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DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL 8-METHOXYQUINOLONES BEARING FUSED PYRROLIDINYL MOIETIES AT THE C-7 POSITION WITH POTENT ANTIBACTERIAL ACTIVITY AGAINST RESPIRATORY PATHOGENS

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Abstract – Novel 8-methoxyquinolones bearing fused pyrrolidinyl moieties at the C-7 position were designed, synthesized, and evaluated for their potent antibacterial activity for the treatment of respiratory tract infections. Compound **5**, possessing a trans-fused octahydroisoindole ring at the C-7 position of the quinolone scaffold, exhibited potent *in vitro* antibacterial activity against nosocomial respiratory pathogens including levofloxacin-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* strains. Furthermore, compound **5** showed a favorable pharmacokinetic profile after a single oral administration in rats.

INTRODUCTION

Given the increasing levels of resistance to β-lactams and macrolides exhibited by community-acquired pathogens, such as multidrug-resistant *Streptococcus pneumoniae* (MDRSP),[1,2] newer quinolones are increasingly being used as first-line antibacterial therapy for respiratory tract infections in clinical settings. Fluoroquinolones such as levofloxacin (LVFX), gatifloxacin (GFLX), and moxifloxacin (MFLX) are beneficial in the empirical treatment of respiratory infections in community settings because of their extended antibacterial spectra, including activity against atypical pathogens, coupled with favorable pharmacokinetic (PK) and safety profiles.[3-5] However, the antibacterial activity of these newer quinolones may be insufficient to prevent the emergence of strains such as quinolone-resistant *S. pneumoniae* and community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA).[6,7] Furthermore, clinical adverse events (e.g., Torsades de Pointes or fatal liver injury) have been shown to be associated with some

quinolones.[8-12] Therefore, novel quinolone antibiotics exhibiting improved activity against respiratory pathogens with few adverse effects are required.

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 (**1**) showed potent activity against respiratory pathogens including penicillin-resistant *S. pneumoniae* (PRSP) and a superior safety profile similar to LVFX as previously reported.[13,14] The results of an *in vitro* metabolic study revealed that compound **1** did not function via mechanism-based inhibition (MBI) of cytochrome P450 (CYP) 3A4. Through this study, it was found that the amino group linked to the *tert*-substituted carbon atom at the C-7 side chain was the key structure to avoid MBI.

Given the increasing need for new drugs with stronger antibacterial activity to overcome resistant bacteria,[6,7] we sought to develop compounds with stronger antibacterial activity against nosocomial respiratory pathogens while maintaining a favorable safety profile. Based on the findings of our previous study,[13-15] we designed scaffolds **A**, having an primary amino group linked to the tertiary carbon atom of the bicycled pyrrolidine ring as the C-7 side chain of the quinolone ring.

Here, we describe the details of the synthesis, *in vitro* antibacterial activity, safety profile, and PK profile of the designed compounds **2-5** shown in Figure 1. Because the absolute stereochemistry of the amino group at the C-7 side chain significantly affects antibacterial activity,[13,14] only the enantiomer exhibiting a strong activity was synthesized.

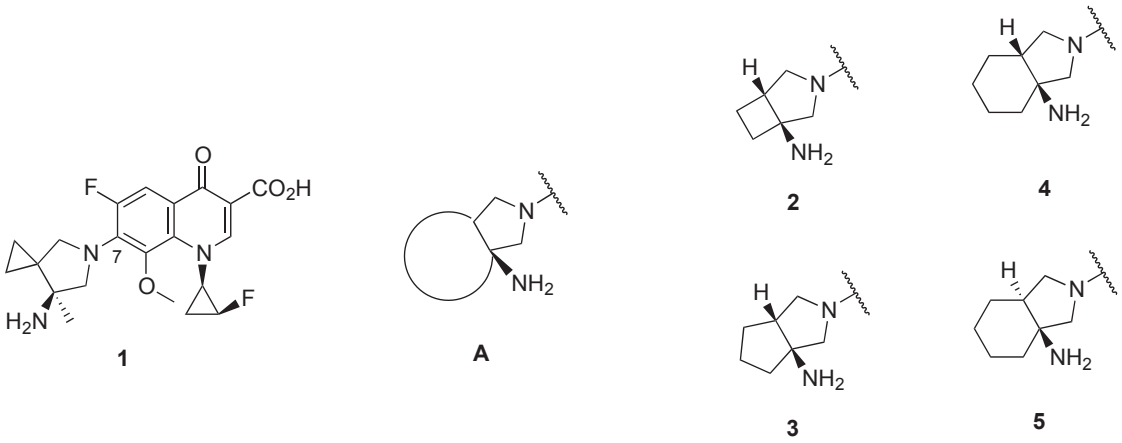


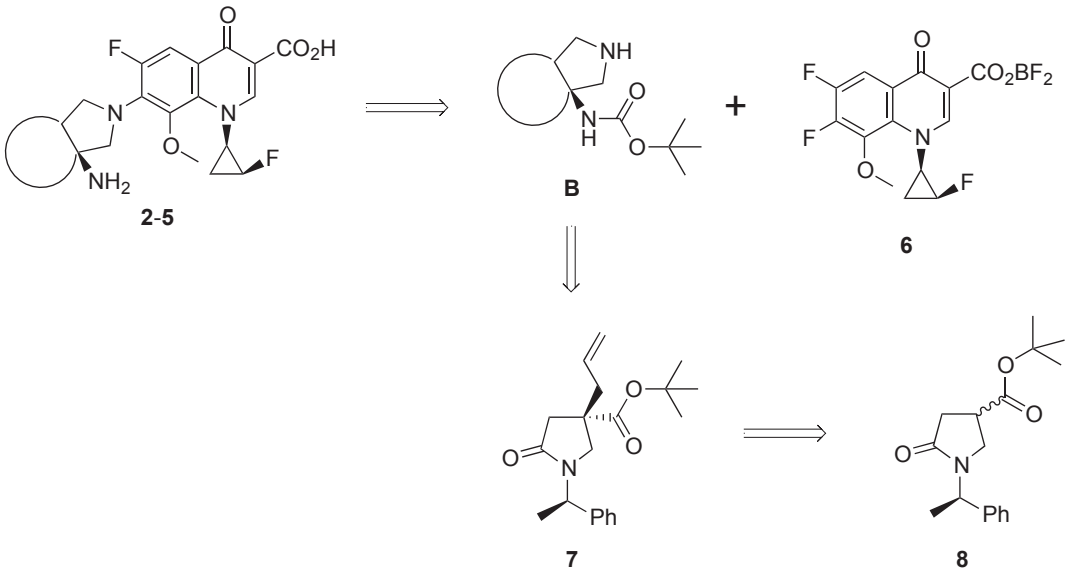
Figure 1. Design of fused pyrrolidine moiety at the C-7 position

RESULTS AND DISCUSSION

1. Chemistry

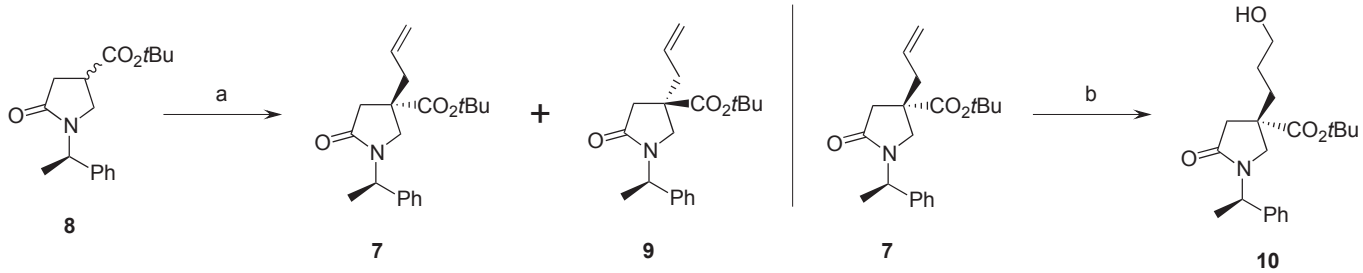
As shown in Scheme 1, we envisioned to synthesize the 7-substituted 8-methoxyquinolone derivatives **2-5** via an aromatic nucleophilic substitution reaction from amine **B** and 6,7-difluoro-1-[(1*R*, 2*S*)-2-fluorocyclopropan-1-yl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid BF_2 chelate

(**6**).[16] The BF_2 chelate was known to improve the reactivity at the C-7 position.[17] We also aimed to prepare amine **B** derived from the common intermediate **7**, which was synthesized from the known compound **8**.[18]



Scheme 1. Retrosynthesis of designed compounds **2-5**

Initially, the allylation of **8**, which was synthesized from (*R*)-phenylethylamine, was performed using sodium hydride as a base to yield two diastereomers, **7** and **9**, which were readily separated by silica-gel column chromatography. The absolute stereochemistry of **7** was determined as follows. Hydroboration of **7** provided the primary alcohol **10**, which was obtained as prisms. X-Ray crystallographic analysis showed that the absolute configuration at C3 position would be (*S*) (Figure 2).



Scheme 2. Reagents and conditions: (a) allyl bromide, NaH, DMF, **7** (36%), **9** (37%); (b) 9-BBN, THF, then 1N aqueous NaOH, 30% aqueous H_2O_2, 46%.

