Studies on 6-Aminoquinolones: Synthesis and Antibacterial Evaluation of 6-Amino-8-methylquinolones[1]

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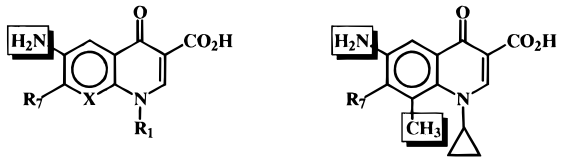
The 6-aminoquinolone had previously been identified as a new class of quinolone antibacterial agents. To continue our structure−activity relationship (SAR) study in this series, novel 6-amino-8-methylquinolone derivatives have now been synthesized and evaluated for in vitro antibacterial activity. We have shown that the coupled presence of a methyl group at the C-8 position with an amino group at C-6 is effective for enhancing antibacterial activity, particularly against Gram-positive bacteria. The SARs associated with the N-1, C-6, and C-7 are discussed. The 1,2,3,4-tetrahydroisoquinolinyl derivative **19v** showed the highest antibacterial activity with MIC values on Gram-positive bacteria superior to that of ciprofloxacin, especially against *Staphylococcus aureus* strains, including those strains which are methicillin- and ciprofloxacin-resistant.

Introduction

Our previous chemometric study on quinolone antibacterials[2] suggested that an amino group might have the appropriate steric and electronic requirements to replace the standard fluorine atom at C-6, one of the major factors indicated as responsible for the increased activity of current quinolones (fluoroquinolones). On the basis of this suggestion, a wide series of 6-aminoquinolones (**1**−**5**) was recently synthesized and evaluated for in vitro antibacterial activity (Figure 1).[3] The results of this study showed that, while the C-6 fluorine atom was still the best substituent, good activity could still be obtained by replacing it with an amino group and reoptimizing the other substituents. These findings prompted us to extend our investigation in order to outline a structure−activity relationship, increase potency, and broaden the spectrum for this new class. Indeed, although the new fluoroquinolones are generally characterized by a broad antimicrobial spectrum, their activities against Gram-positive organisms are limited, with diminished potency especially against staphylococci, streptococci, and enterococci.[4] In addition, the majority of methicillin-resistant staphylococci (especially *Staphylococcus aureus*) are now resistant to all of the currently available quinolones.[4] Therefore, recent efforts have been directed toward the synthesis of compounds which have greater activities against these organisms, such as tosufloxacin,[5,6] clinafloxacin,[6,7] DU 6859,[6,8] Bay 3118,[9] PD 138312,[10] and PD 140248.[10] On the basis of these considerations, we hoped that with the new series of 6-aminoquinolones, we could obtain compounds with strong activity against all Gram-positives and which would also be effective against resistant strains such as methicillin-resistant staphylococci.

Therefore, with these objectives in mind and considering the predictive indications of the previous chemometric study[2b] that indicated a large substituent at C-8, such as a methyl, as optimum to improve the activity against Gram-positive bacteria, we synthesized a large series of 6-amino-8-methyl-1-cyclopropylquinolones (**19a**−**v**) variously substituted in the C-7 position (Figure 1). This was also motivated by an observed favorable antimicrobial effect due to the presence of the C-8 methyl group as reported by Miyamoto et al.[11] In addition, we have included a few data (compounds **20a**−**22a**) employing 4-substituted phenyl groups as N-1 substituent instead of the usual cyclopropyl group. Finally, in order to clarify the importance of the C-6 amino group, we also synthesized and assayed some 1-cyclopropyl-8-methylquinolones bearing a nitro (**24a**,**i**,**j**,**v**), a *N*-methylamino (**26j**), and a *N*,*N*-dimethylamino (**27j**) group, as well as the usual fluorine atom (**30a**,**i**,**v**) at the C-6 position, with which a head to head comparison with 6-amino-8-methylquinolones was made.



1 X=CH R_1=*c*-Pr
2 X=CH R_1=4-FC_6H_4
3 X=CH R_1=*t*-Bu
4 X=CF R_1=*c*-Pr
5 X=N R_1=*c*-Pr
for R_7 see Chart 1

19a-v

R_7= see Chart 1

Figure 1.

Chemistry

Our synthetic approach was focused on the preparation of the key intermediates ethyl 7-fluoro-8-methyl-6-nitro-1-substituted-4-oxo-1,4-dihydroquinoline-3-carboxylates **10**−**12** in order to allow for easy diversification of the substituent at the C-7 position.

The first attempt was achieved starting from 2,6-difluoro-3-nitrotoluene[12] which was reacted with the appropriate amine (R_1NH_2) in order to obtain the nucleophilic displacement of C-6 fluorine atom. The successive reaction with (ethoxymethylene)malonate

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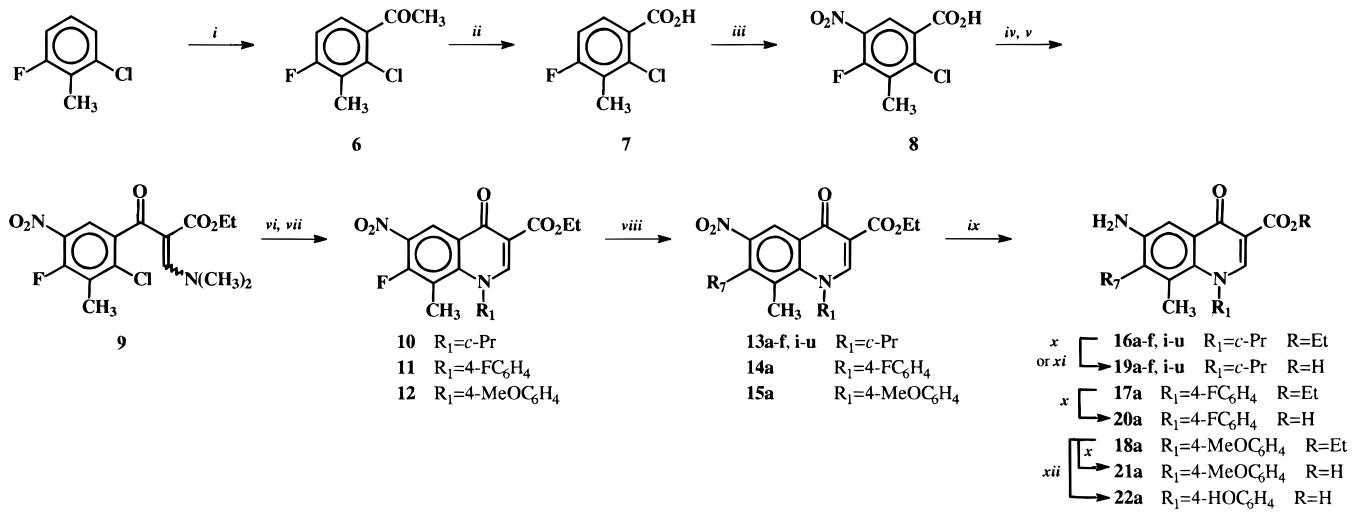
Scheme 1[a]



[a] Reagents: (i) CH_3COCl, $AlCl_3$; (ii) Br_2, NaOH, dioxane; (iii) 99.5% HNO_3, 98% H_2SO_4; (iv) $SOCl_2$; (v) $(CH_3)_2NCH{=}CHCO_2Et$, Et_3N, toluene, 90 °C; (vi) NH_2R_1, EtOH/Et_2O; (vii) K_2CO_3, DMF, 100 °C; (viii) R_7H, methods A−D (see the Experimental Section); (ix) H_2, Raney Ni, $CH_3O(CH_2)_2OH$; (x) 6 N HCl, EtOH, reflux; (xi) 1 N NaOH, reflux; (xii) 48% HBr, reflux.

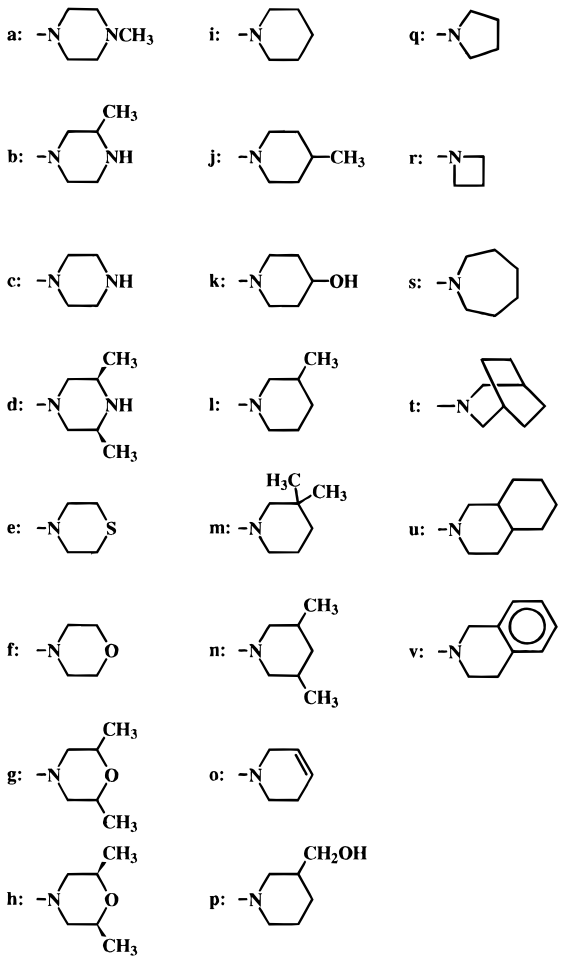
(EMME) and ring closure should have provided the desired key intermediates. Unfortunately, as previously observed,[3] the nucleophilic displacement occurs regiospecifically at the C-2 position without being the least bit influenced by steric hindrance from the methyl group at C-1.

