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In Vitro Antibacterial Activities of AF 3013, the Active Metabolite of Prulifloxacin, against Nosocomial and Community Italian Isolates

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AF 3013, the active metabolite of prulifloxacin, was tested to determine its inhibitory and bactericidal activities against 396 nosocomial and 258 community Italian isolates. Compared with that of ciprofloxacin, its activity (assessed in MIC and minimal bactericidal concentration tests) was generally similar or greater against gram-positive bacteria and greater against gram-negative bacteria. In time-kill assays using selected isolates, its bactericidal activity was comparable to that of ciprofloxacin.

Nonfluorinated quinolones, developed in the 1960s and 1970s, had a spectrum limited to aerobic gram-negative bacteria; they had a poor systemic distribution but reached high concentrations in urine; their use was therefore confined to the treatment of urinary tract infections. The introduction of fluoroquinolones (i.e., molecules fluorinated in the C-6 position) marked a dramatic improvement (2). The earlier drugs of this group, developed in the late 1970s and in the 1980s, were suitable for a far wider clinical use by virtue of a broader spectrum, encompassing gram-positive bacteria, and a good systemic distribution. Other fluoroquinolones, which had further-enhanced activity against gram-positive bacteria and were also variably active against anaerobes, were developed in the 1990s (10). However, several of these newer quinolones ended up being limited in their clinical use due to various, unexpected toxicity problems (1).

AF 3013, a fluoroquinolone formerly called NM394 (5, 8, 11), is the active compound derived from the transformation of the prodrug prulifloxacin (also called NM441 or AF 3012) after its oral administration and intestinal absorption. Prulifloxacin and AF 3013 were developed in the late 1980s in Japan. The in vitro activity of AF 3013 has so far been investigated only in that country, against strains mostly isolated in the 1980s (11). At present, prulifloxacin is being considered for marketing in Italy and other European countries. This prompted us to investigate the in vitro activity of its active form, AF 3013, against a variety of nosocomial and community bacterial strains recently isolated in Italy. AF 3013 was obtained from Angelini ACRAF, Pomezia, Italy. Of six additional fluoroquinolone molecules tested as comparators, two (ciprofloxacin and ofloxacin) were purchased from Sigma Chemical Co. (St. Louis, Mo.), and four (levofloxacin, sparfloxacin, trovafloxacin, and moxifloxacin) were from their respective manufacturers.

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Bacteria. A total of 654 cultures of aerobic bacteria were examined, all freshly isolated (in 1998 to 2000) from clinical material in several Italian laboratories. Multiple isolates from the same patient were avoided. Most of these cultures (151 gram-positive strains and 245 gram-negative strains), isolated from inpatients >48 h after hospital admission, were regarded as being associated with nosocomial infections. The remaining (102 gram-positive and 156 gram-negative) strains, isolated from outpatients, were regarded as being associated with community infections. Most strains were initially identified using commercial and automated biochemical test systems, but the identification of several isolates was confirmed by evaluating additional distinguishing characters relating to the laboratory determination of genus and species (4). Based on MIC tests and breakpoints as recommended by the National Committee for Clinical Laboratory Standards (7), staphylococci were preliminarily characterized as oxacillin susceptible (MICs of $\leq$2 μg/ml for *Staphylococcus aureus* strains and $\leq$0.25 μg/ml for coagulase-negative staphylococci) or oxacillin resistant (MICs of $\geq$4 μg/ml for *S. aureus* strains and $\geq$0.5 μg/ml for coagulase-negative staphylococci).

MIC tests. MICs were determined by standard microdilution broth tests as recommended by the National Committee for Clinical Laboratory Standards (7). Antibiotics were tested at final concentrations, prepared from serial twofold dilutions, ranging from 0.015 to 128 μg/ml. Except for *Haemophilus* strains, the test medium was Mueller-Hinton II broth (BBL Microbiology Systems, Cockeysville, Md.), supplemented with 5% lysed horse blood when streptococci and listeriae were tested. The *Haemophilus* strains were tested in Haemophilus test medium (7). The inoculum was 5×10^5 CFU/ml. The inoculated trays were incubated at 35°C for 18 to 24 h.

