.013
	Tosufloxacin	≤ 0.006 –0.10	≤ 0.006	0.05
	Ofloxacin	0.05–1.56	0.05	0.39
	Ciprofloxacin	0.013–0.78	0.013	0.20
	Sparfloxacin	≤ 0.006 –0.39	0.013	0.39
<i>Neisseria gonorrhoeae</i> (31)	DU-6859a	≤ 0.006 –0.05	≤ 0.006	≤ 0.006
	Tosufloxacin	≤ 0.006 –0.20	≤ 0.006	0.013
	Ofloxacin	≤ 0.006 –0.78	≤ 0.006	0.05
	Ciprofloxacin	≤ 0.006 –0.20	≤ 0.006	≤ 0.006
	Sparfloxacin	≤ 0.006 –0.20	≤ 0.006	0.013
<i>Moraxella catarrhalis</i> (42)	DU-6859a	≤ 0.006 –0.025	0.013	0.025
	Tosufloxacin	≤ 0.006 –0.025	0.013	0.025
	Ofloxacin	0.05–0.20	0.10	0.20
	Ciprofloxacin	0.025–0.20	0.05	0.20
	Sparfloxacin	≤ 0.006 –0.10	0.013	0.10

TABLE 2. Antibacterial activities of DU-6859a and the other compounds against clinical isolates

Organism (no. of isolates)	Compound	MIC ($\mu\text{g/ml}$)	
		50%	90%
<i>Staphylococcus aureus</i> , methicillin resistant (71)	DU-6859a	0.025	0.78
	Tosufloxacin	0.10	12.5
	Ofloxacin	0.78	50
	Ciprofloxacin	1.56	100
	Sparfloxacin	0.10	12.5
	Vancomycin	0.78	1.56
<i>Staphylococcus epidermidis</i> , methicillin resistant (74)	DU-6859a	0.025	0.20
	Tosufloxacin	0.05	6.25
	Ofloxacin	0.39	6.25
	Ciprofloxacin	0.39	12.5
	Sparfloxacin	0.10	3.13
<i>Enterococcus faecium</i> (99)	DU-6859a	0.10	0.78
	Tosufloxacin	0.78	6.25
	Ofloxacin	3.13	25
	Ciprofloxacin	1.56	25
	Sparfloxacin	0.78	12.5
<i>Serratia marcescens</i> (100)	DU-6859a	0.20	1.56
	Tosufloxacin	0.39	6.25
	Ofloxacin	1.56	25
	Ciprofloxacin	0.39	12.5
<i>Pseudomonas aeruginosa</i> (95)	DU-6859a	0.20	0.78
	Tosufloxacin	0.39	1.56
	Ofloxacin	3.13	12.5
	Ciprofloxacin	0.39	0.78
	Sparfloxacin	1.56	6.25
<i>Pseudomonas aeruginosa</i> , ofloxacin resistant (MIC, $\geq 3.13 \mu\text{g/ml}$) (88)	DU-6859a	6.25	12.5
	Tosufloxacin	>100	>100
	Ofloxacin	100	>100
	Ciprofloxacin	50	>100
<i>Burkholderia cepacia</i> (94)	DU-6859a	0.78	3.13
	Tosufloxacin	3.13	6.25
	Ofloxacin	25	50
	Ciprofloxacin	6.25	12.5
	Sparfloxacin	12.5	12.5
<i>Stenotrophomonas maltophilia</i> (51)	DU-6859a	0.20	0.39
	Tosufloxacin	0.39	0.78
	Ofloxacin	6.25	12.5
	Ciprofloxacin	3.13	6.25
	Sparfloxacin	0.78	1.56
<i>Clostridium perfringens</i> (16)	DU-6859a	0.10	0.39
	Tosufloxacin	0.78	1.56
	Ofloxacin	1.56	25
	Ciprofloxacin	12.5	50
	Sparfloxacin	3.13	6.25
<i>Clostridium difficile</i> (21)	DU-6859a	0.10	0.20
	Tosufloxacin	0.39	0.78
	Ofloxacin	3.13	6.25
	Ciprofloxacin	3.13	12.5
	Sparfloxacin	1.56	3.13
<i>Bacteroides fragilis</i> (29)	DU-6859a	0.20	0.39
	Tosufloxacin	0.78	1.56
	Ofloxacin	3.13	12.5
	Ciprofloxacin	6.25	25
	Sparfloxacin	1.56	3.13

