ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1982, p. 548–553
0066-4804/82/100548-06$02.00/0

Vol. 22, No. 4

In Vitro and In Vivo Activity of DL-8280, a New Oxazine Derivative

KENICHI SATO,[1]* YOSHIHARU MATSUURA,[1] MATSUHISA INOUE,[1] TSUTOMU UNE,[2] YASUAKI OSADA,[2] HIDEMASA OGAWA,[2] AND SUSUMU MITSUHASHI[1]

Department of Microbiology, School of Medicine, Gunma University, Maebashi, Japan,[1] and Research Institute, Daiichi Seiyaku Co., Ltd., Tokyo, Japan[2]

Received 3 May 1982/Accepted 26 July 1982

DL-8280, 9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido-(1,2,3-de)1,4-benzoxazine-6-carboxylic acid, is a new nalidixic acid analog with a broad spectrum of antibacterial activity against gram-negative and gram-positive bacteria, including obligate anaerobes. The activity of DL-8280 against *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus influenzae, Neisseria gonorrhoeae*, and *Clostridium perfringens* was roughly comparable to that of norfloxacin and far exceeded that of pipemidic acid and nalidixic acid. DL-8280 had greater activity against *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas maltophilia, Acinetobacter* spp., and *Bacteroides fragilis* than did norfloxacin, pipemidic acid, and nalidixic acid. Nalidixic acid-resistant *Enterobacteriaceae*, ampicillin-resistant gonococci, and clindamycin-resistant obligate anaerobes were also susceptible to DL-8280. The activity of DL-8280 was affected very little by inoculum size, and its action was bactericidal at two times the minimal inhibitory concentrations at most. Administered orally to mice experimentally infected with *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Proteus mirabilis, Serratia marcescens*, or *P. aeruginosa*, DL-8280 was 2 to 7 times more effective than norfloxacin and 7 to more than 50 times more active than pipemidic acid.

Since nalidixic acid was introduced into practical use for treatment of bacterial infections, many analogs have been developed. Oxolinic acid (13), piromidic acid (11), and cinoxacin (7), like nalidixic acid (1), are active against gram-negative bacteria but inactive against gram-positive bacteria and *Pseudomonas aeruginosa*, an etiological agent productive of infections that are especially difficult to control with available antibiotics (2, 10). Pipemidic acid (12) is active against *P. aeruginosa*, as are miloxacin (8), rosoxacin (4, 9), and AM-715 (later designated norfloxacin) (3–5). Against gram-negative bacteria including *P. aeruginosa*, norfloxacin has greater activity than either pipemidic acid or miloxacin (3). In addition, norfloxacin is highly active against most gram-positive bacteria (3, 4). DL-8280, a more recently developed oxazine derivative, has greater activity and a broader spectrum of activity than norfloxacin. This paper gives the results of a study of the in vitro and in vivo antibacterial activities of DL-8280.

MATERIALS AND METHODS

Drugs. DL-8280 (Fig. 1) was synthesized at the Research Institute, Daiichi Seiyaku Co., Ltd., Tokyo, Japan, as were nalidixic acid, pipemidic acid, and norfloxacin. Ampicillin sodium was purchased from Meiji Seika Co., Ltd., Tokyo, Japan, and clindamycin hydrochloride was purchased from Japan Upjohn Co., Ltd., Tokyo, Japan.

Test strains. Standard strains and clinical isolates were acquired from the Laboratory of Drug Resistance of Bacteria, Gunma University, Maebashi, Japan, and the Research Institute, Daiichi Seiyaku Co., Ltd.

Determination of MICs. Minimal inhibitory concentrations (MICs) were determined by the twofold agar dilution method. The media used are listed in Table 1. An overnight broth culture or gonococcal suspension in proteose peptone no. 3 broth (Difco Laboratories, Detroit, Mich.) was adjusted to the density of a 0.5

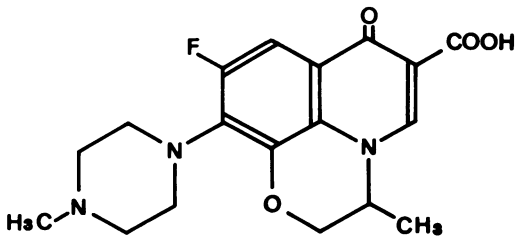

FIG. 1. Chemical structure of DL-8280, 9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido-(1,2,3-de)1,4-benzoxazine-6-carboxylic acid.

