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Studies on 8-Methoxyquinolones: Synthesis and Antibacterial Activity of 7-(3-Amino-4-substituted)pyrrolidinyl Derivatives

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A series of 8-methoxyquinolones bearing 3-amino-4-methylpyrrolidines or 3-amino-4-fluoromethylpyrrolidines at the C-7 position was synthesized and their physicochemical and biological properties were evaluated. All of the compounds synthesized showed more potent activity than LVFX (3) against both gram-positive and -negative bacteria. Increases in lipophilicity of these compounds had desirable effects on their potency of single intravenous toxicity and pharmacokinetic profiles in animals. Among the compounds synthesized, 1-fluorocyclopropyl derivatives 17 and 20, and 7-(*cis*-3-amino-4-fluoromethylpyrrolidinyl) derivative 19 (DC-756h) showed negative responses in the micronucleus test in mice while 1-cyclopropyl-7-(3-aminopyrrolidinyl) derivative 16 showed a positive response. These results suggested that the introduction of a fluorine atom into the 3-aminopyrrolidinyl substituent resulted in favorable influence on genetic toxicity as well as into the N-1 cyclopropyl substituent.

Key words 8-methoxyquinolone; 3-amino-4-fluoromethylpyrrolidine; micronuclei-formation; antibacterial activity; single intravenous toxicity; lipophilicity

The increased frequency of bacterial resistance has become a serious problem in the clinical practice of antimicrobial chemotherapy.[1] In particular, incidence of resistant gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA),[2] vancomycin-resistant enterococci (VRE),[3] and penicillin-resistant *Streptcoccus pneumoniae* (PRSP)[4] is on the increase, and the development of effective drugs for infection caused by the resistant bacteria described above is urgently desired.

Since their introduction in the late 1970s, fluoroquinolone antibacterial agents have been one of the most clinically useful chemotherapeutic drugs.[5] Quinolones are antiinfective agents which selectively inhibit bacterial DNA gyrase[6] and DNA topoisomerase IV,[7] and are characterized by a broad spectrum of activity, high potency, and an excellent oral efficacy. In the investigation of structure–activity relationships (SARs) of quinolones, the introduction of a fluorine atom to the C-6 position of quinolone nucleus turned out to enhance antibacterial activity.[8] The quinolones available in clinical practice are categorized in these fluorinated quinolones such as norfloxacin (NFLX, **1**),[9] ciprofloxacin (CPFX, **2**),[10] levofloxacin (LVFX, **3**),[11] sparfloxacin (SPFX, **4**),[12] and tosufloxacin (TFLX, **5**).[13] As to basic substituents at the C-7 position, 3-aminopyrrolidines are more effective for the activity against gram-positive bacteria than piperazines.[14] Although numerous 6-fluoro-7-(3-aminopyrrolidinyl)-1-cyclopropylquinolones have been synthesized to improve activity against gram-positive bacteria, many 3-aminopyrrolidinyl derivatives showed positive responses in the micronuclei-formation test.

To research novel 7-(3-aminopyrrolidinyl)quinolones, we have been studying the influence of a fluorine atom at the N-1 cyclopropyl substituent on antibacterial activity and physicochemical properties,[15] and also reported that sitafloxacin (STFX, formerly DU-6859a, **6**), optically active N1-*cis*-fluorocyclopropyl-C7-4′-spirocyclopropyl-3-aminopyrrolidinyl quinolone, had a potent activity against both gram-positive and -negative bacteria.[16] STFX (**6**) is a promising fluoroquinolone resulted in showing a negative response in the micronuclei-formation test by introduction of a fluorine atom into the N-1 cyclopropyl substituent,[17] and has been under clinical trial.

These results prompted us to study the effect of introducing a fluorine atom into 3-aminopyrrolidine substituents at the C-7 position to obtain novel 3-aminopyrrolidinyl quinolones with no micronuclei-formation activity.

Regarding the substituent at the C-8 position of quinolone nucleus, a methoxy group plays a significant role in reducing phototoxicity.[18] Therefore, we introduced 3-aminopyrrolidinyl substituents described above into the 8-methoxy quinolone nucleus to obtain novel compounds possessing a potent activity against gram-positive bacteria along with favorable safety profiles.

In this paper, we report the synthesis and SARs of a series of 8-methoxyquinolones bearing optically active 3-amino-4-fluoromethylpyrrolidines and other 3-aminopyrrolidines.

Chemistry

The synthesis of the desired chiral *cis*-4-fluoromethyl-3-aminopyrrolidine **13** is outlined in Chart 1. 4(*S*)-Hydroxymethylpyrrolidone **7**, prepared from itaconic acid and (*R*)-(+)-1-phenylethylamine according to the literature,[19] was treated with methanesulfonyl chloride (MsCl) in the presence of triethylamine (Et_3N) to afford a corresponding mesylate, which was converted to fluoromethyl derivative **8** by reaction with tetra-*n*-butylammonium fluoride (TBAF) in quantitative yield. This fluorination was also achieved directly by treatment of **7** with diethylaminosulfur trifluoride (DAST). Introduction of a hydroxy group to **8** using oxygen in the presence of lithium diisopropylamide (LDA) in tetrahydrofuran (THF) at −78 °C gave the 3-hydroxy derivative **9**. After treatment of **9** in a mixture of MsCl and Et_3N, the resulting mesylate was converted to azide **10** with sodium azide (NaN_3). Catalytic hydrogenation of **10** followed by protection of the resulting amine moiety with di-*tert*-butyl dicarbonate (Boc_2O) afforded *cis*-3-*tert*-butoxycarbonylamino-4-fluoromethylpyrrolidone **11**. Reduction of **11** with borane–THF (BH_3–THF)

