Studies on Quinolone Antibacterials. V.[1] Synthesis and Antibacterial Activity of Chiral 5-Amino-7-(4-substituted-3-amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acids and Derivatives

Toshihiko YOSHIDA,* Yoichi YAMAMOTO, Hitomi ORITA, Masato KAKIUCHI, Yoshie TAKAHASHI, Masakazu ITAKURA, Noriyuki KADO, Shingo YASUDA, Hideo KATO, and Yasuo ITOH

Research and Development Division, Hokuriku Seiyaku Co., Ltd., 37–1–1 Inokuchi, Katsuyama, Fukui 911, Japan.
Received February 13, 1996; accepted April 2, 1996

We previously demonstrated that 5-amino-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (7) has strong *in vitro* antibacterial activity even against quinolone-resistant bacteria. We examined optimization of the 3-aminopyrrolidine moiety of 7 by introduction of C-alkyl (Me, Et, Pr, di-Me, cyclopropyl) and N-alkyl groups (Me, di-Me). C-Alkylation at the 4-position of the 3-aminopyrrolidine moiety enhanced *in vitro* and *in vivo* antibacterial activity. (*S*)-5-Amino-7-(7-amino-5-azaspiro[2.4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (15h) and (3*S*,4*S*)-5-amino-7-(3-amino-4-methyl-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (15b) showed strong antibacterial activity (*in vitro* antibacterial activity including quinolone-resistant bacteria is 4 times more potent than that of ciprofloxacin (CPFX) (1); *in vivo* antibacterial activity is 1.5 to 20 times more potent than that of CPFX (1)) and reduced quinolone toxicity (free from both phototoxicity at a dosage of 30 mg/kg in guinea pigs (i.v.) and convulsion when coadministered with 4-biphenylacetic acid at a dosage of 20 μg in rats (i.c.v.)). Their selectivity between DNA topoisomerase II (derived from eukaryotic cells) and DNA gyrase (derived from bacterial cells) was about 3000-fold.

Key words 5-amino-8-methylquinolone; antibacterial activity; phototoxicity; convulsive activity; DNA gyrase; DNA topoisomerase II

Quinolones such as ciprofloxacin (CPFX, **1**)[2] and ofloxacin (OFLX, **2**)[3] are amphoteric antibacterial drugs, whose chemical structures are characterized by a fluorine atom at the C-6 position and a basic heterocyclic amine at the C-7 position. The heterocyclic amine plays an important role in the antibacterial activity, side-effects, and pharmacokinetics.[4] These amines are classified on the basis of chemical structure into two major groups, piperazine type, such as CPFX (**1**) and OFLX (**2**), and 3-aminopyrrolidine type, such as tosufloxacin (TFLX, **3**).[5] In general, quinolones bearing a 3-aminopyrrolidine moiety show higher *in vitro* antibacterial activity than those bearing a piperazine moiety when the N-1 substituent is the cyclopropyl group, which is favorable for antibacterial activity.[6] In our previous paper, we discussed the structure–activity relationship of 5- and 8-substituted 7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids (**6**) and found that 5-amino-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (**7**) is highly potent.[1]

On the other hand, it was reported that changing the piperazine moiety to a 3-methyl- or 3,5-dimethylpiperazine moiety, as in lomefloxacin (LFLX, **4**)[7] and sparfloxacin (SPFX, **5**),[8] enhanced antibacterial activity and pharmacokinetics. Thus, better 3-aminopyrrolidine derivatives might be found if C-methylation is applied to the 3-aminopyrrolidine moiety. We carried out optimization of substituents by C-methylation around the amino group of the 3-aminopyrrolidine moiety of **7**. This report describes the effect on the antibacterial activity of alkylation at the 3-aminopyrrolidine moiety and the finding of (3*S*,4*S*)-5-amino-7-(3-amino-4-methyl-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (**15b**) and (*S*)-5-amino-7-(7-amino-5-azaspiro[2.4]hept-5-yl)-1-cycloporopyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (**15h**), as candidate drugs.

Chemistry

The known 3-aminopyrrolidine derivatives were prepared essentially according to the literature.[9] The (+)- and (−)-3-*tert*-butoxycarbonylamino-3-methylpyrrolidine (**11**) were prepared by the method shown in Chart 2. Each optical isomer (**9**) was obtained by optical resolution of the L- or D-tartrate of racemic **8**[10] and the optical purity of each enantiomer was checked by HPLC. Treatment of the chiral amine **9** with di-*tert*-butyl dicarbonate gave the N-protected compound (**10**) which was hydrogenated to give **11**.

The compounds for screening were prepared by method A or method B as shown in Chart 3. Treatment of the carboxylic acid (**12**)[6] with boron trifluoride etherate gave the boron chelate (**13**). Condensation of **13** with 3-aminopyrrolidine derivatives, followed by dechelation, afforded 7-aminoquinolones (**14**), which were hydrolyzed to give the desired compounds **15** (method A). Condensation of **12** with 3-aminopyrrolidine derivatives in dimethyl sulfoxide (DMSO) provided the corresponding 7-substituted quinolones (**14**), which were hydrolyzed to give the desired compounds **15** (method B). The physicochemical data of these compounds (**14**, **15**) are listed in

* To whom correspondence should be addressed.

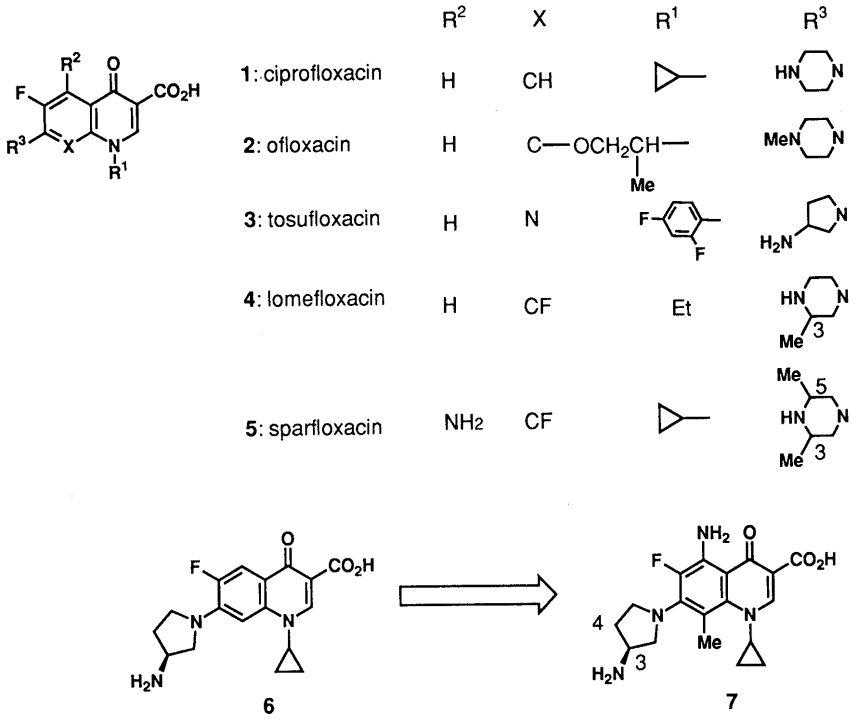
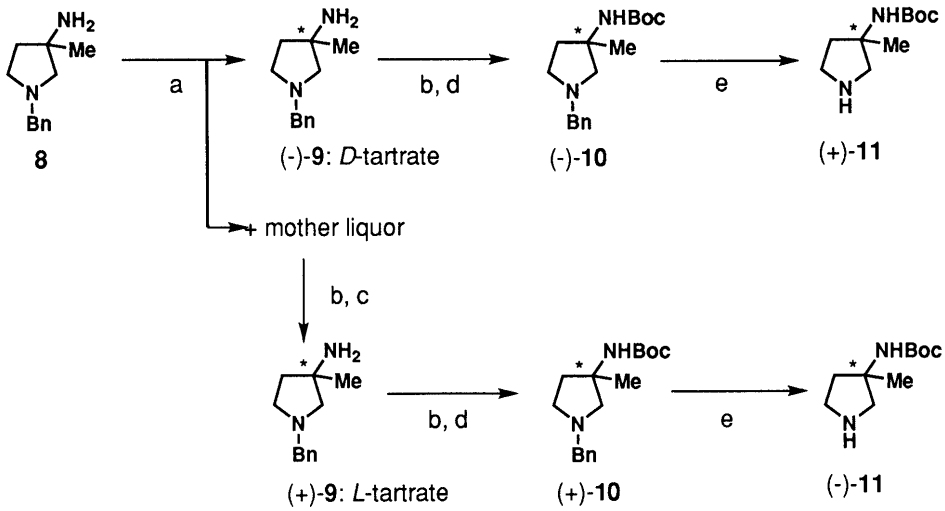


Chart 1



a) *D*-tartaric acid b) aq. NaOH c) *L*-tartaric acid d) Boc_2O e) H_2, 10% Pd/C

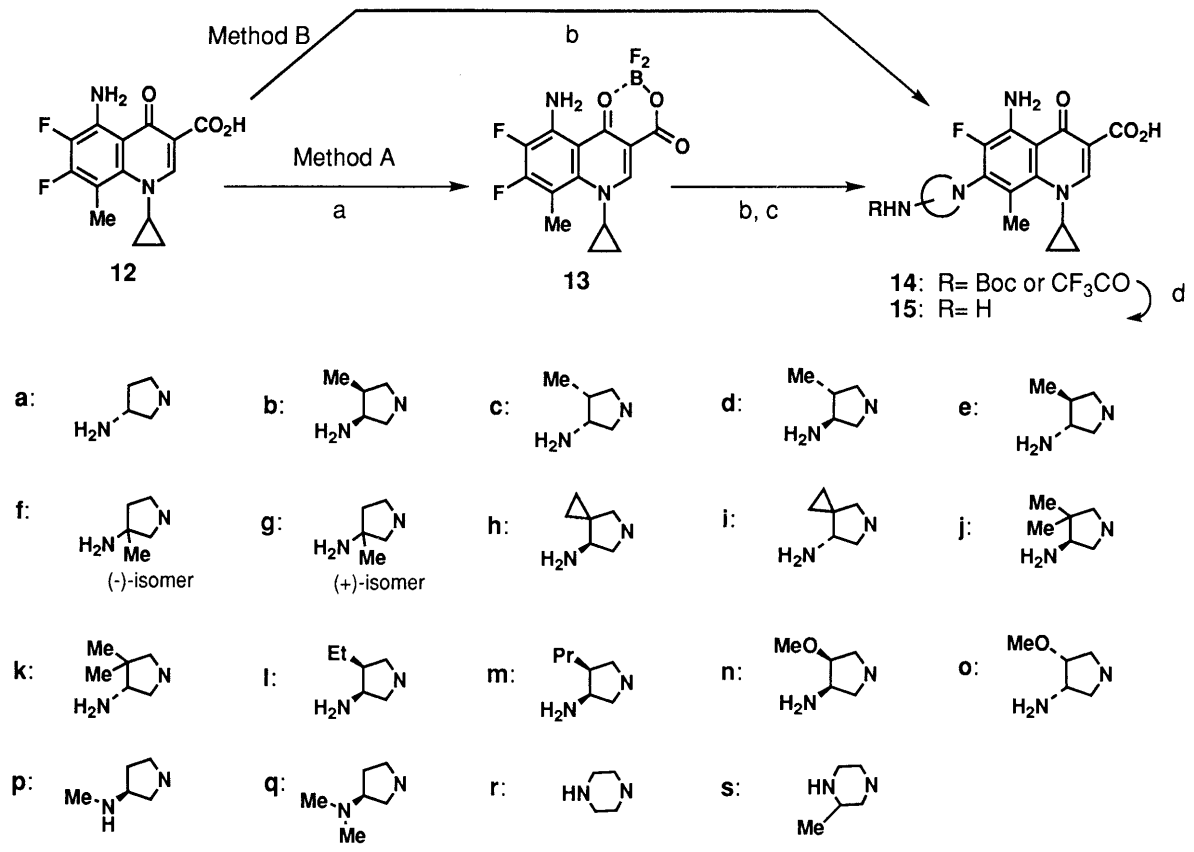
Chart 2

Tables 1 and 2.

