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N-1-*tert*-Butyl-Substituted Quinolones: In Vitro Anti-*Mycobacterium avium* Activities and Structure-Activity Relationship Studies

GILLES KLOPMAN,[1]* DAN FERCU,[1] THOMAS E. RENAU,[2] AND MICHAEL R. JACOBS[3]

Department of Chemistry[1] and School of Medicine,[3] Case Western Reserve University, Cleveland, Ohio 44106, and Parke-Davis Pharmaceutical Research,[2] Division of Warner Lambert Company, Ann Arbor, Michigan 48105

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We determined the MICs of 63 quinolones against 14 selected reference and clinical strains of the *Mycobacterium avium-Mycobacterium intracellulare* complex. Sixty-one of the compounds were selected from the quinolone library at Parke-Davis, Ann Arbor, Mich., including N-1-*tert*-butyl-substituted agents. T 3761 and tosufloxacin were also tested. The activities of all 63 compounds were compared with those of ciprofloxacin and sparfloxacin. The results showed 45 of the quinolones to be active against the *M. avium-M. intracellulare* complex, with MICs at which 50% of the strains were inhibited (MIC_{50}s) of less than 32 μg/ml. Twenty-four of these quinolones had activities equivalent to or greater than that of ciprofloxacin, and nine of them had activities equivalent to or greater than that of sparfloxacin. The most active compounds were the N-1-*tert*-butyl-substituted quinolones, PD 161315 and PD 161314, with MIC_{50}s of 0.25 μg/ml and MIC_{90}s of 1 μg/ml; comparable values for ciprofloxacin were 2 and 4 μg/ml, respectively, while for sparfloxacin they were 1 and 2 μg/ml, respectively. The next most active compounds, with MIC_{50}s of 0.5 μg/ml and MIC_{90}s of 1 μg/ml, were the N-1-cyclopropyl-substituted quinolones, PD 138926 and PD 158804. These values show that the *tert*-butyl substituent is at least as good as cyclopropyl in rendering high levels of antimycobacterial activity. However, none of the quinolones showed activity against ciprofloxacin-resistant laboratory-derived *M. avium-M. intracellulare* complex strains. A MULTICASE program-based structure-activity relationship analysis of the inhibitory activities of these 63 quinolones and 109 quinolones previously studied against the most resistant clinical strain of *M. avium* was also performed and led to the identification of two major biophores and two biophobes.

Among the different classes of antibacterial agents, fluoroquinolones have achieved great success and aroused considerable expectations because of their broad spectrum of activity against gram-positive and gram-negative bacteria and mycobacteria (2, 16). As a result, thousands of fluoroquinolones have been synthesized in the past decade and several have been marketed and are widely used clinically (1). Recently however, some strains that display resistance to quinolones have appeared, and it is therefore important to search for better antibacterial agents capable of dealing with the resistant strains.

Quinolones are bactericidal agents that target the bacterial DNA gyrase enzyme. Many quinolones have pharmacodynamic properties that result in high intracellular concentrations in host inflammatory cells. This results in many quinolones being effective against intracellular pathogens such as mycobacteria. Intracellular concentrations of quinolone agents are dependent on properties such as the partition coefficient between *n*-octanol and water (log P) and aqueous solubility, and these features can be used in structure-activity relationship (SAR) studies to predict not only in vitro or extracellular activity but also intracellular activity (11). Extensive studies of the SARs of antibacterial quinolones have been published and reviewed in the literature (1–8, 12, 13, 15). We have used the multiple-computer automated structure evaluation (MULTICASE; MULTICASE, Inc., Cleveland, Ohio) methodology (10) to study the SARs of quinolones against the *Mycobacterium avium-Mycobacterium intracellulare* complex, as well as against gram-positive and gram-negative bacteria (11–15). A number of crucial features relevant to the respective endpoints were identified by the program and incorporated into quantitative SAR equations. These equations have been successfully used to predict the activity of new quinolones (12) and to identify more potent and less toxic quinolones (11).

