Synthesis and Biological Activity of 5-Alkyl-1,7,8-trisubstituted-6-fluoroquinoline-3-carboxylic Acids

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A series of 5-alkyl-1,7,8-trisubstituted-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids was prepared and evaluated for in vitro and in vivo antibacterial activity. When compared to the 5-hydrogen analogues, the presence of the 5-methyl group enhanced in vitro potency for those compounds containing a cyclopropyl moiety at N_1 but decreased potency for those containing an ethyl group at N_1. Replacing the 5-methyl with a 5-ethyl significantly reduced the efficacy. In general, the 5-methyl and 5-hydrogen analogues were equipotent in vivo. Several of the 5-methyl-1-cyclopropylquinolones displayed excellent in vitro and in vivo activity, warranting further development.

Although the fluoroquinolone antiinfectives have been the focus of extensive structure–activity relationship (SAR) examination and synthetic manipulation, the modification of the 5-position has not been studied systematically until recently.[1-3] It has been shown that the activity of the quinolone class is greatly influenced by the combination of substituents found at C_5, C_7, C_8, and N_1. In particular, an amino moiety at the 5-position increases the potency (especially against Gram-positive organisms) if the substituent at the quinolone ring nitrogen is cyclopropyl; however, if the N_1-substituent is ethyl, the Gram-positive potency decreases while the Gram-negative efficacy essentially remains unchanged. The same general trends are observed when a hydroxy group is situated at C_5. Thus, the addition of a 5-substituent does not affect the potency uniformly, but is dependent on the nature of the other substituents, particularly the substituent at N_1.

This synergy between various substituents is novel in the quinolone literature and has elicited great interest within our laboratories. A previous investigator has claimed that 5-alkyl substitution is deleterious to activity,[4] but the systems studied did not contain optimized functionalities at the crucial positions in the quinolone ring. In this paper we wish to extend our investigation of 5-substitution to the 5-alkyl quinolones which contain those groups (a fluorine at C_6, a hydrogen or halogen atom at C_8, and a cyclopropyl or ethyl moiety at N_1) which have been shown to confer good antibacterial potency.

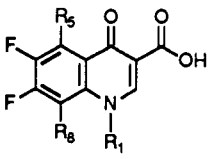
Chemistry

The 5-methyl and 5-hydrogen quinolone precursors were synthesized via literature procedures as shown in Table I. In all cases, the presence of the alkyl group did not significantly affect the chemistry of the ring construction.

The 5-ethylquinolone precursor was prepared according to Scheme I in a route similar to that used to synthesize the 5-methyl derivative.[5] Silylated bromobenzene **2** was deprotonated with lithium diisopropylamide at low temperature and alkylated with ethyl triflate to give fully substituted benzene **3**; the use of milder alkylating agents such as ethyl iodide resulted in the recovery of appreciable amounts of starting material. Metal–halogen exchange followed by carbon dioxide quench gave acid **4**, which was treated with fluoride ion to remove the trimethylsilyl moiety. Elaboration of the benzoic acid **5** to the quinolone **7** was accomplished via the standard methodology.

The piperazinyl and pyrrolidinyl side chains used for this study were either purchased commercially or prepared by using literature procedures (Chart I). The reaction between these side chains and the quinolone precursor is described elsewhere[6,7] and is summarized in Table II and in the Experimental Section. As in the previous paper in this series, the numbering system employed throughout this manuscript correlates the quinolone nucleus (letters

Table I. 5-Substituted-6,7-difluoroquinoline Starting Materials **7** Prepared for This Study



7

compd 7	R_5	R_8	R_1	synthetic ref for known procedures
a	H	H	Et	9
b	H	F	Et	9
c	H	H	2,4-diFPh	10
d	H	H		11
e	H	F		7
f	H	Cl		11
g	CH_3	H	Et	5
h	CH_3	F	Et	5
i	CH_3	H	2,4-diFPh	5
j	CH_3	H		5
k	CH_3	F		5
l	CH_3	Cl		5
m	CH_2CH_3	H		*a*

[a] See Scheme I and Experimental Section.

(1) (a) Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Trehan, A. K. *J. Med. Chem.* 1988, *31*, 503. (b) Nakamura, S.; Minami, A.; Kojima, T.; Fujimoto, K.; Kurobe, N.; Kashimoto, S.; Ohue, T.; Kuona, K.; Hashimoto, M.; Shimizu, H. 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, 1988, Abstr. 1487.

(2) Moran, D. B.; Ziegler, C. B.; Dunne, T. S.; Kuck, N. A.; Lin, Y. *J. Med. Chem.* 1989, *32*, 1313.

(3) See the previous paper in this series: Domagala, J. M.; Bridges, A. J.; Culbertson, T. P.; Gambino, L.; Hagen, S. E.; Karrick, G.; Porter, K.; Sanchez, J. P.; Sesnie, J. A.; Spense, F. G.; Szotek, D.; Wemple, J. *J. Med. Chem.*, preceding paper in this issue.

(4) Cecchetti, V.; Fravolini, A.; Fringuelli, R.; Mascellani, G.; Pagella, M.; Palmioli, M.; Serge, G.; Terni, P. *J. Med. Chem.* 1987, *30*, 465.

