Effect of Lipophilicity at N-1 on Activity of Fluoroquinolones against Mycobacteria†

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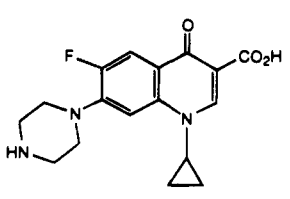
The dramatic increase in drug resistant *Mycobacterium tuberculosis* has caused a resurgence in research targeted toward these organisms. As part of a systematic study to optimize the quinolone antibacterials against mycobacteria, we have prepared a series of N-1-phenyl-substituted derivatives to explore the effect of increasing lipophilicity on potency at this position. The compounds, synthesized by the modification of a literature procedure, were evaluated for activity against Gram-negative and Gram-positive bacteria, *Mycobacterium fortuitum* and *Mycobacterium smegmatis*, and the results correlated with log *P*, pK_a, and other attributes. The activity of the compounds against the rapidly growing, less hazardous organism *M. fortuitum* was used as a measure of *M. tuberculosis* activity. The results demonstrate that increasing lipophilic character by itself does not correlate with increased potency against mycobacteria. Rather, intrinsic activity against Gram-negative and/or Gram-positive bacteria is the governing factor for corresponding activity against mycobacteria.

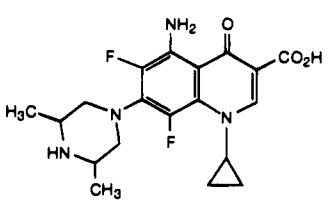
Introduction

The incidence of tuberculosis (TB) in the United States had shown a large and sustained decline from 1953 to 1985, mainly because of the use of antituberculosis chemotherapy. However, from 1985 through 1992, the number of cases has increased 20%.[1] There are a number of epidemiological factors which account for this increase, including an increase in foreign-born cases, an increase in active TB transmission in young children, and a reduced emphasis on TB control programs. Perhaps the most significant factor is the association of TB and HIV/AIDS.[2] HIV infected individuals account for nearly 50% of the excess TB cases in the United States from 1985 through 1992.[1]

The drugs currently used to treat TB are isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin.[3] However, the emergence of multi-drug resistant tuberculosis (MDRTB)[4] and studies showing that TB may be a cofactor in the progression of HIV[5,6] has caused a resurgence in research targeted toward improved drug therapy for *Mycobacterium tuberculosis* infection. For example, several of the quinolone antibacterials, such as ciprofloxacin (**A**) and sparfloxacin (**B**), have been examined as potential chemotherapeutics for *M. tuberculosis* infection;[7,8] however, no large clinical trials are available to assess the effectiveness of these compounds.[3]



Ciprofloxacin (A)



Sparfloxacin (B)

One major factor relevant to the design of new antituberculosis agents is the transport of compounds through the cell wall of mycobacteria. This is difficult since it is well-known that mycolic acids and surface associated lipids of these organisms form a transport barrier when compared to the cell wall of true bacteria.[9] As part of a study attempting to further optimize the quinolone antibacterials against *M. tuberculosis*, we have explored the effect of increasing the lipophilic character of a series of N-1-phenyl-substituted fluoroquinolones on activity against mycobacteria. We have replaced the cyclopropyl group of sparfloxacin and ciprofloxacin with the phenyl group since the phenyl moiety provides, *via* substitution, a ready handle to modulate the lipophilicity of the compounds under study. This approach is not entirely unreasonable since one phenyl-substituted quinolone, temafloxacin, has demonstrated good activity against mycobacteria.[10] In the present report we describe the synthesis and biological evaluation of several key compounds.

Chemistry

The target compounds **6a**–**k** and **7a**–**k** were prepared by the modification of a literature procedure[11] using the seven-step synthesis outlined in Scheme 1. The keto ester **2**, prepared in two steps from 2,4,5-trifluorobenzoic acid (**1**), was converted to the enamine derivatives **4a**–**k** *via* the diethyl (ethoxymethylene)malonate adduct followed by displacement with an appropriate aniline (Table 1, **3a**–**k**). The anilines **3a**–**j** were commercially available while the aniline **3k**[12] was prepared from 2,6-difluorotoluene by nitration followed by reduction of 2,6-difluoro-3-nitrotoluene. Ring closure and acid hydrolysis provided the quinolone acids, **5a**–**k**. Treatment of **5a**–**k** with either piperazine or 2,6-dimethylpiperazine furnished the C-7-substituted analogs **6a**–**k** and **7a**–**k**, respectively. The structural properties of **6a**–**k** and **7a**–**k** were confirmed by NMR, MS, and IR, and the purity of each compound established by either C-H-N or HPLC analysis (Table 2).

