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Design, Synthesis, and Biological Evaluation of Novel 7-[(3a*S*,7a*S*)-3a-Aminohexahydropyrano[3,4-*c*]pyrrol-2(3*H*)-yl]-8-methoxyquinolines with Potent Antibacterial Activity Against Respiratory Pathogens

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.

ABSTRACT.

Novel 7-[(3a*S*,7a*S*)-3a-aminohexahydropyrano[3,4-*c*]pyrrol-2(3*H*)-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **5** (**DS21412020**) was designed and synthesized to obtain potent antibacterial drugs for the treatment of respiratory tract infections. Compound **5** possessing a *trans*-fused pyranose ring on the pyrrolidine moiety at the C-7 position of the quinolone scaffold exhibited potent *in vitro* antibacterial activity against respiratory pathogens, including quinolone-resistant and methicillin-resistant *Staphylococcus aureus* (QR-MRSA) and quinolone-resistant *Escherichia coli* (QR-*E. coli*). Furthermore, compound **5** showed *in vivo* activity against the experimental murine pneumonia model due to penicillin-resistant *Streptococcus pneumoniae* (PRSP) and favorable profiles in preliminary toxicological and nonclinical pharmacokinetic studies. In particular, the reduced lipophilicity and basicity of compound **5** compared to those of the previously synthesized carba-type compound **4** resulted in a significant reduction in the human ether-a-go-go (hERG) related gene channel inhibition which have the potential to prolong the QT interval.

INTRODUCTION

After various antibiotics have been successfully used for decades, the emergence of antibiotic resistance among Gram-positive and Gram-negative bacteria makes a serious threat for public health. Recently, the innovation rate of novel antibiotics is unable to keep up with the emergence of antibiotic-resistant bacteria. In the United States, It is estimated that the impact of antibiotic-resistant bacteria causes over 23,000 deaths, 2 million illnesses, and costs amounting to 20 billion dollars each year.[1] Because of the increasing levels of resistance to β-lactams and macrolides exhibited by community-acquired pathogens, such as the multidrug-resistant *Streptococcus pneumoniae* (MDRSP),[2,3] newer quinolones are increasingly being used as first-line antibacterial therapy for respiratory tract infections in clinical settings. Respiratory fluoroquinolones, including levofloxacin (LVFX) and moxifloxacin (MFLX), have been important therapeutic options for community-acquired bacterial pneumoniae (CABP) due to their enhanced activity against Streptococcus pneumoniae, compared to ciprofloxacin (CPLX), coupled with favorable pharmacokinetic (PK) and safety profiles.[4-6] However, the antibacterial activity of these newer quinolones may be insufficient to prevent the emergence of strains such as quinolone-resistant *S. pneumoniae* (QRSP) and community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).[7,8] Due to their insufficient activity against MRSA, US Food and Drug Administration (FDA) doesn't recommend using fluoroquinolones in the treatment of MRSA-related infections.[9] Furthermore, clinical adverse events (e.g., Torsades de Pointes or fatal liver injury) have been shown to be associated with some quinolones.[10-14] Therefore, novel quinolone antibiotics exhibiting improved activity with minimal adverse effects are required.

As previously reported,[15,16] compound **1** shows potent activity against respiratory pathogens, including PRSP, together with a superior safety profile similar to that of LVFX. As a result of further research,[17] we found that compound **4**, which has a *trans*-fused bicyclic structure at the C-7 position, also has potent activity against respiratory pathogens; however, it inhibits hERG potassium channels.

Generally, the degree of hERG inhibition closely relates to the lipophilicity of the compound, which is expressed as LogD or *P'*.[18] Therefore, we planned to introduce an oxygen atom into the structure of compound **4** at the C-7 pyrrolidine substituent, in order to decrease its *P'* value. Moreover, it is known that the degree of hERG inhibition correlates with the basicity of the compound and that reducing the basicity of a compound is effective in preventing hERG inhibition.[19] According to a report by Morgenthaler et al, the γ-position (Figure 1, γ1 and γ2) from the amino group on the C-7 side chain would be appropriate for the introduction of an oxygen atom.[19] Based on the knowledge that the introduction of an heteroatom into the γ2-position reduces antibacterial activity,[20] compound **5** bearing an oxygen atom at the γ1-position from the amino group at the C-7 side chain was designed.

In this article, we describe the details of the synthesis, *in vitro* and *in vivo* antibacterial activities, safety profile, and pharmacokinetic profile of the designed compound **5**. Since the absolute stereochemistry of the amino group on the C-7 side chain greatly affects the strength of the antibacterial activity,[15,16] the synthesis was carried out only for the enantiomer having more potent activity.

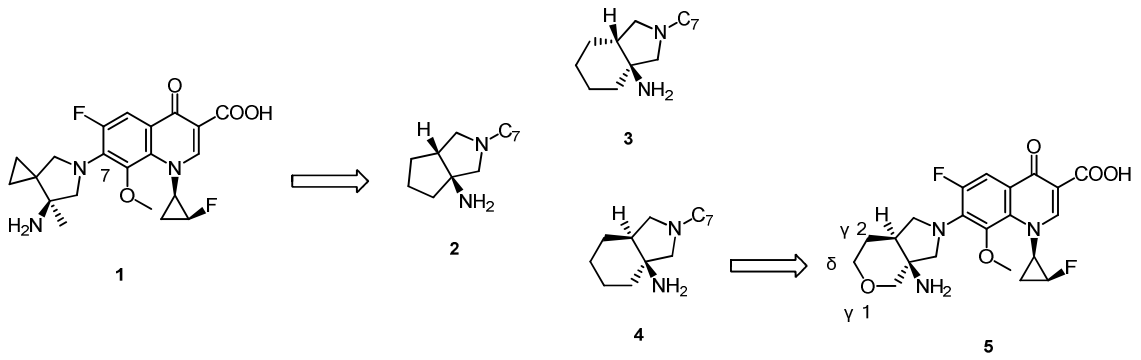
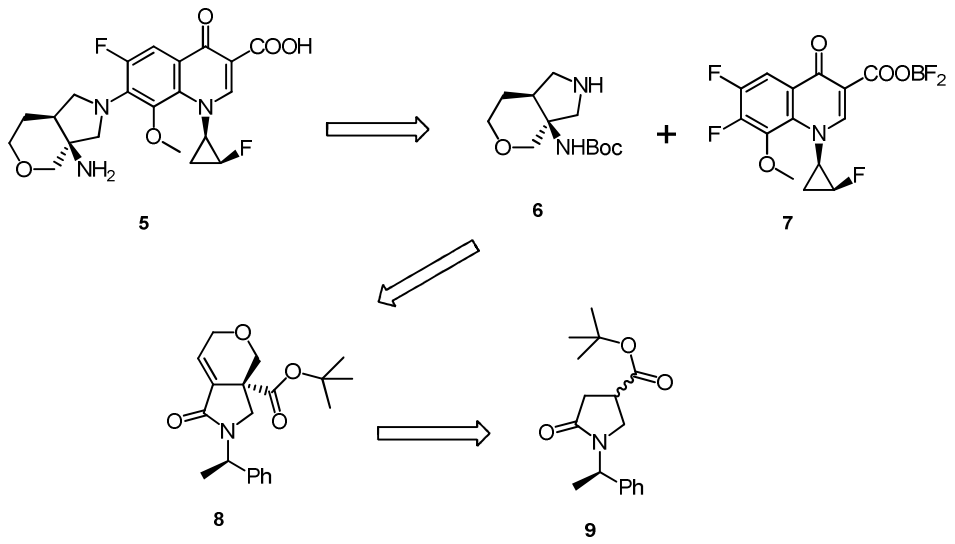


Figure 1. Design of novel compound **5**.

