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Articles

Synthesis of Antimicrobial Agents. 5. *In Vivo* Metabolism of 7-(4-Hydroxypiperazin-1-yl)quinolones

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A series of novel pyridone carboxylic acids having a 4-hydroxypiperazin-1-yl, a 4-hydroxy-3-methylpiperazin-1-yl, and a 4-hydroxy-3,5-dimethylpiperazin-1-yl group was prepared, and their metabolism to corresponding piperazinyl derivatives after oral administration to mice and rats was studied. This reductive metabolism appeared to be more extensive in mice than in rats. Moreover, the introduction of a methyl group into the α-position of the 4-hydroxy group depressed the metabolism in both species.

Introduction

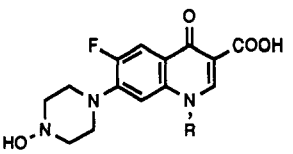
In the previous paper,[1] we reported that 6-fluoro-7-(4-hydroxypiperazin-1-yl) derivatives 1 and 2 showed higher *in vivo* antibacterial activity than the corresponding piperazinyl compounds, norfloxacin (NFLX)[2] and ciprofloxacin (CPFX),[3] respectively, against experimentally induced infections of mice. Our preliminary studies indicated that 1 was metabolized to NFLX, and the higher blood levels of NFLX after oral adminstration of 1 than NFLX itself brought about the above results. These findings led us to synthesize 7-(substituted 4-hydroxypiperazin-1-yl)quinolines and to study the structure–metabolism relationship of the quinolones, particularly the influence of substituents at the α-position of the 4-hydroxyl group.

This paper describes the syntheses of 7-(4-hydroxypiperazin-1-yl) derivative 3, 7-(4-hydroxy-3-methylpiperazin-1-yl) derivative 4, and 7-(4-hydroxy-3,5-dimethylpiperazin-1-yl) derivative 5 and the conversion of these compounds (3, 4, 5) to the corresponding piperazinyl compounds (7, 8, 9) after oral administration to mice and rats.

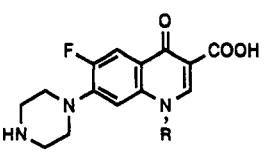
Chemistry

The 6,7,8-trifluoro derivative 6,[4] 7-(4-hydroxypiperazin-1-yl) derivative 3,[4] and compounds 7–9[5] were prepared according to literature procedures. The hydroxylated

Chart I



1: R = CH_2CH_3
2: R = 



NFLX: R = CH_2CH_3
CPFX: R = 

derivatives 4 and 5 were synthesized from the corresponding piperazinyl compounds 8 and 9, respectively, as shown in Scheme I.

Treatment of 8 and 9 with acrylonitrile provided the corresponding 7-[4-(2-cyanoethyl)-3-methylpiperazin-1-yl] derivative 10 and 7-[4-(2-cyanoethyl)-3,5-dimethylpiperazin-1-yl] derivative 11, respectively. Oxidation of 10 and 11 with *m*-chloroperbenzoic acid in chloroform at room temperature afforded the *N*-oxides, which spontaneously rearranged to 4 and 5, respectively, by a reverse Michael addition.[6] Therefore, the unstable *N*-oxides were not isolated.

The physical properties of these compounds are listed in Table I.

Blood Levels after Oral Administration in Mice and Rats. Test compounds suspended in 0.5% sodium

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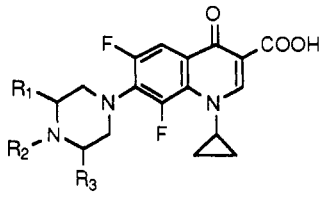
Table I. 6,8-Difluoro-7-(substituted piperazin-1-yl)quinolones



