6-Aminoquinolones: A New Class of Quinolone Antibacterials?[1]

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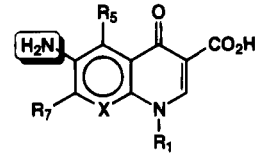
Received May 19, 1994[⊗]

A series of quinolone- and 1,8-naphthyridone-3-carboxylic acids, designed by previous QSAR studies and characterized by an amino group at the C-6 position instead of the usual fluorine atom, were synthesized for the first time and evaluated for *in vitro* antibacterial activity. All of the synthesized compounds maintain good activity against Gram-negative bacteria (*Pseudomonas aeruginosa* excluded), and those compounds having a thiomorpholine group as the C-7 substituent also have good activity against Gram-positive bacteria. Some aspects of structure–activity relationships associated with the C-1, C-5, C-7, and C-8 substituents are also discussed. Derivatives **18g** and **38g** displayed the best activity with geometric mean MICs of 0.45 and 0.66–0.76 μg/mL against Gram-negative and Gram-positive bacteria, respectively. This antimicrobial activity reflects their ability to inhibit bacterial DNA-gyrase. The results of this study show that, while the C-6 fluorine is still the preferred substituent, good activity can still be obtained by replacing it with an amino group.

Introduction

In our continuing search for new potent antibacterial agents, following the development of Rufloxacin[2] in our laboratory which is now marketed as a once-daily quinolone agent, we have taken advantage of a chemometric approach. The use of computer chemistry methodologies, namely the chemometric tools used in QSAR and molecular modeling techniques, constitutes the basis for modern drug design because the behavior of the existing molecules can be understood and the activity of new ones predicted. In two parallel studies directed toward Gram-negative[3] and Gram-positive[4] bacteria we ranked the relative importance of structural features affecting the activity of quinolones and suggested a new active class of compounds. The two key steps in our chemometric studies were (a) the selection of a well-balanced set of varying structures, from which generally valid QSAR models were obtained and (b) the use of the CARSO procedure. In this latter procedure the activity is described as a response surface, obtained by PLS, in terms of some selected structural descriptors, which indicate the substituents that can be used at each substitution site. The results of these two studies indicated that active molecules should preferably bear (a) a rather large substituent (MR ranging between 13 and 20) at N-1, confirming the appropriateness of using cyclopropyl or *tert*-butyl groups; (b) hydrogen or small substituents such as fluorine or endocyclic nitrogen at C-8, but a larger substituent such as methyl (being the largest in the training set) is also good for *Staphylococcus aureus*; and (c) an amino group instead of hydrogen at C-5. The recommended features for substitution at the C-7 position were more difficult to formulate, because the resulting range was too large to precisely define the required structures. However, the most relevant information was that the presence of an amino group at C-6, instead of the usual fluorine atom, could produce interesting, active molecules even though they would not increase activity.



X = CH, N, CF
R_1 = *c*-Pr, *t*-Bu, 4-FC_6H_4
R_5 = H, NH_2
R_7 = see Chart 1

Figure 1.

On the basis of these suggestions, a new study was undertaken on a whole class of 6-aminoquinolones in order to verify if an amino group could actually be substituted for the fluorine at C-6, the optimal feature common to all of the most potent agents. To date the activity of only a few 6-aminoquinolones has been reported.[5] We herein present our initial investigation of the 6-aminoquinolone series (Figure 1).

In our primary strategy, we introduced the amino group at the C-6 position in both the quinoline and naphthyridine nuclei, maintaining the arrangement of the functional groups that gives the highest activity levels[6] and that the QSAR studies indicated as optimal: a cyclopropyl group at N-1, *N*-methylpiperazine at C-7, and a small substituent such as hydrogen, endocyclic nitrogen, or fluorine at C-8. Therefore, 6-aminoquinolone acid **17a**, 6-aminonaphthyridone acid **37a**, and 6-amino-8-fluoroquinolone acid **38a**[7] were synthesized. The fact that the MIC values of these three prototypes were very interesting, even though they were higher than the cyprofloxacin values, prompted us to extend our investigation in an effort to increase potency, broaden the spectrum, and outline a structure–activity relationship for this new class. Therefore, only in the 6-aminoquinolone series, because of its comparative ease of synthesis, were a variety of substituents introduced at C-7 (Chart 1) that were coupled with a cyclopropyl, *tert*-butyl, or 4-fluorophenyl group at N-1. In addition,

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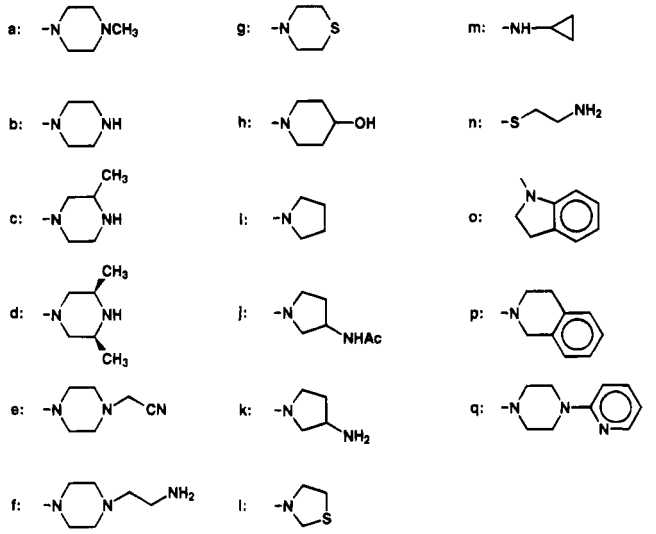
[⊗] Abstract published in *Advance ACS Abstracts*, February 1, 1995.

0022-2623/95/1838-0973$09.00/0

Chart 1. Heterocyclic Side Chains Employed as the R_7 Substituent in This Study



following the QSAR indications, a second amino group was introduced at C-5 to give 5,6-diamino derivatives **45a–47a**.

Other structural modifications on 6-aminoquinolones will be reported in a subsequent paper, after which a thorough rationalization in QSAR terms will be attempted.

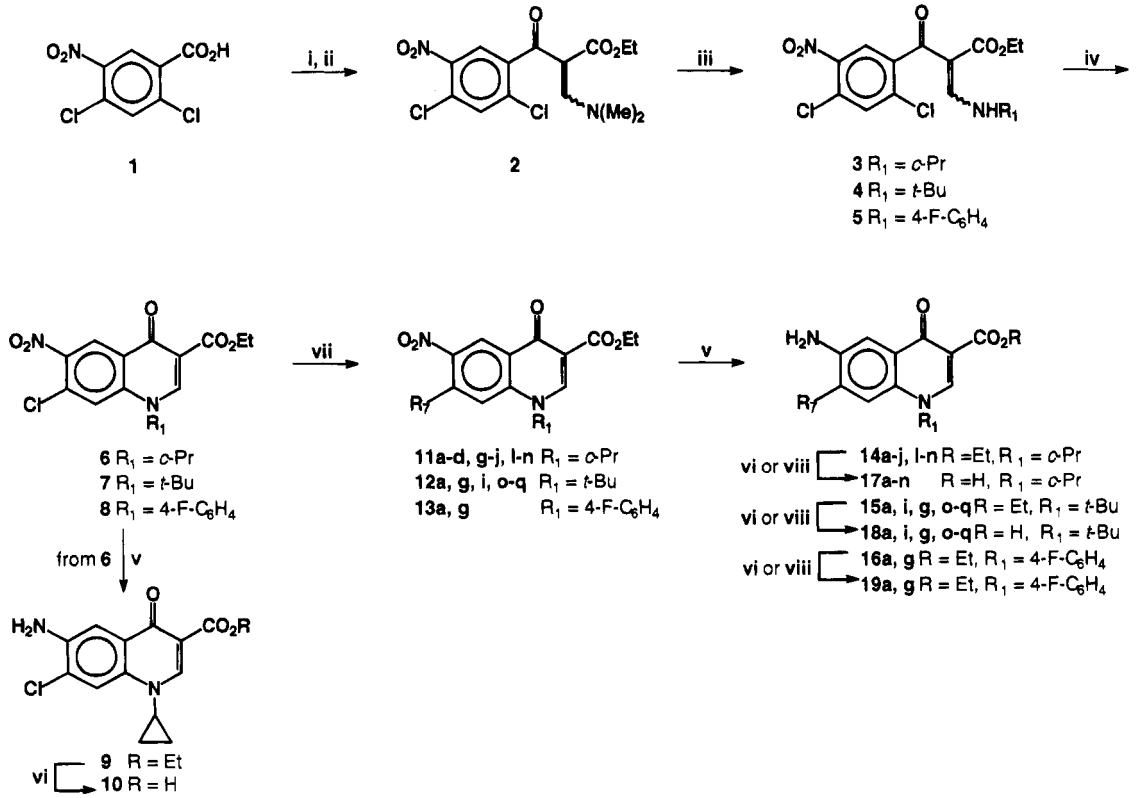
Chemistry

Different synthetic approaches were used to prepare the compounds used in this investigation.

