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Synthesis and antibacterial activity of novel 6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-4-oxoquinoline-3-carboxylic acids bearing cyclopropane-fused 2-amino-8-azabicyclo[4.3.0]nonan-8-yl substituents at the C-7 position

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Abstract—A series of novel 6-fluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-4-oxoquinoline-3-carboxylic acids bearing cyclopropane-fused 2-amino-8-azabicyclo[4.3.0]nonan-8-yl substituents at the C-7 position were synthesized to obtain potent drugs for the treatment of Gram-positive infections. Some compounds exhibited excellent antibacterial activity, and potent inhibitory activity against bacterial DNA topoisomerase IV. In addition, some of the potent compounds showed reduced inhibitory activity against human DNA topoisomerase II compared with the corresponding noncyclopropane-fused compounds.

Multidrug-resistant Gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *S. pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE), have become a serious problem in the medical community.[1] Some antibacterial agents (linezolid,[2] teicoplanin,[3] quinupristin/dalfopristin,[4] etc.) are now available in clinical use as agents for the treatment of infections caused by those resistant bacteria, but they show some problems, for example, resistant mutations and/or side effects.[1d,e,5] Fluoroquinolone antibacterial agents demonstrate good clinical efficacy, which is considered to result from their excellent in vitro antibacterial activity, rapid bactericidal activity, good tissue distribution, etc., and are therefore expected to be developed as agents for such resistant Gram-positive infections. Some newer agents, such as gatifloxacin,[6] moxifloxacin,[7] and gemifloxacin,[8] have been developed over the last several years as agents that are active against such resistant pathogens as *S. pneumoniae*. Their activity against MRSA or VRE are not so potent, however, and considering that the incidence of Gram-positive bacterial resistance to antibacterial agents has been growing, their activity even against *S. pneumoniae* are considered not to be potent enough, and bacteria resistant to those agents will be problematic in the near future.[9,10] For that reason, we focused on exploring new quinolone compounds that are more potent against such resistant Gram-positive bacteria.

Among quinolone antibacterial agents, substituents at the C-7 position greatly influence their potency, spectrum, and safety.[11] Shionogi group reported a series of 7-(2-amino-8-azabicyclo-[4.3.0]nonan-8-yl) quinolone compounds (**1**, Fig. 1) that exhibited very potent antibacterial activity against Gram-positive bacteria.[12] Meanwhile, these derivatives were reported to show strong genotoxicity,[13] which was thought to result from their low enzyme selectivity, that is, between inhibition against bacterial DNA gyrase, or DNA topoisomerase IV and against its mammalian counter-part, topoisomerase II. To reduce the genotoxicity of the derivatives while maintaining the strong activity against Gram-positive bacteria, we designed novel quinolone derivatives that have cyclopropane-fused 2-amino-8-azabicyclo[4.3.0]nonan-8-yl C-7 substituents (**2** and **3**, Fig. 1). The fused cyclopropane can give some rigidity to the