TABLE 3. Inhibitory effects of quinolones on DNA gyrase supercoiling activity

Organism	Drug	MIC ($\mu\text{g/ml}$) ^a	IC ₅₀ ($\mu\text{g/ml}$) ^b
<i>E. coli</i> KL-16	DU-6859a	0.025	0.86
	Tosufloxacin	0.013	1.37
	Ofloxacin	0.05	2.36
	Ciprofloxacin	0.025	1.01
	Sparfloxacin	0.013	1.10
<i>P. aeruginosa</i> PAO1	DU-6859a	0.20	1.05
	Tosufloxacin	0.20	1.71
	Ofloxacin	0.78	3.25
	Ciprofloxacin	0.20	1.30
	Sparfloxacin	0.78	1.47

^a The MICs were determined by the agar dilution method.

^b IC₅₀, 50% inhibitory concentration.

The number of viable cells decreased during incubation with DU-6859a, reducing the viable cell count to undetectable levels within 2 to 4 h at twice the MICs. The regrowth of all organisms tested was not observed at concentrations equal to or greater than two times the MICs after 24 h of incubation with these fluoroquinolones. The studies show that the bactericidal activity of DU-6859a is similar to those of ofloxacin and ciprofloxacin against these strains.

Spontaneous mutation to DU-6859a resistance. The frequencies of occurrence of spontaneous mutants resistant to quinolones in *S. aureus* Smith, *Escherichia coli* ML4707, and *P. aeruginosa* GN11189 were determined by spreading a 0.1-ml sample of an overnight culture of each test organism onto three SDA plates containing drugs at concentrations of two and four times the MIC. The numbers of cells in the overnight cultures of *S. aureus* Smith, *E. coli* ML4707, and *P. aeruginosa* GN11189 were 2.7×10^8 , 3.3×10^9 , and 2.2×10^9 CFU/ml, respectively. After incubation at 37°C for 48 h, the colonies were counted and the frequency of occurrence of spontaneous mutants resistant to the drug was calculated as the ratio of the number of resistant cells to the number of cells inoculated (3).

The frequencies of occurrence of spontaneous mutants of *S. aureus* Smith, *E. coli* ML4707, and *P. aeruginosa* GN11189 resistant to DU-6859a were $\leq 4.0 \times 10^{-9}$ to 1.9×10^{-8} .

Inhibition of DNA gyrase activity. The subunit A and B proteins of DNA gyrase were purified from *E. coli* KL-16 and *P. aeruginosa* PAO1 by the methods reported previously (4, 7). One unit of enzyme was defined as the amount that brought 50% of relaxed pBR322 DNA to the supercoiled form, as described by Gellert et al. (2). In the present study, the specific activities of purified enzyme from *E. coli* and *P. aeruginosa* were 230 and 59 U/mg of protein, respectively. The reactions for DNA supercoiling activity were performed by using the modifications described previously (4, 7). The reaction mixture was incubated for 1 h at 37°C, and the reaction was stopped by the addition of 1% proteinase K (Sigma Chemical, St. Louis, Mo.). The reaction mixture was then subjected to 0.8% agarose gel electrophoresis. Ethidium bromide-stained gels were photographed during UV transillumination, and the photographic negatives were analyzed with a densitometer.

