Potent 6-Desfluoro-8-methylquinolones as New Lead Compounds in Antibacterial Chemotherapy[1]

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In a furtherance of our SAR study on the C-6 position of quinolone antibacterials, a series of 6-desfluoro-8-methylquinolones were synthesized and evaluated for their in vitro antimicrobial activity. As a result of this study, compounds with strong activity against Gram-positive bacteria, including ciprofloxacin-resistant and methicillin-resistant *Staphylococcus aureus*, were identified. The best Gram-positive antibacterial activity was exhibited by piperidinyl derivative **6c**, which was 17 times more potent than ciprofloxacin and displayed extremely high activity against *Streptococcus pneumoniae* with an MIC value of ≤0.016 μg/mL. Thus, we have shown that substituent combinations in the quinolone ring, excluding the C-6 fluorine atom, might produce powerful antibacterial agents.

Introduction

Quinolone antibacterial agents are among the most attractive drugs in the antiinfective chemotherapy field. Progress in the development of quinolones came with the introduction of a fluorine atom at the C-6 position of the fundamental moiety. This modification, with the concomitant presence of an appropriate base at C-7, gave compounds which became dramatically more potent than the 6-desfluoro predecessors. Even if the precise role of the fluorine atom at C-6 has never been clearly defined, it continues to be a fundamental structural feature of all currently synthesized analogues (fluoroquinolones) which have been marketed recently or are currently undergoing clinical development.

Recently, we discovered that good activity can still be retained by replacing the C-6 fluorine atom with an amino group and reoptimizing the other substituents.[2,3] In particular, we have found 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.[4] Moreover, Ledussal et al. recently observed that the C-6 fluorine atom can be shifted to the C-8 position with only a minor loss of activity.[5] These findings prompted us to extend our investigation in the structure activity−relationship (SAR) in order to verify if the the C-6 fluorine atom is essential for a potent antibacterial activity.

In our consequent approach to new quinolones, the usual fluorine atom at the C-6 position was excluded, while a methyl group was instead included at the C-8 position as a potential enhancer of antibacterial activity, especially against Gram-positive bacteria.[3,6] This property is of particular interest since the currently available quinolone agents exhibit only moderate activities against many Gram-positive bacteria. Indeed, recent efforts have been directed toward synthesizing compounds which show greater activity against these organisms.[7] The 6-desfluoro-8-methylquinolones **6a**−**l**, herein reported (Figure 1), differ from their slightly active and surpassed predecessors such as nalidixic acid, pipemidic acid, or piromidic acid, in that they maintain the arrangement of the functional groups that SAR studies indicated as optimal to warrant the highest activity levels. Thus, besides the methyl group in C-8, they bear the "magic" cyclopropyl group at N-1,[8] and a selected heterocyclic base at C-7.

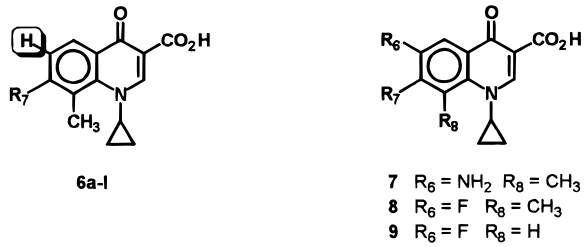

Figure 1.

Chemistry

The synthetic approach used to prepare the target compounds involved the usual intramolecular nucleophilic displacement cyclization to form the quinolone ring, as depicted in Scheme 1. Thus, reaction of the acid chloride of 2-chloro-4-fluoro-3-methylbenzoic acid (**1**) with ethyl (dimethylamino)acrylate, followed by substitution with cyclopropylamine and successive cyclization with potassium carbonate in DMF, gave the ester **3**. This key intermediate was then converted into borine complex **5** using fluoboric acid in order to activate the C-7 position. The successive nucleophilic displacement reaction of the C-7 fluorine atom with the selected heterocyclic side chain (shown in Chart 1) was carried out in DMSO, using triethylamine or an excess of side chain as HF scavenger. The simple treatment with water during the workup of the reaction directly cleaved

