Studies on Quinolone Antibacterials. IV.[1] Structure–Activity Relationships of Antibacterial Activity and Side Effects for 5- or 8-Substituted and 5,8-Disubstituted-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids

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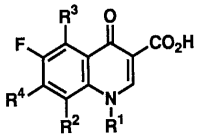
A series of 7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids bearing various substituents (H, F, Cl, Me, OH, OMe, OEt, OCH_2F, $OCHF_2$, OCF_3, SMe) at the C-8 position was prepared and evaluated for *in vitro* antibacterial activity against both standard laboratory strains and bacteria resistant to quinolones such as ciprofloxacin (CPFX, 1) and ofloxacin (OFLX, 2) from clinical isolates. The 8-methyl (8a), 8-fluoro (9a), 8-chloro (10a) and 8-methoxy (12a) compounds were 4 times more potent than CPFX (1) against both gram-positive and gram-negative bacteria. But these four compounds caused injury to the chromosomes of mammalian cells at a concentration of 100 μg/ml. Next, a series of quinolones having various substituents (H, Cl, Me, NH_2, NHMe, NMe_2) at the C-5 position was prepared and evaluated for antibacterial activity and injurious effect on the chromosome. We found that the 5-amino-8-methyl compound (8d) showed strong antibacterial activity (*in vitro* antibacterial activity of 8d is 4 times more potent than that of CPFX (1) against both gram-positive and gram-negative bacteria), reduced injury to the chromosome, and reduced quinolone-type toxicity (free from both phototoxicity at a dosage of 30 mg/kg in guinea pigs (i.v.) and convulsion-inducing activity when coadministered with fenbufen at a dosage of 100 mg/kg in mice (i.p.)).

Key words 5-amino-8-methylquinolone; antibacterial activity; chromosome; phototoxicity; 8-substituted quinolone; 5-substituted quinolone

Quinolone antibacterial agents, such as ciprofloxacin (CPFX, **1**),[2] ofloxacin (OFLX, **2**),[3] and sparfloxacin (SPFX, **3**),[4] are a major class of antibacterial drugs. These quinolones show broad-spectrum antibacterial activity and are widely used to treat patients with infections. Recently the incidence of infections by gram-positive and gram-negative bacteria resistant to these quinolones has been increasing, so that novel quinolone having higher activity against quinolone-resistant bacteria are required.[5] Nevertheless, some quinolones cause injury to the chromosome of eukaryotic cells.[6] In our previous paper, we reported that introduction of substituents at the C-5 and C-8 positions on the 7-(2-aminoalkoxy)quinolone ring improved antibacterial activity against quinolone-resistant bacteria.[1] This finding prompted us to optimize the substituents at the C-5 and C-8 positions on the quinolone ring when the C-7 substituent is the 3-aminopyrrolidine group, which is one of the most potent substituents for antibacterial activity. This report describes the effects on



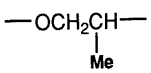
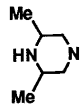
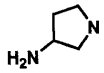
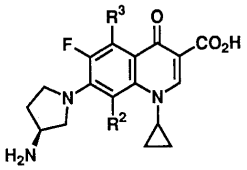
	R^2	R^1	R^3	R^4
1: ciprofloxacin	H		H	
2: ofloxacin			H	
3: sparfloxacin	F		NH_2	
4: lomefloxacin	F	Et	H	
5: AM-1091	Cl		H	
6: AM-1155	OMe		H	

Chart 1



7: R^2= H
8: R^2= Me
9: R^2= F
10: R^2= Cl
11: R^2= OH
12: R^2= OMe
13: R^2= OEt
14: R^2= OCH_2F
15: R^2= $OCHF_2$
16: R^2= OCF_3
17: R^2= SMe

a: R^3= H **b**: R^3= Me **c**: R^3= Cl **d**: R^3= NH_2 **e**: R^3= NHMe **f**: R^3=NMe_2

Chart 2

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antibacterial activity and chromosome-injuring ability of introducing various substituents at the C-5 and C-8 positions on 7-(3-amino-1-pyrrolidinyl)-1-cyclopropylquinolone and the discovery of 5-amino-8-methyl compound (**8d**) as a potent compound with reduced side effects.

Chemistry

The compounds were prepared as shown in Charts 3 and 4. The known compounds (**7a, b, d**, **8a, b**, **9a, b, d**, **10a, b, d**, **12a, b, d**)[7,8] tested were prepared according to the literature.

The 8-methylthio derivative **17a** was synthesized as shown in Chart 3. The reaction of the thiophthalic acid **18**[9] with methyl iodide afforded the methylthiophthalic acid which, without purification, was heated to cause regioselective decarboxylation, affording the methylthiobenzoic acid **19**. Treatment of **19** with oxalyl chloride, followed by condensation of diethyl malonate, and decarboxylation furnished the ketoester **20**. Treatment of **20** with ethyl orthoformate in acetic anhydride followed by reaction with cyclopropylamine gave the enaminoketoester **21**. Cyclization of **21** proceeded smoothly in the presence of sodium hydride to give **22**. Acid hydrolysis of **22** gave **23**, which was condensed with 3-aminopyrrolidine to give **24**. Finally, deacetylation of **24** afforded the desired 8-methylthio derivative **17a**.

The phenol **11a** and ethyl ether derivative **13a** were synthesized as follows. Condensation of the known intermediate **25**[10] with 3-aminopyrrolidine gave **26**. Subsequent treatment of **26** with trimethylsilyl chloride in the presence of sodium iodide provided the phenol **27**, which was hydrolyzed to give **11a**. The reaction of **27** with ethyl iodide gave the 8-ethoxy derivative **28**, which was hydrolyzed to give **13a**.

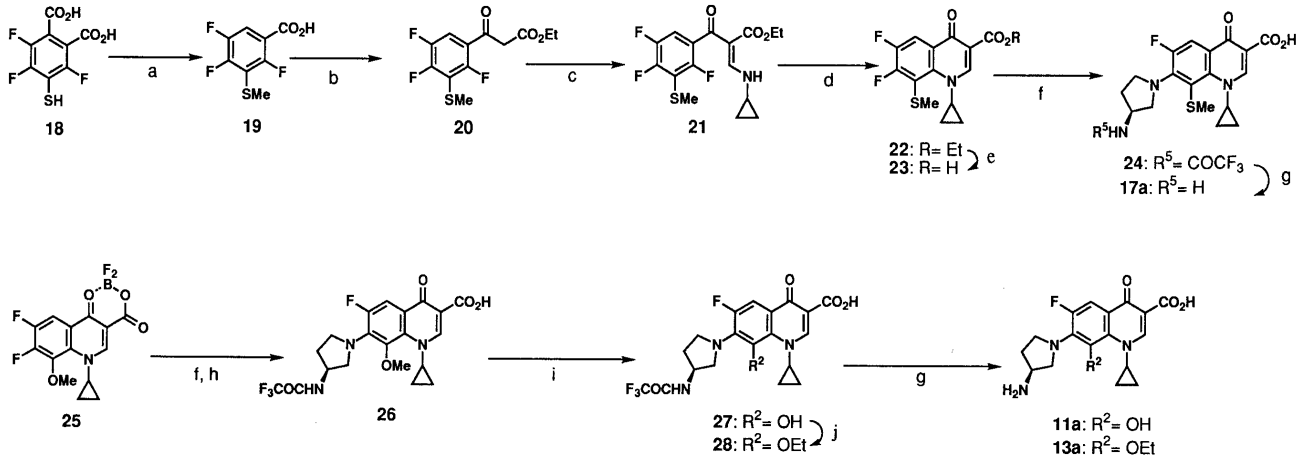
The 5-amino and 5-dimethylamino derivatives (**8d, f**) were synthesized as shown in Chart 4. Nitration of benzoic acid **29**[11] with a mixture of concentrated sulfuric acid and 70% nitric acid gave **30**. In a similar manner to that described for the preparation of **22** from the benzoic acid **19**, the nitrobenzoic acid **30** was converted to the ketoester **31**, which was transformed to the nitro compound **33** *via* the enaminoketoester **32**. Hydrogenation of the nitro group, followed by hydrolysis of **34** gave the 5-amino derivative **35**. Treatment of **35** with boron trifluoride etherate afforded **37**, which was condensed with 3-aminopyrrolidine to afford the desired compound **8d**. Methylation of **35** under the Eschweiler–Clark condition gave **36**, which was converted to **8f** in 2 steps.

The 5-methylamino derivative **8e** was synthesized by way of the 5-chloro compound **47**, as follows. Chlorination of the oxazoline **40**[11] with hexachloroacetone provided the corresponding 2-chloro compound **41**. Acid hydrolysis of **41** unexpectedly gave only the ester **42** in place of the benzoic acid **44**. The carboxylic acid **44** was obtained indirectly by acetylation of **42** with acetic anhydride and subsequent alkaline hydrolysis. In a similar manner to that described for the preparation of **17a** from **19**, the desired compound **8c** was obtained through the sequence of **44**, the ketoester **45**, the enaminoketoester **46**, the ring-closed compound **47**, the carboxylic acid **50**, and the 7-amino compound **52**. Treatment of **47** with *N*-methylbenzylamine provided the 5-substituted compound **48**, which was hydrogenated to give **49**. Finally, the 5-methylamino compound **8e** was synthesized from **49** by way of **51** and **53**.

Results and Discussion

The 5- or 8-substituted and 5,8-disubstituted compounds (**7**—**17**) were tested for antibacterial activity against both standard strains (gram-positive strain, *Staphylococcus aureus* FDA 209P JC-1; gram-negative strain, *Escherichia coli* NIHJ JC-2) and the clinical isolates of bacteria resistant to CPFX (**1**) and OFLX (**2**) (gram-positive bacteria, *Staphylococcus aureus* HPC 292 and *Enterococcus faecalis* HPC 975; gram-negative bacteria, *Klebsiella pneumonia* HNR 828 and *Pseudomonas aeruginosa* HNR 1472) and their minimum inhibitory concentrations (*MIC*s, μg/ml) are shown in Tables 1 and 2. The data for CPFX (**1**) and OFLX (**2**) are included for

8-Substituted Quinolones



a) 1) MeI, K_2CO_3 2) $[Me(CH_2)_7]_3N$ b) 1) $(COCl)_2$ 2) $EtOMgCH(CO_2Et)_2$ 3) *p*-TsOH c) 1) $CH(OEt)_3$ 2) cyclopropylamine d) NaH e) 6 N HCl f) pyrrolidine derivatives g) aq. NaOH h) NEt_3 in MeOH i) TMSCl j) EtI, K_2CO_3

Chart 3

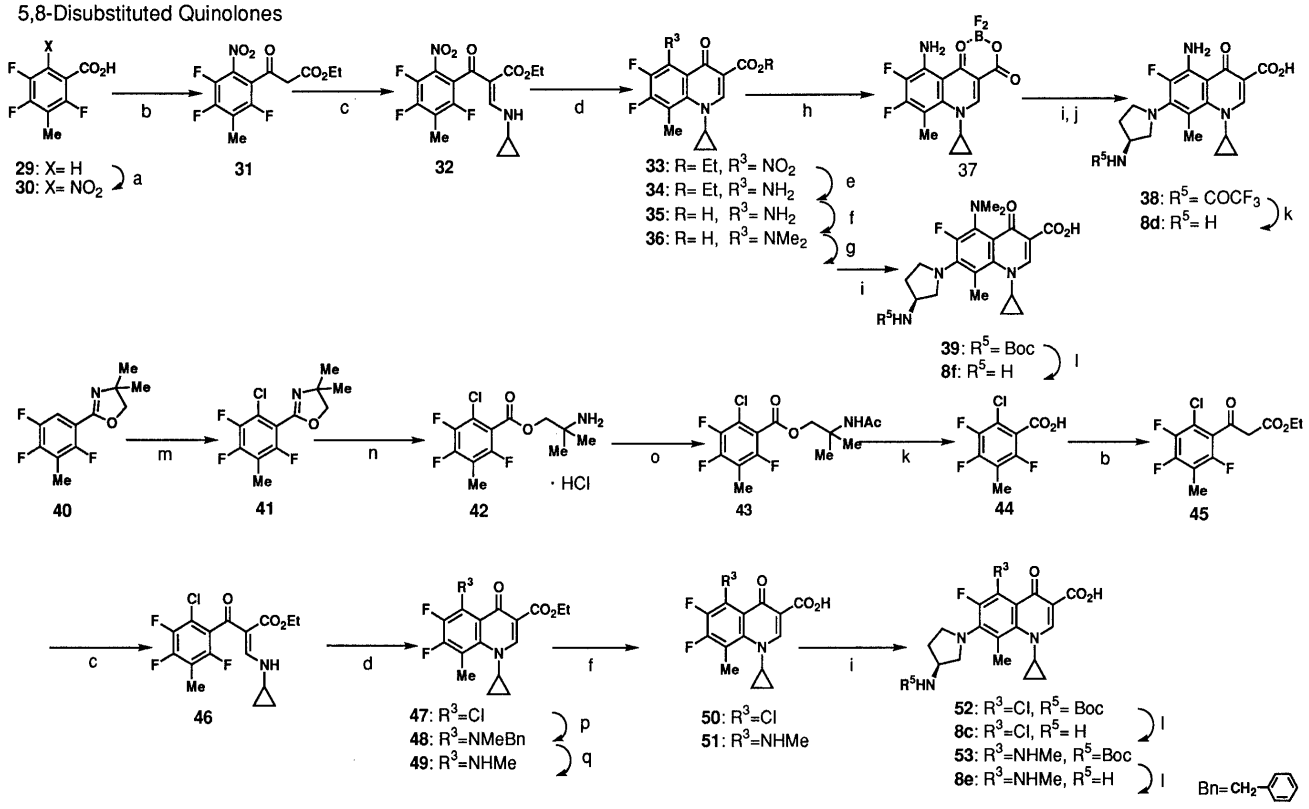


Chart 4

Table 1. *In Vitro* Antibacterial Activity and Chromosome Injury of 8-Substituted Quinolones

