Bioorganic & Medicinal Chemistry 17 (2009) 6879–6889

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluations of novel 7-[3-(1-aminocycloalkyl)pyrrolidin-1-yl]-6-desfluoro-8-methoxyquinolones with potent antibacterial activity against multi-drug resistant Gram-positive bacteria ☆

Rie Miyauchi [a,*], Katsuhiro Kawakami [a], Masao Ito [a], Norikazu Matsuhashi [b], Hitoshi Ohki [a], Hiroaki Inagaki [a], Hisashi Takahashi [a], Makoto Takemura [a]

[a] Medicinal Chemistry Research Laboratories II, Daiichi Sankyo Co., Ltd, 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan
[b] Exploratory Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan

ARTICLE INFO

Article history:
Received 6 July 2009
Revised 11 August 2009
Accepted 13 August 2009
Available online 20 August 2009

Keywords:
6-Desfluoro-quinolones
Type II topoisomerases
Gram-positive bacteria
Antibacterial agents

ABSTRACT

A series of novel 6-desfluoro [des-F(6)] and 6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methoxyquinolones bearing 3-(1-aminocycloalkyl)pyrrolidin-1-yl substituents at the C-7 position (**1–6**) was synthesized to obtain potent drugs for nosocomial infections caused by Gram-positive pathogens. The des-F(6) compounds **4–6** exhibited at least four times more potent activity against representative Gram-positive bacteria than ciprofloxacin or moxifloxacin. Among the derivatives, 7-[(3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl] derivative **4**, which showed favorable profiles in preliminary toxicological and nonclinical pharmacokinetic studies, exhibited potent antibacterial activity against clinically isolated Gram-positive pathogens that had become resistant to one or more antibiotics.

1. Introduction

Multi-drug-resistant Gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE), have become a serious problem in the medical community.[2–5] One particularly alarming sign is the acquisition of resistance to vancomycin (VRE and vancomycin-resistant staphylococcal strains), an antibiotic generally regarded as the agent of last resort for hospital-acquired infections.[2,4–6] In the field of quinolone antibacterial agents, various attempts have been made to obtain potent drugs for resistant Gram-positive bacteria, and trovafloxacin,[7] moxifloxacin,[8] gemifloxacin,[9] and gatifloxacin,[10] etc., have been developed and introduced into clinical use over the last few years. Considering that the incidence of Gram-positive bacterial resistance to antibacterial agents has been growing, however, the antibacterial activities of these newer quinolones are not potent enough and bacteria resistance to these agents will be problematic in the near future.[11,12] There are a few agents other than quinolones, such as teicoplanin,[13] quinupristin/dalfopristin,[14] and linezolid,[15] tigecycline,[16] daptomycin,[17] which are now available in clinical use, but they show some problems, for example, resistance mutations and/or side effects.[5,18] These problems have been the driving force for the development of new antibacterial agents that would be applicable to infections caused by multi-drug-resistant Gram-positive pathogens.

In our previous paper, we reported that several quinolone derivatives bearing 3-(1-amino-1-substituted-methyl)pyrrolidin-1-yl groups, including 3-(1-aminocycloalkyl)pyrrolidin-1-yl substituent, at the C-7 position showed potent antibacterial activity against Gram-positive bacteria.[19] Although 7-[3-(1-aminocycloalkyl)pyrrolidin-1-yl]quinolone derivatives exhibit the most potent activity among them, they possess higher genotoxicity than 7-(piperazin-1-yl) or 7-(3-aminopyrrolidin-1-yl) quinolone derivatives. The high genotoxicity of 7-(3-aminomethylpyrrolidin-1-yl)quinolone derivatives has also been reported by other groups.[20–23] As a method to reduce this genotoxicity, we reported the usefulness of introducing a (1*R*,2*S*)-2-fluorocyclopropan-1-yl substituent into the N-1 position instead of a cyclopropyl substituent.[24–26] Furthermore, 8-methoxyquinolone derivatives were reported to exhibit potent antibacterial activity against Gram-positive bacteria and show reduced phototoxicity in comparison with several quinolone derivatives.[27,28] Therefore, we fully used the scientific knowledge of the structure–activity relationships of quinolone antibacterials and a new 1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methoxyquino-

☆ See Ref. 1.

* Corresponding author. Tel.: +81 3 3680 0151; fax: +81 3 5696 8344.
E-mail address: miyauchi.rie.hr@daiichisankyo.co.jp (R. Miyauchi).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.08.026

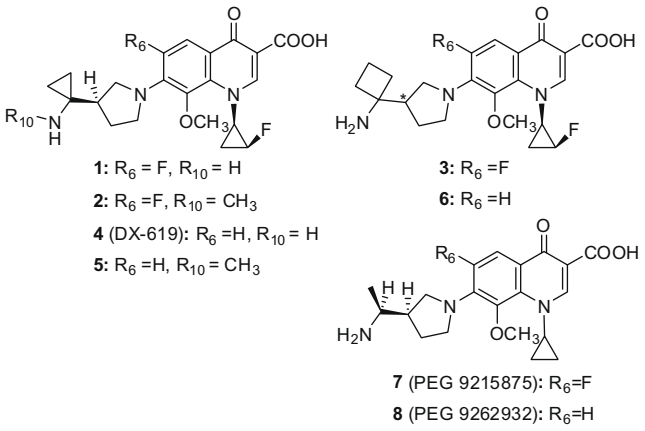


Figure 1. Chemical structure of 8-methoxyquinolones. (* The compound is enantiomeric, although the absolute configuration has not been determined.)

lone having the (3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl substituent at the C-7 position (**1,** Fig. 1) was designed and synthesized to obtain a highly potent compound against Gram-positive bacteria with reduced genotoxicity. As we expected, **1** showed highly potent antibacterial activity, but showed a positive response in a micronucleus test and was toxic in a chromosome injuring test.

Recently, favorable safety profiles of 6-desfluoro [des-F(6)]-quinolones have been elucidated by a number of groups.[29–31] In particular, it was reported by Ledoussal et al. that the genotoxicity of des-F(6)-quinolone **8** (PEG 9262932) was less than that of 6-fluoro (6-F)-quinolone **7** (PEG 9215875).[32] As another approach to reduce the toxicity of 7-[(3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl]quinolone derivatives, we planned to utilize this strategy for compound **1**, and designed and synthesized several des-F(6)-quinolone compounds having the (3*R*)-3-(1-aminocyclopropan-1-yl) pyrrolidin-1-yl substituent **4** (DX-619),[1] (3*R*)-3-(1-methylaminocyclopropan-1-yl)pyrrolidin-1-yl substituent (**5**), or 3-(1-aminocyclobutan-1-yl)pyrrolidin-1-yl substituent (**6**)[33] at the C-7 position.

In this paper, we draw parallels between des-F(6)-quinolones **4–6** and 6-F-quinolones **1–3**. In addition, we report the synthesis, the in vitro antibacterial activity, and the toxicity profiles of the 8-methoxyquinolones **1–6**.

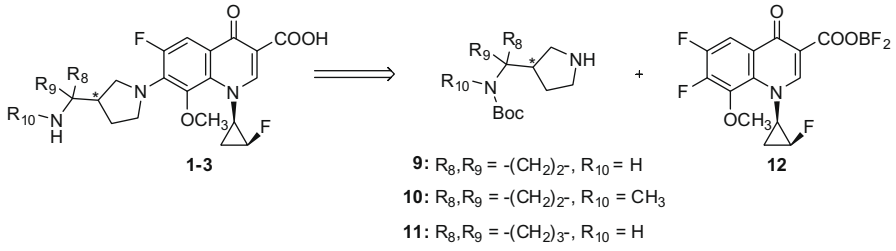
2. Chemistry

We planned to synthesize compounds **1–6** by an aromatic nucleophilic substitution reaction from 8-methoxyquinolonecarboxlic derivatives **12, 13** and appropriate 3-(1-aminoalkyl)pyrrolidines **9–11**, of which the peripheral amino groups were protected by *tert*-butoxycarbonyl groups (Schemes 1 and 2).

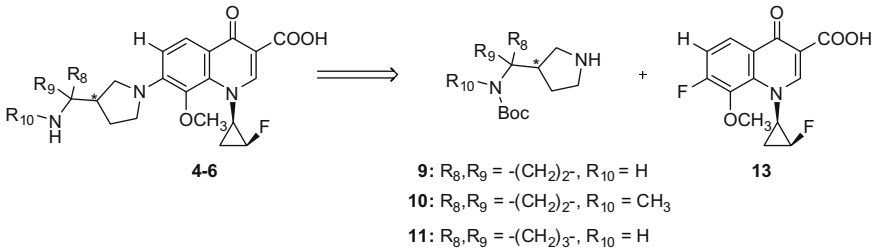
The synthesis of the difluoroboron chelate derivative of the 6,7-difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methoxyquinolone-3-carboxilic acid **12** is illustrated in Scheme 3. Thus, the reaction of the acid chloride of benzoic acid **14** with ethyl 3-(dimethylamino)acrylate, reported by Ataka et al.,[34] followed by substitution of the dimethylamino group with (1*R*,2*S*)-2-fluoro-1-cyclopropylamine, gave crude enaminoketoester. Cyclization of the resultant product with potassium carbonate in *N*,*N*-dimethylformamide yielded ethyl 6,7-difluoro-8-methoxyquinolone-3-carboxylate **15**. The acidic hydrolysis of **15**, followed by treatment of the resultant 6,7-difluoro-8-methoxyquinolone-3-carboxylate with boron trifluoride diethyl etherate in diethyl ether provided difluoroboron chelate **12**.

The synthesis of the 7-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methoxyquinolone-3-carboxilic acid **13** is illustrated in Scheme 4. Treatment of ketoester **17**, reported by Ledoussal et al.[35] with ethyl orthoformate in acetic anhydride followed by the reaction with (1*R*,2*S*)-2-fluoro-1-cyclopropylamine *p*-toluenesulfonate in the presence of triethylamine provided the crude enaminoketoester. Cyclization of the resultant product with sodium hydride in 1,4-dioxane yielded ethyl 6-desfluoro-8-methoxyquinolone-3-carboxylate **18**. Acidic hydrolysis of **18** provided quinolonecarboxylic acid **13**.