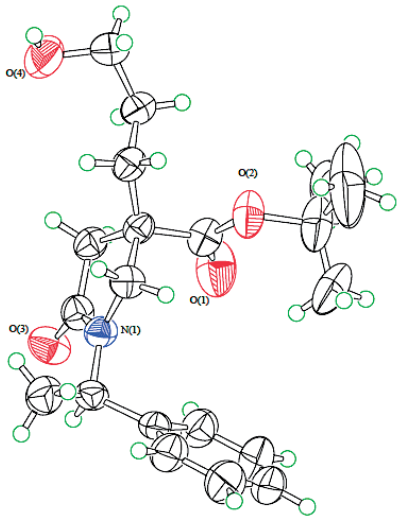
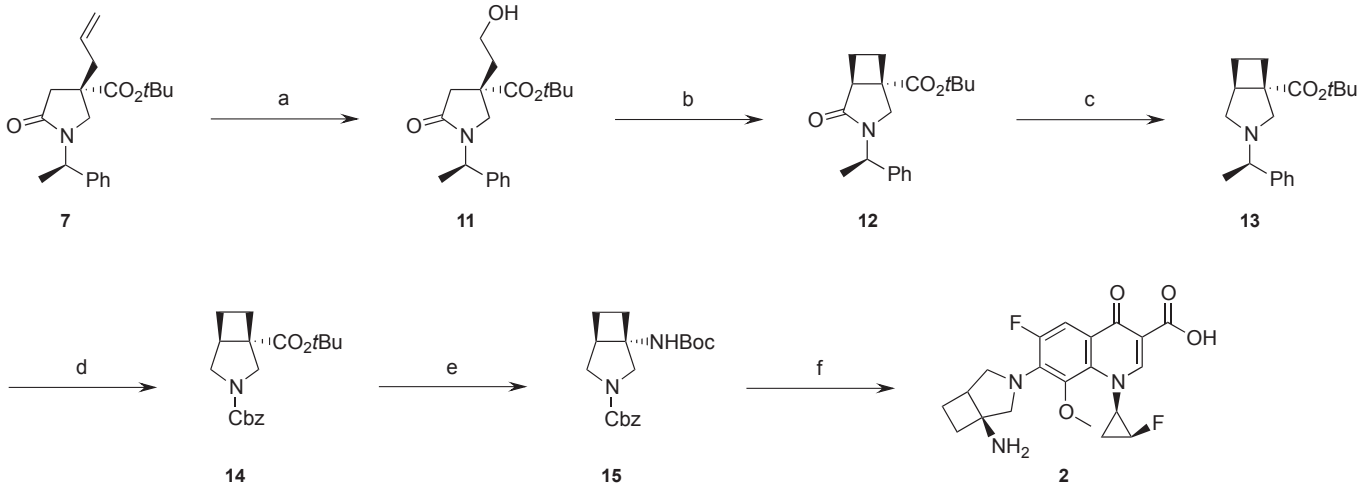


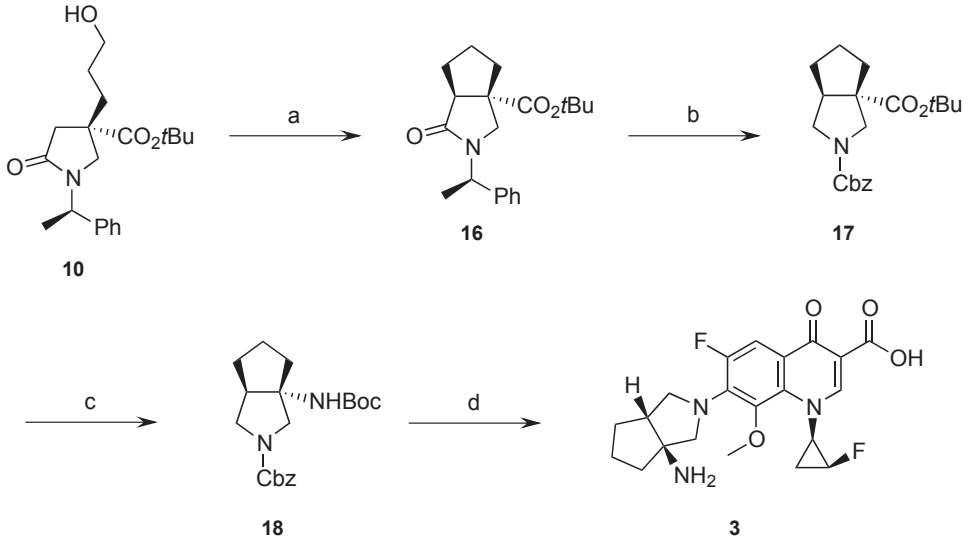
Figure 2. ORTEP of compound **10**

Compound **2** was synthesized as shown in Scheme 3. Ozonolysis of compound **7** followed by reduction with sodium borohydride provided the primary alcohol **11**. After conversion of alcohol **11** to the bromide, intramolecular cyclization was performed by treatment with lithium hexamethyldisilazide (LHMDS). Intermediate **15**, protected by Cbz and Boc groups, was prepared by multistep reactions (reduction with the BH_3-THF complex, hydrogenolysis, formation of benzyl carbamate, removal of *t*-butyl ester, and Curtius rearrangement and simultaneous *t*-BuOH addition). After the Cbz group of **15** was removed by catalytic hydrogenolysis under H_2, the resultant secondary amine was reacted with the BF_2 chelate **6**, followed by dechelation and deprotection of the Boc group to give the desired compound **2**.



Scheme 3. Reagents and conditions: (a) (1) O_3, MeOH −78 °C then Me_2S, (2) $NaBH_4$, MeOH −20 °C, 77%; (b) (1) PPh_3, CBr_4, DCM, (2) LHMDS, THF, −78 °C, 97%; (c) BH_3-THF, THF, 70 °C, 88%; (d) (1) H_2, Pd-C (wet), EtOH, (2) Cbz-Cl, Na_2CO_3, THF, H_2O, 94%; (e) (1) TFA, DCM, (2) DPPA, TEA, *t*-BuOH, 1,4-dioxane, 90 °C, 20%; (f) (1) H_2, Pd-C (wet), EtOH, (2) **6**, TEA, DMF, (3) TEA, 80% aqueous EtOH, reflux, (4) concentrated aqueous HCl, 68%.

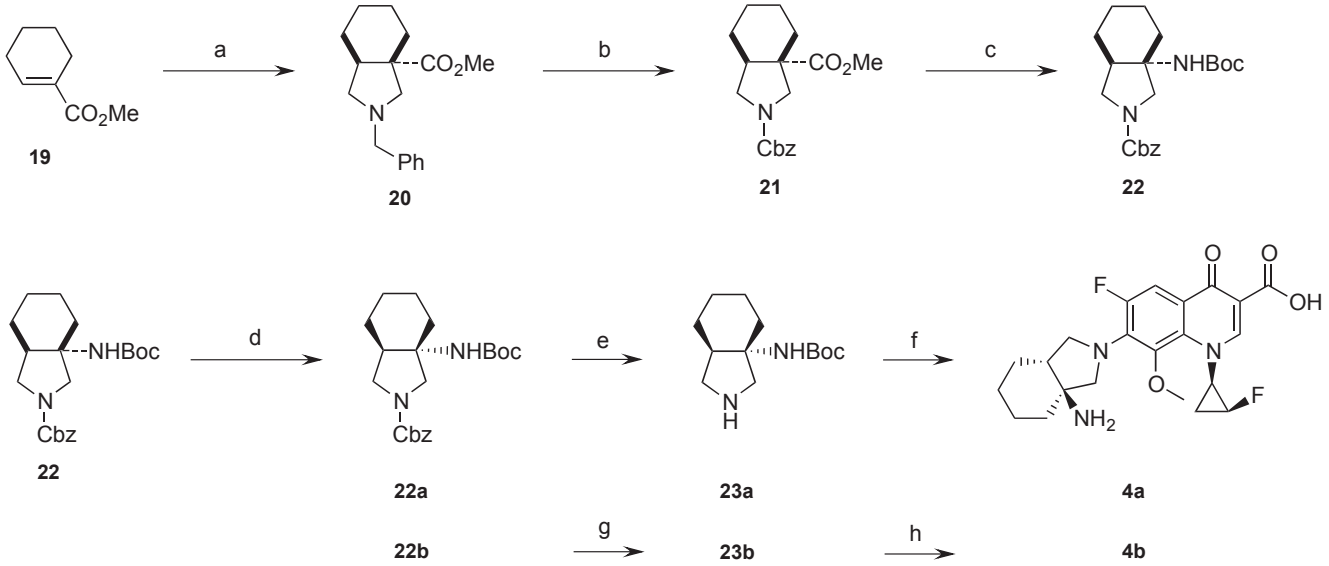
The synthesis of compound **3** from alcohol **10** is illustrated in Scheme 4. The synthesis method was similar to that of compound **2**.



