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

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A series of 8-methoxy or 8-methylquinolones bearing novel 3-aminoctahydrocyclopenta[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-aminoctahydrocyclopenta[c]pyrrole at the C-7 position7) (Fig. 1). The 3-aminoctahydrocyclopenta[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 bioavailability8) 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 values5) of compounds 1, 2, and 3, the antimicrobial activity of compound 7 was increased against most tested bacteria.

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 PA01 | 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). catarrhalis ATCC25238 | 0.05 | 0.025 | 0.025 | 0.025 ≤0.025 |
| S. aureus FDA 209-P | 0.025 | 0.012 | 0.015 | 0.1 | 0.1 |
| S. epidermidis 56500 | 0.1 | 0.1 | 0.1 | 0.39 | 0.2 |
| S. pneumoniae 324 | 0.05 | 0.05 | 0.05 | 0.78 | 0.78 |
| S. pyogenes G-36 | 0.2 | 0.2 | 0.2 | 0.78 | 1.56 |
| E. faecalis 19433 | 0.2 | 0.2 | 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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Compound 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 recovery13) (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 6.15) Compound 7 inhibited $E. coli$ DNA gyrase and topoisomerase IV with significantly lower IC$_{50}$ values, which

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Table 2. Antibacterial Activities (MIC, $\mu$g/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  |
| $P. aeruginosa$ PAOI        | 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  |
| $M(B). caterhalis$ 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    |
| $S. pneumoniae$ 324         | 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.
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. 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. Therefore, we evaluated the long QT, photosensitivity, and convulsion risks associated with compound 7.

Table 8 shows the effect of the drugs on the human ether-a-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 3. Physicochemical and Toxicological Properties of Quinolones

| Compound | $P'$ (CHCl$_3$) | Solubility (µg/mL) | Protein binding (%) | MLD (mg/kg) (mortality) | Micronucleus test (mg/kg)$^d$ |
|----------|-----------------|--------------------|---------------------|-------------------------|-----------------------------|
| 7        | 5.62            | >57.18             | 0                   | 100 (2/2)                | 50 (-)                      |
| LVFX     | 5.11            | >56.41             | NT                  | 100 (1/5)                | 100 (-)                     |
| MFLX     | 53.8            | 21.96              | NT                  | NT                      | NT                          |

$^a$ Apparent partition coefficient, CHCl$_3$-0.1 M phosphate buffer (pH 7.4); $^b$ 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

| Strain | MIC (µg/mL) |
|--------|-------------|
| 7      | LVFX | CPFX | MFLX | STFX |
| 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 |

### Table 5. Antibacterial Activity against Key Pathogenic Species of Clinical Isolates

| Species (#) | MIC$_{90}$ (µg/mL) |
|-------------|--------------------|
| 7 | LVFX | CPFX | MFLX |
| 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$^b$ (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 | 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.

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

Table 8 shows the effect of the drugs on the human ether-a-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.
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 and Table 10, 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 and 4, along with the comparative data 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 S. pneumoniae in murine respiratory tract infection due to S. pneumoniae GEO1085. 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.
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 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 hexamethyldisilazide (KHMDS), gave tert-butyloxycarbonyl 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 (LHMS), 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 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-
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. 1H-NMR spectra were determined on a JEOL JNM-EX400 spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standards. Significant 1H-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.

(3S)-3-[3-(tert-Butyldimethylsilyloxy)-1-propyl]-5-oxo-1-[(1R)-1-phenylethyl]pyrrolidine-3-carboxylic Acid tert-Butyl Ester (9) (3S)-3-(3-Hydroxy-1-propyl)-5-oxo-1-[(1R)-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. 1H-NMR (400 MHz, CDCl3) 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)+. 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. 1H-NMR (400 MHz, CDCl3) 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).

(1S,5R)-5-Fluoro-4-oxo-3-[(1R)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (15a) (3S)-3-[(3-Phenylsulfonyloxy-1-propyl)-4-fluoro-5-oxo-1-[(1R)-1-phenylethyl]pyrrolidine-3-carboxylic acid tert-butyl ester (14a), 4.36 g, 12.5 mmol) was dissolved in DCN (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% 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 as a pale yellow oil.

1H-NMR (400 MHz, CDCl3) 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 (9H, 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 C20H28FN2O3: 363.2084).

\((1R,5S)-1-(tert-Butoxycarbonylamino)-5-fluoro-3-[\{(1R)-1-phenylethyl\}-3-azabicyclo[3.3.0]octane (14a)\)

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 2h 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 2h. 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 = 95:5–9:10) to give 3.69 g (81%) of the title compound as a pale yellow powder. mp: 191–195°C (dec.). 

\([\alpha]_D^{22} +97^\circ (c = 0.100, 0.1 \text{ N NaOH})\).

