The Synthesis, Structure–Activity, and Structure–Side Effect Relationships of a Series of 8-Alkoxy- and 5-Amino-8-alkoxyquinolone Antibacterial Agents

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A series of 1-cyclopropyl-6-fluoro-8-alkoxy (8-methoxy and 8-ethoxy)-quionoline-3-carboxylic acids and 1-cyclopropyl-5-amino-6-fluoro-8-alkoxyquinoline-3-carboxylic acids has been prepared and evaluated for antibacterial activity. In addition, they were also compared to quinolones with classic substitution at C_8 (H, F, Cl) and the naphthyridine nucleus in a phototoxicity and mammalian cell cytotoxicity assay. The series of 8-methoxyquinolones had antibacterial activity against Gram-positive, Gram-negative, and anaerobic bacteria equivalent to the most active 8-substituted compounds (8-F and 8-Cl). There was also a concomitant reduction in several of the potential side effects (i.e., phototoxicity and clonogenicity) compared to the most active quinolones with classic substitution at C-8. The 8-ethoxy derivatives had an even better safety profile but were significantly less active (2–3 dilutions) in the antibacterial assay.

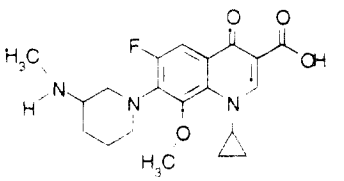
Since Lesher's discovery of nalidixic acid in 1962, a tremendous amount of synthetic effort has been channeled into the synthesis of quinolone antibacterial agents.[1] These research efforts have been rewarded by very significant improvements in antibacterial potency as well as in vivo efficacy. This progress has resulted from changes in the basic quinolone nucleus, the placement of a wide variety of substituents on these nuclei, and, perhaps most importantly, the extensive structure-activity relationship (SAR) studies on the effect of the side chain at C_7 (C_{10} of the benzoxazine types).[2] The contributions of many research facilities have allowed us to reach the point where quinolones are some of the most potent and broadest acting antibacterials known to man.[3]

Some of the various structural modifications which have provided the increased antibacterial activity and efficacy have also resulted in a concomitant increase in toxicity. A review of the structure–activity and structure–side effect relationships for the quinolones by Domagala[4] highlights these trends.

One of the major contributors to two significant side effects of the quinolone antibacterials, phototoxicity and in vitro genetic toxicity, is the substituent at C_8. The highest phototoxicity is seen when the substituent is halogen and the least when the substituent is O-alkyl, with the order of decreasing phototoxicity as CF > CCl > N > CH > CF_3 > COR.[4]

Although genetic toxicity appears to be a function of three factors,[4] the nature of the substituent at C_1, the side chain at C_7, and the group at C_8, it was felt that a beneficial effect on genetic toxicity could be achieved by suitable substitution at C_8. Keeping in mind that the contribution to cytotoxicity for the 8-substituent, in the order of greatest to least, is CF, CCl, COMe > N > CCF_3 > H and ofloxacin type, it was decided to more fully investigate the 8-alkoxy derivatives.

Initial reports on Q-35 (**1**),[5] an 8-methoxyquinolone, concimitant with our preliminary work in this area[6,12a] generally indicated good activity with less photoliability,



Q-35 (**1**)

which intensified our interest. It was decided to prepare an expanded series of 8-alkoxy-substituted quinolones and evaluate them for an improved phototoxic and genetic toxicity spectrum side-by-side with many other quinolones having conventional C_8 substituents.

We had previously reported an extensive SAR study which examined the effect of changes at the 8-position of the quinolone nucleus on antibacterial activity.[7] Keeping all of these variables in mind, we felt that by the correct choice of the side chain at C_7, we could maintain both antibacterial activity and efficacy as well as study the changes in toxicity provided by an 8-alkoxy substituent.

Chemistry

The 8-alkoxyquinolone nucleus was prepared from 3-hydroxy-2,4,5-trifluorobenzoic acid (**2**)[8] using the synthetic sequence outlined in Scheme 1. Alkylation of the phenol and acid moieties followed by saponification of the esters **3a,b** and acidification provided the alkoxy acids **4a,b**. Standard literature procedures[9] led to the formation of the keto esters **6a,b**, which were converted to the quinolone esters **8a,b** via the diethyl (ethoxymethylene)malonate adducts, enamines **7a,b**, and ring closure. The diminished activity to displace the 7-fluoro substituent caused by the 8-alkoxy group was overcome by converting the ethyl ester to the boron difluoride chelates **9a,b** using boron trifluoride etherate in refluxing tetrahydrofuran. The 5-amino-8-alkoxyquinolone substrates were prepared as outlined in Scheme 2. The quinolone esters **8a,b** were nitrated using potassium nitrate in sulfuric acid at ambient temperatures to give the nitro esters **12a,b**. Reduction of the nitro group

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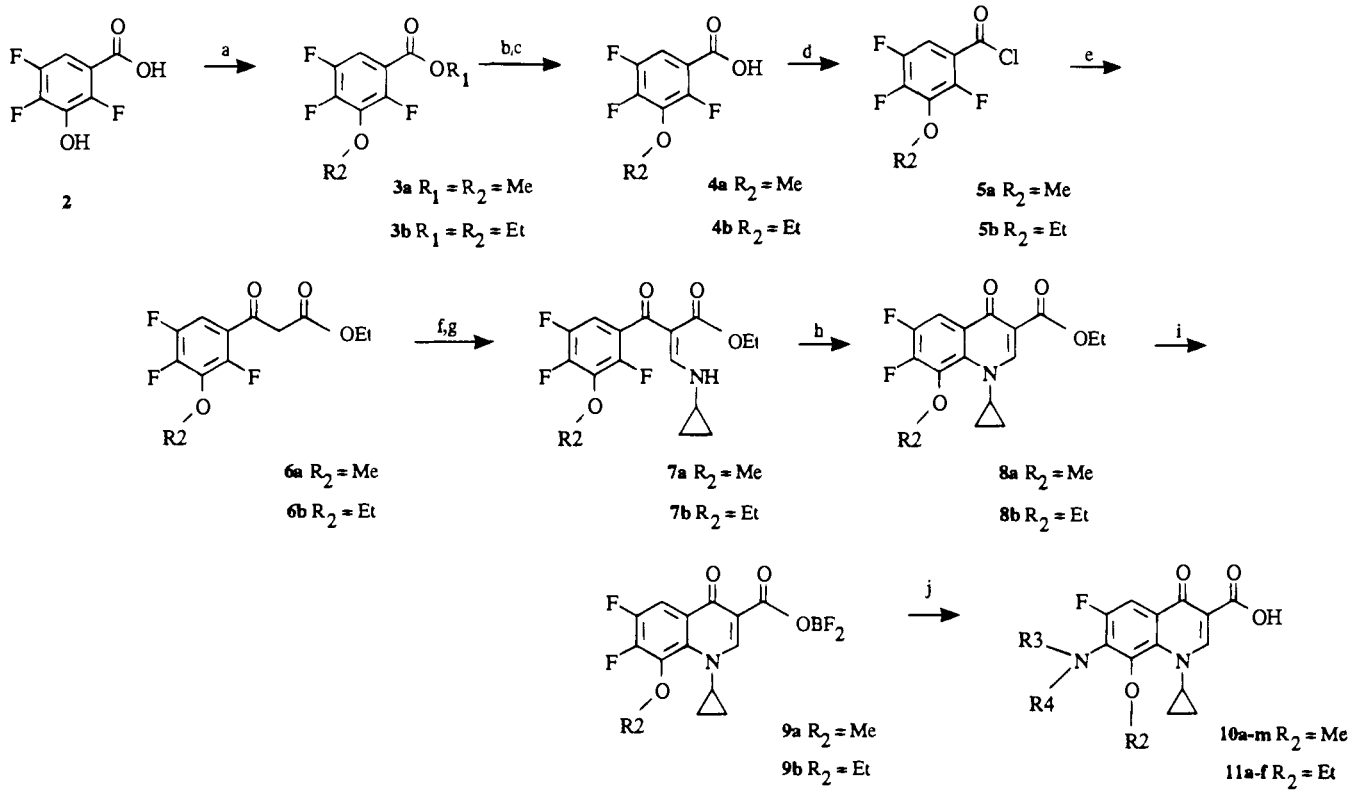
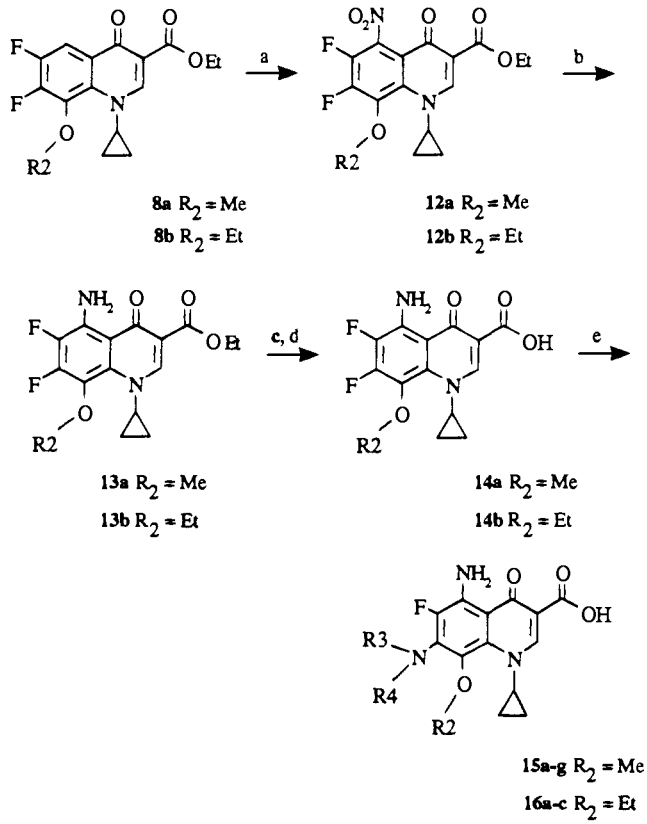
Scheme 1[a]



