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Comparative In Vitro Activity of a New Quinolone, AM-1091

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The in vitro activity of a new quinolone, AM-1091 [7-(3-amino-1-pyrrolidinyl)-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid hydrochloride], was compared with those of ciprofloxacin, ofloxacin, beta-lactams, and gentamicin. AM-1091 inhibited 90% of the isolates of the family *Enterobacteriaceae* at ≤0.12 μg/ml. For many species AM-1091 was 2-fold more active than ciprofloxacin and 2- to 32-fold more active than ofloxacin. It inhibited *Enterobacter*, *Citrobacter*, and *Klebsiella* species resistant to ceftazidime and gentamicin. Ninety percent of *Pseudomonas aeruginosa* isolates were inhibited by 0.5 μg/ml, so for this species AM-1091 was twofold less active than ciprofloxacin. AM-1091 was more active against *Pseudomonas cepacia* and *Xanthomonas maltophilia*, inhibiting isolates resistant to imipenem and gentamicin. Most *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Branhamella catarrhalis* isolates were inhibited by ≤0.06 μg/ml. The MICs for 90% of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis* isolates were 0.06, 0.06, and 2 μg/ml, respectively. AM-1091 inhibited hemolytic streptococci and *Streptococcus pneumoniae* at 0.25 μg/ml and was more active than ciprofloxacin or ofloxacin against gram-positive species. AM-1091 inhibited 90% of the *Bacteroides* species at 0.5 μg/ml. The frequency of spontaneous resistance was $<10^{-10}$ for most organisms, but resistant strains could be selected by repeated subculturing. Although AM-1091 had lower in vitro activity at pH 5.5 and in the presence of high concentrations of Mg^{2+}, it still inhibited most organisms at ≤0.5 μg/ml under these conditions. AM-1091 rapidly killed *Escherichia coli* and *P. aeruginosa* and had a prolonged postantibiotic suppressive effect on these bacteria.

There has been major interest in the synthesis of novel quinolones. Many compounds of the new quinolone class have excellent in vitro activity against members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*, but activity against streptococcal and enterococcal species is borderline, and that against anaerobic species is inadequate (3, 10). Compound AM-1091 is a new quinolone with the chemical structure 7-(3-amino-1-pyrrolidinyl)-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid hydrochloride (Fig. 1). We compared the activity of AM-1091 with those of other quinolones, ceftazidime, imipenem, and gentamicin and determined the effect of various assay conditions on its in vitro activity.

MATERIALS AND METHODS

Drugs and isolates. AM-1091 was a gift from Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan. All other agents were obtained from their respective manufacturers. Quinolones were prepared as described previously (6). Fresh dilutions of all compounds used were prepared daily. Bacterial isolates were obtained from patients hospitalized at The Presbyterian Hospital, New York, N.Y. Only one isolate from each patient was tested to avoid multiple copies of the same strain. Some isolates came from patients who had been subjects in an investigation of the efficacy and safety of new quinolones.

Antimicrobial susceptibility tests. Antimicrobial susceptibility was measured by an agar dilution method with Mueller-Hinton agar in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (8). A replicating spot device applied 10^4 CFU prepared by dilution of fresh overnight broth. Broth dilutions were performed with 5×10^5 CFU in 1-ml tubes. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited the development of visible growth on agar or in the tubes after 18 to 20 h of incubation. The MBC was determined by plating 0.01 ml from clear tubes to agar plates. The MBC was defined as the concentration at which there was a 99.9% reduction in CFU by the method of Pearson et al. (9), considering normal pipetting error. The effects of serum, urine, pH, and ion changes in the medium were determined as described previously (6). All assays were run simultaneously.

The susceptibilities of *Neisseria*, *Branhamella*, and *Haemophilus* spp. were determined with chocolate Mueller-Hinton agar in the presence of 5% CO_2. The susceptibilities of streptococci were determined with Mueller-Hinton agar supplemented with 5% sheep blood. Activity against anaerobic species was determined with brucella agar supple-

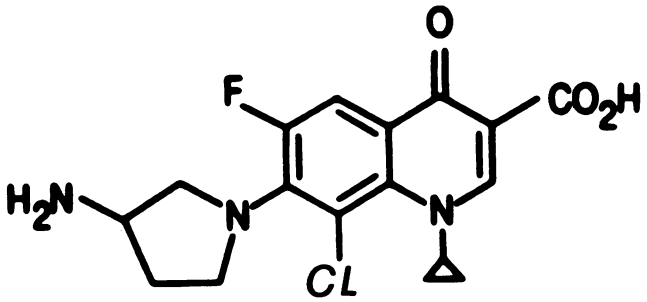

FIG. 1. Structure of AM-1091.

