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Studies on 6-Aminoquinolones: Synthesis and Antibacterial Evaluation of 6-Amino-8-ethyl- and 6-Amino-8-methoxyquinolones

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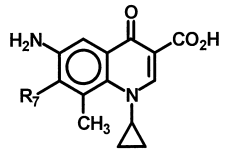
Abstract—From our quantitative structure–activity relationship (QSAR) study on a large set of 6-aminoquinolones, which indicated that a group larger than methyl could be allocated at C-8 position, we have synthesized two new series of 6-aminoquinolones characterized by the presence of an ethyl or a methoxy group at C-8 position. The antibacterial evaluation shows that, while the 8-ethyl derivatives were devoid of any antibacterial activity, the introduction of methoxy group gave compounds with good antibacterial activity, especially against Gram-positive bacteria. A tentative explanation of the different behaviours among the 8-substituted analogues is given taking into account both the length and electronic properties of the C-8 groups.

Introduction

Based on indications from chemometric study,[1,2] we recently developed a new class of antibacterial quinolones characterized by an amino group instead of the typical fluorine atom at C-6 position.[3,4] In this class of 6-aminoquinolones, compounds **1** (Fig. 1) bearing a methyl group at C-8 position show the highest antibacterial activity, particularly against Gram-positive bacteria, including methicillin- and ciprofloxacin-resistant *Staphylococcus aureus*.[4,5] In order to optimize this promising class we carried out a quantitative structure–activity relationship (QSAR) study on a large set of 6-aminoquinolones based on principal component analysis (PCA), projection onto latent structures (PLS), and response surface (RS).[6] The information contained in the response surface model, gave clear information about N-1 and C-7 substituents, while that for C-8 position was less clear. In fact, a large group, such as methyl, was indicated as optimum at C-8, but this information is a biased indication since the methyl group was the largest substituent considered in the set of analyzed molecules.[6] In fact, the quadratic model indicated an open range for the C-8 substituent and perhaps a larger substituent could provide an even greater increase in antibacterial activity. Therefore, to verify whether a larger group could be allocated at C-8, two new series of 6-aminoquinolones **2** and **3**, bearing an 8-ethyl or 8-methoxy group, respectively, were synthesized and tested (Fig. 1). In addition, all of the new compounds bear a cyclopropyl group at N-1 position, as well as *N*-methylpiperazine, tetrahydroisoquinoline, or piperidine, as C-7 side chain, as optimal substituents indicated by QSAR study.



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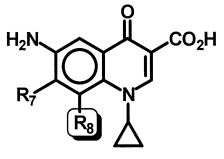

2a-c $R_8 = C_2H_5$
3a-c $R_8 = OCH_3$

Figure 1. For R_7 substituent see Scheme 2.

Chemistry

To prepare both series of 6-amino-8-substituted quinolones the usual intramolecular nucleophilic displacement cyclization method was used, as illustrated in Scheme 1.

Thus, starting from 2,6-dichloroethylbenzene (**5**), prepared by standard procedure, or from commercial 2,6-dichlorofluorobenzene, the nitroacid derivatives **8** and

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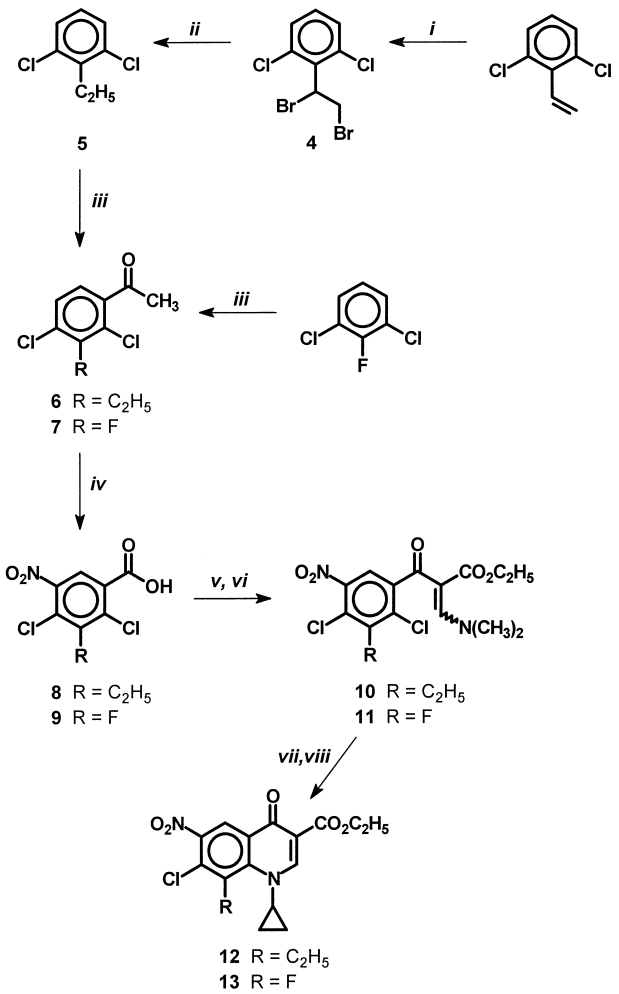