Therefore, we planned an alternative pathway, shown in Scheme 1, involving the Friedel−Crafts acetylation of the 2-chloro-6-fluorotoluene. This reaction afforded the desired acetyl derivative **6**, as well as the other *meta*-isomer, in 95:5 ratio, respectively, on the basis of the ^1H NMR spectrum. The failure to completely remove this impurity forced us to use impure starting material to carry out the following steps: oxidation, nitration, and reaction with ethyl (dimethylamino)-acrylate, after which the pure acrylate **9** was obtained by column chromatography purification. The successive substitution with appropriate amine (R_1NH_2) and ring closure with potassium carbonate afforded the key intermediates **10**−**12**.

The desired acids **19**−**22** (see Table 1) were thus synthesized by replacement of the C-7 fluorine atom of **10**−**12** with the selected heterocyclic side chain (shown in Chart 1) followed by catalytic reduction and acid or base hydrolysis. When 2,6-dimethylmorpholines (**g**,**h**) or 1,2,3,4-tetrahydroisoquinoline (**v**) was used, it was more convenient to carry out the hydrolysis step before the nucleophilic reaction (Scheme 2). Thus, acid **23**, obtained from ester **10**, was reacted with the above heterocyclic bases to directly yield the desired 6-nitroquinolones **24g**,**h**,**v**, which were then reduced to the target 6-aminoquinolones **19g**,**h**,**v**, respectively. The other 6-nitroquinolones (**24a**,**i**,**j**) assayed in this study were, however, obtained from 6-nitro esters **13a**,**i**,**j** by direct basic hydrolysis (Scheme 2).

The ester **16j** was chosen as a convenient intermediate to obtain an example of *N*-substituted 6-amino derivatives. The ester **16j** was elaborated, as in Scheme 3, in two ways: In the first, the C-6 amino group was converted into its trifluoroacetamide derivative **25j** followed by methylation and hydrolysis to give the 6-(methylamino)quinolone **26j**; in the second, the ester

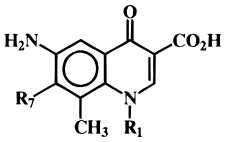
Chart 1. Heterocyclic Side Chains Employed as the R_7 Substituent



was one-step *N*,*N*-dimethylated with trimethyl phosphate and hydrolized to 6-(dimethylamino)quinolone **27j**.

For the preparation of the the known **30a**,[11] as well as the unknown 6-fluoro-8-methylquinolones **30i**,**v** (all used for a comparative purpose), we did not use the laborious synthetic procedure reported by Miyamoto et al.,[11] but we planned a shorter and more convenient

Table 1. Physical Properties for the 6-Amino-8-methyl-4-oxoquinolinecarboxylic Acids Tested in This Study

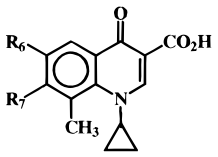


compd	R_1	R_7	coupling reaction method[a] (% yield)	hydrolysis method[a] (% yield)	mp, °C	formula[b]
19a	*c*-Pr		A (75)	F (45)	>300	$C_{19}H_{24}N_4O_3 \cdot 2\ HCl$
19b	*c*-Pr		C (50)	F (50)	291-293	$C_{19}H_{24}N_4O_3 \cdot HCl$
19c	*c*-Pr		B (47)	F (55)	260-261	$C_{18}H_{22}N_4O_3 \cdot HCl$
19d	*c*-Pr		B (90)	F (62)	>300	$C_{20}H_{26}N_4O_3 \cdot HCl$
19e	*c*-Pr		C (15)	F (19)	>300	$C_{18}H_{21}N_3O_4S \cdot HCl$
19f	*c*-Pr		D (33)	F (30)	258-260	$C_{18}H_{21}N_3O_4 \cdot HCl$
19g	*c*-Pr		E (36)	[c]	293-295	$C_{20}H_{25}N_3O_4$
19h	*c*-Pr		E (40)	[c]	269-272	$C_{20}H_{25}N_3O_4$
19i	*c*-Pr		A (68)	F (40)	168-160	$C_{19}H_{23}N_3O_3 \cdot HCl$
19j	*c*-Pr		A (40)	F (60)	244-247	$C_{20}H_{25}N_3O_3 \cdot HCl$
19k	*c*-Pr		B (57)	F (61)	266-268	$C_{19}H_{23}N_3O_4 \cdot HCl$
19l	*c*-Pr		A (45)	F (45)	210-214	$C_{20}H_{25}N_3O_3 \cdot HCl$
19m	*c*-Pr		B (39)	F (55)	220-225	$C_{21}H_{27}N_3O_3 \cdot HCl$
19n	*c*-Pr		B (45)	F (50)	248-257	$C_{21}H_{27}N_3O_3 \cdot HCl$
19o	*c*-Pr		B (42)	F (40)	>300	$C_{19}H_{21}N_3O_3 \cdot HCl$
19p	*c*-Pr		B (63)	F (30)	>300	$C_{20}H_{25}N_3O_4 \cdot HCl$
19q	*c*-Pr		D (48)	F (50)	205-208	$C_{18}H_{21}N_3O_3 \cdot HCl$
19r	*c*-Pr		A (76)	G (50)	230-231	$C_{17}H_{19}N_3O_3 \cdot H_2O$
19s	*c*-Pr		A (45)	F (50)	165-167	$C_{20}H_{25}N_3O_3 \cdot HCl$
19t	*c*-Pr		A (60)	F (48)	273-275	$C_{22}H_{27}N_3O_3 \cdot HCl$
19u	*c*-Pr		C (50)	F (63)	192-195	$C_{23}H_{29}N_3O_3 \cdot HCl$
19v	*c*-Pr		E (38)	[c]	268-270	$C_{23}H_{27}N_3O_3$
20a	4-FC_6H_4		A (88)	F (73)	252-254	$C_{22}H_{23}FN_4O_3 \cdot 2\ HCl$
21a	4-$MeOC_6H_4$		A (90)	F (75)	283-285	$C_{23}H_{26}N_4O_4 \cdot 2HCl$
22a	4-OHC_6H_4		[a]	[a]	>300	$C_{22}H_{24}N_4O_4$

[a] See the Experimental Section. [b] All compounds had elemental analyses within ±0.4% of theoretical values. [c] Yield is that obtained from Raney Ni reduction step as a final step.

alternative pathway, exploiting our key intermediate **10**. Thus, as depicted in Scheme 4, reduction of **10** gave the 6-amino derivative **28** which, by successive Balz–Schiemann reaction, afforded, in only one step, the 6-fluoro derivative **29** as borate complex, indispensable for the success of the following nucleophilic displacement reaction. Finally, the desired 6-fluoro-8-methyl-7-substituted acids **30** were directly obtained by coupling reaction of borate complex **29** with the selected heterocyclic bases.

Table 2. Physical Properties for the 6-Substituted-1-cyclopropyl-8-methyl-4-oxoquinolinecarboxylic Acids Tested in This Study



compd	R_6	R_7	% yield[a]	mp, °C	formula[b]
24a	NO_2		38	>300	$C_{19}H_{22}N_4O_5 \cdot 0.5\ H_2O$
24i	NO_2		53	229-231	$C_{19}H_{21}N_3O_5 \cdot H_2O$
24j	NO_2		51	208-210	$C_{20}H_{23}N_3O_5$
24v	NO_2		38	>300	$C_{23}H_{21}N_3O_5$
26j	$NHCH_3$		58	>300	$C_{21}H_{27}N_3O_3$
27j	$N(CH_3)_2$		70	>300	$C_{22}H_{29}N_3O_3$
30a	F		30	255-259	$C_{19}H_{22}FN_3O_3$
30i	F		35	162-166	$C_{19}H_{21}FN_2O_3$
30v	F		27	229-232	$C_{23}H_{21}FN_2O_3$

[a] Yield is referred to a final step. [b] All compounds had elemental analyses within ±0.4% of theoretical values.

Biological Assays

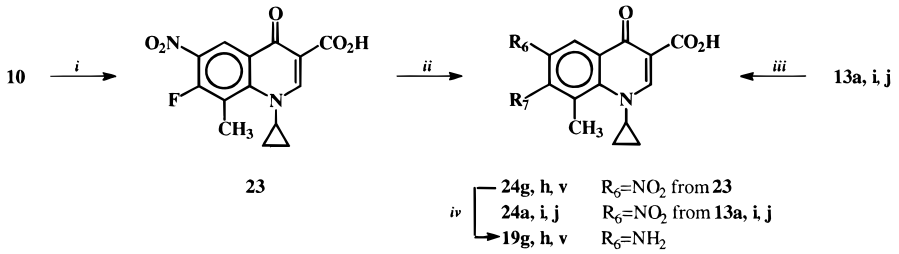
All 6-amino-8-methylquinolone acids (**19a**–**v**, **20a**, **21a**, and **22a**) as well as 6-substituted-8-methylquinolone acids (**24a**,**i**,**j**,**v**, **26j**, **27j**, and **30a**,**i**,**v**) prepared for this study were tested in vitro against an assortment of eight Gram-negative and five Gram-positive organisms by conventional agar dilution procedure.[13] The minimum inhibitory concentrations (MICs, μg/mL) are presented in Table 3. The geometric means of the MICs for both Gram-positive and Gram-negative strains were also calculated to facilitate a comparison of activity. In addition, control drug ciprofloxacin and 6-aminoquinolones **1a**–**e**,**k**,**q**,**v**, **2a**, **4a**, and **5a**, previously reported by us,[3] are included for comparative purposes.