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Scheme 1. Synthesis of Fluoroquinolone Analogs **6a–k** and **7a–k**

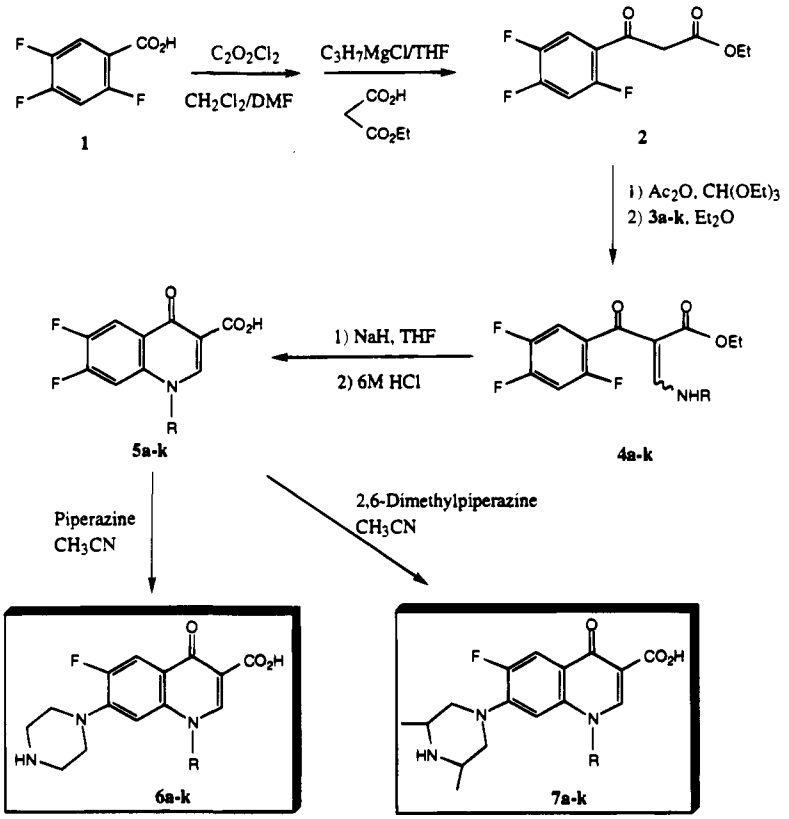
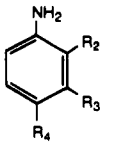


Table 1. Structure of Aniline Reagents, **3a–k**



compd	R_2	R_3	R_4
3a	H	H	H
3b	CH_3	H	H
3c	H	H	CH_3
3d	F	H	H
3e	H	H	F
3f	CH_3	H	CH_3
3g	F	H	F
3h	CH_3	H	F
3i	F	H	CH_3
3j	Cl	H	Cl
3k	F	CH_3	F

Biological Evaluation

The N-1-phenylquinolone derivatives (**6a–k** and **7a–k**) were tested against four representative Gram-negative and Gram-positive bacteria by using standard antibacterial microtitration techniques.[13] The compounds were also tested against *Mycobacterium fortuitum* (ATCC 6841) and *Mycobacterium smegmatis* (ATCC 19420) using a modification of NCCLS procedures designed for rapidly growing mycobacteria.[14] These modifications included the use of cation-adjusted Mueller–Hinton broth (CAMHB) and 0.02% Tween 80 media and an incubation time of 72 h. In both cases, minimum inhibitory concentrations (MICs in μg/mL) for each compound were determined. Aqueous stock solutions of test compounds were prepared as follows: compounds were suspended in 5% dimethylacetamide and solubilized with either NaOH or HCl and brought to final volume with water. Ciprofloxacin and sparfloxacin were used as positive controls for the antibacterial and antimycobacterial assays.

