Synthesis of Possible Metabolites of 1-Cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic Acid (Grepafloxacin, OPC-17116)

Seiji MORITA,*[,a] Kenji OTSUBO,[a] Jun MATSUBARA,[a] Tadaaki OHTANI,[a] Yoshikazu KAWANO,[a] Kazunori OHMORI,[b] Kinue OHGURO,[b] and Minoru UCHIDA[a]

Tokushima Research Institute,[a] and Microbiological Research Institute,[b] Otsuka Pharmaceutical Co., Ltd., Kagasuno 463–10, Kawauchi-cho, Tokushima 771–01, Japan. Received March 10, 1995; accepted August 9, 1995

Grepafloxacin (1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid, OPC-17116) (1) exhibits potent and broad-spectrum *in vitro* and *in vivo* antibacterial activity. In order to identify the structures of the metabolites of grepafloxacin, 17 possible metabolites were prepared. Among them, 6 compounds were found to be actual metabolites (2a, b, 4a, b and 6a, b) of grepafloxacin in rats, dogs and/or humans. The antibacterial activities of these metabolites were found to be weaker than that of grepafloxacin.

Key words metabolite; grepafloxacin; quinolone carboxylic acid; 1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid; antibacterial activity; antibacterial agent

The quinolone carboxylic acid derivative (±)-1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (Grepafloxacin, OPC-17116) (**1**), a new antibacterial agent, was synthesized by Miyamoto and co-workers.[1] Grepafloxacin (**1**) is characterized by possessing a methyl group at the C-5 position on the quinolone ring and exhibits potent antibacterial activity against gram-positive and gram-negative bacteria. It was found that **1** was distributed to various tissues, especially the lungs, in a pharmacokinetic study.[2] The metabolism of quinolone carboxylic acids, nadifloxacin, ciprofloxacin, enoxacin and lomefloxacin, has been studied.[3] In order to identify the metabolites of **1**, 17 possible metabolites (**2a, b**, **3a—d**, **4a, b**, **5a, b**, **6a, b**, **7a—c** and **8a, b**) were synthesized (Fig. 1). These are the piperazine ring-cleaved products (**2a, b**, **3a—d** and **4a, b**), the oxidized derivatives (**5a, b** and **6a, b**) at the methyl group on the piperazine ring and the 5-methyl group on the quinolone ring, and others (**7a—c** and **8a, b**). We wish to report here the synthesis of these compounds and the antibacterial activities of the actual metabolites (**2a, b**, **4a, b** and **6a, b**).[2,4]

Synthesis

Compounds **2a, b**, **3a—d**, **4a, b** and **5a—c** were synthesized as shown in Chart 1. Condensation of the borate complex **9**[5] with various amines in dimethyl sulfoxide (DMSO) afforded the corresponding 7-amino-quinoline derivatives **2a, b**, **5a, c**, **10** and **11**. The 7-ethylenediamino derivative **2a** hydrochloride was prepared by deprotection of the *N-tert*-butoxycarbonyl (Boc) group with 1 N HCl in EtOH in 66% yield from **9**.

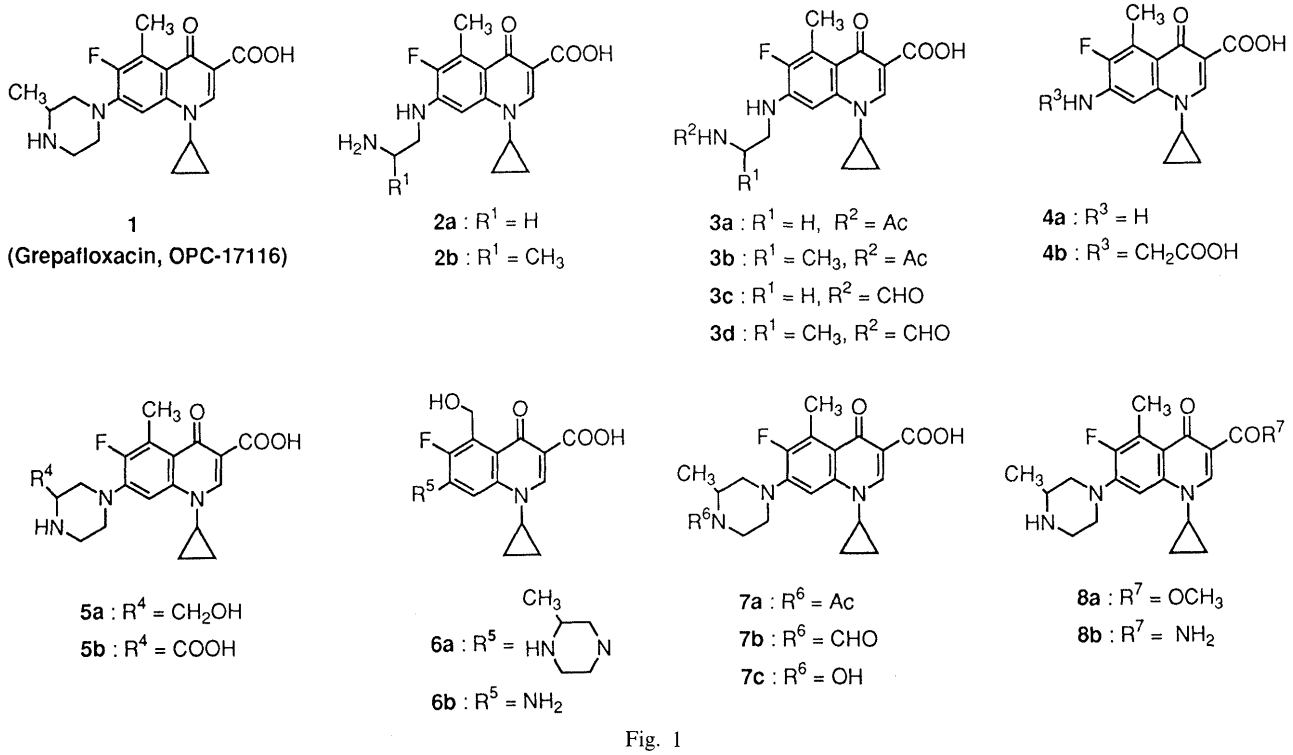


Fig. 1

* To whom correspondence should be addressed.