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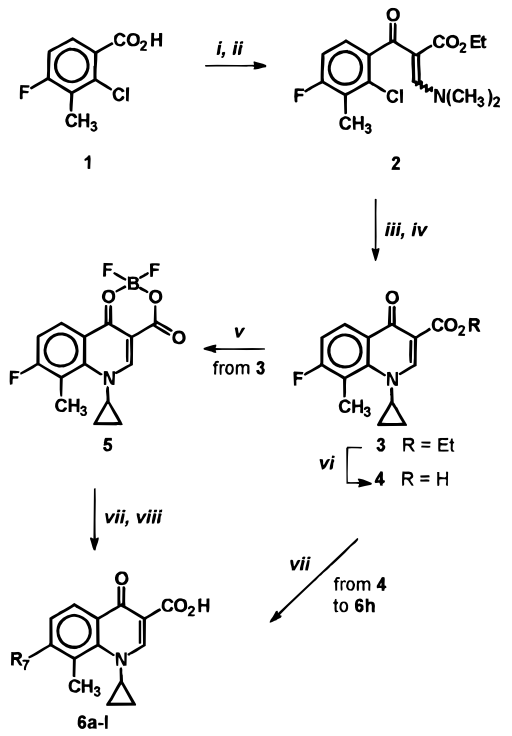
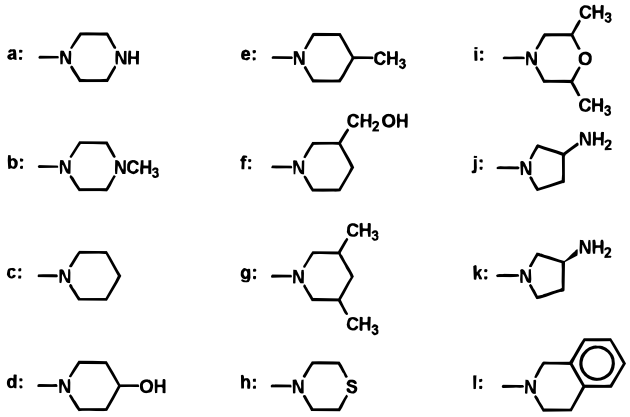
Scheme 1[a]



[a] Reagents: (i) $SOCl_2$, reflux; (ii) $(CH_3)_2NCH{=}CHCO_2Et$, Et_3N, toluene, 90 °C; (iii) c-$PrNH_2$, EtOH/Et_2O; (iv) K_2CO_3, DMF, 100 oC; (v) 48% HBF_4, 90−100 °C; (vi) 6 N HCl; (vii) R_7H, DMSO; (viii) H_2O.

Chart 1. Heterocyclic Side Chains Employed as the R_7 Substituent



the boron ester chelate to produce the target free acids **6** (see Table 1). Only when thiomorpholine (**h**) was used as the nucleophile, was it possible to carry out the coupling reaction starting from the acid **4**, obtained by acid hydrolysis from ester **3**.

Biological Assays

The 6-desfluoroquinolones (**6a**−**l**) prepared in this study were tested in vitro and compared to ciprofloxacin against an assortment of eight Gram-positive and 10 Gram-negative organisms, including some clinical isolates as well as ciprofloxacin-resistant *Escherichia coli* and *Staphylococcus aureus* strains and methicillin-resistant *S. aureus* (MRSA). The minimum inhibitory concentrations (MICs, μg/mL) were determined by the microdilution technique using nutrient broth, according to NCCLS;[9] the data are presented in Table 2. The geometric means of the MICs were also calculated to facilitate a comparison of activity. For a comparative purpose, some 6-amino-8-methyl- (**7b**,**c**,**l**) and 6-fluoro-8-methylquinolones (**8b**,**c**,**l**), previously reported,[3] as well as 6-fluoroquinolones (**9b**,**c**,**l**),[10] were reassayed, and their MIC values are given in Table 3 along with the MICs of selected 6-desfluoro-8-methylquinolones (**6b**,**c**,**l**).

All compounds reported in Table 3 were also tested for their ability to inhibit the supercoiling activity of DNA gyrase by using a previously described protocol.[11] The IC_{50} values are reported in Table 4, together with the CLOG*P* values.[12]

Results and Discussion

The MIC values, reported in Table 2, show that 6-desfluoro-8-methyl derivatives **6** display a very potent Gram-positive antibacterial activity, which is, with the exception of piperazine derivatives (**6a**,**b**), considerably superior to that of ciprofloxacin and well comparable to the activity displayed by the most effective presently available agents.[13] In particular, piperidinyl derivatives **6c**−**g** and thiomorpholinyl derivative **6h** show an excellent activity against all *S. aureus* strains, including ciprofloxacin-resistant and methicillin-resistant isolates. It must be pointed out that they were 62 times more potent than ciprofloxacin against MRSA whose infection today has become a serious medical problem. Among the 6-desfluoro-8-methyl derivatives, the best Gram-positive antibacterial activity was exhibited by piperidinyl derivative **6c** with a geometric mean MIC value of 0.052 μg/mL; the value is 17 times lower than in the case of ciprofloxacin (0.917 μg/mL). In particular, it showed excellent activity against *Streptococcus pneumoniae* with MIC value $\leq$0.016 μg/mL.

In general, the 6-desfluoro-8-methylquinolones **6** display less activity against Gram-negative bacteria than ciprofloxacin. None of them were active against ciprofloxacin-resistant *E. coli*. Nevertheless, the piperazine derivative **6a** and 3-aminopyrrolidinyl derivatives **6j** and **6k** showed geometric mean MICs of 0.187, 0.126, and 0.164 μg/mL, respectively, comparable to that of ciprofloxacin (0.083 μg/mL). Compound **6j** and **6k** showed good balanced activity against Gram-positive and Gram-negative bacteria.