The general method used to prepare the 6-aminoquinolone derivatives, illustrated in Scheme 1, involved the usual intramolecular nucleophilic displacement cyclization which was unimpeded by the presence of the latent C-6 substituent. Thus, a reaction of the acid chloride of 2,4-dichloro-5-nitrobenzoic acid (**1**) with ethyl (dimethylamino)acrylate, followed by substitution with appropriate amine (R_1NH_2) and successive cyclization, allowed the quinolone ring to be built. The crucial key intermediates **6–8**, thus obtained, would allow the substituent at the C-7 position (Chart 1) to be easily diversified. Therefore, the target acids **17a–n**, **18a**, **18i**, **18g**, **18o–q**, **19a**, and **19g** were obtained by nucleophilic substitution with selected bases, successive reduction of nitro group and basic (method A), or acid (method B) hydrolysis.

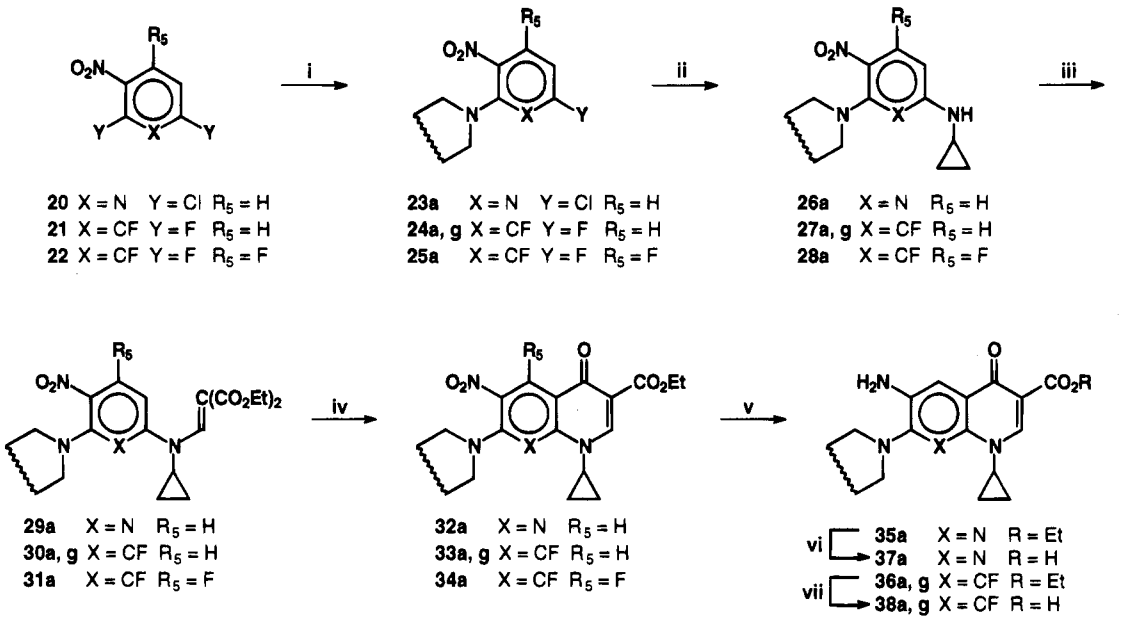
The synthesis of 6-aminonaphthyridone acid **37a** and 6-amino-8-fluoroquinolone acids **38a** and **38g** was achieved, as depicted in Scheme 2, starting from 2,6-dichloro-3-nitropyridine (**20**) and 2,3,4-trifluoronitrobenzene (**21**), respectively. The presence of a nitro group significantly affects the reactivity of the halogen atom at the *ortho* and *para* positions and consequently the chemistry of ring construction in agreement with the observations of Matsumoto et al.[8] The lower reactivity of the *p*-halogen made it impossible to introduce a cyclopropylamine in this position in the first step, as was planned in order to obtain ethyl 1-cyclopropyl-7-halo-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine- (or 8-fluoroquinoline)-3-carboxylate that would have permitted different substitutions at C-7. Therefore, each derivative with a different C-7 substituent had to be prepared by a total synthesis (because of the introduction of the heterocyclic base in the first step). The regiochemistry of *ortho* displacement to give **23a**, **24a**, and **24g** was enhanced by using toluene as solvent while

Scheme 1[a]



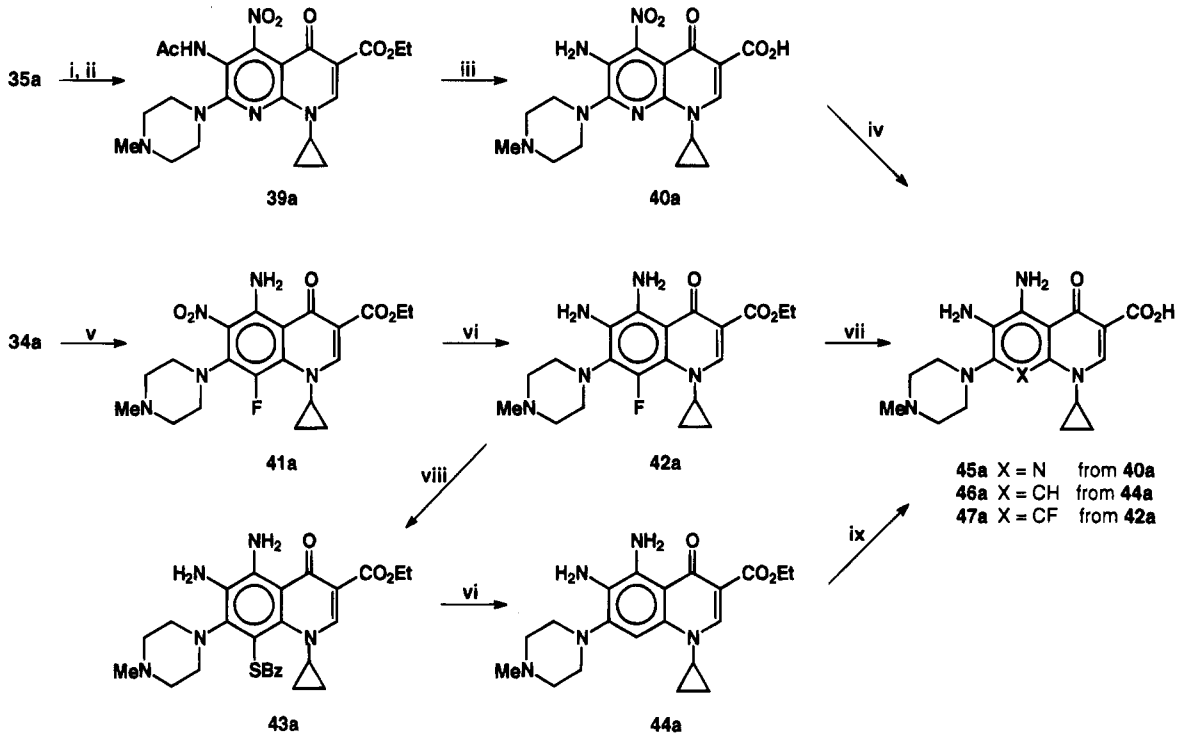
[a] Reagents: (i) $SOCl_2$, reflux; (ii) $(CH_3)_2NCH{=}CHCO_2Et$, Et_3N, toluene, 90 °C; (iii) NH_2R_1, EtOH/Et_2O; (iv) K_2CO_3, DMF, 100 °C; (v) H_2, Raney-Ni, $MeOCH_2CH_2OH$; (vi) 6 N HCl, EtOH, reflux; (vii) R_7H, DMF, 80÷120 °C; (viii) 4% NaOH, reflux.

Scheme 2[a]



[a] Reagents: (i) heterocyclic base, toluene; (ii) *c*-$C_3H_5NH_2$, HMPA; (iii) EMME; (iv) PPA; (v) H_2, Raney-Ni, EtOH; (vi) 6 N HCl, EtOH, reflux; (vii) 4% NaOH, reflux.

Scheme 3[a]



[a] Reagents: (i) Ac_2O/AcOH; (ii) fum. HNO_3/Ac_2O, 0 °C; (iii) 8 N HCl, reflux; (iv) H_2, 10% Pd/C, 6 N HCl, MeOH; (v) 30% NH_4OH, EtOH, 80 °C; (vi) H_2, Raney-Ni, EtOH; (vii) 4% NaOH, reflux; (viii) HSBz, 60% NaH, THF; (ix) 6 N HCl, reflux.

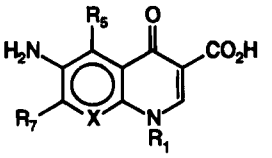
the subsequent *p*-halogen substitution with cyclopropylamine to give **26a**, **27a**, and **27g** was carried out in HMPA. These intermediates were then allowed to react with diethyl (ethoxymethylene)malonate (EMME) to give **29a**, **30a**, and **30g**, which had to be isolated and purified for a successful polyphosphoric acid (PPA) cyclization to the esters **32a**, **33a**, and **33g**. Subsequent catalytic reduction and hydrolysis gave the target acids **37a**, **38a**, and **38g**. We were unable to obtain the desired 6-amino-7-thiomorpholinonaphthyridone acid because the cyclization step, using several of the usual thermal and acid-catalyzed conditions, failed.