Scheme 1. Reagents: (i) Br_2, $CHCl_3$, 5°C; (ii) $NaBH_4$, DMSO, 60°C; (iii) CH_3COCl, $AlCl_3$; (iv) 90% HNO_3, 98% H_2SO_4; (v) $SOCl_2$ reflux; (vi) $(CH_3)_2NCH{=}CHCO_2C_2H_5$, Et_3N, toluene, 90°C; (vii) *c*-$PrNH_2$, $EtOH/Et_2O$; (viii) KF or K_2CO_3, DMF, 160°C.

9, respectively, were prepared via Friedel–Crafts acetylation and oxidative nitration. These intermediates were converted to the quinolone esters **12** and **13** via the (dimethylamine)acrylate adducts **10** and **11**, corresponding cyclopropyl enamines and ring closure in the presence of KF and/or K_2CO_3.

The desired 6-amino-8-ethyl target acids **2a,c** were then synthesized, as illustrated in Scheme 2, by replacing the C-7 chlorine atom of **12** with *N*-methylpiperazine or piperidine followed by catalytic reduction and acid hydrolysis. To obtain 1,2,3,4-tetrahydroisoquinolinyl target acid **2b**, it was more convenient to carry out the hydrolysis step before the nucleophilic reaction.

Similarly, starting from the 8-fluoro key intermediate **13**, the desired 6-amino-8-methoxy target acids **3a–c** were obtained, as illustrated in Scheme 2, following four sequential steps: nucleophilic displacement of C-6 chlorine atom with the requisite side chain, acid hydrolysis, additional nucleophilic reaction with MeONa in MeOH/DMF in order to replace the C-8 fluorine atom with the desired methoxy group, and finally catalytic reduction of the nitro group.

Biological Assay

The 6-amino-8-substituted quinolone acids prepared for this study (**2a–c** and **3a–c**) were tested for antibacterial activity 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* strain and methicillin-resistant *S. aureus* (MRSA). In addition, the control drug ciprofloxacin (CPX) and 6-amino-8-methyl counterparts (**1a–c**) (Fig. 1), previously reported by us,[4] are included for comparative purposes. The minimun inhibitory concentrations (MICs, μg/mL) were determined by microdilution technique using nutrient broth, according to NCCLS;[7] the data are presented in Table 1. The geometric means of the MICs were also calculated to facilitate a comparison of activity.

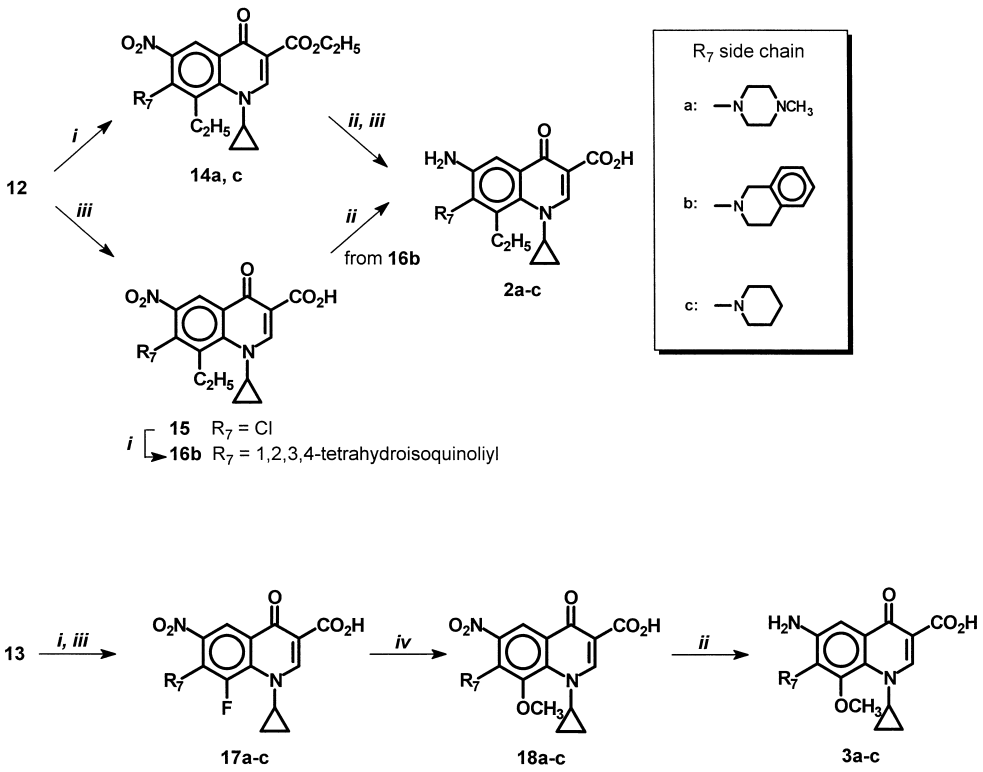
8-Methoxy-7-tetrahydroisoquinolinyl derivative **3b**, as the most active compound, and corresponding 8-ethyl derivative **2b**, were also tested for their ability to inhibit the supercoiling of DNA gyrase, using a previously described protocol.[8] This assay measures the conversion of relaxed closed-circular pUC8 (prepared from *E. coli* strain K12JM83) into the supercoiled form by the intervention of DNA gyrase. The A and B subunits of *E. coli* DNA gyrase were purified from over-expression vector strains N4186 and MK47, respectively. The IC_{50} values were calculated by the quantitative measurement of the supercoiled DNA peak in an agarose gel by densitometric assay.

