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Comparative Activities of Eight Quinolones against Members of the *Bacteroides fragilis* Group

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The in vitro activities of five new quinolones (clinafloxacin [CI-960 or PD-127391], BAY Y 3118, E-4868, E-5065, and E-5068) against 100 *Bacteroides fragilis* group bacterial isolates were compared with those of ciprofloxacin, ofloxacin, and sparfloxacin. Overall, E-5068 was the most active in vitro (MIC for 90% of isolates tested [MIC_{90}], 0.25 μg/ml); this was followed by clinafloxacin and BAY Y 3118 (MIC_{90}, 0.5 μg/ml). Ciprofloxacin, sparfloxacin, and ofloxacin were the least active (MIC_{90}s, 64, 16, and 16 μg/ml, respectively). *B. fragilis* and *Bacteroides caccae* were more susceptible than the other members of the *B. fragilis* group to all of the quinolones tested.

The most important and most frequently isolated anaerobes from clinical infections are those belonging to the *Bacteroides fragilis* group. In the past few years, several reports have emphasized the increasing incidence of resistance of *Bacteroides* species to the currently available antimicrobial agents (1, 7, 9). Consequently, a need has arisen for new, safe, and effective antimicrobial agents that can be used in the treatment of infections caused by anaerobes.

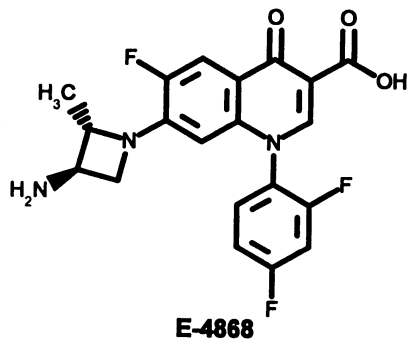
The recent resurgence in the clinical use of quinolones in the treatment of bacterial infections stems from the development of new fluorinated compounds which exhibit both greater and broader ranges of antibacterial activity. These two characteristics allow the use of a single agent in the treatment of infections when a mixed bacterial population is present.

In the work described here, a comparison was made between the in vitro activities of ciprofloxacin and ofloxacin and those of sparfloxacin (a difluoroquinolone with a 7 dimethyl-piperazinyl substituted group), BAY Y 3118 and clinafloxacin (monofluoroquinolones with new substituents at positions 7 and 8), and three mono- or difluorinated naphthyridine derivatives (E-4868, E-5065, and E-5068), which are characterized chemically by the presence at position 7 of an azetidin ring with different C′-3 substituents (Fig. 1), against 100 *B. fragilis* group strains in order to assess their antianaerobic activities.

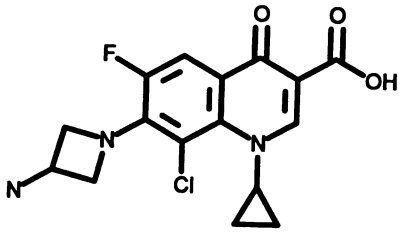
The 100 anaerobic bacteria studied were isolated from the same number of patients at Seville University Hospital during 1992 and 1993 and were identified by standard criteria (14). These included 31 *B. fragilis*, 12 *Bacteroides caccae*, 16 *Bacteroides distasonis*, 13 *Bacteroides ovatus*, 12 *Bacteroides thetaiotaomicron*, and 16 *Bacteroides vulgatus*.

The following antimicrobial agents were provided by the indicated manufacturers: ciprofloxacin and BAY Y 3118, Quimica Farmaceutica Bayer S.A., Barcelona, Spain; ofloxacin, Hoechst Iberica S.A., Barcelona, Spain; sparfloxacin, Rhône Poulenc Farma, Madrid, Spain; clinafloxacin, Parke Davis S.A., Barcelona, Spain; E-4868, E-5065, and E-5068, Laboratorios Esteve SA, Barcelona, Spain.

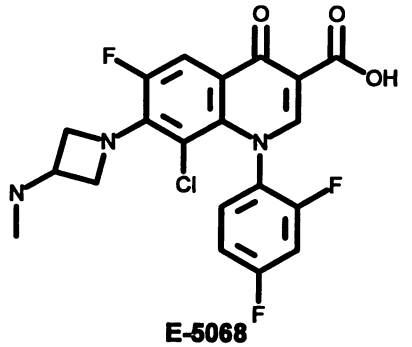
The susceptibilities of the isolates to the test drugs were determined in Wilkins-Chalgren agar by the reference dilution method in accordance with the guidelines of the National



E-4868



E-5065



E-5068

FIG. 1. Chemical structures of compounds E-4868, E-5065, and E-5068.