A representative number of compounds was also tested for their ability to inhibit the supercoiling activity of DNA gyrase by using a previously described protocol.[14] The IC_{50} values are reported in Table 4, together with the clog *P* values.[15]

In addition, the in vitro antibacterial activity against an assortment of Gram-positive clinical isolates, including methicillin- and ciprofloxacin-resistant strains, was evaluated for those 6-amino-8-methylquinolones which showed highest Gram-positive antibacterial activity, coupled with good Gram-negative antibacterial activity. The MICs are presented in Table 5.

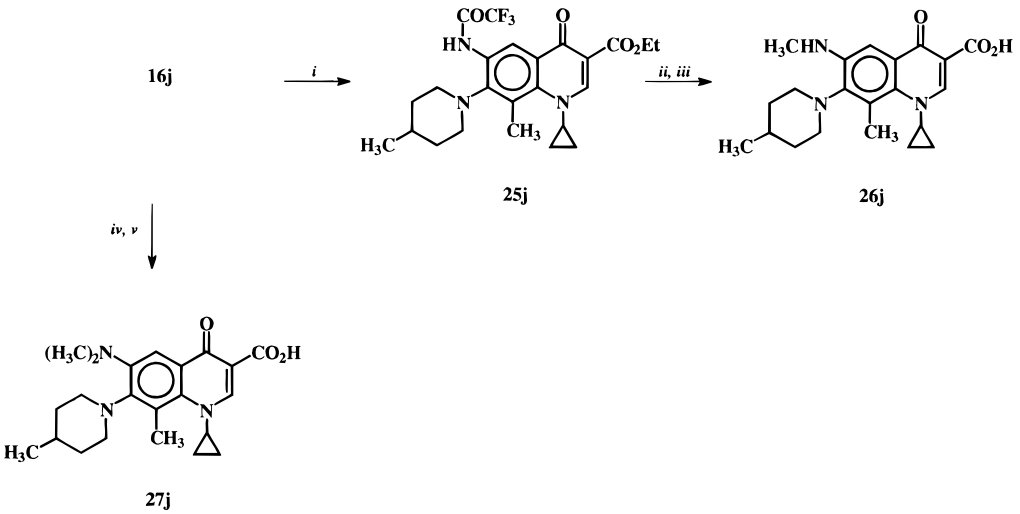
Finally, the in vivo potency of selected **19v**, expressed as the median effective dose (ED_{50}, mg/kg), was determined in the experimental model of septicemia caused

Scheme 2[a]



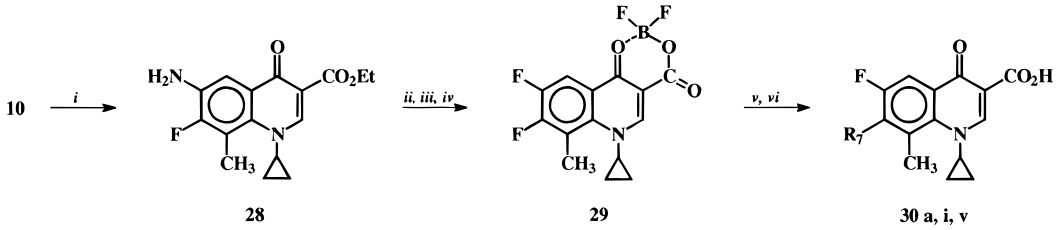
[a] Reagents: (i) 6 N HCl, EtOH, reflux; (ii) R_7H, Et_3N, CH_3CN, reflux; (iii) 1 N NaOH, reflux; (iv) H_2, Raney Ni, $CH_3O(CH_2)_2OH$.

Scheme 3[a]



[a] Reagents: (i) $(CF_3CO)_2O$; (ii) CH_3I, K_2CO_3, acetone, reflux; (iii) 20% NaOH, EtOH, reflux; (iv) $(CH_3O)_3PO$, 150 °C; (v) NaOH, reflux.

Scheme 4[a]



[a] Reagents: (i) H_2, Raney Ni, $CH_3O(CH_2)_2OH$; (ii) $NaNO_2$, 6 N HCl, −5 °C; (iii) 50% HBF_4; (iv) 180−200 °C; (v) R_7H, Et_3N, CH_3CN, reflux; (vi) 6 N HCl.

by *S. aureus* in mice after subcutaneous and oral administration. Results are given in Table 6.

Results and Discussion

In a direct comparison (Table 3) between the 4-methylpiperazinyl derivative of 6-amino-8-methyl series **19a** and the previously reported counterparts having a different C-8 substituent such as **1a** (X = CH), **4a** (X = CF), and **5a** (X = N) (see Figure 1), the 8-methyl derivative **19a** displays an increased potency against both Gram-positives (4−8 times) and Gram-negatives (2.5−3.5 times). In addition, matching the 8-methyl-6-aminoquinolones **19a**−**e**,**k**,**v**, having various C-7 substituents, with the 8-unsubstituted counterparts **1a**−**e**,**k**,**v**, it can be seen that the presence of the methyl group at C-8 increased the potency against Gram-positives, which rises to 37 times for the 4-hydroxypiperidinyl derivative **19k** (vs **1k**) and to over 1000 times for the 1,2,3,4-tetrahydroisoquinolinyl derivative **19v** (vs **1v**).

Among the 8-methyl series, a significant loss of activity was observed when 4-substituted phenyl groups were present at the N-1 position instead of the cyclopropyl group (compare **20a**, **21a**, and **22a** vs **19a**). The drop in activity was less noticeable for the 4-hydroxyphenyl group than for the 4-fluoro- and 4-methoxyphenyl groups.

It should be noted that, relative to the 8-desmethyl compounds **2a** and **1q**, derivatives **20a** and **19q** show greatly diminished Gram-negative activity.

Substituent effects at the C-7 position were extensively studied while maintaining the cyclopropyl group at the N-1 position as well as the characteristic C-8 methyl group. The series of piperazinyl derivatives **19a**−**d** was more active against Gram-negatives than Gram-positives with the 4-methylpiperazinyl derivative **19a** having the best balanced activity. The replacement of the 4-piperazinyl nitrogen atom by a sulfur atom or oxygen atom enhanced the activity against Gram-positive bacteria without reducing their activity against Gram-negatives (compare **19e**,**f** vs **19c**). This enhance-

Table 3. In Vitro Antibacterial Activity (MICs, μg/mL)[a]