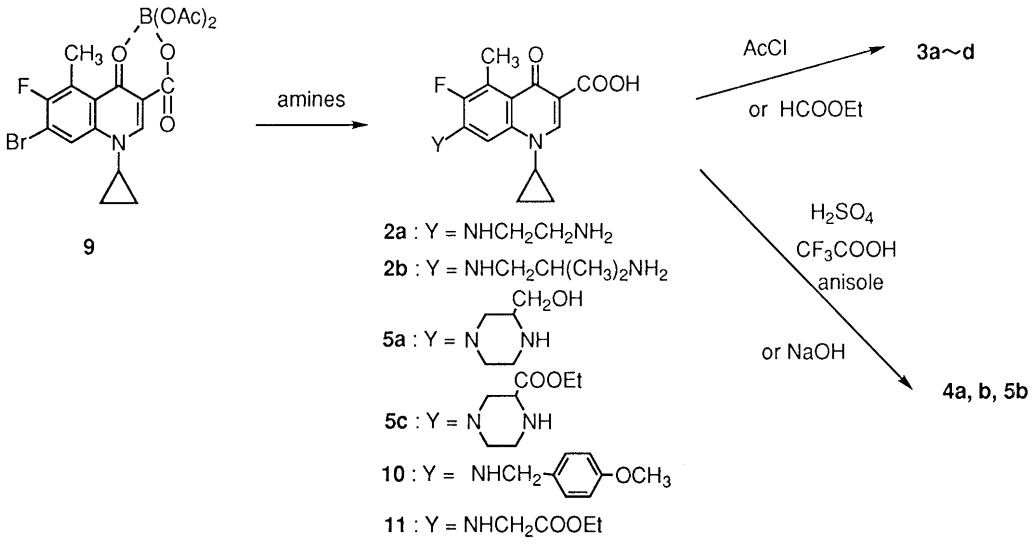
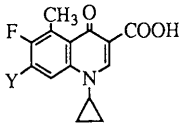
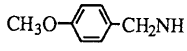


Chart 1

Table 1. Yields and Physical Data for 7-Substituted 1-Cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acids



Compd. No.	Y	Yield (%)[a]	mp (°C)	Appearance (Recrystn. solv.)	Formula	Analysis (%) Calcd (Found)		
						C	H	N
2a·HCl	$HCl \cdot H_2NCH_2CH_2NH$	66	289—291 (dec.)	Colorless needles ($EtOH-H_2O$)	$C_{16}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$	51.41 (51.74)	5.66 5.67	11.24 11.31)
2a	$H_2NCH_2CH_2NH$	89	261—263 (dec.)	Pale yellow powder ($DMF-H_2O$)	$C_{16}H_{18}FN_3O_3$	60.18 (59.82)	5.68 5.72	13.16 13.04)
2b·HCl	$HCl \cdot H_2NCH(CH_3)CH_2NH$	36	298—299 (dec.)	Pale yellow needles ($EtOH-H_2O$)	$C_{17}H_{20}FN_3O_3 \cdot HCl$	54.59 (54.43)	5.79 5.76	11.23 11.18)
						Water content 1.1% corrected		
2b	$H_2NCH(CH_3)CH_2NH$	91	238—239	White powder (EtOH)	$C_{17}H_{20}FN_3O_3$	57.45 (57.20	6.37 6.03	11.82 11.75)
						Water content 6.2% corrected		
5a[b]		11	219—221	Yellow powder (MeOH)	$C_{19}H_{22}FN_3O_4 \cdot 1/4H_2O$	60.07 (60.12	5.97 5.92	11.06 11.15)
5c[b]		50		Yellow oil	$C_{21}H_{24}FN_3O_5$			
10		68	171—175	Yellow powder (MeOH)	$C_{22}H_{21}FN_2O_4$	66.66 (66.21	5.34 5.09	7.07 7.08)
11[c]	$EtOOCCH_2NH$	35		Pale brown powder	$C_{18}H_{19}FN_2O_5$			

a) Isolated yield from **9**. *b*) These starting materials (2-hydroxymethylpiperazine and 2-ethoxycarbonylpiperazine) were prepared by the method described in reference 5. *c*) This **11** was used in the next step without further purification.

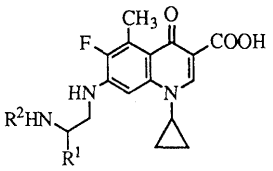
The desired compound **2a** was obtained by neutralization of the hydrochloride with sodium carbonate in water in 89% yield.

Acetylation of **2a** hydrochloride with acetyl chloride in the presence of 1 N NaOH in acetone gave the acetate **3a** in 22% yield. Formylation of **2a** hydrochloride with ethyl formate in the presence of triethylamine (Et_3N) in *N*,*N*-dimethylformamide (DMF) afforded **3c** in 71% yield.

(2-Methyl)ethylenediamine series **3b** and **d** were also prepared by the same methods as described above.

Hydrolysis of **10** with concentrated sulfuric acid in the presence of trifluoroacetic acid in anisole afforded the 7-amino derivative **4a** in 51% yield. The carboxylic acid derivatives **4b** and **5b** were obtained by hydrolysis of the esters **11** and **5c** with NaOH in 12 and 45% overall yields from **9**, respectively.

Table 2. Yields and Physical Data for 7-Substituted Amino 1-Cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acids



Compd. No.	R^1	R^2	Yield (%)	mp (°C)	Appearance (Recrystn. solv.)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
3a	H	Ac	22	225—256	Colorless needles (EtOH)	$C_{18}H_{20}FN_3O_4 \cdot 3/2H_2O$	55.66 (55.52	5.97 6.02	10.82 10.82)
3b	CH_3	Ac	71	237—238	Pale yellow powder (MeOH–Et_2O)	$C_{19}H_{22}FN_3O_4$	60.79 (60.65	5.91 6.04	11.19 11.20)
3c	H	CHO	71	286—289.5	Pale yellow needles (DMF–H_2O)	$C_{17}H_{18}FN_3O_4$	58.78 (58.51	5.22 5.27	12.10 11.93)
3d	CH_3	CHO	74	229—230.5	White powder (MeOH–Et_2O)	$C_{18}H_{20}FN_3O_4$	59.83 (59.59	5.58 5.55	11.63 11.60)

