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Anti-*Mycobacterium avium* Activity of Quinolones: In Vitro Activities

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The MICs of 88 quinolones against 14 selected reference and clinical strains of *Mycobacterium avium-M. intracellulare* complex were determined. Agents tested included ciprofloxacin, sparfloxacin (PD 131501), and 86 other experimental quinolones. Test strains were selected to represent various susceptibilities to ciprofloxacin and other drug resistance profiles. MICs were determined by the microdilution method in 7HSF broth, with incubation for 14 days at 35°C. The results showed 25 of the quinolones to be active against the strains, with MICs for 90% of the strains (MIC_{90}s) of 2 to 32 μg/ml. Ten of these compounds had activities equivalent to or greater than that of ciprofloxacin. The most active compound was PD 125354, with an MIC_{50} of 0.5 μg/ml and an MIC_{90} of 2 μg/ml; comparable values for ciprofloxacin were 4 and 8 μg/ml, respectively. The next most active compounds, with MIC_{90}s of 4 μg/ml, were sparfloxacin (PD 131501), PD 123982, PD 135144, and PD 119421. MIC_{90}s of PD 131575, PD 126889, PD 122642, PD 139586, and PD 143289 were 8 μg/ml. Further evaluation of the most active agents is warranted, as is assessment of structure-activity relationships of active and inactive agents to elucidate the active portions of the compounds and to lead to the development of compounds with enhanced activity.

During the past decade, much attention has been given to the synthesis of new 4-quinolone-3-carboxylates and the evaluation of these agents for antibacterial activity; this revival of interest was generated by the discovery of the antibacterial activity of nalidixic and oxolinic acids (2–8, 11, 12, 19, 20, 30). This renewed effort uncovered a number of 6-fluoro-7-piperazino-4-quinolones noteworthy for both the breadth and intensity of their activities against gram-negative bacilli and cocci in vitro and for their capacity to control experimentally induced systemic infections when administered orally (24, 27). The most active representatives of this class of compounds, designated fluoroquinolones, include norfloxacin, ofloxacin, ciprofloxacin, enoxacin, and pefloxacin (32). The mechanism of action of the quinolone antibacterial agents is believed to result from the combination of their abilities to penetrate into bacterial cells and to inhibit DNA gyrase, an essential bacterial enzyme that maintains superhelical twists in DNA (2, 6, 11, 21, 25). The general structure of quinolones is shown in Fig. 1.

Although a large number of quinolone derivatives have been made and their activities have been tested against several groups of bacteria, such as members of the family *Enterobacteriaceae* and staphylococci, both in vitro and in vivo, the in vitro activity of quinolones against *Mycobacterium avium-M. intracellulare* complex has only recently been evaluated. *M. avium-M. intracellulare* complex is of increasing clinical importance as the most frequent bacterial complication of AIDS and also is resistant to most antimicrobial agents (28).

The fluorinated quinolones show excellent activity in vitro against *Mycobacterium tuberculosis*, *M. kansasii*, *M. xenopi*, and *M. fortuitum* (9, 10, 14, 15, 26, 31). Activity against *M. avium-M. intracellulare* complex, however, is method and strain dependent (9, 10, 14, 15, 17, 22, 26, 30, 31). Approximately one-third of them are susceptible to quinolones, with ciprofloxacin being the most active quinolone currently in clinical use against *M. avium-M. intracellulare* complex (31). It is not clear whether the resistance seen in some *M. avium-M. intracellulare* complex strains is due to poor penetration of the drug through the bacterial cell wall or occurs at the level of the enzyme target, DNA gyrase. The development of quinolones with increased activities against *M. avium-M. intracellulare* complex is a logical and important goal because of the excellent activity of ciprofloxacin against susceptible strains and the synergism with amikacin, excellent tissue penetration, and low toxicity.

As a first step in our effort to discover more active quinolone compounds against *M. avium-M. intracellulare* complex, we have tested the in vitro activities of 88 quinolones against 14 of these strains. Strains were selected from a panel of 34 strains: 17 reference clinical and environmental strains of known serovar (provided by Patrick Brennan and Ian Orme, Colorado State University, Fort Collins, Colo.) and 17 clinical strains isolated from blood cultures of AIDS patients in Cleveland, Ohio. Preliminary testing of these strains against a battery of conventional antimycobacterial agents, including rifampin, ethambuthol, isoniazid, and amikacin, showed that the MICs for 90% of strains tested (MIC_{90}s) of the two groups were identical (Table 1). Ciprofloxacin

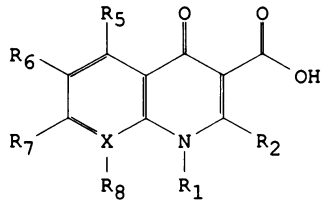

FIG. 1. General structure of the quinolones. X = N or C; R1, R2, R5, R6, R7, and R8 can be any group.