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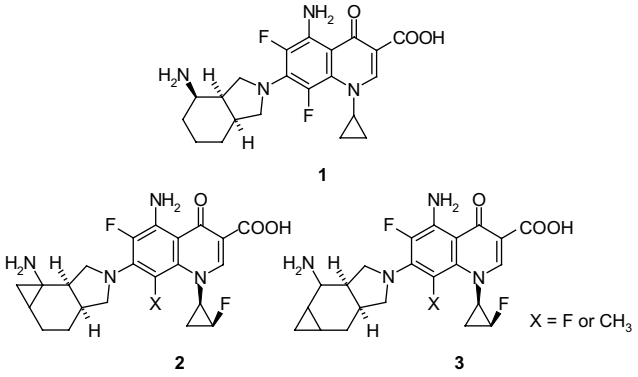
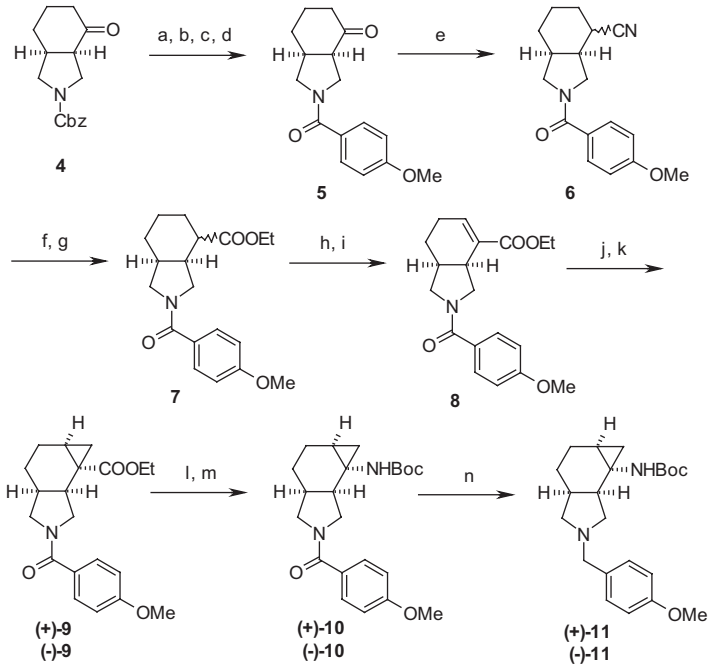


Figure 1. A prototype compound and designed compounds.

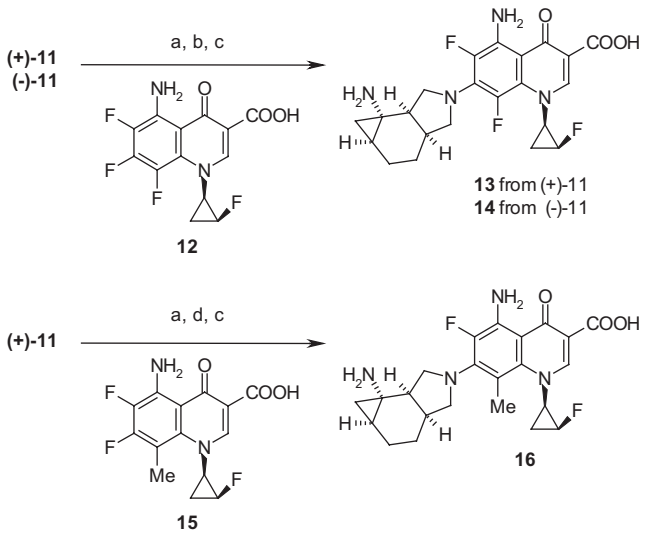
bicyclo[4.3.0]nonane system, and the rigidity is thought to achieve higher enzyme selectivity. In addition to the C-7 substituents, we also utilized a 5-amino-6,8-difluoro-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl] quinolone nucleus or a 5-amino-6-fluoro-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methyl quinolone nucleus, both of which are expected to show potent antibacterial activity against Gram-positive bacteria and to show low genotoxicity.[14]

The C-7 substituent of **2** was synthesized from the 8-azabicyclo[4.3.0]nonan-2-one derivative (**4**) described in Ortho's patent (Scheme 1).[15] The Cbz group of **4** was converted to a 4-methoxybenzoyl group (**5**), and the ketone function was converted to a nitrile function by using tosylmethyl isocyanide (**6**). After the nitrile function was hydrolyzed and converted to an ethyl ester function, the ester derivative **7** was converted to an α,β-unsaturated ester derivative **8** via a phenylselenyl compound. Cyclopropanation of the electron-deficient olefin group of **8** by using trimethylsulfoxonium salt and sodium hydride gave exclusively one diastereomer **9**, and the relative configuration of **9** was determined to be (1*S**,2*R**,6*S**,9*S**) by X-ray crystallographic analysis. The racemic **9** was separated to the two enantiomers (+)-**9** and (−)-**9** by column chromatography with CHIRALPAK AD. The ester groups of (+)-**9** and (−)-**9** were hydrolyzed and converted to *tert*-butoxycarbonylamino groups by the Curtius rearrangement reaction using diphenylphosphoryl azide ((+)-**10** and (−)-**10**). The 4-methoxybenzoyl groups of the resultant compounds were deprotected by two step reactions (reduction with lithium aluminum hydride and catalytic hydrogenation), and the resultant secondary amines were reacted with 5-amino-6,7,8-trifluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**12**)[14a] followed by deprotection of Boc groups to give the target molecules **13** and **14**. Because **13** showed more potent antibacterial activity than **14**, the corresponding amine, synthesized from (+)-**9**, was introduced to the other quinolone nucleus, 5-amino-6,7-difluoro-1-[(1*R*,2*S*)-2-fluoro-cyclopropan-1-yl]-8-methyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**15**) to give **16** (Scheme 2).