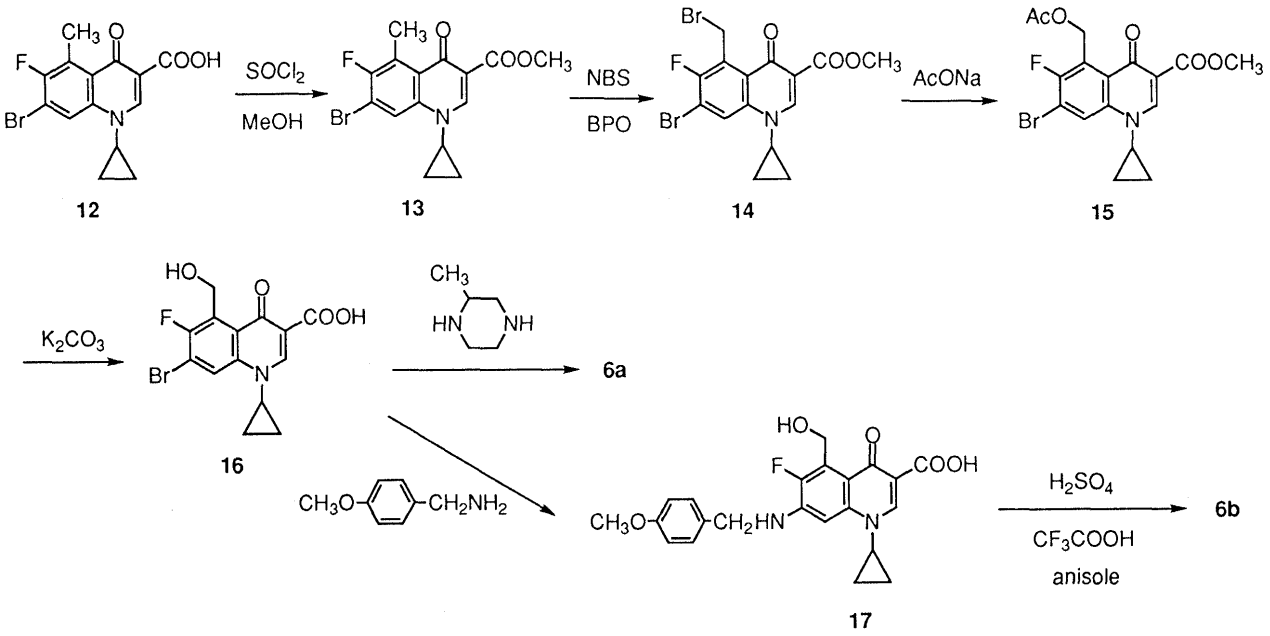


Chart 2

The synthetic route to the 5-hydroxymethyl quinolone derivatives **6a** and **b** is illustrated in Chart 2. The key intermediate **16** was prepared from **12**[5] in four steps. Esterification of **12** with thionyl chloride–methanol afforded the methyl ester **13** in 97% yield, and this was reacted with *N*-bromosuccinimide (NBS) in the presence of benzoyl peroxide (BPO) in carbon tetrachloride (CCl_4) to give the 5-bromomethyl derivative **14** in 53% yield. Treatment of **14** with sodium acetate in DMF gave the 5-acetoxymethyl derivative **15** in 70% yield. This product was hydrolyzed with 10% aqueous potassium carbonate in MeOH to afford **16** in 94% yield. The target compound **6a** was prepared by condensation of **16** with 2-methylpiperazine in pyridine in 60% yield. Compound **6b** was synthesized from **16** by a procedure similar to that used for **4a**.

The syntheses of **7a, b** and **8a, b** were readily accomplished from the mother compound **1** hydrochloride as shown in Chart 3. The acetyl derivative **7a** and formyl

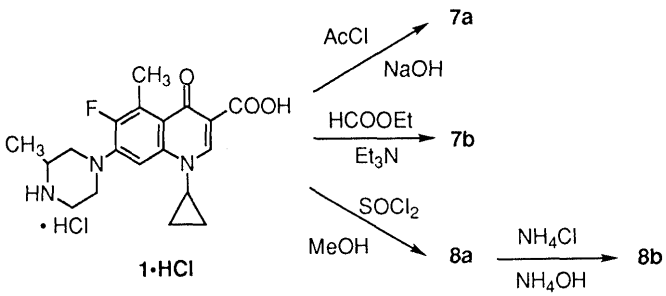


Chart 3

derivative **7b** were synthesized by procedures similar to that used for **3a** and **c**, respectively. The methyl ester **8a** was prepared by esterification of **1** hydrochloride with thionyl chloride–methanol in 77% yield, and the carboxamide **8b** was prepared by ammonolysis of **8a** with 28% ammonia solution in the presence of ammonium chloride in DMF in 55% yield.

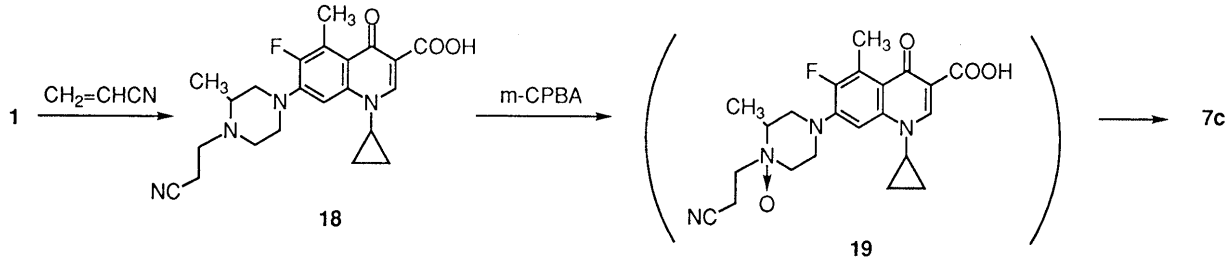


Chart 4

Preparation of 4-hydroxypiperazine derivative **7c** is illustrated in Chart 4. Michael addition of **1** with acrylonitrile in $CHCl_3$ and MeOH provided the corresponding 4-(2-cyanoethyl)piperazine derivative **18** in 94% yield. Oxidation of **18** with *m*-chloroperbenzoic acid (*m*-CPBA) in $CHCl_3$ afforded the N-oxide **19**, which spontaneously transformed to **7c** in 54% yield by reverse Michael addition.[3d]

Biological Results

The antibacterial activities against gram-positive and gram-negative bacteria were measured *in vitro* by the serial agar dilution method.[7] The antibacterial activities of all actual metabolites **2a, b**, **4a, b** and **6a, b** were found to be lower than that of **1**.