An additional amino group was introduced at C-5 in both quinolone and naphthyridone nuclei to obtain 5,6-diamino derivatives **45a–47a** as outlined in Scheme 3.

The 5,6-diaminonaphthyridone acid **45a** was synthesized from ester **35a** by nitration in mild conditions followed by acid hydrolysis and catalytic reduction on Pd/C.

Attempts to repeat this procedure to prepare the 5,6-diaminoquinolone acids **46a** and **47a** failed since nitration of esters **14a** and **36a**, under different reaction conditions, gave an unacceptably low yield of corresponding 5-nitro derivatives. Therefore, an alternative

Table 1. Physical Properties for the 6-Aminoquinolone- and 6-Aminonaphthyridonecarboxylic Acids Tested in This Study



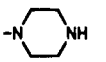
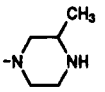
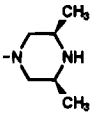
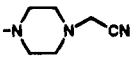
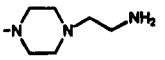
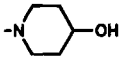
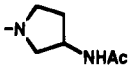
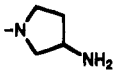
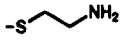
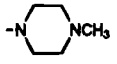
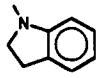
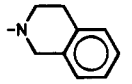
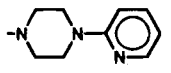
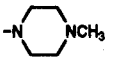
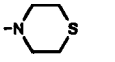
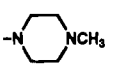
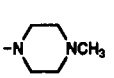
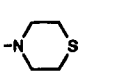
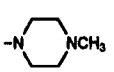
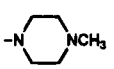
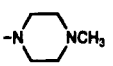
compd	X	R_1	R_5	R_7	method[a]	% yield[b]	mp, °C	formula[c]
10	CH	*c*-Pr	H	Cl	B	75	>300	$C_{13}H_{11}ClN_2O_3 \cdot HCl$
17a	CH	*c*-Pr	H		A	91	255 dec	$C_{19}H_{25}N_4O_3$
17b	CH	*c*-Pr	H		A	54	>300	$C_{18}H_{22}N_4O_3 \cdot 1.5H_2O$
17c	CH	*c*-Pr	H		A	73	>300	$C_{18}H_{22}N_4O_3$
17d	CH	*c*-Pr	H		A	72	>300	$C_{19}H_{24}N_4O_3 \cdot H_2O$
17e	CH	*c*-Pr	H		A	10	>300	$C_{19}H_{21}N_5O_3$
17f	CH	*c*-Pr	H		A	75	>300	$C_{19}H_{25}N_4O_3$
17g	CH	*c*-Pr	H		B	76	295-297	$C_{17}H_{19}N_3O_3S \cdot HCl$
17h	CH	*c*-Pr	H		B	54	250 dec	$C_{18}H_{21}N_3O_4 \cdot HCl$
17i	CH	*c*-Pr	H		A	73	275-276	$C_{17}H_{19}N_3O_3$
17j	CH	*c*-Pr	H		A	86	280 dec	$C_{19}H_{22}N_4O_4$
17k	CH	*c*-Pr	H		A[a]	78	260 dec	$C_{17}H_{20}N_4O_3$
17l	CH	*c*-Pr	H		A	80	>300	$C_{16}H_{17}N_3O_3S \cdot H_2O$
17m	CH	*c*-Pr	H		B	56	>300	$C_{16}H_{17}N_3O_3S \cdot HCl$
17n	CH	*c*-Pr	H		A	90	>300	$C_{15}H_{17}N_3O_3S$
18a	CH	*t*-Bu	H		A	76	>300	$C_{20}H_{27}N_4O_3$
18g	CH	*t*-Bu	H		A	63	>300	$C_{18}H_{23}N_3O_3$
18i	CH	*t*-Bu	H		A	55	>300	$C_{18}H_{23}N_3O_3S$
18o	CH	*t*-Bu	H		A	66	>300	$C_{22}H_{23}N_3O_3$
18p	CH	*t*-Bu	H		A	44	>300	$C_{23}H_{25}N_3O_3$
18q	CH	*t*-Bu	H		A	72	>300	$C_{23}H_{27}N_5O_3 \cdot 0.75H_2O$

Table 1. (Continued)

compd	X	R_1	R_5	R_7	method[a]	% yield[b]	mp, °C	formula[c]
19a	CH	4-FC_6H_4	H		B	70	>300	$C_{21}H_{21}FN_4O_3 \cdot 2HCl$
19g	CH	4-FC_6H_4	H		B	65	>300	$C_{20}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$
37a	N	*c*-Pr	H		B	76	>300	$C_{17}H_{21}N_5O_3 \cdot 2HCl \cdot H_2O$
38a	CF	*c*-Pr	H		A	86	>300	$C_{18}H_{21}FN_4O_3$
38g	CF	*c*-Pr	H		A	73	>300	$C_{17}H_{18}FN_3O_3S$
45a	N	*c*-Pr	NH_2		*a*	32	>300	$C_{17}H_{22}N_6O_3$
46a	CH	*c*-Pr	NH_2		B	68	>300	$C_{18}H_{23}N_5O_3 \cdot 2HCl$
47a	CF	*c*-Pr	NH_2		A	58	281-283	$C_{18}H_{22}FN_5O_3$

[a] See Experimental Section. [b] Yields are those obtained from the final hydrolysis steps, including the salt formation. [c] All compounds had elemental analyses within ±0.4% of theoretical value.

route, which entailed the preparation of the common intermediate **42a**, was planned. Thus, starting from 2,3,4,6-tetrafluoronitrobenzene (**22**), through the intermediates **25a**, **28a**, and **31a**, 5,8-difluoro-6-nitroquinoline derivative **34a** was obtained, as outlined in Scheme 2, by using the same procedure employed to prepare the analogous esters **32a**, **33a**, and **33g**. The structures of compounds **25a** and **28a** were assigned on the basis of their ^{19}F NMR spectra. The intermediate **34a** was converted, as in Scheme 3, to the 5,6-diaminoquinoline key intermediate **42a** by regiospecific displacement of fluorine at C-5 with 30% ammonia in a stainless steel bomb and successive catalytic reduction of nitro group. Basic hydrolysis of **42a** directly gave the desired acid **47a**. On the other hand, acid **46a** was obtained from the same **42a** by catalytic dehalogenation via thiobenzyl intermediate **43a** and successive acid hydrolysis.

Biological Assays

The series of quinolone and naphthyridone acids prepared for this study were tested *in vitro* against an assortment of eight Gram-negative and five Gram-positive organisms by conventional agar dilution procedure.[2a] The minimum inhibitory concentrations (MICs, μg/mL) are presented in Table 2. The geometric means of the MICs for both Gram-positive and Gram-negative strains were also calculated to facilitate a comparison of activity. The fluoroquinolones ciprofloxacin, 1-cyclopropyl-6,8-difluoro-7-(4-thiomorpholinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**48**),[9] and 5-amino-1-cyclopropyl-6,8-difluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**49**)[10] are included for comparative purposes. In addition, a representative number of compounds were tested for their ability to inhibit DNA gyrase, the target enzyme, by using a protocol described previously[11] and using gyrase which was isolated from *Escherichia coli* JM103 and purchased from TopoGEN. The procedure measures the concentration of drug (μg/mL) needed to produce linear DNA from closed circular DNA, and the values obtained are generally accurate to ±1 order of magnitude.

Result and Discussion

The first information obtained in this study is that the three prototypes, quinoline **17a**, naphthyridine **37a**, and 8-fluoroquinoline **38a**, all with a 4-methyl-1-piperazinyl group at C-7 and cyclopropyl at N-1, maintain a good activity against Gram-negative bacteria (*Pseudomonas aeruginosa* excluded) albeit less than the ciprofloxacin used for comparison, while they show a decreased activity against Gram-positive bacteria.