Compd. No.	R^2	Minimum inhibitory concentration (μg/ml)[a]						Chromosome injury[c] (100 μg/ml)
		Standard strains		Clinical isolates[b]				
		S. aureus FDA 209P	*E. coli* NIHJ JC-2	*S. aureus* HPC 292	*E. faecalis* HPC 975	*K. pneumoniae* HNR 828	*P. aeruginosa* HNR 1472	
7a	H	0.05	0.025	12.5	12.5	6.25	6.25	I
8a	Me	0.025	0.012	3.13	3.13	3.13	0.78	IV
9a	F	0.025	0.012	3.13	3.13	1.56	1.56	IV
10a	Cl	0.025	0.012	1.56	1.56	1.56	0.78	IV
11a	OH	6.25	6.25	>100	>100	>100	>100	
12a	OMe	0.025	0.025	1.56	1.56	3.13	1.56	III
13a	OEt	0.10	0.10	3.13	0.78	25	100	
14a	OCH_2F	0.78	0.20	>100	>100	>100	>100	
15a	$OCHF_2$	0.05	0.05	3.13	3.13	6.25	6.25	
16a	OCF_3	0.20	0.10	25	25	25	12.5	
17a	SMe	0.025	0.10	3.13	3.13	12.5	12.5	
CPFX (**1**)		0.20	0.025	50	50	12.5	12.5	I
OPFX (**2**)		0.20	0.10	50	50	25	25	I
AM-1091 (**5**)		0.05	0.025	3.13	3.13	1.56	1.56	

a) Inoculum size, one loopful of 10^6 CFU/ml. *b*) Clinical isolates are resistant to CPFX (**1**) and OFLX (**2**). *c*) The scoring is: I, <10%; II, 10—20%; III, 20—50%; IV, >50%.

comparison. Selected compounds with excellent antibacterial activity were further evaluated for chromosome-injuring action on Chinese hamster lung (CHL) cells at the concentrations of 100 μg/ml. The results are also shown in Tables 1 and 2.

8-Substituted Quinolones The results of the antibacterial activity tests of the 8-substituted compounds are shown in Table 1 and may be summarized as follows. Introduction of the hydroxy, fluoromethoxy and trifluoromethoxy groups (**11a**, **14a**, **16a**) decreased the potency against gram-positive and gram-negative bacteria. In particular **11a** and **14a** lacked antibacterial activity against resistant bacteria. Introduction of the difluoromethoxy and methylthio groups (**15a**, **17a**) increased the potency against gram-positive bacteria and decreased or maintained the potency against gram-negative bacteria. Intro-

duction of the methyl, fluoro, chloro or methoxy groups (**8a**, **9a**, **10a**, **12a**) increased the potency against both gram-positive and gram-negative bacteria. These four compounds (**8a**, **9a**, **10a**, **12a**) were 4 times more potent than CPFX (**1**), which is a quinolone having a good balance of antibacterial activity against both gram-positive and gram-negative bacteria, and were selected for further evaluation. These four compounds have activity similar to that of AM-1091 (**5**)[12] which is one of the most potent quinolones.

To clarify the physicochemical background of the effects of the substituents at the C-8 position on antibacterial activity, we performed quantitative structure–activity analyses by the Hansch–Fujita method.[13] Though the activity against gram-positive bacteria was not correlated with any physicochemical parameter, the antibacterial activity against gram-negative bacteria was well correlated with the steric parameter, $B4$[14] or L[14] (Eqs. 1—3). $B4$ and L are Sterimol parameters representing the maximum width and the length of the substituent, respectively. In the equations, the value in parenthesis under each coefficient is the 95% confidence interval, n is the number of compounds, r is the correlation coefficient, s is the standard deviation and F is the observed F value.

In this analysis, compounds **11a** (R^2=OH) and **14a** (R^2=OCH_2F) were omitted from the calculation because the observed activities of these compounds were lower than the calculated values. The reason is not clear, but the OH group of **11a** is the only acidic group and so the acidic character may have an unfavorable effect upon the antibacterial activity. In the case of **14a**, the true activity could not be observed because of the instability of this compound in the solution.

〈*Escherichia coli* NIHJ JC-2〉

$$\log(1/MIC) = -0.42B4^2 + 1.60B4 + 5.99 \quad (1)$$
$$(0.22)\quad (1.03)\ (1.04)$$
$$(n=9,\ r=0.95,\ s=0.13,\ F_{2,6}=30.09)$$

$$\log(1/MIC) = -0.28L^2 + 1.63L + 5.05 \quad (1')$$
$$(0.24)\quad (1.66)^{*}(2.74)$$
$$(n=9,\ r=0.90,\ s=0.19,\ F_{2,6}=12.56)$$

〈*Klebsiella pneumoniae* HNR 828〉

$$\log(1/MIC) = -0.44L^2 + 2.71L + 1.04 \quad (2)$$
$$(0.24)\quad (1.65)\ (2.73)$$
$$(n=9,\ r=0.92,\ s=0.19,\ F_{2,6}=17.70)$$

$$\log(1/MIC) = -0.50B4^2 + 1.99B4 + 3.35 \quad (2')$$
$$(0.42)\quad (1.95)\ (1.97)$$
$$(n=9,\ r=0.87,\ s=0.25,\ F_{2,6}=9.20)$$

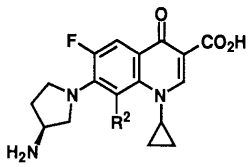
〈*Pseudomonas aeruginosa* HNR 1472〉

$$\log(1/MIC) = -0.73L^2 + 4.62L - 1.70 \quad (3)$$
$$(0.31)\quad (2.12)\ (3.49)$$
$$(n=9,\ r=0.95,\ s=0.25,\ F_{2,6}=26.37)$$

$$\log(1/MIC) = -0.92B4^2 + 3.84B4 + 1.80 \quad (3')$$
$$(0.59)\quad (2.70)\ (2.73)$$
$$(n=9,\ r=0.89,\ s=0.35,\ F_{2,6}=11.85)$$

The quantitative structure–activity analysis of the C-8 substituents of antibacterial quinolones has been studied by Ohta and Koga,[15] who reported that the antibacterial activity against the same *Escherichia coli* NIHJ JC-2 as used in our analysis was well correlated with $B4$. In our analysis, $B4$ also gave the best correlation (Eq. 1), but the other parameters such as L gave poor correlations (Eq. 1′). In contrast, the activities against *Klebsiella pneumoniae* HNR 828 and *Pseudomonas aeruginosa* HNR 1472, clinically resistant bacteria, were better correlated with L than with $B4$, unlike the activity against *Escherichia coli* NIHJ JC-2 (Eqs. 2, 3, 2′ and 3′). The results indicate that the steric size of the C-8 substituent contributes to the activity against gram-negative bacteria, but the nature of the contribution is different among bacteria. Equations 1—3 gave $B4$=1.92 Å, L=3.12 Å and L=3.17 Å as the optimum values, respectively. These values are close to the $B4$ and L values of the methyl, chloro, fluoro and

Table 2. Antibacterial Activity and Physicochemical Parameters of 8-Substituted Quinolones



Compd. No.	R^2	B_4[a]	L[a]	Minimum inhibitory concentration (mol/l)			Minimum inhibitory concentration (mol/l)			Minimum inhibitory concentration (mol/l)		
				Obsd.	Eq. 1		Obsd.	Eq. 2		Obsd.	Eq. 3	
					Calcd	(Δ)[b]		Calcd	(Δ)[b]		Calcd	(Δ)[b]
7a	H	1.00	2.06	7.13	7.17	(−0.04)	4.73	4.78	(−0.05)	4.73	4.74	(0.01)
8a	Me	2.04	2.87	7.48	7.51	(−0.03)	5.07	5.25	(−0.18)	5.67	5.57	(0.10)
9a	F	1.35	2.65	7.47	7.39	(0.08)	5.36	5.18	(0.18)	5.36	5.44	(−0.08)
10a	Cl	1.80	3.52	7.48	7.51	(−0.03)	5.37	5.20	(0.17)	5.67	5.55	(0.12)
12a	OMe	2.87	3.98	7.20	7.14	(0.06)	5.10	4.95	(0.15)	5.41	5.17	(0.24)
13a	OEt	3.36	4.80	6.63	6.65	(−0.02)	4.24	4.05	(0.19)	3.63	3.72	(−0.09)
15a	$OCHF_2$	3.30	3.98	6.92	6.69	(0.23)	4.82	4.95	(−0.13)	4.82	5.17	(−0.35)
16a	OCF_3	3.30	4.57	6.63	6.69	(−0.06)	4.23	4.36	(−0.13)	4.53	4.22	(0.31)
17a	SMe	3.26	4.30	6.58	6.77	(−0.19)	4.48	4.67	(−0.19)	4.48	4.72	(−0.24)

a) Taken from ref. 14. b) Δ, the difference between observed and calculated values.

methoxy groups selected as C-8 substituents.

The four selected compounds (**8a**, **9a**, **10a**, **12a**) with excellent antibacterial activity and the 8-unsubstituted compound (**7a**) were tested for effect on the chromosome. All these compounds were markedly injurious to the chromosome, except **7a**.

5-Substituted Quinolones To evaluate substituent effects at the C-5 position, the four compounds (**8a**, **9a**, **10a**, **12a**) bearing various substituents (H, Me, Cl, NH_2, NHMe, NMe_2) at the C-5 position were evaluated for antibacterial activity and chromosome-injuring action; the results are shown in Table 3. The data for C-5 substituted derivatives (**7b**, **d**) of **7a** are included for comparison.

The introduction of a methyl group (**7b**, **8b**, **9b**, **12b**) increased the potency against gram-positive bacteria and decreased that against gram-negative bacteria. The same trend against standard bacteria was reported by Hagen *et al.*[16] and Suto *et al.*[17] Among the 5-methyl compounds, the 8-fluoro compound **9b** was 4 times more potent than CPFX (**1**) against both gram-positive and gram-negative bacteria. Introduction of a chlorine atom (**8c**) decreased the potency against both gram-positive and gram-negative bacteria. The same trend in the 5-halogeno compounds was reported by Ledoussal *et al.*,[7a] and others.[3,17,18] All 5-amino compounds (**7d**, **8d**, **9d**, **10d**, **12d**) were more potent than CPFX (**1**) and OFLX (**2**); **8d** and **9d** were 4 times more potent than CPFX (**1**) against both gram-positive and gram-negative bacteria. These compounds were classified into four groups, as follows. 1) The 8-unsubstituted compound (**7d**) showed decreased antibacterial activity against gram-positive bacteria and maintained activity against gram-negative bacteria. 2) The 8-methyl compound (**8d**) showed increased antibacterial activity against gram-positive bacteria and maintained activity against gram-negative bacteria. 3) The 8-fluoro compound (**9b**) showed maintained antibacterial activity against gram-positive bacteria and decreased activity against gram-negative bacteria. 4) The 8-chloro and methoxy compounds (**10d**, **12d**) had decreased antibacterial activity against both gram-positive bacteria and gram-negative bacteria. Introduction of a monomethylamino group (**8e**) decreased the potency against both gram-positive and gram-negative bacteria. Furthermore, introduction of a dimethylamino group (**8f**) resulted in loss of antibacterial activity against resistant bacteria. To rationalize the effects of the amino groups, we performed conformation analysis with NEMECIS. The amino group at the C-5 position of **8d** was parallel to the plane of the quinolone ring. The same result was reported in an X-ray diffraction study of SPFX (**3**)[3] having an amino group at the C-5 position. Monomethyl and dimethyl groups on the amino moiety at the C-5 position were above and below the plane of the quinolone ring. This result indicated that the steric bulkiness of the methyl group had an adverse effect on antibacterial activity. Shen *et al.* reported that quinolones form a complex with the base of DNA by hydrogen bonding, and the complex inhibits recombination of DNA by DNA gyrase. This inhibition of DNA gyrase requires at least four quinolone molecules.[19] Thus, the steric bulkines the monomethylamino and dimethylamino groups may interfere with hydrogen bonding between the carbonyl group at the C-3 or C-4 position of the quinolone ring and the base of DNA, or with the interactions between quinolone molecules.