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TABLE 1. Comparative in vitro activities of AM-1091 and other agents against gram-negative organisms

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%[a]	90%
Escherichia coli (30)	AM-1091	≤0.008	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.015	0.008	0.015
	Ofloxacin	0.03–1	0.06	0.12
	Ceftazidime	0.03–0.5	0.12	0.25
	Imipenem	0.06–1	0.12	0.5
	Gentamicin	0.25–16	0.25	1
Klebsiella pneumoniae (30)	AM-1091	≤0.008–0.25	0.03	0.6
	Ciprofloxacin	≤0.008–0.015	≤0.008	0.015
	Ofloxacin	0.03–0.25	0.12	0.25
	Ceftazidime	0.03–1	0.25	1
	Imipenem	0.12–1	0.25	0.5
	Gentamicin	0.25–>16	0.5	>16
Klebsiella oxytoca (20)	AM-1091	≤0.008–0.15	≤0.008	0.015
	Ciprofloxacin	0.015–0.5	0.06	0.12
	Ofloxacin	0.12–1	0.25	0.5
	Ceftazidime	0.12–16	0.5	2
	Imipenem	0.25–4	0.5	2
	Gentamicin	0.5–>16	0.5	>16
Enterobacter aerogenes (25)	AM-1091	≤0.008–0.12	≤0.008	0.12
	Ciprofloxacin	0.015–0.25	0.015	0.03
	Ofloxacin	0.06–1	0.12	0.25
	Ceftazidime	0.06–>64	0.25	16
	Imipenem	0.25–4	2	4
	Gentamicin	0.5–>16	0.5	8
Enterobacter agglomerans (6)	AM-1091	≤0.008–0.03	≤0.008	
	Ciprofloxacin	≤0.008–0.015	≤0.008	
	Ofloxacin	0.015–0.25	0.06	
	Ceftazidime	0.12–1	0.25	
	Imipenem	0.12–2	0.5	
	Gentamicin	0.06–>16	0.12	
Enterobacter cloacae (25)	AM-1091	≤0.008–0.06	≤0.008	0.03
	Ciprofloxacin	≤0.008–0.12	0.015	0.03
	Ofloxacin	0.06–0.5	0.12	0.25
	Ceftazidime	0.06–64	0.5	64
	Imipenem	0.06–8	1	4
	Gentamicin	0.5–>16	0.5	8
Hafnia alvei (10)	AM-1091	≤0.008–0.015	≤0.008	0.015
	Ciprofloxacin	0.015–0.12	0.03	0.12
	Ofloxacin	0.06–0.25	0.12	0.25
	Ceftazidime	0.5–32	4	16
	Imipenem	0.25–0.5	0.5	0.5
	Gentamicin	0.25–1	0.25	1
Citrobacter freundii (30)	AM-1091	≤0.008–0.25	≤0.008	0.12
	Ciprofloxacin	≤0.008–1	0.06	0.06
	Ofloxacin	0.06–1	0.12	0.5
	Ceftazidime	0.12–64	0.5	64
	Imipenem	0.12–2	0.5	2
	Gentamicin	0.25–>16	0.5	2
Citrobacter diversus (10)	AM-1091	≤0.008–0.015	≤0.008	≤0.008
	Ciprofloxacin	≤0.008	≤0.008	≤0.008
	Ofloxacin	0.06–0.12	0.06	0.06
	Ceftazidime	0.06–0.25	0.12	0.12
	Imipenem	0.12–2	0.25	0.25
	Gentamicin	0.25–2	0.25	0.5

