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Synthesis and Biological Properties of Substituted 1,4-Dihydro-5-methyl-4-oxo-3-quinolinecarboxylic Acids

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Abstract—A series of substituted 1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-4-oxo-3-quinoline carboxylic acids was synthesized and tested for their *in vitro* and *in vivo* antibacterial activity. The introduction of a methyl group at the 5-position of quinoline nucleus enhanced characteristically the antibacterial activity against Gram-positive bacteria, including *Streptococcus pneumoniae*, which is a major pathogen in the respiratory tract infection, while retaining Gram-negative activity. Among them, 1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid hydrochloride (grepafloxacin) exhibited potent *in vitro* antibacterial activity against Gram-positive bacteria such as *Streptococcus pneumoniae* and high *in vivo* efficacy on the experimental systemic infections caused by the Gram-positive and -negative bacteria tested. It also showed a high distribution to the lung and bronchoalveolar lavage fluid in comparison to reference drugs and is now undergoing clinical evaluation.

Introduction

In recent years, highly potent and broad-spectrum quinolone antibacterial agents, such as norfloxacin (NFLX),[1] enoxacin (ENX),[2] ciprofloxacin (CPFX)[3] and ofloxacin (OFLX)[4] have been developed and clinically used for the treatment of various infections. They exhibit excellent therapeutic efficacy in the urinary tract infection, but are not always sufficient for therapeutic efficacy in the respiratory tract infection. This may be attributed to comparatively weak antibacterial activity against Gram-positive bacteria, especially *Streptococcus pneumoniae*, which remains the leading cause of death in patients with pneumonia, despite antibiotic therapy.[5] In order to improve the antibacterial activity, a number of investigators have focused on the modification of the side chain at 7-position of quinoline skeleton.[6] On the contrary, our efforts have been directed toward the introduction of a substituent to the quinoline nucleus.[7] As a result, we found that quinolone derivatives possessing a methyl group at the 5-position of quinolone nucleus exhibit more potent antibacterial activity against Gram-positive bacteria than the corresponding 5-unsubstituted compound. Furthermore, a few compounds of 5-methylquinolone derivatives were, surprisingly, found to show a high distribution to the lung in rats, which is favorable for the treatment of the respiratory tract infection.

Now, we wish to report here the synthesis and biological evaluation of a series of 5-methyl quinolone carboxylic acid derivatives. The synthesis and biological activity of a similar series of compounds was disclosed by the Warner-Lambert group,[8] after we applied for a patent.[9]

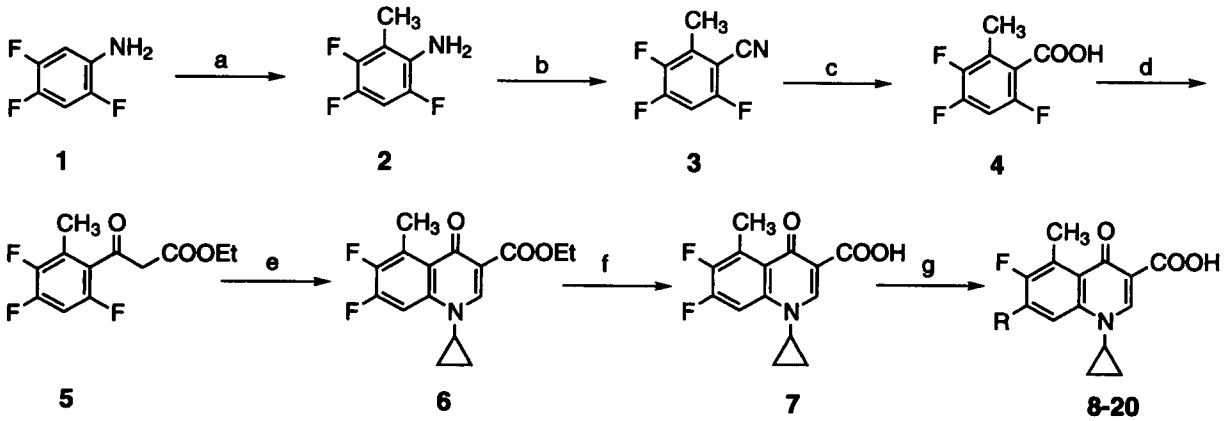
Chemistry

First, the benzoic acid derivative (**4**), which was a key intermediate for the synthesis of the 5-methylquinolone derivatives (**8–20**) were prepared. Methylthiomethylation[10] of 2,4,5-trifluoro aniline (**1**) and successive treatment with activated Raney nickel gave 3,4,6-trifluoro-2-methylaniline (**2**). Diazotization of **2** with sodium nitrite ($NaNO_2$) in conc HCl, followed by the treatment with potassium cyanide (KCN) afforded 3,4,6-trifluoro-2-methylbenzonitrile (**3**). Hydrolysis of **3** with 50% H_2SO_4 gave the desired 3,4,6-trifluoro-2-methylbenzoic acid (**4**). Condensation of acid chloride of **4** with ethoxymagnesium malonic ester, followed by heating with *p*-toluenesulfonic acid (*p*-TsOH) in water afforded ethyl 3,4,6-trifluoro-2-methylbenzoylacetate (**5**). Treatment of **5** with acetic anhydride and triethyl orthoformate, followed by the addition of cyclopropylamine and successive cyclization with 60% sodium hydride gave ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-5-methyl-4-oxo-3-quinolinecarboxylate (**6**). Hydrolysis of **6** with conc HCl in 90% AcOH afforded the corresponding acid (**7**) in 92% yield. Finally, the acid (**7**) was allowed to react with various cyclic amines in *N,N*-dimethylformamide (DMF) at 90 °C to afford the desired 7-amino-derivatives (**8–20**) (Chart 1, Table 1). Moreover, we investigated an alternative route for the industrial-scale synthesis as illustrated in Chart 2. Namely, commercially available 2-methyl-3-nitrobenzoic acid (**21**) was treated with thionyl chloride in MeOH, followed by the hydrogenation with 5% palladium on carbon (5% Pd–C) to give methyl 3-amino-2-methylbenzoate (**22**). Regioselective dibromination of **22** with bromine in AcOH gave methyl 3-amino-4,6-dibromo-2-methybenzoate (**23**) in good yield.