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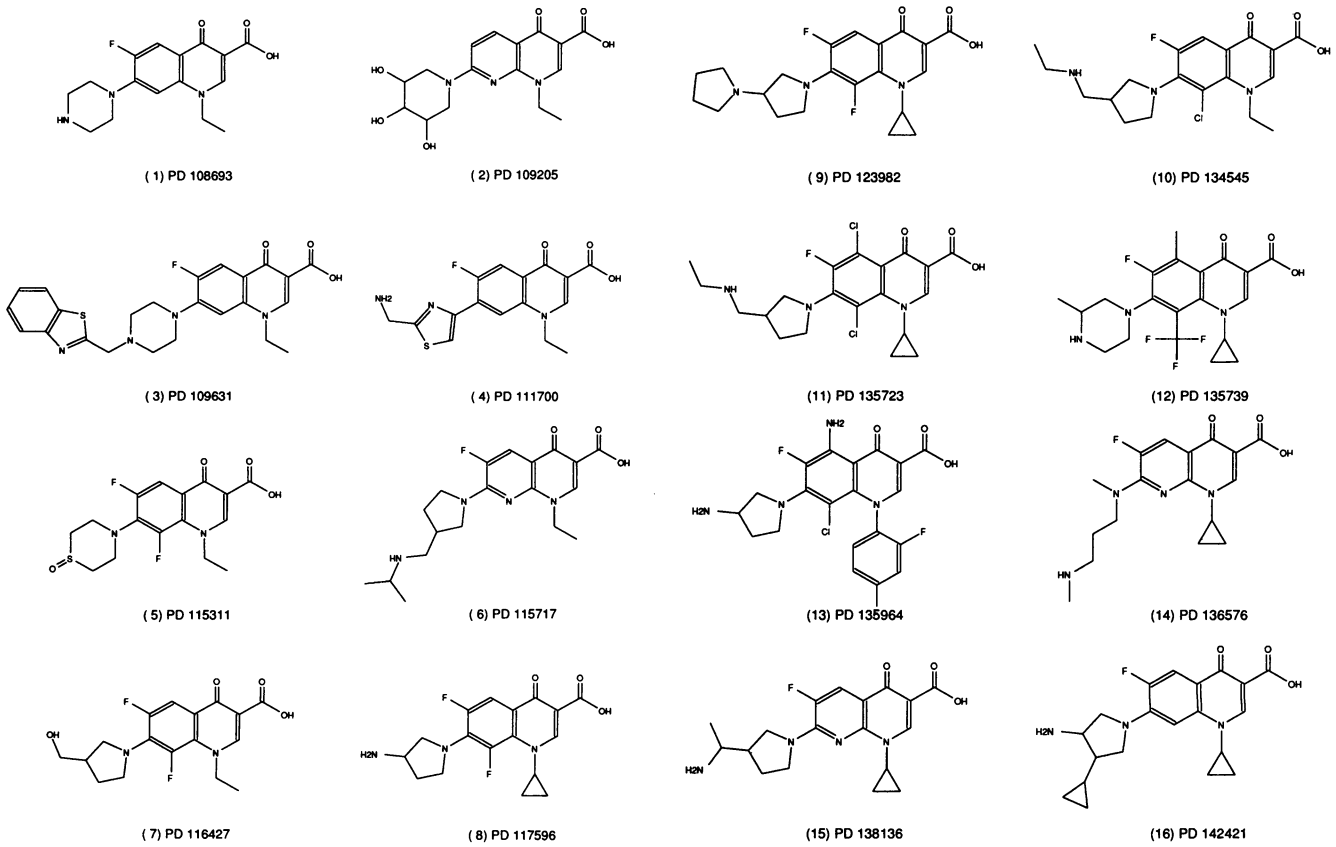

FIG. 2. Structures of the 88 quinolones studied.

floxacin was also tested, and seven strains were chosen from each group to provide strains with the greatest variation in ciprofloxacin activity as well as variation in activity of the other agents tested.

MATERIALS AND METHODS

Antimicrobial agents. Eighty-seven quinolones, selected to include a wide variety of functional groups and physical properties, were provided by Parke-Davis Pharmaceutical Research, Ann Arbor, Mich. These compounds have been previously reported and were prepared by standard methods as described in the literature (3–7, 13, 20, 23). Ciprofloxacin was obtained from Miles Pharmaceuticals, West Haven, Conn. The structures of these compounds are shown in Fig. 2.

Strains. Fourteen strains of *M. avium-M. intracellulare* complex were selected from reference and clinical isolates (see the introduction) to provide the most variation in activity of ciprofloxacin and other agents. Strains were maintained at −70°C and subcultured at least twice on 7H11 agar (Difco, Detroit, Mich.) before testing. If variation in colonial morphology was present, flat transparent colonies were chosen for susceptibility testing.