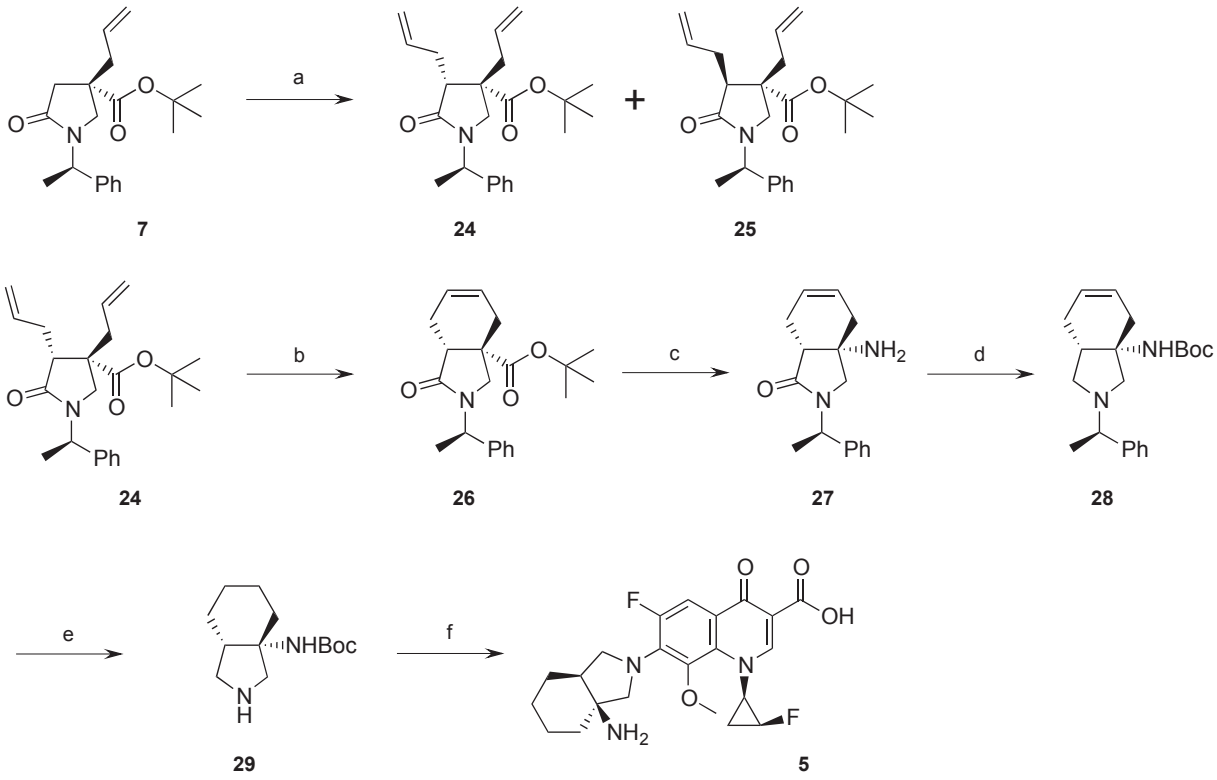
Scheme 4. Reagents and conditions: (a) (1) PPh_3, CBr_4, DCM, (2) LHMDS, THF, −78 °C, 85%; (b) (1) BH_3-THF, THF, 70 °C, (2) H_2, Pd-C (wet), EtOH, (3) Cbz-Cl, Na_2CO_3, THF, H_2O, 83%; (c) (1) TFA, DCM, (2) DPPA, TEA, toluene, 90 °C, (3) 6N aqueous HCl, 1,4-dioxane, 50 °C, (4) Boc_2O, DCM, 54%; (d) (1) H_2, Pd-C (wet), EtOH, (2) **6**, TEA, DMSO, (3) TEA, 80% aqueous EtOH, reflux, (4) concentrated aqueous HCl, 17%.

The 5-6 *cis*-fused compounds **4a** and **4b** were synthesized as shown in Scheme 5. The bicyclic structure was constructed with 1,3-dipolar cycloaddition to give the *dl*-compound **20** as the sole product. After von Brown reaction, removal of methyl ester, and Curtius rearrangement, the key intermediate *dl*-**22** was obtained. This racemic **22** was separated into the enantiomers **22a** and **22b** by column chromatography with CHIRALPAK AD. Both enantiomers **22a** and **22b** were converted to compounds **4a** and **4b**, respectively, in a similar manner as described above. The absolute configuration of **22a** and **22b** have not been determined. Therefore, it was not clear which one of **4a** and **4b** has the desired conformation. The absolute stereochemistry of **4a** and **4b** illustrated in Scheme 5 was the estimated one from the antibacterial activity described later.

The synthesis of the 5-6 *trans*-fused compound **5** is illustrated in Scheme 6. Allylation of **7** furnished an approximately 1:1 mixture of the two diastereomers **24** and **25**, which were easily separated by silica-gel column chromatography. The more polar isomer **24** was cyclized by using a 2nd generation Grubbs' catalyst and treated as described above to yield the secondary amine **29**. Since the ^{1}H-NMR spectrum of **29** did not match that of **23a** (or **23b**), **29** was confirmed to be a desired *trans*-fused compound. Compound **5** was synthesized from **29** in a similar manner. Finally, the ^{1}H-NMR spectrum of **5** was confirmed to be different from that of **4a** and **4b**.



Scheme 5. Reagents and conditions: (a) *N*-benzyl-*N*-methoxymethyl-*N*-trimethylsilylamine, TFA, DCE, 40%; (b) Cbz-Cl, DCM, 71%; (c) (1) 1N aqueous NaOH, THF, MeOH, (2) DPPA, TEA, toluene, 90 °C, (3) 6N aqueous HCl, 1,4-dioxane, 50 °C, (4) Boc_2O, DCM, 80%; (d) CHIRALPAK AD; (e) H_2, Pd-C (wet), EtOH; (f) (1) **6**, TEA, DMSO, (2) TEA, 80% aqueous EtOH, reflux, (3) concentrated aqueous HCl, 73%; (g) H_2, Pd-C (wet), EtOH; (h) (1) **6**, TEA, DMSO, (2) TEA, 80% aqueous EtOH, reflux, (3) concentrated aqueous HCl, 73%.



Scheme 6. Reagents and conditions: (a) allyl bromide, LHMDS, THF, −10 °C, **24** (44%), **25** (38%); (b) Grubbs' catalyst 2nd generation, DCM, 87%; (c) (1) TFA, DCM, (2) DPPA, TEA, toluene, 90 °C, (3) 4N aqueous HCl, 1,4-dioxane, 50 °C, 84%; (d) (1) sodium bis(2-methoxyethoxy)aluminum hydride, toluene, (2) Boc_2O, DCM, 72%; (e) H_2, Pd-C (wet), EtOH; (f) (1) **6**, TEA, DMSO, (2) TEA, 80% aqueous EtOH, reflux, (3) concentrated aqueous HCl, 31%.

2. MIC

The minimum inhibitory concentrations (MICs) of the synthesized compounds **2**-**5** against several representative gram-positive and gram-negative bacteria are summarized in Table 1, along with the corresponding data for LVFX, MFLX, and the previously reported compound **1** for comparison. The 5-4 fused compound **2** and 5-6 *trans*-fused compound **5** exhibited a broad antibacterial spectrum against gram-positive and gram-negative bacteria. In particular, **2** and **5** exhibited an approximately 2–8-fold increased activity against gram-positive bacteria compared with **1**, which had the most potent antibacterial activity among the quinolones we have reported.[13,14] Compounds **2** and **5** exhibited almost identical antibacterial activity against the representative non-resistant bacteria listed in Table 1. Against gram-negative bacteria, the activity of **5** was comparable with that of the other quinolones tested.

Table 1.[a] Antibacterial activities (MIC; μg/mL) of the synthesized compounds and reference quinolones against gram-positive and gram-negative bacteria

Compounds / Organisms	**2**	**3**	**4a**	**4b**	**5**	LVFX	MFLX	**1**
S. aureus SMITH	<0.003	<0.003	0.006	0.05	<0.003	0.1	0.025	0.012
S. pneumoniae J24[b]	0.025	0.05	0.05	0.39	0.025	0.78	0.1	0.05
S. pneumoniae J41[b]	0.05	0.1	0.05	0.39	0.025	1.56	0.1	0.1
S. pyogenes ATCC 12344	0.025	0.025	0.1	-	0.025	0.39	0.2	0.2
E. faecalis ATCC 19433	0.1	0.1	0.2	0.78	0.1	0.78	0.2	0.2
B. subtilis ATCC 6633	0.006	0.012	0.012	0.025	0.006	0.05	0.025	0.012
E. coli NIHJ	0.012	0.025	0.025	0.05	0.006	0.012	0.012	0.012
K. pneumoniae TYPE 1	0.1	0.1	0.1	0.20	0.05	0.05	0.1	0.05
H. influenzae ATCC49247	0.006	0.006	0.025	0.05	0.006	0.012	0.012	0.012
M(B). catarrhalis ATCC25238	0.025	0.025	0.1	0.1	0.025	0.025	0.05	0.05
P. aeruginosa PAO-1	0.39	0.39	1.56	3.13	0.39	0.39	0.78	0.78

[a] Antibacterial activities were determined using a standard microbroth dilution method. Abbreviations: LVFX, levofloxacin; MFLX, moxifloxacin. [b] Penicillin-susceptible *S. pneumoniae* (PSSP).

The antibacterial activities of synthesized compounds and reference quinolones against several resistant bacteria and mutant strains are shown in Table 2. The 5-6 *trans*-fused compound **5** exhibited the strongest antibacterial activity against *MRSA*, *MDRSP*, resistant *E. coli*, and mutant *E. coli* among all compounds tested. Since the 3D structure of the target protein complex (quinolone-DNA-'DNA gyrase' or 'topoisomerase IV' complex) has not been solved, it was not clear whether the direction of the amino group or the conformation of 5-6 *trans*-fused compound **5** was optimal. Because the influence of compound's lipophilicity and membrane permeability on the antibacterial activity could not be ignored. However, the result described above was consistent with the previous knowledge and compound **5** was considered to have the strongest activity not only against representative respiratory pathogens but also against resistant strains.

