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In Vitro and In Vivo Antibacterial Activities of T-3761, a New Quinolone Derivative

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T-3761, a new quinolone derivative, showed broad and potent antibacterial activity. Its MICs for 90% of the strains tested were 0.20 to 100 μg/ml against gram-positive bacteria, including members of the genera *Staphylococcus*, *Streptococcus*, and *Enterococcus*; 0.025 to 3.13 μg/ml against gram-negative bacteria, including members of the family *Enterobacteriaceae* and the genus *Haemophilus*; 0.05 to 50 μg/ml against glucose nonfermenters, including members of the genera *Pseudomonas*, *Xanthomonas*, *Acinetobacter*, *Alcaligenes*, and *Moraxella*; 0.025 μg/ml against *Legionella* spp.; and 6.25 to 25 μg/ml against anaerobes, including *Bacteroides fragilis*, *Clostridium difficile*, and *Peptostreptococcus* spp. The in vitro activity of T-3761 against these clinical isolates was comparable to or 2- to 32-fold greater than those of ofloxacin and norfloxacin and 2- to 16-fold less and 1- to 8-fold greater than those of ciprofloxacin and tosufloxacin, respectively. When administered orally, T-3761 showed good efficacy in mice against systemic, pulmonary, and urinary tract infections with gram-positive and gram-negative bacteria, including quinolone-resistant *Serratia marcescens* and *Pseudomonas aeruginosa*. The in vivo activity of T-3761 was comparable to or greater than those of ofloxacin, ciprofloxacin, norfloxacin, and tosufloxacin against most infection models in mice. The activities of T-3761 were lower than those of tosufloxacin against gram-positive bacterial systemic and pulmonary infections in mice but not against infections with methicillin-resistant *Staphylococcus aureus*. The activities of T-3761 against systemic quinolone-resistant *Serratia marcescens* and *Pseudomonas aeruginosa* infections in mice were 2- to 14-fold greater than those of the reference agents.

The new quinolones in current use, such as norfloxacin (7), ofloxacin (14), ciprofloxacin (1), and tosufloxacin (2), have broad spectra of activity against gram-positive and gram-negative bacteria. These activities are characterized chemically by the presence of piperazine or pyrrolidine derivatives at the C-7 position of 4-oxoquinoline-3-carboxylic acid and 4-oxo-1,8-naphthyridine-3-carboxylic acid or the C-10 position of 7-oxopyrido-[1,2,3-de][1,4]-benzoxazine-6-carboxylic acid. T-3761, which was synthesized by Toyama Chemical Co., Ltd. (Tokyo, Japan), is a new quinolone with a unique substituent, 1-aminocyclopropyl, at the C-10 position of the 7-oxopyrido-[1,2,3-de][1,4]-benzoxazine-6-carboxylic acid. The structural formula of T-3761 is (−)-(*S*)-10-(1-aminocyclopropyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-de][1,4]-benzoxazine-6-carboxylic acid (Fig. 1).

In the study described here, the in vitro and in vivo activities of T-3761 were compared with those of ofloxacin, ciprofloxacin, norfloxacin, and tosufloxacin.

(This work was presented in part at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy [10a].)

MATERIALS AND METHODS

Antimicrobial agents. T-3761 and tosufloxacin were synthesized at the Research Laboratories, Toyama Chemical Co., Ltd. Ofloxacin, ciprofloxacin, and norfloxacin were extracted from commercially available tablets. The purities of ofloxacin, ciprofloxacin, and norfloxacin, as determined by high-performance liquid chromatography, were 99.7, 99.9, and 99.9%, respectively. The agents were dissolved in 0.1 N NaOH before dilution to the desired concentration in distilled water.

Bacterial strains. The bacterial strains used in the present study were clinical isolates obtained from various hospitals in Japan from 1977 to 1989. All organisms were identified by standard procedures at our laboratories and were stored at −135°C.

Susceptibility tests. MICs were determined by the twofold agar dilution method (15) with Mueller-Hinton agar (Eiken Chemical Co., Ltd., Tokyo, Japan) which was supplemented with 10% sheep blood to support the growth of streptococci. Chocolate agar, GAM agar (Nissui Seiyaku Co., Ltd., Tokyo, Japan), and buffered starch-yeast extract agar (13) were used for *Moraxella catarrhalis* and *Haemophilus influenzae*, obligate anaerobes, and *Legionella* spp., respectively. The inocula for most strains were grown overnight in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.). *Haemophilus influenzae*, streptococci, and obligate anaerobes were grown overnight in brain heart infusion broth (Eiken) plus 5% Fildes enrichment (Difco), brain heart infusion broth (Eiken) with 10% sheep blood, and GAM broth (Nissui), respectively. *Moraxella catarrhalis* and *Legionella* spp. were grown overnight on chocolate agar or buffered starch-yeast extract agar plates.