Regarding side effects, the compound with the amino (**7b**) or methyl (**7d**) group at the C-5 position of **7a** induced chromosomal injury. Surprisingly, the compound (**8d**) with a methyl group at C-8 and an amino group at C-5 was

Table 3. *In Vitro* Antibacterial Activity and Chromosome Injury of 5-Substituted Quinolones

Compd. No.	R^3	R^2	Minimum inhibitory concentration (μg/ml)[a]						Chromosome injury[c] (100 μg/ml)
			Standard strains		Clinical isolates[b]				
			S. aureus FDA 209P	*E. coli* NIHJ JC-2	*S. aureus* HPC 292	*E. faecalis* HPC 975	*K. pneumoniae* HNR 828	*P. aeruginosa* HNR 1472	
7a	H	H	0.05	0.025	12.5	12.5	6.25	6.25	I
7b	Me	H	0.05	0.05	6.25	6.25	12.5	12.5	IV
7d	NH_2	H	0.05	0.025	50	25	6.25	12.5	III
8a	H	Me	0.025	0.012	3.13	3.13	3.13	0.78	IV
8b	Me	Me	0.025	0.025	1.56	1.56	6.25	12.5	IV
8c	Cl	Me	0.025	0.025	12.5	6.25	25	50	IV
8d	NH_2	Me	0.025	0.025	1.56	1.56	3.13	3.13	I
8e	NHMe	Me	0.10	0.10	100	100	50	>100	
8f	NMe_2	Me	1.56	1.56	>100	>100	>100	>100	
9a	H	F	0.025	0.012	3.13	3.13	1.56	1.56	IV
9b	Me	F	0.025	0.025	1.56	1.56	3.13	6.25	IV
9d	NH_2	F	0.012	0.006	3.13	3.13	3.13	3.13	IV
10a	H	Cl	0.025	0.012	1.56	1.56	1.56	0.78	IV
10d	NH_2	Cl	0.05	0.012	6.25	6.25	6.25	6.25	IV
12a	H	OMe	0.025	0.025	1.56	1.56	3.13	1.56	III
12b	Me	OMe	0.05	0.10	1.56	1.56	25	50	IV
12d	NH_2	OMe	0.05	0.05	3.13	3.13	12.5	50	IV
CPFX (**1**)			0.20	0.025	50	50	12.5	12.5	I
OFLX (**2**)			0.20	0.10	50	50	25	25	I

a) Inoculum size, one loopful of 10^6 CFU/ml. *b*) Clinical isolates are resistant to CPFX (**1**) and OFLX (**2**). *c*) The scoring is: I, <10%; II, 10—20%; III, 20—50%; IV, >50%.

Table 4. Phototoxicity Test of Selected Quinolones in Guinea Pigs

Compd. No.	Dose (i.v.) (mg/kg)	Number of animals with phototoxicity Score[a]				
		−	+	++	+++	++++
8a	10	1/5	4/5			
8d	10	5/5				
	30	5/5				
9a	10					5/5
9d[b]	10			1/2	1/2	
10a	10				1/5	4/5
12a	10	5/5				
CPFX (**1**)	10	2/5	3/5			
OPFX (**2**)	10	5/5				
	30		5/5			
AM-1091 (**5**)	10					5/5
AM-1155 (**6**)	10	5/5				

a) Scoring is as follows: −, no erythema; +, very slight erythema; ++, well defined erythema; +++, moderate to severe erythema; ++++, severe erythema (beet redness) to slight eschar formation (injuries in depth). *b*) Three animals died.

less damaging to the chromosome (at a concentration of 100 μg/ml), but the other combinations of the 5- and 8-substituents (**7d**, **8b**, **9b**, **d**, **10d**, **12b**, **d**) gave no significant improvement.

In conclusion, the 5-amino-8-methyl compound (**8d**) was an excellent compound with strong antibacterial activity and reduced ability to injure the chromosome.

Toxicity in Animals It was reported that lomefloxacin (LFLX, **4**), synthesized and developed in our laboratories, induces phototoxicity and convulsive activity.[20] These side-effects are important clinically. So, we continued to search for an active quinolone without these side-effects. Therefore, six compounds (**8a**, **d**, **9a**, **d**, **10a**, **12a**), which were 4 times more potent than CPFX (**1**) against both gram-positive and gram-negative bacteria, were evaluated for phototoxicity by intravenous injection in guinea pigs, based on evaluation of the grade of skin reactions in positive animals. The results are shown in Table 4. The data for CPFX (**1**) and OFLX (**2**) are included for comparison.

Phototoxicity of the compounds (**8a**, **9a**, **10a**, **12a**) with a methyl, fluoro, chloro or methoxy group at the C-8 position was as follows. Compound **12a** with a methoxy group was essentially free from phototoxicity at a dosage of 10 mg/kg in guinea pigs (i.v.). Kimura *et al.* also reported that an 8-methoxy compound, AM-1155 (**6**),[21] showed little phototoxicity. The highest phototoxicity (severe erythema to slight eschar formation) was seen with halogen substituents at the C-8 position (fluoro and chloro compounds **9a**, **10a**, and **5**). Domagala also found that 8-halogeno compounds had strong phototoxicity.[22] Compound **8a** with a methyl group showed weak phototoxicity.

The compounds (**8d**, **9d**) with an amino group at the C-5 position exhibited reduced phototoxicity (**8d** *vs.* **8a**, **9d** *vs.* **9a**), and the 5-amino-8-methyl compound (**8d**) was essentially free from phototoxicity at a dosage of 30 mg/kg (i.v.). Even OFLX (**2**), which shows no phototoxicity in man at normally used doses, exhibited toxicity at this concentration (a dosage of 30 mg/kg (i.v.)).

Thus, **8d** is very favorable from the viewpoint of reduced phototoxicity.

It is known that combination of a quinolone and a non-steroidal anti-inflammatory drug (NSAID) can induce convulsion.[23] Compound **8d** was subjected to convulsive activity assay by intraperitoneal injection with fenbufen in six mice. The result with CPFX (**1**) is shown for comparison. Compound **8d** did not cause convulsion when coadministered with fenbufen at a dosage of 100 mg/kg (i.p.). CPFX (**1**) induced convulsions in half of the mice used under the same condition.

In conclusion, we have found that 5-amino-7-(3-amino-1-pyrrolidinyl)-8-methyl-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**8d**) exhibits a reduced ability to injure chromosome, has a good balance of antibacterial activity against both gram-positive and gram-negative bacteria, and shows sufficient antibacterial activity against quinolone-resistant bacteria. It also appears to lack quinolone-type toxicity (free from both phototoxicity at a dosage of 30 mg/kg in guinea pigs (i.v.) and convulsion at a dosage of 100 mg/kg when coadministered with fenbufen in mice (i.p.)). The optimization of this compound at the 7-position will be reported in the future.

Experimental

Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. Elemental analyses were measured with a Yanaco MT-5 elemental analyses apparatus. Infrared (IR) spectra were recorded using a Hitachi 270-30 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured with a JEOL JNM-A 500 spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were measured by using a JEOL DX-300 mass spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter. Column chromatography was carried out with silica gel [Kieselgel 60 (Merck)]. TLC was conducted on 0.25 mm pre-coated silica gel plates ($60F_{245}$, Merck). Organic solutions were dried with Na_2SO_4 and the solvent was removed by rotary evaporation under reduced pressure. The known compounds (**7a**, **b**, **8b**, **9a**, **d**, **10a**, **d**, **12a**) were prepared according to the literature.[7] Compounds **7d**, **8a**, **9b**, **10b**, **d**, **12b**, **d**, **14a**, **15a** and **16a** of which the racemates were known, were prepared according to the literature,[8] and physicochemical data are given in the experimental section.

2,4,5-Trifluoro-3-methylthiobenzoic Acid (19) A suspension of 2,4,5-trifluoro-3-thiophthalic acid **18** (81.7 g, 0.324 mol), methyl iodide (101 ml, 1.62 mol) and K_2CO_3 (89.4 g, 0.648 mol) in dimethylformamide (DMF) (355 ml) was stirred at 30 °C for 30 min. The reaction mixture was poured into ice water and the solution was made acidic with 10% HCl. The acidic solution was extracted with Et_2O. The extract was washed with brine, dried, and concentrated to give crude 2,4,5-trifluoro-3-methylthiophthalic acid (81.0 g). A mixture of the phthalic acid (79.6 g) and trioctylamine (106 g, 0.299 mol) was heated at 140 °C for 3 h. After cooling, the reaction mixture was poured into ice water. The solution was made acidic with 10% HCl and extracted with Et_2O. The ethereal layer was extracted with 10% K_2CO_3, and the aqueous layer was made acidic with concentrated HCl. The acidic solution was extracted with Et_2O. The extract was washed with water, dried and concentrated. The residue was purified by silica gel column chromatography with a mixture of CH_2Cl_2 and MeOH (9 : 1) to afford **19** (24.1 g, 33%). Recrystallization from a mixture of *n*-hexane and iso-Pr_2O gave yellow needles, mp 118—120 °C. IR (KBr): 1698 (CO_2H) cm^{-1}. MS *m/z*: 222 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.53 (3H, s, SMe), 7.79 (1H, dt, J=8.0, 1.5 Hz, Ar-H). *Anal.* Calcd for $C_8H_5F_3O_2S$: C, 43.25; H, 2.27. Found: C, 43.28; H, 2.22.

Ethyl 2,4,5-Trifluoro-3-methylthiobenzoylacetate (20) A suspension of the 3-methylthiobenzoic acid **19** (25.8 g, 116 mmol), $(COCl)_2$ (19.9 ml, 232 mmol) and DMF (a few drops) in CH_2Cl_2 (200 ml) was stirred at room temperature for 2 h, then concentrated to give 2,4,5-trifluoro-3-methylthiobenzoyl chloride. Diethyl malonate (18.0 ml, 11.7 mmol) was

treated with magnesium (3.07 g, 128 mmol) in a mixture of EtOH (17.6 ml), toluene (53.4 ml), and carbon tetrachloride (a few drops) by the conventional method. A solution of 2,4,5-trifluoro-3-methylthiobenzoyl chloride in toluene (31.0 ml) was added dropwise to a solution of Grignard reagent in toluene (31.0 ml) under ice cooling. This mixture was kept for 2 h at room temperature, then 5% aqueous H_2SO_4 (100 ml) was added, and the resulting solution was extracted with Et_2O. The extract was washed with brine, dried, and concentrated to give the malonate as a brown oil. A suspension of this malonate and *p*-TsOH (0.30 g) in water (190 ml) was heated under reflux for 4 h. After cooling, the reaction mixture was extracted with Et_2O and the extract was dried and concentrated. The residue was purified by silica gel column chromatography with a mixture of CH_2Cl_2 and *n*-hexane (1 : 3) to afford **20** (24.6 g, 72%) as a colorless oil. IR (liq): 1746 (CO_2Et), 1696 (CO) cm^{-1}. MS *m/z*: 292 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.27, 1.34 (total 3H, each t, J=7.0 Hz, CH_2CH_3), 2.52, 2.50 (total 3H, each s, SMe), 3.96, 5.85, 12.72 (total 2H, each s, CH_2), 4.22, 4.28 (total 2H, each q, J=7.0 Hz, CH_2CH_3), 7.63—7.76 (1H, m, Ar-H). High-resolution MS *m/z*: Calcd for $C_{12}H_{11}F_3O_3S$: 292.0381. Found: 292.0389.

Ethyl 3-Cyclopropylamino-2-(2,4,5-trifluoro-3-methylthiobenzoyl)acrylate (21) A mixture of the ester **20** (40.0 g, 137 mmol), ethyl orthoformate (29.0 ml, 174 mmol) and Ac_2O (25.0 ml, 265 mmol) was heated under reflux for 5 h, then concentrated to give ethyl 3-ethoxy-2-(2,4,5-trifluoro-3-methylthiobenzoyl)acrylate, as a brown oil. Cyclopropylamine (8.77 ml, 127 mmol) was added dropwise to a solution of the acrylate in CH_2Cl_2 (200 ml) under ice cooling with stirring. The reaction mixture was kept for 30 min at room temperature, then concentrated to give a crystalline residue, which was washed with a mixture of *n*-hexane and iso-Pr_2O to give **21** (21.0 g, 43%). Recrystallization from iso-Pr_2O gave yellow needles, mp 76—77 °C. IR (KBr): 1700 (CO_2Et), 1634 (CO) cm^{-1}. MS *m/z*: 359 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.10 (7H, m, CH_2CH_3, $CH_2\times2$), 2.46 (3H, s, SMe), 2.91—3.05 (1H, m, CH), 3.98, 4.04 (total 2H, each q, J=7.0 Hz, CH_2CH_3), 7.09, 7.22 (total 1H, m, CH), 8.21 (1H, d, J=13.0 Hz, Ar-H). *Anal.* Calcd for $C_{16}H_{16}F_3NO_3S$: C,53.48; H, 4.49; N, 3.90. Found: C, 53.44; H, 4.48; N, 3.94.

Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methylthio-4-oxoquinoline-3-carboxylate (22) Sodium hydride (2.83 g, 70.8 mmol, 60% mineral oil dispersion) was added portionwise to a solution of the acrylate **21** (23.1 g, 64.3 mmol) in tetrahydrofuran (THF) (460 ml) and DMF (10.0 ml) and the mixture was stirred in an ice bath for 1 h. It was poured into ice water (500 ml) and the solution was neutralized with 10% HCl and extracted with AcOEt. The extract was washed with brine, dried and concentrated to give **22** (17.0 g, 70%). Recrystallization from a mixture of *n*-hexane and AcOEt gave colorless needles, mp 178—181 °C. IR (KBr): 1732 (CO_2Et), 1612 (CO) cm^{-1}. MS *m/z*: 339 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.85—1.27 (4H, m, $CH_2\times2$), 1.40 (3H, t, J=7.0 Hz, CH_2CH_3), 2.44 (3H, s, SMe), 4.39 (2H, q, J=7.0 Hz, CH_2CH_3), 4.40—4.48 (1H, m, CH), 8.20 (1H, t, J=9.5 Hz, C_5-H), 8.67 (1H, s, C_2-H). *Anal.* Calcd for $C_{16}H_{15}F_2NO_3S$: C, 56.63; H, 4.46; N, 4.13. Found: C, 56.53; H, 4.59; N, 4.17.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methylthio-4-oxoquinoline-3-carboxylic Acid (23) A mixture of the carboxylate **22** (150 mg, 0.442 mmol) and 6 N HCl (1.50 ml) was heated under reflux for 1 h. After cooling, the crystals that deposited were collected by filtration and washed with water to give **23** (110 mg, 80%). Recrystallization from a mixture of CH_2Cl_2 and MeOH gave a colorless powder, mp 214—222 °C. IR (KBr): 1736 (CO_2H), 1614 (CO) cm^{-1}. MS *m/z*: 311 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.92—0.98 (2H, m, $CH_2\times2$), 1.32—1.37 (2H, m, $CH_2\times2$), 2.48 (3H, d, J=3.0 Hz, SMe), 4.58—4.62 (1H, m, CH), 8.23 (1H, t, J=9.0 Hz, C_5-H), 8.95 (1H, s, C_2-H), *Anal.* Calcd for $C_{14}H_{11}F_2NO_3S$: C, 54.02; H, 3.56; N, 4.50. Found: C, 54.00; H, 3.46; N, 4.59.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methylthio-4-oxo-7-((*S*)-3-trifluoroacetylamino-1-pyrrolidinyl)quinoline-3-carboxylic Acid (24) A mixture of the quinolone **23** (1.00 g, 3.21 mmol), (*S*)-3-trifluoroacetylaminopyrrolidine hydrochloride (2.11 g, 9.65 mmol) and NEt_3 (1.30 g, 12.9 mmol) in acetonitrile (20.0 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was concentrated and the residue was neutralized with 10% HCl. The crystals that deposited were collected by filtration and washed with water to give yellow crystals. Recrystallization from acetonitrile gave **24** (0.73 g, 48%) as yellow prisms, mp 260—262 °C. IR (KBr): 1716 (CO_2H), 1624 (CO) cm^{-1}. MS *m/z*: 473 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.75—0.90 (2H, m, $CH_2\times2$), 1.12—1.25 (2H, m, $CH_2\times2$), 1.91 (3H, s, SMe), 2.05—2.16 (1H, m, CH_2), 2.23—2.35 (1H, m, CH_2), 3.60—3.70 (1H, m, NCH_2), 3.80—3.95 (3H, m, NCH_2, NCH_2), 4.45—4.55 (2H, m, CH, NCH), 7.67 (1H, d, J=14.0 Hz, C_5-H), 8.79 (1H, s, C_2-H), 9.61 (1H, br s, CONH). *Anal.* Calcd for $C_{20}H_{19}F_4N_3O_4S$: C, 50.60; H, 4.01; N, 8.73. Found: C, 50.74; H, 4.04; N, 8.88.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methylthio-4-oxoquinoline-3-carboxylic Acid (17a) A suspension of the quinolone **24** (1.00 g, 2.11 mmol) in 10% NaOH (4.20 ml) was stirred at room temperature for 1 h, then neutralized with 10% HCl. The crystals that deposited were collected by filtration and washed with water. Recrystallization from MeOH gave **17a** (0.34 g, 43%), pale yellow prisms, mp 213.5—215.5 °C. $[\alpha]_D^{20}$ −30.7° (c=0.1, DMF). IR (KBr): 1732 (CO_2H), 1624 (CO) cm^{-1}. MS *m/z*: 377 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.82—0.90 (2H, m, $CH_2\times2$), 1.12—1.22 (2H, m, $CH_2\times2$), 1.72—1.82 (1H, m, CH_2), 1.89 (3H, s, SMe), 2.03—2.14 (1H, m, CH_2), 3.30—3.37 (1H, m, NCH_2), 3.60—3.70 (2H, m, NCH_2, NCH), 3.74—3.82 (1H, m, NCH_2), 3.84—3.92 (1H, m, NCH_2), 4.44—4.52 (1H, m, CH), 7.63 (1H, d, J=14.0 Hz, C_5-H), 8.76 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{20}FN_3O_3S$: C, 57.28; H, 5.34; N, 11.13. Found: C, 57.00; H, 5.18; N, 11.47.

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-((*S*)-trifluoroacetylamino-1-pyrrolidinyl)quinoline-3-carboxylic Acid (26) A mixture of the difluoroboron chelate **25** (5.00 g, 14.6 mmol), (*S*)-3-trifluoroacetylaminopyrrolidine hydrochloride (4.05 g, 18.5 mmol) and NEt_3 (4.79 g, 47.4 mmol) in dimethyl sulfoxide (DMSO) (20.0 ml) was stirred at room temperature for 7.5 h. Ether (125 ml) was added to the reaction mixture and the whole was stirred for 2 d. The crystals that deposited were collected by filtration to give yellow crystals (7.34 g). A mixture of these crystals and NEt_3 (5.60 ml, 76.2 mol) in a mixture of MeOH (260 ml) and $ClCH_2CH_2Cl$ (65.0 ml) was heated under reflux for 2.5 h. After cooling, the reaction mixture was concentrated. Water was added to the residue and the solution was acidified to pH 3 with 10% HCl. The crystals that deposited were collected by filtration to give **26** (4.20 g, 63%). Recrystallization from a mixture of acetonitrile and iso-Pr_2O gave a yellow powder, mp 186—188 °C. $[\alpha]_D^{20}$ −17.0° (c=0.1, DMSO). IR (KBr): 1716 (CO_2H), 1620 (CO) cm^{-1}. MS *m/z*: 457 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.96—1.05 (2H, m, $CH_2\times2$), 1.10—1.15 (2H, m, $CH_2\times2$), 2.02—2.08 (1H, m, CH_2), 2.20—2.26 (1H, m, CH_2), 2.49 (3H, d, J=2.0 Hz, OMe), 3.55—3.60 (1H, m, NCH_2), 3.66—3.89 (3H, m, NCH_2, NCH_2), 4.12—4.17 (1H, m, CH), 4.42—4.46 (1H, m, NCH), 7.68 (1H, d, J=14.0 Hz, C_5-H), 8.66 (1H, s, C_2-H), 9.56 (1H, br s, NHCO). *Anal.* Calcd for $C_{20}H_{19}F_4N_3O_5$: C, 52.52; H, 4.19; N, 9.19. Found: C, 52.36; H, 4.24; N, 9.33.

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-hydroxy-4-oxo-7-((*S*)-trifluoroacetylamino-1-pyrrolidinyl)quinoline-3-carboxylic Acid (27) A suspension of the 8-methoxyquinolone **26** (2.50 g, 5.47 mmol), TMSCl (4.20 ml, 52.8 mmol) and NaI (4.92 g, 32.8 mmol) in DMF (10.0 ml) was heated at 130 °C for 7.5 h. After cooling, the mixture was poured into ice water, and the precipitates were collected by filtration and washed with Et_2O to give **27** (2.10 g, 87%). Recrystallization from AcOEt gave a yellow powder, mp 126—129 °C. $[\alpha]_D^{20}$ −39.0° (c=0.1, DMSO). IR (KBr): 1722 (CO_2H), 1610 (CO) cm^{-1}. MS *m/z*: 443 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.22 (4H, m, $CH_2\times2$), 1.92—2.10 (1H, m, CH_2), 2.32—2.44 (1H, m, CH_2), 3.00—3.30 (2H, m, $NCH_2\times2$), 3.38—3.48 (1H, m, NCH_2), 3.50—3.56 (1H, m, NCH_2), 4.30—4.38 (1H, m, CH), 4.55—4.62 (1H, m, NCH), 7.52 (1H, d, J=12.0 Hz, C_5-H), 8.69 (1H, s, C_2-H), 9.58 (1H, br s, CONH). *Anal.* Calcd for $C_{19}H_{17}F_4N_3O_5$: C, 50.45; H, 4.01; N, 9.29. Found: C, 50.43; H, 3.93; N, 9.27.

Ethyl 1-Cyclopropyl-8-ethoxy-6-fluoro-1,4-dihydro-4-oxo-7-((*S*)-trifluoroacetylamino-1-pyrrolidinyl)quinoline-3-carboxylate (28) A mixture of the 8-hydroxyquinolone **27** (2.00 g, 4.51 mmol), K_2CO_3 (1.25 g, 9.02 mmol) and ethyl iodide (0.90 ml, 11.3 mmol) in DMF (20.0 ml) was heated at 80 °C for 2 h with stirring. Ethyl iodide (0.18 ml, 2.26 mmol) was added and the solution was stirred at the same temperature for 2 h. The reaction mixture was poured into ice water and the whole was acidified to pH 3 with 10% HCl. The precipitates were collected by filtration and washed with water to afford **28** (1.67 g, 74%). Recrystallization from a mixture of iso-PrOH and Et_2O gave pale brown needles, mp 168—169 °C. $[\alpha]_D^{20}$ +54.2° (c=0.1, DMF). IR (KBr): 1724 (CO_2H), 1694 (CONH), 1616 (CO) cm^{-1}. MS *m/z*: 499 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.81—0.93 (2H, m, $CH_2\times$ 2), 1.00—1.10 (2H, m, $CH_2\times2$), 1.26 (3H, t, J=7.0 Hz, CH_2CH_3), 1.27 (3H, t, J=7.0 Hz, CH_2CH_3), 2.00—2.10 (1H, m, CH_2), 2.16—2.28 (1H, m, CH_2), 3.48—3.83 (4H, m, $NCH_2\times2$), 3.76 (2H, q, J=7.0 Hz, CH_2CH_3), 3.94—4.02 (1H, m, CH), 4.21 (2H, q, J=7.0 Hz, CH_2CH_3), 4.34—4.44 (1H,

m, NCH), 7.54 (1H, d, J=14.0 Hz, C_5-H), 8.43 (1H, s, C_2-H), 9.52 (1H, br s, CONH). *Anal.* Calcd for $C_{23}H_{25}F_4N_3O_5$: C, 55.31; H, 5.05; N, 8.41. Found: C, 55.29; H, 5.12; N, 8.34.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-hydroxy-4-oxoquinoline-3-carboxylic Acid (11a) In a similar manner to that described for the preparation of **17a** from **24**, compound **27** was converted to **11a** (64%), pale brown powder, mp 181—185 °C. $[\alpha]_D^{20}$ −59.8° (c=0.1, DMSO). IR (KBr): 1616 (CO) cm^{-1}. MS m/z: 347 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.92—1.10 (4H, m, CH_2×2), 2.10—2.15 (2H, m, CH_2), 2.94—2.96 (1H, m, NC$\underline{H}_2$), 3.05—3.12 (1H, m, NCH), 3.40—3.50 (2H, m, NC$\underline{H}_2$×2), 3.62—3.68 (1H, m, NC$\underline{H}_2$), 4.67—4.75 (1H, m, CH), 6.76 (1H, d, J=9.5 Hz, C_5-H), 8.49 (1H, s, C_2-H). *Anal.* Calcd for $C_{17}H_{18}FN_3O_4 \cdot H_2O$: C, 55.89; H, 5.52; N, 11.50. Found: C, 56.27; H, 5.31; N, 11.32.

7-((*S*)-3-Amino-1-pyrrolidinyl)-8-ethoxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid Hydrochloride (13a) In a similar manner to that described for the preparation of **17a** from **24**, compound **28** was converted to **13a** (64%), pale yellow needles, mp 216.5—219 °C (dec.). $[\alpha]_D^{20}$ +54.2° (c=0.1, DMF). IR (KBr): 1718 (CO_2H), 1620 (CO) cm^{-1}. MS m/z: 375 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.92—1.18 (4H, m, CH_2×2), 1.28 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 2.05—2.16 (1H, m, C$\underline{H}_2$), 2.25—2.35 (1H, m, C$\underline{H}_2$), 3.63—3.95 (5H, m, NCH_2×2, NCH), 3.77 (2H, q, J=7.0 Hz, C$\underline{H}_2CH_3$), 4.11—4.20 (1H, m, CH), 7.67 (1H, d, J=13.5 Hz, C_5-H), 8.68 (1H, s, C_2-H). *Anal.* Calcd for $C_{19}H_{22}FN_3O_4 \cdot HCl \cdot H_2O$: C, 53.09; H, 5.86; N, 9.77. Found: C, 53.05; H, 5.98; N, 9.75.

2,4,5-Trifluoro-3-methyl-6-nitrobenzoic Acid (30) 2,4,5-Trifluoro-3-methylbenzoic acid **29** (110 g, 0.579 mol) was added portionwise to a mixture of concentrated H_2SO_4 (550 ml) and 70% HNO_3 (200 ml) at 55 °C to 70 °C with stirring. After 2 h at room temperature, the reaction mixture was poured into ice and extracted with iso-Pr_2O. The extract was washed with brine, dried and concentrated to afford **30** (55.3 g, 41%). Recrystallization from a mixture of iso-PrOH and *n*-hexane gave colorless needles, mp 170—171 °C. IR (KBr): 1720 (CO_2H) cm^{-1}. MS m/z: 235 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.37 (3H, t, J=2.5 Hz, Me). *Anal.* Calcd for $C_8H_4F_3NO_4$: C, 40.87; H, 1.71; N, 5.96. Found: C, 40.75; H, 1.54; N, 5.98.

