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In Vitro Activity of BAY 12-8039, a New Fluoroquinolone

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The in vitro activity of BAY 12-8039, a new fluoroquinolone, was studied in comparison with those of ciprofloxacin, trovafloxacin (CP 99,219), cefpodoxime, and amoxicillin-clavulanate against gram-negative, gram-positive, and anaerobic bacteria. Its activity against mycobacteria and chlamydia was also investigated. BAY 12-8039 was active against members of the family *Enterobacteriaceae* (MIC at which 90% of strains tested were inhibited [MIC_{90}s] ≤1 μg/ml, except for *Serratia* spp. MIC_{90} 2 μg/ml), *Neisseria* spp. (MIC_{90}s, 0.015 μg/ml), *Haemophilus influenzae* (MIC_{90}, 0.03 μg/ml), and *Moraxella catarrhalis* (MIC_{90}, 0.12 μg/ml), and these results were comparable to those obtained for ciprofloxacin and trovafloxacin. Against *Pseudomonas aeruginosa*, the quinolones were more active than the β-lactam agents but BAY-12-8039 was less active than ciprofloxacin. Strains of *Stenotrophomonas maltophilia* were fourfold more susceptible to BAY 12-8039 and trovafloxacin (MIC_{90}s, 2 μg/ml) than to ciprofloxacin. BAY 12-8039 was as active as trovafloxacin but more active than ciprofloxacin against *Streptococcus pneumoniae* (MIC_{90}, 0.25 μg/ml) and methicillin-susceptible *Staphylococcus aureus* (MIC_{90}s, 0.12 μg/ml). The activity of BAY 12-8039 against methicillin-resistant *S. aureus* (MIC_{90}, 2 μg/ml) was lower than that against methicillin-susceptible strains. BAY 12-8039 was active against anaerobes (MIC_{90}s ≤ 2 μg/ml), being three- to fourfold more active against *Bacteroides fragilis*, *Prevotella* spp., and *Clostridium difficile* than was ciprofloxacin. Against *Mycobacterium tuberculosis*, BAY 12-8039 exhibited activity comparable to that of rifampin (MICs ≤ 0.5 μg/ml). Against *Chlamydia trachomatis* and *Chlamydia pneumoniae* BAY 12-8039 was more active (MICs ≤ 0.12 μg/ml) than either ciprofloxacin or erythromycin and exhibited a greater lethal effect than either of these two agents. The protein binding of BAY 12-8039 was determined at 1 and 5 μg/ml as 30 and 26.4%, respectively. The presence of human serum (at 20 or 70%) had no marked effect on the in vitro activity of BAY 12-8039.

BAY 12-8039 is a new fluoroquinolone derivative with a chemical nomenclature of 1-cyclopropyl-7-[(*S*,*S*)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid. It shares structural similarities with other agents, namely, a cyclopropyl group at position 1 (as ciprofloxacin has), a methoxy group at position 8 (as AM1155 has) (10), and a diazabicyclo group at position 7 (as BAY y3118 has) (5). Preliminary information suggests that BAY 12-8039 has enhanced activity against gram-positive bacterial pathogens (3). In this study, the activity of BAY 12-8039 was compared with that of other fluoroquinolones and the novel naphthyridone compound trovafloxacin (CP 99,219) (2) against a wide range of pathogens.

MATERIALS AND METHODS

Antimicrobial agents. The following agents were employed: BAY 12-8039 and ciprofloxacin (Bayer AG, Wuppertal, Germany), trovafloxacin (Pfizer Inc., Groton, Conn.), cefpodoxime (Roussel Uclaf, Romainville, France), amoxicillin and clavulanic acid (SmithKline Beecham, Worthing, United Kingdom), rifampin (Sigma, Poole, United Kingdom), rifampin (Sigma, Poole, United Kingdom), and erythromycin (Lilly Products, Basingstoke, United Kingdom). All agents were prepared and stored following the manufacturer's instructions.