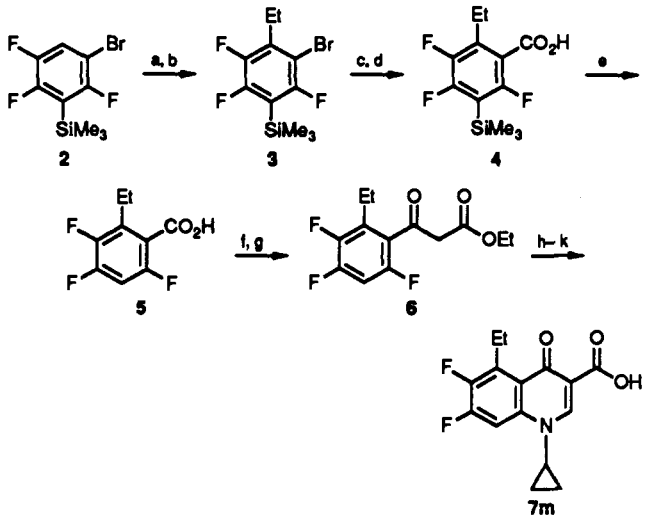
(5) Hagen, S. E.; Domagala, J. M. *J. Heterocyc. Chem.* 1990, *27*, 1609.

(6) Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. *J. Med. Chem.* 1986, *29*, 394.

(7) Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nichols, J. B.; Solomon, M.; Worth, D. F. *J. Med. Chem.* 1988, *31*, 991.

0022-2623/91/1834-1155$02.50/0

Scheme I[a]



[a] (a) LDA, −78 °C; (b) $CF_3SO_3C_2H_5$; (c) nBuLi, −78 °C; (d) CO_2, then HCl; (e) CsF; (f) ClC(O)C(O)Cl; (g) $Li_2(O_2CCHCO_2Et)$; (h) $HC(OEt)_3$, Ac_2O; (i) $\overline{CH_2CH_2CH}$-NH_2; (j) NaH; (k) 6 N HCl.

a through m) with the appropriate heterocycle (numbers 1 through 6) from Chart I.

Biological Assays

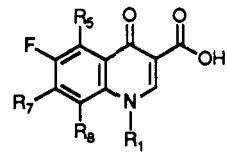
The quinolones prepared for this study were tested against 10 representative Gram-negative and Gram-positive organisms by using standard microtitration techniques.[15] The minimum inhibitory concentrations (MICs, in μg/mL) are presented in Table III. In order to facilitate comparisons in activity between compounds, the geometric means of the MICs were calculated for both Gram-positive and Gram-negative strains and are included in Tables IV–VI. These means were first grouped by common N_1 substituents (N_1 = ethyl, difluorophenyl, or cyclopropyl) and then further divided into the 5-methyl and 5-hydrogen derivatives. By so doing, direct comparisons of activity between the 5-substituted and 5-unsubstituted quinolones have been simplified. The quinolones were also tested for their ability to inhibit DNA gyrase, the target enzyme, by using a process described previously and using gyrase which was isolated from *Escherichia coli* H560.[6] The procedure measures the concentration of drug (in μg/mL) needed to produce linear DNA from closed circular DNA, and the values obtained are generally accurate to ±50%.

For those 5-methylquinolones which were especially active in vitro, the compounds were next tested in vivo. The in vivo potency, expressed as the median protective dose (PD_{50}, in mg/kg), is determined in acute lethal systemic infections in female Charles River CD-1 mice. This procedure is described in detail elsewhere.[14,16] The results obtained for these 5-methylquinolones and their 5-hydrogen analogues are summarized in Table VII.

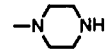
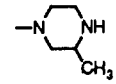
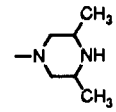
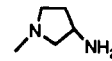
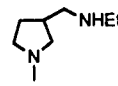
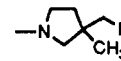
Results and Discussion

In general, the presence of a 5-methyl group on the quinolone ring does not seriously alter those trends in activity previously noted for their 5-amino and 5-hydrogen counterparts. For example, the 5-methylquinolones possessing an aminopyrrolidine at C_7 exhibit better in vitro potency (especially against Gram-positive organisms) than those containing a piperazine. The unalkylated piperazinyl quinolones exhibit better Gram-positive activity than do the mono- or dimethylated analogues when N_1 is ethyl, but when N_1 is cyclopropyl the side chain bulk has little effect. The addition of a fluorine atom to C_8 also increases activity

Chart I. Heterocyclic Side Chains Employed as the R_7 Substituent of 1



1

heterocycle no.	heterocyclic R_7 in 1	synthetic ref for preparation of R_7
1		[a]
2		[a]
3		[a]
4		11
5		14
6		13

[a] Commercially available.

for the *N*-cyclopropyl compounds and has little effect on the *N*-ethyl analogues; since it has been reported that such fluorine substitution is detrimental to potency in the *N*-difluorophenyl series,[8] the 5-methyl-1-(difluorophenyl)-8-fluoro analogues were not synthesized. Therefore, the presence of a 7-aminopyrrolidine, an 8-fluorine, and a 1-cyclopropyl group confer optimal activity for the 5-methylquinolones as they did for their 5-amino and 5-hydrogen counterparts.

In order to best compare the 5-methylquinolones with their 5-hydrogen parents, the compounds prepared for this study were divided into three groups depending on the substitution at N_1. The *N*-ethyl moiety has been shown to confer good, though not excellent, activity to the quinolones[7] (excellent MICs being less than 0.1 mg/mL); therefore, 5-methyl-1-ethyl compounds **1g** (where C_8 is unsubstituted) and **1h** (where C_8 contains a fluorine) were synthesized. Comparison of the resultant Gram-negative and Gram-positive means (Table IV) shows a marked decrease in potency when a methyl group is present at C_5. Although methylated **1g–3** is roughly equipotent with unsubstituted analogue **1a–3**, compounds **1g–1** and **1g–4** are three times less potent than **1a–1** and **1a–4** against Gram-positive strains. Even more dramatically, **1g-5** shows a 7-fold loss in Gram-positive efficacy when compared to **1a–5**. Gram-negative activity also suffers when a 5-methyl group is added: quinolones **1g–1**, **1g–4**, and **1g–5** are 6, 4, and 10 times less active, respectively, than their 5-hydrogen counterparts. Similar results were noted in the 5-amino series where N_1 was an ethyl group.[3]

Addition of a fluorine to C_8 to give *N*-ethylquinolones **1h–1** and **1h–4** does not reverse this trend. Against Gram-positive strains, **1h–1** is slightly less potent than

(8) Chu, D. T. W.; Fernandes, P. B.; Maleczka, R. E.; Nordeen, C. W.; Pernet, A. G. *J. Med. Chem.* **1987**, *30*, 504.