TABLE 1. Media used for preculture and drug susceptibility measurements

Media[a]	Organism
For preculture	
BHIB	*Streptococcus* spp.
BHIB + hemin (10 μg/ml) + β-NAD (2 μg/ml)	*H. influenzae*
GCA + 1% hemoglobin + 1% IsoVitaleX	*N. gonorrhoeae*
GAMB	Obligate anaerobes
MHB	Other organisms
For MIC determination	
Chocolate agar	*Streptococcus* spp. *H. influenzae*
GCA + 1% hemoglobin + 1% IsoVitaleX	*N. gonorrhoeae*
GAMA	Obligate anaerobes
MHA	Other organisms

[a] Abbreviations: BHIB, brain heart infusion broth (Difco); β-NAD, β-nicotinamide adenine dinucleotide (Sigma Chemical Co.); GCA, GC agar (Difco); GAMB, GAM broth (Nissui); MHB, Mueller-Hinton broth (Difco); chocolate agar, Mueller-Hinton agar (Difco) + 10% defibrinated horse blood with heating; GAMA, GAM agar (Nissui); MHA, Mueller-Hinton agar (Difco).

McFarland standard (about 10^8 cells per ml) and then diluted to 10^{-2}. One loopful (5 μl) of the diluted culture, corresponding to about 10^3 cells, was inoculated onto 10-ml drug-containing agar layers in petri dishes. Bacterial growth was observed after 18 h of incubation at 37°C, except for obligate anaerobes, which were incubated for 48 h. *Haemophilus influenzae* and *Neisseria gonorrhoeae* were incubated in a candle jar, and obligate anaerobes were incubated in an anaerobic glove box. The MIC was the lowest drug concentration which prevented visible growth of bacteria.

Determination of MBCs. Minimal bactericidal concentrations (MBCs) were determined by the twofold broth dilution method with a Dynatech MIC2000 system. Volumes (1.5 μl each) of the diluted and undiluted cultures described above, containing 10^3 or 10^5 cells, were inoculated into 0.1 ml of drug-containing Mueller-Hinton broth in microtiter wells. The inoculum sizes were 10^4 and 10^6 cells per ml (final concentration), respectively. After 18 h of incubation at 37°C, the lowest drug concentration that allowed no visible growth was defined as the MIC. The MBC was defined as the lowest concentration that yielded no colony formation after subculture on drug-free Mueller-Hinton agar plates overnight at 37°C after 18 h of exposure to drugs.

In vivo test. In vivo activities were determined against systemic infections in mice. Ten male STD: ddY mice weighing 20 to 25 g were used for each dose level. An overnight culture on heart infusion agar was suspended in physiological saline or in 5% gastric mucin. A 0.2-ml volume of a bacterial suspension, corresponding to 2 or 10 times higher than the minimal lethal dose, was inoculated intraperitoneally. Immediately after infection, mice were treated orally with a

TABLE 2. Antibacterial spectra of DL-8280, norfloxacin, pipemidic acid, and nalidixic acid[a]

