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In Vitro and In Vivo Antibacterial Activities of E-4497, a New 3-Amine-3-Methyl-Azetidinyl Tricyclic Fluoroquinolone

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The in vitro and in vivo antibacterial activities of a new tricyclic fluoroquinolone, E-4497 [*S*(−)-9-fluoro-3-methyl-10-(3-amine-3-methyl-azetidin-1-yl)-7-oxo-2,3-dihydro-*7H*-pyrido-(1,2,3-*de*)-1,4-benzoxazine-6-carboxylic acid], were evaluated in comparison with those of DR-3355 [*S*-(−)-ofloxacin], norfloxacin, and ciprofloxacin. E-4497 was more potent than norfloxacin and as potent as or more potent than DR-3355 and ciprofloxacin against *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus faecalis*. With the exception of *Providencia* spp., E-4497 inhibited 90% of the *Enterobacteriaceae* at ≤0.25 μg/ml. Against enteric bacteria, E-4497 was similar in potency to norfloxacin but less potent than DR-3355 and ciprofloxacin. For *Pseudomonas aeruginosa*, the MICs of E-4497, DR-3355, norfloxacin, and ciprofloxacin for 90% of strains were 2, 2, 4, and 0.5 μg/ml, respectively. Against *Clostridium perfringens* and *Bacteroides fragilis*, E-4497 (MICs for 90% of strains, 2 and 8 μg/ml, respectively) was two- to fourfold more active than norfloxacin and ciprofloxacin. E-4497 activity decreased moderately in the presence of 10 mM Mg^{2+}. Urine at pH 5.5 caused a significant decrease in activity compared with urine at pH 7.2. However, the presence of serum either had no effect or increased the activity of E-4497. In general, E-4497 was bactericidal at the MIC. In systemic infections with *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* in mice, the protective effect of E-4497 was generally greater than that of norfloxacin and comparable to those of DR-3355 and ciprofloxacin.

In recent years many chemical modifications of the quinolone nucleus have been attempted in order to obtain more-active compounds (1, 16, 23). Recently, quinolone antibacterial agents with a broad spectrum of activity, such as norfloxacin, ciprofloxacin, and ofloxacin, have been developed and proven efficacious in the therapy of bacterial infections (24). Nevertheless, most of the new fluoroquinolones have moderate activity against gram-positive and anaerobic bacteria, with MICs approaching or exceeding achievable levels in blood (20). The search for quinolone agents with greater activity against staphylococci, streptococci, enterococci, and anaerobes has been the subject of numerous studies (2–6, 10, 17, 22, 26).

E-4497, *S*(−)-9-fluoro-3-methyl-10-(3-amine-3-methyl-azetidin-1-yl)-7-oxo-2,3-dihydro-*7H*-pyrido-(1,2,3-*de*)-1,4-benzoxazine-6-carboxylic acid (Fig. 1), is a new tricyclic fluoroquinolone similar in structure to DR-3355 [*S*-(−)-ofloxacin] (19) but differing in the 3-amine-3-methyl-azetidin-1-yl substitution at position 10 of the quinolone nucleus.

In this study, we compare the in vitro and in vivo antibacterial activities of E-4497 with those of DR-3355, norfloxacin, and ciprofloxacin.

(This work was presented in part at the 7th Mediterranean Congress of Chemotherapy, Barcelona, Spain, 1990 [13a].)

MATERIALS AND METHODS

Antibacterial agents. E-4497 and DR-3355 were synthesized at the Laboratorios Esteve S.A. (Barcelona, Spain). Norfloxacin was obtained from Chemo-Iberica S.A. (Barcelona, Spain), and ciprofloxacin was provided by Bayer A.G. (Wuppertal, Federal Republic of Germany). For determining the MICs, stock solutions at a concentration of 1.6 mg of quinolone base per ml were prepared in 0.1 N NaOH. For in vivo tests, antibacterial agents were dissolved in 0.1 N NaOH, diluted with sterile water appropriately, and finally mixed in 0.1% carboxymethyl cellulose. The antibiotic doses were expressed as milligrams of base per kilogram.

Organisms. The organisms used in this study were recent clinical isolates randomly obtained from various hospitals of broad geographical distribution in Spain (only one isolate was collected from each patient).