CHEMISTRY

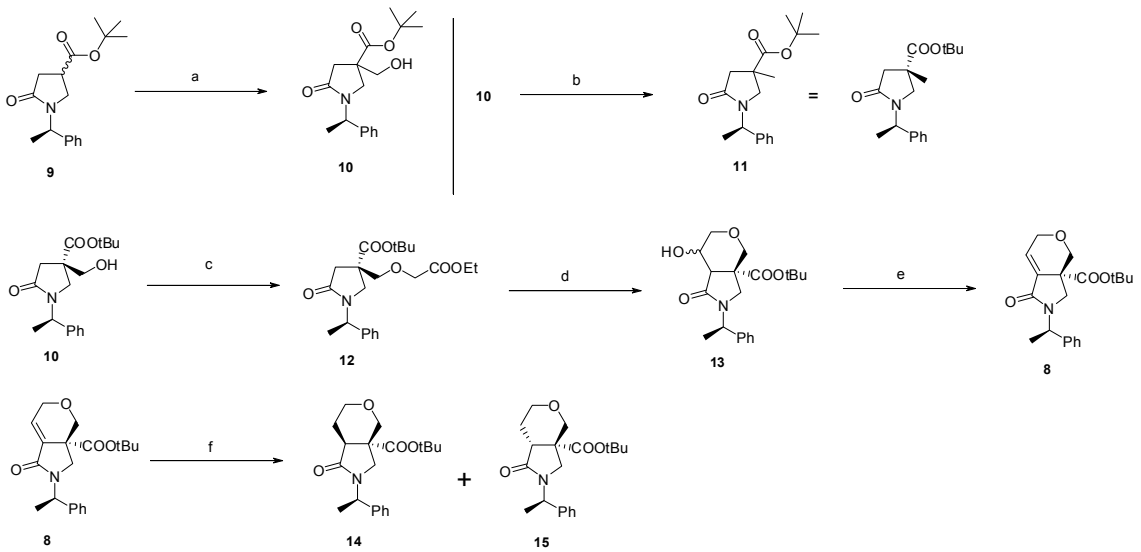
As shown in Scheme 1, we planned to synthesize the desired 7-substituted 8-methoxyquinolone derivative **5** by an aromatic nucleophilic substitution reaction from amine **6** and 6,7-difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid BF_2 chelate **7**,[21] in which the BF_2 chelate was known to improve the reactivity at the C-7 position.[22] We planned to prepare amine **6** by the hydrogenation of conjugated amide **8**, which would be synthesized from known compound **9**.[23]

Scheme 1. Synthetic Route to Compound **5**



The synthesis of intermediates **14** and **15** is illustrated in Scheme 2. Hydroxymethylation of known compound **9** gave **10** as the major product. The minor product obtained in trace amounts could not be isolated due to contamination with an impurity. Deoxygenation of **10** gave methylate **11**, whose ^{1}H-NMR spectrum matched that of the reported compound.[23] Therefore, the absolute stereochemistry of **10** was determined to be the desired (3*S*). After alkylation of **10**, intramolecular cyclization was accomplished by employing lithium hexamethyldisilazide (LHMDS). Subsequent reduction with $NaBH_4$ gave alcohol **13** as a diastereomeric mixture, which was converted to key intermediate **8** by sequential one-pot mesylation and elimination with the use of DBU. Hydrogenation of conjugate amide **8** gave *cis* and *trans* diastereomers **14** and **15** in approximately 4:1 ratio. Compound **14** was obtained as prisms, and its absolute stereochemistry was determined to be 7a*S* by X-Ray crystallographic analysis (Figure 2). Unfortunately, the undesired *cis*-isomer **14** was the major product in the hydrogenation step.

Scheme 2. Synthesis of Key Intermediate **14**[a]



[a]Reagents and conditions: (a) (HCHO)n, NaH, DMF, 23%; (b) (1) PhSSPh, Bu_3P, THF, 65 °C, (2) H_2, Raney-Ni, EtOH, 12%; (c) NaH, ethyl bromoacetate, Bu_4NI, DMF, 84%; (d) (1) LHMDS, THF, –40 °C, (2) $NaBH_4$, (e) (1) MsCl, triethylamine, CH_2Cl_2, –10 °C, (2) DBU, 40 °C, 36% from **12**, (f) H_2, Pd/C (wet), EtOAc, **14** (79%), **15** (21%).

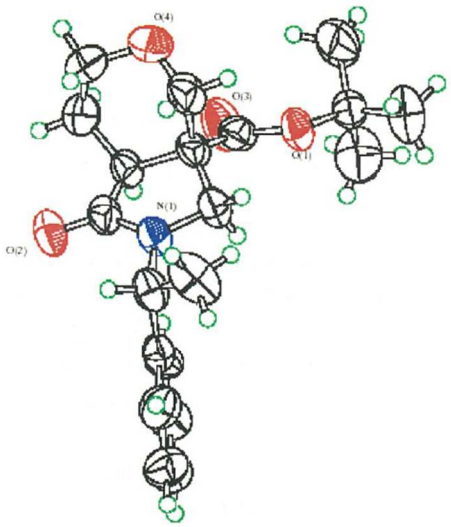


Figure 2. ORTEP of compound **14**.

As described above, the desired *trans*-isomer **15** was the minor product in the Pd-catalyzed reduction process. Therefore, we attempted to investigate the reaction conditions to increase the

ratio of **15**. As a chelate reagent, $MgBr_2$ was selected as the additive with reference to the report by Nagano et al.[24] As shown in Table 1, when $MgBr_2$ (2.5eq.) was added, the reaction did not proceed at all under an atmospheric pressure of hydrogen (entry 2). We speculated that $MgBr_2$ was effectively chelated on the surface of **8**. Thus, increasing the amount of catalyst and hydrogen pressure up to 50 atm facilitated the progress of the reaction, and **15** was obtained as the main product (entry 3). When ethanol was mixed with the reaction solvent for reducing the amount of the catalyst and accelerating the hydrogenation, the reaction proceeded at atmospheric pressure, although the selectivity was slightly reduced (entry 4). There was a positive correlation between the hydrogen pressure and the selectivity, consistent with Le Chatelier's law. Specifically, the equilibrium between the chelated form (single molecule) and the dechelated form (two molecules) was shifted toward the chelate side upon increasing the hydrogen pressure. Finally, an effective synthesis of the oxa *trans*-form **15** was achieved under the conditions shown in entry 6.

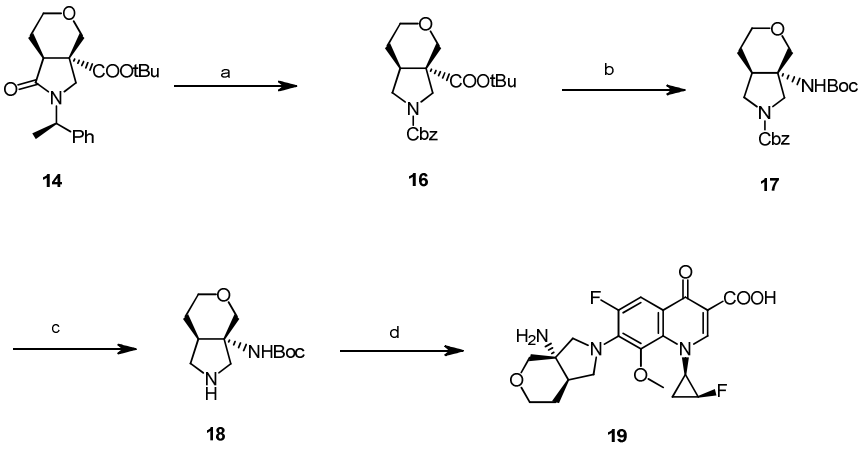
Table 1. Reaction Conditions for Hydrogenation

	Additive ($MgBr_2$)	Solvent	Time	Pressure (atm)	Pd/C (50%wet) (wt%)	**14** (cis)[b]/ **15** (trans)[b]
1	none	EtOAc	1 day	1[a]	50	79/21 %
2	2.5 eq.	EtOAc	1 day	1[a]	50	No Reaction.
3	1 eq.	EtOAc	1 day	50	200	23/77 %
4	1 eq.	EtOAc:EtOH=2:1	3 days	1[a]	50	-[c]/71 %
5	1 eq.	EtOAc:EtOH=2:1	14 h	10	50	-[c]/83 %
6	1 eq.	EtOAc:EtOH=2:1	14 h	30	50	-[c]/89 %[d]

[a]Balloon pressure. [b]Isolated yield. [c]Not isolated. [d]Experimental details described in the experimental section.

Oxa *cis*-fused compound **19** was synthesized as shown in Scheme 3. After the reduction of **14** with a BH_3–THF complex, the phenylethyl group was converted to the benzyloxycarbonyl group. Then, the *t*-Bu group of **16** was removed, and the carboxylic acid was subsequently converted to the carbamoyl group. Furthermore, the carbamoyl group was subjected to Hoffman rearrangement conditions, and Boc-protected amine **17** was obtained as a single product. The benzyloxycarbonyl group of **17** was then removed by catalytic hydrogenolysis, and the resultant crude substance was heated with quinolonecarboxylic acid BF_2 chelate **7** and triethylamine in DMSO; finally oxa *cis*-fused compound **19** was obtained after dechelation and deprotection of the Boc group.