Table 2. Antibacterial activities (MIC; μg/mL) of the synthesized compounds and reference quinolones against resistant bacteria and mutant strains

Organisms \ Compounds	**2**	**3**	**4a**	**4b**	**5**	LVFX	MFLX	**1**
MRSA 870307[a]	0.2	0.78	0.39	3.13	0.1	>6.25	0.78	0.78
MRSA 890325-1[a]	0.39	0.78	1.56	-	0.1	6.25	1.56	0.78
S. pneumoniae 104835[b]	0.2	0.39	0.78	-	0.2	>6.25	3.13	0.39
E. coli DNS5101[c]	>6.25	6.25	>6.25	>6.25	1.56	>6.25	>6.25	6.25
E. coli 5-037042 '98[d]	0.05	0.1	0.1	0.39	0.025	0.1	0.1	0.05

[a] Levofloxacin-resistant and methicillin-resistant *S. aureus* (levofloxacin-r-MRSA). [b] Multidrug-resistant *S. pneumoniae* (MDRSP, quinolone-resistant and penicillin-resistant strains). [c] Quinolone-resistant *E. coli*. [d] *gyrase* A mutation: Asp87→Gly.

3. hERG & *P'*

Table 3 shows the effects of the synthesized compounds on human ether-a-go-go related gene (hERG) potassium current in hERG-transfected cells and on the apparent partition coefficient (*P'*) value. Electrocardiogram studies showed that compounds that inhibit hERG potassium channels have the potential to prolong the QT interval in humans and increase the risk of fatal cardiac arrhythmia.[19,20] At a concentration of 30 μM, **2** and **3** had virtually no effect on hERG currents, while compound **5** slightly inhibited hERG currents. A clear correlation was observed between hERG inhibition and *P'* value (lipophilicity). The hERG inhibition of compound **5** was almost equal to that of MFLX, which is known to cause QT prolongation syndrome at clinical dosage.[21] As we had established a threshold of within 10%

inhibition at a concentration of 30 μM, compound **5** did not meet the criteria. The high lipophilicity of **5** was considered to indicate hERG inhibition.

Table 3. Effects on hERG potassium current in hERG-transfected cells[a] and apparent partition coefficient (P'[b])

Compound	Concentration (μM)		P'
	30	100	
2[c]	4.8	16	9.0
3[c]	5.0	11	5.6
5[c]	19	36	>68
LVFX[d]	−0.9	4.2	5.1
MFLX[d]	22	42	54
1[d]	1.9	2.7	19

[a] Data represent % inhibition.[22,23] [b] Apparent partition coefficient, $CHCl_3$/0.1 M phosphate buffer (pH 7.4).[24] [c] HEK 293 cells. [d] CHO-K1 cells.

4. PK

The pharmacokinetics (PK) profiles of the synthesized compounds, LVFX, MFLX, and **1** following single oral administration in rats are shown in Table 4. Compounds **2** and **3** exhibited lower maximum drug concentration (C_{max}) and area under the time-concentration curve (*AUC*) than compound **1**. Compound **2** and **3** were considered to have poor oral absorbability because of their low lipophilicity. Compound **5**, however, which has high lipophilicity, showed high blood levels after oral administration. The C_{max} and *AUC* values of **5** were equal to those of the other commercially available quinolones. Based on the PK/PD theory of antibacterial drugs,[25] the *in vivo* efficacy of quinolone drugs is known to depend on the value of *AUC/MIC*. Therefore, compound **5**, with favorable *MIC* and high *AUC* values, was expected to show good *in vivo* efficacy.

Table 4. Pharmacokinetic parameters of the synthesized compounds and reference quinolones after an oral dose of 5 mg/kg (n = 3)[a] in rats

PK parameters	**2**	**3**	**5**	LVFX	MFLX[b]	**1**
C_{max} (μg/mL)	0.51	0.049	1.28	1.47	1.49	1.22
$AUC_{0\text{-}8h}$ (μg·h/mL)	0.91	0.21	4.76	3.41	4.46	3.08

[a] Seven-week-old male Crj: CD Rats. The animals were administered drug samples in a single oral dosing (5 mg/kg) as an aqueous solution. [b] Moxifloxacin hydrochloride hydrate was administered.

5. Conclusions

Novel 8-methoxyquinolones bearing fused pyrrolidinyl moieties at the C-7 position were designed, synthesized, and evaluated in this study. Compounds **2** and **5** exhibited approximately 2–8-fold increased antibacterial activity *in vitro* against gram-positive bacteria compared with compound **1**. Compound **5**, in particular, exhibited very strong activity against resistant bacteria strains, including LVFX-resistant *E. coli* strains and *MRSA* strains. Furthermore, compound **5** showed a highly favorable PK profile, indicative of good *in vivo* efficacy, although this compound was considered likely to inhibit hERG.

EXPERIMENTAL

1. General

All melting points were determined on a Yanaco MP-500D or a BUCHI B-545 and are uncorrected. Optical rotations were measured in a 0.5 dm cell at 25 °C and 589 nm with a HORIBA SEPA-300 polarimeter. ^{1}H-NMR spectra were determined using 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. Significant ^{1}H-NMR data 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 Hz. 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 (HRMS) spectra were recorded on a JEOL JMS-100LP spectrometer. IR spectra were recorded on a HITACHI 270-30 or HORIBA FT-720 spactrometer. 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 was performed as flash column chromatography on Merck silica gel 60 (particle size 0.060-0.200 or 0.040-0.063). Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 TLC plates, and compound visualization was performed with a 5% solution of molybdophosphoric acid in EtOH, a UV lamp, iodine, or Wako ninhydrin spray.

2. In vitro antibacterial activity

The minimum inhibitory concentrations (MICs) of the test compounds were determined by two-fold micro dilution using Mueller-Hinton broth (Difco Laboratories, Detroit, MI) and an inoculum size of approximately 10^5 colony-forming units (CFU) per well. The MIC was defined as the lowest concentration that prevented visible bacterial growth after incubation at 35 °C for 18 h.

3. X-Ray crystallographic analysis of 10

A colorless prism-shaped crystal was formed from Et_2O: $C_{20}H_{29}NO_4$; FW = 347.45; sample dimensions, 0.36 mm × 0.18 mm × 0.08 mm. Lattice parameters and intensities were measured on a Rigaku AFC7R diffractometer (Cu*K*α radiation, λ = 1.54178 Å, graphite monochromator, ω-2θ scans, $2\theta_{max}$ = 120.1°); orthorhombic, space group $P2_12_12$(#18); a = 13.281(1), b = 26.689(2), c = 5.859(1), V = 2076.6(5)Å^3, Z = 4; D_{calcd} = 1.11 g/cm^3; F_{000} = 752; μ = 6.19 cm^{-1}. The structure was solved by direct methods using the Sir92 program.[26] The final cycle of full-matrix least-squares refinement was based on 1828 observed reflections and 256 variable parameters and converged at R = 0.070 (Rw = 0.140).

Deposition number CCDC-1823938 for compound **10**. Free copies of the data can be obtained via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

4. Compounds

***tert*-Butyl (3*S*)-5-oxo-1-[(1*R*)-1-phenylethyl]-3-(prop-2-en-1-yl)pyrrolidine-3-carboxylate (7)**

***tert*-Butyl (3*R*)-5-oxo-1-[(1*R*)-1-phenylethyl]-3-(prop-2-en-1-yl)pyrrolidine-3-carboxylate (9)**

To a solution of **8** (2.02 g, 6.98 mmol) and allyl bromide (2.96 g, 24.4 mmol) in DMF (16 mL) was added NaH (60% in oil, 0.70 g, 17.5 mmol) at 5 °C. After stirring for 0.5 h, the mixture was stirred at ambient temperature for 24 h. To the reaction mixture were added saturated aqueous NH_4Cl and EtOAc. The organic layer was washed with H_2O 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 = 3:1 to yield **7** (828 mg, 36%) as a colorless oil and **9** (858 mg, 37%) as a colorless oil.

7: Rf = 0.36 (hexane/EtOAc = 3:1), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35 (9H, s), 1.51 (3H, d, J = 7.3 Hz), 2.37–2.48 (2H, m), 2.38 (1H, d, J = 16.8 Hz), 2.88 (1H, d, J = 17.6 Hz), 3.16 (1H, d, J = 10.3 Hz), 3.28 (1H, d, J = 10.5 Hz), 5.10–5.13 (1H, m), 5.15 (1H, s), 5.49 (1H, q, J = 7.1 Hz), 5.61–5.72 (1H, m), 7.26–7.35 (5H, m). MS (ESI) *m/z*: 330 $(M + H)^+$. $[\alpha]_D^{25}$ 72.9 (c 0.89, $CHCl_3$). High-resolution MS (ESI) calcd for $C_{20}H_{27}NO_3$: 330.2071. Found: 330.2073. IR (ATR): 3064, 3032, 3002, 2977, 2933, 2880, 1723, 1686, 1642, 1604, 1488 cm^{-1}.

9: Rf = 0.39 (hexane/EtOAc = 3:1), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.45 (9H, s), 1.52 (3H, d, J = 7.1 Hz), 2.13–2.31 (2H, m), 2.35 (1H, d, J = 17.1 Hz), 2.80 (1H, d, J = 10.3 Hz), 2.86 (1H, d, J = 17.1 Hz), 3.60 (1H, d, J = 10.3 Hz), 4.78 (1H, dd, J = 17.1, 1.7 Hz), 4.95 (1H, dt, J = 10.2, 0.9 Hz), 5.40–5.55 (2H, m), 7.26–7.38 (5H, m). MS (ESI) *m/z*: 330 $(M + H)^+$. $[\alpha]_D^{25}$ 67.7 (c 0.85, $CHCl_3$). High-resolution MS (ESI) calcd for $C_{20}H_{27}NO_3$: 330.2071. Found: 330.2061. IR (ATR): 3077, 3063, 3031, 2978, 2934, 2881, 1723, 1685, 1642, 1604, 1488 cm^{-1}.