Ethyl 2,4,5-Trifluoro-3-methyl-6-nitrobenzoylacetate (31) A suspension of the 3-methylbenzoic acid **30** (25.0 g, 10.6 mmol), $(COCl)_2$ (19.5 ml, 230 mmol) and DMF (a few drops) in CH_2Cl_2 (270 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated to give 2,4,5-trifluoro-3-methyl-6-nitrobenzoyl chloride. Diethyl malonate (18.0 ml, 11.7 mmol) was treated with magnesium (3.07 g, 128 mmol) in a mixture of EtOH (17.6 ml), toluene (53.4 ml), and carbon tetrachloride (a few drops) by the conventional method. A solution of the above 2,4,5-trifluoro-3-methyl-6-nitrobenzoyl chloride in toluene (31.0 ml) was added dropwise to a solution of the Grignard reagent in toluene (31.0 ml) under ice cooling. The reaction mixture was kept for 2 h at room temperature, then 5% aqueous H_2SO_4 (100 ml) was added and the resulting solution was extracted with Et_2O. The extract was washed with brine, dried and concentrated to give the malonate. A suspension of this malonate and *p*-TsOH (0.77 g) in water (110 ml) was heated under reflux for 50 min. After cooling, the reaction mixture was extracted with Et_2O and the extract was dried and concentrated to afford crude **31** (33.5 g, theoretical amount 32.5 g) as a brown oil. IR (liq): 1748 (CO_2Et), 1648 (CO) cm^{-1}. MS m/z: 305 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.26, 1.34 (total 3H, each t, J=7.0 Hz, $CH_2C\underline{H}_3$), 2.33, 2.35 (total 3H, each t, J=2.5 Hz, Me), 3.91, 5.48, 12.34 (total 2H, each s, CH_2), 4.20, 4.28 (total 2H, each q, J=7.0 Hz, C$\underline{H}_2CH_3$). High-resolution MS m/z: Calcd for $C_{12}H_{10}F_3NO_5$: 305.0511; Found: 305.0531.

Ethyl 3-Cyclopropylamino-2-(2,4,5-trifluoro-3-methyl-6-nitrobenzoyl)-acrylate (32) A mixture of the ester **31** (30.8 g, theoretical amount 29.9 g, 98.0 mmol), ethyl orthoformate (25.2 ml, 152 mmol) and Ac_2O (22.8 ml, 242 mmol) was heated under reflux for 1 h. The reaction mixture was concentrated to give ethyl 3-ethoxy-2-(2,4,5-trifluoro-3-methyl-6-nitrobenzoyl)acrylate (34.0 g) as a brown oil. Cyclopropylamine (7.20 ml, 104 mmol) was added dropwise to a solution of the acrylate (34.0 g) in EtOH (83.3 ml) under ice cooling with stirring. The reaction mixture was kept for 30 min at room temperature, then concentrated, and the residue was purified by silica gel column chromatography with a mixture of *n*-hexane and CH_2Cl_2 (1 : 1) to give **32** (23.3 g, 64% from **29**). Recrystallization from iso-Pr_2O gave yellow needles, mp 115.0—115.5 °C. IR (KBr): 1702 (CO_2Et), 1636 (CO) cm^{-1}. MS m/z: 372 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.80—0.99 (4H, m, CH_2×2), 1.13 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 2.29 (3H, t, J=2.0 Hz, Me), 2.97—3.05 (1H, m, CH), 3.94—4.11 (2H, m, C$\underline{H}_2CH_3$), 8.25 (1H, d, J=14.0 Hz, CH), 10.90—11.00 (1H, br s, NH). *Anal.* Calcd for $C_{16}H_{15}F_3N_2O_5$: C, 51.62; H, 4.06; N, 7.52. Found: C, 51.57; H, 3.92; N, 7.52.

Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-5-nitro-4-oxoquinoline-3-carboxylate (33) 1) Sodium hydride (3.20 g, 80.0 mmol, 60% mineral oil dispersion) was added portionwise to a solution of the acrylate **32** (27.1 g, 72.8 mmol) in 1,4-dioxane (270 ml), and the mixture was stirred at room temperature for 1 h, then poured into ice water (300 ml). The crystals that deposited were collected by filtration to give **33** (19.5 g, 76%). Recrystallization from acetonitrile gave colorless needles, mp 260—261 °C. IR (KBr): 1732 cm^{-1} (CO_2Et). MS m/z: 352 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 1.00—1.06 (2H, m, C$\underline{H}_2$×2), 1.16—1.20 (2H, m, C$\underline{H}_2$×2), 1.27 (3H, t, J=7.5 Hz, $CH_2C\underline{H}_3$), 2.79 (3H, d, J=3.5 Hz, Me), 4.23 (2H, q, J=7.5 Hz, C$\underline{H}_2CH_3$), 4.20—4.25 (1H, m, CH), 8.64 (1H, s, C_2-H). *Anal.* Calcd for $C_{16}H_{14}F_2N_2O_5$: C,54.55; H, 4.01; N, 7.95. Found: C, 54.66; H, 3.77; N, 7.99.

2) A suspension of the acrylate **32** (15.0 g, 40.3 mmol), K_2CO_3 (12.2 g, 88.7 mmol) and 18-crown-6-ether (0.60 g) in THF (150 ml) was heated at 70 °C for 8 h. After cooling, the crystals that deposited were collected by filtration and washed with water. Recrystallization from acetonitrile gave **33** (12.1 g, 85%) as colorless needles. This product was identical with **33** synthesized as above.

Ethyl 5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylate (34) A suspension of the 5-nitroquinolone **33** (37.5 g, 106 mmol) and iron powder (22.0 g, 394 mmol) in AcOH (150 ml) was heated at 90 °C for 4.5 h. After cooling, the inorganic precipitate was filtered off and the filtrate was poured into water (540 ml). The precipitates were collected by filtration and washed with iso-PrOH to give **34** (30.8 g, 90%) as pale a brown powder, mp 182.5—185.5 °C. IR (KBr): 1686 (CO_2Et) cm^{-1}. MS m/z: 322: (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.87—0.96 (2H, m, C$\underline{H}_2$×2), 1.10—1.19 (2H, m, C$\underline{H}_2$×2), 1.39 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 2.53 (3H, d, J=2.5 Hz, Me), 3.83—3.90 (1H, m, CH), 4.37 (2H, q, J=7.0 Hz, CH_2CH_3), 6.80—7.20 (1H, br s, NH_2), 8.51 (1H, s, C_2-H). *Anal.* Calcd for $C_{16}H_{16}F_2N_2O_3$: C, 59.62; H, 5.00; N, 8.69. Found: C, 59.74; H, 5.08; N, 8.60.

5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid (35) The carboxylate **34** (14.8 g, 45.9 mmol) in concentrated HCl (37.2 ml) and AcOH (50.0 ml) was heated under reflux for 2 h. After cooling, the deposited crystals were collected by filtration and washed with water to give **35** (11.8 g, 87%). Recrystallization from DMF gave a yellow powder, mp 290—291 °C. IR (KBr): 1714 (CO_2H) cm^{-1}. MS m/z: 294 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.90—0.96 (2H, m, C$\underline{H}_2$×2), 1.13—1.21 (2H, m, C$\underline{H}_2$×2), 2.57 (3H, d, J=3.0 Hz, Me), 4.20—4.30 (1H, m, CH), 7.50—7.70 (2H, br s, NH_2), 8.68 (1H, s, C_2-H), 14.30—14.50 (1H, br s, CO_2H). *Anal.* Calcd for $C_{14}H_{12}F_2N_2O_3$: C, 57.15; H, 4.11; N, 9.52. Found: C, 57.10; H, 4.03; N, 9.53.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-5-dimethylamino-8-methyl-4-oxoquinoline-3-carboxylic Acid (36) A mixture of the 5-aminoquinolone **35** (10.0 g, 34.0 mmol) and 37% formaldehyde (50.0 ml) in formic acid (50.0 ml) was heated under reflux for 8 h. After cooling, the reaction mixture was poured into ice water and the precipitate was filtered off. The filtrate was concentrated and the residue was neutralized with 10% NaOH. The crystals that deposited were collected by filtration and washed with water to give **36** (10.1 g, 92%). Recrystallization from a mixture of CH_2Cl_2 and *n*-hexane gave yellow needles, mp 164.5—166 °C. IR (KBr): 1722 (CO_2H), 1622 (CO) cm^{-1}. MS m/z: 322 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.81—0.97 (2H, m, C$\underline{H}_2$×2), 1.10—1.24 (2H, m, C$\underline{H}_2$×2), 2.61 (3H, d, J=3.0 Hz, Me), 2.94 (6H, d, J=2.5 Hz, NMe_2), 4.22—4.32 (1H, m, CH), 8.70 (1H, s, C_2-H). *Anal.* Calcd for $C_{16}H_{16}F_2N_2O_3$: C,59.62; H, 5.00; N, 8.69. Found: C, 59.33; H, 4.95; N, 8.66.

[5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylate-O^3,O^4]difluoroboron (37) A mixture of the quinolinecarboxylic acid **36** (5.00 g, 17.0 mmol) and boron trifluoride etherate (3.13 ml: 24.5 mmol) in methyl isobutyl ketone (75.5 ml) was heated under reflux for 1 h. After cooling, the crystals that deposited were collected by filtration and washed with Et_2O to give **37** (5.38 g, 93%) as a yellow powder. IR (KBr): 1696 (CO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.08—1.15 (2H, m, C$\underline{H}_2$×2), 1.21—1.13 (2H, m, C$\underline{H}_2$×2), 2.67 (3H, d, J=2.5 Hz, Me), 4.52—4.59 (1H, m, CH), 7.28 (2H, br s, NH_2), 9.10 (1H, s, C_2-H).

5-Amino-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxo-7-((*S*)-3-trifluoroacetylamino-1-pyrrolidinyl)quinoline-3-carboxylic Acid (38) A mixture of the difluoroboron chelate **37** (2.50 g, 7.31 mmol), (*S*)-3-

trifluoroacetylaminopyrrolidine hydrochloride (3.20 g, 14.6 mol) and NEt_3 (1.02 ml, 7.31 mmol) in DMSO (10.0 ml) was stirred at 30 °C for 3 d. The reaction mixture was made acidic with 10% HCl (10.0 ml), and then concentrated to afford yellow crystals (3.66 g). A mixture of these crystals and NEt_3 (3.80 ml, 27.3 mmol) in MeOH (30.0 ml) was heated under reflux for 3.5 h. The crystals that deposited were collected by filtration to give **38** (0.72 g, 22%). Recrystallization from acetonitrile gave a yellow powder, mp 238.5—240 °C. $[\alpha]_D^{20}$ −28.1° (c=0.1, DMSO). IR (KBr): 1716 (CO_2H), 1628 (CO) cm^{-1}. MS m/z: 456 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.75—0.81 (2H, m, $CH_2 \times 2$), 1.10—1.15 (2H, m, $CH_2 \times 2$), 2.00—2.07 (1H, m, CH_2), 2.23—2.30 (1H, m, CH_2), 3.20 (3H, m, Me), 3.44—3.46 (1H, m, NCH_2), 3.49—3.53 (1H, m, NCH_2), 3.60—3.64 (1H, m, NCH_2), 3.73—3.76 (1H, m, NCH_2), 4.14—4.18 (1H, m, CH), 4.42—4.46 (1H, m, NCH), 7.05—7.22 (2H, br s, NH_2), 8.62 (1H, s, C_2-H), 9.55 (1H, d, J=6.0 Hz, CONH), 14.87 (1H, s, CO_2H). *Anal.* Calcd for $C_{20}H_{20}F_4N_4O_4$: C, 52.63; H, 4.42; N, 12.28. Found: C, 52.64; H, 4.37; N, 12.35.

5-Amino-7-((*S*)-3-amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid Hydrate (8d) A mixture of the quinolone **37** (620 mg, 1.36 mmol) and NaOH (0.57 g, 10.2 mmol) in water (10.0 ml) was stirred at room temperature for 1 h, then neutralized with 10% HCl and concentrated. MeOH was added to the residue, and the precipitates were collected by filtration and washed with iso-PrOH. The crystals were dissolved into a mixture of CH_2Cl_2 and MeOH, insoluble material was filtered off and the filtrate was concentrated to give **8d** (36 mg, 6%). Recrystallization from a mixture of CH_2Cl_2 and MeOH gave a pale yellow powder, mp 202—204 °C (dec.). $[\alpha]_D^{20}$ −11.0° (c=0.1, DMF). MS m/z: 360 (M^+). IR (KBr): 1634 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.70—0.82 (2H, m, $CH_2 \times 2$), 1.04—1.17 (2H, m, $CH_2 \times 2$), 1.61—1.71 (1H, m, CH_2), 2.02—2.12 (1H, m, CH_2), 2.33 (3H, s, Me), 3.10—3.69 (5H, m, $NCH_2 \times 2$, NCH), 4.10—4.19 (1H, m, CH), 7.05 (2H, br s, NH_2), 8.60 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{21}FN_4O_3 \cdot H_2O$: C, 57.13; H, 6.13; N, 14.81. Found: C, 57.36; H, 5.91; N, 14.70.