1b–**1** and **1h**–**4** is 3 times less potent than **1b**–**4**; however, Gram-negative efficacy does not suffer in the presence of a 5-methyl group. In all cases, the substitution at C_7 does not significantly alter the trends in activity: piperazinyl- and pyrrolidinyl-substituted quinolones suffer equally from the detrimental effects of the 1-ethyl-5-methyl substitution pattern.

It is safe to say, then, that the addition of a methyl group to the 5-position of the *N*-ethylquinolones decreases (or, at best, has no effect on) overall activity. When a difluorophenyl moiety is appended to N_1, the differences in efficacy between the 5-methyl and 5-hydrogen compounds are very small (Table V). If a "significant" change in activity is said to be 2-fold or greater, then all the 5-methyl-1-(difluorophenyl)quinolones are equipotent to their 5-hydrogen analogues. Against Gram-positive strains, for example, compounds **1i**–**1**, **1i**–**2**, **1i**–**4**, and **1i**–**5** show no significant variation in potency when compared to **1c**–**1**, **1c**–**2**, **1c**–**4**, and **1c**–**5**. A slight decrease in Gram-negative activity is seen for **1i**–**2** (2.5 times).

It is for the compounds where N_1 is cyclopropyl that the 5-methyl greatly improves Gram-positive efficacy. For those quinolones where C_8 contains either a hydrogen or fluorine atom, the presence of a methyl group at C_5 (e.g., **1j** and **1k**) increases Gram-positive potency by a 3–12-fold margin over 5-hydrogen analogues **1d** and **1e** (Table VI). This improvement in activity is most pronounced for the 7-piperazinyl quinolones: compounds **1j**–**1**, **1j**–**2**, **1k**–**1**, and **1k**–**3** are, respectively, 12, 5, 12, and 8 times more efficacious than their des-methyl counterparts. Although the 5-methyl confers a boost in activity to the 7-pyrrolidinyl compounds as well, the magnitude of the improvement is less striking (approximately 3–4-fold), probably due to the intrinsically high potency of most 7-pyrrolidinyl quinolones. At the same time, the presence of the 5-methyl does not affect Gram-negative potency, nor does it influence the efficacy of the 8-chloroquinolones **1l**–**1** and **1l**–**4**. As with the 5-aminoquinolones, then, the 5-methyl group is beneficial to activity especially when C_8 is a fluorine.

Increasing the steric bulk at C_5 by replacing a methyl group with an ethyl results in a significant decrease in activity. The Gram-negative means for **1m**–**1**, **1m**–**2**, and **1m**–**4** are 20–40 times greater than those for the analogous 5-methyl compounds **1j**–**1**, **1j**–**2**, and **1j**–**4**. Gram-positive efficacy suffers even more: **1m**–**1**, **1m**–**2**, and **1m**–**4** are 300, 200, and 100 times less potent than **1j**–**1**, **1j**–**2**, and **1j**–**4**, respectively. Obviously, any increases in steric bulk over methyl at the 5-position are not tolerated.

In almost all cases, the incorporation of a methyl group at C_5 results in a decrease of potency against the target gyrase enzyme as reflected in an increase in the gyrase cleavage values (Table III). However, for the compounds where N_1 is either a cyclopropyl or difluorophenyl group, the cleavage values still remain below 5 μg/mL, indicating that they are excellent inhibitors of the target enzyme. Only when N_1 is an ethyl group do the cleavage values climb above the 5 μg/mL cutoff. It has been demonstrated that the correlation between gyrase inhibition and antibacterial activity is general,[9] for while very low gyrase numbers are desireable, they do not necessarily correspond to proportionally better in vitro antibacterial potency. Therefore, it is not surprising that some 5-methylquinolones show both an improvement in antibacterial efficacy and a decrease in gyrase activity.

In vivo activity for the most active 5-methylquinolones (the *N*-cyclopropyl series) and their 5-hydrogen counterparts is presented in Table VII. Against the Gram-negative organism *E. coli*, the 5-methyl quinolones were roughly equipotent to their unsubstituted analogues, although in a few cases (**1j**–**4** and **1k**–**5**) the methylated derivative loses some oral efficacy. Since the methylated and unmethylated quinolones display essentially the same Gram-negative potency in vitro, this observation is not unexpected. However, the presence of a methyl group at C_5, shown to greatly enhance Gram-positive efficacy in vitro, does not significantly increase the in vivo potency against *Streptococcus pneumonia* as might be predicted. In almost all cases, the methylated quinolones are equal in Gram-positive activity to the unmethylated compounds in vivo; in only two cases—**1k**–**1** (4 times more potent than **1e**–**1**) and **1k**–**4** (5 times more potent than **1e**–**4**)—does the methyl group confer a substantial boost in oral activity. In other words, the improvement in Gram-positive activity in vitro does not correspond to a similar improvement in vivo.