The bacterial colonies were removed just before use and were suspended in saline. The overnight broth cultures and bacterial suspensions of *Moraxella catarrhalis* and *Legionella* spp. were diluted in buffered saline. About 10^4 CFU was spotted onto each of the agar plates containing the compounds, and the plates were incubated for 20 h at 37°C, but the plate containing *Legionella* spp. was incubated for 48 h. *Moraxella catarrhalis* was incubated in a candle jar. Obligate anaerobes were incubated in an anaerobic cabinet (model 1024 anaerobic system; Farma Scientific Inc., Mari-

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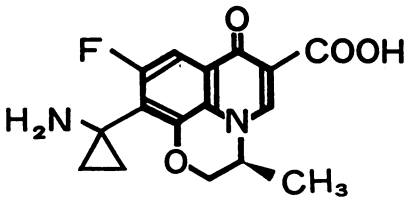

FIG. 1. Structure of T-3761.

etta, Ohio). The MIC was defined as the lowest drug concentration which prevented the visible growth of bacteria.

In vivo activities. The in vivo activities of the agents in mice were evaluated by using systemic, pulmonary, and urinary tract models of infection. Male ICR mice (age, 4 weeks; weight, 18 to 21 g; Japan SLC Inc., Shizuoka, Japan) were used in the systemic and pulmonary infection studies, and female ICR mice (age, 5 weeks; weight, 25 to 30 g; Japan SLC) were used in the urinary tract infection studies. The agents, which were suspended in 0.5% methylcellulose (Wako Chemical Co., Ltd., Tokyo, Japan), were administered orally.

(i) Systemic infection. Ten mice were used for each dosage group. Mice were challenged intraperitoneally with approximately 25- to 100-fold the 50% lethal doses of the respective organisms. These inocula, which were prepared from overnight cultures on brain heart infusion agar (Eiken) at 37°C, were suspended in 1/15 M phosphate buffer (pH 7.0) containing 5% gastric mucin (Nacalai Tesque Co., Ltd., Kyoto, Japan); *Streptococcus pneumoniae* and *Escherichia coli*, however, were suspended in brain heart infusion broth (Eiken) and saline solution, respectively. In all cases except *Streptococcus pneumoniae*, a series of doses of the agents, which were increased by twofold increments, were administered orally once at 1 h after infection (15); with *Streptococcus pneumoniae* the agents were administered twice, at 1 and 3 h after challenge. The total number of surviving mice at day 7 postchallenge was recorded, and the dose of drug that gave protection to 50% of the infected mice was determined by the method of Litchfield and Wilcoxon (9).

(ii) Pulmonary infection. Survival and eradication of the bacteria from the lungs at 7 days postinfection were used as the end points in judging therapeutic efficacy. Viable cells in the lungs were counted by plating lungs homogenized with physiological saline onto heart infusion agar (Eiken).

Pulmonary infection (n = 10 to 20 mice each) was induced by injecting intratracheally 0.03 ml of a *Streptococcus pneumoniae* D-289 suspension at a final inoculum of 2.0×10^6 CFU per lung while the mice were under ether anesthetization. The agents were administered orally twice (25 mg/kg of body weight each time), at 4 and 6 h after infection. Untreated mice died within 5 days after infection.

Pulmonary infection (n = 9 to 15 mice each) was induced by administering a nebulized suspension of *Klebsiella pneumoniae* Y-41 (final inoculum, 1.0×10^5 CFU per lung) in an aerosol exposure apparatus (Ikemoto Scientific Technology Co., Ltd., Tokyo, Japan) (4, 12). The agents were administered orally seven times (5 mg/kg each time), at 4, 22, 28, 46, 52, 70, and 76 h after challenge. Untreated mice died within 3 days after infection.

Cyclophosphamide (Shionogi Pharmaceutical Co., Ltd., Osaka, Japan) was administered intraperitoneally at 250 mg/kg and 4 days later mice were infected with *Pseudomonas aeruginosa* S-406. Pulmonary infection (n = 12 to 35 mice each) was induced by administering a nebulized bacterial suspension (final inoculum, 3.5×10^5 CFU per lung) in an aerosol exposure apparatus (Ikemoto) (4, 10). The agents were administered orally twice (25 mg/kg each time), at 4 and 6 h after infection. Untreated mice died within 2 days after infection.

(iii) Urinary tract infection. Urinary tract infections (n = 8 to 30 mice each) were induced in female mice by injecting 0.1-ml bacterial suspensions of *Proteus mirabilis* T-111, *Serratia marcescens* IID620, *Pseudomonas aeruginosa* S-68, or *Pseudomonas aeruginosa* S-429 (2.0×10^5, 2.4×10^5, 5.0×10^6, or 5.0×10^6 CFU per mouse, respectively) transurethrally (12) into their bladders, after which the distal end of the urethra was clamped for 2 h. The agents were administered orally at 25 mg/kg (12.5 mg/kg for *Serratia marcescens* IID620) twice, at 4 and 24 h (4 and 6 h for *Pseudomonas aeruginosa* S-429) after inoculation. Kidneys were removed at 48 h after inoculation, homogenized, and serially diluted in physiological saline, and aliquots were cultured onto heart infusion agar (Eiken) in order to determine the viable cell count per kidney.

