Synthesis and Structure–Activity Relationships of 7-(3′-Amino-4′-methoxypyrrolidin-1′-yl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids

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A new series of quinolone derivatives 3a—l bearing 3-amino-4-methoxypyrrolidines of different configurations and chirality were synthesized and their antibacterial activities as well as some of their toxicological properties were examined. As predicted by our previous quantitative structure–activity relationships (QSAR) analysis of C-7 heterocyclic amine substituted quinolonecarboxylic acid antibacterial agents, these pyrrolidine derivatives showed higher *in vitro* and *in vivo* antibacterial activities against both gram-positive and gram-negative bacteria than the analogs bearing various 3-substituted azetidines. Furthermore, the amino and methoxy substituent groups on the pyrrolidine ring exhibited strong configurational and chiral effects on the *in vitro* and *in vivo* antibacterial activities of these compounds: (1) *cis* compounds showed higher antibacterial activities against most of the pathogens examined; (2) *N*-methylation of the 3-amino group on the pyrrolidine ring lowered *in vitro* but not *in vivo* antibacterial activities, particularly leading to superior *in vivo* anti-pseudomonal activity; (3) the (3′*S*,4′*R*)-derivative showed substantially higher activity that the (3′*R*,4′*S*)-one. These findings led to the selection of compound 3k for further evaluation as it possessed the highest *in vivo* antibacterial activity and no cytotoxicity.

Keywords antibacterial agent; quinolonecarboxylic acid; chiral pyrrolidine derivative; optical resolution; QSAR analysis

In the preceding paper,[1] we reported a QSAR (quantitative structure–activity relationships) analysis of the quinolone antibacterials **1** possessing various C-3′-substituted azetidinyl groups at the C-7 position of 1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid. We expected this versatile synthon to be beneficial in designing useful new derivatives. In that study, the calculated hydrophobic parameters, CLOG *P*, of these molecules were significantly affected by the C-3′ substituents on the azetidine ring. A good parabolic relationship was observed between the CLOG *P* values and the mean values of relative antibacterial activity indices determined for five gram-negative bacteria, with the CLOG *P* value for the most potent derivative estimated to be near 2.3. As for antibacterial activity against gram-positive bacteria, it remained rather constant and high regardless of the C-3′ substituent on the azetidinyl group. However, the *in vivo* antibacterial activities of azetidine derivatives showed somewhat lesser potencies than those of the pyrrolidine derivatives.

Thus, our interest was shifted from azetidine derivatives to the pyrrolidine derivatives: one earlier report[2] showed that the pyrrolidine derivative 7-(3′-aminopyrrolidinyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**2**) exhibited excellent *in vitro* as well as *in vivo* antibacterial activities against both gram-positive and gram-negative bacteria. Interestingly, the CLOG *P* value of this derivative was 2.11, which was quite close to 2.3, the value predicted for the most potent azetidine derivative. As the development of that pyrrolidine compound as a clinical agent had been abandoned and also because the substituted pyrrolidine derivatives had not been studied much as to their C-7 substituent group, we extended the QSAR information obtained from the 3′-substituted azetidine derivatives to 3′- and/or 4′-substituted pyrrolidine derivatives to find the most promising 3′-amino-4′-methoxypyrrolidine derivative having a CLOG *P* value near 2.3.

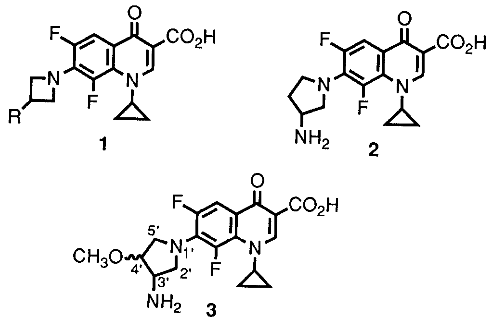


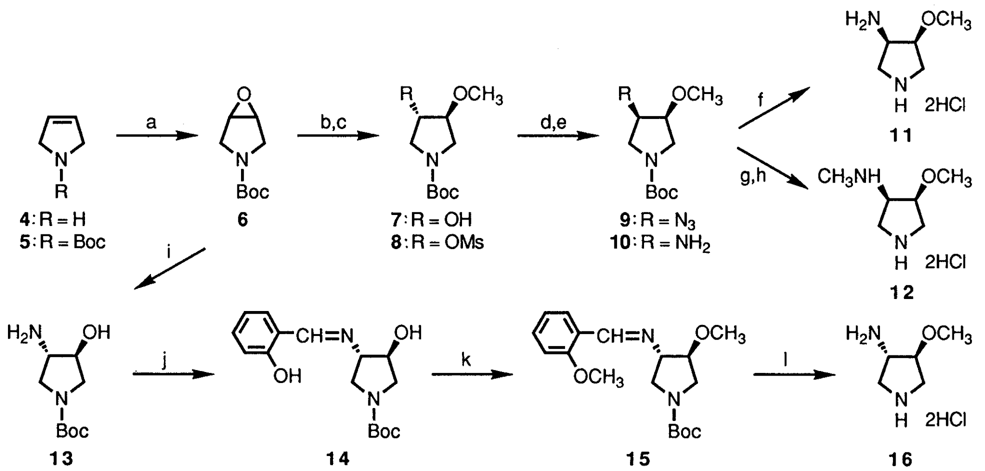
Fig. 1

The main subjects of this work were the synthesis of these new series of quinolone antibacterials **3a—l** having 3′-amino-4′-methoxypyrrolidine derivatives of different configurations and the evaluation of their antibacterial activities as well as pharmacodynamic and toxicological properties. When our work was almost completed, a similar patented work[3] appeared, claiming, in part, the synthesis of the same compound **3a** in a racemic form and the evaluation of its *in vitro* antibacterial activity. However, the work did not described the configurational effects of the amino and methoxy substituent groups on the antibacterial activities, nor did it evaluate the *in vivo* antibacterial activities. Thus, in our present paper, particular emphasis is given to the following: the *in vivo* antibacterial activities of these compounds, the geometrical and chiral effects of the two substituent groups on the antibacterial activities, and the pharmacological and toxicological effects of introducing the 5-amino and the 3-methylamino substituent groups. These studies led us to select the new (3′*S*,4′*R*)-4′-methoxy-3′-methylaminopyrrolidine derivative **3k** for further evaluation of its biological activities.