1H-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 C24H22FN2O4: 361.2084; C, 56.27%; H, 5.22%; F, 12.72%; N, 9.37%. Found: C, 56.03%; H, 5.11%; F, 12.79%; N, 9.66%. IR (ATR) cm\(^{-1}\): 2958, 2871, 1724, 1621, 1513, 1452, 1319, 1056, 929, 804.

tert-Butyl (3S)-4-Chloro-3-(3-hydroxy-1-propyl)-5-oxo-1-
\([\{(1R)-1-phenylethyl\}pyrrolidin-3-3-azabicyclo[3.3.0]octane (12b)\)

Starting with 9 (960 mg, 2.08 mmol) and NCS (333 mg, 2.50 mmol) 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. 1H-NMR (CDCl3) 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 = 10.01 Hz), 3.14 (0.25H, d, J = 10.91 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 (1S,5R)-5-chloro-4-oxo-3-[\{(1R)-1-phenylethyl\}-
\[3-azabicyclo[3.3.0]octane (14b)\]

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.
(CDCl₃) 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: 364.170 (M + H⁺). 1H-NMR (CDCl₃) δ: 7.38 (1H, d, J = 7.1 Hz). HR-MS (ESI) m/z: 364.170 (M + H⁺). IR (ATR) cm⁻¹: 2986, 1730, 1691, 1249, 1239, 1157, 1122.

(1R,5R)-1-[(tert-Butoxycarbonyl)amino]-5-chloro-4-oxo-3-[(1R)-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. 1H-NMR (CDCl₃) δ: 7.38–7.26 (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⁺). HR-MS (ESI) m/z: 379.0000 (M + H⁺). 1H-NMR (CDCl₃) 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⁺). 1H-NMR (CDCl₃) 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⁺). IR (ATR) cm⁻¹: 2977, 1740, 1712, 1670, 1304, 1170, 1151.

(1S,5R)-4,6-DiOxo-3-[(1R)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-3-ylcarboxylic acid tert-Butyl Ester (18) (35.0 g, 93.2 mmol) was dissolved in THF (1 L). A 2M lithium diisopropylamidine–heptane–THF–ethylbenzene solution (100 mL, 200 mmol) was added dropwise in a nitrogen atmosphere at an internal temperature of −96°C over 30 min. After stirring at the same temperature for 1 h, the reaction solution was poured into a 10% hydrochloric acid solution (2 L) in an ice bath, followed by extraction with ethyl acetate (2 L), then 1L. The organic layers were concentrated under reduced pressure, and the precipitated solid was collected by filtration to give 2.88 g 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 2.69 g (65%) of the title compound 18 as colorless crystals.
(19) DMF (2.0 mL) was added to sodium hydride (152 mg, 3.48 mmol) in an argon atmosphere. A solution of (1S,5R)-4,6-dioxo-3-[(1R)-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. 1H-NMR (400 MHz, CDCl₃) 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).

(1S,5R)-6,6-Ethanediyldimercapto-5-methyl-4-oxo-3-[(1R)-1-phenylethyl]-3-azabicyclo[3.3.0]octan-1-ylcarboxylic Acid Methyl Ester (20) (1S,5R)-5-Methyl-4-dioxo-3-[(1R)-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). Toluene sulfonic 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. 1H-NMR (400 MHz, CDCl₃) 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 (2H, 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).

(1S,5S)-1-(tert-Butoxycarbonylamino)-5-methyl-4-oxo-3-[(1R)-1-phenylethyl]-3-azabicyclo[3.3.0]octane (21) (1S,5R)-5-Methyl-4-oxo-3-[(1R)-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. 1H-NMR (400 MHz, CDCl₃) δ: 7.33–7.16 (5H, m), 4.79 (1H, brs), 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).

(1S,5S)-1-(tert-Butoxycarbonylamino)-5-methyl-4-oxo-3-azabicyclo[3.3.0]octane (23) (1S,5S)-1-(tert-Butoxycarbonylamino)-5-methyl-4-oxo-3-[(1R)-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% wt, 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. 1H-NMR (400 MHz, CDCl3) δ: 4.68 (1H, brs), 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-[(1R,5S)-1-Amino-3-aza-5-methylbicyclo[3.3.0]octan-3-yl]-6-fluoro-1-[(1R,2S)-2-fluorocyclopropyl]-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.). [α]D26 +78.2° (c = 0.135, 0.1 N NaOH). 1H-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. Calculated for C22H25F2N3O4·2H2O: C, 62.24%; H, 5.75%; N, 9.01%. IR (ATR) cm⁻¹: 3038, 2976, 1724. HR-MS (ESI) m/z: 386 (M + H)+. 1H-NMR (CDCl3) 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: 138 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+. HR-MS (ESI) m/z: 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+.

Ethyl 2-[(4-Bromo-2,5-difluoro-3-methylbenzoyl)-3-diethylaminocarboxylate (27) 2,5-Difluoro-4-bromo-3-methylbenzoic acid (26, 10.7 g, 42.4 mmol) was dissolved in toluene (160 mL). Thiouyl 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-diethylaminocarboxylate (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 = 1:1) 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. 1H-NMR (CDCl3) δ: 7.81–7.74 (1H, m), 7.27–7.16 (1H, m), 4.00 (2H, q, J = 7.1 Hz), 3.31 (3H, brs), 2.89 (3H, brs), 2.35 (3H, d, J = 2.9 Hz), 0.97 (3H, t, J = 7.1 Hz).