Continued

TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%[a]	90%
Proteus mirabilis (30)	AM-1091	≤0.008–0.03	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.5	0.015	0.12
	Ofloxacin	0.06–2	0.12	1
	Ceftazidime	0.06–0.5	0.06	0.25
	Imipenem	0.5–8	4	4
	Gentamicin	0.5–8	1	4
Morganella morganii (30)	AM-1091	≤0.008–0.5	0.015	0.12
	Ciprofloxacin	≤0.008–0.5	≤0.008	0.06
	Ofloxacin	0.06–0.5	0.12	0.5
	Ceftazidime	0.12–4	0.5	2
	Imipenem	2–8	2	2
	Gentamicin	0.25–16	1	4
Proteus vulgaris (30)	AM-1091	≤0.008–0.5	0.03	0.06
	Ciprofloxacin	≤0.008–4	0.015	0.06
	Ofloxacin	0.03–8	0.12	1
	Ceftazidime	0.03–8	0.06	1
	Imipenem	0.5–32	2	4
	Gentamicin	0.12–>16	0.5	>16
Providencia rettgeri (20)	AM-1091	0.015–0.25	0.06	0.25
	Ciprofloxacin	≤0.008–2	0.5	2
	Ofloxacin	0.25–4	1	2
	Ceftazidime	0.03–16	1	4
	Imipenem	0.12–4	2	4
	Gentamicin	0.12–>16	2	>16
Providencia stuartii (30)	AM-1091	≤0.008–0.5	0.06	0.12
	Ciprofloxacin	0.06–2	0.25	1
	Ofloxacin	0.5–16	1	8
	Ceftazidime	0.12–32	1	4
	Imipenem	1–8	4	4
	Gentamicin	0.5–>16	2	>16
Serratia marcescens (30)	AM-1091	0.03–0.5	0.06	0.12
	Ciprofloxacin	0.06–1	0.12	0.5
	Ofloxacin	0.25–2	0.5	1
	Ceftazidime	0.12–8	0.5	1
	Imipenem	0.5–4	1	2
	Gentamicin	0.5–>16	1	>16
Pseudomonas aeruginosa (80)	AM-1091	0.06–0.5	0.25	0.5
	Ciprofloxacin	0.015–2	0.12	0.25
	Ofloxacin	0.25–32	1	4
	Ceftazidime	0.5–32	2	8
	Imipenem	0.5–32	2	8
	Gentamicin	0.25–>16	4	>16
	Piperacillin	8–>128	32	>128
	Ticarcillin	16–>128	64	>128
Pseudomonas cepacia (15)	AM-1091	≤0.008–0.25	0.06	0.12
	Ciprofloxacin	0.12–8	2	4
	Ofloxacin	0.5–8	4	8
	Ceftazidime	0.5–>128	2	128
	Imipenem	>32	32	>32
	Gentamicin	2–>16	>16	>16
Xanthomonas maltophilia (20)	AM-1091	0.12–0.5	0.25	0.5
	Ciprofloxacin	0.25–8	1	2
	Ofloxacin	1–8	1	4
	Ceftazidime	8–>64	>64	>64
	Imipenem	>32	>32	>32
	Gentamicin	>16	>16	>16