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TABLE 1. Comparative in vitro activities of various fluoroquinolones against members of the *B. fragilis* group

Organism	No. of isolates	Antimicrobial agent	MIC (μg/ml)[a]		
			Range	50%	90%
B. fragilis	31	Ciprofloxacin	1–128	4	16
		Ofloxacin	2–64	1	4
		Sparfloxacin	1–32	2	4
		Clinafloxacin	≤0.03–0.5	0.06	0.25
		BAY Y 3118	0.06–2	0.12	0.25
		E-4868	1–16	4	8
		E-5065	0.25–2	1	1
		E-5068	≤0.015–0.5	≤0.015	0.12
B. caccae	12	Ciprofloxacin	1–128	4	32
		Ofloxacin	0.25–16	2	8
		Sparfloxacin	0.06–16	1	2
		Clinafloxacin	≤0.03–4	0.06	0.12
		BAY Y 3118	0.06–0.5	0.25	0.5
		E-4868	0.12–16	4	4
		E-5065	0.03–4	0.5	1
		E-5068	0.06–2	≤0.03	0.12
B. thetaiotaomicron	12	Ciprofloxacin	4–64	4	64
		Ofloxacin	4–16	8	16
		Sparfloxacin	1–32	2	32
		Clinafloxacin	0.06–0.5	0.12	0.5
		BAY Y 3118	0.12–0.5	0.5	0.5
		E-4868	4–4	4	4
		E-5065	0.5–0.5	0.5	0.5
		E-5068	0.12–0.25	0.12	0.25
B. vulgatus	16	Ciprofloxacin	4–128	4	128
		Ofloxacin	2–64	2	32
		Sparfloxacin	1–32	1	8
		Clinafloxacin	≤0.03–4	4	4
		BAY Y 3118	0.12–2	0.12	2
		E-4868	4–16	4	16
		E-5065	0.12–4	0.12	4
		E-5068	≤0.03–4	0.06	4
B. distasonis	16	Ciprofloxacin	8–64	8	64
		Ofloxacin	4–128	4	32
		Sparfloxacin	1–2	1	2
		Clinafloxacin	0.5–1	0.5	1
		BAY Y 3118	0.25–2	0.25	1
		E-4868	2–16	4	16
		E-5065	0.125–4	0.25	4
		E-5068	0.06–0.5	0.06	0.25
B. ovatus	13	Ciprofloxacin	8–64	8	64
		Ofloxacin	4–32	4	32
		Sparfloxacin	1–16	2	16
		Clinafloxacin	0.06–0.5	0.12	0.5
		BAY Y 3118	0.12–2	0.5	1
		E-4868	1–8	2	8
		E-5065	0.25–4	1	2
		E-5068	0.06–0.5	0.06	0.25
Total	100	Ciprofloxacin	0.06–128	0.25	32
		Ofloxacin	0.25–128	2	16
		Sparfloxacin	0.06–32	2	4
		Clinafloxacin	<0.03–4	0.06	0.5
		BAY Y 3118	0.06–2	0.12	0.5
		E-4868	0.12–16	4	8
		E-5065	0.03–4	1	1
		E-5068	<0.03–4	<0.03	0.25

[a] 50% and 90%, MICs required to inhibit 50 and 90% of isolates, respectively.

Committee for Clinical Laboratory Standards (17) and Barry et al. (4). Plates containing serial doubling dilutions of antimicrobial agents ranging from 0.06 to 128 μg/ml were inoculated with a Steer's replicator to give a final inoculum of 10^5 CFU. They were incubated in an anaerobic chamber (Forma Scientific) for 48 h at 37°C.

The MIC was defined as the lowest concentration of drug that inhibited growth. The appearance of a single colony or a barely visible haze was disregarded (17).

The in vitro activities of the quinolones against members of the *B. fragilis* group are given in Table 1. E-5068 had significantly greater activity than any of the other agents studied against all of the strains tested (MIC for 90% of isolates tested [MIC_{90}], 0.25 μg/ml). The second most active compounds (MIC_{90}s, 0.5 μg/ml) were clinafloxacin and BAY Y 3118; this was followed by E-5065 (MIC_{90}, 2 μg/ml), sparfloxacin (MIC_{90}, 4 μg/ml), E-4858 (MIC_{90}, 8 μg/ml), ofloxacin (MIC_{90}, 16 μg/ml), and ciprofloxacin (MIC_{90}, 64 μg/ml); ofloxacin and ciprofloxacin showed uniformly poor activities against all 100 strains tested. The *B. fragilis* and *B. caccae* species, when considered separately, were generally more susceptible than the other members of the *B. fragilis* group to all of the agents tested.

Clinafloxacin and E-5068 were the most active antimicrobial agents against the *B. fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 131124 control strains (MICs, ≤0.06 μg/ml); this was followed by BAY Y 3118 (MIC, 0.125 μg/ml) and E-5065 (MIC, 0.25 μg/ml). Ofloxacin, sparfloxacin, and E-4868 were more active against *C. perfringens* (MICs, 0.125, 0.125, and 0.5 μg/ml, respectively) than against *B. fragilis* (MICs, 1, 1, and 4 μg/ml, respectively). The MICs of ciprofloxacin were 4 μg/ml for both control strains.