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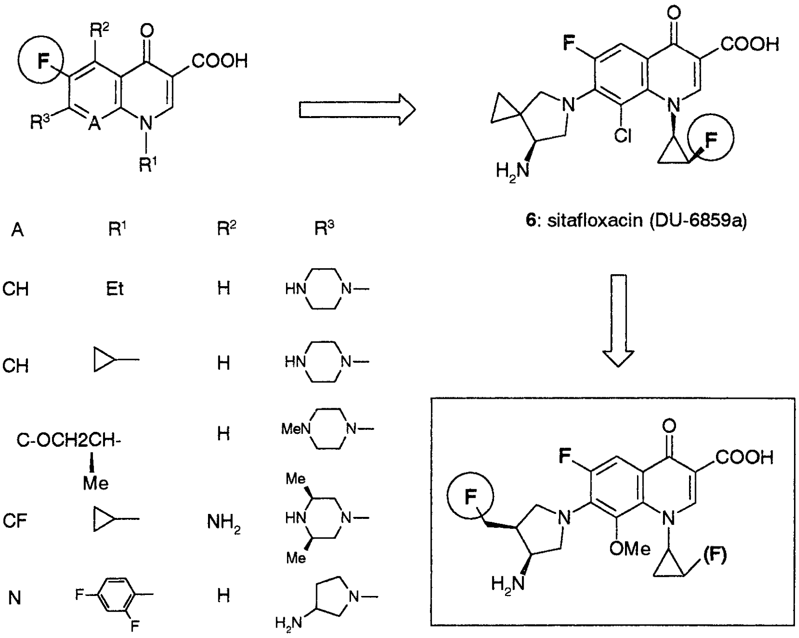
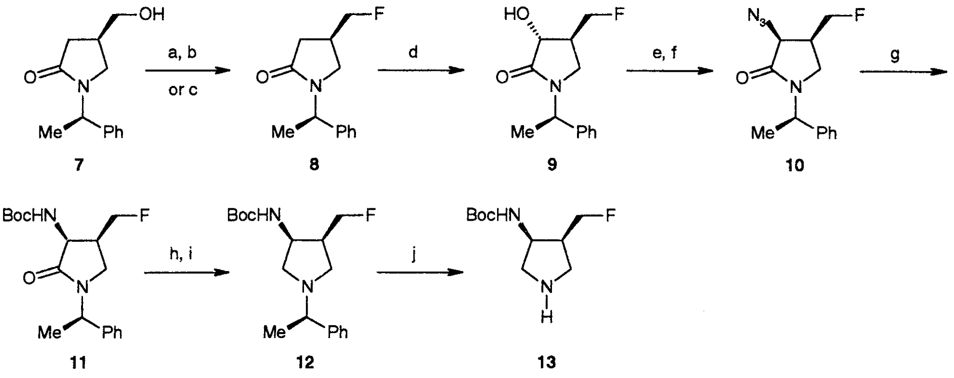


Fig. 1



a) MsCl, Et_3N, CH_2Cl_2 b) n-$Bu_4N^+F^-$, THF c) DAST, CH_2Cl_2 d) LDA, O_2, THF e) MsCl, Et_3N, CH_2Cl_2 f) NaN_3, DMF g) H_2, 10% Pd-C, Boc_2O, EtOH h) BH_3-THF, THF i) Et_3N, 80% aq. EtOH j) H_2, 10% Pd-C, EtOH

Chart 1

complex in THF followed by treatment Et_3N in aqueous ethanol (EtOH) yielded pyrrolidine **12**. Absolute configuration of C-3 and C-4 substituents on the pyrrolidine ring of **12** was determined to be *S* and *S* respectively, based on the X-ray analysis of **12** (Fig. 2). The N-1 phenylethyl group was removed by catalytic hydrogenation to afford target pyrrolidine **13**. The desired 7-substituted 8-methoxyquinolone derivatives **15**—**20** were obtained by treatment of BF_2-chelate **14** with various 3-aminopyrrolidines in dimethyl sulfoxide (DMSO) followed by dechelation with Et_3N in aqueous EtOH and successive deprotection of Boc group of the terminal amino substituent (Chart 2).