compd	R_1	R_3	R_2	yield[a] (%)	mp (°C) dec	formula[b]
3[c]	H	H	OH		223–227	$C_{17}H_{17}F_2N_3O_4$
4	H	CH_3	OH	98.9	224–237	$C_{18}H_{19}F_2N_3O_4$
5	CH_3	CH_3	OH	64.5	214–221	$C_{19}H_{21}F_2N_3O_4$
7	H	H	H	75.1	>350	$C_{17}H_{17}F_2N_3O_3$·HCl
8	H	CH_3	H	73.8	325–330	$C_{18}H_{19}F_2N_3O_3$·HCl
9	CH_3	CH_3	H	72.2	310–315	$C_{19}H_{21}F_2N_3O_3$·HCl
10	H	CH_3	CH_2CH_2CN	80.5	224–226	$C_{21}H_{22}F_2N_4O_3$
11	CH_3	CH_3	CH_2CH_2CN	50.9	238–240	$C_{22}H_{24}F_2N_4O_3$

[a] Yields were not optimized. [b] The elementary analyses for C, H, N were within ±0.3% of the theoretical values. [c] See ref 4.

(carboxymethyl)cellulose solution (0.5% CMC) were administered orally to ddY-strain male mice (20–25 g, three mice per group) or Wister-strain male rats (150–160 g, three rats per group) fasted for 16 h. The dose of the test compound was adjusted to 50 mg/kg of body weight of mice and rats. After 30, 60, 120, and 240 min, mice and rats were killed by bleeding. Blood samples were centrifuged at 3000 rpm for 30 min at 4 °C, and the serum was collected. All speciens were stored at −20 °C until analyzed.

Serum concentrations of test compounds in mice or rats treated with 4-hydroxypiperazinyl compounds **3–5** and their dehydroxylated compounds **7–9** were determined by high-performance liquid chromatography (HPLC), after the serum specimens had been treated with 10% aqueous trichloroacetic acid and centrifuged at 3000 rpm for 15 min to give protein-free specimens. The HPLC system was equipped with a Waters Model 6000A pump, a Waters U6K injector, a Shimadzu SPD-6A spectrophotometric detector (280 nm), and a YMC A-312 ODS column. Mixtures of 5% acetic acid in methanol (v/v **7**, **8**; 80/20, **9**; 75/25, **3**; 70/30, **4**; 60/40, **5**; 50/50) were used as the mobile phase, and the flow rate was 1.0 mL/min. The specimens were assayed against standard solutions of the above test compounds prepared in mouse or rat serum and then treated as described above.

Results and Discussion

The serum levels of the test compounds **3–5** and their corresponding metabolized piperazinyl compounds **7–9** after oral administration to mice and rats are shown in Figures 1–3.

In mice receiving a dose of 50 mg/kg of unsubstituted 4-hydroxypiperazinyl compound **3**, **3** was not detected in the serum but the dehydroxylated compound **7** was found in all runs, as shown in Figure 1. However, in rats given a 50 mg/kg dose of **3**, both **3** and **7** were detected and the serum levels of **3** were approximately 5-fold lower than those of **7**.

On the other hand, after oral administration of the 4-hydroxy-3-methylpiperazinyl compound **4** to mice, **4** and its dehydroxylated derivative **8** were both present, and the mean AUC value of **4** was approximately 4-fold lower than that of **8**. In rats, the AUC values of these compounds were reversed, with the AUC of **8** being approximately twice that of **4**, as shown in Figure 2.

These results suggest that the *in vivo* reduction of the hydroxyl group at the 4-nitrogen atom of the piperazinyl moiety was depressed by the introduction of a methyl group at the α-position of the hydroxyl group. This phenomenon was observed still more clearly after oral administration of the 4-hydroxy-3,5-dimethylpiperazinyl derivative **5** to mice and rats as shown in Figure 3. The major component in the serum after administration of **5** to mice and rats was the unchanged compound **5**.

In vitro and *in vivo* antibacterial activities of compounds **3–5** and **7–9** against several organisms and experimentally induced infection of mice after oral administration are given in Tables II and III. The data on NFLX, CPFX, and OFLX[7] are also included for reference.

The *in vitro* activity of the methyl-substituted derivatives **4**, **5**, **8**, and **9** against *Pseudomonas aeruginosa* was generally less than that of **3** and **7**, while against all other Gram-negative and Gram-positive bacteria compounds **3–9** showed similar activity.