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%[a]	90%
Pseudomonas spp.[b] (40)	AM-1091	≤0.008–0.5	0.03	0.25
	Ciprofloxacin	≤0.008–4	0.12	1
	Ofloxacin	0.12–16	4	8
	Ceftazidime	0.25–32	4	8
	Imipenem	1–>32	1	8
	Gentamicin	0.25–>16	>16	>16
Acinetobacter anitratus (22)	AM-1091	≤0.008–0.12	0.03	0.03
	Ciprofloxacin	0.03–4	0.5	1
	Ofloxacin	0.12–2	0.5	1
	Ceftazidime	0.25–>64	8	64
	Imipenem	0.06–1	0.25	0.25
	Gentamicin	0.25–>16	1	2
Salmonella spp. (25)	AM-1091	≤0.008–0.015	≤0.008	0.015
	Ciprofloxacin	≤0.008–0.12	≤0.008	0.03
	Ofloxacin	0.06–1	0.12	0.12
	Ceftazidime	0.12–4	0.25	4
	Imipenem	0.12–1	0.25	0.5
	Gentamicin	0.25–4	0.5	4
Shigella spp. (30)	AM-1091	≤0.008–0.04	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.03	≤0.008	0.03
	Ofloxacin	0.06–0.25	0.06	0.12
	Ceftazidime	0.06–8	0.06	2
	Imipenem	0.25–0.5	0.25	0.5
	Gentamicin	0.25–2	1	2
Aeromonas sp. (15)	AM-1091	≤0.008–0.12	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.06	≤0.008	0.06
	Ofloxacin	0.015–1	0.03	0.5
	Ceftazidime	0.06–0.25	0.12	0.25
	Imipenem	0.25–0.5	0.25	0.5
	Gentamicin	1–4	2	4
Yersinia enterocolitica (15)	AM-1091	≤0.008–0.03	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.06	0.03	0.3
	Ofloxacin	0.06–0.5	0.12	0.25
	Ceftazidime	0.06–1	0.12	0.5
	Imipenem	0.12–1	0.25	1
	Gentamicin	0.5–4	1	2
Haemophilus influenzae (12)	AM-1091	≤0.008–0.12	≤0.008	0.06
	Ciprofloxacin	≤0.008–0.5	≤0.008	0.03
	Ofloxacin	0.06–4	0.12	0.5
	Ceftazidime	≤0.12–0.25	≤0.12	0.25
	Imipenem	4–>16	8	16
	Gentamicin	1–4	4	4
Branhamella catarrhalis (15)	AM-1091	≤0.008–0.12	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.25	0.06	0.12
	Ofloxacin	0.12–0.25	0.12	0.25
	Ceftazidime	0.12–0.25	0.12	0.25
Neisseria gonorrhoeae (10)	AM-1091	≤0.008–0.12	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.015	≤0.008	≤0.008
	Ofloxacin	≤0.015–0.12	≤0.015	0.03
	Ceftazidime	≤0.12–0.25	≤0.12	0.25
Neisseria meningitidis (10)	AM-1091	≤0.008–0.15	≤0.008	≤0.008
	Ciprofloxacin	≤0.008–0.015	≤0.008	≤0.008

[a] 50%, MIC for 50% of the isolates.

[b] *P. acidovorans* (n = 10), *P. fluorescens* (n = 20), *P. putida* (n = 5), and *P. stutzeri* (n = 5).

mented with 5% sheep blood, vitamin K, and hemin. Incubation was done for 48 h in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.). All tests were run with control strains from the American Type Culture Collection, Rockville, Md.

Selection of resistant isolates. Organisms (5×10^5 CFU) were inoculated into Mueller-Hinton broth containing twofold increasing concentrations of the compound and transferred daily for 14 days. To determine that the resistance was stable, we plated colonies on antibiotic-free medium daily for 1 week and retested them by the broth dilution method. Isolates were subsequently tested after storage for 2 months on agar slants lacking antibiotic.

Mutants spontaneously resistant to the compound were detected by plating overnight cultures, concentrated by centrifugation to yield $\geq 10^{10}$ CFU as the final inoculum, onto Mueller-Hinton agar plates containing the compound at a concentration eight times the MIC: Two isolates of each species were tested.

PAE. Overnight cultures were diluted into fresh medium to yield a final concentration of 5×10^5 CFU. A final concentration of 3 μg of compound AM-1091 per ml was added to Mueller-Hinton broth (pH 7.4) and to serum, and 300 μg of compound AM-1091 per ml was added to urine. The concentrations were based on anticipated peak concentrations in plasma and urine (communication from Kyorin Pharmaceuticals). After 2 h of exposure in a shaking incubator at 35°C, the compound was removed by filtration through a membrane filter (Millipore Corp., Bedford, Mass.). Bacterial cells were washed three times with prewarmed medium and suspended in the respective antibiotic-free medium. Bacterial counts were determined by plating serial dilutions on Mueller-Hinton agar before and after compound AM-1091 was removed. The number of CFU was determined every hour for the first 4 h and every 2 h thereafter up to 10 h. Organisms which had not been exposed to drug were processed in the same manner.

The postantibiotic effect (PAE) was measured as described by Craig and Gudmundsson (2) as the difference in time required for test and control culture CFU to increase by 1 $\log_{10}$ after the antibiotic was removed.

Killing effect. The killing effect was determined by exposing bacteria to the MBC for various periods and removing samples, which were then filtered, washed, and plated to determine the number of CFU. Untreated bacteria (as a control) were processed in the same way.