The supercoiling activities of the DNA gyrases from *E. coli* KL16 and *P. aeruginosa* PAO1 obtained by using plasmid pBR322 were inhibited by DU-6859a, tosufloxacin, ofloxacin, ciprofloxacin, and sparfloxacin (Table 3). The 50% inhibitory concentrations of DU-6859a for the DNA gyrases of *E. coli* and *P. aeruginosa* were 0.86 and 1.05 $\mu\text{g/ml}$, respectively. The

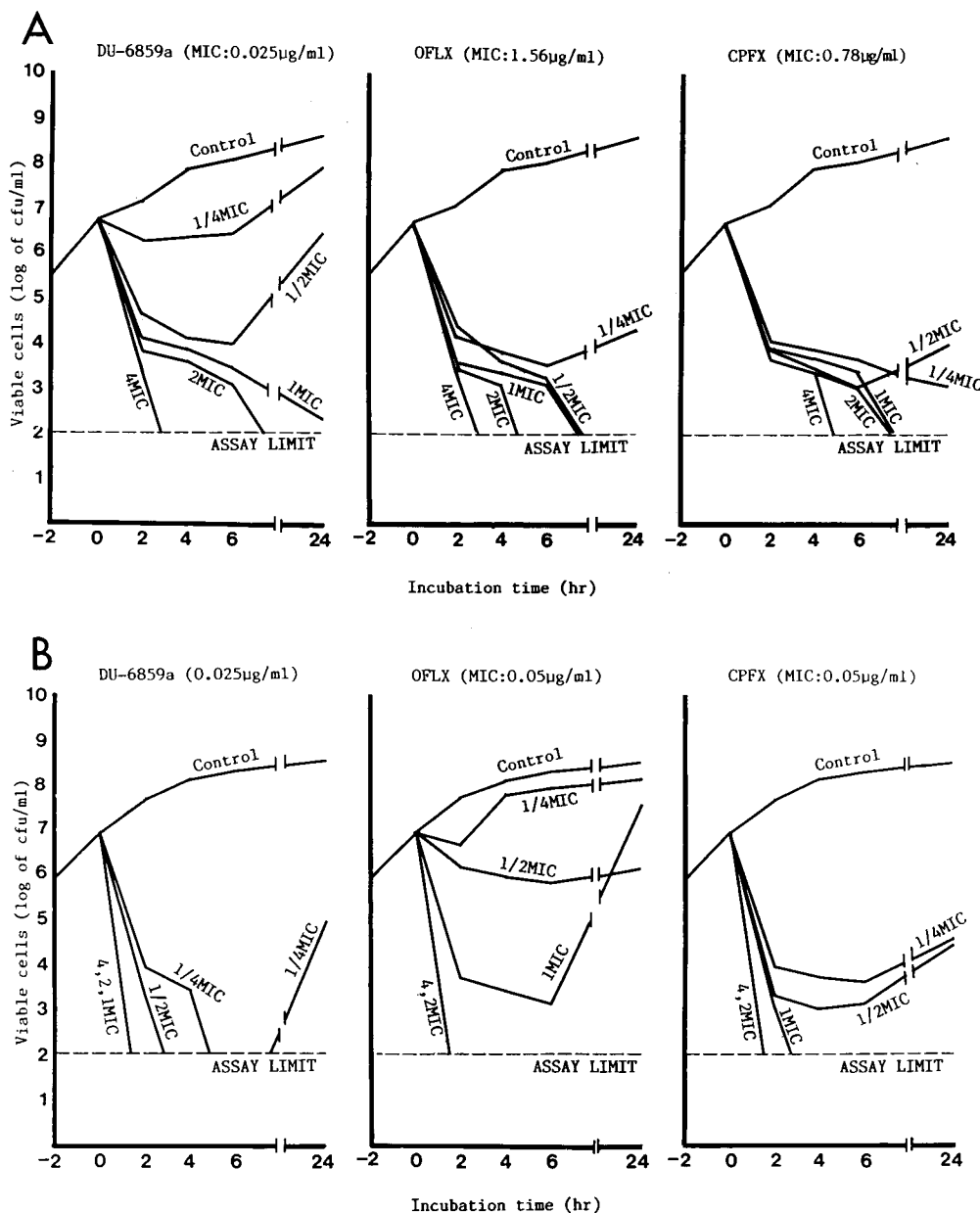


FIG. 1. Bactericidal activities of DU-6859a, ofloxacin (OFLX), and ciprofloxacin (CPFX) against *S. aureus* Smith (A) and *P. aeruginosa* GN1189 (B).

rank order of the 50% inhibitory concentrations against both DNA gyrases roughly paralleled the MICs.

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