In vitro activity. Growth-inhibitory activity was determined on liquid or solid medium by the antibiotic twofold serial dilution technique. For aerobic and facultatively anaerobic organisms, a MIC 2000 Dynatech microdilution system (Dynatech A.G., Denkendorf, Federal Republic of Germany) was used to prepare broth microdilution panels containing twofold dilutions of antibacterial agent in 0.2 ml of Mueller-Hinton (MH) broth (Oxoid Ltd., Basingstoke, England). Panels were inoculated with each test organism to yield a final inoculum of 10^5 CFU per well (5×10^5 CFU/ml). The MIC in liquid medium was defined as the lowest concentration of antibacterial agent that inhibited development of visible growth after 18 h of incubation at 37°C. MBCs, defined as the lowest antibiotic concentration that killed ≥99.9% of the initial inoculum, were determined by subculturing 0.01 and 0.1 ml of broth from the drug-free control well, the first well containing growth, and each clear well on MH agar (Oxoid Ltd.) plates. For *Streptococcus* spp., MH medium was supplemented with 5% horse blood (Oxoid Ltd.).

Anaerobic bacteria were tested by the agar dilution method on Wilkins-Chalgren medium (Oxoid Ltd.) at 35°C

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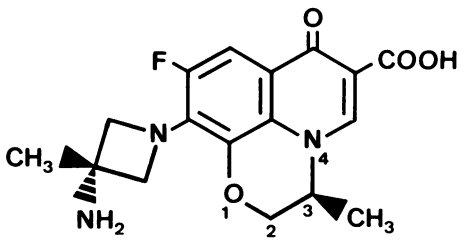

FIG. 1. Chemical structure of E-4497.

for 48 h in anaerobic GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) (14). The plates were inoculated with a Steers-type multipoint inoculator (18), which deposited approximately 10^4 CFU on the agar surface. Two plates of test medium without antibacterial agents were also incubated with bacteria. One was incubated anaerobically to serve as a growth control, and the other was incubated aerobically to detect possible aerobic contamination. The MIC on solid medium was defined as the lowest antibacterial agent concentration that inhibited development of visible growth on agar.

Organisms used as controls included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Clostridium perfringens* ATCC 13124, and *Bacteroides fragilis* ATCC 25285.

Factors affecting in vitro activity. The effects of magnesium concentration, serum, and urine on the in vitro activity of E-4497 against *Staphylococcus aureus* HS-93, *Escherichia coli* HM-42, *Salmonella enteritidis* HS-41, *Klebsiella pneumoniae* HS-28, *Citrobacter freundii* HS-50, *Enterobacter aerogenes* HS-107, *Serratia marcescens* HM-20, *Proteus vulgaris* HS-48, and *Pseudomonas aeruginosa* HS-116 were determined as described above for aerobic and facultatively anaerobic organisms.

(i) Mg^{2+} concentration. The effect of Mg^{2+} was determined with unsupplemented MH broth and MH broth supplemented with 25, 122, or 243 μg of Mg^{2+} as $MgCl_2 \cdot 6H_2O$ per ml (1, 5, and 10 mM, respectively).

(ii) Serum. The effect of serum was studied with MH broth supplemented with horse serum (Oxoid Ltd.) (inactivated at 56°C for 30 min) to final concentrations of 20 or 70% (vol/vol) with the pH adjusted to 7.2. Serum-supplemented wells without antibacterial agents were used as a control.

(iii) Urine. The effect of urine on E-4497 activity was determined in early-morning pooled urine samples obtained from normal human male volunteers. The urine was adjusted to pH 5.5 or 7.2 and subsequently sterilized by passage through a 0.22-μm (pore size) membrane filter (Millipore Corp., Bedford, Mass.).

Systemic infections in mice. The organisms used to establish infections, *Staphylococcus aureus* HS-93, *Streptococcus pyogenes* HM-P152, *Escherichia coli* HM-42, and *Pseudomonas aeruginosa* HS-116, were cultured for 12 h at 37°C on MH agar, except *Streptococcus pyogenes* HM-P152, which was grown on MH agar supplemented with 5% horse blood and incubated for 18 h at 37°C. The resulting growth was washed from the plates with sterile physiological saline solution. The procedures for infection and treatment were as previously described (6). Male HC:CFLP mice weighing approximately 25 g were inoculated intraperitoneally with 0.5 ml of a bacterial suspension adjusted in physiological saline solution to yield five times the minimal lethal dose. Immediately after challenge, the mice were given a single oral administration of serial twofold doses of the test compounds. Four groups of 10 mice each were treated with each test compound at different dose levels of the antibacterial agents. The challenge inoculum was sufficient to kill 100% of untreated control mice, which died within 48 h postinfection, with the exception of mice infected with *Staphylococcus aureus* HS-93 or *Streptococcus pyogenes* HM-P152, which died within 4 days after challenge. The 50% effective dose (ED_{50}) and 95% confidence limits were calculated by probit analysis (12) and by the method of Litchfield and Wilcoxon (11), respectively, at 7 days after infection.