Results and Discussion

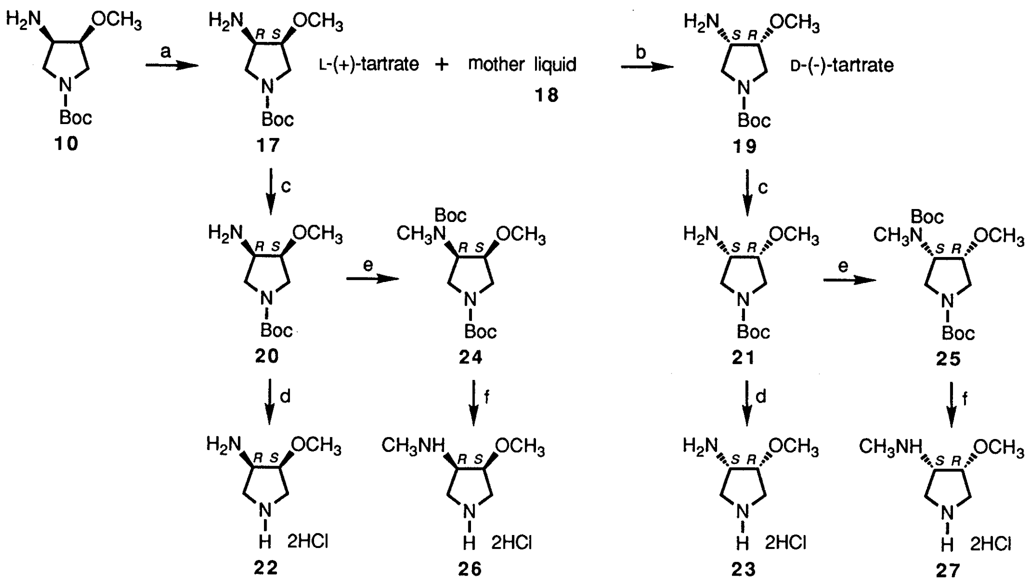
Chemistry The synthesis schemes for quinolone derivatives **3a** and **3c** bearing racemic *cis*- and *trans*-3-amino-4-methoxypyrrolidines (**11**) and (**16**), respectively, are summarized in Chart 1.

The commercially available starting material, 3-pyrroline (**4**), was first treated with di-*tert*-butyl dicarbonate (Boc_2O) to protect the amino group and then with 3-chloroperbenzoic acid to give the epoxide **6**. The epoxide **6** was cleaved with either $LiOCH_3$ or $NaOCH_3$ in methanol, providing mono-methyl ether of *trans*-diol,[4] **7**, in excellent yield. The alcohol **7** was converted into mesylate **8** in the usual manner, followed by treatment with NaN_3 in dimethylformamide (DMF) in the presence of tetrabutylammonium bromide, giving the *cis*-azide **9** with complete inversion of configuration. After reduction of the azide group either with Pd–C catalyzed hydrogenation or with triphenylphosphine,[5] the Boc group was removed by treatment with HCl in methanol to give the desired *cis*-3-amino-4-methoxypyrrolidine dihydrochloride (**11**). The other 3-methylamino derivative **12** was also prepared from **10** by treatment with Boc_2O, followed by metalation with NaH and then methylation with CH_3I. Next, to prepare *trans*-3-amino-4-methoxypyrrolidine dihydrochloride (**16**), the epoxide **6** was cleaved with NH_3 in aqueous methanol to produce the *trans*-amino alcohol[4] **13** in good yield. After protection of its amino group as a Schiff base with salicylaldehyde,[6] the resultant alcohol **14** was methylated in the usual manner by successive treatment with NaH and CH_3I, giving **15**. Two protective groups, the Schiff base and the Boc group, were simultaneously removed from **15** by treatment with HCl in methanol, producing the desired *trans*-3-amino-4-methoxypyrrolidine dihydrochloride (**16**).



a: MCPBA b: LiOMe c: Py-MsCl d: *n*-Bu_4NBr-NaN_3 in DMF e: Pd-C/H_2 f: HCl in MeOH
g: 1) Boc_2O, 2) NaH, 3) CH_3I h: HCl in MeOH i: 28%NH_3aq-MeOH j: salicylaldehyde
k: 1) NaH, 2) CH_3I l: HCl in MeOH

Chart 1



a: L-(+)-tartaric acid b: 1) neut. with K_2CO_3, 2) D-(-)-tartaric acid c: neut. with K_2CO_3 d: HCl in MeOH
e: 1) Boc_2O, 2) NaH, 3) CH_3I f: HCl in MeOH

Chart 2

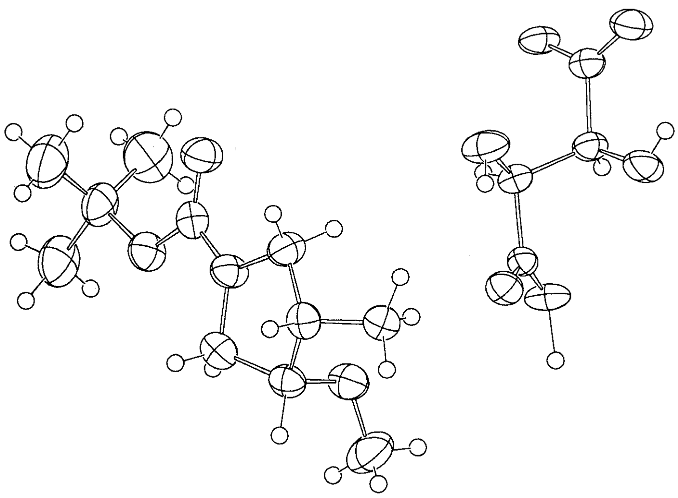


Fig. 2. ORTEP Drawing of **19**

To estimate the effects of chirality on biological activity, optical resolution of racemic *cis*-3-amino-4-methoxypyrrolidine dihydrochloride (**11**) was required. Suitable resolution agents[7] were searched for, and the following resolution method was established for the intermediate **10** using L- and D-tartaric acids as resolution agents. The racemate **10** was first treated with 1.5 equimolar amount of natural type L-tartaric acid in methanol and the crystalline precipitates **17** which showed $[\alpha]_D = +34.1°$ were separated in 79% yield. Filtrate **18** was neutralized with K_2CO_3 and the resultant free amine, after isolation, was treated with 1.5 equimolar amount of unnatural type D-tartaric acid, affording, after recrystallization, the crystalline salt **19** with $[\alpha]_D = -33.4°$ in 71% yield.