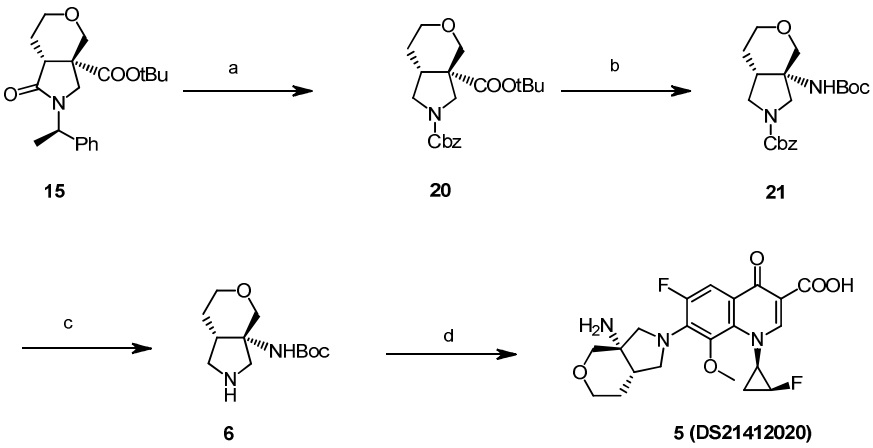
Scheme 3. Synthesis of *Cis*-fused Compound **19**[a]



[a]Reagents and conditions: (a) (1) BH_3–THF complex, THF, (2) CbzCl, 1,2-dichloroethane, 40 °C, 68%; (b) (1) TFA, CH_2Cl_2, (2) 1,1'-carbonylbis-1*H*-imidazole, CH_3CN, 0 °C, then NH_3 gas, (3) $Pb(OAc)_4$, *t*-BuOH, 80 °C, 65%; (c) H_2, Pd/C (wet), MeOH; (d) (1) **7**, TEA, DMSO, (2) TEA, 80% aqueous EtOH, reflux, (3) concentrated aqueous HCl, 67% from **17**. TEA = triethylamine.

Oxa *trans*-fused isomer **15** was converted to Cbz-protected amine **20** by a similar method as that for *cis*-fused isomer **14** (Scheme 4). Initially, we attempted the Hoffmann rearrangement as in the case of the *cis*-fused isomer, but the reaction did not proceed at all. Although the reasons are unknown, the bulkiness of the *trans*-form scaffold is presumed to prevent the reaction. Instead, the Curtius rearrangement proceeded with high yield, and the desired Boc-protected amine **21** was obtained as a single product. The subsequent steps were carried out in the same manner as in the case of the *cis*-fused isomer, and the targeted oxa *trans*-fused compound **5** (**DS21412020**) was synthesized. Finally, the ^{1}H-NMR spectrum of **5** was confirmed to be different from that of **19**.

Scheme 4. Synthesis of *Trans*-fused Compound **5**[a]



[a]Reagents and conditions: (a) (1) BH_3–THF complex, THF, 40 °C, (2) CbzCl, 1,2-dichloroethane, 35 °C, 82%; (b) (1) TFA, CH_2Cl_2, (2) diphenylphosphorylazide, TEA, toluene, 110 °C, (3) 6N aqueous HCl, 1,4-dioxane, 60 °C, (4) Boc_2O, 50 °C, 83%; (c) H_2, Pd/C (wet), MeOH; (d) (1) **7**, TEA, DMSO, (2) TEA, 80% aqueous EtOH, reflux, (3) concentrated aqueous HCl, 72% from **21**.

RESULTS AND DISCUSSION

The minimum inhibitory concentrations (MICs) of the synthesized compounds **5** and **19** against several representative Gram-positive and Gram-negative bacteria are summarized in Table 2, along with the corresponding data for LVFX, MFLX, and the previously reported compounds **1**–**4** for comparison. Oxa *trans*-fused compound **5** showed a broad antibacterial spectrum against Gram-positive and Gram-negative bacteria. In particular, the activity of **5** against Gram-positive bacteria was 2- to 4-fold stronger than that of oxa *cis*-fused compound **19** and control compound **1**, although the activity of **5** against some of the bacteria was slightly weaker than that of carba *trans*-fused compound **4**.

Table 2. Antibacterial Activities MIC (μg/mL) of Synthesized Compounds and Reference Quinolones against Gram-positive and Gram-negative Bacteria[a]

Organisms \ Compounds	5	19	2	3	4	LVFX	MFLX	1
S. aureus SMITH	0.006	0.012	<0.003	0.006	<0.003	0.1	0.025	0.012
S. pneumoniae J24[b]	0.1	0.2	0.05	0.05	0.025	0.78	0.1	0.05
S. pneumoniae J41[b]	0.05	0.2	0.1	0.05	0.025	1.56	0.1	0.1
S. pyogenes ATCC 12344	0.05	0.2	0.025	0.1	0.025	0.39	0.2	0.2
E. faecalis ATCC 19433	0.2	0.39	0.1	0.2	0.1	0.78	0.2	0.2
B. subtilis ATCC 6633	0.006	0.025	0.012	0.012	0.006	0.05	0.025	0.012
E. coli NIHJ	0.006	0.05	0.025	0.025	0.006	0.012	0.012	0.012
K. pneumoniae TYPE 1	0.05	0.2	0.1	0.1	0.05	0.05	0.1	0.05
H. influenzae ATCC49247	0.006	0.012	0.006	0.025	0.012	0.012	0.012	0.012

M(B). catarrhalis ATCC25238	0.025	0.1	0.025	0.1	0.025	0.025	0.05	0.05
P. aeruginosa PAO-1	0.78	3.13	0.39	1.56	0.39	0.39	0.78	0.78

[a]Antibacterial activities were determined using a standard microbroth dilution method, MIC μg/mL. [b]Penicillin-susceptible *S. pneumoniae* (PSSP).

The antibacterial activities against several resistant bacteria and mutant strains are shown in Table 3. Oxa *trans*-fused compound **5** exhibited potent antibacterial activity against *MRSA*, resistant *E. coli*, and mutant *E. coli.* The antibacterial activity of **5** was stronger than that of **1** and slightly weaker than that of **4**; the same tendency was observed against representative bacteria pathogens.

Table 3. Antibacterial Activities MIC (μg/mL) of Synthesized Compounds and Reference Quinolones against Resistant Bacteria and Mutant Strains[a]

Organisms \ Compounds	5	19	2	3	4	LVFX	MFLX	1
MRSA 870307[a]	0.2	0.78	0.78	0.39	0.1	>6.25	0.78	0.78
MRSA 890325-1[a]	0.2	0.78	0.78	1.56	0.1	6.25	1.56	0.78
PRSP 033806[b]	0.12	-	-	-	-	1.56	0.25	0.12
E. coli DNS5101[c]	1.56	>6.25	6.25	>6.25	1.56	>6.25	>6.25	6.25
E. coli 5-037042 '98[d]	0.05	0.39	0.1	0.1	0.025	0.1	0.1	0.05

[a]Levofloxacin-resistant and methicillin-resistant *S. aureus* (levofloxacin-r-*MRSA*). [b]Penicillin-resistant *S. pneumoniae*. [c]Quinolone-resistant *E. coli*. [d]*Gyrase* A mutation: Asp87→Gly.

Finally, the MICs against clinically isolated *MRSA* of compound **5** and reference quinolones are shown in Table 4. The MIC_{90} of compound **5** was 0.5 μg/mL, superior to the other quinolones.

Thus, compound **5** was considered to have potent activity against not only representative respiratory pathogens but also resistant strains.

Table 4. Antibacterial Activities MIC (μg/mL) against *MRSA* (44 strains)[a,b]

	MICrange	MIC_{50}	MIC_{90}
5	0.008 - 8	0.015	0.5
LVFX	0.25 - >64	0.5	8
MFLX	0.015 - 16	0.06	2
GRNX[c]	0.008 - 8	0.03	1
1	0.015 - 4	0.03	1

[a] Clinical Isolates collected in Japan. [b] *S. aureus* ATCC 29213 was used as the quality control strains for MRSA. The quality control strains were included as internal controls throughout the study and the control results were within acceptable ranges defined by CLSI (M100-S16). [c] GRNX : garenoxacin.

Table 5 shows the result of the synthesized compounds on the hERG potassium current in hERG-transfected cells along with the apparent partition coefficient value (*P'*). Electrocardiogram studies revealed that compounds inhibit hERG potassium channels have the potential to prolong the QT interval and increase the risk of fatal cardiac arrhythmia.[25,26] As mentioned above, carba *trans*-fused compound **4** inhibited hERG currents at a concentration of 30 μM, and the hERG inhibition of **4** was almost equal to that of MFLX, which is known to cause QT prolongation syndrome at clinical dosage.[27] On the other hand, oxa compounds **5** and **19** showed almost no hERG inhibitory activity, as was anticipated on the basis of their structures. Although the *P'* value (lipophilicity) of **5** was reduced compared to that of carba *trans*-fused compound **4**, it was almost the same as that of MFLX. Therefore, the decrease in lipophilicity is not the only factor causing the suppression of hERG inhibition. Instead, the reduction in basicity,

achieved by the introduction of an oxygen atom at the γ1-position from the amino group of **5**, may be responsible for this change. We had established an inhibition threshold within 10% at a concentration of 30 μM, so compound **5** and **19** meet this criterion.