The *in vivo* activity against *Staphylococcus aureus* was increased by the introduction of a methyl group into the

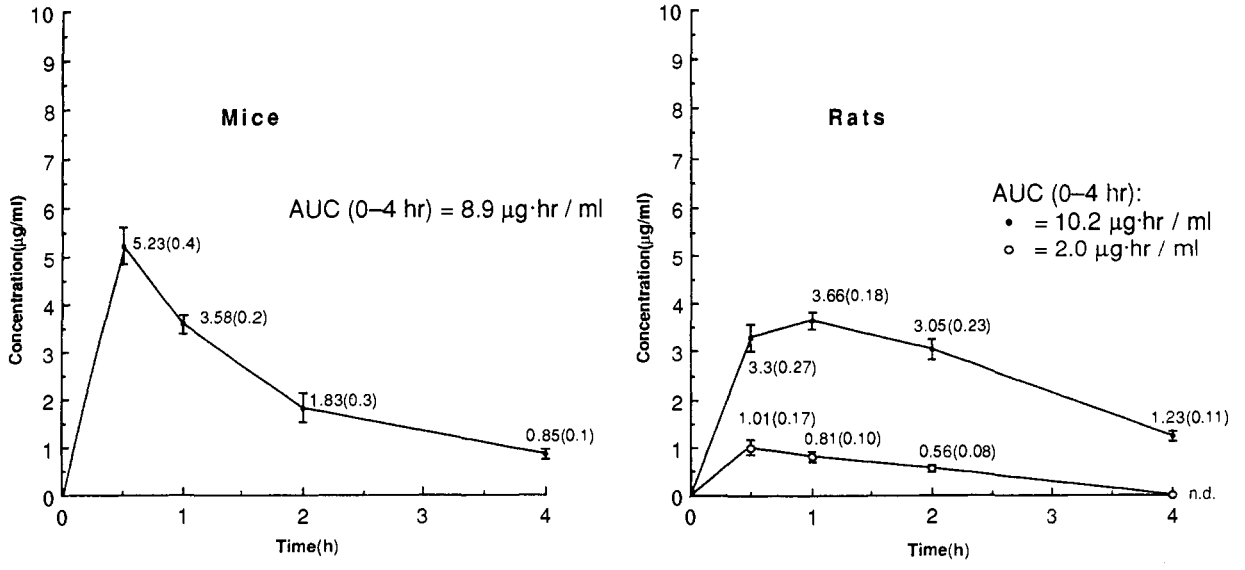
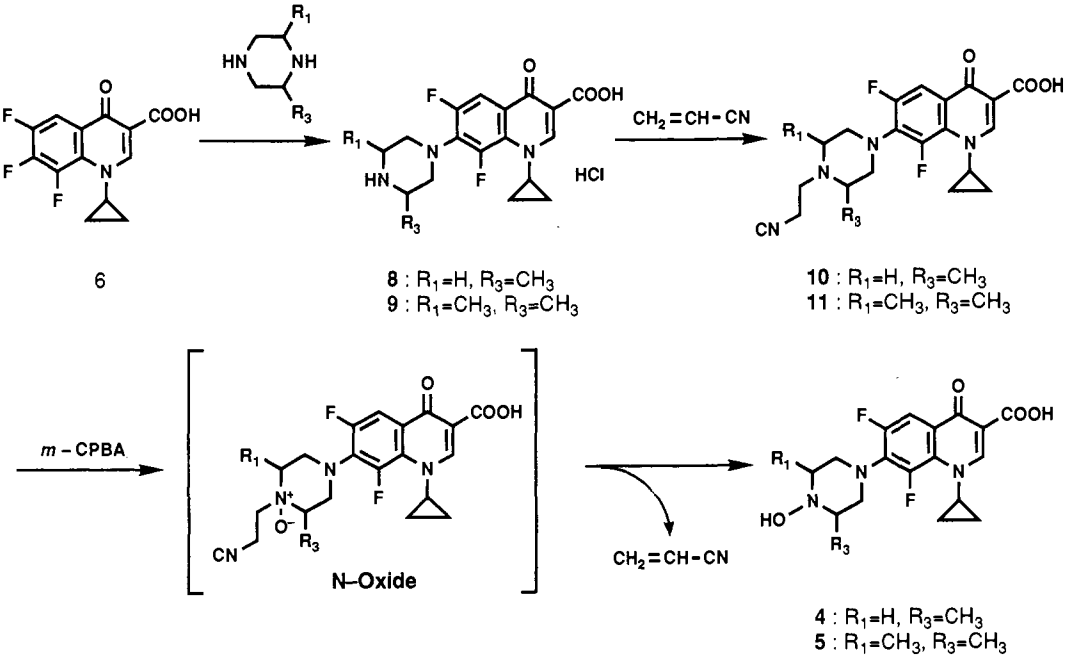