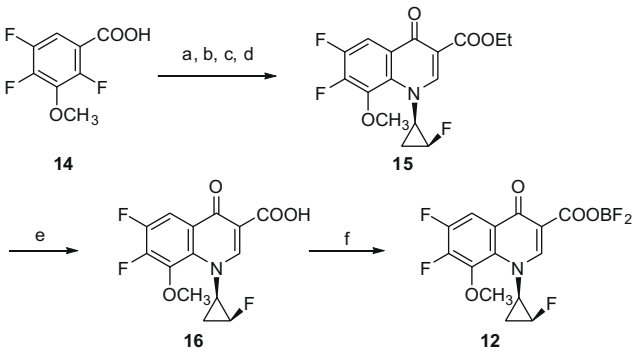
The synthesis of (3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl substituents **9**[36] is illustrated in Scheme 5. We designed a facile synthetic route for **9** by using the intramolecular Horner–Wadsworth–Emmons reaction of ketophosphonate **20**. Treatment of the amino keto ester **19**, reported in our previous paper,[37] with diethylphosphonoacetic acid in the presence of 1,1′-Carbonyldiimidazole provided the condensation product **20**. The subsequent intramolec-



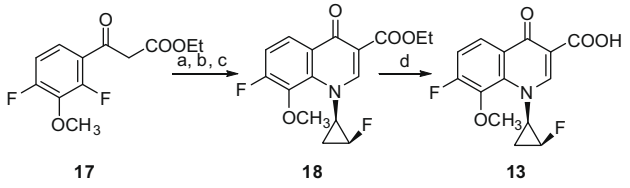
Scheme 1. * The compound is enantiomeric, although the absolute configuration has not been determined (see Ref. 33).



Scheme 2. * The compound is enantiomeric, although the absolute configuration has not been determined (see Ref. 33).

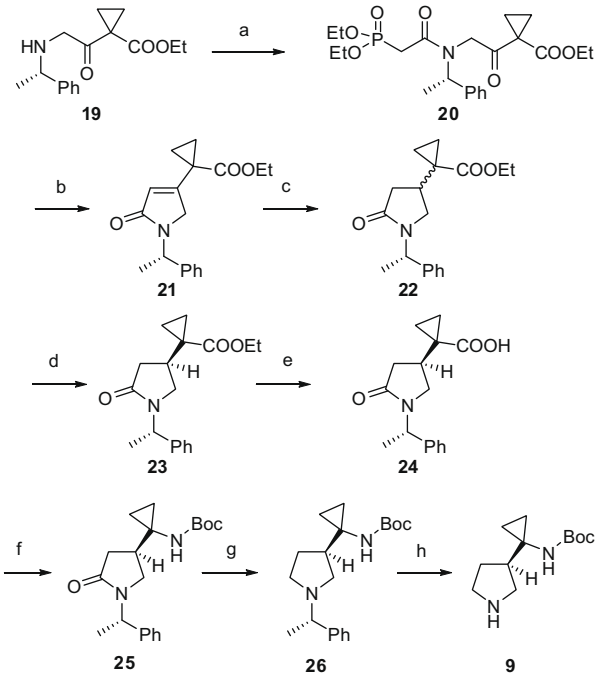


Scheme 3. Reagents and conditions: (a) $SOCl_2$/toluene, reflux; (b) ethyl 3-(dimethylamino)acrylate, Et_2N/THF; (c) (1*R*,2*S*)-2-fluoro-1-cyclopropylamine *p*-toluenesulfonate, Et_3N/CH_2Cl_2; (d) K_2CO_3/DMF; (e) concentrated aqueous HCl, AcOH, reflux; (f) BF_3–OEt_2/Et_2O.

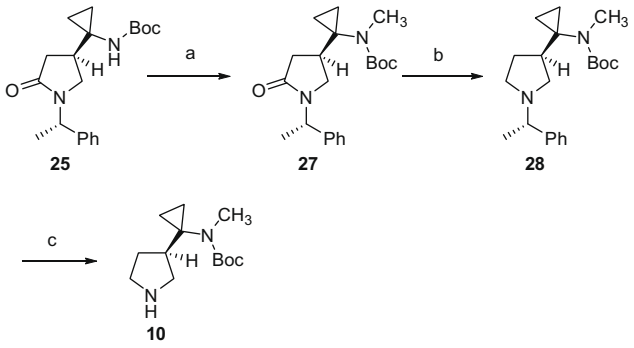


Scheme 4. Reagents and conditions: (a) Ac_2O, $CH(OEt)_3$, reflux; (b) (1*R*,2*S*)-2-fluoro-1-cyclopropylamine *p*-toluenesulfonate, Et_3N/CH_2Cl_2; (c) NaH/1,4-dioxane; (d) concentrated aqueous HCl, AcOH, reflux.

ular Horner–Wadsworth–Emmons reaction was proceeded by using potassium *tert*-butoxide in toluene below 0 °C to yield cyclic enamide **21.**[38] Hydrogenation of **21** catalyzed by platinum carbon gave a mixture of two diastereomers **22** (3.5:1), which were separated by silica gel column chromatography. The major isomer **23** was converted to the *tert*-butoxycarbonylamino compound **25** by hydrolysis



Scheme 5. Reagents and conditions: (a) $(EtO)_2P(O)CH_2COOH$, CDI/THF; (b) *tert*-BuOK/toluene; (c) H_2, 5% Pt–C/EtOAc; (d) silica gel column chromatography; (e) aqueous NaOH/EtOH; (f) diphenylphosphoryl azide, Et_3N/toluene, then *tert*-BuOH; (g) BH_3–THF/THF, then K_2CO_3/H_2O, reflux; (h) H_2, Pd–C/EtOH.



Scheme 6. Reagents and conditions: (a) NaH, CH_3I/DMF; (b) BH_3–THF/THF, then K_2CO_3/H_2O, reflux; (c) H_2, Pd–C/EtOH.

and a subsequent Curtius rearrangement using diphenylphosphoryl azide (DPPA) and *tert*-butyl alcohol. Reduction of the amide moiety of **25** with the borane–tetrahydrofuran complex provided *N*-(1-phenylethyl)pyrrolidine derivative **26**. Deprotection of the 1-phenylethyl group by catalytic hydrogenation in ethanol yielded the C-7 substituent **9**. C-7 substituent **9** was used for the next reaction without purification.

The synthesis of (3*R*)-3-(1-methylaminocyclopropan-1-yl)pyrrolidin-1-yl substituent **10** is illustrated in Scheme 6. Treatment of the amide **25** with methyl iodide in the presence of sodium hydride provided N-methylation product **27**. Reduction of the amide moiety of **27** with the borane–tetrahydrofuran complex provided *N*-(1-phenylethyl)pyrrolidine derivative **28**. Deprotection of the 1-phenylethyl group by catalytic hydrogenation in ethanol yielded the C-7 substituent **10**. C-7 substituent **10** was used for the next reaction without purification.

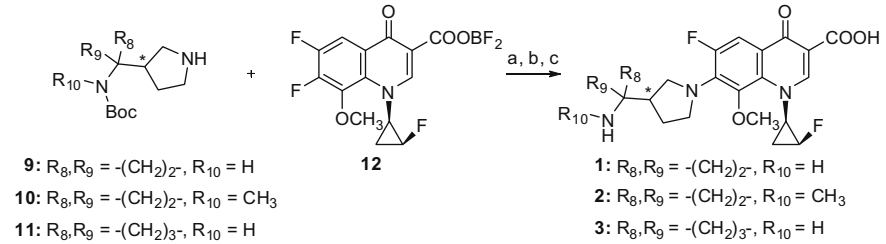
The reaction of the resultant crude products **9,10** with difluoroboron chelate **12**, followed by removal of the chelates under basic conditions and deprotection of the *tert*-butoxycarbonyl groups under acidic conditions, gave the 6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methoxyquinolone-3-carboxilic acid having the 3-(1-aminocyclopropyl)pyrrolidine substituents **1–2**, as shown in Scheme 7. The 7-[3-(1-aminocyclobutan-1-yl)pyrrolidine] compound **3** was synthesized by the same procedure as described in Scheme 7, from C-7 substituent **11**, reported in our previous paper.[33]

Finally, as illustrated in Scheme 8, the pyrrolidines of **9–11** and quinolonecarboxylic acid **13** were heated with triethylamine in dimethylsulfoxide followed by deprotection of *tert*-butoxycarbonyl groups under acidic condition to give des-F(6)-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-quinolone compounds having 3-(1-aminocycloalkyl)pyrrolidin-1-yl substituent, **4–6**, respectively. The hydrochloride of compound **4** was obtained as prisms, and the absolute configuration was determined by X-ray crystallographic analysis.

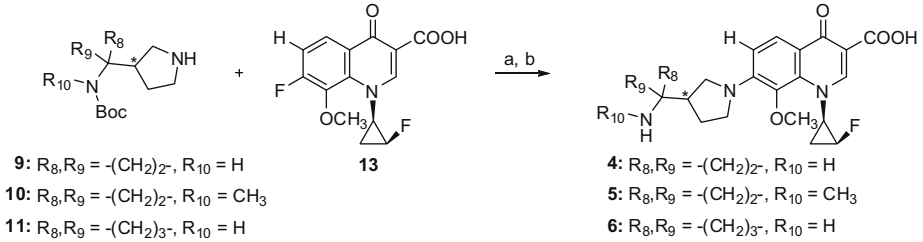
The 1-cyclopropanyl-quinolone compounds **7, 8** were synthesized by the same procedure as described in Scheme 7, reported by Domagala et al.,[39,40] or in Scheme 8, reported by Ledoussal et al., respectively.[35]

3. Results and discussion

The minimum inhibitory concentrations (MICs) of **1–8** against several representative Gram-positive and Gram-negative bacteria are summarized in Table 1, along with the data for garenoxacin,[30] trovafloxacin, moxifloxacin, gatifloxacin, and ciprofloxacin[41] for comparison. The synthesized compounds **1–8** exhibited 4–520 times more potent activity against Gram-positive bacteria than the reference quinolones, trovafloxacin, moxifloxacin, gatifloxacin, or ciprofloxacin. In particular, the des-F(6)-1-[(1*R*,2*S*)-2-fluorocy-



Scheme 7. Reagents and conditions: (a) Et_3N/DMSO; (b) Et_3N/80% aqueous EtOH, reflux; (c) concentrated aqueous HCl.



Scheme 8. Reagents and conditions: (a) Et_3N/DMSO, heat; (b) concentrated aqueous HCl.

clopropan-1-yl]-quinolone compounds **4–6** were at least four times more potent than the newer quinolones, garenoxacin, trovafloxacin, moxifloxacin, or gatifloxacin, which were designed for Gram-positive infections. Against Gram-negative bacteria except for *Pseudomonas aeruginosa*, **1–8** exhibited potency comparable with that of trovafloxacin and ciprofloxacin. The compounds possessing a fluorine atom at the C-6 position **1–3** exhibited 1–4 times more potent activity against both Gram-positive and Gram-negative bacteria than the des-F(6) compounds **4–6.** Thus, concerning substitution at the C-6 position about our compounds **1–6**, the rank of inhibitory activity was fluorine (F) > hydrogen (H). However, compound **7** and des-F(6) compound **8** showed almost identical antibacterial activities, and the introduction of a fluorine atom to the C-6 position of **8** did not affect antibacterial activity substantially.

