# Quinolone Antimicrobial Agents Substituted with Morpholines at the 7-Position. Syntheses and Structure-Activity Relationships[1]

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*Received July 20, 1992*

A series of novel 7-substituted 1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids have been prepared and tested for antibacterial activities and for convulsive activities in combination with nonsteroidal antiinflammatory drug. Structure-activity relationships revealed that 7-(2-(aminomethyl)morpholino) derivative **28** had a better Gram-positive activity than the reference quinolones, such as ciprofloxacin, norfloxacin, and ofloxacin. Its Gram-negative activity was equipotent with those of norfloxacin and ofloxacin but was inferior to that of ciprofloxacin. In mouse systemic infection models, **28** showed an excellent therapeutic efficacy which might result from the potent antibacterial activity and suitable physicochemical properties. Convulsive activities of 7-morpholino derivatives in combination with nonsteroidal antiinflammatory drug fenbufen or its metabolite biphenylacetic acid markedly diminished as compared to those of 7-piperazino derivatives in the electrophysiological, biochemical, and behavioral experiments. These results suggest that **28** (Y-26611) is a novel quinolone with reduced neurotoxic excitatory adverse reaction.

## Introduction

Since the discovery of nalidixic acid, numerous quinolone derivatives have been prepared in search of improved antibacterial activities.[2] In the past decade, the so-called new quinolones such as norfloxacin[3] (NFLX), enoxacin[4] (ENX), ofloxacin[5] (OFLX), ciprofloxacin[6] (CPFX), tosufloxacin[7] (TFLX), and lomefloxacin[8] (LFLX) which possess potent antibacterial activities have been discovered and used clinically. The Gram-positive activities of these quinolones except for TFLX are rather poor. Especially the activities against methicillin resistance *Staphylococcus aureus* (MRSA), which causes many problems in clinical treatment,[2] are not sufficient. The great majority of the new quinolones under development or in clinical use are incorporated with piperazine or pyrrolidine derivatives as the C-7 substituents, whereas quinolones modified with morpholine groups have not been studied systematically until recently. Comparing the physical properties of morpholine to those of piperazine, we noted that the solubilities of both compounds in water are similar, while the lipophilicity of the former is somewhat higher than that of the latter.[9] From these properties, we supposed that the use of morpholine in place of piperazine would improve Gram-positive activity. Therefore, a series of 7-morpholino derivatives have been prepared and tested for antibacterial activities. On the other hand, central nervous system (CNS) stimulation is a characteristic side effect[10] of some members of the new quinolones as manifested by giddiness, dizziness, and convulsion when administered either alone or in combination with nonsteroidal antiinflammatory drugs (NSAID). In regard to CNS effects, the electrophysiological and biochemical studies[11a,b] revealed that some quinolones produced only a little inhibition on the $\gamma$-aminobutyric acid (GABA)-induced chloride currents ($I_{Cl(GABA)}$) in dissociated frog sensory neurons and on the [$^3$H]GABA binding to rat brain synaptic membranes; however, their inhibitory effects were potentiated by coadministration with certain NSAIDs. Akahane et al.[10b] reported that the administration of certain quinolones (e.g. CPFX and ENX) in combination with biphenylacetic acid (BPAA), an active metabolite of fenbufen, potently induced clonic convulsion and subsequent death in mice. These results correlated well with the drugs' potencies to displace the [$^3$H]muscimol binding to rat brain synaptic membranes. These data are consistent with their hypothesis that quinolones mimic as the GABA-receptor antagonist and cause CNS overexcitation such as convulsion by reducing the influence of the inhibitory neurotransmitter system. They postulated that the epileptogenic activity of some quinolones possibly relates to the GABA-like structure of C-7 piperazine such quinolones. In this study, we have investigated the structure-activity relationships of quinolones incorporated with morpholine (not GABA-like structure) or piperazine derivatives at the C-7 position and their CNS effects in the electrophysiological, biochemical, and behavioral experiments.