Table 5. Effects on hERG Potassium Current in hERG Transfected Cells[a] and Apparent Partition Coefficient (*P'*)[b]

Compound	Concentration (μM)		*P'*
	30	100	
5[c]	-2.2	-2.5	49
19[d]	-6.4	-7.5	37
4[d]	19	36	>68
LVFX[c]	-0.9	4.2	5.1
MFLX[c]	22	42	54
1[c]	1.9	2.7	19

[a]Data represent % inhibition.[28,29] [b]Apparent partition coefficient, $CHCl_3$/0.1M phosphate buffer (pH 7.4).[30] [c]CHO-K1 cells. [d]HEK293 cells.

The metabolic stability, protein binding, and mechanism-based inhibition (MBI) against CYP3A4 are shown in Table 6. Both **5** and **19** showed high metabolic stability in human microsomes. The protein binding rate was very low for both compounds. In particular, the protein binding rate for compound **5** was almost 0%. Furthermore, the potential inhibitory effects of compound **5** on the MBI against CYP3A4 was investigated. Compound **5** did not show any inhibitory effect on the MBI against CYP3A4.

Table 6. Metabolic Stability, Protein Binding, and MBI against CYP3A4[a]

Compound	Metabolic Stability[a]	Protein Binding[b]	CYP3A4 MBI[c]
5	100	0	97.4
19	100	12	-
LVFX	95	14	104
MFLX	92	1.3	97.9
1	97	5.4	78.4[d]

[a]The % remaining value using human microsomes. 1 μM, 30 min. [b]Protein binding (%) to human albumin [c]The % remaining value at a 10 μM concentration of compounds reacted with CYP3A4 probe substrates after 30 min preincubation in human liver microsomes. [d]As a result of detailed examination, it was judged to be negative.[15]

The pharmacokinetics (PK) profiles of the synthesized compounds, LVFX, MFLX, and **1** following single oral administration in rats are shown in Table 7. The maximum drug concentration (C_{max}) and area under the time–concentration curve (*AUC*) for compound **5** were almost equal to those of compound **1**. Furthermore, investigation of the PK profile in monkeys revealed that compound **5** had a higher blood concentration than compound **1**, LVFX, and MFLX (Table 8). The urinary recovery rate of **5** was as high as that of LVFX. Thus, compound **5** is expected to be effective in the treatment of urinary tract infections.

Table 7. Pharmacokinetic Parameters of Synthesized Compounds and Reference Quinolones in Rats After an Oral Dose of 5 mg/kg (n = 3)[a]

PK parameters	**5**	**2**	**4**	LVFX	MFLX[b]	**1**
C_{max} (μg/mL)	0.88	0.049	1.28	1.47	1.49	1.22

$AUC_{0\text{-}8h}$ (μg· h/mL)	3.14	0.21	4.76	3.41	4.46	3.08

[a]Animal: 7-week-old male Crj:CD rats. [b]Moxifloxacin hydrochloride hydrate was administered.

Table 8. Pharmacokinetic Parameters of Synthesized Compounds and Reference Quinolones in Monkeys after a Dose of 5 mg/kg (n = 3)[a]

Route	PK parameters		**5**[b]	**1**	LVFX	MFLX[c]
p.o.	Serum	C_{max} (μg/mL)	3.7	2.2	1.92	1.03
		$AUC_{0\text{-}24h}$ (μg· h/mL)	19.8	16.9	15.2	6.63
		$t_{1/2}$ (h)	4.6	4.8	3.5	5.3
	Urinary recovery$_{0\text{-}24h}$ (%)[d]		64.1	61.3	73.9	8.1
	Bioavailability (%)		75	71	-	61
i.v.	Serum	C_{5min} (μg/mL)	7.0	3.66	4.17[e]	2.36
		$AUC_{0\text{-}8h}$ (μg· h/mL)	22.9	23.8	15.5[e]	10.9
		$t_{1/2\alpha}$ (h)	3.5	4.8	3.2[f]	4.8
	Urinary recovery$_{0\text{-}24h}$ (%)[d]		65.0	69.8	80.7[f]	11.0

[a]Animal: female cynomolgus monkeys. [b]p.o.: MsOH salt (0.5 H_2O), see experimental section (**5a**), i.v.: free (**5**). [c]Moxifloxacin hydrochloride hydrate was administered. [d]The values were calculated by using the concentrations of unchanged quinolones. [e]The values result from the division of data for 10 mg/kg intravenous administration by levofloxacin. [f]The values are the results for a 10 mg/kg intravenous administration.

The therapeutic efficacies of oxa *trans*-fused compound **5** in experimental murine pneumonia models due to penicillin-resistant *S. pneumoniae* (PRSP033806) are compared with the efficacies of moxifloxacin and compound **1** in Figure 3. The therapeutic efficacy of subcutaneously administrated **5** was superior to that of moxifloxacin and equal to that of compound **1**. Based on

the PK/PD theory of antibacterial drugs,[31] the efficacy of compound 5 after oral administration can be expected sufficiently for clinical treatment.

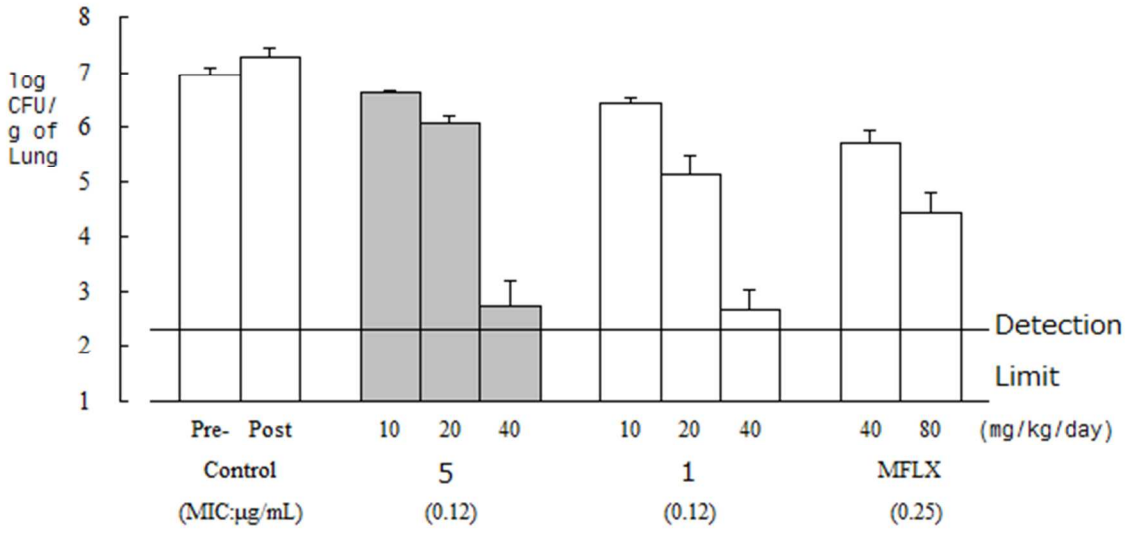


Figure 3. Therapeutic effects on pneumococcal pneumonia (PRSP) in mice. Animals: CBA/JNCrj mice (n = 4). Bacteria: *S. pneumoniae* 033806 (PRSP). Test drugs were administered subcutaneously twice a day (2 h and 8 h after infection).

The result of convulsion incidence test was shown in Table 9. As a representative side effect of quinolone drugs, it is known that there is a case of rarely enhancing convulsion induction when used in combination with NSAIDs[32]. The convulsion inducing activity of compound **5** itself was weaker than the other quinolones. Furthermore, when BPAA was used in combination, **5** did not show the effect of enhancing convulsion induction as much as compound **1**.

Table 9. Effect of Ciprofloxacin (CPFX), LVFX and compounds on Convulsion Incidence in Mice

Compound	Dose (μg/5μl/mouse, i.cist.)	Incidence of Convulsion (Death)	
		Without BPAA	With BPAA[a]
CPFX	5	0/6-1/6 (0/6)	6/6 (6/6)
	15	4/6-6/6 (1/6-0/6)	6/6 (6/6)
	50	6/6 (2/6-5/6)	6/6 (6/6)
LVFX	5	0/6	1/6
	15	1/6	6/6
1	5	0/6 (0/6)	0/6 (0/6)
	15	2/6 (0/6)	3/6 (0/6)
	50	6/6 (0/6)	5/6 (0/6)
5	5	0/6 (0/6)	0/6 (0/6)
	15	0/6 (0/6)	0/6 (0/6)
	50	4/6 (0/6)	1/6 (0/6)

[a]BPAA : 4-Biphenylacetic Acid (reactive metabolite of fenbufen). 30 minutes before the drug solution administration, BPAA 400 mg/kg was orally administered at a volume of 10 mL/kg.

