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Design, Synthesis, and Biological Evaluations of Novel 7-[7-Amino-7-methyl-5-azaspiro[2.4]heptan-5-yl]-8-methoxyquinolines with Potent Antibacterial Activity against Respiratory Pathogens[1]

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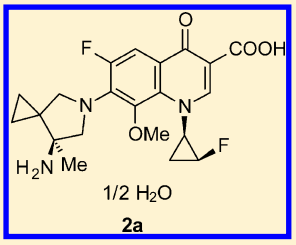
Supporting Information

ABSTRACT: Novel 7-[7-amino-7-methyl-5-azaspiro[2.4]heptan-5-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]- 8-methoxy-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **2a** and **2b** were designed and synthesized to obtain potent antibacterial drugs for the treatment of respiratory tract infections. Among these, compound **2a** possessing (*S*)-configuration for the asymmetrical carbon on the pyrolidine moiety at the C-7 position of the quinolone scaffold exhibited potent in vitro antibacterial activity against respiratory pathogens including Gram-positive (*Streptococcus pneumoniae* and *Staphylococcus aureus*), Gram-negative (*Haemophilus influenzae* and *Moraxcella catarrhalis*), and atypical strains (*Chalmydia pneumoniae* and *Mycoplasma pneumoniae*), as well as multidrug-resistant *Streptococcus pneumoniae* and quinolone-resistant and methicillin-resistant *Staphylococcus aureus*). Furthermore, compound **2a** showed excellent in vivo activity against the experimental murine pneumonia model due to multidrug resistant *Streptococcus pneumoniae* (MDRSP) and favorable profiles in preliminary toxicological and nonclinical pharmacokinetic studies.



INTRODUCTION

As community-acquired pathogens are exhibiting increasing levels of resistance to β-lactams and macrolides, newer quinolones have emerged as one of the first-line antibacterial drugs for respiratory tract infections in the medical community. Meanwhile, multidrug-resistant *Streptococcus pneumoniae* (MDRSP) and community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are emerging as key pathogens in community-acquired respiratory infections.[2,3] Fluoroquinolones such as levofloxacin (LVFX), gatifloxacin (GFLX), and moxifloxacin (MFLX) are beneficial for the empirical treatment of respiratory infections in the community because of their extended antibacterial spectra, including atypical pathogens, with preferable pharmacokinetic (PK) and safety profiles;[4] however, the antibacterial activity of these newer quinolones are not potent enough, and bacteria resistance such as quinolone-resistant *S. pneumoniae* and CA-MRSA to these drugs will be problematic in the near future.[5] On the other hand, clinical adverse events (e.g., torsades de pointes, or fatal liver injury) by some quinolones have been reported.[6,7] Thus, the new respiratory quinolone providing both improved activity against respiratory pathogens including MDRSP and a good safety profile is keenly required.

As previously reported,[8] compound **1** showed potent activity against respiratory pathogens including penicillin-resistant *S. pneumoniae* (PRSP), together with a superior safety profile similar to levofloxacin (LVFX). However, the results of the in vitro metabolic study revealed that compound **1** exerted mechanism-based inhibition (MBI) of cytochrome (CYP) 3A4. CYP3A4 is the most abundant CYP isozyme that accepts a great number of medicines as substrates. Although beneficial combination therapy utilizing CYP3A4 inhibition has been reported, clinical drug−drug interaction (DDI) due to CYP3A4 inhibition often resulted in serious adverse events.[9,10] Therefore, we judged compound **1** to be inappropriate as a new candidate for further development.

To solve the MBI problem, we focused on the chemical structure at the C-7 pyrrolidine substituent of compound **1**. According to the report by Silverman,[11] the chemical structure like the cyclopropylmethylamine unit may have a risk to inactivate the enzyme monoamine oxidase (MAO) and the inactivation begins with the formation of iminium intermediate. That is to say, the conversion to the structure that cannot form an iminium intermediate may prevent the exertion of MBI. On the other hand, trovafloxacin is reported to show numerous cases of hepatotoxicity, which limits its clinical usefulness. It possesses two substructural elements that have the potential to generate reactive intermediates: a cyclopropylamine moiety and a difluoroanilino system. Evidence has been reported that trovafloxacin-induced hepatotoxicity may be mediated through oxidation of the cyclopropylamine substructure to reactive intermediates that may form covalent adducts to hepatic proteins, resulting in damage to liver tissue.[12] Therefore, we planned to introduce a methyl group onto the carbon atom at the root of the C7-amino moiety of compound **1**, thereby aiming to avoid the formation of the reactive intermediate.

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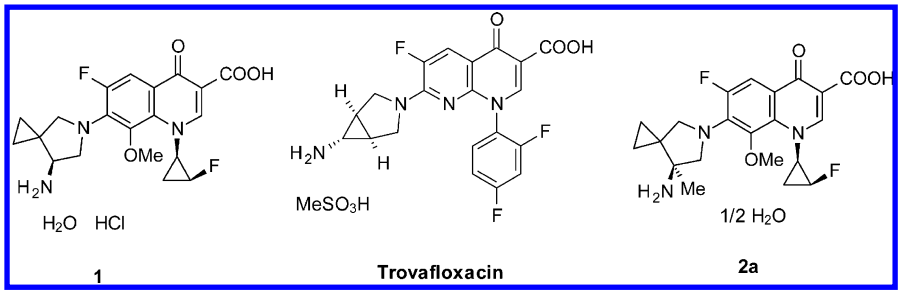
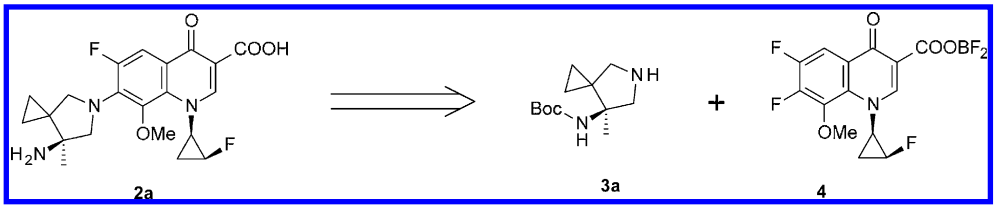
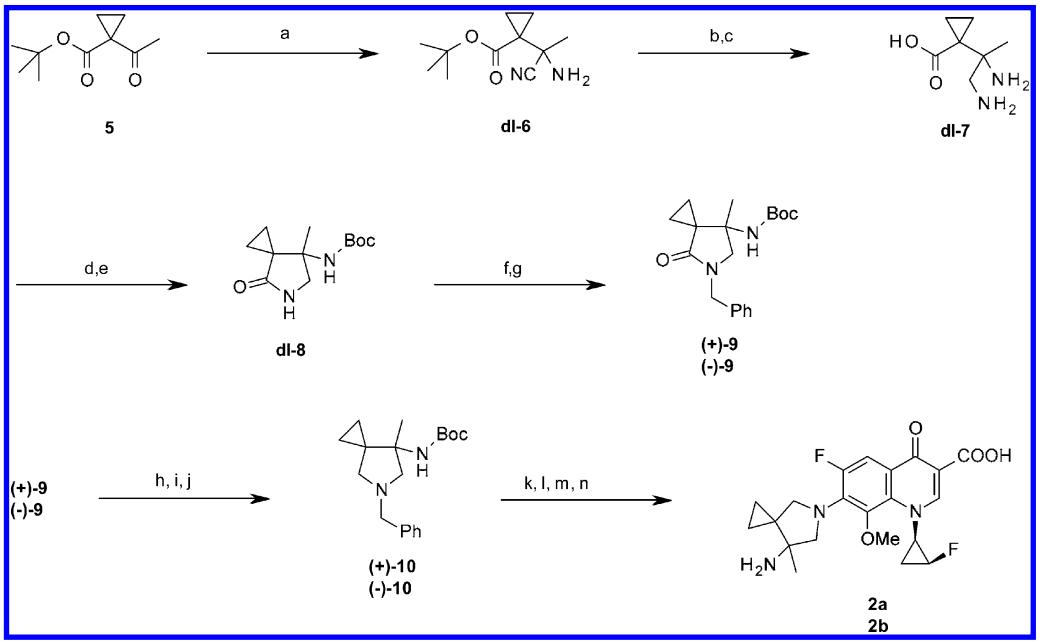


Figure 1.