In an attempt to clarify the importance of the C-6 substituent, some selected 6-desfluoro-8-methyl derivatives (**6**) were compared to their 6-amino-8-methyl (**7**) and 6-fluoro-8-methyl (**8**) counterparts. In addition to the "usual" *N*-methylpiperazinyl side chain (**b**), the piperidinyl (**c**) and the 1,2,3,4-tetrahydroisoquinolinyl (**l**) were chosen as C-7 substituents, because of their remarkable contribution to activity in 6-desfluoro-8-methyl- and the 6-amino-8-methylquinolone series. The MIC values are summarized in Table 3. A varied trend was observed in the 4-methylpiperazinyl derivatives: the 6-fluoro derivative **8b** was 4 times more potent than the 6-desfluoro analogue **6b** against both Gram-negative and Gram-positive bacteria, while the latter showed the same Gram-negative activity as its 6-amino counterpart **7b**, but was three times more potent against Gram-positives. On the other hand, in the piperidinyl derivatives (**6c**, **7c**, **8c**) and in the tetrahydroisoquinolinyl derivatives (**6l**, **7l**, **8l**) there is a clear rank order of activity against both Gram-positive and Gram-negative bacteria: F $\geq$ H > NH_2, and F $\cong$ NH_2 > H, respectively.

Table 1. Physical Properties for 6-Desfluoro-1-cyclopropyl-8-methyl-7-substituted-4-oxo-1,4-dihydroquinolone-3-carboxylic Acids Tested in This Study

compd	R_7	Method of prep,[a] base[b]/temp/ react. time	purification method[c]	% yield	mp, ° C	formula[d]
6a		-/80°/10 h	A	35	304-307	$C_{18}H_{21}N_3O_3 \cdot HCl$
6b		-/80°/8 h	B	40	273-274	$C_{19}H_{23}N_3O_3$
6c		Et_3N/80°/5 h	B	40	208-210	$C_{19}H_{22}N_2O_3$
6d		Et_3N/90°/5 h	B	30	226-229	$C_{19}H_{22}N_2O_4$
6e		Et_3N/90°/20 h	B	45	249-250	$C_{20}H_{24}N_2O_3$
6f		-/80°/30 h	B	38	228-230	$C_{20}H_{24}N_2O_4$
6g		-/90°/30 h	B	29	252-255	$C_{21}H_{26}N_2O_3$
6h		Et_3N/120°/20 h[e]	B	40	250-252	$C_{18}H_{20}N_2O_3S$
6i		-/90°/12 h	B	27	269-272	$C_{20}H_{24}N_2O_4$
6j		*1.* Et_3N/80°/5 h[f] *2.* NaOH, AcOH	B	*1.* 34 *2.* 88	275-277	$C_{18}H_{21}N_3O_3$
6k		*1.* Et_3N/120°/14 h[f] *2.* NaOH, AcOH	B	*1.* 31 *2.* 85	212-213	$C_{18}H_{21}N_3O_3 \cdot CH_3CO_2H$
6l		-/70°/30 h	C	25	215-217	$C_{23}H_{22}N_2O_3$

[a] All reaction were carried out in DMSO and monitored for completion by TLC. [b] Where no base is given, excess side chain was employed as base. [c] (A) Trituration with EtOAc followed by filtration, solubilization in $CHCl_3$/MeOH and treatment of the resulting solution with HCl to obtain a precipitate; (B) trituration with EtOAc; (C) column chromatography eluting with $CHCl_3$ followed by trituration with Et_2O. [d] All compounds had elemental analyses within 0.4% of the theoretical values. [e] The acid 4 was employed as starting material. [f] The 3-acetamidopyrrolidine was employed as side chain requiring an basic deprotection step.