CONCLUSION

Novel oxa *trans*-fused compound **5** (**DS21412020**), a 6-fluoro-8-methoxyquinolone possessing a [(3a*S*,7a*S*)-3a-aminohexahydropyrano[3,4-*c*]pyrrol-2(3*H*)-yl] group at the C-7 position of the scaffold, was designed, synthesized, and evaluated. Compound **5** exhibited about 2- to 4-fold better antibacterial activity *in vitro* against Gram-positive bacteria as compared with the previously reported compound **1**.[15] Compound **5** also showed high AUCs in rats and monkeys, and stronger *in vivo* antibacterial activity against penicillin-resistant *S. pneumoniae* infection in mice than that of MFLX. Furthermore, oxa compound **5**, which was designed to present lower lipophilicity and basicity than does carba compound **4**, solved the problem of strong hERG

inhibition. Finally, we developed an efficient method for the synthesis of the oxa *trans*-fused intermediate by chelate control using $MgBr_2$.

EXPERIMENTAL SECTION

General. All melting points were recorded on a Yanaco MP-500D melting point apparatus and reported 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 recorded on a JEOL JNM-EX400 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standards. Significant ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant(s) in hertz. 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). High-resolution mass (HRMS) spectra were recorded on a JEOL JMS-100LP spectrometer. Elemental analyses are indicated only by the symbols of the elements; analytical results were within 0.4% of the theoretical values. Purities of ≥95% were determined by elemental analysis (all tested compounds). Column chromatography refers to flash column chromatography conducted on Merck silica gel 60, 230–400 mesh ASTM. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F254 TLC plates, and compound visualization was effected with a 5% solution of molybdophosphoric acid in ethanol, UV lamp, iodine, or Wako ninhydrin spray. All experimental procedures for the animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

***In Vitro* Antibacterial Activity.** The MICs of the compounds tested in this study were determined by the two-fold micro 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 35 °C for 18 h.

***In Vivo* Efficacy against *S. pneumoniae* Infection in Mice**. Three-week-old male CBA/JNCrj mice (4 mice/group) were used. The bacteria used for infection was quinolone-sensitive *S. pneumoniae* 033806 (penicillin-resistant, QS-PRSP), which was inoculated by nasal drops (Inoculam Size : 10^6 CFU/mouse, Inoculation Volume : 0.05 mL). One day after the infection, drugs were administered subcutaneously twice a day (2 h and 8 h after infection, administration volume : 0.1 mL / 10 g body weight). The number of bacteria in the lungs was examined on the day after the final administration of drug. The lungs were removed aseptically and weighed, and then the viable bacterial counts were determined. The detection limit was ≥2.30 $\log_{10}$ CFU/g of lung.

7-[(3a*S*,7a*S*)-3a-Aminohexahydropyrano[3,4-*c*]pyrrol-2(3*H*)-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (5)

To a solution of **21** (610 mg, 1.62 mmol) in MeOH (20 mL) was added 10% Pd/C (200 mg, containing 50% water), and the mixture was stirred for 3 h at ambient temperature under a hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was mixed with **7** (566 mg, 1.57 mmol), TEA (0.621 mL, 4.48 mmol), and DMSO (8 mL). The mixture was stirred for 18 h at 40 °C. To this solution were added 90% aqueous EtOH (150 mL) and TEA (15 mL), and the resultant mixture was stirred at 80 °C for 2.5 h, and then concentrated *in vacuo* to give the residue, which was diluted with EtOAc. The organic layer was washed with 10% aqueous citric acid solution, water, and brine dried over anhydrous

Na_2SO_4, and concentrated *in vacuo*. To the residue was added concentrated aqueous HCl (4 mL) at 0 °C, and the mixture was stirred for 20 min at ambient temperature. The aqueous solution was washed with $CHCl_3$ and made alkaline with saturated aqueous NaOH at 0 °C. The pH of the solution was adjusted to 7.4 with concentrated aqueous HCl and then with diluted aqueous HCl. The resultant solution was extracted with $CHCl_3$ (3 ×), the combined organic solution dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant solid was recrystallized from EtOH to yield **5** (470mg, 72%) as a pale yellow powder, m.p. 190–192 °C. ^{1}H NMR (0.1 N NaOD/D_2O): δ 1.32–1.36 (1H, m), 1.48–1.54 (1H, m), 1.71–1.88 (2H, m), 2.11–2.14 (1H, m), 3.24 (1H, d, J = 10.3 Hz), 3.51–3.67 (8H, m), 3.95–4.02 (2H, m), 4.09 (1H, dd, J = 11.4, 4.0 Hz), 5.06 (1H, dd, J = 64.1, 3.5 Hz), 7.65 (1H, d, J = 14.4 Hz), 8.36 (1H, d, J = 3.7 Hz). ^{13}C NMR (0.1N NaOD/D_2O): δ 13.92, 14.01, 22.80, 38.08, 39.59, 39.66, 42.88, 52.49, 52.55, 54.87, 58.88, 58.92, 60.49, 66.68, 71.67, 73.44, 74.43, 106.5, 106.7, 115.9, 119.6, 119.7, 133.9, 136.0, 136.1, 139.8, 139.9, 149.7, 151.8, 153.8, 172.2, 174.9, 175.0. MS (ESI) *m/z*: 436$(M + H)^+$. $[\alpha]_D^{25}$ = –160.882° (*c* 0.27, 0.1N aqueous NaOH). Anal. calcd for $C_{21}H_{23}F_2N_3O_5 \cdot 0.25H_2O$, C 57.33, H 5.38, F 8.64, N 9.55; found, C 57.28, H 5.39, F 8.48, N 9.27. IR (ATR): 3502, 3374, 3091, 2948, 2881, 2850, 1716, 1617, 1513 cm^{-1}.

7-[(3a*S*,7a*S*)-3a-Aminohexahydropyrano[3,4-*c*]pyrrol-2(3*H*)-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, Methanesulfonic acid salt (5a·MsOH salt)

To a solution of **21** (1.79 g, 4.75 mmol) in MeOH (40 mL) was added 10% Pd/C (900 mg, containing 50% water), and the mixture was stirred for 7 h at ambient temperature under a hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*.

The residue was mixed with **7** (1.67 g, 4.64 mmol), TEA (1.84 mL, 13.3 mmol), and DMSO (6.5 mL). The mixture was stirred for 4 h at 40 °C. To this solution were added water (30 mL), and the mixture was stirred for 0.5 h at ambient temperature. Then, the precipitate was filtered and washed with water (20 mL). To a suspension of the resultant solid in 90% aqueous EtOH (100 mL) was added TEA (10 mL), and the mixture was stirred for 2 h at 80 °C. After concentration *in vacuo*, EtOAc and 10% aqueous citric acid solution were added to the residue. The organic solution was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To the residue were added isopropanol (4.7 mL) and methanesulfonic acid (847 mg, 8.81 mmol) at ambient temperature, and the mixture was stirred for 3 h at 50 °C and at 40 °C for 18 h. After the mixture was stirred for 1 h at 0 °C, the precipitate was filtered and washed with cold isopropanol (10 mL) to yield **5a** (2.06 g, 88%) as a pale yellow powder, m.p. >280°C. ^{1}H NMR (0.1N NaOD/D_2O): δ 1.41–1.51 (2H, m), 1.78–1.93 (2H, m), 2.53–2.56 (1H, m), 2.70 (3H, s), 3.49–3.53 (1H, m), 3.53 (3H, s), 3.56 (1H, d, J = 12.7 Hz), 3.71 (1H, dd, J = 11.0, 5.4 Hz), 3.77 (1H, d, J = 12.2 Hz), 3.84 (1H, t, J = 9.0 Hz), 3.89 (1H, d, J = 12.2 Hz), 4.02–4.07 (1H, m), 4.12 (1H, dd, J = 11.5, 4.6 Hz), 4.17 (1H, d, J = 12.2 Hz), 5.02 (1H, d, J = 64.0 Hz), 7.20 (1H, d, J = 13.7 Hz), 8.60 (1H, d, J = 3.7 Hz). MS (ESI) *m/z*: 436$(M + H)^+$. Anal. calcd for $C_{21}H_{23}F_2N_3O_5 \cdot MsOH \cdot 0.5H_2O$, C 48.88, H 5.22, F 7.03, N 7.77, S 5.93; found, C 48.93, H 5.17, F 7.07, N 7.59, S 6.12..

tert-Butyl (3a*S*)-1-Oxo-2-[(1*R*)-1-phenylethyl]-1,2,3,6-tetrahydropyrano[3,4-*c*]pyrrole-3a(4*H*)-carboxylate (8)

To a mixture of LHMDS (0.835mL, 1.3M in THF) and THF (3mL) was added a solution of **12** (200 mg, 493 mmol) in THF (3 mL) at –40 °C under a nitrogen atmosphere. The mixture was

stirred for 20 min at –40 °C and at 0 °C for 30 min, and then saturated aqueous NH_4Cl (30 mL) and EtOAc (30 mL) were added. The resulting mixture was stirred for 30 min at ambient temperature, then $NaBH_4$ (224 mg, 594 mmol) was added, and the reaction mixture was stirred for another 10 h at ambient temperature. The organic solution was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 33:67 to yield **13** as a colorless oil (98.0 mg, diastereomixture ca 4 : 1).

