Regular Article

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Discovery of Novel 7-[(1*R*,5*S*)-1-Amino-5-fluoro-3-azabicyclo[3.3.0]octan-3-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropane]-8-(methoxy or methyl)-quinolones

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Received August 30, 2018; accepted October 16, 2018

A series of 8-methoxy or 8-methylquinolones bearing novel 3-aminooctahydrocyclopenta[*c*]pyrrole derivatives at the C-7 position was synthesized, and the pharmacological, physicochemical, and toxicological properties of the individual compounds were evaluated. Novel 8-methylquinolone 7, which includes a 3-amino-7-fluorooctahydrocyclopenta[*c*]pyrrole moiety at the C-7 position, showed potent antibacterial activity against both Gram-positive and negative pathogens. Compound 7 also demonstrated favorable pharmacokinetic and pharmacodynamic properties and an acceptably safe toxicological profile. Consequently, compound 7 was selected as a clinical candidate.

Key words 8-methylquinolone; antibacterial activity; multidrug-resistant microorganism; multidrug-resistant Gram-negative pathogen; multidrug-resistant *Acinetobacter baumannii*

Introduction

Increasing antibiotic-resistant infections caused by drug-resistant microorganisms and the shortage of clinically available antibacterial agents present a major global public health threat. Pathogenic bacteria include not only multidrug-resistant Gram-positive pathogens like multidrug-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus*, and community-acquired methicillin-resistant *Staphylococcus aureus*, but also multidrug-resistant Gram-negative pathogens like multidrug-resistant *Acinetobacter baumannii*.[1–4)]

In February 2017, the WHO published its first ever list of antibiotic-resistant "priority pathogens"—a catalog of 12 families of bacteria that pose the greatest threat to human health. In this news release, carbapenem-resistant *Acinetobacter baumannii* was identified as one of the pathogens for which new antibiotics are urgently needed.[5)]

Novel antibacterial compound **1** (Table 1), as previously reported,[6)] exhibits potent *in vitro* and *in vivo* activity against several quinolone-resistant pathogens. Furthermore, compound **1** shows excellent pharmacokinetic (PK) and safety profiles. Compound **1** possesses a unique tertiary alkyl amine structure that reduces the risk of mechanism-based inactivation.[6)]

We recently reported novel quinolone compound **2** bearing 3-aminooctahydrocyclopenta[*c*]pyrrole at the C-7 position[7)] (Fig. 1). The 3-aminooctahydrocyclopenta[*c*]pyrrole unit features a tertiary alkyl amine structure. Similar to compound **1**, compound **2** exhibits a low mechanism-based inactivation risk (data not shown). Moxifloxacin (**3**: MFLX) is a commercially available respiratory quinolone (Fig. 1) that exhibits potent antibacterial activity and good PK and safety profiles.

The minimum inhibitory concentrations (MICs) of **1**, **2**, and **3** against several representative Gram-positive and negative bacteria are summarized in Table 1, along with the data for levofloxacin (LVFX) and ciprofloxacin (CPFX) for comparison. Compound **2** exhibited a broad-spectrum antibacterial effect against Gram-positive organisms, and its potency against Gram-positive pathogens was higher than that of compound **1** or **3** (Table 1). Unfortunately, the results of our PK and safety studies with compound **2** indicated low bioavailability[8)] and relatively high single-dose toxicity. In previous structure–PK relationship studies of quinolones, it is already reported that the structure of the substituent at the C-5, C-6, C-7, or C-8 position has a great influence.[9–11)] In order to improve the PK profile of compound **2**, we substituted unique pyrrolidine derivatives at the C-7 position and identified the substituent that allowed excellent activity, safety, and PK.

Comparing the p values[12)] of compounds **1**, **2**, and **3**, com-

Table 1. Antibacterial Activities (MIC, μg/mL) of Compounds **1**, **2**, and **3** and Reference Quinolones against Gram-Positive and Negative Bacteria

Bacteria/Compound	**1**	**2**	**3**: MFLX	LVFX	CPFX
E. coli NIHJ	0.012	0.025	0.012	0.012	≤0.003
K. pneumoniae TYPE 1	0.05	0.1	0.1	0.05	0.025
P. aeruginosa PAO1	0.78	0.78	0.78	0.39	0.05
H. influenzae ATCC49247	0.012	0.006	0.012	0.012	0.006
M(B). caterrhalis ATCC25238	0.05	0.025	0.05	0.025	0.025
S. aureus FDA 209-P	0.025	0.012	0.05	0.1	0.1
S. epidermidis 56500	0.1	0.05	0.1	0.39	0.2
S. pneumoniae J24	0.05	0.05	0.1	0.78	0.78
S. pyogenes G-36	0.2	0.1	0.2	0.78	1.56
E. faecalis 19433	0.2	0.1	0.2	0.78	0.78
S. aureus 870307 (MRSA)	0.39	0.78	0.78	6.25	>6.25
S. pneumoniae 104835	0.39	0.39	3.13	>6.25	>6.25

MFLX, moxifloxacin; LVFX, levofloxacin; CPFX, ciprofloxacin; MRSA, methicillin-resistant *Staphylococcus aureus*.

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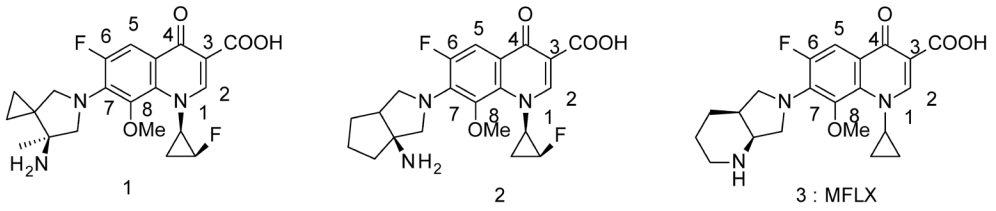


Fig. 1. Structures of Compounds **1**, **2**, and Moxifloxacin (MFLX) (**3**)

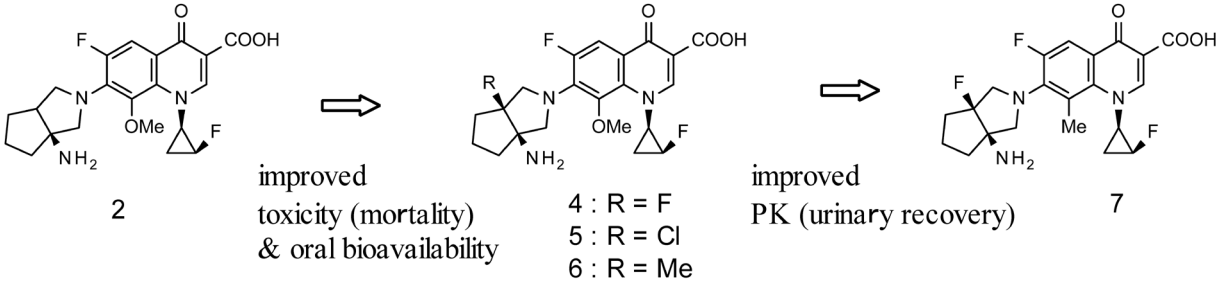


Fig. 2. Design of 8-Methyl Quinolone Derivative **7**

Table 2. Antibacterial Activities (MIC, μg/mL) of Compounds **2**, **4**, **5**, **6**, and **7** and Reference Quinolones against Gram-Positive and Negative Bacteria

Bacteria/Compound (Ex. No.)	**2**	**4**	**5**	**6**	**7**	LVFX	CPFX
E. coli NIHJ	0.025	0.006	0.1	0.05	≤0.003	0.012	≤0.003
K. pneumoniae TYPE 1	0.1	0.05	0.39	0.2	0.012	0.05	0.025
P. aeruginosa PAO1	0.78	0.78	3.13	1.56	0.2	0.39	0.05
H. influenzae ATCC49247	0.006	≤0.003	0.025	0.05	≤0.003	0.012	0.006
M(B). caterrhalis ATCC25238	0.025	0.025	0.05	0.1	0.006	0.025	0.025
S. aureus FDA 209-P	0.012	0.012	0.025	0.05	0.006	0.1	0.1
S. epidermidis 56500	0.05	0.05	0.1	0.1	0.025	0.39	0.2
S. pneumoniae J24	0.05	0.05	0.2	0.05	0.025	0.78	0.78
S. pyogenes G-36	0.1	0.1	0.2	0.2	0.025	0.78	1.56
E. faecalis 19433	0.1	0.2	0.2	0.2	0.05	0.78	0.78
S. aureus 870307 (MRSA)	0.78	0.39	0.78	0.78	0.2	6.25	>6.25
S. pneumoniae 104835	0.39	0.39	—	—	0.1	>6.25	>6.25

LVFX, levofloxacin; CPFX, ciprofloxacin; MRSA, methicillin-resistant *Staphylococcus aureus*.

pound **2** ($p = 5.62$) had much lower p value than compound **1** ($p = 19.2$) or MFLX (**3**) ($p = 53.8$), and such low lipophilicity is thought to lead to poor permeability causing low bioavailability. Therefore, we introduced a halogen or a methyl group at the C-7 position to increase the lipophilicity (Fig. 2).

Synthesis and biological evaluation of a series of 8-methoxyquinoline-3-carboxylic acid derivatives with modified C-7 substituents showed that fluoro compound **4** exhibited good antibacterial activity, reduced toxicity (mortality), and improved oral bioavailability, but weak activity against *P. aeruginosa*. In addition, compound **4** exhibited low urinary recovery[13] (Fig. 2). We also designed its 8-methylquinoline-3-carboxylic acid analog. Replacement of the 8-methoxy group with an 8-methyl group on this scaffold afforded higher antibacterial activity against Gram-negative pathogens and higher urinary recovery probably owing to its higher hydrophilicity (Fig. 2).

Results and Discussion

Biology The MICs of compounds **2**, **4**, **5**, **6**, and **7** against several representative Gram-positive and negative bacteria are summarized in Table 2, along with the corresponding data for LVFX and CPFX. The physicochemical and toxicological properties of compounds **2**, **4**, **5**, **6**, and **7** are summarized in Table 3, along with the data for LVFX and MFLX.