RESULTS

Antibacterial activity of T-3761 against clinical isolates. The antibacterial activity of T-3761 against clinical isolates is shown in Table 1. The MICs at which 90% of the isolates were inhibited (MIC_{90}s) of T-3761 were 0.2 to 0.39 μg/ml for *Staphylococcus aureus* and *Staphylococcus epidermidis*, including methicillin-resistant strains. T-3761 was as active or was 2 to 16 times more active than ofloxacin, ciprofloxacin, and norfloxacin but was 2 to 4 times less active than tosufloxacin against these isolates. Against ofloxacin- and methicillin-resistant *Staphylococcus aureus* strains, all the agents tested had significantly weak activities (MIC_{90}s, >25 μg/ml); the MIC_{50} of T-3761 was 6.25 μg/ml, which was 2 to 16 times less than those of the other agents. The MIC_{90}s of T-3761 for streptococci and *Enterococcus faecalis* were 3.13 μg/ml, which was as active or 2 to 16 times less active than ofloxacin, ciprofloxacin, and tosufloxacin. Against various members of the family *Enterobacteriaceae*, including *Escherichia coli*, *Salmonella enteritidis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, and *Providencia rettgeri*, the MIC_{90}s of T-3761 ranged from 0.025 to 3.13 μg/ml and were roughly comparable to those of ciprofloxacin. Thus, T-3761 was two to eight times more active than ofloxacin and norfloxacin and was either two to eight times more active or slightly less active than tosufloxacin against these strains. Against nonfermenting gram-negative bacteria, the MIC_{90}s of T-3761 were comparable to or two times less than those of the reference agents against imipenem- or gentamicin-resistant *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Xanthomonas maltophilia*, and *Alcaligenes faecalis*. Against *Pseudomonas aeruginosa*, T-3761 was less active than ciprofloxacin, norfloxacin, and tosufloxacin but was more active than ofloxacin. *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Legionella* spp. were highly susceptible to T-3761, with MIC_{90}s ranging from 0.025 to 0.05 μg/ml. Against *Bacteroides fragilis*, *Clostridium difficile*, and *Peptostreptococcus* spp., the MIC_{90}s of T-3761 were 6.25, 6.25, and 25 μg/ml, respectively, and were comparable to those of ofloxacin and ciprofloxacin but less than that of tosufloxacin.

Activity of T-3761 against systemic infections. The in vivo activity of T-3761 against systemic infections with *Staphylococcus aureus*, *Streptococcus pneumoniae*, members of

TABLE 1. Antibacterial activities of T-3761 and other agents against clinical isolates

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Methicillin-susceptible *Staphylococcus aureus* (25)	T-3761	0.10–0.39	0.20	0.39
	Ofloxacin	0.20–0.78	0.39	0.39
	Ciprofloxacin	0.20–0.78	0.39	0.39
	Norfloxacin	0.78–3.13	1.56	3.13
	Tosufloxacin	≤0.013–0.20	0.05	0.10
Ofloxacin-susceptible MRSA (40)[a]	T-3761	0.10–0.39	0.20	0.39
	Ofloxacin	0.10–3.13	0.20	0.78
	Ciprofloxacin	0.10–6.25	0.39	1.56
	Norfloxacin	0.39–25	1.56	6.25
	Tosufloxacin	≤0.013–0.2	0.05	0.10
Ofloxacin-resistant MRSA (26)[b]	T-3761	3.13–>100	6.25	100
	Ofloxacin	12.5–>100	25	>100
	Ciprofloxacin	12.5–>100	50	>100
	Norfloxacin	50–>100	>100	>100
	Tosufloxacin	3.13–>25	12.5	25
Staphylococcus epidermidis (25)	T-3761	0.10–0.39	0.20	0.20
	Ofloxacin	0.10–0.78	0.20	0.39
	Ciprofloxacin	0.05–0.78	0.20	0.39
	Norfloxacin	0.39–6.25	0.78	3.13
	Tosufloxacin	≤0.013–0.10	0.05	0.10
Methicillin-resistant *Staphylococcus epidermidis* (18)[c]	T-3761	0.10–0.39	0.20	0.20
	Ofloxacin	0.10–0.39	0.39	0.39
	Ciprofloxacin	0.05–0.20	0.20	0.20
	Norfloxacin	0.78–1.56	0.78	1.56
	Tosufloxacin	0.025–0.10	0.05	0.10
Streptococcus pyogenes (25)	T-3761	0.78–6.25	1.56	3.13
	Ofloxacin	0.78–3.13	0.78	1.56
	Ciprofloxacin	0.20–1.56	0.39	1.56
	Norfloxacin	0.78–25	1.56	6.25
	Tosufloxacin	0.10–0.39	0.20	0.20
Streptococcus agalactiae (18)	T-3761	1.56–3.13	3.13	3.13
	Ofloxacin	0.78–1.56	0.78	1.56
	Ciprofloxacin	0.39–0.78	0.78	0.78
	Norfloxacin	3.13–6.25	3.13	6.25
	Tosufloxacin	0.20–0.39	0.39	0.39
Streptococcus pneumoniae (25)	T-3761	1.56–6.25	1.56	3.13
	Ofloxacin	0.78–3.13	1.56	1.56
	Ciprofloxacin	0.39–3.13	0.78	0.78
	Norfloxacin	1.56–25	3.13	6.25
	Tosufloxacin	0.10–0.39	0.20	0.20
Enterococcus faecalis (25)	T-3761	1.56–3.13	1.56	3.13
	Ofloxacin	0.78–3.13	1.56	3.13
	Ciprofloxacin	0.39–1.56	0.78	1.56
	Norfloxacin	1.56–6.25	3.13	6.25
	Tosufloxacin	0.10–0.39	0.39	0.39
Escherichia coli (25)	T-3761	0.013–0.05	0.025	0.025
	Ofloxacin	0.013–0.10	0.05	0.05
	Ciprofloxacin	0.003–0.025	0.013	0.013
	Norfloxacin	0.013–0.20	0.05	0.10
	Tosufloxacin	0.006–0.05	0.013	0.025
Salmonella enteritidis (25)	T-3761	0.013–0.025	0.025	0.025
	Ofloxacin	0.025–0.10	0.10	0.10
	Ciprofloxacin	0.006–0.025	0.025	0.025
	Norfloxacin	0.006–0.025	0.10	0.10
	Tosufloxacin	0.006–0.05	0.05	0.05