Table 2. Synthetic and Physical Data of Analogs **6a–k** and **7a–k**

compd	mp (°C)	formula	method of purification[a]	yield (%)
6a[11]	260–261	$C_{20}H_{18}N_3O_3F \cdot {}^1/_2H_2O$	A	85
6b	174–175	$C_{21}H_{20}N_3O_3F$[b]	B	66
6c	250–252	$C_{21}H_{20}N_3O_3F \cdot {}^1/_2H_2O$	B	90
6d	135–137	$C_{20}H_{17}N_3O_3F_2$[b]	B	100
6e[11]	275 dec	$C_{20}H_{17}N_3O_3F_2$	A	83
6f	255 dec	$C_{22}H_{22}N_3O_3F \cdot {}^3/_4H_2O$	B	65
6g	231–232	$C_{20}H_{16}N_3O_3F_3$[b]	A	46
6h	267–269	$C_{21}H_{19}N_3O_3F_2$	A	77
6i	238–239	$C_{21}H_{19}N_3O_3F_2 \cdot 2{}^1/_4H_2O$	A	92
6j	272 dec	$C_{20}H_{16}N_3O_3FCl_2 \cdot {}^1/_2H_2O$	A	90
6k	244–245	$C_{21}H_{18}N_3O_3F_3$	A	82
7a	242–243	$C_{22}H_{22}N_3O_3F \cdot {}^1/_4H_2O$	A	77
7b	224–225	$C_{23}H_{24}N_3O_3F \cdot {}^1/_4H_2O$	A	92
7c	254–255	$C_{23}H_{24}N_3O_3F \cdot {}^1/_4H_2O$	A	46
7d	214–215	$C_{22}H_{21}N_3O_3F_2$[b]	B	69
7e	268–269	$C_{22}H_{21}N_3O_3F_2 \cdot H_2O$	B	69
7f	222–223	$C_{24}H_{26}N_3O_3F$	B	69
7g	227–228	$C_{22}H_{20}N_3O_3F_3$	A	86
7h	233–234	$C_{23}H_{23}N_3O_3F_2 \cdot 2{}^1/_2H_2O$	B	79
7i	253–254	$C_{23}H_{23}N_3O_3F_2$	A	71
7j	228–229	$C_{22}H_{20}N_3O_3FCl_2$	A	81
7k	220–221	$C_{23}H_{22}N_3O_3F_3 \cdot {}^1/_4H_2O$	A	69

[a] A: CH_3CN, H_2O wash. B: isoelectric precipitation. [b] CHN off. Structure confirmed by NMR and MS; purity (≥99%) established in two separate solvent systems by HPLC.

Determination of pK_a, *c* log *P* and log *D* Values

The pK_a and log *D* values for compounds **6a–k** and **7a–k** were determined by Robertson Microlit Laboratories, Madison, NJ. The log *D* values, determined experimentally, are defined as the log of the distribution of test compound in 1-octanol to buffer at pH 7.4. A

Table 3. Activity of **6a–k** and **7a–k**, reported as MIC (μg/mL), against Gram-Negative and Gram-Positive Bacteria, *Mycobacterium fortuitum* and *Mycobacterium smegmatis*, and Related pK_a, *c* log *P*, and log *D* Values[a]