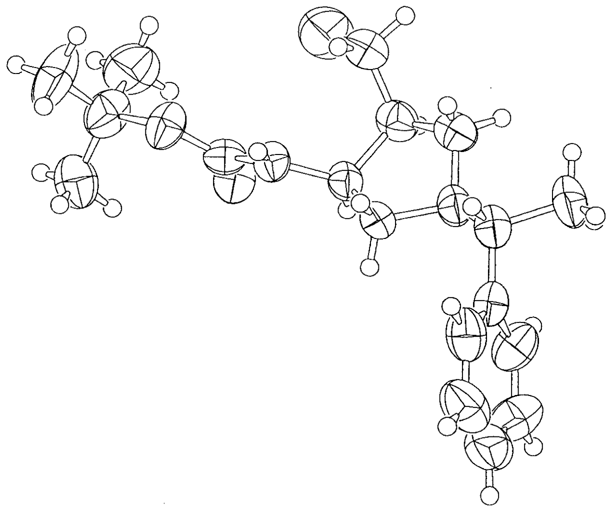


Fig. 2

Results and Discussion

The activity of compounds **15**—**20** was tested against five representative gram-positive (*Staphylococcus aureus* 209P, *Staphylococcus epidermidis* 56556, *Streptcoccus pyogenes* G36, *Streptcoccus pneumoniae* J24, and *Enterococcus faecalis* ATCC19433) and three representative gram-negative (*Escherichia coli* NIHJ, *Klebsiella pneumoniae* Type 1, and

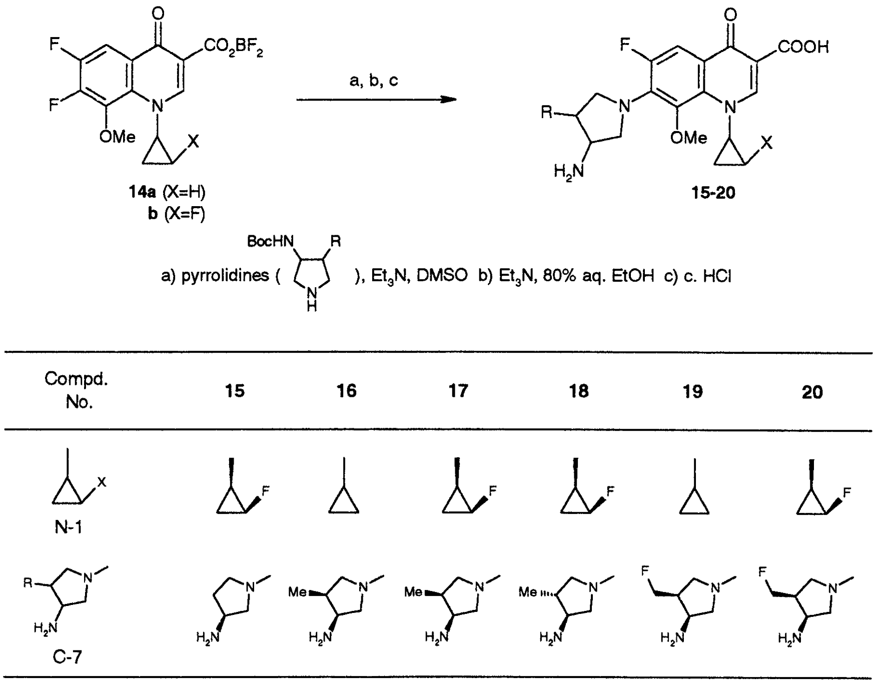


Chart 2

Table 1. *In Vitro* Antibacterial Activity of 7-(3-Aminopyrrolidinyl)-8-methoxyquinolones (MIC: μg/ml)[a]

Organism	15	16	17	18	19	20	LVFX (3)
S. aureus FDA 209P	0.013	0.013	0.013	0.013	0.013	0.013	0.20
S. epidermidis 56556	0.025	0.013	0.013	0.025	0.025	0.013	0.20
S. pyogenes G36	0.20	0.05	0.05	0.10	0.10	0.10	0.78
S. pneumoniae J24	0.05	0.025	0.025	0.05	0.025	0.025	0.78
E. faecalis ATCC19433	0.10	0.10	0.10	0.20	0.10	0.10	0.78
E. coli NIHJ	0.013	≦0.003	0.006	0.013	≦0.003	≦0.003	0.013
K. pneumoniae Type 1	0.10	0.025	0.025	0.05	0.025	0.05	0.10
P. aeruginosa PAO1	0.10	0.20	0.20	0.10	0.20	0.20	0.39

a) See Experimental section.

Table 2. Physicochemical Properties of 7-(3-Aminopyrrolidinyl)-8-methoxyquinolones **15**—**20**

Compd. No.	15	16	17	18	19	20
P'[a]	1.3	34.5	8.7	8.0	39.6	9.6
Solubility[b]	383	1112	833	582	932	186

a) Apparent partition coefficient, $CHCl_3$/0.1 M phosphate buffer (pH 7.4). *b*) Solubility to water (μg/ml).

Pseudomonas aeruginosa PAO1) organisms. The minimum inhibitory concentrations (MIC, μg/ml) of these compounds compared with those of LVFX (**3**) are summarized in Table 1.