***tert*-Butyl (3*S*)-3-(3-hydroxypropyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylate (10)**

To a solution of **7** (373 mg, 1.13 mmol) in THF (10 mL) was added 9-BBN 0.5 M in THF (3.4 mL, 1.70 mmol) at ambient temperature. After stirring for 5 h, to the mixture were added 1N NaOH aq. (4.3 mL) and 30% aqueous H_2O_2 (0.43 mL) at 5 °C. The mixture was stirred at ambient temperature for 0.5 h. To the reaction mixture were added saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was washed with H_2O 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 EtOAc to yield **10** (180 mg, 46%) as a colorless oil. Recrystallization from Et_2O gave a colorless solid, mp 106–108 °C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.33 (9H, s), 1.47–1.54 (1H, m), 1.51 (3H, d, J = 7.1 Hz), 1.67–1.88 (3H, m), 2.33 (1H, d, J = 16.8 Hz), 2.95 (1H, d, J = 17.1 Hz), 3.14 (1H, d, J = 10.3 Hz), 3.34 (1H, d, J = 10.3 Hz), 3.62 (2H, t, J = 6.2 Hz), 5.48 (1H, q, J = 7.3 Hz), 7.24–7.35 (5H, m).

MS (ESI) *m/z*: 348 $(M + H)^+$. $[\alpha]_D^{25}$ 44.3 (*c* 0.93, $CHCl_3$). High-resolution MS (ESI) calcd for $C_{20}H_{29}NO_4$: 348.2177. Found: 348.2176. IR (ATR): 3389, 3031, 2976, 2930, 2867, 1721, 1668, 1604, 1488 cm^{-1}.

***tert*-Butyl (3*S*)-3-(2-hydroxyethyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylate (11)**

To a solution of **10** (11.5 g, 34.8 mmol) in MeOH (115 mL) at −78 °C, O_3 gas bubbled for 5.5 h, and then O_2 gas bubbled for 1.5 h. Me_2S (10.8 g, 174 mmol) was added to the reaction mixture at 5 °C. The resultant mixture was stirred for 9 h at ambient temperature. The reaction mixture was diluted with EtOAc, and the organic solution was washed with 10% sodium thiosulfate aqueous solution 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 and eluted with hexane/EtOAc = 1:3 to yield aldehyde. To the solution of aldehyde in MeOH (260 mL) was added $NaBH_4$ (1.21 g, 32.0 mmol) at −20 °C. After stirring for 1 h, the reaction mixture was poured into saturated aqueous NH_4Cl and extracted with EtOAc. The organic solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to yield **11** (8.89 g, 77%) as a pale yellow amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.32 (9H, s), 1.51 (3H, d, J = 7.3 Hz), 1.88–1.95 (1H, m), 2.02–2.09 (1H, m), 2.40 (1H, d, J = 17.8 Hz), 2.96 (1H, d, J = 17.3 Hz), 3.23 (1H, d, J = 9.8 Hz), 3.38 (1H, d, J = 10.1 Hz), 3.63–3.73 (2H, m), 5.48 (1H, q, J = 6.8 Hz), 7.24–7.35 (5H, m). MS (ESI) *m/z*: 334 $(M + H)^+$.

***tert*-Butyl (1*S*,5*S*)-4-oxo-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.2.0]heptane-1-carboxylate (12)**

To a solution of **11** (8.89 g, 26.7 mmol) and triphenylphosphine (8.63 g, 32.9 mmol) in CH_2Cl_2 (210 mL) under N_2 atmosphere at 5 °C was added carbon tetrabromide (10.9 g, 32.9 mmol). The mixture was stirred for 18 h at ambient temperature. The solution was concentrated *in vacuo*. The resultant mixture

was roughly purified by short silica gel column chromatography, eluting with hexane/EtOAc = 2:1 to yield bromide intermediate.

To a solution of bromide in THF (185 mL) under N_2 atmosphere at −78 °C was added 1 M LHMDS THF solution (52 mL) dropwise. After stirring for 4.5 h, to the reaction mixture were added 5% citric acid aqueous solution and EtOAc. The organic solution 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/EtOAc = 1:2 to yield **12** (7.90 g, 97%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.42 (9H, s), 1.59 (3H, d, J = 7.8 Hz), 2.07–2.18 (2H, m), 2.50–2.67 (2H, m), 3.15–3.20 (1H, m), 3.23 (1H, d, J = 10.3 Hz), 3.32 (1H, d, J = 10.3 Hz), 5.57 (1H, q, J = 7.3 Hz), 7.27–7.37 (5H, m). MS (ESI) *m/z*: 316 $(M + H)^+$.

***tert*-Butyl (1*S*,5*S*)-3-[(1*R*)-1-phenylethyl]-3-azabicyclo[3.2.0]heptane-1-carboxylate (13)**

To a solution of **12** (7.90 g, 25.1 mmol) in dry THF (17 mL) at ambient temperature under N_2 atmosphere was added 1 M BH_3-THF complex in THF (75 mL). The mixture was warmed to 70 °C and stirred for 2.5 h. Then, the mixture was concentrated *in vacuo*. To the resultant residue were added 10% aqueous EtOH (68 mL) and triethylamine (6.8 mL). The mixture was stirred at 70 °C for 5 h and concentrated *in vacuo*. After CH_2Cl_2 and H_2O were added to the residue, the organic solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography and eluted with hexane/EtOAc = 20:1 to yield **13** (5.35 g, 88%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, d, J = 6.6 Hz), 1.40 (9H, s), 1.74–1.82 (1H, m), 1.93–2.00 (1H, m), 2.08–2.17 (2H, m), 2.25–2.37 (2H, m), 2.66 (1H, d, J = 12.2 Hz), 2.88–2.93 (1H, m), 3.05 (1H, d, J = 8.8 Hz), 3.28 (1H, q, J = 7.1 Hz), 7.20–7.41 (5H, m). MS (ESI) *m/z*: 302 $(M + H)^+$.

3-Benzyl 1-*tert*-butyl (1*S*,5*S*)-3-azabicyclo[3.2.0]heptane-1,3-dicarboxylate (14)

To a solution of **13** (6.60 g, 21.9 mmol) in EtOH (66 mL) at ambient temperature under H_2 atmosphere was added 10% Pd-C (50% wet, 1.90 g). The mixture was stirred for 4 days. After removal of catalyst, the filtrate was concentrated *in vacuo*. To the resultant residue were added THF (25 mL), H_2O (25 mL), and Na_2CO_3 (4.62 g, 43.58 mmol). At 0 °C a solution of benzyl chloroformate (5.58 g, 32.7 mmol) in THF (13 mL) was added to the mixture and stirred for 18 h at ambient temperature. To the reaction mixture were added EtOAc and H_2O. The organic solution was washed with 10% citric acid aqueous solution and brine. The solution was dried over Na_2SO_4 and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 5:1 to yield **14** (6.85 g, 94%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.45 (9H, s), 1.63–1.74 (1H, m), 1.87–1.98 (1H,

m), 2.12–2.23 (1H, m), 2.46–2.54 (1H, m), 3.00–3.07 (1H, m), 3.35–3.42 (1H, m), 3.55–3.85 (3H, m), 5.18 (2H, s), 7.29–7.40 (5H, m). MS (ESI) *m/z*: 332 $(M + H)^+$.

Benzyl (1*S*,5*R*)-1-[(*tert*-butoxycarbonyl)amino-3-azabicyclo[3.2.0]heptane-3-carboxylate (15)

To a solution of **14** (6.80 g, 20.52 mmol) in CH_2Cl_2 (80 mL) was added trifluoroacetic acid (10 mL) at 0 °C. The mixture was stirred for 18 h at ambient temperature. After evaporation, EtOAc and brine were added to the residue. The organic solution was dried over Na_2SO_4 and concentrated *in vacuo*. To the solution of the resultant residue and triethylamine (3.1 mL, 22.24 mmol) in 1,4-dioxane (100 mL) were added diphenylphosphoryl azide (4.86 mL, 22.24 mmol) and *tert*-BuOH (4.0 mL, 41.82 mmol). The reaction mixture was stirred for 14 h at 90 °C and evaporated *in vacuo*. To the resultant residue were added $CHCl_3$ and H_2O. The organic solution was washed with 5% citric acid aqueous solution and brine. After removal of solvent, the residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 3:1 to yield **15** (1.41 g, 20%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.53–1.60 (1H, m), 2.15–2.21 (3H, m), 2.85–2.94 (1H, m), 3.44–3.67 (3H, m), 3.87–3.92 (1H, m), 4.78–4.81 (1H, m), 5.15 (2H, s), 7.22–7.52 (5H, m). *m/z*: 347 $(M + H)^+$.