Organism	MIC (μg/ml)			
	DL-8280	Norfloxacin	Pipemidic acid	Nalidixic acid
Staphylococcus aureus Smith	0.19	0.39	12.5	25
Staphylococcus epidermidis 56500	0.78	3.13	100	>100
Streptococcus pyogenes Cook	1.56	3.13	100	>100
Streptococcus mitis IID 685	3.13	6.25	100	>100
Streptococcus faecalis ATCC 19433	3.13	6.25	>100	>100
Escherichia coli NIH JC-2	0.05	0.05	1.56	6.25
Shigella flexneri 2a 5503	≦0.025	0.10	1.56	6.25
Klebsiella pneumoniae PCI 600	≦0.025	≦0.025	1.56	3.13
Salmonella typhi 901	≦0.025	≦0.025	1.56	12.5
Salmonella enteritidis G14	≦0.025	≦0.025	1.56	12.5
Enterobacter cloacae 963	0.10	0.10	1.56	12.5
Proteus mirabilis IFO 3849	0.19	0.10	3.13	12.5
Proteus morganii IFO 3848	0.05	≦0.025	1.56	0.78
Serratia marcescens IAM 1184	0.19	0.10	1.56	6.25
Pseudomonas aeruginosa NCTC 10490	0.78	0.78	25	>100
Pseudomonas maltophilia IID 1275	1.56	12.5	50	25
Flavobacterium meningosepticum ATCC 13253	1.56	12.5	100	12.5
Acinetobacter calcoaceticus ATCC 19606	0.78	25	>100	50
Alcaligenes faecalis ATCC 19018	0.78	3.13	12.5	3.13
Haemophilus influenzae ATCC 9332	0.05	0.10	3.13	3.13
Neisseria gonorrhoeae IID 844	≦0.025	≦0.025	1.56	1.56
Bacteroides fragilis NCTC 9343	1.56	100	>100	>100
Fusobacterium symbiosum ATCC 14940	1.56	100	>100	>100
Clostridium perfringens 22	3.13	3.13	50	>100
Peptococcus asaccharolyticus VPI 5045	1.56	12.5	100	>100

[a] Agar dilution method; 10^3 cells.

TABLE 3. Antibacterial activities of DL-8280, norfloxacin, pipemidic acid, nalidixic acid, ampicillin, and clindamycin against fresh clinical isolates[a]

Organism (no. of strains)	Drug	MIC (μg/ml)		
		Range	For 50% of isolates	For 90% of isolates
S. aureus (100)	DL-8280	0.10–0.78	0.39	0.39
	Norfloxacin	0.19–6.25	1.56	3.13
	Pipemidic acid	3.13–100	50	100
	Nalidixic acid	12.5–100	25	50
S. epidermidis (50)	DL-8280	0.19–1.56	0.39	0.78
	Norfloxacin	1.56–12.5	1.56	3.13
	Pipemidic acid	12.5–>100	25	50
	Nalidixic acid	25–>100	100	>100
S. pyogenes (99)	DL-8280	0.78–3.13	1.56	3.13
	Norfloxacin	0.78–50	3.13	12.5
	Pipemidic acid	>100	>100	>100
	Nalidixic acid	>100	>100	>100
S. faecalis (50)	DL-8280	0.39–3.13	0.78	1.56
	Norfloxacin	1.56–6.25	3.13	6.25
	Pipemidic acid	>100	>100	>100
	Nalidixic acid	>100	>100	>100
E. coli (100)	DL-8280	≦0.025–1.56	0.05	0.19
	Norfloxacin	≦0.025–0.78	0.05	0.19
	Pipemidic acid	0.39–50	1.56	3.13
	Nalidixic acid	0.39–100	3.13	6.25
K. pneumoniae (100)	DL-8280	≦0.025–1.56	0.10	0.19
	Norfloxacin	≦0.025–1.56	0.10	0.19
	Pipemidic acid	0.78–50	3.13	3.13
	Nalidixic acid	0.78–100	3.13	12.5
C. freundii (99)	DL-8280	≦0.025–25	0.10	3.13
	Norfloxacin	≦0.025–100	0.10	3.13
	Pipemidic acid	0.78–>100	1.56	50
	Nalidixic acid	0.39–>100	6.25	>100
E. cloacae (100)	DL-8280	≦0.025–25	0.10	0.78
	Norfloxacin	≦0.025–50	0.10	0.78
	Pipemidic acid	0.78–>100	1.56	12.5
	Nalidixic acid	1.56–>100	3.13	100
P. mirabilis (98)	DL-8280	≦0.025–0.78	0.10	0.19
	Norfloxacin	≦0.025–0.39	0.05	0.19
	Pipemidic acid	0.78–12.5	3.13	3.13
	Nalidixic acid	3.13–>100	3.13	6.25
Indole-positive *Proteus* spp. (181)[b]	DL-8280	0.05–3.13	0.10	0.78
	Norfloxacin	≦0.025–3.13	0.05	0.39
	Pipemidic acid	0.78–50	1.56	6.25
	Nalidixic acid	0.39–>100	3.13	50
S. marcescens (100)	DL-8280	≦0.025–12.5	0.39	6.25
	Norfloxacin	≦0.025–100	0.78	12.5
	Pipemidic acid	0.39–>100	6.25	>100
	Nalidixic acid	0.19–>100	6.25	>100
Nalidixic acid-resistant *Enterobacteriaceae* (91)[c]	DL-8280	0.10–25	1.56	12.5
	Norfloxacin	0.10–100	1.56	25
	Pipemidic acid	3.13–>100	25	>100
	Nalidixic acid	100–>100	>100	>100
P. aeruginosa (100)	DL-8280	0.19–6.25	0.78	3.13
	Norfloxacin	0.19–6.25	0.78	1.56
	Pipemidic acid	3.13–100	12.5	50
	Nalidixic acid	25–>100	>100	>100
P. maltophilia (50)	DL-8280	0.78–6.25	3.13	6.25
	Norfloxacin	6.25–>100	25	100
	Pipemidic acid	50–>100	100	>100
	Nalidixic acid	6.25–100	25	50
Acinetobacter spp. (48)	DL-8280	0.10–3.13	0.39	0.78
	Norfloxacin	0.78–50	1.56	6.25
	Pipemidic acid	3.13–>100	50	>100
	Nalidixic acid	3.13–100	6.25	25