The novel quinolone **7** exhibited a broad-spectrum antibacterial effect against Gram-positive and negative bacteria. Moreover, the p value and mortality associated with compound **7** were significantly lower than those associated with compound **2**. In the micronucleus test, compound **7** exhibited negative responses at a dose of 150 mg/kg (IV) in mice (Table 3).

The MICs of compound **7** against several anaerobic species are summarized in Table 4,[14] along with the corresponding data for LVFX, CPFX, MFLX, and sitafloxacin (STFX). Compound **7** exhibited equivalent or higher activities than STFX against Gram-positive and negative species. The MIC_{90} values of compound **7** against major pathogens that cause hospital- and community-acquired infections are summarized in Table 5,[14] along with the corresponding data for LVFX, CPFX, and STFX. Compound **7** also exhibited potent activities against these pathogens, including quinolone-resistant organisms.

The IC_{50} values of compound **7** and LVFX against *Escherichia coli* and human topoisomerases are summarized in Table 6.[14] Compound **7** inhibited *E. coli* DNA gyrase and topoisomerase IV with significantly lower IC_{50} values, which

Table 3. Physicochemical and Toxicological Properties of Quinolones

	3	4	5	6	7	LVFX	3:MFLX
R^7=							
R^8=							
R^1=							
P' ($CHCl_3$)[a]	5.62	>57.18	>56.41	21.96	15.51	5.1	53.8
Solubility (μg/mL)[b]	>500	>960	NT	388	302	>11900	777
Protein binding (%)	0	32	NT	26	44	14	1.3
MLD (mg/kg) (mortality)[c]	100 (2/2)	100 (1/5)	NT	NT	>150	>200	200 (2/2)
Micronucleus test (mg/kg)[d]	50 (-)	100 (-)	100 (-)	NT	150 (-)	200 (-)	150 (-)

a) Apparent partition coefficient, $CHCl_3$–0.1 M phosphate buffer (pH 7.4)[8]; *b*) aqueous solubility; *c*) mortality = (the number of dead mice)/(the number of tested mice); *d*) using the bone marrow of surviving animals in the IV single-dose toxicity test. MLD, minimum lethal dose.

Table 4. Antibacterial Activity against Anaerobic Species[a]

	Strain	MIC (μg/mL)				
		7	LVFX	CPFX	MFLX	STFX
Gram-negative	*B. fragilis* PA-2-II	≦0.05	3.13	6.25	0.78	0.1
	B. fragilis NCTC9343	≦0.05	0.78	3.13	0.2	≦0.05
	F. varium ATCC 8501	0.78	12.5	25	12.5	0.78
	F. nucleatum IPP 143	0.78	100	50	25	0.78
	P. intermedia ATCC25611	0.03	1	2	1	0.06
	P. melaninogenica JCM6325	0.03	1	2	1	0.06
Gram-positive	*C. perfringens* 22	≦0.05	0.78	0.78	0.78	0.1
	C. difficile ATCC9689	0.1	6.25	12.5	1.56	0.2
	C. difficile GAI-0547	0.2	12.5	25	3.13	0.39
	C. difficile GAI-0796	0.2	12.5	50	3.13	0.2
	P. acnes X-18	≦0.05	0.78	0.78	0.78	≦0.05

a) Microbroth dilution method. LVFX, levofloxacin; CPFX, ciprofloxacin; MFLX, moxifloxacin; STFX, sitafloxacin.

Table 5. Antibacterial Activity against Key Pathogenic Species of Clinical Isolates[a]

Species (#)	MIC_{90} (μg/mL)			
	7	LVFX	CPFX	STFX
P. aeruginosa[b] (18)	8	64	32	4
A. baumannii (20)	1	8	64	2
E. coli (50)	1	8	16	1
K. pneumoniae (26)	0.25	0.5	0.25	0.12
S. pneumoniae (24)	0.25	32	64	1
S. pyogenes (26)	0.03	2	4	0.06
VGS[c] (12)	0.5	64	>64	2
MRSA (49)	4	>64	>64	8
E. faecalis (26)	1	32	32	2

a) Clinical isolates from Eurofins' surveillance (worldwide) and LVFX-surveillance in Japan; *b*) not included highly resistant *P. aeruginosa*; *c*) viridans group streptococci; LVFX, levofloxacin; CPFX, ciprofloxacin; MFLX, moxifloxacin; STFX, sitafloxacin.

Table 6. IC_{50} Values of Compound **7** and Levofloxacin (LVFX) against *E. coli* and Human Topoisomerases

Compound	*E. coli*		Human
	DNA gyrase (μg/mL)	Topoisomerase IV (μg/mL)	Topoisomerase II (μg/mL)
7	0.499	0.0428	>1024
LVFX	1.88	0.735	>1024

LVFX, levofloxacin.

were approximately 4- and 17-fold, respectively, lower than those for LVFX. However, compound **7** had no effect on the activity of human topoisomerase II, similar to LVFX.

The PK profiles of compound **7** after oral administration (5 mg/kg) to rats and oral (5 mg/kg) or IV (5 mg/kg) administration to monkeys are shown in Table 7.[14] Compound **7** exhibited a higher tissue to plasma drug concentration ratio (K_p value) after oral administration in rats, as well as good oral bioavailability in monkeys.

In the clinical study of fluoroquinolones, cardiotoxicity, skin toxicity, and central nervous system (CNS) toxicity have often become safety concerns.[15–18] Therefore, we evaluated the long QT, photosensitivity, and convulsion risks associated with compound **7**.

Table 8[14] shows the effect of the drugs on the human ether-à-go-go-related gene (hERG) channel current and the prolongation of action potential duration at 90% (APD_{90}), both of which are indicators of cardiotoxicity. Compound **7** produced a lower hERG current than MFLX, while its grade in the APD_{90} prolongation test was almost equal to that of LVFX.

Table 7. Pharmacokinetic (PK) Parameters of Compound **7** and Levofloxacin (LVFX) in Rats[a] and Monkeys[b] (5 mg/kg, $n = 3$)

Rats		PK parameters	**7**	LVFX
p.o.	Serum	C_{max} (μg/mL)	1.01	1.47
		AUC_{0-8h} (μg·h/mL)	2.00	3.41
	Lung	AUC_{0-8h} (μg·h/mL)	4.56	5.57
		K_p[c]	2.3	1.6
	Liver	AUC_{0-8h} (μg·h/mL)	28.5	16.4
		K_p[c]	14.3	4.8
	Kidney	AUC_{0-8h} (μg·h/mL)	14.9	19.3
		K_p[c]	7.5	5.6
		Urinary recovery$_{0-24h}$ (%)	19.2	80.0
Monkeys		PK parameters	7	LVFX
p.o.	Serum	C_{max} (μg/mL)	1.31	1.68[d]
		AUC_{0-8h} (μg·h/mL)	5.11	15.3[d]
		Urinary recovery$_{0-24h}$ (%)	15.1	49.1[e]
IV	Serum	C_{max} (μg/mL)	3.55	
		AUC_{0-8h} (μg·h/mL)	7.12	
		Urinary recovery$_{0-24h}$ (%)	38.1	
		Bioavailability (%)	71.8	

a) Male Crj:CD rats (seven weeks); *b*) female cynomolgus monkeys; *c*) K_p values indicate tissue–serum concentration ratio; *d*) value obtained after 20 mg/kg oral administration, which are then divided by 4; *e*) value obtained after 20 mg/kg oral administration.

Table 8. Effect on Human Ether-à-go-go-Related Gene (hERG) Channel Current in hERG-Transfected CHO-K1 Cells and the Prolongation of APD_{90}

Compound	Inhibition in channel current (%)[a]			Prolongation in APD_{90} (%)[a]	
	30 μM	100 μM	300 μM	100 μM	300 μM
7	4.5	8.2	26.9	1.5	−2.1
LVFX	0.5	1.5	7.3	1.3	7.3
MFLX	17.7	34.6	65.6	14.9	44.2

a) All values represent the mean of four replicates. LVFX, levofloxacin; MFLX, moxifloxacin.

Table 9. Phototoxicity of Compound **7** and Ciprofloxacin (CPFX) in Mice[a]

Compounds	Dose (mg/kg, IV)	No. of animals	Auricular		Histopathology	
			Erythema	Thickening	Auriculae	Eye
7	100	6	−	−	−	−
CPFX	5	6	+	+	+	−

a) Female BALB/cAnNCrlCrlj mice (initial age: 6–7 weeks) were exposed to UV A at 1.5 mW/cm^2 for 4 h (21.6 J/cm^2) immediately after single IV dosing.

These results indicated that compound **7** possesses a low risk of QTc prolongation.

Phototoxicity data and the convulsant activity of compound **7** are summarized in Table 9[14] and Table 10,[14] respectively, along with the corresponding data for CPFX. Compound **7** possessed lower phototoxicity and convulsive potential than CPFX.

The *in vivo* antibacterial activities of compound **7** are shown in Figs. 3[14] and 4,[14] along with the comparative data

Table 10. Convulsive Activity of Compound **7** and Ciprofloxacin (CPFX) in Mice[a]

Compounds	Dose (μg/5 μL/mouse, i.cist.)	Incidence			
		without BPAA[b]		with BPAA	
		Convulsion	Mortality	Convulsion	Mortality
7	5	2/6	0/6	0/6	0/6
	15	6/6	2/6	6/6	0/6
	50	6/6	0/6	6/6	1/6
CPFX	5	1/6	0/6	6/6	6/6
	15	6/6	3/6	6/6	6/6
	50	6/6	4/6	6/6	6/6

a) Determined by intracisternal (i.cist.) administration in 3-week-old male Slc:ddY mice (5 μg/mouse). *b*) 4-Biphenylacetic acid (BPAA). CPFX (5 μg/mouse) was administered 30 min after the oral administration of BPAA (400 mg/kg).