[a] (a) RI, DMF, NaH; (b) 1.0 N NaOH/MeOH; (c) 6.0 M HCl; (d) $(COCl)_2$, CH_2Cl_2, cat·DMF; (e) $[O_2CCHCO_2Et]^{2-}2Li^+$, THF; (f) $(EtO)_3CH$, Ac_2O; (g) cyclopropanamine, EtOH (h) NaH, THF; (i) $BF_3{\cdot}Et_2O$, THF, reflux; (j) R_3NHR_4, CH_3CN, reflux.

Scheme 2[a]



[a] (a) KNO_3, H_2SO_4, room temperature; (b) H_2, Raney Ni, MeOH; (c) 1.0 N NaOH, EtOH; (d) 6.0 M HCl; (e) R_3NHR_4, CH_3CN, reflux.

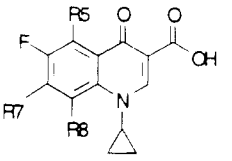
using hydrogen and Raney nickel followed by saponification and acidification provided the 5-amino-8-alkoxyquinolone substrates **14a,b**. In this case, the displacement by the side chain could be accomplished without conversion to the boron chelate. The title 8-alkoxy (**10a–m** and **11a–f**) and 5-amino-8-alkoxyquinolone (**15a–g** and **16a–c**) compounds were prepared by displacing the 7-fluoro substituent of **9a,b** and **14a,b**, respectively, with the requisite side chains. In the cases of the 5-hydro-8-alkoxy compounds, the boron ester chelates were cleaved using aqueous triethylamine to produce the free acids. Boc-protected side chains used in the synthesis (3-aminopyrrolidine, 3-aminopiperidine, and 3-(aminomethyl)-3-methylpyrrolidine) were also cleaved using trifluoroacetic acid to provide the free amines. The physical properties or references for all quinolones tested in this study are summarized in Table 1.

Biological Assays

In Vitro Antibacterial. The series of 8-alkoxy- and 5-amino-8-alkoxyquinolones as well as selected reference agents were tested against 11 representative Gram-positive and Gram-negative organisms and five representative anaerobic organisms using standard microtitraiton techniques,[10] and their minimum inhibitory concentrations (MICs in μg/mL) were compared in multiple experiments and recorded in Tables 2 and 3. Clinafloxacin (**17c**), ciprofloxacin (**22**), and three highly active 3-amino-pyrrolidine derivatives (**17a**, **17b**, and **17e**) were used as controls and are included in Tables 2–4.

Phototoxicity. Screening for photoliability[11] was also performed on selected compounds to determine trends in photosafety. Compounds were tested for the induction of phototoxic skin reaction in depilated, female CD-1 mice which were exposed to UVA radiation (320–400 nm) for a 3 h period, beginning 1 h after a single oral or subcutaneous dose of drug. This regimen was continued for up to 4 consecutive days. Results are

Table 1. Synthetic and Physical Data of the Quinolone Antibacterials Prepared for This Study



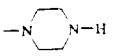
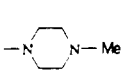
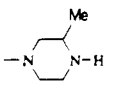
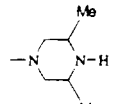
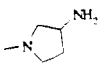
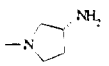
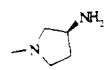
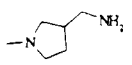
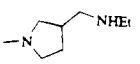
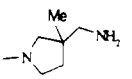
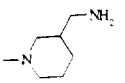
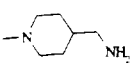
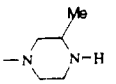
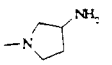
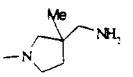
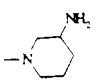
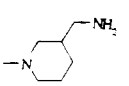
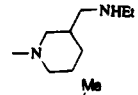
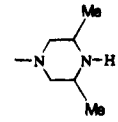
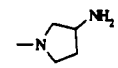
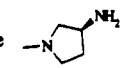
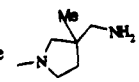
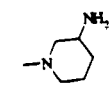
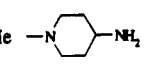
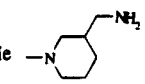
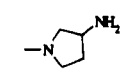
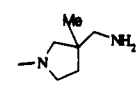
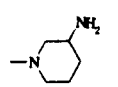
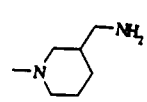
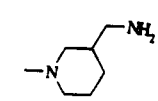
Compound No.	R_5	R_8	R_7	Method of prep[a]	mp, °C	Formula(anal.)[b]	purificn[c]	Yield%[d]
10a	H	OMe		A	191-193	$C_{18}H_{20}FN_3O_4 \cdot 3.0H_2O$ (C, H, N)	isoelectric pptn	80
10b	H	OMe		A	205-207	$C_{19}H_{22}FN_3O_4 \cdot 1.5H_2O$ (C, H, N)	isoelectric pptn	37
10c	H	OMe		A	159-162	$C_{19}H_{22}FN_3O_4 \cdot 0.75H_2O$ (C, H, N)	isoelectric pptn	52
10d	H	OMe		A	270-271	$C_{20}H_{24}FN_3O_4 \cdot 0.8HCl \cdot 1.0H_2O$ (C, H, N, F, Cl)	isoelectric pptn	64
10e	H	OMe		B	200-202	$C_{18}H_{20}FN_3O_4 \cdot 3.0H_2O$ (C, H, N)	isoelectric pptn	89
10f	H	OMe		B	198-201	$C_{18}H_{20}FN_3O_4 \cdot 1.25HCl \cdot 2.0H_2O$ (C, H, N)	lyophilization	83
10g	H	OMe		B	179-180	$C_{18}H_{20}FN_3O_4 \cdot 1.0HCl \cdot 2.25H_2O$ (C, H, N)	lyophilization	68
10h	H	OMe		A	>300	$C_{19}H_{22}FN_3O_4 \cdot 2.0HCl \cdot 3.0H_2O$ (C, H, N, Cl)	lyophilization	54
10i	H	OMe		A	207-208	$C_{21}H_{26}FN_3O_4 \cdot 1.0H_2O$ (C, H, N, F)	isoelectric pptn	32
10j	H	OMe		B	179-182	$C_{20}H_{24}FN_3O_4 \cdot 0.5H_2O$ (C, H, N)	isoelectric pptn	75
10k	H	OMe		B	181-182	$C_{19}H_{22}FN_3O_4 \cdot 1.0H_2O$ (C, H, N, F)	isoelectric pptn	71
10l	H	OMe		B	183-188	$C_{20}H_{24}FN_3O_4 \cdot 1.0H_2O$ (C, H, N)	isoelectric pptn	46
10m	H	OMe		B	241-244	$C_{20}H_{24}FN_3O_4 \cdot 1.0HCl \cdot 1.75H_2O$ (C, H, N)	recryst EtOH/H_2O	34
11a	H	OEt		A	168-169	$C_{20}H_{24}FN_3O_4 \cdot 1.0HCl$ (C, H, N)	isoelectric pptn	50
11b	H	OEt		B	222-223	$C_{19}H_{22}FN_3O_4 \cdot 0.9HCl \cdot 1.25H_2O$ (C, H, N, F, Cl)	trit MeOH	52
11c	H	OEt		B	201-203	$C_{21}H_{26}FN_3O_4$ (C, H, N, F)	isoelectric pptn	49
11d	H	OEt		B	189-191	$C_{20}H_{24}FN_3O_4 \cdot 1.25H_2O$ (C, H, N)	isoelectric pptn	57
11e	H	OEt		B	171-172	$C_{21}H_{26}FN_3O_4 \cdot 2.5H_2O$ (C, H, N)	isoelectric pptn	24