Scheme 1



Scheme 2[a]



[a]Reagents: (a) NaCN, NH_3 aq, NH_4Cl, NH_3/MeOH; (b) H_2, Raney Ni/EtOH; (c) concentrated aqueous HCl; (d) 1,1,1,3,3,3-hexamethyldisilazane/MeCN; (e) Boc_2O/1.4-dioxane; (f) BnBr, NaH/DMF; (g) Chiralpak AD; (h) TFA/CH_2Cl_2; (i) $LiAlH_4$/THF; (j) Boc_2O/CH_2Cl_2; (k) H_2, Pd−C/MeOH; (l) **4**, Et_3N/DMSO; (m) Et_3N/80% aqueous EtOH; (n) concentrated aqueous HCl.

In this article, we describe the details of the synthesis, in vitro and in vivo antibacterial activities, safety profile, and pharmacokinetic profile of the designed compound **2a** (Figure 1).

CHEMISTRY

As shown in Scheme 1, we envisioned synthesizing the desired 7-substituted 8-methoxyquinolone derivative **2a** by aromatic nucleophilic substitution reaction from amine **3a** and 6,7-difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid BF_2 chelate (**4**).[13]

The synthesis of **2** is illustrated in Scheme 2. Amine DL-**6** was prepared by the Strecker reaction of **5**.[14] Hydrogenation with Raney Ni and the removal of the *tert*-butyl group under an acidic condition gave diaminocarboxylic acid DL-**7**. Whne DL-**7** was heated with 1,1,1,3,3,3-hexamethyldisilazane in acetonitrile (MeCN), the desired cyclization was accomplished. The remaining amino moiety was protected by di-*tert*-butyl dicarbonate (Boc_2O) to provide lactam DL-**8**. Treatment of DL-**8** with sodium hydride (NaH) in *N*,*N*-dimethylformamide (DMF) followed by a reaction with benzyl bromide (BnBr) provided benzylamide DL-**9**. This racemic **9** was separated into the two enantiomers (+)-**9** and (−)-**9** by column chromatography with Chiralpak AD. After the temporary removal of *tert*-butoxycarbonyl groups of (+)-**9** and (−)-**9**, the amide moieties were reduced by lithium aluminum hydride ($LiAlH_4$). Then the primary amino groups were protected again by Boc_2O to give benzylamines (+)-**10** and (−)-**10**. The benzyl groups were

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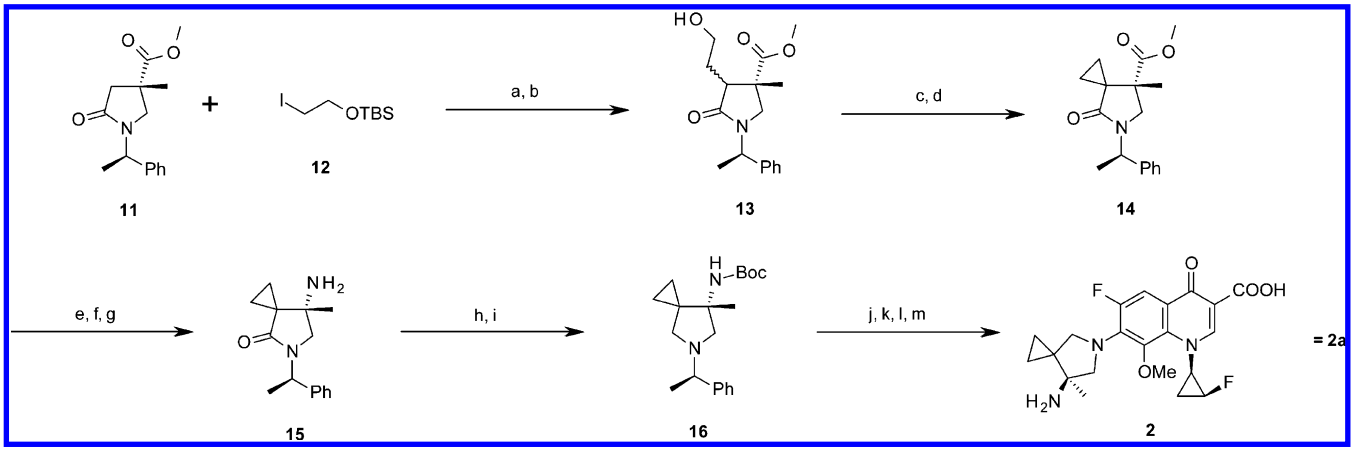
Scheme 3[a]



[a]Reagents: (a) LiHMDS/THF; (b) TBAF/THF; (c) $PhSO_2Cl$, Et_3N, DMAP/CH_2Cl_2; (d) NaHMDS/THF; (e) TFA/CH_2Cl_2; (f) diphenylphosphoryl azide, Et_3N/toluene; (g) 6 N aqueous HCl/1,4-dioxane; (h) sodium bis(2-methoxyethoxy)aluminum hydride/toluene; (i) Boc_2O/toluene; (j) H_2, Pd–C/MeOH; (k) **4**, Et_3N/DMSO; (l) Et_3N/80% aqueous EtOH; (m) concentrated aqueous HCl.

removed by catalytic hydrogenolysis. The resultant crudes and quinolonecarboxylic acid BF_2 chelate **4** were heated with triethylamine in dimethyl sulfoxide (DMSO), followed by dechelation and deprotection to give compounds **2a** and **2b**.

The absolute configurations of **2a** and **2b** were determined by an alternative synthesis (Scheme 3). Alkylation of compound **11**,[15] which was synthesized from (*R*)-phenylethylamine, with use of lithium hexamethyldisilazide (LHMDS), followed by the treatment with tetrabutylammonium fluoride (TBAF) provided alcohol **13** as a diastereomixture. After the conversion of the primary alcohol of **13** to the benzenesulfonyloxy group, the cyclization was performed by treatment with sodium hexamethyldisilazide (NaHMDS). Compound **14** was obtained as prisms, and the absolute configuration was determined to be 7*S* by X-ray crystallographic analysis (Figure 2). Phenylethylamine **16** was prepared by multistep reactions (removal of *tert*-butyl ester, Curtius rearrangement, acidic hydrolysis, reduction with sodium bis(2-methoxyethoxy)-aluminum hydride, and formation of *tert*-butyl carbamate). After the phenylethyl group of **16** was removed by catalytic hydrogenolysis, the resultant secondary amine was reacted with BF_2 chelate **4** followed by the above-mentioned method to give the target compound **2**. The analytical data of **2** corresponded with those of **2a**.