Table 2. In Vitro Antibacterial Activity

	(MICs, mg/mL)												
organism[a]	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**	**6k**	**6l**	CPX[b]
Gram-Positives													
S. au. ATCC 29213	0.5	0.25	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.125	0.125	0.125	≤0.016	0.5
S. au. MPR 5	0.25	0.25	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.125	0.125	0.03	0.25
S. au. M-R[c] POMM 6214	0.5	0.5	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.03	0.25	0.25	0.25	1
S. au. CPX-R[d] OBT 687	16	8	1	0.5	1	1	4	2	1	4	4	2	>16
S. py. OMNFI BI	2	2	0.03	2	0.125	0.5	2	0.5	0.5	1	0.25	0.25	0.5
S. pn. I 0.43	2	2	≤0.016	0.25	0.25	0.5	0.5	1	0.5	0.06	0.25	1	1
E. fe. LEP Br	2	2	1	0.5	0.5	0.5	1	1	1	1	0.5	1	1
E. fe. UCMC 39690	2	1	0.03	0.125	0.03	0.25	1	0.5	0.25	0.5	0.25	0.5	0.5
geometric means	1.414	1.090	0.052	0.126	0.081	0.138	0.252	0.194	0.210	0.384	0.324	0.272	0.917
Gram-Negatives													
E. co. ATCC 25922	0.03	0.06	0.03	0.06	0.03	0.125	1	0.125	1	≤0.016	0.03	1	≤0.016
E. co. 120	0.03	0.06	0.03	0.06	0.06	0.25	1	0.25	0.5	≤0.016	≤0.016	2	≤0.016
E. co CPX-R OBT 431	>16	>16	>16	>16	>16	>16	>16	>16	>16	16	16	>16	8
E. cl. OMNFI 174	0.26	0.25	0.25	0.25	0.25	0.5	4	1	2	≤0.016	0.25	0.03	≤0.016
P. mi. OBT 505	0.25	2	4	2	2	4	16	4	16	0.5	0.06	>16	0.06
P. vu. CNUR 6	2	8	8	8	4	8	>16	16	>16	1	2	>16	0.5
K. pn. ATCC 10031	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.06	0.5	≤0.016	0.125	≤0.016	≤0.016	0.125	≤0.016
P. ae. ATC1C 9027	0.125	2	4	2	2	4	8	4	16	0.125	0.125	>16	0.06
P. ae. OBT 307	2	16	16	16	8	16	>16	16	16	1	0.5	>16	1
H. in.	0.03	0.06	≤0.016	0.25	≤0.016	≤0.016	0.25	≤0.016	1	≤0.016	0.6	0.06	≤0.016
geometric means	0.187	0.609	0.534	0.703	0.434	0.931	3.482	0.937	3.249	0.126	0.164	1.851	0.083

[a] Organisms selected are as follows: *S. au., Staphylococcus aureus; S. py., Streptococcus pyogenes; S. pn., Streptococcus pneumoniae; E. fe., Enterococcus faecalis; E. co., Escherichia coli; E. cl., Enterobacter cloacae; P. mi., Proteus mirabilis; P. vu., Proteus vulgaris; K. pn., Klebsiella pneumoniae; P. ae., Pseudomonas aeruginosa; H. in., Haemophilus influenzae.* [b] CPX = ciprofloxacin. [c] M-R = methicillin resistant. [d] CPX-R = ciprofloxacin resistant.

In addition, a comparison of 6-fluoro-8-methylquinolones **8** with their 8-desmethyl analogues **9** shows that the introduction of the C-8 methyl group clearly improves the Gram-positive MIC whereas it does not affect the activity against Gram-negative bacteria to an appreciable extent.

Table 3. Comparative in Vitro Antibacterial Activity for Selected 6-Desfluoro-8-methylquinolones (**6**), Prepared in This Study, and Their Reference Agents (**7**, **8**, **9**; See Figure 1)

	(MICs, μg/mL)											
organism[a]	**6b**	**7b**	**8b**	**9b**	**6c**	**7c**	**8c**	**9c**	**6l**	**7l**	**8l**	**9l**
						Gram-Positives						
S. au. ATCC 29213	0.25	1	0.125	0.125	≤0.016	0.05	≤0.016	0.03	≤0.016	≤0.016	≤0.016	≤0.016
S. au. MPR 5	0.25	1	0.06	0.125	≤0.016	≤0.016	≤0.016	0.03	0.03	≤0.016	≤0.016	≤0.016
S. au. M-R[b] POMM 6214	0.5	4	0.125	1	≤0.016	0.125	≤0.016	0.25	0.25	≤0.016	≤0.016	0.25
S. au. CPX-R[c] OBT 687	8	>16	2	16	1	8	0.125	>16	2	2	1	8
S. py. OMNFI BI	2	4	0.25	2	0.03	0.5	≤0.016	8	0.25	≤0.016	0.03	2
S. pn. I 043	2	8	0.25	0.5	≤0.016	2	≤0.016	1	1	0.25	0.25	0.25
E. fe. LEP Br	2	2	1	0.5	1	0.5	0.03	0.5	1	0.25	0.5	0.25
E. fe. UCMC 39690	1	4	0.25	0.25	0.03	1	≤0.016	0.25	0.5	0.125	0.06	0.06
geometric means	1.090	3.364	0.271	0.648	0.052	0.376	0.022	0.495	0.272	0.075	0.074	0.210
						Gram-Negatives						
E. co. ATCC 25922	0.06	0.03	≤0.016	≤0.016	0.03	0.125	≤0.016	0.125	1	0.125	0.125	0.25
E. co. 120	0.06	0.06	≤0.016	≤0.016	0.03	0.5	≤0.016	0.25	2	0.125	0.25	0.25
E. co CPX-R OBT 431	>16	>16	4	>16	>16	>16	16	>16	16	>16	>16	>16
E. cl. OMNFI 174	0.25	0.125	0.125	≤0.016	0.25	1	0.5	0.5	0.03	0.5	0.5	1
P. mi. OBT 505	2	1	0.5	0.25	4	4	1	2	>16	>16	4	4
P. vu. CNUR 6	8	8	0.5	0.5	8	>16	2	>16	>16	8	4	>16
K. pn. ATCC 10031	≤0.016	0.03	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.125	0.03	0.06	0.125
P. ae. ATC1C 9027	2	2	0.5	0.5	4	4	1	4	>16	8	4	4
P. ae. OBT 307	16	8	2	0.25	16	16	4	4	>16	>16	16	8
H. in.	0.06	0.03	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.06	≤0.016	≤0.016	≤0.016
geometric means	0.609	0.459	0.155	0.110	0.534	1.005	0.290	0.710	1.851	0.998	0.869	1.151