In the previous paper in this series, the 5-hydroxy and 5-amino groups were shown to enhance in vitro Gram-positive potency in the N_1-cyclopropylquinolones without appreciable effect on in vivo efficacy. In this paper, the same trends are observed when C_5 is substituted with a methyl group. The presence of any substituent at the 5-position decreases in vitro potency when an ethyl group is appended to the quinolone ring nitrogen; when N_1 is substituted with a difluorophenyl moiety, the 5-methyl group has no appreciable effect on activity. Fluorine (and, to a lesser extent, chlorine) substitution at C_8 is generally beneficial to efficacy, as was the presence of an aminopyrrolidine at C_7. Bulky substituents at the C_5 position (or, to a lesser extent, on the piperazine ring at C_7) decrease overall potency.

In summary, the substituent at C_5 (be it hydroxy, methyl, or amino) exerts no uniform influence on antibacterial potency but is very dependent on the substitution pattern at C_7, C_8, and N_1. If the 5-methyl quinolones are compared directly to the analogous 5-amino derivatives, it becomes apparent that the methyl group imparts a boost in activity equal to, if not greater than, that of the amino group. Indeed, compounds **1j**–**1**, **1k**–**1**, **1k**–**2**, **1k**–**4**, and **1l**–**4** all possess excellent broad-spectrum activity against Gram-positive and Gram-negative pathogens. Further study and development of these quinolones are in progress.

Experimental Section

Melting points were taken on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr on a Nicolet FT IR SX-20 spectrophotometer. Proton magnetic resonance (NMR) were recorded on either a

(9) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
(10) Chu, D. T. W.; Fernandes, P. B.; Clairborne, H. K.; Maleczka, R. E.; Klock, P.; Shen, L.; Patel, J.; Pernet, A. 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, 1986, Abstr. 428.
(11) Sanchez, J. P.; Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Nichols, J. B.; Trehan, A. K. *J. Med. Chem.* **1988**, *31*, 983.
(12) Narita, H.; Konishi, Y.; Nitta, J.; Nagaki, H.; Kobayashi, Y.; Watanabe, Y.; Minami, S.; Saikawa, I. *Yakugaka Zasshi* **1986**, *106*, 795.
(13) Hagen, S. E.; Domagala, J. M.; Heifetz, C. L.; Sanchez, J. P.; Solomon, M *J. Med. Chem.* **1990**, *33*, 849.
(14) Domagala, J. M.; Heifetz, C. L.; Mich, T. F.; Nichols, J. B. *J. Med. Chem.* **1986**, *29*, 445.
(15) Cohen, M. A.; Griffin, T. J.; Bien, P. H.; Heifetz, C. L.; Domagala, J. M. *Antimicrob. Agents Chemother.* **1985**, *28*, 766.
(16) Miller, L. C.; Tainter, M. L. *Proc. Exp. Biol. Med.* **1944**, *57*, 261.

Table II. Physical Properties for the Quinolones Tested in This Study[a]

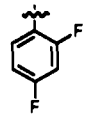
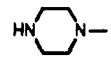
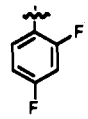
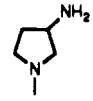
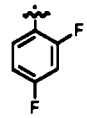
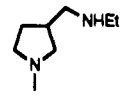
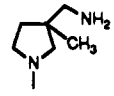
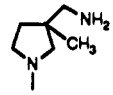
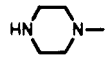
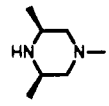
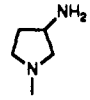
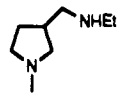
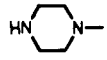
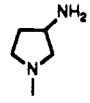
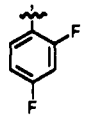
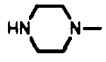
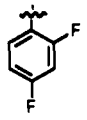
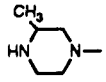
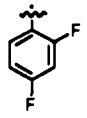
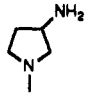
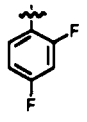
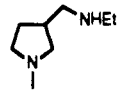
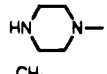
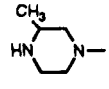
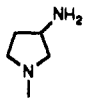
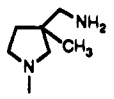
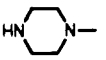
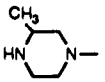
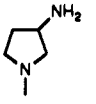
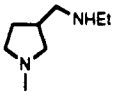
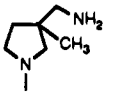
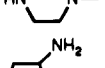
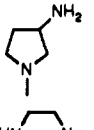
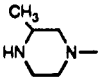
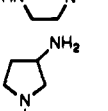
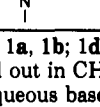
compd no.	R_5	R_8	R_1	R_7	method of prep,[b] base[c] reaction time	method of purification of final product[d]	yield % (from 7)	mp, °C	formula[e]
1c-1	H	H			ref 12				
1c-4	H	H			ref 12				
1c-5	H	H			NEt_3/8 h	isoelec prec	75	227–229	$C_{23}H_{22}F_3N_3O_3 \cdot 2.2H_2O$
1d-6	H	H			−/3h (pyr)[f]	EtOH wash	70	250–252	$C_{19}H_{22}FN_3O_3 \cdot H_2O$
1e-6	H	F			ref 13				
1g-1	CH_3	H	Et		−/8 h	EtOH wash	70	225–227	$C_{17}H_{20}FN_3O_3 \cdot 0.9H_2O$
1g-3	CH_3	H	Et		−/8 h	EtOH wash	50	219–221	$C_{19}H_{24}FN_3O_3 \cdot 2H_2O$
1g-4	CH_3	H	Et		1. NEt_3/2 h[h] 2. HCl, HOAc	isoelec prec	66	210–213	$C_{17}H_{20}FN_3O_3 \cdot 0.3H_2O$
1g-5	CH_3	H	Et		NEt_3/3 h	none	91	180–182	$C_{20}H_{26}FN_3O_3 \cdot 2.7H_2O$
1h-1	CH_3	F	Et		−/3 h	none	86	223–225	$C_{17}H_{19}F_2N_3O_3 \cdot 2.5H_2O$
1h-4	CH_3	F	Et		1. NEt_3/6 h[h] 2. HCl, AcOH	isoelec prec	82	230–232	$C_{17}H_{19}F_2N_3O_3 \cdot 4H_2O$
1i-1	CH_3	H			1. −/18 h[g] 2. HCl/3 h	isoelec prec	72	118–120	$C_{21}H_{18}F_3N_3O_3 \cdot {}^1/_4H_2O$
1i-2	CH_3	H			1. −/18 h[g] 2. HCl/3 h	isoelec prec	67	189–191	$C_{22}H_{20}F_3N_3O_3 \cdot {}^1/_4H_2O$
1i-4	CH_3	H			1. NEt_3/18 h[g,h] 2. HCl/3 h	isoelec prec	70	230–233	$C_{21}H_{18}F_3N_3O_3 \cdot 1.7H_2O$
1i-5	CH_3	H			NEt_3/3 h	recryst EtOH	79	198–201	$C_{24}H_{24}F_3N_3O_3 \cdot 0.2H_2O$
1j-1	CH_3	H			−/15 h	none	98	226–228	$C_{18}H_{20}FN_3O_3 \cdot 4H_2O$
1j-2	CH_3	H			−/5 h	isoelec prec	90	190–192	$C_{19}H_{22}FN_3O_3 \cdot 2H_2O$