Susceptibility testing. A total of 684 recent clinical isolates, 11 control strains, and 10 well-characterized β-lactamase-producing strains were studied. The control strains used were *Escherichia coli* NCTC 10418 and ATCC 25922, *Pseudomonas aeruginosa* NCTC 10662 and ATCC 27853, *Staphylococcus aureus* NCTC 6571 and ATCC 29213, *Streptococcus pneumoniae* NCTC 7465 and ATCC 49619, *Haemophilus influenzae* NCTC 11931 and ATCC 49247, and *Enterococcus faecalis* ATCC 29212. Susceptibilities were determined by a standard agar plate dilution method following recommendations in reference 1. Briefly, Iso-Sensitest agar (pH 7.2; Unipath, Basingstoke, United Kingdom) was employed for aerobic bacteria, supplemented with 50 μg of 1-(4-nitrophenyl)-glycerol (BDH, Poole, United Kingdom) per ml where necessary to prevent swarming. Supplements of 5% horse blood (Bradsure Biologicals, Loughborough, United Kingdom) and 20 μg of NAD (Sigma) per ml were added to support growth of fastidious bacteria. For anaerobic bacteria, Wilkins-Chalgren agar (Unipath) supplemented with 50 μg of 1-(4-nitrophenyl)-glycerol per ml and 5% horse blood was used. All strains were tested at a final inoculum of 10^4 CFU and for a few selected strains at an increased inoculum of 10^6 CFU, using a multipoint inoculator (Denley Instruments, Billingshurst, United Kingdom). Plates were incubated at 35 to 37°C for 18 to 24 h in air; or, for fastidious bacteria, in an atmosphere enriched with 4 to 6% carbon dioxide; or, for anaerobic bacteria, in an anaerobic cabinet (Don Whitley, Shipley, United Kingdom) in an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen.

The MIC was defined as the lowest antibiotic concentration at which no more than two colonies were observed. Amoxicillin and clavulanic acid were combined in a ratio of 2:1, and the results were recorded in terms of the amoxicillin MIC.

Mycobacterium susceptibility testing. The activity of BAY 12-8039 against mycobacteria was studied by an agar incorporation method using rifampin as a comparative agent. Recent clinical isolates of *Mycobacterium tuberculosis* (three resistant to one or more of the commonly used antimycobacterial agents and one susceptible strain) were studied. For both antibiotics a concentration range of 0.015 to 128 μg/ml (doubling dilutions up or down from 1 μg/ml) incorporated into Middlebrook 7H10 medium (Difco, Detroit, Mich.), containing 10% Middlebrook oleic acid-albumin-dextrose-catalase enrichment as a supplement, was used. Plates were incubated at 37°C in 5 to 10% carbon dioxide for 21 days. The lowest concentration of antibiotic that inhibited more than 99% of the bacterial population was considered to be the MIC (8).

Chlamydia susceptibility testing. The activity of BAY 12-8039 against one strain of *Chlamydia pneumoniae* and 3 strains of *Chlamydia trachomatis* was investigated in comparison with those of ciprofloxacin and erythromycin. The method employed was an adaptation of that of Webberley et al. (11). The MIC was taken as the lowest concentration to inhibit the development of inclusion bodies, and the minimum lethal concentration (MLC) was defined by the absence of inclusion bodies after a further 48-h incubation in drug-free medium.

Serum effect. The effect of human serum on the MIC and minimum bactericidal concentration (MBC) of BAY 12-8039 was determined for two strains each of *Streptococcus pyogenes*, *S. pneumoniae*, methicillin-sensitive *S. aureus* (MSSA), *Moraxella catarrhalis*, *E. coli*, and *Klebsiella pneumoniae*. A microdilution method was employed using Iso-Sensitest broth (Unipath) containing 20 or 70% human serum (Bradsure Biologicals) and supplemented for fastidious bacteria with 5% lysed horse blood and 20 μg of NAD per ml. Concentration ranges (doubling dilutions up or down from 1 μg/ml) of BAY 12-8039 were 0.008 to 8 μg/ml or 0.03 to 32 μg/ml (for fastidious bacteria). A final inoculum of 10^5 CFU/ml was used. Following incubation at 35 to 37°C in air or 4 to 6% carbon dioxide (for fastidious bacteria), 50 μl of broth culture was subcultured onto appropriate antibiotic-free medium for MBC determinations. The MIC was defined as the

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TABLE 1. The in vitro activity of BAY 12-8039 in comparison with those of other antimicrobial agents