## Chemistry

2-(Aminomethyl)morpholine derivatives **7** and **11** were prepared according to Scheme I. 2-(Acetamidomethyl)-morpholine (**7**) was synthesized by method A. The reaction of 4-benzyl-2-(chloromethyl)morpholine (**5**)[12] with potassium phthalimide gave 4-benzyl-2-(phthalimidomethyl)-morpholine, which was treated with hydrazine monohydrate to give **6**.[13b] Subsequent N-acetylation[13b] and hydrogenation afforded **7**. 2-(((Ethoxycarbonyl)amino)-methyl)morpholine (**11**) was synthesized by method B. Hydration of 4-benzyl-2-(cyanomethyl)morpholine (**8**)[13b] afforded 4-benzyl-2-(carbamoylmethyl)morpholine (**9**), which was reacted with sodium hypochlorite in ethanol to give 4-benzyl-2-(((ethoxycarbonyl)amino)methyl)morpholine (**10**). Hydrogenation of **10** gave **11**. The intermediate **9** was also prepared from ethyl 4-benzylmorpholine-2-acetate (**12**)[12] (method C). Hydrolysis of **12** gave 4-benzylmorpholine-2-acetic acid (**13**). Subsequent reaction with phosphorus pentachloride and ammonolysis of the resulting acid chloride gave **9**.

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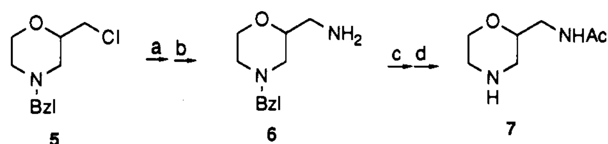
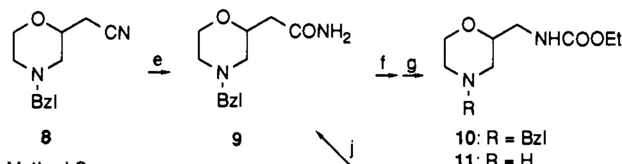
**Scheme I**[a]

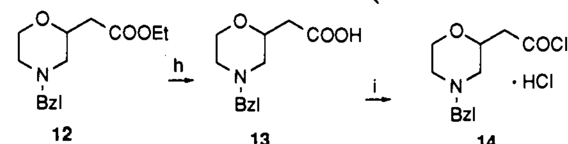
Method A



Method B

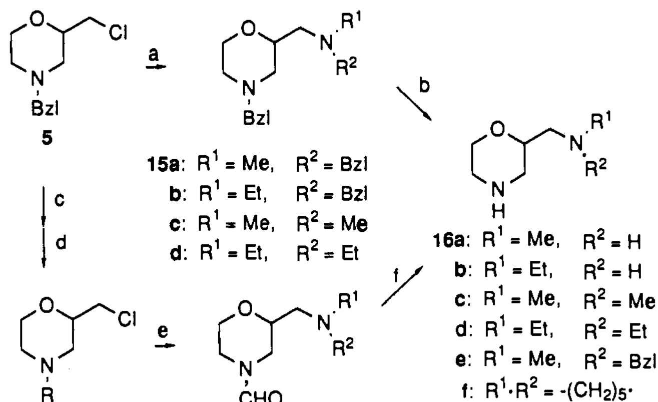


Method C



[a] (a) Potassium phthalimide; (b) $NH_2NH_2 \cdot H_2O$; (c) $Ac_2O$; (d) $NH_2NH_2 \cdot H_2O$, Pd/C; (e) c-$H_2SO_4$; (f) NaOCl/EtOH; (g) $H_2$, Pd/C; (h) KOH, $H_2O$; (i) $PCl_5$; (j) $NH_3$.

**Scheme II**[a]

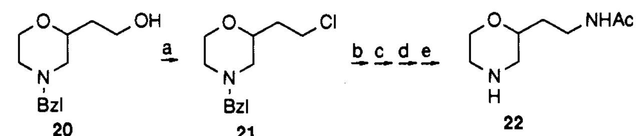


17: R = H
18: R = CHO

19a: $R^1$ = Me, $R^2$ = Bzl
b: $R^1$-$R^2$ = -$(CH_2)_5$-

[a] (a) $R^1R^2NH$; (b) $H_2$, Pd/C; (c) $H_2$, Pd/C; (d) HCOOEt; (e) $R^1R^2NH$; (f) HCl.

**Scheme III**[a]

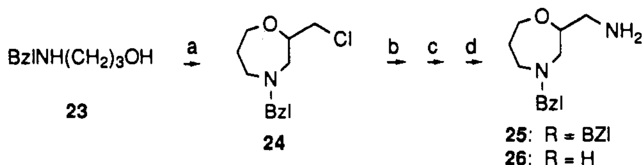


[a] (a) $SOCl_2$; (b) potassium phthalimide; (c) $NH_2NH_2 \cdot H_2O$; (d) $Ac_2O$; (e) $NH_2NH_2 \cdot H_2O$, Pd/C.