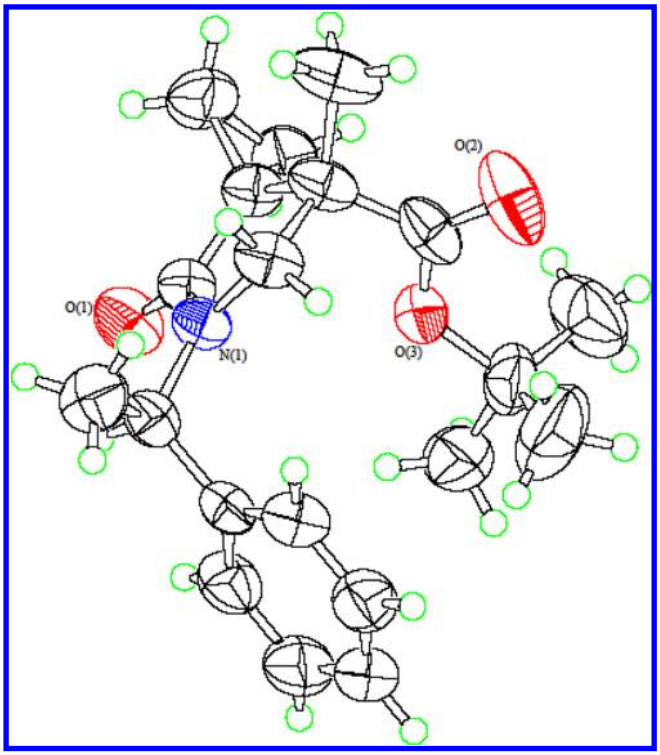


Figure 2.

RESULTS AND DISCUSSION

The minimum inhibitory concentrations (MICs) of **2a** and **2b** against several representative Gram-positive and Gram-negative bacteria are summarized in Table 1, along with the data for levofloxacin, moxifloxacin, ciprofloxacin, and the previously reported compound **1** for comparison. The synthesized compounds **2a** and **2b** exhibited a broad antibacterial spectrum against Gram-positive and Gram-negative bacteria. A novel quinolone **2a**, which has the (7*S*)-amino group of the C-7 substituent, exhibited about 4 to >16 times better activity against Gram-positive bacteria compared with the activity of LVFX, although activities of **2a** against some bacteria were slightly inferior to those of **1**. Particularly, **2a** was more potent than an existing respiratory quinolone, moxifloxacin, against MDRSP and levofloxacin-resistant MRSA strains. Against Gram-negative bacteria except *Pseudomonas aeruginosa*, the activity of **2a** was comparable to those of other quinolones tested. For example, **2a** showed potent activities against *Haemophilus influenzae* and *Moraxcella catarrhalis*.

Next, the activity of **2a** and representative respiratory quinolones against atypical organisms are listed in Table 2. A newer quinolone **2a** exhibited potent antibacterial activity against *Mycoplasma pneumoniae* and *Chlamydophilia pneumoniae* strains. The activity of **2a** was comparable with those of moxifloxacin and gemifloxacin and was 4–10 times higher than that of levofloxacin.

Finally, the MICs against clinically isolated levofloxacin-intermediate and -resistant *Streptococcus pneumoniae* of compound **2a** and reference antibacterial agents are shown in Table 3. Compound **2a** showed more potent activity than the other quinolone antibacterial agents (levofloxacin, ciproflox-

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Table 1. Antibacterial Activities MIC (μg/mL) of Compounds 2a and 2b and Reference Quinolones against Gram-Positive and Gram-Negative Bacteria[a]

	compd					
organism	**2a**	**2b**	LVFX	CPFX	MFLX	**1**
S. aureus FDA 209-P	0.025	0.05	0.1	0.1	0.05	0.012
S. aureus 870307[b]	0.39	1.56	>6.25	>6.25	0.78	0.39
S. epidermidis 56500	0.1	0.2	0.39	0.2	0.1	0.05
S. pneumoniae J24[c]	0.05	0.39	0.78	0.78	0.1	0.05
S. pneumoniae 104835[d]	0.39		>6.25	>6.25	3.13	0.20
S. pyogenes G-36	0.2	0.78	0.78	1.56	0.2	0.05
E. faecalis ATCC 19433	0.2	0.78	0.78	0.78	0.2	0.1
B. subtilis IID 685	0.1	0.39	1.56	3.12	0.2	0.05
E. coli NIHJ	0.012	0.025	0.012	<0.003	0.012	0.006
K. pneumoniae TYPE 1	0.05	0.1	0.05	0.025	0.1	0.05
H. influenzae ATCC49247	0.012	0.006	0.012	0.006	0.012	<0.003
M(B). catarrhalis ATCC25238	0.05	0.05	0.025	0.025	0.05	0.012
P. aeruginosa PAO-1	0.78	0.78	0.39	0.05	0.78	0.2

[a]Antibacterial activities were determined using a standard microbroth dilution method. Abbreviations are as follows: LVFX, levofloxacin; CPFX, ciprofloxacin; MFLX, moxifloxacin. [b]Levofloxacin-resistant and methicillin-resistant *S. aureus* (levofloxacin-r-MRSA). [c]Penicillin-susceptible *S. pneumoniae* (PSSP). [d]Multidrug-resistant *S. pneumoniae* (MDRSP, quinolone-resistant and penicillin-resistant strains).

Table 2. Antibacterial Activities MIC (μg/mL) of 2a and Reference Quinolones against Atypical Organisms *Mycoplasma pneumoniae* and *Chlamydophilia pneumoniae*[a]

	compd				
organism	**2a**	LVFX	MFLX	GRNX	GMFX
M. pneumoniae 18-1	0.12	2	0.25	0.12	0.25
M. pneumoniae ATCC 29085	0.12	1	0.12	0.06	0.25
C. pneumoniae AR-39	0.125	0.5	0.125	0.063	0.125
C. pneumoniae CM-1	0.063	0.5	0.125	0.016	0.063

[a]Antibacterial activities of *M. pneumoniae* and *C. pneumoniae* strains were determined using a standard microbroth dilution method and standard recommended techniques, respectively. Abbreviations are as follows: LVFX, levofloxacin; MFLX, moxifloxacin; GRNX, garenoxacin; GMFX, gemifloxacin.

Table 3. Antibacterial Activities MIC (μg/mL) of 2a and Reference Drugs against Clinical Isolates of Levofloxacin-Intermediate and -Resistant *Streptococcus pneumoniae*[a]

	MIC (μg/mL)[b]			
compd	range	MIC_{50}	MIC_{80}	MIC_{90}
Organism[c] (No. of Strains): LVFX-Intermediate and -Resistant *S. pneumoniae* (14)				
2a	0.25−2	1	1	1
LVFX	4−32	8	16	16
CPFX	8−32	16	32	32
MFLX	0.25−4	2	4	4
GRNX	0.06−1	0.25	0.5	1
AZM	0.12 to >32	>32	>32	>32
CAM	0.06 to >32	>32	>32	>32
AMPC	0.03−4	0.06	2	2
PCG	0.03−8	0.06	2	2

[a]Antibacterial Aactivities were determined using a standard microbroth dilution method. Abbreviations are as follows: LVFX, levofloxacin; CPFX, ciprofloxacin; MFLX, moxifloxacin; GRNX, garenoxacin; AZM, azythromycin; CAM, clarithromycin; AMPC, amoxicillin; PCG, benzylpenicillin. [b]MIC_{50} = MIC at which 50% of isolates are inhibited. MIC_{80} = MIC at which 80% of isolates are inhibited. MIC_{90} = MIC at which 90% of isolates are inhibited. [c]The strains were collected by the Levofloxacin Surveillance Group from patients in Japan in 2004.

acin, or moxifloxacin) and the other categories of drugs (azythromycin, clarithromycin, or amoxicillin) that were used generally in the clinic as antibacterials for the treatment of respiratory tract infections. The MIC_{90} of **2a** was 1 μg/mL, equal to that of garenoxacin. Thus, **2a** exhibited potent and well balanced antibacterial activity against representative respiratory pathogens, including atypical strains and MDRSP.