Organism (no.)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
E. coli (39)	BAY 12-8039	0.06	1	0.03–32
	Trovafloxacin	0.06	1	0.015–>128
	Ciprofloxacin	0.015	0.5	0.008–64
	Cefpodoxime	0.25	4	0.12–>128
	Amoxicillin-clavulanate	2	16	0.5–32
Klebsiella spp. (30)	BAY 12-8039	0.12	0.5	0.06–4
	Trovafloxacin	0.12	0.5	0.06–8
	Ciprofloxacin	0.03	0.25	0.015–4
	Cefpodoxime	0.25	8	0.12–64
	Amoxicillin-clavulanate	4	8	1–32
P. mirabilis (30)	BAY 12-8039	0.25	0.25	0.12–0.5
	Trovafloxacin	0.25	0.25	0.12–0.5
	Ciprofloxacin	0.03	0.03	0.008–0.03
	Cefpodoxime	0.06	0.06	0.03–0.06
	Amoxicillin-clavulanate	0.5	4	0.25–8
P. vulgaris (15)	BAY 12-8039	0.25	0.25	0.06–0.5
	Trovafloxacin	0.25	0.5	0.06–1
	Ciprofloxacin	0.03	0.03	0.008–0.03
	Cefpodoxime	0.12	0.5	0.03–0.5
	Amoxicillin-clavulanate	2	8	0.5–8
M. morganii (15)	BAY 12-8039	0.12	0.25	0.03–0.25
	Trovafloxacin	0.25	0.5	0.06–1
	Ciprofloxacin	0.008	0.015	0.004–0.015
	Cefpodoxime	0.12	4	0.015–16
	Amoxicillin-clavulanate	64	64	16–128
Serratia spp. (20) [*S. marcescens* (15);	BAY 12-8039	0.5	2	0.03–16
S. liquefaciens (4)]	Trovafloxacin	0.5	4	0.06–64
	Ciprofloxacin	0.12	1	0.015–16
	Cefpodoxime	4	64	1–>128
	Amoxicillin-clavulanate	64	128	8–>128
Acinetobacter spp. (15) [*A. baumannii* (11);	BAY 12-8039	0.06	2	0.008–16
A. haemolyticus (3)]	Trovafloxacin	0.03	1	0.004–16
	Ciprofloxacin	0.25	8	0.015–128
	Cefpodoxime	16	>128	1–>128
	Amoxicillin-clavulanate	8	64	2–>128
P. aeruginosa (15)	BAY 12-8039	2	8	0.12–64
	Trovafloxacin	0.5	8	0.03–128
	Ciprofloxacin	0.25	4	0.015–32
	Cefpodoxime	>128	>128	128–>128
	Amoxicillin-clavulanate	128	>128	32–>128
S. maltophilia (13)	BAY 12-8039	0.5	2	0.06–2
	Trovafloxacin	0.5	2	0.12–8
	Ciprofloxacin	2	8	0.25–16
	Cefpodoxime	>128	>128	64–>128
	Amoxicillin-clavulanate	128	>128	64–>128
Enterobacter spp. (5)	BAY 12-8039			0.12
	Trovafloxacin			0.06–0.12
	Ciprofloxacin			0.015–0.03
	Cefpodoxime			1–>128
	Amoxicillin-clavulanate			4–128
Citrobacter spp. (5) [*C. diversus* (3);	BAY 12-8039			0.03–0.25
C. freundii (2)]	Trovafloxacin			0.03–0.25
	Ciprofloxacin			0.008–0.06
	Cefpodoxime			0.25–2
	Amoxicillin-clavulanate			1–32
Salmonella spp. (5)	BAY 12-8039			0.06–1
	Trovafloxacin			0.06–1
	Ciprofloxacin			0.03–0.25
	Cefpodoxime			0.5–2
	Amoxicillin-clavulanate			0.5–16
Shigella spp. (5)	BAY 12-8039			0.03–0.06
	Trovafloxacin			0.015–0.06
	Ciprofloxacin			0.015
	Cefpodoxime			0.25–0.5
	Amoxicillin-clavulanate			1–8
Providencia spp. (15) [*P. stuartii* (11);	BAY 12-8039	0.25	0.5	0.06–1
P. retgerii (2); *P. alcalifaciens* (2)]	Trovafloxacin	0.12	0.25	0.06–1