TABLE 3—*Continued*

Organism (no. of strains)	Drug	MIC (μg/ml)		
		Range	For 50% of isolates	For 90% of isolates
H. influenzae (20)	DL-8280	≦0.05–1.56	0.39	1.56
	Norfloxacin	≦0.05–3.13	0.39	1.56
	Pipemidic acid	1.56–50	6.25	25
	Nalidixic acid	1.56–25	3.13	12.5
N. gonorrhoeae (48)	DL-8280	≦0.025–0.05	≦0.025	0.05
	Norfloxacin	≦0.025–0.10	0.05	0.10
	Pipemidic acid	≦0.025–3.13	0.78	1.56
	Nalidixic acid	0.78–3.13	1.56	3.13
	Ampicillin	≦0.025–25	0.19	0.78
B. fragilis (42)	DL-8280	0.78–12.5	1.56	6.25
	Norfloxacin	12.5–>100	25	>100
	Pipemidic acid	50–>100	100	>100
	Nalidixic acid	100–>100	>100	>100
	Clindamycin	≦0.05–>100	0.10	>100
C. perfringens (17)	DL-8280	0.39–12.5	0.39	0.78
	Norfloxacin	0.78–12.5	1.56	1.56
	Pipemidic acid	12.5–50	25	50
	Nalidixic acid	12.5–>100	25	100
	Clindamycin	≦0.05–>100	0.78	25

[a] Agar dilution method; 10^3 cells.
[b] 50 *P. morganii*, 50 *P. rettgeri*, and 81 *P. vulgaris* strains.
[c] 5 *E. coli*, 13 *E. cloacae*, 36 *S. marcescens*, 2 *K. pneumoniae*, 3 *P. mirabilis*, 3 *P. morganii*, 7 *P. rettgeri*, and 22 *C. freundii* strains.

single-dose regimen of DL-8280, norfloxacin, or pipemidic acid by using intragastric tubes. 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 (6).