The C-7 substituent of **3** was synthesized from 1-trimethylsiloxy-1,3-butadiene (**17**) and *N*-benzyl-maleimide (**18**) (Scheme 3). The Dields–Alder reaction



Scheme 1. Reagents and conditions: (a) ethylenegylcol, cat TsOH, heat; (b) H_2, Pd–C/MeOH; (c) *p*-methoxybenzoylchloride, 2 N NaOH aq/THF; (d) 80% AcOH aq/THF, heat (four steps, 70%); (e) tosylmethyl isocyanide, *t*-BuOK/DME, *t*-BuOH; (f) NaOH aq/EtOH, heat; (g) concd H_2SO_4/EtOH, heat (three steps, 48%); (h) LDA, $Zr(C_5H_5)_2Cl_2$/THF; PhSeBr (99%); (i) *m*CPBA/CH_2Cl_2 (86%); (j) trimethylsulfoxonium iodide, NaH/DMSO (75%); (k) separation of enantiomers (CHIRALPAK AD); (l) NaOH aq/THF, MeOH; (m) DPPA, Et_3N/toluene, *t*-BuOH, heat (two steps, (+)-**10**: 90%, (−)-**10**: 82%); (n) LAH/THF ((+)-**11**: 84%, (−)-**11**: 79%).



Scheme 2. Reagents and conditions: (a) H_2, $Pd(OH)_2$–C/MeOH; (b) Et_3N/MeCN, heat; (c) concd HCl aq; (d) Et_3N/DMSO, heat (three steps, **13**: 70%, **14**: 26%, **16**: 49%).

between these two compounds gave **19**. Cyclopropanation of the olefin part of **19** was performed by diazomethane in the presence of a catalytic amount of palladium acetate. After the trimethylsilyl group of the resultant product was removed, the obtained hydroxyimide derivative **20** was separated to the two enantiomers (+)-**20** and (−)-**20** by column chromatography with CHIRALCEL OD. Reduction of the imide functions of (+)-**20** and (−)-**20** with lithium aluminum hydride and oxidation of the hydroxy groups provided ketone derivatives, which were converted to methoxyimino derivatives **21a** and **21b**, respectively by treatment with *O*-methylhydroxylamine. The methoxyimino functions of **21a** and **21b** were reduced to amine functions by a borane tetrahydrofuran complex, and the amine functions were then protected with Boc groups to give **22a** and **22b** as diastereomixtures. After the benzyl protecting groups of **22a** and **22b** were converted to Cbz groups by the von Braun conditions, each of the diastereomixtures was separated to the two epimers by column chromatography to afford (+)-**23a** and (+)-**23b** from (+)-**22**, (−)-**23a** and (−)-**23b** from (−)-**22**, respectively. The relative configurations of these four compounds were assigned on the basis of NOE experiments, that is, (+)-**23a** and (−)-**23a** were (1*R**,2*S**,3*S**,7*R**,9*S**), and (+)-**23b** and (−)-**23b** were (1*R**,2*R**,3*S**,7*R**,9*S**). After deprotection of Cbz groups, these four C-7 substituents were introduced to the quinolone nucleus **12** to afford the target molecules **24**, **25**, **26**, and **27**.