A comparison of the MICs of AF 3013 and the other fluoroquinolones for gram-positive bacteria is detailed in Table 1. It is worth stressing that the MICs of AF 3013 for 50% (MIC_{50}) and 90% (MIC_{90}) of *S. aureus* strains were identical (0.25 μg/ml) for oxacillin-susceptible isolates and were 16 and 32 μg/ml, respectively, for oxacillin-resistant isolates. The MIC_{50}

TABLE 1. Comparative activities of AF 3013, ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, trovafloxacin, and moxifloxacin against 253 (151 nosocomial and 102 community) isolates of gram-positive bacteria

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)			MBC (μg/ml)		
		Range	50%	90%	Range	50%	90%
Nosocomial isolates (151)							
Staphylococcus aureus, oxacillin susceptible (30)	AF 3013	0.03–0.5	0.25	0.25	0.12–2	0.25	0.5
	Ciprofloxacin	0.12–0.5	0.25	0.5	0.25–2	0.5	1
	Ofloxacin	0.12–4	0.25	0.5	0.12–16	0.5	1
	Levofloxacin	0.06–0.25	0.12	0.25	0.12–2	0.25	0.5
	Sparfloxacin	0.03–0.12	0.06	0.12	0.06–0.25	0.12	0.12
	Trovafloxacin	≤0.015–0.25	0.06	0.12	0.03–0.5	0.12	0.12
	Moxifloxacin	≤0.015–0.5	0.06	0.12	0.03–1	0.12	0.12
Staphylococcus aureus, oxacillin resistant (31)	AF 3013	0.12–>128	16	32	0.25–>128	32	128
	Ciprofloxacin	0.25–>128	32	32	0.5–>128	64	>128
	Ofloxacin	0.25–>128	16	32	0.5–>128	32	128
	Levofloxacin	0.12–64	8	16	0.25–>128	16	32
	Sparfloxacin	0.06–32	16	16	0.12–128	32	128
	Trovafloxacin	0.06–8	2	4	0.06–16	4	4
	Moxifloxacin	0.06–16	2	4	0.12–16	4	4
Coagulase-negative staphylococci, oxacillin susceptible (14)[a]	AF 3013	0.03–16	0.12	8	0.03–64	0.12	32
	Ciprofloxacin	0.03–32	0.25	16	0.03–64	0.25	64
	Ofloxacin	0.12–16	0.25	8	0.12–128	0.5	64
	Levofloxacin	0.03–8	0.12	8	0.06–64	0.12	32
	Sparfloxacin	≤0.015–8	0.12	8	0.03–32	0.25	16
	Trovafloxacin	≤0.015–2	0.06	1	0.03–4	0.06	2
	Moxifloxacin	≤0.015–2	0.06	1	0.03–4	0.12	1
Coagulase-negative staphylococci, oxacillin resistant (22)[b]	AF3013	0.25–>128	16	64	0.25–>128	32	>128
	Ciprofloxacin	1–128	32	64	1–>128	64	>128
	Ofloxacin	2–>128	16	64	2–>128	32	128
	Levofloxacin	0.25–128	8	16	0.25–128	16	64
	Sparfloxacin	2–64	8	16	2–128	16	64
	Trovafloxacin	0.12–32	2	8	0.5–64	4	16
	Moxifloxacin	0.12–32	2	8	0.25–32	2	16
Enterococcus faecalis (26)	AF 3013	0.12–64	0.5	2	0.25–128	1	16
	Ciprofloxacin	0.25–64	1	2	0.5–128	2	8
	Ofloxacin	0.5–128	2	8	1–128	4	16
	Levofloxacin	0.25–32	0.5	2	0.5–32	1	16
	Sparfloxacin	0.12–32	0.25	1	0.25–128	0.5	2
	Trovafloxacin	0.03–16	0.25	2	0.25–16	0.5	4
	Moxifloxacin	≤0.015–16	0.25	0.5	0.12–16	0.5	2
Enterococcus faecium (18)	AF 3013	0.03–>128	0.5	16	0.5–>128	1	128
	Ciprofloxacin	0.5–>128	1	16	1–>128	4	128
	Ofloxacin	2–>128	2	32	4–>128	8	128
	Levofloxacin	0.12–>128	0.5	16	1–>128	2	64
	Sparfloxacin	0.12–64	0.5	16	0.5–128	2	128
	Trovafloxacin	0.06–64	0.5	2	0.25–128	2	16
	Moxifloxacin	0.03–64	0.25	4	0.25–128	1	16
Listeria monocytogenes (10)	AF 3013	0.06–1	0.5	1	0.5–4	0.5	2
	Ciprofloxacin	0.25–4	1	4	0.5–8	1	8
	Ofloxacin	0.5–4	1	4	2–16	4	16
	Levofloxacin	0.5–2	1	2	1–4	2	4
	Sparfloxacin	0.03–2	1	2	1–4	2	4
	Trovafloxacin	0.06–0.25	0.12	0.25	0.25–2	0.5	0.5
	Moxifloxacin	0.06–0.5	0.12	0.5	0.5–2	0.5	0.5
Community isolates (n = 102)							
Staphylococcus aureus, oxacillin-susceptible (26)	AF3013	0.12–0.5	0.25	0.25	0.12–2	0.25	0.5
	Ciprofloxacin	0.12–0.5	0.25	0.5	0.25–2	0.5	0.5
	Ofloxacin	0.06–1	0.25	0.5	0.12–2	0.25	0.5
	Levofloxacin	0.06–0.25	0.12	0.25	0.12–0.5	0.25	0.5
	Sparfloxacin	0.03–0.12	0.06	0.06	0.06–0.25	0.12	0.25
	Trovafloxacin	≤0.015–0.12	0.03	0.06	≤0.015–0.25	0.06	0.12
	Moxifloxacin	≤0.015–0.25	0.03	0.06	0.03–0.25	0.06	0.12