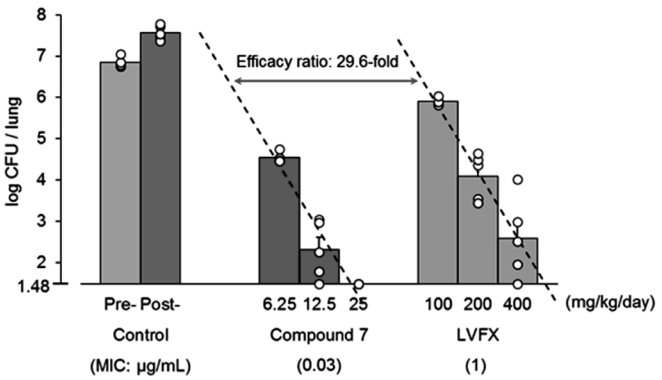


Fig. 3. Therapeutic Efficacy of Compound **7** and Levofloxacin (LVFX) in Murine Respiratory Tract Infection Due to *S. pneumoniae* GE01085

Circles represent the pulmonary bacterial counts in each mouse ($n = 5$). Bars represent the mean ± S.E.M. of the bacterial counts. Dashed lines represent the regression line calculated by a parallel line analysis.

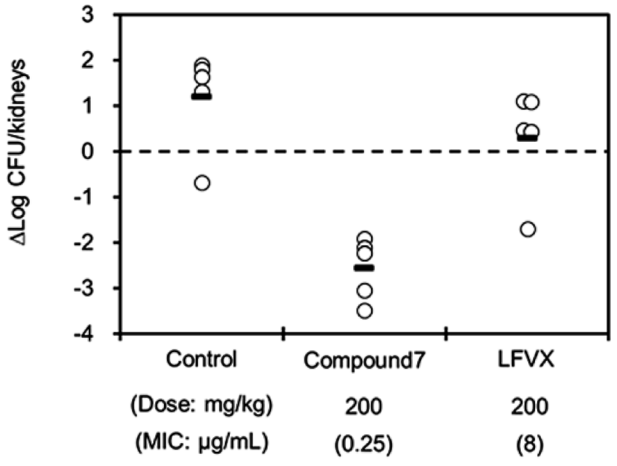
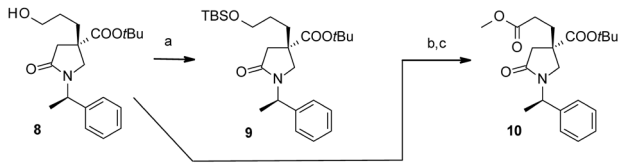


Fig. 4. Therapeutic Efficacy of Compound **7** and Levofloxacin (LVFX) in Rodent Urinary Tract Infection Due to *E. coli* GK00432

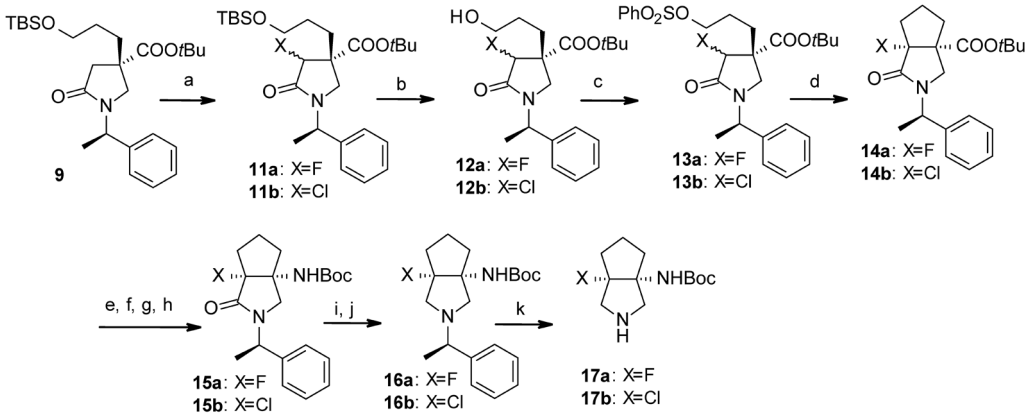
Circles represent the difference between each renal bacterial count and the mean renal bacterial counts at the onset of treatment (6.08 log CFU/kidney). The mean renal bacterial count at the onset of treatment is represented as a broken horizontal line. The black short bars represent the mean of the renal bacterial counts ($n = 5$).

for LVFX. The antibacterial activity and therapeutic efficacy of compound **7** against *S. pneumoniae* were 32- and 30-fold, respectively, better than those of LVFX. Compound **7** also showed more potent *in vivo* bactericidal activity against



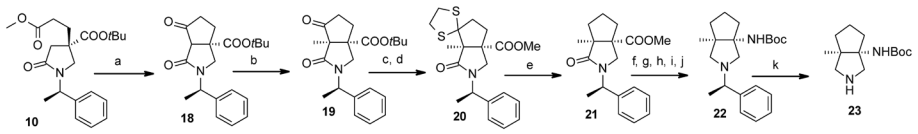
Reagents and conditions: (a) TBS-Cl, imidazole–DMF, 49%; (b) $RuCl_3 \cdot H_2O$, NaIO–CCl_4, CH_3CN, H_2O; (c) MeI, $NaHCO_3$–DMF, 85% from **8**.

Chart 1. Synthesis of pPyrrolidine Intermediates **9** and **10**



Reagents and conditions: (a) LHMDS–THF, then *N*-fluorobenzenesulfonimide (for **11a**) or *N*-chlorosuccinimide (for **11b**), 26% (**11a**); (b) TBAF, AcOH–THF, 93% (**12a**) or 64% (**12b** from **9**); (c) phenylsulfonyl chloride, Et_3N, DMAP–DCM, 69% (**13a**); (d) KHMDS–THF, 58% (**14a**) or 28% (**14b** from **12b**); (e) trifluoroacetic acid–DCM; (f) DPPA, Et_3N, toluene, reflux; (g) 6 N HCl_{aq}–1,4-dioxane, heat; (h) $(Boc)_2O$, heat, 81% (**15a** from **14a**) or 64% (**15b** from **14b**); (i) BH_3–THF–THF; (j) Et_3N, 90% $EtOH_{aq}$, reflux, 94% (**16a** from **15a**) or 32% (**16b** from **15b**); (k) 10% Pd-C (wt), H_2, EtOH, heat, 91% (**17a**) or crude (**17b**).

Chart 2. Synthesis of Amines **17a** and **17b**



Reagents and conditions: (a) LHMDS–THF, 76%; (b) NaH–DMF, then MeI, 76%; (c) ethanedithiol, *p*-toluenesulfonic acid-H_2O–toluene, reflux; (d) trimethylsilyldiazomethane–MeOH, THF, 87% from **19**; (e) Raney-Ni–EtOH, reflux, 65%; (f) $NaOH_{aq}$–MeOH; (g) DPPA, Et_3N, toluene, reflux; (h) 6 N HCl_{aq}–1,4-dioxane, heat; (i) Red-Al, heat; (j) $(Boc)_2O$–DCM, MeOH, 52% from **21**; (k) 10% Pd-C (wt), H_2, EtOH, heat, 95%.

Chart 3. Synthesis of Amine **23**

LVFX-resistant *E. coli* in the kidneys of a rodent urinary tract infection (UTI) model than LVFX. Compound **7** exhibited about 30-fold better antibacterial activity *in vitro* than LVFX in both a murine respiratory tract infection (RTI) and a rodent UTI model. These results reveal that compound **7** has the same target tissue penetration as LVFX. Since it has already been known that LVFX shows good PK in humans, it can be expected that compound **7** will also exhibit favorable efficacy in humans.

Chemistry The preparation of key chiral intermediates **9** and **10** is shown in Chart 1. Silylation of the chiral alcohol **8**[7] with *tert*-butyldimethylsilyl chloride (TBS-Cl) afforded key chiral intermediate **9**. Oxidation of **8** followed by esterification gave another key intermediate **10**.

Preparation of amines **17a** and **17b** is shown in Chart 2. Fluorination or chlorination of **9**, followed by desilylation, phenylsulfonylation, and cyclization with potassium hexamethyldisilazide (KHMDS), gave *tert*-butyl ester **14a** or **14b**. Deprotection of the *tert*-butyl ester **14a** or **14b**, followed by diphenylphosphoryl azide (DPPA)-mediated sequential amine formation reactions (acid azide formation, the Curtius rearrangement, and hydrolysis of the resulting isocyanate), and *tert*-butoxycarbonylation gave the corresponding *N*-Boc derivative **15a** or **15b**. Finally, BH_3 reduction of the *N*-Boc derivative **15a** or **15b**, followed by hydrogenation, gave amine **17a** or **17b**, respectively.

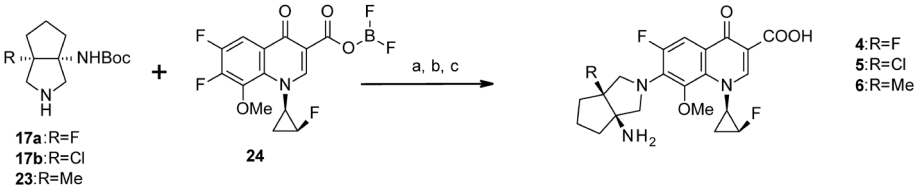
The preparation of amine **23** is depicted in Chart 3. Cyclization of ester **10** with lithium hexamethyldisilazide (LHMDS), followed by methylation and a 3-step decarbonylation, gave methyl ester **21**. Hydrolysis of methyl ester **21** followed by the same amine formation, reduction with Red-Al, *tert*-butoxycarbonylation, and hydrogenation afforded amine **23**.

The secondary amine (**17a**, **17b**, or **23**) and quinolonecarboxylic acid BF_2 chelate **24**[19] were heated with triethylamine in dimethyl sulfoxide (DMSO) followed by dechelation and deprotection to afford 8-methoxyquinolone **4**, **5**, or **6** (Chart 4).

