Pyridonecarboxylic Acids as Antibacterial Agents. IX.[1a] Synthesis and Structure–Activity Relationship of 3-Substituted 10-(1-Aminocyclopropyl)-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic Acids and Their 1-Thio and 1-Aza Analogues[2]

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Received June 24, 1994; accepted August 1, 1994

A series of the title compounds listed in Chart 1 have been synthesized to study the effects of 3-alkyl substituents on the antibacterial potency and *in vivo* efficacy of 10-(1-aminocyclopropyl)-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid and its 1-thio and 1-aza variants. Compound (*S*)-1, which proved most active *in vitro* against five representative gram-positive and gram-negative organisms, was assayed *in vivo* using *Staphylococcus aureus* and *Pseudomonas aeruginosa* mouse infection models. It exhibited an excellent *in vivo* efficacy, being superior to ofloxacin and ciprofloxacin, and was then assayed for convulsion-inducing activity, mammalian cell cytotoxicity, and topoisomerase II inhibition. The biological results showed that (*S*)-1 displayed antibacterial and toxicological advantages over ofloxacin and ciprofloxacin. Compound (*S*)-1 and its methanesulfonate showed high serum concentrations after oral and intravenous administrations to mice.

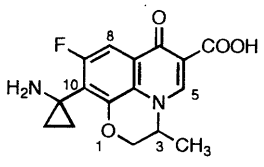
Keywords antibacterial agent; fluoroquinolone; structure–activity relationship; aminocyclopropyl substituent; toxicological property; pharmacokinetic property

In the preceding paper,[1a] we disclosed that compound **1**, a 10-(1-aminocyclopropyl) analogue of ofloxacin (OFLX),[3] exhibits potent *in vitro* antibacterial activity against both gram-positive and gram-negative bacteria, comparable to that of OFLX or ciprofloxacin (CPFX),[4] and we showed that **1** is considerably less toxic than OFLX and CPFX in terms of acute toxicity, cytotoxicity, and convulsion-inducing activity. This encouraging discovery prompted us to synthesize both enantiomers of **1** and a series of its 3-alkyl variants (**2a—g**), as well as 1-thio and 1-aza analogues of **1** (**2h, i**) (Chart 1). This paper describes the synthesis of these new quinolones and the results of their biological evaluation. Among the compounds synthesized in this study, the (*S*) enantiomer of **1** has been found to be the best compound, being superior to OFLX and CPFX, in terms of *in vivo* antibacterial activity and toxicological and pharmacokinetic properties.

Chemistry

All of the designed molecules were synthesized from the *N*-benzyloxycarbonyl (Z) derivative **3**[1a] of ethyl 4-(1-aminocyclopropyl)-2,3,5-trifluorobenzoylacetate utilizing the pyridone annulation methodology[5] reported in the synthesis of OFLX and its analogues (Chart 2).

Reaction of the β-keto ester **3** with *N*,*N*-dimethylformamide dimethyl acetal followed by treatment of the resulting (α-dimethylamino)methylene derivative with 2-aminoalcohols (**4a—h**) afforded the corresponding enamines **5a—h**.[6] These compounds were subjected to a double cyclization by heating in *N*,*N*-dimethylformamide (DMF) in the presence of potassium carbonate to give pyridobenzoxazines **6a—h**[6] in 61—89% overall yield,



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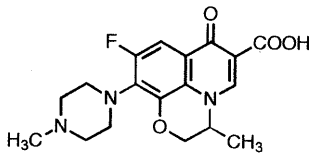
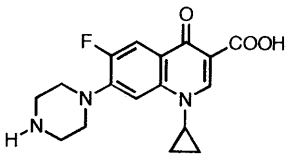
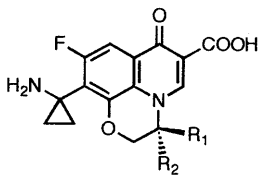


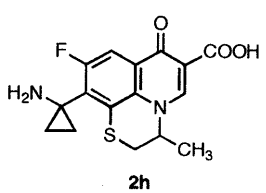
ofloxacin (OFLX)



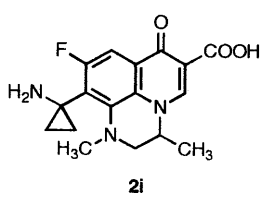
ciprofloxacin (CPFX)



	R_1	R_2
(*R*)-1	H	CH_3
(*S*)-1	CH_3	H
(*R,S*)-2a	C_2H_5	H
(*R,S*)-2b	*n*-C_3H_5	H
(*R,S*)-2c	CH_2F	H
(*R,S*)-2d	CH_2OH	H
2e	CH_3	CH_3
2f	$-CH_2-CH_2-$	
2g	$-CH_2-$	