Morpholine derivatives **16a–f** were prepared according to Scheme II. Substitution of the chlorine atom of **5** with appropriate amines afforded **15a–d**, which were hydrogenated to give **16a–d**. Compounds **16e** and **16f** were also prepared according to the alternative route shown in Scheme II. Hydrogenation of **5** afforded **17**, which was formylated to give **18**. Subsequent amination with appropriate amines gave **19a** and **19b**, which were converted to **16e** and **16f** by hydrolysis in aqueous hydrochloric acid.

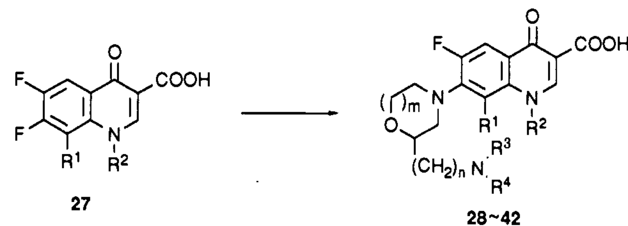
2-(2-Acetamidoethyl)morpholine (**22**) was prepared from 4-benzyl-2-morpholineethanol (**20**)[13b] (Scheme III). Chlorination of **20** with thionyl chloride gave the chloride **21**. Subsequent reaction with potassium phthalimide, hydrazine monohydrate, and acetic anhydride followed by hydrogenation gave **22**.

**Scheme IV**[a]



[a] (a) (i) Epichlorohydrin; (ii) $H_2SO_4$, Δ; (b) potassium phthalimide; (c) $NH_2NH_2 \cdot H_2O$; (d) $NH_2NH_2 \cdot H_2O$, Pd/C.

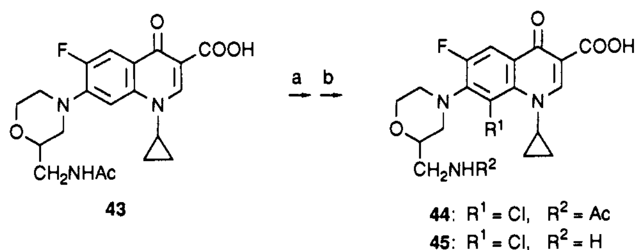
**Scheme V**



27 a: $R^1$ = F, $R^2$ = c-$C_3H_5$
b: $R^1$ = H, $R^2$ = c-$C_3H_5$
c: $R^1$ = F, $R^2$ = $C_2H_5$
d: $R^1$ = F, $R^2$ = $CH_2CH_2F$
e: $R^1$ = F, $R^2$ = $C_6H_3F_2$
f: $R^1$-$R^2$ = -$OCH_2CH(CH_3)$-

(Abbreviations are summarized in Table III)

**Scheme VI**[a]



[a] (a) $SO_2Cl_2$; (b) HCl.

2-(Aminomethyl)hexahydro-1,4-oxazepine (**26**) was synthesized according to the route shown in Scheme IV. The reaction of 3-(benzylamino)propanol (**23**) with epichlorohydrin, followed cyclization of the product with sulfuric acid, gave 4-benzyl-2-(chloromethyl)hexahydro-1,4-oxazepine (**24**). Reaction of **24** with potassium phthalimide and subsequent reaction with hydrazine monohydrate gave 4-benzyl-2-(aminomethyl)hexahydro-1,4-oxazepine (**25**). Hydrogenation of **25** gave **26**.

The quinolone intermediates **27a–f** were prepared according to the reported procedures.[13c,14] The 7-fluorine atoms of **27a–f** were replaced with morpholine derivatives to yield the desired quinolones **28–42** (Scheme V) ($R^1$–$R^4$, *m*, and *n* are summarized in Table IV).

When an acetylated aminoalkylmorpholine was used as the C-7 substituent, the acetyl group was removed by refluxing in hydrochloric acid. 8-Chloroquinolone derivative **45** was synthesized from 8-unsubstituted quinolone **43** by chlorination with sulfuryl chloride and followed deacetylation (Scheme VI).