RESULTS

Activity of AM-1091. Compound AM-1091 was an extremely active agent. The MIC for 90% (MIC_{90}) of the members of the family *Enterobacteriaceae* was ≤0.12 μg/ml (Table 1). The only member of the family *Enterobacteriaceae* for which the MIC_{90} was higher was *Providencia rettgeri*, inhibited by 0.25 μg/ml. Against some isolates AM-1091 was two- to eightfold more active than ciprofloxacin, whereas ciprofloxacin was more active against others. Against many of the members of the family *Enterobacteriaceae* AM-1091 was 2- to 128-fold more active than ofloxacin, depending on the species. AM-1091 inhibited isolates of *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Citrobacter freundii* which were resistant to ceftazidime, and it inhibited isolates of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Providencia* spp. which were gentamicin resistant. AM-1091 inhibited *Enterobacter*, *Citrobacter*, and *Providencia* spp. for which imipenem MICs were 2 to 4

TABLE 2. Comparative in vitro activities of AM-1091 and other agents against gram-positive and anaerobic organisms

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%[a]	90%
Staphylococcus aureus, methicillin susceptible (25)	AM-1091	0.015–0.6	0.03	0.06
	Ciprofloxacin	0.25–1	0.5	1
	Ofloxacin	0.25–2	0.5	0.5
Staphylococcus aureus, methicillin resistant (25)	AM-1091	0.03–0.12	0.12	0.12
	Ciprofloxacin	0.25–2	0.5	1
	Ofloxacin	0.25–2	0.5	1
Coagulase-negative staphylococci, methicillin susceptible (25)	AM-1091	≤0.008–0.06	0.03	0.03
	Ciprofloxacin	0.25–1	0.25	0.5
	Ofloxacin	0.25–1	0.5	1
Coagulase-negative staphylococci, methicillin resistant (25)	AM-1091	0.03–0.12	0.06	0.06
	Ciprofloxacin	0.12–1	0.5	0.5
	Ofloxacin	0.25–1	0.5	1
Streptococcus pyogenes (20)	AM-1091	0.03–1	0.06	0.25
	Ciprofloxacin	0.25–2	0.5	1
	Ofloxacin	1–2	1	2
Streptococcus agalactiae (20)	AM-1091	0.12–1	0.25	0.25
	Ciprofloxacin	1–4	1	4
	Ofloxacin	1–4	1	2
Viridans group streptococci (20)	AM-1091	0.06–0.25	0.25	0.25
	Ciprofloxacin	0.5–4	1	2
	Ofloxacin	1–4	2	2
Streptococcus bovis (18)	AM-1091	0.12–0.25	0.25	0.25
	Ciprofloxacin	0.5–2	2	2
	Ofloxacin	0.5–4	2	2
Streptococcus groups C, F, and G (30)	AM-1091	0.12–2	0.25	0.5
	Ciprofloxacin	0.25–4	1	2
	Ofloxacin	0.5–8	2	4
Streptococcus pneumoniae (22)	AM-1091	0.03–0.12	0.06	0.12
	Ciprofloxacin	0.25–2	1	2
	Ofloxacin	0.25–2	2	2
Enterococcus faecalis (30)	AM-1091	0.06–2	1	2
	Ciprofloxacin	0.5–2	1	2
	Ofloxacin	1–4	2	2
Listeria monocytogenes (20)	AM-1091	0.12–0.25	0.25	0.25
	Ciprofloxacin	1–2	2	2
	Ofloxacin	0.5–2	2	2
Corynebacterium group JK (10)	AM-1091	0.25–0.5	0.25	0.5
	Ciprofloxacin	0.25–2	0.25	2
	Ofloxacin	0.5–2	1	2
Bacteroides fragilis (25)	AM-1091	0.12–1	0.25	0.5
	Ciprofloxacin	4–32	8	16
	Ofloxacin	2–16	4	8
	Clindamycin	0.12–4	0.25	1
	Cefoxitin	2–64	8	32
Bacteroides spp.[b] (20)	AM-1091	0.12–0.25	0.25	0.25
Clostridium perfringens (15)	AM-1091	0.06–1	0.12	0.5
	Ciprofloxacin	0.5–16	1	8
	Ofloxacin	0.25–16	1	8
Clostridium spp.[c] (15)	AM-1091	0.15–0.5	0.25	0.5
Peptococci and peptostreptococci[d] (10)	AM-1091	0.15–0.5	0.25	0.5

[a] 50%, MIC for 50% of the isolates.
[b] *B. melaninogenicus*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus*.
[c] *C. ramosum*, *C. septicum*, *C. difficile*, and *C. innocuum*.
[d] Peptococci, five isolates; peptostreptococci, five isolates.