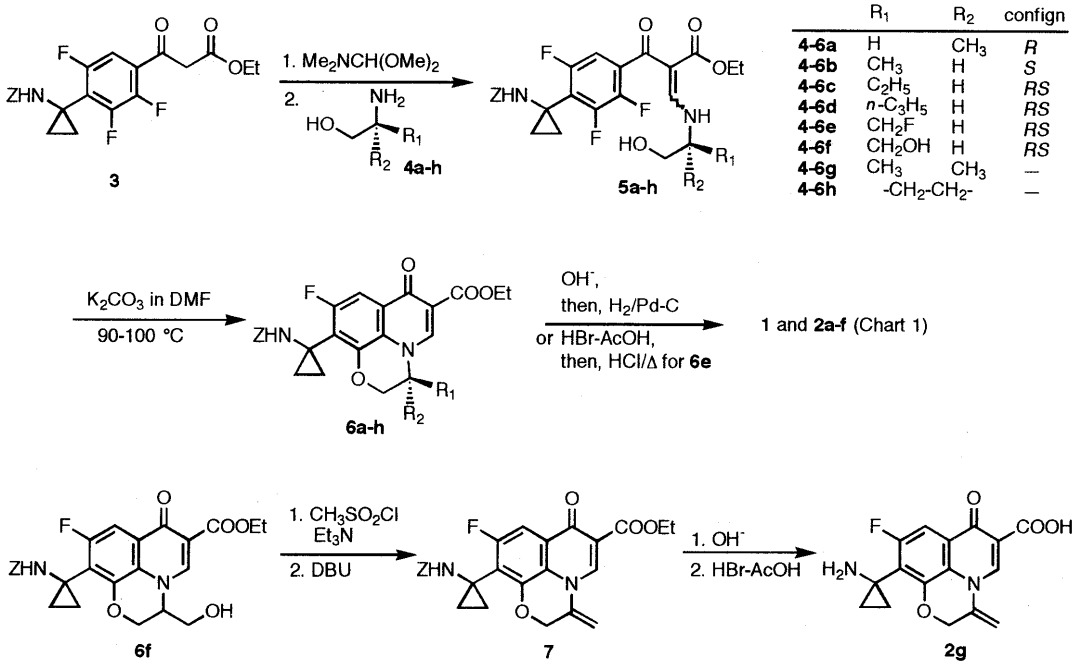


2h



2i

Chart 1



	R_1	R_2	confign
4-6a	H	CH_3	*R*
4-6b	CH_3	H	*S*
4-6c	C_2H_5	H	*RS*
4-6d	*n*-C_3H_5	H	*RS*
4-6e	CH_2F	H	*RS*
4-6f	CH_2OH	H	*RS*
4-6g	CH_3	CH_3	—
4-6h	-CH_2-CH_2-		—

Chart 2

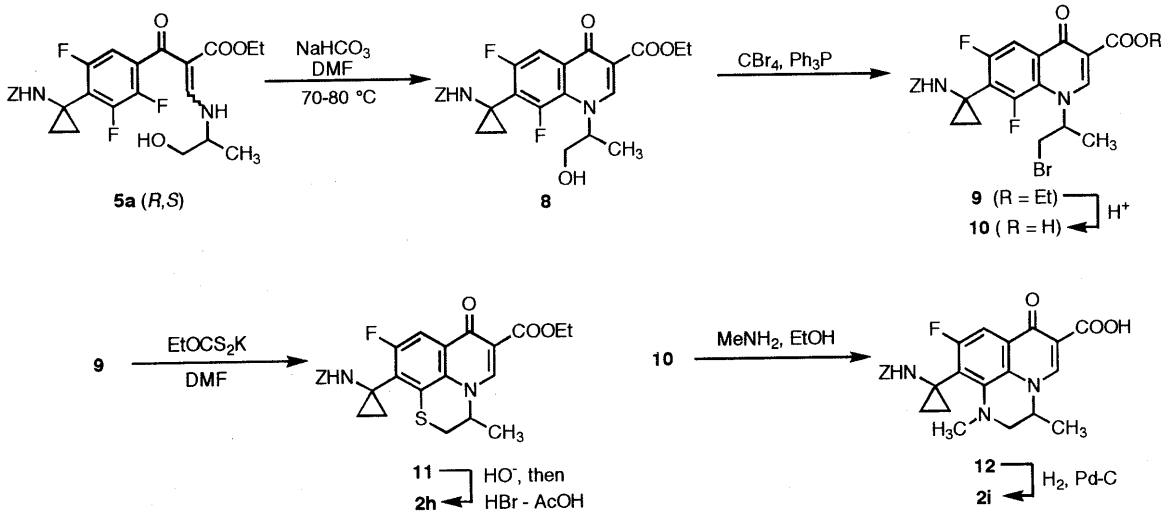


Chart 3

except for the fluoromethyl compound **6e** (15%). These tricyclic compounds were converted to **1** and **2a**—**f** by ester hydrolysis with ethanolic NaOH and subsequent removal of the Z-protecting group by catalytic hydrogenolysis, except for base-sensitive **6e**, where acidic conditions were used for this step: treatment with HBr in acetic acid for *N*-deprotection and ester hydrolysis by heating with 6 N HCl. The 3-methylene-1,4-oxazine compound **2g** was obtained from the alcohol **6f** by treatment of its *O*-methanesulfonate with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), then subjecting the resultant olefin **7** to removal of *O*- and *N*-protecting groups.

The 1,4-thiazine and 1,4-diazine analogues **2h** and **2i** were prepared from **5a** *via* the common intermediate, *N*-1-[(1-bromomethyl)ethyl]quinolone **9** obtained by bromination of the pyridone annulation product **8** (26% overall yield) (Chart 3). To obtain **2h**, the bromide **9** was first subjected to a thiazine-ring formation by treatment with potassium ethyl xanthate in DMF, then the remaining ester and Z-amide groups were hydrolyzed under basic and acidic conditions, respectively, to give **2h** in 54% overall yield. On the other hand, **2i** was derived from **9** by carrying out a three-step sequence of reactions: acid-catalyzed ester hydrolysis to **10**; cyclization to **12** with methylamine; and catalytic hydrogenolysis of the Z-group (32% overall yield). Characterization data for the new quinolones obtained above are recorded in Table I.