Results and Discussion

***In Vitro* Antibacterial Activity** Compounds **15a—s** were tested for *in vitro* antibacterial activity against both standard strains (gram-positive strain, *Staphylococcus aureus* FDA 209P JC-1; gram-negative strain, *Escherichia coli* NIHJ JC-2) and clinical isolates resistant to CPFX (**1**) and OFLX (**2**) (gram-positive bacteria, *Staphylococcus aureus* HPC 292, *Enterococcus faecalis* HPC 975; gram-negative bacteria, *Klebsiella pneumoniae* HNR 828, *Pseudomonas aeruginosa* HNR 1472) and the minimum inhibitory concentrations (*MIC*, μg/ml) are shown in Table 3. The data for **7**, CPFX (**1**), and OFLX (**2**) are included for comparison.

We tested the effect of C-methylation at the 3 or 4 position of the 3-aminopyrrolidine moiety on the *in vitro* antibacterial activity. Among the 4-methyl derivatives (**15b—e**), **15b, d** having the (*S*)-3-aminopyrrolidine moiety were 2 to 16 times more potent than their enantiomers (**15c, e**). Similar results were obtained with other compounds tested (**7** *vs.* **15a**, **15h** *vs.* **15i**, **15j** *vs.* **15k**). Previously, a similar tendency was reported for 7-(3-amino-1-pyrrolidinyl)quinolone and 7-(3-amino-1-pyrrolidinyl)-naphthyridone bearing phenyl,[5b] cyclopropyl[11] and *tert*-butyl groups[9b] at the N-1 position. On the other hand, essentially no difference in antibacterial activities was observed between the diastereomers depending on the



a) $BF_3 \cdot Et_2O$ b) Heterocyclic amine c) NEt_3 in MeOH d) conc. HCl or aq. KOH

Chart 3

configuration of the methyl group (**15b**=**15d**, **15c**=**15e**). This result was different from Di Cesare's report,[9b] which showed that 1-*tert*-butylnaphthyridone having the (3*S*,4*R*)-3-amino-4-methylpyrrolidine moiety was the most potent compound among the four isomers. With regard to the effect of the methyl group, compounds **15b, d** having (*S*)-3-aminopyrrolidine were **2** to **4** times more potent than **7** against gram-positive bacteria and maintained the potency of **7** against gram-negative bacteria. Compounds **15c, e** having (*R*)-3-aminopyrrolidine were 2 to 16 times less potent than **7** against both gram-positive and gram-negative bacteria.

Among the 3-methyl derivatives (**15f, g**), the (−)-enantiomer (**15f**) was 2 to 4 times more active than the (+)-enantiomer (**15g**). Compound **15f** showed similar activity to **7** against gram-positive bacteria, but was 2 to 4 times less potent than **7** against gram-negative bacteria. Recently, Hagen *et al.* reported that racemic 7-(3-amino-3-methylpyrrolidinyl)-1-cyclopropyl-6,8-difluoroquinolone was less potent than the corresponding 3-aminopyrrolidine analog.[12]

This result prompted us to test the effect of several substituents at the 4 position of the (*S*)-3-aminopyrrolidine moiety. We firstly chose symmetrical substituent groups, such as spiro cyclopropyl (**15h**) and dimethyl (**15j**), without the asymmetric carbon at the 4 position, because the configuration of the methyl group did not influence antibacterial activity. Compound **15h** was more potent than **7** against *S. aureus*, and maintained the excellent potency of **7** against gram-negative bacteria. Compound **15h** showed similar activity to the methyl derivatives (**15b, d**), the most potent members of this series. The dimethyl derivative (**15j**) was 4 to 8 times less potent than **7** against gram-negative bacteria. Next, replacing the methyl (**15b**) group with ethyl (**15l**) or propyl (**15m**) maintained the potency against gram-positive bacteria, but decreased the potency against gram-negative bacteria with increasing chain length.

To evaluate the influence of steric bulkiness around the amino group, we synthesized mono- and dimethylamino-pyrrolidine derivatives (**15p, q**). The introduction of mono- and dimethyl groups decreased the potency with increasing number of substituent groups against both gram-positive and gram-negative bacteria. Thus, we consider that the primary amino group on the pyrrolidine moiety is important for *in vitro* antibacterial activity against gram-positive bacteria and gram-negative bacteria. A similar tendency was reported for some quinolone skeletons.[5a,9b]

Okada *et al.*[9g] reported that 5-amino-8-fluoroquinolone having (3*S*,4*R*)-3-amino-4-methoxypyrrolidine was more potent than its enantiomer. This is in contrast to the active configuration of the amino group on 3-amino-4-methylpyrrolidine obtained in our study. To evaluate the effect of 3-amino-4-methoxypyrrolidine on the 5-amino-8-methylquinolone ring, we synthesized 4-methoxy derivatives (**15o, n**). The (*S*)-enantiomer (**15o**) was 2 times more potent than the (*R*)-enantiomer (**15n**). Though it is known

Table 1. Physical and Analytical Data for Intermediate 5-Amino-7-substituted-8-methylquinolones (**14**)

Compd. No.	Protecting group (R)	Method	Yield (%)	mp (°C) (Recryst. solv.)	$[\alpha]_D$[a] (Solv.)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
14a	Boc	A	29	135—137 (CH_2Cl_2–MeOH)	+28.1° (DMF)	$C_{23}H_{29}FN_4O_5$	59.99 (60.09)	6.35 (6.32)	12.17 (12.09)
14b	Boc	A	35	178—180 (Acetone–iso-Pr_2O)	−141.9° ($CHCl_3$)	$C_{24}H_{31}FN_4O_5$	60.75 (60.59)	6.58 (6.55)	11.81 (11.73)
14c	Boc	A	31	179—180 (Acetone–iso-Pr_2O)	+142.6° ($CHCl_3$)	$C_{24}H_{31}FN_4O_5$	60.75 (60.54)	6.58 (6.61)	11.81 (11.69)
14d	Boc	A	33	196—197 (Acetone–iso-Pr_2O)	+44.0° ($CHCl_3$)	$C_{24}H_{31}FN_4O_5$	60.75 (60.70)	6.58 (6.66)	11.81 (11.68)
14e	Boc	A	35	195—196 (Acetone–iso-Pr_2O)	−41.1° ($CHCl_3$)	$C_{24}H_{31}FN_4O_5$	60.75 (60.62)	6.58 (6.68)	11.81 (11.73)
14f	Boc	A	22	213—215 (CH_3CN)	+47.0° (MeOH)	$C_{24}H_{31}FN_4O_5$	60.75 (60.63)	6.58 (6.55)	11.81 (11.80)
14g	Boc	A	18	214—216 (CH_3CN)	−47.8° (MeOH)	$C_{24}H_{31}FN_4O_5$	60.75 (60.85)	6.58 (6.57)	11.81 (11.76)
14h	Boc	A	31	217—219 (CH_2Cl_2–MeOH)	−96.6° (DMF)	$C_{25}H_{31}FN_4O_5$	61.72 (61.71)	6.42 (6.48)	11.52 (11.39)
		B	74						
14i	Boc	B	56	217—218 (CH_2Cl_2–MeOH)	+91.0° (DMF)	$C_{25}H_{31}FN_4O_5$	61.72 (61.65)	6.42 (6.44)	11.52 (11.50)
14j	CF_3CO	A	9	253—255 (MeOH)	+42.6° (MeOH)	$C_{22}H_{24}F_4N_4O_4$	54.54 (54.33)	4.99 (4.88)	11.57 (11.63)
14k	CF_3CO	A	7	256—258 (MeOH)	−44.0° (MeOH)	$C_{22}H_{24}F_4N_4O_4$	54.54 (54.41)	4.99 (5.02)	11.57 (11.45)
14l[b]	Boc	B	61		−213.1° ($CHCl_3$)	$C_{25}H_{33}FN_4O_5$[c]		488.2435 (488.2428)	
14m[b]	Boc	B	51		−216.7° ($CHCl_3$)	$C_{26}H_{35}FN_4O_5$[c]		502.2592 (502.2599)	
14n	CF_3CO	A	15	214—216 (CH_2Cl_2–MeOH)	+107.1° ($CHCl_3$)	$C_{21}H_{22}F_4N_4O_5$	51.85 (51.78)	4.56 (4.69)	11.52 (11.49)
14o	CF_3CO	A	13	214—216 (CH_2Cl_2–MeOH)	−115.8° ($CHCl_3$)	$C_{21}H_{22}F_4N_4O_5$	51.85 (51.81)	4.56 (4.68)	11.52 (11.51)
14p	CF_3CO	A	16	188—190 (MeOH)	−12.0° ($CHCl_3$)	$C_{21}H_{22}F_4N_4O_4$	53.62 (53.57)	4.71 (4.62)	11.91 (11.97)

a) Concentration is $c=0.1$. b) **14l, m** were amorphous compounds. c) High-resolution MS (m/z).

that *in vitro* antibacterial activity depends on the combination of substituents at the N-1, C-5, C-7, and C-8 positions on the quinolone ring,[13] our result is similar to the tendency reported by Okada.[9g] Compound **15o** exhibited excellent antibacterial activity, comparable to that of **7**, against gram-positive bacteria, but was 4 to 8 times less potent than **7** against gram-negative bacteria.

Finally, we synthesized and evaluated two compounds (**15r, s**), for comparison, having piperazine at the C-7 position of the quinolone ring. These compounds (**15r, s**) were 4 to 8 times less potent than **7** against both gram-positive and gram-negative bacteria.

Based on the *in vitro* antibacterial activity studies, we chose three compounds (**15b, d, h**) which were similar in potency to **7** against both gram-positive and gram-negative bacteria to evaluate *in vivo* antibacterial activity.