The conversion of **23** to the corresponding fluoroarene (**24**) through diazonium salt was achieved by photo-irradiation[11] in high yield. Hydrolysis of **24** with 10% NaOH gave the benzoic acid (**25**). Treatment of **25** with thionyl chloride gave the acid chloride, which was condensed with monoethyl malonate in the presence of magnesium ethoxide to afford ethyl 4,6-dibromo-3-fluorobenzoyl acetate (**26**) in 92% yield. 7-Bromo-1-cyclopropyl-1,4-dihydro-5-methyl-4-oxo-3-quinoline carboxylic acid (**28**) was prepared from **26** in the same manner as described for the synthesis of **7**, except cyclization with K_2CO_3 as a base in DMF. Finally, compound (**28**) was condensed with 2-methylpiperazine by heating in dimethyl sulfoxide (DMSO) to afford the desired compound (**10**) in 56% yield.

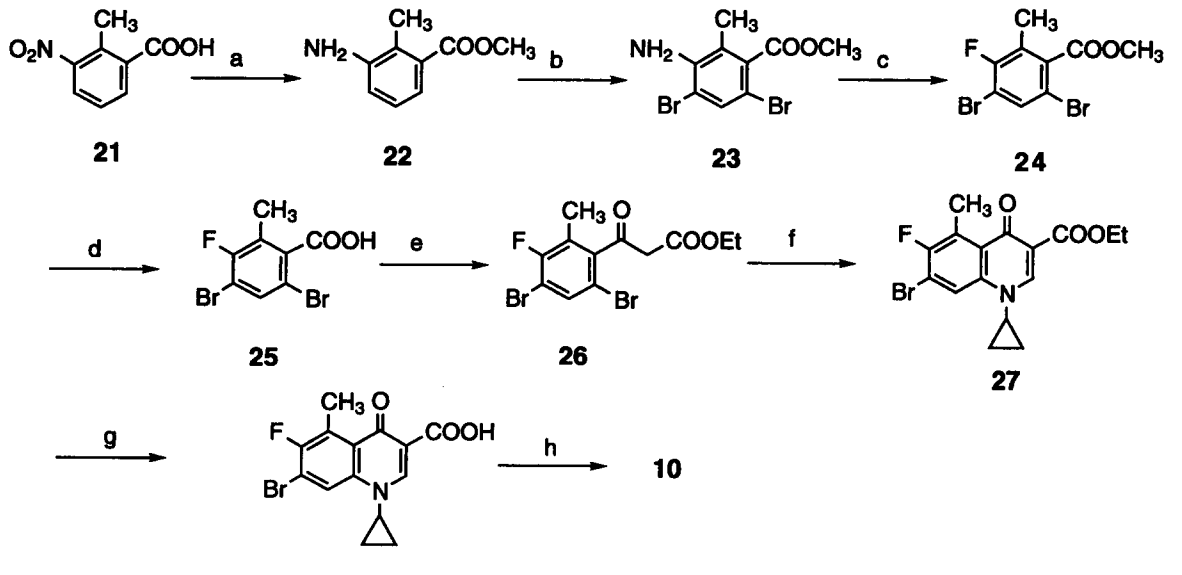
Results and Discussion

The compounds (**8–20**) prepared in this investigation were tested for *in vitro* antibacterial activity against Gram-positive (*Staphylococcus aureus* 209P, *Streptococcus pneumoniae* type II and *Enterococcus faecalis* IFO 12580) and Gram-negative (*Escherichia coli* NIHJ JC-2, *Klebsiella pneumoniae* NCTC 9632, *Haemophilus influenzae* ATCC 9327, *Pseudomonas aeruginosa* ATCC 10145 and *Acinetobacter calcoaceticus* Ac-54) bacteria by serial dilution method.[12] The results are summarized in Table 2. The antibacterial activity of NFLX, ENX, CPFX and OFLX are included for comparison. The antibacterial activity of the compound **8** possessing a methyl group at the 5-position of quinoline ring against Gram-positive bacteria including *S. pneumoniae* was more potent than those of the corresponding 5-unsubstituted compound (CPFX). Furthermore, the addition of a methyl group at the 3-position of piperazine ring increased the activity against *S. aureus* (**8** and **10**). Aminopyrrolidine derivative (**13**) had about the same activity against both Gram-positive and -negative



a: (1) CH_3SCH_3, NCS (2) Et_3N (3) Raney Ni. b: (1) $NaNO_2$ (2) KCN. c: 50% H_2SO_4. d: (1) $SOCl_2$ (2) $EtOMgCH(COOC_2H_5)_2$ (3) *p*-TsOH. e: (1) Ac_2O, $CH(OC_2H_5)_3$ (2) *c*-$C_3H_5NH_2$ (3) 60% NaH. f: HCl. g: RH.