Biological Assays and Discussion

***In Vitro* and *in Vivo* Antibacterial Activity** All of the tricyclic (1-aminocyclopropyl)quinolones (Chart 1) syn-

TABLE I. Physical Data for 1,4-Oxazino-, 1,4-Thiazino-, and 1,4-Diazinoquinolones

Compd.	mp (dec. °C)	Recryst. solvent	Yield[a] (%)	Formula	Analysis (%) Calcd (Found) C	H	N	IR (KBr) cm^{-1}	^{1}H-NMR (CF_3COOD) δ (ppm)
(*R*)-**1**[b]	269—273	$CHCl_3$–MeOH	74	$C_{16}H_{15}FN_2O_4$	60.38 (60.40)	4.75 4.82	8.80 8.86)	1711	1.3—2.3 (7H, m), 4.6—5.5 (3H, m), 8.05 (1H, d, J=9.5 Hz), 9.43 (1H, s)
(*S*)-**1**[c]	269—272	$CHCl_3$–MeOH	63	$C_{16}H_{15}FN_2O_4$	60.38 (60.30)	4.75 4.67	8.80 9.06)	1711	1.3—2.2 (7H, m), 4.5—5.6 (3H, m), 8.06 (1H, d, J=9.5 Hz), 9.42 (1H, s)
2a	261—265	$CHCl_3$–EtOH	53	$C_{17}H_{17}FN_2O_4$	61.44 (61.42)	5.16 5.22	8.43 8.49)	1713	0.9—2.4 (9H, m), 4.5—5.3 (3H, m), 8.05 (1H, d, J=9.5 Hz), 9.37 (1H, s)
2b	223—226	EtOH	67	$C_{18}H_{19}FN_2O_4 \cdot 1/2H_2O$	60.84 (60.62	5.67 5.54	7.88 7.92)	1718	0.9—2.4 (11H, m), 4.7—5.3 (3H, m), 8.05 (1H, d, J=9.5 Hz), 9.36 (1H, s)
2c	240—245	6 N HCl–EtOH	12	$C_{16}H_{14}F_2N_2O_4 \cdot HCl \cdot 3/4H_2O$	49.75 (49.62	4.31 4.25	7.25 7.24)	1718	1.3—2.3 (4H, m), 4.4—5.8 (5H, m), 8.08 (1H, d, J=9.5 Hz), 9.39 (1H, s)
2d	255—260	6 N HCl–EtOH	23	$C_{16}H_{15}FN_2O_5 \cdot HCl \cdot 1/2H_2O$	50.60 (50.55	4.51 4.30	7.38 7.45)	1711	1.0—1.8 (4H, m), 4.3—5.2 (5H, m), 7.64 (1H, d, J=10 Hz), 8.84 (1H, s)
2e	269—272	$CHCl_3$–EtOH	67	$C_{17}H_{17}FN_2O_4 \cdot 1/4H_2O$	60.62 (60.90	5.24 5.21	8.32 8.56)	1639	1.3—2.3 (10H, m), 4.71 (2H, s), 8.08 (1H, d, J=10 Hz), 9.48 (1H, s)
2f	255—260	$CHCl_3$–EtOH	49	$C_{17}H_{15}FN_2O_4 \cdot 1/2H_2O$	60.17 (60.44	4.75 4.51	8.26 8.19)	1723	1.2—2.4 (8H, m), 4.81 (2H, s), 8.06 (1H, d, J=9 Hz), 9.07 (1H, s)
2g	275—280	EtOH–MeOH	19	$C_{16}H_{13}FN_2O_4 \cdot HBr$	48.38 (48.37	3.55 3.59	7.05 7.05)	1715	1.3—2.3 (4H, m), 5.34 (2H, s), 5.95 (1H, d, J=4.5 Hz), 6.28 (1H, d, J=4.5 Hz), 8.04 (1H, d, J=9.5 Hz), 9.55 (1H, s)
2h	276—280	EtOH	14	$C_{16}H_{15}FN_2O_3S$	46.28 (46.24	3.88 4.00	6.75 6.63)	1701	1.4—2.4 (7H, m), 3.2—4.3 (2H, m), 5.2—5.8 (1H, m), 8.24 (1H, d, J=9.5 Hz), 9.51 (1H, s)
2i	256—260	6 N HCl–EtOH	8	$C_{17}H_{18}FN_3O_3 \cdot HCl$	55.52 (55.47	5.21 5.44	11.42 11.28)	1687	1.2—2.4 (7H, m), 3.3—4.2 (5H, m), 4.8—5.4 (1H, m), 7.89 (1H, d, J=10 Hz), 9.29 (1H, s)

a) Overall yield from **3**. b) $[\alpha]_D^{25}$ +80.3° (c=1.0, 1.0 N NaOH). c) $[\alpha]_D^{25}$ −80.0° (c=1.0, 1.0 N NaOH).

thesized in this study and the two reference drugs (OFLX, CPFX) were tested for *in vitro* antibacterial activity against five selected gram-positive and gram-negative bacteria. Their minimum inhibitory concentrations (MICs, μg/ml) determined by conventional agar dilution procedures[1c,7] are recorded in Table II. Of the three 3-methyl compounds (**1**, **2h**, **2i**; all racemates) which differ only in the heteroatom at the 1-position, the oxazine compound **1** is evidently more potent than the other two, the activity order being **1** > **2h** ≫ **2i**. Among the 3-substituted 1,4-oxazinoquinolones other than **1**, the 3-fluoromethyl compound **2c** displayed the best antibacterial profile, but its MICs against the tested organisms are equal to those of **2h**. The best overall antimicrobial activity (equal to CPFX) was obtained with (*S*)-**1**, which was more active than (*R*,*S*)-**1** against *Staphylococcus aureus* (two-fold MIC). It is interesting that (*R*)-**1** is much less active than (*S*)-**1**, particularly against *S. aureus* and *P. aeruginosa*.

Compound (*S*)-**1**, which displayed excellent *in vitro* antibacterial activity, was tested for *in vivo* efficacy against acute, lethal systemic infections due to gram-positive *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa* in male ICR-strain mice. The effective dose (ED_{50}, mg/mouse) for (*S*)-**1** and those of the reference drugs (OFLX, CPFX) are listed in Table III, in which MICs against the corresponding organisms are included for reference. Comparison of the oral (*p.o.*) efficacy data indicates that (*S*)-**1** is 1.5—2.5 times more active orally than the reference quinolones in the *S. aureus* and *P. aeruginosa* infection models. On subcutaneous (s.c.) administration, on the other hand, (*S*)-**1** is 2.1—3.9 times more active than OFLX, but shows the same order of activity as CPFX. A noteworthy difference between *in vitro* and *in vivo* activity is seen in the oral efficacy data with *P. aeruginosa*: while (*S*)-**1** is less active than CPFX *in vitro*, its *in vivo* activity is 1.6 times superior to that of CPFX.