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TABLE 1—*Continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)			MBC (μg/ml)		
		Range	50%	90%	Range	50%	90%
Streptococcus pyogenes (21)	AF 3013	0.12–2	0.25	1	0.12–4	0.25	2
	Ciprofloxacin	0.25–2	0.25	1	0.25–4	0.5	4
	Ofloxacin	0.25–4	0.5	4	0.5–8	1	4
	Levofloxacin	0.25–2	0.25	1	0.25–2	0.5	2
	Sparfloxacin	0.06–1	0.25	0.5	0.12–2	0.25	1
	Trovafloxacin	0.03–0.5	0.06	0.5	0.06–2	0.12	0.5
	Moxifloxacin	0.06–0.5	0.12	0.25	0.12–1	0.12	0.5
Streptococcus pneumoniae (36)	AF 3013	≤0.015–2	0.5	1	≤0.015–4	0.5	2
	Ciprofloxacin	≤0.015–2	0.5	1	≤0.015–4	1	2
	Ofloxacin	0.25–2	1	2	0.5–4	2	2
	Levofloxacin	≤0.015–1	0.5	1	≤0.015–2	0.5	1
	Sparfloxacin	≤0.015–0.5	0.25	0.5	≤0.015–1	0.25	0.5
	Trovafloxacin	≤0.015–0.5	0.12	0.25	≤0.015–0.5	0.12	0.5
	Moxifloxacin	≤0.015–0.25	0.12	0.12	≤0.015–0.25	0.12	0.25
Enterococcus spp. (19)[c]	AF 3013	0.03–1	0.5	1	0.12–4	1	4
	Ciprofloxacin	0.06–2	0.5	1	0.12–4	2	2
	Ofloxacin	0.06–2	2	2	0.25–4	2	4
	Levofloxacin	0.03–1	0.5	1	0.12–4	1	2
	Sparfloxacin	0.06–0.5	0.25	0.5	0.12–2	0.5	1
	Trovafloxacin	0.06–1	0.25	0.25	0.06–2	0.25	1
	Moxifloxacin	0.03–1	0.25	0.25	0.06–1	0.25	0.5

[a] Seven strains of *S. epidermidis*, two strains of *S. haemolyticus*, two strains of *S. hominis*, one strain of *S. capitis*, one strain of *S. saprophyticus*, and one strain of *S. simulans*.
[b] Sixteen strains of *S. epidermidis*, four strains of *S. haemolyticus*, one strain of *S. hominis*, and one strain of *S. simulans*.
[c] Sixteen strains of *E. faecalis*, two strains of *E. faecium*, and one strain of *E. durans*.

and MIC_{90} for *Streptococcus pneumoniae* were 0.5 and 1 μg/ml. Compared with the respective values of AF 3013, the MIC_{50}s and MIC_{90}s of ciprofloxacin and ofloxacin for the gram-positive bacteria tested ranged from identical to four times higher; those of levofloxacin and sparfloxacin ranged from identical to four times lower (but to two times higher for *L. monocytogenes* strains); and those of trovafloxacin and moxifloxacin ranged from identical to eight times lower.