All of the compounds synthesized showed more potent activities against both gram-positive and -negative bacteria than LVFX (**3**), in particular exhibiting 4 to 32 times more potent activity against gram-positive organisms. Compound **17** having a methyl group at the C-4 position on the pyrrolidine ring was more active than non-alkylated derivative **15** against both gram-positive and -negative bacteria. This result suggests that alkylation at this position enhances antibacterial activity. In the configuration of the methyl group on the pyrrolidine ring, 3,4-*cis* derivative **17** showed approximately 2 times more potent activity than 3,4-*trans* isomer **18** against both gram-positive and -negative organisms. Introduction of a fluorine atom into the C-7 substituent had little effect on antibacterial activity (**16** *vs.* **19**, **17** *vs.* **20**). Introduction of a fluorine into the cyclopropyl group at the N-1 position also had no significant effect on the antibacterial activities (**16** *vs.* **17**, **19** *vs.* **20**).[16a]

In the next study, we tested the effect of the fluorine atom in the N-1 or C-7 substituent on physicochemical properties. These results are summarized in Table 2. Introduction of a fluorine to cyclopropyl group at the N-1 position decreased lipophilicity of the compounds. Fluorocyclopropyl derivatives showed about a quarter of the apparent partition coefficients (P') compared with the corresponding cyclopropyl derivatives (**16** *vs.* **17**, **19** *vs.* **20**). This result substantiated our previous report dealing with 8-chloroquinolones.[16a] On the other hand, 4-fluoromethylpyrrolidinyl derivatives showed slightly higher P' values than 4-methylpyrrolidinyl compounds (**16** *vs.* **19**, **17** *vs.* **20**).

Although the lipophilicity of a series of the compounds had little influence on antibacterial activity, it had a considerable effect on their toxicity by a single intravenous injection in mice (Table 3). Non-substituted 3-aminopyrrolidinyl derivative **15** had the highest toxicity among the compounds tested, while 4-methyl or 4-fluoromethyl derivatives with higher lipophilicity showed lower toxicity. Their toxic order (from the highest to the lowest) was **15**>**17**≧**20**>**18**≧**16**≧**19**. This result was consistent with our previous report concerning pyridobenzoxazines.[20] In the micronucleus test in the bone marrow of mice (Table 3), N-1 fluorocyclopropyl derivatives **17** and **20** did not induce micronuclei, while N-1 cyclopropyl derivative **16** showed such an effect. However, N-1 cyclopropyl derivative **19** bearing 3-amino-4-fluoromethylpyrrolidinyl substituent showed a negative response in this test. These results indicate that the introduction of a fluorine atom into the basic substituent at the C-7 position also contributes to the disappearance of actions of micronuclei-formation as well as into the N-1 cyclopropyl group.[17]

The pharmacokinetic profiles of 4-fluoromethylpyrrolidinyl derivatives **19** and **20** after oral administration (20 mg/kg) to rats are given in Table 4. Compound **19** with more lipophilicity showed both higher peak plasma concentration ($C_{\max}$) and area under the time-concentration curve (AUC) than compound **20** with less lipophilicity. This result suggests that the more lipophilic compound has the advantage of absorption after oral administration.

In conclusion, we have synthesized optically active *cis*-3-amino-4-fluoromethylpyrrolidine successfully, and then introduced it to 1-cyclopropyl-8-methoxyquinolone substrate to prepare novel 7-(3-aminopyrrolidinyl)quinolones. The introduction of a fluorine atom to the 3-aminopyrrolidine moiety resulted in a favorable effect on their genetic toxicity, while the antibacterial activity was equipotent to those of corresponding non-fluorinated derivatives. We found that compound **19** showed a negative response in the micronucleus test and exhibited well balanced and potent antibacterial activity, which was 4 to 8 times more active than SPFX (**4**) and TFLX (**5**) against gram-positive bacteria including MRSA, and 2 to 8 times more active than CPFX (**2**) and LVFX (**3**) against gram-negative bacteria except *P. aeruginosa* (Table 5). Compound **19** (DC-756h) showed a good pharmacokinetic profile in rats and was finally selected for clinical evaluation.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus MP-500D and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on 400 MHz with a JEOL JNM-EX400 spectrometer. Chemical shifts are expressed in ppm (δ) with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Elemental analyses are indicated only by the symbols of the elements; analytical results were within ±0.4% of the theoretical values unless otherwise noted. Optical rotations were measured at 589 nm with a Horiba SEPA-300 polarimeter. All organic solvent extracts were dried over anhydrous sodium sulfate. Column chromatography was carried out with Merck Silica gel 60 (230—400 mesh). Thin layer chromatography (TLC) was performed on Merck Silica gel 60 F254 TLC plates.

4(*S*)-Fluoromethyl-1-[*N*-1(*R*)-phenylethyl]-2-pyrrolidone (8) (i)

Table 4. Pharmacokinetic Parameters of Selected Compounds after Oral Administration to Rats (20 mg/kg)[a)]

Compd. No.	$C_{\max}$ (μg/ml)	$AUC_{0-6\,\mathrm{h}}$ (μg·h/ml)	P'[b)]
19	3.1	6.9	39.6
20	1.8	2.2	9.6

a) See Experimental section. *b*) Apparent partition coefficient, $CHCl_3$/0.1 M phosphate buffer (pH 7.4).

Table 3. Single Intravenous Toxicity in Mice and Micronucleus Test of Selected Compounds[a)]

Compd. No.			**15**	**16**	**17**	**18**	**19**	**20**
		150	2/2	2/5	2/2	3/5	1/5	2/3
Mortality	Dose (mg/kg)	100	2/2	N.T.	1/5	N.T.	N.T.	1/5
		50	0/5	N.T.	N.T.	N.T.	N.T.	N.T.
Micronucleous Test	Dose (mg/kg)	100	N.T.	+	−	N.T.	−	−

a) See Experimental section. The mortality rate is expressed as number of animals dead/number of animals treated. The results of the micrinucleus test are expressed as symbols (+: positive, −: negative). N.T.: Not tested.