TABLE II. *In Vitro* Antibacterial Activity (MICs, μg/ml)[a]

Compd.	Microorganism[b] Gram-positive	Gram-negative			
	S. au.	*E. c.*	*K. pn.*	*Pr. v.*	*P. ae.*
(*R*)-**1**	50	1.56	1.56	1.56	100
(*S*)-**1**	0.2	≤0.05	≤0.05	≤0.05	0.39
(*R*,*S*)-**1**	0.39	≤0.05	≤0.05	≤0.05	0.39
2a	1.56	≤0.05	≤0.05	≤0.05	0.78
2b	6.25	0.1	0.2	0.1	3.13
2c	0.78	≤0.05	≤0.05	≤0.05	1.56
2d	12.5	0.39	0.39	0.39	12.5
2e	3.13	0.1	0.1	0.2	12.5
2f	3.13	0.1	0.2	0.2	3.13
2g	3.13	0.1	0.2	0.2	3.13
2h	0.78	≤0.05	≤0.05	≤0.05	1.56
2i	6.25	0.2	0.39	0.39	12.5
OFLX	0.39	≤0.05	≤0.05	≤0.05	0.78
CPFX	0.2	≤0.05	≤0.05	≤0.05	0.39

a) Inoculation was performed with one loopful of 10^6 cells/ml. b) *S. au.* = *Staphylococcus aureus* FDA 209P, *E. c.* = *Escherichia coli* NIHJ, *K. pn.* = *Klebsiella pneumoniae* Y-50, *Pr. v.* = *Proteus vulgaris* GN 3027, *P. ae.* = *Pseudomonas aeruginosa* IFO 3445.

Toxicological Properties of (*S*)-1 Compound (*S*)-**1**, which showed an excellent antibacterial activity, was evaluated for acute toxicity[1b] and convulsion-inducing activity[1c,8] in male ICR-strain mice by intravenous and intracerebral administrations, respectively. The dose–mortality ratios data are recorded in Table IV, together

TABLE III. *In Vivo* Efficacy against Systemic Infections in Mice[a]

Organism	Compd.	MIC (μg/ml)	ED_{50} mg/mouse (95% confidence limits)	
			p.o.	s.c.
S. aureus SMITH[b]	(*S*)-**1**	0.20	0.056 (0.033—0.095)	0.021 (0.015—0.030)
	OFLX	0.39	0.11 (0.05—0.23)	0.044 (0.029—0.067)
	CPFX	0.39	0.14 (0.08—0.26)	0.026 (0.020—0.034)
P. aeruginosa S-68[c]	(*S*)-**1**	0.39	0.16 (0.11—0.23)	0.056 (0.035—0.089)
	OFLX	0.78	0.39 (0.26—0.58)	0.22 (0.15—0.33)
	CPFX	0.20	0.26 (0.23—0.29)	0.044 (0.031—0.063)

a) Male ICR-strain mice (18—21 g body weight). *b*) Challenge dose (for oral dosing, 2.2×10^7 CFU/mouse; for subcutaneous dosing, 2.4×10^7 CFU/mouse). *c*) Challenge dose (for oral dosing, 4.9×10^6 CFU/mouse; for subcutaneous dosing, 1.6×10^7 CFU/mouse). CFU, colony forming unit.

TABLE IV. Acute Toxicity and Convulsion Induction in Male ICR-Strain Mice

Compd.	Acute toxicity (i.v.)[a]		Convulsion induction (i.c.)[b]		
	Dose (mg/kg)	Mortality (after 7 d)	Clonic seizure	Tonic seizure	Mortality (after 24 h)
(*S*)-**1**	500	0/5	0/8	0/8	0/8
OFLX	250	3/5	10/10	8/10	8/10
CPFX	250	1/5	10/10	10/10	10/10

a) Intravenous dose to 18—24 g body weight mice. *b*) Intracerebral dose of 50 μg to 20—25 g body weight mice.

TABLE V. Assays for Chinese Hamster V79 Cell Cytotoxicity and for Inhibition of DNA Gyrase and Topoisomerase II

Compd.	IC_{50} (μg/ml)		
	V-79 cell	DNA gyrase[a]	Topoisomerase II[b]
(*S*)-**1**	295	0.68	2010
OFLX	120	0.70	1358
CPFX	70	0.35	236

a) Isolated and purified from *E. coli* KL-16. *b*) Isolated and purified from calf thymus.

with the data obtained with two reference drugs. The acute toxicity data indicate that (*S*)-**1** is much less toxic ($LD_{50} = > 500$ mg/kg) than OFLX and CPFX. Furthermore, convulsion-inducing action of (*S*)-**1** proved to be significantly lower than that of the reference drugs in both clonic and tonic seizures, 100% survival being observed at the 50 μg dose level.

The clonogenic cytotoxicity of (*S*)-**1** was determined in Chinese hamster V-79 cells according to the method reported in the preceding paper,[1a] and the 50%-inhibitory concentrations (IC_{50}, μg/ml) obtained are recorded in Table V. The data reveal that (*S*)-**1** is significantly less toxic than the two reference drugs against these mammalian cells.