Table II (Continued)

compd no.	R_5	R_8	R_1	R_7	method of prep,[b] base[c] reaction time	method of purification of final product[d]	yield % (from 7)	mp, °C	formula[e]
1j-4	CH_3	H			1. NEt_3/5 h[h] 2. HCl/AcOH	trit 2-PrOH	95	>300	$C_{18}H_{20}FN_3O_3 \cdot 2.1HCl \cdot 0.5H_2O$
1j-6	CH_3	H			1. NEt_3/8 h 2. HCl/AcOH	trit 2-PrOH	79	250–252	$C_{20}H_{24}FN_3O_3 \cdot HCl \cdot 0.5\ H_2O$
1k-1	CH_3	F			-/5 h	none	88	205–206	$C_{18}H_{19}F_2N_3O_3 \cdot 0.4H_2O$
1k-2	CH_3	F			-/3 h	EtOH wash	75	187–188	$C_{19}H_{21}F_2N_3O_3 \cdot 1.35H_2O$
1k-4	CH_3	F			1. NEt_3/6 h[h] 2. AcOH, HCl	trit 2-PrOH	79	>300	$C_{18}H_{19}F_2N_3O_3 \cdot HCl \cdot 1.5H_2O$
1k-5	CH_3	F			NEt_3/3 h	recryst EtOH	83	198–199	$C_{21}H_{25}F_2N_3O_3 \cdot 0.6H_2O$
1k-6	CH_3	F			NEt_3/3 h	EtOH wash	77	203–205	$C_{20}H_{23}F_2N_3O_3 \cdot 0.3H_2O$
1l-1	CH_3	Cl			-/8 h	EtOH wash	70	234–235	$C_{18}H_{19}ClFN_3O_3 \cdot 1.1H_2O$
1l-4	CH_3	Cl			1. NEt_3/8 h[h] 2. HCl, AcOH	isoelec prec	55	124–126	$C_{18}H_{19}ClFN_3O_3 \cdot 1.5\ H_2O$
1m-1	Et	H			-/5 h	EtOH wash	74	222–223	$C_{19}H_{22}FN_3O_3 \cdot 2.5H_2O$
1m-2	Et	H			NEt_3/5 h	isoelec prec	63	118–120	$C_{20}H_{24}FN_3O_3 \cdot 2.5H_2O$
1m-4	Et	H			1. NEt_3/5 h 2. HOAc, HCl	trit 2-PrOH	88	278–280	$C_{19}H_{22}FN_3O_3 \cdot 2.5HCl \cdot H_2O$

[a] Data for reference compounds **1a**, **1b**; **1d-1**, **1d-2**, **1d-4**, **1e-1**, **1e-2**, 1e-4, **1e-5**, **1f-1**, and **1f-4** can be found in the preceding paper in this series. [b] All reactions were carried out in CH_3CN. [c] If no base is given, excess side chain was used. [d] Isoelectric precipitation (isoelec prec) refers to dissolving the solid in aqueous base, adjusting the pH to 7.2, and filtering the solid that precipitates. Trituration (trit) refers to grinding of the solids under solvent to produce a fine powder. [e] In addition to this analytical data, all samples were >97% by HPLC. [f] Pyridine was used as the solvent. [g] The ethyl ester of **7i** was employed as starting material. [h] The 3-[(*tert*-butoxycarbonyl)amino]-pyrrolidine was used as the side chain, requiring an acid deprotection step.

Varian XL-200 or IBM 100 WP100SY spectrometer. Chemical shifts are reported in δ units relative to internal tetramethylsilane. Column chromatography was performed with E. Merck silica gel, 230–400 mesh ASTM; solutions were dried over magnesium sulfate and concentrations were performed in vacuo at 10–30 mmHg. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer, and all compounds had analytical results ±0.4% of theoretical values. Final compounds **1a–m** were assayed for purity by using a Perkin-Elmer LC-95 HPLC system equipped with a 5-μm Ultrasphere ODS column and a mobile phase consisting of 25% THF/75% 0.05 M $NH_4H_2PO_4$ (adjusted to pH 5.0 with H_3PO_4); in all cases, the purity exceeded 97.0%. *tert*-Butyl alcohol was distilled from CaH_2, THF was distilled from Na/benzophenone, and all other solvents were used without purification. The structures of all compounds were consistent with their spectral properties.