[a–c] See footnotes *a*, *c*, *d*, respectively, in Table 2.

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-Desfluoro-8-methylquinolones (**6**) and Their Reference Agents (**7**, **8**, **9,** See Figure 1)

		geometric mean MICs		
compd	IC_{50}	Gram-positives	Gram-negatives	CLOG*P*
6b	28.5	1.090	0.609	0.373
6c	3.3	0.052	0.534	3.663
6l	7.8	0.272	1.851	4.373
7b	3.8	3.364	0.459	−0.695
7c	3.2	0.376	1.005	2.595
7l	3.6	0.075	0.998	3.305
8b	5.9	0.271	0.155	0.549
8c	2.4	0.022	0.290	3.839
8l	3.0	0.074	0.869	4.549
9b	1.5	0.648	0.110	−0.011
9c	32.1	0.495	0.710	3.300
9l	10.8	0.210	1.151	4.010
CPX[b]	0.68	0.917	0.083	−0.805

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

The IC_{50} values reported in Table 4 show a conserved order of activity: **c** > **l** > **b** for compounds **6**, **7**, and **8**. This is not the case for the 8-desmethylquinolones as **9c** is almost inactive. These results confirm a mutual influence between substitution at C-7 and C-8 positions, which eventually leads to remarkable biological responses. In particular, it is clear that the introduction of a methyl group at C-8 plays a negative role in a simultaneous presence of a basic substituent, like *N*-methylpiperazinyl at C-7. A possible cause for this behavior could be a displacement of the C-7 ring due to steric hindrance generated by the C-8 methyl group. As a result, an unfavorable localization of the basic substituent in the enzymatic pocket could occur. However, our preliminary molecular modeling studies, not shown, seem to rule out steric interference between C-8 and C-7 substituents. Hence, other molecular mechanisms, such as changes in electron density (and pK_a), could be responsible for the observed behavior.

Concerning substitution at the C-6 position, the rank of inhibitory activity is F ≥ NH_2 > H. These data suggest a possible direct involvment of substituents at this position not only in cellular uptake, but also in gyrase poisoning.[14] Moreover, an electron rich group like amino seems to represent a good substitute for fluorine in gyrase inhibition experiments.

A good correlation was found comparing MIC values for Gram-negative bacteria to gyrase IC_{50}, whereas it was not the case when the comparison was made between IC_{50} and Gram-positive MICs. This might indicate an involvement of factors other than impairment of gyrase activity in the biological response. On the other hand, a good correlation links CLOG*P* to Gram-positive MICs, indicating an important role of hydrophobic effects in the mechanism of action. The decreasing values of MIC when increasing CLOG*P* are in line with the higher activity observed when the methyl group is introduced at the C-8 position. It is finally interesting to note that a satisfactory proportionality relationship ties both IC_{50} and Gram-negative MICs to CLOG*P*.

In conclusion, the results of the present study as well as of our previous ones[2,3] indicate that substituent combinations in the quinolone ring, not including fluorine at C-6 position, might produce powerful antibacterial agents such as the piperidinyl derivatives, including 1-cyclopropyl-7-piperidinyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6c)** as the most potent. Thus, the C-6 fluorine substituent may no longer be a "must" in the future design of active compounds of the quinolone family.

Therefore, derivative **6c** is an interesting lead compound for further development, which should particularly take into account an evaluation of genetic toxicity and phototoxicity. In fact, important side effects have been observed when using polyfluorinated quinolones,[15] and it would be of interest to determine whether the lack of C-6 fluorine would tend to reduce these side effects.

In addition, it is well-known that the C-8 substituent also contributes to toxicity of quinolone antibacterials.[16] Since no data are reported about the role played by a C-8 methyl group in such a context, dissecting this question will represent an additional meaningful target for further research.

Experimental Section

Thin layer chromatography (TLC) was performed on precoated sheets of silica gel $60F_{254}$ (Merck) and visualized by using UV. Column chromatography separations were carried out on Merck silica gel 40 (mesh 70−230). 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 200 MHz (Bruker AC-200) with Me_4Si as internal standard, and chemical shifts are given in ppm (δ). The spectral data are consistent with the assigned structures. Reagents and solvents were purchased from common commercial suppliers and were used as received. 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 target acid derivative are summarized in Table 1.