Results and Discussion

The MIC values reported in Table 1 indicate that the introduction of ethyl group at C-8 position causes a marked decrease in antibacterial activity, while with a methoxy group at C-8 position, the resulting compounds maintain good activity. Compared with the 8-methyl counterparts **1a–c**, the compounds are about one dilution less potent but have the same trend: the (1-methyl)piperazinyl derivative **3a** is more potent against Gram-negative bacteria, while the 1,2,3,4-tetrahydroisoquinolinyl derivative **3b** and piperidinyl derivative **3c** are more potent against Gram-positive. It is noteworthy that, against Gram-positive, compound **3c** is as potent as CPX, and compound **3b** is 5 times more potent than CPX with a MIC value against methicillin-resistant *S. aureus* of 0.016 μg/mL.

The DNA gyrase inhibitory activity evaluated for all 6-aminoquinolones having a tetrahydroisoquinolinyl group at C-7 position gave an IC_{50} value of 65 μg/mL for 8-ethyl derivative **2b**, an IC_{50} value of 11.3 μg/mL for the 8-methoxy analogue **3b**, and an IC_{50} value of 3.6 μg/mL for the 8-methyl counterpart **1b** (CPX $IC_{50} = 0.68$ μg/mL) which confirmed the trend observed in the MIC values.

The fact that the presence of 8-methoxy group in the 6-aminoquinolone class gives compounds with good antibacterial activity, particularly against Gram-positive, is in agreement with what has already been reported in the



Scheme 2. Reagents: (i) R_7H, CH_3CN, reflux; (ii) H_2, Raney-Ni, DMF or $CH_3OCH_2CH_2OH$; (iii) 6 N HCl, EtOH, reflux; (iv) CH_3ONa, CH_3OH/DMF, 80°C.

Table 1. Comparative in vitro antibacterial activity for 6-amino-8-ethyl (**2**) and 6-amino-8-methoxyquinolones (**3**), prepared in this study, and 8-methyl analogues (**1**)

Organisms[a]	(MICs, μg/mL)									
	1a	**2a**	**3a**	**1b**	**2b**	**3b**	**1c**	**2c**	**3c**	CPX[b]
Gram-positives										
S. au. ATCC 29213	1	>16	2	≤0.016	4	≤0.016	0.06	8	0.06	0.5
S. au. MPR 5	1	>16	1	≤0.016	4	≤0.016	≤0.016	8	0.03	0.25
S. au. M-R[c] POMM 6214	4	>16	4	≤0.016	4	≤0.016	0.125	16	0.125	1
S. au. CPX-R[d] OBT 687	>16	>16	>16	2	>16	2	8	>16	16	>16
S. py. OMNFI BI	4	>16	8	≤0.016	>16	0.25	0.5	>16	4	0.5
S. pn. I 043	8	>16	16	0.25	>16	4	2	>16	4	1
E. fe. LEP Br	2	>16	16	0.25	>16	0.5	0.5	>16	4	1
E. fe. UCMC 39690	4	>16	8	0.125	0.5	0.125	1	8	2	0.5
Geometric means	3.364	>16	6.169	0.075	6.169	0.164	0.385	12.338	0.908	0.917
Gram-negatives										
E. co. ATCC 25922	0.03	>16	0.125	0.125	16	0.25	0.125	16	0.5	≤0.016
E. co. 120	0.06	>16	0.125	0.125	>16	0.5	0.5	16	0.5	≤0.016
E. co. CPX-R[d] OBT 431	>16	>16	>16	>16	>16	>16	>16	>16	>16	8
E. cl. OMNFI 174	0.125	>16	0.5	0.5	>16	2	1	>16	4	≤0.016
P. mi. OBT 505	1	>16	4	>16	>16	8	4	>16	8	0.06
P. vu. CNUR 6	8	>16	>16	8	>16	>16	>16	>16	>16	0.5
K. pn. ATCC 10031	0.03	>16	0.08	0.03	16	0.25	≤0.016	4	0.08	≤0.016
P. ae. ATCC 9027	2	>16	1	8	>16	16	4	>16	8	0.06
P. ae. OBT 307	8	>16	>16	>16	8	8	16	>16	>16	1
H. in.	0.03	>16	≤0.016	≤0.016	2	0.03	≤0.016	8	0.03	≤0.016
Geometric means	0.459	>16	0.834	0.998	12.126	1.858	1.005	12.996	1.905	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.