1-Bromo-2-ethyl-3,4,6-trifluoro-5-(trimethylsilyl)benzene (3). A solution of 3.5 g (35 mmol) of diisopropylamine in 125 mL of dry THF was cooled to −78 °C under argon, treated dropwise with 12.2 mL of 2.5 M *n*-butyllithium (30 mmol), and stirred for 15 min. To this LDA solution was added a solution of 8.5 g (30 mmol) of **2** in 30 mL of dry THF, and the solution was stirred for 1.5 h at −78 °C. Neat ethyl trifluoromethanesulfonate (9.7 mL; 70 mmol) was added all at once. The reaction mixture was allowed to warm to −5 °C, treated with saturated $NaHCO_3$, and extracted with EtOAc. The organic phase was washed with water, dried, and concentrated to give 9.5 g of **3** as a yellow oil which was used without purification.

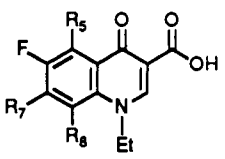
2-Ethyl-3,4,6-trifluoro-5-(trimethylsilyl)benzoic Acid (4). A solution of 8.2 g (26 mmol) of **3** in 150 mL of ether was cooled to −78 °C under argon, treated dropwise with 14.6 mL of 1.8 M *n*-butyllithium (26 mmol), and stirred for 5 min. The solution was then poured into a mixture of dry ice and ether and allowed to warm to room temperature. The mixture was quenched with 6 M HCl and extracted with EtOAc; the organic phase was concentrated to a yellow solid which was suspended in water. The suspension was basified to pH 12, washed with ether, acidified to pH 2, and extracted with ether. The organic extract was washed with water, dried over magnesium sulfate, and concentrated to give a white solid which was recrystallized from hexane to give 4.2 g (58%) of **4**: IR 1708 cm^{-1}; NMR ($CDCl_3$); δ 2.8 (m, 2 H, CH_2CH_3), 1.25 (t, 3 H, CH_2CH_3), 0.40 (s, 9 H, $Si(CH_3)_3$).

Table III. Biological Testing Results from the Antibacterial Screen and the DNA-Gyrase Supercoiling Inhibition Assay

	antibacterial activity (MICs),[a] μg/mL										
	Gram-negative organisms					Gram-positive organisms					Gyrase[b] DNA cleavage, μg/mL
						S. aeurus					
compd	E. cloac. MA2646	E. coli Vogel	K. pneum. MGH-2	P. rettg. M1771	P. aerug. UI-18	H228	UC76	S. faecalis MGH-2	S. pneum. SV-1	S. pyog. C203	
1a-1	0.1	0.025	0.05	0.025	0.2	0.8	0.05	1.6	1.6	0.8	1.0
1a-3	0.2	0.2	0.4	0.8	6.3	1.6	0.4	1.6	1.6	1.6	
1a-4	0.1	0.1	0.2	0.2	0.8	0.8	0.1	0.8	0.4	0.4	2.5
1a-5	0.4	0.1	0.4	1.6	3.1	0.2	0.1	0.2	0.2	0.1	2.5
1b-1	0.1	0.1	0.1	0.2	0.8	1.6	0.4	1.6	3.1	3.1	2.5
1b-4	0.1	0.1	0.2	0.2	0.4	0.4	0.1	0.4	0.4	0.2	2.5
1c-1	0.05	0.05	0.1	0.1	0.8	0.4	0.05	0.4	0.1	0.2	
1c-2	0.05	0.05	0.1	0.2	1.6	0.1	0.025	0.2	0.1	0.1	0.75
1c-4	0.05	0.025	0.05	0.1	0.4	0.05	0.013	0.1	0.025	0.025	
1c-5	0.40	0.2	0.8	1.6	3.1	0.1	0.025	0.2	0.1	0.1	
1d-1	0.013	0.013	0.05	0.05	0.4	1.6	0.2	0.8	0.8	0.8	0.5
1d-2	0.025	0.025	0.05	0.10	0.4	0.4	0.1	0.4	0.2	0.2	
1d-4	0.025	0.025	0.05	0.10	0.2	0.1	0.025	0.1	0.1	0.1	0.1
1d-6	0.8	0.2	0.4	0.8	0.8	0.1	0.013	0.1	0.025	0.05	3.0
1e-1	0.05	0.05	0.05	0.1	0.2	0.4	0.1	0.4	0.8	0.8	0.5
1e-2	0.05	0.025	0.025	0.1	0.4	0.2	0.1	0.4	0.4	0.4	1.0
1e-4	0.013	0.013	0.025	0.05	0.1	0.05	0.013	0.1	0.05	0.05	0.25
1e-5	0.1	0.05	0.1	0.2	0.4	0.05	0.013	0.025	0.025	0.025	0.25
1e-6	0.025	0.05	0.1	0.2	0.4	0.013	0.003	0.013	0.006	0.025	2.6
1f-1	0.025	0.025	0.05	0.1	0.4	0.1	0.05	0.4	0.1	0.2	0.5
1f-4	0.025	0.025	0.025	0.025	0.05	0.025	0.013	0.05	0.025	0.05	0.5
1g-1	0.2	0.1	0.4	0.4	1.6	3.1	0.4	1.6	3.1	6.3	3.0
1g-3	0.4	0.2	0.8	1.6	3.1	1.6	0.2	1.6	1.6	3.1	8.8
1g-4	0.2	0.2	0.8	1.6	3.1	1.6	0.4	1.6	1.6	3.1	6.3
1g-5	3.1	1.6	6.3	12.5	25	1.6	0.4	1.6	0.8	1.6	3
1h-1	0.05	0.05	0.2	0.4	1.6	0.8	0.2	3.1	6.3	12.5	7.5
1h-4	0.05	0.05	0.2	0.4	1.6	0.4	0.1	0.8	1.6	3.1	3.0
1i-1	0.05	0.05	0.1	0.2	0.8	0.1	0.025	0.2	0.05	0.1	3.0
1i-2	0.2	0.1	0.4	0.8	1.6	0.1	0.025	0.4	0.05	0.1	1.75
1i-4	0.05	0.05	0.1	0.2	0.8	0.05	0.013	0.1	0.025	0.05	0.37
1i-5	0.8	0.4	0.8	1.6	3.1	0.1	0.025	0.2	0.025	0.025	0.75
1j-1	0.013	0.013	0.05	0.1	0.2	0.1	0.025	0.05	0.05	0.10	0.30
1j-2	0.025	0.013	0.05	0.2	0.8	0.1	0.025	0.1	0.025	0.05	3.75
1j-4	0.05	0.025	0.1	0.1	0.4	0.1	0.025	0.4	0.013	0.025	0.38
1j-6	0.1	0.1	0.4	0.8	1.6	0.025	0.006	0.1	0.006	0.013	0.75
1k-1	0.025	0.025	0.025	0.05	0.2	0.025	0.025	0.05	0.025	0.05	2.75
1k-2	0.025	0.025	0.05	0.1	0.4	0.025	0.013	0.10	0.025	0.05	3.0
1k-4	0.006	0.006	0.013	0.025	0.4	0.013	0.003	0.025	0.003	0.013	3.0
1k-5	0.1	0.05	0.1	0.2	0.8	0.013	0.003	0.025	0.003	0.006	3.0
1k-6	0.05	0.05	0.1	0.2	0.8	0.006	0.003	0.025	0.003	0.003	3.75
1l-1	0.05	0.025	0.1	0.2	0.2	0.05	0.025	0.2	0.1	0.2	1.50
1l-4	0.05	0.013	0.05	0.05	0.2	0.025	0.013	0.05	0.025	0.05	0.3
1m-1	0.8	0.4	1.6	6.3	12.5	25	6.3	>25	12.5	25	
1m-2	0.8	0.4	1.6	6.3	6.3	6.3	3.1	>25	12.5	25	
1m-4	0.8	0.4	1.6	6.3	12.5	12.5	3.1	25	1.6	3.1	