The potential inhibitory effects of compounds **1** and **2a** on a mechanism-based inhibition (MBI) of CYP3A4 were investigated using the general procedure described in the Experimental Section. The results are illustrated in Figures 3 and 4. Compound **1** inhibited the activity of CYP3A4 for 1′-hydroxylation of midazolam dose-dependently; however, **2a** did not show an inhibitory effect on the MBI of CYP3A4 over the range of concentrations tested. According to these experimental findings, introduction of the methyl group into the geminal position of the 7-amino group on the cyclopropylmethylamine system is effective to reduce the MBI potential. Meanwhile, compound **2a** did not exhibit any inhibitory effects on the enzymatic activities of other human CYP isoforms over the range of concentrations tested.[16]

Table 4 shows effects on human ether-a-go-go-related gene (hERG) potassium current in hERG transfected CHO-K1 cells. Compounds that inhibit hERG potassium channels have been shown to prolong the QT interval of electrocardiogram in man and increase the risk of fatal cardiac arrhythmia.[17] Compound **2a** at a dose 30 and 100 μM had no effect on hERG currents. Comparative studies using other quinolones indicated a potential of **2a** for hERG current that is lower than those of moxifloxacin and compound **1**. The grade of compound **2a** was

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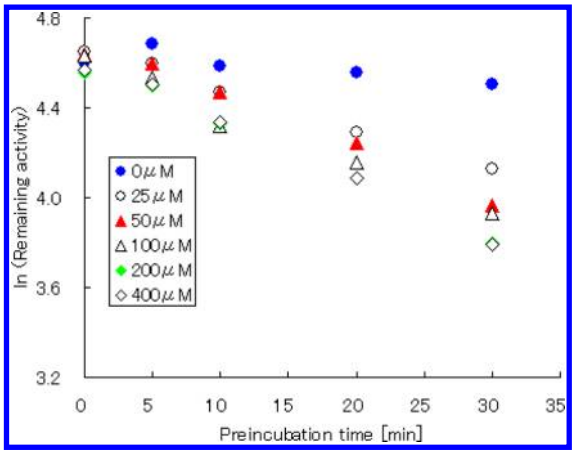

Figure 3. Mechanism-based inhibition against CYP3A4 by **1**. Shown are effects of preincubation of CYP3A4 with **1** for 1′-hydroxylation of midazolam in pooled human liver microsomal system.

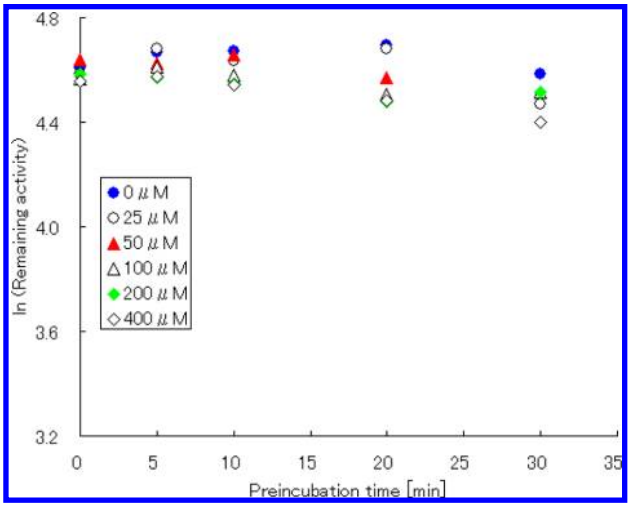

Figure 4. Mechanism-based inhibition against CYP3A4 by **2a**. Shown are effects of preincubation of CYP3A4 with **2a** for 1′-hydroxylation of midazolam in pooled human liver microsomal system.

Table 4. Effects on hERG Potassium Current in hERG Transfected CHO-K1 Cells[a]

	% inhibition	
compd	30 μM	100 μM
2a	1.9	2.7
LVFX	−0.9	4.2
MFLX	21.5	42.3
1	5.2	14.1

[a]Abbreviations are as follows: LVFX, levofloxacin; MFLX, moxifloxacin.

approximately equal to that of levofloxacin, which is known not to cause QT prolongation syndrome at clinical dosage.[18]

The apparent partition coefficients (P'),[19] the solubility to water,[20] and in vitro plasma protein binding ratios in human of **2a**, **1** (as free base substance), and levofloxacin are listed in Table 5. Introduction of a methyl group into the geminal position of the 7-amino group on the C7-substituent significantly increased the P' of parent compound **1**. The P' of **2a** was 2 times higher than that of **1**, while the solubility in water of **2a** was slightly improved. On the other hand, the

Table 5. Physicochemical Properties and in Vitro Protein Binding Ratios of 1, 2a, LVFX, and MFLX

	2a	**1**[a]	LVFX[b]	MFLX[b]
P'[c]	19.2	8.8	5.1	53.8
solubility (μg/mL)[d]	620	446	>11900	777
protein binding ratio (%)[e]	34.7	41.8	32.2[f]	47.8

[a]The physicochemical parameters (apparent partition coefficient and aqueous solubility) of **1** and moxifloxacin were assessed by using a free base quinolone compound. [b]Abbreviation are as follows: LVFX, levofloxacin; MFLX, moxifloxacin. [c]Apparent partition coefficient, $CHCl_3$/0.1 M phosphate buffer (pH 7.4). [d]Aqueous solubility. [e]In vitro human plasma protein binding ratios of the quinolones were measured by an ultrafiltration method at 5 μg/mL compounds. The data were expressed as the mean values ± SD of three experiments. [f]The value was examined at 10 μg/mL.

protein binding ratio of **2a** was almost the same as the value of levofloxacin.

The pharmacokinetic profiles of **2a**, **1**, levofloxacin, and moxifloxacin after single oral administration to rats are displayed in Table 6. Compound **2a** exhibited higher peak

Table 6. Pharmacokinetic Parameters of 2a and Reference Quinolones in Rats after an Oral Dose of 5 mg/kg (n = 3)[a]

PK parameter/route: po		**2a**	**1**	LVFX	MFLX[d]
serum	C_{max} (μg/mL)	1.22	0.816	1.47	1.49
	AUC_{0-8h} (μg·h/mL)	3.08	1.59	3.41	4.46
lung	AUC_{0-8h} (μg·h/mL)	13.7	6.77	5.57	15.2
	K_p[b]	4.4	4.3	1.6	3.4
liver	AUC_{0-8h} (μg·h/mL)	32.1	15.0	16.4	24.5
	K_p[b]	10.4	9.4	4.8	5.5
kidney	AUC_{0-8h} (μg·h/mL)	27.8	19.3	19.3	30.3
	K_p[b]	9.0	12.2	5.6	6.8
urinary recovery$_{0-24h}$ (%)[c]		32.0	23.1	80.0	25.7

[a]Animal: 7-week-old male Crj:CD rats. [b]K_p values are tissue/serum concentration ratio. [c]The values were calculated by using the concentrations of unchanged quinolones. [d]Moxifloxacin hydrochloride hydrate was administered.

plasma concentration (C_{max}) and area under the time–concentration curve (AUC) than those of **1**. Compound **2a** also exhibited a favorable profile of distribution. It was distributed to the lung, a major target organ, very effectively; $AUC_{(lung)}/AUC_{(serum)}$ ratio (K_p) of compound **2a** was higher than the value of existing respiratory quinolone, moxifloxacin.