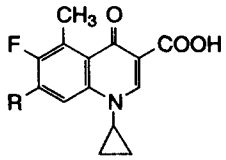
Chart 1.

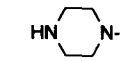
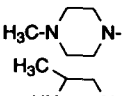
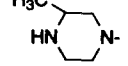
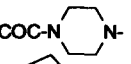
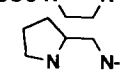
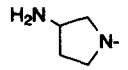
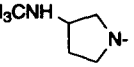
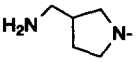
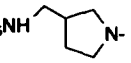
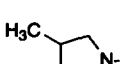
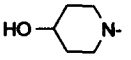
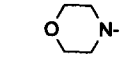


a: (1) $SOCl_2$, MeOH (2) H_2, 5% Pd–C. b: Br_2, AcONa. c: (1) $NaNO_2$, HPF_6 (2) BF_3·Et_2O, hv. d: NaOH. e: (1) $SOCl_2$ (2) $HOOCCH_2COOC_2H_5$, $Mg(OEt)_2$. f: (1) Ac_2O, $CH(OC_2H_5)_3$ (2) *c*-$C_3H_5NH_2$ (3) K_2CO_3. g: HCl. h: 2-methylpiperazine.

Chart 2.

Table 1. Substituted 1,4-dihydro-5-methyl-4-oxoquinoline-3-carboxylic acids



Compd	R	Recryst. solvent	Yield* (%)	Mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
8		EtOH-H_2O	68	> 300	$C_{18}H_{20}FN_3O_3$ ·HCl	56.62 (56.28	5.28 5.54	11.00 10.87)
9		EtOH	63	229–232	$C_{19}H_{22}FN_3O_3$	63.50 (63.46	6.17 6.19	11.69 11.64)
10		EtOH-H_2O	56†	> 300	$C_{19}H_{22}FN_3O_3$ ·HCl·3/2H_2O	53.97 (53.80	6.20 5.94	9.94 10.02)
11		EtOH-AcOEt	65‡	261–263	$C_{20}H_{22}FN_3O_4$ ·1/4H_2O	61.29 (61.36	5.79 6.02	10.72 10.63)
12		EtOH	60	203–205	$C_{21}H_{26}FN_3O_3$	65.10 (65.25	6.76 6.50	10.85 10.90)
13		EtOH	47	258–261 (dec.)	$C_{18}H_{20}FN_3O_3$ ·HCl·3/2H_2O	52.88 (52.83	5.67 5.65	10.28 10.27)
14		EtOH	32	257–259 (dec.)	$C_{19}H_{22}FN_3O_3$ ·HCl·H_2O	55.14 (54.92	5.84 5.87	10.15 10.01)
15		MeOH-H_2O	55	280–283 (dec.)	$C_{19}H_{22}FN_3O_3$ ·HCl	57.65 (57.46	5.60 5.79	10.60 10.52)
16		EtOH	47	236–239	$C_{21}H_{26}FN_3O_3$ ·HCl·3/2H_2O	55.93 (56.30	6.15 6.37	9.32 9.33)
17	 *trans*	EtOH-AcOEt	45	272–275 (dec.)	$C_{19}H_{22}FN_3O_3$ ·HCl·H_2O	57.65 (57.66	5.60 5.87	10.61 10.60)
18	*cis*	EtOH-AcOEt	54	280–284 (dec.)	$C_{19}H_{22}FN_3O_3$ ·HCl·H_2O	55.14 (55.22	5.84 5.97	10.15 10.11)
19		MeOH	60	220–221	$C_{19}H_{21}FN_2O_4$ ·1/4H_2O	63.32 (63.02	5.87 6.17	7.77 7.66)
20		EtOH	75	245–247	$C_{18}H_{19}FN_2O_4$ ·H_2O	59.33 (59.47	5.81 5.86	7.69 8.08)

*Yield from **7**.
†Yield from **28**.
‡Yield from **8**.

bacteria as piperazine derivative (**8**). The introduction of 4-hydroxypiperidine (**19**) and morpholine (**20**) at the 7-position retained the activity against *S. aureus*, but they markedly decreased the activity against *S. pneumoniae* and *P. aeruginosa*. Among the 5-methyl quinolone derivatives, the compounds **8** and **10** exhibited the more potent activity against Gram-positive bacteria such as *S. pneumoniae*, which is a major pathogen in the respiratory tract infection, than that of reference drugs, and about the same Gram-negative activity as CPFX, which has the highest activity against Gram-negative bacteria among the commercialized quinolones.

The *in vivo* efficacy of the selected compounds **8**, **10** and reference drugs in the experimental systemic infections caused by *S. aureus* Smith, *S. pneumoniae* type III and *E. coli* No. 29 in mice after oral dosing is shown in Table 3. Efficacy of each compound was expressed as 50% effective dose value (ED_{50}) calculated by probit method. The ED_{50} of **10** against *S. aureus* Smith was 1.66 mg kg^{-1}, which is 3.8 to 12.7 times lower than that of reference drugs, reflecting the *in vitro* antibacterial activity. In particular, the compound **10** was highly active against *S. pneumoniae* type III, which were not susceptable to **8**, NFLX, ENX and CPFX. *In vivo* potency of **10** against *E. coli* No. 29 was slightly superior to that of **8**, OFLX and CPFX.