RESULTS

In vitro activity. The antibacterial activities of E-4497, DR-3355, norfloxacin, and ciprofloxacin against groups of clinical isolates are shown in Table 1. Against *Staphylococcus aureus*, E-4497 was as active as DR-3355, twofold more active than ciprofloxacin, and eightfold more active than norfloxacin. E-4497 was also as active as DR-3355 and ciprofloxacin and showed fourfold more activity than norfloxacin did against *Staphylococcus epidermidis*. Against *Streptococcus pneumoniae*, E-4497 was more active than all reference quinolones; E-4497 was 2-, 4-, and 16-fold more active than DR-3355, ciprofloxacin, and norfloxacin, respectively. Furthermore, E-4497 was as active as DR-3355, 4-fold more active than ciprofloxacin, and 32-fold more active than norfloxacin against oral streptococci (Table 1). E-4497 and ciprofloxacin were equally active against *Enterococcus faecalis* and showed two- and fourfold more activity than DR-3355 and norfloxacin did, respectively.

E-4497 was active against most of the species of *Enterobacteriaceae*, with MICs for 90% of the isolates tested of ≤0.25 μg/ml. The exception was the *Providencia* spp., for which the MICs of E-4497, DR-3355, norfloxacin, and ciprofloxacin for 90% of the isolates tested were 4, 4, 8, and 2 μg/ml, respectively. Against enteric bacteria, the antibacterial activities of E-4497 were comparable to those of norfloxacin, equal to or up to 4-fold lower than those of DR-3355, and 2- to 16-fold lower than those of ciprofloxacin.

E-4497 and DR-3355 were equally active against *Pseudomonas aeruginosa*, being twofold more active than norfloxacin and fourfold less active than ciprofloxacin.

Against pathogenic anaerobes such as *Clostridium perfringens* and *Bacteroides fragilis*, the activities of E-4497 were two- and fourfold higher, respectively, than those of ciprofloxacin, eightfold higher than those of norfloxacin, and twofold lower than those of DR-3355.

Factors affecting in vitro activity. The effects of magnesium concentration, serum, and urine on E-4497 activity are shown in Tables 2 and 3.

(i) Effect of magnesium concentration. Table 2 shows the effect of increasing concentration of Mg^{2+} on the MICs and MBCs of E-4497. The MICs of E-4497 in MH broth supplemented with 1 mM Mg^{2+} were unchanged, with the exception of the MIC for *Citrobacter freundii* HS-50. Minimum changes also occurred in the presence of magnesium ions at 5 mM. The activity of E-4497 was unchanged or decreased up to fourfold at 10 mM Mg^{2+} for the organisms tested. In general, there was no difference between the MICs and MBCs, or at most there was a twofold difference (Table 2).

(ii) Effect of serum. In medium containing 20 or 70% horse serum, E-4497 was two- to fourfold more active, compared with the activities observed in broth against most species tested (Table 3). An exception was *Citrobacter freundii*