The pharmacokinetic profiles of compound **2a** and reference quinolones after oral or intravenous administration to monkeys are summarized in Table 7. After single oral administration, the C_{max} and AUC values of **2a** were higher than those of the other quinolones tested. Similarly, **2a** administered intravenously further showed a high AUC value. The bioavailability of compound **2a** was about 70% in monkeys. Compound **2a** was thus expected to exhibit a high plasma concentration and a good distribution to lung in humans, thereby furnishing superior efficacy for the respiratory infections. Moreover, above 60% of an intact **2a** was excreted in the urine. Taken together with the profile of pharmacokinetics and the antibacterial activity, compound **2a** will be expected to show excellent efficacy against urinary tract infections.

The results of the intravenous single-dose toxicity study and the micronucleus test are summarized in Table 8. An approximate lethal dose in mice was greater than 100 mg/kg.

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Table 7. Pharmacokinetic Parameters of 2a and Reference Quinolones in Monkeys after a Dose of 5 mg/kg (n = 3)[a]

PK parameter	2a	1	LVFX	MFLX[b]
	Route: po			
serum				
C_{max} (μg/mL)	2.20	1.27	1.92	1.03
AUC_{0-24h} (μg·h/mL)	16.9	9.45	15.2	6.63
$t_{1/2}$ (h)	4.8	3.1	3.5	5.3
urinary recovery$_{0-24h}$ (%)[c]	61.3	41.0	73.9	8.1
bioavailability (%)	71	49		61
	route: iv			
serum				
C_{5min} (μg/mL)	3.66	4.75	4.17[d]	2.36
AUC_{0-8h} (μg·h/mL)	23.8	19.4	15.5[d]	10.9
$t_{1/2\alpha}$ (h)	4.8	2.1	3.2[e]	4.8
urinary recovery$_{0-24h}$ (%)[d]	69.8	50.7	80.7[e]	11.0

[a]Animal: female cynomolgus monkeys. [b]Moxifloxacin hydrochloride hydrate was administered. [c]The values were calculated by using the concentrations of unchanged quinolones. [d]These values result from the division of data for 10 mg/kg intravenous administration by levofloxacin. [e]Those values are the results for a 10 mg/kg intravenous administration.

Compound **2a** showed negative responses in the micronucleus induction at a dose of 150 mg/kg in mice.

Table 8. Intravenous Single-Dose Toxicity and Micronuclei-Forming Toxicity of 2a in Mice

	dose (mg/kg)		
	50	100	150
intravenous single-dose toxicity/mortality[a]	0/5	0/5	1/5
micronuclei-forming toxicity test/result[b]	negative	negative	negative

[a]Mortality = (the number of dead mice)/(the number of tested mice). [b]Using the bone marrow of surviving animals in the intravenous single-dose toxicity test.

The therapeutic efficacies of compound **2a** in experimental murine pneumonia models due to quinolone-resistant (QR) or -sensitive (QS) penicillin-resistant *Streptococcus pneumoniae* (PRSP) are compared with the efficacies of moxifloxacin and compound **1** in Figure 5 and Figure 6. The therapeutic efficacy of subcutaneously administrated **2a** was superior to that of moxifloxacin, while the one in oral administration was

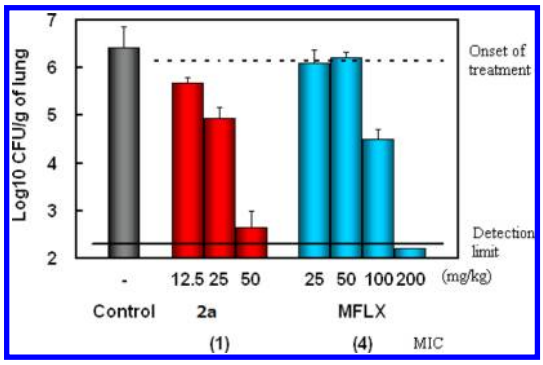

Figure 5. Therapeutic effects on pneumococcal pneumonia (QR-PRSP) in mice. Animals were CBA/JNCrj mice (n = 4). Bacteria were *S. pneumoniae* (QR-PRSP). Test drugs were administered subcutaneously twice a day.

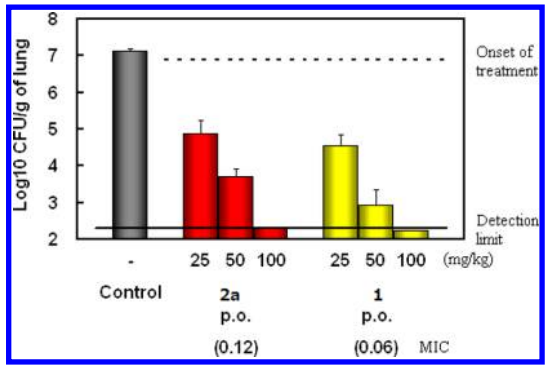

Figure 6. Therapeutic effects on pneumococcal pneumonia (QS-PRSP) in mice. Animals were CBA/JNCrj mice (n = 4). Bacteria were *S. pneumoniae* (QR-PRSP). Test drugs were administered subcutaneously twice a day.

comparable to that of compound **1**, although possessing 2-fold more potency in vitro compared with **2a**. The comparison with GRNX has been previously reported.[21]

CONCLUSION

Compound **2a**, 6-fluoro-8-methoxyquinolone possessing the (7*S*)-7-amino-7-methyl-5-azabicyclo[2.4]heptan-5-yl group at the C-7 position of the scaffold, was designed, synthesized, and evaluated. Compound **2a** exhibited about 4- to 8-fold better antibacterial activity in vitro against Gram-positive bacteria compared with the activity of LVFX. Compound **2a** also showed excellent in vivo antibacterial activity against *Streptococcus pneumoniae* infection in mice, which was supported and rationalized by a good pharmacokinetic profile. In addition, compound **2a** did not exert mechanism-based inhibition on CYP3A4, which was a serious setback of compound **1**.

Thus, compound **2a** has been selected for further evaluation as the promising candidate for treating community-acquired pneumonia.

EXPERIMENTAL SECTION

General. All melting points were taken on a Yamato MP-500D melting point apparatus and are uncorrected. Optical rotations were measured in a 0.5 dm cell at 25 °C at 589 nm with a HORIBA SEPA-300 polarimeter. ^{1}H NMR spectra were determined on a JEOL JNM-EX400 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standards. Signficant ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant(s) in hertz. High-resolution mass spectra were obtained on a JEOL JMS-700 mass spectrometer under electron impact ionization conditions (EI), electron spray ionization conditions (ESI), or fast atom bombardment ionization conditions (FAB). The high-resolution mass spectra were recorded on a JEOL JMS-100LP spectrometer. Elemental analyses are indicated only by the symbols of the elements; analytical results were within 0.4% of the theoretical values. Purities of ≥95% were determined by elemental analysis (all tested compounds). Column chromatography refers to flash column chromatography conducted on Merck silica gel 60, 230−400 mesh ASTM. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F254 TLC plates, and compound visualization was effected with a 5% solution of molybdophosphoric acid in ethanol, UV lamp, iodine, or Wako ninhydrin spray.