The results of the intravenous single-dose toxicity study and the micronucleus test are summarized in Tables 2 and 3. Concerning intravenous single-dose toxicity, des-F(6) compounds **4–6** or **8** were less toxic than compounds **1–3** or **7**, which possesses a fluorine atom at the C-6 position. In particular, compounds **4** and **5**, which have the (3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl substituent at the C-7 position, were less toxic than compound **6**, which has the 3-(amincyclobutan-1-yl)pyrrolidin-1-yl substituent, or **8**, which has the (3*R*,1′*S*)-3-(1-aminoethyl) pyrrolidin-1-yl substituent. Concerning micronuclei-forming toxicity, compounds **4** and **5** showed different profiles according to their N-substituent. Compound **5**, which has *N*-methyl substituent, was more toxic than compound **4**, which has (3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl substituent. Thus, compound **5** showed a positive response, even though it has a combination of des-F(6) and the (1*R*,2*S*)-2-fluorocyclopropan-1-yl group at the N-1 position, which are reported to reduce genotoxicity.[23–28] The des-F(6) compounds **4, 6** and **8** showed negative responses as we expected. The results indicate the advantage of removing the fluorine atom at the C-6 position for reducing intravenous single-dose toxicity and micronuclei-forming toxicity.

Table 4 shows the solubilities to water and apparent partition coefficients of these compounds. The des-F(6) compounds **4, 6** or **8** were more soluble than the fluorinated compounds **1, 3** or **7**. However, des-F(6) compounds(**4, 5** or **8**) and the corresponding fluorinated compounds(**1, 2** or **7**) showed the almost same apparent partition coefficients. These data indicate that the removing

Table 1
Antibacterial activity MIC (μg/ml) of the compounds **1–8** and reference compounds

Compound	Organism								
	S. aureus FDA 209-P	*S. epidermidis* 56,500	*S. pneumoniae* J-24	*S. pyogenes* G-36	*S. mitis* IID685	*E. faecalis* ATCC19433	*E. coli* NIHJ	*K. pneumoniae* type II	*P. aeruginosa* PAO1
1	⩽0.003	⩽0.003	⩽0.003	⩽0.003	0.003	0.012	⩽0.003	0.012	0.2
2	⩽0.003	0.006	⩽0.003	⩽0.003	0.003	0.025	⩽0.003	0.012	0.2
3	⩽0.003	⩽0.003	⩽0.003	⩽0.003	0.003	0.025	⩽0.003	0.025	0.39
4	⩽0.003	0.006	0.006	0.006	0.003	0.025	⩽0.003	0.05	0.39
5	⩽0.003	0.012	0.006	0.012	0.003	0.05	0.006	0.05	0.39
6	⩽0.003	0.012	0.006	0.006	0.003	0.05	0.012	0.05	0.39
7	0.0006	0.012	N.T.	0.012	0.025	0.05	0.012	0.05	N.T.
8	⩽0.003	0.006	⩽0.003	0.012	0.006	0.05	0.012	0.1	0.2
TVFX[a]	0.013	0.05	0.05	0.2	0.1	0.2	⩽0.003	0.025	0.2
GRNX[a]	0.025	0.05	0.025	0.1	0.05	0.2	0.012	0.05	0.78
MFLX[a]	0.013	0.1	0.025	0.2	0.1	0.2	0.006	0.05	0.78
GFLX[a]	0.05	0.2	0.2	0.39	0.2	0.39	0.006	0.05	0.78
CPFX[a]	0.05	0.2	0.39	1.56	0.78	0.78	⩽0.003	0.025	0.05

[a] Abbreviations are as follows: TVFX = trovafloxacin; GRNX = garenoxacin; MFLX = moxifloxacin; GFLX = gatifloxacin; CPFX = ciprofloxacin.

Table 2
Intravenous single-dose toxicity of **1–8** in mice

Dose (mg/kg)	Mortality (dead/tested)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
150	2/2	2/2	2/2	1/3	1/4	2/2	1/1	2/2
100	2/2	2/2	2/3	0/5	0/2	0/5	4/4	2/2
50	5/5	5/5	N.T.[a]	N.T.	N.T.	0/5	4/4	0/5

[a] Not tested.

Table 3
Micronuclei-forming toxicity of **1–8** in mice

Result (dose, mg/kg)							
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
N.T.[a]	N.T.	N.T.	−[b] (150)	+[c] (100)	− (100)	N.T.	− (50)

[a] Not tested.
[b] Negative.
[c] Positive.

fluorine atom at the C-6 position contributes to improving the solubility in water with similar lipophilicity.

The MICs against clinically isolated resistant Gram-positive bacteria of compound **4**, which exhibited the lowest single-dose toxicity and a negative response in the micronucleus test, were determined and the results are shown in Table 5. Compound **4** exhibited more potent activity than the other quinolone antibacterial agents (gatifloxacin, moxifloxacin, or garenoxacin), or the other categories of drugs (vancomycin, teicoplanin, or linezolid) and the activity was that of with quinupristin/dalfopristin against ciprofloxacin-resistant MRSA (Bacterial strains were collected by the Levofloxacin Surveillance Group from patients in Japan in 2000[42]). Against penicillin-intermediate resistant *S. pneumoniae* (PISP)+PRSP, **4** exhibited the most potent activity among the listed compounds. Compound **4** also exhibited the most potent activity against both strains of VRE (*Enterococcus faecalis* and *Enterococcus faecium*) among the listed compounds.

The bacterial strains were collected by the Levofloxacin Surveillance Group from patients in Japan in 2007. The MICs against levofloxacin-resistant MRSA of compound **4** were determined and the results are shown in Table 6, along with the data for garenoxacin, compound **8**, moxifloxacin, ciprofloxacin, levofloxacin and vancomycin for comparison. The MIC at which 90% of isolates are inhibited (MIC_{90}) of compound **4** for levofloxacin-resistant MRSA was 1 μg/ml, which was superior to those of the reference quinolones garenoxacin, compound **8**, moxifloxacin, ciprofloxacin, and levofloxacin. In particular, compound **4** was 64 times and eight times more potent than the des-F(6) quinolones garenoxacin and compound **8**, which were designed for Gram-positive infections. Com-

Table 6
Antibacterial activities of **4** and reference compounds against clinically isolated levofloxacin-resistant MRSA (LVFX-r MRSA: The bacterial strains were collected by the Levofloxacin Surveillance Group from patients in Japan in 2007) (MIC: μg/ml)

Compound	LVFX-r MRSA[a] (24[b])			
	Range	MIC_{50}[c]	MIC_{80}[c]	MIC_{90}[c]
4	⩽0.03–1	0.12	1	1
GRNX[c]	0.25–64	2	64	64
8	0.12–8	0.5	8	8
MFLX[c]	1–32	4	32	32
CPFX[c]	8–>64	>64	>64	>64
LVFX[c]	4–>64	16	>64	>64
VCM[c]	0.5–2	1	1	1

[a] The bacterial strains were collected by the Levofloxacin Surveillance Group from patients in Japan in 2007.
[b] No. of strains.
[c] Abbreviations are as follows: GRNX = garenoxacin; MFLX = moxifloxacin; CPFX = ciprofloxacin; LVFX = levofloxacin; VCM = vancomycin MIC_{50} = MIC at which 50% of isolates are inhibited; MIC_{80} = MIC at which 80% of isolates are inhibited; MIC_{90} = MIC at which 90% of isolates are inhibited.

Table 4
Physicochemical profiles of **1–8**

	Compound							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Aqueous solubility (μg/ml)	221	173	180	480	161	604	89	>10,000
P′[a]	74.1	93.9	26.9	75.9	>85.9	9.9	9.9	9.5

[a] Apparent partition coefficient, $CHCl_3$—0.1 M phosphate buffer (pH 7.4).

Table 5
Antibacterial activities of **4** and reference compounds against clinically isolated ciprofloxacin-resistant MRSA (CPFX-r MRSA), PRSP, and VRE (MIC: μg/ml)

Compound	Organisms											
	CPFX-r MRSA[a] (99[b])			PISP+PRSP[c] (50[b])			VRE (*E. faecalis*) (18[b])			VRE (*E. faecium*) (19[b])		
	Range	MIC_{50}[d]	MIC_{90}[d]	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
4	⩽0.03–1	0.06	0.5	0.008–0.06	0.03	0.03	0.015–0.5	0.25	0.5	0.06–4	0.25	2
GFLX[d]	1–128	4	64	0.25–1	0.5	0.5	0.25–32	16	32	0.5–32	4	32
MFLX[d]	1–64	2	32	0.12–0.5	0.25	0.5	0.12–32	16	32	0.5–32	4	32
GRNX[d]	0.25–64	1	32	0.03–0.25	0.12	0.12	0.06–8	4	8	0.25–32	8	32
ABK[d]	4–>128	8	32	16–64	32	64	16–>128	2	2	2–64	16	32
VCM[d]	0.5–2	1	2	0.25–1	0.5	0.5	8–>128	>128	>128	32–>128	>128	>128
TEIC[d]	0.25–4	1	1	N.T.			0.25–128	64	128	0.5–64	32	64
QPR/DPR[d]	0.12–4	0.5	0.5	0.5–4	1	1	0.5–16	8	16	0.5–4	0.5	2
LNZ[d]	0.5–2	1	1	1–2	1	2	2	2	2	2	2	2

[a] MIC range of ciprofloxacin is 8–>64 μg/ml. (Bacterial strains were collected by the Levofloxacin Surveillance Group from patients in Japan in 2000.[42])
[b] No. of strains.
[c] MIC range of benzylpenicillin is 1–4 μg/ml.
[d] Abbreviations are as follows: GFLX = gatifloxacin; MFLX = moxifloxacin; GRNX = garenoxacin; ABK = arbekacin; VCM = vancomycin; TEIC = teicoplanin; QPR/DPR = quinupristin/dalfopristin; LNZ = linezolid; MIC_{50} = MIC at which 50% of isolates are inhibited; MIC_{90} = MIC at which 90% of isolates are inhibited.

pound **4** showed excellent antibacterial activity, even against the latest (2007) clinically isolated levofloxacin-resistant MRSA.

The potent antibacterial activity of compound **4** described in this paper is due, in part, to its potent inhibitory activity against bacterial type II topoisomerases. The inhibitory effects of compound **4** against type II topoisomerases of *S. aureus* were determined as 50% inhibitory concentrations (IC_{50}s) and the results are shown in Tables 7 and 8. Against wild-type topoisomerase IV and altered topoisomerase IV with quinolone-resistant mutation (GrlA; S80F) of *S. aureus*, IC_{50}s of compound **4** were 0.309 μg/ml and 1.76 μg/ml, respectively (Table 7). Against DNA gyrase and altered DNA gyrase with quinolone-resistant mutation (GyrA; S84L) of *S. aureus*, IC_{50}s of compound **4** were 0.835 μg/ml and 7.81 μg/ml, respectively (Table 8). Compound **4** showed the most potent inhibitory activities against wild type and altered quinolone-resistant target enzymes. Inhibitory activities of **4** against altered enzymes were the same level as those of comparator quinolones against wild type enzyme. The ratio of the IC_{50}s of **4** against wild-type enzyme to altered one was also the lowest among the quinolone tested. From these results the potent antibacterial activity of compound **4** against various types of quinolone-resistant MRSA may due to the potent inhibitory activity against both target enzymes.