Susceptibility testing. MICs were determined by the broth microdilution method in 7HSF medium (the broth equivalent of 7H11 agar) (28). The medium was prepared by supplementing Bacto Middlebrook 7H9 broth (Difco) with 0.5% glycerol and 100 ml of Bacto Middlebrook OADC enrichment (Difco) per liter. Quinolones were dissolved in 1 N NaOH, diluted in water, incorporated into the medium at concentrations of 0.25 to 32 μg/ml, and dispensed into 96-well microdilution trays, which were frozen at −70°C until needed. Inocula were prepared from cultures of strains in 4 ml of 7HSF broth which were incubated at 35°C for 1 week. The day before the susceptibility test was done, the 1-week-old culture was diluted 1:20 in fresh 7HSF broth and incubated overnight at 35°C. The overnight culture was mixed by inverting the tube 10 times and then diluted 1:100

TABLE 1. MIC_{90}s of a battery of nine reference antimycobacterial agents against a panel of 17 reference and 17 clinical strains of *M. avium-M. intracellulare* complex

Strains	MIC_{90} (μg/ml)								
	Rifampin	Amikacin	Ciprofloxacin	Ethambutol	Isoniazid	Erythromycin	Clarithromycin	Rifabutin	Clofazimine
Reference	>16	8	16	16	>16	>16	16	2	1
Clinical	>16	16	16	16	>16	>16	16	4	2
All	>16	8	16	16	>16	>16	16	4	2

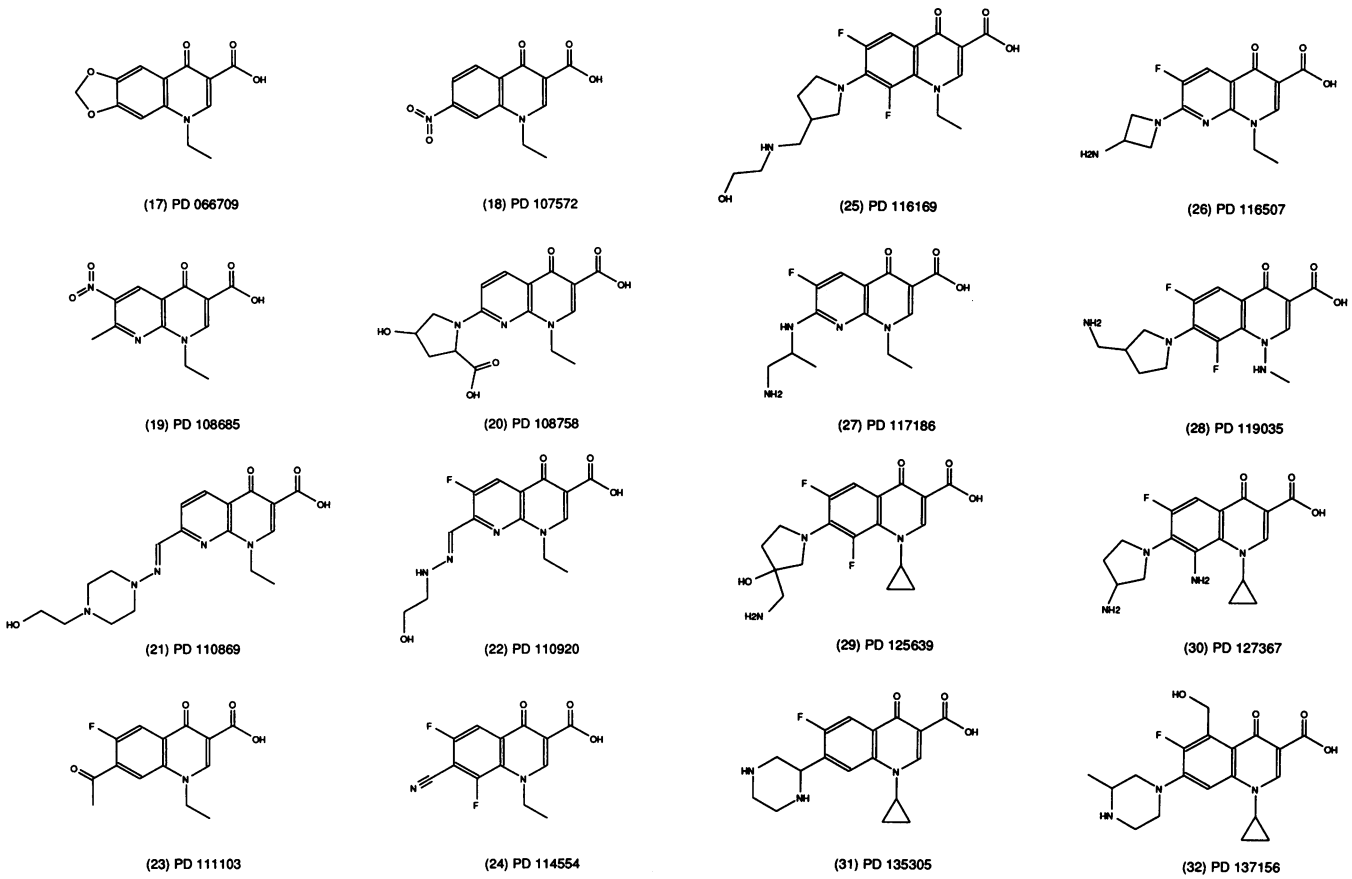

FIG. 2—*Continued.*

in 7HSF broth just prior to the inoculation of microdilution test panels. This procedure yielded an inoculum of ca. 5×10^5 CFU/ml when wells were inoculated with a multiwell inoculator delivering 5 μl per well. Viable counts were determined for inocula to confirm that the inoculum size was correct. Trays were covered with lids, placed in plastic bags, incubated at 35°C in ambient atmosphere, and read after 7 and 14 days of incubation. MICs were read in indirect light with a Dynatech reading stand with mirror (Dynatech Laboratories, Alexandria, Va.). The MIC was defined as the lowest concentration of antimicrobial agent at which the organism showed no visible growth. Growth and sterility wells were included in each tray.