***In Vivo* Antibacterial Activity** The three compounds (**15b, d, h**) were tested for *in vivo* antibacterial activity against both gram-positive bacteria (*Staphylococcus aureus* Smith) and gram-negative bacteria (*Escherichia coli* KC-14) and the ED_{50} values (mg/kg) of these tested compounds are shown in Table 4. The ED_{50} values by the oral route were determined based on the survival rates on day 7 after infection in mice. The data for **7** and CPFX (**1**) are included for comparison.

Against gram-positive bacteria, the ED_{50} values of the three compounds (**15b, d, h**) having an alkyl group at the 4 position of the (*S*)-3-aminopyrrolidine moiety were about 4.5 to 20 times smaller than that of CPFX (**1**). The (3*S*,4*S*)-3-amino-4-methylpyrrolidine compound (**15b**), which was about 3 times more potent than its diastereomer (**15d**), was the most potent. No clear difference of the effective doses was observed between CPFX (**1**) and **7**.

Against gram-negative bacteria, the ED_{50} values of compounds **15b, d, h** having alkyl groups were more potent than that of CPFX (**1**). Though the *in vitro* antibacterial activity of **7** against gram-negative bacteria was more potent than that of CPFX (**1**), the *in vivo* effect of **7** was lower than that of CPFX (**1**). These results showed a clear improvement of *in vivo* antibacterial activity against both gram-positive and gram-negative bacteria by C-alkylation at the 4 position of the (*S*)-3-aminopyrrolidine moiety.

Next, **15b, d, h** were tested for urinary recovery and the results are shown in Table 4. Urine was collected by placing mice in metabolic cages and microbiologically active compounds in the urine were assayed with *E. coli* KP. The data for **7** and CPFX (**1**) are included for comparison.

The urinary recoveries (0—48 h) of all compounds tested after oral administration were less than that of CPFX (**1**).

Table 2. Physical and Analytical Data for 5-Amino-7-substituted-8-methylquinolones (**15**)

Compd. No.	Yield (%)	mp (°C) (Recryst. solv.)	$[\alpha]_D$[a] (Solv.)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
15a	76	201—205 (dec.) (CH_2Cl_2–MeOH)	+6.0° (DMF)	$C_{18}H_{21}FN_4O_3 \cdot H_2O$	57.13 (57.34)	6.13 (6.09)	14.81 (14.74)
15b	68	212—214 (EtOH–Et_2O)	−163.9° (DMF)	$C_{19}H_{23}FN_4O_3 \cdot 1/4H_2O$	60.23 (60.32)	6.25 (6.32)	14.79 (14.47)
15c	95	212—215 (EtOH–Et_2O)	+173.1° (DMF)	$C_{19}H_{23}FN_4O_3 \cdot 1/2H_2O$	59.52 (59.67)	6.31 (6.31)	14.61 (14.37)
15d	56	187—189 (H_2O)	+180.6° (DMF)	$C_{19}H_{23}FN_4O_3 \cdot 1/2H_2O$	59.52 (59.74)	6.31 (6.07)	14.61 (14.57)
15e	33	187—189 (EtOH)	−178.4° (DMF)	$C_{19}H_{23}FN_4O_3 \cdot 1/2H_2O$	59.52 (59.60)	6.31 (6.20)	14.61 (14.63)
15f	54	261—263 (EtOH–H_2O)	−50.6° (MeOH)	$C_{19}H_{23}FN_4O_3 \cdot CH_3SO_3H$	51.05 (50.89)	5.78 (5.93)	11.91 (11.78)
15g	45	260—262 (EtOH–H_2O)	+46.6° (MeOH)	$C_{19}H_{23}FN_4O_3 \cdot CH_3SO_3H$	51.05 (50.75)	5.78 (5.88)	11.91 (11.69)
15h	50	263—265 (dec.) (EtOH–H_2O)	−93.6° (MeOH)	$C_{20}H_{23}FN_4O_3 \cdot CH_3SO_3H$	52.27 (52.00)	5.64 (5.91)	11.61 (11.42)
15i	55	215—217 (MeOH)	+42.7° (DMF)	$C_{20}H_{23}FN_4O_3$	62.16 (62.05)	6.00 (5.80)	14.50 (14.34)
15j	48	191—193 (CH_3CN)	+190.9° (0.1 N NaOH)	$C_{20}H_{25}FN_4O_3$	61.84 (61.70)	6.49 (6.51)	14.42 (14.32)
15k	77	188—190 (MeOH)	−173.2° (0.1 N NaOH)	$C_{20}H_{25}FN_4O_3 \cdot 1/4H_2O$	61.13 (61.14)	6.54 (6.68)	14.26 (14.09)
15l	59	222—225 (MeOH–H_2O)	−244.8° (0.1 N HCl)	$C_{20}H_{25}FN_4O_3 \cdot 5/4H_2O$	58.45 (58.22)	6.74 (6.52)	13.63 (13.68)
15m	45	183—185 (MeOH–AcOEt)	−223.1° (MeOH)	$C_{21}H_{27}FN_4O_3 \cdot 1/2H_2O$	61.30 (61.29)	6.86 (6.92)	13.62 (13.52)
15n	64	195—197 (CH_3CN)	+231.3° (MeOH)	$C_{19}H_{23}FN_4O_4$	58.45 (58.40)	5.94 (5.89)	14.35 (14.29)
15o	50	191—193 (CH_3CN)	−228.4° (MeOH)	$C_{19}H_{23}FN_4O_4$	58.45 (58.30)	5.94 (5.89)	14.35 (14.37)
15p	59	274—276 (dec.) (EtOH–H_2O)	+2.0° (MeOH)	$C_{19}H_{23}FN_4O_3 \cdot HCl \cdot 1/2H_2O$	54.35 (54.43)	6.00 (5.89)	13.34 (13.32)
15q[b]	12	204—206 (CH_3CN)	+156.0° (MeOH)	$C_{20}H_{25}FN_4O_3$	61.84 (61.72)	6.49 (6.46)	14.42 (14.44)
15r[c]	17	>300 (H_2O)		$C_{18}H_{21}FN_4O_3 \cdot HCl \cdot 3/2H_2O$	51.01 (51.16)	5.94 (5.72)	13.22 (13.21)
15s[c]	4	>300 (H_2O)		$C_{19}H_{23}FN_4O_3 \cdot HCl \cdot 1/4H_2O$	54.94 (55.02)	5.95 (5.88)	13.49 (13.61)

a) Concentration is $c=0.1$. b) **15q** was synthesized by method A. c) **15r, s** were synthesized by method B.

The (3*S*,4*R*)-3-amino-4-methylpyrrolidine derivative (**15d**) showed the lowest urinary recovery (1.8%) among the all compounds tested. Though **15b** and **15h**[14] gave about 3 times lower urinary recovery than CPFX (**1**), these compounds, with potent *in vitro* antibacterial activity, may be suitable for the treatment of urinary tract infections. Generally, quinolone antibacterial agents show high urinary recoveries; for example, that of CPFX (**1**) is about 50% in man.[15] In some cases, blood concentration of quinolone agents in patients with renal functional disorder (aged people or patients with renal failure) increased rapidly, leading to various side-effects.[15] Next, we selected two compounds (**15b, h**)[14] with sufficient *in vivo* antibacterial activity and adequate urinary recovery.

Toxicity in Animal We tested **15b** and **15h** for convulsive activity and phototoxicity. Convulsion is one of the side-effects in the central nervous system (CNS), and it is known that combinations of quinolone and non-steroidal anti-inflammatory drugs (NSAID) induce convulsions.[16] Compounds **15b, h** were subjected to convulsive activity assay by intraperitoneal injection in mice and intracerebroventricular administration in rats with fenbufen, as an NSAID. The results are summarized in Table 5, in which the data for **7**, CPFX (**1**), and OFLX (**2**) are included for comparison.

Compounds **15b, h** and **7**, whose structures are characterized by the (*S*)-3-aminopyrrolidine moiety at the C-7 position on the quinolone ring, were shown to be essentially free from convulsion-inducing activity under the test conditions.

Akahane *et al.* reported that convulsive activity induced by combination of quinolone and NSAID correlated with γ-aminobutyric acid receptor ($GABA_A$) binding inhibition.[16b] We tested **15b** and **15h** by $GABA_A$ receptor binding assay in the presence or absence of 4-biphenylacetic acid (BPAA), a major active metabolite of fenbufen. The results are presented in Table 5 and Fig. 1, in which the data for **7**, CPFX (**1**), and OFLX (**2**) are included for comparison.

Little inhibitory effect of BPAA was seen on

Table 3. *In Vitro* Antibacterial Activity of 5-Amino-7-substituted-8-methylquinolones

Compd. No.	MIC (μg/ml)[a]					
	Standard strains		Clinically isolated bacteria[b]			
	S. aureus FDA 209P	*E. coli* NIHJ JC-2	*S. aurerus* HPC 292	*E. faecalis* HPC 975	*K. pneumoniae* HNR 828	*P. aeruginosa* HNR 1472
15a	0.025	0.025	6.25	3.13	3.13	12.5
15b	0.006	0.006	0.78	0.78	3.13	3.13
15c	0.012	0.025	6.25	3.13	12.5	50
15d	0.006	0.006	0.78	0.78	3.13	3.13
15e	0.025	0.012	6.25	6.25	6.25	25
15f	0.025	0.05	3.13	3.13	6.25	12.5
15g	0.05	0.05	12.5	12.5	12.5	25
15h	0.025	0.012	1.56	1.56	3.13	3.13
15i	0.10	0.10	50	50	25	>100
15j	0.025	0.05	1.56	1.56	12.5	25
15k	0.012	0.05	3.13	6.25	25	>100
15l	0.006	0.012	0.78	0.78	3.13	12.5
15m	0.012	0.05	3.13	1.56	25	100
15n	0.05	0.05	3.13	3.13	12.5	25
15o	0.025	0.05	1.56	1.56	6.25	25
15p	0.012	0.012	3.13	3.13	6.25	12.5
15q	0.05	0.05	6.25	6.25	25	50
15r	0.10	0.05	25	12.5	12.5	25
15s	0.05	0.05	12.5	12.5	12.5	25
7	0.025	0.025	3.13	1.56	3.13	3.13
CPFX (**1**)	0.20	0.025	50	50	12.5	12.5
OFLX (**2**)	0.20	0.10	50	50	25	25

a) Inoculum size, one loopful of 10^6 CFU/ml. *b*) Clinical isolates resistant to CPFX (**1**) and OFLX (**2**).