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TABLE 1—*Continued*

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Citrobacter freundii (25)	T-3761	0.025–0.39	0.10	0.39
	Ofloxacin	0.05–1.56	0.20	0.78
	Ciprofloxacin	0.006–0.78	0.025	0.78
	Norfloxacin	0.025–1.56	0.10	0.78
	Tosufloxacin	0.003–0.39	0.05	0.20
Klebsiella pneumoniae (25)	T-3761	0.025–0.39	0.05	0.20
	Ofloxacin	0.025–0.39	0.10	0.39
	Ciprofloxacin	0.006–0.20	0.05	0.10
	Norfloxacin	0.05–1.56	0.20	1.56
	Tosufloxacin	0.013–0.20	0.05	0.10
Enterobacter cloacae (25)	T-3761	0.025–0.10	0.05	0.10
	Ofloxacin	0.05–0.39	0.10	0.20
	Ciprofloxacin	0.006–0.05	0.013	0.05
	Norfloxacin	0.025–0.39	0.10	0.20
	Tosufloxacin	0.013–0.05	0.025	0.05
Serratia marcescens (50)	T-3761	0.025–6.25	0.78	3.13
	Ofloxacin	0.10–25	3.13	12.5
	Ciprofloxacin	0.025–6.25	0.78	3.13
	Norfloxacin	0.05–25	3.13	25
	Tosufloxacin	0.025–6.25	0.78	6.25
Proteus mirabilis (25)	T-3761	0.025–0.05	0.025	0.05
	Ofloxacin	0.05–0.39	0.10	0.20
	Ciprofloxacin	0.013–0.05	0.025	0.025
	Norfloxacin	0.05–0.10	0.05	0.10
	Tosufloxacin	0.05–0.20	0.10	0.20
Proteus vulgaris (25)	T-3761	0.013–0.025	0.025	0.025
	Ofloxacin	0.025–0.20	0.10	0.10
	Ciprofloxacin	0.006–0.025	0.025	0.025
	Norfloxacin	0.025–0.10	0.05	0.05
	Tosufloxacin	0.013–0.10	0.05	0.10
Morganella morganii (25)	T-3761	0.013–0.05	0.025	0.025
	Ofloxacin	0.025–0.39	0.10	0.20
	Ciprofloxacin	0.013–0.025	0.013	0.025
	Norfloxacin	0.025–0.10	0.05	0.10
	Tosufloxacin	0.013–0.39	0.10	0.20
Providencia rettgeri (24)	T-3761	0.013–1.56	0.05	0.39
	Ofloxacin	0.025–1.56	0.20	1.56
	Ciprofloxacin	0.006–0.39	0.05	0.39
	Norfloxacin	0.025–1.56	0.10	0.78
	Tosufloxacin	0.013–1.56	0.10	0.39
Pseudomonas aeruginosa (50)	T-3761	0.10–12.5	0.39	3.13
	Ofloxacin	0.20–25	1.56	6.25
	Ciprofloxacin	0.025–3.13	0.10	0.39
	Norfloxacin	0.20–25	0.39	1.56
	Tosufloxacin	0.025–3.13	0.20	0.78
Imipenem-resistant *Pseudomonas aeruginosa* (23)[d]	T-3761	0.20–0.78	0.39	0.39
	Ofloxacin	0.39–3.13	1.56	1.56
	Ciprofloxacin	0.10–0.39	0.20	0.39
	Norfloxacin	0.39–1.56	0.78	1.56
	Tosufloxacin	0.20–0.78	0.39	0.78
Gentamicin-resistant *Pseudomonas aeruginosa* (46)[e]	T-3761	0.39–>100	3.13	50
	Ofloxacin	1.56–>100	12.5	100
	Ciprofloxacin	0.20–>100	1.56	50
	Norfloxacin	0.78–>100	6.25	100
	Tosufloxacin	0.20–>25	1.56	>25