Next, we examined the distribution to the lung and bronchoalveolar fluid at a dose of 20 mg kg^{-1} in rats. The results are shown in Table 4. The concentration of

Table 2. *In vitro* antibacterial activity (MIC*, $\mu g\ mL^{-1}$)

Compd	Microorganism†							
	Sa	Sp	Ef	Ec	Kp	Hi	Pa	Ac
8	0.1	0.2	0.39	0.024	0.024	0.006	0.39	0.1
9	0.1	0.78	0.78	0.1	0.1	0.006	0.78	0.2
10	0.024	0.2	0.2	0.012	0.012	0.006	0.39	0.05
11	0.1	1.56	1.56	0.78	0.78	0.39	3.13	1.56
12	0.05	0.39	0.39	0.1	0.1	0.024	1.56	0.1
13	0.1	0.1	0.39	0.05	0.012	0.006	0.39	0.1
14	0.1	0.39	0.78	0.1	0.05	0.024	0.39	0.2
15	0.1	0.024	0.39	0.1	0.2	0.024	0.78	0.1
16	0.2	0.1	0.78	0.39	0.39	0.2	3.13	0.39
17	0.2	0.2	0.78	0.1	0.05	0.024	0.39	0.05
18	0.2	0.1	0.78	0.2	0.1	0.024	0.78	0.2
19	0.05	1.56	1.56	0.2	0.39	0.1	1.56	0.78
20	0.1	1.56	0.78	0.2	0.2	0.1	1.56	0.78
NFLX	3.13	3.13	1.56	0.2	0.05	0.1	0.78	3.13
ENX	12.5	12.5	3.13	0.2	0.2	0.1	1.56	1.56
CPFX	0.78	0.78	0.39	0.024	0.012	0.012	0.2	0.39
OFLX	0.2	1.56	1.56	0.1	0.05	0.024	1.56	0.39

*Minimum inhibitory concentration.
†Sa *Staphylococcus aureus* FDA 209 P; Sp, *Streptococcus pneumoniae* type II; Ef, *Enterococcus faecalis* IFO 12580; Ec, *Escherichia coli* NIHJ JC-2; Kp, *Klebsiella pneumoniae* NCTC 9632; Hi, *Haemophilus influenzae* ATCC 9327; Pa, *Pseudomonas aeruginosa* ATCC 10145; Ac, *Acinetobacter calcoaceticus* Ac-54.

Table 3. Protective effect against systemic infections in mice

Organisms	Compd	MIC ($\mu g\ mL^{-1}$)*	ED_{50} ($mg\ kg^{-1}$)†	95% Confidence limits ($mg\ kg^{-1}$)
S. *aureus* Smith (3% mucin)	**8**	0.05	2.79	2.32–3.62
	10	0.024	1.66	1.30–2.09
	NFLX	0.39	21.16	17.18–27.20
	ENX	0.78	15.17	12.03–19.19
	CPFX	0.20	7.57	4.73–12.69
	OFLX	0.2	6.41	4.80–8.64
S. *pneumoniae* type III	**8**	0.05	> 50	–
	10	0.2	23.64	17.30–32.55
	NFLX	6.25	> 200	–
	ENX	12.5	> 200	–
	CPFX	1.56	100.63	66.69–168.14
	OFLX	1.56	57.2	41.42–80.40
E coli No.29 (3% mucin)	**8**	0.024	0.89	0.67–1.15
	10	0.012	0.60	0.30–0.78
	NFLX	0.05	3.53	2.50–4.89
	ENX	0.1	2.50	1.99–3.16
	CPFX	0.012	0.95	0.75–1.20
	OFLX	0.05	1.01	0.80–1.18

*MIC was determined by agar dilution method.
†ED_{50} was calculated by probit method.

Table 4. Distribution in the lung and bronchoalveolar lavage fluid (BAL) at a dose of 20 $mg\ kg^{-1}$ *

Compd	Plasma (P) ($\mu g\ mL^{-1}$)†	Lung (L) ($\mu g\ g^{-1}$)†	BAL (B)	L:P Ratio	B:P Ratio
8	1.16	2.81	NT§	2.4	-
10	1.03	7.07	0.92	6.86	0.89
NFLX	0.32	0.25	0.02	0.78	0.06
ENX	1.30	1.48	< 0.10	1.14	-
CPFX	0.32	0.34	< 0.10	1.06	-
OFLX	3.85	2.63	0.69	0.68	0.18

*The concentrations of **10** and reference drugs were measured by the HPLC method and **8** was measured by the bioassay method.
†Maximum concentration.
§Not tested.

10 in the lungs was significantly higher than those of the other compounds and was about seven times more than serum level. The compound **10** (grepafloxacin) also showed a high distribution to bronchoalveolar lavage fluid, and can be expected to demonstrate an excellent therapeutic effect in respiratory tract infection. It is now undergoing clinical evaluation.

Experimental

All the melting points were determined on a Yanaco MP-500D apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC200 spectrometer using tetramethylsilane as internal standard.