fluoroquinolone class. In fact, some 6-fluoro-8-methoxy derivatives are in clinical use or preclinical phase (e.g. gatifloxacin,[9] balofloxacin,[10] and moxifloxacin[11]).

Turning our attention to 6-aminoquinolones, the simplest explanation for the different behaviour in the three series (**1**, **2**, and **3**) could be due to different steric size of the substituents at C-8 which can affect the conformational orientation of the groups in the N-1 and C-7 positions. The presence of CH_3, C_2H_5 and OCH_3 groups at C-8 position in reality did not cause different conformational orientations of the substituents at N-1 and C-7 positions. In fact, the graphic analysis of the molecular structures[12] (data not shown) shows how the width of the atom bonded at C-8 of the aromatic ring is almost identical for the three considered groups, such that the conformations of the three series of derivatives can almost be superimposed. Therefore, the discriminating factor for the activity could be the substituent length. This hypothesis could well explain the drastic decrease in activity of the 8-ethyl derivatives (**2a**–**c**) when compared with the shorter methyl (**1a**–**c**) but does not explain the good activity of the 8-methoxy derivative (**3a**–**c**), given that the methoxy group is as long as the ethyl one. This apparent contradiction could be explained by considering the different electronic properties of the ethyl and methoxy groups. If it is true that the increased length of the substituents causes a decrease in activity, the presence of an oxygen atom bonded at C-8 may provide the electronic requirement that compensates, in part, for the loss of activity. The oxygen lone pair of the C-8 bonded group may interact with the π electron of the aromatic ring of quinolone thereby affecting the quinolone staking, as has been suggested in the various proposed modes of quinolone/DNA/DNA-gyrase interactions.[13,14]

Experimental

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.

1,3-Dichloro-2-(1,2-dibromoethyl)benzene (4). Bromine (5.1 g, 31.9 mmol) in $CHCl_3$ (10 mL) was added dropwise to a solution of 2,6-dichlorostyrene (5.0 g, 29 mmol) in $CHCl_3$ (15 mL) maintained at 5°C, and the mixture was allowed to react at room temperature for 30 min. The mixture was then washed with a solution of saturated $Na_2S_2O_3$ to eliminate the bromine excess, and the organic layer was dried, and evaporated to dryness. The whitish semisolid **4** (9.46 g, 98%) was used without further purification in the subsequent reaction: ^{1}H NMR ($CDCl_3$) δ 4.00 (1H, dd, $J = 8$ and 16 Hz, CH_2), 4.72 (1H, t, $J = 16$ Hz, CHBr), 6.08 (1H, d, $J = 8$ and 16 Hz, CH_2), 7.15–7.45 (3H, m, aromatic H).

1,3-Dichloro-2-ethylbenzene (5). A solution of **4** (5 g, 15 mmol) in dry DMSO (10 mL) was added to a suspension of $NaBH_4$ (2.3 g, 60 mmol) in dry DMSO (20 mL) maintained at 60°C and the mixture was allowed to react for 40 min. After cooling, the reaction mixture was poured into ice–water and extracted several times with cyclohexane. The combined organic layers were dried and evaporated to dryness to give **5** (1.65 g, 63%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 1.20 (3H, t, $J = 7$ Hz, CH_2CH_3), 2.90 (2H, q, $J = 7$ Hz, CH_2CH_3), 6.95 (1H, t, $J = 9$ Hz, H-5), 7.20 (2H, d, $J = 9$ Hz, H-4 and H-6).