Figure 1. Serum concentrations (mean standard error) after a 50 mg/kg oral dose of 4-hydroxypiperazinyl derivative **3** to mice and rats. Open and closed circles represent **3** and piperazinyl derivative **7**, respectively.

Scheme I



piperazinyl moiety, but the activity against *P. aeruginosa* was decreased.

Furthermore, the *N*-hydroxypiperazinyl derivatives **3–5** showed more potent activity than the dehydroxylated compounds **7–9** against *S. aureus*.

However, the activity of the methyl-substituted *N*-hydroxypiperazinyl derivatives **4** and **5** against *P. aeruginosa* was lower than that of the dehydroxylated compounds **8** and **9**, respectively. Since reduction of **5** to **9** apparently did not occur *in vivo* in mice, the lower potency of **5** vs **9** is consistent with the *in vitro* potencies. Metabolic reduction of **4** to the more potent **8**, however, does occur readily in mice, suggesting that the lack of *in vivo* potency of **4** is due to disposition or distribution characteristics of the compound.

From the studies on the pharmacokinetics of the novel *N*-hydroxypiperazinyl derivatives **3–5**, metabolic reduction of the *N*-hydroxyl group was considered to be reduced by increased steric hindrance. This effect appeared to be stronger in rats than in mice.

Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus, and all melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined at 100 MHz on a Nihon Denshi PS-100 NMR spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were measured with a Hitachi M-60 (70 eV).

NFLX,[2] CPFX,[3] OFLX,[7] 6,7,8-trifluoro derivative **6**,[4] 7-(4-hydroxypiperazin-1-yl) derivative **3**,[4] 7-(piperazin-1-yl) derivative **7**,[5] 7-(3-methylpiperazin-1-yl) derivative **8**,[5] and 7-(3,5-dimethylpiperazin-1-yl) derivative **9**[5] were prepared according to the literature procedures.

1-Cyclopropyl-7-[4-(2-cyanoethyl)-3-methylpiperazin-1-yl]-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (10). To a suspension of **8** (2.5 g) in water (45 mL) was added 20% aqueous sodium hydroxide slowly, and the pH of the mixture was adjusted to 7.0 to give a solid. The solid was collected by