7-((*S*)-3-*tert*-Butoxycarbonylamino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-5-dimethylamino-4-oxoquinoline-3-carboxylic Acid (39) A suspension of the 5-dimethylaminoquinolone **36** (2.00 g, 6.21 mmol), (*S*)-3-*tert*-butoxycarbonylaminopyrrolidine (1.74 g, 9.32 mmol) and NEt_3 (0.87 ml, 6.21 mmol) in DMSO (8.0 ml) was stirred at 100 °C under a nitrogen atmosphere for 62 h. The reaction mixture was poured into water, then the solution was acidified to pH 4 with 10% HCl and extracted with CH_2Cl_2. The extract was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography with a mixture of CH_2Cl_2 and MeOH (9 : 1) to afford **39** (1.10 g, 36%). Recrystallization from a mixture of acetone and iso-Pr_2O gave pale yellow needles, mp 215—216 °C. $[\alpha]_D^{20}$ −61.9° (c=0.1, $CHCl_3$). IR (KBr): 1734 (CO_2H), 1688 (CONH), 1616 (CO) cm^{-1}. MS m/z: 488 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.64—0.78 (2H, m, $CH_2 \times 2$), 1.06—1.15 (2H, m, $CH_2 \times 2$), 1.41 (9H, s, $C(CH_3)_3$), 1.82—1.95 (1H, m, CH_2), 2.10—2.21 (1H, m, CH_2), 2.39 (3H, s, Me), 2.88 (6H, d, J=2.5 Hz, NMe_2), 3.25—3.70 (4H, m, $NCH_2 \times 2$), 4.05—4.21 (2H, m, CH, NCH), 7.06 (1H, br s, CONH), 8.64 (1H, s, C_2-H). *Anal.* Calcd for $C_{25}H_{33}FN_4O_5$: C, 61.46; H, 6.81; N, 11.47. Found: C, 61.39; H, 6.87; N, 11.48.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-5-dimethylamino-4-oxoquinoline-3-carboxylic Acid (8f) A suspension of the quinolone **39** (1.00 g, 2.05 mmol) in concentrated HCl (1.70 ml) was stirred for 5 min at room temperature. Water (1.70 ml) was added, and the reaction mixture was stirred for 15 min then adjusted to pH 11 with 10% NaOH. After 20 min, the reaction mixture was neutralized with 10% HCl and extracted with CH_2Cl_2. The extract was washed with water and brine, dried and concentrated. AcOEt was added to the residue and the crystals that deposited were collected by filtration and washed with a mixture of AcOEt and Et_2O to afford **8f** (0.38 g, 48%). Recrystallization from a mixture of AcOEt and Et_2O gave a yellow powder, mp 178—179.5 °C. $[\alpha]_D^{20}$ +23.0° (c=0.1, MeOH). IR (KBr): 1724 (CO_2H), 1616 (CO) cm^{-1}. MS m/z: 388 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.67—0.78 (2H, m, $CH_2 \times 2$), 1.04—1.15 (2H, m, $CH_2 \times 2$), 1.62—1.72 (1H, m, CH_2), 2.05—2.15 (1H, m, CH_2), 2.38 (3H, m, Me), 2.88 (6H, d, J=2.5 Hz, NMe_2), 3.10—3.29 (1H, m, NCH_2), 3.42—3.68 (4H, m, NCH_2, NCH_2, NCH), 4.11—4.20 (1H, m, CH), 8.64 (1H, s, C_2-H). *Anal.* Calcd for $C_{20}H_{25}FN_4O_3$: C, 61.84; H, 6.49; N, 14.42. Found: C, 61.88; H, 6.58; N, 14.26.

(2-Chloro-2,4,5-trifluoro-3-methylphenyl)-4,4-dimethyl-2-oxazoline (41) *n*-BuLi (19.1 ml, 1.62 M solution in *n*-hexane, 30.9 mmol) was added dropwise to a solution of iso-Pr_2NH (3.54 g, 35.0 mmol) in THF (50.0 ml) at −50 °C under a nitrogen atmosphere and the resulting solution was stirred at the same temperature for 30 min. A solution of the oxazoline **40** (5.00 g, 20.6 mmol) in THF (20.0 ml) was added dropwise to the above solution at −60 °C and stirring was continued for 1 h at the same temperature. Hexachloroacetone (15.2 g, 57.7 mmol) was added dropwise to the above solution at −65 °C and stirring was continued at −70 °C for 1 h and then at −20 °C for 2 h. Saturated NH_4Cl was added to the reaction mixture to quench the reaction. The organic layer was separated, and the aqueous layer was extracted with Et_2O. The combined organic extracts were washed, dried and concentrated to leave a residue, which was purified by silica gel column chromatography with a mixture of CH_2Cl_2 and *n*-hexane (3 : 1) to afford **40** (4.49 g, 78%) as a pale yellow oil, bp 105—110 °C (5 mmHg). IR (liq): 1678 cm^{-1}. MS m/z: 227, 229 (M^+, 3 : 1). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (6H, s, Me × 2), 2.23 (2H, t, J=2.0 Hz, Me), 4.15 (2H, s, CH_2). High-resolution MS m/z: Calcd for $C_{12}H_{11}ClF_3NO$: 277.0482, 279.0451 (3 : 1). Found: 277.0505, 279.0430 (3 : 1).

2-Amino-2-methylpropyl-2-chloro-3,4,6-trifluoro-5-methylbenzoate Hydrochloride (42) A mixture of the oxazoline **41** (17.7 g, 63.7 mmol) and 1 N HCl (177 ml) was heated at 70 °C with stirring for 3.5 h. After cooling, the reaction mixture was washed with Et_2O, and extracted with CH_2Cl_2. The extract was dried and concentrated to leave a crystalline residue, which was washed with iso-Pr_2O to afford **42** (15.8 g, 75%). Recrystallization from a mixture of CH_2Cl_2 and *n*-hexane gave colorless scales, mp 186.5—188.5 °C. IR (KBr): 1746 (CO_2) cm^{-1}. MS m/z: 280, 282 ($M^+ - NH_2 + H$, 3 : 1). ^{1}H-NMR (DMSO-d_6) δ: 1.35 (6H, s, Me × 2), 2.24 (3H, t, J=2.0 Hz, Me), 4.42 (2H, s, CH_2), 8.41 (3H, br s, NH_3^+). *Anal.* Calcd for $C_{12}H_{13}ClF_3NO_2 \cdot HCl$: C, 43.39; H, 4.25; N, 4.22. Found: C, 43.27; H, 4.32; N, 4.34.

2-Acetylamino-2-methylpropyl-2-chloro-3,4,6-trifluoro-5-methylbenzoate (43) A suspension of the benzoate **42** (15.8 g, 0.476 mol) and pyridine (8.50 ml) in Ac_2O (63.0 ml) was heated at 50 °C with stirring for 1 h, then concentrated. The residue was taken up in ice water and extracted with iso-Pr_2O. The extract was washed with saturated $NaHCO_3$ and water, dried and concentrated to afford **43** (16.0 g, 100%). Recrystallization from a mixture of CH_2Cl_2 and *n*-hexane gave colorless needles, mp 108—110 °C. IR (KBr): 1744 (CO_2), 1646 (AcNH) cm^{-1}. MS m/z: 280, 282 ($M^+ - AcNH + H$, 3 : 1). ^{1}H-NMR (DMSO-d_6) δ: 1.41 (6H, s, Me × 2), 1.93 (3H, s, CH_3CO), 2.25 (3H, t, J=2.0 Hz, Me), 4.53 (2H, s, CH_2). *Anal.* Calcd for $C_{14}H_{15}ClF_3NO_3$: C, 49.79; H, 4.48; N, 4.15. Found: C, 49.60; H, 4.47; N, 4.16.

2-Chloro-3,4,6-trifluoro-5-methylbenzoic Acid (44) A suspension of the benzoate **43** (16.0 g, 47.4 mmol) in 1 N NaOH (80 ml) was heated at 100 °C for 2 h. After cooling, the mixture was acidified to pH 1 with 10% HCl, and extracted with iso-Pr_2O. The extract was washed with brine, dried and concentrated to afford **44** (10.1 g, 95%). Recrystallization from a mixture of iso-Pr_2O and *n*-hexane gave colorless prisms, mp 139—142 °C. IR (KBr): 1706 (CO_2H) cm^{-1}. MS m/z: 224 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 2.27 (3H, t, J=2.0 Hz, Me). *Anal.* Calcd for $C_8H_4ClF_3O_2$: C, 42.79; H, 1.80. Found: C, 43.09; H, 1.72.

Ethyl 3-Cyclopropylamino-2-(6-chloro-3,4,5-trifluoro-3-methylbenzoyl)acetate (45) In a similar manner to that described for the preparation of **20** from **19**, compound **44** was converted to **45** (81%), a pale yellow oil. IR (liq): 1746 (CO_2Et), 1644 (CO) cm^{-1}. MS m/z: 294, 296 (M^+, 3 : 1). ^{1}H-NMR ($CDCl_3$) δ: 1.23—1.36 (3H, m, CH_2CH_3), 2.24, 2.25 (total 3H, each s, OMe), 4.16—4.22, 12.47 (total 4H, m, CH_2CH_3, CH_2). High-resolution MS m/z: Calcd for $C_{12}H_{10}ClF_3O_3$: 294.0263, 296.0240 (3 : 1). Found: 294.0262, 296.0236 (3 : 1).

Ethyl 3-Cyclopropylamino-2-(2-chloro-3,4,5-trifluoro-3-methylbenzoyl)acrylate (46) In a similar manner to that described for the preparation of **21** from **20**, compound **45** was converted to **46** (58%), colorless needles, mp 110—111 °C. IR (KBr): 1706 (CO_2Et), 1630 (CO) cm^{-1}. MS m/z: 361 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.82—0.97 (4H, m, $CH_2 \times 2$), 1.05 (3H, t, J=7.0 Hz, CH_2CH_3), 2.20 (3H, d, J=2.0 Hz, Me), 2.97—3.05 (1H, m, CH), 3.95, 4.02 (total 2H, each q, J=7.0 Hz, CH_2CH_3), 8.29, 8.42 (1H, d, J=14.0 Hz, CH), 11.15 (1H, br s, NH). *Anal.* Calcd for $C_{16}H_{15}ClF_3NO_3$: C, 53.12; H, 4.18; N, 3.87. Found: C, 53.03; H, 4.09; N, 3.91.

Ethyl 5-Chloro-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylate (47) In a similar manner to that described for the preparation of **22** from **21**, compound **46** was converted to **47** (100%), colorless needles, mp 191—192 °C. IR (KBr): 1736 (CO_2Et)

cm^{-1}. MS *m/z*: 341 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.83—0.88 (2H, m, $CH_2 \times 2$), 1.08—1.14 (2H, m, $CH_2 \times 2$), 1.27 (3H, t, J=7.0 Hz, CH_2CH_3), 2.68 (3H, d, J=3.5 Hz, Me), 4.13 (1H, m, CH), 4.22 (2H, q, J=7.0 Hz, CH_2CH_3), 8.49 (1H, s, C_2-H). *Anal.* Calcd for $C_{16}H_{14}ClF_2NO_3$: C, 56.23; H, 4.13; N, 4.10. Found: C, 56.08; H, 4.11; N, 4.14.

Ethyl 5-(*N*-Benzyl-*N*-methyl)amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylate (48) A mixture of the 5-chloroquinolone **47** (1.00 g, 2.93 mmol) and *N*-methylbenzylamine (3.78 ml, 29.3 mmol) in acetonitrile (20.0 ml) was heated under reflux for 7 d. The reaction mixture was concentrated and water was added to the residue. The solution was made acidic with 10% HCl, and the crystals that deposited were collected by filtration and washed with iso-PrOH to afford **48** (0.80 g, 64%). Recrystallization from MeOH gave pale brown prisms, mp 153.5—154 °C. IR (KBr): 1728 (CO_2H), 1626 (CO) cm^{-1}. MS *m/z*: 426 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.69—0.76 (2H, m, $CH_2 \times 2$), 1.04—1.11 (1H, m, $CH_2 \times 2$), 1.29 (3H, t, J=7.0 Hz, CH_2CH_3), 2.56 (3H, d, J=3.0 Hz, Me), 2.08 (3H, s, NMe), 4.01—4.09 (1H, m, CH), 4.23 (2H, q, J=7.0 Hz, CH_2CH_3), 4.24 (2H, s, CH_2), 7.14—7.32 (5H, m, Ar-H), 8.38 (1H, s, C_2-H). *Anal.* Calcd for $C_{24}H_{24}F_2N_2O_3$: C, 67.59; H, 5.67; N, 6.57. Found: C, 67.56; H, 5.66; N, 6.59.

Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-5-methylamino-4-oxoquinoline-3-carboxylate (49) A suspension of the quinolone **48** (17.0 g, 39.9 mmol) and 5% Pd/C (1.70 g) in AcOH (120 ml) and EtOH (170 ml) was stirred at room temperature under a hydrogen atmosphere for 3 h. The catalyst was removed by filtration and washed with MeOH. The filtrate was concentrated to afford **49** (12.5 g, 93%). Recrystallization from MeOH give pale yellow needles, mp 164.5—165 °C. IR (KBr): 1734 (CO_2H), 1636 (CO) cm^{-1}. MS *m/z*: 336 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.85—0.92 (2H, m, $CH_2 \times 2$), 1.03—1.15 (2H, m, $CH_2 \times 2$), 1.26 (3H, t, J=7.0 Hz, CH_2CH_3), 2.42—2.53 (3H, m, Me), 3.05 (3H, dd, J=6.5, 5.5 Hz, Me), 4.02—4.11 (1H, m, CH), 4.20 (2H, q, J=7.0 Hz, CH_2CH_3), 8.41 (1H, s, C_2-H), 9.99 (1H, br s, NH). *Anal.* Calcd for $C_{17}H_{18}F_2N_2O_3$: C, 60.71; H, 5.39; N, 8.33. Found: C, 60.49; H, 5.35; N, 8.32.

5-Chloro-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid (50) In a similar manner to that described for the preparation of **23** from **22**, compound **47** was converted to **50** (97%), pale brown prisms, mp 255.5—257.0 °C. IR (KBr) 1728 (CO_2H) cm^{-1}. MS *m/z*: 313, 315 (M^+, 3:1). ^{1}H-NMR ($CDCl_3$) δ: 0.90—1.05 (2H, m, $CH_2 \times 2$), 1.15—1.25 (2H, m, $CH_2 \times 2$), 2.78 (3H, d, J=3.0 Hz, Me), 4.06—4.12 (1H, m, CH), 8.89 (1H, s, C_2-H). *Anal.* Calcd for $C_{14}H_{10}ClF_2NO_3$: C, 53.61; H, 3.21; N, 4.47. Found: C, 53.65; H, 3.24; N, 4.46.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-5-methylamino-4-oxoquinoline-3-carboxylic Acid (51) In a similar manner to that described for the preparation of **23** from **22**, compound **49** was converted to **51** (97%), yellow needles, mp 230—231.5 °C. IR (KBr): 1730 (CO_2H), 1636 (CO) cm^{-1}. MS *m/z*: 308 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.78—0.99 (2H, m, $CH_2 \times 2$), 1.08—1.25 (2H, m, $CH_2 \times 2$), 2.55 (3H, d, J=3.0 Hz, Me), 3.12 (3H, dd, J=7.0, 5.0 Hz, Me), 4.18—4.28 (1H, m, CH), 8.68 (1H, s, C_2-H), 9.36 (1H, br s, NH). *Anal.* Calcd for $C_{15}H_{14}F_2N_2O_3$: C, 58.44; H, 4.58; N, 9.09. Found: C, 58.38; H, 4.44; N, 9.04.

7-((*S*)-3-*tert*-Butoxycarbonylamino-1-pyrrolidinyl)-5-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid (52) In a similar manner to that described for the preparation of **39** from **36**, compound **50** was converted to **52** (52%), pale yellow needles, mp 200.5—202 °C. $[\alpha]_D^{20}$ −48.9° (c=0.1, $CHCl_3$). IR (KBr): 1714 (CO_2H), 1616 (CO) cm^{-1}. MS *m/z*: 409, 501 (M^+, 3:1). ^{1}H-NMR ($CDCl_3$) δ: 0.75—0.80 (2H, m, $CH_2 \times 2$), 1.11—1.28 (2H, m, $CH_2 \times 2$), 1.47 (9H, s, $C(CH_3)_3$), 1.94—2.04 (1H, m, CH_2), 2.27—2.40 (1H, m, CH_2), 2.51 (3H, s, Me), 3.32—3.88 (4H, m, $NCH_2 \times 2$), 3.99—4.05 (1H, m, NCH), 4.24—4.45 (1H, m, CH), 4.82 (1H, br s, CONH), 8.84 (1H, s, C_2-H). *Anal.* Calcd for $C_{23}H_{27}ClFN_3O_5$: C, 57.56; H, 5.67; N, 8.76. Found: C, 57.40; H, 5.69; N, 8.75.

7-((*S*)-3-Amino-1-pyrrolidinyl)-5-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid Hydrochloride (8c) In a similar manner to that described for the preparation of **8f** from **39**, compound **52** was converted to **8c** (33%), pale brown crystals, mp 202—203 °C. $[\alpha]_D^{20}$ +22.0° (c=0.1, MeOH). IR (KBr): 1700 (CO_2H), 1614 (CO) cm^{-1}. MS *m/z*: 379, 381 (M^+, 3:1). ^{1}H-NMR (DMSO-d_6) δ: 0.73—0.88 (2H, m, $CH_2 \times 2$), 1.10—1.20 (2H, m, $CH_2 \times 2$), 2.05—2.16 (1H, m, CH_2), 2.28—2.40 (1H, m, CH_2), 2.54 (3H, s, Me), 3.52—3.96 (5H, m, $NCH_2 \times 2$, NCH), 4.22—4.30 (1H, m, CH), 8.45 (1H, br s, NH_2), 8.76 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{19}ClFN_3O_3 \cdot HCl \cdot 3/4H_2O$: C, 50.30; H, 5.04; N, 9.78. Found: C, 50.24; H, 5.08; N, 9.79.

7-((*S*)-3-*tert*-Butoxycarbonylamino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-5-methylamino-4-oxoquinoline-3-carboxylic Acid (53) In a similar manner to that described for the preparation of **39** from **36**, compound **51** was converted to **53** (31%), yellow needles, mp 200—201 °C. $[\alpha]_D^{20}$ −54.9° (c=0.1, $CHCl_3$). IR (KBr): 1688 (CO_2H), 1624 (CO) cm^{-1}. MS *m/z*: 474 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.69—0.83 (2H, m, $CH_2 \times 2$), 1.04—1.18 (2H, m, $CH_2 \times 2$), 1.41 (9H, s, $C(CH_3)_3$), 1.83—1.95 (1H, m, CH_2), 2.09—2.21 (1H, m, CH_2), 2.33 (3H, s, Me), 3.03 (3H, dd, J=6.5, 5.5 Hz, Me), 3.25—3.33 (1H, m, NCH_2), 3.43—3.70 (3H, m, NCH_2, NCH_2), 4.04—4.20 (2H, m, CH, NCH), 7.05 (1H, br s, NH), 8.62 (1H, s, C_2-H), 8.87 (1H, br s, NH). *Anal.* Calcd for $C_{24}H_{31}FN_4O_5$: C, 60.75; H, 6.58; N, 11.81. Found: C, 60.86; H, 6.76; N, 11.75.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-5-methylamino-4-oxoquinoline-3-carboxylic Acid (8e) In a similar manner to that described for the preparation of **8f** from **39**, compound **53** was converted to **8e** (43%), yellow prisms. mp 108.5—109 °C. $[\alpha]_D^{20}$ +30.9° (c=0.1, MeOH). IR (KBr): 1628 (CO_2H) cm^{-1}. MS *m/z*: 374 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.69—0.80 (2H, m, $CH_2 \times 2$), 1.05—1.19 (2H, m, $CH_2 \times 2$), 1.61—1.72 (1H, m, CH_2), 2.01—2.13 (1H, m, CH_2), 2.32 (3H, s, Me), 3.03 (3H, dd, J=6.5, 5.5 Hz, Me), 3.08—3.68 (5H, m, $NCH_2 \times 2$, NCH), 4.10—4.19 (1H, m, CH), 8.61 (1H, s, C_2-H), 8.08—8.90 (1H, m, NH). *Anal.* Calcd for $C_{19}H_{23}FN_4O_3 \cdot 1/2H_2O$: C, 59.52; H, 6.31; N, 14.61. Found: C, 59.77; H, 6.27; N, 14.67.

Compounds **7d**, **8a**, **9b**, **10b**, **d**, **12b**, **d**, **14a**, **15a** and **16a** were prepared as reported for the corresponding known racemates, and physicochemical data are listed below.

5-Amino-7-((*S*)-3-amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (7d) mp 226—229 °C (dec.). $[\alpha]_D^{20}$ −7.8° (c=0.1, DMSO). IR (KBr): 1708 (CO_2H), 1640 (CO) cm^{-1}. MS *m/z*: 346 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 1.01—1.12 (2H, m, $CH_2 \times 2$), 1.19—1.30 (2H, m, $CH_2 \times 2$), 1.67—1.78 (1H, m, CH_2), 2.00—2.10 (1H, m, CH_2), 3.06—3.75 (6H, m, $NCH_2 \times 2$, NCH, CH), 6.31 (1H, d, J=7.5 Hz, C_8-H), 7.07 (2H, br s, NH_2), 8.42 (1H, s, C_2-H). *Anal.* Calcd for $C_{17}H_{19}FN_4O_3 \cdot 3/4H_2O$: C, 56.74; H, 5.74; N, 15.57. Found: C, 56.49; H, 5.62; N, 15.45.

5-Amino-7-((*S*)-3-amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid (8a) mp 192—194 °C (dec.). $[\alpha]_D^{20}$ −19.1° (c=0.1, DMF). IR (KBr): 1618 (CO) cm^{-1}. MS *m/z*: 345 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.80—0.95 (2H, m, $CH_2 \times 2$), 1.13—1.25 (2H, m, $CH_2 \times 2$), 1.68—1.79 (1H, m, CH_2), 2.06—2.15 (1H, m, CH_2), 2.57 (3H, s, Me), 3.14—3.24 (1H, m, NCH_2), 3.46—3.73 (4H, m, NCH_2, NCH_2, NCH), 4.25—4.35 (1H, m, CH), 7.68 (1H, d, J=14.0 Hz, C_5-H), 8.75 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{20}FN_3O_3 \cdot H_2O$: C, 59.49; H, 6.10; N, 11.56. Found: C, 59.59; H, 5.95; N, 11.51.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-5-methyl-4-oxoquinoline-3-carboxylic Acid (9b) mp 230—232 °C. $[\alpha]_D^{20}$ −14.0° (c=0.1, DMF). IR (KBr): 1714 (CO_2H), 1620 (CO) cm^{-1}. MS *m/z*: 363 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.98—1.20 (4H, m, $CH_2 \times 2$), 1.60—1.74 (1H, m, CH_2), 1.95—2.18 (1H, m, CH_2), 2.66 (3H, d, J=3.5 Hz, Me), 3.26—3.40 (1H, m, NCH_2), 3.49—3.58 (1H, m NCH), 3.65—3.90 (3H, m, NCH_2, NCH_2), 4.00—4.13 (1H, m, CH), 8.56 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{19}F_2N_3O_3$: C, 59.50; H, 5.27; N, 11.56. Found: C, 59.54; H, 5.23; N, 11.62.

5-Amino-7-((*S*)-3-amino-1-pyrrolidinyl)-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid Hydrochloride (10d) mp 237—240 °C (dec.). $[\alpha]_D^{20}$ −9.8° (c=0.05, H_2O). IR (KBr): 1628 (CO) cm^{-1}. MS *m/z*: 380, 382 (M^+, 3:1). ^{1}H-NMR (DMSO-d_6) δ: 0.79—0.87 (2H, m, $CH_2 \times 2$), 1.05—1.17 (2H, m, $CH_2 \times 2$), 2.00—2.13 (1H, m, CH_2), 2.25—2.37 (1H, m, CH_2), 3.68—4.00 (5H, m, $NCH_2 \times 2$, NCH), 4.25—4.30 (1H, m, CH), 7.50 (2H, br s, NH_2), 8.66 (1H, s, C_2-H). *Anal.* Calcd for $C_{17}H_{18}ClFN_4O_3 \cdot HCl$: C, 48.93; H, 4.59; N, 13.43. Found: C, 49.22; H, 4.55; N, 13.37.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-5-methyl-4-oxoquinoline-3-carboxylic Acid Hydrochloride (12b) mp 185—188 °C (dec.). $[\alpha]_D^{20}$ +39.0° (c=0.1, DMF). IR (KBr): 1694 (CO_2H), 1616 (CO) cm^{-1}. MS *m/z*: 375 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.80—0.93 (2H, m, $CH_2 \times 2$), 1.00—1.14 (2H, m, $CH_2 \times 2$), 2.03—2.13 (1H, m, CH_2), 2.25—2.37 (1H, m, CH_2), 2.70 (3H, m, J=3.5 Hz, CH_3), 3.53 (3H, s, OMe), 3.64—3.95 (5H, m, $NCH_2 \times 2$, NCH), 4.12—4.20 (1H, m, CH), 8.36 (2H, br s, NH_2), 8.65 (1H, s, C_2-H). *Anal.* Calcd for $C_{19}H_{22}FN_3O_4 \cdot HCl \cdot 2H_2O$: C, 50.95; H, 6.08; N, 9.38.

Found: C, 50.99; H, 5.76; N, 9.43.