Inhibitory activity against DNA gyrase and mammalian topoisomerase II was determined with the enzymes isolated and purified from *Escherichia coli* KL-16 and calf thymus cells, respectively, according to the procedures of Hoshino and his co-workers.[9] The IC_{50} data included in Table V indicate that the DNA gyrase supercoiling inhibition activity of (*S*)-**1** is less potent than that of CPFX but is at the same level as that of OFLX. An important finding

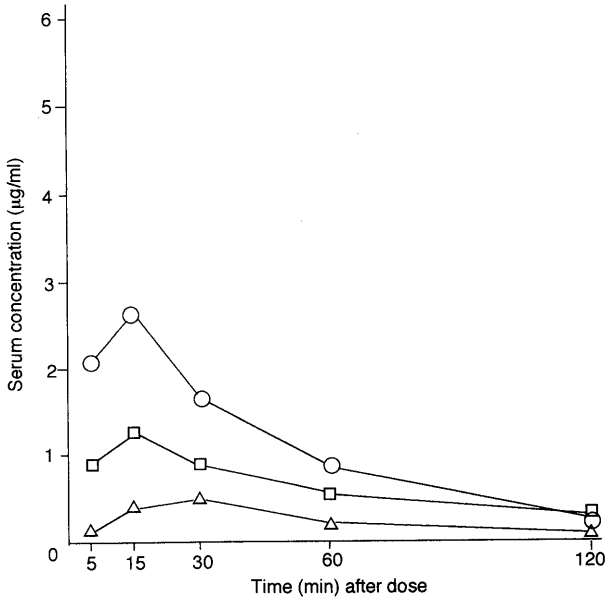


Fig. 1. Mean Serum Concentrations after Oral Administration of 5 mg/kg to Mice ($n = 5$)
○, (*S*)-**1**; □, OFLX; △, CPFX.

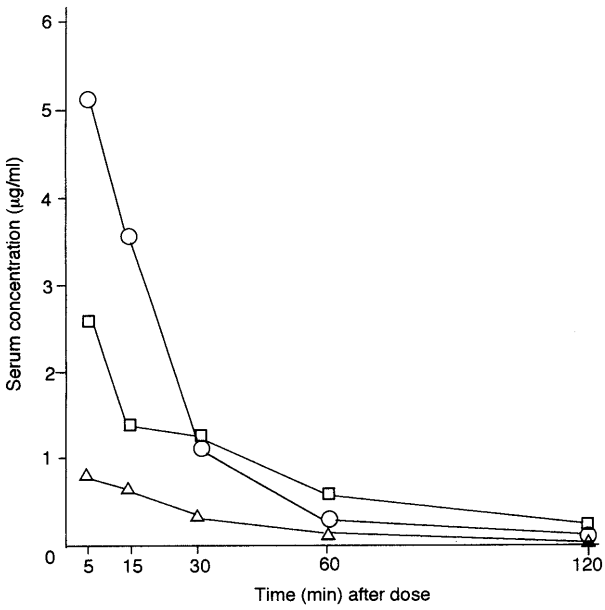


Fig. 2. Mean Serum Concentrations after Intravenous Administration of 5 mg/kg to Mice ($n = 5$)
○, methanesulfonate of (*S*)-**1**; □, OFLX; △, CPFX.

is that the inhibitory activity of (*S*)-**1** against mammalian topoisomerase II is 1.5 or 8.5 times weaker than that of OFLX or CPFX, respectively. Thus the selectivity index (the value obtained by division of IC_{50} for topoisomerase II by IC_{50} for DNA gyrase) is the best for (*S*)-**1** (2956), being 1.5 or 4.4 times greater than those of OFLX (1940) and CPFX (674), respectively.

Pharmacokinetic Properties of (*S*)-1 and Its Methanesulfonate Serum concentrations of (*S*)-**1** as well as of its methanesulfonate, which has a good water solubility (> 200 mg/ml at 25 °C), were determined by oral and intravenous administration to mice. The results for oral

administration of (*S*)-**1** and the reference agents (Fig. 1) indicate that the maximum serum concentration (C_{max}) of (*S*)-**1** (obtained 15 min after dosing) is 2.1 and 5.5 times those of OFLX and CPFX, respectively. The half-life time of (*S*)-**1** (30 min) was, however, about 50% shorter than those of the reference agents, and approximately 27% of the administered drug was recovered from urine over 24 h. On intravenous administration of (*S*)-**1** methanesulfonate, on the other hand, the serum concentration five minutes after dosing (5.12 μg/ml) was 2.0 and 6.3 times higher than those of OFLX and CPFX, respectively, (Fig. 2) and approximately 42% was recovered from the urine over 24 h.

In conclusion, we have obtained a new, highly potent quinolone antibacterial agent (*S*)-**1** (T-3761) simply by replacing the 10-piperazinyl substituent of OFLX with the hitherto unexplored 1-aminocyclopropyl group. Our new drug is safer than most existing quinolones, yet has a broad antibacterial spectrum. Clinical trials of both T-3761 and its methanesulfonate (T-3762) as oral and intravenous drugs, respectively, are in progress.

Experimental[10]

Ethyl 3-Alkyl-10-[(1-benzyloxycarbonylamino)cyclopropyl]-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylates (6a—h) These compounds were prepared from **3** by the procedure reported in the previous paper.[1a]

6a: 89% yield. mp 183—185 °C (colorless prisms from AcOEt). $[\alpha]_D^{25}$ +57.3° (c=1.0, $CHCl_3$). IR (KBr): 1722, 1696, 1677 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.1—1.8 (10H, m), 4.1—4.7 (5H, m), 5.02 (2H, s), 5.60 (1H, s), 7.27 (5H, s), 7.64 (1H, d, J=10.5 Hz), 8.31 (1H, s). *Anal.* Calcd for $C_{26}H_{25}FN_2O_6$: C, 64.99; H, 5.24; N, 5.83. Found: C, 64.85; H, 5.33; N, 5.91.