To discover more potent quinolones against the *M. avium-M. intracellulare* complex, we previously tested 88 quinolones (14). The MULTICASE SAR analysis of the in vitro inhibitory activities of these 88 quinolones against 14 increasingly resistant strains of the *M. avium-M. intracellulare* complex (15) led to the identification of a number of structural constraints required to overcome the resistance of most of these strains. It was found that structural features, called biophores, required for activity against increasingly resistant strains become increasingly large while keeping the same substructural backbone. This suggested that the increased resistance of the strains that were previously studied was probably not due to a sudden, new kind of resistance mechanism but rather to a gradual increase in the restrictions imposed on the structure of the active quinolones (15). In a subsequent study (12), 19 new quinolones qualifying under the above structural constraints were identified by MULTICASE and their predicted activities were found to be in good agreement with subsequently derived experimental results.

As part of a National Cooperative Drug Discovery Group for Opportunistic Infections, and in collaboration with researchers at Warner Lambert Parke-Davis, we evaluated several new quinolone compounds against the *M. avium-M. intracellulare* complex. These agents were prepared as part of a study to optimize the quinolone antibacterial agents for mycobacterial infections, particularly those caused by *Mycobacte-*

* Corresponding author. Mailing address: Department of Chemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-7078. Phone: (216) 368-3618. Fax: (216) 368-3006.