[a] See ref 15. [b] See ref 6.

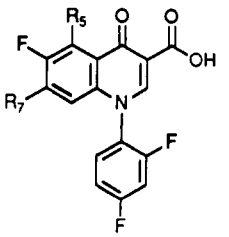
Table IV. Mean MICs for 1-Ethyl Analogues



		mean MICs (Gram-negative/Gram-positive), μg/mL	
R_7[a]	R_8	R_5 = H (cmpd no.)	R_5 = CH_3 (cmpd no.)
1	H	0.057/0.61 (1a-1)	0.35/2.08 (1g-1)
3	H	0.60/1.21 (1a-3)	0.79/1.20 (1g-3)
4	H	0.20/0.40 (1a-4)	0.69/1.38 (1g-4)
5	H	0.60/0.15 (1a-5)	6.28/1.06 (1g-5)
1	F	0.17/1.58 (1b-1)	0.20/2.08 (1h-1)
4	F	0.17/0.27 (1b-4)	0.20/0.69 (1h-4)

[a] Numbers refer to the piperazine or pyrrolidine side chain as defined in Figure 1.

Table V. Mean MICs for 1-Difluorophenyl Analogues

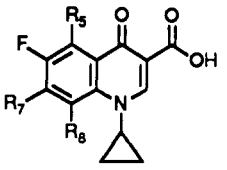


	mean MICs (Gram-negative/Gram-positive), μg/mL	
R_7[a]	R_5 = H (cmpd no.)	R_5 = CH_3 (cmpd no.)
1	0.11/0.17 (1c-1)	0.13/0.076 (1l-1)
2	0.15/0.087 (1c-2)	0.40/0.087 (1i-2)
4	0.076/0.033 (1c-4)	0.13/0.038 (1i-4)
5	0.79/0.087 (1c-5)	1.05/0.05 (1i-5)

[a] Numbers refer to the piperazine or pyrrolidine side chain as defined in Figure 1.

2-Ethyl-3,4,6-trifluorobenzoic Acid (5). To a solution of 3.9 g (14 mmol) of 4 in 50 mL of CH_3CN was added 2.6 g (17 mmol) of CsF, and the solution was stirred at room temperature for 18 h. The mixture was diluted with water and extracted with EtOAc; the extract was washed with water, dried, and concentrated to

Table VI. Mean MICs for 1-Cyclopropyl Analogues



R_7[a]	R_8	mean MICs (Gram-negative/Gram-positive), μg/mL	
		R_5 = H (cmpd no.)	R_5 = CH_3 (cmpd no.)
1	H	0.044/0.70 (**1d-1**)	0.044/0.057 (**1j-1**)
2	H	0.066/0.23 (**1d-2**)	0.076/0.050 (**1j-2**)
4	H	0.057/0.075 (**1d-4**)	0.087/0.050 (**1j-4**)
6	H	0.53/0.044 (**1d-6**)	0.35/0.016 (**1j-6**)
1	F	0.076/0.40 (**1e-1**)	0.043/0.033 (**1k-1**)
2	F	0.066/0.26 (**1e-2**)	0.066/0.033 (**1k-2**)
4	F	0.029/0.044 (**1e-4**)	0.021/0.0082 (**1k-4**)
5	F	0.13/0.025 (**1e-5**)	0.15/0.0071 (**1k-5**)
6	F	0.10/0.095 (**1e-6**)	0.13/0.0053 (**1k-6**)
1	Cl	0.066/0.13 (**1f-1**)	0.087/0.087 (**1l-1**)
4	Cl	0.029/0.029 (**1f-4**)	0.05/0.029 (**1l-4**)
1	H	0.044/0.70 (**1d-1**)	1.91/16.2[b] (**1m-1**)
2	H	0.066/0.23 (**1d-2**)	1.83/10.9[b] (**1m-2**)
4	H	0.057/0.075 (**1d-4**)	2.09/5.45[b] (**1m-4**)