6b: 83% yield. mp 182—184 °C (colorless prisms from AcOEt). $[\alpha]_D^{25}$ −58.1° (c=1.0, $CHCl_3$). IR (KBr): 1722, 1695, 1677 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.2—1.8 (10H, m), 4.2—4.6 (5H, m), 5.03 (2H, s), 5.61 (1H, s), 7.27 (5H, s), 7.62 (1H, d, J=10.5 Hz), 8.30 (1H, s). *Anal.* Calcd for $C_{26}H_{25}FN_2O_6$: C, 64.99; H, 5.24; N, 5.83. Found: C, 65.06; H, 5.22; N, 5.67.

6c: 72% yield. mp 107—109 °C (colorless prisms from aqueous EtOH). IR (KBr): 1717 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.7—2.1 (12H, m), 3.8—4.7 (5H, m), 5.02 (2H, s), 5.60 (1H, s), 7.27 (5H, s), 7.62 (1H, d, J=10.5 Hz), 8.26 (1H, s). *Anal.* Calcd for $C_{27}H_{27}FN_2O_6 \cdot 1/2H_2O$: C, 64.41; H, 5.61; N, 5.56. Found: C, 64.38; H, 5.89; N, 5.59.

6d: 89% yield. mp 148—149 °C (colorless prisms from aqueous EtOH). IR (KBr): 1716 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.7—2.1 (14H, m), 3.9—4.8 (5H, m), 5.02 (2H, s), 5.60 (1H, s), 7.27 (5H, s), 7.65 (1H, d, J=10.5 Hz), 8.26 (1H, s). *Anal.* Calcd for $C_{28}H_{29}FN_2O_6$: C, 66.13; H, 5.75; N, 5.51. Found: C, 66.20; H, 5.81; N, 5.60.

6e: 15% yield. mp 192—194 °C (colorless prisms from aqueous EtOH). IR (KBr): 1724, 1687 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.0—1.7 (7H, m), 4.2—5.3 (9H, m), 5.57 (1H, s), 7.27 (5H, s), 7.59 (1H, d, J=10.5 Hz), 8.27 (1H, s). *Anal.* Calcd for $C_{26}H_{24}F_2N_2O_6$: C, 62.65; H, 4.85; N, 5.62. Found: C, 62.61; H, 4.91; N, 5.69.

6f: 61% yield. mp 229—231 °C (colorless needles from EtOH). IR (KBr): 1732, 1715 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.0—1.8 (7H, m), 3.8—4.8 (7H, m), 5.02 (2H, s), 5.59 (1H, s), 6.00 (1H, br s), 6.43 (1H, d, J=10.5 Hz), 7.26 (5H, s), 8.43 (1H, s). *Anal.* Calcd for $C_{26}H_{25}FN_2O_7 \cdot 1/4H_2O$: C, 62.33; H, 5.13; N, 5.59. Found: C, 62.43; H, 5.35; N, 5.88.

6g: 81% yield. mp 194—196 °C (colorless needles from aqueous EtOH). IR (KBr): 1719 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.1—1.8 (13H, m), 4.14 (2H, s), 4.41 (2H, q, J=7.5 Hz), 5.03 (2H, s), 5.61 (1H, s), 7.27 (5H, s), 7.72 (1H, d, J=11 Hz), 8.56 (1H, s). *Anal.* Calcd for $C_{27}H_{27}FN_2O_6 \cdot 1/2H_2O$: C, 64.41; H, 5.61; N, 5.56. Found: C, 64.33; H, 5.88; N, 5.63.

6h: 64% yield. mp 175—177 °C (a white powder from aqueous EtOH). IR (KBr): 1722, 1694, 1683 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.0—1.8 (11H, m), 4.1—4.6 (4H, m), 5.03 (2H, s), 5.62 (1H, s), 7.29 (5H, s), 7.69 (1H, d, J=10.5 Hz), 8.14 (1H, s). *Anal.* Calcd for $C_{27}H_{25}FN_2O_6 \cdot 1/4H_2O$: C, 65.25; H, 5.17; N, 5.64. Found: C, 65.48; H, 5.19; N, 5.74.

(*S*)-10-(1-Aminocyclopropyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic Acid ((*S*)-1) A solution of the ethyl ester **6b** (7.00 g, 14.6 mmol) in a mixture of EtOH (70 ml) and dioxane (70 ml) was stirred at room temperature for 2 h after addition of 1 N NaOH (70 ml). The mixture was poured into a two-layer mixture of H_2O (200 ml) and $CHCl_3$ (200 ml), and the whole was acidified to pH 2 by addition of 6 N HCl. The aqueous layer was separated, and the organic layer was washed successively with water and saturated brine, dried, and concentrated. The solid residue was washed with Et_2O to give the corresponding carboxylic acid (6.34 g, 96%). An analytical sample was obtained by recrystallization from EtOH as colorless prisms, mp 181—183 °C. $[\alpha]_D^{25}$ −45.8° (c=1.0, $CHCl_3$). IR (KBr): 1731, 1715 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.0—1.9 (7H, m), 4.1—4.7 (3H, m), 5.01 (2H, s), 5.63 (1H, s), 7.28 (5H, s), 7.70 (1H, d, J=10 Hz), 8.66 (1H, s), 14.62 (1H, br s). *Anal.* Calcd for $C_{24}H_{21}FN_2O_6$: C, 63.71; H, 4.68; N, 6.19. Found: C, 63.79; H, 4.67; N, 6.04.

A solution of this material (6.30 g, 13.9 mmol) in AcOH (126 ml) was stirred under atmospheric pressure of hydrogen after addition of 5% Pd–C (1.26 g). After 2 h, the catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The resulting amino compound was isolated as its hydrochloride by concentration of a solution in 6 N HCl (21 ml) followed by crystallization of the residue from EtOH. This material was dissolved in 40% aqueous EtOH (100 ml) containing KOH (1.80 g, 32.1 mmol), and the solution was saturated with CO_2. The resulting precipitate was collected by filtration to give (*S*)-**1** (3.85 g, 87%).