3,4,6-Trifluoro-2-methylaniline (2)

N-Chlorosuccinimide (40.1 g, 0.3 mol) was added portionwise to a mixture of 2,4,5-trifluoroaniline (**1**) (35.0 g, 0.2 mol) and dimethyl sulfide (22.0 mL, 0.3 mol) in CH_2Cl_2 (530 mL) below 5 °C and the mixture was stirred at 5 °C for 30 min. Triethylamine (30.3 g, 0.3 mol) was added and the mixture was refluxed for 12 h. The solution was washed with 10% NaOH and CH_2Cl_2 solution was concentrated. A suspension of activated Raney Ni (200 g) in EtOH (400 mL) was added to the residue, and the mixture was stirred at room temperature for 30 min. The catalyst was filtered off and the filtrate was concentrated. The residue was distilled under reduced pressure to give **2** (15.3 g, 40%) as colorless oil, bp 78 °C at 13 mmHg. NMR ($CDCl_3$) δ: 2.13 (3H, *d*, *J* = 2.1 Hz), 3.56 (2H, *br s*), 6.63–6.84 (1H, *m*). Anal. calcd for $C_7H_6F_3N$: C, 52.18; H, 3.75; N, 8.69. Found: C, 52.18; H, 3.70; N, 8.76.

3,4,6-Trifluoro-2-methylbenzonitrile (3)

A solution of $NaNO_2$ (5.5 g, 80 mmol) in water (20 mL) was added dropwise to a mixture of **2** (10.6 g, 66 mmol), conc H_2SO_4 (13.8 mL) and water (46 mL) at 0–5 °C. After 30 min of stirring, the solution was added dropwise to a mixture of $CuSO_4 \cdot 5H_2O$ (41g, 0.2 mol), KCN (45 g, 0.7 mol) and 28% ammonia water (60 mL) in water (260 mL) at 10–30 °C. The mixture was stirred at 30 °C for 1 h, and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (eluent, CH_2Cl_2:hexane, 1:4) to give **3** (4.3 g, 38%) as colorless oil, bp 74 °C at 12 mmHg. NMR ($CDCl_3$) δ: 2.52 (3H, *d*, *J* = 2.5 Hz), 6.86–7.05 (1H, *m*). Anal. calcd for $C_8H_4F_3N$: C, 56.15; H, 2.36; N, 8.19. Found: C, 55.95; H, 2.09; N, 8.03.

3,4,6-Trifluoro-2-methylbenzoic acid (4)

A mixture of **3** (9.3 g, 54 mmol) and 50% H_2SO_4 (80 mL) was heated at 150 °C for 7 h. After cooling, the reaction mixture was diluted with water, and the solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over $MgSO_4$ and concentrated. The residue was recrystallized from ether–hexane to give **4** (8.1 g, 79 %) as colorless needles, mp 116–117 °C. NMR ($CDCl_3$) δ: 2.47 (3H, *t*, *J* = 2.6 Hz), 6.88 (1H, *dt*, *J* = 6.3, 9.6 Hz), 10.75 (1H, *br s*). Anal. calcd for $C_8H_5F_3O_2$: C, 50.54; H, 2.65. Found: C, 50.45; H, 2.61.

Ethyl 3,4,6-trifluoro-2-methylbenzoylacetate (5)

A mixture of **4** (3.2 g, 17 mmol) and thionyl chloride (7 mL, 0.1 mol) was heated at 80 °C for 1 h, and then concentrated to give acid chloride. A few drops of CCl_4 was added to a suspension of magnesium (0.5 g, 19 mmol) in absolute EtOH (0.9 mL). When the reaction was started, a solution of diethyl malonate (3.0 g, 19 mmol) was added dropwise to the suspension below 60 °C. After 30 min of stirring, the reaction mixture was cooled at 0 °C. A solution of acid chloride obtained above in toluene (5 mL) was added dropwise to the reaction mixture below 10 °C, and then stirred at room temperature for 30 min. A solution of conc H_2SO_4 (0.4 mL) in water (20 mL) was added to the mixture under ice-cooling. The organic layer was separated, washed with 2% $NaHCO_3$ and concentrated. A solution of *p*-TsOH (30 mg) in water (20 mL) was added to the residue, and the mixture was refluxed for 3 h. After cooling, the mixture was extracted with ether and the ether solution was concentrated. The residue was purified by silica gel column chromatography (eluent, CH_2Cl_2:hexane, 1:4) to give **5** (3.3 g, 59%) as colorless oil. NMR ($CDCl_3$) δ: 1.23 (2.1H, *t*, *J* = 7.2 Hz), 1.34 (0.9H, *t*, *J* = 7.2 Hz), 2.34 (3H, *d*, *J* = 7.2 Hz), 3.88 (1.4H, *d*, *J* = 2.2 Hz), 4.18 (1.4H, *q*, *J* = 7.2 Hz), 4.28 (0.6H, *q*, *J* = 7.2 Hz), 5.24 (0.3H, *d*, *J* = 1.0 Hz), 6.70–6.95 (1H, *m*), 12.34 (0.3H, *s*). Anal. calcd for $C_{12}H_{11}F_3O_3$: C, 55.39; H, 4.26. Found: C, 55.36; H, 4.12.

Ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-5-methyl-4-oxo-3-quinolinecarboxylate (6)

A mixture of **5** (3.2 g, 12 mmol), acetic anhydride (3.0 g, 29 mmol) and triethyl orthoformate (2.7 g, 18 mmol) was heated at 150 °C for 1 h, and then concentrated. EtOH (25 mL) was added to the residue, and then cyclopropylamine (0.84 mL, 12 mmol) was added to the EtOH solution. The mixture was stirred for 30 min and concentrated. Absolute dioxane (26 mL) was added to the residue, and then 60% sodium hydride (0.56 g, 14 mmol) was added portionwise to the dioxane solution. After 30 min of stirring, the reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The CH_2Cl_2 solution was concentrated, and the residue was recrystallized from EtOH to give **6** (2.0 g, 53%) as colorless needles, mp 185–187 °C. NMR ($CDCl_3$) δ: 1.03–1.45 (4H, *m*), 1.40 (3H, *t*, *J* = 7.1 Hz), 2.88 (3H, *d*, *J* = 3.0 Hz), 3.28–3.54 (1H, *m*), 4.38 (2H, *q*, *J* = 7.1 Hz), 7.60 (1H, *dd*, *J* = 6.8, 11.4 Hz), 8.48 (1H, *s*). Anal. calcd for $C_{16}H_{15}F_2NO_3$: C, 62.54; H, 4.92; N, 4.56. Found: C, 62.48; H, 5.14; N, 4.51.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-5-methyl-4-oxo-3-quinolinecarboxylic acid (7)

A mixture of **6** (1.9 g, 6.2 mmol), conc HCl (5 mL) and

90% AcOH (20 mL) was refluxed for 2 h. After cooling, the resulting precipitates were collected by the filtration to give **7** (1.6 g, 92%) as colorless needles, mp 294–298 °C. NMR (CF_3COOD) δ: 1.40–1.83 (4H, *m*), 3.60 (3H, *d*, *J* = 2.8 Hz), 4.04–4.22 (1H, *m*), 8.40 (1H, *dd*, *J* = 6.8, 10.3 Hz), 9.46 (1H, *s*). Anal. calcd for $C_{14}H_{11}F_2NO_3$: C, 60.22; H, 3.97; N, 5.02. Found: C, 60.29; H, 4.27; N, 5.01.

Methyl 3-amino-2-methylbenzoate (22)

Thionyl chloride (10 mL, 0.14 mol) was added to a solution of 2-methyl-3-nitrobenzoic acid (**21**) (10.0 g, 55 mmol), and the mixture was refluxed for 2 h. The mixture was poured into ice-water and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water, dried over $MgSO_4$ and concentrated. To the residue was added AcOH (50 mL) and 5% Pd–C (1 g), and the suspension was stirred at room temperature under atmospheric pressure of hydrogen until the absorbtion of hydrogen ceased. The catalyst was filtered off and the filtrate was basified with 10% K_2CO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over $MgSO_4$ and concentrated to give **22** (8.6 g, 95%) as brown oil, which was used in the next reaction step without further purification. NMR ($CDCl_3$) δ: 2.33 (3H, *s*), 3.72 (2H, *br s*), 3.87 (3H, *s*), 6.80 (1H, *d*, *J* = 7.8 Hz), 7.04 (1H, *t*, *J* = 7.8 Hz), 7.20 (1H, *d*, *J* = 7.8 Hz).

Methyl 3-amino-4,6-dibromo-2-methylbenzoate (23)

A solution of bromine (3.1 g, 20 mmol) in AcOH (5 mL) was added dropwise to a mixture of **22** (1.6 g, 10 mmol) and sodium acetate (1.6 g, 20 mmol) in AcOH (20 mL) below 20 °C. The mixture was stirred at room temperature for 30 min. The reaction mixture was poured into ice-water and extracted with Et_2O. The Et_2O solution was washed with 10% K_2CO_3, dried over $MgSO_4$ and concentrated. The residue was recrystallized from hexane to give **23** (2.8 g, 90%) as colorless prisms, mp 41–42 °C. NMR ($CDCl_3$) δ: 2.14 (3H, *s*), 3.94 (3H, *s*), 4.14 (2H, *br s*), 7.52 (1H, *s*). Anal. calcd for $C_9H_9Br_2NO_2$: C, 33.47; H, 2.81; N, 4.34. Found: C, 33.46; H, 2.70; N, 4.44.

Methyl 4,6-dibromo-3-fluoro-2-methylbenzoate (24)

A solution of **23** (20.0 g, 62 mmol) in EtOH (100 mL) was added to a solution of 60% hexafluorophosphoric acid (54 g, 0.22 mol) in EtOH (100 mL) at room temperature. A solution of $NaNO_2$ (4.6 g, 67 mmol) in water (10 mL) was added dropwise to the mixture at 0–5 °C and then stirred for 1.5 h. The resulting precipitates were collected by filtration, washed with EtOH and then ether to give diazonium salt (34.2 g). A stirred suspension of the diazonium salt (5.9 g) and boron trifluoride diethyl ether complex (500 mL) was irradiated with ultraviolet light for 4 h at 27–42 °C by using a high pressure mercury lamp (Riko Kagaku Sangyou K. K. type UVL-100P). The reaction mixture was diluted with ethyl acetate and poured into ice-water. The organic layer was dried over $MgSO_4$, concentrated and distilled to give **24** (2.7 g, 85%) as colorless oil, bp 120–124 °C at 6 mmHg. NMR ($CDCl_3$) δ: 2.27 (3H, *d*, *J* = 2.5 Hz), 3.95 (3H, *s*), 7.63 (1H, *d*, *J* = 5.9 Hz). Anal. calcd for $C_9H_7Br_2FO_2$: C, 33.16; H, 2.06. Found: C, 32.95; H, 2.03.

