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Structure-Activity Relationships of Quinolone Agents against Mycobacteria: Effect of Structural Modifications at the 8 Position

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A series of quinolones with substitutions at the 8 position has been prepared as part of a study to examine the relationship between structural modifications at this position and activity against mycobacteria. The compounds were prepared by procedures described in the literature and were evaluated for their activities against *Mycobacterium fortuitum* and *Mycobacterium smegmatis*. The activities of the compounds against these two organisms were used as a measure of *Mycobacterium tuberculosis* activity. The results demonstrate that the contribution of the 8 position to antimycobacterial activity was dependent on the substituent at N-1 and was in the order (i) COMe ≈ CBr > CCl > CH ≈ CF ≈ COEt > N > CCF_3 when N-1 was cyclopropyl; (ii) N ≈ CH > CF > COMe when N-1 was 2,4-difluorophenyl; (iii) N ≥ CH when N-1 was *tert*-butyl; and (iv) N > CH when N-1 was ethyl. In general, derivatives with piperazine substitutions at C-7 were slightly less active against mycobacteria than the analogs with pyrrolidine substitutions, regardless of the pattern of substitution at the 8 position. Several of the best compounds were evaluated for their potential side effects as well as their activities against *Mycobacterium aurum*, *Mycobacterium avium-M. intracellulare*, and *M. tuberculosis*. These agents exhibited biological profiles similar to or better than those of the positive controls ciprofloxacin and sparfloxacin.

As part of a study to optimize the quinolone agents against mycobacterial infections, specifically *Mycobacterium tuberculosis*, we recently described the antimycobacterial activity of several quinolones with substitutions at N-1 and C-7 (19). Several compounds, such as compounds A and B, were as active in our primary screen as ciprofloxacin and sparfloxacin (Fig. 1). Our previous work (19, 20) was primarily undertaken to address the issue of the dramatic rise in resistance of *M. tuberculosis* to conventional drug therapy and the need for the development of alternative chemotherapeutic agents to treat infection with this organism (1, 13, 27). The key conclusion from the initial study was that activity against mycobacteria was more strongly related to antibacterial activity than to other physical chemical factors such as lipophilicity. With an understanding of how the N-1 and C-7 substituents affect mycobacterial activity and knowing that modifications at the 8 position can affect antibacterial activity in general, we have prepared a variety of 8-substituted quinolones to examine the role modifications of the 8 position have in antimycobacterial activity. These compounds have been evaluated in our facile and robust primary assay which includes the organisms *Mycobacterium fortuitum* and *Mycobacterium smegmatis*, and, as before, the activity of the compounds against these organisms has been used as a predictive measure of *M. tuberculosis* activity (19). Thus, in the present report, we describe the biological activities of several key compounds from our latest study and illustrate the overall effect on antimycobacterial activity of structural changes at the N-1, C-7, and 8 positions. We also show the validation of the testing strategy by selecting several of the best compounds for further evaluation against *M. tuberculosis* and several other organisms, such as *Mycobacterium avium* and *Mycobacterium aurum*. Additionally, toxicity profiles are included for several selected agents with good antimycobacterial activity.

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MATERIALS AND METHODS

Chemicals. The quinolone and naphthyridine nuclei used in the study are referred to as series 1 to 12 and are shown in Table 1. The amine side chains employed for this study, designated by letters a to f, are illustrated in Fig. 2. The compounds evaluated in this study were prepared by procedures described in the literature (2–4, 8, 10, 11, 15, 17, 18, 21–23). The structural properties of the compounds were confirmed by nuclear magnetic resonance, mass spectrometry, and infrared, and the purity of each compound was established by either elemental analysis or high-performance liquid chromatography analysis.

Antibacterial and antimycobacterial assays. The series of 8-substituted quinolones and naphthyridines along with the reference agents ciprofloxacin, sparfloxacin, and compounds A and B were tested against *M. fortuitum* (ATCC 6841) and *M. smegmatis* (ATCC 19420) as previously described (19). Several selected compounds were also tested against *M. aurum* (ATCC 23366) by the procedures described for *M. fortuitum* and *M. smegmatis*. Additionally, several analogs were tested against 14 strains of *M. avium* by previously published procedures (14). These same compounds were tested for their activities against *M. tuberculosis* by the conventional BACTEC procedure (12). All of the compounds were tested against two gram-negative and two gram-positive bacteria by standard microtitration techniques (6). In all cases, MICs (in micrograms per milliliter) were determined for each compound and are recorded in Tables 2 and 3.