By subjecting **6a, c, d, f—h** to the same two-step deprotection procedure, (*R*)-**1** and **2a, b, d—f** were obtained. Base-sensitive **2c** was obtained from **6e** by successive treatment with 30% HBr–AcOH at room temperature for 1 h and with 6 N HCl at reflux temperature.

10-(1-Aminocyclopropyl)-9-fluoro-3-methylene-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic Acid (2g) Methanesulfonyl chloride (260 mg, 2.27 mmol) was added dropwise to a stirred suspension of **6f** (380 mg, 0.77 mmol) and triethylamine (230 mg, 2.27 mmol) in CH_2Cl_2 (4 ml) at 5 to 10 °C. After the addition was completed, the mixture was stirred at room temperature for 30 min, then poured into a two-layer mixture of ice-water (10 ml) and CH_2Cl_2 (10 ml), and the whole was acidified to pH 1 with 2 N HCl. The organic layer was separated, washed successively with water and saturated brine, dried, and concentrated. The resultant crude *O*-mesylate was dissolved in benzene (4.4 ml) containing DBU (175 mg, 1.15 mmol), and the solution was heated under reflux for 1 h. The reaction mixture was cooled to room temperature, and poured into a two-layer mixture of H_2O (20 ml) and AcOEt (20 ml), and the whole was acidified to pH 1 with 2 N HCl. The organic layer was washed successively with water and saturated brine, dried, and concentrated. The residue was purified by chromatography (silica gel 10 g, toluene : AcOEt = 3 : 2) to give **7** (260 mg, 71%). An analytical sample was obtained by recrystallization from EtOH as colorless prisms, mp 195—197 °C. IR (KBr): 1724, 1700, 1687 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.2—1.7 (7H, m), 4.41 (2H, q, J=7.5 Hz), 4.78 (2H, s), 5.02 (2H, s), 5.12 (1H, d, J=2.5 Hz), 5.45 (1H, d, J=2.5 Hz), 5.60 (1H, s), 7.27 (5H, s), 7.66 (1H, d, J=10.5 Hz), 8.64 (1H, s). *Anal.* Calcd for $C_{26}H_{23}FN_2O_6$: C, 65.27; H, 4.85; N, 5.85. Found: C, 65.24; H, 4.89; N, 5.93.

The ester **7** (220 mg, 0.46 mmol) was saponified by the same procedure as described for **6b**, and the resultant carboxylic acid was treated with 30% HBr–AcOH (8 ml) at 5 to 10 °C for 1 h. The whole was concentrated under reduced pressure, and the residue was treated with Et_2O to induce crystallization. The crude hydrobromide of **2g** obtained by filtration was recrystallized from MeOH–EtOH.

Ethyl 7-[(1-Benzyloxycarbonylamino)cyclopropyl]-1-[(2-bromo-1-methyl)ethyl]-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (9) A solution of (*R,S*)-**5a** (5.60 g, 10.8 mmol) in DMF (56 ml) was stirred at 70—80 °C for 12 h after addition of $NaHCO_3$ (0.99 g, 11.8 mmol). The mixture was then poured into a two-layer mixture of ice-water (200 ml) and AcOEt (150 ml), and the whole was adjusted to pH 2 with 6 N HCl. The organic layer was separated, washed successively with water and saturated brine, dried, and concentrated. The residue was subjected to chromatography (silica gel 100 g, toluene : AcOEt = 1 : 3) to give **8** (1.73 g, 32%). An analytical sample was obtained by recrystallization from AcOEt as colorless prisms, mp 157—159 °C. IR (KBr): 1725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.0—1.9 (10H, m), 3.6—4.6 (5H, m), 5.04 (2H, s), 5.2—5.7 (2H, m), 7.0—7.5 (6H, m), 8.64 (1H, s). *Anal.* Calcd for $C_{26}H_{26}F_2N_2O_6$: C, 62.40; H, 5.24; N, 5.60. Found: C, 62.50; H, 5.28; N, 5.67.

Carbon tetrabromide (500 mg, 1.51 mmol) and triphenylphosphine (390 mg, 1.49 mmol) were added to a stirred suspension of **8** (500 mg, 1.00 mmol) in CH_2Cl_2 (5 ml). After being stirred at room temperature for 5 h, the mixture was concentrated under reduced pressure, and the residue was subjected to chromatography (silica gel 20 g, toluene: AcOEt = 1 : 1) to give **9** (460 mg, 82%). An analytical sample was obtained by recrystallization from iso-Pr_2O–AcOEt as colorless needles, mp 148—149 °C. IR (KBr): 1730, 1692 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.1—2.0 (10H, m), 3.68 (2H, d, J=4 Hz), 4.40 (2H, q, J=7 Hz), 5.04 (2H, s), 5.2—5.8 (2H, m), 7.28 (5H, s), 8.02 (1H, dd, J=10, 2 Hz), 8.55 (1H, s). *Anal.* Calcd for $C_{26}H_{25}BrF_2N_2O_5$: C, 55.43; H, 4.47; N, 4.97. Found: C, 55.41; H, 4.54; N, 5.00.

10-(1-Aminocyclopropyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzothiazine-6-carboxylic Acid (2h) Potassium ethyl xanthate (290 mg, 1.81 mmol) was added to a stirred solution of **9** (250 mg, 0.44 mmol) in DMF (5 ml) at room temperature. After 12 h, the reaction mixture was poured into a two-layer mixture of ice-water (40 ml) and AcOEt (30 ml), and the whole was acidified to pH 2 with 2 N HCl. The organic layer was separated, washed successively with water and saturated brine, dried, and concentrated. The residue was purified by chromatography (silica gel 5 g, $CHCl_3$: EtOH = 5 : 1) to give **11** (120 mg, 54%). An analytical sample was obtained by recrystallization from EtOH as pale yellow needles, mp 209—211 °C. IR (KBr): 1719, 1695, 1679 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.0—1.8 (10H, m), 2.7—3.7 (2H, m), 4.1—4.8 (3H, m), 5.05 (2H, s), 5.81 (1H, s), 7.29 (5H, s), 7.90 (1H, d, J=10 Hz), 8.37 (1H, s). *Anal.* Calcd for $C_{26}H_{25}FN_2O_5S$: C, 62.89; H, 5.07; N, 5.64. Found: C, 62.52; H, 5.05; N, 5.76.