The pharmacokinetic profiles of compound **4** (20 mg/kg) to rats and monkeys are shown in Table 9. Compound **4** exhibited high plasma concentration and area under the time–concentration curve (AUC) and showed a good distribution pattern in the liver, the kidney, and especially the lung. Compound **4** was expected to exhibit a high plasma concentration and a long half-life time in humans. The excellent in vitro activities (Tables 1, 5 and 6) and pharmacokinetic profiles (Table 7) of compound **4** showed a promising result compound **4** exhibited potent in vivo efficacies on gram-positive bacteria such as PRSP in animal models of pneumonia. Indeed, in the recent study of Kohno and co-workers,[43] compound **4** had high degrees of efficacy against penicillin-susceptible *S. pneumoniae* (PSSP) and PRSP in a mouse lung infection model.

Table 7
Inhibitory activities of **4** and reference compounds against wild-type and altered topoisomerase IV of *Staphylococcus aureus* (*S. aureus*)

Compound	IC_{50}[a] (μg/ml)		
	Wild-type (A)	Ser80Phe[b] (B)	B/A ratio
4	0.309	1.76	5.70
GRNX[c]	1.14	20.7	18.16
MFLX[c]	1.36	24.2	17.79
GFLX[c]	1.85	22.5	12.16
LVFX[c]	2.67	57.1	21.38
CPFX[c]	1.24	22.4	18.06

[a] 50% Inhibitory concentration.
[b] Altered topoisomerase IV with quinolone-resistant mutation (GrlA; S80F) of *S. aureus*.
[c] Abbreviations are as follows: GRNX = garenoxacin; MFLX = moxifloxacin; GFLX = gatifloxacin; CPFX = ciprofloxacin; LVFX = levofloxacin.

Table 8
Inhibitory activities of **4** and reference compounds against wild-type and altered DNA gyrase of *Staphylococcus aureus* (*S. aureus*)

Compound	IC_{50}[a] (μg/ml)		
	Wild-type (A)	Ser84Leu[b] (B)	B/A ratio
4	0.835	7.81	9.35
GRNX[c]	3.59	>512	>143
MFLX[c]	4.38	>512	>117
GFLX[c]	3.22	287	89.1
LVFX[c]	9.41	>512	>54.4
CPFX[c]	13.4	>256	>19.1

[a] 50% Inhibitory concentration.
[b] Altered DNA gyrase with quinolone-resistant mutation (GyrA; S84L) of *S. aureus*.
[c] Abbreviations are as follows: GRNX = garenoxacin; MFLX = moxifloxacin; GFLX = gatifloxacin; CPFX = ciprofloxacin; LVFX = levofloxacin.

4. Conclusion

We synthesized a series of novel des-F(6) and 6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-metoxylquinolones bearing 3-(1-aminocycloalkyl)pyrrolidin-1-yl substituents at the C-7 position. The des-F(6) compound **4–6** exhibited slightly inferior antibacterial activity comparable with that of the 6-fluorinated compounds **1–3**. The des-F(6) compounds **4,6** had at least four times more potent activities against Gram-positive bacteria than the reference quinolones. In addition, they showed no micronuclei-forming toxicity. Among the des-F(6) compounds, (3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl derivative **4** showed reduced intravenous single-dose toxicity in comparison with **6** and good pharmacokinetic profiles in rats and monkeys. As well, compound **4** exhibited comparable or greater antibacterial activity against clinically isolated Gram-positive bacteria that had become resistant to one or more antibacterial agents in comparison with the other quinolones tested, vancomycin, teicoplanin, quinupristin/dalfopristin, or linezolid. Compound **4** has been found to exhibit excellent antibacterial activity against other clinically isolated bacteria[44] in addition to those we describe in this paper, and was selected for further preclinical and clinical evaluation. Thus, compound **4** should be a promising candidate for the treatment of seri-

Table 9
Pharmacokinetic parameters of **4** in rats and monkeys at a dose of 20 mg/kg

Animal	Route	Tissue	Parameters (units)[a]				
			C_{5min} (μg/mL or μg/g)	$t_{1/2}$ (h)	AUC_{0-6h} (μg h/mL or μg h/g)	AUC ratio (tissue/serum)	BA[b]
Rats	po	Serum	6.7[c]	1.3	9.8[d]		67.4%
	iv	Serum	11.0	1.2	14.5[d]	1.0	
		Liver	48.5	1.1	66.1[d]	4.6	
		Kidney	47.2	1.4	64.4[d]	4.4	
		Lung	32.4	1.2	45.5[d]	3.1	
Monkeys	po	Serum	3.7[c]	6.0	37.5[e]		58.2%
	iv	Serum	12.3	4.4	64.4[e]		

[a] Mean values of four animals.
[b] Bioavailability, calculated from AUC ratio.
[c] C_{max}.
[d] 0–6 h.
[e] 0–24 h.

ous human infections due to multi-drug resistant gram-positive bacteria. The clinical trials of compound **4** are in progress.

5. Experimental

5.1. Chemistry

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were taken on a Yanako MP-500D melting point apparatus and are uncorrected. Optical rotations were measured in a 0.5-dm cell at 25 °C at 589 nm with a HORIBA SEPA-300 polarimeter. ^{1}H NMR spectra were determined on a JEOL JNM-EX400 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulphonate as internal standards. Significant ^{1}H NMR data are tabulated in the order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant(s) in hertz. Infrared (IR) spectra were obtained on a HORIBA FT-720 spectrometer or a JASCO FT/IR-6100 typeA. 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). Elemental analyses are indicated only by the symbols of the elements; analytical results were within 0.4% of the theoretical values unless otherwise noted, which indicates $\geqslant$95% purity of the tested compounds. Column chromatography refers to flash column chromatography conducted on Merck Silica Gel 60, 230–400 mesh ASTM. Thin-layer chromatography (TLC) was performed with Merck Silica Gel 60 F_{254} TLC plates, and compound visualization was effected with a 5% solution of molybdophosphoric acid in ethanol, UV-lamp, iodine, or Wako Ninhydrin Spray. Reagents and solvents were purchased from Sigma Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku and used without purification.

5.1.1. Ethyl 6,7-difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (15)

To a solution of 2,4,5-trifluoro-3-methoxybenzoic acid **14** (206 g, 1.00 mol) in 2000 mL of toluene at ambient temperature, DMF (2 mL) and thionyl chloride (109 ml, 1.50 mol) were added dropwise. The mixture was stirred at 80 °C for 16 h. The mixture was concentrated in vacuo to give a brown oil. To a stirred solution of ethyl 3-(dimethylamino)acrylate[34] (172 g, 1.20 mol) and triethylamine (184 mL, 1.32 mol) in 1500 mL of THF in an ice bath was added dropwise a solution of the brown oil in 500 mL of THF. The mixture was refluxed for 5 h, and concentrated in vacuo. The residue was diluted with CH_2Cl_2, and the solution was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo to give a brown oil. To a stirred solution of the brown oil in 2200 mL of CH_2Cl_2 in an ice bath was added (1*R*,2*S*)-2-fluorocyclopropylamine *p*-toluenesulfonic acid salt (224 g, 904 mmol), and then a solution of triethylamine (139 mL, 995 mmol) in 300 mL of CH_2Cl_2 was added dropwise. Stirring was continued for 2 h in the ice bath. The mixture was diluted with CH_2Cl_2, and the solution was washed with water, brine, dried over Na_2SO_4, and concentrated. To a stirred solution of the residue in 2000 mL of DMF on an ice bath was added K_2CO_3 (276 g, 764 mmol) portionwise. The mixture was stirred at ambient temperature for 72 h. After the mixture was poured into ice-cooled 2 N HCl aq, the precipitate was collected by filtration and washed with water (3×), EtOH, and Et_2O to give **15** (213 g, 82%) as a colorless powder. mp 161–163 °C. IR (KBr pellet) cm^{-1}: 3431, 3092, 3002, 2984, 2964, 2936, 2910, 2872, 1724, 1618, 1604, 1570, 1495, 1471, 1405, 1346, 1330, 1288, 1265, 1224, 1175, 1143, 1111, 1095, 1068, 1054, 1019. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 1.56–1.68 (2H, m), 3.83–3.88 (1H, m), 4.10 (3H, d, J = 2.2 Hz), 4.39 (2H, q, J = 7.1 Hz), 4.85 (1H, br d, J = 63.0 Hz), 8.05 (1H, dd, J = 8.6, 10.0 Hz), 8.57 (1H, d, J = 1.2 Hz). $[\alpha]_D$: −24.3 (*c* 0.989, $CHCl_3$). Anal. Calcd for $C_{16}H_{14}F_3NO_4$: C, 56.31; H, 4.13; F, 16.70; N, 4.10. Found: C, 56.09; H, 4.11; F, 16.90; N, 4.22.

5.1.2. 6,7-Difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (16)

A mixture of **15** (201 g, 590 mmol), 700 mL of AcOH, and 700 mL of concd HCl aq was heated for reflux for 12 h. After cooling the mixture, 2000 mL of cold water was added, and the precipitate was collected by filtration. The resultant solid was washed with water (2×), EtOH and Et_2O, to give crude product. Recrystallization from acetone/EtOH gave **16** (91.3 g, 49%) as a colorless powder. mp 184–186 °C. IR (KBr pellet) cm^{-1}: 3458, 3105, 3088, 3025, 2958, 2923, 2851, 2733, 1733, 1621, 1569, 1508, 1487, 1460, 1401, 1380, 1365, 1346, 1320, 1284, 1246, 1229, 1185, 1135, 1115, 1104, 1063, 1057, 1015. ^{1}H NMR ($CDCl_3$) δ: 1.64–1.75 (2H, m), 3.97–4.00 (1H, m), 4.17 (3H, d, J = 2.2 Hz), 4.91 (1H, br d, J = 63.2 Hz), 8.05 (1H, dd, J = 8.6, 10.0 Hz), 8.84 (1H, s), 14.31 (1H, s). $[\alpha]_D$: −9.4 (*c* 0.953, $CHCl_3$). Anal. Calcd for $C_{14}H_{10}F_3NO_4$: C, 53.68; H, 3.22; F, 18.20; N, 4.47. Found: C, 53.69; H, 3.18; F, 18.01; N, 4.54.

5.1.3. {6,7-Difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-yl}carboxyl difluoroborate (12)

Compound 16 (90.3 g, 288 mmol) was suspended in 1000 mL of Et_2O and 653 mL of boron trifluoride ethyl etherate was added to the suspension and the suspension was stirred at ambient temperature for 24 h. The precipitate was collected by filtration. The resultant solid was washed with Et_2O to give **12** (96.5 g, 93%) as a colorless powder. mp 206–208 °C. IR (KBr pellet) cm^{-1}: 3433, 3065, 2969, 2857, 1724, 1628, 1579, 1555, 1498, 1480, 1412, 1363, 1329, 1267, 1241, 1223, 1192, 1139, 1113, 1068, 1053. ^{1}H NMR ($CDCl_3$) δ: 1.77–1.98 (2H, m), 4.30 (3H, d, J = 2.9 Hz), 4.38–4.44 (1H, m), 5.03 (1H, br d, J = 62.5 Hz), 8.17 (1H, dd, J = 8.1, 8.8 Hz), 9.14 (1H, s). $[\alpha]_D$: +166.2 (*c* 0.157, $CHCl_3$). Anal. Calcd for $C_{14}H_9BF_5NO_4$: C, 46.58; H, 2.51; F, 26.31; N, 3.88. Found: C, 46.43; H, 2.54; F, 26.22; N, 3.90.