*NMR data of diastereomixture **13**: ^{1}H NMR ($CDCl_3$): δ 1.45 (9H, s), 1.53 (3H, d, J = 7.1 Hz), 2.82 (0.8H, d. J = 10.8 Hz), 2.89 (0.2H, d, J = 5.4 Hz), 2.97 (0.8H, d, J = 10.7 Hz), 3.08 (0.8H, s), 3.30-3.25 (1.4H, m), 3.45 (0.8H, d, J = 12.4 Hz), 3.83 (0.2H, m), 3.92 (0.8H, J = 12.2 Hz), 3.95–4.02 (0.2H, m), 4.06–4.13 (3H, m), 5.52 (1H, q, J = 7.2 Hz), 7.26–7.38 (5H, m). MS (ESI) *m/z*: 362 $(M + H)^+$.

To a solution of **13** and TEA (0.056 mL, 407 mmol) in CH_2Cl_2 (4 mL) was added methanesulfonyl chloride (0.030 mL, 352 mmol) at –10 °C under a nitrogen atmosphere. After stirring for 30 min, MeOH (0.030 mL) was added, and the reaction mixture stirred for 10 min at ambient temperature. 1,8-Diazabicyclo[5.4.0]-7-undecene (0.202 mL, 1.36 mmol) was then added, and the resultant mixture was stirred for 10 h at 40 °C. Afterwards, 10 wt% citric acid aqueous solution and EtOAc were added. The organic solution was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude was purified by silica gel column chromatography, eluting with hexane/EtOAc = 33:67 to yield **8** (60.5 mg, 36 % from **12**) as a colorless solid, mp 132–134 °C.^{1}H NMR (CDCl3): δ 1.27 (9H, s), 1.51 (3H, d, J = 7.1 Hz), 3.13 (1H, d, J = 10.0 Hz), 3.21 (1H, d, J = 10.0 Hz), 3.26 (1H, d, J = 10.3 Hz), 4.24 (1H, dd, J = 18.6, 2.4 Hz), 4.45–4.50 (2H, m), 5.57 (1H, d, J = 7.2 Hz), 6.60 (1H, t, J = 2.3 Hz), 7.32–7.29

(5H, m). MS (ESI) *m/z*: 344 $(M + H)^+$. $[\alpha]_D^{25}$ = –4.124° (*c* 1.0, $CHCl_3$). Anal. calcd for $C_{20}H_{25}NO_4$-0.25·H_2O: C 69.04, H 7.39, N 4.03; found, C 69.01, H 7.42, N 4.03.

***tert*-Butyl 3-(Hydroxymethyl)-5-oxo-1-[(1*R*)-1-phenylethyl]pyrrolidine-3-carboxylate (10).** To a solution of **9** (4.0g, 13.8 mmol) in DMF (40 mL) were added paraformaldehyde (0.83 g, 27.7 mmol) and NaH (60% in oil, 0.60 g, 13.8 mmol) at ambient temperature. After stirring for 0.5 h, the mixture was poured into 10% citric acid aqueous solution at 5 °C. After dilution with EtOAc, the organic solution was washed with water (3×) and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 1:1 to yield **10** (1.0 g, 23%) as a colorless solid, mp 88–90 °C. Another stereoisomer was obtained as a mixture with an impurity. ^{1}H NMR ($CDCl_3$): δ 1.35 (9H, s), 1.53 (3H, d, *J* = 7.3 Hz), 2.40 (1H, d, *J* = 17.3 Hz), 2.51 (1H, dd, *J* = 7.8, 5.4 Hz), 2.78 (1H, d, *J* = 17.1 Hz), 3.21 (1H, d, *J* = 10.3 Hz), 3.39 (1H, d, *J* = 10.5 Hz), 3.61 (1H, dd, *J* = 11.2, 7.8 Hz), 3.77 (1H, dd, *J* = 11.2, 5.4 Hz), 5.51 (1H, q, *J* = 7.2 Hz), 7.28–7.34 (5H, m). MS (ESI) *m/z*: 320 $(M + H)^+$. $[\alpha]_D^{25}$ = 95.350° (*c* 1.0, $CHCl_3$). HRMS (ESI) calcd for $C_{18}H_{26}NO_4$: 320.1862. Found: 320.1860.; Anal. calcd for $C_{18}H_{25}NO_4$, C 67.69, H 7.89, N 4.39; found, C 67.34, H 7.82, N 4.47.

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

To a solution of **10** (165 mg, 0.516 mmol) in THF (10 mL) were added diphenyl disulfide (169 mg, 0.775 mmol) and Bu_3P (0.26 mL, 1.03 mmol) at ambient temperature. The mixture was stirred at 65 °C for 15 h. The reaction mixture was allowed to cool to ambient temperature, and diluted with EtOAc; then, the organic solution was washed with 1N aqueous NaOH solution, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The resultant residue was purified by

silica gel column chromatography, eluting with hexane/EtOAc = 3:7. To a solution of resultant residue (31 mg) in EtOH (6 mL) was added Raney nickel (about 50%, 1 mL) and the mixture was stirred at ambient temperature for 6 h under a hydrogen atmosphere. The resultant suspension was filtered through Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 2:1 to yield **11** (16.4 mg, 12% from **10**) as a colorless solid. The ^{1}H-NMR spectrum data of **11** corresponded with the reported data.[20]

^{1}H NMR ($CDCl_3$): δ ppm: 1.34 (12H, s), 1.52 (3H, d, J = 7.10 Hz), 2.27 (1H, d, J = 17.0 Hz), 2.93 (1H, d, J = 17.0 Hz), 3.05 (1H, d, J = 10.1 Hz), 3.32 (1H, d, J = 10.1 Hz), 5.50 (1H, q, J = 7.1 Hz), 7.23-7.38 (5H, m).

***tert*-Butyl (3*S*)-3-[(2-Ethoxy-2-oxoethoxy)methyl]-5-oxo-1-[(*1R*)-1-phenylethyl]pyrrolidine-3-carboxylate (12)**

To a solution of **10** (10.0 g, 31.3 mmol), ethyl bromoacetate (7.8 g, 47.0 mmol), and tetra-*n*-butylammonium iodide (0.58 g, 1.6 mmol) in THF (300mL) was added NaH (60% in oil, 1.6 g, 38 mmol) at 0 °C. The mixture was stirred for 0.5 h at 0 °C and then at ambient temperature for 18 h. After the reaction mixture was diluted with EtOAc, the organic solution was washed with saturated aqueous NH_4Cl and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 4:6 to yield **12** (10.6 g, 84%) as a colorless oil.^{1}H NMR ($CDCl_3$): δ 1.28 (3H, t, J = 7.2 Hz), 1.35 (9H, s), 1.53 (3H, d, J = 7.3 Hz), 2.56 (1H, d, J = 17.1 Hz), 2.80 (1H, d, J = 17.1 Hz), 3.31 (1H, d, J = 10.2 Hz), 3.46 (1H, d, J = 10.2Hz), 3.68 (2H, brs), 4.08 (2H, s), 4.21 (2H, q, J = 7.2Hz), 5.49 (1H, q, J = 7.0 Hz), 7.28–7.34 (5H, m). MS (ESI) *m/z*: 406

$(M + H)^+$. HRMS (ESI) calcd for $C_{22}H_{32}NO_6$: 406.2229. Found: 406.2240. $[\alpha]_D^{25}$ = 56.560° (*c* 0.531, $CHCl_3$).

tert-Butyl (3a*S*,7a*S*)-1-Oxo-2-[(1*R*)-1-phenylethyl]hexahydropyrano[3,4-*c*]pyrrole-3a(4*H*)-carboxylate (14) and _tert_-Butyl (3a*S*,7a*R*)-1-Oxo-2-[(1*R*)-1-phenylethyl]hexahydropyrano[3,4-*c*]pyrrole-3a(4*H*)-carboxylate (15)

To a solution of **8** (231 mg, 0.67 mmol) in EtOAc (12 mL) was added 10% Pd/C (50% wet, 116 mg), and the mixture was stirred for 4 h under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 4:1–1:1 to yield **14** (183 mg, 79%) and **15** (48.5 mg, 21%) as colorless solids, respectively.