Table 4. Efficacy on Systemic Infections in Mice and Urinary Recovery in Rats after Oral Administration of Selected Quinolones

Compd. No.	*S. aureus* Smith ED_{50} (mg/kg) [95% confidence limit]		*E. coli* KC-14 ED_{50} (mg/kg) [95% confidence limit]		Cumulative recovery[a] (0—48 h, %)
15b	0.52	[0.33—0.74]	1.14	[0.78—1.63]	6.5
15d	1.77	[1.43—2.19]	1.30	[0.87—1.99]	1.8
15h	2.42	[1.66—3.50]	1.26	[0.72—2.17]	6.7
7	2.87	[1.44—16.33]	2.92	[1.73—5.44]	4.8
CPFX (**1**)	10.84	[6.98—17.69]	1.75	[1.22—2.52]	19.1

a) Urinary recovery was determined by microbiological assay with *E. coli* KP. ($n=5$)

Table 5. Convulsive Activity Tests of Selected Quinolones

Compd. No.	Convulsion: Number of animals with convulsions		$GABA_A$ receptor binding: [^{3}H]SR95531 binding (%) in rat cerebral cortex Mean±S.E. (10^{-5} M)	
	Mice[a] (i.p.) (100 mg/kg)	Rat[b] (i.c.v.) (20 μg)	Alone	with BPAA[c]
15b	0/6	0/3	95.2±3.2	76.0±5.7
15h	0/6	0/3	91.5±3.4	89.4±4.6
7	0/6	0/3	80.6±1.8	68.7±2.9
CPFX (**1**)	3/6	3/3	89.0±1.9	14.1±0.5
OFLX (**2**)	0/6	0/3	92.4±5.9	31.7±6.3

a) Orally coadministered with fenbufen at a dose of 100 mg/kg to male ICR mice. *b*) Intraperitoneally coadministered with fenbufen at a dose of 50 mg/kg to male Wistar rats. *c*) BPAA (4-biphenylacetic acid) 100 μM.

[^{3}H]SR95531 binding of the three compounds (**15b, h, 7**) having an (*S*)-3-aminopyrrolidine moiety. In particular, binding of **15h** was not inhibited even at a high concentration (10^{-5} M) although that of CPFX (**1**) bearing a piperazine moiety was strongly inhibited by BPAA. These results confirm that compounds bearing the (*S*)-3-aminopyrrolidine moiety at the C-7 position should have little convulsive activity in animals when coadministered with an NSAID such as fenbufen.

Next, **15b** and **15h** were subjected to a phototoxicity test by means of intravenous injection in guinea pigs. The criterion is the grade of skin reaction in positive animals, and the results are shown in Table 6, in which data for **7** and CPFX (**1**) are included for comparison. In this test, **15b, h** and **7**, having both an amino group at the C-5 position and a methyl group at the C-8 position were free from phototoxicity in the dosage range of 10—30 mg/kg, and it was confirmed that **15b** and **15h** had less phototoxicity than OFLX (**2**), which does not show phototoxic side-effects at clinically used doses. We showed in our previous paper[1] that the 5-amino-8-methylquinolone skeleton has reduced phototoxicity.

Inhibition of Topoisomerase II (Topo II) Activity The Topo II in eukaryotic cells is required for maintenance of chromosome structure, condensation and separation. It also appears to play a role in DNA replication, transcription and recombination. The bacterial Topo II is called DNA gyrase, and has the same role as Topo II in eukaryotic cells. DNA gyrase is a target enzyme of quinolones. Thus, evaluation of the inhibitory activity against Topo II is important in the development of novel quinolones. Compounds **15b, h** were tested for inhibition of the activity of Topo II (Topo GEN) from human

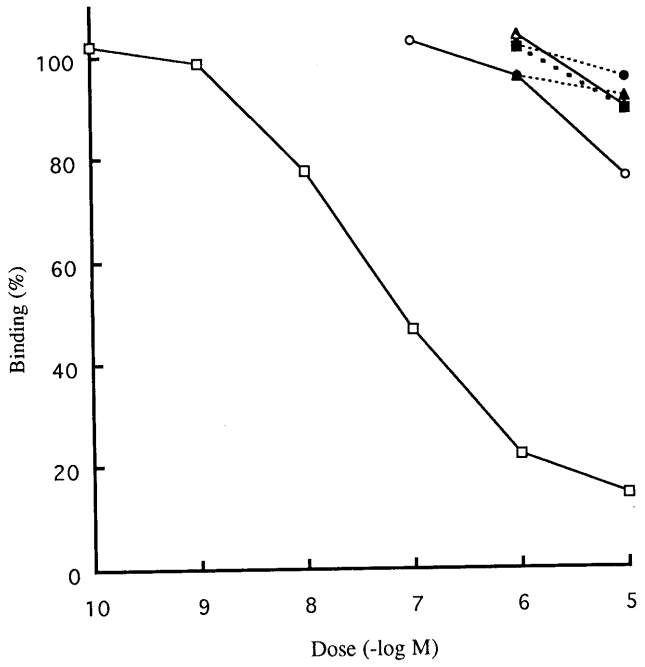


Fig. 1. Inhibitory Effect of Selected Quinolones on $GABA_A$ Receptor Binding

●, **15b**; ○, **15b** + BPAA (100 μM); ▲, **15h**; △, **15h** + BPAA (100 μM); ■, CPFX (**1**); □, CPFX (**1**) + BPAA (100 μM).

Table 6. Phototoxicity Test of Selected Quinolones in Guinea Pigs

Compd. No.	Dose (i.v.) (mg/kg)	Number of animals with phototoxicity Score[a]				
		−	+	++	+++	++++
15b	10	5/5				
	30	5/5				
15h	10	5/5				
	30	5/5				
7	10	5/5				
	30	5/5				
CPFX (**1**)	10	2/5	3/5			
	30		4/5	1/5		
OFLX (**2**)	10	5/5				
	30		5/5			

a) Scoring is as follows: −, no erythema; +, very slight erythema; ++, well defined erythema; +++, moderate to severe erythema; ++++, severe erythema (beet redness) to slight eschar formation (injuries in depth).

Table 7. Selectivity against Topo II of Selected Quinolones

Compd. No.	IC_{50} (μg/ml)		Selectivity Topo II/ DNA gyrase
	DNA gyrase[a]	Topo II[b]	
15b	0.12	401	3342
15h	0.13	393	3023

a) Purified from *E. coli* KL-16. *b*) Purified from human placenta (Topo GEN).

placenta, and DNA gyrase from *E. coli* KL-16. The IC_{50} values (μg/ml) on Topo II were determined by means of relaxing assay and those on DNA gyrase were determined by supercoiling assay. The results are summarized in Table 7.

The IC_{50} values of both **15b** and **15h** against DNA gyrase are about 0.1 μg/ml. These results imply that **15b** and **15h** have sufficient activity against bacterial cells. On the other hand, the IC_{50} values of **15b** and **15h** against Topo II are about 400 μg/ml. Quinolone concentrations in most tissues were found to be in the range of 1—10 μg/ml, so at least a 40-fold selectivity of **15b** and **15h** should exist. The selectivities of both **15b** and **15h** between eukaryotic cells and bacterial cells (ratio of Topo II/DNA gyrase) were more than 3000-fold.

In conclusion, we have sought to find novel quinolones with excellent *in vitro* and *in vivo* antibacterial activity, including activity against quinolone-resistant bacteria, reduced quinolone-type toxicity (particularly phototoxicity and convulsive activity), and sufficient selectivity between Topo II and DNA gyrase by optimization of the 3-aminopyrrolidine moiety at the C-7 position of the 5-amino-8-methylquinolone ring. We found that C-alkylation at the 4 position of the 3-aminopyrrolidine moiety improved the *in vitro* and *in vivo* antibacterial activity, a 3-aminopyrrolidine moiety reduced convulsive activity, and a 5-amino-8-methylquinolone ring reduced phototoxicity. Two of the most potent members of this series, **15b, h** had good selectivity between Topo II and DNA gyrase. Compound **15h** (HSR-903) is presently under clinical study.

Experimental

Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. Elemental analyses were done with a Yanaco MT-5 elemental analysis apparatus. Infrared (IR) spectra were recorded using a Hitachi 270-30 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured with a JEOL JNM-A 500 spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were measured by using a JEOL DX-300 mass spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter. HPLC was performed with a JASCO BIP-1 pumping system and a JASCO UNIDEC-100-V ultraviolet detector. Column chromatography was carried out with silica gel [Kieselgel 60 (Merck)]. TLC was conducted on 0.25 mm pre-coated silica gel plates ($60F_{254}$, Merck). The extracted solvents were dried with Na_2SO_4 and removed by a rotary evaporation under reduced pressure.

(−)-3-Amino-1-benzylpyrrolidine D-Tartrate ((−)-9) A mixed solution of the racemate **8** (55.0 g, 298 mmol) and D-tartaric acid (54.2 g, 361 mmol) in MeOH (150 ml) was allowed to stand at room temperature. The crystalline precipitates that deposited were collected by filtration, washed with MeOH, and recrystallized twice from a mixture of EtOH and water (1 : 1) to afford (−)-**9** (23.3 g, 47%) as colorless needles, mp 192—193 °C. $[\alpha]_D^{20}$ −18.8° (*c* = 0.5, H_2O). MS *m/z*: 190 (M^+). NMR (CD_3OD) δ: 1.48 (3H, s, CH_3), 2.01—2.20 (2H, m, C_4-H_2), 2.60 (1H, d, *J* = 11.0 Hz, C_2-H), 2.70—2.80 (1H, m, C_5-H), 3.09 (1H, d, *J* = 11.0 Hz, C_2-H), 3.15—3.22 (1H, m, C_5-H), 3.82, 3.91 (each 1H, AB-d, *J* = 13.0 Hz, CH_2Ph), 4.40 (2H, s, CH × 2 (tartrate)), 7.25—7.45 (5H, m, Ar-H).

A solution of (−)-**9** in water (100 ml) was neutralized with 10% NaOH and extracted with CH_2Cl_2. The extract was dried and concentrated to afford the free base of (−)-**9** (11.0 g, 40% from **8**) as a colorless oil. $[\alpha]_D^{20}$ +8.2° (*c* = 0.5, MeOH). MS *m/z*: 190 (M^+). NMR ($CDCl_3$) δ: 1.25 (3H, s, CH_3), 1.60—1.90 (2H, m, C_4-H_2), 2.30 (1H, d, *J* = 9.0 Hz, C_2-H), 2.38—2.43 (1H, m, C_5-H), 2.50 (1H, d, *J* = 9.0 Hz, C_2-H), 2.80—2.90 (1H, m, C_5-H), 3.57, 3.61 (each 1H, AB-d, *J* = 13.5 Hz, CH_2Ph), 7.20—7.35 (5H, m, Ar-H). High-resolution MS *m/z*: Calcd for $C_{12}H_{18}N_2$: 190.1471. Found: 190.1476.

(+)-3-Amino-1-benzylpyrrolidine L-Tartrate ((+)-9) The mother liquor of the D-tartrate of **9** was concentrated and the residue was dissolved in water. After neutralization with 10% NaOH, the free amine was extracted with CH_2Cl_2. The extract was dried and concentrated. The resultant amine (38.0 g) was added to a solution of L-tartaric acid (45.0 g, 300 mmol) in MeOH (190 ml), and the mixture was allowed to

stand at room temperature. The crystalline precipitates that deposited were collected by filtration and recrystallized twice from a mixture of EtOH and water (1 : 1) to afford (+)-**9** (16.5 g, 34% from **8**) as colorless needles, mp 190—191 °C. $[\alpha]_D^{20}$ +17.7° (c=0.5, H_2O). MS m/z: 190 (M^+). NMR (CD_3OD) δ: 1.48 (3H, s, CH_3), 2.03—2.20 (2H, m, C_4-H_2), 2.61 (1H, d, J=11.0 Hz, C_2-H), 2.70—2.80 (1H, m, C_5-H), 3.10 (1H, d, J=11.0 Hz, C_2-H), 3.15—3.25 (1H, m, C_5-H), 3.82, 3.91 (each 1H, AB-d, J=13.0 Hz, CH_2Ph), 4.40 (2H, s, CH×2 (tartrate)), 7.25—7.45 (5H, m, Ar-H).