Table 5. *In Vitro* Antibacterial Activity of **19** (DC-756h) and Reference Quinolones (MIC: μg/ml)[a)]

Organism	**19** (DC-756h)	CPFX (**2**)	LVFX (**3**)	SPFX (**4**)	TFLX (**5**)
S. aureus FDA 209P	0.013	0.10	0.20	0.05	0.05
S. aureus 870307[b)]	0.39	>6.25	>6.25	3.13	0.78
S. aureus 891185[b)]	0.78	>6.25	>6.25	6.25	>6.25
S. epidermidis 56556	0.025	0.20	0.20	0.05	0.10
S. pyogenes G36	0.10	1.56	0.78	0.78	0.39
S. mitis IID685	0.05	0.78	0.39	0.20	0.10
S. pneumoniae J24	0.025	0.39	0.78	0.10	0.20
S. pneumoniae 57664	0.05	0.39	0.78	0.20	0.20
E. faecalis ATCC19433	0.10	0.78	0.78	0.39	0.39
E. coli NIHJ	≦0.003	≦0.003	0.013	≦0.003	0.006
E. coli KL16	0.006	0.013	0.05	0.013	0.013
K. pneumoniae Type 1	0.025	0.025	0.10	0.05	0.05
K. pneumoniae Type 2	0.013	0.025	0.05	0.025	0.013
S. marcescens 10104	0.025	0.05	0.05	0.05	0.10
P. aeruginosa PAO1	0.20	0.05	0.39	0.39	0.10
P. aeruginosa 32234	0.20	0.05	0.20	0.20	0.10

a) See Experimental section. *b*) MRSA strains.

Method A: To a solution of 4-(*S*)-hydroxymethyl-1-[*N*-1(*R*)-phenylethyl]-2-pyrrolidone **7** (5.0 g, 22.80 mmol) and Et_3N (6.36 ml, 45.63 mmol) in dichloromethane (CH_2Cl_2) (100 ml) was added dropwise MsCl (2.65 ml, 34.24 mmol) at 0 °C, and then the mixture was stirred at the same temperature for 30 min. The reaction mixture was washed with 10% aqueous citric acid and brine. The organic layer was dried and concentrated to give a mesylate as slightly yellow oil, which was dissolved in THF (100 ml). To this mesylate solution was added TBAF [1.0 M solution in THF, 114 ml], and the mixture was refluxed for 1.5 h. After cooling, the reaction mixture was poured into 10% aqueous citric acid and concentrated under reduced pressure. The residue was extracted with $CHCl_3$ and the organic layer was separated, dried and concentrated. The resulting residue was chromatographed on silica gel using *n*-hexane–ethyl acetate (AcOEt) (1 : 3) as an eluent to afford **8** (5.05 g, quantitatively) as slightly yellow oil. NMR ($CDCl_3$) δ: 1.52 (3H, d, J=7.33 Hz), 2.24—2.29 (1H, m), 2.52—2.63 (2H, m), 3.10 (1H, t, J=9.76 Hz), 3.20 (1H, dd, J=5.37, 9.76 Hz), 4.26—4.47 (2H, m), 5.50 (1H, q, J=7.32 Hz), 7.26—7.36 (5H, m). $[\alpha]_D$ +176.7° (c=0.958, $CHCl_3$).

(ii) Method B: To a solution of **7** (2.0 g, 9.12 mmol) in CH_2Cl_2 (50 ml) was added DAST (1.9 ml, 14.38 mmol) at −78 °C, and the mixture was stirred at the same temperature for 30 min and allowed to stand for 15 h at room temperature. The reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was chromatographed on silica gel using $CHCl_3$–methanol (MeOH) (97 : 3) as an eluent to afford **8** (1.11 g, 55%) as slightly yellow oil, which ^{1}H-NMR data agreed with that of **8** obtained by method A described above.

4(*S*)-Fluoromethyl-3(*R*)-hydroxy-1-[*N*-1(*R*)-phenylethyl]-2-pyrrolidone (9) A solution of diisopropylamine (1.65 ml, 11.75 mmol) in THF (20 ml) was cooled to −78 °C under a nitrogen atmosphere, treated dropwise with *n*-butyllithium [1.66 M solution in *n*-hexane, 7.08 ml, 11.75 mmol], and stirred for 5 min at 0 °C. To a solution of **8** (2.0 g, 9.04 mmol) in THF (20 ml) was added dropwise this LDA solution at −78 °C under a nitrogen atmosphere and the mixture was stirred for 15 min at the same temperature. After replacement of nitrogen to oxygen, the reaction mixture was stirred for 30 min at the same temperature under an oxygen atmosphere and quenched with 5% aqueous sodium thiosulfate. The solution was concentrated under reduced pressure and the resulting residue was extracted with AcOEt. The extract was washed with brine, dried and concentrated. The residue was chromatographed on silica gel using $CHCl_3$–methanol (MeOH) (97 : 3) as an eluent to afford **9** (1.57 g, 73%) as a colorless solid. mp 101—109 °C. NMR ($CDCl_3$) δ: 1.52 (3H, d, J=7.32 Hz), 2.31—2.48 (1H, m), 3.05—3.10 (1H, m), 3.16—3.21 (1H, m), 4.29 (1H, d, J=9.37 Hz), 4.53—4.67 (2H, m), 5.48 (1H, q, J=7.33 Hz), 7.26—7.37 (5H, m). $[\alpha]_D$ +280.2° (c=0.955, $CHCl_3$).