Phototolerance and cytotoxicity assays. Screening for phototolerance was performed on several compounds as previously described (24). Briefly, CF1 mice, whose backs were depilated 24 h earlier, were dosed by an appropriate route with various amounts of a drug. One hour postdosing, the mice were exposed to light in the UVA range for a 3-h period. The dosing-exposure regimen was repeated daily for 4 days. The mice were observed daily for any phototoxic reactions, such as erythema and edema. The results, recorded in Table 4, are reported as the dose necessary to induce a phototoxic reaction in 50% of the mice as determined by using the probit method and doses of 30, 100, and 300 mg/kg of body weight. Compounds evaluated for their phototolerance were also examined in a mam-

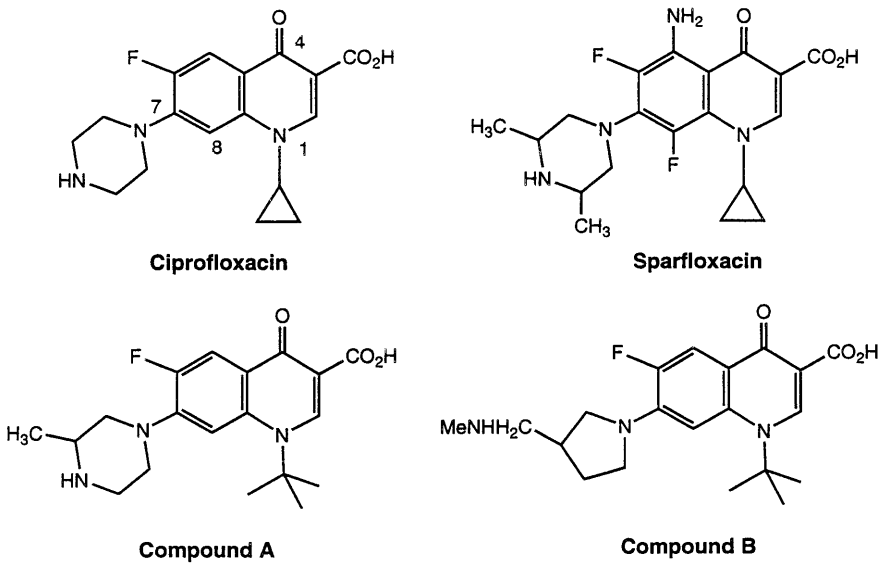


FIG. 1. Structures of several fluoroquinolones examined in this study.

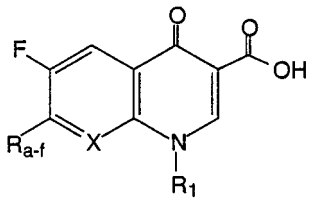
malian cell clonogenic cytotoxicity assay to estimate their clastogenic potential (25). The drug concentration (in micrograms per milliliter) inhibiting colony formation by 50% relative to the control was determined and is recorded in Table 4.

RESULTS

Effect of structural modifications at the 8 position. To examine how modifications at the 8 position affect antimycobacterial activity, we chose to substitute at C-7 and N-1 with optimal groups which we had identified from our initial study (19). The results, recorded in Table 2, demonstrate that the contribution of the 8 position to antimycobacterial activity is highly dependent upon the substituent at N-1. For example, within the series of compounds substituted at N-1 with a cyclopropyl group (series 1 to 7), the 8-OMe derivatives (series 5) were among the most active, with MICs against *M. fortuitum* and *M. smegmatis* of ≤0.03 to 0.13 μg/ml. However, within the series of compounds substituted at N-1 with a 2,4-difluorophenyl group (series 10 to 12), the 8-OMe derivatives (series 11) were the least active (MICs, 0.5 to 16 μg/ml). Interestingly, the opposite holds true in the same series of derivatives when the 8-OMe is exchanged for an 8-N. In that case, the naphthyridines substituted at N-1 with a 2,4-difluorophenyl (series 12) were more active than the 8-methoxy or 8-fluoro compounds whereas in the N-1 cyclopropyl series the naphthyridines (series 7) were among the least active analogs. Compounds substituted with CCF_3 at the 8 position (series 4) were among the least active derivatives in the N-1 cyclopropyl series. Overall, the compounds most active against both *M. fortuitum* and *M. smegmatis* were derivatives from series 3 (8-Br), 5 (8-OMe), and 9 (the N_1 *t*-butyl naphthyridines).