The preparation of 8-methylquinolone **7** is shown in Chart 5. Buchwald–Hartwig coupling of the secondary amine **17a** and 7-bromo-8-methylquinolone **25**, followed by hydrolysis and deprotection, gave **7**. 7-Bromo-8-methylquinolone **25** was prepared from commercially available benzoic acid **26** by introduction of aminoacrylate, then cyclopropyl amine, and cyclization (Chart 6).

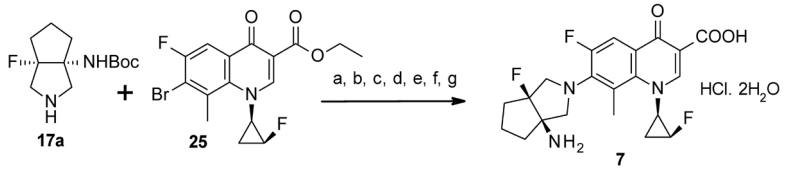
Conclusion

8-Methoxy- or 8-methylquinolone derivatives bearing bicy-



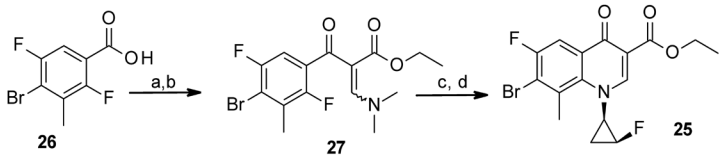
Reagents and conditions: (a) Et_3N, DMSO, heat; (b) conc. HCl; (c) pH 7.4, 32% (**4**) or 41% (**5** from **16b**) or 46% (**6**).

Chart 4. Synthesis of 8-Methoxyquinolones **4**–**6**



Reagents and conditions: (a) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3–1,4-dioxane, heat; (b) 1 N $NaOH_{aq}$–EtOH; (c) conc. HCl; (d) pH 7.4; (e) crystallization; (f) 1 N HCl (1 eq); (g) recrystallization, 38% from **17a**.

Chart 5. Synthesis of 8-Methylquinolone **7**



Reagents and conditions: (a) Thionyl chloride–DMF, reflux; (b) ethyl 3-dimethylaminoacrylate, Et_3N–THF, reflux, 71% from **26**; (c) (1*R*,2*S*)-2-fluorocyclopropylamine, Et_3N–DCM; (d) Cs_2CO_3–DMF, 26% from **27**.

Chart 6. Synthesis of 7-Bromo-8-methylquinolone **25**

clic amines at the C-7 position were synthesized, evaluated for antibacterial activities, and assessed for physicochemical and pharmacokinetic properties and preliminary safety. These analogs exhibited broad-spectrum activity against pathogens of major hospital- and community-acquired infections including infections by drug-resistant strains.

Specifically, compound **7** exhibited better activity than LVFX and MFLX against streptococci, staphylococci, enterococci, *E. coli*, *A. baumannii*, and anaerobes. Compound **7** also showed potent *in vivo* antibacterial activity in a murine RTI model and a rodent UTI model. Moreover, compound **7** exhibited excellent PK and toxicological profiles. As already reported by Higuchi *et al.*,[20] compound **7** showed excellent *in vitro* and *in vivo* antibacterial activity against multidrug-resistant *A. baumannii*. Thus, compound **7** was selected as a candidate for further evaluation as a new-generation, broad-spectrum quinolone antibiotic. We also expect compound **7** to potentially become an option for antibacterial therapy against multidrug-resistant *A. baumannii*.

Experimental

General Melting points were recorded on a Yanaco MP-500D melting point apparatus and were 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 JNMEX400 spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standards. Significant ^{1}H-NMR peaks are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), and coupling constant(s) in hertz. High-resolution (HR)-MS were obtained on a JEOL JMS-700 mass spectrometer under electron impact ionization, electrospray ionization, or FAB ionization conditions. The high-resolution mass spectra were recorded on a JEOL JMS-100LP spectrometer. Elemental analyses are indicated by the symbols of the elements; analytical results were within 0.4% of the theoretical values. Purities of ≥95% were determined by elemental analysis for all tested compounds. Column chromatography refers to flash column chromatography conducted on Merck silica gel 60, 230–400 mesh ASTM. TLC was performed with Merck silica gel 60 F_{254} TLC plates, and compound visualization was accomplished with a 5% solution of molybdophosphoric acid in ethanol, a UV lamp, iodine, or Wako ninhydrin spray.

(3*S*)-3-[3-(*tert*-Butyldimethylsilyloxy)-1-propyl]-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic Acid *tert*-Butyl Ester (9) (3*S*)-3-(3-Hydroxy-1-propyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic acid *tert*-butyl ester (**8**, 46 g, 0.13 mol) and imidazole (11.9 g, 0.13 mol) were dissolved in *N*,*N*-dimethylformamide (DMF, 600 mL). After addition of TBS-Cl (23.2 g, 0.15 mol) under ice-cooling, the mixture was stirred at room temperature for 59.5 h. The reaction solution was extracted with a 10% citric acid solution and ethyl acetate. The organic layer was then sequentially washed with saturated sodium bicarbonate, water, and brine; dried over anhydrous sodium sulfate; and filtered. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 9 : 1–2 : 1) to give 29.7 g (49%) of the title compound (**9**) as a pale yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37–7.22 (5H, m), 5.48 (1H, q, J = 7.11 Hz), 3.58 (2H, t, J = 6.13 Hz),

3.34 (1H, d, $J = 10.05$ Hz), 3.12 (1H, d, $J = 10.05$ Hz), 2.94 (1H, d, $J = 16.91$ Hz), 2.31 (1H, d, $J = 17.16$ Hz), 1.86–1.74 (1H, m), 1.72–1.62 (1H, m), 1.51 (3H, d, $J = 7.11$ Hz), 1.49–1.24 (2H, m), 1.33 (9H, s), 0.88 (9H, s), 0.03 (6H, s). MS (electrospray ionization (ESI)) *m/z*: 462 $(M + H)^+$. HR-MS (ESI) *m/z*: 462.3078 $(M + H)^+$ (Calcd for $C_{26}H_{44}NO_4Si$: 462.3039). IR (attenuated total reflectance (ATR)) cm^{-1}: 2948, 2928, 2857, 1721, 1681, 1426, 1299, 1250, 1219, 1156, 1116, 1089, 1080, 1034.

(3*S*)-3-[3-(*tert*-Butyldimethylsilyloxy)-1-propyl]-4-fluoro-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic Acid *tert*-Butyl Ester (11a) (3*S*)-3-[3-(*tert*-Butyldimethyl silyloxy)-1-propyl]-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic acid *tert*-butyl ester (**9**, 30 g, 65 mmol) was dissolved in tetrahydrofuran (THF, 280 mL), and the atmosphere was replaced with argon. Then, lithium hexamethyldisilazide (1.0 M solution in THF) (78.0 mL) was added dropwise at −15°C, and the mixture was stirred at −5°C for 30 min. After cooling again to −15°C, a solution of *N*-fluorobenzenesulfonimide (26.6 g, 84 mmol) in THF (220 mL) was added dropwise, and the mixture was stirred at room temperature for 17 h. The reaction solution was extracted with a 10% citric acid solution and ethyl acetate. Then, the organic layer was washed with brine, dried over anhydrous sodium sulfate, and filtered. The solvent was then evaporated under reduced pressure. The residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 9 : 1–8 : 2) to give 8.15 g (26%) of the title compound (**11a**) as a pale yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37–7.23 (5H, m), 5.53–5.44 (1H, m), 5.18 (1H, d, $J = 51.72$ Hz), 3.64–3.52 (2H, m), 3.32–3.19 (2H, m), 1.92–1.65 (2H, m), 1.55 (3H, d, $J = 4.66$ Hz), 1.33 (9H, s), 0.88 (9H, s), 0.03 (6H, s). MS (FAB) *m/z*: 480 $(M + H)^+$. IR (ATR) cm^{-1}: 3421, 2977, 2935, 2877, 1698, 1454, 1369, 1309, 1249, 1153, 1058, 1035, 1006, 842.

(3*S*)-4-Fluoro-3-(3-hydroxy-1-propyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic Acid *tert*-Butyl Ester (12a) (3*S*)-3-[3-(*tert*-Butyldimethylsilyloxy)-1-propyl]-4-fluoro-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic acid *tert*-butyl ester (**11a**, 8.15 g, 17 mmol) was dissolved in THF (25.0 mL). Acetic acid (22.0 mL) and tetrabutylammonium fluoride (TBAF, 1.0 M solution in THF, 25.0 mL) were added under ice-cooling, and the mixture was stirred at room temperature for 21.5 h. The reaction solution was extracted with a 10% citric acid solution and ethyl acetate. The organic layer was then sequentially washed with saturated sodium bicarbonate, water, and brine; dried over anhydrous sodium sulfate; and filtered. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 9 : 1–8 : 2–1 : 1) to give 5.77 g (93%) of the title compound (**12a**) as a pale yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37–7.22 (5H, m), 5.48 (1H, q, $J = 7.03$ Hz), 5.20 (1H, d, $J = 51.48$ Hz), 3.69–3.59 (2H, m), 3.31–3.21 (2H, m), 1.95–1.72 (2H, m), 1.68–1.43 (2H, m), 1.56 (3H, d, $J = 7.11$ Hz), 1.33 (9H, s).

(3*S*)-3-(3-Phenylsulfonyloxy-1-propyl)-4-fluoro-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic Acid *tert*-Butyl Ester (13a) (3*S*)-4-Fluoro-3-(3-hydroxy-1-propyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic acid *tert*-butyl ester (**12a**, 12.20 g, 33.4 mmol) was dissolved in dichloromethane (DCM, 400 mL). Phenylsulfonyl chloride (9.06 mL, 79.6 mmol), triethylamine (10.7 mL, 76.8 mmol), and 4-dimethylaminopyridine (DMAP, 2.04 g, 16.7 mmol) were added under ice-cooling, and the mixture was stirred at room temperature for 12.5 h. Saturated aqueous sodium bicarbonate was added to the reaction solution, and the mixture was stirred for 30 min, followed by extraction with DCM. The organic layer was sequentially washed with a 10% citric acid solution and brine, dried over anhydrous sodium sulfate, and filtered. The solvent was then evaporated under reduced pressure. The residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 8 : 2–1 : 1) to give 11.7 g (69%) of the title compound (**13a**) as a pale yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.94–7.87 (2H, m), 7.71–7.63 (1H, m), 7.60–7.53 (2H, m), 7.37–7.23 (5H, m), 5.46 (1H, q, $J = 7.11$ Hz), 5.15 (1H, d, $J = 51.48$ Hz), 4.10–3.98 (2H, m), 3.26–3.15 (2H, m), 1.88–1.50 (4H, m), 1.55 (3H, s), 1.30 (9H, s).