3(*S*)-Azide-4(*S*)-fluoromethyl-1-[*N*-1(*R*)-phenylethyl]-2-pyrrolidone (10) To a solution of **9** (2.61 g, 11.0 mmol) and Et_3N (3.07 ml, 22.02 mmol) in CH_2Cl_2 (40 ml) was added dropwise MsCl (1.28 ml, 16.54 mmol) at −10 °C, and then the mixture was stirred at the same temperature for 30 min. The reaction mixture was washed with 10% aqueous citric acid and brine. The organic layer was dried and concentrated to give mesylate as slightly yellow oil, which was dissolved in *N*,*N*-dimethylformamide (DMF) (80 ml). To this mesylate solution was added NaN_3 (2.86 g, 44.0 mmol), and the mixture was heated at 100 °C for 16 h. After cooling, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was separated ,washed with brine, dried and concentrated. The residue was chromatographed on silica gel using *n*-hexane–AcOEt (3 : 1) as an eluent to afford **10** (1.81 g, 63%) as slightly yellow oil. NMR ($CDCl_3$) δ: 1.56 (3H, d, J=7.32 Hz), 2.67—2.75 (1H, m), 3.02 (1H, dd, J=7.32, 10.25 Hz), 3.23 (1H, dd, J=4.39, 10.25 Hz), 4.27 (1H, d, J=8.30 Hz), 4.38 (1H, ddd, J=7.81, 9.28, 46.39 Hz), 4.59 (1H, ddd, J=5.86, 9.28, 46.37 Hz), 5.48 (1H, q, J=7.32 Hz), 7.26—7.37 (5H, m). $[\alpha]_D$ +32.3° (c=0.896, $CHCl_3$).

3(*S*)-*tert*-Butoxycarbonylamino-4(*S*)-fluoromethyl-1-[*N*-1(*R*)-phenylethyl]-2-pyrrolidone (11) A mixture of **10** (1.81 g, 6.90 mmol), 50% aqueous–10% palladium on carbon (1.80 g) and Boc_2O (3.01 g, 13.79 mmol) in EtOH (100 ml) was shaken for 15 h under a hydrogen atmosphere (1.0 atm.) at room temperature. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel using *n*-hexane–AcOEt (2 : 1) as an eluent to afford **11** (1.68 g, 72%) as a colorless solid. mp 128—130 °C. NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.55 (3H, d, J=7.10 Hz), 2.80—2.96 (1H, m), 3.06 (1H, dd, J=6.86, 10.53 Hz), 3.31 (1H, d, J=10.53 Hz), 4.30—4.56 (3H, m), 5.10 (1H, br s), 5.49 (1H, q, J=7.10 Hz), 7.25—7.37 (5H, m). $[\alpha]_D$ +158.2° (c=1.052, $CHCl_3$).

3(*S*)-*tert*-Butoxycarbonylamino-4(*S*)-fluoromethyl-1-[*N*-1(*R*)-phenylethyl]pyrrolidine (12) To a solution of **11** (1.68 g, 4.99 mmol) in THF (60 ml) was added dropwise BH_3–THF complex [1 M solution in THF, 19.98 ml] at 0 °C, and then the mixture was stirred at room temperature for 18 h. After evaporation of THF, the residue was dissolved in 80% aqueous EtOH (40 ml) and refluxed in the presence of Et_3N (8.0 ml) for 2 h and then cooled. The solution was concentrated and dissolved in $CHCl_3$, which was washed with brine, dried and then evaporated to dryness. The residue was chromatographed on silica gel using *n*-hexane–AcOEt (3 : 1) as an eluent to afford **12** (1.54 g, 96%) as a colorless solid. mp 108—109 °C. NMR ($CDCl_3$) δ: 1.35 (3H, d, J=6.59 Hz), 1.43 (9H, s), 2.35—2.82 (5H, m), 3.24 (1H, q, J=6.59 Hz), 4.30—4.59 (3H, m), 4.85 (1H, d, J=9.03 Hz), 7.20—7.35 (5H, m). $[\alpha]_D$ +37.1° (c=1.030, $CHCl_3$).

3(*S*)-*tert*-Butoxycarbonylamino-4(*S*)-fluoromethylpyrrolidine (13) A mixture of **12** (484 mg, 1.50 mmol) and 50% aqueous–10% palladium on carbon (500 mg) in EtOH (50 ml) was shaken under a hydrogen atmosphere (1.0 atm.) at 50 °C for 15 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a crude **13** (328 mg, quantitatively) as an amorphous foam, which was used in the next reaction without further purification. NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.69 (1H, br s), 2.45—2.53 (1H, m), 2.66 (1H, dd, J=5.37, 10.74 Hz), 2.90—2.95 (1H, m), 3.18 (2H, dd, J=7.81, 10.74 Hz), 4.18—4.27 (1H, m), 4.44—4.53 (1H, m), 4.56—4.65 (1H, m). $[\alpha]_D$ +28.6° (c=1.035, $CHCl_3$).