μg/ml. In general, AM-1091 was twofold less active than ciprofloxacin but fourfold more active than ofloxacin against *P. aeruginosa*. Among the *P. aeruginosa* isolates were those resistant to ceftazidime, imipenem, amikacin, and piperacillin. AM-1091 was more active than ciprofloxacin and ofloxacin against *Pseudomonas cepacia* and *Xanthomonas maltophilia*, inhibiting these species at concentrations of 0.12 and 0.5 μg/ml, respectively. These isolates were resistant to ceftazidime, imipenem, and gentamicin. Among the other *Pseudomonas* species tested, namely, *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Pseudomonas diminuta*, *Pseudomonas putida*, and *Pseudomonas acidovorans*, all were inhibited by AM-1091 at ≤0.5 μg/ml. AM-1091 also inhibited *Acinetobacter* spp., including ceftazidime- and gentamicin-resistant isolates. AM-1091 had activity comparable to that of ciprofloxacin against *Haemophilus influenzae* and *Neisseria gonorrhoeae*, including isolates which were ampicillin resistant, and against *Neisseria meningitidis*. It was more active than ciprofloxacin against *Branhamella* spp., *Legionella pneumophila*, and *Campylobacter* spp., including *Campylobacter jejuni*, *Campylobacter intestinalis*, and *Campylobacter pylori*, which were inhibited (five isolates each) by ≤0.12 μg/ml (data not shown).

The MIC_{90} of AM-1091 for *Staphylococcus aureus* isolates, both methicillin susceptible and methicillin resistant, was ≤0.06 μg/ml (Table 2). The compound was equally active against coagulase-negative staphylococci, including *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, and *Staphylococcus hemolyticus*. The MIC_{90} of AM-1091 for *Streptococcus pyogenes* was 0.25 μg/ml, as compared with 1 μg of ciprofloxacin and 2 μg of ofloxacin per ml. AM-1091 inhibited hemolytic streptococci belonging to groups C, F, and G at concentrations of 0.12 to 2 μg/ml. The MIC_{90} of AM-1091 for *Streptococcus pneumoniae* was 0.12 μg/ml, but 2 μg/ml was required to inhibit 100% of *Enterococcus faecalis* and *Enterococcus faecium* isolates (five isolates each) (data not shown). The MIC_{90} of AM-1091 for *Bacteroides fragilis* isolates was ≤0.5 μg/ml, as compared with 8 μg of ofloxacin and 16 μg of ciprofloxacin per ml. The *Clostridium* spp., including *Clostridium perfringens*, *Clostridium difficile*, and *Clostridium sordellii*, were inhibited by 1 μg/ml.

Effect of various conditions on activity. The activity of AM-1091 was identical to or within twofold of that determined on Mueller-Hinton agar for assays done on nutrient agar, Columbia agar, and Trypticase soy agar (BBL Microbiology Systems) when the pH of the media was adjusted to 7.4 for five isolates each of *Escherichia coli*, *K. pneumoniae*, *C. freundii*, *P. aeruginosa*, and *Serratia marcescens*. Table 3 shows the effect of various concentrations of cations on the activity of AM-1091. At 9 mM magnesium the MIC for the isolates increased eightfold over the MIC determined in

TABLE 3. Effect of various cations on the activity of AM-1091

Organism	MIC and MBC (μg/ml) in:			
	MHB[a]	MHB + 4.5 mM Ca^{2+}	MHB + 3 mM Mg^{2+}	MHB + 9 mM Mg^{2+}
E. coli 5800	0.004 and 0.004	0.008 and 0.015	0.008 and 0.015	0.03 and 0.03
E. coli 6351	0.008 and 0.008	0.008 and 0.015	0.015 and 0.015	0.03 and 0.06
K. pneumoniae 5563	0.008 and 0.015	0.015 and 0.03	0.015 and 0.03	0.06 and 0.12
K. pneumoniae 8708	0.008 and 0.015	0.015 and 0.03	0.03 and 0.03	0.06 and 0.12
C. freundii 5821	0.015 and 0.015	0.015 and 0.015	0.015 and 0.015	0.015 and 0.06
C. freundii 10346	0.008 and 0.015	0.008 and 0.015	0.015 and 0.03	0.03 and 0.03
P. aeruginosa 153	0.06 and 0.12	0.06 and 0.25	0.06 and 0.25	0.25 and 0.5
P. aeruginosa 158	0.06 and 0.25	0.12 and 0.25	0.12 and 0.5	0.25 and 2.0
S. marcescens 186	0.03 and 0.03	0.03 and 0.06	0.06 and 0.06	0.12 and 0.25
S. marcescens 207	0.03 and 0.06	0.12 and 0.12	0.06 and 0.06	0.12 and 0.25