Both tartaric acid salts **17** and **19** were treated with base to liberate free amines **20** and **21**, respectively. They were proven to be optically pure by both NMR spectroscopic measurement and HPLC analysis of the diastereoisomeric ratios of their amide derivatives[8] obtained from the coupling reaction with Mosher's reagent, (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid. Furthermore, the absolute configuration of the crystals **19** were unambiguously determined as (3*S*,4*R*)-(−)-3-amino-1-*tert*-butoxycarbonyl-4-methoxypyrrolidine D-tartaric acid salt by X-ray crystallographic analysis as shown in Fig. 2.

Thus, our resolution method proved to be very efficient, giving each diastereomer in pure form by a single fractional recrystallization. Removal of the Boc group from **20** and **21** by treatment with HCl in methanol gave the hydrochloric acid slats of the desired optically pure (3*R*,4*S*)- and (3*S*,4*R*)-3-amino-4-methoxypyrrolidine dihydrochlorides (**22** and **23**), which showed $[\alpha]_D$ values of +54.5° and −54.4°, respectively. In the same way as described for racemic compound **12**, optically pure (3*R*,4*S*)- and (3*S*,4*R*)-4-methoxy-3-methylaminopyrrolidine hydrochlorides (**26** and **27**) were prepared from **20** and **21** *via* **24** and **25**, respectively. All the racemic and chiral pyrrolidine derivatives heretofore prepared, **11**, **12**, **16**, **22** and **23**, were subjected to the well estabilished reaction at the C-7 position of 1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid,[9] giving the desired quinolone derivatives **3a**—**e** as shown in Table I.

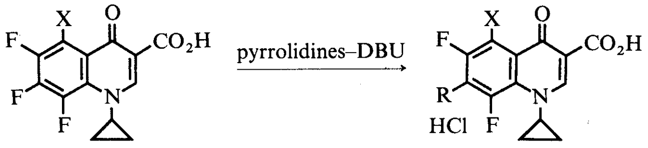
To study the effect of introducing the amino group onto the C-5 position of the quinolone skeleton,[10] we prepared the corresponding C-5 amino derivatives, **3f**—**k**, also shown in Table I. In additions, for comparison of antibacterial activity with that of 4′-methoxy-3′-methylamino-pyrrolidine derivative **3g**, the 4′-ethoxy-3′-methylamino-pyrrolidine derivative **3l** was prepared. These compounds were subjected to biological assay for their antibacterial activities.

Biological Results Tables II and III summarize both *in vitro* and *in vivo* antibacterial activities of all the compounds prepared, **3a**—**l**, presenting *MIC* (minimum inhibitory concentration) and ED_{50} (median effective doses) values against pathogens examined. The ED_{50} by the oral route were determined for acute lethal infection in mice. Bóth *in vitro* and *in vivo* assay methods empolyed in the present work were essentially the same as previously reported (see ref. 11). The characteristic features of the results shown in Tables II and III were as follows. As predicted in our previous work,[1] both pyrrolidine derivatives **3a** and **3c**, when compared with azetidine derivatives, showed higher *in vitro* as well as *in vivo* antibacterial activities against gram-positive and gram-negative bacteria. Comparison of the *MIC* and ED_{50} values of racemic *cis*- and *trans*-3′-amino-4′-methoxypyrrolidine derivatives, **3a** and **3c**, clearly demonstrated a higher activity for the *cis* derivative **3a** than the *trans* **3c** against all pathogens. Their ED_{50} values paralleled the *MIC* values, suggesting similar bioavailability of the two compounds *in vivo*.

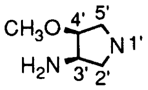
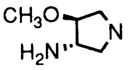
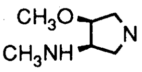
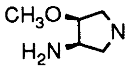
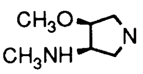
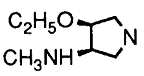
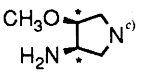
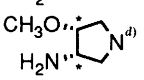
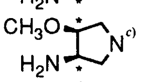
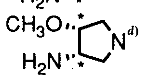
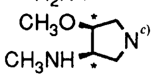
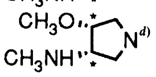
On the other hand, *N*-methyl compound **3b** showed poorer *MIC* values than **3a** against all types of pathogens, revealing that the *in vitro* antibacterial activity was significantly decreased by *N*-methylation of the 3-amino group on the pyrrolidine ring. However, *in vivo*, **3b** and **3a** showed almost the same activity, thus suggesting substantially improved bioavailability for the *N*-methyl compound **3b**. Particularly noteworthy was the superiority of the *in vivo* antibacterial activity of **3b** against *Pseudomonas aeruginosa* SR24. In addition, *N*-methylation clearly abolished the substantial cytotoxicity observed for the unsubstituted compound **3a**. Anothr noteworthy finding was the fact that introduction of the amino group onto the 5-position of the quinolone skeleton improved the *in vitro* antibacterial activity against gram-positive bacteria but not the *in vivo* activity, *e.g.* **3a** *versus* **3f** and **3b** *versus* **3g**. Alkyl-chain elongation from the methoxy derivative **3g** to the ethoxy one **3l** notably diminished both the *in vitro* and *in vivo* antibacterial activities against all pathogens examined.