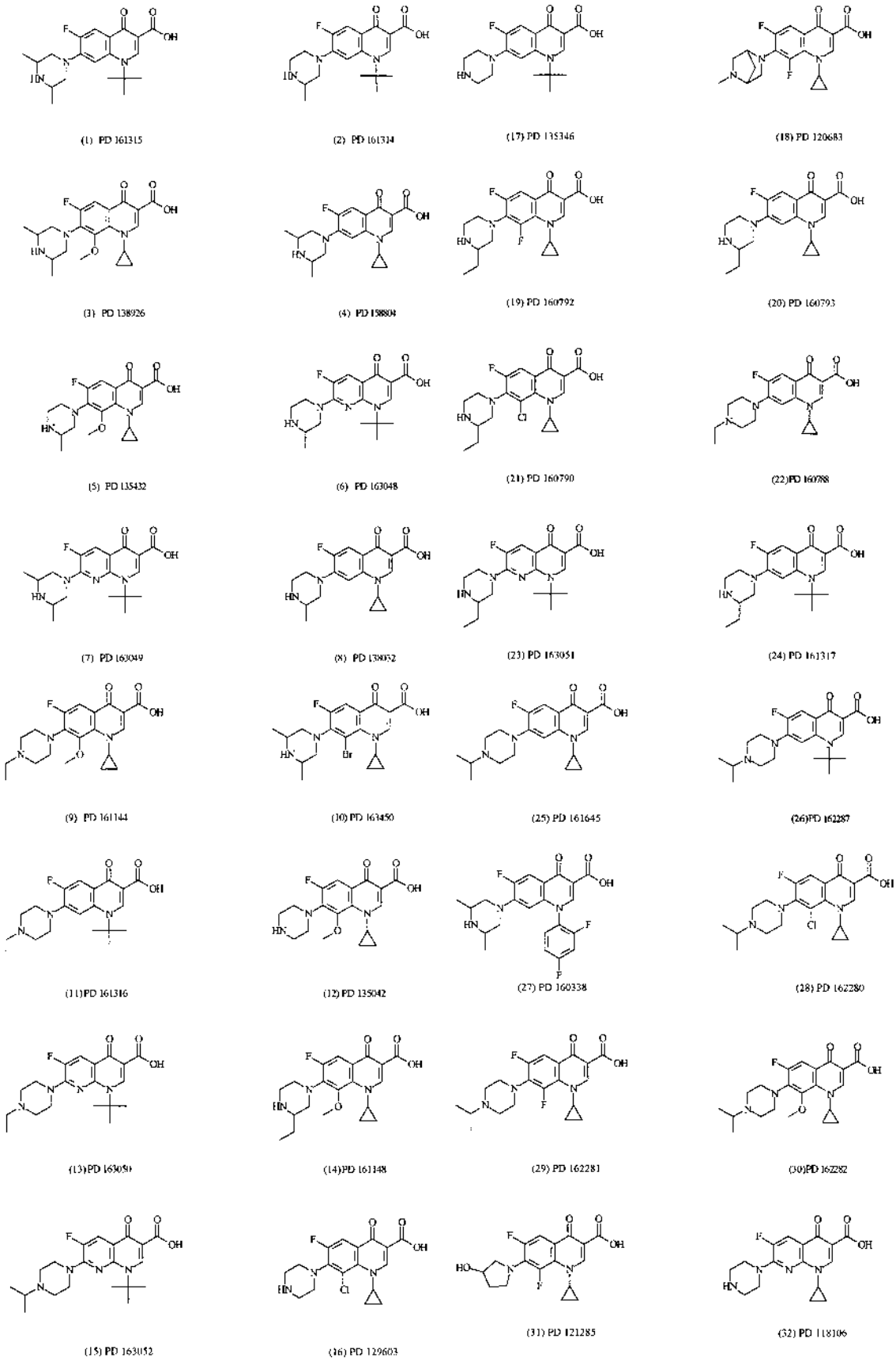


FIG. 1. Structures of the 63 tested quinolones.