Ethyl 2-(2-Chloro-4-fluoro-3-methylbenzoyl)-3-(dimethylamino)acrylate (**2**). The mixture of 2-chloro-4-fluoro-3-methylbenzoic acid [3] (**1**) (26 g, 0.13 mmol) and thionyl chloride (20 g) 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] (18.6 g, 0.13 mmol) and dry Et_3N (28 g, 0.27 mmol). The resulting solution was heated at 90° C for 2 h. After cooling and filtration of the insoluble material, the solvent was washed several times with water, dried, and evaporated to dryness. The obtained thick residue, after treatment with cyclohexane, gave a solid which was washed with Et_2O and dried to give **2** (23 g, 56%): mp 110−114 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, t, J = 7 Hz, CH_2CH_3), 2.30 (3 H, d, J = 3 Hz, CH_3), 2.95 and 3.35 (each 3 H, bs, NCH_3), 3.95 (2 H, q, J = 7 Hz, CH_2CH_3), 6.95 (1 H, t, J = 8.9 Hz, H-5), 7.20 (1 H, dd, J = 6.7 and 8.9 Hz, H-6), 7.80 (1 H, s,vinyl H). Anal. ($C_{15}H_{17}ClFNO_3$) C, H, N.

Ethyl 1-Cyclopropyl-7-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3**). A stirred solution of **2** (20 g, 64 mmol) in EtOH/Et_2O (1:2) (350 mL) was treated dropwise with cyclopropylamine (3.65 mL, 64 mmol). After 15 min at room temperature the solvent was evaporated to dryness and the residue dissolved in dry DMF (50 mL) and treated with K_2CO_3 (8.82 g, 63.9 mmol). The resulting mixture was heated at 100 °C for 3 h and, after cooling, poured into ice water. The solid so obtained was filtered, washed with water, and dried to give **3** (15 g, 81%): mp 164−166 °C; ^{1}H NMR ($CDCl_3$) δ 0.90−1.05 and 1.15−1.30 (each 2 H, m, cyclopropyl CH_2), 1.40 (3 H, t, J = 7.1 Hz, CH_2CH_3), 2.72 (3 H, d, J = 2.7 Hz, CH_3), 3.90−4.05 (1 H, m, cycloproyl CH), 4.40 (2 H, q, J = 7.1 Hz, CH_2CH_3), 7.12 (1 H, t, J = 8.9 Hz, H-6), 8.35 (1 H, dd, J = 6.7 and 8.9 Hz, H-5), 8.65 (1 H, s, H-2). Anal. ($C_{16}H_{16}FNO_3$) C, H, N.

1-Cyclopropyl-7-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (**4**). The mixture of **3** (2 g, 6.9 mmol) and 6 N HCl (10 mL) was refluxed for 2 h. After cooling, the crystalline-precipitate solid was filtered off, washed with water, and dried to give **4** (1.4 g, 80%): mp 236−237 °C; ^{1}H NMR (DMSO-d_6) δ 1.05−1.35 (4 H, m, cyclopropyl CH_2), 2.80 (3 H, d, J = 3.2 Hz, CH_3), 4.30−4.50 (1 H, m, cyclopropyl CH), 7.50 (1 H, t, J = 9 Hz, H-6), 8.25 (1 H, dd, J = 6.3, 9 Hz, H-5), 8.85 (1 H, s, H-2), 14.80 (1 H, bs, CO_2H). Anal. ($C_{14}H_{12}FNO_3$) C, H, N.

1-Cyclopropyl-7-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid BF_2 Chelate (**5**). The mixture of **3** (0.8 g, 2.7 mmol) and 48% HBF_4 in water (10 mL) was heated at 90−100 °C for 8 h. After cooling, the mixture was poured into ice water and the solid filtered, washed with water, and dried to give **5** (0.7 g, 84%) as a white solid: mp 276−278 °C; ^{1}H NMR (DMSO-d_6) δ 1.20−1.40 (4 H, m, cyclopropyl CH_2), 2.90 (3 H, d, J = 3.2 Hz, CH_3), 4.10−4.30 (1 H, m, cyclopropyl CH), 7.80 (1 H, t, J = 9.1 Hz, H-6), 8.45 (1 H, dd, J = 6.3, 9.1 Hz, H-5), 8.65 (1 H, s, H-2). Anal. ($C_{14}H_{11}BF_3NO_3$) C, H, N.

General Procedure for Coupling Reaction. The mixture of borine complex **5,** appropiate heterocyclic amine (1.5 equiv), and Et_3N (1.5 equiv) in dry DMSO was heated at 70−120 °C until no starting material was present by TLC (usually 5−30 h). Occasionally, excess side chain was used as the base instead of Et_3N. The reaction mixture was then poured into ice−water and extracted with $CHCl_3$. The combined organic layers were washed several times with water and dried. The crude product is then purified as defined in footnote *c* in Table 1.