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TABLE 1—*Continued*

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Pseudomonas cepacia (25)	T-3761	0.20–100	1.56	3.13
	Ofloxacin	0.39–>100	6.25	12.5
	Ciprofloxacin	0.20–100	3.13	6.25
	Norfloxacin	0.78–>100	6.25	12.5
	Tosufloxacin	0.10–>25	1.56	6.25
Xanthomonas maltophilia (25)	T-3761	0.78–3.13	1.56	3.13
	Ofloxacin	0.78–6.25	3.13	6.25
	Ciprofloxacin	0.78–6.25	3.13	6.25
	Norfloxacin	6.25–50	12.5	50
	Tosufloxacin	0.10–6.25	0.78	6.25
Acinetobacter calcoaceticus (25)	T-3761	0.025–0.78	0.39	0.39
	Ofloxacin	0.05–0.78	0.20	0.78
	Ciprofloxacin	0.025–0.78	0.20	0.78
	Norfloxacin	0.10–12.5	3.13	12.5
	Tosufloxacin	0.006–0.10	0.05	0.10
Alcaligenes faecalis (25)	T-3761	0.20–6.25	0.78	6.25
	Ofloxacin	0.39–100	1.56	25
	Ciprofloxacin	0.20–50	0.78	50
	Norfloxacin	1.56–>100	12.5	>100
	Tosufloxacin	0.10–>25	3.13	>25
Haemophilus influenzae (25)	T-3761	0.013–0.05	0.025	0.025
	Ofloxacin	0.025–0.05	0.05	0.05
	Ciprofloxacin	0.013–0.025	0.025	0.025
	Norfloxacin	0.05–0.10	0.10	0.10
	Tosufloxacin	0.006–0.025	0.006	0.013
Moraxella catarrhalis (25)	T-3761	0.025–0.10	0.05	0.05
	Ofloxacin	0.10–0.20	0.10	0.10
	Ciprofloxacin	0.05–0.10	0.10	0.10
	Norfloxacin	0.10–0.39	0.20	0.39
	Tosufloxacin	0.013–0.025	0.025	0.025
Legionella spp. (13)	T-3761	≤0.006–0.025	0.013	0.025
	Ofloxacin	0.025–0.10	0.05	0.05
	Ciprofloxacin	0.013–0.05	0.025	0.05
	Norfloxacin	0.025–0.20	0.10	0.20
	Tosufloxacin	≤0.006–0.025	≤0.006	0.013
Bacteroides fragilis (25)	T-3761	1.56–6.25	3.13	6.25
	Ofloxacin	0.78–3.13	1.56	3.13
	Ciprofloxacin	3.13–25	3.13	6.25
	Norfloxacin	25–>100	25	100
	Tosufloxacin	0.20–0.78	0.39	0.39
Clostridium difficile (25)	T-3761	6.25	6.25	6.25
	Ofloxacin	12.5	12.5	12.5
	Ciprofloxacin	6.25–12.5	12.5	12.5
	Norfloxacin	50	50	50
	Tosufloxacin	1.56	1.56	1.56
Peptostreptococcus spp. (25)	T-3761	0.78–50	6.25	25
	Ofloxacin	0.20–25	6.25	25
	Ciprofloxacin	0.20–6.25	1.56	6.25
	Norfloxacin	0.78–12.5	6.25	12.5
	Tosufloxacin	0.05–1.56	0.20	1.56

[a] MIC of methicillin, ≥12.5 μg/ml.
[b] MIC of ofloxacin, ≥12.5 μg/ml.
[c] MIC of methicillin, ≥12.5 μg/ml.
[d] MIC of imipenem, ≥6.25 μg/ml.
[e] MIC of gentamicin, ≥6.25 μg/ml.

TABLE 2. Therapeutic effects of various compounds on experimental systemic infections in mice