7-[(1*S*)-1-Amino-3-azabicyclo[3.2.0]heptan-3-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 **15** (1.40 g, 4.04 mmol) in EtOH (20 mL) was added 10% Pd-C (50% wet, 0.45 g). The mixture was stirred at ambient temperature under H_2 atmosphere for 22 h. After removal of catalyst by filtration, the filtrate was concentrated *in vacuo*. The solution of the resultant residue, triethylamine (1.08 mL, 8.08 mmol) and **6** (1.41 g, 3.91 mmol) in DMF (10 mL) was stirred for 2 days at ambient temperature and for 7 h at 40 °C. To the mixture were added EtOH (260 mL), water (66 mL), and triethylamine (33 mL). The resultant mixture was heated to reflux for 4 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 12 mL of concentrated aqueous HCl at 0 °C, and the mixture was stirred for 10 min at ambient temperature. The aqueous solution was washed with $CHCl_3$ and rendered 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 $CHCl_3$ (× 3). The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant solid was washed with EtOH and Et_2O to yield **2** (1.09 g, 68%) as a pale yellow powder, mp 229–231 °C. ^{1}H-NMR (0.1N NaOD/D_2O) δ: 1.47–1.67 (3H, m), 2.08–2.14 (3H, m), 2.56–2.58 (1H, m), 3.19 (1H, d, J = 10.3 Hz), 3.49 (1H, dd, J = 8.5, 5.4 Hz), 3.61–3.68 (5H, m), 4.04–4.07 (1H, m),

4.88–5.05 (1H, m), 7.71 (1H, d, J = 13.7 Hz), 8.48 (1H, s). MS (ESI) *m/z*: 406 $(M + H)^+$. $[\alpha]_D^{25}$ 109.8 (*c* 0.58, 0.1N aqueous NaOH); Anal. Calcd for $C_{20}H_{21}F_2N_3O_4$, C 59.25, H 5.22, F 9.37, N 10.37. Found, C 58.92, H 5.23, F 9.42, N 10.17. IR (ATR): 3432, 3387, 3365, 3101, 3079, 3057, 3009, 2971, 2053, 2938, 2871, 2837, 2650, 2595, 2122, 1725, 1620, 1547, 1512 cm^{-1}.

tert-Butyl (3a*S*,6a*S*)-1-oxo-2-[(1*R*)-1-phenylethyl]hexahydrocyclopenta[*c*]pyrrole-3a(1*H*)-carboxylate (16)

To a solution of **10** (1.50 g, 4.32 mmol) in CH_2Cl_2 (40 mL) were added triphenylphosphine (1.36 g, 5.19 mmol) and carbon tetrabromide (1.72 g, 5.19 mmol) at ambient temperature. After stirring for 13 h, the solution was purified by silica gel column chromatography, eluting with hexane/EtOAc = 2:1 to yield bromide (1.50 g) as a colorless oil.

To a solution of bromide in THF (30 mL) was added 1 M LHMDS in THF solution (9.2 mL) dropwise at −78 °C under N_2 atmosphere. After the solution was stirred for 7 h, 5% citric acid aqueous solution and EtOAc were added. 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/EtOAc = 2:1 to yield **16** (1.21 g, 85%) as a colorless solid, mp 97–99 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (9H, s), 1.51 (3H, d, J = 7.3 Hz), 1.69–1.84 (2H, m), 1.92–2.02 (1H, m), 2.10–2.19 (2H, m), 3.06 (1H, d, J = 10.4 Hz), 3.13 (1H, dd, J = 9.5, 2.1 Hz), 3.40 (1H, d, J = 10.4 Hz), 5.50 (1H, q, J = 7.1 Hz), 7.25–7.52 (5H, m). MS (ESI) *m/z*: 330 $(M + H)^+$. $[\alpha]_D^{25}$ 114.8 (*c* 0.45, $CHCl_3$); Anal. Calcd for $C_{20}H_{27}NO_3 \cdot 0.25H_2O$, C 71.93, H 8.30, N 4.19. Found, C 71.58, H 8.35, N 4.27. IR (ATR): 3423, 3052, 3028, 3004, 2974, 2958, 2881, 1720, 1669, 1633 cm^{-1}.

2-Benzyl 3a-_tert_-butyl (3a*S*,6a*S*)-tetrahyrdocyclopenta[*c*]pyrrole-2,3a(1*H*,3*H*)-dicarboxylate (17)

To a solution of **16** (1.20 g, 3.64 mmol) in THF (26 mL) was added 1 M BH_3-THF solution (10.9 mmol) at 0 °C under N_2 atmosphere. The solution was warmed to 70 °C and stirred for 2.5 h. After concentration *in vacuo*, to the resultant residue were added EtOH (9 mL), H_2O (1 mL) and triethylamine (1 mL). The mixture was stirred for 7 h at 70 °C and concentrated *in vacuo*. The residue was purified by short silica gel column chromatography, eluting with hexane/EtOAc = 9:1. To the solution of the residue in EtOH (20 mL) was added 10% Pd-C (50% wet, 300 mg). The mixture was stirred at ambient temperature under H_2 atmosphere for 24 h. After removal of catalyst by filtration, the filtrate was concentrated *in vacuo*. To the solution of the resultant residue in THF (4 mL) and H_2O (4 mL) were added Na_2CO_3 (626 mg, 5.90 mmol) and benzyl chloroformate (756 mg, 4.43 mmol) at 0 °C. The reaction mixture was stirred for 18 h at ambient temperature. After addition of EtOAc and H_2O, the organic solution was washed with 3% citric acid aqueous solution and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The

resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 5:1 to yield **17** (980 mg, 83%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.42 (9H, s), 1.66–1.81 (2H, m), 1.91–2.01 (1H, m), 2.12–2.19 (1H, m), 2.78–2.88 (1H, m), 3.23–3.43 (2H, m), 3.62–3.71 (1H, m), 3.91 (1H, d, *J* = 12.0 Hz), 5.13 (2H, s), 7.28–7.37 (5H, m). MS (ESI) *m/z*: 346 $(M + H)^+$.

Benzyl (3a*S*,6a*R*)-3a-[(*tert*-butoxycarbonyl)amino]hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate (18)

To a solution of **17** (980 mg, 2.84 mmol) in CH_2Cl_2 (10 mL) was added trifluoroacetic acid (1 mL) at 0 °C. The reaction mixture was stirred for 60 h at ambient temperature. The solution was concentrated *in vacuo* and azeotroped with toluene.

To the solution of the resultant residue in toluene (15 mL) were added triethylamine (0.79 mL, 5.67 mmol) and diphenylphosphoryl azide (0.80 mL, 3.71 mmol) at 0 °C under N_2 atmosphere. The reaction solution was stirred for 2 h at ambient temperature and for 0.5 h at 90 °C. The solution was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, water, and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. To the resultant residue were added 1,4-dioxane (10 mL) and 6N aqueous HCl (10 mL). The mixture was stirred for 2 h at 50 °C. The reaction mixture was concentrated *in vacuo* and azeotroped with EtOH. After the residue was dissolved with CH_2Cl_2 (13 mL), to the solution was added Boc_2O (990 mg, 4.54 mmol) and the mixture was stirred for 2 h at ambient temperature. After the mixture was diluted with EtOAc, the organic solution was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 3:1 to yield **18** (557 mg, 54%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.43 (9H, s), 1.76 (2H, m), 1.88–2.04 (3H, m), 3.23–3.31 (1H, m), 3.61 (1H, d, *J* = 11.0 Hz), 3.70 (1H, d, *J* = 10.5 Hz), 3.73 (1H, d, *J* = 7.8 Hz), 4.63–4.73 (1H, m), 5.12 (2H, s), 7.28–7.37 (5H, m). MS (ESI) *m/z*: 361 $(M + H)^+$.

7-[(3a*S*,6a*R*)-3a-Aminohexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3)

To a solution of **18** (575 mg, 1.60 mmol) in MeOH (20 mL) was added 10% Pd-C (115 mg, 50% wet) at ambient temperature. The mixture was stirred under H_2 atmosphere for 2 h. After removal of catalyst by filtration, the filtrate was concentrated *in vacuo*. The solution of the resultant residue, **6** (576 mg, 1.60 mmol) and triethylamine (0.67 mL, 4.79 mmol) in DMSO (4 mL) was stirred for 16 h at 40 °C. To the mixture were added EtOH (40 mL), water (10 mL) and triethylamine (5 mL). The resultant mixture was heated to reflux for 3 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 rendered 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 $CHCl_3$ (× 3). The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. To the resultant residue were added EtOH and 28% NH_3 aqueous solution and heated to reflux to yield **3** (111 mg, 17%) as a pale yellow powder, mp 169–172 °C. ^{1}H-NMR (400 MHz, 0.1N NaOD/D_2O) δ: 1.47–1.89 (7H, m), 2.04 (1H, m), 2.30 (1H, m), 3.35 (1H, dd, *J* = 9.8, 4.9 Hz), 3.52 (2H, s), 3.63 (3H, s), 3.77 (1H, t, *J* = 9.0 Hz), 4.04 (1H, m), 4.80–5.05 (1H, m), 7.68 (1H, d, *J* = 13.9 Hz), 8.47 (1H, s). MS (ESI) *m/z*: 419(M)$^+$. $[\alpha]_D^{25.1}$ +103.5 (*c* 0.23, 0.1N aqueous NaOH); Anal. Calcd for $C_{21}H_{23}F_2N_3O_4 \cdot 0.75H_2O \cdot 0.25EtOH$, C 58.10, H 5.90, F 8.55, N 9.45. Found, C 57.87, H 5.51, F 8.60, N 9.11. IR (ATR): 2952, 2873, 2831, 2177, 1712, 1614, 1577, 1535 cm^{-1}.