Table 1 (Continued)

Compound No.	R_5	R_8	R_7	Method of prep[a]	mp, °C	Formula(anal.)[b]	purifcn:	Yield%[d]
11f	H	OEt		B	229-230	$C_{23}H_{30}FN_3O_4 \cdot 2.25H_2O$ (C, H, N, F)	isoelectric pptn	33
15a	NH_2	OMe		C	>300	$C_{20}H_{25}FN_4O_4 \cdot 1.0HCl \cdot 0.75H_2O$ (C, H, N, F, Cl)	trit THF/MeOH	57
15b	NH_2	OMe		C	196-197	$C_{18}H_{21}FN_4O_4 \cdot 3.0H_2O$ (C, H, N)	isoelectric pptn	26
15c	NH_2	OMe		C	202-204	$C_{18}H_{21}FN_4O_4$ (C, H, N)	chromat.	18
15d	NH_2	OMe		C	188-191	$C_{20}H_{25}FN_4O_4 \cdot 0.5H_2O$ (C, H, N)	isoelectric pptn	52
15e	NH_2	OMe		C	218-219	$C_{19}H_{23}FN_4O_4 \cdot 1.0H_2O$ (C, H, N)	isoelectric pptn	41
15f	NH_2	OMe		C	224-225	$C_{19}H_{23}FN_4O_4 \cdot 4.0H_2O$ (C, H, N)	isoelectric pptn	33
15g	NH_2	OMe		C	203-204	$C_{20}H_{25}FN_4O_4 \cdot 1.75H_2O$ (C, H, N, F)	isoelectric pptn	27
16a	NH_2	OEt		C	198-201	$C_{19}H_{23}FN_4O_4 \cdot 1.0HCl \cdot 2.5H_2O$ (C, H, N)	isoelectric pptn	26
16b	NH_2	OEt		C	212-215	$C_{21}H_{27}FN_4O_4 \cdot 1.0HCl \cdot 0.75H_2O$ (C, H, N, F, Cl)	isoelectric pptn	64
16c	NH_2	OEt		C	226-228	$C_{20}H_{25}FN_4O_4 \cdot 3.5H_2O$ (C, H, N, F)	isoelectric pptn	47
21a	H	H		C	219-222	$C_{19}H_{22}FN_3O_3 \cdot 1.25H_2O$ (C, H, N)	isoelectric pptn	39
21b	H	F		C	232-234	$C_{19}H_{21}F_2N_3O_3 \cdot 0.75H_2O$ (C, H, N)	isoelectric pptn	46
21c	H	Cl		C	265-266	$C_{19}H_{21}ClFN_3O_3 \cdot 1.25H_2O$ (C, H, N)	isoelectric pptn	20
21d	H	N		C	219-220	$C_{18}H_{21}FN_4O_3 \cdot 1.0HCl \cdot 3.0H_2O$ (C, H, N)	lyophilization	30
21e	NH_2	F		C	235-236	$C_{19}H_{22}F_2N_4O_3 \cdot 1.0HCl \cdot 1.5H_2O$ (C, H, N)	recryst MeOH/Et_2O	36

[a] Refers to the general method used and is described in the Experimental Section. [b] Symbols refer to those elements analyzed. Analyses were ±0.4% of the theoretical values. [c] Trituration (trit) refers to grinding of the solids under solvent to produce a fine powder. Isoelectric precipitation refers to dissolving the solid in aqueous base, adjusting the pH to 7.2, and filtering the solid that precipitates. [d] Yields are those obtained from the coupling step to final product isolation, including hydrolyses, reductions, and deprotections where applicable.

reported as the dose necessary to induce a phototoxic reaction in 50% of the mice, PTD_{50}, by the probit method using initial doses of 30, 100, and 300 mg/kg. The raw data was also used to assess the first day of positive reaction and the no effect dose. The results are recorded in Table 3.

Mammalian Cell Cytotoxicity. Representative quinolones were also evaluated in a mammalian cell cytotoxicity assay[12] to estimate their clastogenic potential.[13] The clonogenic cytotoxicity was determined in Chinese hamster V-79 cells. The cells were grown overnight and treated with drug for 3 h at 37 °C, at which time the compound-containing media was replaced with the fresh media. The cells were then incubated for 5 days and examined for colony formation. The concentration of drug inhibiting colony formation by 50% (IC_{50}, μg/mL) relative to control was determined and recorded in Table 3.

Results and Discussion

A comparison of the MICs of the 8-alkoxy- and 5-amino-8-alkoxyquinolones against clinafloxacin (**17c**), ciprofloxacin (**22**), and three very active quinolones with the 3-aminopyrrolidine side chain (**17a**, **17b**, and **17e**)