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TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
	Ciprofloxacin	0.03	0.25	0.015–0.25
	Cefpodoxime	0.03	1	0.03–16
	Amoxicillin-clavulanate	128	128	2–128
MSSA (54)	BAY 12-8039	0.06	0.12	0.03–0.12
	Trovafloxacin	0.03	0.06	0.015–0.12
	Ciprofloxacin	0.5	1	0.12–2
	Cefpodoxime	2	4	0.5–4
	Amoxicillin-clavulanate	0.25	0.5	0.12–1
MRSA (20)	BAY 12-8039	2	2	2
	Trovafloxacin	2	2	2
	Ciprofloxacin	128	128	32–128
	Cefpodoxime	>128	>128	128–>128
	Amoxicillin-clavulanate	16	16	16–32
S. epidermidis (29)	BAY 12-8039	0.06	2	0.03–2
	Trovafloxacin	0.03	4	0.15–4
	Ciprofloxacin	0.25	8	0.12–32
	Cefpodoxime	1	16	0.5–>128
	Amoxicillin-clavulanate	0.12	2	0.12–64
S. saprophyticus (30)	BAY 12-8039	0.12	0.25	0.12–0.25
	Trovafloxacin	0.06	0.12	0.06–0.12
	Ciprofloxacin	0.5	0.5	0.25–0.5
	Cefpodoxime	4	8	2–8
	Amoxicillin-clavulanate	0.25	0.5	0.12–0.5
S. pneumoniae (32)	BAY 12-8039	0.12	0.25	0.06–8
	Trovafloxacin	0.12	0.25	0.06–8
	Ciprofloxacin	1	16	0.5–128
	Cefpodoxime	0.5	4	0.03–8
	Amoxicillin-clavulanate	0.12	1	0.015–1
S. milleri (30)	BAY 12-8039	0.12	0.25	0.06–0.25
	Trovafloxacin	0.12	0.25	0.06–0.25
	Ciprofloxacin	0.5	1	0.5–8
	Cefpodoxime	0.25	0.5	0.03–32
	Amoxicillin-clavulanate	0.12	0.12	0.03–0.25
Group A streptococci (20)	BAY 12-8039	0.25	0.25	0.06–0.25
	Trovafloxacin	0.12	0.25	0.06–0.25
	Ciprofloxacin	0.5	1	0.25–1
	Cefpodoxime	0.015	0.015	0.015
	Amoxicillin-clavulanate	0.015	0.015	0.015
Group B streptococci (20)	BAY 12-8039	0.25	0.25	0.06–0.5
	Trovafloxacin	0.25	0.25	0.12–0.5
	Ciprofloxacin	1	1	0.5–2
	Cefpodoxime	0.06	0.06	0.03–0.06
	Amoxicillin-clavulanate	0.06	0.06	0.06
E. faecalis (30)	BAY 12-8039	0.25	0.5	0.12–4
	Trovafloxacin	0.25	0.5	0.12–8
	Ciprofloxacin	2	2	1–32
	Cefpodoxime	8	>128	1–>128
	Amoxicillin-clavulanate	0.5	0.5	0.12–16
E. faecium (20)	BAY 12-8039	2	2	0.25–4
	Trovafloxacin	0.5	2	0.25–8
	Ciprofloxacin	2	4	1–8
	Cefpodoxime	>128	>128	0.5–>128
	Amoxicillin-clavulanate	4	16	0.12–16
H. influenzae (36)	BAY 12-8039	0.03	0.03	0.015–0.06
	Trovafloxacin	0.008	0.015	0.004–0.03
	Ciprofloxacin	0.015	0.015	0.008–0.015
	Cefpodoxime	0.06	0.12	0.03–0.5
	Amoxicillin-clavulanate	0.5	2	0.25–4
M. catarrhalis (35)	BAY 12-8039	0.06	0.12	0.06–0.12
	Trovafloxacin	0.03	0.03	0.008–0.06
	Ciprofloxacin	0.06	0.06	0.03–0.06
	Cefpodoxime	0.5	1	0.12–16
	Amoxicillin-clavulanate	0.12	0.25	0.015–1
N. gonorrhoeae (34)	BAY 12-8039	0.008	0.015	0.004–0.12
	Trovafloxacin	0.004	0.008	0.002–0.03
	Ciprofloxacin	0.004	0.004	0.001–0.12