Organism, challenge dose (CFU/mouse)	Compound	MIC (μg/ml)	ED_{50} (mg/kg) (95% confidence limit)[a]
Staphylococcus aureus Smith, 2.2×10^7	T-3761	0.20	2.8 (1.6–4.8)
	Ofloxacin	0.39	5.5 (2.7–11)
	Ciprofloxacin	0.39	7.0 (3.8–13)
	Norfloxacin	1.56	28 (14–55)
	Tosufloxacin	0.025	0.65 (0.36–1.2)
Staphylococcus aureus F-1282 (MRSA), 4.0×10^7	T-3761	0.20	4.5 (3.1–6.5)
	Ofloxacin	0.78	21 (15–29)
	Ciprofloxacin	1.56	73 (58–92)
	Norfloxacin	12.5	>400
	Tosufloxacin	0.20	9.0 (6.0–14)
Streptococcus pneumoniae D-289, 1.5×10^5[b]	T-3761	3.13	150 (99–230)
	Ofloxacin	3.13	140 (98–200)
	Ciprofloxacin	0.78	>400
	Norfloxacin	6.25	>400
	Tosufloxacin	0.20	11 (7.6–16)
Escherichia coli TK-16, 1.2×10^7	T-3761	0.013	0.15 (0.11–0.21)
	Ofloxacin	0.025	0.65 (0.42–1.0)
	Ciprofloxacin	0.006	0.38 (0.27–0.53)
	Norfloxacin	0.05	2.3 (1.9–2.9)
	Tosufloxacin	0.013	0.15 (0.11–0.21)
Klebsiella pneumoniae Y-193, 7.0×10^3	T-3761	0.05	5.5 (4.0–7.6)
	Ofloxacin	0.10	16 (11–23)
	Ciprofloxacin	0.05	10 (6.9–14)
	Norfloxacin	0.10	93 (64–130)
	Tosufloxacin	0.025	7.0 (4.9–10)
Proteus mirabilis T-111, 3.0×10^7	T-3761	0.025	0.55 (0.40–0.75)
	Ofloxacin	0.10	1.3 (1.0–1.7)
	Ciprofloxacin	0.025	1.5 (1.1–2.1)
	Norfloxacin	0.20	7.5 (5.8–9.7)
	Tosufloxacin	0.20	1.3 (0.90–1.7)
Proteus vulgaris T-319, 7.6×10^7	T-3761	0.025	0.25 (0.14–0.44)
	Ofloxacin	0.10	2.1 (1.6–2.8)
	Ciprofloxacin	0.025	3.0 (1.8–4.9)
	Norfloxacin	0.05	8.0 (6.0–11)
	Tosufloxacin	0.05	2.1 (1.1–3.9)
Serratia marcescens IID620, 1.0×10^7	T-3761	0.05	0.70 (0.49–0.99)
	Ofloxacin	0.20	1.4 (1.1–1.8)
	Ciprofloxacin	0.025	0.95 (0.62–1.5)
	Norfloxacin	0.10	4.6 (3.6–5.9)
	Tosufloxacin	0.10	2.3 (1.7–3.1)
Serratia marcescens W-196, 1.5×10^7	T-3761	0.10	1.1 (0.73–1.7)
	Ofloxacin	0.20	3.6 (2.7–4.9)
	Ciprofloxacin	0.05	3.1 (2.1–4.5)
	Norfloxacin	0.20	7.0 (5.2–9.5)
	Tosufloxacin	0.10	4.5 (3.1–6.6)
Serratia marcescens W-217, 4.3×10^6	T-3761	1.56	9.0 (4.9–17)
	Ofloxacin	3.13	39 (29–52)
	Ciprofloxacin	1.56	62 (42–91)
	Norfloxacin	12.5	>130
	Tosufloxacin	3.13	82 (58–120)
Pseudomonas aeruginosa S-68, 4.9×10^6	T-3761	0.39	8.0 (5.5–12)
	Ofloxacin	0.78	20 (13–30)
	Ciprofloxacin	0.20	13 (12–15)
	Norfloxacin	1.56	43 (32–58)
	Tosufloxacin	0.20	8.0 (5.4–12)

Continued on following page

TABLE 2—*Continued*

Organism, challenge dose (CFU/mouse)	Compound	MIC (μg/ml)	ED_{50} (mg/kg) (95% confidence limit)[a]
Pseudomonas aeruginosa S-406, 4.0×10^5	T-3761	0.39	10 (6.3–16)
	Ofloxacin	0.78	38 (28–52)
	Ciprofloxacin	0.10	17 (13–23)
	Norfloxacin	0.78	74 (50–110)
	Tosufloxacin	0.20	12 (7.8–18)
Pseudomonas aeruginosa S-916, 4.3×10^6	T-3761	3.13	50 (37–68)
	Ofloxacin	12.5	>130
	Ciprofloxacin	1.56	>130
	Norfloxacin	6.25	>130
	Tosufloxacin	3.13	120 (87–170)

[a] ED_{50}, 50% effective dose, estimated by the method of Litchfield and Wilcoxon (9).
[b] For *Streptococcus pneumoniae* infections only, each of the agents was administered orally twice after infection, and the 50% effective doses are given as half of the total doses.