RESULTS

Antibacterial spectrum. The antibacterial spectra of DL-8280, norfloxacin, pipemidic acid, and nalidixic acid are shown in Table 2. DL-8280 exhibited a broad spectrum of activity. Gram-positive bacteria such as *Staphylococcus* spp. and *Streptococcus* spp., most of which were resistant to pipemidic acid and nalidixic acid, were susceptible to DL-8280 and norfloxacin. Gram-negative bacteria such as various species of *Enterobacteriaceae*, *P. aeruginosa*, *H. influenzae*, and *N. gonorrhoeae* proved to be re-

TABLE 4. Influence of inoculum size on bactericidal activities of DL-8280 and norfloxacin against fresh clinical isolates[a]

Organism (no. of strains)	Drug	Inoculum size (cells/ml)	MIC (μg/ml)		MBC (μg/ml)	
			Range	For 90% of isolates	Range	For 90% of isolates
E. coli (50)	DL-8280	10^4	≦0.05–1.56	0.39	≦0.05–3.13	0.39
		10^6	≦0.05–1.56	0.39	≦0.05–3.13	0.39
	Norfloxacin	10^4	≦0.05–1.56	0.39	≦0.05–6.25	0.39
		10^6	≦0.05–3.13	0.78	≦0.05–6.25	0.78
K. pneumoniae (50)	DL-8280	10^4	0.10–1.56	0.39	0.10–1.56	0.78
		10^6	0.10–1.56	0.78	0.10–1.56	0.78
	Norfloxacin	10^4	≦0.05–1.56	0.39	0.10–1.56	0.39
		10^6	0.10–1.56	0.78	0.10–1.56	0.78
P. aeruginosa (50)	DL-8280	10^4	0.10–6.25	1.56	0.10–12.5	1.56
		10^6	0.19–12.5	1.56	0.39–12.5	3.13
	Norfloxacin	10^4	0.10–6.25	0.78	0.39–25	6.25
		10^6	0.10–12.5	0.78	0.78–50	6.25
S. aureus (50)	DL-8280	10^4	0.19–0.39	0.39	0.19–0.78	0.39
		10^6	0.19–0.78	0.39	0.39–1.56	0.78
	Norfloxacin	10^4	0.39–3.13	1.56	0.78–25	3.13
		10^6	0.78–6.25	3.13	0.78–25	6.25

[a] Broth dilution method.

TABLE 5. Protective effect of DL-8280, norfloxacin, and pipemidic acid on systemic infections in mice

Organism	Inoculum size[a]	Drug[b]	MIC (μg/ml)	50% effective dose (mg/kg)	95% confidence limit
S. aureus E46	2.6×10^8 cells (2 × MLD)	DL-8280	0.39	10.3	7.6–13.8
		Norfloxacin	1.56	61.7	48.4–78.6
		Pipemidic acid	50	>500	
S. pyogenes G36	2.7×10^7 cells (2 × MLD)	DL-8280	1.56	75.1	48.5–123
		Norfloxacin	6.25	>500	
		Pipemidic acid	>100	>500	
E. coli ML4707	6.3×10^7 cells (10 × MLD)	DL-8280	0.05	0.7	0.4–1.3
		Norfloxacin	0.05	1.5	0.9–2.6
		Pipemidic acid	1.56	21.9	12.9–37.0
P. mirabilis GN4757	4.5×10^6 cells (2 × MLD)	DL-8280	0.19	1.9	1.0–3.5
		Norfloxacin	0.39	8.3	5.9–11.7
		Pipemidic acid	6.25	80.0	56.7–113
S. marcescens 13001	8.3×10^7 cells (2 × MLD)	DL-8280	0.10	1.2	0.5–3.5
		Norfloxacin	0.05	5.7	3.1–10.4
		Pipemidic acid	1.56	23.4	16.3–33.5
P. aeruginosa GN11189	6.0×10^6 cells (10 × MLD)	DL-8280	1.56	27.5	17.2–44.2
		Norfloxacin	0.78	63.1	40.2–99.0
		Pipemidic acid	12.5	>200	

[a] Administered intraperitoneally without gastric mucin, except *P. mirabilis* and *P. aeruginosa*, which were administered with gastric mucin. MLD, Minimal lethal dose.
[b] Single oral regimen immediately after infection.

markably susceptible to DL-8280 as well as norfloxacin, but less susceptible to pipemidic acid and nalidixic acid. Glucose-nonfermenting gram-negative bacteria other than *P. aeruginosa*, such as *Pseudomonas maltophilia, Flavobacterium meningosepticum, Acinetobacter calcoaceticus*, and *Alcaligenes faecalis*, and obligate anaerobes such as *Bacteroides fragilis, Fusobacterium symbiosum*, and *Peptococcus asaccharolyticus*, were also susceptible to DL-8280; they were less susceptible or resistant to the reference drugs, including norfloxacin. All of the test strains were inhibited by 3.13 μg or less of DL-8280 per ml.