Modifications at the C-7 position, examined in the quinoline series, showed that the piperazinyl derivatives **17b** (direct analogue of ciprofloxacin), **17c**, and **17d** showed good activity against Gram-negatives which was on the same order as the prototype **17a**, but derivative **17b** showed increased MIC values against Gram-positives. A dramatic drop of activity was observed for 4-substituted piperazinyl derivatives **17e** and **17f**, as well as for the derivatives **17m** and **17n** having less conventional groups at C-7. A significant loss of activity, particularly against Gram-positive bacteria, also occurred when a five-membered heterocyclic base was linked to C-7 with a MIC value of 128 μg/mL for pyrrolidinyl derivatives **17i**, **17j**, and **17k**. This is in agreement with what was observed by Matsumoto et al. who, describing 6-amino-7-substituted-1-ethylnaphthyridines, found the pyrrolidinyl derivative completely inactive.[5a] Therefore, it can be deduced that the C-7 1-pyrrolidinyl group decreases the antibacterial activity in 6-aminoquinolones, also against Gram-positives, in contrast to what observed for the 6-fluoro analogues.[5a,12]

Table 2. *In Vitro* Antibacterial Activity (MICs, μg/mL)[a]

	Gram-negative organisms								Gram-positive organisms					geometric means	
	E. co.		*E. cl.*	*A. ca.*	*P. st.*	*K. pn.*		*P. ae.*	*S. au.*		*S. ep.*				
compd	ATCC 8739	ISF 432	OMNFI 174	OSMPV 113	CNUR 5	ATCC 10031	*S. en.*	ATCC 9027	MPR 5	ATCC 6538	HCF Berset C	CPHL A2	*S. fe.* LEP Br	Gram- (−)	Gram- (+)
10	2	2	16	128	64	2	16	128	128	128	64	128	128	14.67	111.43
17a	0.25	0.03	0.25	4	8	0.03	0.25	4	2	2	4	2	32	0.45	4.00
17b	0.25	0.03	0.25	16	32	0.25	0.5	2	64	64	16	16	128	0.84	42.22
17c	0.03	0.03	0.25	16	16	0.03	0.25	2	16	16	4	4	32	0.41	10.55
17d	0.03	0.03	0.5	8	4	0.03	0.25	2	8	8	4	2	32	0.35	5.96
17e	4	2	2	64	64	2	2	32	64	64	64	64	64	7.34	64.00
17f	0.5	0.5	2	32	128	0.5	2	32	128	128	32	64	128	4.00	84.45
17g	0.03	0.03	0.5	1	1	0.03	0.5	2	0.5	0.5	2	1	8	0.25	1.32
17h	0.03	0.03	2	2	8	0.03	2	8	4	4	4	2	8	0.58	4.00
17i	0.25	0.25	8	128	128	0.25	16	128	128	128	128	128	128	6.73	128.00
17j	4	4	128	128	128	4	128	128	128	128	128	128	128	34.90	128.00
17k	1	0.5	16	128	128	1	16	16	128	128	128	128	128	8.72	128.00
17l	0.5	1	16	128	128	0.5	32	128	128	64	64	64	128	11.31	84.45
17m	2	4	64	128	128	2	64	128	128	128	128	128	128	24.67	128.00
17n	1	1	128	128	128	2	128	128	128	128	128	128	128	22.63	128.00
18a	0.06	0.06	1	4	4	0.06	1	4	2	2	4	2	32	0.58	4.00
18g	0.06	0.03	2	0.5	4	0.03	2	4	0.25	0.25	0.5	0.5	8	0.45	0.66
18i	0.12	0.12	16	16	64	0.12	32	64	8	4	8	8	128	3.94	12.13
18o	0.12	0.12	8	8	4	0.12	8	32	2	2	4	4	64	1.81	5.28
18p	0.12	0.12	8	2	4	0.12	8	32	0.5	0.25	1	0.5	8	1.52	0.87
18q	0.5	0.5	64	1	128	0.25	64	128	1	1	2	1	16	6.73	2.00
19a	0.12	0.12	4	16	4	0.12	4	8	4	8	8	8	64	1.39	10.56
19g	0.06	0.03	4	4	4	0.03	4	128	1	0.5	4	4	128	1.07	4.00
37a	0.12	0.03	0.12	4	2	0.03	0.12	2	4	4	4	4	32	0.26	6.06
38a	1	0.05	0.25	8	4	0.05	0.25	2	4	4	8	4	64	0.56	8.00
38g	0.12	0.06	1	1	0.5	0.03	4	4	0.25	0.5	1	0.5	4	0.45	0.76
45a	0.25	0.01	0.25	0.5	0.5	0.01	0.25	8	2	2	2	2	8	0.20	2.64
46a	16	16	128	128	128	32	128	128	128	128	128	128	128	64.00	128.00
47a	0.03	0.03	0.12	2	2	0.03	0.25	4	2	2	4	4	4	0.24	3.03
48	0.01	0.008	0.25	1	0.12	0.008	0.25	1	0.03	0.03	0.06	0.06	0.5	0.09	0.07
49	0.004	0.002	0.03	0.05	0.06	0.002	0.03	0.25	0.06	0.06	0.25	0.12	0.5	0.02	0.14
CPX[b]	0.03	0.01	0.01	0.06	0.06	0.03	0.03	0.06	0.12	0.12	0.12	0.06	0.5	0.03	0.14

[a] Organisms selected are as follows: *E. co.*, *Escherichia coli*; *E. cl.*, *Enterobacter cloacae*; *A. ca.*, *Acinetobacter calcoaceticus*; *P. st.*, *Providencia stuardii*; *K. pn.*, *Klebsiella pneumoniae*; *S. en.*, *Shigella enteritidis*; *P. ae.*, *Pseudomonas aeruginosa*; *S. au.*, *Staphylococcus aureus*; *S. ep.*, *Staphylococcus epidermidis*; *S. fe.*, *Streptococcus faecalis*. [b] CPX = ciprofloxacin.

The reduced activity was less noticeable when the pyrrolidine was coupled with the *tert*-butyl group at N-1, as in the derivative **18i**. Among the C-7 substituents, the best activity was displayed by 1-thiomorpholinyl group. Indeed, the derivatives **17g**, **18g**, and **19g**, variously substituted at N-1, and 8-fluoroquinoline **38g**, when compared with 4-methylpiperazinyl analogues, showed an equal activity against Gram-negatives while they were 3−10 times more potent against Gram-positives (compare **17g** vs **17a**, **18g** vs **18a**, **19g** vs **19a**, and **38g** vs **38a**), with **38g** being the most potent. The increased Gram-positive antibacterial activity observed for 7-thiomorpholinyl derivative is in line with what was already observed for the 6-fluoro analogues.[13]

Like the MICs, the DNA cleavage data from Table 2 also show ciprofloxacin > 6-aminoquinolones, even though the magnitudes of differences are not close. In general, 6-aminoquinolones tested are 25−6 times less potent as DNA gyrase inhibitors than ciprofloxacin, while their MICs against *E. coli* ATCC 8739 or ISF 432 show a decrease in potency of 8−2 times (**38a** excluded) or 6−3 times, respectively. This effect could lead us to hypothesize that the 6-aminoquinolones have a better cell permeability than the 6-fluoroquinolone ciprofloxacin. Among the 6-aminoquinolones, the thiomorpholinyl derivatives **17g**, **18g**, and **38g** are more potent DNA gyrase inhibitors than the corresponding 4-methyl-1-piperazinyl analogues **17a**, **18a**, and **38a**.

Looking at N-1 substituent, the cyclopropyl and *tert*-butyl groups were more effective than 4-fluorophenyl

Table 3. DNA-Gyrase Inhibition and *in Vitro* Antibacterial Activity Against *E. coli* by Select 6-Aminoquinolones and 6-Aminonaphthyridones

compd	cleavage concn,[a] μg/mL	MIC, μg/mL	
		ATCC 8739	ISF 432
17a	25	0.25	0.03
18a	25	0.06	0.06
37a	25	0.12	0.03
38a	25	1	0.05
17g	10	0.03	0.03
18g	5.8	0.06	0.03
38g	5.8	0.12	0.06
CPX[b]	1	0.03	0.01

[a] Refers to the minimum concentration at which cleaved linear DNA is observed. [b] CPX = ciprofloxacin.

(compare **17a** and **18a** vs **19a**). Like the 6-fluoro analogues,[14] the *tert*-butyl group in N-1 enhanced the activity against Gram-positive bacteria without reducing its activity against Gram-negatives. The 1-*tert*-butyl-7-thiomorpholinyl **18g** and 1-*tert*-butyl-7-tetrahydroisoquinolinyl derivative **18p** showed the lowest MIC (0.25 μg/mL) against *S. aureus*.

The introduction of another NH_2 group in C-5, in both the 8-fluoroquinolone and naphthyridone series, showed an increased activity against the Gram-positives compared to the monoamino derivatives (**45a** vs **37a** and **47a** vs **38a**) while the Gram-negative activity remained fairly constant and with significant geometric mean MICs of 0.20 and 0.24 μg/mL for **45a** and **47a**, respectively. On the contrary, the 5,6-diaminoquinoline derivative **46a** showed an unexpected decrease in activ-

ity against both Gram-positive and Gram-negative bacteria with geometric mean MICs of 64 and 128 μg/mL, respectively. These results do not clarify the importance of NH_2 at C-5.

A direct comparison between the 6-aminoquinolones **17b**, **38g**, and **47a** and the corresponding 6-fluoroquinolones ciprofloxacin, **48** and **49**, shows a minor activity of the former compared to the latter. However, it must be pointed out that, while **17b** is 28–300 times less potent than ciprofloxacin, this values is reduced to 4.5–10 for **38g** vs **48** and 10–21 for **47a** vs **49**. This fact indicates that the efficacy of the 6-amino derivatives can be enhanced by reoptimizing the substituents in all the other positions of the quinolone nucleus.