Conclusion

Among the 17 compounds prepared in this study, compounds **2a, b**, **4a, b** and **6a, b** were found to be identical with the actual metabolites based on comparisons of the nuclear magnetic resonance (NMR) spectra, mass spectra (MS) and high-performance liquid chromatographic (HPLC) behavior. All the actual metabolites had very weak antibacterial activities. It is considered that the contribution of the metabolites to the activity of the mother compound **1** is small. The 4′-*O*-glucuronide of **7c** was isolated from biological fluids of monkey and human.[4] Therefore, **7c** is presumed to be a metabolic intermediate though **7c** itself was not found. The mother compound **1** was mainly metabolized at the methylpiperazine ring or the 5-methyl group on the quinoline ring. Compound **2** was the main metabolite in humans and the other metabolites were conjugated derivatives of **1**.[4]

Experimental

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IR-810 spectrometer. NMR spectra were recorded on a Bruker AC-200 spectrometer. MS were obtained on a Shimadzu GCMS-QP-1000 instrument. Silica gel (Merck Art 7734) was used for column chromatography. Preparative thin layer chromatography (PLC) was carried out on plates (20 × 20 cm, 0.5 mm, thickness) precoated with silica gel ($60F_{254}$, Merck Art 5744).

The yields, melting points and elemental analysis data of **2a, b**·HCl, **2a, b**, **5a, c**, **10** and **11** are listed in Table 1. The ^{1}H-NMR, IR and MS data are listed in Table 3.

7-[(2-Aminoethyl)amino]-1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acid (2a) A Typical Procedure: A mixture of borate complex (**9**) (48.21 g, 103 mmol) and [*N*-(*tert*-butoxycarbonyl)amino]ethylamine[8] (57.52 g, 359 mmol) in DMSO (635 ml) was stirred at 100—110 °C for 22 h. The reaction mixture was allowed to cool to 50 °C and poured into ice-water. The precipitated crystals were suspended in EtOH (700 ml) and water (200 ml), and 6 N HCl (150 ml) was added dropwise to the suspension. The mixture was stirred at 70—75 °C for 2 h, then the solvent was evaporated off. The residue was collected by filtration and washed with CH_2Cl_2. Recrystallization from EtOH–H_2O gave **2a**·HCl (48.10 g, 66%) as colorless needles, mp 289—291 °C (dec.). Sodium carbonate (0.24 g, 2.25 mmol) was added to a stirred solution of **2a**·HCl (1.07 g, 3 mmol) in water (220 ml), and the mixture was stirred at room temperature for 2 h. The precipitated crystals were collected by filtration. Recrystallization from DMF–H_2O gave **2a** (0.85 g, 89%) as a pale yellow powder, mp 261—263 °C (dec.).

Compounds **2b**·HCl, **2b**, **5a, c**, **6a**, **10**, **11** and **17** were obtained by a similar procedure to that described for **2a**.

6a: 60% yield, pale yellow needles from EtOH–H_2O, mp 155—157 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.05 (3H, d, J=6.02 Hz), 1.10—1.45 (4H, m), 2.75—3.10 (5H, m), 3.45—3.70 (2H, m), 3.75—3.95 (1H, m), 4.82 (1H, t, J=6.60 Hz), 4.95—5.15 (2H, m), 7.55 (1H, d, J=7.98 Hz), 8.70 (1H, s). IR (KBr): 3400, 1619, 1579, 1466, 1294 cm^{-1}. MS *m/z* (%): 375 (87, M^+), 329 (89), 287 (58), 57 (59), 45 (54), 44 (100). *Anal.* Calcd for $C_{19}H_{22}FN_3O_4 \cdot 3/2H_2O$: C, 56.71; H, 6.26; N, 10.44. Found : C, 56.72; H, 5.92; N, 10.40.

17: 41% yield, brown prisms from MeOH, mp 215—219 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.85—1.10 (2H, m), 1.10—1.25 (2H, m), 3.25—3.45 (1H, br), 3.81 (3H, s), 4.53 (2H, d, J=5.34 Hz), 5.30 (2H, s), 5.54 (1H, br s), 6.90 (2H, d, J=11.64 Hz), 6.91 (1H, d, J=8.02 Hz), 7.29 (2H, d, J= 11.64 Hz), 8.71 (1H, s), 15.30 (1H, s). IR (KBr): 3430, 1718, 1630, 1523, 1458, 1383, 1325, 1302, 1246, 833 cm^{-1}. MS *m/z* (%): 412 (10, M^+), 366 (20), 311 (28), 309 (25), 277 (25), 231 (59), 121 (100). *Anal.* Calcd for $C_{22}H_{21}FN_2O_4 \cdot 1/2H_2O$: C, 62.70; H, 5.26; N, 6.65. Found: C, 62.91; H, 5.08; N, 6.65.

7-{[(2-Acetylamino)ethyl]amino}-1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acid (3a) Acetyl chloride (55 mg, 0.7 mmol) was added dropwise to a stirred and ice-cooled solution of **2a**·HCl (0.18 g, 0.5 mmol) in 1 N NaOH (1.5 ml, 1.5 mmol), water (3 ml) and acetone (2 ml). The reaction mixture was stirred at 0 °C for 3 h and at room temperature for 3 h, then poured into 10% aqueous citric acid, and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$, and the solvent was evaporated off *in vacuo*. Recrystallization from EtOH gave **3a** (40 mg, 22%) as colorless needles, mp 255—256 °C.

Compounds **3b** and **7a** were obtained by a similar procedure to that described for **3a**.

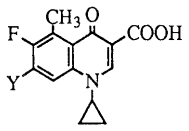
7a: 84% yield, a white powder from MeOH–Et_2O, mp 235—237 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.25 (2H, m), 1.38 (3H, d, J=6.40 Hz), 1.40—1.60 (2H, m), 2.17 (3H, s), 2.82 (3H, d, J=3.08 Hz), 2.80—3.30 (3H, m), 3.40—4.20 (4H, m), 4.55—5.10 (1H, m), 7.28 (1H, d, J=7.44 Hz), 8.73 (1H, s), 15.55 (1H, s). IR (KBr) : 3440, 1723, 1644, 1623, 1428, 1339, 1228 cm^{-1}. MS *m/z* (%): 402 (41), 401 (44, M^+), 41 (100), 355 (30), 221 (36), 70 (61), 43 (61), 42 (45), 41 (100). *Anal.* Calcd for $C_{21}H_{24}FN_3O_4$: C, 62.83; H, 6.03; N, 10.47. Found: C, 62.92; H, 6.05; N, 10.47.