Antimycobacterial activities of several compounds. Several compounds from each series (3b, 5d, 9b, and 9c) were further evaluated against other species of mycobacteria, and the results of these studies are presented in Table 3. Data for ciprofloxacin, sparfloxacin, and compound A are included for comparative purposes. The chosen compounds were initially tested against *M. aurum* since a recent report described this organism as a predictor of *M. tuberculosis* activity (5). The MICs for *M. aurum* were very similar to the MICs obtained for *M. fortuitum* and *M. smegmatis*. The same derivatives were also tested against 14 strains of *M. avium*, and although quinolones are typically less active against *M. avium* than against other mycobacterial species such as *M. fortuitum* and *M. tuberculosis*, the compounds were generally as active as or more active than both ciprofloxacin and sparfloxacin, with MICs at which 50% of the isolates were inhibited ranging from 0.25 to 1.0 μg/ml. The activity of the compounds against *M. tuberculosis* was slightly less than the activity of ciprofloxacin, with MICs ranging from 0.39 to 0.78 μg/ml. In contrast, sparfloxacin was 6.5- to 13-fold more active than the compounds examined. Overall, the correlation between activity against *M. fortuitum* and *M. smegmatis* and activity against *M. tuberculosis* was less good for the new compounds than for ciprofloxacin and sparfloxacin.

TABLE 1. Quinolone substrates used in this study[a]



Series	R_1	X
1	c-C_3H_5	CF
2	c-C_3H_5	CCl
3	c-C_3H_5	CBr
4	c-C_3H_5	CCF_3
5	c-C_3H_5	COMe
6	c-C_3H_5	COEt
7	c-C_3H_5	N
8	CH_2CH_3	N
9	$(CH_3)_3C$	N
10	2,4-F_2Ph	CF
11	2,4-F_2Ph	COMe
12	2,4-F_2Ph	N

[a] c-C_3H_5, cyclopropyl; CH_2CH_3, ethyl; $(CH_3)_3C$, *tert*-butyl; 2,4-F_2Ph, 2,4-difluorophenyl. R_{a-f}, see Fig. 2.