the family *Enterobacteriaceae*, and *Pseudomonas aeruginosa* in mice was compared with those of ofloxacin, ciprofloxacin, norfloxacin, and tosufloxacin (Table 2). Against *Staphylococcus aureus* Smith and *Streptococcus pneumoniae* D-289, T-3761 either had activity comparable to those of the reference agents or was more effective than the reference agents with the exception of tosufloxacin, which was more active. Against methicillin-resistant *Staphylococcus aureus* (MRSA) F-1282, T-3761 was more effective than tosufloxacin and the other agents. Against *Escherichia coli* TK-16, *Proteus mirabilis* T-111, *Proteus vulgaris* T-319, *Serratia marcescens* IID620, and *Serratia marcescens* W-196, T-3761 demonstrated protection, with 50% effective doses of 0.15, 0.55, 0.25, 0.70, and 1.1 mg/kg, respectively. This degree of protection was either comparable to or greater than those of the reference agents. The 50% effective doses of T-3761 against *Klebsiella pneumoniae* Y-193, norfloxacin-resistant (MIC, 12.5 μg/ml) *Serratia marcescens* W-217, *Pseudomonas aeruginosa* S-68, *Pseudomonas aeruginosa* S-406, and ofloxacin-resistant (MIC, 12.5 μg/ml) *Pseudomonas aeruginosa* S-916 were 5.5, 9.0, 8.0, 10, and 50 mg/kg, respectively. Thus, the in vivo activity of T-3761 against some quinolone-resistant isolates was lower than that against quinolone-susceptible members of the family *Enterobacteriaceae*. Against quinolone-resistant *Serratia marcescens* W-217 and *Pseudomonas aeruginosa* S-916, the activities of T-3761 were 2 to 14 times greater than those of ofloxacin, ciprofloxacin, norfloxacin, and tosufloxacin.

Activity of T-3761 against pulmonary infections. The activity of T-3761 against pulmonary infections with *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* was compared with those of ofloxacin, ciprofloxacin, norfloxacin, and tosufloxacin (Table 3).

Against *Streptococcus pneumoniae* D-289, tosufloxacin showed the greatest efficacy, with 93% survival and 53% eradication rates; this was followed by T-3761 and ofloxacin. Norfloxacin and ciprofloxacin were less efficacious.

Against *Klebsiella pneumoniae* Y-41, T-3761 and tosufloxacin were the most active, with 53 and 40% eradication rates, respectively; this was followed by ofloxacin and ciprofloxacin. Norfloxacin was notably less active.

Against *Pseudomonas aeruginosa* S-406, T-3761 showed

TABLE 3. Therapeutic effect of T-3761 on experimental pulmonary infections in mice

Organism, challenge dose (CFU/lung)	Compound	MIC (μg/ml)	Efficacy (%)[a]	
			Survival	Eradication
Streptococcus pneumoniae D-289, 2.0×10^6[b]	T-3761	3.13	50	30
	Ofloxacin	3.13	50	10
	Ciprofloxacin	0.78	25	0
	Norfloxacin	6.25	40	0
	Tosufloxacin	0.20	93	53
Klebsiella pneumoniae Y-41, 1.0×10^5[c]	T-3761	0.05	87	53
	Ofloxacin	0.10	80	20
	Ciprofloxacin	0.025	80	7
	Norfloxacin	0.10	10	0
	Tosufloxacin	0.05	89	40
Pseudomonas aeruginosa S-406, 3.5×10^5[d]	T-3761	0.39	94	94
	Ofloxacin	0.78	55	55
	Ciprofloxacin	0.10	83	83
	Norfloxacin	0.78	33	33
	Tosufloxacin	0.20	74	74

[a] Percent efficacy was calculated as follows: (number of surviving or culture-negative mice in the lungs/number of mice tested) × 100.
[b] Therapy (25 mg/kg, orally) was done at 4 and 6 h after infection.
[c] Therapy (5 mg/kg, orally) was started at 4 h after infection and was continued twice per day for 3 days.
[d] Therapy (25 mg/kg, orally) was done at 4 and 6 h after infection.

TABLE 4. Therapeutic effect of T-3761 on experimental urinary tract infections in mice

Organism, challenge dose (CFU/mouse)	Compound	MIC (μg/ml)	Log CFU/kidney (mean ± SD)
Proteus mirabilis T-111, 2.0×10^{5}[a]	None		8.3 ± 1.2
	T-3761	0.025	3.8 ± 1.9
	Ofloxacin	0.10	4.3 ± 3.0
	Ciprofloxacin	0.025	4.7 ± 2.8
	Norfloxacin	0.20	4.8 ± 2.0
	Tosufloxacin	0.20	4.8 ± 1.8
Serratia marcescens IID620, 2.4×10^{5}[b]	None		6.3 ± 0.5
	T-3761	0.05	2.5 ± 1.0
	Ofloxacin	0.20	4.1 ± 1.9
	Ciprofloxacin	0.025	2.9 ± 1.1
	Norfloxacin	0.10	4.2 ± 2.0
	Tosufloxacin	0.10	2.8 ± 1.5
Pseudomonas aeruginosa S-68, 5.0×10^{6}[a]	None		8.1 ± 1.6
	T-3761	0.39	3.5 ± 2.5
	Ofloxacin	0.78	4.3 ± 3.1
	Ciprofloxacin	0.20	4.5 ± 1.6
	Norfloxacin	1.56	6.0 ± 2.0
	Tosufloxacin	0.20	4.3 ± 3.3
Pseudomonas aeruginosa S-429, 5.0×10^{6}[c]	None		6.1 ± 2.0
	T-3761	0.05	2.9 ± 1.3
	Ofloxacin	0.20	4.5 ± 1.8
	Ciprofloxacin	0.05	4.0 ± 1.5
	Norfloxacin	0.20	4.7 ± 1.7
	Tosufloxacin	0.05	4.6 ± 2.4

[a] Therapy (25 mg/kg, orally) was done at 4 and 24 h after infection.
[b] Therapy (12.5 mg/kg, orally) was done at 4 and 24 h after infection.
[c] Therapy (25 mg/kg, orally) was done at 4 and 6 h after infection.

the most potent efficacy, with 94% survival and eradication rates; this was followed by ciprofloxacin and tosufloxacin. Ofloxacin and norfloxacin were less efficacious.