		antibacterial[b]									
		Gram-negative		Gram-positive		*Mycobacterium*					
R	compd	EC-1	PA-7	SA-13	SP1–1	*fort.*	*smeg.*	pK_{a1}	pK_{a2}	*c* log *P*	log *D*
Ph	**6a**[11]	0.1	0.8	0.8	3.1	0.5	2	6.06	8.72	3.50	−0.97
2-MePh	**6b**	0.4	1.6	3.1	>3.1	4	8	5.98	8.59	4.00	−0.83
4-MePh	**6c**	0.4	1.6	3.1	>3.1	2	4	6.06	8.65	4.00	−0.73
2-FPh	**6d**	0.1	0.8	0.8	1.6	0.25	2	5.86	8.99	3.66	−1.19
4-FPh	**6e**[11]	0.1	0.2	0.4	0.4	0.25	1	6.02	8.59	3.66	−1.18
2,4-Me_2Ph	**6f**	0.8	3.1	>3.1	>3.1	16	32	5.88	8.42	4.49	−0.07
2,4-F_2Ph	**6g**	0.1	0.2	0.4	0.4	0.25	0.5	5.87	8.31	3.81	−0.48
2-Me-4-FPh	**6h**	0.2	0.4	0.8	1.6	1	4	6.11	8.37	4.16	−0.43
2-F-4-MePh	**6i**	0.2	1.6	1.6	3.1	1	2	5.96	8.34	4.16	0.31
2,4-Cl_2Ph	**6j**	0.4	3.1	>3.1	>3.1	2	4	5.94	8.58	4.95	−0.55
2,4-F_2-3-MePh	**6k**	0.8	3.1	1.6	3.1	0.5	8	5.87	8.54	4.31	−1.12
Ph	**7a**	0.4	1.6	1.6	3.1	2	4	6.12	8.58	4.53	−0.12
2-MePh	**7b**	1.6	>3.1	>3.1	>3.1	1	8	6.01	8.55	5.03	−0.17
4-MePh	**7c**	0.8	3.1	3.1	>3.1	1	4	6.10	8.61	5.03	0.16
2-FPh	**7d**	0.4	1.6	1.6	3.1	0.5	2	5.88	8.51	4.70	−0.23
4-FPh	**7e**	0.2	0.8	0.4	0.8	0.25	1	5.97	8.56	4.70	−1.11
2,4-Me_2Ph	**7f**	3.1	>3.1	>3.1	>3.1	4	16	6.05	8.24	5.53	0.02
2,4-F_2Ph	**7g**	0.4	0.8	0.4	0.8	0.25	0.5	5.94	8.26	4.85	−0.73
2-Me-4-FPh	**7h**	0.4	1.6	1.6	>3.1	2	2	6.16	8.16	5.20	−1.05
2-F-4-MePh	**7i**	0.8	3.1	3.1	>3.1	1	2	5.89	8.34	5.20	0.31
2,4-Cl_2Ph	**7j**	1.6	>3.1	>3.1	>3.1	2	4	5.75	8.37	5.99	−0.03
2,4-F_2-3-MePh	**7k**	3.1	>3.1	1.6	>3.1	1	4	5.94	8.47	5.35	−1.36
ciprofloxacin	**A**	0.05	0.2	0.8	0.4	0.06	0.25	6.19	8.54	2.04	−0.94
sparfloxacin	**B**	0.025	0.8	0.05	0.1	0.06	0.13	nd	nd	2.42	nd

[a] Assay techniques and determination of pK_a, *c* log *P*, and log *D* values described in text. [b] EC-1, *Escherichia coli* Vogel; PA-7, *Pseudomonas aeruginosa* U1–18; SA-13, *Staphlococcus aureus* H-228; SP1-1, *Streptococcus pyogenes* C203. nd = not determined.

computational model was used to determine the theoretical distribution coefficients (*c* log *P*) for each compound.

Results and Discussion

The MICs of **6a–k** and **7a–k** against several representative Gram-positive and Gram-negative organisms,*M. fortuitum* and *M. smegmatis*, are summarized in Table 3. Because of the potential hazards of using *M. tuberculosis* in the laboratory, it was necessary to find an alternative, reliable and rapid method of screening the compounds for activity against *M. tuberculosis*. An examination of the literature suggested that the activity of quinolone analogs against the rapidly growing, less hazardous organism *M. fortuitum* correlated closely with the activity aginst *M. tuberculosis*.[8,10,15,16] We confirmed these observations by showing that MICs obtained for sparfloxacin and ciprofloxacin on *M. fortuitum* were essentially the same values already obtained for these compounds on *M. tuberculosis*.[17,18] Thus, we have used the activity of **6a–k** and **7a–k** against *M. fortuitum* as a barometer of potential activity against *M. tuberculosis*. Compounds of interest could be examined further in a secondary assay which would involve the more routine BACTEC procedure.[19] The compounds have also been tested against the rapidly growing organism *M. smegmatis* for comparative purposes.