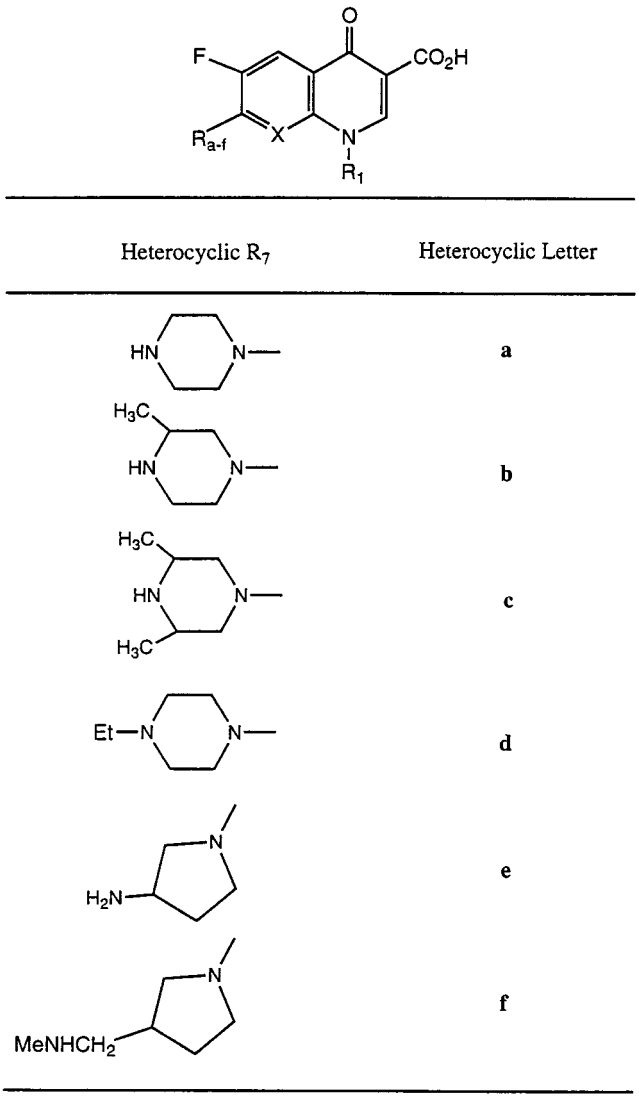


FIG. 2. Side chain substituents used in this study.

Phototolerance and cytotoxicity of several compounds. Safety issues are important in the development of any fluoroquinolone for tuberculosis, so several of the best compounds were evaluated for their potential side effects; the results are recorded in Table 4. Data for ciprofloxacin, sparfloxacin, and several additional agents are included for comparison. In all cases, the profiles were the same as data currently available for structurally similar fluoroquinolones (7). For example, the 8-OMe derivatives, a representative sample being 5b, were the least phototoxic, while the *tert*-butyl derivatives, such as 9b, 9f, A, and B, were the least cytotoxic. The 8-Br derivatives, such as 3b, were as phototoxic as sparfloxacin.

DISCUSSION

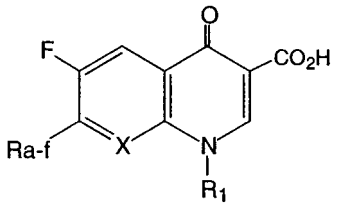
We initially evaluated all the 8-substituted quinolones against *M. fortuitum* and *M. smegmatis* in a high-throughput screen. This primary screen was a rapid broth susceptibility test which used nonpathogenic organisms, avoided radioactive waste, and offered the possibility of screening a substantial number of compounds in an assay which is predictive of *M. tuberculosis* activity (19, 20). Since good antibacterial activity is a prerequisite for good antimycobacterial activity (19, 20), the compounds were also tested against several representative gram-negative and gram-positive bacteria for comparative purposes. One of the most active compounds in this study was 5d. Interestingly, another 6-fluoro-8-methoxy quinolone (AM-1155 [5b]) has shown excellent antimycobacterial activity (26), and a report of its pharmacokinetic properties in humans was recently published (16).

The correlation between N-1 substitution and antimycobacterial activity in the present study was essentially the same as what was observed in our previous work (19). For instance, the analogs with *tert*-butyl and cyclopropyl substitutions at N-1 were again the most active agents. Substitution at N-1 with an ethyl (series 8) or 2,4-difluorophenyl (series 10 to 12) gave, as before, significantly less active compounds despite the greater lipophilicity of these agents, a feature expected to favor antimycobacterial potency. In the case of the N-1 cyclopropyl derivatives (series 1 to 7), halogen substitution at the 8 position (series 1 to 3) typically gave compounds very active against mycobacteria, and similar trends were observed for these compounds in the antibacterial screen. The 8-Br compounds showed better antimycobacterial activity than the 8-fluoro or 8-chloro derivatives even though the latter two were more active in the standard antibacterial assays. Replacement of carbon with nitrogen at the 8 position in the case of the N-1 *tert*-butyl derivatives (series 9) gave agents slightly more active than compound B. Analogs from series 9 were some of the most active compounds studied, suggesting a particular importance of the N_1 *tert*-butyl group for antimycobacterial activity.