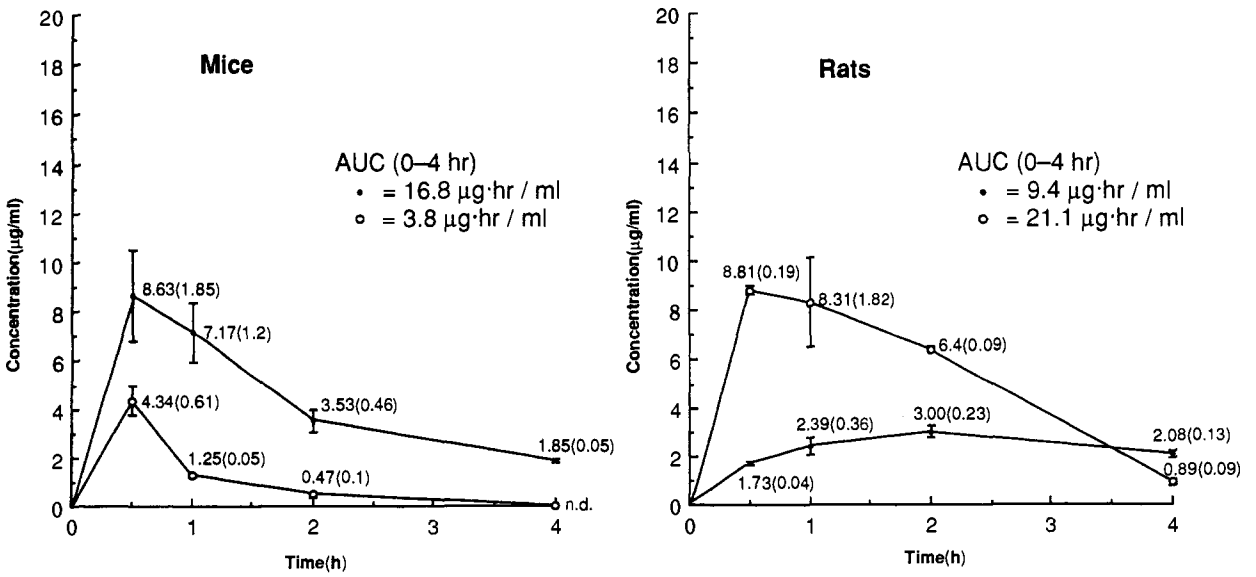


Figure 2. Serum concentrations (mean standard error) after a 50 mg/kg oral dose of 4-hydroxy-3-methylpiperazinyl derivative **4** to mice and rats. Open and closed circles represent **4** and 3-methylpiperazinyl derivative **8**, respectively.

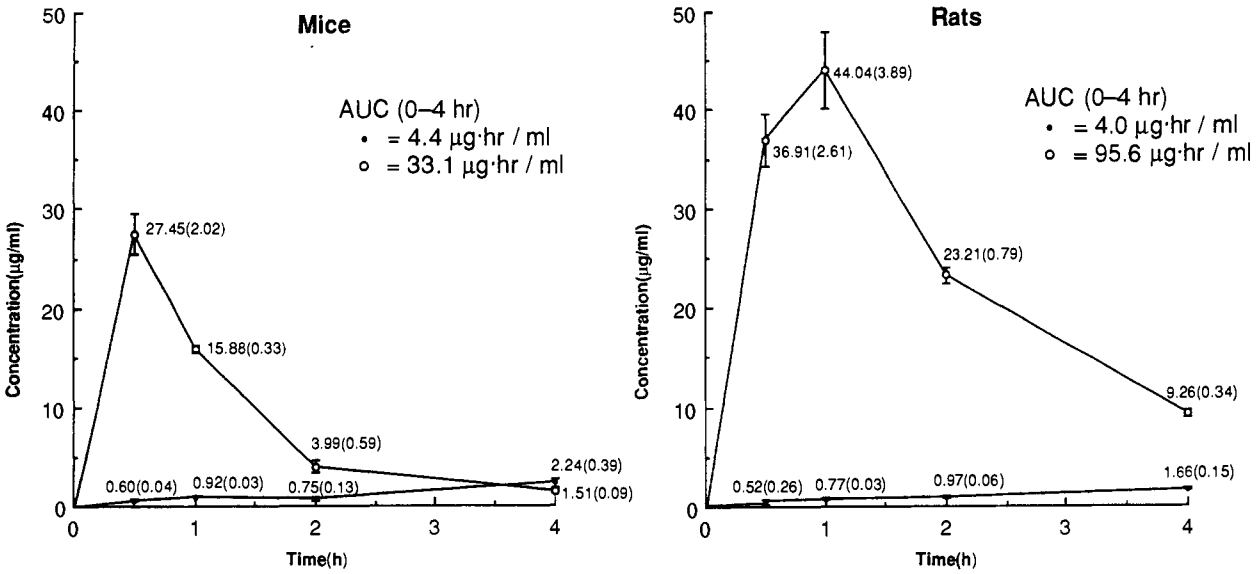


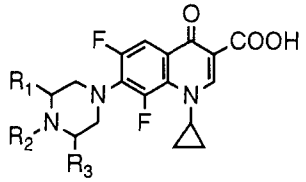
Figure 3. Serum concentrations (mean standard error) after a 50 mg/kg oral dose of 3,5-dimethyl-4-hydroxypiperazinyl derivative **5** to mice and rats. Open and closed circles represent **5** and 3,5-dimethylpiperazinyl derivative **9**, respectively.