In Vitro Antibacterial Activity. The MICs of the compounds tested in this study were determined by the 2-fold microdilution method using Mueller−Hinton broth (Difco Laboratories, Detroit,

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MI) with an inoculum size of approximately 10^5 CFU per well. The MIC was defined as the lowest concentration that prevented visible bacterial growth after incubation at 35 °C for 18 h.

In Vivo Efficacy against *S. pneumoniae* Infection in Mice. Three-week-old male CBA/JNCrlj mice (4 mice/group) were used. The bacteria used for infection were quinolone-resistant *S. pneumoniae* 104835 (penicillin-resistant, QR-PRSP) and quinolone-sensitive *S. pneumoniae* 033806 (penicillin-resistant, QS-PRSP), both of which were inoculated by nasal drop. One day after the infection, drugs were administered subcutaneously or orally twice a day. The number of bacteria in the lungs was examined on the day after the final administration of drug. The lungs were removed aseptically and weighed, and then the viable bacterial counts were determined. The detection limit was ≥2.30 $\log_{10}$ CFU/g of lung.

Single Intravenous Dose Toxicity. The test compounds were dissolved in 0.1 N NaOH in saline at different concentrations. The solution was administered intravenously to male Slc:ddY mice, 6 weeks of age at a dose level of 50, 100, and 150 mg/kg (10 mL/kg, 0.2 mL/min). The number of dead mice was counted on day 7.

Bone Marrow Micronucleus Test. The test compounds were dissolved in 0.1 N NaOH in saline at different concentrations. The solution was administered intravenously to male Slc:ddY mice, 6 weeks of age at a dose level of 50, 100, and 150 mg/kg (10 mL/kg, 0.2 mL/min). At 24 and 48 h after dosing of the compounds, approximately 5 μL of peripheral blood was collected from a tail blood vessel of each mice. The blood was dropped onto an acridine orange coated glass slide and covered immediately with a coverslip. For each animal, 1000 reticulocytes were examined for micronuclei by fluorescence microscopy, and the frequency of micronucleated reticulocytes (MNRET) was expressed as a percentage. Statistical analysis was performed by the Kastenbaum and Bowman method.

Pharmacokinetic Studies. Seven-week-old male Crj:CD rats (n = 4) were used. The animals were administered drug samples in a single intravenous dose (5 mg/kg) as an aqueous solution. The concentrations of the compounds were determined by a microbiological assay (agar well dilution method) using *B. subtilis* ATCC 6051. The mean values of the four rats were shown.

X-ray Crystallographic Analysis of 14. A colorless prism-shaped crystal was formed from AcOEt: $C_{20}H_{27}NO_3$; FW = 329.44; sample dimensions, 0.40 mm × 0.30 mm × 0.34 mm. The lattice parameters and intensities were measured on a Rigaku AFC7R diffractometer (Cu Kα radiation, λ = 1.541 78 Å, graphite monochromator, ω–2θ scans, $2\theta_{max}$ = 120.1°): orthorhombic, space group $P2_12_12_1$; a = 16.396(2) Å, b = 17.122(2) Å, c = 6.800(1) Å, V = 1908.9(5) Å^3, Z = 4; D_{calcd} = 1.15 g/cm^3; F_{000} = 712; μ = 6.09 cm^{-1}. The structure was solved by direct methods with the program Sir92.[22] The final cycle of full-matrix least-squares refinement was based on 1665 observed reflections and 245 variable parameters and converged at R = 0.063 (Rw = 0.191).

1-(1,2-Diamino-1-methylethyl)-1-cyclopropanecarboxylic Acid Dihydrochloride (7). To a solution of **5** (9.21 g, 50.0 mmol) in 7 N NH_3–MeOH (300 mL) were added aqueous NH_3 (28%, 90 mL), ammonium chloride (53.5 g, 1.00 mol), and sodium cyanide (4.90 g, 100 mmol) at 0 °C, and the mixture was stirred at ambient temperature for 18 h. The mixture was concentrated in vacuo. Water (100 mL) was added to the residue, and then the aqueous solution was extracted with CH_2Cl_2 (3×). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated to give **6** (10.15 g) as a pale brown oil. To a solution of **6** (1.12 g, 5.30 mmol) in EtOH (50 mL) was added Raney nickel catalyst (about 50%, 10 mL), and the mixture was stirred at ambient temperature for 6 h under hydrogen atmosphere. The resultant suspension was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was dissolved in concentrated aqueous HCl (5 mL), and the mixture was stirred at ambient temperature for 0.5 h. After the mixture was diluted with water, the resultant solution was concentrated in vacuo. The resultant residue was mixed with EtOH and concentrated in vacuo (2×) to give **7** (0.82 g, 65%) as a yellow amorphous product. ^{1}H NMR (CD_3OD): δ 1.20–1.26 (1H, m), 1.28 (3H, s), 1.32–1.43 (2H, m), 1.58–1.62 (1H, m), 3.46 (1H, d, J = 13.4 Hz), 3.80 (1H, d, J = 13.4 Hz). High-resolution MS (ESI) calcd for $C_7H_{15}N_2O_2$: 159.1134. Found: 159.1125.

***tert*-Butyl (7-Methyl-4-oxo-5-azaspiro[2.4]hept-7-yl)-carbamate (8).** To a solution of **7** (800 mg, 3.46 mmol) in 70 mL of CH_3CN was added 1,1,1,3,3,3-hexamethyldisilazane (7.38 mL, 34.6 mmol) under nitrogen atmosphere, and then the mixture was heated to reflux for 4 h. After the mixture was cooled at ambient temperature, MeOH (70 mL) was added. The resultant solution was concentrated in vacuo. The residue was mixed with Boc_2O (1.53 g, 7.00 mmol) and 1,4-dioxane (20 mL), and the mixture was stirred for 5 h at ambient temperature. Water was added, and the aqueous solution was extracted with $CHCl_3$ (2×). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was treated with light petroleum ether and the solid was collected by filtration to give **8** (502 mg, 60%) as a colorless powder, mp 221–223 °C. ^{1}H NMR ($CDCl_3$): δ 0.77–0.82 (1H, m), 0.94–1.04 (2H, m), 1.16–1.23 (1H, m), 1.28 (3H, s), 1.43 (9H, s), 3.29 (1H, d, J = 10.3 Hz), 4.12 (1H, m), 4.60 (1H, br s), 5.82 (1H, br s). High-resolution MS (ESI) calcd for $C_{12}H_{21}N_2O_3$: 241.1552. Found: 241.1538. Anal. Calcd for $C_{12}H_{20}N_2O_3$: C 59.88, H 8.39, N 11.66. Found: C 59.80, H 8.43, N 11.74.

***tert*-Butyl (5-Benzyl-7-methyl-4-oxo-5-azaspiro[2.4]hept-7-yl)carbamate (9).** To a solution of **8** (3.12 g, 13.0 mmol) in 65 mL of DMF on an ice bath was added NaH (55% mineral oil dispersion, 566 mg, 13.0 mmol) carefully. After the mixture was stirred for 40 min at the same temperature, BnBr (1.85 mL, 15.6 mmol) was added dropwise and the mixture was stirred for 1.5 h at ambient temperature. The reaction mixture was diluted with 300 mL of AcOEt and washed with water (2×) and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 4:1 to yield **9** (4.20 g, 98%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.76–0.81 (1H, m), 0.93–1.06 (2H, m), 1.21–1.29 (4H, m), 1.37 (9H, s), 3.14 (1H, d, J = 10.3 Hz), 3.92–3.98 (1H, m), 4.44, 4.56 (each 1H, ABq, J = 14.9 Hz), 4.56 (1H, br s), 7.22–7.33 (5H, m). High-resolution MS (ESI) calcd for $C_{19}H_{27}N_2O_3$: 331.2022. Found: 331.2020.