TABLE 1. Antibacterial activities of E-4497 and reference fluoroquinolones

Organism (no. of isolates)	Antibacterial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus (26)	E-4497	0.06–1	0.06	0.12
	DR-3355	0.06–1	0.06	0.12
	Norfloxacin	0.25–2	0.5	1
	Ciprofloxacin	0.06–0.5	0.12	0.25
Staphylococcus epidermidis (18)	E-4497	0.015–0.12	0.06	0.12
	DR-3355	≤0.004–0.12	0.06	0.12
	Norfloxacin	0.25–16	0.25	0.5
	Ciprofloxacin	0.015–0.12	0.06	0.12
Streptococcus pneumoniae (14)	E-4497	0.25–1	0.5	0.5
	DR-3355	0.25–1	0.5	1
	Norfloxacin	0.5–8	4	8
	Ciprofloxacin	0.25–2	0.5	2
Oral streptococci[b] (22)	E-4497	0.25–4	0.5	1
	DR-3355	0.25–2	0.5	1
	Norfloxacin	1–32	8	32
	Ciprofloxacin	0.25–8	1	4
Enterococcus faecalis (25)	E-4497	0.25–4	1	1
	DR-3355	0.25–2	1	2
	Norfloxacin	2–16	2	4
	Ciprofloxacin	0.25–4	0.5	1
Escherichia coli (47)	E-4497	0.03–2	0.06	0.12
	DR-3355	0.015–1	0.03	0.03
	Norfloxacin	0.03–1	0.06	0.12
	Ciprofloxacin	≤0.004–0.5	0.008	0.015
Enterobacter cloacae (22)	E-4497	0.03–0.5	0.06	0.12
	DR-3355	0.008–0.12	0.03	0.06
	Norfloxacin	0.03–1	0.12	0.25
	Ciprofloxacin	0.008–0.25	0.015	0.03
Serratia species[c] (18)	E-4497	0.03–0.5	0.12	0.25
	DR-3355	0.015–0.25	0.06	0.12
	Norfloxacin	0.03–0.5	0.12	0.25
	Ciprofloxacin	0.008–0.12	0.06	0.06
Citrobacter freundii (21)	E-4497	0.03–0.5	0.06	0.12
	DR-3355	0.008–0.25	0.03	0.06
	Norfloxacin	0.03–1	0.06	0.5
	Ciprofloxacin	≤0.004–0.12	0.008	0.03
Klebsiella species[d] (35)	E-4497	0.015–0.25	0.06	0.12
	DR-3355	0.015–0.25	0.03	0.06
	Norfloxacin	0.03–0.5	0.06	0.25
	Ciprofloxacin	0.008–0.06	0.015	0.06
Salmonella enteritidis (22)	E-4497	0.12–0.12	0.12	0.12
	DR-3355	0.03–0.06	0.06	0.06
	Norfloxacin	0.12–0.25	0.12	0.12
	Ciprofloxacin	0.015–0.03	0.015	0.015
Providencia species[e] (10)	E-4497	0.03–4	0.06	4
	DR-3355	0.015–4	0.03	4
	Norfloxacin	0.03–16	0.12	8
	Ciprofloxacin	≤0.004–2	0.008	2
Proteus species[f] (31)	E-4497	0.015–0.5	0.03	0.12
	DR-3355	0.015–0.5	0.03	0.12
	Norfloxacin	0.03–1	0.06	0.25
	Ciprofloxacin	0.004–0.25	0.015	0.03
Morganella morganii (17)	E-4497	0.03–0.12	0.06	0.06
	DR-3355	0.015–0.12	0.03	0.06

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibacterial agent	MIC (μg/ml)[a]		
		Range	50%	90%
	Norfloxacin	0.015–0.12	0.06	0.12
	Ciprofloxacin	≤0.004–0.03	0.008	0.015
Pseudomonas aeruginosa (21)	E-4497	0.25–2	0.5	2
	DR-3355	0.12–>4	0.5	2
	Norfloxacin	0.25–16	1	4
	Ciprofloxacin	0.06–0.5	0.12	0.5
Clostridium perfringens (18)	E-4497	0.06–4	1	2
	DR-3355	0.06–2	0.25	1
	Norfloxacin	0.25–32	2	16
	Ciprofloxacin	0.12–16	1	4
Bacteroides fragilis (17)	E-4497	0.5–16	4	8
	DR-3355	0.25–16	2	4
	Norfloxacin	2–64	32	64
	Ciprofloxacin	2–32	8	32

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Include 12 isolates of *Streptococcus milleri*, 4 isolates of *Streptococcus mitior*, 4 isolates of *Streptococcus sanguis*, and 2 isolates of *Streptococcus salivarius*.
[c] Include 13 isolates of *Serratia marcescens* and 5 isolates of *Serratia liquefaciens*.
[d] Include 23 isolates of *Klebsiella pneumoniae* and 12 isolates of *Klebsiella oxytoca*.
[e] Include seven isolates of *Providencia stuartii*, two isolates of *Providencia rettgeri*, and one isolate of *Providencia alcalifaciens*.
[f] Include 21 isolates of *Proteus mirabilis* and 10 isolates of *Proteus vulgaris*.

HS-50, for which the MIC and MBC were not affected by the presence of serum. In general, there was a proportional decrease of MICs and MBCs; however, against *Staphylococcus aureus* HS-93 and *Pseudomonas aeruginosa* HS-116 there was an up to fourfold difference between both parameters (Table 3).