In a similar manner to that described for the preparation of the free base from (−)-**9**, compound (+)-**9** was converted to its free base (7.70 g, 28% from **8**) as a colorless oil. $[\alpha]_D^{20}$ −8.5° (c=0.5, MeOH). MS m/z: 190 (M^+). NMR ($CDCl_3$) δ: 1.25 (3H, s, CH_3), 1.60—1.90 (2H, m, C_4-H_2), 2.30 (1H, d, J=9.0 Hz, C_2-H), 2.38—2.43 (1H, m, C_5-H), 2.50 (1H, d, J=9.0 Hz, C_2-H), 2.80—2.90 (1H, m, C_5-H), 3.57, 3.61 (each 1H, AB-d, J=13.5 Hz, CH_2Ph), 7.20—7.35 (5H, m, Ar-H). High-resolution MS m/z: Calcd for $C_{12}H_{18}N_2$: 190.1471. Found: 190.1463.

(−)-1-Benzyl-3-*tert*-butoxycarbonylamino-3-methylpyrrolidine ((−)-10) A mixture of (+)-3-aminopyrrolidine (free base of (−)-**9**; 10.5 g, 55.2 mmol) and di-*tert*-butyl dicarbonate (12.0 g, 55.2 mmol) in CH_2Cl_2 (105 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography with Et_2O to afford (−)-**10** (16.0 g, 100%) as a pale yellow oil. $[\alpha]_D^{20}$ −4.6° (c=0.5, MeOH). MS m/z: 290 (M^+). NMR ($CDCl_3$) δ: 1.43 (9H, s, $C(CH_3)_3$), 1.45 (3H, s, CH_3), 1.80—1.90 (2H, m, C_4-H), 2.00—2.10 (1H, m, C_4-H), 2.48 (1H, d, J=9.0 Hz, C_2-H), 2.55—2.60 (1H, m, C_5-H), 2.70—2.78 (1H, m, C_5-H), 2.74 (1H, d, J=9.0 Hz, C_2-H), 3.57, 3.62 (each 1H, AB-d, J=13.5 Hz, CH_2Ph), 4.71 (1H, br s, CONH), 7.20—7.30 (5H, m, Ar-H). High-resolution MS m/z: Calcd for $C_{17}H_{26}N_2O_2$: 290.1996. Found: 290.1984.

(+)-1-Benzyl-3-*tert*-butoxycarbonylamino-3-methylpyrrolidine ((+)-10) In a similar manner to that described for the preparation of (−)-**10** from (−)-**9**, compound (+)-**9** was converted to (+)-**10** (98%) as a colorless oil. $[\alpha]_D^{20}$ +4.4° (c=0.5, MeOH). MS m/z: 290 (M^+). NMR ($CDCl_3$) δ: 1.43 (9H, s, $C(CH_3)_3$), 1.45 (3H, s, CH_3), 1.80—1.90 (2H, m, C_4-H), 2.00—2.10 (1H, m, C_4-H), 2.48 (1H, d, J=9.0 Hz, C_2-H), 2.55—2.60 (1H, m, C_5-H), 2.70—2.78 (1H, m, C_5-H), 2.74 (1H, d, J=9.0 Hz, C_2-H), 3.57, 3.62 (each 1H, AB-d, J=13.5 Hz, CH_2Ph), 4.71 (1H, br s, CONH), 7.20—7.30 (5H, m, Ar-H). High-resolution MS m/z: Calcd for $C_{17}H_{26}N_2O_2$: 290.1996. Found: 290.2000.

(+)-3-*tert*-Butoxycarbonylamino-3-methylpyrrolidine ((+)-11) A suspension of the 1-benzylpyrrolidine ((−)-**10**; 15.5 g, 53.4 mmol) and 10% Pd/C (3.74 g) in EtOH (160 ml) was stirred at 65 °C under a hydrogen atmosphere (50 kgf/cm^2) for 4 h. The catalyst was removed by filtration and washed with MeOH. The filtrate was concentrated to afford (+)-**11** (10.5 g, 98%) as a pale yellow oil. $[\alpha]_D^{20}$ +8.1° (c=0.5, MeOH). IR (liq.): 1696 (CONH) cm^{-1}. MS m/z: 201 (M^++H). NMR (CD_3Cl) δ: 1.41 (3H, s, CH_3), 1.44 (9H, s, $C(CH_3)_3$), 1.38—1.45 (1H, m, C_4-H), 2.01 (1H, br s, NH), 2.71 (1H, d, J=11.5 Hz, C_2-H), 2.90—2.95 (1H, m, C_5-H), 3.07—3.12 (1H, m, C_4-H), 3.17 (1H, d, J=11.5 Hz, C_2-H), 4.61 (1H, br s, CONH). High-resolution MS m/z: Calcd for $C_{10}H_{21}N_2O_2$: 201.1602. Found; 201.1601.

(−)-3-*tert*-Butoxycarbonylamino-3-methylpyrrolidine ((−)-11) In a similar manner to that described for the preparation of (+)-**11** from (−)-**10**, compound (+)-**10** was converted to (−)-**11** (98%) as a colorless oil. $[\alpha]_D^{20}$ −8.2° (c=0.5, MeOH). IR (liq.): 1696 (CONH) cm^{-1}. MS m/z: 201 (M^++H). NMR (CD_3Cl) δ: 1.41 (3H, s, CH_3), 1.44 (9H, s, $C(CH_3)_3$), 1.38—1.45 (1H, m, C_4-H), 2.01 (1H, br s, NH), 2.71 (1H, d, J=11.5 Hz, C_2-H), 2.90—2.95 (1H, m, C_5-H), 3.07—3.12 (1H, m, C_4-H), 3.17 (1H, d, J=11.5 Hz, C_2-H), 4.61 (1H, br s, CONH). High-resolution MS m/z: Calcd for $C_{10}H_{21}N_2O_2$: 201.1602. Found; 201.1604.

[5-Amino-1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylate-O^3,O^4]difluoroboron (13) A mixture of quinolinecarboxylic acid (**12**; 5.00 g, 17.0 mol) and boron trifluoride etherate (3.13 ml, 24.5 mol) in methyl isobutyl ketone (75.5 ml) was heated under reflux for 1 h. After cooling, the precipitates that deposited were collected by filtration and washed with Et_2O to give **13** (5.38 g, 93%) as yellow crystals. IR (KBr): 1696 (CO_2) cm^{-1}. NMR (DMSO-d_6) δ: 1.08—1.15 (2H, m, CH_2), 1.21—1.13 (2H, m, CH_2), 2.67 (3H, d, J=2.5 Hz, Me), 4.52—4.59 (1H, m, CH), 7.28 (2H, br s, NH_2), 9.10 (1H, s, C_2-H).

Representative Procedure (Method A); (*S*)-5-Amino-7-(7-*tert*-butoxylcarbonylamino-5-azaspiro[2.4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid (14h) A mixture of difluoroboron chelate (**13**; 6.00 g, 17.5 mmol) and (*S*)-7-*tert*-butoxylcarbonylamino-5-azaspiro[2.4]heptane (5.59 g, 26.3 mmol) and iso-Pr_2NEt (3.06 ml, 17.5 mmol) in DMSO (24.0 ml) was stirred at 30 °C for 3 d. The reaction mixture was neutralized with 10% HCl, and the solution was extracted with CH_2Cl_2. The extract was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography with a mixture of CH_2Cl_2 and MeOH (100 : 1) to afford yellow crystals (3.17 g). A mixture of these crystals and NEt_3 (3.09 ml, 22.1 mol) in MeOH (62.0 ml) was heated under reflux for 14 h. The reaction mixture was concentrated and the residue was dissolved in water. The solution was made acidic with 10% HCl, and the precipitates that deposited were collected by filtration to give **14h** (2.79 g, 31%). Recrystallization from a mixture of CH_2Cl_2 and MeOH gave pale yellow needles. The physical and spectral data are listed in Tables 1 and 8.

Compounds **14a—g, j, k, n—p** were prepared in a similar manner. The physical and spectral data are listed in Tables 1 and 8. Compound **15q** was also prepared in a similar manner. The physical and spectral data are listed in Tables 2 and 9.

Representative Procedure (Method B); (*S*)-5-Amino-7-(7-*tert*-butoxylcarbonylamino-5-azaspiro[2.4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid (14h) A suspension of 7-halogenoquinolone (**13**; 15.0 g, 51.0 mmol), (*S*)-7-*tert*-butoxylcarbonylamino-5-azaspiro[2.4]heptane (32.8 g, 76.5 mmol) and NEt_3 (7.10 ml, 51.0 mmol) in DMSO (60.0 ml) was stirred at 100 °C under a nitrogen atmosphere for 87 h. The reaction mixture was poured into water and the solution was acidified to pH 5 with 10% HCl. The precipitates that deposited were collected by filtration and washed with water and MeOH to give **14h** (18.5 g, 74%), which was identical with the compound (**14h**) synthesized by method A.

Compound **14i, l, n** were prepared in a similar manner. The physical and spectral data are listed in Tables 1 and 8. Compounds **15r, s** were also prepared in a similar manner. The physical and spectral data are listed in Tables 2 and 9.

(*S*)-5-Amino-7-(3-amino-4,4-dimethyl-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid (15j) A mixture of quinolone (**14j**; 0.47 g, 0.97 mmol) and KOH (0.32 g, 4.9 mmol) in water (3.2 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with 10% HCl and the precipitates that deposited were collected by filtration and washed with water to give **15j** (0.18 g, 48%) as yellow crystals. The physical and spectral data are listed in Tables 2 and 9.

Compounds **15k, n—p** were prepared in a similar manner, and the physical and spectral data are listed in Tables 2 and 9.

(*S*)-5-Amino-7-(7-amino-5-azaspiro[2.4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic Acid Methanesulfonate (15h) A suspension of the quinolone **14h** (40.5 g, 83.2 mmol) in concentrated HCl (69.0 ml) was stirred for 5 min at room temperature. Water (69.0 ml) was added to the reaction mixture and the whole was stirred for 10 min. The solution was filtered and the filtrate was made alkaline (pH 11) with 10% NaOH. The solution was stirred for 30 min, and the precipitates were collected by filtration and washed with saturated NaCl. The precipitates were dissolved in water (190 ml), and the solution was neutralized with 10% HCl. The resultant precipitates were collected by filtration, and washed with water to afford **15h** (24.5 g, 76%). The methanesulfonate salt was obtained in the usual manner. The physical and spectral data are listed in Tables 2 and 9.