5-Amino-7-((*S*)-3-amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid Hydrochloride (12d) mp 229—231 °C (dec.). $[\alpha]_D^{20}$ +26.9° (*c*=0.1, MeOH). IR (KBr): 1694 (CO_2H), 1630 (CO) cm^{-1}. MS *m/z*: 376 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.80—1.01 (4H, m, $CH_2 \times 2$), 2.01—2.13 (1H, m, CH_2), 2.23—2.33 (1H, m, CH_2), 3.46 (3H, s, OCH_3), 3.63—3.91 (5H, m, $NCH_2 \times 2$, NCH), 3.99—4.08 (1H, m, CH), 7.05 (2H, br s, NH_2), 8.46 (2H, br s, NH_2), 8.52 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{21}FN_4O_4 \cdot HCl \cdot 1/2H_2O$: C, 51.25; H, 5.50; N, 13.28. Found: C, 51.31; H, 5.44; N, 13.48.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-8-fluoromethoxy-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (14a) mp >300 °C. $[\alpha]_D^{20}$ −20.9° (*c*=0.1, DMF). IR (KBr): 1726 (CO_2H), 1622 (CO) cm^{-1}. MS *m/z*: 379 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.88—1.20 (4H, m, $CH_2 \times 2$), 1.63—1.75 (1H, m, CH_2), 1.99—2.10 (1H, m, CH_2), 3.14—3.80 (5H, m, $NCH_2 \times 2$, NCH), 4.09—4.16 (1H, m, CH), 5.54 (2H, d, *J*=53.0 Hz, OCH_2F), 7.72 (1H, d, *J*=14.0 Hz, C_5-H), 8.67 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{19}F_2N_3O_4 \cdot 1/2H_2O$: C, 55.67; H, 5.19; N, 10.82. Found: C, 55.51; H, 4.99; N, 10.78.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-8-difluoromethoxy-1,4-dihydro-5-methyl-4-oxoquinoline-3-carboxylic Acid (15a) mp 211—212 °C. $[\alpha]_D^{20}$ −27.3° (*c*=0.1, DMF). IR (KBr): 1730 (CO_2H), 1624 (CO) cm^{-1}. MS *m/z*: 397 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.96—1.08 (2H, m, $CH_2 \times 2$), 1.12—1.22 (2H, m, $CH_2 \times 2$), 1.66—1.71 (1H, m, CH_2), 1.98—2.09 (1H, m, CH_2), 3.24—3.35 (1H, m, NCH_2), 3.53—3.86 (4H, m, NCH_2, NCH_2, NCH), 4.01—4.10 (1H, m, CH), 6.82 (1H, t, *J*=74.0 Hz, $OCHF_2$), 7.78 (1H, d, *J*=14.0 Hz, C_5-H), 8.69 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{18}F_3N_3O_4 \cdot 3/4H_2O$: C, 52.62; H, 4.78; N, 10.23. Found: C, 52.38; H, 4.50; N, 10.18.

7-((*S*)-3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-8-trifluoromethoxy-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (16a) mp 202—204 °C. $[\alpha]_D^{20}$ +5.0° (*c*=0.1, DMF). IR (KBr): 1626 (CO) cm^{-1}. MS *m/z*: 415 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 0.97—1.07 (2H, m, CH_2), 1.12—1.20 (2H, m, $CH_2 \times 2$), 1.68—1.77 (1H, m, $CH_2 \times 2$), 1.99—2.10 (1H, m, CH_2), 3.05—3.37 (1H, m, NCH_2), 3.54—3.88 (4H, m, NCH_2, NCH_2, NCH), 3.91—3.98 (1H, m, CH), 7.83 (1H, d, *J*=14.0 Hz, C_5-H), 8.72 (1H, s, C_2-H). *Anal.* Calcd for $C_{18}H_{17}F_4N_3O_4 \cdot 1/2H_2O$: C, 50.95; H, 4.28; N, 9.90. Found: C, 50.95; H, 3.98; N, 9.93.

***In Vitro* Studies** These studies were conducted according to the method of the Japan Society of Chemotherapy.[24] The minimum inhibitory concentrations (*MIC*, μg/ml) were determined by an agar dilution method with Muller-Hinton agar (MHA, Difco Laboratories, Detroit, Mich). Bacterial suspensions for inocula were prepared by diluting overnight cultures of organisms to give a final concentration of 10^6 CFU/ml, and one loopful (5 μl) of an inoculum, corresponding to about 5×10^3 CFU per spot was inoculated on drug-containing agar plates. The plates were incubated for 18 h at 37 °C. The MIC was defined as the lowest drug concentration which prevented visible growth of bacteria.

Test of Injury to Chromosome A fibroblast cell line from the lung of a newborn Chinese hamster, CHL, was purchased from JCRB and injury to chromosomes at a test compound concentration of 100 μg/ml was examined as follows. The cells in 5 ml of cell suspension (8×10^3/ml) were seeded in a 60 mm plastic petri dish and cultured for 3 d. The medium was replaced with fresh medium, then a solution of a test compound or AF-2 (positive control) or solvent alone (negative control) was added to the culture. Cells were treated for 6 h, washed with Dulbecco's phosphate-buffered saline solution, and then recultured for 18 h with fresh medium. At 2 h before harvesting, 100 μl of 10 μg/ml colcemid was added to all cultures. Hypotonic potassium chloride solution (75 mM) was added to the cells harvested using 1 ml of 0.05% trypsin–0.53 mM EDTA·4Na, and then the cells were incubated for 20 min in a water bath at 37 °C. After having been fixed with a mixture of MeOH–AcOH (3 : 1) fixative, the cell suspension was dropped on slides and stained with Giemsa solution.

The cells in 2 dishes per group were treated with each test compound, and 2 slides of chromosomal preparations were made from each dish. A hundred well-spread metaphases per group were analyzed. The final judgment was based on the incidence of total cells with aberrations. Scoring was as follows; I is less than 10%, II is from 10% to less than 20%, III is from 20% to less than 50%, IV is 50% or more.

Phototoxicity Test The photoxicity test was carried out according to the Adjutant-Strip method.[25] Five- to 6-week-old male Hartley guinea pigs (350—400 g body weight) were irradiated with a UV lamp (20 joules/cm^2, Toshiba FL2·BLB) for 90 min after intravenous administration of a test compound. Phototoxicity was evaluated at 24 h after irradiation. The scoring criteria for skin reactions were as follows: − is no erythema, + is very slight erythema, ++ is well defined erythema, +++ is moderate to severe erythema, ++++ is severe erythema (beet redness) to slight eschar formation (injuries in depth).

Convulsion Induced by Coadministration with NSAID A quinolone (100 mg/kg) was administered intraperitoneally 30 min after oral administration of fenbufen (100 mg/kg) in male ICR mice (5-week-old, 21—27 g body weight). Mice were observed for occurrence of convulsions for 4 h after administration.

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References and Notes

1) *a*) Part II: Yoshida T., Yamamoto Y., Yagi N., Takahashi Y., Yasuda S., Katoh H., Itoh Y., *Yakugaku Zasshi*, **111**, 19—31 (1991); *b*) Part III: Yoshida T., Yamamoto Y., Yagi N., Takahashi Y., Yasuda S., Katoh H., Itoh Y., *ibid.*, **111**, 386—392 (1991).
2) Wise R., Andrews J. M., Edwards L. J., *Antimicrob. Agents Chemother.*, **23**, 559—564 (1983).
3) Hayakawa I., Hiramitsu T., Tanaka Y., *Chem. Pharm. Bull.*, **32**, 4907—4913 (1984).
4) Miyamoto T., Matsumoto J., Chiba K., Egawa H., Shibamori K., Minamida A., Nishimura Y., Okada H., Kataoka M., Fuzita M., Hirose T., Nakano J., *J. Med. Chem.*, **33**, 1645—1656 (1990).
5) Ueda Y., Shimizu K., Konno M., Matsumoto F., (ed.), "The Quinolones," Life Science Publisher, Tokyo, 1991, pp. 128—145.
6) *a*) Gootz T. D., Barrett J. F., Sutcliffe J. A., *Antimicrob. Agents Chemother.*, **34**, 8—12 (1990); *b*) Shimada H., Itoh S., Program and Abstracts of 31st ICAAC 1991, American Society for Microbiology, Washington DC, Abstr. no. 1507.
7) *a*) Ledoussal B., Bouzard D., Coroneos E., *J. Med. Chem.*, **35**, 198—200 (1992); *b*) Petersen U., Schenke T., Schriewer M., Krebs A., Grohe K., Haller I., Metzger K. G., Endermann R., Zeiler H. J., European Patent 391169 (1990) [*Chem. Abstr.*, **114**, 185466*v* (1991)]; *c*) Ciavavino V., Suto M. J., Theiss J. C., *Mutat. Res.*, **298**, 227—236 (1993) [*Chem. Abstr.*, **118**, 182640*u* (1993)].
8) *a*) Masuzawa K., Suzue S., Hirai K., Ishizaki T., European Patent 237955 (1987) [*Chem. Abstr.*, **108**, 75234*m* (1988)]; *b*) Iwata M., Kimura T. Inoue T., Fujihara Y., Katsube T., European Patent 352123 (1988) [*Chem. Abstr.*, **113**, 78178*k* (1990)]; *c*) Iwata M., Kimura T. Inoe T., Fujiwara Y., Katsube T., Japan Kokai Tokkyo Koho, Japan. Patent 05255183 (1993) [*Chem. Abstr.*, **120**, 217312*j* (1994)]; *d*) Domagala J. M., Bridges A. J., Culbertson T. P., Gambino L., Hagen S. E., Karrick G., Porter K. Sanchez J. P., Sesnie J. A. Spense G. Szotek D., Wemple J., *J. Med. Chem.*, **34**, 1142—1154 (1991); *e*) Domagala J. M., Mich T. F., Sanchez Joseph P., European Patent 265230 (1988) [*Chem. Abstr.*, **109**, 92826*u* (1988)]; *f*) Ueda H., Miyamoto H., Yamashita H., Tone H., European Patent 287951 (1988) [*Chem. Abstr.*, **110**, 173109*k* (1989)]; *g*) Domagala J. M., Hagen S. E., Kiely J. S., World Intellectual Property Organization Patent 8906649 (1989) [*Chem. Abstr.*, **112**, 98508*a* (1990)].
9) *a*) Ishikawa R., Kaieda O., Takatani N., Japan Kokai Tokkyo Koho, Japan. Patent 62185066 (1987) [*Chem. Abstr.*, **108**, 21511*f* (1988)]; *b*) Hiromoto K., Otsubo A., Japan Kokai Tokkyo Koho, Japan. Patent 63162669 (1988) [*Chem. Abstr.*, **110**, 57311*w* (1989)].
10) *a*) Ataka K., Oku M., Omori K., Kimura T., Iwata M., Japan Kokai Tokkyo Koho, Japan. Patent 63316757 (1989) [*Chem. Abstr.*, **111**, 97105*j* (1989)]; *b*) Hayakawa I., Kimura Y., Japan Kokai Tokkyo Koho, Japan. Patent 0372476 (1991) [*Chem. Abstr.*, **115**, 71622*h* (1991)].
11) Hagen S. E., Domagala J. M., *J. Heterocyclic Chem.*, **27**, 1609—1616 (1990).
12) Wemple J. N., Karrick G. L., Spence F. G., U. S. Patent 4885386 (1989) [*Chem. Abstr.*, **112**, 216450*a* (1990)].
13) Fujita T., Iwasa J., Hansch C., *J. Am. Chem. Soc.*, **86**, 5175—5180 (1964).
14) Verloop A., Hoogenstraaten. W., Tipker J., "Drug Design," Vol.

VII, ed., by Ariens E. J., Academic Press Publisher, New York, 1976, pp. 180—185.
15) Ohta M., Koga H., *J. Med. Chem.*, **34**, 131—139 (1991).
16) Hagen S. E., Domagala J. M. Heifetz C. L., Johnson J., *J. Med. Chem.*, **34**, 1155—1161 (1991).
17) Suto M. J., Domagala J. M., Roland G. E., Mailloux G. B., Cohen M. A., *J. Med. Chem.*, **35**, 4745—4750 (1992).
18) Moran D. B., Ziegler C. B., Dunne T. S., Kuck N. A., Lin Y., *J. Med. Chem.*, **32**, 1313—1318 (1989).
19) *a*) Shen L. L., Kohlbrenner W. E., Weigl D., Baranowski J., *J. Biol. Chem.*, **264**, 2973—2978 (1989); *b*) Shen L. L., Mitscher L. A., Sharma P. N., O'Donnell T. T., Chu D. W. T., Cooper C. S., Rosen T., Pernet A. G., *Biochemistry*, **28**, 3886—3894 (1989).
20) Itoh Y., Kato H., Ogawa N., Koshinaka E., Suzuki T., Yagi N., Ger. Offen. Patent 3433924 (1985) [*Chem. Abstr.*, **103**, 123517*b* (1985)].
21) Kimura U., Yamada M., Nii S., Kumon Y., Omori H., *Chemotherapy*, **43**, 357—360 (1995).
22) Domagala J. M., *J. Antimicrob. Chemother.*, **33**, 685—706 (1994).
23) *a*) Ueda Y., Shimizu K., Konno M., Matsumoto F., (ed.), "The Quinolones," Life Science Publisher, Tokyo, 1991, pp. 315—326; *b*) Akahane K., Sekiguchi M., Une T., Osada Y., *Antimicrob. Agents Chemother.*, **33**, 1704—1708 (1989).
24) Goto S., Jo K. Kawakita T., Kosakai N., Mitsuhashi S., Nishino T. Ohsawa N. Tanami H. *Chemotherapy*, **29**, 76—79 (1981).
25) Satou E., Katumura Y., Itikawa H., Kobayasi T., Nakazima K., *Nishinihon J. Dermatol.*, **42**, 831—837 (1980).