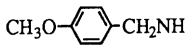
1-Cyclopropyl-1,4-dihydro-6-fluoro-7-{[(2-formylamino)ethyl]amino}-5-methyl-4-oxo-3-quinolinecarboxylic Acid (3c) Ethyl formate (6 ml) was added to a stirred solution of **2a**·HCl (50 mg, 0.141 mmol) and Et_3N (43 mg, 0.423 mmol) in DMF (3 ml), and the mixture was stirred at 70 °C for 6 h. The solvent was evaporated off and the residue was dissolved in $CHCl_3$. The extracts were washed with 20% aqueous citric acid and water, and dried over $MgSO_4$. After evaporation of the solvent, the residue was recrystallized from DMF–H_2O to give **3c** (35 mg, 71%) as pale yellow needles, mp 286—289.5 °C.

Compounds **3d** and **7b** were obtained by a similar procedure to that described for **3c**.

7b: 73% yield, colorless needles from CH_2Cl_2–Et_2O, mp 275—277 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.30 (2H, m), 1.30—1.70 (2H, m), 2.85 (3H, d, J=3.24 Hz), 2.80—3.20 (2H, m), 3.30—4.10 (5H, m), 4.20—4.90 (1H, m), 7.28 (1H, d, J=7.26 Hz), 8.10 (0.45H, s) and 8.21 (0.55H, s), 8.76 (1H, s), 15.48 (1H, s). IR (KBr): 3450, 1722, 1675, 1621,

Table 3. Spectral Data for 7-Substituted 1-Cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acids



Compd. No.	Y	^{1}H-NMR (solvent) δ ppm (J in Hz)	IR (KBr) cm^{-1}	MS m/z (%)
2a·HCl	HCl·$H_2NCH_2CH_2NH$	(DMSO-d_6) 1.00—1.20 (2H, m), 1.30—1.50 (2H, m), 2.74 (3H, d, 3.04), 3.00—3.25 (2H, br), 3.50—3.70 (2H, m), 3.70—3.85 (1H, m), 7.00—7.20 (1H, br), 7.10 (1H, d, 7.82), 8.00 (3H, br s), 8.57 (1H, s), 16.06 (1H, s)	3422, 3284, 2990, 1680, 1621, 1526, 1468, 1313	319 (100, M$^+$), 276 (22), 275 (55), 273 (30), 245 (46), 220 (20)
2a	$H_2NCH_2CH_2NH$	(DMSO-d_6) 1.05—1.15 (2H, m), 1.25—1.40 (2H, m), 2.71 (3H, d, 3.04), 2.81 (2H, t, 6.70), 6.85—7.00 (1H, br), 7.07 (1H, d, 7.86), 8.53 (3H, s)	3342, 2975, 1628, 1567, 1515, 1365, 1304	319 (100, M$^+$), 289 (17), 275 (31), 273 (34), 272 (25), 245 (47), 243 (16), 216 (25)
2b·HCl	HCl·$H_2NCH(CH_3)CH_2NH$	(DMSO-d_6) 1.05—1.20 (2H, m), 1.28 (3H, d, 5.72), 1.30—1.50 (2H, m), 2.74 (3H, d, 3.02), 3.40—3.70 (1H, m), 3.70—3.90 (1H, m), 7.12 (1H, d, 7.88), 7.14 (1H, br), 8.08 (3H, br), 8.57 (1H, s), 16.05 (1H, s)	1627, 1526, 1461, 1322, 1219	333 (9, M$^+$), 290 (17), 272 (18), 216 (13), 44 (100)
2b	$H_2NCH(CH_3)CH_2NH$	(DMSO-d_6) 1.00—1.20 (5H, m), 1.25—1.40 (2H, m), 2.73 (3H, d, 2.94), 3.00—3.50 (5H, br), 3.60—3.80 (1H, br), 6.85—7.05 (1H, br), 7.06 (1H, d, 7.88), 8.54 (1H, s)	3562, 1627, 1586, 1515, 1310	333 (52, M$^+$), 290 (100), 272 (90), 271 (16), 245 (15), 244 (15), 243 (31), 217 (18), 216 (15)
5a[a]		(DMSO-d_6) 1.00—1.25 (2H, m), 1.25—1.50 (2H, m), 2.50—3.10 (5H, m), 2.75 (3H, d, 3.22), 3.30—3.90 (5H, m), 4.72 (1H, t, 5.70), 7.45 (1H, d, 8.10), 8.62 (1H, s)	3310, 1728, 1628, 1512, 1462, 1379, 1270	375 (30, M$^+$), 344 (31), 303 (41), 259 (49), 163 (72), 47 (100), 44 (58), 43 (86)
5c[a]		($CDCl_3$) 1.00—1.25 (2H, m), 1.33 (3H, t, 7.14), 1.30—1.55 (2H, m), 2.76 (3H, d, 3.22), 3.00—3.60 (6H, m), 3.65—3.85 (2H, m), 4.26 (2H, q, 7.14), 7.34 (1H, d, 7.60), 8.68 (1H, s), 15.50—16.50 (1H, d, 7.58)		418 (35), 417 (100, M$^+$), 373 (28), 344 (83), 303 (56), 300 (38), 259 (92), 245 (33), 149 (29), 56 (78), 41 (43)
10		(DMSO-d_6) 0.85—1.10 (2H, m), 1.10—1.25 (2H, m), 2.82 (3H, d, 3.04), 3.15—3.35 (1H, m), 3.81 (3H, m), 4.49 (2H, d, 5.34), 5.15—5.35 (1H, m), 6.86 (1H, d, 7.50), 6.91 (2H, d, 8.72), 7.29 (2H, d, 8.72), 8.65 (1H, s), 16.00 (1H, s)	3430, 1718, 1630, 1523, 1458, 1302, 1246, 833	396 (13, M$^+$), 352 (9), 350 (8), 122 (10), 121 (100), 91 (9), 78 (10), 77 (10)
11[b]	EtOOCCH$_2$NH	($CDCl_3$) 1.10—1.25 (2H, m), 1.25—1.45 (5H, m), 2.84 (3H, d, 3.00), 3.35—3.60 (1H, m), 4.06 (2H, d, 5.24), 4.31 (2H, q, 7.14), 5.35—5.50 (1H, br), 6.86 (1H, d, 7.46), 8.72 (1H, s), 15.86 (1H, s)		

a) These starting materials (2-hydroxymethylpiperazine and 2-ethoxycarbonylpiperazine) were prepared by the method described in reference 6. *b*) This **11** was used in the next step without further purification.