In conclusion, these results show that the C-6 fluorine atom in antibacterial quinolones is not absolutely necessary for good antimicrobial activity. We have shown that the replacement of C-6 fluorine atom with an NH_2 group produces derivatives with broad and good *in vitro* antibacterial activity even though they are less potent than the corresponding 6-fluoro analogues.

Regarding the importance of the fluorine atom in the C-6 position, other authors have also recently reported that it is not essential: in fact the shift of the fluorine atom from the C-6 to the C-8 position gives an equivalent *in vitro* activity, and even the complete removal results in active compound.[15]

To better elucidate the quinolone SAR and following QSAR suggestions, further modifications in 6-aminoquinolones, such as introducing a methyl group at C-8, are now in progress and will be described in a subsequent paper.

Experimental Section

Melting points were determined in capillary tubes (Buchi melting point apparatus) and are uncorrected. Elemental analyses were performed on a Carlo Erba elemental analyzer, Model 1106, and the data for C, H, and N are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded at 90 MHz (Varian EM 390) or 200 MHz (Bruker AC-200) with Me_4Si as internal standard. ^{19}F NMR spectra were taken at 200 MHz (Bruker AC-200) with C_6F_6 as internal standard. Chemical shifts are given in ppm (δ), and the spectral data are consistent with the assigned structures. Reagents and solvents were purchased from common commercial suppliers and were used as received. Column chromatography separations were carried out on Merck silica gel 40 (mesh 70–230) and flash chromatography on Merck silica gel 60 (mesh 230–400). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated with a Büchi rotary evaporator at low pressure. Yields are of purified product and were not optimized. All starting materials were commercially available unless otherwise indicated. The physical properties of the target acid derivatives are summarized in Table 1.

Ethyl 2-(2,4-Dichloro-5-nitrobenzoyl)-3-(dimethylamino)acrylate (2). A mixture of 2,4-dichloro-5-nitrobenzoic acid (**1**)[16] (5 g, 21 mmol) and thionyl chloride (20 mL) was refluxed for 3 h. The excess thionyl chloride was removed by distillation under reduced pressure to give a mobile oil residue which was dissolved in dry toluene (50 mL) and added to ethyl 3-(dimethylamino)acrylate[17] (3.2 g, 22 mmol) and triethylamine (3 g, 30 mmol). The resulting solution was heated at 90 °C for 1 h. After cooling and filtration of the insoluble material, the solvent was evaporated to dryness and the oil residue was purified by flash chromatography, eluting with gradient of cyclohexane/EtOAc (9:1) to cyclohexane/EtOAc (7:3) to give **2** (2.1 g, 42%) as semisolid: ^{1}H NMR ($CDCl_3$) δ 1.00 (3 H, t, J = 7 Hz, CH_2CH_3), 3.00 and 3.40 (each 3 H, bs, NCH_3), 3.95 (2 H, q, J = 7 Hz, CH_2CH_3), 7.50 (1 H, s, H-3), 7.85 (2 H, s, vinyl H and H-6). Anal. ($C_{14}H_{14}Cl_2N_2O_5$) C, H, N.

Ethyl 2-(2,4-Dichloro-5-nitrobenzoyl)-3-(cyclopropylamino)acrylate (3). A stirred solution of **2** (0.72 g, 2 mmol) in EtOH/Et_2O (1:2) (7.5 mL) was treated dropwise with cyclopropylamine (0.175 g, 3 mmol). After 15 min at room temperature, the precipitate was filtered and washed with Et_2O to give **3** (0.71 g, 94%) as a yellowish solid: mp 123–125 °C; ^{1}H NMR ($CDCl_3$) δ 0.75–1.15 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 2.75–3.10 (1 H, m, cyclopropyl CH), 3.90 (2 H, q, J = 7 Hz, CH_2CH_3), 7.40 (1 H, s, H-3), 7.65 (1 H, s, H-6), 8.18 (1 H, d, J = 12 Hz, vinyl H), 10.80–11.00 (1 H, m, NH). Anal. ($C_{15}H_{14}Cl_2N_2O_5$) C, H, N.

By using the same procedure, compounds **4** and **5** were prepared from **2**, replacing cyclopropylamine with *tert*-butylamine or 4-fluoroaniline respectively: **4**, oil (96%); **5**, oil (85%).

Ethyl 1-Cyclopropyl-7-chloro-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6). A mixture of **3** (0.5 g, 1.3 mmol) and K_2CO_3 (0.2 g, 1.4 mmol) in dry DMF (8 mL) was heated at 100 °C for 1 h. After cooling, the resulting solid was filtered washing with water and dried to give **6** (0.4 g, 92%) as yellowish solid: mp 255–257 °C; ^{1}H NMR (TFA/DMSO-d_6) δ 1.30–1.75 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 4.10–4.40 (1 H, m, cyclopropyl CH), 4.70 (2 H, q, J = 7 Hz, CH_2CH_3), 8.85 (1 H, s, H-8), 9.10 (1 H, s, H-5), 9.40 (1 H, s, vinyl H). Anal. ($C_{15}H_{13}ClN_2O_5$) C, H, N.

By this procedure compounds **7** and **8** were obtained from **4** and **5** respectively: **7**, mp >300 °C (70%); **8**, mp >300 °C (90%).

Ethyl 1-Cyclopropyl-7-(4-methyl-1-piperazinyl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11a). A mixture of ester **6** (0.34 g, 1 mmol) and *N*-methylpiperazine (0.2 g, 2 mmol) in dry DMF (5 mL) was heated at 100 °C for 1 h. The solvent was removed, and the residue was triturated with water (10 mL) and filtered, washing with EtOH to give **11a** (0.3 g, 75%): mp 218–221 °C; ^{1}H NMR (TFA) δ 1.30–1.80 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 3.15 (3 H, bs, NCH_3), 3.50–4.30 (9 H, m, piperazine CH_2 and cyclopropyl CH), 4.65 (2 H, q, J = 7 Hz, CH_2CH_3), 8.05 (1 H, s, H-8), 9.10 (1 H, s, H-5), 9.30 (1 H, s, vinyl H). Anal. ($C_{20}H_{24}N_4O_5$) C, H, N.

Compounds **11b–d**, **11g–j**, and **11l–n** were prepared from intermediate **6**, while **12a**, **12g**, **12i**, and **12o–q** were prepared from **7**, and **13a** and **13g** were prepared from **8** by reaction with appropriate amine, according to this procedure.

Ethyl 1-Cyclopropyl-7-(4-(cyanomethyl)-1-piperazinyl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11e). A mixture of **11b** (0.6 g, 1.5 mmol), chloroacetonitrile (0.15 g, 1.5 mmol), and Et_3N (0.25 g, 2.5 mmol) was heated at 100 °C for 1 h. The solution was then evaporated to dryness, and the residue was triturated with EtOH, filtered, washed with EtOH, and dried to afford **11e** (0.6 g, 94%): mp 253–256 °C; ^{1}H NMR (DMSO-d_6/TFA) δ 1.15–1.50 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 3.35–3.85 (9 H, m, piperazine CH_2 and cyclopropyl CH), 4.15–4.55 (4 H, m, CH_2CH_3 and CH_2CN), 7.65 (1 H, s, H-8), 8.70 (1 H, s, H-5), 8.80 (1 H, s, H-2). Anal. ($C_{21}H_{23}N_5O_5$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (14a). A stirred solution of **11a** (0.3 g, 0.75 mmol) in 2-methoxyethanol (50 mL) was hydrogenated over Raney nickel (0.06 g) at room temperature and atmospheric pressure for 3 h. The mixture was filtered over Celite, and the filtrate was concentrated to half the initial volume. After cooling, the precipitate solid was filtered off and washed with EtOH, giving **14a** (0.2 g, 72%) as a white solid: mp 282–285 °C; ^{1}H NMR (TFA) δ 1.25–1.75 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 3.20 (3 H, bs, NCH_3), 3.55–4.08 (8 H, m, piperazine CH_2), 4.10–4.35 (1 H, m, cyclopropyl CH), 4.75 (2 H, q, J = 7 Hz, CH_2CH_3), 8.45 (1 H, s, H-8), 8.75 (1 H, s, H-5), 9.35 (1 H, s, vinyl H). Anal. ($C_{20}H_{26}N_4O_3$) C, H, N.

In an analogous procedure, compounds **9**, **14b–d**, **14g–j**, **14l–n**, **15a**, **15g**, **15i**, **15o–q**, **16a**, and **16g** were prepared from the corresponding nitro esters **6**, **11**, **12**, and **13**, respectively, while nitro ester **11e** afforded to **14f**.