	Gram-negative organisms								Gram-positive organisms						
	E. co.		*E. cl.*	*P. vu.*	*P. st.*	*K. pn.*		*P. ae.*	*S. au.*		*S. ep.*			geometric means	
compd	ATCC 8739	ISF 432	OMNFI 174	CNUR 5	CNUR 5	ATCC 10031	*S. en.*	ATCC 9027	MPR 5	ATCC 6538	HCF Berset C	CPHL A2	*S. fe.* LEP Br	Gram-(−)	Gram-(+)
19a	0.03	0.03	0.125	4	2	0.03	0.06	2	0.5	0.5	1	1	4	0.205	1.000
19b	0.125	0.03	0.125	4	2	0.03	0.06	1	1	1	1	1	4	0.225	1.319
19c	0.03	0.03	0.06	4	2	0.03	0.03	1	4	2	8	4	16	0.158	5.278
19d	0.06	0.03	0.5	8	2	0.03	0.25	2	1	1	2	1	16	0.348	2.000
19e	0.25	0.03	0.25	32	2	0.02	0.125	1	0.06	0.06	0.125	0.125	0.5	0.362	0.123
19f	0.03	0.03	0.125	8	0.5	0.03	0.125	1	0.125	0.125	0.125	0.25	1	0.189	0.218
19g	0.06	0.03	1	16	0.5	0.03	1	4	0.06	0.06	0.125	0.06	1	0.451	0.122
19h	0.008	0.008	1	8	1	0.08	1	4	0.03	0.03	0.125	0.125	1	0.336	0.107
19i	0.03	0.03	0.25	8	0.5	0.03	0.25	1	0.06	0.06	0.125	0.125	0.5	0.225	0.123
19j	0.03	0.03	0.5	8	0.5	0.03	0.5	1	0.06	0.06	0.125	0.125	0.5	0.268	0.122
19k	1	0.03	0.5	16	1	0.03	0.25	2	0.125	0.06	0.06	0.06	0.5	0.495	0.106
19l	0.03	0.03	1	32	0.25	0.03	1	4	0.06	0.03	0.25	0.125	2	0.414	0.162
19m	0.5	0.5	16	>128	8	0.5	16	64	0.5	0.5	1	1	16	6.168	1.132
19n	0.125	0.03	2	16	0.5	0.06	2	4	0.03	0.03	0.06	0.06	1	0.641	0.079
19o	1	1	>128	>128	>128	1	>128	>128	128	128	>128	>128	>128	20.749	128.000
19p	0.016	0.016	2	64	4	0.016	1	2	0.125	0.125	0.25	0.25	2	0.504	0.287
19q	32	1	64	>128	64	0.5	64	>128	4	4	16	16	64	22.627	12.120
19r	16	0.25	16	>128	64	0.25	16	>128	8	8	16	16	>128	11.313	18.379
19s	2	0.03	1	32	0.5	0.03	1	4	0.125	0.125	0.2	0.25	2	0.763	0.275
19t	4	0.3	4	64	1	0.03	3	16	0.03	0.03	1	0.25	4	2.141	0.246
19u	4	4	4	64	1	0.125	2	64	0.06	0.06	0.125	0.25	2	4.000	0.186
19v	0.008	0.008	0.25	2	0.06	0.06	0.25	0.5	0.008	0.008	0.03	0.016	0.25	0.105	0.024
20a	1	1	16	>128	16	1	16	128	128	64	128	128	128	9.513	111.430
21a	>128	16	>128	128	128	16	>128	>128	64	64	64	128	128	76.109	84.448
22a	0.125	0.125	8	>128	8	0. 25	4	8	16	16	16	8	64	2.378	18.370
24a	16	4	8	64	32	16	1	64	4	4	4	2	16	13.454	4.595
24i	32	4	64	64	64	64	64	64	1	32	8	8	32	41.498	9.189
24j	32	2	64	64	64	64	64	64	2	32	4	4	32	38.054	8.000
24v	64	4	64	64	64	64	64	64	0.25	64	2	4	4	45.254	3.482
26j	1	0.5	64	>128	16	0.5	64	>128	1	1	4	2	32	11.313	3.031
27j	64	64	64	64	64	64	64	64	16	64	8	32	32	64.000	24.251
30a	0.03	0.03	0.03	2	2	0.03	0.03	2	0.03	0.06	0.25	0.25	1	0.145	0.162
30i	0.125	0.03	16	2	2	0.125	0.5	1	0.03	0.03	0.03	0.03	0.25	0.591	0.046
30v	0.06	0.03	4	4	2	0.03	0.25	4	0.03	0.03	0.03	0.03	0.25	0.451	0.046
1a	0.25	0.03	0.25	64	8	0.03	0.25	4	2	2	4	2	32	0.642	4.000
1b	0.03	0.03	0.25	32	16	0.03	0.25	2	16	16	4	4	32	0.451	10.556
1c	0.25	0.03	0.25	32	32	0.25	0.25	2	64	64	16	16	128	0.836	42.224
1d	0.03	0.03	0.5	64	4	0.03	0.25	2	8	8	4	2	32	0.451	6.964
1e	0.03	0.03	0.5	128	1	0.03	0.5	2	0.5	0.5	2	1	8	0.451	1.320
1k	0.03	0.03	2	128	8	0.03	2	8	4	4	4	2	8	0.984	4.000
1q	0.25	0.25	8	128	128	0.25	16	128	128	128	128	128	128	6.727	128.000
1v	128	32	128	128	128	128	128	128	4	128	16	16	128	107.635	27.857
2a	0.125	0.125	4	128	4	0.125	4	8	4	8	8	8	64	1.834	10.556
4a	1	0.06	0.25	32	4	0.06	0.25	2	4	4	8	4	64	0.699	8.000
5a	0.125	0.06	0.125	64	2	0.03	0.125	2	4	4	4	4	32	0.416	6.063
CPX[b]	0.015	0.015	0.015	0.5	0.06	0.015	0.015	0.06	0.06	0.06	0.06	0.06	0.5	0.033	0.092

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

Table 4. Inhibitory Effect (IC_{50}, μg/mL)[a] on Gyrase Supercoiling Activity from *E. coli*, in Vitro Antibacterial Activity (Geometric Mean MICs, μg/mL), and clog *P* by Selected 6-Amino-8-methylquinolones

		geometric mean MICs		
compd	IC_{50}	Gram-negatives	Gram-positives	clog *P*
19a	3.78	0.205	1	0.695
19e	1.42	0.362	0.123	1.672
19f	5.64	0.189	0.218	0.951
19h	≥50	0.336	0.107	1.989
19i	3.22	0.225	0.123	2.595
19j	5.86	0.268	0.123	3.114
19k	5.74	0.495	0.106	0.508
19v	3.58	0.105	0.024	3.305
30v	3.14	0.450	0.046	4.549
CPX[b]	0.68	0.033	0.092	−0.805

[a] Calculated by the quantitative measurement of the supercoiled DNA peak in an agarose gel by densitometric assay. [b] CPX = ciprofloxacin.

Table 5. In Vitro Antibacterial Activity (MIC, μg/mL)[a] of Selected 6-Amino-8-methylquinolones on Clinical Gram-Positive Isolates

compd	*S. aureus*	*S. aureus*[b]	*S. pneumoniae*	*E. faecalis*	*E. faecium*
19e	0.06−0.13	2−8	1−2	0.5	2
19f	0.06−0.13	4−8	1−2	0.25−0.5	0.5−2
19h	0.06−0.13	2−4	2	0.05	1−32
19i	0.016−0.13	2−4	1−2	0.13−0.5	1−2
19j	0.016−0.13	1−2	1−2	0.13−0.25	0.13−2
19k	0.06−0.13	4−32	0.5−2	0.25−0.5	0.25−4
19v	0.004−0.03	0.05−1	0.13	0.06	0.06−1
CPX[c]	0.13−2	4−128	0.5−2	0.5−1	1−4

[a] The range of MIC values for five isolates for each species. [b] Methicillin- and ciprofloxacin-resistant isolates (seven strains). [c] CPX = ciprofloxacin.

ment in Gram-positive antibacterial activity is in agreement with what was already observed for both the thiomorpholinyl group, as in the case of our previous 8-unsubstituted-6-aminoquinolones **1e**,[3] and the morpholinyl group, as in the case of its 6-fluoro-8-methyl counterpart[11] and Kanebo products.[16] The 2,6-dialkylation of the morpholinyl moiety, as in **19g** and *cis*-derivative **19h**, has a somewhat favorable effect on the activity against Gram-positives (which reaches those of

Table 6. Efficacy of **19v** in the Experimental Model of Septicemia Caused by *S. aureus*[a] in Mice

compd	MIC, μg/mL	ED_{50}, mg/kg (95% confidence limits) po	sc
19v	0.016	5 (3.8−6.6)	1.3 (1.1−1.5)
CPX[b]	0.13	7 (5.1−9.7)	0.9 (0.8−1.1)

[a] See the Experimental Section. [b] CPX = ciprofloxacin.

ciprofloxacin) but an unfavorable effect against Gram-negatives. The same good antibacterial activity was also displayed by piperidinyl derivatives **19i**−**l**,**n**,**p** with the 3,5-dimethyl derivative **19n** showing a geometric mean MIC of 0.079 μg/mL against Gram-positives on the same order as those of ciprofloxacin (0.092 μg/mL). On the contrary, the presence of *gem*-dimethyl group in the C-3 position of piperidine moiety had a deleterious effect on both Gram-positives and Gram-negatives (compare **19m** vs its monomethyl analogue **19l**), as well as the 3,4-dehydrogenation which gave completely inactive **19o** (compare vs **19i**). Considering the piperidine moiety, it was observed that the ring contraction to pyrrolidinyl (**q**) or azetidinyl (**r**) groups markedly decreased all antibacterial activity (compare **19q**,**r** vs **19i**) with the pyrrolidinyl group again showing itself to be an unsuitable C-7 substituent for the 6-aminoquinolones.[3] On the contrary, a ring expansion to homopiperidine (**s**) permitted the activity to be maintained (compare **19s** vs **19i**). Among the C-7 substituents, the best overall antibacterial activity was displayed by bicyclic 1,2,3,4-tetrahydroisoquinolinyl group. Indeed, compound **19v** was more potent against Gram-positives than ciprofloxacin, with MIC values against *S. aureus* of 0.008 and 0.06 μg/mL, respectively. While, its activity against Gram-negatives was the best of all 6-aminoquinolones, it was not as good as that of ciprofloxacin. The saturation of 1,2,3,4-tetrahydroisoquinolinyl nucleus to (±)-*trans*-perhydroisoquinoline, as in **19u**, caused a marked decline in potency, particularly against Gram-negatives.

We next turned our attention to the C-6 amino group, and in an attempt to explore the importance of amino group in the C-6 position, we prepared and assayed the 6-methylamino and 6-dimethylamino acids **26j** and **27j**, some 6-nitro acids (**24a**,**i**,**j**,**v**) which were intermediates in the preparation of the 6-amino series, and 6-fluoro analogues **30a**,**i**,**v** (Table 3). When compared with **19j**, the *N*-monomethylation of the C-6 amino group, as in **26j**, lowered the antibacterial activity which disappeared completely against Gram-negatives with the *N*,*N*-dimethylation, as in **27j**. The reduced hydrophilicity caused by introducing a methyl group may account for this diminished potency. The replacement of the electron-donating C-6 amino group with the electron-withdrawing nitro group resulted in a considerable loss of activity against Gram-positives and the complete disappearance of activity against Gram-negatives (compare **24a**,**i**,**j**,**v** vs **19a**,**i**,**j**,**v**).

When the 6-amino-8-methyl derivatives **19a**,**i**,**v** were matched with the 6-fluoro-8-methyl counterparts **30a**,**i**,**v**, contrasting indications were obtained. It can be seen that the 6-amino-8-methyl-7-tetrahydroisoquinoline derivative **19v** was more active than its fluorurated analogue **30v** against both Gram-negative and Gram-positive bacteria. This result was however reversed in the case of the piperazinyl and piperidinyl derivatives.

The DNA supercoiling assay (Table 4) shows that, in general, the 6-amino-8-methylquinolones tested are 2−9 times less potent than ciprofloxacin as DNA gyrase inhibitors, with the exception of **19h** which is quite inactive. Such a decrease in activity does not seem to depend on the amino group at the C-6 position since the 6-amino derivative **19v** and its 6-fluoro counterpart **30v** have the same inhibitory potency.