A comparison of the MICs of AF 3013 and the other fluoroquinolones against gram-negative bacteria is detailed in Table 2. In particular, against nosocomial isolates of the different *Enterobacteriaceae* genera, the MIC_{50}s of AF 3013 ranged from ≤0.015 to 0.03 μg/ml, and the MIC_{90}s ranged from 0.5 to 8 μg/ml. Against community isolates of *Enterobacteriaceae*, the MIC_{50}s of AF 3013 were consistently ≤0.015 μg/ml, whereas the MIC_{90}s for the different genera ranged from 0.12 to 2 μg/ml. Compared with AF 3013, the MIC_{50}s of ciprofloxacin against *Enterobacteriaceae* isolates ranged from identical to four times higher, and the MIC_{90}s ranged from two to four times higher; the other fluoroquinolones usually exhibited similar or greater differences. Differences between AF 3013 and the other drugs being compared were of little significance for those organisms, such as *Pseudomonas aeruginosa* and *Acinetobacter* isolates, against which the MIC_{90}s of all drugs fell well in the range of resistance. All fluoroquinolones tested were highly active against *Moraxella catarrhalis* and *Haemophilus* isolates.

MBC tests. Minimal bactericidal concentrations (MBCs) were established by extending the MIC procedure to the evaluation of bactericidal activity (6). After the MIC was read, 0.01-ml volumes were drawn with an Eppendorf pipette from the wells showing no growth and spotted onto suitable agar plates. These plates were incubated at 35°C for 24 to 48 h. The MBC was read as the lowest antibiotic concentration which resulted in ≤0.1% survival in the subculture.

The MBCs of AF 3013 for gram-positive isolates (Table 1) were usually identical to or twofold the respective MICs; higher MBC-to-MIC ratios were observed less frequently. The MBCs of AF 3013 for gram-negative isolates (Table 2) usually ranged from identical to fourfold the respective MICs. Comparable MBC-to-MIC ratios were generally yielded by the other fluoroquinolones.

Time-kill assays. AF 3013 and ciprofloxacin were investigated for their killing kinetics against four test strains (A430, an oxacillin-susceptible *S. aureus* isolate; C330, a ciprofloxacin-susceptible *Escherichia coli* isolate; C1061, a ciprofloxacin-resistant *E. coli* isolate; and M578, a *Proteus mirabilis* isolate) (Fig. 1). Time-kill experiments were performed by standard procedures (6, 9) using tubes containing 10 ml of the test medium used in MIC assays. Three doubling concentrations of each antibiotic (1, 2, and 4 times the MIC) were tested. Drug-free control tubes were included in each run. To inoculate each tube of serially diluted antibiotic, 50 μl of diluted inoculum was delivered by pipette beneath the surface of the broth. The mixture was then vortexed and plated for viability counts (0 h) as reported below. Only tubes containing an initial inoculum within the range of 5×10^5 to 1×10^6 CFU/ml were considered acceptable. Cultures were incubated at 35°C with shaking. At 4-, 8-, and 24-h intervals, viable counts were performed in

TABLE 2. Comparative activities of AF 3013, ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, trovafloxacin, and moxifloxacin against 401 (245 nosocomial and 156 community) isolates of gram-negative bacteria