5.1.4. Ethyl 7-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (18)

A mixture of ethyl 2,4-difluoro-3-methoxybenzoylacetate (**17**[35]) (141 g, 546 mmol), ethyl orthoformate (227 mL, 1.37 mol) and 500 mL of Ac_2O was heated at 120 °C for 6 h. The mixture was concentrated in vacuo to give a brown oil. To a stirred solution of the brown oil in 1000 mL of CH_2Cl_2 in an ice bath was added (1*R*,2*S*)-2-fluorocyclopropylamine *p*-toluenesulfonic acid salt (165 g, 668 mmol), and then a solution of triethylamine (118 mL, 846 mmol) in 382 mL of CH_2Cl_2 was added dropwise. Stirring was continued at ambient temperature for 6 h. The mixture was diluted with CH_2Cl_2, and the solution was washed with water, brine (2×), dried over Na_2SO_4, and concentrated. To a stirred solution of the residue in 1200 mL of 1,4-dioxane on an ice bath was added NaH (60% mineral oil dispersion, 31.2 g, 780 mmol) portionwise. The mixture was stirred at ambient temperature for 1 h. After the mixture was poured into ice-cooled 1 N HCl aq, the precipitate was collected by filtration and washed with water (3×), EtOH and Et_2O (3×) to gave **18** (131 g, 75%) as a yellow powder. mp 153–155 °C dec.. IR (KCl pellet) cm^{-1}: 3429, 3094, 3071, 2981, 2950, 2906, 2839, 1727, 1631, 1611, 1595, 1564, 1497, 1446, 1401, 1373, 1350, 1329, 1313, 1271, 1252, 1192, 1172, 1128, 1076, 1043, 1017. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 1.55–1.65 (2H, m), 3.85–3.90 (1H, m), 4.03 (3H, d, J = 2.0 Hz), 4.40 (2H, q, J = 7.1 Hz), 4.87 (1H, br d, J = 62.3 Hz), 7.20 (1H, dd, J = 9.0, 10.5 Hz), 8.24 (1H, dd, J = 6.1, 9.0 Hz), 8.57 (1H, s). $[\alpha]_D$: −27.2 (*c*

0.464, $CHCl_3$). Anal. Calcd for $C_{16}H_{15}F_2NO_4$: C, 59.44; H, 4.68; F, 11.75; N, 4.33. Found: C, 59.40; H, 4.67; F, 11.58; N, 4.42.

5.1.5. 7-Fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (13)

A mixture of **18** (131 g, 406 mmol), 1000 mL of AcOH, and 1000 mL of concd HCl aq was heated for reflux for 6 h. After cooling the mixture, 3000 mL of cold water was added, and the precipitate was collected by filtration. The resultant solid was washed with water (2×), EtOH and Et_2O, to give crude product. Recrystallization from MeCN gave **13** (92.8 g, 77%) as a colorless powder. mp 232–234 °C. IR (KCl pellet) cm^{-1}: 3427, 3090, 3060, 3019, 2949, 2858, 2589, 1726, 1616, 1560, 1515, 1476, 1443, 1400, 1375, 1320, 1269, 1230, 1206, 1186, 1171, 1133, 1123, 1105, 1069, 1044, 1015. ^1H NMR ($CDCl_3$) δ: 1.55–1.73 (2H, m), 4.01 (1H, m), 4.10 (3H, d, J = 2.2 Hz), 4.90 (1H, br d, J = 62.7 Hz), 7.35 (1H, dd, J = 9.0, 10.2 Hz), 8.27 (1H, dd, J = 5.9, 9.0 Hz), 8.76 (1H, s). $[\alpha]_D$: 40.0 (*c* 0.165, 0.1 N NaOH aq). Anal. Calcd for $C_{14}H_{11}F_2NO_4$: C, 56.95; H, 3.76; F, 12.87; N, 4.74. Found: C, 56.87; H, 3.76; F, 12.63; N, 4.82.

5.1.6. *N*-[(1-Ethoxycarbonylcycloprop-1-yl)carbonylmethyl]-*N*-[(*S*)-1-phenylethyl]-2-(diethoxy- phosphoryl)acetamide (20)

To a solution of diethoxyphosphorylacetic acid (15.1 g, 76.8 mmol) in 120 mL of THF on an ice bath was added 1,1′-carbonyldiimidazole (13.7 g, 84.5 mmol). The mixture was stirred at ambient temperature for 1 h, and then a solution of ethyl 1-{*N*-[(*S*)-1-phenylethyl]aminoacetyl}cyclopropanecarboxylate (**19**)[37] (17.6 g, 64.0 mmol) in 30 mL of THF was added dropwise over 20 min below 0 °C. After stirring the solution for 1 h at ambient temperature, the solution was diluted with AcOEt, and washed with aqueous 1 N HCl solution, aqueous saturated $NaHCO_3$ solution, and brine. The resultant solution was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography, eluting with AcOEt/hexane = 2:1 to yield crude **20** (28.7 g) as a yellow oil. ^1H NMR ($CDCl_3$) δ: 1.14–1.68 (16H, m), 2.85–4.69 (2H, m), 3.18–4.55 (2H, m), 4.06–4.22 (6H, m), 5.42–6.05 (1H, m), 7.26–7.37 (5H, m). MS (ESI) *m/z* 454 ($M+H^+$). High-resolution MS (ESI) Calcd for $C_{22}H_{32}NO_7P$+H: 454.1995. Found: 454.1993. $[\alpha]_D$: −30.3 (*c* 0.945, $CHCl_3$).

5.1.7. Ethyl 1-{5-oxo-1-[(*S*)-1-phenylethyl]-2,5-dihydro-1*H*-pyrrol-3-yl}cyclopropanecarboxylate (21)

To a solution of **20** (25.0 g, 55.2 mmol) in 250 mL of toluene was added potassium *tert*-butoxide (7.40 g, 66.2 mmol) portionwise over 1 h below 0 °C. After the solution was stirred for 15 min at ambient temperature, an aqueous 10% citric acid solution was added dropwise to the solution at 0 °C. The resultant suspension was diluted with AcOEt and filtered through Celite, and then the organic layer of the filtrate was separated and washed with aqueous saturated $NaHCO_3$ solution, and brine. The resultant solution was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography, eluting with AcOEt/hexane = 1:2 to yield **21** (12.1 g, 73%) as a red-yellow oil. The spectral data of **21** obtained by this procedure were identical to those reported.[38] ^1H NMR ($CDCl_3$) δ: 1.13–1.15 (2H, m), 1.18 (3H, t, J = 6.8 Hz), 1.60 (3H, d, J = 7.3 Hz), 1.61–1.64 (2H, m), 3.80 (1H, d, J = 19.5 Hz), 4.09 (2H, q, J = 6.8 Hz), 4.13 (1H, q, J = 19.5 Hz), 5.56 (1H, q, J = 7.3 Hz), 5.85 (1H, t, J = 1.5 Hz), 7.25–7.37 (5H, m). MS (ESI) *m/z* 300 ($M+H^+$). High-resolution MS (ESI) Calcd for $C_{18}H_{21}NO_3$+H: 300.1600. Found: 300.1587. $[\alpha]_D$: −45.7 (*c* 1.104, $CHCl_3$).

5.1.8. Ethyl 1-{(3*S*)-5-oxo-1-[(*S*)-1-phenylethyl]pyrrolidin-3-yl}cyclopropanecarboxylate (23)

To a solution of **21** (12.1 g, 40.5 mmol) in 300 mL of EtOAc was added 5% Pt/C (2.40 g, containing 50% water), and the mixture was stirred at ambient temperature for 17 h under a hydrogen atmosphere. The mixture was filtrated and concentrated in vacuo to give a colorless oil. The oil was separated to two diastereomers by column chromatography eluting with AcOEt/hexane = 2:3. **23** (9.0 g, 74%) as a pale yellow oil. The spectral data of **23** obtained by this procedure were identical to those reported.[38] ^1H NMR ($CDCl_3$) δ: 0.63–0.65 (2H, m), 1.13 (3H, t, J = 7.1 Hz), 1.12–1.19 (2H, m), 1.52 (3H, d, J = 7.3 Hz), 2.17 (1H, dd, J = 9.0, 16.8 Hz), 2.46 (1H, dd, J = 9.3, 16.3 Hz), 2.67–2.76 (2H, m), 3.47 (1H, t, J = 8.3 Hz), 3.96–4.11 (2H, m), 5.51 (1H, q, J = 7.3 Hz), 7.26–7.35 (5H, m). MS (ESI) *m/z* 302 ($M+H^+$). High-resolution MS (ESI) Calcd for $C_{18}H_{23}NO_3$+H: 302.1756. Found: 302.1745. $[\alpha]_D$: −93.4 (*c* 1.136, $CHCl_3$).

5.1.9. 1-{(3*S*)-5-Oxo-1-[(*S*)-1-phenylethyl]pyrrolidin-3-yl}cyclopropanecarboxylic acid (24)

To a solution of **23** (10.5 g, 34.9 mmol) in 70 mL of EtOH was added 1 N NaOH aq (70 mL, 70.0 mmol) at ambient temperature, and the mixture was stirred at this temperature for 15.5 h, and then at 40 °C for 3 h. The reaction mixture was concentrated in vacuo, and the residual aqueous solution was washed with AcOEt. Then the aqueous layer was acidified with concd HCl aq under ice cooling, and the mixture was extracted with $CHCl_3$ (3×). The combined organic layer was dried over Na_2SO_4, and concentrated in vacuo to give **18** (9.4 g, 99%) as a white solid. The spectral data of **24** obtained by this procedure were identical to those reported.[38] mp 184–186 °C. IR (KBr pellet) cm^{-1}: 3418, 3091, 3008, 2991, 2945, 2921, 2887, 2711, 2579, 2538, 2511, 2094, 1972, 1852, 1704, 1627, 1581, 1498, 1455, 1422, 1385, 1376, 1358, 1331, 1309, 1292, 1258, 1240, 1219, 1191, 1159, 1093, 1067, 1035, 1006. ^1H NMR ($CDCl_3$) δ: 0.72–0.74 (2H, m), 1.21–1.23 (2H, m), 1.52 (3H, d, J = 7.3 Hz), 2.17 (1H, dd, J = 8.8, 16.8 Hz), 2.48 (1H, dd, J = 9.5, 16.8 Hz), 2.66–2.78 (2H, m), 3.50 (1H, t, J = 9.3 Hz), 5.51 (1H, q, J = 7.3 Hz), 7.25–7.34 (5H, m). $[\alpha]_D$: −116.8 (*c* 0.787, $CHCl_3$). Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 69.91; H, 6.75; N, 5.06.