Matching the Gram-positive MIC and DNA gyrase values, we might think that 6-amino-8-methylquinolones have a better cell permeability against Gram-positives than ciprofloxacin. Although it is now generally recognized that the more hydrophobic a compound, the better its diffusion into Gram-positives,[17] no close correlation was found between MICs and clog *P* values (Table 4); in fact, the last lipophilic agent in our study, ciprofloxacin (clog $P = -0.805$), is the third best agent against Gram-positives.

The interaction with topoisomerase II is central to the activity of both quinolone and antitumor agents, so it seemed of interest, from a safety standpoint,[18] to also determine the effects of 6-amino-8-methylquinolones, tested on DNA-gyrase, on mammalian topoisomerase II. All tested compounds resulted inactive in the decatenation assay.

Since our main objective was to identify quinolones with strong activity against Gram-positives, we broadened our study on clinical isolates such as *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *S. aureus*, and methicillin- and ciprofloxacin-resistant *S. aureus* that cause considerable clinical problems. For this purpose, we selected the 6-amino-8-methylquinolones, having excellent Gram-positive antibacterial activity coupled with good Gram-negative activity. The results reported in Table 5 show that all of the tested compounds have extremely high activity against staphylococci. The activity of **19v** was also excellent against methicillin- and ciprofloxacin-resistant *S. aureus*. It must be pointed out that its MIC values on *S. pneumoniae* isolates were 4−15 times lower than those of ciprofloxacin and are probably better than any other available agent.

In a preliminary in vivo study, a model of *S. aureus* septicemia in mouse was used to evaluate the activity of compound **19v** (Table 6). Following oral administration, **19v** protected mice to the same extent as ciprofloxacin. Considering that **19v** had MIC 10 times lower than ciprofloxacin on *S. aureus* in vitro, thought to have been due to poor bioavailability, a subcutaneous study was undertaken. Once again both compounds had a similar effect. Therefore, further studies are needed in order to clarify the in vivo efficacy of compound **19v**.

In conclusion, in this study the amino group was confirmed to be a good replacement for the C-6 fluorine atom. In particular, as predicted by our previous chemometric study,[2b] the introduction of a methyl group at the C-8 position in the 6-aminoquinolones increased the antibacterial activity, especially against Gram-positives, and allowed some very potent antibacterial agents to be identified. Among them, 6-amino-1-cyclopropyl-8-methyl-7-(1,2,3,4-tetrahydroisoquinolinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**19v**) (MF 5137) is characterized by an extremely high activity against staphylococci as well as those strains which are methicillin- and ciprofloxacin-resistant. In addition, its

activity against all streptococci is very high with MIC values against isolated *S. pneumoniae* as much as 15 times lower than those of ciprofloxacin which makes it probably better than any other agent around. An expanded description of the microbiological, toxicological, and pharmacokinetic profiles of **19v** will be reported elsewhere.

Experimental Section

Melting points were determined in capillary tubes (Büchi 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 spectrometer) or 200 MHz (Bruker AC-200 spectrometer) with Me_4Si 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 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 were 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 Tables 1 and 2.

1-(2-Chloro-4-fluoro-3-methylphenyl)ethanone (6). Some drops of acetyl chloride were added to a mixture of 2-chloro-4-fluorotoluene (40 g, 0.28 mol) and $AlCl_3$ (73.86 g, 0.55 mol) which was then heated to 40 °C to trigger the reaction (generate HCl). After cooling to room temperature, acetyl chloride (22 g, 0.28 mol) was added dropwise and the reaction mixture was allowed to react for 2 h. The mixture was then poured into ice−water and acidified with 2 N HCl. The solution was extracted several time with CH_2Cl_2, and the combined organic layers were washed with water, dried, and evaporated to dryness to give a yellow oil (48.2 g, 93%) containing **6**, as well as the other *meta*-isomer, 1-(4-chloro-2-fluoro-3-methylphenyl)ethanone, in 95:5 ratio, respectively, on the basis of ^{1}H NMR spectrum. The material was used as is in the next step: ^{1}H NMR ($CDCl_3$) δ 2.35 (3 H, d, J = 2.3 Hz, CH_3), 2.60 (3 H, s, $COCH_3$), 7.00 (0.95 H, t, J = 8.5 Hz, H-5 of the major isomer), 7.20 (0.05 H, d, J = 8.5 Hz, H-5 of the minor isomer), 7.35 (0.95 H, dd, J = 6, 8.5 Hz, H-6 of the major isomer), 7.65 (0.05 H, t, J = 8.1, H-6 of the minor isomer).

2-Chloro-4-fluoro-3-methylbenzoic Acid (7). Bromine (102.6 g, 0.64 mol) was added dropwise to a solution of NaOH (85.6 g, 2.14 mol) in water (400 mL) mantained at 10 °C. The mixture was then cooled to 0 °C, and a solution of **6** (40 g, 0.21 mol) in dioxane (400 mL) was added dropwise. After stirring for 1 h at room temperature, the reaction mixture was poured into water and extracted with $CHCl_3$. The aqueous solution was acidified with 37% HCl, and the white precipitated solid was filtered off, washed with water and then with Et_2O, and dried to give **7** (39.5 g, 98%) (presumably slightly impure, for the other isomer not detectable by ^{1}H NMR spectrum) which was used as is in the subsequent reaction: ^{1}H NMR ($CDCl_3$) δ 2.40 (3 H, d, J = 2.3 Hz, CH_3), 7.05 (1 H, t, J = 8.5 Hz, H-5), 7.90 (1 H, dd, J = 6, 8.5 Hz, H-6).

2-Chloro-4-fluoro-3-methyl-5-nitrobenzoic Acid (8). A mixture of 99.5% HNO_3 (4.7 mL), 98% H_2SO_4 (100 mL), and acid **7** (20 g, 10.61 mol) was allowed to react at room temperature for 1 h. The mixture was poured into ice−water, and the resulting precipitate was filtered, dried, and recrystallized from benzene to give a solid (22.8 g, 92%) containing **8**, as well as the isomer 4-chloro-2-fluoro-3-methyl-5-nitrobenzoic acid, in 95:5 ratio, respectively, based on ^{1}H NMR spectrum: ^{1}H NMR ($CDCl_3$) δ 2.45 (3 H, d, J = 2.3 Hz, CH_3), 8.50 (0.05 H, d, J = 7.5 Hz, H-6 of the minor isomer), 8.60 (0.95 H, d, J = 7.5 Hz, H-6 of the major isomer), 9.25 (1 H, bs, CO_2H).

Ethyl 2-(2-Chloro-4-fluoro-3-methyl-5-nitrobenzoyl)-3-(dimethylamino)acrylate (9). A mixture of **8** (5 g, 21.4 mmol) and thionyl chloride (15 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 toulene (10 mL) and added to ethyl 3-(dimethylamino)acrylate[19] (3.06 g, 21.4 mmol) and dry Et_3N (3.24 g, 32 mmol). The resulting solution was heated at 90 °C for 2 h. After cooling and filtering the insoluble material, the solvent was evaporated to dryness and the residue was purified by flash column chromatography eluting with the gradient cyclohexane/EtOAc (90:10−30:70) to give **9** (4.60 g, 60%) as yellowish solid: mp 110−112 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t, J = 7 Hz, CH_2CH_3), 2.40 (3 H, d, J = 3 Hz, CH_3), 3.05 and 3.40 (each 3 H, bs, NCH_3), 3.55 (2 H, q, J = 7 Hz, CH_2-CH_3), 7.90 (1 H, d, J = 7.5 Hz, H-6), 8.00 (1 H, s, vinyl H). Anal. ($C_{15}H_{16}ClFN_2O_5$) C, H, N.

Ethyl 1-Cyclopropyl-7-fluoro-8-methyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (10). A stirred solution of **9** (1.5 g, 4 mmol) in EtOH (48 mL) and Et_2O (18 mL) was treated dropwise with cyclopropylamine (0.37 g, 6.5 mmol). After 15 min at room temperature, the mixture was evaporated to dryness to give a residue which was solubilized in dry DMF (5 mL). To this solution was added K_2CO_3 (1 g, 7.2 mmol), and the mixture was heated at 100 °C for 40 min. After cooling, the reaction mixture was poured into ice−water. The precipitated solid was filtered off, washed with water, and dried to give **10** (1.3 g, 97%): mp 198−200 °C; ^{1}H NMR ($CDCl_3$) δ 1.00−1.10 and 1.25−1.35 (each 2 H, m, cyclopropyl CH_2), 1.45 (3 H, t, J = 7.5 Hz, CH_2CH_3), 2.80 (3 H, d, J = 3 Hz, CH_3), 3.90−4.10 (1 H, m, cyclopropyl CH), 4.40 (2 H, q, J = 7.5 Hz, CH_2CH_3), 8.65 (1 H, s, H-2), 8.95 (1 H, d, J = 7.5 Hz, H-5). Anal. ($C_{16}H_{15}FN_2O_5$) C, H, N.

According to this procedure, compounds **11** and **12** were prepared from **9**, replacing cyclopropylamine with 4-fluoroaniline and 4-methoxyaniline, respectively. **11**: mp 195−197 °C (95%). **12**: mp 205−207 °C (90%).