RESULTS

Results of determinations of MICs of the 25 most active of the 88 quinolones against the panel of 14 reference strains are shown as MIC ranges, MIC_{50}s, and MIC_{90}s in Table 2. All other quinolones tested had MIC_{50}s of ≥32 μg/ml and MIC_{90}s of >32 μg/ml, although MICs for the most susceptible strains were 0.25 to 2 μg/ml with 25 of the agents and 4 to 16 μg/ml with 8 of the agents. The remaining 30 agents showed no activity, with all MICs against all strains being >32 μg/ml.

As can be seen, besides ciprofloxacin, at least 10 other quinolones show good in vitro activity against the *M. avium-M. intracellulare* complex strains. Among them, PD 125354 (no. 53 in Fig. 2) was found to have the best in vitro activity, on the basis of an MIC_{50} eightfold lower than that of

TABLE 2. MICs of the 25 most active quinolones against 14 strains of *M. avium-M. intracellulare* complex[a]

Agent[b]	MIC range	MIC_{50}	MIC_{90}
PD 125354 (53)	≤0.25–8	0.5	2
PD 123982 (9)	≤0.25–8	1	4
Sparfloxacin (PD 131501) (82)	≤0.25–4	1	4
PD 119421 (71)	≤0.25–4	2	4
PD 135144 (56)	≤0.25–8	4	4
PD 131575 (83)	≤0.25–8	2	8
PD 126889 (76)	≤0.25–8	2	8
PD 122642 (73)	≤0.25–16	2	8
Ciprofloxacin (33)	≤0.25–8	4	8
PD 139586 (59)	≤0.25–8	4	8
PD 143289 (62)	≤0.25–8	4	8
PD 144881 (88)	≤0.25–32	4	16
PD 115311 (5)	≤0.25–16	8	16
PD 117596 (8)	≤0.25–16	8	16
PD 135739 (12)	≤0.25–32	8	16
PD 142421 (16)	≤0.25–16	8	16
PD 126592 (54)	≤0.25–16	8	16
PD 131413 (81)	≤0.25–32	8	16
PD 119805 (72)	0.5–32	8	32
PD 108693 (1)	1–32	16	32
PD 138136 (15)	≤0.25–32	16	32
PD 116169 (25)	0.5–32	16	32
PD 137156 (32)	0.5–32	16	32
PD 138029 (86)	≤0.25–>32	16	32
PD 125999 (75)	≤0.25–32	16	32

[a] Expressed as micrograms per milliliter. MIC_{90}s of the other quinolones were all ≥32 μg/ml.

[b] Numbers in parentheses are the compound numbers in Fig. 2.