[a] MHB, Mueller-Hinton broth.

supplemented Mueller-Hinton broth. For example, the MIC for a *P. aeruginosa* isolate increased from 0.06 to 0.25 μg/ml, while the MBC increased from 0.12 to 0.5 μg/ml. Geometric mean MBCs were identical to or within twofold of the MBCs for *E. coli*, *K. pneumoniae*, *C. freundii*, *S. marcescens*, *P. aeruginosa*, *E. faecalis*, and *S. aureus* (five isolates each). The effect of pH on the MICs of AM-1091 was determined at pHs 5.5, 6.5, and 7.5. The optimal pH of the compound was 7.5 for members of the family *Enterobacteriaceae*, with a 16-fold increase in the MIC for *E. coli* at pH 5.5. The geometric mean MIC for *P. aeruginosa* was only 0.37 μg/ml, even at pH 5.5 (Table 4). Although an increase in inoculum size from 10^5 CFU to 10^7 CFU increased the MICs, the increase was only to 0.38 μg/ml for *P. aeruginosa* and to 0.01 μg/ml for *E. coli*. In general, except for *P. aeruginosa* at 10^7 CFU, the MICs determined at 10^7 CFU only increased twofold over the MICs determined at 10^5 CFU.

Activity against permeability mutants. Differences in susceptibility to quinolones because of outer membrane changes have been shown by Hirai et al. (5). The MICs and MBCs were determined for isolates of *E. coli*, provided by H. Nakaido, in which either OmpC or OmpF was deficient. The MIC and MBC for both the $OmpF^+C^+$ strain and the $OmpF^+C^-$ strain were 0.008 and 0.015 μg/ml, respectively. The MIC and MBC for the $OmpF^-C^+$ strain were 0.015 and 0.015 μg/ml, respectively. When *P. aeruginosa* 799K (11) was used, the MIC for the permeable mutant was 0.06 μg/ml, as compared with 0.25 μg/ml for the parent strain.

Development of resistance to AM-1091. The development of spontaneous resistance to AM-1091 was determined for two isolates each of *E. coli*, *K. pneumoniae*, *C. freundii*, *E. cloacae*, *S. marcescens*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*. For all of these organisms the frequency of resistance to a concentration eight times the MIC was $<10^{-9}$, and in most situations it was $<10^{-10}$.

Repeated subculturing in the presence of AM-1091 resulted in increases in MICs. The MIC for *E. coli* 4017 increased from 0.004 to 0.03 μg/ml, that for *K. pneumoniae* increased from 0.015 to 2 μg/ml, that for *P. aeruginosa* increased from 0.06 to 2 μg/ml, and that for *S. aureus* increased from 0.015 to 0.03 μg/ml. The increase in MICs was stable. MICs of ciprofloxacin, ofloxacin, and norfloxacin also increased (Table 5). These isolates did not show cross-resistance to ureido-penicillins or aminothiazoly-cephalosporins or aminoglycosides. Susceptibilities to tetracycline and chloramphenicol were not determined.

Killing curves and PAE. Exposure of a *P. aeruginosa* strain for which the AM-1091 MIC was 0.25 μg/ml to the MIC for 15, 30, and 60 min produced 1.15-, 1.4-, and 2.23-$\log_{10}$ reductions in CFU, respectively. A 60-min exposure of *P. aeruginosa* to a concentration of AM-1091 eight times the MIC in urine produced a 4.1-$\log_{10}$ reduction in CFU. AM-1091 at eight times the MIC produced a PAE of 5 h for *P. aeruginosa* and a PAE of 6 h for *E. coli* in Mueller-Hinton broth (pH 7.4) with an Mg^{2+} concentration similar to that in human serum. Similarly, in urine AM-1091 at a concentration 16 times the MIC produced a PAE of 5 h for both *E. coli* and *P. aeruginosa*.