The hydrobromide of **2h** was obtained from **11** by two-step deprotection as performed with **7**.

10-(1-Aminocyclopropyl)-9-fluoro-1,3-dimethyl-7-oxo-2,3-dihydro-1*H*,7*H*-pyrido[1,2,3-*de*]quinoxaline-6-carboxylic Acid (2i) The ester **9** (400 mg, 0.71 mmol) was added to a mixture of dioxane (4 ml) and 6 N HCl (4 ml), and the mixture was heated under reflux for 30 min. The reaction mixture was poured into a two-layer mixture of H_2O (50 ml) and $CHCl_3$ (50 ml), and the organic layer was separated, washed successively with water and saturated brine, dried, and concentrated. The solid residue was treated with Et_2O and collected by filtration to give **10** (180 mg, 47%). An analytical sample was obtained by recrystallization from iso-Pr_2O–AcOEt as colorless prisms, mp 167—169 °C. IR (KBr): 1737 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.0—2.0 (7H, m), 3.70 (2H, d, J=4.5 Hz), 5.03 (2H, s), 5.2—5.8 (2H, m), 7.28 (5H, s), 8.03 (1H, dd, J=9.5, 2 Hz), 8.77 (1H, s), 14.13 (1H, br s). *Anal.* Calcd for $C_{24}H_{21}BrF_2N_2O_5$: C, 53.85; H, 3.95; N, 5.23. Found: C, 54.02; H, 4.00; N, 5.45.

A solution of **10** (150 mg, 0.28 mmol) in 30% $MeNH_2$–EtOH (7.5 ml) was stirred at 45—55 °C for 12 h, then concentrated under reduced pressure. The residue was taken up in H_2O (50 ml) and $CHCl_3$ (30 ml), the whole was acidified to pH 2 with 6 N HCl, and the aqueous layer was separated. The organic layer was washed successively with water and saturated brine, dried, and concentrated. The solid residue was treated with Et_2O and collected by filtration to give **12** (90 mg, 69%). An analytical sample was obtained by recrystallization from EtOH as pale yellow needles, mp 218—220 °C. IR (KBr): 1711 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.1—1.8 (7H, m), 3.2—3.7 (5H, m), 4.2—4.6 (1H, m), 5.03 (2H, s), 5.80 (1H, s), 7.31 (5H, s), 7.70 (1H, d, J=11 Hz), 8.66 (1H, s), 14.75 (1H, br s). *Anal.* Calcd for $C_{25}H_{24}FN_3O_5$: C, 64.51; H, 5.20; N, 9.03. Found: C, 64.60; H, 5.16; N, 9.11.

Catalytic hydrogenolysis of **12** by the same procedure as described for **6b** afforded **2i**.

Methanesulfonate of (*S*)-1 Methanesulfonic acid (0.31 g, 3.22 mmol) was added to a stirred suspension of (*S*)-**1** (1.00 g, 3.14 mmol) in EtOH (10 ml) at 50 °C, then the mixture was allowed to cool to room temperature. Precipitates were collected by filtration to give the methanesulfonate of (*S*)-**1** (1.22 g, 94%), mp 258—259 °C (dec.), colorless prisms. $[\alpha]_D^{20}$ −64.2° (c=1.0, 1.0 N NaOH). IR (KBr): 1704 cm^{-1}. ^{1}H-NMR (CF_3COOD) δ: 1.3—2.2 (7H, m), 3.09 (1H, s), 4.6—5.5 (3H, m), 8.05 (1H, d, J=9.5 Hz), 9.43 (1H, s). *Anal.* Calcd for $C_{16}H_{15}FN_2O_4 \cdot CH_4O_3S$: C, 49.27; H, 4.62; N, 6.76. Found: C, 49.32; H, 4.66; N, 6.75.

***In Vivo* Efficacy** The *in vivo* assay was carried out according to the method reported in the previous paper.[11] Male ICR mice (18—21 g body weight, 10 mice per group) were used in this assay. Each mouse received 0.5-ml volume of bacterial suspension in 5% gastric mucin intraperitoneally. Test compounds, which were suspended in 0.5% aqueous methylcellulose or dissolved in dilute NaOH (*ca.* 1 eq), were administered orally or subcutaneously at 1 h after infection. Fifty percent effective dose values (ED_{50}) were calculated from the cumulative mortalities on the seventh day after infection.

Determination of Serum Concentrations and Urinary Recovery Male ICR mice (18—21 g body weight, 5 mice per group) which had been starved for 18 h were used in this assay. For the oral dosing test, each sample (10 mg) was suspended in 20 ml of 0.5% aqueous methylcellulose. For the intravenous dosing test, each sample (10 mg) was dissolved in 20 ml of water or in 20 ml of dilute NaOH (*ca.* 1 eq). These preparations (corresponding to 5 mg/kg) were administered orally or intravenously to mice. Serum samples were obtained at 5, 15, 30, 60, 120, and 240 min after dosing. Urine was collected over the period of 24 h after dosing. Serum concentrations and urinary excretion were determined by microbiological assay with *E. coli* Kp.

Acknowledgment The authors are grateful to Professor E. Yoshii, Toyama Medical and Pharmaceutical University, for his help and useful suggestions during the preparation of the manuscript.

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