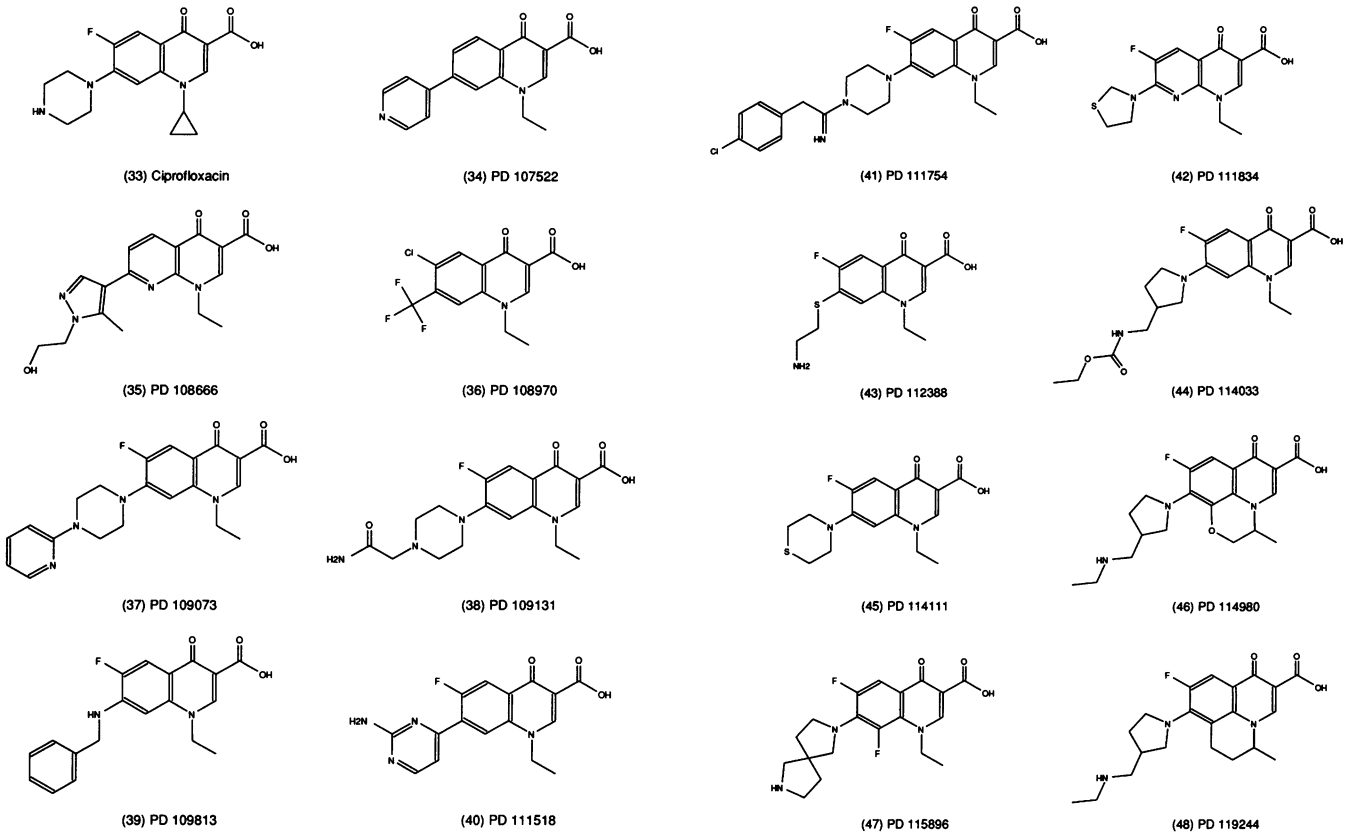

FIG. 2—*Continued.*

ciprofloxacin and an MIC_{90} fourfold lower. Compounds PD 123982 (no. 9 in Fig. 2), PD 135144 (no. 56 in Fig. 2), sparfloxacin (PD 131501, no. 82 in Fig. 2), PD 119421 (no. 71 in Fig. 2), PD 131575 (no. 83 in Fig. 2), PD 126889 (no. 76 in Fig. 2), PD 139586 (no. 59 in Fig. 2), PD 143289 (no. 62 in Fig. 2), and PD 122642 (no. 73 in Fig. 2) all have MIC_{50}s and MIC_{90}s comparable to or better than those of ciprofloxacin.

The conventional approach to evaluate the efficacy of drugs is, as shown in Table 2, to take the MIC_{50}s and MIC_{90}s of each compound as the activity input. However, we felt that we would gain more understanding of the biological events related to drug resistance if we were to treat each strain as a separate data base. For comparison purposes we arbitrarily considered quinolone MICs of ≤16 μg/ml as active and those of >16 μg/ml as inactive against a given strain. With this assumption, we tabulated the number of active compounds for each strain (Table 3). As can be seen, strains TMC 1403 and PI 2/8 are the two most susceptible strains, with more than 63% of the 88 quinolones tested being active against these two strains. Strains 1695757 and 1779564 are very susceptible strains as well; more than 50% of the 88 compounds are active against these two strains. Strains 1958339 and PI 44/4 have medium susceptibility; about 45% of the compounds are active against these two strains. Strains PI 2/6, TMC 1461, and 34540W are more resistant strains; about 30% of the 88 compounds are active against these strains. The other five strains, 1988557, PI 1239, 1760694, 1772733, and 1915112, are very resistant; only 21 to 25% of the 88 compounds are active against these five strains.

We also evaluated the hypothesis that agents with activity against resistant strains are indeed the most active against sensitive strains. To test this hypothesis, we compared the activities against the three strains most susceptible to ciprofloxacin (TMC 1403, PI 2/8, and 1695757) with those against the three strains most resistant to ciprofloxacin (PI 4/44, 1772733, and 1915112) for the 87 quinolones (Fig. 3). MICs for the susceptible and resistant strains were compared for the 54 instances for which MICs of both groups were on

TABLE 3. Distributions of active and inactive compounds for the 14 tested strains[a]

Strain	No. (%) of active agents
TMC 1403	56 (63.6)
PI 2/8	56 (63.6)
1695757	48 (54.5)
1779564	46 (52.2)
PI 44/4	41 (46.6)
1958339	38 (43.2)
PI 2/6	29 (32.9)
TMC 1461	29 (32.9)
34540W	27 (30.7)
PI 12/39	22 (25.0)
1760694	22 (25.0)
1988557	21 (23.9)
1915112	19 (21.6)
1772733	19 (21.6)

[a] Agents with MICs of ≤16 μg/ml were regarded as active, while those with MICs of >16 μg/ml were regarded as inactive.