The contribution of the 8 position to antimycobacterial activity is much less dependent upon modifications at C-7 than upon changes at N-1. In general, piperazine-substituted derivatives were slightly less active than pyrrolidine-substituted derivatives, regardless of modifications at the 8 position. Since pyrrolidine- and piperazine-substituted derivatives are nearly equally effective against mycobacteria, it is apparent that compounds containing a pyrrolidine side chain, with their increased cytoliability (7, 25), are much less attractive in the development of a quinolone for the treatment of tuberculosis. This is primarily due to the fact that the eradication of tuberculosis requires a lengthy course of treatment, and the need for an agent with a high margin of safety becomes a primary concern.

In general, there was good agreement between antimycobacterial activity and antibacterial activity. These results are supported by a recent study which demonstrated that many physiological aspects of quinolone action in *Escherichia coli*, such as detectable breaks in DNA, are also seen in mycobacteria (9). These results further reinforce our previous observations (19, 20) that some level of intrinsic antibacterial activity is extremely important for corresponding activity against mycobacteria. In one case, however, the correlation between antibacterial and antimycobacterial activity was not as strong. Specifically, compounds from series 9 have only modest to weak antibacterial activity but excellent antimycobacterial activity, with MICs ranging from 0.03 to 0.13 μg/ml. This comparison is more apparent upon examination of the biological profiles of ciprofloxacin and several compounds from series 9. The antibacterial activity of ciprofloxacin is superior to that of 9d, for example, even though 9d is slightly more active than ciprofloxacin against *M. fortuitum* and *M. smegmatis*. Similar observations have been made with several other N-1 *tert*-butyl derivatives, such as compounds A and B (19). The weaker correlation between antibacterial and antimycobacterial activity was not as striking with analogs A and B compared with the

TABLE 2. Biological testing results of the antibacterial and mycobacteria assays[a]