(iii) Effect of urine. The effect of urine on the in vitro activity of E-4497 is shown in Table 3. The activity of E-4497 was decreased two- to eightfold when tested in fresh urine at pH 7.2 against *Staphylococcus aureus* HS-93, *Salmonella enteritidis* HS-41, *Klebsiella pneumoniae* HS-28, *Enterobacter aerogenes* HS-107, *Serratia marcescens* HM-20, *Proteus vulgaris* HS-48, and *Pseudomonas aeruginosa* HS-116. For *Escherichia coli* HM-42 and *Citrobacter freundii* HS-50, the MICs in urine at pH 7.2 were unchanged. At a pH of 5.5, E-4497 was 4- to 32-fold less active compared with the activities in urine at pH 7.2 and 16- to 64-fold less active compared with the activities in broth. MBCs were usually equal to or twofold, but occasionally up to fourfold, higher than MICs.

Systemic infections in mice. The ability of E-4497 to protect mice against otherwise lethal systemic infections was compared with those of DR-3355, norfloxacin, and ciprofloxacin (Table 4). The ED_{50}s of E-4497 for infections with *Staphylococcus aureus* HS-93 and *Streptococcus pyogenes* HM-P152 were 23.3 and 75.3 mg/kg, respectively. The protective effect of E-4497 against these gram-positive bacterial infections was higher than that of norfloxacin and comparable to those of DR-3355 and ciprofloxacin. The protective effect of E-4497 was also comparable to those of DR-3355 and ciprofloxacin and higher than that of norfloxacin against *Pseudomonas aeruginosa* HS-116 infections. E-4497 showed a high protective effect against infections caused by *Escherichia coli* HM-42, with an ED_{50} of 5.9 mg/kg, which was comparable to that of norfloxacin but approximately twofold lower than those of DR-3355 and ciprofloxacin.

TABLE 2. Effect of Mg^{2+} on activity of E-4497

Organism	Concn (μg/ml) in:							
	MHB alone		MHB containing Mg^{2+} at:					
			1 mM		5 mM		10 mM	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus HS-93	0.12	0.25	0.12	0.12	0.12	0.25	0.12	0.5
Escherichia coli HM-42	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Salmonella enteritidis HS-41	0.12	0.12	0.12	0.12	0.12	0.12	0.25	0.25
Klebsiella pneumoniae HS-28	0.12	0.12	0.12	0.12	0.25	0.5	0.5	0.5
Citrobacter freundii HS-50	0.25	0.25	0.5	0.5	0.5	1	1	2
Enterobacter aerogenes HS-107	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5
Serratia marcescens HM-20	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5
Proteus vulgaris HS-48	0.12	0.12	0.12	0.12	0.12	0.25	0.12	0.25
Pseudomonas aeruginosa HS-116	0.5	0.5	0.5	1	1	2	2	4

TABLE 3. Effect of serum and urine on activity of E-4497

Organism	Concn (μg/ml) in:							
	Serum				Urine			
	20%		70%		pH 5.5		pH 7.2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus HS-93	0.03	0.12	0.03	0.06	4	8	0.25	0.5
Escherichia coli HM-42	0.06	0.06	0.06	0.06	4	4	0.12	0.12
Salmonella enteritidis HS-41	0.06	0.06	0.06	0.12	8	8	0.5	0.5
Klebsiella pneumoniae HS-28	0.06	0.06	0.12	0.12	8	8	1	4
Citrobacter freundii HS-50	0.25	0.25	0.25	0.25	8	8	0.25	0.5
Enterobacter aerogenes HS-107	0.12	0.12	0.12	0.12	4	8	1	2
Serratia marcescens HM-20	0.06	0.12	0.12	0.12	8	8	1	4
Proteus vulgaris HS-48	0.06	0.12	0.03	0.03	8	8	1	4
Pseudomonas aeruginosa HS-116	0.12	0.25	0.25	1	32	>32	2	4

DISCUSSION

E-4497, a new tricyclic fluoroquinolone active against staphylococci, streptococci, enterococci, enteric bacteria, *Pseudomonas aeruginosa*, and anaerobes, is an optically active *S* isomer derived from a methyl group at the C-3 position in the oxazine ring, which provides an asymmetric center at this position (Fig. 1). Ofloxacin has two optically active isomers, DR-3355 [*S*-(−)-ofloxacin] and DR-3354 [*R*-(+)-ofloxacin]. DR-3355 is 8- to 128-fold more active than DR-3354 and approximately twice as active as the 50:50 racemic mixture of ofloxacin (8, 19). Gerster et al. (7) also reported that the *S* isomer of the tricyclic quinolone analog containing a piperidine ring was much more active than the *R* isomer and about twice as active as the racemic mixture. The difference in activity between *S*-(−)-ofloxacin and *R*-(−)-ofloxacin seems to be related to their anti-DNA gyrase activity (9, 13, 25).