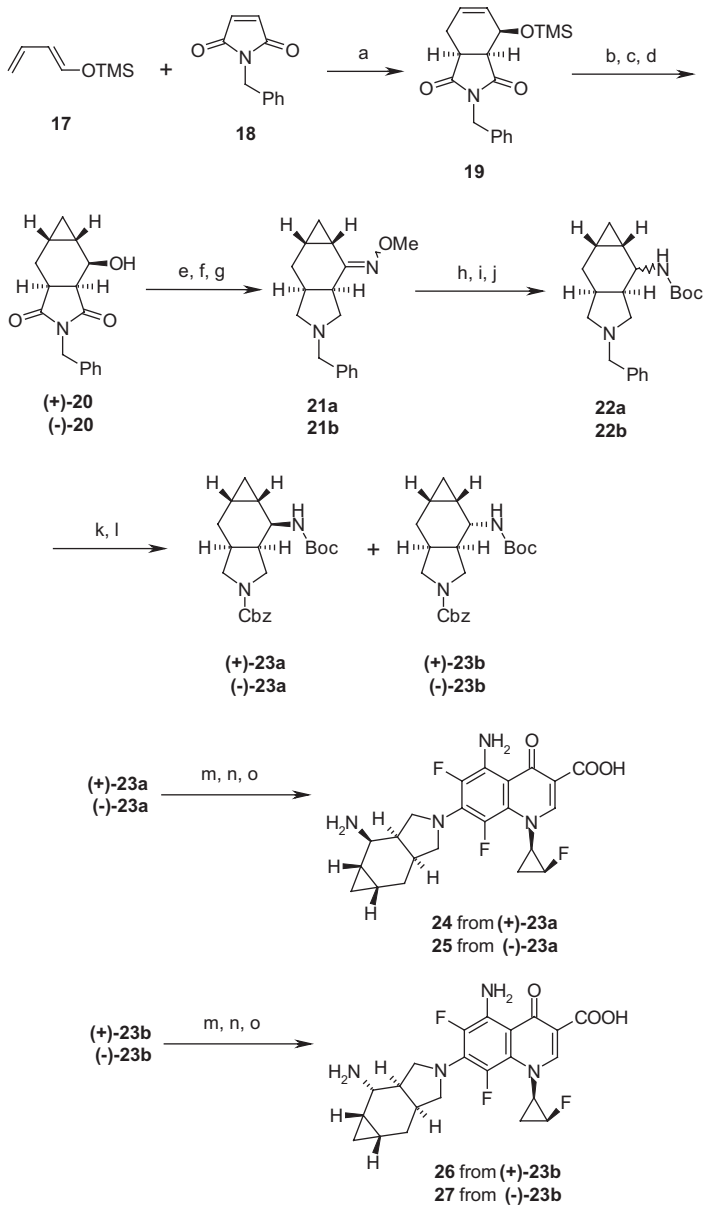
To prepare reference compounds, the C-7 substituent of **1**, reported by Shionogi group,[12] was separated to the two enantiomers. And they were introduced to quinolone nucleus **12** or **15** to give **28**, **29**, and **30** (Fig. 2).

The minimum inhibitory concentrations (MICs) of the synthesized compounds **13**, **14**, **16**, and **24**–**30** against several representative bacteria are listed in Table 1, along with the data for DQ-113,[14f] levofloxacin (LVFX), moxifloxacin (MFLX), vancomycin (VCM), and linezolid (LZD) for comparison.

All of the synthesized compounds exhibited potent antibacterial activity against each species of bacteria, not only against Gram-positive bacteria but also against Gram-negative bacteria. And especially, the compounds synthesized from the dextrorotatory C-7 substituent **13**, **16**, **24**, and **26** exhibited very potent antibacterial activity. The activity of these compounds was comparable with that of DQ-113 and superior to those of clinically available LVFX, MFLX, VCM, or LZD. And the activity was superior to those of the compounds **14**, **25** and **27**, which were synthesized from the levorotatory C-7 substituents. The same tendency was observed with the reference compounds **28** and **30**. In addition, the antibacterial activities of **13**, **16**, **24**, and **26** were comparable with those of **28** and **30**, and the fused cyclopropane rings did not substantially influence the antibacterial activity. Concerning the configurations of the peripheral amino functions (C-2 position in the C-7 substituent) of compounds **24**–**27**, **24**, and **26** showed almost the same activity although **25** showed about 8 times more potent activity than **27**.