(1*S*,5*R*)-5-Fluoro-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic Acid *tert*-Butyl Ester (14a) (3*S*)-3-(3-Phenylsulfonyloxy-1-propyl)-4-fluoro-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic acid *tert*-butyl ester (**13a**, 10.9 g, 21.6 mmol) was dissolved in THF (350 mL), and the atmosphere was replaced with argon. Then, potassium hexamethyldisilazide (0.5 M solution in toluene, 86.5 mL) was added dropwise at −15°C, and the mixture was stirred at 0°C for 1.5 h. After cooling to −10°C, saturated aqueous ammonium chloride (100 mL) was added dropwise, and the mixture was stirred at room temperature for 30 min. The reaction solution was extracted with a 10% citric acid solution and ethyl acetate. The organic layer was then washed with brine, dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 9 : 1–7 : 1) to give 4.36 g (56%) of the title compound (**14a**) as a pale yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.38–7.25 (5H, m), 5.58–5.49 (1H, m), 3.63 (1H, d, $J = 10.3$ Hz), 2.91 (1H, dd, $J = 10.3$, 3.2 Hz), 2.67–2.56 (1H, m), 2.50–2.38 (1H, m), 2.26–2.09 (1H, m), 2.06–1.94 (1H, m), 1.74–1.66 (1H, m), 1.54 (3H, d, $J = 7.1$ Hz), 1.50–1.40 (1H, m), 1.34 (9H, s). MS (ESI) *m/z*: 348 $(M + H)^+$. HR-MS (ESI) *m/z*: 348.1983 $(M + H)^+$ (Calcd for $C_{20}H_{27}FNO_3$: 348.1975). IR (ATR) cm^{-1}: 2974, 1729, 1686, 1278, 1242, 1156, 1122, 850.

(1*R*,5*R*)-1-(*tert*-Butoxycarbonylamino)-5-fluoro-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (15a) (1*S*,5*R*)-5-Fluoro-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]-octan-1-ylcarboxylic acid *tert*-butyl ester (**14a**, 4.36 g, 12.5 mmol) was dissolved in DCM (70 mL). Trifluoroacetic acid (70 mL) was added dropwise, and the mixture was stirred at room temperature for 6 h. The solvent was evaporated under reduced pressure, and then the residue was azeotropically distilled with toluene to give carboxylic acid (3.70 g). The resulting carboxylic acid was dissolved in toluene. Triethylamine (3.51 mL, 25.2 mmol) and diphenylphosphoryl azide (2.98 mL, 13.8 mmol) were added, and the mixture was heated to reflux for 5 h. The solvent was evaporated under reduced pressure. Then, 1,4-dioxane (110 mL) and 6 N hydrochloric acid (110 mL) were added to the residue, and the mixture was stirred at 60°C for 2.5 h. After extraction with water and ethyl acetate, the aqueous layer was made alkaline with a saturated sodium hydroxide solution and extracted twice with chloroform. The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and then the solvent was evaporated under reduced pressure. Di-*tert*-butyl dicarbonate (11.05 g, 50.6 mmol) was added to the residue, and the mixture was stirred at 75°C

for 6 h. The reaction solution was concentrated under reduced pressure, and then the residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 9 : 1–1 : 1) to give 3.69 g (81%) of the title compound (**15a**) as a pale yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37–7.23 (5H, m), 5.50 (1H, q, J = 7.1 Hz), 5.22 (1H, brs), 3.34 (2H, s), 2.49–2.37 (1H, m), 2.32–2.03 (3H, m), 2.02–1.90 (1H, m), 1.51 (3H, d, J = 7.1 Hz), 1.55–1.48 (1H, m), 1.35 (9H, s). MS (ESI) m/z: 363 $(M+H)^+$. HR-MS (ESI) m/z: 363.2110 $(M+H)^+$ (Calcd for $C_{20}H_{28}FN_2O_3$: 363.2084).

(1*R*,5*S*)-1-(*tert*-Butoxycarbonylamino)-5-fluoro-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (16a) (1*R*,5*R*)-1-(*tert*-Butoxycarbonylamino)-5-fluoro-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (**15a**, 3.69 g, 10.2 mmol) was dissolved in THF (200 mL). A 1.20 M solution of a borane–THF complex in THF (42.4 mL, 50.9 mmol) was added dropwise under ice-cooling, and the mixture was stirred for 2 h while gradually warming to room temperature. The solvent was evaporated under reduced pressure. Under ice-cooling, 90% aqueous ethanol (100 mL) and triethylamine (100 mL) were added to the residue, and the mixture was heated to reflux for 2 h. The solvent was evaporated under reduced pressure, and the residue was extracted with saturated aqueous sodium bicarbonate and DCM. The target substance was extracted from the aqueous layer with DCM. The organic layers were combined, washed with brine, and dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure, and the resulting residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 95 : 5–90 : 10) to give 3.33 g (94%) of the title compound (**16a**) as a pale yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.32–7.18 (5H, m), 5.38 (1H, brs), 3.22 (1H, q, J = 6.37 Hz), 2.92–2.57 (4H, m), 2.12–1.86 (4H, m), 1.80–1.67 (1H, m), 1.63–1.52 (3H, m), 1.42 (9H, s), 1.32 (3H, d, J = 6.37 Hz). MS (ESI) m/z: 349 $(M+H)^+$. HR-MS (ESI) m/z: 349.2315 $(M+H)^+$ (Calcd for $C_{20}H_{30}FN_2O_2$: 349.2291). IR (ATR) cm^{-1}: 3458, 2972, 1711, 1487, 1365, 1249, 1161.

(1*R*,5*S*)-1-(*tert*-Butoxycarbonylamino)-5-fluoro-3-azabicyclo[3.3.0]octane (17a) (1*R*,5*S*)-1-(*tert*-Butoxycarbonylamino)-5-fluoro-3-[(1*R*)-1-phenylethyl]-3-azabicyclo-[3.3.0]octane (**16a**, 700 mg, 2.0 mmol) was dissolved in ethanol (30 mL). Ten percent palladium–carbon (50% wet, 1.01 g) was added, and the mixture was stirred under a hydrogen atmosphere at 50°C for 15 h. The catalyst was removed by filtration, and then the filtrate was concentrated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (DCM–methanol = 98 : 2–>95 : 5) to give 446 mg (91%) of the title compound (**17a**) as a pale yellow oil.

$[\alpha]_D^{23}$ −15° (c = 0.100, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.29 (1H, brs), 3.47–3.18 (2H, m), 2.93–2.79 (2H, m), 2.15–1.71 (6H, m), 1.45 (9H, s). MS (FAB) m/z: 245 $(M+H)^+$. HR-MS (ESI) m/z: 245.16453 $(M+H)^+$ (Calcd for $C_{12}H_{22}FN_2O_2$: 245.16653). IR (ATR) cm^{-1}: 3280, 3212, 2952, 1702, 1560, 1284, 1245, 1176, 1101, 1035, 1016, 966, 917, 889, 867, 701.

7-[(1*R*,5*S*)-1-Amino-5-fluoro-3-azabicyclo[3.3.0]-octan-3-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (4) (1*R*,5*S*)-1-(*tert*-Butoxycarbonylamino)-5-fluoro-3-azabicyclo[3.3.0]-octane (**17a**, 1.32 g, 5.40 mmol), 6,7-difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid-BF_2 chelate (**24**, 2.93 g, 8.12 mmol), and triethylamine (3.30 mL, 23.7 mmol) were dissolved in DMSO (30 mL), and the solution was heated with stirring in an oil bath at 40°C. After 19 h of stirring, 6,7-difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid-BF_2 chelate (**24**, 1.00 g, 2.77 mmol) was added, then this reaction mixture was heated with stirring in an oil bath at 40°C for 16 h. A mixed solution of ethanol–water = 4 : 1 (50 mL) and triethylamine (5 mL) was added to the reaction solution, and the mixture was heated to reflux in an oil bath at 90°C for 1.5 h. The reaction solution was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with a 10% citric acid solution, water, and brine. The organic layer was dried over anhydrous sodium sulfate and filtered, and then the solvent was evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (DCM–methanol = 99 : 1) to afford 3.30 g of the amine coupling product as a yellow oil. The amine coupling compound was dissolved in concentrated hydrochloric acid (20 mL) under ice-cooling. After stirring at room temperature for 1 h, the reaction solution was washed with chloroform. The aqueous layer was adjusted to pH 12.0 with a 10 M sodium hydroxide solution under ice-cooling and then adjusted to pH 7.4 with hydrochloric acid. The target substance was then extracted from the aqueous layer with chloroform. The organic layers were dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure, and the resulting residue was purified by recrystallization from ethanol to give 769 mg (32%) of the title compound (**4**) as a pale yellow powder. mp: 191–195°C (dec.). $[\alpha]_D^{24}$ +97° (c = 0.100, 0.1 N NaOH). ^{1}H-NMR (400 MHz, 0.1 N NaOD) δ: 8.48 (1H, d, J = 1.23 Hz), 7.70 (1H, d, J = 13.73 Hz), 5.07–4.85 (1H, m), 4.10–4.02 (1H, m), 3.93–3.78 (2H, m), 3.70–3.53 (2H, m), 3.66 (3H, s), 2.21–2.04 (2H, m), 2.01–1.83 (2H, m), 1.83–1.46 (4H, m). MS (FAB) m/z: 438 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{22}F_3N_3O_4 \cdot 0.6H_2O$: C, 56.27%; H, 5.22%; F, 12.72%; N, 9.37%. Found: C, 56.03%; H, 5.51%; F, 12.79%; N, 9.66%. IR (ATR) cm^{-1}: 2958, 2871, 1724, 1621, 1513, 1452, 1319, 1056, 929, 804.