Compound	R_1[c]	X	MIC (μg/ml) for organism[b]					
			Gram negative		Gram positive		*M. fortuitum*	*M. smegmatis*
			Vogel	U1-18	H-228	C203		
1a	c-C_3H_5	CF	0.05	0.2	0.4	0.8	0.06	0.25
1b	c-C_3H_5	CF	0.025	0.4	0.4	0.8	0.06	0.25
1c	c-C_3H_5	CF	0.05	0.8	0.4	>3.1	0.06	0.13
1d	c-C_3H_5	CF	0.1	0.8	0.4	1.6	0.13	0.25
1e	c-C_3H_5	CF	0.013	0.1	0.05	0.05	0.06	0.06
1f	c-C_3H_5	CF	0.05	0.4	0.03	0.03	0.06	0.13
2a	c-C_3H_5	CCl	0.05	0.2	0.1	0.4	0.06	0.13
2b	c-C_3H_5	CCl	0.05	0.4	0.2	0.4	0.06	0.13
2c	c-C_3H_5	CCl	0.1	1.6	0.4	3.1	0.13	0.25
2d	c-C_3H_5	CCl	0.1	0.8	0.2	0.4	0.13	0.25
2e	c-C_3H_5	CCl	0.013	0.05	0.05	0.1	≤0.03	≤0.03
2f	c-C_3H_5	CCl	0.2	0.4	0.03	0.05	≤0.03	0.06
3a	c-C_3H_5	CBr	0.05	0.4	0.4	0.4	≤0.03	0.06
3b	c-C_3H_5	CBr	0.05	0.8	0.2	0.4	≤0.03	0.06
3c	c-C_3H_5	CBr	0.2	3.1	0.4	1.6	0.06	0.13
3d[d]	c-C_3H_5	CBr	0.1	1.6	0.4	0.8	0.06	0.13
3f	c-C_3H_5	CBr	0.1	0.8	0.03	0.03	≤0.03	≤0.03
4a	c-C_3H_5	CCF_3	0.1	1.6	0.4	0.8	0.25	0.5
4b	c-C_3H_5	CCF_3	0.2	3.1	0.4	1.6	1.0	2.0
4c	c-C_3H_5	CCF_3	0.4	>3.1	0.4	1.6	0.5	1.0
4d	c-C_3H_5	CCF_3	0.4	>3.1	0.8	3.1	1.0	2.0
4e	c-C_3H_5	CCF_3	0.1	1.6	0.2	0.8	0.13	0.25
4f	c-C_3H_5	CCF_3	0.4	>3.1	0.1	0.1	8.0	4.0
5a	c-C_3H_5	COMe	0.05	0.4	0.2	0.2	0.06	0.13
5b	c-C_3H_5	COMe	0.05	0.8	0.2	0.8	0.06	0.06
5c	c-C_3H_5	COMe	0.2	0.8	0.2	0.4	0.06	0.13
5d	c-C_3H_5	COMe	0.1	0.8	0.2	>3.1	≤0.03	≤0.03
5e	c-C_3H_5	COMe	0.05	0.4	0.05	0.05	0.06	0.06
5f	c-C_3H_5	COMe	0.2	0.8	0.03	0.03	≤0.03	0.06
6a	c-C_3H_5	COEt	0.1	1.6	0.4	0.8	0.06	0.25
6b	c-C_3H_5	COEt	0.1	1.6	0.2	0.4	0.06	0.13
6c	c-C_3H_5	COEt	0.2	3.1	0.2	0.4	0.06	0.13
6d	c-C_3H_5	COEt	0.4	3.1	0.4	1.6	0.25	0.5
6e	c-C_3H_5	COEt	0.1	0.8	0.1	0.4	0.06	0.13
6f	c-C_3H_5	COEt	0.4	3.1	0.05	0.1	0.13	0.25
7a	c-C_3H_5	N	0.05	0.2	1.6	1.6	0.13	0.5
7b	c-C_3H_5	N	0.05	0.4	1.6	3.1	0.13	0.5
7c	c-C_3H_5	N	0.1	0.8	3.1	>3.1	0.13	0.5
7d	c-C_3H_5	N	0.1	0.8	0.8	3.1	0.25	0.25
7e	c-C_3H_5	N	0.025	0.1	0.2	3.1	0.06	0.13
7f	c-C_3H_5	N	0.1	0.8	0.2	0.8	0.13	0.25
8a	CH_2CH_3	N	0.2	0.8	3.1	6.3	0.4	1.6
8b	CH_2CH_3	N	0.2	1.6	3.1	>3.1	0.25	1.0
8c	CH_2CH_3	N	0.8	>3.1	>3.1	>3.1	0.5	2.0
8d	CH_2CH_3	N	0.4	3.1	1.6	>3.1	0.5	2.0
8e	CH_2CH_3	N	0.1	0.4	0.4	0.8	0.25	1.0
8f	CH_2CH_3	N	0.8	3.1	0.4	0.8	0.25	1.0
9a	$(CH_3)_3C$	N	0.2	0.8	0.4	1.6	0.06	0.13
9b	$(CH_3)_3C$	N	0.2	0.8	0.8	>3.1	0.03	0.06