Ethyl 6-Amino-1-cyclopropyl-7-(4-(cyanomethyl)-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (14e). A stirred mixture of **11e** (0.6 g, 1.4 mmol) and reduced-

iron powder (0.57 g) in AcOH (8 mL) was heated at 50 °C for 4 h. The reaction mixture was then poured into ice–water, basified with 10% Na_2CO_3 solution, and extracted with EtOAc. The combined organic layers were washed with brine, dried, and evaporated to dryness to give **14e** (0.08 g, 31%), which was used in the next step without further purification: mp 257–260 °C; ^{1}H NMR (DMSO-d_6) δ 0.95–1.45 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 2.60–2.90 and 2.95–3.25 (each 4 H, m, piperazine CH_2), 3.40–3.65 (1 H, m, cyclopropyl CH), 3.85 (2 H, s, CH_2CN), 4.20 (2 H, q, J = 7 Hz, CH_2CH_3), 5.15 (2 H, bs, NH_2), 7.45 (2 H, s, H-5 and H-8), 8.30 (1 H, s, H-2). Anal. ($C_{21}H_{25}N_5O_3$) C, H, N.

6-Amino-1-cyclopropyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (17a). Method A. The suspension of **14a** (0.2 g, 5.4 mmol) in 4% NaOH (2 mL) was refluxed for 30 min. After cooling at room temperature, the obtained solution was diluted with water (4 mL) and neutralized with 2 N HCl. The resulting precipitate was filtered and washed with water to give **17a** (0.16 g, 91%): mp 255 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.10–1.40 (4 H, m, cyclopropyl CH_2), 2.35 (3 H, s, NCH_3), 2.45–2.80 and 2.95–3.20 (each 4 H, m, piperazine CH_2), 3.65–4.00 (1 H, m, cyclopropyl CH), 5.35 (2 H, bs, NH_2), 7.50 (1 H, s, H-8), 7.55 (1 H, s, H-5), 8.50 (1 H, s, vinyl H). Anal. ($C_{18}H_{22}N_4O_3$) C, H, N.

In an analogous procedure, compounds **17b–f**, **17i–j**, **17l**, **17n**, **18a**, **18g**, **18i**, and **18o–q** were prepared starting from the corresponding amino esters **14** and **15**, respectively, as well as **38a** and **38g** from **36a** and **36g**.

6-Amino-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (17k). 17k was obtained from ester **14j** by basic hydrolysis via method A, employing 10% NaOH instead of 4% NaOH in order to remove the *N*-acetyl along with the ester. Compound **17k** was isolated in 78% yield: mp 260 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.00–1.50 (4 H, m, cyclopropyl CH_2), 4.32–4.10 (10 H, m, pyrrolidine H, NH_2 and cyclopropyl CH), 5.30 (2 H, bs, NH_2), 7.45 (1 H, s, H-8), 7.50 (1 H, s, H-5), 8.50 (1 H, s, H-2). Anal. ($C_{17}H_{20}N_4O_3$) C, H, N.

6-Amino-1-cyclopropyl-7-(1-thiomorpholinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Hydrochloride (17g). Method B. A solution of **14g** (0.2 g, 0.54 mmol) in EtOH (2.5 mL) and 6 N HCl (2.5 mL) was refluxed for 6 h. After cooling, the precipitated solid was filtered off, washed with dry EtOH, and dried to give **17g** (0.15 g, 75%): mp 295–297 °C; ^{1}H NMR (DMSO-d_6) δ 1.10–1.40 (4 H, m, cyclopropyl CH_2), 2.80–3.05 and 3.20–3.40 (each 4 H, m, thiomorpholine H), 3.75–4.00 (1 H, m, cyclopropyl CH), 5.55 (2 H, bs, NH_2), 7.50 (2 H, s, H-5 and H-8), 8.60 (1 H, s, H-2). Anal. ($C_{17}H_{19}N_3O_3S$·HCl) C, H, N.

In an analogous procedure, compounds **10**, **17h**, **17m**, **19a**, and **19g** were prepared starting from the corresponding amino esters **9**, **14h**, **14m**, **16a**, and **16g**, respectively.

6-Chloro-2-(4-methyl-1-piperazinyl)-3-nitropyridine (23a). A solution of *N*-methylpiperazine (1.04 g, 10.3 mmol) in toluene (10 mL) was added dropwise to a solution of 2,6-dichloro-3-nitropyridine (**20**) (2 g, 10.3 mmol) in toluene (15 mL). The mixture was stirred at room temperature for 5 h. The precipitated solid was filtered and washed with MeOH to give **23a** (2.42 g, 92%): mp 241–243 °C; ^{1}H NMR ($CDCl_3$) δ 2.80 (3 H, s, NCH_3), 3.00–3.40 and 3.45–3.85 (each 4 H, m, piperazine CH_2), 7.05 (1 H, d, J = 9 Hz, H-5), 8.35 (1 H, d, J = 9 Hz, H-4). Anal. ($C_{10}H_{13}ClN_4O_2$) C, H, N.

According to this procedure, compounds **24a** and **24g** were prepared from **21** while compound **25a** was prepared from **22** by reaction with the appropriate amine: **24a**, oil (53%); **24g**, semisolid (59%); **25a**, mp 100–104 °C (33%); ^{19}F NMR ($CDCl_3$) δ 36.90 (1 F, dd, J = 16 and 4 Hz, F-6), 33.60 (1 F, dd, J = 20 and 4 Hz, F-4), 14.25 (1 F, dd, J = 20 and 12 Hz, F-3).

6-(Cyclopropylamino)-2-(4-methyl-1-piperazinyl)-3-nitropyridine (26a). Cyclopropylamine (0.67 g, 11.6 mmol) was added at room temperature to a stirred solution of **23a** (2 g, 7.8 mmol) in HMPA (10 mL). The reaction mixture was warmed at 45 °C for 3 h, then cooled to room temperature, and poured into water. The solution was extracted several time with EtOAc, and the combined organic layers were washed with water, dried, and evaporated to dryness to give **26a** (2 g, 93%) as a yellow clear solid: mp 132–134 °C; ^{1}H NMR ($CDCl_3$) δ 0.45–1.00 (4 H, m, cyclopropyl CH_2), 2.35 (3 H, s, NCH_3), 2.45–2.80 (5 H, m, piperazine CH_2 and cyclopropyl CH), 3.30–3.60 (4 H, m, piperazine CH_2), 5.45 (1 H, bs, NH), 6.15 (1 H, d, J = 9 Hz, H-5), 8.20 (1 H, d, J = 9 Hz, H-4). Anal. ($C_{13}H_{19}N_5O_2$) C, H, N.

Cyclopropylamino derivatives **27a**, **27g**, and **28a** were prepared in an analogous procedure starting from **24a**, **24g**, and **25a**, respectively. Slight modifications were necessary for compound **28a** only; the reaction was carried out at room temperature using an equimolar amount of cyclopropylamine: **27a**, semisolid (92%); **27g**, mp 141–143 °C (92%); **28a**, mp 129–131 °C (54%), ^{19}F NMR ($CDCl_3$) δ 36.40 (1 F, d, J = 11.7 Hz, F-6), 13.20 (1 F, d, J = 11.7 Hz, F-3).

Diethyl [[*N*-Cyclopropyl-*N*-[2-(4-methyl-1-piperazinyl)-3-nitropyridyl]amino]methylene]malonate (29a). A mixture of **26a** (0.5 g, 1.8 mmol) and EMME (0.586 g, 2.7 mmol) was heated at 140 °C for 36 h. After cooling, the reaction mixture was purified by column chromatography, eluting with cyclohexane/$CHCl_3$ (1:1) to give **29a** (0.4 g, 50%) as a semisolid: ^{1}H NMR ($CDCl_3$) δ 1.15–1.60 (10 H, m, CH_2CH_3 and cyclopropyl CH_2), 2.35 (3 H, s, NCH_3), 2.45–2.70 (4 H, m, piperazine CH_2), 2.85–3.00 (1 H, m, cyclopropyl CH), 3.40–3.65 (4 H, m, piperazine CH_2), 4.15–4.45 (4 H, m, CH_2CH_3), 6.65 (1 H, d, J = 9 Hz, H-5), 8.25 (1 H, d, J = 9 Hz, H-4), 8.85 (1 H, s, vinyl H). Anal. ($C_{21}H_{29}N_5O_6$) C, H, N.

Diethyl (aminomethylene)malonate derivatives **30a**, **30g**, and **31a** were prepared according to this procedure starting from **27a**, **27g**, and **28a**, respectively: **30a**, oil (50%); **30g**, oil (45%); **31a**, semisolid (39%).

Ethyl 1-Cyclopropyl-7-(4-methyl-1-piperazinyl)-6-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (32a). A mixture of **29a** (0.4 g, 0.9 mmol) and PPA (2 g) was heated at 90 °C for 2 h. After cooling, the reaction mixture was poured into ice–water and basified with 10% Na_2CO_3 solution. The precipitated solid was filtered off, washed with water, dried, and recrystallized from EtOAc, giving **32a** (0.35 g, 90%) as a dark yellow solid: mp 187–188 °C; ^{1}H NMR ($CDCl_3$) δ 1.00–1.55 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 2.35 (3 H, s, NCH_3), 2.50–2.70 (4 H, m, piperazine CH_2), 3.40–3.80 (5 H, m, piperazine CH_2 and cyclopropyl CH), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 8.50 (1 H, s, H-5), 8.95 (1 H, s, vinyl H). Anal. ($C_{19}H_{23}N_5O_5$) C, H, N.