***tert*-Butyl (3*S*)-4-Chloro-3-(3-hydroxy-1-propyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylate (12b)** Starting with **9** (960 mg, 2.08 mmol) and NCS (333 mg, 2.50 mmol) and following the procedure for the preparation of **11a** gave **11b** as a pale yellow solid. No further purification was attempted on this compound, which was used directly in the next step. Starting with **11b** and following the procedure for the preparation of **12a** afforded **12b** (506 mg, 64% from **9**) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.34–7.26 (5H, m), 5.49–5.40 (1H, m), 4.75 (1H, s), 3.68–3.61 (2H, m), 3.39–3.34 (2H, m), 3.24 (0.75H, d, J = 10.0 Hz), 3.14 (0.25H, d, J = 10.9 Hz), 1.95–1.80 (2H, m), 1.68–1.40 (2H, m), 1.52 (3H, d, J = 7.1 Hz), 1.28 (9H, s). MS (ESI) m/z: 382 $(M+H)^+$.

***tert*-Butyl (1*S*,5*R*)-5-chloro-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylate (14b)** Starting with **12b** (270 mg, 0.71 mmol) and following the procedure for the preparation of **13a** gave **13b** as a pale yellow solid. No further purification was attempted on this compound, which was used directly in the next step. Starting with **13b** and following the procedure for the preparation of **14a** afforded **14b** (73 mg, 28% from **12b**) as a pale yellow solid. ^{1}H-NMR

($CDCl_3$) δ: 7.37–7.26 (5H, m), 5.54 (1H, q, $J=7.1$ Hz), 3.51 (1H, d, $J=10.7$ Hz), 3.02 (1H, d, $J=10.7$ Hz), 2.76–2.71 (1H, m), 2.52–2.45 (1H, m), 2.38–2.30 (1H, m), 2.02–1.96 (1H, m), 1.74–1.60 (2H, m), 1.53 (3H, d, $J=7.1$ Hz), 1.45 (9H, s). MS (ESI) *m/z*: 364 $(M+H)^+$. HR-MS (ESI) *m/z*: 364.1710 $(M+H)^+$ (Calcd for $C_{20}H_{27}ClNO_3$: 364.1680). IR (ATR) cm^{-1}: 2968, 1730, 1691, 1249, 1239, 1157, 1122.

(1*R*,5*R*)-1-(*tert*-Butoxycarbonylamino)-5-chloro-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (15b) Starting with **14b** (332 mg, 0.912 mmol) and following the procedure for the preparation of **15a** gave **15b** (222 mg, 64%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 7.37–7.26 (5H, m), 5.50 (1H, q, $J=6.9$ Hz), 5.25 (1H, brs), 3.66 (1H, brd, $J=10.0$ Hz), 2.96 (1H, d, $J=6.8$ Hz), 2.76–2.69 (1H, m), 2.55–2.51 (1H, m), 2.18–2.08 (1H, m), 1.98–1.84 (2H, m), 1.51 (3H, d, $J=7.1$ Hz), 1.65–1.50 (1H, m), 1.40 (9H, s). MS (ESI) *m/z*: 379 $(M+H)^+$.

(1*R*,5*R*)-1-(*tert*-Butoxycarbonylamino)-5-chloro-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (16b) Starting with **15b** (217 mg, 0.573 mmol) and following the procedure for the preparation of **16a** gave **16b** (67.3 mg, 32%) as a colorless oil. $[\alpha]_D^{25.1}$ +103.5° ($c=0.228$, 0.1 N NaOH). ^{1}H-NMR ($CDCl_3$) δ: 7.29–7.19 (5H, m), 5.53 (1H, brs), 3.22 (1H, q, $J=6.8$ Hz), 3.07 (1H, brd, $J=8.8$ Hz), 2.93 (1H, brd, $J=8.7$ Hz), 2.82–2.71 (1H, m), 2.64–2.57 (1H, m), 2.27–2.15 (2H, m), 2.09–2.06 (2H, m), 1.74–1.67 (2H, m), 1.55 (9H, s), 1.31 (3H, d, $J=6.6$ Hz). MS (ESI) *m/z*: 365 $(M+H)^+$. HR-MS (ESI) *m/z*: 365.2000 $(M+H)^+$ (Calcd for $C_{20}H_{30}ClN_2O_2$: 365.1996). IR (ATR) cm^{-1}: 3428, 2971, 2927, 1716, 1481, 1453, 1365, 1160.

7-[(1*R*,5*R*)-1-Amino-5-chloro-3-azabicyclo[3.3.0]octan-3-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (5) Starting with **16b** (63.0 mg, 0.17 mmol) and following the procedure for the preparation of **17a** gave **17b** as a colorless oil. No further purification was attempted on this compound, which was used directly in the next step. Starting with **17b** and following the procedure for the preparation of **4** afforded **5** (33.9 mg, 41% from **16b**) as a colorless powder. mp: 161–163°C. $[\alpha]_D^{24}$ +100.8° ($c=0.104$, 0.1 N NaOH). ^{1}H-NMR (400 MHz, 0.1 N NaOD) δ: 8.48 (1H, s), 7.70 (1H, d, $J=14.2$ Hz), 5.07–4.85 (1H, m), 4.19–4.08 (2H, m), 3.92 (1H, d, $J=11.8$ Hz), 3.76–3.63 (5H, m), 2.48–2.41 (1H, m), 2.30–2.25 (1H, m), 2.08–1.87 (4H, m), 1.70–1.51 (2H, m), 1.19 (1.8H, t, $J=7.2$ Hz). *Anal.* Calcd for $C_{21}H_{22}ClF_4N_3O_3\cdot 0.6EtOH$: C, 55.38%; H, 5.36%; N, 8.73%; F, 7.89%; Cl, 7.36%. Found: C, 55.24%, H, 4.91%; N, 8.85%; F, 8.27%; Cl, 6.92%. MS (ESI) *m/z*: 454 $(M+H)^+$. HR-MS (ESI) *m/z*: 454.1359 $(M+H)^+$ (Calcd for $C_{21}H_{23}ClF_2N_3O_4$: 454.1345). IR (ATR) cm^{-1}: 3384, 3075, 2880, 1728, 1621, 1513, 1453, 1360, 1318, 1188, 1136, 1120, 1103, 1056.

(3*S*)-3-(2-Methoxycarbonyl-1-ethyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic Acid *tert*-Butyl Ester (10) Carbon tetrachloride (550 mL), acetonitrile (550 mL), and water (550 mL) were dissolved in (3*S*)-3-(3-hydroxy-1-propyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic acid *tert*-butyl ester (**8**, 38.2 g, 0.110 mol), and ruthenium (III) chloride hydrate (456 mg, 2.20 mmol) and sodium periodide (94.1 g, 0.440 mol) were sequentially added. The mixture was stirred in a water bath with a thermostatic circulator at 15°C for 2.5 h while maintaining the internal temperature at 20–25°C. The reaction solution was cooled in an ice bath and a 1 N hydrochloric acid solution (2.2 L) was added at an internal temperature of 10°C or lower, followed by extraction with 2 L of chloroform. The aqueous layer was extracted with chloroform (1 L×3). Then, the organic layers were combined, washed with brine (2 L×2), and dried over anhydrous sodium sulfate. After filtration, the solvent was concentrated under reduced pressure. The resulting crude product was dissolved in DMF (370 mL). Sodium bicarbonate (37.9 g, 0.451 mol) and methyl iodide (70.7 g, 0.498 mol) were added with stirring at room temperature, and the mixture was stirred for 3 d. The reaction solution was poured into ice water (1.8 L), followed by extraction with ethyl acetate (1.8, 0.5 L). The organic layers were combined, washed with brine, and then dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated under reduced pressure; the residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 1 : 1), and the fraction containing the target substance was concentrated under reduced pressure. The resulting solid was dissolved in ethyl acetate, washed with a 10% sodium thiosulfate solution, and dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated under reduced pressure, and the resulting solid was dried to give 35.0 g (85%) of the title compound **10** as a pale yellow brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.35–7.25 (5H, m), 5.48 (1H, q, $J=7.1$ Hz), 3.68 (3H, s), 3.33 (1H, d, $J=10.3$ Hz), 3.13 (1H, d, $J=10.3$ Hz), 2.93 (1H, d, $J=16.8$ Hz), 2.34–2.20 (3H, m), 2.13–1.94 (2H, m), 1.51 (3H, d, $J=7.1$ Hz), 1.32 (9H, s). MS (ESI) *m/z*: 376 $(M+H)^+$. HR-MS (ESI) *m/z*: 376.2140 $(M+H)^+$ (Calcd for $C_{21}H_{30}NO_5$: 376.2124). IR (ATR) cm^{-1}: 2977, 1740, 1712, 1670, 1304, 1170, 1151.