## Biology

**Determination of Minimum Inhibitory Concentrations (MICs).** According to the method of the MIC Committee of the Japan Society of Chemotherapy,[15] MICs

were determined by the 2-fold serial agar dilution method. The media used for preculture and MIC determination were Mueller-Hinton broth and Mueller-Hinton agar (Difco), respectively. The plates were inoculated by an inoculating apparatus (Sakuma, Tokyo, Japan) with one loopful (about 5 $\mu$L) of approximately $1.0 \times 10^6$ colony-forming units (CFU) of organisms cultured in broth per milliliter. The plates were incubated at 37 °C for 18 h. The MIC was defined as the lowest drug concentration at which visible bacterial growth was inhibited.

**Systemic Infection in Mice.** Eight male ICR mice weighing 18–22 g each were used for each dose level. The culture and infectious doses were prepared as follows: *Staphylococcus aureus* Smith ($3.5 \times 10^6$ CFU/mL in 5% hog gastric mucin), *Escherichia coli* KC-14 ($1.0 \times 10^4$ CFU/mL in 5% hog gastric mucin). The infection was initiated by injecting 0.5 mL of the culture intraperitoneally. Drugs were suspended in 0.5% methyl cellulose and administered orally at 1 h postinfection. All untreated mice died within 48 h. The 50% effective dose was calculated by the probit method[16] from the survival rates at 7 days postinfection.

**Partition Coefficient.** The solutions (10 $\mu$g/mL) of quinolones were made in 0.1 M phosphate buffer (pH 7.4). After shaking with an equal volume of chloroform at 25 °C for 1 h and centrifuging at 1000*g* for phase separation, the concentration of quinolones in the aqueous phase were determined by the spectrophotometric assay. The partition coefficients were expressed as the ratio of the concentration in the organic phase to that in the aqueous phase. Furukawa[17a] et al. reported that partition coefficients of quinolones between chloroform and water correlated to the oral absorption in human. In addition, we observed that partition coefficients of some quinolones in chloroform/$H_2O$ had good correlation with those in octanol/$H_2O$.[17b] From these facts, we used conveniently chloroform as the nonpolar phase in this study.

**Solubility in Water.** An excess of the quinolone was vigorously shaken with distilled water at 25 °C for 2 h. The mixture was centrifuged at 15000*g* for 20 min. The clear supernatant was diluted with water, and the concentrations of quinolone in the diluent was determined by the spectrophotometric assay.

**Inhibition of GABA-Induced Chloride Current.** The experiment was carried out according to the previously reported method.[11,18] Dorsal root ganglion (DRG) isolated from bull frog were digested in normal Ringer solution containing 0.3% collagenase and 0.05% trypsin at pH 7.4 for 15–20 min at 37 °C. During the enzyme treatment, the preparation was gently agitated by bubbling with 95% $O_2$ and 5% $CO_2$. Thereafter, single neurons were mechanically isolated from the digested ganglia with fine pins under a biocular microscope. Isolated neurons were maintained in a solution which contained equal parts of Eagle's minimum essential medium and normal Ringer solution at room temperature of 22–25 °C. Isolated neuronal cell bodies were perfused internally and externally by a suction pipette technique with respective test solutions for recording the chloride current ($I_{Cl}$). The external solution contained Tris-Cl 89, CsCl 2, $MgCl_2$ 5, TEA-Cl 25, glucose 5, and HEPES 10 (each unit, mM) and was adjusted at pH 7.4 with appropriate Tris-base. The internal solution contained CsCl 95, Cs-aspartate 10, TEA-Cl 25, HEPES 10 (each unit, mM) and EGTA-$Ca^{2+}$ $10^{-8}$ M and was adjusted at pH 7.2. Neurons were voltage-clamped at a holding membrane potential of −50 mV with a single electrode. Quinolones were applied by the use of a concentration-clamp technique.