Compound **14**: m.p. 88–90 °C. ^{1}H NMR ($CDCl_3$): δ 1.41 (9H, s), 1.53 (3H, d, *J* = 7.1 Hz), 2.01–2.03 (2H, m), 2.91 (2H, dd, *J* = 18.3, 10.2 Hz), 3.02 (1h, t, *J* = 4.6 Hz), 3.29 (1H, d, *J* = 11.7 Hz), 3.39 (1H, m), 3.77–3.82 (1H, m), 4.12 (1H, *J* = 11.7 Hz), 5.54 (1H, d, *J* = 7.1 Hz), 7.26–7.36 (5H, m). ^{13}C NMR ($CDCl_3$): δ 16.00, 21.80, 27.89, 41.44, 45.67, 45.89, 49.06, 65.66, 70.23, 82.09, 127.1, 127.7, 128.7, 139.4, 171.9, 173.0. MS (ESI) *m/z*: 346$(M + H)^+$. $[\alpha]_D^{25}$ = 106.722° (*c* 1.0, $CHCl_3$). Anal. calcd for $C_{20}H_{27}NO_4$, C 69.54, H 7.88, N 4.06; found, C 69.30, H 7.84, N 4.08.

Compound **15**: m.p. 183–185 °C. ^{1}H NMR ($CDCl_3$): δ 1.23 (9H, s), 1.46 (3H, d, *J* = 7.3 Hz), 1.92–1.96 (1H, m), 2.19–2.28 (2H, m), 3.08 (1H, d, *J* = 10.0 Hz), 3.17 (1H, d, *J* = 9.8 Hz), 3.33–3.38 (2H, m), 4.10 (1H, dd, *J* = 11.0, 4.4 Hz), 5.48 (1H, d, *J* = 7.2 Hz), 7.24–7.29 (5H, m). ^{13}C NMR ($CDCl_3$): δ 15.98, 23.07, 27.67, 47.12, 48.45, 48.99, 50.40, 68.16, 73.61, 81.78, 127.4,

127.5, 128.5, 139.9, 170.6, 172.7. MS (ESI) *m/z*: 346(M + H)$^+$. $[\alpha]_D^{25}$ = 83.118° (*c* 1.0, $CHCl_3$). Anal. calcd for $C_{20}H_{27}NO_4$, C 69.54, H 7.88, N 4.06; found, C 69.40, H 7.91, N 4.10.

X-ray Crystallographic Analysis of 14. A colorless prism-shaped crystal was formed from Et_2O: $C_{20}H_{27}NO_4$; FW = 345.44; sample dimensions, 0.38 mm × 0.30 mm × 0.20 mm. The lattice parameters and intensities were measured on a Rigaku AFC7R diffractometer (Cu*K*α radiation, λ = 1.54178 Å, graphite monochromator, ω–2θ scans, $2\theta_{max}$ = 120.1°); monoclinic, space group $P2_1$(#4); a = 6.486(1), b = 10.763(3), c = 13.994(2), V = 970.8(3)Å^3, Z = 2; D_{calcd} = 1.18 g/cm^3; F_{000} = 372; μ = 6.62 cm^{-1}. The structure was solved by direct methods with the program Sir92.[33] The final cycle of full-matrix least-squares refinement was based on 1684 observed reflections and 254 variable parameters and converged at R = 0.086 (Rw = 0.161).

These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?, CCDC1834361.

Improved method Using $MgBr_2$ (Table 1, entry 6)

To a solution of **8** (400 mg, 1.16 mmol) in EtOAc (7 mL) and EtOH (3.5 mL) was added $MgBr_2$ (225 mg, 1.22 mmol). The mixture was stirred until a clear solution was obtained. The resultant solution was introduced in a stainless-steel autoclave, and 10% Pd/C (50%wet, 200 mg) was added. The autoclave was closed, purged with hydrogen gas, and then pressurized with hydrogen up to 30 atm. After stirring the mixture at ambient temperature for 14 h, the pressure was released and water (3.5 mL) was added to the reaction solution. After stirring for 15 min, the catalyst was removed by filtration and washed with EtOAc. The filtrate was washed with 1N

aqueous NaOH and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To the resultant solid were added diisopropyl ether (3 mL) and *n*-hexane (2 mL), and the mixture was stirred for 15 min. The mixture was filtered to yield pure **15** (357 mg, 89%) as a colorless solid.

2-Benzyl 3a-*tert*-Butyl (3a*S*,7a*S*)-tetrahydropyrano[3,4-*c*]pyrrole-2,3a(3*H*,4*H*)-dicarboxylate (16)

To a solution of **14** (3.36 g, 9.73 mmol) in THF (50 mL) was added BH_3-THF complex (48.6 mL, 1 M in THF) at ambient temperature under a nitrogen atmosphere. After stirring for 3 days, EtOH (27 mL), water (3 mL), and TEA (3 mL) were added, and the reaction mixture was stirred at 80 °C for 2 h. After evaporation, the residue was diluted with EtOAc. The organic solution was washed with saturated aqueous NH_4Cl, water, and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by on a short silica gel column, eluting with hexane/EtOAc = 65:35.

The residue was dissolved in 1,2-dichloroethane (18 mL) and benzyloxycarbonyl chloride (3.40 g, 19.9 mmol) was added. The mixture was stirred at 40 °C for 24 h. The mixture was purified by silica gel column chromatography, eluting with hexane/EtOAc = 65:35 to yield **16** (2.39 g, 68%) as a colorless oil.^{1}H NMR ($CDCl_3$): δ 1.44 (9H, s), 1.80–1.93 (1H, m), 2.70–2.81 (1H, m), 3.33–3.41 (1H, m), 3.48 (1H, t, J = 10.5 Hz), 3.54–3.77 (6H, m), 3.88 (1H, dd, J = 20.6, 11.8 Hz), 5.14 (2H, s), 7.24–7.39 (5H, m). MS (ESI) *m/z*: 384 $(M + Na)^+$. HRMS (ESI) calcd for $C_{20}H_{28}NO_5$: 362.1969. Found: 362.1985. $[\alpha]_D^{25}$ = 21.676° (*c* 0.95, $CHCl_3$).

Benzyl (3a*S*,7a*R*)-3a-[(*tert*-Butoxycarbonyl)amino]hexahydropyrano[3,4-*c*]pyrrole-2(3*H*)-carboxylate (17)

To a solution of **16** (2.30 g, 6.94 mmol) in CH_2Cl_2 (20 mL) was added TFA (10 mL). The mixture was stirred for 24 h at ambient temperature. After evaporation, to the residue was added 1N aqueous NaOH. The aqueous solution was washed with $CHCl_3$, and acidified by adding concentrated aqueous HCl. After extraction from $CHCl_3$ (2×), the organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was dissolved in CH_3CN (40 mL) and 1,1'-carbonylbis-1*H*-imidazole (1.55g, 9.53 mmol) was added at 0 °C under a nitrogen atmosphere. After stirring for 1 h, NH_3 gas was bubbled into the solution for 30 min. The reaction solution was then diluted with EtOAc; the organic layer washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by short silica gel column chromatography, eluting with EtOAc. The resultant residue was dissolved in *tert*-BuOH (50 mL) and lead (IV) acetate (3.98 g, 8.97 mmol) was added at ambient temperature under a nitrogen atmosphere. Then, the reaction mixture was warmed up to 80 °C and stirred for 1 h. To the solution were added Na_2CO_3 (3.52 g, 41.9 mmol) and EtOAc. After filtration through Celite, the filtrate was washed with saturated aqueous Na_2CO_3 and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography, eluting with hexane/EtOAc = 70:30 to yield **17** (1.70 g, 65%) as a colorless oil.^{1}H NMR ($CDCl_3$): δ 1.43 (9H, s), 1.50–1.54 (1H, m), 1.79–1.87 (1H, m), 2.55–2.63 (1H, m), 3.34–3.38 (1H, m), 3.57–3.86 (7H, m), 4.66 (1H, d, J = 7.6 Hz), 5.13 (2H, s), 7.31–7.36 (5H, m). MS (ESI) *m/z*: 399 $(M + Na)^+$. $[\alpha]_D^{25}$ = 36.895° (*c* 1.00, $CHCl_3$). HRMS (ESI) calcd for $C_{20}H_{28}NO_5$: 377.2078. Found: 377.2099.