(1*S*,5*R*)-4,6-Dioxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic Acid *tert*-Butyl Ester (18) (3*S*)-3-(2-Methoxycarbonyl-1-ethyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylic acid *tert*-butyl ester (**10**, 35.0 g, 93.2 mmol) was dissolved in THF (1 L). A 2 M lithium diisopropylamide–heptane–THF–ethylbenzene solution (100 mL, 200 mmol) was added dropwise in a nitrogen atmosphere at an internal temperature of −69°C over 30 min. After stirring at the same temperature for 1 h, the reaction solution was poured into a 1 N hydrochloric acid solution (2 L) in an ice bath, followed by extraction with ethyl acetate (2 L, then 1 L). The organic layers were combined, washed with brine, and dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated under reduced pressure, and the precipitated solid was collected by filtration to give 22.2 g (69%) of the title compound **18** as pale red crystals. The filtrate was further concentrated to give 2.88 g of the title compound as pale red crystals. The filtrate was concentrated under reduced pressure, and the residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 1 : 1) to give 2.09 g (6.5%) of the title compound **18** as colorless crystals. $[\alpha]_D^{25}$ −46.5° ($c=1.009$, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37–7.26 (5H, m), 5.50 (1H, q, $J=7.1$ Hz), 3.38 (1H, d, $J=10.5$ Hz), 3.23 (1H, d, $J=10.5$ Hz), 2.55–2.35 (3H, m), 2.05–1.94 (1H, m), 1.53 (3H, d, $J=7.1$ Hz), 1.38 (9H, s). MS (ESI) *m/z*: 344 $(M+H)^+$. HR-MS (ESI) *m/z*: 344.1870 $(M+H)^+$ (Calcd for $C_{20}H_{26}NO_4$: 344.1862). IR (ATR) cm^{-1}: 3442, 2975, 1742, 1732, 1671, 1424, 1366, 1261, 1244, 1221, 1170, 1155, 1144.

(1*S*,5*R*)-5-Methyl-4,6-dioxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic Acid *tert*-Butyl Ester

(19) DMF (2.0 mL) was added to sodium hydride (152 mg, 3.48 mmol) in an argon atmosphere. A solution of (1*S*,5*R*)-4,6-dioxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]-octan-1-ylcarboxylic acid *tert*-butyl ester (**18**, 1.00 g, 2.91 mmol) in DMF (8.0 mL) was added dropwise to this suspension under ice-cooling, and the mixture was stirred at 0°C for 30 min. Subsequently, methyl iodide (0.217 mL, 3.49 mmol) was added dropwise under ice-cooling, and the mixture was stirred at room temperature for 2.5 h. The reaction solution was ice-cooled, and then the reaction was quenched with water, followed by extraction with ethyl acetate. The organic layer was then washed with water and brine, dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 2 : 1–1 : 1) to give 0.79 g (76%) of the title compound **19** as a pale yellow solid. No further purification was attempted on this compound, which was used directly in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.41–7.23 (5H, m), 5.48 (1H, q, J = 6.62 Hz), 3.40 (1H, d, J = 10.54 Hz), 3.13 (1H, d, J = 10.54 Hz), 2.63–2.40 (3H, m), 1.96–1.83 (1H, m), 1.54 (3H, d, J = 7.11 Hz), 1.39 (9H, s), 1.22 (3H, s).

(1*S*,5*R*)-6,6-Ethanediyldimercapto-5-methyl-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic Acid Methyl Ester (20) (1*S*,5*R*)-5-Methyl-4,6-dioxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic acid *tert*-butyl ester (**19**, 0.28 g, 0.78 mmol) was dissolved in toluene (14 mL). Toluenesulfonic acid monohydrate (155 mg, 0.81 mmol) and ethanedithiol (0.14 mL, 1.7 mmol) were added, and the mixture was heated to reflux for 9 h. The solvent was evaporated under reduced pressure, and the resulting residue was subjected to silica gel column chromatography (DCM–methanol = 98 : 2) to give the target 1-position carboxylic acid (289 mg) as a pale yellow solid. The carboxylic acid (289 mg) was dissolved in THF (10 mL) and methanol (3.0 mL). Trimethylsilyldiazomethane (1.7 mL) was added under ice-cooling, and the mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, and the resulting residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 3 : 2) to give 267 mg (87%) of the title compound **20** as a pale yellow solid. No further purification was attempted on this compound, which was used directly in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.39–7.23 (5H, m), 5.52 (1H, q, J = 6.86 Hz), 3.60 (3H, s), 3.55 (1H, d, J = 10.05 Hz), 3.44–3.31 (1H, m), 3.28–3.19 (1H, m), 3.16 (1H, d, J = 10.05 Hz), 2.79–2.70 (1H, m), 2.54–2.44 (1H, m), 2.26–2.15 (1H, m), 1.81–1.70 (1H, m), 1.59–1.52 (2H, m), 1.56 (3H, d, J = 7.11 Hz), 1.33 (3H, s).

(1*S*,5*R*)-5-Methyl-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic Acid Methyl Ester (21) (1*S*,5*R*)-6,6-Ethanediyldimercapto-5-methyl-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic acid methyl ester (**20**, 266 mg, 0.68 mmol) was dissolved in ethanol (10 mL). Raney nickel (2.0 mL) was added dropwise, and the mixture was heated to reflux for 5.5 h. The catalyst was removed by filtration, and then the filtrate was concentrated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (hexane–ethyl acetate = 7 : 3–1 : 1) to give 133 mg (65%) of the title compound **21** as a pale yellow solid. No further purification was attempted on this compound, which was used directly in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.39–7.24 (5H, m), 5.56–5.44 (1H, m), 3.64 (3H, s), 3.51 (1H, d, J = 10.05 Hz), 3.02–2.96 (1H, m), 2.49–2.26 (2H, m), 1.91–1.44 (4H, m), 1.52 (3H, d, J = 7.35 Hz), 1.12 (3H, s).

(1*S*,5*S*)-1-(*tert*-Butoxycarbonylamino)-5-methyl-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (22) (1*S*,5*R*)-5-Methyl-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic acid methyl ester (**21**, 130 mg, 0.43 mmol) was dissolved in methanol (5.0 mL). A 1 N NaOH solution (1.5 mL) was added dropwise under ice-cooling, and the mixture was stirred at room temperature for 4 h. Then, a 1 N NaOH solution (1.5 mL) was added dropwise, and the mixture was stirred at room temperature for 15 h. NaOH (93 mg) was again added, and the mixture was stirred at room temperature for 6 h. NaOH (90 mg) was added again, and the mixture was stirred at room temperature for 4 h, and then at 50°C for 1 h. The reaction solution was made weakly acidic with hydrochloric acid, and the solvent was evaporated under reduced pressure. The resulting residue was extracted with DCM and dilute hydrochloric acid. The organic layer was dried over anhydrous sodium sulfate and filtered, and then the solvent was evaporated under reduced pressure. The resulting residue was dissolved in toluene (5.0 mL). Triethylamine (0.132 mL, 0.95 mmol) and diphenylphosphoryl azide (0.111 mL, 0.52 mmol) were added, and the mixture was heated to reflux for 3 h. The reaction solution was extracted with ethyl acetate and saturated aqueous sodium bicarbonate. Then, the organic layer was washed with brine, dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure. 1,4-Dioxane (2.0 mL) and 6 N hydrochloric acid (2.0 mL) were added to the resulting residue, and the mixture was stirred at 50°C for 15 h. After extraction with water and ethyl acetate, the aqueous layer was made alkaline with a saturated NaOH solution and extracted with chloroform twice. The organic layers were combined, dried over anhydrous sodium sulfate, and filtered, and then the solvent was evaporated under reduced pressure. Toluene (3.0 mL) and Red-Al™ (65% solution in toluene, 0.50 mL) were sequentially added to the resulting residue, and the mixture was stirred at 80°C for 2.5 h. A 3 N NaOH solution was added to the reaction solution under ice-cooling, and the layers were separated with toluene. The organic layer was dried over anhydrous sodium sulfate and filtered, and then the solvent was evaporated under reduced pressure. The resulting residue was dissolved in DCM (10 mL) and methanol (5.0 mL). Di-*tert*-butyl dicarbonate (560 mg, 2.57 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction solution was subjected to silica gel column chromatography (DCM–methanol = 98 : 2) to give 77 mg (52%) of the title compound **22** as a pale yellow solid. No further purification was attempted on this compound, which was used directly in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.33–7.16 (5H, m), 4.79 (1H, br s), 3.17–3.00 (1H, m), 2.74–2.58 (2H, m), 2.53–2.44 (1H, m), 2.27–2.13 (1H, m), 2.08–1.89 (2H, m), 1.74–1.62 (2H, m), 1.60–1.24 (2H, m), 1.41 (9H, s), 1.28 (3H, d, J = 6.59 Hz), 1.07 (3H, s).

(1*S*,5*S*)-1-(*tert*-Butoxycarbonylamino)-5-methyl-4-oxo-3-azabicyclo[3.3.0]octane (23) (1*S*,5*S*)-1-(*tert*-Butoxycarbonylamino)-5-methyl-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (**22**, 77 mg, 0.22 mmol) was dissolved in ethanol (6.0 mL). 10% palladium–carbon (50% wet, 69 mg)

was added, and the mixture was stirred in a hydrogen atmosphere at 45°C for 19.5 h. After removing the catalyst by filtration, the filtrate was concentrated under reduced pressure to give 50 mg (95%) of the title compound **23** as a pale yellow oil. No further purification was attempted on this compound, which was used directly in the next step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.68 (1H, br s), 3.36–3.19 (1H, m), 3.00–2.58 (4H, m), 2.40–1.89 (4H, m), 1.81–1.26 (2H, m), 1.44 (9H, s), 1.07 (3H, s).

7-[(1*R*,5*R*)-1-Amino-3-aza-5-methylbicyclo[3.3.0]octan-3-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropane]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (6) Starting with **23** (79.1 mg, 0.22 mmol) and following the procedure for the preparation of **4** afforded **6** (47 mg, 46%) as a pale yellow solid. mp: 109–113°C (dec.). $[\alpha]_D^{24}$ +78.2° ($c = 0.135$, 0.1 N NaOH). ^{1}H-NMR (400 MHz, 0.1 N NaOD) δ: 8.45 (1H, s), 7.66 (1H, d, $J = 14.5$ Hz), 4.79–4.85 (1H, m), 4.00–4.10 (1H, m), 3.61 (3H, s), 3.51–3.75 (3H, m), 3.34–3.44 (1H, m), 1.94–2.07 (1H, m), 1.43–1.93 (7H, m), 1.10 (3H, s). MS (FAB) *m/z*: 434 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{25}F_2N_3O_4 \cdot 2H_2O$: C, 56.28%; H, 6.23%; F, 8.09%; N, 8.95/%. Found: C, 56.57%; H, 6.24%; F, 8.19%; N, 9.01%. IR (ATR) cm^{-1}: 2942, 2877, 1612, 1573, 1448, 1434, 1392, 1349, 1342, 1311, 1301, 1290, 1272, 821, 804.