**[$^3$H]Muscimol Binding to GABA$_A$ Receptor.** The experimental methods were similar to those described by Zukin et al.[19] The cerebral cortex from the brain of Wistar rats were homogenized in 15 volumes of ice-cold 0.32 M sucrose. The homogenate was centrifuged at 1000*g* for 10 min, and the supernatant was further centrifuged at 20000*g* for 20 min. The resultant crude membrane pellet was resuspended in 20 volumes of 50 mM Tris hydrochloride buffer (pH 7.1) by dispersion with Biotron and was centrifuged at 48000*g* for 20 min. The pellet was suspended in 0.05% Triton X-100, incubated for 30 min at 37 °C, and washed three times with the buffer. The final suspension (2.5 mg of protein/mL) was stored at −80 °C without loss of binding capacity for 7 days. The standard binding assay preparation (1 mL), which contained 850 $\mu$L of the membrane suspension, 50 $\mu$L of [$^3$H]muscimol (2 nM, specific activity, 20 Ci/mmol), 50 $\mu$L of the buffer or the test quinolone, and 50 $\mu$L of the buffer solution of BPAA ($10^{-4}$ M), was incubated at 0 °C for 30 min. The preparations were filtered through glass filters (GF/B; Whatman Inc.). The filters were then quickly washed three times with 3 mL of ice-cold buffer. The filters were placed in vials containing 3 mL of ACS-II aqueous counting scintillant and counted by a liquid scintillation counter. Specific binding was defined as the difference between the levels of binding observed in the presence and absence of a large excess ($2 \times 10^{-4}$ M) of unlabeled GABA. The concentration required to inhibit 50% of specific binding ($IC_{50}$) for quinolone with or without BPAA was derived from the concentration-response curve.

**Convulsive Activities in Mice.** The male ddY mice, weighing 23–34 g and aged 5 weeks, were quarantined for at least 7 days before use. A group of eight mice was allocated to each test compound. Control mice received either 400 mg/kg of fenbufen or a quinolone. A quinolone alone or in combination with fenbufen was administered orally. Their convulsive activities in mice were observed for up to 6 h after administration.

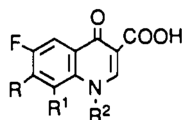
## Results and Discussion

In the initial stages of this study, six typical quinolones having piperazine (NFLX, CPFX, and **3**) or morpholine (**1**, **2**, and **4**) at the C-7 position were synthesized in order to evaluate the influence of the C-7 substituents on the antibacterial and convulsive activities.

The piperazine series (NFLX, CPFX, and **3**[14]) had better Gram-negative activities than the corresponding morpholine series (**1**,[20] **2**,[21] and **4**[22]), while the anti-staphylococcal activities of the piperazine series were slightly inferior to those of the morpholine series as shown in Table I. Compound **4** exhibited much higher activity against *S. aureus* than compound **3**. The potent activity of **4** against MRSA suggested its potential for clinical use, although its Gram-negative activity was about one-eighth of that of CPFX and similar to that of NFLX (Table I).

In the course of the studies on CNS effects, the relationships between convulsive activities and quinolones having piperazine or morpholine at the C-7 position were investigated in the electrophysiological, biochemical, and behavioral experiments. In the electrophysiological studies, the effects of quinolones with or without BPAA on the $I_{Cl(GABA)}$ were examined in the dissociated frog DRG cells. NFLX, CPFX, and **3** in piperazine series and BPAA at a

Table I. In Vitro Antibacterial Activity of Typical Quinolones




| compd | R | R[1] | R[2] | MIC (μg/mL) *S. a.*[a] | *S. a.* (R)[b] | *S. pn.* | *E. f.* | *E. c.* | *P. a.* |
|---|---|---|---|---|---|---|---|---|---|
| NFLX | —N NH | H | Et | 0.39 | 12.5 | 3.13 | 0.78 | 0.10 | 0.39 |
| 1 | —N O | H | Et | 0.20 | 0.39 | 12.5 | 3.13 | 0.78 | 3.13 |
| CPFX | —N NH | H | —◁ | 0.10 | 1.56 | 0.39 | 0.39 | 0.012 | 0.1 |
| 2 | —N O | H | —◁ | 0.05 | 0.10 | 3.13 | 0.78 | 0.20 | 1.56 |
| 3 | —N NH | F | —◁ | 0.10 | 0.78 | 0.39 | 0.20 | 0.006 | 0.10 |
| 4 | —N O | F | —◁ | 0.025 | 0.05 | 0.39 | 0.78 | 0.10 | 0.78 |

[a] *S. a.*, *S. aureus* FDA209P; *S. pn.*, *S. pneumoniae* Type I; *E. f.*, *E. faecalis* LS-101; *E. c.*, *E. coli* NIHJ JC-2; *P. a.*, *P. aeruginosa* U-31. [b] MRSA.