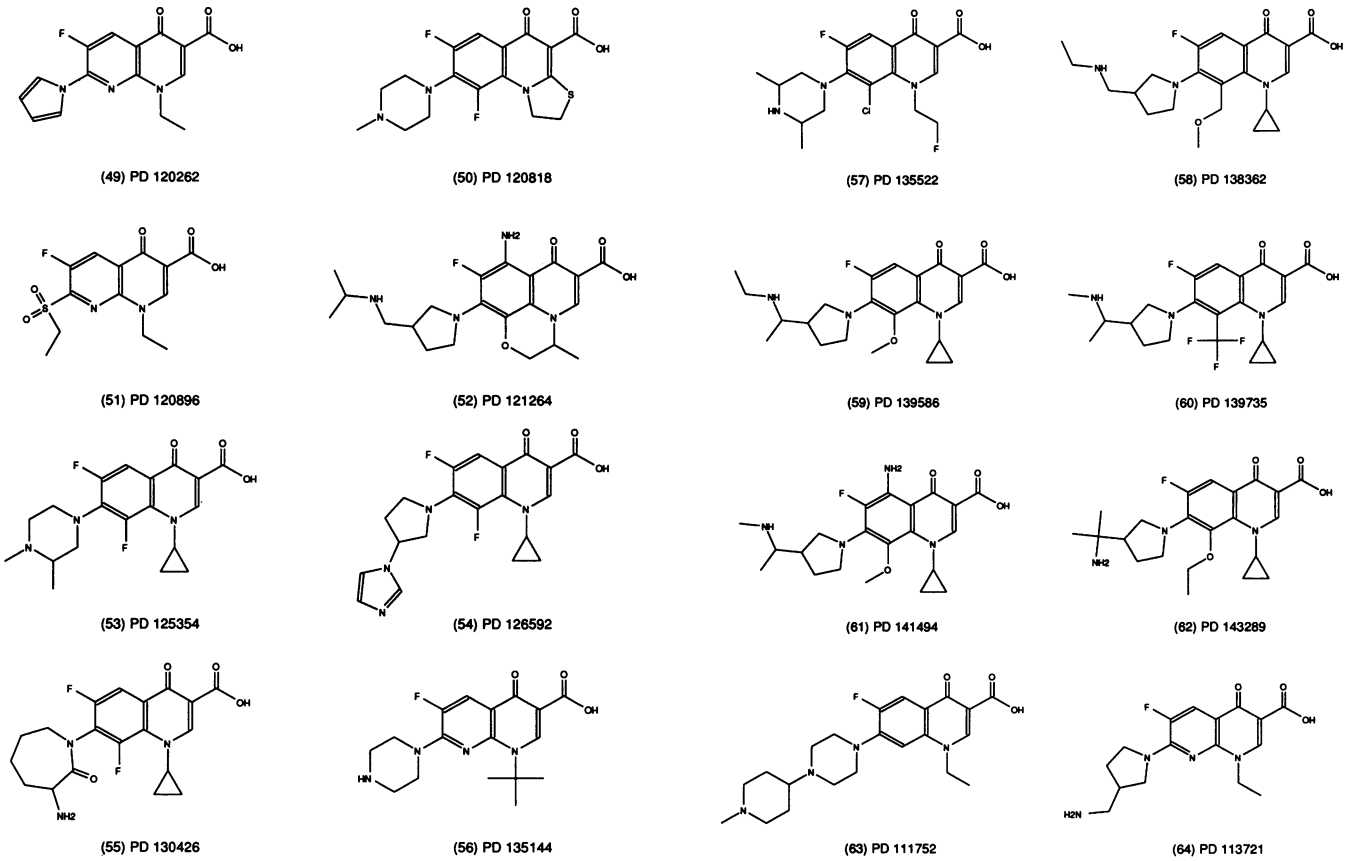

FIG. 2—*Continued.*

scale. MICs for the susceptible strains were ≥32-fold lower than those for the resistant strains in 11 instances, 4- to 16-fold lower in 36 instances, and ≤2-fold lower in 7 instances. Overall we find that activity against susceptible strains is a poor predictor of activity against resistant strains. This is shown by the clustering of values along the *y* axis of Fig. 3. However, good activity against resistant strains guarantees good activity against susceptible strains, as shown by the fact that all the values fall in a triangular area around the *y* axis of Fig. 3.

We also compared the activities of the agents against the most susceptible strain overall (TMC 1403) and the most resistant strain (PI 12/39). Twenty agents were highly active against the susceptible strain (MICs, ≤0.25 μg/ml), but only one agent had an MIC of ≤0.5 μg/ml and only five had MICs of ≤2 μg/ml against the resistant strain. Therefore, activity against resistant strains is a valid indicator of activity against all strains.

DISCUSSION

While ciprofloxacin is generally regarded as the most active of the quinolones currently in clinical use against *M. avium-M. intracellulare* complex strains, almost 40% of strains are regarded as resistant to the current clinical breakpoint of 2 μg/ml (28). Of other quinolones, WIN 57273 has been reported to have activity against *M. avium-M. intracellulare* complex, with MIC_{90}s in two studies of 1 and 4 μg/ml, compared with 1 and 16 μg/ml for ciprofloxacin (1, 11); however, this agent is not being developed further for clinical use. Sparfloxacin (PD 131501, also known as AT-4140) has also been evaluated for activity against *M. avium-M. intracellulare* complex, with findings of MIC_{90}s of 0.5 to 4 μg/ml, compared with 1 to 16 μg/ml for ciprofloxacin (1, 8, 15, 26, 29). Sparfloxacin has also shown good efficacy in a human macrophage model (22).