Compounds **15a—g, i, l, m** were prepared in a similar manner. The physical and spectral data are listed in Tables 2 and 9.

***In Vitro* Antibacterial Test** According to the method of the Japan Society of Chemotherapy,[17] the *MIC* (μg/ml) was determined by the agar dilution method with Muller–Hinton agar (MHA, Difco Laboratories, Detroit, MI). Bacterial suspensions for inocula were prepared by diluting overnight cultures of organisms to give a final concentration of 10^6 CFU/ml, and one loopful (5 μl) of an inoculum corresponding to about 5×10^3 CFU per spot was inoculated on drug-containing agar plates. The plates were incubated for 18 h at 37 °C. The *MIC* was defined as the lowest drug concentration that prevented visible growth of bacteria.

***In Vivo* Antibacterial Test** Four-weeks old male ICR mice (18—21 g body weight) were infected intraperitoneally with bacterial suspension. The bacteria used for infection were *S. aureus* SMITH (2.4×10^7 CFU per mouse) and *E. coli* KC-14 (8.4×10^7 CFU per mouse). Following infection, graded doses of compounds were administered orally to mice in groups of 10 each. The ED_{50}, including 95% confidence limits, was calculated by the probit method[18] from the survival rates on day 7 after

Table 8. Spectral Data for Intermediate 5-Amino-7-substituted-8-methylquinolones (**14**)

Compd. No.	MS (*m/z*)	IR (KBr) cm^{-1}	NMR (DMSO-d_6) δ
14a	460 (M^+)	1728 (CO_2H) 1632 (CO)	0.72—0.83 (2H, m, $CH_2 \times 2$), 1.10—1.18 (2H, m, $CH_2 \times 2$), 1.41 (9H, s, $C(CH_3)_3$), 1.83—1.92 (1H, m, CH_2), 2.10—2.20 (1H, m, CH_2), 2.34 (3H, s, CH_3), 3.28—3.70 (4H, m, $NCH_2 \times 2$), 4.06—4.20 (2H, m, NCH, CH), 7.01—7.17 (3H, m, NH_2, CONH), 8.61 (1H, s, C_2-H)
14b	474 (M^+)	1716 (CO_2H) 1630 (CO)	0.70—0.85 (2H, m, $CH_2 \times 2$), 0.98 (3H, d, J=7.0 Hz, CH_3), 1.06—1.18 (2H, m, $CH_2 \times 2$), 1.41 (9H, s, $C(CH_3)_3$), 2.33 (3H, s, CH_3), 2.39—2.55 (1H, m, CH), 3.27—3.38 (2H, m, $NCH_2 \times 2$), 3.50—3.62 (1H, m, NCH_2), 3.75—3.85 (1H, m, NCH_2), 4.10—4.20 (2H, m, NCH, CH), 6.95—7.11 (3H, m, NH_2, CONH), 8.60 (1H, s, C_2-H)
14c	474 (M^+)	1716 (CO_2H) 1628 (CO)	0.70—0.85 (2H, m, $CH_2 \times 2$), 0.98 (3H, d, J=7.0 Hz, CH_3), 1.09—1.20 (2H, m, $CH_2 \times 2$), 1.41 (9H, s, $C(CH_3)_3$), 2.33 (3H, s, CH_3), 2.40—2.50 (1H, m, CH), 3.30—3.38 (2H, m, $NCH_2 \times 2$), 3.52—3.61 (1H, m, NCH_2), 3.78—3.85 (1H, m, NCH_2), 4.10—4.20 (2H, m, NCH, CH), 6.90—7.12 (3H, m, NH_2, CONH), 8.60 (1H, s, C_2-H)
14d	474 (M^+)	1738 (CO_2H) 1626 (CO)	0.68—0.85 (2H, m, $CH_2 \times 2$), 1.00—1.25 (2H, m, $CH_2 \times 2$), 1.07 (3H, d, J=6.5 Hz, CH_3), 1.41 (9H, s, $C(CH_3)_3$), 2.10—2.23 (1H, m, CH), 2.33 (3H, s, CH_3), 3.11—3.25 (1H, m, NCH_2), 3.35—3.43 (1H, m, NCH_2), 3.53—3.75 (3H, m, $NCH_2 \times 2$, NCH), 4.10—4.20 (1H, m, CH), 6.95—7.15 (3H, m, NH_2, CONH), 8.61 (1H, s, C_2-H)
14e	474 (M^+)	1718 (CO_2H) 1628 (CO)	0.70—0.86 (2H, m, $CH_2 \times 2$), 1.00—1.20 (2H, m, $CH_2 \times 2$), 1.07 (3H, d, J=6.5 Hz, CH_3), 1.41 (9H, s, $C(CH_3)_3$), 2.15—2.25 (1H, m, CH), 2.33 (3H, s, CH_3), 3.13—3.44 (2H, m, $NCH_2 \times 2$), 3.55—3.78 (3H, m, $NCH_2 \times 2$, NCH), 4.05—4.20 (1H, m, CH), 6.99-7.20 (3H, m, NH_2, CONH), 8.61 (1H, s, C_2-H)
14f	474 (M^+)	1714 (CO_2H) 1646 (CO)	0.69—0.85 (2H, m, $CH_2 \times 2$), 1.04—1.16 (2H, m, $CH_2 \times 2$), 1.39 (9H, s, $C(CH_3)_3$), 1.42 (3H, s, CH_3), 1.83—1.93 (1H, m, CH_2), 2.23—2.37 (1H, m, CH_2), 2.33 (3H, s, CH_3), 3.40—3.77 (4H, m , $NCH_2 \times 2$), 4.07—4.20 (1H, m, CH), 6.85 (1H, br s, CONH), 7.07 (2H, br s, NH_2), 8.61 (1H, s, C_2-H)
14g	474 (M^+)	1714 (CO_2H) 1646 (CO)	0.70—0.85 (2H, m, $CH_2 \times 2$), 1.08—1.18 (2H, m, $CH_2 \times 2$), 1.39 (9H, s, $C(CH_3)_3$), 1.42 (3H, s, CH_3), 1.81—1.95 (1H, m, CH_2), 2.25—2.38 (1H, m, CH_2), 2.33 (3H, s, CH_3), 3.38—3.78 (4H, m, $NCH_2 \times 2$), 4.10—4.20 (1H, m, CH), 6.85 (1H, br s, CONH), 7.07 (2H, br s, NH_2), 8.61 (1H, s, C_2-H)
14h	486 (M^+)	1704 (CO_2H) 1640 (CO)	0.53—0.83 (6H, m, $CH_2 \times 2$, $CH_2 \times 2$), 1.06—1.16 (2H, m, $CH_2 \times 2$), 1.39 (9H, s, $C(CH_3)_3$), 2.37 (3H, s, CH_3), 3.28 (1H, d, J=9.5 Hz, NCH_2), 3.40—3.50 (1H, m, NCH_2), 3.69 (1H, d, J=9.5 Hz, NCH_2), 3.80—3.92 (2H, m, NCH_2, NCH), 4.08—4.21 (1H, m, CH), 6.90—7.18 (3H, m, NH_2, CONH), 8.62 (1H, s, C_2-H)
14i	486 (M^+)	1704 (CO_2H) 1642 (CO)	0.48—0.84 (6H, m, $CH_2 \times 2$, $CH_2 \times 2$), 1.05—1.18 (2H, m, $CH_2 \times 2$), 1.39 (9H, s, $C(CH_3)_3$), 2.37 (3H, s, CH_3), 3.28 (1H, d, J=9.5 Hz, NCH_2), 3.40—3.50 (1H, m, NCH_2), 3.69 (1H, d, J=9.5 Hz, NCH_2), 3.83—3.95 (2H, m, NCH_2, NCH), 4.10—4.20 (1H, m, CH), 6.94—7.15 (3H, m, NH_2, CONH), 8.61 (1H, s, C_2-H)
14j	484 (M^+)	1720 (CO_2H) 1628 (CO)	0.71—0.86 (2H, m, $CH_2 \times 2$), 1.00—1.25 (2H, m, $CH_2 \times 2$), 1.04 (3H, s, CH_3), 1.15 (3H, s, CH_3), 2.37 (3H, s, CH_3), 3.12—3.86 (4H, m, $NCH_2 \times 2$), 4.10—4.38 (2H, m, NCH, CH), 7.09 (2H, br s, NH_2), 8.62 (1H, s, C_2-H), 9.38 (1H, d, J=8.5 Hz, CONH)
14k	484 (M^+)	1718 (CO_2H) 1628 (CO)	0.70—0.83 (2H, m, $CH_2 \times 2$), 1.05—1.20 (2H, m, $CH_2 \times 2$), 1.04 (3H, s, CH_3), 1.14 (3H, s, CH_3), 2.36 (3H, s, CH_3), 3.32—3.49 (2H, m, NCH_2), 3.58—3.70 (1H, m, NCH_2), 3.73—3.85 (1H, m, NCH_2), 4.08—4.20 (1H, m, CH), 4.22—4.35 (1H, m, NCH), 7.09 (2H, br s, NH_2), 8.62 (1H, s, C_2-H), 9.38 (1H, d, J=8.5 Hz, CONH)
14l	488 (M^+)	1712 (CO_2H) 1626 (CO)	0.66—0.75 (1H, m, CH_2), 0.80—0.85 (1H, m, CH_2), 0.92 (3H, t, J=7.5 Hz, CH_2CH_3), 1.03—1.19 (2H, m, $CH_2 \times 2$), 1.30—1.55 (2H, m, CH_2CH_3), 1.41 (9H, s, $C(CH_3)_3$), 2.16—2.25 (1H, m, CH), 2.32 (3H, s, CH_3), 3.13—3.29 (1H, m, NCH_2), 3.41—3.52 (2H, m, NCH_2), 3.83—3.93 (1H, m, NCH_2), 4.10—4.29 (2H, m, NCH, CH), 6.93—7.10 (3H, m, NH_2, CONH), 8.60 (1H, s, C_2-H)
14m	502 (M^+)	1714 (CO_2H) 1628 (CO)	0.62—0.72 (1H, m, CH_2), 0.79—0.95 (1H, m, CH_2), 0.90 (3H, t, J=7.0 Hz, CH_3), 1.04—1.19 (2H, m, $CH_2 \times 2$), 1.29—1.50 (4H, m, CH_2CH_2), 1.41 (9H, s, $C(CH_3)_3$), 2.22—2.38 (1H, m, CH), 2.32 (3H, s, CH_3), 3.10—3.30 (1H, m, NCH_2), 3.39—3.49 (2H, m, NCH_2), 3.86—3.92 (1H, m, NCH_2), 4.09—4.26 (2H, m, NCH, CH), 6.95—7.10 (3H, m, NH_2, CONH), 8.06 (1H, s, C_2-H)
14n	486 (M^+)	1724 (CO_2H) 1628 (CO)	0.75—0.89 (2H, m, $CH_2 \times 2$), 1.05—1.20 (2H, m, $CH_2 \times$ 2), 2.35 (3H, s, CH_3), 3.34 (3H, s, OCH_3), 3.48—3.57 (1H, m, NCH_2), 3.64—3.86 (3H, m, NCH_2, NCH_2), 4.08—4.20 (2H, m, CH × 2), 4.53—4.60 (1H, m, NCH), 7.11 (2H, br s, NH_2), 8.62 (1H, s, C_2-H), 9.40 (1H, d, J=6.5 Hz, CONH)
14o	486 (M^+)	1724 (CO_2H) 1628 (CO)	0.72—0.88 (2H, m, $CH_2 \times 2$), 1.08—1.21 (2H, m, $CH_2 \times 2$), 2.35 (3H, s, CH_3), 3.34 (3H, s, OCH_3), 3.48—3.56 (1H, m, NCH_2), 3.68—3.88 (3H, m, NCH_2, NCH_2), 4.08—4.20 (2H, m, CH × 2), 4.50—4.60 (1H, m, NCH), 7.11 (2H, br s, NH_2), 8.63 (1H, s, C_2-H), 9.40 (1H, d, J=6.5 Hz, CONH)
14p	470 (M^+)	1719 (CO_2H) 1642 (CO)	0.72—0.83 (2H, m, $CH_2 \times 2$), 1.06—1.20 (2H, m, $CH_2 \times 2$), 2.07—2.35 (2H, m, CH_2), 2.41, 2.42 (total 3H, each s, CH_3), 3.01, 3.13 (total 3H, each s, CH_3), 3.45—3.70 (4H, m, $NCH_2 \times 2$), 4.10—4.23 (1H, m, CH), 4.65—4.75, 4.96—5.05 (total 1H, each m, NCH), 7.14 (2H, br s, NH_2), 8.64 (1H, s, C_2-H)