Since the introduction of the fluoroquinolones in 1982 (15), innovative developments have yielded several antimicrobial agents with increased activities and broader antibacterial spectra. However, those in clinical use, such as ciprofloxacin or ofloxacin, have MICs for anaerobic organisms that approach or exceed the achievable levels of those agents in human serum (6, 19).

Because ciprofloxacin is widely used clinically, many recent studies of new fluoroquinolones have used it as a reference agent (3, 12) and, as in the present work, have found its in vitro activity against *B. fragilis* to be very poor.

Ofloxacin possesses an oxazine ring that results in slightly improved activity against anaerobic organisms (8, 12). Some investigators have found that strains of *B. fragilis* were susceptible to ofloxacin (MIC_{90}, 4 μg/ml) but that most other species of the *B. fragilis* group were resistant to this agent. In the current study, it was found that the MIC_{90} of ofloxacin for *B. fragilis* was 4 μg/ml; the ofloxacin MICs for other species tested in the present study were greater.

Our results, that sparfloxacin is more active than ofloxacin, with overall MIC_{90}s of 4 μg/ml, agree with those of Barry and Fuchs (3) and Goldstein and Citron (12). It should be noted that, in our study, the MIC_{90} of sparfloxacin for *B. thetaiotaomicron* was 16 times greater (32 μg/ml) than those observed for *B. caccae* and *B. fragilis* (MIC_{90}s, 2 μg/ml).

As reported previously (3, 4, 8), clinafloxacin has very good activity against anaerobes. King et al. (16) and Goldstein and Citron (13) found interspecies differences, as we did, since the MIC_{90} of clinafloxacin for *B. vulgatus* was 4 μg/ml, or 33 times higher than that for *B. caccae* (0.12 μg/ml).

BAY Y 3118 had the same favorable activity as clinafloxacin against the *B. fragilis* group, with both compounds having overall MIC_{90}s of 0.5 μg/ml, and interspecies differences were observed. These results are in agreement with those of other investigators (5, 10, 18), even though such interspecies differences were not reported. Clinafloxacin resembles BAY Y 3118 very closely in terms of its potency and range of activity against *B. fragilis* group strains. Both agents also have identical structures except for the moiety at position 7.

The Esteve compounds showed different activities against the members of the *B. fragilis* group. E-4868 was the least active of the three, with an overall MIC_{90} greater than that of sparfloxacin. E-5065 had an MIC_{90} of 2 μg/ml, and E-5068 had an MIC_{90} of 0.25 μg/ml for all 100 strains. Barrett et al. (2) reported an E-4868 MIC_{90} for the *B. fragilis* group of 4 μg/ml. The activities of these antimicrobial agents against the different species of the *B. fragilis* group also varied in our study. Thus, E-4868 had an MIC_{90}s of 4 μg/ml for *B. caccae* and *B. thetaiotaomicron* and 8 μg/ml for *B. fragilis* and *B. ovatus*. *B. vulgatus* and *B. distasonis* proved to be the most resistant; the MIC_{90} of E-4868 was 16 μg/ml. E-5065 was most active against *B. thetaiotoaomicron* (MIC_{90}, 0.5 μg/ml); this was followed by its activity against *B. fragilis* and *B. caccae* (MIC_{90}s, 1 μg/ml) and *B. vulgatus* and *B. distasonis* (MIC_{90}s, 4 μg/ml). E-5068 was the most active of the three drugs, with MIC_{90}s of between 0.12 and 4 μg/ml. The most striking feature was the difference between the MIC_{90}s of E-5068 for *B. caccae* and *B. fragilis* (0.12 μg/ml) and that for *B. vulgatus* (4 μg/ml). The same phenomenon was observed with clinafloxacin.

Gargallo-Viola et al (11) studied different azetidin derivatives from the Esteve Laboratories and demonstrated that two of them, E-4502 and E-4500, displayed considerable activity against anaerobes. The differences in the activities of the compounds tested in the present work may be due to the different C′-3 substituents of the azetidin group at position 7 of the molecule.

A comparison of the MIC_{90}s of the eight compounds that we studied shows the enhanced activities that were achieved with E-5068, BAY Y 3118, and clinafloxacin in comparison with those of ciprofloxacin and ofloxacin against members of the *B. fragilis* group. Their very low MICs for these common anaerobic pathogens suggest that they may be useful for the treatment of infections caused by these organisms; anaerobes have previously represented a significant gap in the spectra of the currently available quinolones. The increased levels of activity of these novel compounds suggest that they will have a role to play in the treatment of mixed and anaerobic infections. Their therapeutic usefulness will depend on the results of additional in vitro susceptibility studies involving a larger number of strains, pharmacokinetic and toxicological studies, and clinical trials.

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