The need for development of quinolones with improved activity against *M. avium-M. intracellulare* complex is obvious, and several promising candidates have been uncovered in this study, particularly PD 125354, which has an MIC_{90} of 2 μg/ml, fourfold lower than that of ciprofloxacin and twofold lower than that of sparfloxacin.

It is clear from our results that *M. avium-M. intracellulare* complex strains exhibit variable and progressive resistance to the drugs rather than an abrupt change as would be expected if the drugs were all becoming inactive or if the strains were in two different categories. Fluoroquinolone resistance in bacteria such as staphylococci is due to changes in the DNA gyrase or drug penetration (8, 21); the mechanism responsible for the variation seen in *M. avium-M. intracellulare* complex is unknown. Most of the 88 quinolones tested showed at least some activity against a few strains at the concentrations tested, although activity was typically 4- to 16-fold lower against resistant strains compared with susceptible strains (Fig. 3). While low MICs against resistant strains are found for compounds that are extremely active against susceptible strains, activity of an agent against susceptible strains is not an indication that the agent will be active against resistant strains.

In a study reported in the accompanying paper (18a), we

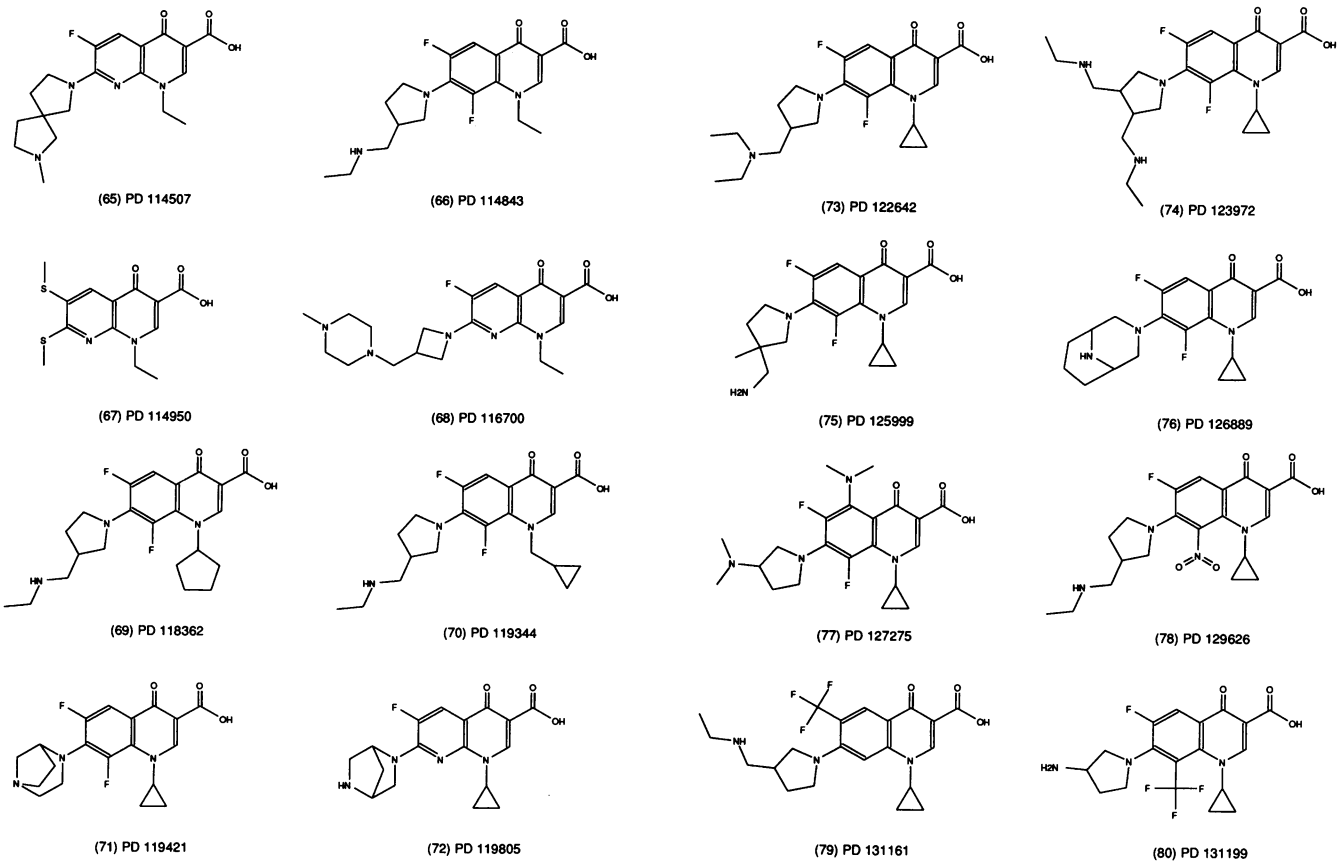

FIG. 2—*Continued.*

evaluated the molecular structural constraints that may be relevant to this increasing resistance to drugs in an effort to identify either a universal drug or a combination of agents that would be effective throughout the whole spectrum of strains. The results were evaluated in an attempt to find a possible link between the structure and activity of these quinolones by using the MULTICASE methodology, a computer automated structure evaluation program to help identify the relevant structural factors and develop adequate quantitative structure-activity relationships (6, 16, 18). Indeed, as the key structural features relevant to activity are identified, they can be used to efficiently optimize the structure of the compounds to achieve optimal activity.