Ethyl 2-(4-Bromo-2,5-difluoro-3-methylbenzoyl)-3-dimethylaminoacrylate (27) 2,5-Difluoro-4-bromo-3-methylbenzoic acid (**26**, 10.7 g, 42.4 mmol) was dissolved in toluene (160 mL). Thionyl chloride (5.00 mL, 63.9 mmol) and DMF (5.0 mL) were added, and the mixture was heated to reflux for 2 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in THF (300 mL). Ethyl 3-dimethylaminoacrylate (7.30 mL, 50.9 mmol) and triethylamine (7.60 mL, 54.5 mmol) were added, and the mixture was heated to reflux for 3 h. The solvent was evaporated under reduced pressure, and DCM and water were added to the residue to separate the layers. Then, the organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was subjected to flash column chromatography (hexane–ethyl acetate = 2 : 1–1 : 1–1 : 2) to give the title compound (**27**, 11.4 g, 71%) as a yellow oil. No further purification was attempted on this compound, which was used directly in the next step. ^{1}H-NMR ($CDCl_3$) δ: 7.81–7.74 (1H, m), 7.27–7.16 (1H, m), 4.00 (2H, q, $J = 7.1$ Hz), 3.31 (3H, br s), 2.89 (3H, br s), 2.35 (3H, d, $J = 2.9$ Hz), 0.97 (3H, t, $J = 7.1$ Hz).

Ethyl 7-Bromo-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylate (25) Ethyl 2-(4-bromo-2,5-difluoro-3-methylbenzoyl)-3-dimethylaminoacrylate (**27**, 11.4 g, 30.2 mmol) was dissolved in DCM (200 mL). (1*R*,2*S*)-2-Fluorocyclopropylamine tosylate (8.24 g, 33.3 mmol) was added, and the mixture was cooled to −25°C. Triethylamine (6.60 mL, 47.4 mmol) was added dropwise to the reaction solution at −25°C, and the mixture was stirred at −15°C for 1 h and at 0°C for 2.5 h. The solvent was evaporated under reduced pressure, and ethyl acetate and water were added to the residue to separate the layers. The organic layer was washed with brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give an aminoacrylate as a yellow oil. The resulting aminoacrylate was dissolved in DMF (350 mL). Cesium carbonate (19.8 g, 60.9 mmol) was added, and the mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure, and ethyl acetate and water were added to the residue to separate the layers. The organic layer was washed with brine and dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was subjected to flash column chromatography (hexane–ethyl acetate = 9 : 1–1 : 1–1 : 2) to give the title compound (**25**, 2.98 g, 26%) as a colorless powder. mp: 191–195°C (dec.). $[\alpha]_D^{25}$ −166.5° ($c = 0.159$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.56 (1H, d, $J = 3.2$ Hz), 8.06 (1H, d, $J = 8.1$ Hz), 4.98–4.73 (1H, m), 4.40 (2H, q, $J = 7.1$ Hz), 3.91–3.82 (1H, m), 2.85 (3H, s), 1.61–1.22 (2H, m), 1.41 (3H, t, $J = 7.1$ Hz). MS (ESI) *m/z*: 386 $(M+H)^+$. HR-MS (ESI) *m/z*: 386.0229 $(M+H)^+$ (Calcd for $C_{16}H_{15}BrF_2NO_3$: 386.0203). IR (ATR) cm^{-1}: 3083, 2976, 1724, 1624, 1610, 1456, 1404, 1387, 1312, 1239, 1173, 1126, 1047, 1031, 1022, 1006.

7-[(1*R*,5*S*)-1-Amino-5-fluoro-3-azabicyclo[3.3.0]octan-3-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropane]-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid Hydrochloride Dihydrate (7) To a solution of tris(dibenzylideneacetone)-dipalladium (0) (7.75 g, 8.46 mmol), 4,5-bis(diphenyl)phosphino-9,9-dimethylxanthene (14.7 g, 25.4 mmol), ethyl 7-bromo-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (**25**, 14.2 g, 36.8 mmol) and (1*R*,5*S*)-1-(*tert*-butoxycarbonyl amino)-5-fluoro-3-azabicyclo-[3.3.0]octane (**17a**, 6.90 g, 28.2 mmol) in 1,4-dioxane (345 mL) was added cesium carbonate (18.4 g, 56.5 mmol), and the mixture was stirred at 110°C for 23 h under a nitrogen atmosphere. The reaction mixture was diluted with water (400 mL) and extracted with ethyl acetate (600 mL×1, 250 mL×1). The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (chloroform–methanol = 100 : 0–99 : 1) to afford a solid. To a solution of this solid in ethanol (273 mL) was added a 1 N aqueous solution of NaOH (68.2 mL) in an ice bath, and the mixture was stirred at room temperature for 1 h. To this reaction solution was added ethanol (327 mL) and a 1 N aqueous solution of NaOH (27.3 mL), and the mixture was stirred at room temperature for 13 h. To this reaction solution was added a 1 N aqueous solution of hydrochloric acid (95.5 mL) in an ice bath, and the organic layer was concentrated under reduced pressure. The aqueous solution was diluted with water (150 mL) and extracted with chloroform (250 mL×1, 150 mL×1). The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate = 100 : 0–2 : 1–1 : 5–0 : 100) to afford a pale yellow solid. The solid was dissolved in concentrated hydrochloric acid (30 mL) in an ice bath, and the aqueous solution was washed with chloroform (100 mL×5). To the aqueous layer was added a saturated solution of NaOH to adjust the pH to 12.0. To the solution was added water (1.8 L), and the pH of the basic aqueous solution was adjusted with hydrochloric acid to pH 7.4. The solution was extracted with chloroform (1.5 L×1, 800 mL×1). The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by recrystallization from 2-propanol–methanol = 10 : 1 to afford the crude (6.14 g, 52%) of 7-[(1*R*,5*S*)-1-amino-5-fluoro-3-azabicyclo[3.3.0]-octan-3-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropane]-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid.

Subsequently, the crude product (3.05 g, 7.24 mmol) was dissolved in 1 N hydrochloric acid (7.19 mL). The reaction mixture was stirred at 40°C for 30 min. The mixture was diluted with water (7 mL) and distilled under reduced pressure to afford 3.48 g of crude product. This (1.16 g) was recrystallized from 10% H_2O–isopropanol to afford 0.87 g (73%) of the title compound **7** as colorless powder. mp: 227–230°C (dec.). $[\alpha]_D^{24}$ −153.9° ($c = 0.104$, 0.1 N NaOH). ^{1}H-NMR (400 MHz, 0.1 N NaOD) δ: 8.49 (1H, s), 7.72 (1H, d, $J = 13.8$ Hz), 5.10–4.90 (1H, m), 4.15–4.05 (1H, m), 3.95–3.80 (1H, m), 3.60–3.40 (2H, m), 3.35 (1H, d, $J = 10.5$ Hz), 2.63 (3H, s), 2.00–2.15 (3H, m), 1.90–1.80 (1H, m), 1.80–1.55 (3H, m), 1.35–1.20 (1H, m). MS (FAB) *m/z*: 422 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{22}F_3N_3O_3 \cdot HCl \cdot 2H_2O$: C, 51.07%; H, 5.51%; F, 11.54%; N, 8.51%; Cl, 7.18%. Found: C, 50.85%; H, 5.39%; F, 11.51%; N, 8.48%; Cl, 7.31%. IR (ATR) cm^{-1}: 3477, 3333, 2861, 1696, 1616, 1519, 1450, 1373, 1361, 1317, 1138, 1124, 1026, 981, 969, 805.

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

Animal Experiments The care and use of animals and the experimental protocols were approved by the Experimental Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

Single IV Dose Toxicity The test compounds were dissolved in 0.1 N NaOH in saline at various concentrations. Solutions were administered IV to male Slc:ddY mice (six weeks old) at dose levels 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 (six weeks old) at dose levels of 50, 100, and 150 mg/kg (10 mL/kg, 0.2 mL/min). At 24 and 48 h after dosing of the compounds, *ca.* 5 μL of peripheral blood was collected from the tail vein of each mouse. 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 is expressed as a percentage. Statistical analysis was performed by the Kastenbaum and Bowman method.

Topoisomerase Inhibition Assay *E. coli* DNA gyrase supercoiling and topoisomerase IV decatenation and human topoisomerase II (Topogen) decatenation assays were carried out as described previously.[21,22] IC_{50} values were calculated by the linear regression analysis.

PK Studies Seven-week-old male Crj:CD rats ($n = 4$) were used. The animals were administered drug samples at a single IV 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* ATC C6051. The mean values of four rats are reported.

***In Vivo* Antibacterial Activity** *S. pneumoniae* GE01085 and *E. coli* GK00432 were used as causative pathogens for the models of murine RTI and rodent UTI, respectively. For the murine RTI model, bacteria were intranasally inoculated in six-week-old male CBA/JNCrlj mice. The mice ($n = 5$/group) were subcutaneously injected with the dissolved compounds at 2 and 8 h after inoculation. For the rodent UTI model, bacteria were transurethrally inoculated into the bladders of seven-week-old female Crl:CD(SD)(IGS) rats and then, the urethrae were clamped for 2 h to prevent urine flow. The rats ($n = 5$/group) were infused with the dissolved compounds through the tail vein for 2 h at 4 h post-inoculation. The bacterial numbers in the lungs (mice) or kidneys (rats) were examined on the day following the inoculations.

Acknowledgments We thank Dr. Kiyoshi Takasuna and Dr. Koichi Goto for assistance with the safety assessment, Ms. Megumi Chiba, Dr. Ryo Okumura, Dr. Saito Higuchi, and Dr. Yuichi Kurosaka for assistance with biological testing. We are grateful to Dr. Makoto Takemura for helpful discussion.

Conflict of Interest The authors declare no conflict of interest.

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