Table 2. Test Results for the Compounds in This Study

	minimum inhibitory concentrations (MIC's, μg/mL)[a,b]										
	Gram-negative organisms						Gram-positive organisms				
compd no.	*E. cloacae* HA 2646	*E. coli* Vogel	*E. coli* H560	*K. pneumonia* MGH-2	*P. rettgeri* H1771	*P. aeruginosa* UI-18	*S. aureus* H228	*S. aureus* UC-76	*S. faecalis* MGH-2	*S. pneumonia* SV-1	*S. pyogenes* C203
10a	0.025	0.05	0.025	0.1	0.1	0.8	0.2	0.05	0.2	0.1	0.1
10b	0.1	0.1	0.1	0.2	0.4	0.8	0.1	0.05	0.2	0.2	0.2
10c	0.05	0.05	0.05	0.1	0.2	0.8	0.2	0.05	0.2	0.1	0.1
10d	0.1	0.1	0.05	0.2	0.8	3.1	0.1	0.05	0.2	0.1	0.1
10e	0.025	0.025	0.013	0.05	0.1	0.4	0.05	0.013	0.05	0.025	0.025
10f	0.025	0.025	0.025	0.025	0.05	0.8	0.05	0.025	0.05	0.05	0.05
10g	0.025	0.025	0.025	0.05	0.1	0.4	0.05	0.013	0.05	0.025	0.025
10h	0.05	0.05	0.025	0.1	0.1	0.8	0.025	0.003	0.013	0.003	0.003
10i	0.1	0.1	0.05	0.2	0.4	1.6	0.025	0.006	0.05	0.013	0.013
10j	0.1	0.1	0.05	0.2	0.4	1.6	0.025	0.006	0.025	0.013	0.013
10k	0.1	0.1	0.05	0.2	0.4	1.6	0.05	0.006	0.025	0.013	0.013
10l	0.2	0.2	0.1	0.4	0.8	3.1	0.05	0.006	0.05	0.006	0.006
10m	0.1	0.1	0.1	0.2	0.4	1.6	0.1	0.05	0.05	0.025	0.025
11a	0.1	0.1	0.05	0.2	0.4	3.1	0.2	0.05	0.2	0.05	0.2
11b	0.1	0.05	0.05	0.2	0.4	3.1	0.05	0.013	0.1	0.1	0.2
11c	0.2	0.2	0.2	0.4	0.8	1.6	0.05	0.013	0.1	0.1	0.1
11d	0.4	0.2	0.2	0.4	0.8	3.1	0.1	0.025	0.2	0.2	0.2
11e	0.4	0.2	0.1	0.8	1.6	3.1	0.05	0.013	0.05	0.025	0.025
11f	1.6	0.8	0.8	3.1	>3.1	>3.1	0.2	0.05	0.2	0.1	0.1
15a	0.2	0.2	0.1	0.4	0.8	3.1	0.1	0.025	0.2	0.05	0.1
15b	0.05	0.025	0.025	0.1	0.2	0.8	0.05	0.006	0.05	0.05	0.1
15c	0.05	0.05	0.05	0.1	0.2	0.8	0.025	0.013	0.1	0.025	0.05
15d	0.1	0.1	0.05	0.2	0.8	3.1	0.025	0.006	0.05	0.013	0.025
15e	0.1	0.1	0.05	0.2	0.4	1.6	0.025	0.006	0.05	0.006	0.013
15f	0.1	0.1	0.05	0.2	0.4	1.6	0.05	0.013	0.05	0.013	0.025
15g	0.2	0.1	0.1	0.4	0.8	3.1	0.05	0.006	0.025	0.025	0.025
16a	0.1	0.05	0.05	0.2	0.4	3.1	0.05	0.013	0.1	0.05	0.1
16b	0.4	0.2	0.1	0.8	1.6	3.1	0.1	0.025	0.1	0.05	0.1
16c	0.2	0.2	0.1	0.4	0.8	3.1	0.05	0.013	0.1	0.025	0.05
17a[c]	0.025	0.025	0.05	0.1	0.1	0.1	0.025	0.1	0.05	0.1	0.006
17b[c]	0.025	0.013	0.006	0.025	0.05	0.1	0.05	0.025	0.05	0.05	0.05
17c[c]	0.025	0.013	0.006	0.025	0.025	0.05	0.05	0.013	0.05	0.05	0.05
17e[c]	0.006	0.006	0.003	0.006	0.025	0.05	0.013	0.006	0.025	0.006	0.025
21a	0.4	0.4	0.4	0.8	1.6	3.1	0.8	0.1	0.2	0.1	0.05
21b	0.05	0.05	0.05	0.2	0.2	0.4	0.1	0.013	0.05	0.013	0.013
21c	0.2	0.1	0.05	0.2	0.2	1.6	0.025	0.003	0.05	0.003	0.003
21d	0.4	0.4	0.4	0.8	1.6	6.3	1.6	0.2	0.4	0.2	0.2
21e	0.05	0.05	0.025	0.1	0.2	0.8	0.025	0.003	0.013	0.003	0.13
22[c]	0.05	0.05	0.025	0.1	0.1	0.4	3.1	0.2	0.8	1.6	0.8

[a] Standard microdilution techniques; see ref 10. [b] All values for **10a–m** through **17a–e** are accurate to ±50% and have been obtained from duplicate or triplicate experiments. See ref 10. [c] Reference 7.

is summarized in Table 2. The 8-methoxy compounds with the 3-aminopyrrolidine side chain (**10e**, **10f**, and **10g**) have activity equivalent to clinafloxacin vs Gram-positive and Gram-negative organisms. Compounds **10h–l**, **11e**, **15b–g**, and **16c** (see Table 1 for structures), while less active than clinafloxacin vs Gram-negative organisms, were equipotent against Gram-positive organisms.

Phototoxicity. Data in Table 4 summarize the effect of varying the 8-position of the quinolones on phototoxicity potential. For analogues with either the 3-amino- or the 3-(aminomethyl)-3-methylpyrrolidine side chain at C-7, the rank order (least to most toxic) of photoxicity liability for 8-position variants is OMe = N = H ≫ Cl > F. A limited comparison of analogues having the 3-aminopiperidine or *cis*-3,5-dimethylpiperazine side chain shows the order of safety to be OMe = H ≫ F.

The 8-OMe and 8-OEt groups also decrease the phototoxic liability of quinolones having a 5-amino group (with an 8-F substituent), as can be seen in the cases of the 3-aminopyrrolidine and *cis*-3,5-dimethylpiperazine (sparfloxacin) series.

Clonogenic Cytotoxicity. The effect of the 8-position on clonogenicity (IC_{50}) is exemplified by the 3-aminopyrrolidine side chain at C_7 (Table 4). For this series, the rank order (least to most toxic) of clonogenic cytotoxicity liability for 8-position variants is OEt > N > H > OMe > F > Cl. The 3-(aminomethyl)-3-methylpyrrolidine derivatives had the greatest clonogenic liability, with safety in the order OEt > OMe > N = H > F = Cl. The 8-alkoxy-7-[3-(aminomethyl)piperidine] compounds had reduced colonogenic safety with the order for the series being N > H > OEt > F > OMe > Cl. The narrow series of cis-3,5-dimethylpiperazine compounds reinforced the clonogenic safety of the 8-alkoxy compounds with OMe = H > F. In all of the series studied (3-aminopyrrolidine, 3-aminopiperidine, and 3-(aminomethyl)piperidine), the 8-OMe had much greater clonogenic safety than the 8-F derivatives.

Anaerobic Activity. A comparison of the activity of the 8-alkoxy derivatives vs compounds with the standard substitution at the 8-position (H, F, Cl, N) and the 5-NH_2 derivative of the 8-F analog, against five representative anaerobic bacteria is summarized in Table 4. The 8-OMe with the 3-aminopyrrolidine side chain at C_7 (**10e**) has comparable activity to the most active compounds of the series, those with an 8-F (**17b**) or 8-Cl (**17c**) substituent. The substitution with an 8-OEt (**11b**) lowers the activity to be equivalent to the 8-H (**17a**) and naphthyridine (**17d**) derivatives. The activity of the 5-amino-8-alkoxy compounds **15b** and **16a** drops off when compared to the 5-amino-8-fluoro