Methyl 2-benzyloctahydro-3a*H*-isoindole-3a-carboxylate (20, cis)

To a solution of methyl cyclohex-1-ene-1-carboxylate (**19**, 25.0 g, 178 mmol) and *N*-benzyl-*N*-methoxymethyl-*N*-trimethylsilylamine (46.6 g, 196 mmol) in 1,2-dichloroethane (178 mL) was added trifluoroacetic acid (0.14 mL, 1.78 mmol) at ambient temperature. After the reaction solution was stirred for 2 h, to the mixture were added saturated aqueous $NaHCO_3$ and $CHCl_3$. The organic solution 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/EtOAc = 3:1 to yield **20** (21.3 g, 40%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.21–1.53 (6H, m), 1.65–1.79 (2H, m), 1.90–1.96 (1H, m), 2.68–2.73 (3H, m), 2.92 (1H, d, *J* = 9.3 Hz), 3.65–3.70 (5H, m), 7.24–7.32 (5H, m). MS (ESI) *m/z*: 274 (M + H)$^+$.

2-Benzyl 3a-methyl tetrahydro-1*H*-isoindole-2,3a(3*H*,4*H*)-dicarboxylate (21, cis)

To a solution of **20** (21.3 g, 77.9 mmol) in CH_2Cl_2 (260 mL) was added benzyl chloroformate (33.4 mL, 234 mmol) at ambient temperature under N_2 atmosphere. After stirring for 15 h, the solution was concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 3:1 to yield **21** (17.5 g, 71%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.38–1.63 (6H, m), 1.70–1.79 (1H, m), 1.93 (1H, m), 2.64–2.72 (1H, m), 3.28–3.52 (3H, m), 3.63 (1H, dd, *J* = 10.9, 8.2 Hz), 3.71 (3H, d, *J* = 3.2 Hz), 5.13 (2H, m), 7.29–7.37 (5H, m). MS (ESI) *m/z*: 318 (M + H)$^+$.

Benzyl 3a-[(*tert*-butoxycarbonyl)amino]octahydro-2*H*-isoindole-2-carboxylate (22, cis)

To a solution of **21** (7.50 g, 23.6 mmol) in MeOH (80 mL) and THF (80 mL) was added 1N aqueous NaOH solution (70 mL) dropwise at ambient temperature. After stirring for 3 days, the solution was concentrated *in vacuo*. The resultant residue was acidified by addition of 3N aqueous HCl and extracted with $CHCl_3$. The organic solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To the solution of the resultant residue and triethylamine (6.17 mL, 44.2 mmol) in toluene (110 mL) was added diphenylphosphoryl azide (6.19 mL, 28.7 mmol) at 0 °C under N_2 atmosphere. The mixture was stirred for 40 min at ambient temperature and for 1 h at 90 °C. The reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, H_2O, and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To the resultant residue were added 1,4-dioxane (55 mL) and 6N aqueous HCl (55 mL). After stirring for 2 h at 50 °C, the reaction mixture was concentrated *in vacuo* and azeotroped with EtOH. To a solution of the residue in CH_2Cl_2 (110 mL) were added triethylamine (15.4 mL 110 mmol) and Boc_2O (9.65 g, 44.2 mmol). The solution was stirred for 15 h at ambient temperature. After concentration *in vacuo*, the residue was diluted with EtOAc and the organic solution was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 2:1 to yield **22** (6.65 g, 80%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.43–1.66 (17H, m), 1.99–2.03 (1H, m), 3.25–3.35 (1H, m), 3.45–3.58 (2H, m), 3.69 (1H, d, J = 11.3 Hz), 4.55 (1H, d, J = 14.0 Hz), 5.13 (2H, s), 7.29–7.37 (5H, m). MS (ESI) *m/z*: 374 $(M + H)^+$.

<Optical resolution>

Racemic **22** (870 mg) was separated into its enantiomers by semipreparative HPLC using a Chiralpak AD column (Daicel Chemical Industries, Ltd.; 250 × 20 mm, 5 μm; flow, 20 mL/min; solvents, hexane/isopropanol 95:5; 50 mg/run; UV detection at 254 nm) to give **22a** (427 mg, 49%, t_R = 14.2 min) and **22b** (415 mg, 48%, t_R = 19.4 min) as a colorless oil.

7-(3a-Aminooctahydro-2*H*-isoindol-2-yl)-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4a, cis, derived from 22a)

To a solution of **22a** (400 mg, 1.07 mmol) in MeOH (11 mL) was added 10% Pd-C (80 mg, 50% wet) at ambient temperature. The mixture was stirred under H_2 atmosphere for 1 h. After removal of catalyst by filtration, the filtrate was concentrated *in vacuo* to yield **23a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.43 (9H, s), 1.43–1.57 (8H, m), 2.01 (1H, br s), 2.16 (1H, br s), 2.81 (1H, dd, J = 10.7, 6.7 Hz), 3.02 (1H, d, J = 11.3 Hz), 3.12–3.18 (2H, m), 4.61 (1H, br s).

A solution of **23a**, **6** (351 mg, 0.972 mmol) and triethylamine (0.407 mL, 2.92 mmol) in DMSO (2 mL) was stirred for 17 h at 35 °C. To the mixture were added EtOH (16 mL), water (4 mL) and triethylamine (2 mL). The resultant mixture was heated to reflux for 1 h, and then concentrated *in vacuo* to give the residue, which was diluted with EtOAc. The organic solution was washed with 10% aqueous citric acid solution, water, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was roughly purified by silica gel column chromatography, eluting with $CHCl_3$/MeOH = 98:2. To the residue was added 3 mL of concentrated aqueous HCl at 0 °C, and the mixture was stirred for 15 min at ambient temperature. The aqueous solution was rendered 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 $CHCl_3$/MeOH = 90:10. The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. To the resultant residue was recrystallized from EtOH to yield **4a** (271 mg, 73%) as a pale yellow powder, mp 218–220 °C. ^{1}H-NMR (400 MHz, 0.1N NaOD/D_2O) δ: 1.40–1.60 (8H, m), 1.77 (2H, m), 2.01 (1H, m), 3.36 (1H, d, *J* = 8.3 Hz), 3.57 (3H, s), 3.59–3.64 (1H, m), 3.68–3.72 (1H, m), 3.81–3.87 (1H, m), 4.00–4.05 (1H, m), 4.47–5.07 (1H, m), 7.65 (1H, d, *J* = 14.7 Hz), 8.42 (1H, d, *J* = 2.0 Hz). MS (ESI) *m/z*: 434 $(M+H)^+$. $[\alpha]_D^{25.0}$ +42.3 (*c* 1.0, 0.1N aqueous NaOH); Anal. Calcd for $C_{22}H_{25}F_2N_3O_4 \cdot 0.5H_2O$, C 59.72, H 5.92, F 8.59, N 9.50. Found, C 59.91, H 5.97, F 8.68, N 9.39. IR (ATR): 2927, 2856, 1724, 1616, 1508 cm^{-1}.

7-(3a-Aminooctahydro-2*H*-isoindol-2-yl)-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4b, cis, derived from 22b)

To a solution of **22b** (415 mg, 1.11 mmol) in MeOH (11 mL) was added 10% Pd-C (83 mg, 50% wet) at ambient temperature. The mixture was stirred under H_2 atmosphere for 1 h. After removal of catalyst by filtration, the filtrate was concentrated *in vacuo* to yield **23b**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.43 (9H, s), 1.36–1.63 (8H, m), 2.00 (1H, s), 2.15 (1H, br s), 2.81 (1H, br s), 3.02 (1H, d, *J* = 11.5 Hz), 3.12–3.17 (2H, m), 4.60 (1H, br s).

A solution of **23b**, **6** (364 mg, 1.01 mmol) and triethylamine (0.422 mL, 3.03 mmol) in DMSO (2 mL) was stirred for 17 h at 35 °C. To the mixture were added EtOH (16 mL), water (4 mL) and triethylamine (2 mL). The resultant mixture was heated to reflux for 1 h, and then concentrated *in vacuo* to give the residue, which was diluted with EtAOc. The organic solution was washed with 10% aqueous citric acid solution, water, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was roughly purified by silica gel column chromatography, eluting with $CHCl_3$/MeOH = 98:2. To the residue was added 3 mL of concentrated aqueous HCl at 0 °C, and the mixture was stirred for 15 min at ambient temperature. The aqueous solution was rendered 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 $CHCl_3$/MeOH = 90:10. The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. To the resultant residue was recrystallized from EtOH to yield **4b** (315 mg, 73%) as a pale yellow powder, mp 221–223 °C. ^{1}H-NMR (400 MHz, 0.1N NaOD/D_2O) δ: 1.33 (2H, m), 1.46–1.67 (6H, m), 1.73 (1H, m), 1.83 (1H, m), 1.94 (1H, m), 3.20 (1H, d, *J* = 8.8 Hz), 3.42 (1H, d, *J* = 10.5 Hz), 3.57 (3H, s), 3.88 (1H, dd, *J* = 10.5, 2.2 Hz), 3.99–4.06 (2H, m), 4.84–5.04 (1H, m), 7.64 (1H, d, *J* = 15.0 Hz), 8.45 (1H, d, *J* = 1.5 Hz). MS (ESI) *m/z*: 434 $(M+H)^+$. $[\alpha]_D^{25.0}$ +99.4 (*c* 1.0, 0.1N aqueous NaOH); Anal. Calcd for $C_{22}H_{25}F_2N_3O_4 \cdot 0.75H_2O$, C 59.12, H 5.98, F 8.50, N 9.40. Found, C 59.05, H 6.12, F 8.36, N 9.20. IR (ATR): 2927, 2859, 1724, 1616, 1573, 1509 cm^{-1}.