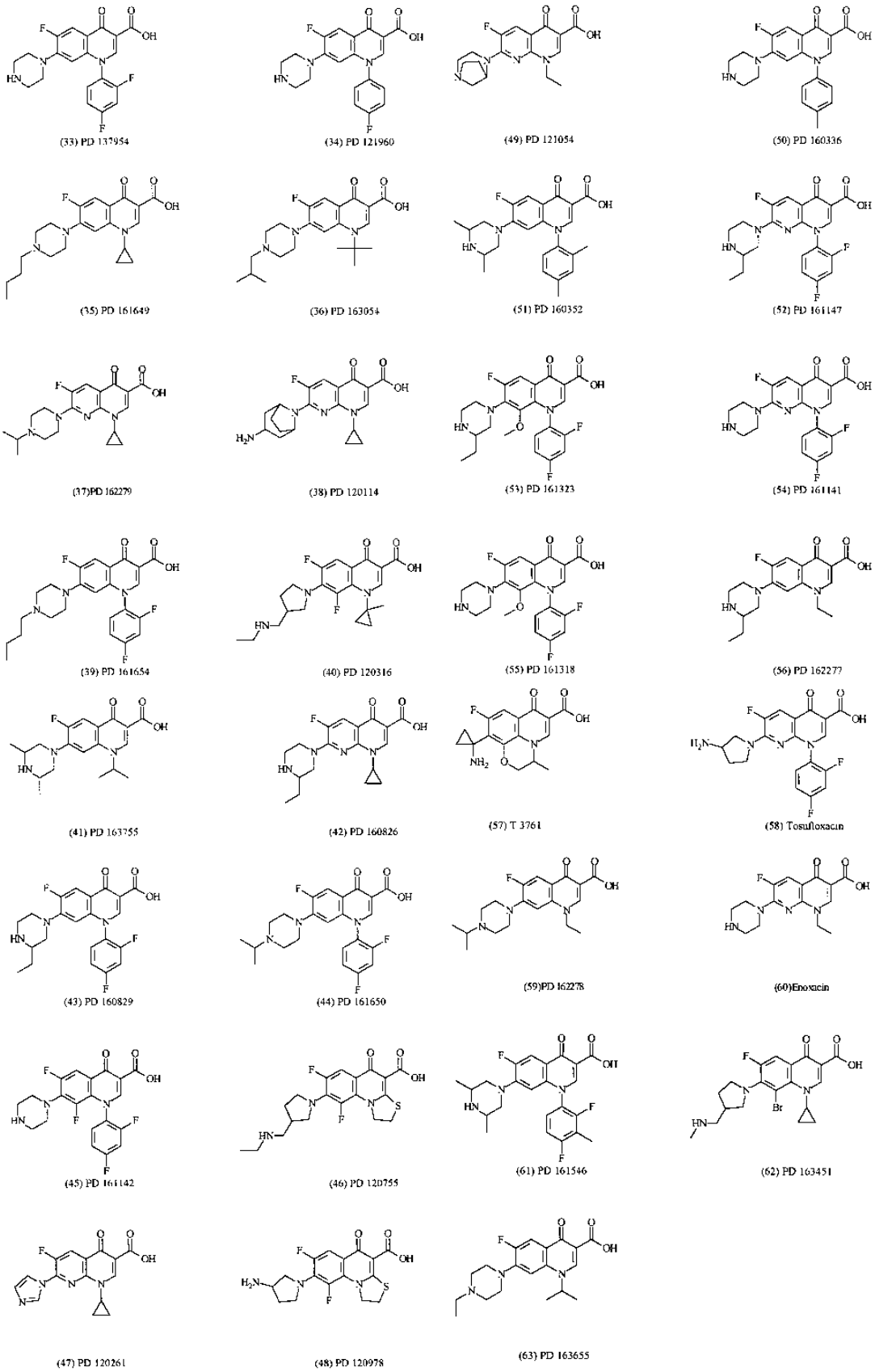


FIG. 1—*Continued.*

TABLE 1. MICs of the 45 quinolones most active against 14 strains of the *M. avium-M. intracellulare* complex

Agent (compound no.[a])	MIC (μg/ml)		
	Range	50%[b]	90%
PD 161315 (1)	0.015–2	0.25	1
PD 161314 (2)	0.03–2	0.25	1
PD 138926 (3)	0.015–2	0.5	1
PD 158804 (4)	0.03–2	0.5	1
PD 135432 (5)	0.03–2	0.25	2
PD 163048 (6)	0.015–4	0.5	2
PD 163049 (7)	0.03–4	0.5	2
PD 138032 (8)	0.06–4	0.5	2
PD 161144 (9)	0.06–4	0.5	2
PD 163450 (10)	0.03–8	0.5	4
PD 161316 (11)	0.06–8	0.5	4
PD 135042 (12)	0.06–4	1	4
PD 163050 (13)	0.06–8	1	4
PD 161148 (14)	0.12–4	1	4
PD 163052 (15)	0.12–8	1	4
PD 129603 (16)	0.06–4	2	4
PD 135346 (17)	0.06–4	2	4
PD 120683 (18)	0.12–4	2	4
PD 160792 (19)	0.12–4	2	4
PD 160793 (20)	0.12–4	2	4
PD 160790 (21)	0.12–8	2	4
PD 160788 (22)	0.12–8	2	4
PD 163051 (23)	0.12–8	2	4
PD 161317 (24)	0.25–4	2	4
PD 161645 (25)	0.25–8	2	8
PD 162287 (26)	0.25–8	2	8
PD 160338 (27)	0.25–16	2	8
PD 162280 (28)	0.5–8	2	8
PD 162281 (29)	0.5–16	2	8
PD 162282 (30)	0.5–16	2	8
PD 121285 (31)	0.12–8	4	8
PD 118106 (32)	0.25–8	4	8
PD 137954 (33)	0.25–16	4	8
PD 121960 (34)	0.5–16	4	8
PD 161649 (35)	0.5–16	4	8
PD 163054 (36)	0.25–16	4	16
PD 162279 (37)	0.5–16	4	16
PD 120114 (38)	0.5–32	4	16
PD 161654 (39)	0.5–32	4	16
PD 120316 (40)	0.25–16	8	16
PD 163755 (41)	2–16	16	16
PD 160826 (42)	0.5–>32	8	32
PD 160829 (43)	1–32	8	32
PD 161650 (44)	1–>32	8	>32
PD 161142 (45)	0.5–>32	16	>32
Ciprofloxacin[c]	0.06–8	2	4
Sparfloxacin[c]	0.015–8	1	2