1462, 1434, 1266 cm^{-1}. MS m/z (%): 387 (100, M$^+$), 343 (46), 341 (88), 70 (52), 44 (61), 42 (46), 41 (44). *Anal.* Calcd for $C_{20}H_{22}FN_3O_4$: C, 62.01; H, 5.72; N, 10.85. Found: C, 61.69; H, 5.63; N, 10.87.

The yields, melting points and elemental analysis data of **3a, b, c** and **d** are listed in Table 2. The ^{1}H-NMR, IR and MS data are listed in Table 4.

7-Amino-1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acid (4a) Trifluoroacetic acid (5 ml) and concentrated H_2SO_4 (0.5 ml) were added to a stirred suspension of **10** (80 mg, 0.2 mmol) in anisole (5 ml), and the mixture was stirred at 120 °C for 7 h. After evaporation of the trifluoroacetic acid, 10% aqueous KOH (10 ml) was added to the residue, and the whole was extracted with CH_2Cl_2. The aqueous layer was separated, acidified by addition of concentrated HCl (10 ml), and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$, and concentrated to dryness *in vacuo*. The residue was recrystallized from DMF–H_2O to give **4a** (28 mg, 51%) as a white powder, mp > 300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.05—1.20 (2H, m), 1.20—1.35 (2H, m), 2.72 (3H, d, J = 3.04 Hz), 3.55—3.75 (1H, m), 6.66 (1H, br s), 7.29 (1H, d, J = 8.10 Hz), 8.52 (1H, s), 16.13 (1H, s). IR (KBr): 3430, 3342, 1650, 1616, 1484, 1433, 1374 cm^{-1}. MS m/z (%): 333 (52, M$^+$), 290 (100), 272 (90), 271 (16), 245 (15), 244 (15), 243 (31), 217 (18), 216 (52). *Anal.* Calcd for $C_{14}H_{13}FN_2O_3$: C, 60.87; H, 4.74; N, 10.14. Found: C, 60.93; H, 4.65; N, 10.11.

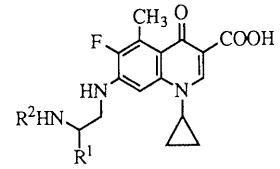
Compound **6b** was obtained by a similar procedure to that described for **4a**.

6b: 35% yield, a pale brown powder from DMF–H_2O, mp 291—294 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.05—1.45 (4H, m), 3.55—3.80 (1H, m), 4.75—5.00 (3H, br), 6.60—7.00 (2H, br), 7.40 (2H, d, J = 8.02 Hz), 8.59 (1H, s), 15.66 (1H, s). IR (KBr): 3346, 3232, 1732, 1655, 1624, 1474, 1458, 1334, 1024 cm^{-1}. MS m/z (%): 292 (25, M$^+$), 247 (16), 246 (100), 217 (10), 202 (10), 189 (17), 149 (11), 122 (14), 107 (12). *Anal.* Calcd for $C_{14}H_{13}FN_2O_4$: C, 57.53; H, 4.48; N, 9.58. Found: C, 57.22; H, 4.17; N, 9.44.

7-[(Carboxymethyl)amino]-1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acid (4b) A mixture of **11** (2.91 g, 8.3 mmol) and 5 N NaOH (10 ml) in EtOH (30 ml) was gently refluxed with stirring for 3 h. After evaporation of the solvent, 10% aqueous citric acid (3 ml) was added to the residue, which was extracted with CH_2Cl_2. The extracts were washed with water, dried over $MgSO_4$, and concentrated to dryness *in vacuo*. The residue was purified by column chromatography (silica gel, eluent, CH_2Cl_2 : MeOH : AcOH = 10 : 1 : 0.1) to give **4b** (0.96 g, 35%) as a pale yellow powder. Recrystallized from DMF–H_2O as a pale yellow powder, mp 303—304 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.20 (2H, m), 1.20—1.40 (2H, m), 2.74 (3H, d, J = 2.70 Hz), 3.65 (1H, br), 4.04 (1H, br s), 6.99 (1H, d, J = 7.88 Hz), 7.14 (1H, br), 8.54 (1H, s), 16.09 (1H, s). IR (KBr): 3460, 1738, 1622, 1526, 1462 cm^{-1}. MS m/z (%) : 334 (69, M$^+$), 290 (64), 289 (28), 288 (100), 245 (29), 216 (32), 214 (22). *Anal.* Calcd for $C_{16}H_{15}FN_2O_5$: C, 57.49; H, 4.52; N, 8.38. Found: C, 57.26; H, 4.53; N, 8.26.

Compound **5b** was obtained by a similar procedure to that described for **4b**.

Table 4. Spectral Data for 7-Substituted Amino 1-Cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acids



Compd. No.	R^1	R^2	^{1}H-NMR (solvent) δ ppm (J in Hz)	IR (KBr) cm^{-1}	MS m/z (%)
3a	H	Ac	($CDCl_3$) 1.05—1.15 (2H, m), 1.35—1.55 (2H, m), 2.04 (3H, s), 2.81 (3H, d, 3.06), 3.49 (2H, t, 5.92), 3.57 (2H, t, 5.92), 5.30—5.50 (1H, br), 5.85—6.05 (1H, br), 7.11 (1H, d, 7.58), 8.69 (1H, s), 16.05 (1H, s)	3308, 1622, 1530, 1470, 1452, 1379, 1316	362 (41), 361 (100, M^+), 317 (58), 315 (63), 245 (93), 44 (49), 43 (57), 41 (55)
3b	CH_3	Ac	($CDCl_3$) 1.00—1.20 (2H, m), 1.31 (3H, d, 6.76), 1.40—1.50 (2H, m), 2.03 (3H, m), 2.81 (3H, d, 3.06), 3.10—3.30 (1H, m), 3.45—3.65 (2H, m), 4.20—4.40 (1H, m), 5.35—5.50 (1H, m), 5.56 (1H, d, 6.74), 7.23 (1H, d, 7.66), 8.68 (1H, s), 16.07 (1H, s)	3356, 1705, 1641, 1532, 1462	376 (30), 375 (88, M^+), 331 (32), 329 (26), 272 (37), 271 (60), 245 (46), 44 (100)
3c	H	CHO	(DMSO-d_6) δ: 1.05—1.25 (2H, m), 1.25—1.50 (2H, m), 2.73 (3H, d, 2.73), 3.00—3.50 (4H, m), 3.60—3.85 (1H, m), 7.09 (1H, br), 7.21 (1H, d, 7.92), 7.95 (1H, d, 1.62), 8.26 (1H, s), 8.55 (1H, s), 16.14 (1H, s)	3320, 1681, 1628, 1532, 1461, 1318	361 (100, M^+), 303 (64), 301 (73), 245 (82), 216 (25), 91 (35), 44 (57), 41 (52)
3d	CH_3	CHO	($CDCl_3$) 1.00—1.20 (2H, m), 1.31 (3H, d, 6.76), 1.40—1.50 (2H, m), 2.03 (3H, m), 2.81 (3H, d, 3.06), 3.10—3.30 (1H, m), 3.45—3.65 (2H, m), 4.20—4.40 (1H, m), 5.35—5.50 (1H, m), 5.56 (1H, d, 6.74), 7.23 (1H, d, 7.66), 8.68 (1H, s), 16.07 (1H, s)	3356, 1705, 1641, 1532, 1462	376 (30), 375 (88, M^+), 331 (32), 329 (26), 272 (37), 271 (60), 245 (46), 44 (100)