7-[3(*S*)-Amino-4(*S*)-fluoromethyl-1-pyrrolidinyl]-6-fluoro-1-cyclopropyl-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (19) A solution of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid BF_2-chelete (**14a**, 8.31 g, 24.2 mmol), **13** (6.35 g, 29.3 mmol) and Et_3N (6.50 ml, 46.7 mmol) in DMSO (60 ml) was stirred at room temperature for 17 h. After removal of Et_3N under reduced pressure, water was added to the residue with ice-water cooling, and then the mixture was stirred at room temperature for 1 h. The precipitate was collected by filtration and washed with water, then was dissolved in 80% aqueous EtOH (500 ml). Et_3N (10 ml) was added to the solution and the mixture was refluxed for 4 h. After concentration, the residue was dissolved in $CHCl_3$, which was washed with 10% aqueous citric acid and brine, dried and then evaporated to dryness. The residue was dissolved in concentrated HCl (30 ml) with ice-water cooling and stirred for 5 min at room temperature. The mixture was adjusted to pH 11 with 20% aqueous NaOH with ice-water cooling, and then was neutralized with 10% aqueous HCl to pH 7.4. The precipitate was collected by filtration to give a crude **19**, which was recrystallized from EtOH–28% NH_4OH to give **19** (8.17 g, 86%) as slightly yellow solid. mp 247—255 °C (dec.). NMR (0.1 N NaOD) δ: 0.90—1.12 (4H, m), 2.73—2.78 (1H, m), 3.41—3.44 (1H, m), 3.58 (3H, s), 3.64—3.73 (3H, m), 3.90—3.96 (1H, m), 4.03—4.09 (1H, m), 4.66—4.82 (1H, m), 7.56 (1H, d, J=14.65 Hz), 8.49 (1H, s). *Anal.* Calcd for $C_{19}H_{21}F_2N_3O_4 \cdot 1/2HCl$: C, 55.44; H, 5.26; N, 10.21. Found: C, 55.59; H, 5.31; N, 10.18. $[\alpha]_D$ −92.0° (c=0.275, 0.1 N NaOH).

An analogous procedure was used to obtain compounds **15**—**18** and **20** using corresponding 3-aminopyrrolidine derivatives prepared according to the literatures.[21]

7-[3(*S*)-Amino-1-pyrrolidinyl]-6-fluoro-1-[2(*S*)-fluoro-1(*R*)-cyclopropyl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (15) **15** was prepared in 87% from **14b** as slightly yellow solid. mp 213—216 °C. NMR (0.1 N NaOD) δ: 1.46—1.71 (3H, m), 1.72—1.79 (1H, m), 2.16—2.22 (1H, m), 3.34—3.38 (1H, m), 3.54—3.60 (1H, m), 3.56 (3H, s), 3.61—3.73 (3H, m), 4.02—4.08 (1H, m), 4.83—4.90 (0.5H, m), 5.02—5.10 (0.5H, m), 7.66 (1H, d, J=14.64 Hz), 8.47 (1H, s). *Anal.* Calcd for $C_{18}H_{19}F_2N_3O_4$: C, 56.99; H, 5.05; N, 11.08. Found: C, 56.73; H, 5.00; N, 11.08. $[\alpha]_D$ +92.4° (c=0.552, 0.1 N NaOH).

7-[3(*S*)-Amino-4(*S*)-methyl-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (16) **16** was prepared in 61% from **14a** as colorless solid. mp 210—212 °C. NMR (0.1 N NaOD) δ: 0.79—1.14 (4H, m), 1.02 (3H, d, J=6.84 Hz), 2.30—2.37 (1H, m), 3.31—3.35 (1H, m), 3.41—3.48 (2H, m), 3.50 (3H, s), 3.65 (1H, ddd, J=1.96, 7.32, 10.25 Hz), 3.85 (1H, ddd, J=2.44, 5.37, 10.35 Hz), 3.98—4.04 (1H, m), 7.58 (1H, d, J=14.65 Hz), 8.42 (1H, s). *Anal.* Calcd for $C_{19}H_{22}FN_3O_4 \cdot 1/4H_2O$: C, 60.07; H, 5.97; N, 11.06. Found: C, 60.10; H, 5.89; N, 11.01. $[\alpha]_D$ −111.4° (c=0.972, 0.1 N NaOH).

7-[3(*S*)-Amino-4(*S*)-methyl-1-pyrrolidinyl]-6-fluoro-1-[2(*S*)-fluoro-1(*R*)-cyclopropyl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (17) **17** was prepared in 69% from **14b** as slightly yellow solid. mp 221—226 °C (dec.). NMR (0.1 N NaOD) δ: 1.09 (3H, d, J=7.00 Hz), 1.48 (1H, dddd, J=3.00, 6.00, 9.50, 27.00 Hz), 1.60 (1H, ddt, J=6.00, 9.00, 17.50 Hz), 2.36—2.42 (1H, m), 3.36—3.53 (3H, m), 3.57 (3H, s), 3.73—3.78 (1H, m), 3.81—3.85 (1H, m), 4.04 (1H, dt, J=5.50, 9.00 Hz), 4.91—5.11 (1H, m), 7.67 (1H, d, J=14.50 Hz), 8.44 (1H, s). *Anal.* Calcd for $C_{19}H_{21}F_2N_3O_4$: C, 57.96; H, 5.38; N, 10.68. Found: C, 57.66; H, 5.36; N, 10.56. $[\alpha]_D$ $-28.6°$ (c=0.620, 0.1 N NaOH).