Table 3. Anaerobe Testing, Clonogenicity, and Phototoxicity

compd no.	*Bact frag* BFA	*Clost diff* CD 1-1	*Clost perf* CP 3-1	*Propion acnes* PA 5-1	*Pepto asac* PA 3-1	clono IC_{50}[a]	photo. PTD_{50}[b]	no effect dose[b]
10a	0.8	1.6	0.4	0.4	3.1	82		
10b	0.2	0.8	0.2	0.4	1.6			
10c	0.4	1.6	0.4	0.4	0.8	85		
10d	0.8	3.1	0.4	0.4	0.8	>500	>100	100
10e	0.2	0.4	0.1	0.2	0.8	45	>100	100
10f	0.4	0.8	0.5	0.2	0.4			
10g	0.2	0.2	0.2	0.4	0.8	32		
10h	0.2	0.2	0.1	0.2	0.2	10		
10i	0.4	0.2	0.05	0.4	0.2	22		
10j	0.4	0.8	0.2	0.4	0.8	38	>100	100
10k	0.4	0.8	0.4	0.2	0.8	69		
10l						22		
10m	0.8	0.8	0.4	0.4	0.4	94		
11a	3.1	6.3	0.8	3.1	1.6			
11b	1.6	3.1	0.8	1.6	0.8	210		
11c	1.6	3.1	0.8	3.1	0.8	140	>100	100
11d						210		
11e	3.1	3.1	0.4	3.1	0.4	63		
11f	3.1	3.1	0.4	3.1	0.4	460		
15a	1.6	3.1	0.4	1.6	0.8		>100	100
15b	1.6	3.1	0.2	0.8	0.8	100	>100	100
15c	1.6	3.1	0.8	1.6	1.6			
15d	1.6	1.6	0.8	1.6	0.8	140		
15e	1.6	1.6	0.2	0.4	0.8	290	>100	100
15f	3.1	3.1	0.8	3.1	1.6	60		
15g	3.1	1.6	0.2	1.6	0.8	140		
16a	3.1	12.5	0.4	3.1	0.8	220	>100	100
16b	1.6	6.3	0.8	6.3	0.8			
16c	3.1	6.3	0.4	3.1	0.8			

[a] For the colongenic cytotoxicity studies, the IC_{50} is in μg/mL. [b] The phototoxicity studies are reported in mg.

derivative **17e**. This trend continues for the 3-(aminomethyl)-3-methylpyrrolidine side chain with the 8-Cl derivative **18c** being the most active and the 8-OMe (**10j**) and 8-F (**18b**) derivatives having identical activity. With a 3-aminopiperidine side chain, the 8-methoxy compound **10k** has equivalent activity to the most active compound of the series, the 8-Cl compound **19c**. The compounds with the 3,5-dimethylpiperazine side chain (C-7), including sparfloxacin (**20d**), have equivalent activity. This activity is noticeably reduced when compared to the pyrrolidine and piperidine analogs. The trend is sustained for the 3-(aminomethyl)piperidine analogs with the rank order being 8-F = 8-OMe > 8-OEt.

Summary. Within the series of 5-unsubstituted-8-alkoxy- and 5-amino-8-alkoxyquinolones, the activity against Gram-positive and Gram-negative aerobic bacteria was in the order 8-OMe ≥ 5-NH_2-8-OMe > 8-OEt = 5-NH_2-8-OEt. The best side chain in all cases was the 3-aminopyrrolidine. The overall activity of the compounds prepared for this study was better against Gram-positive organisms than against Gram-negative organisms.

The summary of phototoxicity testing from Table 4 shows the trend in photosafety to be 8-OMe = 8-OEt = naphthyridine = CH >>> F, Cl. The 8-alkoxy substituent even improved the photoliability of the 5-NH_2 compounds to make them as safe as the 5-H and naphthyridine derivatives.

The activity of the 8-alkoxy series against anaerobes was comparable to the most active compounds tested without the phototoxicity and genetic liability inherent in the 8-F and 8-Cl analogs. The 8-alkoxy derivatives provided diminished in vitro genetic toxicity compared to the 8-F and 8-Cl compounds and were usually equivalent to the 8-H and naphthyridine analogs. The 8-alkoxy compounds had comparable activity to the 5-amino-8-alkoxy analogs vs Gram-positive and Gram-negative aerobic bacteria and were slightly more active vs anaerobes. They had equivalent photosafety, and the introduction of the 5-amino group diminished the in vitro genetic toxicity.

Therefore, compounds **10e**, **15b**, **10k**, and **15e** having the following substitution, 8-methoxy-7-(3-aminopyrrolidine), 5-amino-8-methoxy-7-(3-aminopyrrolidine), 8-methoxy-7-(3-aminopiperidine), and 5-amino-8-methoxy-7-(3-aminopiperidine), were chosen as derivatives for human therapeutics. This is based on their activity against aerobic and anaerobic bacteria along with their relative safety in the phototoxicity and clonogenic cytotoxicity testing.

Experimental Section

Melting points were determined on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr on a Nicolet FT IR SX-20 instrument. Proton magnetic resonance (NMR) spectra were recorded a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts (δ) are expressed in ppm, relative to internal tetramethylsilane. Elemental analyses were performed on a Perkin-Elmer Model 240 elemental analyzer. All compounds had analytical results within ±0.4% of their theoretical values. All organic solutions were dried over magnesium sulfate, and all concentrations of solutions and drying of precipitated solids were performed in vacuo at 10–30 mmHg and ambient temperatures. Flash column or medium pressure chromatography was performed using silica gel (230 to 400 mesh) and the solvent systems indicated in the experimental details.

Methyl 3-Methoxy-2,4,5-trifluorobenzoate (3a). A solution of 20 g (104 mmol) of 3-hydroxy-2,4,5-trifluorobenzoic acid (**2**),[8] 62.5 g (440 mmol) of iodomethane, and 600 mL of DMF was cooled to 0 °C in a salt-ice bath and treated, portionwise, with 9.1 g (228 mmol) of 60% sodium hydride–mineral oil (washed twice with 25 mL portions of hexane) over 1 h. The reaction was stirred at ice bath temperatures for 18 h and the solvent was evaporated. The residue was partitioned between H_2O/$CHCl_3$ (200 mL each) and adjusted to pH 7.0 with HOAc. The organic layer was washed with 0.5 N NaOH and water, dried, and evaporated to give 21.0 g (91%) of **3a** as an oil: NMR ($CDCl_3$) δ 3.94 (s, 3H, ester CH_3), 4.06 (s, 3H, ether CH_3), 7.53 (m, 1H). Anal. ($C_9H_7F_3O_3$) C, H.

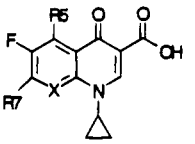
Ethyl 3-Ethoxy-2,4,5-trifluorobenzoate (3b). The above reaction was repeated using 35.0 g (182 mmol) of **2**, 113.5 g (728 mmol) of iodoethane, and 22.0 g (550 mmol) of 60% sodium hydride–mineral oil in 1050 mL of DMF to give 41.6 g (92%) of **3b** as an oil: NMR ($CDCl_3$) δ 1.36 (overlap. t, 6H), 4.14 (q, 2H), 4.25 (q, 2H), 7.54 (m, 1H). Anal ($C_{11}H_{11}F_3O_3$) C, H.

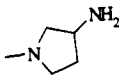
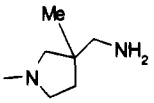
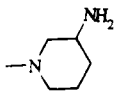
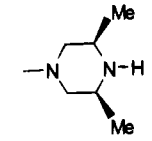
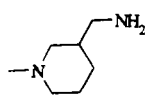
3-Methoxy-2,4,5-trifluorobenzoic Acid (4a). A solution of 135 g (655 mmol) of **3a**, 670 mL (670 mmol) of 1.0 N NaOH, and 1.1 L of MeOH was stirred at room temperature for 3 h. The MeOH was removed in vacuo and the aqueous residue was washed with hexane (2 × 100 mL), acidified to pH 2.0 with 6.0 M HCl, and diluted with water as necessary to permit stirring. The solids were filtered, washed with water, and dried to give 83.0 g (61%) of **4a**: mp 116–118 °C; NMR ($CDCl_3$) δ 4.08 (s, 3H), 7.55 (m, 1H). Anal. ($C_8H_5F_3O_3$) C, H.

3-Ethoxy-2,4,5-trifluorobenzoic Acid (4b). The above reaction was repeated using 41.6 g (168 mmol) of **3b**, 200 mL (200 mmol) of 1.0 N NaOH, and 700 mL of MeOH to give 29.3 g (79%) of **4b**: mp 97–98 °C; NMR ($CDCl_3$) δ 1.43 (t, 3H), 4.28 (q, 2H), 7.56 (m, 1H). Anal. ($C_9H_7F_3O_3$) C, H.