The stereochemical effects of the amino and methoxy substituent groups upon antibacteial activity were examined. First, the activity of the racemic *cis* compound **3a** lacking the 5-amino substituent group on the quinolone skeleton was compared with those of the corresponding chiral (3′*R*,4′*S*)-(+)-3′-amino-4′-methoxy derivative, **3d**. Surprisingly, no significant differences in activities were noted. However, distinct differences were observed between the corresponding derivatives with the 5-amino substituent, whether or not the 3′-amino group was methylated. Thus, the chiral (3′*S*,4′*R*)-(−)-derivatives, **3i** and **3k**, showed substantially higher *in vitro* antibacterial activities than the corresponding racemates, **3f** and **3g**, whereas the antipode (3′*R*,4′*S*)-(+)-derivatives, **3h** and **3j**, showed

TABLE I. Physical Data of **3**



28a: X = H
28b: X = NH_2
3a—k

No.	R	X	Yield (%)	mp (dec.) °C	Formula	Analysis (%) Calcd (Found) C	H	Cl	F	N	^{1}H-NMR (CD_3OD, J = Hz) or $[\alpha]_D$ (in H_2O)
3a		H	74	261—270[a]	$C_{18}H_{20}ClF_2N_3O_4 \cdot H_2O$	49.83 (50.11	5.11 5.24	8.17 7.98	8.76 8.77	9.69 9.71)	1.25 (4H, m), 3.52 (3H, s), 3.90—4.20 (6H, m), 4.25 (1H, br s), 7.70 (1H, d, J = 14), 8.70 (1H, s)
3c		H	72	255—261[a]	$C_{18}H_{20}ClF_2N_3O_4 \cdot 0.4H_2O$	51.11 (51.29	4.96 4.99	8.38 8.09	8.98 8.99	9.93 10.20)	1.25 (4H, m), 3.49 (3H, s), 3.70—3.95 (4H, m), 4.05—4.30 (3H, m), 7.75 (1H, dd, J = 2, 14), 8.72 (1H, s)
3b		H	77	250—253[a]	$C_{19}H_{22}ClF_2N_3O_4 \cdot 0.2H_2O$	52.65 (52.60	5.21 5.21	8.18 7.89	8.77 8.73	9.64 9.72)	1.25 (4H, m), 2.82 (3H, s), 3.52 (3H, s), 3.85—4.20 (6H, m), 4.30 (1H, br s), 7.77 (1H, d, J = 14), 8.85 (1H, s)
3f		NH_2	88	260—263[b]	$C_{18}H_{21}ClF_2N_4O_4 \cdot 0.5H_2O$	49.15 (49.38	5.04 4.87	8.06 8.34	8.64 8.75	12.74 12.82)	1.15 (4H, m), 3.52 (3H, s), 3.80—4.07 (6H, m), 4.22 (1H, br s), 8.51 (1H, s)
3g		NH_2	82	266—270[b]	$C_{19}H_{23}ClF_2N_4O_4 \cdot 0.4H_2O$	50.48 (50.62	5.31 5.48	7.84 7.87	8.40 8.21	12.39 12.18)	1.15 (4H, m), 2.82 (3H, s), 3.53 (3H, s), 3.84—4.08 (6H, m), 4.27 (1H, br s), 8.53 (1H, s)
3l		NH_2	91	278—286[b]	$C_{20}H_{25}ClF_2N_4O_4 \cdot 0.5H_2O$	51.34 (51.61	5.60 5.71	7.58 7.38	8.12 7.75	11.97 12.08)	1.20 (4H, m), 1.31 (3H, dd, J = 7, 7), 2.84 (3H, s), 3.65 (1H, dq, J = 7, 7), 3.80 (1H, dq, J = 7, 7), 3.85—4.15 (6H, m), 4.38 (1H, br s), 8.53 (1H, s)
3d		H	77	275—280[a]	$C_{18}H_{20}ClF_2N_3O_4 \cdot 0.5H_2O$	50.89 (51.14	4.98 4.90	8.35 8.61	8.94 8.71	9.89 10.03)	$[\alpha]_D^{24}$ +233.1° (c = 2.01)
3e		H	76	277—283[a]	$C_{18}H_{20}ClF_2N_3O_4 \cdot 0.5H_2O$	50.89 (50.85	4.98 4.79	8.35 8.52	8.94 9.11	9.89 9.96)	$[\alpha]_D^{24}$ −234.2° (c = 2.01)
3h		NH_2	76	260—265[b]	$C_{18}H_{21}ClF_2N_4O_4 \cdot H_2O$	48.17 (48.26	5.16 5.20	7.90 7.81	8.47 8.27	12.48 12.51)	$[\alpha]_D^{25}$ +287.2° (c = 2.01)
3i		NH_2	69	261—265[b]	$C_{18}H_{21}ClF_2N_4O_4 \cdot H_2O$	48.17 (48.10	5.16 5.21	7.90 7.73	8.47 8.28	12.48 12.46)	$[\alpha]_D^{25}$ −286.3° (c = 2.00)
3j		NH_2	79	260—265[b]	$C_{19}H_{23}ClF_2N_4O_4 \cdot 0.5H_2O$	50.28 (50.44	5.33 5.54	7.81 7.60	8.37 8.00	12.34 12.16)	$[\alpha]_D^{25}$ +361.1° (c = 1.00)
3k		NH_2	77	264—270[b]	$C_{19}H_{23}ClF_2N_4O_4 \cdot 0.5H_2O$	50.28 (50.56	5.33 5.50	7.81 7.70	8.37 8.18	12.34 12.22)	$[\alpha]_D^{25}$ −364.8° (c = 1.00)

a) Colorless crystal. *b*) Yellow crystal. *c*) (3′*R*,4′*S*). *d*) (3′*S*,4′*R*).

lower activities than the racemates, **3f** and **3g**. Interestingly, these chirality effects were stronger on the *in vivo* activity. The findings from these biological tests showed that compound **3k** had the highest *in vivo* and excellent *in vitro* antibacterial activity against all pathogens. Furthermore, this compound did not display the cytotoxicity observed with **3a**, **3f**, **3h** and **3i**. We therefore selected **3k** as our candidate for further evaluation of biological activities.