5.1.10. (3*R*)-3-[1-(*tert*-Butoxycarbonylamino)cyclopropan-1-yl]-5-oxo-1-[(*S*)-1-phenylethyl]pyrrolidine (25)

To a solution of **24** (9.40 g, 34.4 mmol) in 95 mL of toluene was added triethylamine (9.60 mL, 69.0 mmol) followed by diphenylphosphoryl azide (10.4 g, 37.9 mmol) dropwise at ambient temperature. The mixture was stirred at ambient temperature for 1 h and then heated to reflux for 1.5 h. To the mixture was added 95 mL of *tert*-butylalcohol, and the mixture was heated for reflux for another 15 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography eluting with $CHCl_3$/methanol = 50:1 to yield **25** (10.7 g, 90%) as a colorless oil. Recrystallization from *n*-hexane–CH_2Cl_2 gave a colorless powder. mp 130–132 °C. IR (KBr pellet) cm^{-1}: 3379, 3080, 3064, 3025, 3007, 2978, 2940, 2913, 2147, 1694, 1680, 1584, 1501, 1453, 1428, 1390, 1365, 1303, 1277, 1248, 1226, 1166, 1155, 1094, 1072, 1055, 1027. ^1H NMR ($CDCl_3$) δ: 0.56–0.84 (4H, m), 1.37 (9H, s), 1.51 (3H, d, J = 7.3 Hz), 2.32–2.44 (3H, m), 2.79 (1H, dd, J = 7.3, 10.0 Hz), 3.36 (1H, m), 4.66 (1H, br s), 5.50 (1H, q, J = 7.3 Hz), 7.26–7.34 (5H, m). $[\alpha]_D$: −114.7 (*c* 0.716, $CHCl_3$). Anal. Calcd for $C_{20}H_{28}N_2O_3 \cdot 0.1H_2O$: C, 69.38; H, 8.21; N, 8.09. Found: C, 69.06; H, 7.81; N, 8.38.

5.1.11. (3*R*)-3-[1-(*tert*-Butoxycarbonylamino)cyclopropan-1-yl]-1-[(*S*)-1-phenylethyl]pyrrolidine (26)

To a solution of **25** (10.4 g, 30.2 mmol) in 95 mL of THF was added 1 N BH_3–THF (90.7 mL, 90.7 mmol) dropwise at 0 °C. After the solution was stirred for 16 h at ambient temperature, K_2CO_3 (25.0 g, 181 mmol) solution in 72 mL of water was added dropwise to the solution. The mixture was heated for reflux for another 1.5 h. The solvent was removed in vacuo, and the residue was diluted

with AcOEt, and washed with brine. The resultant solution was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography eluting with $CHCl_3$/methanol = 30:1 to yield **26** (8.20 g, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.62 (2H, br s), 0.75–0.88 (2H, m), 1.35 (3H, d, J = 6.6 Hz), 1.41 (9H, s), 1.63 (2H, br s), 1.88–1.92 (1H, m), 2.14–2.17 (1H, m), 2.27–2.34 (2H, m), 2.63 (2H, br s), 3.15 (1H, t, J = 6.6 Hz), 5.10 (1H, br s), 7.23–7.33 (5H, m). MS (ESI) m/z 331 ($M+H^+$). High-resolution MS (ESI) Calcd for $C_{20}H_{30}N_2O_2$+H: 331.2386. Found: 331.2422. $[\alpha]_D$: −27.9 (c 0.531, $CHCl_3$).

5.1.12. (3*R*)-3-[1-(*tert*-Butoxycarbonylamino)cyclopropan-1-yl]pyrrolidine (9)

To a solution of **26** (270 mg, 0.817 mmol) in 15 mL of EtOH was added 10% Pd/C (270 mg, containing 52.0% water), and the mixture was stirred at 40 °C for 3 h under a hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was used for the next reaction without purification. The crude product **9** (185 mg) was yielded as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.69 (2H, br s), 0.79 (2H, br s), 1.42 (9H, s), 1.43–1.50 (1H, m), 1.86–1.88 (1H, m), 2.15–2.19 (1H, m), 2.68–2.72 (1H, m), 2.90–3.07 (3H, m), 4.92 (1H, br s). MS (ESI) m/z 227 ($M+H^+$). High-resolution MS (ESI) Calcd for $C_{12}H_{22}N_2O_2$+H: 227.1760. Found: 227.1772. $[\alpha]_D$: +6.9 (c 1.055, $CHCl_3$).

5.1.13. (3*R*)-3-[1-(*tert*-Butoxycarbonyl-*N*-methylamino) cyclopropan-1-yl]-5-oxo-1-[(*S*)-1-phenylethyl]pyrrolidine (27)

To a stirred solution of **25** (7.87 g, 22.8 mmol) in 100 mL of DMF on an ice bath was added NaH (60% mineral oil dispersion, 1.40 g, 30.2 mmol) portionwise. After the solution was stirred for 5 min at ambient temperature, methyl iodide (8.11 mL, 130 mmol) was added dropwise to the solution. The mixture was stirred at 40 °C for 24 h, and then satd NH_4Cl aq was added dropwise at 0 °C. The mixture was extracted with AcOEt (2×), and the organic layers were combined and washed with water (2×), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel column chromatography eluting with $CHCl_3$/methanol = 30:1 to yield **27** (7.53 g, 92%) as a colorless oil. Recrystallization from n-hexane–CH_2Cl_2 gave a colorless powder. mp 80–82 °C. IR (KBr pellet) cm^{-1}: 3443, 3088, 2978, 2927, 1692, 1677, 1494, 1477, 1452, 1423, 1363, 1270, 1252, 1232, 1170, 1152, 1058, 1040, 1031, 1014. ^{1}H NMR ($CDCl_3$) δ: 0.82–0.84 (4H, m), 1.32 (9H, s), 1.38 (3H, s), 1.51 (3H, d, J = 7.1 Hz), 1.61 (3H, d, J = 16.6 Hz), 2.43 (1H, m), 2.68–2.81 (3H, m), 3.21 (1H, m), 5.48–5.50 (1H, m), 7.26–7.36 (5H, m). $[\alpha]_D$: −102.2 (c 0.968, $CHCl_3$). Anal. Calcd for $C_{21}H_{30}N_2O_3$: C, 70.36; H, 8.44; N, 7.81. Found: C, 70.33; H, 8.30; N, 7.78.

5.1.14. (3*R*)-3-[1-(*tert*-Butoxycarbonyl-*N*-methylamino) cyclopropan-1-yl]-1-[(*S*)-1-phenylethyl]pyrrolidine (28)

To a solution of **27** (7.53 g, 21.0 mmol) in 70 mL of THF was added 1 N BH_3–THF (63.0 mL, 63.0 mmol) dropwise at 0 °C. After the solution was stirred for 20 h at ambient temperature, K_2CO_3 (7.22 g) solution in 72 mL of water was added dropwise to the solution. The mixture was heated for reflux for another 1.5 h. The solvent was removed in vacuo, and the residue was diluted with AcOEt, and washed with brine. The resultant solution was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography eluting with $CHCl_3$/methanol = 50:1 to yield **28** (7.19 g, 99%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.72–0.74 (4H, m), 1.35 (9H, s), 1.36 (3H, s), 1.61–1.62 (1H, m), 1.84–1.85 (1H, m), 1.96–1.98 (1H, m), 2.26–2.27 (1H, m), 2.50–2.58 (2H, m), 2.79 (3H, s), 2.98–2.99 (1H, m), 3.14–3.19 (1H, m), 7.27–7.30 (5H, m).

5.1.15. (3*R*)-3-[1-(*tert*-Butoxycarbonyl-*N*-methylamino)cyclopropan-1-yl]pyrrolidine (10)

To a solution of **28** (7.19 g, 20.9 mmol) in 78 mL of EtOH was added 10% Pd/C (3.9 g, containing 50.0% water), and the mixture was stirred at 40 °C for 4 h under a hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was used for the next reaction without purification. The crude product **10** (4.38 g) was yielded as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.80 (4H, br s), 1.46 (9H, s), 1.80–1.81 (1H, m), 2.05 (1H, br s), 2.28–2.42 (1H, m), 2.54 (1H, br s), 2.84 (3H, s), 2.88–2.96 (3H, m).

5.1.16. 7-[(3*R*)-3-(1-Aminocyclopropan-1-yl)pyrrolidin-1-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinolin-3-carboxylic acid (1)

To a solution of crude **9** (1.20 mmol) in 10 mL of DMSO was added **12** (217 mg, 0.6 mmol), triethylamine (0.174 mL, 1.25 mmol), and the mixture was stirred at ambient temperature for 25 h under a nitrogen atmosphere. The solvent was removed in vacuo, and the residue was added to water, and then the mixture was stirred at ambient temperature for 1 h. The precipitate was collected by filtration and washed with water, and then was dissolved in 20 mL of MeOH/water = 4:1. To the solution was added triethylamine (0.3 mL), and the mixture was heated for reflux for another 4.5 h. The solvent was removed in vacuo, and the residue was dissolved $CHCl_3$, and the solution was washed with 10% citric acid aq and brine. The resultant solution was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. To the residue was added 10 mL of concd HCl aq at 0 °C, and the mixture was stirred for 1 h at ambient temperature. The solution was washed with CH_2Cl_2, alkalified with satd NaOH aq at 0 °C, and then the pH of the solution was adjusted to 7.4 with concd HCl aq and dil HCl aq The resultant solution was extracted with chloroform (4×), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by semi-separable TLC eluting with an under layer solution of $CHCl_3$/MeOH/water = 7:3:1 to give the crude product. The crude product was recrystallized from EtOH to yield **1** (181 mg, 72%) as a pale yellow powder. mp 195–197 °C. IR (KBr pellet) cm^{-1}: 3369, 3311, 3094, 3052, 3028, 3004, 2977, 2953, 2933, 2893, 2869, 1729, 1623, 1516, 1458, 1402, 1370, 1355, 1338, 1318, 1296, 1231, 1207, 1187, 1161, 1122, 1097, 1050, 1039, 1015. ^{1}H NMR (0.1 N NaOD/D_2O) δ: 0.60 (4H, s), 1.34–1.60 (2H, m), 1.71–1.82 (1H, m), 1.99–2.07 (1H, m), 2.20–2.29 (1H, m), 3.46–3.65 (2H, m), 3.60 (3H, s), 3.69–3.78 (1H, m), 3.98–4.07 (1H, m), 4.93–4.96, 5.12–5.15 (1H, m), 7.60 (1H, d, J = 13.7 Hz), 8.43 (1H, d, J = 2.9 Hz). $[\alpha]_D$: −123.1 (c 0.515, 0.1 N NaOH aq). Anal. Calcd for $C_{21}H_{23}F_2N_3O_4$: C, 60.14; H, 5.53; N, 10.02. Found: C, 60.02; H, 5.45; N, 9.92.