[a] Compound numbers correspond to those in Fig. 1.
[b] MIC_{50}s of the other quinolones were all ≥32 μg/ml.
[c] MICs include results from previous studies and are shown for comparison.

rium tuberculosis. Our goal was to test compounds from this study against the *M. avium-M. intracellulare* complex and, by using MULTICASE, to quantitatively analyze the structural features related to the observed antimycobacterial activity of these agents for the purpose of optimizing activity.

MATERIALS AND METHODS

Antimicrobial agents. A total of 63 fluoroquinolones, possessing a wide variety of structural modifications and physical properties, were tested for antimycobacterial activity. Of these quinolones, 61 were synthesized (18) at the Parke-Davis Pharmaceutical Research Laboratories in Ann Arbor, Mich., quinolone T 3761 was obtained from the Green Cross Corp., Osaka, Japan, and tosufloxacin was obtained from Toyama Chemical Company, Toyama, Japan. Ciprofloxacin and sparfloxacin were included as positive controls. The structures of all the test compounds are shown in Fig. 1.

Strains. Fourteen strains of the *M. avium-M. intracellulare* complex, selected from references and clinical isolates to elicit the most variation in activity from ciprofloxacin and other agents, were used (14). Six ciprofloxacin-resistant laboratory-derived mutant *M. avium-M. intracellulare* complex strains, provided by Howard Takiff, were also included in the study. Strains were maintained at −70°C and subcultured at least twice on 7H11 agar (Difco, Detroit, Mich.) prior to testing.

Susceptibility testing. MICs were determined by the broth microdilution method in 7HSF medium (the broth equivalent of 7H11 agar) (22) as previously described (14). Quinolones were dissolved in 1 N NaOH, diluted in water, and incorporated into the medium at concentrations ranging from 0.015 to 32 μg/ml.

MULTICASE methodology. The MULTICASE program, an artificial intelligence system capable of identifying structural descriptors that may be associated with the properties of the molecules examined, was used as previously described (9, 10).

RESULTS

In vitro activities. All 63 quinolones were initially tested against the panel of 14 reference *M. avium-M. intracellulare* complex strains at concentrations of 32 μg/ml. Eighteen of these quinolones had MICs at which 50% of the strains were inhibited (MIC_{50}s) of >32 μg/ml and were not studied further. Both T 3761 and tosufloxacin were in that category. The results of determinations of MICs of the remaining 45 agents are shown as MIC ranges, MIC_{50}s, and MIC_{90}s in Table 1. Of the 45 active compounds, 24 showed in vitro activity comparable to that of ciprofloxacin, while 9 were as good as or better than sparfloxacin.

MULTICASE analysis of the entire quinolone database. We combined all quinolones for which we had experimental in vitro results and ended up with a database of 172 compounds. Biophores were identified by MULTICASE analyses for each of the 14 172-molecule databases derived from the 14 strains. However, only the results for the most resistant strain overall, 1760694, are analyzed in this paper. The two top biophores, as well as the biophobes, from this analysis are presented in Fig. 2. The first biophore (biophore 1) existed in 74 (43%) of the compounds of the database (55 active and 19 inactive compounds). These compounds are 1-cyclopropyl-5-unsubstituted-6-fluoro quinolone derivatives, with an average activity of 11 μg/ml. The modulators associated with the first biophore are given in Table 2. They give information about the C-7 and C-8 regions, thought to be the part of the quinolone molecule that binds to DNA gyrase. The second biophore, containing the *tert*-butyl group, is embedded in 14 compounds, all active, with an average activity of 7 μg/ml.