1-(2,4-Dichloro-3-ethylphenyl)ethanone (6). Some drops of acetyl chloride were added to a mixture of **2** (1 g, 5.71 mmol) and $AlCl_3$ (1.52 g, 11.42 mmol) which was heated to 40°C to trigger the reaction (to generate HCl). After cooling to room temperature, acetyl chloride (0.45 g, 5.71 mmol) was added dropwise and the reaction mixture was allowed to react for 2 h. The mixture was poured into ice–water and acidified with 2 N HCl. The solution was extracted several times with CH_2Cl_2 and the combined organic layers were washed with water, dried, and evaporated to dryness and the residue was purified by column chromatography eluting with petroleum ether to give **6** (0.82 g, 67%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.20 (3H, t, $J = 7$ Hz, CH_2CH_3), 2.60 (3H, s, CH_3), 3.00 (2H, q, $J = 7$ Hz, CH_2CH_3), 7.20 (1H, d, $J = 9.5$ Hz, H-5), 7.35 (1H, d, $J = 9.5$ Hz, H-6). Anal. ($C_{10}H_{10}Cl_2O$) C, H.

1-(2,4-Dichloro-3-fluorophenyl)ethanone (7). The title compound was prepared starting from 1,3-dichloro-2-fluorobenzene, using the procedure as described for **6**, except that the reaction mixture was heated at 120°C for 2 h and when the mixture was poured in water, compound **7** was obtained as a white solid in 61% yield: mp 146–147°C; ^{1}H NMR ($CDCl_3$) δ 2.60 (3H, s, CH_3), 7.20–7.40 (2H, m, H-5 and H-6). Anal. ($C_8H_5Cl_2FO$) C, H.

2,4-Dichloro-3-ethyl-5-nitrobenzoic acid (8). 90% HNO_3 (1.22 g, 19.2 mmol) was added to a solution of **6** (1.04 g, 4.8 mmol) in 98.5% H_2SO_4 (10.5 mL) maintained at 20°C. After heating for 45 min at 50–60°C, the mixture was poured in ice–water and the resulting precipitate was filtered off, solubilized in diethyl ether and extracted with 5% NaOH solution. After treatment of this solution with 2 N HCl a precipitate was obtained which was filtered off, washed with water, and dried to give **8** (0.89 g, 70.5%) as a white solid: mp 136–139°C; ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, t, $J = 7$ Hz, CH_2CH_3), 3.20 (2H, q, $J = 7$ Hz, CH_2CH_3), 8.25 (1H, s, H-6), 9.10 (1H, bs, COOH). Anal. ($C_9H_7Cl_2NO_4$) C, H, N.

2,4-Dichloro-3-fluoro-5-nitrobenzoic acid (9). The title compound was prepared using the procedure as described for **8** starting from **7**. It was obtained in 70% yield as a white solid: mp 47–49°C; ^{1}H NMR ($CDCl_3$) δ 8.50 (1H, bs, H-5), 10.50 (1H , bs, CO_2H). Anal. ($C_7H_2Cl_2FNO_4$), C, H, N.

Ethyl 2-(2,4-dichloro-3-ethyl-5-nitrobenzoyl)-3-(dimethylamino)acrylate (10). A mixture of **8** (0.6 g, 2.3 mmol) and thionyl chloride (3 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 (10 mL) and added to ethyl 3-(dimethylamino)acrylate[15] (0.427 g, 2.99 mmol) and dry Et_3N (0.35 g, 3.45 mmol). The resulting solution was heated at 90°C for 2 h. After cooling and filtering off the insoluble material, the solvent was evaporated to dryness and the residue was purified by column chromatography eluting with EtOAc:petroleum ether (3:7) to give **10** (0.64 g, 72%) as a pale yellow solid: mp 127–129°C; ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, $J = 7$ Hz, CH_2CH_3), 1.20 (3H, t, $J = 7$ Hz, OCH_2CH_3), 3.00 (3H, s, NCH_3), 3.10 (2H, q, $J = 7$ Hz, CH_2CH_3), 3.40 (3H, s, NCH_3), 3.90 (2H, q, $J = 7$ Hz, OCH_2CH_3), 7.60 (1H, s, vinyl H), 8.00 (1H , s, H-6). Anal.($C_{16}H_{18}Cl_2N_2O_5$) C, H, N.

Ethyl 2-(2,4-dichloro-3-fluoro-5-nitrobenzoyl)-3-(dimethylamino)acrylate (11). The title compound was prepared using the procedure as described for **10** starting from **9**. It was obtained in 65% yield as a yellow solid: mp 76–78°C; ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, t, $J = 7$ Hz, CH_2CH_3), 3.00 and 3.40 (each 3H, s, NCH_3), 4.00 (2H, q, $J = 7$ Hz, CH_2CH_3), 7.80 (1H, d, $J = 2$ Hz, H-6), 8.00 (1H, s, vinyl H). Anal. ($C_{14}H_{13}Cl_2FN_2O_5$) C, H, N.