Table II. In Vitro Antibacterial Activities of 6,8-Difluoro-7-(substituted piperazin-1-yl) Derivatives

	organism; minimum inhibitory concentration (MIC)[a] (μg/mL)								
compd	Sa	Se	Bs	Ec	Kp	Pv	St	Sm	Pa
3	0.39	0.78	0.05	0.10	0.025	0.05	0.025	0.78	1.56
7	0.20	0.78	0.10	0.05	0.025	0.05	0.025	0.39	0.78
4	0.39	0.78	0.025	0.10	0.025	0.10	0.05	0.78	3.13
8	0.39	0.78	0.05	0.05	0.0125	0.05	0.025	0.39	1.56
5	0.20	0.39	0.05	0.78	0.10	0.39	0.78	6.25	12.5
9	0.39	0.78	0.10	0.10	0.025	0.10	0.10	0.78	3.13
NFLX	0.39	3.13	0.39	0.39	0.10	0.20	0.10	0.78	3.13
CPFX	0.39	1.56	0.10	0.10	0.025	0.05	0.025	0.39	0.78
OFLX	0.39	0.78	0.20	0.20	0.10	0.10	0.10	0.78	3.13

[a] The MICs were determined by the 2-fold agar dilution on sensitivity test agar. Organisms selected for inclution in the table: Sa, *S. Aureus* FDA 209P JC-1; Se, *Staphylococcus epidermidis* IAM 12896; Bs, *Bacillus subtilis* ATCC 6633; Ec, *Escherichia coli* NIHJ JC-2; Kp, *Klebsiella pneumoniae* PCl-602; Pv, *Proteus vulgaris* OX-19; St, *Salmonella typhimurium* IID 971; Sm, *Serratia marcescens* IAM 1184; Pa, *P. aeruginosa* IFO 3445.

Table III. In Vitro and In Vivo Antibacterial Activities of 6,8-Difluoro-7-(substituted piperazin-1-yl) Derivatives



				S. aureus IID 803		*P. aeruginosa* E-2	
compd	R_1	R_3	R_2	MIC[a] (μg/mL)	ED_{50}[b] (mg/kg),[c] po	MIC[a] (μg/mL)	ED_{50}[b] (mg/kg),[c] po
3	H	H	OH	0.39	3.34 (2.38–4.70)	1.56	4.81 (3.32–6.97)
7	H	H	H	0.39	5.82 (4.15–8.18)	0.78	6.70 (4.77–9.47)
4	H	CH_3	OH	0.20	1.67 (1.19–2.35)	3.13	22.9 (15.1–35.1)
8	H	CH_3	H	0.20	2.21	1.56	8.84
5	CH_3	CH_3	OH	0.20	1.67 (1.19–2.35)	12.5	35.4
9	CH_3	CH_3	H	0.39	2.21 (1.49–3.27)	3.13	11.7 (7.52–18.1)
NFLX				1.56	91.7 (59.9–14.0)	3.13	107 (76.3–151)
CPFX				0.78	35.4	0.78	30.8 (23.3–40.6)
OFLX				0.78	8.84 (5.97–13.1)	3.13	54.5 (34.4–86.4)

[a] See Table II, footnote a. [b] See Experimental Section. [c] 95% confidence limits.

filtration and dried at 80 °C. The solid was suspended in chloroform–methanol (1/1 v/v, 50 mL), and acrylonitrile (2.4 mL) was added. After refluxing for 20 h the reaction mixture was concentraetd to give a pale yellow solid. The resulting solid was collected by filtration and washed with methanol. The solid was dried to give **10** (2.1 g).