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TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
	Cefpodoxime	0.008	0.015	0.002–0.03
	Amoxicillin-clavulanate	0.25	1	0.06–1
N. meningitidis (10)	BAY 12-8039	0.008	0.015	0.004–0.015
	Trovafloxacin	0.004	0.008	0.004–0.008
	Ciprofloxacin	0.008	0.008	0.004–0.008
	Cefpodoxime	0.004	0.004	0.002–0.008
	Amoxicillin-clavulanate	0.06	0.12	0.03–0.12
Peptostreptococcus spp. (20)	BAY 12-8039	0.12	1	0.06–1
	Trovafloxacin	0.5	1	0.06–2
	Ciprofloxacin	1	2	0.12–2
	Cefpodoxime	1	4	0.25–64
	Amoxicillin-clavulanate	0.06	0.25	0.06–16
B. fragilis (25)	BAY 12-8039	0.25	0.25	0.12–1
	Trovafloxacin	1	1	0.5–1
	Ciprofloxacin	2	4	1–4
	Cefpodoxime	64	>128	2–>128
	Amoxicillin-clavulanate	0.5	2	0.5–4
Prevotella spp. (3)	BAY 12-8039			0.12–0.25
	Trovafloxacin			0.25–1
	Ciprofloxacin			2
	Cefpodoxime			2–>128
	Amoxicillin-clavulanate			2
Clostridium perfringens (10)	BAY 12-8039	0.5	0.5	0.25–1
	Trovafloxacin	0.5	1	0.5–1
	Ciprofloxacin	0.5	0.5	0.25–0.5
	Cefpodoxime	16	32	1–32
	Amoxicillin-clavulanate	0.25	0.25	0.06–0.25
C. difficile (10)	BAY 12-8039	1	2	1–2
	Trovafloxacin	2	2	2
	Ciprofloxacin	16	16	16
	Cefpodoxime	>128	>128	128–>128
	Amoxicillin-clavulanate	0.5	1	0.25–2

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

lowest antibiotic concentration at which there was no visible growth, and the MBC was defined as the lowest antibiotic concentration to reduce growth to five colonies or fewer (equivalent to 99.9% lethality) (7a).

Protein binding determinations. The protein binding of BAY 12-8039 at two concentrations (1 and 5 μg/ml) in pooled human serum (Bradsure Biologicals) was investigated. The method employed Centrifree ultrafiltration units (Amicon, Stonehouse, United Kingdom). Ultrafiltrates were assayed against BAY 12-8039 phosphate buffer (pH 7) calibrators by a microbiological plate assay.

RESULTS

The activity of BAY 12-8039 against members of the family *Enterobacteriaceae* (MIC at which 90% of strains tested were inhibited [MIC_{90}] ≤ 1 μg/ml, and for *Serratia* spp. MIC_{90} = 2 μg/ml) was similar to that observed for trovafloxacin (Table 1). Both these agents were generally one-half as active as ciprofloxacin, except against *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Enterobacter* spp., and *Citrobacter* spp., where ciprofloxacin was 8 to 16 times more active. In general, the quinolones were more active than either of the β-lactam agents against members of the *Enterobacteriaceae*. BAY 12-8039 was equally active against β-lactamase-producing and -nonproducing strains of *E. coli.*

BAY 12-8039 was shown to be more active against *Acinetobacter* spp. (MIC_{90}, 2 μg/ml) than ciprofloxacin (MIC_{90}, 8 μg/ml). Against *P. aeruginosa* and *Stenotrophomonas maltophilia* the quinolones, including BAY 12-8039, were more active (MIC_{90} ≤ 8 μg/ml) than the β-lactam agents (MIC_{90}s > 128 μg/ml). Both BAY 12-8039 and trovafloxacin were more active against *S. maltophilia* (MIC_{90}s, 2 μg/ml) than ciprofloxacin (MIC_{90}s, 8 μg/ml).