3-Methoxy-2,4,5-trifluorobenzoyl Chloride (5a). A solution of 82.0 g (397 mmol) of **4a**, 700 mL of CH_2Cl_2, and 1 mL of DMF was cooled to 0 °C and treated dropwise with 62.8 g (495 mmol) of oxalyl chloride. The reaction was allowed to come to room temperature, where it was stirred for 18 h. The solvent was removed in vacuo, and the residue was dissolved in 200 mL of CH_2Cl_2 and concentrated in vacuo to give 92.3g (>100%) of **5a**, which was used without further purification.

Table 4. Summary of Anaerobic Activity, Clonogenicity and Phototoxicity of 8-Alkoxyquinolones Compared to Quinolones with Classical Substitution at C-8



Comound No.	R_7	R_5	X	Clono IC_{50}	Photo PTD_{50}/No Effect Dose	*Bact frag* BFA	*Clost diff* CD 1-1	*Clost perf* CP 3-1	*Propion acnes* PA 5-1	*Pepto asac* PA 3-1
17a[a]		H	CH	72	>300/300	1.6	12.5	0.1	1.6	1.6
17b[a]		H	CF	30	7.5/3	0.2	0.4	0.05	0.2	0.4
17c[a]	clinafloxacin	H	CCl	26	23/18	0.1	0.4	0.1	0.2	0.4
17d[a]		H	N	98	>100/100	1.6	6.3	0.1	0.8	1.6
10e		H	COMe	45	>100/100	0.2	0.4	0.1	0.2	0.8
11b		H	COEt	210	-	1.6	3.1	0.8	1.6	0.8
17e[b]		NH_2	CF	15	52/30	0.4	1.6	0.025	0.1	0.2
15b		NH_2	COMe	100	>100/100	1.6	3.1	0.2	0.8	0.8
16a		NH_2	COEt	220	>100/100	3.1	12.5	0.4	3.1	0.8
18a[d]		H	CH	23	>100/100	6.3	6.3	0.2	1.6	1.6
18b[c]		H	CF	7.7	<30/<30	0.4	1.6	0.1	0.4	0.4
18c[e]		H	CCl	8.6	<30/<30	0.05	0.2	0.05	0.2	0.2
18d[d]		H	N	25	>300/300	6.3	25.0	0.2	1.6	1.6
10j		H	COMe	38	>100/100	0.4	0.8	0.2	0.4	0.8
11c		H	COEt	140	>100/100	1.6	3.1	0.8	3.1	0.8
18e[d]		NH_2	CF	-	>30/30	0.8	1.6	0.025	0.4	0.2
15d		NH_2	COMe	140	-	1.6	1.6	0.8	1.6	0.8
16b		NH_2	COEt	-	-	1.6	6.3	0.8	6.3	0.8
19a[e]		H	CH	560	>100/100	-	-		-	- -
19b[e]		H	CF	53	55/30	1.6	1.6	0.2	0.4	1.6
19c[e]		H	CCl	24	-	0.2	0.4	0.1	0.2	0.4
19d[e]		H	N	100	-	25	>25	0.8	6.3	6.3
10k		H	COMe	69	-	0.4	0.8	0.4	0.2	0.8
11d		H	COEt	210	-	-	-	-	-	-
19e		NH_2	CF	19	-	-	-	-	-	-
15e		NH_2	COMe	290	>100/100	1.6	1.6	0.2	0.4	0.8
16c		NH_2	COEt	-	-	3.1	6.3	0.4	3.1	0.8
20a[e]		H	CH	>500	<100	1.6	6.3	0.2	0.8	0.4
20b[e]		H	CF	150	55/30	1.6	3.1	0.2	0.8	1.6
10d		H	COMe	>500	>100/100	0.8	3.1	0.4	0.4	0.8
20d[b]	sparfloxacin	NH_2	CF	370	28/18	1.6	6.3	0.2	0.4	0.4
15a		NH_2	COMe	-	>100/100	1.6	3.1	0.4	1.6	0.8
21a		H	CH	220	-	-	-	-	-	-
21b		H	CF	43	-	1.6	0.8	0.2	0.8	0.4
21c		H	CCl	17	-	-	-	-	-	-
21d		H	N	290	-	-	-	-	-	-
10l		H	COMe	22	-	0.8	0.8	0.4	0.4	0.4
11e		H	COEt	63	-	3.1	3.1	0.4	3.1	0.4
21e		NH_2	CF	38	-	-	-	-	-	-
15g		NH_2	COMe	140	-	3.1	1.6	0.2	1.6	0.8
22	ciprofloxacin	-	-	>320	172/100	3.1	6.3	0.4	0.8	1.6

[a] Reference 7. [b] Reference 17. [c] Reference 16. [d] Reference 6. [e] Reference 12a.

3-Ethoxy-2,4,5-trifluorobenzoyl Chloride (5b). The above reaction was repeated using 29.0 g (132 mmol) of **4b**, 1 mL of DMF, 250 mL of CH_2Cl_2, and 24.0 g (189 mmol) of oxalyl chloride to give 32.1 g (>100%) of **5b**, which was used without further purification.

Ethyl (3-Methoxy-2,4,5-trifluorobenzoyl)acetate (6a). A solution of 103.0 g (780 mmol) of monoethyl malonate and 0.1 g of 2,2'-bipyridyl in 1.6 L of dry THF was cooled to −20 °C and treated dropwise with 592 mL (1.54 mol) of 2.6 M *n*-butyllithium in hexanes, and the temperature was allowed to reach −5 °C during the addition. After the addition was complete (as indicated by a persistent light pink color, after a total addition time of 1.5 h), the reaction mixture was cooled to −78 °C and treated with a solution of 92.3 g (assume 397 mmol) of **5a** in 1.6 L of dry THF. After the addition was complete (2 h), the cooling bath was removed and the reaction mixture was allowed to warm to −30 °C, where it was stirred an additional 2 h. It was then quenched by being poured into 4 L of water containing 90 mL (1.08 mol) of concentrated HCl. The mixture was extracted with EtOAc (3 × 750 mL), and the combined organic layers were washed with water, dried, and concentrated. The residue was recrystallized from EtOAc to give 100.1 g (91%) of **6a** as a semisolid: NMR ($CDCl_3$) δ 1.27 (overlapping t, 3H), 3.95 (d, 0.9H, keto form), 4.02 (s, 3H), 4.21 (overlapping q, 4H), 7.46 (m, 1H), 12.71 (s, 0.1H enol). Anal. ($C_{12}H_{11}F_3O_4$) C, H.

Ethyl (3-Ethoxy-2,4,5-trifluorobenzoyl)acetate (6b). The above reation was repeated using 32.1 g (assume 132 mmol) of **5b**, 0.1 g of 2,2'-bipyridyl, 34.7 g (263 mmol) of monoethyl malonate, and 222 mL (578 mmol) of 2.6 M *n*-butyllithium in a total of 1.5 L of dry THF to give a total yield of 32.2 g (85%) of **6b** as an oil which was used without further purification: NMR ($CDCl_3$) δ 1.37 (overlapping t, 6H), 3.93 (d, 0.8 H, keto form), 4.22 (overlapping q, 4H), 7.45 (m, 1H), 12.69 (s, 0.2H enol). Anal. ($C_{13}H_{13}F_3O_4$) C, H.