Two important biophobes (detrimental to activity) were found by the MULTICASE program (Fig. 2). One is 3-(alkylaminomethyl)-pyrrolidinyl, while the other is a methylamino moiety. Both are essentially secondary amino-substituted pyrrolidines at the C-7 position.

DISCUSSION

The need for the development of pharmacological agents with improved antimycobacterial activity is urgent. Several promising candidates have been uncovered in this study, particularly PD 161314 and PD 161315, each of which has an MIC_{50} of 0.25 μg/ml and an MIC_{90} of 1 μg/ml, which are four- to eightfold lower than those of ciprofloxacin and two- to fourfold lower than those of sparfloxacin.

Unfortunately, the six ciprofloxacin-resistant laboratory-derived mutant *M. avium-M. intracellulare* complex strains were found to be totally resistant to all 63 quinolones. This observation is in contrast to our previous observation (15) that the clinical strains exhibit progressive resistance due to a gradual increase in the complexity of the structural fragments required

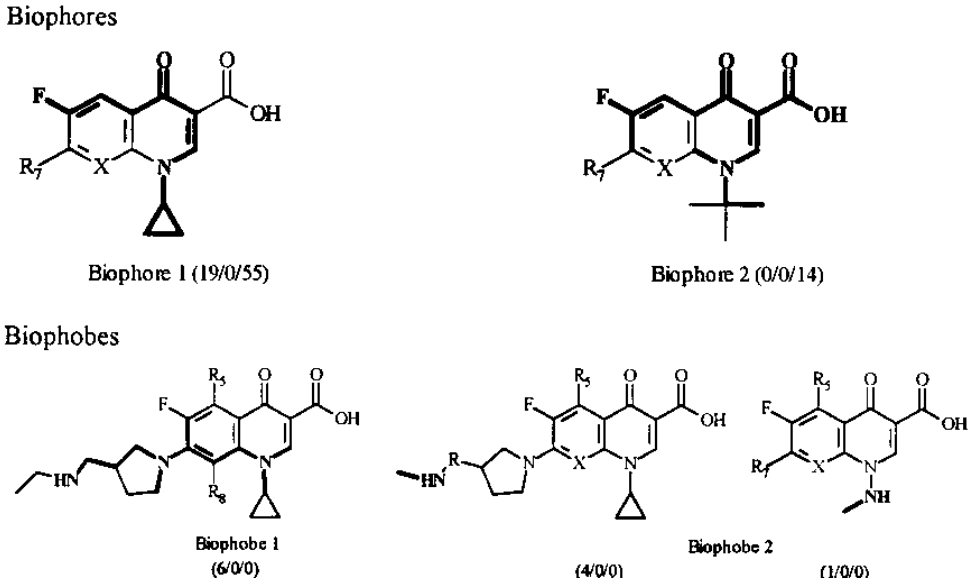


FIG. 2. Biophores and biophobes for anti-*M. avium* (strain 1760694) activity identified by the MULTICASE program. Biophores and biophobes are shown with boldface lines and characters in the molecules. The distribution among inactive, marginal, and active compounds (in that order) is shown in parentheses.

to be present in the active molecules. It is therefore likely that in the laboratory-derived, totally resistant strains, a new resistance mechanism has developed, and if this is true, the likelihood that modified quinolones that are active against these strains could be found is small.

The two most active compounds, PD 161314 (quinolone 2 in Fig. 1) and PD 161315 (quinolone 1 in Fig. 1), feature a *tert*-butyl group at the N-1 position (biophore 2) rather than the common cyclopropyl group found in most active quinolones. Moreover, the C-7 substituent (3,5-dimethylpiperazinyl) in PD 161315 is the same as that in sparfloxacin and in QT 5 (1-cyclopropyl-6,8-difluoro-7-(3,5-dimethylpiperazinyl)-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid, the quinolone previously designed and synthesized in our laboratory [12]). No substituent is present at the C-8 position.