Table II. Effects of Quinolone Alone and in Combination with either Fenbufen or Biphenylacetic Acid on the GABA-Induced Chloride Currents, [$^3$H]Muscimol Binding, and Convulsive Activities[a]

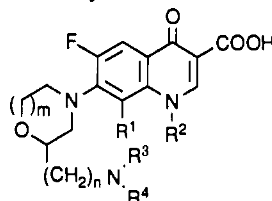
| | % of GABA-induced chloride currents | | | IC$_{50}$ (×10$^{-6}$ M) of [$^3$H]muscimol binding | | % of mice with convulsion | |
|---|---|---|---|---|---|---|---|
| quinolone | alone (10$^{-4}$ M) | with 10$^{-5}$ M BPAA | with 10$^{-4}$ M BPAA | alone (10$^{-4}$ M) | with 10$^{-4}$ M BPAA | alone (400 mg/kg) | with 400 mg/kg fenbufen |
| vehicle | | 95 | 82 | | >100 | | 0 |
| NFLX | 38 | 0 | – | 13 | 0.02 | 0 | 100 |
| 1 | 100 | 100 | 92 | >100 | >100 | 0 | 0 |
| CPFX | 64 | 0 | – | 100 | 0.41 | 0 | 63 |
| 2 | 100 | 99 | 91 | >100 | >100 | 0 | 0 |
| 3 | 81 | 11 | 0 | 64 | 0.12 | 0 | 88 |
| 4 | 91 | 91 | 71 | >100 | >100 | 0 | 0 |
| 28 | 90 | 90 | 72 | >100 | >100 | 0 | 0 |
| 29 | 94 | 90 | 78 | >100 | >100 | 0 | 0 |
| 31 | 91 | 89 | 83 | >100 | >100 | 0 | 0 |
| 37 | 93 | 90 | 81 | >100 | >100 | 0 | 0 |
| 38 | 94 | 89 | 79 | >100 | >100 | 0 | 0 |
| OFLX | 94 | 87 | 39 | >100 | 11 | 0 | 0 |

[a] BPAA, 4-biphenylacetic acid; –, not tested.

concentration of 10$^{-4}$ M reduced the $I_{Cl(GABA)}$ to 38, 64, 81, and 82% of the control, respectively. While BPAA alone at 10$^{-4}$ M reduced the $I_{Cl(GABA)}$, it had no effect at 10$^{-5}$ M. In combination of NFLX, CPFX, or 3 at 10$^{-4}$ M with BPAA at 10$^{-5}$ M, they markedly reduced the $I_{Cl(GABA)}$ to 0, 0, and 11% of the control, respectively. In contrast, the morpholine series quinolones 1, 2, and 4 with or without BPAA at 10$^{-5}$ M had little inhibitory actions on the $I_{Cl(GABA)}$, even at 10$^{-4}$ M. In the biochemical studies, the effects of quinolones with or without BPAA on the [$^3$H]muscimol binding to GABA$_A$ receptor of rat synaptic membranes were investigated. The concentrations of NFLX, CPFX, and 3 required to inhibit 50% of the specific binding of [$^3$H]muscimol (IC$_{50}$) were 1.3 × 10$^{-5}$, 1.0 × 10$^{-4}$, and 6.4 × 10$^{-5}$ M, respectively. However, IC$_{50}$ for NFLX, CPFX, and 3 with BPAA at 10$^{-4}$ M lowered by 2 × 10$^{-8}$, 4.1 × 10$^{-7}$, and 1.2 × 10$^{-7}$ M, respectively. In contrast, IC$_{50}$ values for 1, 2, and 4 with or without BPAA were more than 10$^{-4}$ M. In the behavioral studies, the convulsive activities of quinolones with or without fenbufen were examined in mice after oral administration. Neither NFLX, CPFX, and 3 nor fenbufen (each dose 400 mg/kg) exhibited any epileptogenic activity; however, the three quinolones evoked convulsions in combination with fenbufen to 100, 63, and 88% of the treated mice, respectively. On the other hand, 1, 2, and 4 did not evoke any epileptogenic activities with or without fenbufen (each dose 400 mg/kg). From these results, we perceived that morpholine series of quinolones have no convulsive activities, but the piperazine series have properties to evoke convulsion (Table II).