Nitro ester derivatives **33a**, **33g**, and **34a** were prepared according to this procedure starting from **30a**, **30g**, and **31a**, respectively: **33a**, mp 184–187 °C (62%); **33g**, mp 129–131 °C (34%); **34a**, mp 206–208 °C (79%).

Ethyl 6-Amino-1-cyclopropyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (35a). A stirred solution of **32a** (0.5 g, 1.2 mmol) in absolute EtOH (70 mL) was hydrogenated over Raney nickel (0.1 g) at room temperature and atmospheric pressure for 1 h. The mixture was filtered over Celite, and the filtrate was evaporated to dryness, giving a thick residue. When a mixture of CCl_4 and cyclohexane was added to it, a dark pink solid immediately formed. The precipitate was filtered off, dried, and recrystallized from EtOAc, giving **35a** (0.35 g, 79%): mp 231–232 °C; ^{1}H NMR ($CDCl_3$) δ 0.90–1.30 (4 H, m, cyclopropyl CH_2), 1.40 (3 H, t, J = 7 Hz, CH_2CH_3), 2.40 (3 H, s, NCH_3), 2.55–2.70 (4 H, m, piperazine CH_2), 3.35–3.65 (5 H, m, piperazine CH_2 and cyclopropyl CH), 3.95 (2 H, bs, NH_2), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 7.90 (1 H, s, H-5), 8.45 (1 H, s, vinyl H). Anal. ($C_{19}H_{25}N_5O_3$) C, H, N.

Amino ester derivatives **36a** and **36g** were prepared according to this procedure starting from **33a** and **33g**, respectively: **36a**, mp 234–237 °C (70%); **36g**, mp 198–200 °C (55%).

6-Amino-1-cyclopropyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid Dihydrochloride (37a). Compound **37a** was prepared from **35a** by acid hydrolysis via method B with the exception that the reaction time was 12 h; it was obtained in 76% yield as a pale yellow solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.05–1.43 (4 H, m, cyclopropyl CH_2), 2.85 (3 H, d, J = 4.5 Hz, $NHCH_3$),

3.35–3.65 (9 H, m, piperazine CH_2 and cyclopropyl CH), 7.70 (1 H, s, H-5), 8.55 (1 H, s, vinyl H). Anal. ($C_{17}H_{21}N_5O_3$·2HCl) C, H, N.

Ethyl 6-(Acetylamino)-1-cyclopropyl-7-(4-methyl-1-piperazinyl)-5-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (39a). A mixture of **35a** (0.3 g, 0.8 mmol) in AcOH (3.6 mL) was treated with Ac_2O (1.2 mL) and then stirred at room temperature for 1 h. After cooling below 0 °C, more Ac_2O (1.05 mL) and a solution of fuming HNO_3/Ac_2O (1:2) (1.125 mL) were added to the reacting mixture. Stirring was continued for 1 h at −5 to 0 °C, and the reacting mixture was poured into ice–water, basified with saturated aqueous $NaHCO_3$ solution, and extracted with CH_2Cl_2. The combined organic layers were washed with water, dried, and evaporated to dryness. The resulting residue was purified by column chromatography, eluting with a gradient of $CHCl_3$ to 7% MeOH/$CHCl_3$ to give **39a** (0.13 g, 35%) as yellow solid: mp 245–246 °C; ^{1}H NMR ($CDCl_3$) δ 1.00–1.50 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 2.20 (3 H, s, NCH_3), 2.40 (3 H, s, $COCH_3$), 2.45–2.70 (4 H, m, piperazine CH_2), 3.35–3.80 (1 H, m, cyclopropyl CH), 3.80–4.05 (4 H, m, piperazine CH_2), 4.25 (2 H, q, J = 7 Hz, CH_2CH_3), 8.40 (1 H, s, vinyl H), 8.80 (1 H, bs, NH). Anal. ($C_{21}H_{26}N_6O_6$) C, H, N.

6-Amino-1-cyclopropyl-7-(4-methyl-1-piperazinyl)-5-nitro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (40a). A suspension of **39a** (0.25 g, 0.5 mmol) in 8 N HCl (4.5 mL) was refluxed for 12 h. After cooling, the precipitate was filtered off, dried, and recrystallized from MeOH to give **40a** (0.1 g, 43%) as a pink amorphous solid: mp 275–284 °C; ^{1}H NMR (DMSO-d_6) δ 1.10–1.25 (4 H, m, cyclopropyl CH_2), 2.80 (3 H, s, NCH_3), 3.20–3.40 (8 H, m, piperazine CH_2), 3.45–3.60 (1 H, m, cyclopropyl CH), 4.00 (2 H, bs, NH_2), 8.65 (1 H, s, vinyl H). Anal. ($C_{17}H_{20}N_6O_5$·HCl) C, H, N.

1-Cyclopropyl-5,6-diamino-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (45a). A stirred solution of **40a** (0.1 g, 0.23 mmol) in MeOH (8 mL) and 6 N HCl (0.3 mL) was hydrogenated over 10% Pd/C (0.02 g) at room temperature and atmospheric pressure for 7 h. The mixture was filtered over Celite, neutralized with saturated aqueous $NaHCO_3$ solution, and evaporated to dryness. The resulting residue was crystallized from DMF to give **45a** (0.03 g, 32%) as a yellowish solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.00–1.30 (4 H, m, cyclopropyl CH_2), 2.80 (3 H, s, NCH_3), 3.30–3.70 (12 H, m, piperazine CH_2 and NH_2), 4.30–4.50 (1 H, m, cyclopropyl CH), 8.70 (1 H, s, vinyl H). Anal. ($C_{17}H_{22}N_6O_3$) C, H, N.

Ethyl 5-Amino-1-cyclopropyl-8-fluoro-7-(4-methyl-1-piperazinyl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (41a). A solution of **34a** (1 g, 2.3 mmol) in EtOH (20 mL) and 30% NH_4OH (3 mL) was heated in a stainless steel bomb at 80 °C for 50 min. After cooling, the mixture was poured into water and extracted with EtOAc. The combined organic layers were dried and evaporated to dryness to afford a solid which was recrystallized from EtOAc to give **41a** (0.88 g, 95%) as a dark red solid: mp 102–104 °C; ^{1}H NMR ($CDCl_3$) δ 0.90–1.55 (7 H, m, CH_2CH_3 and cyclopropyl CH_2), 2.45 (3 H, s, NCH_3), 2.50–2.75 and 3.25–3.45 (each 4 H, m, piperazine CH_2), 3.85–4.10 (1 H, m, cyclopropyl CH), 4.50 (2 H, q, J = 7 Hz, CH_2CH_3), 8.40 (1 H, s, vinyl H), 9.10 (2 H, bs, NH_2); ^{19}F NMR ($CDCl_3$) δ 16.00 (1 F, s). Anal. ($C_{20}H_{24}FN_5O_5$) C, H, N.

Ethyl 1-Cyclopropyl-5,6-diamino-8-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (42a). It was prepared from **41a** by following the procedure described for the conversion of **32a** to **35a**. Compound **42a** was obtained in 52% yield as a yellow solid: mp 218–220 °C; ^{1}H NMR ($CDCl_3$) δ 0.70–1.25 (4 H, m, cyclopropyl CH_2), 1.40 (3 H, t, J = 7 Hz, CH_2CH_3), 2.35 (3 H, s, NCH_3), 2.40–3.60 (8 H, m, piperazine CH_2), 3.70–4.20 (3 H, m, cyclopropyl CH and NH_2), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 6.35 (2 H, bs, NH_2), 8.35 (1 H, s, vinyl H). Anal. ($C_{20}H_{26}FN_5O_3$) C, H, N.

1-Cyclopropyl-5,6-diamino-8-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (47a). Compound **47a** was prepared from **42a** by basic hydrolysis following method A with the exception that the precipitation of amino acid **47a** occurred at pH 8; it was obtained in 58% yield as a yellow solid: mp 281–283 °C; ^{1}H NMR (DMSO-d_6) δ 1.00–1.30 (4 H, m, cyclopropyl CH_2), 2.35 (3 H, s, NCH_3), 2.60–3.90 (8 H, m, piperazine CH_2), 3.95–4.15 (1 H, m, cyclopropyl CH), 5.05 and 6.95 (each 2 H, bs, NH_2), 8.35 (1 H, s, vinyl H). Anal. ($C_{18}H_{22}FN_5O_3$) C, H, N.