Thiomorpholinyl derivative **6h** was synthesized by a similar procedure starting from the acid **4**, instead of borine complex **5**.

To prepare 3-aminopyrrolidinyl derivatives **6j** and **6k**, the corresponding 3-acetamidopyrrolidine was employed as nucleophile. The protective acetyl group was then removed by basic hydrolysis as follows: a mixture of 3-acetamido derivative (0.1 g, 0.27 mmol), 20% NaOH (2 mL), and EtOH (1 mL) was refluxed for 14 h. After cooling, the solution was acidified to pH 4.5−6.5 with AcOH, and the precipitated solid was filtered, washed with water and then with Et_2O, and dried.

Further experimental details are given in Table 1, while their spectral data are enumerated below.

Compound **6a**: ^{1}H NMR ($CDCl_3$) δ 0.55−0.70 and 1.00−1.20 (each 2 H, m, cyclopropyl CH_2), 2.50 (3 H, s, CH_3), 3.10−3.40 (8 H, m, piperazine CH_2), 3.95−4.10 (1 H, m, cyclopropyl CH), 6.85 (1 H, d, J = 8.8 Hz, H-6), 7.45 (1 H, d, J = 8.8 Hz, H-5), 8.50 (1 H, s, H-2). Anal. ($C_{18}H_{21}N_3O_3$·HCl) C, H, N.

Compound **6b**: ^{1}H NMR ($CDCl_3$) δ 0.90−1.00 and 1.20−1.30 (each 2 H, m, cyclopropyl CH_2), 2.35−2.85 (10H, m, piperazine CH_2, NCH_3, and CH_3), 3.10−3.30 (4 H, m, piperazine CH_2), 4.05−4.20 (1 H, m, cyclopropyl CH), 7.20 (1 H, d, J = 8.8 Hz, H-6), 8.30 (1 H, d, J = 8.8 Hz, H-5), 8.90 (1 H, s, H-2), 14.95 (1 H, bs, CO_2H). Anal. ($C_{19}H_{23}N_3O_3$) C, H, N.

Compound **6c**: ^{1}H NMR ($CDCl_3$) δ 0.80−0.95 and 1.10−1.25 (each 2 H, m, cyclopropyl CH_2), 1.50−1.85 (6 H, m, piperidine CH_2), 2.65 (3 H, s, CH_3), 2.95−3.10 (4 H, m, piperidine CH_2), 3.95−4.15 (1 H, m, cyclopropyl CH), 7.10 (1 H, d, J = 8.8 Hz, H-6), 8.20 (1 H, d, J = 8.8 Hz, H-5), 8.80 (1 H, s, H-2), 15.00 (1 H, bs, CO_2H). Anal. ($C_{19}H_{22}N_2O_3$) C, H, N.

Compound **6d**: ^{1}H NMR ($CDCl_3$) δ 0.90−1.05 and 1.20−1.35 (each 2 H, m, cyclopropyl CH_2), 1.60−1.95 (3 H, m, piperidine CH_2 and OH), 2.05−2.20 (2 H, m, piperidine CH_2), 2.75 (3 H, s, CH_3), 2.95−3.13 and 3.30−3.50 (each 2 H, m, piperidine CH_2), 3.90−4.20 (2 H, m, cyclopropyl CH and piperidine CH), 7.20 (1 H, d, J = 8.8 Hz, H-6), 8.25 (1 H, d, J = 8.8 Hz, H-5), 8.95 (1 H, s, H-2), 15.00 (1 H, bs, CO_2H). Anal. ($C_{19}H_{22}N_2O_4$) C, H, N.

Compound **6e**: ^{1}H NMR ($CDCl_3$) δ 0.85−0.95 (2 H, m, cyclopropyl CH_2), 1.05 (3 H, d, J = 5.5 Hz, piperidine CH_3), 1.20−1.30 (2 H, m, cyclopropyl CH_2), 1.40−1.90 (5 H, m, piperidine CH and CH_2), 2.70 (3 H, s, CH_3), 2.90−3.00 and 3.25−3.40 (each 2 H, m, piperidine CH_2), 4.05−4.20 (1 H, m, cyclopropyl CH), 7.20 (1 H, d, J = 8.8 Hz, H-6), 8.25 (1 H, d, J = 8.8 Hz, H-5), 8.80 (1 H, s, H-2), 15.00 (1 H, bs, CO_2H). Anal. ($C_{20}H_{24}N_2O_3$) C, H, N.