Antibacterial activity. The antibacterial activities of DL-8280, norfloxacin, pipemidic and nalidixic acids against fresh clinical isolates are shown in Table 3. The minimum concentration at which 90% of isolates were inhibited (MIC_{90}) of DL-8280 for *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, and *Streptococcus faecalis* ranged from 0.39 to 3.13 μg per ml. At this performance level, DL-8280 was 4 to 8 times more active than norfloxacin and 32 to 256 times more active than pipemidic and nalidixic acids.

Against various species of *Enterobacteriaceae*, the activity of DL-8280 was equal to or slightly greater than that of norfloxacin. DL-8280 inhibited most isolates of *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis*, and indole-positive *Proteus* spp. at a concentration of 0.78 μg/ml or less. *Citrobacter freundii* and *Serratia marcescens* were less susceptible than the other species of *Enterobacteriaceae* to DL-8280. All the nalidixic acid-resistant isolates of *Enterobacteriaceae* tested were susceptible to DL-8280, but some of them were resistant to norfloxacin and pipemidic acid.

At the MIC_{90} level, DL-8280 was slightly less active than norfloxacin but more active than pipemidic acid against *P. aeruginosa*. The activity of DL-8280 against *P. maltophilia* and *Acinetobacter* spp. was the greatest among the drugs tested, with MIC_{90}s of 0.78 μg/ml or less for each species tested.

N. gonorrhoeae strains, including those resistant to ampicillin, were extremely susceptible to DL-8280; the MIC_{90} was 0.05 μg/ml. *H. influenzae* was also susceptible to the compound, with an MIC_{90} of 1.56 μg/ml.

At the MIC_{90} level, DL-8280 was more than 16 times as active as norfloxacin against *B. fragilis* and twice as active as norfloxacin against *Clostridium perfringens*. DL-8280 was also active against clindamycin-resistant isolates of both species.

Bactericidal activity. The comparative bactericidal activities of DL-8280 and norfloxacin are shown in Table 4. The minimum concentrations at which 90% of isolates were killed (MBC_{90}s) of both DL-8280 and norfloxacin against *E. coli, K. pneumoniae*, and *S. aureus* were at most twice the MIC_{90}s. The MBC_{90}s and MIC_{90}s of DL-8280 against *P. aeruginosa* were essentially

identical, whereas the MBC_{90}s of norfloxacin were four to eight times higher than the MIC_{90}s. The effects of inoculum size on the activities of DL-8280 and norfloxacin were small. The MIC_{90}s and MBC_{90}s of both compounds were either unchanged or doubled when the inoculum was increased from 10^4 to 10^6 cells per ml.

In vivo antibacterial activity. The protective effects of DL-8280 on systemic infections in mice are shown in Table 5. The 50% effective doses of DL-8280 against *S. aureus* E46 and *S. pyogenes* G36 infections were 10.3 and 75.1 mg/kg, respectively. DL-8280 displayed about six or seven times greater activity than norfloxacin. The activities of DL-8280 against *E. coli* ML4707, *P. mirabilis* GN4757, *S. marcescens* 13001, and *P. aeruginosa* GN11189 infections were 2 to 4 times greater than those of norfloxacin and 7 to 50 times the activities of pipemidic acid.