7-[3(*S*)-Amino-4(*R*)-methyl-1-pyrrolidinyl]-6-fluoro-1-[2(*S*)-fluoro-1(*R*)-cyclopropyl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (18) **18** was prepared in 86% from **14b** as colorless solid. mp 269—271 °C (dec.). NMR (0.1 N NaOD) δ: 1.01 (3H, d, J=6.60 Hz), 1.46—1.64 (2H, m), 1.80—1.88 (1H, m), 2.89 (1H, dd, J=8.06, 15.38 Hz), 3.39—3.44 (2H, m), 3.46 (3H, s), 3.46—3.62 (2H, m), 3.94—3.99 (1H, m), 4.89—4.97 (0.5H, m), 7.57 (1H, d, J=14.40 Hz), 8.43 (1H, s). *Anal.* Calcd for $C_{19}H_{21}F_2N_3O_4 \cdot 1H_2O$: C, 55.47; H, 5.64; N, 10.21. Found: C, 55.31; H, 5.88; N, 10.01. $[\alpha]_D$ $-28.0°$ (c=0.620, 0.1 N NaOH).

7-[3(*S*)-Amino-4(*S*)-fluoromethyl-1-pyrrolidinyl]-6-fluoro-1-[2(*S*)-fluoro-1(*R*)-cyclopropyl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (20) **20** was prepared in 70% from **14b** as colorless solid. mp 232—238 °C. NMR (0.1 N NaOD) δ: 1.41—1.62 (2H, m), 2.62—2.79 (1H, m), 3.44—3.47 (1H, m), 3.57 (3H, s), 3.60—3.65 (1H, m), 3.70—3.73 (1H, m), 3.77—3.88 (2H, m), 4.00—4.05 (1H, m), 4.55—5.08 (3H, m), 7.66 (1H, d, J=14.16 Hz), 8.42 (1H, s). *Anal.* Calcd for $C_{19}H_{20}F_3N_3O_4 \cdot 1/4H_2O$: C, 54.87; H, 4.97; N, 10.10. Found: C, 54.78; H, 4.83; N, 10.00. $[\alpha]_D$ $-13.2°$ (c=0.174, 0.1 N NaOH).

***In Vitro* Antibacterial Activity** The MICs of the compounds tested in this study were measured according to the two-fold micro broth dilution method using Mueller–Hinton broth (Difco Laboratories, Detroit, MI) with an inoculum size of approximately 10^5 cfu/ml. The MIC was defined as the lowest concentration which prevented visible bacterial growth after incubation at 37 °C for 18 h.

Determination of Apparent Partition Coefficients and Aqueous Solubility The apparent partition coefficients and aqueous solubility of the compounds tested in this study were measured according to the method reported previously.[12,16a)]

Pharmacokinetic Studies The test compounds were administered in solution by oral gavage to groups of four animals each. Blood samples were obtained 0.25, 0.5, 1, 2, 4 and 6 h after dosing. Serum levels of the test compounds were determined by a microbiological assay. *Bacillus subtilis* ATCC 6051 was used as the test organism.

Single Intravenous Toxicity Test The test compounds were dissolved in 0.1 N NaOH in saline at different concentrations. The solution was administered intravenously to five week-old male ddY mice at a speed of 0.2 ml/min. The total volume of administration was adjusted to 10 ml/kg of body weight. The number of dead mice was counted 7 d after administration.

Micronucleus Test Six week-old male ddy mice were used in this test. The test compounds were dissolved in 0.1 N NaOH–saline and the solution was administered intravenously (100 mg/kg) in each group of five mice. Mice were sacrificed at 24 h after the treatment, and the smears of bone marrow cells were prepared and stained with acridine orange. For each animal, the numbers of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes (PCEs) and of PCEs per 1000 erythrocytes were counted under a fluorescent microscope. Statistical analysis was performed by the Kastenbaum and Bowman method.[22)]

X-Ray Crystallographic Analysis of 12 **12** was crystallized from MeOH–H_2O to give a colorless plate of $C_{18}H_{27}FN_2O_2$ having approximate dimensions of 0.20×0.25×0.05 mm. The lattice parameters and intensities were measured on a Rigaku AFC7R diffractometer with graphite monochromated Cu$K\alpha$ radiation using ω–2θ scan technique. The compound was crystallized in monoclinic space group $P2_1$ with cell dimensions a=5.306(1) Å, b=12.091(2) Å, c=14.269(1) Å, β=92.74(1) Å, and V=914.4(2) $Å^3$. For Z=2 and F.W.=322.42, the calculated density was 1.171 g/cm^3. The structure was solved by the direct method with the program *sir92*.[23)] The final R value was 0.059. A perspective view of the molecule is shown in Fig. 2.

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