The inhibitory activities against bacterial topoisomerase IV[16] and human topoisomerase II[17] of compounds **13**, **16**, **24**, **26**, **28**, and **30** were summarized in Table 2, along with the data for DQ-113, which had very potent antibacterial activity against Gram-positive bacteria and diminished genotoxicity.[14f] Compounds **13**, **16**, **26**, and **30** exhibited potent inhibitory activity against *S. aureus* topoisomerase IV comparable with that of DQ-113, and the enzyme inhibitory activities were consistent with their respective antibacterial activity. Compounds **24** and **28** demonstrated about the same antibacterial activity as did **13**, **16**, **26**, **30**, and DQ-113 against *S. aureus* FDA 209P, but their inhibitory activity against *S. aureus* topoisomerase IV was about twice more potent. In addition, the 5-amino-8-fluoro derivatives **13** and **28** showed more potent activity than the corresponding 5-amino-8-methyl derivatives **16** and **30** against *S. aureus* topoisomerase IV.

Concerning the inhibitory activity against human topoisomerase II, the cyclopropane-fused compounds **13**, **16**, and **24**, other than **26**, demonstrated inhibitory activity less than those of the reference compounds **28** and **30**, which do not have fused cyclopropane rings. But the activity was more than that of DQ-113. These results indicate that the cyclopropane fusing strategy, which give some rigidity to the C-7 substituents, could be effective for reducing the genotoxicity. Interestingly, compound **26** showed potent inhibitory activity against human topoisomerase II comparable with those of **28** and **30**. This activity was about three times higher than that of the epimer **24**, although **26** was less active than **24** against *S. aureus* topoisomerase IV. Furthermore, the selectivity between topoisomerase IV and topoisomerase II of compound **24** was about 150, and was comparable with that of DQ-113, which exhibited no genotoxicity even in vivo. These results imply that the configuration of the peripheral amino groups could



Scheme 3. Reagents and conditions: (a) toluene, heat; (b) CH_2N_2, cat $Pd(OAc)_2/Et_2O$; (c) HCl aq/MeOH (three steps, 85%); (d) separation of enantiomers (CHIRALCEL OD); (e) LAH/THF; (f) $(COCl)_2$, DMSO/CH_2Cl_2; Et_3N; (g) $MeONH_2$-HCl/pyridine (three steps, **21a**: 78%, **21b**: 83%); (h) BH_3-THF/THF; (i) Et_3N/aq EtOH, heat; (j) Boc_2O, $NaHCO_3$/THF, H_2O (three steps, **22a**: 57%, **22b**: 60%); (k) Cbz-Cl/CH_2Cl_2; (l) separation of epimers ((+)-**23a**: 22%, (+)-**23b**: 41%; (−)-**23a**: 21%, (−)-**23b**: 33%); (m) H_2, Pd–C/MeOH; (n) **12**, Et_3N/MeCN, heat; (o) concd HCl aq (three steps, **24**: 59%, **25**: 72%, **26**: 61%, **27**: 64%).

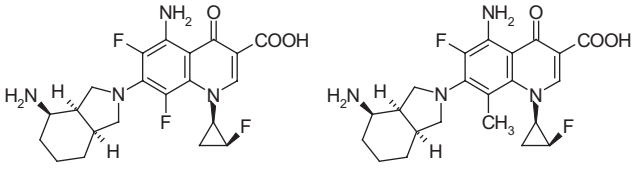


Figure 2. Reference compounds.

influence the selectivity between the antibacterial activity and genotoxicity.