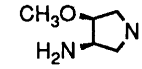
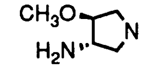
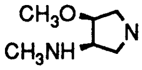
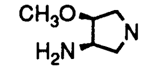
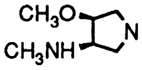
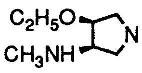
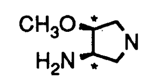
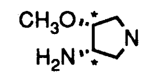
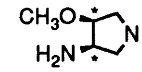
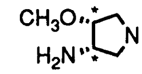
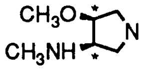
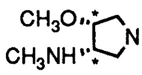
Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and were not corrected. IR spectra were recorded on a Hitachi 260-10 IR spectrophotometer. The ^{1}H-NMR spectra were taken on a Varian VXR-200 spectrometer for organic and D_2O solutions using tetramethylsilane (TMS) and 3-trimethylsilyl-1-propanesulfonic acid sodium salt (DSS) as an internal standard, respectively, and their chemical shifts were given on a ppm scale. The optical rotations were measured on a Perkin-Elmer model 241 polarimeter. Column chromatography was performed on Merck Silica gel 60 (230—400 or 70—230 mesh).

1-*tert*-Butoxycarbonyl-3,4-epoxypyrrolidine (6) Into an ice-cooled solution of 3-pyrroline (**4**) (50.0 g, 0.723 mol) in 300 ml of MeOH was added dropwise di-*tert*-butyl dicarbonate (173.5 g, 0.795 mol) and the reaction mixture was kept at room temperature for 20 h. After evaporation of the solvent, the residue was dissolved in 800 ml of CH_2Cl_2 and then 3-chloroperbenzoic acid (purity of 80%, 250 g, 1.160 mol) was added over a period of 5.0 h at room temperature. After stirring at room temperature for 20 h, the precipitates that appeared were filtered off and the filtrate was successively washed with aqueous $NaHSO_3$, $NaHCO_3$ and water, dried over $MgSO_4$, concentrated *in vacuo*. The residue was column chromatographed on silica gel using toluene–AcOEt (5 : 1) as an eluent, giving **6** (109.0 g, 74%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (9H, s, *tert*-Bu), 3.27 (2H, dd, J = 13, 2 Hz, C_3-H, C_4-H), 3.45—3.83 (4H, m, C_2-H, C_5-H).

1-*tert*-Butoxycarbonyl-*trans*-3-hydroxy-4-methoxypyrrolidine (7) The ring opening of epoxide **6** was achieved by stirring the solution of **6** (54.9 g, 0.296 mol) in 2 N LiOMe in MeOH (670 ml, 1.35 mol) for 3 d at room

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

No.	R	X	Sa(S)[b)]	Sa(R)[c)]	Sp[d)]	Sn[e)]	Ec(S)[f)]	Ec(R)[g)]	Kp[h)]	Pv[i)]	Ecl[j)]	Pa[k)]
3a		H	0.05	0.1	0.4	0.2	0.05	0.4	0.1	0.2	0.1	0.8
3c		H	0.1	0.1	0.8	0.8	0.05	0.8	0.1	0.2	0.2	1.6
3b		H	0.2	0.2	0.8	0.4	0.1	1.6	0.2	0.4	0.2	3.2
3f		NH_2	0.025	0.05	0.2	0.2	0.025	0.2	0.1	0.2	0.1	0.4
3g		NH_2	0.05	0.05	0.4	0.1	0.05	0.4	0.1	0.4	0.2	1.6
3l		NH_2	0.1	0.1	0.8	0.2	0.1	1.6	0.2	0.8	0.4	3.2
3d		H	0.05	0.1	0.2	0.2	0.05	0.4	0.1	0.1	0.1	0.8
3e		H	0.05	0.05	0.2	0.1	0.025	0.2	0.1	0.1	0.1	0.8
3h		NH_2	0.05	0.05	0.4	0.2	0.025	0.4	0.1	0.1	0.1	0.4
3i		NH_2	0.025	0.025	0.2	0.05	0.025	0.2	0.1	0.1	0.05	0.4
3j		NH_2	0.1	0.1	0.8	0.2	0.2	0.4	0.2	0.4	0.2	1.6
3k		NH_2	0.05	0.05	0.4	0.1	0.05	0.4	0.1	0.4	0.2	0.8

a) *MIC* (minimum inhibitory concentrations) were determined by the agar dilution method. Inoculation was performed with one loopful of 10^6 cells per ml. *b)* *Staphylococcus aureus* SMITH. *c)* *Staphylococcus aureus* C-14. *d)* *Streptococcus pyogenes* C-203. *e)* *Streptococcus pneumoniae* type 1. *f)* *Escherichia coli* EC-14. *g)* *Escherichia coli* SR73. *h)* *Klebsiella pneumoniae* SR1. *i)* *Proteus vulgaris* CN-329. *j)* *Enterobacter cloacae* SR233. *k)* *Pseudomonas aeruginosa* SR24.

TABLE III. *In Vivo* Antibacterial Activity ED_{50} (mg/kg)[a)]

No.	Organism			
	Sa(S)	Sp	Ec(S)	Pa
3a	1.68 (0.05)	10.5 (0.4)	0.46 (0.05)	11.5 (0.8)
3c	2.77 (0.1)	33.1 (0.8)	1.30 (0.05)	18.0 (1.6)
3b	1.80 (0.2)	19.0 (0.8)	0.57 (0.1)	6.78 (3.2)
3f	1.52 (0.025)	29.0 (0.2)	0.85 (0.025)	17.2 (0.4)
3g	1.14 (0.05)	28.4 (0.4)	0.38 (0.05)	10.5 (1.6)
3l	1.63 (0.1)	N.D.[b)]	0.63 (0.1)	N.D.[b)]
3d	1.71 (0.05)	10.5 (0.2)	0.49 (0.05)	10.2 (0.8)
3e	1.52 (0.05)	12.5 (0.2)	0.34 (0.025)	11.5 (0.8)
3h	4.53 (0.05)	65.1 (0.4)	1.70 (0.025)	38.1 (0.4)
3i	0.84 (0.025)	21.3 (0.2)	0.46 (0.025)	16.8 (0.4)
3j	2.78 (0.1)	67.0 (0.8)	1.13 (0.2)	19.0 (1.6)
3k	0.76 (0.05)	14.5 (0.4)	0.35 (0.05)	7.46 (0.8)

a) Median effective dose. Compounds were administered orally 1 h after intraperitoneal infection of mice. Each value in parenthesis shows *MIC* from Table II. *b)* Not done.