7-[(3a*S*,7a*R*)-3a-Aminohexahydropyrano[3,4-*c*]pyrrol-2(3*H*)-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (19)

To a solution of **17** (401 mg, 1.07 mmol) in MeOH (20 mL) was added 10% Pd/C (200 mg, containing 50% water), and the mixture was stirred for 2.5 h at ambient temperature under a hydrogen atmosphere. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue containing crude **18** was mixed with **7** (252 mg, 1.04 mmol), TEA (0.433 mL, 3.12 mmol), and DMSO (8 mL), and the mixture was stirred for 17 h at 40 °C. To this solution were added 90% aqueous EtOH (30 mL) and TEA (3 mL), and the resultant mixture was heated to reflux for 5 h, and then concentrated *in vacuo* to give a residue, which was diluted with EtOAc. The organic solution was washed with 10% aqueous citric acid solution, water (2×) and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. To the residue was added 4 mL of concentrated aqueous HCl at 0 °C, and the mixture was stirred for 20 min at ambient temperature. The aqueous solution was washed with $CHCl_3$ and made alkaline with saturated aqueous NaOH at 0 °C. The pH of the solution was adjusted to 7.4 with concentrated aqueous HCl and then with diluted aqueous HCl. The resultant solution was extracted with $CHCl_3$ (3×). The combined organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant solid was washed with EtOH and Et_2O to yield **19** (302 mg, 67% from 17) as a pale yellow powder, mp 132–134 °C. ^{1}H NMR (0.1 N NaOD/D_2O): δ 1.47–1.57 (3H, m), 1.91–1.93 (1H, m), 2.20–2.30 (1H, m), 3.37 (1H, d, J = 11.0 Hz), 3.51 (d, J = 11.0 Hz), 3.59 (3H, s), 3.62–3.68 (2H, m), 3.82 (2H, t, J = 11.2 Hz), 3.88–3.91 (1H, m), 4.02–4.04 (2H, m), 4.99 (d, J = 63.9 Hz), 7.66 (1H, d, J = 14.6 Hz), 8.41 (1H, s). ^{13}C NMR (0.1N NaOD/D_2O): δ 14.16, 14.25, 25.03, 39.82, 39.89, 41.04, 54.09, 54.16, 54.81, 58.02, 58.06, 60.66, 65.21, 70.16, 71.63, 73.38, 106.4, 106.6, 116.4, 119.4, 119.5, 134.2, 135.9, 136.0, 140.1, 149.4, 152.0, 154.9, 168.0, 170.7, 172.4, 175.0. MS (ESI) *m/z*: 436$(M + H)^+$. $[\alpha]_D^{25}$ = –45.410° (*c* 0.16, 0.1N aqueous

NaOH). Anal. calcd for $C_{21}H_{23}F_2N_3O_5 \cdot 2H_2O$, C 53.50, H 5.77, F 8.06, N 8.91; found, C 53.59, H 5.70, F 8.02, N 8.79.

2-Benzyl 3a-*tert*-Butyl (3a*S*,7a*R*)-tetrahydropyrano[3,4-*c*]pyrrole-2,3a(3*H*,4*H*)-dicarboxylate (20)

To a solution of **15** (12.1 g, 35.1 mmol) in THF (180 mL) was added BH_3-THF complex (105 mL, 1M in THF) at ambient temperature under a nitrogen atmosphere. After stirring for 3 days at 40 °C, EtOH (135 mL), water (15 mL), and TEA (15 mL) were added and the resulting solution was stirred at 80 °C for 4 h. After evaporation, the residue was diluted with EtOAc, and the organic layer was washed with saturated aqueous NH_4Cl, water, and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resultant residue was purified by short silica gel column chromatography, eluting with hexane/EtOAc = 65:35. The residue was dissolved in 1,2-dichloroethane (70 mL) and benzyloxycarbonyl chloride (18.0 g, 105 mmol) was added. The mixture was stirred at 35 °C for 3 h. The mixture was purified by silica gel column chromatography, eluting with hexane/EtOAc = 60:40 to yield **20** (10.4 g, 82%) as a colorless solid, m.p. 119-121°C. ^{1}H NMR ($CDCl_3$): δ 1.44 (9H, s), 1.60–1.63 (1H, m), 1.96–1.98 (1H, m), 2.21–2.24 (1H, m), 3.07 (1H, dd, J = 11.0, 3.4 Hz), 3.22 (1H, dd, J = 10.5, 6.8 Hz), 3.38 (1H, td, J = 11.7, 2.8 Hz), 3.52–3.62 (2H, m), 3.77 (1H, dd, J = 24.1, 11.1 Hz), 4.07–4.10 (1H, m), 4.48 (1H, dd, J = 20.8, 10.5 Hz), 5.13 (2H, d, J = 3.4 Hz), 7.28–7.33 (5H, m). MS (ESI) *m/z*: 384 $(M + Na)^+$. $[\alpha]_D^{25}$ = 36.170° (*c* 1.00, $CHCl_3$). Anal. calcd for $C_{20}H_{27}NO_5 \cdot 0.25H_2O$, C 65.64, H 7.57, N 3.83; found, C 65.78, H 7.50, N 3.97.

Benzyl (3a*S*,7a*S*)-3a-[(*tert*-Butoxycarbonyl)amino]hexahydropyrano[3,4-*c*]pyrrole-2(3*H*)-carboxylate (21)

To a solution of **20** (725 mg, 2.01 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL). The mixture was stirred for 15 h at ambient temperature. After evaporation, 1N aqueous NaOH was added to the residue. The aqueous solution was washed with $CHCl_3$, and acidified by adding concentrated aqueous HCl. After extraction with $CHCl_3$ (2×), the organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was dissolved in toluene (20 mL) and TEA (406 mg, 4.01 mmol) and diphenylphosphorylazide (740 mg, 2.61 mmol) were added to the solution at ambient temperature under a nitrogen atmosphere. After stirring for 1.5 h at 110 °C, the solution was concentrated *in vacuo*, the resultant residue was dissolved in 1,4-dioxane (20 mL) and 6N aqueous HCl (20 mL) was added. The mixture was stirred for 2 h at 50 °C. After evaporation, the residue was made alkaline by adding saturated aqueous NaOH at 0 °C. After extraction using $CHCl_3$ (2×), the organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Boc_2O (2.19 g, 10.1 mmol) was added to the resultant residue, and the mixture was stirred for 1.5 h at 50 °C and purified by silica gel column chromatography, eluting with hexane/EtOAc = 40:60 to yield **21** (623 mg, 83%) as a colorless solid, m.p. 106-108 °C. ^{1}H NMR ($CDCl_3$): δ 1.43 (9H, s), 1.62–1.69 (2H, m), 1.97–2.02 (1H, m), 3.09–3.16 (3H, m), 3.34–3.36 (1H, m), 3.62–3.70 (1H, m), 4.08–4.13 (1H, m), 4.25 (1H, t, J = 12.4 Hz), 4.45 (1H. d. J = 10.7 Hz), 4.55 (1H, dd, J = 19.5, 11.2 Hz), 5.13 (2H, s), 7.26–7.52 (5H, m). MS (ESI) *m/z*: 399 $(M + Na)^+$. $[\alpha]_D^{25}$ = 55.467° (c 1.0, $CHCl_3$). Anal. calcd for $C_{20}H_{28}N_2O_5$: C 63.81, H 7.50, N 7.44; found: C 63.76, H 7.48, N 7.43.

Metabolic Stability. Metabolic stability and permeability were measured as previously described.[34]

Protein Binding. Protein binding was measured as previously described.[35]

MBI Assay. Mechanism based inactivation against CYP3A4 is estimated as the percentage of the enzymatic activity (1′-hydroxylation of midazolam) remaining, after the 30 min preincubation of the test compounds in pooled human liver microsomes.[36]

Convulsion Incidence test in Mice. BPAA suspended in 0.5% CMC was orally administered to male Slc:ddY mice (4 weeks) at 400 mg/kg (10 mL/kg), then each drug solution or 0.5% lactic acid solution as a vehicle was intra-cisternally administered to animals at 5, 15, or 50 μg/5 μL/mouse 30 minutes after the administration of BPAA. Just after the dosing of each compound, animal conditions were observed for 30 minutes.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge. X-Ray data of compound **14** and IR spectrum data.

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Author Contributions

T.O. mainly contributed to this work. The project was designed by H.T. with the help of all authors. The manuscript was written by T.O. through contributions from all authors. T.O. carried out the synthesis of compound **5**. H.I., M.N., T.K. and S.K. each gained important hints to

achieve the synthesis of compound **5**. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

TEA, triethylamine;

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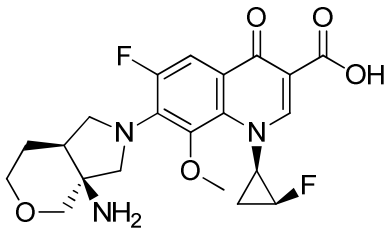
Table of Contents graphic



5 (DS21412020)

MIC (ug/mL)
S.Pneumoniae J41 : 0.05
MRSA 870307 : 0.2

hERG : negative
MBI : negative