DISCUSSION

DL-8280 showed excellent activities, comparable to those of norfloxacin, against gram-negative aerobes including *N. gonorrhoeae*. Its activities against gram-positive aerobes were 4 to 16 times greater than those of norfloxacin. It was also active against obligate anaerobes. DL-8280 was also highly active against *P. aeruginosa*, nalidixic acid-resistant *Enterobacteriaceae*, ampicillin-resistant *N. gonorrhoeae*, and clindamycin-resistant obligate anaerobes.

DL-8280 was well absorbed after oral administration to human volunteers and was excreted in the urine as active compound. At the doses used in this study, DL-8280 did not provoke significant side effects (Y. Osada, M. Tsumura, H. Tachizawa, T. Une, and M. Sano, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21th, Chicago, Ill., abstr. no. 562, 1981). Thus it is reasonable to expect that this oxazine derivative would be therapeutically useful not only in the treatment of urinary tract infections and gonorrhoeae, but also of systemic infections. Clinical studies on DL-8280 are in progress.

LITERATURE CITED

1. **Deitz, W. H., J. H. Bailey, and E. J. Froelich.** 1964. In vitro antibacterial properties of nalidixic acid, a new drug active against gram-negative organisms, p. 583–587. Antimicrob. Agents Chemother. 1963.
2. **Finland, M.** 1970. Changing ecology of bacterial infections as related to antibacterial therapy. J. Infect. Dis. **122**:419–431.
3. **Ito, A., K. Hirai, M. Inoue, H. Koga, S. Suzue, T. Irikura, and S. Mitsuhashi.** 1980. In vitro antibacterial activity of AM-715, a new nalidixic acid analog. Antimicrob. Agents Chemother. **17**:103–108.
4. **Khan, M. Y., Y. Siddiqui, and R. P. Gruninger.** 1981. Comparative in vitro activity of MK-0366 and other selected oral antimicrobial agents against *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **20**:265–266.
5. **King, A., C. Warren, K. Shannon, and I. Phillips.** 1982. In vitro antibacterial activity of norfloxacin (MK-0366). Antimicrob. Agents Chemother. **21**:604–607.
6. **Litchfield, J. T., and F. Wilcoxon.** 1948. A simplified method of evaluating dose-effect experiments. J. Pharmacol. **92**:99–113.
7. **Lumish, R. M., and C. W. Norden.** 1975. Cinoxacin: in vitro antibacterial studies of a new synthetic organic acid. Antimicrob. Agents Chemother. **7**:159–163.
8. **Nagate, T., S. Kurashige, and S. Mitsuhashi.** 1980. In vitro and in vivo antibacterial activity of AB206, a new naphthyridine derivative. Antimicrob. Agents Chemother. **17**:203–208.
9. **O'Connor, J. R., R. A. Dobson, P. E. Came, and R. B. Wagner.** 1980. Rosoxacin, a new synthetic agent for the treatment of systemic gram-negative infections, p. 440–442. *In* J. D. Nelson, and C. Grassi (ed.), Current chemotherapy and infectious diseases. American Society for Microbiology, Washington, D.C.
10. **Reynolds, H. Y., A. S. Levine, R. E. Wood, C. H. Zierdt, D. C. Dale, and J. E. Pennington.** 1975. Pseudomonas infections: persisting problems and current research to find new therapies. Ann. Intern. Med. **82**:819–831.
11. **Shimizu, M., S. Nakamura, and Y. Takase.** 1971. Piromidic acid, a new antibacterial agent: antibacterial properties, p. 117–122. Antimicrob. Agents Chemother. 1970.
12. **Shimizu, M., Y. Takase, S. Nakamura, H. Katae, A. Minami, K. Nakata, S. Inoue, M. Ishiyama, and Y. Kubo.** 1975. Pipemidic acid, a new antibacterial agent active against *Pseudomonas aeruginosa*: in vitro properties. Antimicrob. Agents Chemother. **8**:132–138.
13. **Turner, F. J., S. M. Ringel, J. F. Martin, P. J. Storino, J. M. Daly, and B. S. Schwartz.** 1968. Oxolinic acid, a new synthetic antimicrobial agent. I. In vitro and in vivo activity, p. 475–479. Antimicrob. Agents Chemother. 1967.