tempeature. After neutralization with AcOH (85 ml) under ice-cooling, the solvent was evaporated and the residue was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel using toluene–AcOEt (1 : 1) as an eluent and crystallized from *n*-hexane–ether to give **7** (53.3 g, 83%) as colorless prisms, mp 74—75 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s, *tert*-Bu), 2.45 (1H, br s, OH), 3.37 (3H, s, CH_3O), 3.30—3.62 (4H, m, C_2-H, C_5-H), 3.70 (1H, m, C_4-H), 4.24 (1H, m, C_3-H). *Anal.* Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H. 8.81; N, 6.54. Found: C, 55.04; H, 8.64; N, 6.38.

1-*tert*-Butoxycarbonyl-*trans*-3-methanesulfonyloxy-4-methoxypyrrolidine (8) Methanesulfonyl chloride (14.9 ml, 0.193 mol) was added to a mixed solution of **7** (38.0 g, 0.175 mol) in CH_2Cl_2 (200 ml) and pyridine (100 ml) at 0 °C. After stirring for 4 h at room temperature, the reaction mixture was washed with water, dried over $MgSO_4$, and concentrated to give **8** (50.1 g, 97%) as a light brown oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, *tert*-Bu), 3.08 (3H, s, CH_3SO), 3.42 (3H, s, CH_3O), 3.42—3.80 (4H, m, C_2-H, C_5H), 4.00 (1H, m, C_4-H), 5.05 (1H, m, C_3-H).

1-*tert*-Butoxycarbonyl-*cis*-3-azido-4-methoxypyrrolidine (9) A mixture of mesylate **8** (50.0 g, 0.169 mol), NaN_3 (22.0 g, 0.338 mol) and tetrabutylammonium bromide (2.70 g, 0.008 mol) in DMF (150 ml) was stirred at 100 °C for 20 h. The reaction mixture was poured into water and extracted with toluene. The organic layer was washed with water, dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel using toluene–AcOEt (5 : 1) as an eluent to give **9** (31.1 g, 76%) as a colorless oil. IR (neat): 2100, 1698, 1404 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.46 (9H, s, *tert*-Bu), 3.46 (3H, s, CH_3O), 3.25—3.58 (4H, m, C_2-H, C_5-H), 3.80—3.95 (2H, m, C_3-H, C_4-H).

1-*tert*-Butoxycarbonyl-*cis*-3-amino-4-methoxypyrrolidine (10) Azide **9** (31.0 g, 0.126 mol) was hydrogenated under 4 atm over 10% Pd–C (2.0 g) in MeOH (150 ml) at room temperature. The catalyst was filtered off and the filtrate was concentrated to give **10** (28.2 g, 75%) as a light yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s, *tert*-Bu), 1.64 (2H, br s, NH_2), 3.08 (1H, m, C_3-H), 3.40 (3H, s, CH_3O), 3.35—3.55 (4H, m, C_2-H, C_5-H), 3.65 (1H, m, C_4-H).

***cis*-3-Amino-4-methoxypyrrolidine Dihydrochloride (11)** Methoxyamine **10** (1.00 g, 4.62 mmol) was added to the methanol solution of HCl (1.7 M 20 ml) and the mixture was stirred at room temperature for 18 h. After evaporation of the solvent, the resultant crystals were washed with MeOH, giving **11** (690 mg, 79%) as hygroscopic crystals, mp 220—223 °C (dec.). ^{1}H-NMR (D_2O) δ: 3.40—3.85 (4H, m, C_2-H, C_5-H), 4.19 (1H, m, C_3-H), 4.33 (1H, m, C_4-H). *Anal.* Calcd for $C_5H_{14}Cl_2N_2O$: C, 31.76; H, 7.46; N, 14.82. Found: C, 31. 47; H, 7.46; N, 14.61.

1-*tert*-Butoxycarbonyl-*trans*-3-amino-4-hydroxypyrrolidine (13) A solution of epoxide **6** (2.00 g, 10.8 mmol) in a mixture of 28% NH_4OH (20 ml) and MeOH (30 ml) was allowed to stand at room temperature for 7 d. After evaporation of the solvent, the residue was crystallized from ether to give **13** (1.92 g, 88%) as colorless needles, mp 115—117 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s, *tert*-Bu), 2.10 (3H, br s, NH_2, OH), 3.12 (1H, m, C_3-H), 3.30 (2H, m, C_2-H), 3.66 (2H, m, C_5-H), 4.00 (1H, m, C_4-H). *Anal.* Calcd for $C_9H_{18}N_2O_3$: C, 53.45; H, 8.97; N, 13.85. Found: C, 53.29; H, 8.99; N, 13.90.

1-*tert*-Butoxycarbonyl-*trans*-3-(2′-hydroxy)benzylideneamino-4-hydroxypyrrolidine (14) A mixed solution of aminoalcohol **13** (1.50 g, 7.42 mmol) and salicylaldehyde (866 μl, 8.16 mmol) in dry EtOH (50 ml) was refluxed for 4 h. After evapration of the solvent, the residue was crystallized from ether, giving **14** (2.07 g, 91%) as yellow crystals, mp 125—126 °C. ^{1}H-NMR δ: 1.46 (9H, s, *tert*-Bu), 3.30—3.55 (2H, m, C_2-H), 3.72—3.89 (3H, m, C_3-H, C_5-H), 4.26 (1H, m, C_4-H), 6.8—7.9 (4H, m, aromatic), 8.42 (1H, s, CH=N).