General Procedures for Coupling Reaction. Method A: Preparation of Ethyl 1-Cyclopropyl-8-methyl-6-nitro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (13a). The mixture of ester **10** (0.70 g, 2 mmol) and *N*-methylpiperazine (0.80 g, 8 mmol) in dry pyridine (10 mL) was heated at 100 °C for 4 h. After removing the solvent, the residue was triturated with EtOH and filtered, washing with EtOH, to give **13a** (0.62 g, 75%) which was reduced without further purification: mp 210−213 °C; ^{1}H NMR ($CDCl_3$) δ 0.75−1.50 (7 H, m, CH_2CH_3, cyclopropyl CH_2), 2.40 (3 H, s, NCH_3), 2.40−2.60 (5 H, m, piperazine CH_2, cyclopropyl CH), 2.70 (3 H, s, CH_3), 2.95−3.25 (4 H, m, piperazine CH_2), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 8.50 (1 H, s, H-2), 8.60 (1 H, s, H-5).

In an analogous procedure, compounds **13i**,**j**,**l**,**r**−**t** were prepared from **10**, while compounds **14a** and **15a** were prepared from **11** and **12**, respectively, by reaction with the appropiate heterocylic amine.

Method B: **Preparation of Ethyl 1-Cyclopropyl-8-methyl-6-nitro-7-(3,3-dimethyl-1-piperidinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (13m).** A mixture of ester **10** (0.8 g, 2.4 mmol), dry Et_3N (0.96 g, 9.5 mmol), and 3,3-dimethylpiperidine (1.08 g, 9.5 mmol) in dry CH_3CN (15 mL) was refluxed for 24 h. The solvent was then evaporated to dryness, and the residue, treated with EtOH, gave a solid which was purified by flash chromatography eluting with $CHCl_3$/MeOH (98:2) to give **13m** (0.40 g, 39%): mp 192−193 °C; ^{1}H NMR ($CDCl_3$) δ 0.85−0.95 (2 H, m, cyclopropyl CH_2), 1.00 (6 H, s, piperidine CH_3), 1.15−1.30 (2 H, m, cyclopropyl CH_2), 1.45 (3 H, t, J = 7 Hz, CH_2CH_3), 1.55−1.90 (4 H, m, piperidine CH_2), 2.55−2.70 (2 H, m, piperidine CH_2), 2.75 (3 H, s, CH_3), 3.10−3.25 (2 H, m, piperidine CH_2), 3.90−4.05 (1 H, m, cyclopropyl CH), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 8.60 (1 H, s, H-2), 8.70 (1 H, s, H-5). Anal. ($C_{23}H_{29}N_3O_5$) C, H, N.

By a similar procedure, compounds **13c**,**d**,**k**,**n**−**p** were obtained from **10** by reaction with the appropiate heterocylic amine. For piperazine derivative **13c**, formylpiperazine was employed as nucleophile and the protective formyl group was removed in the successive hydrolysis step.

Method C: Preparation of Ethyl 1-Cyclopropyl-8-methyl-6-nitro-7-(3-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (13b). The mixture of ester **10**

(0.90 g, 2.7 mmol), 2-methylpiperazine (0.81 g, 8.1 mmol), and K_2CO_3 (1.12 g, 8.1 mmol) in dry DMF (6 mL) was heated at 90 °C for 2 h. After cooling, the reaction mixture was poured into water and extracted with CH_2Cl_2. The combined organic layers were washed with water, dried, and evaporated to dryness. The solid residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (97:3) to give **13b** (0.56 g, 50%): mp 126−128 °C; ^{1}H NMR ($CDCl_3$) δ 0.75−0.85 (2 H, m, cyclopropyl CH_2), 1.00−1.30 (5 H, m, cyclopropyl CH_2, piperazine CH_3), 1.40 (3 H, t, J = 7 Hz, CH_2CH_3), 2.70 (3 H, s, CH_3), 2.80−3.20 (7 H, m, piperazine CH_2, CH, NH), 3.85−4.10 (1 H, m, cyclopropyl CH), 4.35 (2 H, q, J = 7 Hz, CH_2-CH_3), 8.45 (1 H, s, H-2), 8.60 (1 H, s, H-5). Anal. ($C_{21}H_{26}N_4O_5$) C, H, N.

By a similar procedure, compounds **13e**,**u** were obtained from **10** by reaction with the appropiate heterocylic amine.

Method D: **Preparation of Ethyl 1-Cyclopropyl-8-methyl-6-nitro-7-(4-morpholinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (13f).** The mixture of ester **10** (0.9 g, 2.7 mmol) and morpholine (0.47 g, 5.4 mmol) in dry DMF (7 mL) was heated at 75 °C for 10 h. After cooling, the precipitate was separated by filtration, washed with water and then with EtOH, and dried to give **13f** (0.35 g, 32%): mp >330 °C; ^{1}H NMR ($CDCl_3$) δ 0.75−0.95 and 1.05−1.25 (each 2 H, m, cyclopropyl CH_2), 1.40 (3 H, t, J = 7 Hz, CH_2CH_3), 2.70 (3 H, s, CH_3), 3.00−3.25 (4 H, m, morpholine CH_2), 3.80−4.10 (5 H, m, morpholine CH_2, cyclopropyl CH), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 8.55 (1 H, s, H-2), 8.65 (1 H, s, H-5). Anal. ($C_{20}H_{23}N_3O_6$) C, H, N.

By a similar procedure, compound **13q** was prepared from **10** by reaction with pyrrolidine.

Method E: Preparation of 1-Cyclopropyl-8-methyl-6-nitro-7-(1,2,3,4-tetrahydro-2-isoquinolinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (24v). The mixture of acid **23** (4 g, 13 mmol), 1,2,3,4-tetrahydroisoquinoline (7.5 g, 56 mmol), and Et_3N (7.5 g, 74 mmol) in dry CH_3CN (200 mL) was heated at reflux for 40 h. After cooling, the resulting solid was filtered and washed with CH_3CN and then with EtOH to give **24v** (2.1 g, 38%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 0.85−1.45 (4 H, m, cyclopropyl CH_2), 2.75 (3 H, s, CH_3), 2.85−3.10 and 3.30−3.60 (each 2 H, m, isoquinoline CH_2), 4.25−4.50 (3 H, m, isoquinoline CH_2, cyclopropyl CH), 7.20 (4 H, bs, isoquinoline aromatic H), 8.50 (1 H, s, H-2), 8.90 (1 H, s, H-5). Anal. ($C_{23}H_{21}N_3O_5$) C, H, N.

By a similar procedure, compounds **24g**,**h** were prepared from acid **23** by reaction with the appropiate heterocyclic amine.

General Procedure for Reduction of 6-Nitro Group ($NO_2 \Rightarrow NH_2$): Preparation of Ethyl 6-Amino-1-cyclopropyl-8-methyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (16a). A stirred solution of **13a** (0.24 g, 0.58 mmol) in 2-methoxyethanol (5 mL) was hydrogenated over Raney nickel (0.1 g) at room temperature and atmospheric pressure for 1 h. The mixture was then filtered over Celite, and the filtrate was evaporated to dryness. The solid residue was triturated with EtOH giving **16a** (0.20 g, 90%): mp 223−225 °C; ^{1}H NMR ($CDCl_3$) δ 0.80−1.55 (7 H, m, CH_2CH_3, cyclopropyl CH_2), 2.40 (3 H, s, NCH_3), 2.60 (3 H, s, CH_3), 2.80−3.60 (8 H, m, piperazine CH_2), 3.75−4.00 (1 H, m, cyclopropyl CH), 4.20 (2 H, bs, NH_2), 4.40 (2 H, q, J = 7 Hz, CH_2CH_3), 7.60 (1 H, s, H-5), 8.60 (1 H, s, H-2). Anal. ($C_{21}H_{28}N_4O_3$) C, H, N.

In an analogous procedure, compounds **16b**−**f**,**i**−**n**,**p**−**u**, **17a**, and **18a** were prepared starting from the corresponding nitro esters **13b**−**f**,**i**−**n**,**p**−**u**, **14a**, and **15a**, while **19g**,**h**,**v** were prepared from corresponding nitro acids **24g**,**h**,**v**.

By a similar procedure intermediate **28** was also obtained from **10**. Compound **28**: mp 219−221 °C (92%); ^{1}H NMR ($CDCl_3$) δ 0.90−1.35 (4 H, m, cyclopropyl CH_2), 1.40 (3 H, t, J = 7 Hz, CH_2CH_3), 2.70 (3 H, d, J = 3 Hz, CH_3), 3.80−3.95 (1 H, m, cyclopropyl CH), 4.10 (2 H, bs, NH_2), 4.35 (2 H, q, J = 7 Hz, CH_2CH_3), 7.80 (1 H, d, J = 7.5 Hz, H-5), 8.60 (1 H, s, H-2). Anal. ($C_{16}H_{17}FN_2O_3$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-8-methyl-7-(1,2,3,6-tetrahydropyridinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (16o). Ammonium formate (0.4 g, 6.25 mmol) and 10% Pd/C (0.5 g) were added to a solution of **13o** (0.5 g, 1.25 mmol) in dry MeOH (15 mL), cooled to 0 °C. The stirred mixture was maintained at this temperature for 30 min and then filtered over charcoal. The solvent was evaporated to dryness, and the resulting residue was purified by column chromatography eluting with a gradient of $CHCl_3$ to $CHCl_3$/MeOH (95:5) to give **16o** (0.15 g, 33%): mp 273−275 °C; ^{1}H NMR ($CDCl_3$) δ 0.80−1.25 (4 H, m, cyclopropyl CH_2), 1.45 (3 H, t, J = 7 Hz, CH_2CH_3), 2.00−2.20 (4 H, m, pyridine CH_2), 3.00 (3 H, s, CH_3), 3.10−3.20 (2 H, m, pyridine CH_2), 3.95−4.10 (1 H, m, cyclopropyl CH), 4.20 (2 H, bs, NH_2), 4.45 (2 H, q, J = 7 Hz, CH_2-CH_3), 4.50−4.60 (2 H, t, J = 5.5 Hz, pyridine CH), 8.50 (1 H, s, H-5), 8.70 (1 H, s, H-2). Anal. ($C_{21}H_{25}N_3O_3$) C, H, N.