Ethyl 2-(3-Methoxy-2,4,5-trifluorobenzoyl)-3-(cyclopropylamino)acrylate (7a). A solution of 100.1 g (362 mmol) of **6a**, 81.5 g (550 mmol) of triethyl orthoformate, and 950 mL of acetic anhydride was heated at reflux for 22 h and concentrated. The residue was triturated with toluene (2 × 200 mL), which was also evaporated, and then concentrated in high vacuo at 40−45 °C. The residue was dissolved in 500 mL of EtOH, cooled to 5 °C, and treated dropwise with 30.7 g (540 mmol) of cyclopropylamine over 1 h. When the addition was complete, the reaction mixture was allowed to come to room temperature, where it was stirred for 18 h. The resulting precipitate was filtered, washed with EtOH, and dried to give 75.1 g of **7a**, mp 57−59 °C. The filtrate was concentrated and the residue was chromatographed, eluting first with 95/5 $CHCl_3$/acetone and then with 90/10 of the same mixture, and fractions were combined on the basis of TLC to give an additional 27.9 g of **7a**, mp 56−58 °C. The combined yield was 103.0 g (83%): NMR ($CDCl_3$) δ 0.86 (m, 2H), 0.95 (m, 2H), 1.08 (t, 3H), 2.97 (m, 1H), 4.00 (s, 3H), 4.06 (q, 2H), 6.87 (m, 1H), 8.18 (d, 0.25H), 8.21 (d, 0.75H), 10.85 (bd, 1H). Anal. ($C_{16}H_{16}F_3NO_4$) C, H, N.

Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylate (8a). A solution of 103.0 g (300 mmol) of **7a** in 2 L of dry THF was cooled to 5 °C and treated portionwise with 12.6 g (315 mmol) of 60% sodium hydride−mineral oil over 2 h. A precipitate developed during the addition and the reaction was stirred for 18 h after the addition was complete. The reaction mixture was treated with water and HOAc to acidify it and the solid was removed by filtration. After washing with cold THF, the precipitate was dissolved in CH_2Cl_2, washed with water, dried, and evaporated to give 67.9 g of **8a**, mp 179−181 °C. The filtrate from the original precipitate was evaporated and the residue was recrystallized from THF (twice) to give an additional 10.1 g of **8a**. The total yield was 78.0 g (81%): NMR ($CDCl_3$) δ 1.06 (m, 2H), 1.22 (m, 2H), 1.43 (t, 3H), 3.99 (m, 1H), 4.10 (s, 3H), 4.39 (q, 2H), 8.04 (t, 1H, C_5H), 8.61 (s, 1H, C_2H). Anal. ($C_{16}H_{15}F_2NO_4$) C, H, N.

Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-ethoxy-4-oxo-3-quinolinecarboxylate (8b). A solution of 12.2 g (42.1 mmol) of **6b**, 9.8 g (69.0 mmol) of triethyl orthoformate, and 85 mL of acetic anhydride was heated at reflux for 18 h. The solvent was removed and the residue was triturated twice with toluene (100 mL), which was also removed in vacuo. The residue was heated in high vacuo at 45 °C for 2 h and dissolved in 60 mL of EtOH. The mixture was cooled to 0 °C in a salt−ice bath and treated dropwise with a solution of 3.6 g (63.0 mmol) of cyclopropylamine in 5 mL of ethanol. The mixture was allowed to stir for 18 h without additional cooling. The solvent was removed and the residue was dissolved in 200 mL of dry THF and cooled to 5 °C. The solution was treated portionwise with 1.8 g (45.0 mmol) of 60% sodium hydride−mineral oil and stirred to room temperature for 5 h. The reaction mixture was treated with 100 mL of water and acidified with HOAc. The solution was evaporated to dryness and the residue was dissolved in CH_2Cl_2, washed with water, dried, and concentrated. The residue was recrystallized from EtOAc/heptane to give 5.6 g (39%) of **8b**: mp 138−139 °C: NMR ($CDCl_3$) δ 1.05 (m, 2H), 1.22 (m, 2H), 1.41 (t, 3H), 1.43 (t, 3H), 3.98 (m, 1H), 4.28 (q, 2H), 4.32 (q, 2H), 8.02 (t, 1H, C_5H), 8.61 (s, 1H, C_2H). Anal. ($C_{17}H_{17}F_2NO_4$) C, H, N, F.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic Acid−Boron Difluoride Chelate (9a). A solution of 10.0 g (30.9 mmol) of **8a**, 50 mL of freshly distilled boron trifluoride etherate, and 150 mL of THF was heated at reflux for 18 h. The reaction was cooled to room temperature and diluted with 150 mL of Et_2O, and the precipitate was filtered, washed with Et_2O, and dried to give 8.9 g (84%) of **9a**: mp 225−227 °C; NMR ($CDCl_3$) δ 1.05 (m, 2H), 1.22 (m, 2H), 1.41 (t,3H), 1.43 (t, 3H), 3.98 (m, 1H), 4.28 (q, 2H), 4.32 (q, 2H), 8.02 (t, 1H, C_5H), 8.61 (s, 1H, C_2H). Anal. ($C_{14}H_{10}F_4NO_4B$) C, H, N.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-ethoxy-4-oxo-3-quinolinecarboxylic Acid−Boron Difluoride Chelate (9b). The above reaction was repeated using 2.5 g (7.4 mmol) of **8b**, 10 mL of freshly distilled $BF_3{\cdot}Et_2O$ and 20 mL of THF to give 1.9 g (72%) of **9b**, mp 217−218 °C; NMR ($CDCl_3$ + Me_2SO-d_6) δ 1.37 (m, 2H), 1.44 (m, 2H), 1.56 (t, 3H), 4.51 (q, 2H), 4.51 (m, 1H), 8.14 (t, 1H, C_5H), 9.21 (s, 1H, C_2H). Anal. ($C_{15}H_{12}F_4NO_4B$) C, H, N.

Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-5-nitro-4-oxo-3-quinolinecarboxylate (12a). A solution of 8.3 g (25.6 mmol) of **8a** in 65 mL of concentrated H_2SO_4 was treated portionwise with 3.9 g (38.6 mmol) of solid KNO_3 over 2 h. The reaction was stirred at room temperature for 18 h and poured onto 600 mL of ice water, and the resulting precipitate was removed by filtration, washed with water, and dissolved in CH_2Cl_2. The resulting solution was washed with water and 5% $NaHCO_3$, dried, and evaporated. The residue was recrystallized from MeOH to give 6.6 g (70%) of **12a**: mp >290 °C; NMR ($CDCl_3$) δ 1.11 (m, 2H), 1.22 (m, 2H), 1.36 (t, 3H), 4.06 (m, 1H), 4.19 (s, 3H), 4.32 (q, 2H), 8.60 (s, 1H, C_2H). Anal. ($C_{16}H_{14}F_2N_2O_6$) C, H, N.

Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-ethoxy-5-nitro-4-oxo-3-quinolinecarboxylate (12b). The above reaction was repeated using 13.0 g (38.5 mmol) of **8b**, 5.5 g (54.4 mmol) of KNO_3, and 100 mL of concentrated H_2SO_4 to give 9.0 g (61%) of **12b**: mp 271−273 °C; NMR ($CDCl_3$) δ 0.84 (m, 2H), 1.08 (m, 2H), 1.10 (t, 3H), 1.28 (t, 3H), 3.84 (m, 1H), 4.14 (overlapping q, 4H), 8.40 (s, 1H, C_2H). Anal. ($C_{17}H_{16}F_2N_2O_6$) C, H, N, F.

Ethyl 5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylate (13a). A solution of 5.5 g (14.9 mmol) of **12a** was dissolved in 250 mL of THF, treated with 2.0 g of Raney nickel, and shaken in a hydrogen atmosphere at temperatures of 21−28 °C and pressures of 19−50 psi for 7 h. The solvent was concentrated and the residue was chromatographed eluting with CH_2Cl_2/THF 95/5 to give 1.8 g (36%) of **13a**: mp 209−210 °C; NMR (Me_2SO-d_6) δ 0.94 (m, 2H), 1.05 (m, 2H), 1.26 (t, 3H), 3.80 (s, 3H), 3.96 (m, 1H), 4.19 (q, 2H), 7.69 (bs, 2H), 8.36 (s, 1H, C_2H). Anal. ($C_{16}H_{16}F_2N_2O_4$) C, H, N, F.