The next two most active compounds, PD 138926 (quinolone 3 in Fig. 1) and PD 158804 (quinolone 4 in Fig. 1) have MIC_{50}s and MIC_{90}s which are fourfold lower than those of ciprofloxacin. Both of these compounds contain a cyclopropyl group at the N-1 position (biophore 1) and the same activating 3,5-dimethylpiperazinyl substituent at the C-7 position. The nature of the substituent at the C-8 position does not seem to be very important because compounds 3 (8-methoxy) and 4 (unsubstituted) have similar MIC_{50}s and MIC_{90}s. It should be noted, however, that the 8-methoxy quinolone seems to be more active against the more susceptible strains.

Overall, it appears that the *tert*-butyl group is at least as good as the cyclopropyl group as a substituent at the N-1 position of the quinolone. This discovery casts some doubts on at least two hypotheses made to explain the previous belief that the cyclopropyl substituent at the N-1 position conveys unusually high activity to the quinolones that contain this feature.

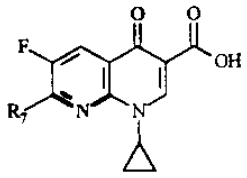
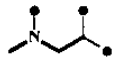
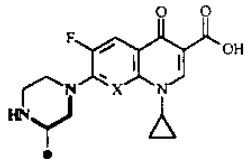
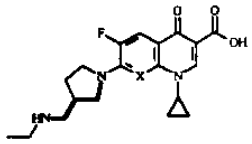
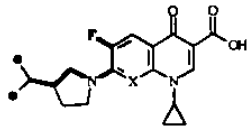
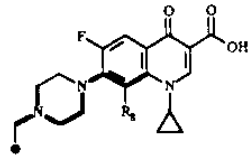
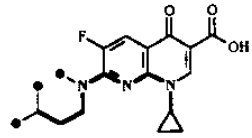
One hypothesis is based on the belief that the antibacterial activity of quinolones is related to the amount of un-ionized drug that is able to penetrate the cell membranes. This in turn was associated with a highly acidic carboxyl group and a less basic C-7 amino substituent (17). The carboxyl group is more acidic if the N-1 substituent is electron withdrawing. The greater activity of cyclopropyl-substituted quinolones was therefore associated with the electron-withdrawing effect of this moiety. However, the *tert*-butyl substituent is an electron-releasing substituent. Therefore, the suggestion that cyclopropyl is better than other alkyl substituents because of its ability to activate the carboxylic acid at C-3 does not appear to be valid.

Our group previously hypothesized that the cyclopropyl-substituted quinolones act as suicide inactivators of bacterial cytochrome P-450 methylamine dehydrogenase (12), an enzyme believed to be involved in the maintenance of the cell membrane. Both such inactivation mechanisms, hydrogen abstraction and cation radical formation, as proposed by Tullman and Hanzlik (21), require the presence of a hydrogen atom on the carbon linked to the amino group. The intermediate metabolites from both of these mechanisms might lead to enzyme inactivation or release of some active metabolite (potential enzyme inactivator) into the solution (12). Assuming that the mechanism of action of the *tert*-butyl-substituted quinolones is the same as that of compounds with a substituted cyclopropyl group at the N-1 position, then they cannot undergo inactivation by the proposed mechanism(s) because there is no hydrogen α to the N-1 atom of the quinolone. Moreover, in an effort to find out if cyclopropyl amine is involved in the quinolone mode of action, we tested it for in vitro antimycobacterial activity and found it to be inactive against all *M. avium-M. intracellulare* complex strains. Therefore, we assume that this kind of suicide inactivation is not involved in the mechanism of action of these quinolones.