Ethyl 1-cyclopropyl-7-chloro-8-ethyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (12). A stirred solution of **10** (0.9 g, 2.31 mmol) in EtOH (15 mL) and Et_2O (10 mL) was treated dropwise with cyclopropylamine (0.211 g, 3.7 mmol). After 30 min at room temperature, the mixture was evaporated to dryness to give a residue which was solubilized in dry DMF (10 mL). KF (0.40 g, 6.9 mmol) was added to this solution and the mixture was heated at 160°C for 4 h. After cooling, the reaction mixture was poured into ice–water, neutralized with saturated solution of $NaHCO_3$ and extracted with $CHCl_3$. The combined organic layers were dried and evaporated to dryness to give a residue which was purified by column chromatography eluting with EtOAc:cyclohexane (2:8) to give **12** (0.56 g, 61%) as a yellow solid: mp 187–189°C; ^{1}H NMR ($CDCl_3$) δ 0.90–1.00 (2H, m, cyclopropyl CH_2), 1.20 (3H, t, $J = 7$ Hz, CH_2CH_3), 1.25–1.35 (2H, m, cyclopropyl CH_2), 1.45 (3H, t, $J = 7$ Hz, OCH_2CH_3), 3.70 (2H, q, $J = 7$ Hz, CH_2CH_3), 3.90–4.05 (1H, m, cyclopropyl CH), 4.40 (2H, q, $J = 7$ Hz, OCH_2CH_3), 8.65 (1H, s, H-2), 8.75 (1H , s, H-5). Anal. ($C_{17}H_{17}ClN_2O_5$) C, H, N.

Ethyl 7-chloro-1-cycloproyl-8-fluoro-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (13). The title compound was prepared using the procedure as described for **12** starting from **11** except that in the cyclization step K_2CO_3 was used instead of KF. It was obtained in 65% yield as a yellow solid: mp 150–152°C (dec); ^{1}H NMR (DMSO-d_6) δ 1.10–1.35 (7H, m, cyclopropyl CH_2 and CH_2CH_3), 3.95–4.10 (1H, m, cyclopropyl CH), 4.25 (2H, q, $J = 7$ Hz, CH_2CH_3), 8.50 (1H, s, H-5), 8.60 (1H, s, H-2). Anal. ($C_{15}H_{12}ClFN_2O_5$) C, H, N.

6-Amino-1-cyclopropyl-8-ethyl-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (2a). A mixture of **12** (0.30 g, 0.823 mmol), Et_3N (0.332 g, 3.3 mmol) and *N*-methylpiperazine (0.33 g, 3.3 mmol) in dry CH_3CN (5 mL) was refluxed for 24 h. The solvent was then evaporated to dryness and the residue was triturated with Et_2O to give **14a** (0.283 g, 80.3%) as a yellow solid: mp 221–223°C; ^{1}H NMR ($CDCl_3$) δ 0.80–1.30 (7H, m, cyclopropyl CH_2 and CH_2CH_3), 1.45 (3H, t, $J = 7$ Hz, OCH_2CH_3), 2.40 (3H, s, piperazine CH_3), 2.45–2.70 and 3.10–3.30 (each 4H, m, piperazine CH_2), 3.40 (2H, q, $J = 7$ Hz, CH_2CH_3), 3.80–3.95 (1H, m, cyclopropyl CH), 4.35 (2H, m, $J = 7$ Hz, OCH_2CH_3), 8.50 (1H, s, H-2), 8.65 (1H, s, H-5).

A stirred solution of **14a** (0.10 g, 0.23 mmol) in 2-methoxyethanol (30 mL), was hydrogenated over a catalytic amount of Raney nickel, at room temperature and 2.05 atm pressure for 4 h. The mixture was then filtered over Celite, and the filtrate was evaporated to dryness to give a residue which was solubilized in EtOH:HCl 6 N (1:1) (4 mL) and refluxed for 2 h. After cooling, the crystalline precipitate was filtered off, dried and crystallized by EtOH to give **2a** (0.076 g, 82%) as white solid: 299–300°C; ^{1}H NMR (DMSO-d_6) δ 0.80–1.25 (7H, m, cyclopropyl CH_2 and CH_2CH_3), 2.70 (3H, s, piperazine CH_3), 3.10–3.40 (4H, m, piperazine CH_2), 3.50–3.70 (6H, m, piperazine CH_2 and CH_2CH_3), 4.05–4.20 (1H, m, cyclopropyl CH), 7.50 (1H, s, H-5), 8.70 (1H, s, H-2), 10.50 (1H, bs, COOH). Anal. ($C_{20}H_{26}N_4O_3$·HCl) C, H, N.