Ethyl 5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-ethoxy-4-oxo-3-quinolinecarboxylate (13b). The above reaction was repeated using 2.8 g (7.3 mmol) of **12b**, 100 mL of DMF, 0.5 g of Raney nickel, temperatures of 24−30 °C, and pressures of 24−30 psi for 4 h. Chromatography using a gradient of CH_2Cl_2/THF (98/2, 96/4, 94/6) gave 2.3 g (89%) of **13b**: mp 228−229 °C; NMR (Me_2SO-d_6) δ 0.94 (m, 2H),

1.14 (m, 2H), 1.38 (overlapping t, 6H), 3.90 (m, 1H), 3.98 (q, 2H), 4.39 (q, 2H), 6.89 (bs, 2H), 8.46 (s, 1H, C_2H). Anal. ($C_{17}H_{18}F_2N_2O_4$) C, H, N, F.

5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic Acid (14a). A solution of 1.6 g (5.0 mmol) of **13a**, 8.0 mL (8.0 mmol) of 1.0 N NaOH, and 25 mL of EtOH was stirred at room temperature for 18 h. The solvent was removed and the residue was dissolved in 30 mL of water and filtered through a fiber glass pad to clarify it. The filtrate was acidified to pH 2.2 with 6.0 M HCl and cooled to 5 °C and the solid removed by filtration, washed with water, and dried to give 1.3 g (90%) of **14a**: mp 290–292 °C; NMR (Me_2SO-d_6) δ 0.81 (m, 2H), 1.03 (m, 2H), 3.76 (s, 3H), 3.90 (m, 1H), 7.69 (bs, 2H), 8.37 (s, 1H, C_2H), 14.52 (s, 1H). Anal. ($C_{14}H_{12}F_2N_2O_4 \cdot 0.5H_2O$) C, H, N, F.

5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-ethoxy-4-oxo-3-quinolinecarboxylic Acid (14b). The above reaction was repeated using 1.5 g (5.1 mmol) of **13b**, 8.0 mL of 1.0 N NaOH, and 25 mL of EtOH to give 1.2 g (90%) of **14b**: mp 283–285 °C; NMR (Me_2SO-d_6) δ 1.09 (m, 4H), 1.37 (t, 3H), 4.05 (q, 2H), 4.18 (m, 1H), 7.72 (bs, 2H), 8.41 (s, 1H, C_2H), 14.61 (s, 1H). Anal. ($C_{15}H_{14}F_2N_2O_4$) C, H, N, F.

General Method A. Preparation of 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-[(ethylamino)methyl]-1-pyrrolidinyl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (10i). A solution of 1.0 g (2.9 mmol) of boron difluoride ester **9a**, 0.9 g (7.0 mmol) of *N*-ethyl-3-pyrrolidinemethanamine,[14] and 20 mL of CH_3CN was stirred at 50 °C for 18 h and then concentrated in vacuo. The residue was dissolved in a mixture of 15 mL of Et_3N and 150 mL of EtOH and stirred at reflux for 18 h. The solvent was removed and the residue was dissolved in 25 mL of EtOH, which was also removed in vacuo. The residue was suspended in water and the pH was adjusted to 11.0 with 1.0 N NaOH. The solution was filtered through a fiber glass pad to clarify it and the pH was adjusted to 7.0 with 6.0 M HCl. The resulting precipitate was filtered, washed with water, and dried to give 0.39 g (33%) of **10i**: mp 207–208 °C; NMR (Me_2SO-d_6 + TFA) δ 1.09 (m, 4H), 1.23 (t, 3H), 1.80 (m, 1H), 2.19 (m, 1H), 2.60 (m, 1H), 3.03 (overlapping CH_2, 4H), 3.58 (s, 3H), 3.68 (m, 4H), 4.15 (m, 1H), 7.68 (d, 1H, C_5H), 8.59 (bs, 1H, NH), 8.68 (s, 1H, C_2H). Anal. ($C_{21}H_{26}FN_3O_4 \cdot H_2O$) C, H, N.

General Method B. 1-Cyclopropyl-6-fluoro-8-ethoxy-7-(3-amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid Hydrochloride Hydrate (11b). A solution of 0.8 g (2.1 mmol) of **9b**, 1.56 g (8.4 mmol) of 3-[(*tert*-butoxycarbonyl)amino]pyrrolidine,[15] and 35 mL of CH_3CN was stirred at 50 °C for 18 h. The solvent was removed and the residue was dissolved in 15 mL of CH_2Cl_2, cooled to 5 °C, and treated with 15 mL of TFA. The mixture was stirred at ice bath temperatures for 4 h and room temperature for 0.5 h. The reaction mixture was concentrated, the residue dissolved in 150 mL of 80% aqueous EtOH treated with 15 mL of Et_3N and refluxed for 18 h. The solvent was evaporated and the residue was dissolved in water which was also concentrated to remove Et_3N. The residue was dissolved in water, adjusted to pH 11.0 with 1.0 N NaOH. The solution was filtered through a fiber glass pad to clarify it and the filtrate was adjusted to pH 6.5 with 6.0 M HCl. The resulting precipitate was filtered, washed with ice–water and Et_2O, and dried to give 0.4 g (51%) of **11b**: mp 222–223 °C; NMR (Me_2SO-d_6 + TFA) δ 0.98 (m, 2H), 1.11 (m, 2H), 1.28 (t, 3H), 2.10 (m, 1H), 2.28 (m, 1H), 3.67–3.89 (m, 5H), 3.76 (q, 2H), 4.15 (m, 1H), 7.66 (d, 1H, C_5H), 8.51 (bs, 3H), 8.68 (s, 1H, C_2H). Anal. ($C_{19}H_{22}FN_3O_4 \cdot HCl \cdot 1.25H_2O$) C, H, N, F, Cl.

General Method C. 5-Amino-1-cyclopropyl-6-fluoro-8-methoxy-7-[(3-aminomethyl)-3-methyl-1-pyrrolidinyl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid Hemihydrate (15d). A suspension of 0.9 g (2.9 mmol) of **14a**, 0.9 g (4.2 mmol) of (3-*tert*-butoxycarbonyl-3-methyl)pyrrolidinemethanamine,[16] 1.0 g (10.0 mmol) of triethylamine, 17 mL of DMSO, and 17 mL of CH_3CN was stirred at room temperature for 18 h. The CH_3CN was removed and the resulting solution was filtered through a fiber glass pad into 150 mL of water. The resulting precipitate was filtered, washed with water, and dried in vacuo at 40 °C for 18 h. The solid was dissolved in 15 mL of CH_2Cl_2 cooled to 5 °C in an ice bath and treated with 15 mL of TFA. The mixture was stirred at ice bath temperatures for 0.5 h and then at room temperature for 0.5 h and concentrated. The residue was suspended in water, the pH adjusted to 11.0 with 1.0 N NaOH, and the solution filtered through a fiber glass pad. The filtrate was adjusted to pH 7.0 with 6.0 M HCl and the resulting precipitate was filtered, washed with water and Et_2O, and dried to give 0.6 g (56%) of **15d**: mp 188–190 °C; NMR (Me_2SO-d_6 + TFA) δ 0.86 (m, 2H), 1.08 (m, 2H), 1.23 (s, 3H). 1.84 (M, 1H), 1.93 (m, 1H), 2.98 (bs, 2H), 3.40 (m, 1H), 3.45 (s, 3H), 3.64 (m, 3H), 4.06 (m, 1H), 7.87 (bs, 2H), 8.56 (s, 1H, C_2H). Anal. ($C_{20}H_{25}FN_4O_4 \cdot 0.5H_2O$) C, H, N.

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