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)			MBC (μg/ml)		
		Range	50%	90%	Range	50%	90%
Nosocomial isolates (245)							
Escherichia coli (41)	AF 3013	≤0.015–16	≤0.015	4	≤0.015–32	0.03	16
	Ciprofloxacin	≤0.015–32	≤0.015	16	≤0.015–128	0.03	32
	Ofloxacin	0.03–64	0.06	32	0.03–128	0.12	64
	Levofloxacin	≤0.015–64	0.06	32	≤0.015–128	0.06	32
	Sparfloxacin	≤0.015–128	0.06	16	≤0.015–128	0.06	16
	Trovafloxacin	≤0.015–128	0.03	16	≤0.015–128	0.06	16
	Moxifloxacin	≤0.015–128	0.03	32	≤0.015–128	0.12	32
Klebsiella spp. (33)[a]	AF 3013	≤0.015–4	≤0.015	2	≤0.015–4	0.03	4
	Ciprofloxacin	≤0.015–16	0.03	8	≤0.015–32	0.03	16
	Ofloxacin	≤0.015–32	0.12	16	≤0.015–32	0.12	16
	Levofloxacin	≤0.015–16	0.06	8	≤0.015–16	0.06	16
	Sparfloxacin	≤0.015–16	0.06	8	≤0.015–32	0.12	16
	Trovafloxacin	≤0.015–16	0.06	4	≤0.015–32	0.12	16
	Moxifloxacin	0.03–16	0.12	8	0.03–32	0.25	16
Enterobacter spp. (31)[b]	AF 3013	≤0.015–64	≤0.015	8	≤0.015–128	≤0.015	16
	Ciprofloxacin	≤0.015–>128	0.03	32	≤0.015–>128	0.03	64
	Ofloxacin	≤0.015–128	0.12	32	≤0.015–128	0.25	64
	Levofloxacin	≤0.015–64	0.03	32	≤0.015–64	0.03	32
	Sparfloxacin	≤0.015–128	0.03	64	0.03–>128	0.12	64
	Trovafloxacin	0.03–128	0.12	64	0.06–128	0.12	64
	Moxifloxacin	0.03–128	0.06	32	0.03–128	0.12	64
Serratia spp. (19)[c]	AF 3013	≤0.015–16	0.03	0.5	≤0.015–64	0.03	0.5
	Ciprofloxacin	≤0.015–32	0.12	1	≤0.015–64	0.12	1
	Ofloxacin	0.03–32	0.25	2	0.03–32	0.25	2
	Levofloxacin	0.03–8	0.12	1	0.03–8	0.12	2
	Sparfloxacin	0.06–32	0.25	4	0.12–32	0.5	8
	Trovafloxacin	0.06–16	0.25	2	0.25–32	2	8
	Moxifloxacin	0.06–16	0.25	2	0.12–16	2	8
Citrobacter spp. (12)[d]	AF 3013	≤0.015–2	≤0.015	2	≤0.015–2	1	2
	Ciprofloxacin	≤0.015–32	0.03	4	0.03–32	0.5	8
	Ofloxacin	0.12–32	0.12	16	0.5–32	4	16
	Levofloxacin	≤0.015–16	0.03	4	0.06–16	2	8
	Sparfloxacin	0.06–64	0.25	16	0.12–64	4	16
	Trovafloxacin	0.03–64	0.12	8	0.12–64	4	16
	Moxifloxacin	0.03–64	0.12	16	0.06–64	4	16
Proteus, *Providencia*, and *Morganella* spp. (44)[e]	AF 3013	≤0.015–4	≤0.015	0.5	≤0.015–4	≤0.015	0.5
	Ciprofloxacin	≤0.015–16	0.03	2	≤0.015–32	0.03	2
	Ofloxacin	≤0.015–16	0.12	2	0.03–16	0.25	4
	Levofloxacin	≤0.015–8	0.06	0.5	≤0.015–8	0.06	2
	Sparfloxacin	0.03–64	0.12	8	0.03–128	0.25	16
	Trovafloxacin	≤0.015–16	0.25	4	≤0.015–32	0.5	8
	Moxifloxacin	0.03–16	0.25	4	0.06–32	1	8
Pseudomonas aeruginosa (45)	AF 3013	0.03–64	2	64	0.06–128	4	64
	Ciprofloxacin	0.06–128	8	128	0.06–128	8	128
	Ofloxacin	0.25–>128	16	>128	1–>128	32	>128
	Levofloxacin	0.12–>128	8	128	0.5–>128	32	>128
	Sparfloxacin	0.12–>128	16	>128	0.25–>128	32	>128
	Trovafloxacin	0.25–>128	16	>128	0.25–>128	32	>128
	Moxifloxacin	0.25–>128	16	>128	1–>128	64	>128
Stenotrophomonas maltophilia (10)	AF 3013	0.25–16	2	4	1–32	8	8
	Ciprofloxacin	0.25–8	2	4	1–32	4	8
	Ofloxacin	1–16	2	16	1–32	4	32
	Levofloxacin	0.25–8	1	4	1–32	2	8
	Sparfloxacin	0.25–8	1	4	0.5–16	1	8
	Trovafloxacin	0.25–4	1	2	0.5–16	1	4
	Moxifloxacin	0.12–4	0.5	2	0.25–16	1	4
Acinetobacter spp. (10)[f]	AF 3013	0.12–32	4	32	0.25–64	8	64
	Ciprofloxacin	0.06–64	4	16	0.12–64	8	64
	Ofloxacin	0.03–16	4	8	0.12–32	8	32
	Levofloxacin	0.06–16	2	8	0.12–16	4	16
	Sparfloxacin	≤0.015–32	0.25	32	≤0.015–32	2	32
	Trovafloxacin	0.06–16	0.5	16	0.25–32	4	16
	Moxifloxacin	0.06–16	0.5	8	0.25–16	2	16
Community isolates (n = 156)							
Escherichia coli (37)	AF 3013	≤0.015–1	≤0.015	0.12	≤0.015–1	0.03	0.5
	Ciprofloxacin	≤0.015–8	≤0.015	0.5	≤0.015–16	0.03	1
	Ofloxacin	≤0.015–16	0.06	0.25	≤0.015–16	0.06	1
	Levofloxacin	≤0.015–4	0.03	0.25	≤0.015–8	0.06	0.5