5.1.17. 6-Fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-7-[(3*R*)-3-(1-methylaminocyclopropan-1-yl)pyrrolidin-1-yl]-4-oxoquinolin-3-carboxylic acid (2)

Following the procedures described for **1**, the title compound was prepared in 83% yield from **10** and **12** as a pale yellow powder. mp 208–209 °C. IR (KBr pellet) cm^{-1}: 3348, 3092, 3040, 3004, 2970, 2937, 2873, 2790, 2671, 1730, 1622, 1515, 1456, 1367, 1356, 1317, 1272, 1228, 1188, 1158, 1134, 1122, 1093, 1055, 1019, 1009. ^{1}H NMR (0.1 N NaOD/D_2O) δ: 0.53–0.69 (4H, m), 1.32–1.59 (3H, m), 1.91–2.02 (1H, m), 2.34 (3H, s), 2.85–2.95 (1H, m), 3.29–3.38 (1H, m), 3.51–3.62 (2H, m), 3.57 (3H, s), 3.70–3.79 (1H, m), 3.98–4.07 (1H, m), 4.95–4.98, 5.09–5.13 (1H, m), 7.66 (1H, d, J = 14.2 Hz), 8.39 (1H, d, J = 2.9 Hz). $[\alpha]_D$: −123.4 (c 0.525, 0.1 N NaOH aq). Anal. Calcd for $C_{22}H_{25}F_2N_3O_4$: C, 60.96; H, 5.81 N, 9.69. Found: C, 60.79; H, 5.73; N, 9.55.

5.1.18. 7-[(3*R*)-3-(1-Aminocyclobutan-1-yl)pyrrolidin-1-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinolin-3-carboxylic acid (3)

Following the procedures described for **1**, the title compound was prepared in 27% yield from **11**[32] and **12** as a pale yellow powder. mp 123–139 °C. ^{1}H NMR (0.1 N NaOD/D_2O) δ: 1.33–1.40 (1H, m), 1.50–1.60 (1H, m), 168–1.79 (2H, m), 1.86–1.88 (3H, m), 2.03–2.07 (1H, m), 2.14 (2H, br s), 2.40–2.49 (1H, m), 3.50–3.52 (3H, m), 3.56 (3H, s), 3.67–3.71 (1H, m), 3.98–4.03 (1H, m), 7.66 (1H, d, J = 14.6 Hz), 8.42 (1H, d, J = 2.9 Hz). Anal. Calcd for $C_{22}H_{25}F_2N_3O_4{\cdot}0.75H_2O$: C, 59.12; H, 5.98; N, 9.40. Found: C, 58.94; H, 5.70; N, 9.13.

5.1.19. 7-[(3*R*)-3-(1-Aminocyclopropan-1-yl)pyrrolidin-1-yl]-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methyl-4-oxoquinolin-3-carboxylic acid (4 = DX-619)

To a solution of crude **9** (0.731 mmol) in 2 mL of DMSO was added triethylamine (0.5 mL) and **13** (180 mg, 0.61 mmol), and the mixture was heated at 100 °C for 13 h under a nitrogen atmosphere. The solvent was removed in vacuo, and the residue was dissolved with $CHCl_3$, and the solution was washed with 10% citric acid aq and brine. The resultant solution was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. To the residue was added 5 mL of concd HCl aq at 0 °C, and the mixture was stirred for 1 h at ambient temperature. The solution was washed with CH_2Cl_2, alkalified with satd NaOH aq at 0 °C, and then the pH of the solution was adjusted to 7.4 with concd HCl aq and dil HCl aq The resultant solution was extracted with chloroform (4×), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by semi-separable TLC eluting with an under layer solution of $CHCl_3$/MeOH/water = 7:3:1 to give the crude product. The crude product was recrystallized from 2-propanol to yield **4** (146 mg, 60%) as a pale yellow powder. mp 189–192 °C. IR (KBr disk) cm^{-1}: 3373, 3315, 3091, 3003, 2976, 2935, 2836, 1903, 1714, 1618, 1518, 1439, 1371, 1313, 1261, 1219. ^{1}H NMR (0.1 N NaOD/D_2O) δ: 0.56 (4H, br s), 1.31–1.37 (1H, m), 1.50–1.56 (1H, m), 1.77–1.78 (1H, m), 2.02–2.04 (1H, m), 2.19–2.21 (1H, m), 3.31–3.32 (1H, m), 3.49–3.51 (3H, m), 3.50 (3H, s), 4.00–4.02 (1H, m), 4.93–4.94, 5.09–5.10 (1H, m), 7.01 (1H, s), 7.90 (1H, d, J = 9.03 Hz), 8.39 (1H, d, J = 3.2 Hz). $[\alpha]_D$: −50.8 (c = 0.240, 0.1 N NaOH aq). Anal. Calcd for $C_{21}H_{24}FN_3O_4$: C, 62.83; H, 6.03; N, 10.47. Found: C, 62.50; H, 6.04; N, 10.26.

5.1.20. 7-[(3*R*)-3-(1-Aminocyclopropan-1-yl)pyrrolidin-1-yl]-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methyl-4-oxoquinolin-3-carboxylic acid hydrochloride (hydrochloride of 4)

To a solution of **4** (13.0 g, 32.3 mmol) in 130 mL of EtOH was added 1 N HCl aq (34.0 mL) dropwise at 0 °C. The solution was stirred for 5 min at 0 °C and concentrated in vacuo. The residue was recrystallized from EtOH to yield the hydrochloride of **4** (13.4 g, 83%) as a pale yellow powder. mp 225–227 °C. IR (ATR) cm^{-1}: 2887, 2665, 1705, 1610, 1514, 1431, 1363, 1311, 1232, 1200. $[\alpha]_D$: −69.2 (c 0.404, MeOH). Anal. Calcd for $C_{21}H_{24}FN_3O_4{\cdot}HCl{\cdot}EtOH{\cdot}H_2O$: C, 55.03; H, 6.63; N, 8.37. Found: C, 55.20; H, 6.25; N, 7.99.

5.1.21. 1-[(1*R*,2*S*)-2-Fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-7-[(3*R*)-3-(1-methylaminocyclopropan-1-yl)pyrrolidin-1-yl]-4-oxoquinolin-3-carboxylic acid (5)

Following the procedures described for **4**, the title compound was prepared in 50% yield from **10** and **13** as a pale yellow powder. mp 213–215 °C. IR (KBr disk) cm^{-1}: 3352, 3095, 3051, 2939, 2837, 2787, 1716, 1699, 1616, 1520, 1439, 1358, 1319, 1259, 1221. ^{1}H NMR (0.1 N NaOD/D_2O) δ: 0.57–0.61 (4H, m), 1.33–1.40 (1H, m), 1.56–1.58 (2H, m), 1.99–2.01 (1H, m), 2.34 (3H, s), 2.87–2.89 (1H, m), 3.15–3.17 (1H, m), 3.521–3.54 (3H, m), 3.53 (3H, s), 4.00–4.02 (1H, m), 5.02 (1H, br d, J = 64.5 Hz), 7.03 (1H, s), 7.92 (1H, d, J = 7.0 Hz), 8.39 (1H, s). $[\alpha]_D$: −119.7 (c 0.295, 0.1 N NaOH aq). Anal. Calcd for $C_{22}H_{26}FN_3O_4$: C, 63.60; H, 6.31; N, 10.11. Found: C, 63.36; H, 6.31; N, 9.97.

5.1.22. 7-[(3*R*)-3-(1-Aminocyclobutan-1-yl)pyrrolidin-1-yl]-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methoxy-4-oxoquinolin-3-carboxylic acid (6)

Following the procedures described for **4**, the title compound was prepared in 93% yield from **11**[32] and **13** as a pale yellow powder. mp 174–176 °C. ^{1}H NMR (DMSO-d_6) δ: 1.30–1.45 (1H, m), 1.45–1.60 (1H, m), 1.60–1.70 (1H, m), 1.70–2.50 (8H, m), 3.30–3.40 (1H, m), 3.40–3.50 (1H, m), 3.50 (3H, s), 3.56–3.60 (2H, m), 4.03–4.09 (1H, m), 5.00–5.22 (1H, m), 7.09 (1H, d, J = 9.1 Hz), 7.92 (1H, d, J = 9.1 Hz), 8.57 (1H, d, J = 3.4 Hz). MS (ESI) m/z 416 (M+H$^+$). $[\alpha]_D$: −42.5 (c 1.01, 0.1 N NaOH aq). Anal. Calcd for $C_{22}H_{26}FN_3O_4{\cdot}0.75H_2O$: C, 61.60; H, 6.46; F, 4.43; N, 9.80. Found: C, 61.39; H, 6.41; F, 4.43; N, 9.73.

5.2. X-ray crystallographic study

All measurements were made on a Rigaku AFC7R diffractometer (Cu Kα radiation; λ = 1.54178 Å, graphite monochrometer, $\omega - 2\theta$ scans, $2\theta_{max}$ = 120.1°). The crystal data and parameters are summarized below. The structures were solved by direct methods and refined by full-matrix least-square and Fourier techniques. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were refined isotropically (d_{C-H} = 0.95 Å) and hydrogen positions were calculated assuming ideal geometries. Acidic hydrogen atom of the hydrochloride of **4** was refined with respect to its coordinates. All calculations were performed by using the CrystalStructure 3.6.0 crystallographic software package of Rigaku/MSC and Rigaku Corporation.

5.2.1. Crystal data and structure analysis. The hydrochloride of 4

A colorless prism-shaped crystal was formed from EtOH: $C_{21}H_{24}FN_3O_4{\cdot}HCl$; FW = 437.89; sample dimensions, 0.30 × 0.30 × 0.18 mm; orthorhombic, space group $P2_12_12_1$; a = 12.0699(21) Å, b = 6.7639(15) Å, c = 16.4648(13) Å, V = 1340.7(4) Å^3, Z = 2; d_{calcd} = 1.20 g/cm^3; F_{000} = 508.00; μ = 16.86 cm^{-1}; Flack parameter = −0.02(3). The final cycle of full-matrix least-squares refinement was based on 4611 observed reflections and 327 variable parameters and converged at R = 0.111 (R_w = 0.239). Lists of the atomic coordinates, anisotropic thermal parameters, and bond lengths and angles are stored at the Cambridge Crystal Crystallographic Data Centre, UK, CCDC-743566.

5.3. Biology

5.3.1. In vitro antibacterial activity

The MICs of the compounds tested in this study were determined by the twofold micro broth dilution method using Mueller-Hinton broth (Difco Laboratories, Detroit, MI) with an inoculum size of approximately 10^5 CFU per well. The MIC was defined as the lowest concentration that prevented visible bacterial growth after incubation at 37 °C for 18 h.

5.3.2. Intravenous single-dose toxicity

The test compounds were dissolved in 0.1 N NaOH in saline at different concentrations. The solution was administered intravenously to five week-old male Slc:ddY mice (n = 5 for each dose) at a speed of 0.2 mL/min. The total volume of administration was adjusted to 10 mL/kg of body weight. The number of dead mice was counted on day 7.