(−)-*tert*-Butyl (5-Benzyl-7-methyl-4-oxo-5-azaspiro[2.4]-hept-7-yl)carbamate ((−)-9) and (+)-*tert*-Butyl (5-Benzyl-7-methyl-4-oxo-5-azaspiro[2.4]hept-7-yl)carbamate ((+)-9). An amount of 2.25 g of racemic **9** was separated into its enantiomers by semipreparative HPLC by using a Chiralpak AD column (Daicel Chemical Industries, Ltd.; 250 mm × 20 mm, 5 μm; flow, 20 mL/min; solvents, hexane/isopropanol 90:10; 50 mg/run; UV detection at 254 nm) to give (−)-**9** (997 mg, 44%, t_R = 7.0 min) and (+)-**9** (957 mg, 43%, t_R = 11.3 min) as a colorless powder, respectively. (−)-**9**: mp 109–111 °C. $[\alpha]_D^{25}$ −113.9° (c 0.180, $CHCl_3$). Anal. Calcd for $C_{19}H_{26}N_2O_3$: C 69.09, H 7.93, N 8.48. Found: C 68.80, H 7.99, N 8.51. (+)-**9**; mp 108–110 °C. $[\alpha]_D^{25}$ +108.8° (c 0.249, $CHCl_3$). Anal. Calcd for $C_{19}H_{26}N_2O_3$: C 69.09, H 7.93, N 8.48. Found: C 68.99, H 8.01, N, 8.48.

(−)-*tert*-Butyl (5-Benzyl-7-methyl-5-azaspiro[2.4]hept-7-yl)-carbamate ((−)-10). To a solution of (−)-**9** (950 mg, 2.88 mmol) in CH_2Cl_2 (15 mL) was added trifluoroacetic acid (7.5 mL) at ambient temperature. The mixture was stirred for 40 min and then concentrated in vacuo. The resultant residue was mixed with toluene and concentrated in vacuo (2×). After saturated aqueous $NaHCO_3$ was added, the aqueous layer was extracted with $CHCl_3$ (2×). The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated in vacuo. To the solution of the residue in THF (30 mL) was added $LiAlH_4$ (218 mg, 5.75 mmol) at 0 °C, and the mixture was stirred for 1 h at same temperature. After addition of $LiAlH_4$ (109 mg, 2.87 mmol), the mixture was stirred for 3 h at ambient temperature and stirred at 0 °C again. To the mixture, water (0.31 mL), 15 wt % aqueous NaOH (0.31 mL), and water (0.93 mL) were added dropwise. The resultant mixture was stirred at ambient temperature for 14 h and dried over anhydrous $MgSO_4$. The suspension was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was mixed with Boc_2O (1.26 g, 5.75 mmol) and CH_2Cl_2 (15 mL), and the mixture was stirred for 20 h at ambient temperature and then

concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with $CHCl_3$/MeOH/triethylamine = 95:5:1 to yield (−)-**10** (586 mg, 64%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.40−0.45 (1H, m), 0.50−0.55 (1H, m), 0.63−0.69 (1H, m), 0.80−0.85 (1H, m), 1.20 (3H, s), 1.43 (9H, s), 2.44 (1H, d, J = 8.8 Hz), 2.59 (1H, d, J = 9.5 Hz), 2.83 (1H, d, J = 8.8 Hz), 3.33 (1H, m), 3.57 (1H, d, J = 13.2 Hz), 3.68 (1H, d, J = 13.2 Hz), 4.75 (1H, br s), 7.20−7.37 (5H, m). High-resolution MS (ESI) calcd for $C_{19}H_{29}N_2O_2$: 317.2229. Found: 317.2253. $[\alpha]_D^{25}$ −63.6° (c 0.129, $CHCl_3$).

7-(7-Amino-7-methyl-5-azaspiro[2.4]hept-5-yl)-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (2a). To a solution of (−)-**10** (581 mg, 1.84 mmol) in MeOH (40 mL) was added 10% Pd/C (M, 290 mg, containing 50% water), and the mixture was stirred for 5 h at ambient temperature under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was mixed with **4** (663 mg, 1.84 mmol), triethylamine (0.795 mL, 5.52 mmol), and DMSO (5 mL), and the mixture was stirred at 40 °C for 14 h. To this solution were added 80% aqueous EtOH (50 mL) and triethylamine (5 mL), and the resultant mixture was heated to reflux for 2 h and then concentrated in vacuo to give the residue, which was diluted with AcOEt. The organic solution was washed with 10% aqueous citric acid solution, water (2×) and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. To the residue was added 10 mL of concentrated aqueous HCl at 0 °C, and the mixture was stirred for 20 min at ambient temperature. The aqueous solution was washed with $CHCl_3$ and made alkaline with saturated aqueous NaOH at 0 °C. The pH of the solution was adjusted to 7.4 with concentrated aqueous HCl and then diluted aqueous HCl. The resultant solution was extracted with $CHCl_3$ (2×) and $CHCl_3$/MeOH = 9:1. The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was recrystallized from EtOH to yield **2a** (644 mg, 84%) as a pale pinkish powder, mp 198−200 °C. ^{1}H NMR (0.1 N NaOD/D_2O): δ 0.49−0.56 (2H, m), 0.67−0.76 (2H, m), 1.12 (3H, s), 1.43−1.64 (2H, m), 3.56 (3H, s), 3.59−3.71 (4H, m), 3.99−4.04 (1H, m), 4.80−5.03 (1H, m), 7.65 (1H, d, J = 13.9 Hz), 8.45 (1H, s). $[\alpha]_D^{25}$ +40.8° (c 0.147, 0.1 N aqueous NaOH). Anal. Calcd for $C_{21}H_{23}F_2N_3O_4$·0.75EtOH·0.5H_2O: C 58.37, H 6.20, F 8.20, N 9.08. Found: C 58.23, H 5.99, F 8.09, N 9.02.

(+)-*tert*-Butyl (5-Benzyl-7-methyl-5-azaspiro[2.4]hept-7-yl)-carbamate ((+)-10). With the procedures as described for (−)-**10**, the title compound was prepared in 69% yield from (+)-**9** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.40−0.45 (1H, m), 0.50−0.55 (1H, m), 0.63−0.69 (1H, m), 0.80−0.85 (1H, m), 1.20 (3H, s), 1.43 (9H, s), 2.44 (1H, d, J = 8.8 Hz), 2.59 (1H, d, J = 9.5 Hz), 2.83 (1H, d, J = 8.8 Hz), 3.33 (1H, m), 3.57 (1H, d, J = 13.2 Hz), 3.68 (1H, d, J = 13.2 Hz), 4.75 (1H, br s), 7.20−7.37 (5H, m). High-resolution MS (ESI) calcd for $C_{19}H_{29}N_2O_2$: 317.2229. Found: 317.2249. $[\alpha]_D^{25}$ +76.2° (c 0.290, $CHCl_3$).