Activity of T-3761 against urinary tract infections. The activity of T-3761 against urinary tract infections with *Proteus mirabilis*, *Serratia marcescens*, and *Pseudomonas aeruginosa* was compared with those of ofloxacin, ciprofloxacin, norfloxacin, and tosufloxacin (Table 4).

Bacterial numbers in the untreated groups increased to $10^{6.1}$ to $10^{8.3}$ CFU per kidney. Twice-daily therapy with T-3761 at doses of 12.5 or 25 mg/kg reduced the numbers of each organism to $10^{2.5}$ to $10^{3.8}$ CFU per kidney. The decrease in bacterial numbers in the kidneys was, in general, more pronounced in T-3761-treated mice than in mice treated with the reference agents.

DISCUSSION

T-3761 showed broad and potent antibacterial activity against gram-positive bacteria, including members of the genera *Staphylococcus*, *Streptococcus*, and *Enterococcus*; gram-negative bacteria, including members of the family *Enterobacteriaceae* and the genera *Haemophilus* and *Legionella*; nonfermenters, including *Pseudomonas aeruginosa*; and obligate anaerobic bacteria, including members of the genera *Bacteroides*, *Clostridium*, and *Peptostreptococcus*. In general, the in vitro antibacterial activity of T-3761 was greater than that of norfloxacin, T-3761 was more active or had activity comparable to that of ofloxacin, and T-3761 had activity comparable to or less than those of ciprofloxacin and tosufloxacin.

Against ofloxacin-susceptible MRSA, the MIC_{90}s of ofloxacin, ciprofloxacin, and norfloxacin were 0.78, 1.56, and 6.25 μg/ml, respectively; this activity was two to four times less than that against methicillin-susceptible *Staphylococcus aureus*, while the MIC_{90}s of T-3761 and tosufloxacin for ofloxacin-susceptible MRSA were equal to those for methicillin-susceptible *Staphylococcus aureus*. The MIC_{90} (0.1 μg/ml) of tosufloxacin was lower than that (0.39 μg/ml) of T-3761 against this group. However, against ofloxacin-resistant MRSA, the MIC_{50} of T-3761 was 6.25 μg/ml, which was two times or more active than tosufloxacin and the other agents, although the MIC_{90}s of all of the agents were high. These results for T-3761 are similar to those reported for sparfloxacin, indicating activity against some quinolone-resistant MRSA (8).

Moreover, against imipenem-resistant *Pseudomonas aeruginosa* (23 strains), the MIC_{90} of T-3761 was 0.39 μg/ml, which was equal to that of ciprofloxacin; this was followed by tosufloxacin and norfloxacin or ofloxacin. While the MIC_{90} of T-3761 was 3.13 μg/ml, for *Pseudomonas aeruginosa* isolates (50 strains) selected at random, it was less active than ciprofloxacin (MIC_{90}, 0.39 μg/ml) and tosufloxacin (MIC_{90}, 0.78 μg/ml); this was followed by norfloxacin and ofloxacin. Because few strains were used in the present study, it is not apparent whether T-3761 and ciprofloxacin or tosufloxacin show different potencies against imipenem-resistant *Pseudomonas aeruginosa*.

The in vivo activity of T-3761 was evaluated by using experimental models of infection in mice. The activity of T-3761 was less than that of tosufloxacin against systemic infections caused by gram-positive bacteria except MRSA and pulmonary infections caused by *Streptococcus pneumoniae*. The efficacy of T-3761 was comparable to or greater than that of tosufloxacin, however, in the other experimental infection models. The in vivo activity of T-3761 was compa-

rable to or greater than those of the other reference agents against the systemic, pulmonary, and urinary tract infections caused by gram-positive and gram-negative bacteria, including quinolone-resistant *Serratia marcescens* and *Pseudomonas aeruginosa*.

We have reported that T-3761 has high levels of absorption and excretion following oral administration to several animals, including mice (3). In contrast, ofloxacin (11), ciprofloxacin (6), norfloxacin (5), and tosufloxacin (16) had lower levels of absorption and excretion than T-3761, and the maximum concentration of each of the agents in serum following oral administration to mice was less than one-fourth that of T-3761. It is possible that these pharmacokinetic differences combined with the vitro potency of T-3761 reflect the overall good efficacy of this quinolone in mouse infection models. Therefore, T-3761 may prove to be a useful quinolone, and clinical trials of T-3761 are in progress in Japan.

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