On the other hand, the fact that the *tert*-butyl group is a better electron-donating group and possesses higher hydrophobicity than the cyclopropyl group provides support for the cooperative-binding model for the inhibition of DNA gyrase proposed by Shen, in which two quinolone molecules self-associate by π-π ring stacking and tail-to-tail hydrophobic interactions between the N-1 substituents (20). However, a higher lipophilicity of the N-1 group was not found to correlate with increased antimicrobial activity (19).

Most of the modulators of biophore 1 deal with the substituents at C-7. Considerable variations in activity are produced by the nature of these substituents. Those that are deactivating are acyclic secondary amines and primary amines bound to a

TABLE 2. Activity contributions of the modulators associated with the first biophore for the most resistant strain (1760694)[a]

Fragment[b]	Quinolone substituent containing fragment	Distribution (I, M, A)[c]	Activity contribution[d]
H_2N—CH—	R_7	5, 0, 3	−38.3
H_2N—CH_2—	R_7	1, 0, 3	−16.6
S—CH_2—	R_7	1, 0, 0	−20.0
	X (naphthyridines)	5, 0, 4	−29.0
	R_7	0, 0, 3	+10.6
	R_7 (3-substituted piperazines)	1, 0, 15	+11.1
	R_7 (3-alkylaminomethyl-pyrrolidines)	5, 0, 0	−39.5
	R_7	3, 0, 2	−10.5
	R_7	0, 0, 2	+10.6
	R_7, R_8	0, 0, 2	+29.3

[a] Average activity (in MULTICASE internal units) of compounds containing the biophore, 43.
[b] ●, nonhydrogen substituent.
[c] I, M, A, number of compounds that were inactive, marginal, and active, respectively.
[d] Values are in MULTICASE internal units and reflect the importance of the modulator's ability to improve the MIC. The activity contribution of water solubility is +6.6.

primary or secondary carbon but not to a tertiary carbon (fragment H_2H—C—, containing a tertiary carbon, is embedded in two 1-cyclopropyl quinolones, both active, with an average activity of 8 μg/ml). On the other hand, the activating modulators are tertiary and cyclic secondary amines. This observation suggests that the activity of quinolones is unlikely to be strongly dependent on the pK_a of the amino group at the C-7 position, as the pK_a values of ethylamine (with a primary carbon linked to the amino group), isopropylamine (with a secondary carbon), and *tert*-butylamine (with a tertiary carbon) are about the same (≅10.7).

Our data show at least three compounds (PD 115311 [14], QT 3, and QT 7 [12]) in which the NH group on the C-7 piperazinyl substituent is replaced by either SO or SO_2. These three compounds have MICs of 8, 4, and 4 μg/ml, respectively, for the most resistant strain overall, 1760694 (for comparison, the MIC range of the whole database is 1 to 32 μg/ml for this particular strain). On the other hand, there are two totally inactive quinolones (MICs >32 μg/ml) in which the NH group on the C-7 piperazinyl substituent is replaced by sulfur. Sulfur alone is unlikely to give H bonds, while both SO and SO_2 groups are strong H-bond acceptors. This supports the idea that it is not the basicity of the amino group that is relevant to activity but rather its potential involvement as an H-bond acceptor in interacting with DNA gyrase.

In conclusion, our results for the *M. avium-M. intracellulare*

complex strains show that (i) *tert*-butyl is as good as, if not better than, cyclopropyl at the N-1 position of the quinolone nucleus, (ii) the pK_a of the amino group at the C-7 position is unlikely to be relevant to antimycobacterial activity, and (iii) a strong H-bond acceptor rather than a basic group, within certain steric requirements, is needed at the C-7 position. Similar results for both of these types of compounds have recently been observed with *Mycobacterium fortuitum* and *Mycobacterium smegmatis* (18). The N-1-*tert*-butyl-substituted quinolones appear to have some potential for clinical use as antibacterial and antimycobacterial agents and warrant further evaluation. None of the compounds, however, shows activity against totally ciprofloxacin-resistant *M. avium-M. intracellulare* complex strains.

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