General Procedure for Hydrolysis Reaction. Method F: Preparation of 6-Amino-1-cyclopropyl-8-methyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Dihydrochloride (19a). A solution of **16a** (0.20 g, 0.52 mmol) in EtOH (2 mL) and 6 N HCl (2 mL) was refluxed for 2 h. After cooling, the crystalline-precipitated solid was filtered off, washed with dry EtOH, and dried to give **19a** (0.1 g, 45%) as a white solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 0.70−1.30 (4 H, m, cyclopropyl CH_2), 2.60 (3 H, s, CH_3), 2.80 (3 H, bd, $NHCH_3$), 3.20−3.70 (9 H, m, piperazine CH_2, cyclopropyl CH), 7.50 (1 H, s, H-5), 8.65 (1 H, s, H-2), 10.80 (1 H, bs, CO_2H). Anal. ($C_{19}H_{24}N_4O_3$·2HCl) C, H, N.

Using the above general procedure, target acids **19b**−**f**,**i**−**q**,**s**−**u**, **20a**, and **21a** were prepared from corresponding esters **16b**−**f**,**i**−**q**,**s**−**u**, **17a**, and **18a**, as well as intermediate acid **23** from ester **10**. Compound **23**: mp 176−180 °C (90%); ^{1}H NMR (DMSO-d_6) δ 1.00−1.30 (4 H, m, cyclopropyl CH_2), 2.85 (3 H, d, J = 3 Hz, CH_3), 4.30−4.50 (1 H, m, cyclopropyl CH), 8.80 (1 H, d, J = 9 Hz, H-5), 8.85 (1 H, s, H-2), 13.00 (1 H, bs, CO_2H). Anal. ($C_{14}H_{11}FN_2O_5$) C, H, N.

Method G: Preparation of 6-Amino-7-azetidinyl-1-cyclopropyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (19r). The suspension of **16r** (0.1 g, 2.7 mmol) in 1 N NaOH (2 mL) was refluxed for 3 h (until the suspension became a solution). After cooling at room temperature, the solution was filtered, diluted with water (4 mL), and brought to pH 6 by adding a solution of 2 N HCl. The resulting precipitate was filtered and washed with water to give **19r** (0.046 g, 50%): mp 230−231 °C; ^{1}H NMR (DMSO-d_6) δ 0.85−1.00 and 1.15−1.30 (each 2 H, m, cyclopropyl CH_2), 2.10−2.30 (2 H, m, azetidine CH_2), 2.40 (3 H, s, CH_3), 4.15−4.35 (5 H, m, cyclopropyl CH, azetidine CH_2), 4.95 (1 H, bs, NH_2), 7.30 (1 H, s, H-5), 8.55 (1 H, s, H-2). Anal. ($C_{17}H_{19}N_3O_3$·H_2O) C, H, N.

In an analogous procedure, compounds **24a**,**i**,**j** were prepared starting from the corresponding nitro esters **13a**,**i**,**j**.

6-Amino-1-(4-hydroxyphenyl)-8-methyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (22a). A solution of **18a** (0.25 g, 0.55 mmol) in 48% HBr (2 mL) was refluxed for 5 h. After cooling, the precipitate was separated by filtration and solubilized in a minimum amount of water. The aqueous solution was made basic (pH 7.5−8) by adding a saturated solution of Na_2CO_3, and the precipitated solid was filtered off and dried to give **22a** (0.15 g, 63%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.65 (3 H, s, CH_3), 2.25 (3 H, s, NCH_3), 2.40−3.60 (8 H, m, piperazine CH_2), 5.40 (2 H, bs, NH_2), 6.90 and 7.35 (each 2 H, d, J = 8 Hz, aromatic H), 7.50 (1 H, s, H-5), 8.35 (1 H, s, H-2), 8.90 (1 H, bs, OH), 10.00 (1 H, bs, CO_2H). Anal. ($C_{22}H_{24}N_4O_4$) C, H, N.

Ethyl 1-Cyclopropyl-8-methyl-7-(4-methyl-1-piperidinyl)-6-[(trifluoroacetyl)amino]-4-oxo-1,4-dihydroquinoline-3-carboxylate (25j). The mixture of **16j** (0.2 g, 0.5 mmol) and $(CF_3CO)_2O$ (3 mL) was stirred at room temperature for 30 min and then poured into water, basified to pH 7 with a saturated solution of Na_2CO_3, and extracted with $CHCl_3$. The combined organic layers were evaporated to dryness to give **25j** (0.2 g, 80%) which was hydrolyzed without further purification: mp 248−250 °C; ^{1}H NMR ($CDCl_3$) δ 0.80−1.20 (7 H, m, cyclopropyl CH_2, piperidine CH_3), 1.40 (3 H, t, J = 7 Hz, CH_2CH_3), 1.55−1.95 (5 H, m, piperidine CH_2, CH), 2.65 (3 H, s, CH_3), 2.95−3.10 and 3.20−3.40 (each 2 H, m, piperidine CH_2), 3.85−4.00 (1 H, m, cyclopropyl CH), 4.40 (2

H, q, $J = 7$ Hz, CH_2CH_3), 8.60 (1 H, s, H-5), 9.05 (1 H, s, H-2), 9.70 (1 H, bs, NH).

1-Cyclopropyl-6-(methylamino)-8-methyl-7-(4-methyl-1-piperidinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (26j). A mixture of **25j** (0.2 g, 0.4 mmol), CH_3I (0.113 g, 0.8 mmol), and K_2CO_3 (0.1 g, 0.8 mmol) in dry acetone (15 mL) was refluxed with stirring for 30 min. After removing the solvent, the residue was taken up in $CHCl_3$ to remove the inorganic salts. The organic solvent was evaporated to dryness and the residue dissolved in a mixture of EtOH/20% NaOH (1:1) (4 mL). The mixture was then refluxed for 2 h. After cooling, the solution was diluted with water and neutralized with 2 N HCl. The precipitated solid so obtained was filtered off, dried, and purified by recrystallization from EtOH to give **26j** (0.10 g, 58%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 0.75−0.85 (2 H, m, cyclopropyl CH_2), 1.00 (3 H, bs, piperidine CH_3), 1.10−1.25 (2 H, m, cyclopropyl CH_2), 1.35−1.75 (5 H, m, piperidine CH_2, CH), 2.60 (3 H, s, CH_3), 2.82 (3 H, bd, $NHCH_3$), 2.95−3.25 (4 H, m, piperidine CH_2), 4.15−4.30 (1 H, m, cyclopropyl CH), 5.30 (1 H, bq, NH), 7.05 (1 H, s, H-5), 8.60 (1 H, s, H-2). Anal. ($C_{21}H_{27}N_3O_3$) C, H, N.

1-Cyclopropyl-6-(dimethylamino)-8-methyl-7-(4-methyl-1-piperidinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (27j). A mixture of **25j** (0.2 g, 0.52 mmol) and trimethyl phosphate (0.073 g, 0.52 mmol) was heated at 150 °C for 30 min. Then a solution of NaOH (0.062 g, 1.55 mmol) in water (1 mL) was added, and the mixture was refluxed for 20 min. After cooling, the mixture was diluted with water and extracted with $CHCl_3$. The combined organic layers were dried and evaporated to dryness to give a residue which, after recrystallization with EtOAc, gave **27j** (0.15 g, 70%): mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 0.85−0.95 (2 H, m, cyclopropyl CH_2), 1.00 (3 H, d, $J = 5.5$ Hz, piperidine CH_3), 1.10−1.20 (2 H, m, cyclopropyl CH_2), 1.35−1.90 (5 H, m, piperidine CH_2, CH), 2.60 (3 H, s, CH_3), 2.75 (6 H, s, NCH_3), 3.20−3.40 (4 H, m, piperidine CH_2), 4.00−4.15 (1 H, m, cyclopropyl CH), 7.85 (1 H, s, H-5), 8.85 (1 H, s, H-2), 15.35 (1 H, s, CO_2H). Anal. ($C_{22}H_{29}N_3O_3$) C, H, N.

1-Cyclopropyl-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Difluoroborate Chelate (29). A solution of $NaNO_2$ (0.45 g, 6.5 mmol) in water (3 mL) was added portionwise to a solution of **28** (1.5 g, 4.9 mmol) in 6 N HCl (8 mL), maintained at −5 °C. After 20 min at this temperature, a 50% solution in water of HBF_4 (4 mL) was added. The resulting yellow solid was filtered off, washed successively with cold water, EtOH, and Et_2O, and dried below 50 °C under reduced pressure giving the diazonium tetrafluoroborate salt (1.6 g, 72%). This material was thermally decomposed at 180−200 °C for some minutes (until no fumes occurred). The obtained dark solid was then extracted with refluxing EtOAc (2 × 400 mL). The organic solvent was evaporated to dryness, and the residue was purified by flash column chromatography eluting with a gradient of cyclohexane/EtOAc (1:1) to EtOAc to give **29** (0.335 g, 21%): mp 195−197 °C; ^{1}H NMR ($CDCl_3$) δ 1.22−1.35 and 1.45−1.60 (each 2 H, m, cyclopropyl CH_2), 3.00 (3 H, d, $J = 3$ Hz, CH_3), 4.45−4.62 (1 H, m, cyclopropyl CH), 8.30 (1 H, t, $J = 9$ Hz, H-5), 9.30 (1 H, s, H-2). Anal. ($C_{14}H_{10}BF_4NO_3$) C, H, N.