6-Amino-1-cyclopropyl-8-ethyl-7-(1-piperidinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (2c). It was obtained using the same procedure employed for the preparation of **2a**, starting from nitroester **12** and using piperidine as nucleophile: mp 294–296°C; ^{1}H NMR (DMSO-d_6) δ 0.80–0.85 (2H, m, cyclopropyl CH_2), 1.05 (3H, t, $J = 7$ Hz, CH_2CH_3), 1.15–1.30 (2H, m, cyclopropyl CH_2), 1.70–1.85 (6H, m, piperidine CH_2), 3.05–3.20 (4H, m, piperidine CH_2), 3.40 (2H, q, $J = 7$ Hz, CH_2CH_3), 3.80–3.95 (1H, m, cyclopropyl CH), 5.60 (2H, bs, NH_2), 7.50 (1H, s, H-5), 8.60 (1H, s, H-2). Anal. ($C_{20}H_{25}N_3O_3$·HCl) C, H, N.

6-Amino-1-cyclopropyl-8-ethyl-7-(1,2,3,4-tetrahydro-2-isoquinolinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2b). The mixture of ester **12** (0.123 g, 0.337 mmol) in EtOH (2 mL) and 6 N HCl (2 mL) was refluxed for 3 h. After cooling, the crystalline precipitate was filtered off, washed with dry EtOH, and dried to give acid **15** (0.105 g, 93%): mp 228–230°C; ^{1}H NMR ($CDCl_3$) δ 0.95–1.05 (2H, m, cyclopropyl CH_2), 1.15 (3H, t, $J = 7$ Hz, CH_2CH_3), 1.20–1.30 (2H, m, cyclopropyl CH_2), 3.70 (2H, q, $J = 7$ Hz, CH_2CH_3), 4.20–4.35 (1H, m, cyclopropyl CH), 8.70 (1H, s, H-2), 8.90 (1H, s, H-5).

A mixture of acid **15** (0.12 g, 0.36 mmol) Et_3N (0.216 g, 214 mmol) and 1,2,3,4-tetrahydroisoquinoline (0.24 g,

1.78 mmol) in dry Me_3CN (5 mL) was refluxed for 30 h. The solvent was then evaporated to dryness and the residue was purified by column chromatography eluting with $CHCl_3$ to give **16b** (0.08 g, 52%): mp 287–291°C; ^{1}H NMR ($CDCl_3$) δ 0.90–1.30 (5H, m, cyclopropyl CH_2 and CH_2CH_3), 1.50–1.70 (2H, m, cyclopropyl CH_2), 2.90–3.05 (2H, m, isoquinoline CH_2), 3.30–3.50 (4H, m, isoquinoline CH_2 and CH_2CH_3), 3.90–4.05 (1H, m, cyclopropyl CH), 4.55 (2H, bs, isoquinoline CH_2), 7.00–7.30 (4H, m, isoquinoline CH), 8.70 (1H, s, H-2), 8.90 (1H, s, H-5), 14.20 (1H, bs, COOH).

A stirred solution of **16b** (0.080 g, 0.184 mmol) in a mixture of EtOH:DMF (9:1) (50 mL) was hydrogenated over catalytic amount of Raney nickel at room temperature and 2.05 atm pressure for 6 h. The mixture was then filtered over Celite, and the filtrate was evaporated to dryness. The solid residue was treated with petroleum ether to give **2b** (0.034 g, 46%) as white solid: mp 287–291°C; ^{1}H NMR (DMSO-d_6) δ 0.90–1.10 (5H, m, cyclopropyl CH_2 and CH_2CH_3), 1.15–1.30 (2H, m, cyclopropyl CH_2), 2.90–3.05 (2H, m, isoquinoline CH_2), 3.35–3.60 (4H, m, isoquinoline CH_2 and CH_2CH_3), 3.90–4.05 (1H, m, cyclopropyl CH), 4.40–4.55 (2H, m, isoquinoline CH_2), 5.50 (2H, bs, NH_2), 7.20–7.50 (4H, m, CH isoquinoline), 7.70 (1H, s, H-5), 8.70 (1H, s, H-2), 14.20 (1H, bs, COOH). Anal. ($C_{24}H_{25}N_3O_3$) C, H, N.