The results demonstrate that the most active mycobacterial agents, such as the 4-fluorophenyl- and 2,4-difluorophenyl-substituted analogs **6e**, **7e** and **6g**, **7g**, respectively, are also the best antibacterials. The 4-fluorophenyl- and 2,4-difluorophenyl-substituted analogs are known to enhance antibacterial activity,[20] and they seem to offer the best activity against mycobacteria as well. The activity of **6e**, **7e** and **6g**, **7g**, as well as the activity of the other compounds studied, was similar against both *M. fortuitum* and *M. smegmatis*; however, none of the compounds examined were as active as or more active than ciprofloxacin or sparfloxacin. Based upon the results obtained for **6a–k** and **7a–k** against *M. fortuitum*, we predict that **6e**, **7e** and **6g**, **7g** would also be moderately active against *M. tuberculosis*. The biological results suggest that DNA gyrase, the site of action of fluoroquinolones, is similar in both mycobacteria and bacteria, and recent studies examining resistance and sequence patterns support this observation.[21,22]

The lipophilicity of the fluoroquinolones is well-known to be important for penetration of these compounds into bacterial cells.[23] Assuming that the issue of penetration is even more crucial for quinolone activity against mycobacteria,[24] our results demonstrate that simply increasing the lipophilic character at N-1 does not directly correlate with an increase in activity against mycobacteria. For example, structural modifications of the phenyl group aimed at increasing the lipophilicity, such as the substitution of a methyl group between the two fluoros on the phenyl group of **6g**, **7g** to give **6k**, **7k**, resulted in reduced biological activity. Furthermore, several of the compounds expected to be the most lipophilic, such as the 2,4-dimethylphenyl- and the 2,4-dichlorophenyl-substituted derivatives (**6f**, **7f** and **6j**, **7j**, respectively), were weakly active or inactive in both screens. Clearly, the fact that quinolones exist as zwitterions at physiological pH complicates this issue (see pK_a values, Table 3). Thus, when compared to ciprofloxacin and sparfloxacin, we did achieve our goal of increasing the lipophilicity of **6a–k** and **7a–k** *via* substitution of the phenyl ring, but changes in the *c* log *P* of these compounds did not directly correlate with either the log *D*s determined at pH 7.4 or the biological data. Therefore, our results argue that the lipophilicity of quinolone analogs at N-1, specifically of N-1-phenyl-

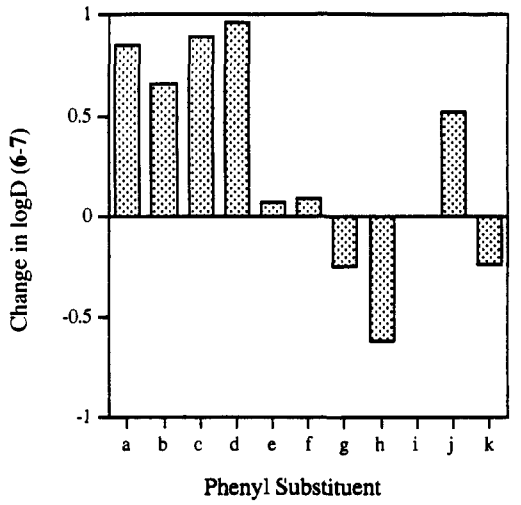


Figure 1. Change in log *D* values of compounds **6a–k** and **7a–k**.

substituted derivatives, is much less important than intrinsic antibacterial activity for corresponding activity against mycobacteria.

Interestingly, while the dimethylpiperazines **7a–k** were generally less active than the corresponding piperazines **6a–k** in the antibacterial screen, this loss in activity did not normally correlate with a decrease in activity against mycobacteria. A comparison of the log *D* values for the dimethylpiperazine analogs (**7a–k**) with the corresponding values for the piperazine derivatives (**6a–k**) shows that **7a–k** are generally more lipophilic than **6a–k** at physiological pH (Figure 1). Furthermore, the addition of two methyl groups on the piperazine side chain of **7a–k** increased the *c* log *P* by 1 log unit. These data would suggest that increasing the lipophilic side chain at C-7 may be more important for antimycobacterial activity than attempts to increase the lipophilic character at N-1 and that structural modifications of the quinolone nucleus to increase the *c* log *P* or log *D* at positions other than N-1 may be an alternative approach to the one reported herein. A recent report supports this observation,[25] and we are currently investigating the importance of more substituted C-7 side chains as we continue to examine ways to optimize the quinolone antibacterials against mycobacteria.

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