DISCUSSION

AM-1091 differs from a number of the currently available fluoroquinolone compounds. It possesses at position N-1 a cyclopropyl group, but the piperazinyl group at position C-7 has been replaced by a 3-amino-1-pyrrolidinyl group, and at position C-8 there is a chlorine. It would appear from this study that these structural modifications of the quinolone compound have resulted in enhanced antibacterial activity against gram-positive bacteria and anaerobic species. For example, the MIC_{90} of AM-1091 for methicillin-susceptible and methicillin-resistant *S. aureus* isolates was 0.06 μg/ml, as compared with 1 to 2 μg/ml for ciprofloxacin. The activity of AM-1091 against streptococci and *S. pneumoniae* was also better than that of the other quinolone agents currently available (3, 10). In this study *B. fragilis* and other *Bacteroides* and *Clostridium* spp. were also inhibited by <1 μg/ml, in contrast to the much higher concentrations required for other agents. The increase in activity against the gram-positive species, particularly streptococci, and against anaerobes has not resulted in a loss of activity against members of the family *Enterobacteriaceae* and *P. aeruginosa*, as occurred with CI-934 (7), since these organisms were inhibited at concentrations lower than or similar to those of the currently most active quinolone, ciprofloxacin. Ciprofloxacin remains the most active agent tested against *P. aeruginosa* on an overall basis, although some strains were inhibited by a twofold-lower concentration of AM-1091.

TABLE 4. Effect of pH on the MICs of AM-1091

Organism[a]	Geometric mean MIC (μg/ml) at pH:		
	5.5	6.5	7.5
E. coli	0.03	0.002	0.002
K. pneumoniae	0.27	0.024	0.013
S. marcescens	0.61	0.05	0.04
P. aeruginosa	0.37	0.27	0.25

[a] Seven isolates each.

TABLE 5. MICs of other quinolone compounds for bacteria repeatedly exposed to AM-1091 for 14 days

Organism	MIC/MBC (μg/ml)			
	AM-1091	Ciprofloxacin	Ofloxacin	Norfloxacin
E. coli 4017				
Wild type	0.004/0.004	0.004/0.008	0.3/0.3	0.015/0.015
Mutant[a]	0.03/0.12	0.5/1	0.5/2	1/4
K. pneumoniae 5561				
Wild type	0.015/0.5	0.015/0.03	0.12/0.25	0.12/0.12
Mutant	2/2	8/8	16/64	16/64
P. aeruginosa 153				
Wild type	0.06/0.25	0.12/1	0.5/4	0.25/2
Mutant	2/4	4/8	32/64	16/32
S. aureus				
Wild type	0.015/0.015	0.25/0.25	ND[b]	ND
Mutant	0.03/0.12	0.5/2	ND	ND

[a] Mutant, Isolate selected after 14 days of subculturing in increasing concentrations of AM-1091.
[b] ND, Not determined.

AM-1091 inhibited imipenem-, ceftazidime-, amikacin-, and piperacillin-resistant *P. aeruginosa*. It was more active than other quinolones or other agents against *X. maltophilia* and *P. cepacia*. Of particular note was the excellent activity against the *Acinetobacter* spp., most of which were resistant to gentamicin and ceftazidime and for which the MIC_{90}s of both ciprofloxacin and ofloxacin were 1 μg/ml.

Like the activity of other quinolones (6), the activity of AM-1091 was reduced at pH 5.5, but its activity was minimally decreased by high Mg^{2+} concentrations. At concentrations anticipated in urine or in serum it showed rapid killing and prolonged PAE even for pathogens for which the MICs were higher. These results are similar to what we have shown for ciprofloxacin and compound T-3262 (A-60969) (1, 4).

The frequency of spontaneous resistance to AM-1091 was low, but isolates for which MICs were higher could be selected by repeated passage in the drug. This resistance was stable, although the MICs of AM-1091 were appreciably lower than the MICs of ciprofloxacin, ofloxacin, and norfloxacin for these organisms.

Because of its excellent in vitro activity, AM-1091 certainly should undergo further evaluation to determine its pharmacology and potential for clinical use.

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