5b: 90% yield, a white powder from MeOH, mp 271—273 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.05—1.20 (2H, m), 1.20—1.40 (2H, m), 2.76 (3H, d, J=2.76 Hz), 2.70—4.00 (8H, m), 7.46 (1H, d, J=7.96 Hz), 8.61 (1H, s). IR (KBr) 3445, 1739, 1622, 1564 cm^{-1}. MS m/z (%): 358 (23, M^+) 346 (31), 343 (56), 259 (43), 149 (21), 56 (58), 44 (100). *Anal.* Calcd for $C_{19}H_{20}FN_3O_5 \cdot 1/2H_2O$: C, 57.28; H, 5.31; N, 10.55. Found: C, 57.58; H, 5.01; N, 10.57.

Methyl 7-Bromo-1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylate (13) Thionyl chloride (9.0 ml, 125 mmol) was added dropwise to a stirred and ice-cooled suspension of **12** (17.01 g, 50 mmol) in MeOH (200 ml), and the reaction mixture was refluxed with stirring for 4 h. After evaporation of the solvent, the residue was poured into ice-water. The precipitated crystals were collected by filtration. Recrystallization from CH_2Cl_2–MeOH gave **13** (17.15 g, 97%) as colorless needles, mp 244—246 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.05—1.20 (2H, m), 1.30—1.50 (2H, m), 2.89 (3H, d, J=2.62 Hz), 3.30—3.45 (1H, m), 3.92 (3H, s), 8.03 (1H, d, J=5.92 Hz), 8.52 (1H, s). IR (KBr) 1697, 1637, 1474, 1449, 1435, 1259, 1242 cm^{-1}. MS m/z (%): 355 (48), 353 (47, M^+), 295 (100), 294 (33), 293 (99), 267 (16), 265 (16), 145 (17). *Anal.* Calcd for $C_{15}H_{13}BrFNO_3$: C, 50.87; H, 3.70; N, 3.95. Found: C, 50.56; H, 3.37; N, 3.91.

Compound **8a** was obtained by a similar procedure to that described for **13**.

8a: 77% yield, a white powder from MeOH–Et_2O, mp 210—212 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.05—1.25 (2H, m), 1.19 (3H, d, J=6.38 Hz), 1.25—1.45 (2H, m), 2.48 (1H, t, J=10.30 Hz), 2.84 (3H, d, J=3.23 Hz), 2.85—3.25 (4H, m), 3.30—3.45 (1H, m), 3.45—3.60 (2H, m), 3.92 (3H, s), 7.19 (1H, d, J=7.63 Hz), 8.49 (1H, s). IR (KBr): 3450, 3314, 1727, 1632, 1478, 1272 cm^{-1}. MS m/z (%): 373 (100, M^+), 318 (21), 317 (100), 56 (16), 42 (25), 41 (21). *Anal.* Calcd for $C_{20}H_{24}FN_3O_3$: C, 64.33; H, 6.48; N, 11.25. Found : C, 63.87; H, 6.51; N, 11.19.

Methyl 7-Bromo-5-bromomethyl-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-3-quinolinecarboxylate (14) A mixture of **13** (11.73 g, 40 mmol), NBS (8.54 g, 48 mmol) and benzoyl peroxide (0.97 g, 4 mmol) in CCl_4 (400 ml) was refluxed for 6 h. After removal of the precipitates by filtration, the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent, CH_2Cl_2: MeOH = 50 : 1) to give **14** (9.09 g, 53%) as a yellow powder. Recrystallized from CH_2Cl_2 as colorless needles, mp 194—198 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 1.05—1.20 (2H, m), 1.30—1.50 (2H, m), 3.35—3.50 (1H, m), 3.93 (3H, s), 5.50 (2H, d, J=2.84 Hz), 8.21 (1H, d, J=6.02 Hz), 8.56 (1H, s). IR (KBr): 1730, 1628, 1470, 1445, 1264 cm^{-1}. MS m/z (%): 435 (26), 433 (53), 431 (26, M^+), 375 (50), 373 (100), 371 (52), 353 (62), 351 (55), 145 (68), 82 (88), 81 (59), 80 (91). *Anal.* Calcd for $C_{15}H_{12}Br_2FNO_3$: C, 41.60; H, 2.79; N, 3.23. Found: C, 41.74; H, 2.76; N, 3.27.

Methyl 5-Acetoxymethyl-7-bromo-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-3-quinolinecarboxylate (15) A mixture of **14** (4.56 g, 12.2 mmol) and sodium acetate (2.00 g, 24.4 mmol) in DMF (50 ml) was stirred at 90 °C for 1.5 h. After evaporation of the solvent, the residue was poured into ice-water and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and concentrated to dryness *in vacuo*. The residue was purified by column chromatography (silica gel, eluent, CH_2Cl_2 : MeOH = 50 : 1) to give **15** (3.50 g, 70%) as a pale brown powder. Recrystallization from MeOH as yellow needles, mp 224—227 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.20 (2H, m), 1.30—1.50 (2H, m), 2.06 (3H, s), 3.35—3.50 (1H, m), 3.91 (3H, s), 5.92 (2H, d, J=2.86 Hz), 8.26 (1H, d, J=5.94 Hz), 8.55 (1H, s). IR (KBr): 3670, 1736, 1624, 1474, 1444, 1268, 1246, 1230 cm^{-1}. MS m/z (%): 413 (14), 411 (13, M^+), 371 (28), 370 (58), 369 (57), 368 (54), 338 (98), 336 (100), 311 (33), 309 (35), 294 (10). *Anal.* Calcd for $C_{17}H_{15}BrFNO_5$: C, 49.53; H, 3.67; N, 3.40. Found: C, 49.03; H, 3.33; N, 3.22.