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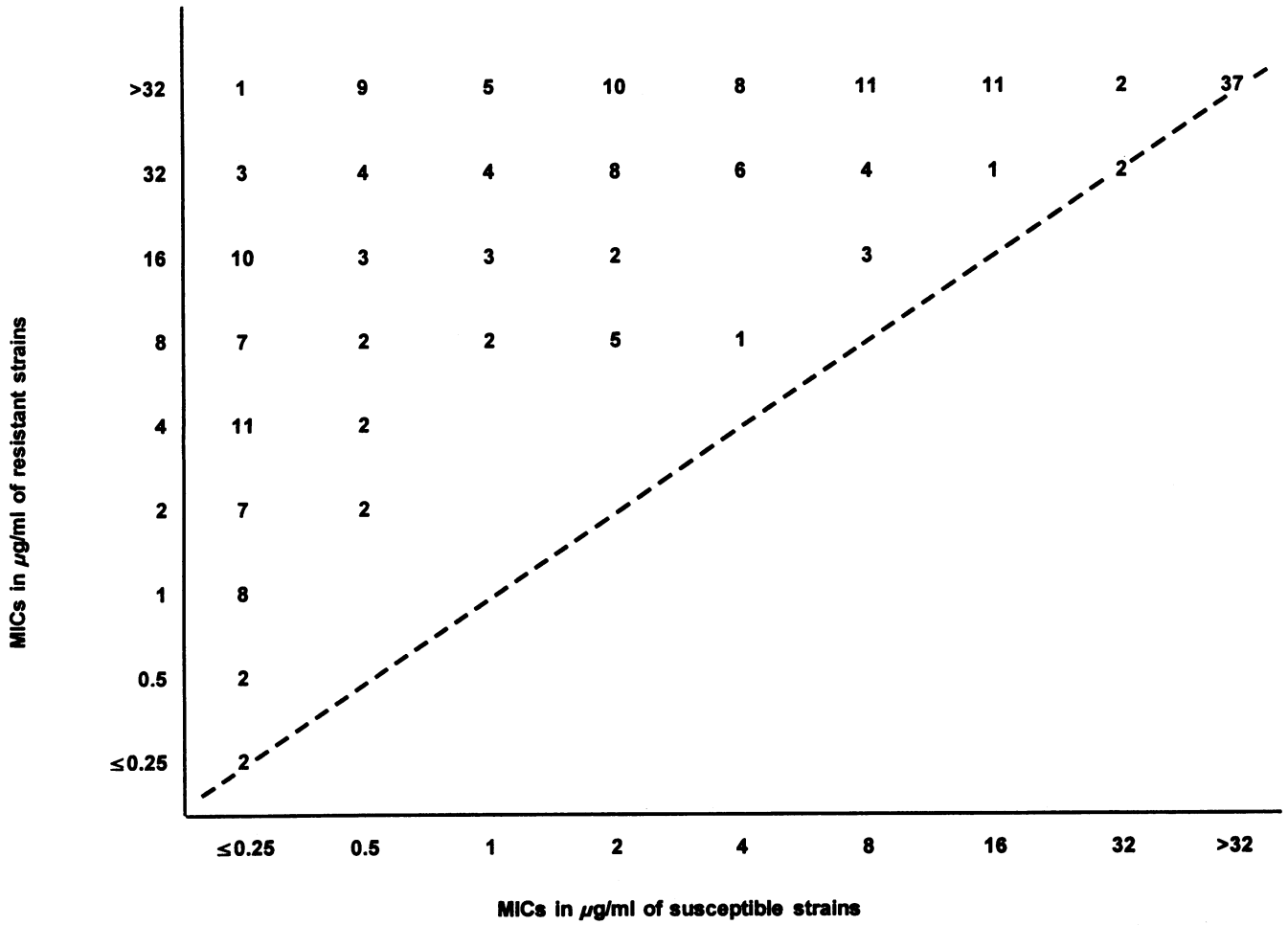

FIG. 3. MICs of the 87 quinolones against the three strains most susceptible to ciprofloxacin (horizontal axis) plotted against MICs of the three strains most resistant to ciprofloxacin (vertical axis). Data were plotted from paired data from susceptible and resistant strains (TMC 1403 versus 1772733, PI 2/8 versus PI 4/44, and 1695757 versus 1915112). Of the 54 on-scale values, MICs for susceptible strains were ≥32-fold lower than those for resistant strains in 11 instances, 4- to 16-fold lower in 36 instances, and ≤2-fold lower in 7 instances. Almost all on-scale points fell above the dashed line, indicating higher MICs for the resistant strains with all agents, with none along the dashed line, where they would be if MICs were equivalent. No points fell into the area below the dashed line, where they would be if any MICs against susceptible strains were higher than those against resistant strains.

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