***trans*-3-(2′-Methoxy)benzylideneamino-4-methoxypyrrolidine (15)** Into a stirred solution of imine **14** (1.50 g, 4.89 mmol) in tetrahydrofuran (THF) (50 ml) was added NaH (60% oil dispersion, 468 mg, 11.7 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. Next, MeI (5.0 ml) was added to the reaction mixture which was then stirred for 2 h. After evaporation of the solvent, AcOEt and water were added to the residue. The AcOEt layer was separated, dried over $MgSO_4$ and concentrated *in vacuo* to give **15** (1.52 g, 93%) as a light yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, *tert*-Bu), 3.38 (3H, s, CH_3O), 3.30—3.55 (2H, m, C_2-H), 3.60—4.02 (4H, m, C_3-H, C_4-H, C_5-H), 3.87 (3H, s, CH_3O-Ar), 6.9—8.0 (4H, m, aromatic), 8.71 (1H, s, CH=N).

***trans*-3-Amino-4-methoxypyrrolidine Dihydrochloride (16)** HCl in MeOH (3.0 M, 3 ml) was added to **15** (1.00 g, 3.00 mmol) at 0 °C and the solution was stirred at room temperature for 4 h. During this time, precipitation of crystalline products was completed and resultant crystal were collected by filtration and dried, giving **16** (520 mg, 92%) as colorless prisms, mp 270—275 °C (dec.). ^{1}H-NMR (D_2O) δ: 3.44 (3H, s, CH_3O), 3.42—4.04 (4H, m, C_2-H, C_5-H), 4.12 (1H, m, C_3-H), 4.36 (1H, m, C_4-H). *Anal.* Calcd for $C_5H_{14}Cl_2N_2O$: C, 31.76; H, 7.46; N, 14.82. Found: C, 31.78; H, 7.26; N, 14.62.

Optical Resolution of 1-*tert*-Butoxycarbonyl-*cis*-3-amino-4-methoxypyrrolidine (10) A mixed solution of *cis* racemate **10** (10.0 g, 46 mmol) and L-tartaric acid (10.0 g, 67 mmol) in MeOH (50 ml) was allowed to stand at room temperature for 3 h. The resulting crystalline precipitates were collected, washed with MeOH, and recrystallized from MeOH, giving L-tartaric acid salt **17** (6.12 g). The mother liquid **18** was combined, concentrated *in vacuo*, and dissolved in water. After neutralization with K_2CO_3, the resultant free amine was extracted with AcOEt, and the AcOEt layer was concentrated *in vacuo*. The resultant amine residue (6.16 g) was added to a solution of D-tartaric acid (6.00 g, 40 mmol) in MeOH (40 ml), and the mixture was allowed to stand for 3 h at room temperature. The resultant crystalline precipitates were collected by filtration and recrystallized from MeOH, giving D-tartaric acid salt **19** (5.62 g). The mother liquid was treated again in the manner described, the gave additional L- and D-tartaric acid salts, **17** (0.57 g) and **19** (0.37 g), respectively. L-Tartaric acid salt **17**: 79% yield, mp 197—198 °C (dec.). $[\alpha]_D^{25}$ +34.1° (c=1.00, MeOH). *Anal.* Calcd for $C_{10}H_{20}N_2O_3 \cdot C_4H_6O_6$: C, 45.90; H, 7.15; N, 7.65. Found: C, 45.69; H, 6.96; N, 7.63. D-Tartaric acid salt **19**: 71% yield, mp 197—198 °C (dec.). $[\alpha]_D^{25}$ −33.4° (c=1.05, MeOH). *Anal.* Calcd for $C_{10}H_{20}N_2O_3 \cdot C_4H_6O_6$: C, 45.90; H, 7.15; N, 7.65. Found: C, 45.76; H, 6.89; N, 7.63.

(3*R*,4*S*)-(+)-3-Amino-1-*tert*-butoxycarbonyl-4-methoxypyrrolidine (20) A solution of L-tartaric acid salt **17** (6.69 g, 18 mmol) in saturated NaCl (30 ml) was neutralized with K_2CO_3 and extracted with AcOEt. The AcOEt layer was dried over $MgSO_4$ and concentrated *in vacuo*, giving **20** (3.80 g, 96%) as colorless oil. $[\alpha]_D^{25}$ +28.5° (c=1.41, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s, *tert*-Bu), 2.27 (2H, br s, NH_2), 3.10 (1H, m, C_3-H), 3.28—3.64 (4H, m, C_2-H, C_5-H), 3.40 (3H, s, CH_3O), 3.70 (1H, m, C_4-H).

(3*S*,4*R*)-(−)-3-Amino-1-*tert*-butoxycarbonyl-4-methoxypyrrolidine (21) D-Tartaric acid salt **19** was converted to **21** as described above: $[\alpha]_D^{25}$ −28.2° (c=1.16, MeOH).

(3*R*,4*S*)-(+)-3-Amino-4-methoxypyrrolidine Dihydrochloride (22) Following the procedure described for **11**, compound **20** was converted to hydroscopic crystals **22** (1.58 g, 90%): mp 207—208 °C. $[\alpha]_D^{24}$ +54.5° (c=1.11, MeOH). *Anal.* Calcd for $C_{15}H_{14}Cl_2N_2O$: C, 31.76; H, 7.46; Cl, 37.50; N, 14.82. Found: C, 31.43; H, 7.34; Cl, 37.51; N. 14.83.

(3*S*,4*R*)-(−)-3-Amino-4-methoxypyrrolidine Dihydrochloride (23) In the same way, compound **21** was converted to hygroscopic crystals **23** (1.63 g, 93%): mp 208—209 °C. $[\alpha]_D^{24}$ −54.4° (c=0.81, MeOH). *Anal.* Calcd for $C_{15}H_{14}Cl_2O$: C, 31.76; H, 7.46; Cl, 37.50; N, 14.82. Found: C, 31.43; H, 7.31; Cl, 37.51; N, 14.58.