4,6-Dibromo-3-fluoro-2-methylbenzoic acid (25)

A mixture of **24** (7.3 g, 23 mmol) and 10% NaOH (90 mL) in EtOH (90 mL) was refluxed for 2 h. After cooling, the mixture was extracted with Et_2O, and the aqueous solution was acidified with dilute HCl. The suspension was extracted with Et_2O, and the Et_2O solution was concentrated. Recrystallization from ether–hexane gave **25** (5.9 g, 86%) as colorless prisms, mp 144–146 °C. NMR ($CDCl_3$) δ: 2.39 (3H, *d*, *J* = 2.5 Hz), 7.68 (1H, *d*, *J* = 5.9 Hz). Anal. calcd for $C_8H_5Br_2FO_2$: C, 30.80; H, 1.62. Found: C, 30.81; H, 1.49.

Ethyl 4,6-dibromo-3-fluoro-2-methylbenzoylacetate (26)

A mixture of **25** (5.0 g, 15 mmol) and thionyl chloride (6.6 mL, 90 mmol) was heated at 80 °C for 1 h, and then concentrated to give acid chloride. A mixture of monoethyl malonate (5.0 g, 38 mmol) and magnesium ethoxide (6.5 g, 57 mmol) in toluene (75 mL) was refluxed for 4 h. A solution of acid chloride obtained above in toluene (5 mL) was added dropwise to the reaction mixture, and then refluxed for 1 h. A solution of conc H_2SO_4 (7 mL) and H_2O (75 mL) was added to the reaction mixture under ice-cooling. The toluene solution was washed with water, dried with $MgSO_4$ and concentrated. The residue was recrystallized from EtOH–H_2O to give **26** (5.3 g, 92%) as colorless prisms, mp 46–47 °C. NMR ($CDCl_3$) δ: 1.27 (1.5H, *t*, *J* = 7.2 Hz), 1.35 (1.5H, *t*, *J* = 7.2Hz), 2.26 (1.5H, *d*, *J* = 2.8 Hz), 2.33 (1.5H, *d*, *J* = 2.8 Hz), 3.89 (1H, *s*), 4.20 (1H, *q*, *J* = 7.2 Hz), 4.29 (1H, *q*, *J* = 7.2 Hz), 7.63 (0.5H, *d*, *J* = 6.4 Hz), 7.67 (0.5H, *d*, *J* = 6.4 Hz), 12.30 (0.5H, *s*). Anal. calcd for $C_{12}H_{11}BrF_2O_3$: C, 37.73; H, 2.90. Found: C, 37.74; H, 2.81.

Ethyl 7-bromo-1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-4-oxo-3-quinolinecarboxylate (27)

A mixture of **26** (6.4 g, 17 mmol), acetic anhydride (3.7 g, 25 mmol) and triethyl orthoformate (4.1 g, 40 mmol) was heated at 150 °C for 1 h, and then concentrated. EtOH was added to the residue, and cyclopropylamine (1.5 g, 26 mmol) was added to the EtOH solution. The mixture was stirred at room temperature for 30 min, and concentrated. DMF (100 mL) and K_2CO_3 (2.8 g, 20 mmol) was added to the residue, and the suspension was heated at 140 °C for 30 min. After cooling, water was added to the reaction mixture, and the resulting precipitates were collected by filtration. Recrystallization from EtOH gave **27** (5.1 g, 83%) as colorless needles, mp 195–197 °C. NMR ($CDCl_3$) δ: 1.08–1.20 (2H, *m*), 1.30–1.45 (2H, *m*), 1.40 (3H, *t*, *J* = 7.1 Hz), 2.89 (3H, *d*, *J* = 2.7 Hz), 3.35–3.46 (1H, *m*), 4.40 (2H, *q*, *J* = 7.1 Hz), 8.01 (1H, *d*, *J* = 5.9 Hz), 8.48 (1H, *s*). Anal. calcd for $C_{16}H_{15}BrFNO_3$: C, 52.19; H, 4.11; N, 3.80. Found: C, 52.22; H, 3.99; N, 3.93.

7-Bromo-1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-4-oxo-3-quinolinecarboxylic acid (28)

Compound **28** (27.2 g, 91%) was obtained from **27** (32.5 g) by the same procedure as described for **7**, mp 237–239 °C. NMR ($CDCl_3$) δ: 1.15–1.30 (2H, *m*), 1.38–1.55 (2H, *m*), 2.91 (3H, *d*, *J* = 2.4 Hz), 3.33–3.66 (1H, *m*), 8.21 (1H, *d*, *J* = 6.0 Hz), 8.81 (1H, *s*), 14.89 (1H, *br s*). Anal. calcd for $C_{14}H_{11}BrFNO_3 \cdot 1/4H_2O$: C, 48.79; H, 3.36; N, 4.06. Found: C, 48.47; H, 3.13; N, 3.97.

1-Cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (9)

A mixture of **7** (0.15 g, 0.54 mmol) and *N*-methyl piperazine (0.26 g, 2.6 mmol) in DMF (2 mL) was heated at 90 °C for 30 min. The mixture was concentrated, and then ether was added to the residue. The resulting precipitates were collected by the filtration and recrystallized from EtOH to give **9** (0.12 g, 63%) as pale yellow prisms, mp 294–298 °C. NMR ($CDCl_3$) δ: 1.05–1.46 (4H, *m*), 2.39 (3H, *s*), 2.55–2.70 (4H, *m*), 2.80 (3H, *d*, *J* = 3.2 Hz), 3.20–3.40 (4H, *m*), 3.40–3.58 (1H, *m*), 7.29 (1H, *d*, *J* = 6.2 Hz), 8.71 (1H, *s*), 14.43 (1H, *br s*). The melting point and elemental analysis data are given in Table 1.