Continued on following page

TABLE 2—*Continued*

Compound	R_1[c]	X	MIC (μg/ml) for organism[b]					
			Gram negative		Gram positive		*M. fortuitum*	*M. smegmatis*
			Vogel	U1-18	H-228	C203		
9c	$(CH_3)_3C$	N	0.4	>3.1	>3.1	>3.1	0.03	0.13
9d	$(CH_3)_3C$	N	0.4	3.1	0.4	3.1	0.13	0.13
9e	$(CH_3)_3C$	N	0.05	0.2	0.05	0.1	≤0.03	≤0.03
9f	$(CH_3)_3C$	N	0.2	0.8	0.05	0.05	0.06	0.13
10a	2,4-F_2Ph	CF	0.2	1.6	3.1	0.16	0.5	1.0
10b	2,4-F_2Ph	CF	0.4	1.6	3.1	3.1	0.5	2.0
10c	2,4-F_2Ph	CF	0.8	3.1	1.6	>3.1	0.5	2.0
10d	2,4-F_2Ph	CF	1.6	>3.1	0.8	>3.1	1.0	2.0
10e	2,4-F_2Ph	CF	0.1	0.8	0.2	0.4	0.25	0.5
10f	2,4-F_2Ph	CF	0.2	1.6	0.1	0.2	0.25	0.5
11a	2,4-F_2Ph	COMe	0.2	0.8	0.4	>3.1	0.5	2.0
11b	2,4-F_2Ph	COMe	0.4	3.1	3.1	3.1	1.0	4.0
11c	2,4-F_2Ph	COMe	>3.1	>3.1	>3.1	>3.1	16	16
11d	2,4-F_2Ph	COMe	0.8	3.1	0.8	>3.1	2.0	2.0
11f	2,4-F_2Ph	COMe	0.4	3.1	0.4	1.6	1.0	2.0
12a	2,4-F_2Ph	N	0.1	0.4	0.4	0.8	0.13	0.5
12b	2,4-F_2Ph	N	0.2	1.6	0.8	3.1	0.13	0.5
12c	2,4-F_2Ph	N	0.8	3.1	3.1	>3.1	0.25	2.0
12d	2,4-F_2Ph	N	0.8	3.1	0.4	>3.1	0.25	0.5
12e	2,4-F_2Ph	N	0.05	0.2	0.1	0.8	0.13	0.13
12f	2,4-F_2Ph	N	0.2	0.8	0.1	0.1	0.5	0.5
A[e]			0.1	0.8	0.4	>3.1	0.03	0.06
B[f]			0.4	1.6	0.2	0.2	0.13	0.25
Ciprofloxacin			0.05	0.2	0.8	0.4	0.06	0.25
Sparfloxacin			0.025	0.8	0.05	0.1	0.06	0.13

[a] Assay techniques are described in the text. Ra-f, see Fig. 2.
[b] Vogel, U1-18, H-228, and C203 are *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes* strains, respectively.
[c] c-C_3H_5, cyclopropyl; CH_2CH_3, ethyl; $(CH_3)_3C$, *tert*-butyl; 2,4-F_2Ph, 2,4-difluorophenyl.
[d] *N*-Methylpiperazine side chain.
[e] Referred to in reference 19 as compound 4c.
[f] Referred to in reference 19 as compound 4k.

compounds from series 9, suggesting that derivatives from series 9 may have the appropriate blend of structural features to elicit selective antimycobacterial activity.

In summary, we have identified, using a rapid primary assay, several key classes of 8-substituted fluoroquinolones, such as compounds from series 5 and 9, which demonstrate good activity against *M. tuberculosis* and display low activity in tests that may predict adverse effects. Several agents, such as 5d and 9b, should be evaluated further in more-extensive studies.

TABLE 3. Antimycobacterial activities of several of the best compounds from this study[a]

Compound	MIC (μg/ml) for organism				
	M. aurum	*M. avium*[b]	*M. fortuitum*	*M. smegmatis*	*M. tuberculosis*
3b	0.06		≤0.03	0.06	0.78
5d	0.06	1.0	≤0.03	≤0.03	0.39
9b		0.5	0.03	0.06	0.78
9c	0.06	0.5	0.03	0.13	0.78
A[c]	0.06	0.25	0.03	0.06	0.39
Ciprofloxacin	0.06	2.0	0.06	0.25	0.25
Sparfloxacin	0.06	1.0	0.06	0.13	0.06

[a] Assay techniques for the determination of MICs are described in the text.
[b] MICs at which 50% of the 14 strains of *M. avium* were inhibited.
[c] Referred to in reference 19 as compound 4c.

TABLE 4. Clonotoxicity and phototolerance of several compounds from this study[a]

Compound	Clonotoxicity[b] (IC_{50}[b] [μg/ml])	Phototolerance (PTD_{50}[c] [mg/kg/dose])
1b	15	43
1f	11	<30
2b	45	
2f	15	
3b	110	<30
3f	36	
5b	86	>100
5f	35	
9b	500	55
9f	170	
A[d]	340	120
B[e]	170	
Ciprofloxacin	320	>300
Sparfloxacin	370	28

[a] Assay techniques for the clonotoxicity and phototoxicity studies are described in the text.
[b] IC_{50}, 50% inhibitory concentration.
[c] PTD_{50}, dose necessary to induce a phototoxic reaction in 50% of the mice.
[d] Referred to in reference 19 as compound 4c.
[e] Referred to in reference 19 as compound 4k.

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