6-Amino-1-cyclopropyl-8-methoxy-7-(1-piperidinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c). The mixture of ester **13** (1.59 g, 4.64 mmol) and piperidine (1.8 mL, 18.5 mmol) in dry MeCN (45 mL) was refluxed for 2 h. After cooling the precipitate solid was filtered, washed with Et_2O, suspended in EtOH:HCl 6 N (1:1) (20 mL) and refluxed for 2 h. After cooling, the precipitate was filtered, washed with water, then with Et_2O and dried to give **17c** (0.96 g, 55%): mp 237–238°C; ^{1}H NMR (DMSO-d_6) δ 1.15–1.30 (4H, m, cyclopropyl CH_2), 1.25–1.55 (6H, m, piperidine CH_2), 3.10–3.25 (4H, m, piperidine CH_2), 4.05–4.20 (1H, m, cyclopropyl CH), 8.45 (1H, d, $J = 2$ Hz, H-5), 8.70 (1H, s, H-2), 14.20 (1H , bs, COOH).

A solution of MeONa (0.45 g, 8.4 mmol) in dry DMF (8 mL) and dry MeOH (5 mL) was added to a suspension of 8-fluoro derivative **17c** (0.47 g, 1.2 mmol) in dry DMF (20 mL). The mixture was heated to 80–90°C for 40 h, then cooled and poured into ice–water. The solution was made acid with 2 N HCl, and extracted with EtOAc. The combined organic layers were dried, evaporated to dryness and the residue obtained was crystallized by acetone to give **18c** (0.2 g, 43.5%) as a white solid: mp 224–225°C; ^{1}H NMR ($CDCl_3$) δ 0.90–1.05 and 1.20–1.35 (each 2H, m, cyclopropyl CH_2), 1.70–1.85 (6H, m, piperidine CH_2), 3.20–3.35 (4H, m, piperidine CH_2), 3.80 (3H, s, OCH_3), 3.95–4.10 (1H, m, cyclopropyl CH), 8.50 (1H, s, H-5), 8.85 (1H, s, H-2), 14.30 (1H, bs, COOH).

The solution of **18c** (0.24 g, 0.6 mmol) in MeOH (30 mL) was hydrogenated over catalytic amount of Raney nickel at room temperature and 2.05 atm pressure for 1 h. The mixture was then filtered over Celite. The filtrate was evaporated to dryness and the residue was triturated with a mixture of $CHCl_3/Et_2O$ to give **3c** (0.13 g, 60%): mp 262–263°C; ^{1}H NMR (DMSO-d_6) δ 0.90–1.15 (4H, m, cyclopropyl CH_2), 1.55–1.80 (6H, m, piperidine CH_2), 3.00–3.15 (4H, m, piperidine CH_2), 3.60 (3H, s, OCH_3), 4.00–4.15 (1H, m, cyclopropyl CH), 5.40 (2H, bs, NH_2), 7.35 (1H, s, H-5), 8.50 (1H, s, H-2), 15.65 (1H, bs, COOH). Anal. ($C_{19}H_{23}N_3O_4$) C, H, N.

6-Amino-1-cyclopropyl-8-methoxy-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3a). This compound was synthesized using the same synthetic procedure employed for the preparation of **3c**, starting from nitro ester **13** and using *N*-methylpiperazine as nucleophile: mp 264–266°C; ^{1}H NMR (DMSO-d_6) δ 0.90–1.20 (4H, m, cyclopropyl CH_2), 2.70 (3H, s, piperazine CH_3), 3.10–3.20 and 3.40–3.65 (each 4H, m, piperazine CH_2), 3.65 (3H, s, OCH_3), 4.05–4.20 (1H, m, cyclopropyl CH), 5.40 (2H, bs, NH_2), 7.40 (1H, s, H-5), 8.55 (1H, s, H-2), 15.60 (1H, bs, COOH). Anal. ($C_{19}H_{24}N_4O_4$) C, H, N.

6-Amino-1-cyclopropyl-8-methoxy-7-(1,2,3,4-tetrahydro-2-isoquinolinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3b). This compound was synthesized using the same synthetic procedure employed for the preparation of **3c**, starting from nitro ester **13** and using 1,2,3,4-tetrahydroisoquinoline as nucleophile: mp 258–260°C; ^{1}H NMR (DMSO-d_6) δ 0.90–1.20 (4H, m, cyclopropyl CH_2), 2.95–3.05 and 3.40–3.55 (each 2H, m, isoquinoline CH_2), 3.70 (3H, s, OCH_3), 4.30 (2H, bs, isoquinoline CH_2), 4.05–4.20 (1H, m, cyclopropyl CH), 5.40 (2H, bs, NH_2), 7.05–7.25 (4H, m, isoquinoline aromatic H), 7.40 (1H, s, H-5), 8.55 (1H, s, H-2), 15.55 (1H, bs, COOH). Anal. ($C_{23}H_{23}N_3O_4$) C, H, N.

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