1-Cyclopropyl-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (30v). A mixture of **29** (0.3 g, 0.92 mmol), 1,2,3,4-tetrahydroisoquinoline (1.27 g, 9.5 mmol), and dry Et_3N (0.96 g, 9.5 mmol) in dry CH_3CN (6 mL) was refluxed for 4 days. After cooling, the solvent was removed and the residue was treated with water, acidified with 6 N HCl to pH 5−6, and extracted with $CHCl_3$. The combined organic layers were evaporated to dryness, and the residue was filtered by column chromatography eluting with $CHCl_3$ and then recrystallized from EtOAc to give **30v** (0.165 g, 27%): mp 229−232 °C; ^{1}H NMR ($CDCl_3$) δ 0.90−1.05 and 1.20−1.35 (each 2 H, m, cyclopropyl CH_2), 2.80 (3 H, s, CH_3), 3.00−3.20 and 3.55−3.65 (each 2 H, m, isoquinoline CH_2), 4.05−4.20 (1 H, m, cyclopropyl CH), 4.45 (2 H, s, isoquinoline CH_2), 7.05−7.40 (4 H, m, isoquinoline aromatic H), 8.00 (1 H, d, $J = 11.5$ Hz, H-5), 8.90 (1 H, s, H-2), 14.70 (1 H, s, CO_2H). Anal. ($C_{23}H_{21}FN_2O_3$) C, H, N.

In an analogous procedure, 6-fluoro derivatives **30a**,**i** were prepared from **29** by reaction with the appropiate amine.

In Vitro Antibacterial Activity. The method has been described previously.[13]

Inhibitory Effect on Supercoiling Activity of DNA Gyrase. This assay was carried out according to a method reported previously with slight modifications.[14]

log *P* Calculation. The partition coefficient values, log *P* (octanol/water), were calculated by MedChem Software Release 3.5[15] and are listed as clog *P* values in Table 4.

In Vivo Efficacy on Systemic Infection in Mice. Mouse protection tests were performed against *S. aureus* Smith ATCC19636. Male and female CD-1 mice (Charles River) weighing 18−22 g were infected intraperitoneally with 0.5 mL of a bacterial suspension from an overnight culture (in brain−heart infusion; Difco) diluted in 5% bacteriological mucin (Difco). The test compounds were solubilized in DMSO. Subsequent dilutions were performed using 0.5% methocel. Micronized powders were administered as suspensions in 0.5% methocel plus 0.0025% of poly(oxyethylene) fatty acid esters 40 (P40). Ciprofloxacin was administered suspended in 0.5% methocel. Three to five groups, of six mice each, were subcutaneously or orally treated once, within 10 min after infection, with each test compound at different dose levels. The 50% effective dose (ED_{50}) and 95% confidence limits were calculated by the Spearmen−Kärber method[20] from the percentages of animals surviving to day 7 at each dose.

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Supporting Information Available: ^{1}H NMR data of target acids not reported in the test (6 pages). Ordering information is given on any current masthead page.

References

(1) This work was previously presented in part; see: (a) Wise, R.; Pagella, P. G.; Cecchetti, V.; Fravolini, A.; Tabarrini, O. In Vitro Activity of MF 5137, a New Potent 6-Aminoquinolone. 5th International Symposium on New Quinolones, Singapore, August 1994; p 11. (b) Cecchetti, V.; Fravolini, A.; Lorenzini, C.; Tabarrini, O.; Terni, P.; Wise, R.; Xin, T. A New Series of 6-Aminoquinolones with Potent Antimicrobial Activity. XIIIth International Symposium on Medicinal Chemistry, Paris, France, September 1994; p 148.

(2) (a) 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. (b) Bonelli, D.; Cecchetti, V.; Clementi, S.; Cruciani, G.; 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.

(3) Cecchetti, V.; Clementi, S.; Cruciani, G.; Fravolini, A.; Pagella, P. G.; Savino, A.; Tabarrini, O. 6-Aminoquinolones: A New Class of Quinolone Antibacterials? *J. Med. Chem.* **1995**, *38*, 973−982.

(4) (a) Peterson, L. P. Quinolone Resistance in Clinical Practice: Occurrence and Importance. In *Quinolone Antimicrobial Agents*, 2nd ed.; Hooper, D. C., Wolfson, J. S., Eds.; American Society for Microbiology: Washington, DC, 1993; pp 119−137. (b) Moellering, R. C., Jr. Quinolone Antimicrobial Agents: Overview and Conclusions. *Ibid.* pp 527−535.

(5) Maple, P. A. C.; Hamilton-Miller, J. M. T.; Brumfitt, W. Differing Activities of Quinolones against Ciprofloxacin-Susceptible and Ciprofloxacin-Resistant, Methicillin-Resistant *Sthaphylococcus aureus*. *Antimicrob. Agents Chemother.* **1991**, *35*, 345−350.

(6) Piddok, L. J. V. New Quinolones and Gram-Positive Bacteria. *Antimicrob. Agents Chemother.* **1994**, *38*, 163−169.

(7) Forstall, G. J.; Knapp, C. C.; Washington, J. A. Activity of New Quinolones against Ciprofloxacin-Resistant Staphylococci. *Antimicrob. Agents Chemother.* **1991**, *35*, 1679−1681.

(8) Korten, V.; Tomayko, J. F.; Murray, B. E. Comparative In Vitro Activity of DU-6859a, a New Fluoroquinolone Agent, against Gram-Positive Cocci. *Antimicrob. Agents Chemother.* **1994**, *38*, 611−615.

(9) Bremm, K. D.; Peterson, U.; Metzger, K. G.; Endermann, R. In Vitro Evaluation of Bay Y3118, a New Full-Spectrum Fluoroquinole. *Chemotherapy (Basel)* **1992**, *38*, 376−387.

(10) Huband, M. D.; Cohen, M. A.; Meservey, M. A.; Roland, G. E.; Yoder, S. L.; Dazer, M. E.; Domagala, J. M. In Virto Antibacterial Activities of PD 138312 and PD 140248, New Fluoronaphthyridines with Outstanding Gram-Positive Potency. *Antimicrob. Agents Chemother.* **1993**, *37*, 2563−2570.
(11) Miyamoto, H.; Ueda, H.; Otsuka, T.; Aki, S.; Tamaoka, H.; Tominaga, M.; Nakagawa, K. Studies on Antibacterial Agents. III. Synthesis and Antibacterial Activities of Substituted 1,4-Dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acids. *Chem. Pharm. Bull.* **1990**, *38*, 2472−2475.
(12) Ueda, H.; Miyamoto, H.; Yamashita, H.; Tone, H. Eur. Pat. Appl. EP287951; *Chem. Abstr.* **1989**, *110*, 17310.
(13) Cecchetti, V.; Fravolini, A.; Fringuelli, R.; Mascellani, G.; Pagella, P. G.; Palmioli, M.; Segre, G.; Terni, P. Quinolinecarboxylic Acids. 2. Synthesis and Antibacterial Evaluation of 7-Oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylic Acids. *J. Med. Chem.* **1987**, *30*, 465−473.
(14) Antonello, C.; Uriarte, E.; Palumbo, M.; Valisena, S.; Parolin, C.; Palù, G. Synthesis and Biological Activity of New Quinolone Derivatives. *Eur. J. Med. Chem.* **1993**, *28*, 291−296.
(15) *CLOGP3 User Guide*, MedChem Software Release 3.5; Daylight Chemical Information Systems, Inc.: Irvine, CA, 1988.
(16) Inoue, Y.; Kondo, H.; Taguchi, M.; Jinbo, Y.; Sakamoto, F.; Tsukamoto, G. Synthesis and Antibacterial Activity of Thiazolopyrazine-Incorporated Tetracyclic Quinolone Antibacterials. *J. Med. Chem.* **1994**, *37*, 586−592.
(17) Bazile, S.; Moreau, N.; Bouzard, D.; Essiz, M. Relationships among Antibacterial activity, Inhibition of DNA Gyrase, and Intracellular Accumulation of 11 Fluoroquinolones. *Antimicrob. Agents Chemother.* **1992**, *36*, 2622−2627.
(18) Domagala, J. M.; Hagen, S. E.; Joannides, T.; Kiely, J. S.; Laborde, E.; Schroeder, M. C.; Sesnie, J. A.; Shapiro, M. A.; Suto, M. J.; Vanderroest, S. Quinolones Antibacterial Containing the New 7-[3-(1-Aminoethyl)-1-pyrrolidinyl] Side Chain: The Effects of the 1-Aminoethyl Moiety and Its Stereochemical Configurations on Potency and in Vivo Efficacy. *J. Med. Chem.* **1993**, *36*, 871−882.
(19) Lang, S. A., Jr.; Cohen, E. β-Aminocinnamonitriles as Potential Antiinflammatory Agents. *J. Med. Chem.* **1975**, *18*, 441−443.
(20) Gibaldi, M.; Perrier, D. *Drugs and Pharmaceutical Sciences, Vol.15: Pharmacokinetics*, 2nd ed.; Marcell Dekker Inc.: New York, 1982.

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