(3*R*,4*S*)-(+)-1-*tert*-Butoxycarbonyl-3-(*tert*-butoxycarbonyl)methylamino-4-methoxypyrrolidine (24) Di-*tert*-butyldicarbonate (2.0 ml, 8.67 mmol) was added to a solution of **20** (1.50 g, 6.93 mmol) in MeOH (10 ml) at 0 °C. After being stirred for 30 min at room temperature, the mixture was concentrated *in vacuo* and well tritulated with *n*-hexane to leave a crystalline product. The crystals were added to a suspension of NaH (60% oil dispersion, 414 mg, 10.4 mmol) in DMF (10 ml) at 0 °C. After being stirred at room temperature for 10 min, the reaction mixture was cooled to 0 °C and CH_3I (4.3 ml) was added. After being stirred at room temperature for 30 min, the mixture was poured into water and extraced with AcOEt. The organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The result was column chromatographed on silica gel using toluene–AcOEt (7:1) as an eluent, giving **24** (2.25 g, 98%) as a colorless oil. $[\alpha]_D^{25}$ +53.7° (c=1.00, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, *tert*-Bu), 1.48 (9H, *tert*-Bu), 2.91 (3H, s, CH_3N), 3.34 (3H, s, CH_3O), 3.42—3.61 (4H, m, C_2-H, C_5-H), 3.92 (1H, m, C_3H), 4.55 (1H, m, C_4-H).

(3*S*,4*R*)-(−)-1-*tert*-Butoxycarbonyl-3-(*tert*-butoxycarbonyl)methylamino-4-methoxypyrrolidine (25) In the same way, compound **21** was converted to **25** as a colorless oil. $[\alpha]_D^{25}$ −54.4° (c=1.07, MeOH).

(3*R*,4*S*)-(+)-4-Methoxy-3-methylaminopyrrolidine Dihydrochloride (26) According to the procedure described for **11**, two Boc groups of compound **24** (2.10 g, 6.4 mmol) were removed and the resultant product was recrystallized from EtOH, giving hygroscopic crystals **26** (1.25 g, 97%), mp 180—182 °C. $[\alpha]_D^{24}$ +54.1° (c=0.97, MeOH). ^{1}H-NMR (CD_3OD) δ: 2.81 (3H, s, CH_3N), 3.47 (3H, s, CH_3O), 3.27—3.88 (4H, m, C_2-H, C_5-H)), 4.05 (1H, m, C_3-H), 4.35 (1H, m, C_4-H). *Anal.* Calcd for $C_6H_{16}Cl_2N_2O \cdot 0.2H_2O$: C, 34.86; H, 8.00; Cl, 34.30; N, 13.55. Found: C, 35.06; H, 7.90; Cl, 34.58; N, 13.52.

(3*S*,4*R*)-(−)-4-Methoxy-3-methylaminopyrrolidine Dihydrochloride (27) In the same way, compound **25** was converted to hygroscopic crystals **27**, mp 181—182 °C. $[\alpha]_D^{24}$ −53.1° (c=1.03, MeOH). *Anal.* Calcd for $C_6H_{16}Cl_2N_2O \cdot 0.2H_2O$: C, 34.86; H, 8.00; Cl, 34.30, N, 13.55. Found: C, 35.11; H, 7.75; Cl, 34.49; N, 13.47.

General Procedure for the Preparation of 7-Substituted Quinolone Derivatives All the quinolone derivatives, **3a**—**l**, were prepared in the manner described below for compound **3k**. Their analytical and physical data are summarized in Table I.

(3′*S*,4′*R*)-(−)-1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(4′-methoxy-3′-methylaminopyrrolidine-1′-yl)-4-oxoquinoline-3-carboxylic Acid Hydrochloride (3k) A mixed solution of trifluoroquinolonecarboxylic acid **28b** (298 mg, 1.00 mmol), (−)-amine HCl salt **27** (305 mg, 1.50 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (600 μl, 4.00 mmol) in CH_3CN (4.0 ml) was refluxed for 1.0 h. After cooling, the resultant crystals were collected and washed with CH_3CN. The obtained crystals (352 mg, mp 251—253 °C) were dissolved in 2 N HCl (1.0 ml), and concentrated. The residue was crystallized from a MeOH–EtOH mixture to give **3k** (350 mg, 77%) as yellow needles: mp 264—270 °C (dec.). $[\alpha]_D^{25}$ −364.8° (c=1.00, H_2O). *Anal.* Calcd for $C_{19}H_{23}ClF_2N_4O_4 \cdot 0.5H_2O$: C, 50.28; H, 5.33; Cl, 7.81; F, 8.37; N, 12.34. Found: C, 50.56; H, 5.50; Cl, 7.70; F, 8.18; N, 12.22. ^{1}H-NMR (CD_3OD) δ: 1.15 (4H, m, C_2-H and C_3-H of cyclopropyl), 2.82 (3H, s, CH_3N), 3.53 (3H, s, CH_3O), 3.82—4.15 (6H, m, pyrrolidyl), 4.27 (1H, br s, C_1-H of cyclopropyl), 8.53 (1H, s, C_2H).

X-Ray Crystallographic Analysis Suitable crystals of **19** for X-ray crystallographic analysis were grown from a MeOH solution. A crystal with dimensions of $0.4 \times 0.4 \times 0.2$ mm^3 was used for data collection. Diffraction measurements were carried out on a Rigaku AFC-5R diffractometer using graphite-monochromated CuK_α radiation (λ= 1.54178 Å). The crystal data are as follows: $C_{10}H_{21}N_2O_3^+ \cdot C_4H_5O_6^-$, M_r=366.36, monoclinic, space group $P2_1$, a=13.916(1) Å, b=9.431(1) Å, c=7.169(1) Å, β=104.75(1)°, Z=2, D_c=1.337 g/cm^3. A total of 1771 independent reflections in the range of $2\theta < 140°$ were measured and corrected for Lorenz and polarization factors, but not for absorption effects. The structure was solved by a direct method, and atomic parameters were refined by a full-matrix least-squares method. The final R value was 0.036 for the 1721 observed reflections $[F_O > 3\sigma(F_O)]$.

Supplementary Material Available Tables of final atomic positional parameters, atomic thermal parameters, and bond length and angles of compound **19** are available. Ordering information is given on the current masthead page.

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References and Notes

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