Table 9. Spectral Data for 5-Amino-7-substituted-8-methylquinolones (**15**)

Compd. No.	MS (*m/z*)	IR (KBr) cm^{-1}	NMR (DMSO-d_6) δ
15a	360 (M^+)	1713 (CO_2H) 1634 (CO)	0.74—0.81 (2H, m, $CH_2 \times 2$), 1.09—1.20 (2H, m, $CH_2 \times 2$), 1.61—1.72 (1H, m, CH_2), 2.01—2.13 (1H, m, CH_2), 2.33 (3H, s, CH_3), 3.07—3.69 (5H, m, $NCH_2 \times 2$, NCH), 4.10—4.19 (1H, m, CH), 7.05 (2H, br s, NH_2), 8.60 (1H, s, C_2-H)
15b	374 (M^+)	1710 (CO_2H) 1628 (CO)	0.68—0.84 (2H, m, $CH_2 \times 2$), 0.97—1.20 (2H, m, $CH_2 \times 2$), 1.03 (3H, d, J=6.5 Hz, CH_3), 2.31 (3H, s, CH_3), 2.20—2.38 (1H, m, CH), 3.08—3.53 (4H, m, NCH_2, NCH_2, NCH), 3.70—3.80 (1H, m, NCH_2), 4.09—4.18 (1H, m, CH), 7.03 (2H, br s, NH_2), 8.06 (1H, s, C_2-H)
15c	374 (M^+)	1708 (CO_2H) 1628 (CO)	0.69—0.86 (2H, m, $CH_2 \times 2$), 0.99—1.20 (2H, m, $CH_2 \times 2$), 1.03 (3H, d, J=6.5 Hz, CH_3), 2.10—2.38 (1H, m, CH), 2.31 (3H, s, CH_3), 3.00—3.54 (4H, m, NCH_2, NCH_2, NCH), 3.72—3.80 (1H, m, NCH_2), 4.08—4.18 (1H, m, CH), 7.03 (2H, br s, NH_2), 8.61 (1H, s, C_2-H)
15d	374 (M^+)	1710 (CO_2H) 1630 (CO)	0.67—0.89 (2H, m, $CH_2 \times 2$), 0.96—1.22 (2H, m, $CH_2 \times 2$), 1.07 (3H, d, J=6.5 Hz, CH_3), 1.83—1.99 (1H, m, CH), 2.31 (3H, s, CH_3), 2.91—3.70 (5H, m, $NCH_2 \times 2$, NCH), 4.05—4.20 (1H, m, CH), 7.04 (2H, br s, NH_2), 8.60 (1H, s, C_2-H)
15e	374 (M^+)	1634 (CO)	0.69—0.85 (2H, m, $CH_2 \times 2$), 1.01—1.18 (2H, m, $CH_2 \times 2$), 1.07 (3H, d, J=6.0 Hz, CH_3), 1.85—1.95 (1H, m, CH), 2.31 (3H, s, CH_3), 2.93—3.03 (1H, m, NCH), 3.10—3.34 (2H, m, $NCH_2 \times 2$), 3.54—3.68 (2H, m, $NCH_2 \times 2$), 4.09—4.17 (1H, m, CH), 7.04 (2H, br s, NH_2), 8.59 (1H, s, C_2-H)
15f	374 (M^+)	1716 (CO_2H) 1628 (CO)	0.70—0.86 (2H, m, $CH_2 \times 2$), 1.07—1.21 (2H, m, $CH_2 \times 2$), 1.51 (3H, s, CH_3), 2.01—2.26 (2H, m, CH_2), 2.34 (3H, s, CH_3), 2.41 (3H, s, CH_3), 3.44—3.70, (4H, m, $NCH_2 \times 2$), 4.14—4.23 (1H, m, CH), 7.14 (1H, br s, SO_3H), 8.15 (2H, br s, NH_2), 8.67 (1H, s, C_2-H)
15g	374 (M^+)	1716 (CO_2H) 1628 (CO)	0.73—0.83 (2H, m, $CH_2 \times 2$), 1.08—1.23 (2H, m, $CH_2 \times 2$), 1.50 (3H, s, CH_3), 2.06—2.25 (2H, m, CH_2), 2.33 (3H, s, CH_3), 2.40 (3H, s, CH_3), 3.46—3.73 (4H, m, $NCH_2 \times 2$), 4.15—4.25 (1H, m, CH), 7.15 (1H, br s, SO_3H), 8.13 (2H, br s, NH_2), 8.67 (1H, s, C_2-H)
15h	386 (M^+)	1704 (CO_2H) 1630 (CO)	0.68—1.20 (8H, m, $CH_2 \times 4$), 2.32 (3H, s, CH_3), 2.43 (3H, s, CH_3), 3.11 (1H, d, J=10.0 Hz, NCH_2), 3.48—3.51 (1H, m, NCH), 3.60 (1H, d, J=11.0 Hz, NCH_2), 3.90 (1H, d, J=10.0 Hz, NCH_2), 4.09—4.22 (2H, m, NCH_2, CH), 7.15 (1H, br s, SO_3H), 8.00 (2H, br s, NH_2), 8.65 (1H, s, C_2-H)
15i	386 (M^+)	1716 (CO_2H) 1638 (CO)	0.38—0.90 (6H, m, $CH_2 \times 2$, $CH_2 \times 2$), 1.04—1.20 (2H, m, $CH_2 \times 2$), 2.35 (3H, s, CH_3), 3.01—3.32 (2H, m, NCH_2 NCH), 3.42 (1H, d, J=10.0 Hz, NCH_2), 3.60 (1H, d, J=10.0 Hz, NCH_2) 3.75—3.89 (1H, m, NCH_2), 4.08—4.20 (1H, m, CH), 7.05 (2H, br s, NH_2), 8.61 (1H, s, C_2-H)
15j	388 (M^+)	1718 (CO_2H) 1628 (CO)	0.72—1.20 (4H, m, $CH_2 \times 2$), 0.99 (3H, s, CH_3), 1.05 (3H, s, CH_3), 2.30 (3H, s, CH_3), 3.01—3.45 (4H, m, NCH_2, NCH_2, NCH), 3.54—3.60 (1H, m, NCH_2), 4.09—4.19 (1H, m, CH), 7.03 (2H, br s, NH_2), 8.60 (1H, s, C_2-H)
15k	388 (M^+)	1718 (CO_2H)	0.70—0.88 (2H, m, $CH_2 \times 2$), 0.99 (3H, s, CH_3), 1.00—1.20 (2H, m, $CH_2 \times 2$), 1.05 (3H, s, CH_3), 2.30 (3H, s, CH_3), 3.03—3.33 (3H, m, $NCH_2 \times 2$, NCH), 3.40—3.46 (1H, m, NCH_2), 3.52—3.60 (1H, m, NCH_2), 4.09—4.18 (1H, m, CH), 7.03 (2H, br s, NH_2), 8.60 (1H, s, C_2-H)
15l	388 (M^+)	1636 (CO)	0.66—0.89 (2H, m, $CH_2 \times 2$), 0.96 (3H, t, J=7.5 Hz, CH_2CH_3), 1.05—1.20 (2H, m, $CH_2 \times 2$), 1.38—1.49 (1H, m, CH_2CH_3), 1.53—1.64 (1H, m, CH_2CH_3), 2.20—2.29 (1H, m, CH), 2.34 (3H, s, CH_3), 3.01—3.59 (3H, m, NCH_2, NCH_2), 3.67—3.72 (1H, m, NCH), 3.90—3.99 (1H, m, NCH_2), 4.11—4.19 (1H, m, CH), 7.06 (2H, br s, NH_2), 8.62 (1H, s, C_2-H)
15m	402 (M^+)	1628 (CO)	0.65—0.73 (1H, m, CH_2), 0.78—0.89 (1H, m, CH_2), 0.93 (3H, t, J=7.0 Hz, CH_3), 1.02—1.21 (2H, m, $CH_2 \times 2$), 1.29—1.59 (4H, m, CH_2CH_2), 2.08—2.19 (1H, m, CH), 2.30 (3H, s, CH_3), 3.08—3.59 (4H, m, NCH_2, NCH_2, NCH), 3.82—3.90 (1H, m, NCH_2), 4.09—4.18 (1H, m, CH), 7.02 (2H, br s, NH_2), 8.59 (1H, s, C_2-H)
15n	390 (M^+)	1728 (CO_2H) 1630 (CO)	0.71—0.88 (2H, m, $CH_2 \times 2$), 1.05—1.20 (2H, m, $CH_2 \times 2$), 2.30 (3H, s, CH_3), 3.29—3.40 (1H, m, NCH_2), 3.37 (3H, s, OCH_3), 3.48—3.57 (3H, m, $NCH_2 \times 2$, NCH), 3.69—3.82 (2H, m, NCH_2, CH), 4.10—4.19 (1H, m, CH), 7.05 (2H, br s, NH_2), 8.60 (1H, s, C_2-H)
15o	390 (M^+)	1712 (CO_2H) 1632 (CO)	0.71—0.86 (2H, m, $CH_2 \times 2$), 1.08—1.20 (2H, m, $CH_2 \times 2$), 2.30 (3H, s, CH_3), 3.29—3.39 (1H, m, NCH_2), 3.37 (3H, s, OCH_3), 3.48—3.55 (3H, m, $NCH_2 \times 2$, NCH), 3.69—3.80 (2H, m, NCH_2, CH), 4.09—4.18 (1H, m, CH), 7.05 (2H, br s, NH_2), 8.60 (1H, s, C_2-H)
15p	374 (M^+)	1702 (CO_2H) 1628 (CO)	0.70—0.81 (2H, m, $CH_2 \times 2$), 1.08—1.20 (2H, m, $CH_2 \times 2$), 2.07—2.19 (1H, m, CH_2), 2.25—2.40 (1H, m, CH_2), 2.41 (3H, s, CH_3), 2.61 (3H, s, CH_3), 3.40—3.89 (5H, m, $NCH_2 \times 2$, NCH), 4.13—4.22 (1H, m, CH), 7.15 (2H, br s, NH_2), 8.64 (1H, s, C_2-H)
15q	388 (M^+)	1724 (CO_2H) 1630 (CO)	0.68—0.85 (2H, m, $CH_2 \times 2$), 1.04—1.20 (2H, m, $CH_2 \times 2$), 1.72—1.86 (1H, m, CH_2), 2.05—2.16 (1H, m, CH_2), 2.21 (6H, s, $CH_3 \times 2$), 2.34 (3H, s, CH_3), 2.78—2.89 (1H, m, NCH_2), 3.37—3.66 (4H, m, NCH_2, NCH_2, NCH), 4.09—4.20 (1H, m, CH), 7.08 (2H, br s, NH_2), 8.61 (1H, s, C_2-H)
15r[a]	360 (M^+)	1710 (CO_2H) 1640 (CO)	0.70—0.83 (2H, m, $CH_2 \times 2$), 1.14—1.25 (2H, m, $CH_2 \times 2$), 2.52 (3H, s, CH_3), 3.39—3.63 (8H, m, $NCH_2 \times 4$), 4.07—4.19 (1H, m, CH), 8.62 (1H, s, C_2-H)
15s	374 (M^+)	1712 (CO_2H)	0.71—0.85 (2H, m, $CH_2 \times 2$), 1.06—1.23 (2H, m, $CH_2 \times 2$), 1.31 (3H, d, J=6.5 Hz, CH_3), 2.45—2.55 (3H, m, CH_3), 3.12—3.58 (7H, m, $NCH_2 \times 3$, NCH), 4.18—4.28 (1H, m, CH), 7.23 (2H, br s, NH_2), 8.66 (1H, s, C_2-H)