In conclusion, we designed and synthesized the novel 6-difluoro-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]quinolone compounds having cyclopropane-fused 2-amino-8-azabicyclo[4.3.0]nonan-8-yl C-7 substituents and uncovered SARs of these derivatives. Of the synthesized compounds, the compounds synthesized from the dextrorotatory C-7 substituent, **13**, **16**, **24**, and **26** exhibited highly potent activity against Gram-positive bacteria including resistant strains, and also highly potent inhibitory activity against *S. aureus* topoisomerase IV. Some of them demonstrated reduced inhibitory activity against human topoisomerase II, and the results indicated that the cyclopropane fusing strategy, which give

Table 1. Antibacterial activity of **13**, **14**, **16**, **24**–**30**, DQ-113 and clinically available antibacterials (MIC, μg/mL)

Compds	*S. aureuss* FDA 209P	MRSA 870307	*S. epidermidis* 56500	PRSP J-24	*S. pyogenes* G-36	*S. mitis* IID685	*E. faecalis* ATCC19433	*E. coli* NIHJ	*K. pneumoniae* type II
13	⩽0.003	0.006	⩽0.003	⩽0.003	⩽0.003	⩽0.003	0.025	⩽0.003	0.012
14	⩽0.003	0.20	0.025	0.025	0.05	0.025	0.20	0.006	0.05
16	⩽0.003	0.012	⩽0.003	0.006	⩽0.003	⩽0.003	0.025	⩽0.003	0.012
24	⩽0.003	0.012	⩽0.003	⩽0.003	⩽0.003	⩽0.003	0.025	⩽0.003	0.012
25	0.006	1.56	0.05	0.10	0.39	0.10	0.20	0.012	0.10
26	⩽0.003	0.025	⩽0.003	⩽0.003	0.006	⩽0.003	0.025	⩽0.003	0.012
27	⩽0.003	0.20	0.006	0.006	0.012	0.006	0.025	⩽0.003	0.012
28	⩽0.003	0.006	⩽0.003	⩽0.003	0.003	⩽0.003	0.025	⩽0.003	0.012
29	0.025	0.78	0.20	0.10	0.10	0.10	0.78	0.10	0.39
30	0.006	0.012	⩽0.003	⩽0.003	⩽0.003	⩽0.003	0.025	0.006	0.025
DQ-113	⩽0.003	0.025	⩽0.003	⩽0.003	⩽0.003	⩽0.003	0.025	⩽0.003	0.012
LVFX[a]	0.20	>6.25	0.39	0.78	0.78	0.39	0.78	0.012	0.05
MFLX[a]	0.025	0.78	0.10	0.025	0.20	0.10	0.20	0.006	0.05
VCM[a]	0.20	0.39	1.56	0.20	0.39	0.39	0.39	>6.25	>6.25
LZD[a]	0.78	1.56	1.56	0.78	3.13	1.56	3.13	6.25	>6.25

[a] Abbreviations as follows: LVFX = levofloxacin; MFLX = moxifloxacin; VCM = vancomycin; LZD = linezolid.

Table 2. Inhibitory activity against *S. aureus* topoisomerase IV[a] and human topoisomerase II (IC_{50}, μg/mL)[b] of compounds **13**, **16**, **24**, **26**, **28**, **30**, and DQ-113

Compds	*S. aureus* Topo IV[a]	Human Topo II[b]	Selectivity (Human Topo II/ *S. aureus* Topo IV)
13	0.307	21.89	71.3
16	0.367	34.21	93.2
24	0.149	22.47	150.8
26	0.384	6.65	17.3
28	0.183	7.00	38.3
30	0.317	4.66	14.7
DQ-113	0.320	50.22	156.9

[a] *S. aureus* DNA Topoisomerase IV, Ref. 16.
[b] Human DNA Topoisomerase II, Ref. 17.

some rigidity to the C-7 substituents, could be effective for reducing genotoxicity.

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17. Using topoisomerase IIα of human (QIAGEN) and 50% inhibitory concentrations against decatenation activity were determined.