7-(7-Amino-7-methyl-5-azaspiro[2.4]hept-5-yl)-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (2b). With the procedures as described for **2a**, the title compound was prepared in 78% yield from (+)-**10** as a pale pinkish powder, mp 212−214 °C. ^{1}H NMR (0.1 N NaOD/D_2O): δ 0.52 (2H, m), 0.73 (2H, m), 1.07 (3H, s), 1.42−1.64 (2H, m), 3.45 (1H, d, J = 10.3 Hz), 3.52−3.56 (1H, m), 3.55 (3H, s), 3.73 (1H, dd, J = 2.2, 10.0 Hz), 3.85 (1H, d, J = 9.0 Hz), 3.99−4.04 (1H, m), 4.82−5.02 (1H, m), 7.64 (1H, d, J = 14.4 Hz), 8.45 (1H, s). $[\alpha]_D^{25}$ +128.8° (c 0.163, 0.1 N aqueous NaOH). Anal. Calcd for $C_{21}H_{23}F_2N_3O_4$·1.0EtOH·0.5H_2O: C 58.22, H 6.37, F 8.01, N 8.86. Found: C 58.02, H 6.13, F 8.05, N 9.02.

***tert*-Butyl (3*S*)-4-(2-Hydroxyethyl)-3-methyl-5-oxo-1-[(*R*)-1-phenylethyl]pyrrolidine-3-carboxylate (13).** Under nitrogen atmosphere, to a solution of **11** (30.0 g, 98.9 mmol) and **12** (36.8 g, 129 mmol) in anhydrous THF (288 mL) was added 1 M LiHMDS/THF (129 mL) dropwise at −4 °C. After the mixture was stirred at 2 °C for 3.5 h, saturated aqueous NH_4Cl and AcOEt were added. The separated organic solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. To a solution of the residue in THF (450 mL) on an ice bath was added 1 M TBAF/THF (148 mL) dropwise, and the mixture was stirred for 2 h at ambient temperature. After concentration in vacuo, AcOEt and 10% aqueous $NaHCO_3$ were added to the mixture. The organic solution was washed with 10% aqueous citric acid and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography, eluting with hexane/AcOEt = 1:1 to yield diastereomixture **13** (29.1 g, 85%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.28 (3H, s), 1.40 (9H, s), 1.51−1.53 (1H, m), 1.53 (3H, d, J = 7.1 Hz), 1.78−1.94 (2H, m), 2.90−3.08 (2H, m), 3.67−3.75 (1H, m), 3.80−3.91 (1H, m), 4.85−4.89 (1H, m), 5.43−5.53 (1H, m), 7.27−7.37 (5H, m). High-resolution MS (ESI) calcd for $C_{20}H_{30}NO_4$: 348.2175. Found: 348.2177.

***tert*-Butyl (7*S*)-7-Methyl-4-oxo-5-[(*R*)-1-phenylethyl]-5-azaspiro[2.4]heptan-7-carboxylate (14).** To a solution of **13** (29.1 g, 83.9 mmol) in CH_2Cl_2 (280 mL) on an ice bath were added TEA (15.2 mL, 109 mmol), benzenesulfonyl chloride (11.8 mL, 92.3 mmol), and DMAP (1.02 g, 8.39 mmol). The mixture was stirred at ambient temperature for 19 h and diluted with AcOEt. This solution was washed with saturated aqueous NH_4Cl, 1 N aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. To a solution of the residue in anhydrous THF (470 mL) on an ice bath was added 1 M NaHMDS/THF (109 mL) under nitrogen atmosphere, and the mixture was stirred at ambient temperature for 1 h. Aqueous saturated NH_4Cl was added to the mixture, and the resultant solution was extracted with AcOEt (2×). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The resultant residue was purified by silica gel column chromatography, eluting with hexane/AcOEt = 3:1 to yield **14** (24.6 g, 89%) as a colorless crystal, mp 55−57 °C. ^{1}H NMR ($CDCl_3$): δ 0.72−0.77 (1H, m), 0.85−0.90 (1H, m), 1.04−1.13 (2H, m), 1.18 (3H, s), 1.32 (9H, s), 1.54 (3H, d, J = 7.1 Hz), 3.08 (1H, d, J = 9.8 Hz), 3.53 (1H, d, J = 7.1 Hz), 5.52 (1H, q, J = 7.1 Hz), 7.26−7.34 (5H, m). High-resolution MS (FAB) calcd for $C_{20}H_{28}NO_3$: 330.2069. Found: 330.2069. $[\alpha]_D^{25}$ +122.1° (c 0.517, $CHCl_3$). Anal. Calcd for $C_{20}H_{27}NO_3$: C 72.92, H 8.26, N 4.25. Found: C 72.64, H 8.27, N 4.06.

(7*S*)-7-(*tert*-Butoxycarbonylamino)-7-methyl-5-[(*R*)-1-phenylethyl]-5-azaspiro[2.4]heptane (16). To a solution of **14** (24.5 g, 74.4 mmol) in CH_2Cl_2 (120 mL) on an ice bath was added trifluoroacetic acid (120 mL), and the mixture was stirred at ambient temperature for 2 h and concentrated in vacuo. The residue was mixed with toluene, and the solution was concentrated in vacuo. After the residue was dissolved in 1 N aqueous NaOH at 0 °C, the aqueous solution was washed with AcOEt and acidified with concentrated aqueous HCl under ice-cooling. The mixture was extracted with $CHCl_3$ (2×), and the combined organic solution was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. To a solution of the residue in toluene (200 mL) were added triethylamine (20.7 mL, 149 mmol) and diphenylphosphoryl azide (17.6 mL, 81.8 mmol) at ambient temperature. The mixture was heated to reflux for 1 h and then concentrated in vacuo. The residue was mixed with 1,4-dioxane (180 mL), water (90 mL), and concentrated aqueous HCl (90 mL), and the mixture was stirred at 50 °C for 1 h. Water (200 mL) was added to the mixture, and the resultant solution was washed with AcOEt and made alkaline with 10 N aqueous NaOH at 0 °C and extracted with toluene (2×). The combined organic solution was washed with brine and dried over anhydrous Na_2SO_4 and concentrated in vacuo. To a solution of the residue in toluene (82 mL) was added Red-Al (65% w/w in toluene, 77.6 mL, 259 mmol) dropwise over 15 min, and the mixture was stirred at 80 °C for 10 min. After the mixture was cooled to 0 °C, 25 wt % aqueous NaOH (158 mL) was dropped into the mixture, which was extracted with toluene. To the organic solution which was washed with brine was added Boc_2O (15.6 g, 71.2 mmol), and the mixture was stirred at ambient temperature for 3 h and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with hexane/AcOEt = 3:1 to yield **16** (18.0 g, 73%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.37−0.49 (2H, m), 0.62−0.68 (1H, m), 0.77−0.82 (1H, m), 1.20 (3H, s), 1.32 (3H, d, J = 6.6 Hz), 1.44 (9H, s), 2.46

(2H, dd, J = 33.2, 9.3 Hz), 2.68 (1H, d, J = 8.8 Hz), 3.27 (1H, q, J = 6.6 Hz), 3.31−3.34 (1H, m), 4.71 (1H, s), 7.19−7.34 (5H, m). High-resolution MS (ESI) calcd for $C_{20}H_{31}N_2O_2$: 331.2385. Found: 331.2406. $[\alpha]_D^{25}$ −23.817° (c 0.786, $CHCl_3$).

7-[(7*S*)-7-Amino-7-methyl-5-azaspiro[2.4]hept-5-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (2). To a solution of **16** (18.0 g, 54.5 mmol) in MeOH (180 mL) was added 10% Pd/C (M, 9.0 g, containing 50% water), and the mixture was stirred at ambient temperature for 18 h and then at 40 °C for 5.5 h under hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated in vacuo, followed by the procedures as described for **2a**. The title compound was prepared in 79% yield from **16** as a pale pinkish powder. The analytical data of this compound corresponded with those of compound **2a**.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Crystallographic details for **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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