Compounds **8**, **12**, **19** and **20** were obtained by the same procedure as described for **9**. The yield, melting point and elemental analysis data are given in Table 1.

1-Cyclopropyl-7-(3-ethylaminomethyl-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-5-methyl-4-oxo-3-quinolinecarboxylic acid hydrochloride (16)

A mixture of **7** (0.1 g, 0.36 mmol) and 3-(*N*-ethyl-*N*-*tert*-butoxycarbonylamino)methylpyrrolidine (0.23 g, 1.0 mmol) in DMF (2 mL) was heated at 90 °C for 30 min. The mixture was concentrated, acidified with dilute HCl and extracted with CH_2Cl_2. The CH_2Cl_2 solution was concentrated, and a mixture of EtOH (2 mL) and 10% HCl (4 mL) was added to the residue. The mixture was refluxed for 30 min, and then concentrated. The residue was recrystallized from EtOH to give **16** (70 mg, 47%) as pale yellow needles. NMR ($CDCl_3$) δ: 0.92–1.45 (7H, *m*), 1.70–1.95 (1H, *m*), 2.04–2.30 (1H, *m*), 2.65 (3H, *d*, *J* = 3.2 Hz), 2.55–2.80 (1H, *m*), 2.82–3.90 (9H, *m*), 6.94 (1H, *d*, *J* = 8.0 Hz), 8.46 (1H, *s*), 9.07 (2H, *br s*), 14.05 (1H, *br s*). The melting point and elemental analysis data are given in Table 1.

Compounds **13–15**, **17** and **18** were obtained by the same procedure as described for **16**. The yield, melting point and elemental analysis data are given in Table 1.

7-(4-Acetyl-1-piperazinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-4-oxo-3-quinolinecarboxylic acid (11)

Acetic anhydride (0.1 mL, 1.0 mmol) was added to a solution of **8** (40 mg, 0.12 mmol) in 5% NaOH (2 mL), and then the mixture was stirred at room temperature for 1 h. The reaction mixture was acidified with dilute HCl and extracted with CH_2Cl_2. The CH_2Cl_2 solution was concentrated, and the residue was recrystallized from EtOH to give **11** (29 mg, 65%) as white powder. NMR ($CDCl_3$) δ: 1.05–1.48 (4H, *m*), 2.17 (3H, *s*), 2.81 (3H, *d*, *J* = 3.2 Hz), 3.15–3.97 (9H, *m*), 7.29 (1H, *d*, *J* = 7.2 Hz), 8.71 (1H, *s*), 15.46 (1H, *br s*). The melting point and elemental analysis data are given in Table 1.

1-Cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-4-oxo-7-(3-methyl-1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride (10)

A mixture of **28** (4.0 g, 11.8 mmol) and 2-methylpiperazine (11.8 g, 0.118 mol) in DMSO (80 mL) was heated at 130 °C for 30 min and then concentrated. EtOH (40 mL) and 10% HCl (8 mL) was added to the residue and concentrated. The residue was recrystallized from EtOH–H_2O to give **10** (2.6 g, 56%) as white powder. NMR (DMSO–d_6) δ: 1.04–1.48 (4H, *m*), 1.35 (3H, *d*, *J* = 6.5 Hz), 2.75 (3H, *d*, *J* = 3.0 Hz), 3.04–3.86 (8H, *m*), 7.49 (1H, *d*, *J* = 7.9 Hz), 8.62 (1H, *s*), 9.65 (2H, *br s*), 15.56 (1H, *br s*). The melting point and elemental analysis data are given in Table 1.

In vitro *antibacterial activity*

Minimum inhibitory concentration (MIC) was determined by the two-fold agar dilution method with Müller–Hinton agar (Difco Laboratories, Detroit, MN U.S.A.). The overnight broth cultures were diluted to approximately 10^6 CFU mL^{-1} with fresh broth, and an inoculum of 10^4 CFU per spot was applied to agar plates containing graded concentrations of each compound with an incubating apparatus (Microplanter: Sakuma Seisakusyo, Tokyo, Japan). After incubation at 37 °C for 18 h, the MIC was defined as the minimum drug concentraion which inhibited the growth of bacteria.

In vivo *antibacterial activity*

In vivo activity was determined against the experimental systemic infections caused by Gram-positive and -negative pathogens. ICR strain mice (male, 20–25 g) were divided into groups of 10. *S. aureus* Smith and *E. coli* No. 29 were preincubated in nutrient broth and *S. pneumoniae* type III was preincubated in brain heart infusion broth containing 5% of horse serum for 18 h at 37 °C. The bacteria were suspended in the same fresh media and mucin was added before injection. Mice were infected intraperitonally with 0.5 mL of the respective pathogens. 1 h Following injection of the bacteria, a single dose of each compound was administered orally to mice. The survival rate on day seven was calculated and the ED_{50} value was determined by the probit method.

Pharmacokinetics

The concentration of **10** and reference drugs in the plasma and lungs of rats (*n* = 5) were determined by HPLC following the administration of a single oral 20

mg kg^{-1} dose. After blood collection, the rats were perfused with normal saline, and 2 mL of saline was injected into the bronchi through the trachea. The bronchoalveolar fluid was collected from the trachea by syringe and the concentration of drugs was measured. After homogenizing excited tissue, the concentration in the lungs was determined.

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