Ethyl 7-bromo-6-fluoro-1-[(1R,2S)-2-fluorocyclopropyl]-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylate (25) Ethyl 2-[(4-bromo-2,5-difluoro-3-methylbenzoyl)-3-diethylaminocarboxylate (27, 11.4 g, 30.2 mmol) was dissolved in DCM (200 mL). (1R,2S)-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 aminocarboxylic acid as a yellow oil. The resulting aminocarboxylic acid 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) to give the title compound (25, 2.98 g, 26%) as a colorless powder. mp: 191–195°C (dec.). [α]D26 −166.5° (c = 0.159, CHCl3). 1H-NMR (CDCl3) 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 C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+, 386.0203 (M + H)+. HR-MS (ESI) m/z: 386.0229 (M + H)+ (Calcd for C22H25F2N3O4·2H2O: M + H)+.
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% H2O–isopropanol to afford 0.87 g (73%) of the title compound 7 as colorless powder. mp: 227–230°C (dec.). [α]D 20 = −153.9° (c = 0.104, 0.1 N NaOH). 1H-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 C23H20F5N4O6·HCl·2H2O: 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, 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 10⁵ 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. The MICs of the compounds were determined by the 2-fold dilution method using Mueller–Hinton broth (Difco Laboratories, Detroit, MI, U.S.A.) with an inoculum size of 10⁵ 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 administrated 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 acidic 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 IC₅₀ 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 ATCC6051. 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 murine RTI model 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 Crj:CD(SD)(IJS) 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.

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Conflict of Interest The authors declare no conflict of interest.

References and Notes

1) Tong S. Y. C., McDonald M. J., Hohl D. C., Currie B. J., Clin. Infect. Dis., 46, 1871–1878 (2008).
2) Rice L. B., J. Infect. Dis., 197, 1079–1081 (2008).
3) Roulet D. H., Bouchier H. W., Taiлот G. H., Bradley J. S., Edwards J. E., Gilbert D., Rice L. B., Scheld M., Spellberg B., Bartlett J., Clin. Infect. Dis., 48, 1–12 (2009).
4) Larmin M., Lancet Infect. Dis., 3, 322 (2003).
5) WHO published the list of bacteria for which new antibiotics are urgently needed on February 7, 2017.
6) Odagiri T., Inagaki H., Sugimoto Y., Nagamochi M., Miyauichi R., Kuroyanagi J., Kitamura T., Komoriya S., Takahashi H., J. Med. Chem., 56, 1974–1983 (2013).
7) Odagiri T., Inagaki H., Nagamochi M., Kitamura T., Komoriya S., Takahashi H., Heterocycles, 96, 858–881 (2018).
8) PK data of compound 2: Rat, p.o., 5 mg/kg, Serum Cmax 0.05 μg/mL, AUC0–24 h 0.21 μg h/mL.
9) Domagal J. M., J. Antimicrob. Chemother., 33, 685–706 (1994).
10) Gootz T. D., Brighty K. E., Clin. Infect. Dis., 41, 37–42 (2005).
11) Van Bambek F., Michot J. M., Van Eldere J., Tulkens P. M., Clin. Microbiol. Infect., 11, 256–280 (2005).
12) Atarashi S., Imamura M., Kimura Y., Yoshida A., Hayakawa I., J. Med. Chem., 36, 3444–3448 (1993).
13) PK data of compound 4: Rat, p.o., 5 mg/kg, Serum Cmax 1.05 μg/mL, AUC0–24 h 4.40 μg h/mL, Urinary recovery 0–24 h 6.2%.
14) 52th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, F-2035, 2037, 2038, 2041, 2042 (2012).
15) Stahlmann R., Lode H., Drugs, 58 (Suppl. 2), 37–42 (1999).
16) Oliphant C. M., Greeth O. M., Am. Fam. Physician, 65, 455–464 (2002).
17) Owens R. C. Jr., Ambrose P. G., Clin. Infect. Dis., 41 (Suppl. 2), S144–S157 (2005).
18) Stahlmann R., Lode H., Drugs Aging, 27, 193–209 (2010).
19) Miyazaki R., Kawakami K., Ito M., Matsuhashi N., Ohki H., Inagaki H., Takahashi H., Takemura M., Bioorg. Med. Chem., 17, 6879–6889 (2009).
20) Higuchi S., Kurosaka Y., Uoyama S., Yoshida K., Chiba M., Ishii C., Fujikawa K., Karibe Y., Hoshino K., J. Infect. Chemother., 20, 312–316 (2014).
21) Onodera Y., Uchida Y., Tanaka M., Sato K., J. Antimicrob. Chemother., 44, 533–536 (1999).
22) Akasaka T., Kurosaka S., Uchida Y., Tanaka M., Sato K., Hayakawa I., Antimicrob. Agents Chemother., 42, 1284–1287 (1998).