7-Bromo-1-cyclopropyl-1,4-dihydro-6-fluoro-5-hydroxymethyl-4-oxo-3-quinolinecarboxylic Acid (16) A mixture of **15** (1.73 g, 4.2 mmol) and 10% aqueous K_2CO_3 (18 ml) in MeOH (60 ml) was stirred at room temperature overnight. After evaporation of the solvent, the residue was extracted with CH_2Cl_2. The extracts were washed with 10% aqueous citric acid and water, and dried over $MgSO_4$. The extracts were concentrated to dryness *in vacuo*, and the residue was recrystallized from CH_2Cl_2–MeOH to give **16** (1.40 g, 94%) as colorless needles, mp 241—244 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.35 (2H, m), 1.45—1.60 (2H, m), 3.55—3.75 (1H, m), 4.60 (1H, t, J=8.16 Hz), 5.10 (2H, dd, J=2.44, 8.16 Hz), 8.40 (1H, d, J=5.82 Hz), 8.94 (1H, s), 14.40 (1H, m). IR (KBr): 1736, 1618, 1514, 1439, 1329, 1029 cm^{-1}. MS m/z (%): 357 (10), 355(11, M^+), 312 (16), 311 (99), 310 (22), 309 (100), 282 (11), 280 (12), 252 (10), 173 (14), 172 (12), 133 (10). *Anal.* Calcd for $C_{14}H_{11}BrFNO_4$: C, 47.21; H, 3.11; N, 3.93. Found: C, 46.94; H, 3.11; N, 4.02.

1-Cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxamide (8b) A mixture of **8a** (0.11 g, 0.3 mmol), NH_4Cl (19 mg, 0.36 mmol) and 25% aqueous NH_4OH (2 ml) in DMF (2 ml) was stirred at 110 °C for 6 h in an autoclave tube. The reaction mixture was poured into ice-water. The resulting precipitates were collected by filtration and recrystallized from MeOH–Et_2O to give **8b** (60 mg, 55%) as a pale yellow powder, mp 213—214 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.95—1.20 (2H, m), 1.03 (3H, d, J=6.20 Hz), 1.20—1.40 (2H, m), 2.03—2.65 (1H, m), 2.74 (3H, d, J=3.04 Hz), 2.70—3.15 (4H,

m), 3.35—3.60 (2H, m), 3.60—3.80 (1H, m), 7.35 (1H, d, J=8.20 Hz), 7.38 (1H, br s), 8.57 (1H, s), 9.29 (1H, d, J=3.00 Hz). IR (KBr): 3450, 3314, 1727, 1632, 1478, 1272 cm^{-1}. MS m/z (%): 358 (23, M$^+$), 303 (21), 302 (100), 70 (11). *Anal.* Calcd for $C_{19}H_{23}FN_4O_2 \cdot H_2O$: C, 60.62; H, 6.69; N, 14.88. Found: C, 60.70; H, 6.51; N, 14.97.

7-[4-(2-Cyanoethyl)-3-methyl-1-piperazinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-4-oxo-3-quinolinecarboxylic Acid (18) A mixture of **1** (0.54 g, 1.5 mmol) and acrylonitrile (0.79 ml, 12 mmol) in $CHCl_3$ (5 ml) and MeOH (5 ml) was refluxed with stirring for 20 h. The mixture was concentrated to give a white solid, which was collected by filtration and washed with MeOH. The solid was recrystallized from DMF–H_2O to give **18** (0.58 g, 94%) as a white powder. mp 197—198 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, d, J=5.78 Hz), 1.00—1.25 (2H, m), 1.25—1.45 (2H, m), 2.50—3.30 (9H, m), 2.75 (3H, d, J=2.77 Hz), 3.40—3.60 (2H, m), 3.70—3.90 (1H, m), 7.46 (1H, d, J=8.04 Hz), 8.63 (1H, s), 15.69 (1H, s). MS m/z (%): 413 (26), 412 (100, M$^+$), 372 (26), 368 (34), 366 (39), 303 (69), 259 (41), 242 (21), 109 (34), 84 (47), 71 (33), 70 (26). *Anal.* Calcd for $C_{22}H_{25}FN_3O_4$: C, 64.66; H, 6.11; N, 13.58. Found: C, 64.07; H, 5.97; N, 13.58

1-Cyclopropyl-1,4-dihydro-6-fluoro-7-(4-hydroxy-3-methyl-1-piperazinyl)-5-methyl-4-oxo-3-quinolinecarboxylic Acid (7c) *m*-Chloroperbenzoic acid (80%, 55 mg, 0.255 mmol) was added to a stirred solution of **18** in $CHCl_3$ (10 ml) at 0 °C. The reaction mixture was stirred at room temperature, and the solvent was evaporated off. The residue was purified by column chromatography (silica gel, eluent, CH_2Cl_2 : MeOH = 30 : 1) to give **7c** (30 mg, 53%) as a white powder. Recrystallized from CH_2Cl_2–MeOH as a white powder, mp 205—207 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, d, J=5.50 Hz), 1.10—1.40 (4H, m), 2.50—2.90 (2H, m), 2.76 (3H, d, J=3.14 Hz), 3.00—3.50 (4H, m), 3.60—3.90 (2H, m), 7.45 (1H, d, J=7.96 Hz), 8.17 (1H, s) 8.63 (1H, s), 15.70 (1H, s). IR (KBr): 3420, 1705, 1618, 1512, 1450, 1379, 1260 cm^{-1}. MS m/z (%): 375 (35, M$^+$), 359 (45), 316 (93), 303 (82), 259 (100), 70 (93), 44 (76). *Anal.* Calcd for $C_{19}H_{22}FN_3O_4$: C, 60.79; H, 5.91; N, 11.19. Found: C, 60.68; H, 6.07; N, 11.12.

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