5.3.3. Micronucleus test

Five-week-old male Slc:ddY mice were used in this test. The test compounds were dissolved in 0.1 N NaOH in saline, and the solution was administered intravenously in each group of five mice. At 24 h and 48 h after treatment, approximately 5 μL of peripheral blood was collected from a tail blood vessel of each mouse. The blood was dropped onto an acridine orange-coated glass slide and covered immediately with a coverslip. For each animal, 1000 reticulocytes were examined for micronuclei by fluorescence microscopy, and the frequency of micronucleated reticulocytes (MNRET) was expressed as a percentage. Statistical analysis was performed by the Kastenbaum and Bowman method.[45]

5.3.4. Anti-topoisomerase activity

To determine the inhibitory activities of quinolones against target enzymes, GyrA and GyrB proteins of DNA gyrase, and GrlA and GrlB proteins of topoisomerase IV were purified separately using a protein fusion and purification system (New England Biolabs, MA). DNA gyrase activity was measured by supercoiling assay using relaxed pBR322 as a substrate and topoisomerase IV activity was measured by decatenation assay using kinetoplast DNA as a substrate. The 50% inhibitory concentration (IC_{50}) was determined as the drug concentration that reduced the supercoiling and the decatenation activity seen with drug-free control by 50%, respectively. The altered GyrA (a substitution of Leu for Ser-84) and GrlA (a substitution of Phe for Ser-80) were purified as the same procedure after mutation was introduced by site-directed mutagenesis.

5.3.5. Pharmacokinetic studies

Seven-week-old male Crj:CD rats or five/six-year-old female cynomolgus monkeys ($n = 4$) were used. The animals were administered drug samples in a single intravenous or oral dosing (20 mg/kg) as an aqueous solution. The concentrations of the compounds were determined by a microbiological assay (agar well dilution method) using *Bacillus subtilis* ATCC 6051 or ATCC 6633. The mean values of the 4 animals are shown.

Acknowledgements

We thank the Medicinal Safety Research Laboratories for the safety assessment, as well as, Biological Research Laboratories IV for the biological evaluation. In particular, we thank Dr. Katsuko Fujikawa and Ms. Megumi Chiba for the biological tests against levofloxacin-resistant MRSA, as well as, Dr. Mayumi Tanaka, Dr. Takaaki Akasaka and Dr. Hiroko Kanda for the determination of inhibitory activities against target enzymes. We also thank Mr. Hidetaka Sakurai for the X-ray crystal analyses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.08.026.

References and notes

1. *Abstracts of papers*, No. F-1054, This paper is based upon work: DX-619, a Novel Des-F(6)-Quinolone: Synthesis and In Vitro Activity against Multi-Drug Resistant Gram-positive Bacteria, presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, 43rd Chicago, 2003.
2. Cunha, B. A. *Crit. Care Clin.* **1998**, *14*, 309.
3. Dalhoff, A. *Infection* **1994**, *22*, S111.
4. Witte, W.; Cuny, C.; Klare, I.; Nübel, U.; Strommenger, B.; Werner, G. *Int. J. Med. Microbiol.* **2008**, *298*, 365.
5. Gould, I. M. *J. Antimicrob. Agents* **2008**, *32S*, S2.
6. Appelbaum, P. C. *Clin. Microbiol. Infect.* **2006**, *12*, 16.
7. Gootz, T. D.; Brighty, K. E.; Anderson, M. R.; Haskell, S. L.; Sutcliffe, J. A.; Castaldi, M. J.; Miller, S. A. *Abstracts of papers*, No. 751, 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Anaheim, 1992.
8. Petersen, U.; Bremm, K. D.; Dalhoff, A.; Endermann, R.; Heilmann, W.; Krebs, A.; Schenke, T. *Abstracts of papers*, No. F1, 36th ICAAC, New Orleans, 1996.
9. Hong, C. Y.; Kim, Y. K.; Chang, J. H.; Kim, S. H.; Choi, H.; Nam, D. H.; Kim, Y. Z.; Kwak, J. H. *J. Med. Chem.* **1997**, *40*, 3584.
10. Hirai, K.; Hosaka, M.; Niwata, Y.; Yasue, T.; Fukuda, H.; Ishizaki, T.; Suzue, S.; Nishino, K. *Abstracts of papers*, No. 385, 30th ICAAC, Atlanta, 1990.
11. Ho, P. L.; Yung, R. W. H.; Tsang, D. N. C.; Que, T. L.; Ho, M.; Seto, W. H.; Ng, T. K.; Yam, W. C.; Ng, W. W. S. *J. Antimicrob. Chemother.* **2001**, *48*, 659.
12. Hooper, D. C. *Lancet Infect. Dis.* **2001**, *2*, 530.
13. Cynamon, M. H.; Granato, P. A. *Antimicrob. Agents Chemother.* **1982**, *21*, 504.
14. Barriere, J. C.; Bouanchaud, D. H.; Harris, N. V.; Paris, J. M.; Rolin, O.; Smith, C. *Abstracts of papers*, No. 768, 30th ICAAC, Atlanta, 1990.
15. Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673.
16. Kelesidis, T.; Karageorgopoulos, D. E.; Isosif, K.; Falagas, M. E. *J. Antimicrob. Chemother.* **2008**, *62*, 895.
17. Falagas, M. E.; Giannopoulou, K. P.; Ntziora, F.; Vardakas, K. Z. *J. Antimicrob. Chemother.* **2007**, *60*, 7.
18. Rubinstein, E.; Prokocimer, P.; Talbot, G. H. *J. Antimicrob. Chemother.* **1999**, *44*, 37.
19. Kimura, Y.; Atarashi, S.; Takahashi, M.; Hayakawa, I. *Chem. Pharm. Bull.* **1994**, *42*(7), 1442.
20. Domagala, J. M.; Hagen, S. E.; Joannides, T.; Kiely, J. S.; Laborde, E.; Schroeder, M. C.; Sesnie, J. A.; Shapiro, M. A.; Suto, M. J.; Vanderroest, V. *J. Med. Chem.* **1993**, *36*, 871.
21. Akahane, K.; Hoshino, K.; Sato, K.; Kimura, Y.; Une, T.; Osada, Y. *Chemotherapy* **1991**, *37*, 224.
22. Suto, M. J.; Domagala, J. M.; Roland, G. E.; Mailloux, G. B.; Cohen, M. A. *J. Med. Chem.* **1992**, *35*, 4745.
23. Domagala, J. M. *J. Antimicrob. Chemother.* **1994**, *33*, 685.
24. Atarashi, S.; Imamura, M.; Kimura, Y.; Yoshida, A.; Hayakawa, I. *J. Med. Chem.* **1993**, *36*, 3444.
25. Kimura, Y.; Atarashi, S.; Kawakami, K.; Sato, K.; Hayakawa, I. *J. Med. Chem.* **1994**, *37*, 3344.
26. Hoshino, K.; Sato, K.; Kitamura, A.; Hayakawa, I.; Sato, M.; Osada, Y. *Abstracts of papers*, No. 1506, 31st ICAAC, Chicago, 1991.
27. Man, I.; Murphy, J.; Ferguson, J. *J. Antimicrob. Chemother.* **1999**, *43*, 77.
28. Yabe, K.; Goto, K.; Jindo, T.; Sekiguchi, M.; Furuhama, K. *Toxicol. Lett.* **2005**, *157*, 203.
29. Cecchetti, V.; Fravolini, A.; Palumbo, M.; Sissi, C.; Tabarrini, O.; Terni, P.; Xin, T. *J. Med. Chem.* **1996**, *39*(25), 4952.
30. Lawrence, L. E.; Wu, P.; Fan, L.; Gouveia, K. E.; Card, A.; Casperson, M.; Denbleyker, K. h.; Barrett, J. F. *J. Antimicrob. Chemother.* **2001**, *48*, 195.
31. Hayashi, K.; Takahata, M.; Kawamura, Y.; Todo, Y. *Arzneim.-Forsch./Drug Res.* **2002**, *52*, 903.
32. Ledoussal, B.; Almstead, J. K.; Flam, S. M.; Gallagher, C. P.; Gray, J. L.; Hu, X. E.; Kim, N. K.; Mekeever, H. D.; Miley, C. J.; Twinem, T. L.; Zheng, S. X. *Abstracts of papers*, No. F-751, 40th ICAAC, Toronto, Canada, 2000.
33. The C-7 substituent moiety of compound **3** or **6** was prepared by our reported method. (Takemura, M.; Kimura, Y.; Kawakami, K.; Kimura, K.; Ohki, H.; Matsuhashi, N.; Kawato, H. PCT Int. WO9623782, 1996.) The quinolone derivatives having this isomer (isomer **11**) of the C-7 substituent have been become clear that the compounds are higher activity the corresponding quinolones bearing an enantiomer of **11**.
34. Ataka, K.; Oku, M.; Omori, K.; Kimura, T.; Iwata, M. Jpn. Kokai Tokkyo Koho JP63316757, 1988.
35. Ledoussal, B.; Almstead, J. K.; Gray, J. L.; Hu, X. E. PCT Int. WO9914214, 1999.
36. The racemate of compound **9** was reported by Chu, D. T. (Chu, D. T.; Li, Q.; Cooper, C. S.; Fung, A. K. L.; Lee, C. M.; Plattner, J. J.; Ma, Z.; Wang, W.-B. PCT Int. WO9639407, 1996.).
37. Inagaki, H.; Takeda, T.; Miyauchi, R.; Kawakami, K.; Takahashi, H.; Takemura, M. *Heterocycles* **2004**, *63*, 699.
38. Compounds **21**–**24** were reported in our previous paper. (Inagaki, H.; Miyauchi, S.; Miyauchi, R.; Kawato, H.; Ohki, H.; Matsuhashi, N.; Kawakami, K.; Takahashi, H.; Takemura, M. *J. Med. Chem.* **2003**, *46*, 1005.).
39. Domagala, J. M.; Hagen, S. E.; Joannides, T.; Kiely, J. S.; Laborde, E.; Schroeder, M. C.; Sesnie, J. A.; Shapiro, M. A.; Suto, M. J.; Vanderroest, S. *J. Med. Chem.* **1993**, *36*(7), 871.
40. Compound **7** (PEG9215875) was also reported in our previous patent. (Hayakawa, I.; Kimura, Y. Jpn. Kokai Tokkyo Koho JP03072476, 1991.).
41. Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.
42. Yamaguchi, K.; Ohno, A.; Kashitani, F.; Iwata, M.and the Levofloxacin Surveillance Group *Jpn. J. Antibiot.* **2003**, *56*, 5.
43. Fukuda, Y.; Yanagihara, K.; Ohno, H.; Higashiyama, Y.; Miyazaki, Y.; Tsukamoto, K.; Hirakata, Y.; Tomono, K.; Mizuta, Y.; Tashiro, T.; Kohno, S. *Antimicrob. Agents Chemother.* **2006**, *50*, 121.
44. Fujikawa, K.; Chiba, M.; Tanaka, M.; Sato, K. *Antimicrob. Agents Chemother.* **2005**, *49*, 3040.
45. Kastenbaum, K.; Bowman, K. *Mutat. Res.* **1970**, *9*, 527.