a) NMR was measured in D_2O.

infection.

Urinary Recovery Six- to 7-weeks old male SD rats (170—210 g body weight) which had been starved for 20 h were given a single oral dose of 5 mg/kg of body weight. Following administration, animals were housed in metabolism cages. Urine was collected over the period of 48 h after administration. The concentrations of compounds in urine were determined by an agar diffusion method by using *E. coli* KP as a test organism. Cumulative urinary excretion was calculated as the percentage of the dose excreted in urine obtained from 0 to 48 h after administration.

Phototoxicity Test The phototoxicity test was carried out according to the Adjutant-Strip method.[19] Five- to 6-weeks old male Hartley guinea pigs (350—400 g body weight) were irradiated using a UV lamp (20 J/cm^2, Toshiba FL2·BLB) for 90 min after intravenous administration of the test compound. Phototoxicity was evaluated at 24 h after irradiation.

Convulsion Induced by Coadministration with NSAID A quinolone (100 mg/kg) was administered intraperitoneally 30 min after oral administration of fenbufen (100 mg/kg) in male ICR mice (5 weeks old, 21—27 g body weight). Alternatively, a quinolone (20 μg) was administered intracerebroventricularly 30 min after intraperitoneal administration of fenbufen (50 mg/kg) in male Wistar rats (7—8 weeks old, 215—315 g body weight). Those mice or rats were observed for occurrence of convulsions for 4 h after administration.

$GABA_A$ Receptor-Binding Assay This assay was carried out according to the method of Heaulme *et al.* with minor modifications.[20] Membrane fraction of cerebral cortices from Wistar rats (7 weeks old, 241—280 g body weight) was used for binding assay. Aliquots of membranes were incubated with 1 nM [^{3}H]SR95531 (specific activity; 57.3 Ci/mmol, purchased from Dupont/NEN). Reactions were terminated by rapid filtration using a Brandel cell harvester with Whatman GF/B filters. The filters were then washed 3 times with Tris HCl buffer (pH 7.4) and dried. The filter-bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined as binding in the presence of 100 μM SR95531 and assays were conducted in duplicate.

Enzyme Assays The A and B subunits of DNA gyrase were purified separately from *Escherichia coli* KL-16 through novobiocin-epoxy-activated Sepharose 6B. Topo II from human placenta was purchased from Topo GEN, Inc. Inhibitory effects of compounds on DNA gyrase were evaluated by supercoiling assay according to the method of Otter and Cozzarelli[21] and those on Topo II, by relaxing assay according to the method of kit.[22]

Acknowledgements We are grateful to Dr. Eiichi Okezaki and Yasuji Aoki for encouragement, Tomio Suzuki and Akemi Nakagawa for excellent technical assistance, Koichi Omichi and Keiko Yamazaki for measuring the antibacterial activities of the compounds, Dr. Masafumi Oshita for $GABA_A$ receptor binding assay, and Tomiko Suzuki for measuring the phototoxicity of the compounds.

References and Notes

1) Part IV: Yoshida T., Yamamoto Y., Orita H., Kakiuchi M., Takahashi Y., Itakura M., Kado N., Mitani K., Yasuda S., Kato H., Itoh Y., *Chem. Pharm. Bull.*, **44**, 1074—1085 (1996).
2) Wise R., Andrews J. M., Edwards L. J., *Antimicrob. Agents Chemother.*, **23**, 559—564 (1983).
3) Hayakawa I., Hiramitsu T., Tanaka Y., *Chem. Pharm. Bull.*, **32**, 4907—4913 (1984).
4) Ueda Y., Shimizu K., Konno M., Matsumoto F. (ed.), "The Quinolones," Life Science Publisher, Tokyo, 1991, pp. 1—14.
5) *a*) Narita H., Konishi Y., Nitta J., Kitayama I., Miyajima M., Watanabe Y., Yotsuji A., Saikawa I., *Yakugaku Zasshi*, **106**, 802—807 (1986); *b*) Rosen T., Chu D. T. W., Lico I. M., Fernandes P. B., Shen L., Borodkin S., Pernet A. G., *J. Med. Chem.*, **31**, 1586—1590 (1988).
6) Sanchez J. P., Domagala J. M., Hagen S. E., Heifetz C. L., Hutt M. P., Nichols J. B., *J. Med. Chem.*, **31**, 983—991 (1988).
7) *a*) Itoh Y., Kato H., Ogawa N., Koshinaka E., Suzuki T., Ger. Offen. Patent 3433924 (1985) [*Chem. Abstr.*, **103**, 123517*b* (1985)]; *b*) Okezaki E., Terasaki T., Nakamura M., Nagata O., Kato H., Tsuji A., *Drug Metab. Dispos.*, **16**, 856—874 (1988).
8) Miyamoto T., Matsumoto J., Chiba K., Egawa H., Shibamori K., Minamida A., Nishimura Y., Okada H., Kataoka M., Fujita M., Hirose T., Nakano J., *J. Med. Chem.*, **33**, 1645—1656 (1990).
9) *a*) Yoshida T., Takeshita M., Orita H., Kado N., Yasuda S., Kato H., Itoh Y., *Chem. Pharm. Bull.*, **44**, 1128—1131 (1996); *b*) Di Cesare P., Bouzard D., Essiz M., Jacquet J. P., Ledoussal B., Kiechel J. R., Remuzon P., Kessler R. E., Fung-Tomc J., *J. Med. Chem.*, **35**, 4205—4213 (1992); *c*) Asahina Y., Fukuda Y., Fukuda H., European Patent 443498 (1990) [*Chem. Abstr.*, **115**, 256018*j* (1991)]; *d*) Hirabayashi S., Ike K., Zanka A., Kawakami T., Ichihara M., World Intellectual Property Organization 9220652 (1992) [*Chem. Abstr.*, **119**, 180649*a* (1993)]; *e*) Kimura Y., Atarashi S., Kawakami K., Sato K., Hayakawa I., *J. Med. Chem.*, **37**, 3344—3352 (1994); *f*) Ito Y., Kato H., Yasuda S., Kato N., Yoshida T., Ueshima M., Japan Kokai Tokkyo Koho, Japan. Patent 05345777 (1994) [*Chem. Abstr.*, **120**, 298485*t* (1994)]; *g*) Okada T., Sato H., Tsuji T., Tsushima T., Nakai H., Yoshida T., Matsuura S., *Chem. Pharm. Bull.*, **41**, 132—138 (1993).
10) Matsumoto J., Miyamoto K., Egawa H., Nakamura S., Japan Kokai Tokkyo Koho, Japan. Patent 61137885 (1986) [*Chem Abstr.*, **106**, 4996*j* (1987)].
11) Sanchez J. P., Domagala J. M., Heifetz C. L., Priebe S. R., Sesnie J. A., Trehan A. K., *J. Med. Chem.*, **35**, 1764—1773 (1992).
12) Hagen S. E., Domagala J. M., Heifetz C. L., Sanchez J. P., Solomon M., *J. Med. Chem.*, **33**, 849—854 (1990).
13) Ohta M., Koga H., *J. Med. Chem.*, **34**, 131—139 (1991).
14) Pharmacokinetics study showed that ^{14}C-**15h** (^{14}C-HSR-903) had a good absorption and high biliary excretion. These results will be reported elsewhere.
15) Ueda Y., Shimizu K., Konno M., Matsumoto F., (ed.), "The Quinolones," Life Science Publisher, Tokyo 1991, pp. 275—286.
16) *a*) Ueda Y., Shimizu K., Konno M., Matsumoto F., (ed.), "The Quinolones," Life Science Publisher, Tokyo 1991, pp. 315—326; *b*) Akahane K., Sekiguchi M., Une T., Osada Y., *Antimicrob. Agents Chemother.*, **33**, 1704—1708 (1989).
17) Goto S., Jo K., Kawakita T., Kosakai N., Mitsuhasi S., Nishino T., Ohsawa N., Tanami H., *Chemotherapy*, **29**, 76—79 (1981).
18) Bliss C. I., *Method Probit. Science*, **79**, 38—39 (1934).
19) Satou E., Katumura Y., Itikawa H., Kobayasi T., Nakazima K., *Nishinihon Hifuka*, **42**, 831—837 (1980).
20) Heaulme M., Chambon J. P., Leyris R., Wermuth C. G., Biziere K., *J. Neurochem.*, **48**, 1677—1686 (1987).
21) Otter R., Cozzarelli N. R., *Methods Enzymol.*, **100**, 171—180 (1983).
22) Manual for Topoisomerase II Drug Screening Kit by Topo GEN, Inc.