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TABLE 2—*Continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)			MBC (μg/ml)		
		Range	50%	90%	Range	50%	90%
	Sparfloxacin	≤0.015–16	≤0.015	0.25	≤0.015–16	0.03	1
	Trovafloxacin	≤0.015–16	0.03	0.25	≤0.015–16	0.03	1
	Moxifloxacin	≤0.015–16	0.03	0.5	≤0.015–32	0.06	1
Klebsiella spp. (15)[g]	AF 3013	≤0.015–0.25	≤0.015	0.12	≤0.015–0.25	0.06	0.12
	Ciprofloxacin	≤0.015–0.5	0.03	0.25	≤0.015–0.5	0.06	0.25
	Ofloxacin	≤0.015–1	0.12	0.5	0.06–1	0.12	0.5
	Levofloxacin	0.03–0.5	0.06	0.25	0.03–0.5	0.06	0.5
	Sparfloxacin	≤0.015–0.5	0.06	0.25	≤0.015–0.5	0.06	0.25
	Trovafloxacin	≤0.015–0.5	0.06	0.25	≤0.015–0.5	0.06	0.25
	Moxifloxacin	0.03–1	0.12	0.25	0.03–1	0.12	0.25
Enterobacter spp. (10)[h]	AF 3013	≤0.015–8	≤0.015	2	≤0.015–16	≤0.015	4
	Ciprofloxacin	≤0.015–32	≤0.015	8	≤0.015–64	0.03	8
	Ofloxacin	0.06–64	0.06	16	0.12–64	0.12	16
	Levofloxacin	≤0.015–32	0.03	8	≤0.015–64	0.03	8
	Sparfloxacin	≤0.015–64	0.06	16	0.03–64	0.06	16
	Trovafloxacin	0.03–64	0.06	16	0.03–64	0.12	16
	Moxifloxacin	0.03–32	0.06	16	0.03–64	0.12	16
Serratia spp. (5)[i]	AF 3013	≤0.015–0.03			≤0.015–0.03		
	Ciprofloxacin	≤0.015–0.03			≤0.015–0.12		
	Ofloxacin	0.06–0.12			0.06–0.12		
	Levofloxacin	0.03–0.06			0.03–0.06		
	Sparfloxacin	≤0.015–0.12			≤0.015–0.12		
	Trovafloxacin	≤0.015–0.12			0.03–0.12		
	Moxifloxacin	0.03–0.12			0.03–0.25		
Citrobacter spp. (10)[j]	AF 3013	≤0.015–2	≤0.015	0.25	≤0.015–4	≤0.015	0.5
	Ciprofloxacin	≤0.015–4	≤0.015	1	≤0.015–4	0.03	2
	Ofloxacin	0.03–8	0.12	2	0.03–8	0.12	4
	Levofloxacin	≤0.015–4	0.06	1	≤0.015–8	0.12	4
	Sparfloxacin	≤0.015–16	0.03	2	≤0.015–32	0.12	4
	Trovafloxacin	≤0.015–16	0.06	4	0.03–16	0.12	4
	Moxifloxacin	≤0.015–16	0.03	4	0.03–32	0.12	4
Proteus, Providencia, and *Morganella* spp. (23)[k]	AF 3013	≤0.015–2	≤0.015	0.5	≤0.015–8	≤0.015	1
	Ciprofloxacin	≤0.015–2	0.03	1	≤0.015–16	0.03	4
	Ofloxacin	≤0.015–2	0.06	1	0.03–16	0.25	4
	Levofloxacin	≤0.015–2	0.06	0.5	≤0.015–8	0.06	2
	Sparfloxacin	0.03–4	0.12	2	0.06–8	0.12	8
	Trovafloxacin	0.03–4	0.12	4	0.06–16	0.25	8
	Moxifloxacin	0.06–8	0.25	4	0.12–16	0.5	8
Pseudomonas aeruginosa (16)	AF 3013	0.06–64	1	32	0.25–128	2	128
	Ciprofloxacin	0.06–128	2	64	0.25–128	4	128
	Ofloxacin	0.12–>128	8	128	0.5–>128	16	>128
	Levofloxacin	0.5–128	4	64	1–>128	8	128
	Sparfloxacin	0.12–>128	8	128	0.5–>128	32	>128
	Trovafloxacin	0.25–>128	32	128	1–>128	32	>128
	Moxifloxacin	0.5–>128	64	128	2–>128	64	>128
Moraxella catarrhalis (16)	AF 3013	≤0.015–0.03	≤0.015	0.03	≤0.015–0.03	0.03	0.03
	Ciprofloxacin	All ≤0.015	≤0.015	≤0.015	≤0.015–0.03	0.03	0.03
	Ofloxacin	≤0.015–0.06	≤0.015	0.06	0.03–0.12	0.06	0.06
	Levofloxacin	≤0.015–0.03	≤0.015	0.03	≤0.015–0.03	0.03	0.03
	Sparfloxacin	All ≤0.015	≤0.015	≤0.015	All ≤0.015	≤0.015	≤0.015
	Trovafloxacin	≤0.015–0.03	≤0.015	0.03	≤0.015–0.03	≤0.015	0.03
	Moxifloxacin	All ≤0.015	≤0.015	≤0.015	≤0.015–0.03	≤0.015	≤0.015
Haemophilus spp. (24)[l]	AF 3013	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.03	≤0.015	0.03
	Ciprofloxacin	All ≤0.015	≤0.015	≤0.015	≤0.015–0.03	≤0.015	0.03
	Ofloxacin	≤0.015–0.06	≤0.015	0.06	≤0.015–0.06	0.03	0.06
	Levofloxacin	≤0.015–0.06	≤0.015	0.03	≤0.015–0.06	≤0.015	0.03
	Sparfloxacin	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.06	≤0.015	0.03
	Trovafloxacin	≤0.015–0.03	≤0.015	0.03	≤0.015–0.03	≤0.015	0.03
	Moxifloxacin	≤0.015–0.06	≤0.015	0.03	≤0.015–0.06	≤0.015	0.03