BAY 12-8039 exhibited activity against *Staphylococcus saprophyticus* (MIC_{90}, 0.25 μg/ml) and *Staphylococcus epidermidis* (MIC_{90}, 2 μg/ml), the MIC_{90}s of ciprofloxacin being 0.5 and 8 μg/ml, respectively. The activity of BAY 12-8039 against MSSA (MIC_{90}, 0.12 μg/ml) was similar to that of trovafloxacin (MIC_{90}, 0.06 μg/ml) but greater than that of ciprofloxacin (MIC_{90}, 1 μg/ml). BAY 12-8039 was less active against methicillin-resistant *S. aureus* (MRSA) (MIC_{90}, 2 μg/ml) than against methicillin-susceptible strains (MIC_{90}, 0.12 μg/ml). However, it was more active than ciprofloxacin (MIC_{90}, 128 μg/ml), cefpodoxime (MIC_{90}, >128 μg/ml), and amoxicillin-clavulanate (16 μg/ml) against the MRSA.

TABLE 2. In vitro activity of BAY 12-8039 in comparison with rifampin against *M. tuberculosis*

Strain	Resistance pattern to commonly used antimycobacterial agents	MIC (μg/ml) of:	
		BAY 12-8039	Rifampin
1	Fully sensitive	0.5	0.25
2	Isoniazid and streptomycin resistant	0.25	0.25
3	Isoniazid and rifampin resistant	0.12	ND[a]
4	Streptomycin resistant	0.25	0.5

[a] ND, not determined.

TABLE 3. MIC and MLC of BAY 12-8039 and comparator agents for *C. trachomatis* and *C. pneumoniae*[a]

Strain	BAY 12-8039		Ciprofloxacin		Erythromycin	
	MIC	MLC	MIC	MLC	MIC	MLC
C. trachomatis 6/96	0.06	0.12	2.0	2.0	0.25	2.0
C. trachomatis 7/96	0.12	0.12	2.0	2.0	0.5	4.0
C. trachomatis 8/96	0.06	0.12	1.0	2.0	0.5	4.0
C. pneumoniae TW183	0.06	0.06	2.0	2.0	0.25	0.5

[a] Values are given in micrograms per milliliter.

BAY 12-8039 exhibited activity against *Streptococcus milleri* and group A and group B streptococci (MIC_{90}s, 0.25 μg/ml), and this was comparable to that of trovafloxacin. The activity of BAY 12-8039 against *S. pneumoniae* (MIC_{90}, 0.25 μg/ml) was also similar to that of trovafloxacin but was considerably greater than that of ciprofloxacin (MIC_{90}, 16 μg/ml). A strain inhibited by 16 μg of ciprofloxacin per ml was inhibited by 0.12 and 0.25 μg of BAY 12-8039 and trovafloxacin per ml, respectively. BAY 12-8039 was also shown to be active against *E. faecalis* (MIC_{90}, 0.5 μg/ml) and *Enterococcus faecium* (MIC_{90}, 2 μg/ml).

BAY 12-8039, in common with the other quinolones, was highly active against *Neisseria gonorrhoeae* and *Neisseria meningitidis* (MIC_{90}, 0.015 μg/ml), *H. influenzae* (MIC_{90}, 0.03 μg/ml), and *M. catarrhalis* (MIC_{90}, 0.12 μg/ml).

BAY 12-8039 was found to be active against all the strains of anaerobic bacteria studied (MIC_{90}s $\leq$ 2 μg/ml). BAY 12-8039 was three or fourfold more active against *Bacteroides fragilis*, *Prevotella* spp., and *Clostridium difficile* than ciprofloxacin.

BAY 12-8039 exhibited an activity comparable to that of rifampin for all strains of *M. tuberculosis* (Table 2).

Against both *C. trachomatis* and *C. pneumoniae* (Table 3) BAY 12-8039 was shown to be more active (MICs of 0.06 to 0.12 μg/ml) than either ciprofloxacin (MICs of 1 to 2 μg/ml) or erythromycin (MICs of 0.25 to 0.5 μg/ml). BAY 12-8039 exhibited a high lethal effect against both *C. trachomatis* and *C. pneumoniae*, with the MLCs being equal to, or within one dilutional step of, the MICs.