It is well-known that the chemotherapeutic efficacies of quinolones in clinical treatment are influenced not only by their in vitro antibacterial activities but also by their pharmacokinetic profiles. Their oral bioavailabilities will be mainly dependent on their physicochemical properties, such as lipophilicity and water solubility. Since the physicochemical property of 4 appeared to be extremely lipophilic and sparingly soluble in water, we presumed that it might be metabolized in vivo to the nonactive glucuronide. Domagala et al. have shown that introduction of an aminomethyl group at the C-7 pyrrolidine ring improves the Gram-positive activities.[23] Other C-7 heterocyclic substituents[24] having aminomethyl groups were also reported. From these facts, we expected that introduction of aminoalkylated morpholines at the C-7 position would result in favorable antibacterial activities and

Table III. Physical Data of Quinolones Prepared in This Study



| no. | $R^1$ | $R^2$ | $NR^3R^4$ | yield % | mp, °C | formula |
|---|---|---|---|---|---|---|
| 28 | F | c-$C_3H_5$ | $NH_2$ ($m$ = 1, $n$ = 1) | 50 | 183–185 | $C_{18}H_{19}F_2N_3O_4 \cdot 2H_2O$ |
| 29 | F | c-$C_3H_5$ | $NHCH_3$ | 62 | 251–254 | $C_{19}H_{21}F_2N_3O_4$ |
| 30 | F | c-$C_3H_5$ | $NHC_2H_5$ | 60 | 215–216 | $C_{20}H_{23}F_2N_3O_4$ |
| 31 | F | c-$C_3H_5$ | $N(CH_3)_2$ | 76 | 182–183 | $C_{20}H_{23}F_2N_3O_4$ |
| 32 | F | c-$C_3H_5$ | $N(C_2H_5)_2$ | 20 | 155–157 | $C_{22}H_{27}F_2N_3O_4$ |
| 33 | F | c-$C_3H_5$ | piperidinyl | 14 | 160–162 | $C_{23}H_{27}F_2N_3O_4 \cdot {}^1/_2H_2O$ |
| 34 | F | c-$C_3H_5$ | $NHCOCH_3$ | 96 | 230–233 | $C_{20}H_{21}F_2N_3O_5$ |
| 35 | F | c-$C_3H_5$ | $NCH_2C_6H_5(CH_3)$ | 34 | 155–157 | $C_{26}H_{27}F_2N_3O_4$ |
| 36 | –$OCH_2CH(CH_3)$– | | $NH_2$ | 49 | 115–118 | $C_{18}H_{20}FN_3O_5 \cdot {}^1/_4H_2O$ |
| 37 | H | c-$C_3H_5$ | $NH_2$ | 50 | 252–254 | $C_{18}H_{20}FN_3O_4$ |
| 38 | F | $C_2H_5$ | $NH_2$ | 51 | 208–210 | $C_{17}H_{19}F_2N_3O_4$ |
| 39 | F | $CH_2CH_2F$ | $NH_2$ | 49 | 279–280 dec | $C_{17}H_{18}F_3N_3O_4 \cdot HCl \cdot {}^1/_4H_2O$ |
| 40 | F | $C_6H_3F_2$ | $NH_2$ | 63 | 244–246 | $C_{21}H_{17}F_4N_3O_4 \cdot HCl \cdot H_2O$ |
| 41 | F | c-$C_3H_5$ | $NH_2$ ($m$ = 2, $n$ = 1) | 8 | 207–210 | $C_{19}H_{21}F_2N_3O_4$ |
| 42 | F | c-$C_3H_5$ | $NH_2$ ($m$ = 1, $n$ = 2) | 34 | 261–263 | $C_{19}H_{21}F_2N_3O_4 \cdot HCl \cdot {}^1/_2H_2O$ |
| 45 | Cl | c-$C_3H_5$ | $NH_2$ ($m$ = 1, $n$ = 1) | 32 | 265–266 dec | $C_{18}H_{19}ClFN_3O_4 \cdot HCl \cdot {}^1/_2H_2O$ |