[a] Twenty nine strains of *K. pneumoniae* and four strains of *K. okytoca*.
[b] Twenty strains of *E. cloacae* and 11 strains of *E. aerogenes*.
[c] Fourteen strains of *S. marcescens* and five strains of the *S. liquefaciens* group.
[d] Nine strains of *C. freundii* and three strains of *C. koseri*.
[e] Twenty six strains of *Proteus mirabilis*, four strains of *P. vulgaris*, four strains of *Providencia stuartii*, and 10 strains of *M. morganii*.
[f] Eight strains of *A. baumannii* and two strains of *A. lwoffii*.
[g] Twelve strains of *K. pneumoniae* and three strains of *K. okytoca*.
[h] Six strains of *E. cloacae*, three strains of *E. aerogenes*, and one strain of *E. sakazakii*.
[i] Four strains of *S. marcescens* and one strain of the *S. liquefaciens*-group.
[j] Nine strains of *C. freundii* and one strain of *C. koseri*.
[k] Seven strains of *Proteus mirabilis*, one strain of *P. vulgaris*, five strains of *Providencia stuartii*, three strains of *P. rettgeri*, and seven strains of *M. morganii*.
[l] Sixteen strains of *H. influenzae* and six strains of *H. parainfluenzae*.

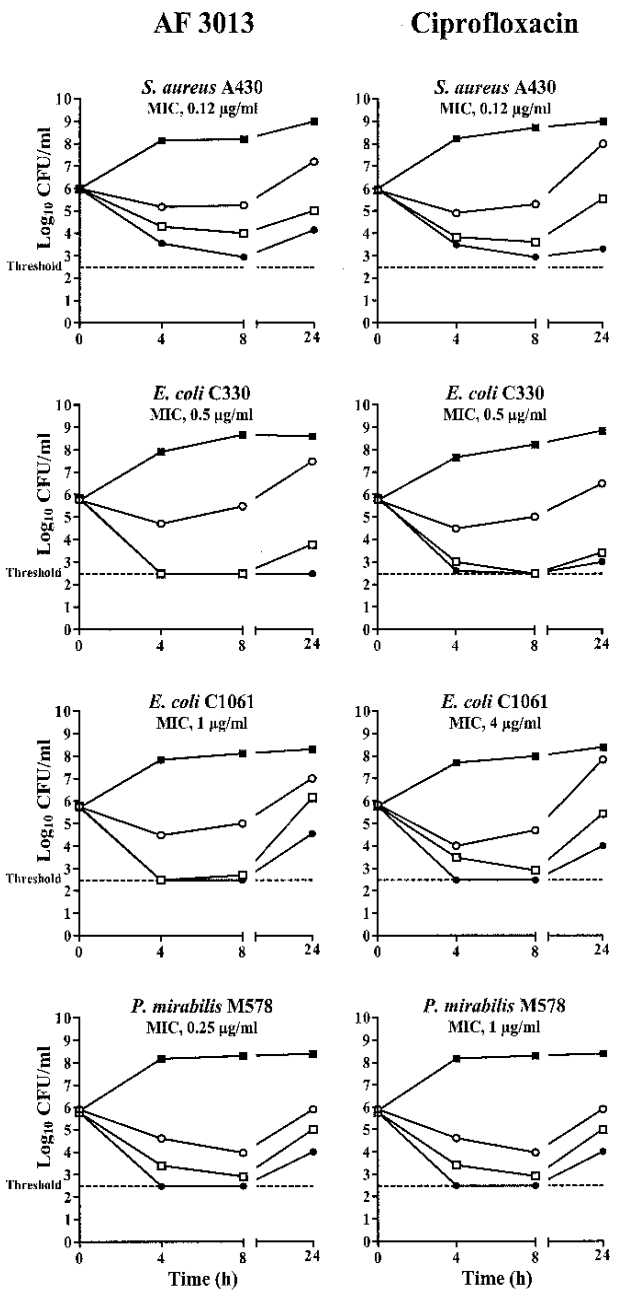

FIG. 1. Killing kinetics of AF 3013 and ciprofloxacin against four clinical isolates: *S. aureus* A430 (oxacillin susceptible), *E. coli* C330 (ciprofloxacin susceptible), *E. coli* C1061 (ciprofloxacin resistant), and *P. mirabilis* M578. ■, growth control; ○, 1× MIC; □, 2× MIC; ●, 4× MIC. All experiments were done in triplicate. Standard deviations of the reported values were below 1 log.

triplicate by spreading aliquots of 0.1 ml of the suitable dilutions onto plates of Trypticase soy agar (BBL). Plates were incubated for up to 48 h, and colony counts were performed on plates yielding 30 to 300 colonies (3). A threshold corresponding to the lower end of the range (30 colonies, i.e., 300 CFU/ml) was therefore used. Antibiotics were considered bactericidal when the original inoculum was reduced by ≥ 3 $\log_{10}$ CFU/ml ($\geq 99.9\%$).

At concentrations equal to the MIC, no bactericidal effect was found with either AF 3013 or ciprofloxacin, the viable count reductions in the first 4 to 8 h being around 1 or 2 logs. At a concentration two times the MIC, AF 3013 was bactericidal at 4 and 8 h against both the ciprofloxacin-susceptible and ciprofloxacin-resistant *E. coli* isolates, and at 8 h it was also bactericidal against the *P. mirabilis* isolate; viable count