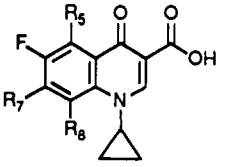
[a] Numbers refer to the piperazine or pyrrolidine side chain as defined in Figure 1. [b] R_5 = Et.

give 2.8 g (95%) of **5**: mp 113–115 °C; NMR ($CDCl_3$) δ 6.9 (m, 1 H), 2.9 (m, 2 H), 1.25 (t, 3 H).

Ethyl 2-Ethyl-3,4,6-trifluoro-β-oxobenzenepropanoate (6). Compound **6** was prepared as described in ref 3 to give an orange oil which was chromatographed, eluting with 1:1 EtOAc/hexane, to give 3.0 g (83%) of the title compound: IR (LF) 1705, 1747 cm^{-1}; NMR ($CDCl_3$) δ 12.35 (s, 0.4 H, O*H* from enol tautomer), 6.8 (m, 1 H), 5.2 (d, 0.4 H, vinyl proton from enol tautomer), 4.3–4.1 (m, 2 H, OCH_2CH_3), 3.9 (d, 1.2 H, CH_2CO_2Et from keto tautomer), 2.7 (m, 2 H, CH_2CH_3), 1.35–1.15 (m, 6H, OCH_2CH_3 and CH_2CH_3).

1-Cyclopropyl-5-ethyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (7). To a solution of 3.0 g (11 mmol) of **6** in 40 mL of Ac_2O was added 2.7 mL (16 mmol) of triethyl orthoformate. The solution was refluxed for 2.5 h, cooled, and concentrated. The residue was dissolved in 40 mL of ether, cooled to 5 °C, and treated dropwise with 0.62 g (11 mmol) of cyclopropylamine. The solution was stirred at room temperature for 18 h, then concentrated to a yellow oil. This oil was dissolved in 100 mL of dry THF, treated portionwise with 0.45 g (11 mmol) of 60% sodium hydride, and stirred at room temperature for 4 h. Glacial acetic acid (5 mL) was added; the solution was concentrated to a small volume and diluted with 75 mL of water. The solids that formed were filtered and washed with water to give the crude quinolone ester. This material was suspended in 50 mL of 6 M HCl, refluxed for 4 h, and stirred at room temperature for 18 h. The solids were filtered, washed with water and ether, and dried to give 1.3 g (45% from **6**) of white powder: mp 234–235 °C; IR 1726, 1626 cm^{-1}; NMR (DMSO) δ 15.2 (s, 1 H, CO_2H), 8.75 (s, 1 H, C_2H), 8.25 (m, 1 H, C_8H), 3.8 (m, 1 H, cyclopropyl), 3.4 (m, 2 H, CH_2CH_3), 1.35 (m, 2 H, cyclopropyl), 1.2 (t, 3 H, CH_2CH_3), 1.15 (m, 2 H, cyclopropyl). Anal. Calcd for $C_{15}H_{13}F_2NO_3 \cdot 0.1HCl$: C, 60.68; H, 4.44; N, 4.71. Found: C, 60.82; H, 4.05; N, 4.65.

Table VII. Biological Testing Results from Mouse Protection Assay



R_8	R_7	in vivo *E. coli* Vogel[a]		in vivo *S. pneumonia*[a]	
		R_5 = H (cmpd no)	R_5 = CH_3 (cmpd no.)	R_5 = H	R_5 = CH_3
H	1	1/0.25 (**1d-1**)	1.5/0.4 (**1j-1**)	100/19	100/21
H	2	1/0.4 (**1d-2**)	1.5/0.5 (**1j-2**)	39/12	24/8
H	4	3.4/0.5 (**1d-4**)	9.5/0.7 (**1j-4**)	–	–
F	1	0.45/0.25 (**1e-1**)	0.4/0.2 (**1k-1**)	59/29	14/13.5
F	2	– (**1e-2**)	0.75/0.4 (**1k-2**)	–	12/11
F	4	1/0.4 (**1e-4**)	2/0.5 (**1k-4**)	15/6	3/1.6
F	5	4.2/1 (**1e-5**)	14/3 (**1k-5**)	3.3/2	2.8/1.6

[a] PO/SC ratio, where PO indicates oral administration by gavage and SC indicates subcutaneous injection. The values are in mg/kg.

General Procedure for the Conversion of Derivatives 7 into Final Compounds 1. The quinolone acid **7** was added to a suspension of the appropriate aminopyrrolidine or piperazine (1.1 equiv), triethylamine (2 equiv), and CH_3CN; occasionally, excess side chain was used as the base and no triethylamine was added. The mixture was then refluxed until no starting material was present by TLC (usually 5–8 h). The solution was cooled to room temperature and filtered, and the solids were washed with H_2O and ether. The crude product could be purified via recrystallization, trituration, or isoelectric precipitation as defined in footnote d in Table II. Further experimental details are given in Table II.