E-4497 has proven active against gram-positive organisms and anaerobes. Against *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus faecalis*, this new agent was similar in potency to DR-3355 and ciprofloxacin and more potent than norfloxacin. Ninety percent of the anaerobic bacteria were susceptible to ≤8 μg of E-4497 per ml. Tricyclic fluoroquinolones (E-4497 and DR-3355) seemed more potent than norfloxacin and ciprofloxacin against anaerobes. In addition, E-4497 was equal to or slightly less active than DR-3355 (as mentioned above, DR-3355 is approximately twice as active as ofloxacin) and more active than norfloxacin against *Pseudomonas aeruginosa* and most species of the *Enterobacteriaceae*.

E-4497 activity was minimally affected by Mg^{2+} concentrations similar to those found in urine. Like most fluoroquinolones, E-4497 had a significant reduction of activity in urine at pH 5.5. However, urine at pH 7.2 had much less effect on the activity of this agent. In contrast, the presence of serum generally increased the activity of E-4497. Similar findings with lomefloxacin (21) and enoxacin (15) have been previously reported by others.

TABLE 4. Protective effects of E-4497 and reference fluoroquinolones against systemic infections in mice

Organism	Challenge dose[a] (CFU/mouse)	Test compound[b]	MIC (μg/ml)	MBC (μg/ml)	ED_{50}[c] (mg/kg)	
					Dose	95% Confidence limit
Staphylococcus aureus HS-93	5.5×10^9	E-4497	0.12	0.25	23.3	13.1–41.5
		DR-3355	0.12	0.25	12.9	9.4–17.5
		Norfloxacin	1	4	>100	
		Ciprofloxacin	0.12	0.5	40.8	15.9–104.3
Streptococcus pyogenes HM-P152	2.0×10^9	E-4497	1	1	75.3	46.9–120.8
		DR-3355	0.5	0.5	46.1	16.1–120.4
		Norfloxacin	4	4	>100	
		Ciprofloxacin	0.5	0.5	>100	
Escherichia coli HM-42	2.5×10^8	E-4497	0.12	0.12	5.9	4.0–8.8
		DR-3355	0.06	0.06	2.5	1.8–3.6
		Norfloxacin	0.12	0.12	6.5	4.9–8.7
		Ciprofloxacin	0.008	0.008	3.0	2.2–4.0
Pseudomonas aeruginosa HS-116	1.5×10^7	E-4497	0.5	0.5	107.5	67.1–172.2
		DR-3355	0.5	0.5	79.8	53.8–118.3
		Norfloxacin	0.5	1	>200	
		Ciprofloxacin	0.06	0.25	75.9	17.3–332.5

[a] Mice were inoculated intraperitoneally with 0.5 ml of bacterial suspension, approximately five times the minimal lethal dose.
[b] Single oral administration immediately after bacterial challenge.
[c] Calculated by probit analysis (12); 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (11).

The in vivo antibacterial activity of E-4497 was generally greater than that of norfloxacin and similar to those of DR-3355 and ciprofloxacin. In general, there was a good correlation of the relative activities of the compounds tested when the ED_{50}s and MICs or MBCs were compared. However, the protective effect of E-4497 was also comparable to that of ciprofloxacin against infections caused by *Escherichia coli* HM-42 or *Pseudomonas aeruginosa* HS-116, which were less susceptible to E-4497 than to ciprofloxacin in vitro. This suggests that factors other than in vitro activity are responsible for the good in vivo activity of E-4497. According to pharmacokinetic studies in mice, the area under the curve achieved after a single oral administration (50 mg/kg of body weight) of E-4497 was almost fourfold greater than that of norfloxacin or ciprofloxacin and comparable to that obtained after a similar dose of DR-3355 (13a).

In summary, the potent broad-spectrum in vitro activity as well as the protective effect in treating systemic infections should encourage further studies with E-4497.

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