reductions of about 2 logs were recorded at 4 and 8 h with the *S. aureus* isolate. At the same concentration relative to the MIC, ciprofloxacin was bactericidal at 8 h against the ciprofloxacin-susceptible *E. coli* isolate and the *P. mirabilis* isolate (at 4 h the viable count reductions were just below 3 logs). The reductions in viable count produced by ciprofloxacin for the ciprofloxacin-resistant *E. coli* isolate were lower than those caused by AF 3013; similar reductions were recorded for the *S. aureus* isolate. At concentrations four times the MIC, both AF 3013 and ciprofloxacin were bactericidal at 4 and 8 h against the two *E. coli* and the *P. mirabilis* isolates and at 8 h also against the *S. aureus* isolate. Regrowth at 24 h, frequently observed with both drugs, should probably be attributed to the easy selection for resistant mutants by fluoroquinolones (1, 2).

Conclusion. On the whole, the in vitro activity of AF 3013 against gram-positive bacteria appeared to be greater than that of ofloxacin, similar to or greater than that of ciprofloxacin, similar to or at a lower level than those of levofloxacin and sparfloxacin, and at a lower level than those of trovafloxacin and moxifloxacin. Against gram-negative bacteria (with the exception of *Stenotrophomonas maltophilia* and *Acinetobacter* isolates), AF 3013 was more active than ciprofloxacin and generally even more active than the other compared drugs. With reference to the earlier Japanese study (11), the comparison with ofloxacin and sparfloxacin for gram-positive bacteria and with ciprofloxacin for both gram-positive and gram-negative bacteria appears to be more in favor of AF 3013 for recently collected Italian isolates. Time-kill experiments using selected isolates confirmed that the bactericidal activity of AF 3013 was at least similar to that of ciprofloxacin. Altogether, the excellent inhibitory and bactericidal activities exhibited by AF 3013 in vitro, in addition to the favorable characteristics shown by its prodrug (prulifloxacin) in preliminary in vivo studies—experimental infections in mice (8), pharmacokinetic investigations with mice and dogs (8), and pharmacokinetic and safety studies with healthy human volunteers (5)—warrant further in vitro and in vivo studies and appropriate clinical trials addressing in particular the treatment of urinary infections.

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