Ethyl 1-Cyclopropyl-5,6-diamino-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (44a). Benzyl mercaptan (0.09 g, 0.77 mmol) was added to a solution of **42a** (0.3 g, 0.77 mmol) in dry THF (15 mL) cooled at 0 °C. Then 60% NaH in an oil suspension (0.03 g, 0.77 mmol) was added portionwise, and the mixture was stirred for 1 h at room temperature under a nitrogen atmosphere. The resulting precipitate was filtered off and washed with EtOH to give benzyl mercapto derivative **43a** (0.3 g, 79%) as yellow ochre: mp 296–238 °C; ^{1}H NMR ($CDCl_3$) δ 0.85–1.50 (7 H, m, cyclopropyl CH_2 and CH_2CH_3), 2.35 (3 H, s, NCH_3), 2.80–3.05 and 3.30–3.50 (each 2 H, m, piperazine CH_2), 3.10 (2 H, s, CH_2Ph), 3.75–3.95 (2 H, m, piperazine CH_2), 4.00–4.20 (3 H, m, piperazine CH_2, and cyclopropyl CH), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 4.75 and 6.35 (each 2 H, bs, NH_2), 7.30–7.40 (5 H, m, aromatic H), 8.40 (1 H, s, vinyl H).

Benzyl mercapto derivative **43a** was converted to **44a** by following the procedure employed for the preparation of **35a**, except that the reaction time and temperature were 10 min and 78 °C, respectively. Compound **44a** was obtained in 65% yield as a solid: mp 215–218 °C; ^{1}H NMR ($CDCl_3$) δ 0.90–1.20 (4 H, m, cyclopropyl CH_2), 1.40 (3 H, t, J = 7 Hz, CH_2CH_3), 2.40 (3 H, s, NCH_3), 2.55–3.60 (8 H, m, piperazine CH_2), 3.80–4.00 (1 H, m, cyclopropyl CH), 4.15 (2 H, bs NH_2), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 6.55 (2 H, bs, NH_2), 7.40 (1 H, s, H-8), 8.40 (1 H, s, vinyl H). Anal. ($C_{20}H_{27}N_5O_3$) C, H, N.

1-Cyclopropyl-5,6-diamino-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Dihydrochloride (46a). 46a was obtained from **44a** by acid hydrolysis following method B in 68% yield: mp >300 °C; ^{1}H NMR (TFA) δ 1.15–1.65 (4 H, m, cyclopropyl CH_2), 3.20 (3 H, s, NCH_3), 3.45–4.25 (9 H, m, piperazine CH_2 and cyclopropyl CH), 7.40 (1 H, s, H-8), 8.90 (1 H, s, vinyl H). Anal. ($C_{18}H_{23}N_5O_3$·2HCl) C, H, N.

Acknowledgment. We are grateful to Mr. Roberto Bianconi for skillful synthetic assistance.

Supplementary Material Available: Table of physical properties for intermediates **9** and **11–16** and ^{1}H NMR data of target acids not reported in the text (6 pages). Ordering information is given on any current masthead page.

References

(1) This work was previously presented in part: Cecchetti, V.; Fravolini, A.; Pagella, G.; Savino, A. F.; Tabarrini, O. A New Generation of Quinolones: 6-Aminoquinolones. X Convegno Nazionale della Divisione di Chimica Farmaceutica della S.C.I., Siena, Italy, September 1991, p 40.

(2) (a) Cecchetti, V.; Fravolini, A.; Fringuelli, R.; Mascellani, G.; Pagella, P.; Palmioli, M.; Segre, G.; Terni, P. Quinolinecarboxylic Acids. 2. Synthesis and Antibacterial Evaluation of 7-Oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acids. *J. Med. Chem.* **1987**, *30*, 465–473. (b) Mediolanum. Rufloxacin Hydrochloride. *Drugs Future* **1991**, *16*, 678–680.

(3) Bonelli, D.; Cecchetti, V.; Clementi, S.; Cruciani, G.; Fravolini, A.; Savino, A. F. The Antibacterial Activity on Quinolones against *E. coli*: a Chemometric Study. *Quant. Struct.-Act. Relat.* **1991**, *10*, 333–343.

(4) Bonelli, D.; Cecchetti, V.; Clementi, S.; Fravolini, A.; Savino, A. F. Chemometric Rationalization of the Structural Features Affecting the Antibacterial Activity of Quinolones against *Staphylococcus aureus*. *Pharm. Pharmacol. Lett.* **1993**, *3*, 13–16.

(5) (a) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. Pyridonecarboxylic Acid as Antibacterial Agents. 2. Synthesis and Structure-Activity Relationships of 1,6,7-Trisubstituted 1,4-Dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids, Including Enoxacin, a New Antibacterial Agent. *J. Med. Chem.* **1984**, *27*, 292–301. (b) Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon M. New Structure-Activity Relationship of the Quinolone Antibacterial Using the Target Enzyme. The Develop-

ment and Application of a DNA Gyrase Assay. *J. Med. Chem.* **1986**, *29*, 394–404. (c) Masuzawa, K.; Suzue, S.; Hirai, K.; Ishizaki, T. Jpn. Kokai Tokkyo Koho JP 62 26, 272; *Chem. Abstr.* **1987**, *107*, 96604b.

(6) (a) Chu, D. T. W.; Fernandes, P. B. Recent Developments in the Field of Quinolone Antibacterial Agents. *Adv. Drug Res.* **1990**, *21*, 39–144. (b) Asahina, Y.; Ishizaki, T.; Suzue, S. Recent Advances in Structure Activity Relationships in New Quinolones. *Progr. Drug Res.* **1992**, *38*, 57–106. (c) Mitscher, L. A.; Devasthale, P.; Zavord, R. Structure-Activity Relationships. In *Quinolone Antimicrobial Agents*, 2nd ed.; Hooper, D. C., Wolfson, J. S., Eds.; American Society for Microbiology: Washington, D. C., 1993; pp 3–52.

(7) This compound was previously described in ref 5c.

(8) Matsumoto, J.; Miyiamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, Y. Synthesis of Fluorinated Pyridines by the Balz-Schiemann Reaction. An Alternative Route to Enoxacin, a New Antibacterial Pyridonecarboxylic Acid. *J. Heterocycl. Chem.* **1984**, *21*, 673–679.

(9) Grohe, K.; Peterson, U.; Zeiler, H. J.; Metzger, K. 7-Amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids and Their Antibacterial Use. Ger. Offen. DE 3 318 145; *Chem. Abstr.* **1985**, *102*, 78744q.

(10) Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Trehan, A. K. 7-Substituted 5-Amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids: Synthesis and Biological Activity of New Class of Quinolone Antibacterials. *J. Med. Chem.* **1988**, *31*, 503–506.

(11) Barrett, J. F.; Sutcliffe, J. A.; Gootz, T. D. In Vitro Assays Used to Measure the Activity of Topoisomerases. *Antimicrob. Agents Chemother.* **1990**, *34*, 1–7.

(12) Domagala, J. M.; Heifetz, C. L.; Mich, T. F.; Nicols, J. B. 1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid. New Quinolone Antibacterial with Potent Gram-Positive Activity. *J. Med. Chem.* **1986**, *29*, 445–448.

(13) (a) Wentland, M. P.; Bailey, D. M.; Cornettt, J. B.; Dobson, R. A.; Powles, R. G.; Wagner, R. B. Novel Amino-Substituted 3-Quinolinecarboxylic Acid Antibacterial Agents: Synthesis an Structure-Activity Relationships. *J. Med. Chem.* **1984**, *27*, 1103–1108. (b) Culbertson, T. P.; Domagala, J. M.; Hagen, S. E.; Hutt, M. P.; Nichols, J. B.; Mich, T. F.; Sanchez, J. P.; Schroeder, M. C.; Solomon, M.; Worth, D. F. Structure-Activity Relationships of the Quinolone Antibacterials. The Nature of the C_7-Side Chain. In *International Telesymposium on Quinolones*; Fernandes, P. B., Ed.; J. R. Prous Science Publishers: Barcelona, 1989; pp 47–61.

(14) Bouzard, D.; Di Cesare, P.; Essiz, M.; Jacquet, J. P.; Remuzon, P.; Weber, A.; Oki, T.; Masuyoshi, M. Fluoronaphthyridines and Quinolones as Antibacterial Agents. 1. Synthesis and Structure-Activity Relationships of New 1-Substituted Derivatives. *J. Med. Chem.* **1989**, *32*, 537–542.

(15) (a) Ledoussal, B.; Bouzard, D.; Coroneos, E. Potent Non-6-Fluoro-Substituted Quinolone Antibacterials: Synthesis and Biological Activity. *J. Med. Chem.* **1992**, *35*, 198–200. (b) Clairefond, P.; Bouzard, D.; Ledoussal, B.; Coroneos, E.; Bazile, S.; Moreau, N. DNA-Gyrase Inhibition and Antibacterial Activity of Fluoro-Quinolones: Influence of the Position of the Fluorine(s). *Bioorg. Med. Chem. Lett.* **1992**, *2*, 643–646.

(16) Goldstein, H.; Schaaf, E. 5-Nitro and 3-Nitro-2,4-dichlorobenzoic Acids. *Helv. Chim. Acta* **1957**, *40*, 1187–1188.

(17) Lang, S. A., Jr.; Cohen, E. β-Aminocinnamonitriles as Potential Antiinflammatory Agents. *J. Med. Chem.* **1975**, *18*, 441–443.

JM940319S