^{1}H NMR ($CDCl_3$) δ: 1.08–1.35 (4H, m, cyclopropyl), 1.13 (3H, d, J = 6.0 Hz, methyl), 2.40–3.50 (11H, m, piperazine and CH_2-CH_2CN), 3.82–4.16 (1H, m, cyclopropyl), 7.86 (1H, dd, J = 2.0, 12.0 Hz, C_5-H), 8.74 (1H, s, C_2-H), 14.46 (1H, br s, COOH). MS m/e: 416 (M^+).

1-Cyclopropyl-7-[4-(2-cyanoethyl)-3,5-dimethylpiperazin-1-yl]-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (11). To a suspension of **9** (5.2 g) in water (90 mL) was slowly added 20% aqueous sodium hydroxide, and the pH of the mixture was adjusted to 7.0 to give a solid. The solid was collected by filtration and dried at 80 °C. The solid was suspended in chloroform–methanol (1/1 v/v, 100 mL), and acrylonitrile (50

mL) was added. After refluxing for 68 h the reaction mixture was evaporated to give a solid. The resulting solid was collected by filtration and washed with methanol. The solid was dried to give 11 (2.9 g). ^{1}H NMR (DMSO-d_6) δ: 1.00–1.35 (4H, m, cyclopropyl), 1.07 (6H, d, J = 7.0 Hz, two methyls), 2.50–3.15 (10H, m, piperazine and CH_2CH_2CN), 4.12 (1H, m, cyclopropyl), 7.83 (1H, dd, J = 2.0, 12.0 Hz, C_5-H), 8.67 (1H, s, C_2-H), 14.75 (1H, br s, COOH). MS m/e: 430 (M^+).

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(4-hydroxy-3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (4). To an ice-cooled solution of 10 (1.0 g) in chloroform (30 mL) was gradually added m-chloroperbenzoic acid (0.71 g) and the mixture stirred for 30 min. The reaction mixture was concentrated to about 3 mL, and then methanol (10 mL) was added. The resulting solid was filtered, washed with ether, and dried to give 4 (0.9 g) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.08 (3H, d, J = 7.0 Hz, methyl), 1.00–1.32 (4H, m, cyclopropyl), 2.40–3.65 (7H, m, piperazine), 4.10 (1H, m, cyclopropyl), 7.74 (1H, dd, J = 2.0, 12.0 Hz, C_5-H), 8.04 (1H, s, OH), 8.58 (1H, s, C_2-H), 14.45 (1H, br s, COOH). MS m/e: 379 (M^+), 363 ($M^+ - O$).

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(4-hydroxy-3,5-dimethylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (5). To an ice-cooled solution of 11 (431 mg) in chloroform (30 mL) was added a chloroform solution (30 mL) of m-chloroperbenzoic acid (296 mg) dropwise. After addition, the reaction mixture was stirred at room temperature for 1 h and then concentrated to about 3 mL. Methanol (5 mL) was added. The resulting solid was collected, washed with methanol, and dried to give 5 (254 mg) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.18 (6H, d, J = 7.0 Hz, two methyls), 1.00–1.30 (4H, m, cyclopropyl), 2.40–3.60 (6H, m, piperazine), 4.10 (1H, m, cyclopropyl), 7.74 (1H, dd, J = 2.0, 12.0 Hz, C_5-H), 7.87 (1H, s, OH), 8.59 (1H, s, C_2-H), 15.54 (1H, br s, COOH). MS m/e: 393 (M^+), 377 ($M^+ - O$).

***In Vitro* Antibacterial Activity.** As described by Goto et al.,[8] the MICs of the compounds were determined by the agar dilution method, using Mueller–Hinton agar. The results are summarized in Table II.

***In Vivo* Antibacterial Activity.** The *in vivo* assay was carried out according to a previously reported method.[9] The test compounds were suspended in 0.5% sodium (carboxymethyl) cellulose (CMC) and administered orally at 1 h after infection.

ED_{50} values were calculated from the cumulative mortalities on the 7th day after infection by using the shortened version of the Weil method.[10] The results are summarized in Table III.

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