Compound **6f**: ^{1}H NMR ($CDCl_3$) δ 0.90−1.00 and 1.15−1.35 (each 2 H, m, cyclopropyl CH_2), 1.60−2.00 (5 H, m, piperidine CH and CH_2), 2.50 (1 H, m, OH), 2.70−3.60 (9 H, m, piperidine CH_2 and CH_3), 4.10−4.30 (1 H, m, cyclopropyl CH), 7.20 (1 H, d, J = 8.8 Hz, H-6), 8.20 (1 H, d, J = 8.8 Hz, H-5), 8.90 (1 H, s, H-2), 15.00 (1 H, bs, CO_2H). Anal. ($C_{20}H_{24}N_2O_4$) C, H, N

Compound **6g**: ^{1}H NMR ($CDCl_3$) δ 0.80−1.00 (8 H, m, cyclopropyl CH_2 and piperidine CH_3), 1.20−1.30 (2 H, m, cyclopropyl CH_2), 1.80−2.00 (4 H, m, piperidine CH_2), 2.30−2.50 (2 H, m, piperidine CH_2), 2.70 (3 H, s, CH_3), 3.15−3.30 (2 H, m, piperidine CH_2), 4.00−4.20 (1 H, m, cyclopropyl CH),

7.10 (1 H, d, J = 8.8 Hz, H-6), 8.20 (1 H, d, J = 8.8 Hz, H-5), 8.90 (1 H, s, H-2), 15.00 (1 H, bs, CO_2H). Anal. ($C_{21}H_{26}N_2O_3$) C, H, N.

Compound **6h**: ^{1}H NMR ($CDCl_3$) δ 0.90−1.30 (4 H, m, cyclopropyl CH_2), 2.70 (3 H, s, CH_3), 2.80−3.00 and 3.35−3.50 (each 4 H, m, thiomorpholine CH_2), 4.05−4.25 (1 H, m, cyclopropyl CH), 7.20 (1 H, d, J = 8.8 Hz, H-6), 8.30 (1 H, d, J = 8.8 Hz, H-5), 8.95 (1 H, s, H-2), 14.95 (1 H, bs, CO_2H). Anal. ($C_{18}H_{20}N_2O_3S$) C, H, N.

Compound **6i**: ^{1}H NMR ($CDCl_3$) δ 0.90−1.05 (2 H, m, cyclopropyl CH_2), 1.20−1.45 (8 H, m, cyclopropyl CH_2 and morpholine CH_3), 2.70−2.85 (5 H, m,CH_3 and morpholine CH_2), 3.10−3.25 (2 H, m, morpholine CH_2), 3.80−4.20 (3 H, m, cyclopropyl CH and morpholine CH), 7.15 (1 H, d, J = 8.8 Hz, H-6), 8.30 (1 H, d, J = 8.8 Hz, H-5), 8.9 0 (1 H, s, H-2), 14.95 (1 H, bs, CO_2H). Anal. ($C_{20}H_{24}N_2O_4$) C, H, N.

Compound **6j**: ^{1}H NMR (DMSO-d_6) δ 0.80−1.00 and 1.05−1.35 (each 2 H, m, cyclopropyl CH_2), 2.00−2.40 (2 H, m, pyrrolidine CH_2), 2.50 (3 H, s, CH_3), 3.35−4.05 (5 H, m, cyclopropyl CH and pyrrolidine CH_2), 4.25−4−40 (1 H, m, pyrrolidine CH), 7.15 (1 H, d, J = 8.8 Hz, H-6), 8.05 (1 H, d, J = 8.8 Hz, H-5), 8.8 0 (1 H, s, H-2), 10.00 (3 H, bs, NH_3^+). Anal. ($C_{18}H_{21}N_3O_3$) C, H, N.

Compound **6k**: ^{1}H NMR (DMSO-d_6) δ 0.85−0.95 and 1.15−1.30 (each 2 H, m, cyclopropyl CH_2), 1.65−1.85 (1 H, m, pyrrolidine CH_2), 1.90 (3 H, s, $CH_3CO_2^-$), 2.00−2.20 (1 H, m, CH_2 pyrrolidine), 2.55 (3 H, s, CH_3), 3.05−3−20 (1 H, m, pyrrolidine CH), 3.35−3.70 (4 H, m, pyrrolidine CH_2), 4.20−4.35 (1 H, m, cyclopropyl CH), 7.05 (1 H, d, J = 9 Hz, H-6), 7.95 (1 H, d, J = 9 Hz, H-5), 8.75 (1 H, s, H-2), 6.20 (3 H, bs, NH_3^+). Anal. ($C_{18}H_{21}N_3O_3 \cdot CH_3CO_2H$) C, H, N.

Compound **6l**: ^{1}H NMR ($CDCl_3$) δ 0.85−1.00 and 1.15−1.20 (each 2 H, m, cyclopropyl CH_2), 2.70 (3 H, s, CH_3), 3.00−3.15 and 3.40−3.50 (each 2 H, m, isoquinoline CH_2), 4.00−4.15 (1 H, m, cyclopropyl CH), 4.35 (2 H, bs, isoquinoline CH_2), 7.05−7.25 (5 H, m, H-6 and aromatic H), 8.25 (1 H, d, J = 8.8 Hz, H-5), 8.9 0 (1 H, s, H-2), 14.95 (1 H, bs, CO_2H). Anal. ($C_{23}H_{22}N_2O_3$) C, H, N.

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