***tert*-Butyl (3*S*,4*R*)-5-oxo-1-[(1*R*)-1-phenylethyl]-3,4-di(prop-2-en-1-yl)pyrrolidine-3-carboxylate (24)**
***tert*-Butyl (3*S*,4*S*)-5-oxo-1-[(1*R*)-1-phenylethyl]-3,4-di(prop-2-en-1-yl)pyrrolidine-3-carboxylate (25)**
To a solution of **7** (4.50 g, 12.3 mmol) and allyl bromide (1.36 mL, 16.1 mmol) in THF (41 mL) was added 1 M LHMDS in THF solution (16.0 mL) dropwise at −10 °C under N_2 atmosphere. After stirring for 15 min, saturated aqueous NH_4Cl and EtOAc were added to the reaction solution. 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/EtOAc = 3:1 to yield **24** (2.02 g, 44%) and **25** (1.73 g, 38%) as a colorless oil.
24: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.40 (9H, s), 1.50 (3H, d, *J* = 7.1 Hz), 2.27 (1H, dd, *J* = 13.7, 8.3 Hz), 2.40 (3H, m), 2.56 (1H, dd, *J* = 14.3, 6.0 Hz), 3.09 (1H, d, *J* = 10.8 Hz), 3.21 (1H, d, *J* = 10.5 Hz), 4.90–5.16 (4H, m), 5.48 (1H, q, *J* = 7.1 Hz), 5.62–5.77 (2H, m), 7.27–7.32 (5H, m). MS (ESI) *m/z*: 370 $(M + H)^+$.
25: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.39 (9H, s), 1.49 (3H, d, *J* = 7.1 Hz), 2.16 (1H, dd, *J* = 14.1, 8.5 Hz), 2.37–2.45 (1H, m), 2.54–2.63 (2H, m), 2.85 (1H, t, *J* = 6.9 Hz), 3.15 (1H, d, *J* = 10.3 Hz), 3.21 (1H, d, *J* = 10.0 Hz), 5.02–5.17 (4H, m), 5.48 (1H, q, *J* = 7.2 Hz), 5.66–5.76 (1H, m), 5.94–6.04 (1H, m), 7.27–7.35 (5H, m). MS (ESI) *m/z*: 370 $(M + H)^+$.

***tert*-Butyl (3a*S*,7a*R*)-1-oxo-2-[(1*R*)-1-phenylethyl]-1,2,3,4,7,7a-hexahydro-3a*H*-isoindole-3a-carboxylate (26)**
To a solution of **24** (2.00 g, 5.41 mmol) in CH_2Cl_2 (54 mL) was added Grubbs' catalyst 2nd generation (91.9 mg, 0.108 mmol) at ambient temperature under N_2 atmosphere. After stirring for 1 h, the reaction solution was concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 1:1 to yield **26** (1.61 g, 87%) as a colorless oil. ^{1}H-NMR

(400 MHz, $CDCl_3$) δ: 1.18 (9H, s), 1.48 (3H, d, *J* = 7.1 Hz), 2.08–2.15 (1H, m), 2.42 (1H, m), 2.49 (1H, d, *J* = 5.4 Hz), 2.54–2.62 (1H, m), 2.74 (1H, dd, *J* = 16.4, 5.2 Hz), 3.19–3.25 (2H, m), 5.49 (1H, q, *J* = 7.2 Hz), 5.62–5.67 (1H, m), 5.75 (1H, m), 7.34–7.21 (5H, m). MS (ESI) *m/z*: 342 $(M + H)^+$.

(3a*S*,7a*R*)-3a-Amino-2-[(1*R*)-1-phenylethyl]-2,3,3a,4,7,7a-hexahydro-1*H*-isoindol-1-one (27)

To a solution of **26** (2.04 g, 5.99 mmol) in CH_2Cl_2 (18 mL) was added trifluoroacetic acid (18 mL) at ambient temperature. The reaction mixture was stirred for 15 h, concentrated *in vacuo* and azeotroped with toluene. To the solution of the resultant residue in toluene (29 mL) were added trimethylamine (1.61 mL, 11.5 mmol) and diphenylphosphoryl azide (1.62 mL, 7.52 mmol) at 0 °C under N_2 atmosphere. The mixture was stirred for 0.5 h at 100 °C, concentrated *in vacuo*, and azeotroped with EtOH. To the solution of the residue in 1,4-dioxane (14 mL) was added 4N aqueous HCl (14 mL). The mixture was stirred for 6 h at 50 °C. After concentration *in vacuo*, to the residue was added 1N aqueous NaOH to render it alkaline. The aqueous solution was extracted with $CHCl_3$. The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield **27** (1.24 g, 84%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.50 (3H, d, *J* = 7.1 Hz), 2.10 (1H, dd, *J* = 16.8, 5.0 Hz), 2.16–2.27 (1H, m), 2.33 (1H, m), 2.45 (2H, m), 2.96 (1H, d, *J* = 9.8 Hz), 3.23 (1H, d, *J* = 9.8 Hz), 3.70 (2H, s), 5.53 (1H, q, *J* = 7.1 Hz), 5.62–5.67 (1H, m), 5.77–5.81 (1H, m), 7.39–7.23 (5H, m). MS (ESI) *m/z*: 257 $(M + H)^+$.

***tert*-Butyl {(3a*S*,7a*S*)-2-[(1*R*)-1-phenylethyl]-1,2,3,4,7,7a-hexahydro-3a*H*-isoindol-3a-yl}carbamate (28)**

To a solution of **27** (612 mg, 2.39 mmol) in toluene (12 mL) was added sodium bis(2-methoxyethoxy)aluminum hydride (65% w/w in toluene, 2.87 mL, 9.56 mmol) at ambient temperature under N_2 atmosphere. After the reaction solution was stirred for 1 h at 80 °C, the mixture was cooled in ice-bath. To the reaction mixture were added 5 M aqueous NaOH and toluene. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. To a solution of the resultant residue in CH_2Cl_2 (9 mL) was added Boc_2O (689 mg, 3.16 mmol) at ambient temperature. The solution was stirred for 16 h and then concentrated *in vacuo*. The residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 1:1 to yield **28** (456 mg, 72%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.32 (3H, d, *J* = 6.6 Hz), 1.44 (9H, s), 1.83–1.99 (2H, m), 2.08–2.26 (2H, m), 2.52 (1H, dd, *J* = 11.3, 9.3 Hz), 2.60 (1H, d, *J* = 11.0 Hz), 2.89 (1H, d, *J* = 18.4 Hz), 3.04 (1H, dd, *J* = 9.0, 7.2 Hz), 3.53 (1H, d, *J* = 10.5 Hz), 3.62 (1H, q, *J* = 6.5 Hz), 4.43 (1H, s), 5.59–5.69 (2H, m), 7.29–7.18 (5H, m). MS (ESI) *m/z*: 343 $(M + H)^+$.

7-[(3a*S*,7a*S*)-3a-Aminooctahydro-2*H*-isoindol-2-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5)

To a solution of **28** (406 mg, 1.18 mmol) in EtOH (12 mL) was added 10% Pd-C (406 mg, 50% wet) at ambient temperature. The mixture was stirred under H_2 atmosphere for 7 h at 40 °C. After removal of catalyst by filtration, the filtrate was concentrated *in vacuo* to yield **29**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.44 (9H, s), 1.53–1.79 (9H, m), 2.52 (1H, d, *J* = 11.3 Hz), 2.62–2.67 (2H, m), 3.01 (1H, dd, *J* = 9.8, 7.6 Hz), 3.59 (1H, br s), 4.24 (1H, br s).

A solution of **29**, **6** (387 mg, 1.07 mmol) and triethylamine (0.449 mL, 3.22 mmol) in DMSO (2 mL) was stirred for 15 h at 35 °C. To the mixture were added EtOH (16 mL), water (4 mL) and triethylamine (2 mL). The resultant mixture was heated to reflux for 1.5 h, and then concentrated *in vacuo* to give the residue, which was diluted with EtOAc. The organic solution was washed with 10% aqueous citric acid solution, water, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was roughly purified by silica gel column chromatography, eluting with $CHCl_3$/MeOH = 98:2. To the residue was added 3 mL of concentrated aqueous HCl at 0 °C, and the mixture was stirred for 15 min at ambient temperature. The aqueous solution was rendered 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 $CHCl_3$/MeOH = 90:10. The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was recrystallized from EtOH to yield **5** (126 mg, 31%) as a pale yellow powder, mp 135–138 °C. ^{1}H-NMR (400 MHz, 0.1N NaOD/D_2O) δ: 1.28–1.40 (3H, m), 1.45–1.56 (3H, m), 1.67–1.72 (2H, m), 1.79–1.88 (3H, m), 3.27 (1H, d, *J* = 9.6 Hz), 3.44 (1H, t, *J* = 8.5 Hz), 3.54 (3H, s), 3.57–3.67 (2H, m), 3.96–4.02 (1H, m), 4.96–5.16 (1H, m), 7.65 (1H, d, *J* = 14.7 Hz), 8.35 (1H, d, *J* = 3.9 Hz). MS (ESI) *m/z*: 434 $(M+H)^+$. $[\alpha]_D^{25.0}$ −262.5 (*c* 0.025, 0.1N aqueous NaOH); Anal. Calcd for $C_{22}H_{25}F_2N_3O_4 \cdot 0.25H_2O$, C 60.33, H 5.87, F 8.68, N 9.59. Found, C 60.27, H 5.84, F 8.60, N 9.58. IR (ATR): 2929, 2859, 1722, 1617, 1508, 1432 cm^{-1}.

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