An increase in inoculum size from 10^4 to 10^6 did not affect the MICs for the *E. coli* strains studied (data not shown). For *K. pneumoniae*, however, one strain was affected, and in this case the MIC increased fourfold. The majority of *P. mirabilis* strains tested at an increased inoculum showed a twofold increase in MIC. For *S. marcescens*, two of five strains showed a threefold increase in MIC.

The presence of human serum had no marked effect on the MICs or MBCs determined for BAY 12-8039 at either 20 or 70% (Table 4), with the exception of one strain of group A streptococci for which the MBC was 0.25 μg/ml in the absence of serum and 1 μg/ml in the presence of 70% serum. The protein binding of BAY 12-8039 was determined at 1 and 5 μg/ml as 30 and 26.4%, respectively.

DISCUSSION

The results presented here generally agree with preliminary information on BAY 12-8039 which indicates improved in vitro activity against gram-positive bacteria (3). In this study, BAY 12-8039 was found to be more active than ciprofloxacin against *S. pneumoniae*, MSSA, and MRSA. In addition, the activity of BAY 12-8039 equalled that of trovafloxacin, which has previously been shown to possess improved activity against gram-positive bacteria (2, 4). It should be noted that BAY 12-8039 was less active against MRSA than against MSSA. The strains of MRSA used in this study were recent clinical isolates, and it is therefore likely that some were ciprofloxacin-resistant epidemic MRSA (6). In the clinical situation resistance to ciprofloxacin by MRSA appears to be rapidly acquired (7, 9), and it is possible that the mechanism(s) of resistance to ciprofloxacin also applies to BAY 12-8039.

In common with other fluoroquinolones, BAY 12-8039 exhibited activity against the *Enterobacteriaceae*. Against *Acinetobacter* spp. BAY 12-8039 was shown to be more active than ciprofloxacin. In addition, BAY 12-8039 was generally found to have improved activity compared to that of ciprofloxacin against anaerobic bacteria.

BAY 12-8039 was shown to be active against common respiratory pathogens, such as *M. catarrhalis* and *H. influenzae*. Against *M. tuberculosis* BAY 12-8039 was found to be as active as rifampin. This activity is similar to that of ciprofloxacin and improved compared to that of trovafloxacin (2). BAY 12-8039 was shown to be slightly more active against *Mycobacterium avium-Mycobacterium intracellulare* compared to rifampin. Against strains of *Chlamydia* spp. BAY 12-8039 was found to be more active than either erythromycin or ciprofloxacin.

The protein binding of BAY 12-8039 was similar to that of many other fluoroquinolones (<50%) but less than that of trovafloxacin, which we found to be approximately 85% em-

TABLE 4. Effect of human serum on the in vitro activity of BAY 12-8039

Organism type[a]	Agar MIC (μg/ml)	Broth MIC (μg/ml)	MBC (μg/ml)	20% Human serum		70% Human serum	
				MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Group A streptococci	0.25	0.25	0.25	0.25	0.25	0.5	1
	0.25	0.12	0.25	0.25	0.25	0.25	0.25
S. pneumoniae	0.12	0.25	0.25	0.12	0.12	0.12	0.5
	0.25	0.25	0.25	0.25	0.25	0.25	0.5
M. catarrhalis	0.12	0.06	0.12	0.03	0.12	ND[b]	0.25
	0.12	0.06	0.12	ND	0.12	0.03	0.25
S. aureus	0.03	0.03	0.06	0.06	0.12	0.06	0.12
	0.06	0.03	0.06	0.06	0.12	0.06	0.12
E. coli	0.06	0.03	0.06	0.03	0.03	0.03	0.03
	0.03	0.03	0.03	0.015	0.03	0.03	0.03
K. pneumoniae	0.06	0.06	0.12	0.12	0.12	0.12	0.12
	0.12	0.06	0.06	0.03	0.06	0.03	0.06

[a] Two strains of each organism were studied.
[b] ND, not determined.

ploying similar methodologies (2). The presence of serum had, as expected, little or no effect upon the in vitro antimicrobial activity of the new compound.

BAY 12-8039 has a broad spectrum of activity which includes gram-negative and gram-positive bacteria, *Chlamydia* spp., *M. tuberculosis*, and anaerobes and therefore has considerable clinical potential in a wide range of infections.

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