Table IV. In Vitro Antibacterial Activity of Quinolones Prepared in This Study

| | MIC (μg/mL) | | | | |
|---|---|---|---|---|---|
| no. | *S. a.*[a] | *S. pn.* | *E. f.* | *E. c.* | *P. a.* |
| 28 | 0.025 | 0.05 | 0.05 | 0.10 | 0.78 |
| 29 | 0.025 | 0.10 | 0.10 | 0.10 | 3.13 |
| 30 | 0.10 | 0.05 | 0.20 | 0.10 | 3.13 |
| 31 | 0.025 | 0.05 | 0.39 | 0.20 | 3.13 |
| 32 | 0.10 | 0.10 | 0.39 | 0.39 | 6.25 |
| 33 | 0.78 | 6.25 | 25 | 3.13 | 50 |
| 34 | 0.20 | 0.78 | 0.78 | 1.56 | 12.5 |
| 35 | 0.39 | 0.78 | 1.56 | 1.56 | 25 |
| 36 | 0.78 | 12.5 | 3.13 | 3.13 | 25 |
| 37 | 0.20 | 0.39 | 0.20 | 0.20 | 1.56 |
| 38 | 0.20 | 0.39 | 0.39 | 1.56 | 3.13 |
| 39 | 0.78 | 3.13 | 0.78 | 6.25 | 25 |
| 40 | 0.20 | 0.20 | 0.39 | 0.39 | 6.25 |
| 41 | 0.78 | 1.56 | 1.56 | 0.39 | 6.25 |
| 42 | 0.20 | 0.39 | 0.39 | 1.56 | 12.5 |
| 45 | 0.012 | 0.05 | 0.05 | 0.10 | 1.56 |
| OFLX | 0.20 | 0.78 | 0.78 | 0.10 | 0.78 |

[a] *S. a., S. aureus* FDA209P; *S. pn., S. pneumoniae* Type I; *E. f., E. faecalis* LS-101; *E. c., E. coli* NIHJ JC-2; *P. a., P. aeruginosa* U-31.

physicochemical properties, because of their high hydrophilicities and capabilities of zwitterionic structure formations.

The results showed that some of these substituents contributed significantly to decrease lipophilicities as evidenced by partition coefficients, increase water solubilities, and enhance anti-*Streptococcal* activities of the corresponding quinolones. They are shown in Tables III–V.

The typical compound **28**, having a 2-(aminomethyl)-morpholine at the C-7 position, exhibited enhanced Gram-positive activity over **4**, especially the potent activities against *Streptococcus pneumoniae* and *Enterococcus faecalis* were remarkable. On the other hand, its Gram-negative activity was equipotent with that of **4**, NFLX, and OFLX. The compound **29**, 7-(2-(methylamino)-methyl)morpholino) derivative, displayed comparable potency, relatively increased partition coefficient, and similar water solubility as compared with **28**. Incorporation of the dimethylamino function (analogue **31**) reduced the in vitro potency compared to the nonmethylated analogue **28**, while apparent increases of partition coefficient and water solubility were observed. Modification of the primary amine of **28** with bulky groups had a tendency to decrease the activities against both Gram-positive and Gram-negative bacteria. For example, acetylamino and benzylamino analogues **34** and **35** were weak in potency. Neither ring expansion of morpholine nor lengthening of the aminoalkyl side chain afforded efficacious antibacterial activities, e.g., **41** and **42**. Pyridobenzoxazine derivative **36** also exhibited poor activity. Several quinolones having an alternative N-1 substituent in place of cyclopropyl group, such as ethyl **38**, fluoroethyl **39**, and difluorophenyl **40**, were not notable in their activities. Accordingly the cyclopropyl group seemed to be optimal as the N-1 substituent of morpholine series quinolones. Three compounds, **28**, **29**, and **31** prepared in this study were selected for evaluation of their in vivo efficacies, and the relationships among their physicochemical properties and in vivo efficacies were examined compared to those of the reference compounds NFLX, CPFX, and OFLX. In addition, convulsive activities of these compounds were investigated in the electrophysiological, biochemical, and behavioral experiments (Tables II and V).

Among the quinolones shown in Table V, **28**, **29**, and **31** showed more potent activities against *S. aureus* compared to the others, and their in vitro activities reflected the relative results on their in vivo efficacies when administered orally. As for the MICs against *E. coli*, CPFX showed the lowest value (<0.006 μg/mL), while the other quinolones (**28**, **29**, **31**, and OFLX) had similar value (0.05 μg/mL). Their in vivo efficacies appeared to be inconsistent with their MICs; that is, the most potent CPFX showed comparable $ED_{50}$ to that of less potent OFLX or **31**. In order to elucidate the inconsistency, we attempted to relate their partition coefficients and solubilities in water to their in vivo efficacies. However, there was no clear correlation. Nevertheless, the results indicate that in vivo efficacies of zwitterionic quinolones seem to be improved with increasing partition coefficients. In vivo efficacies of **37**, **38**,

Table V. In Vitro Activity, In Vivo Efficacy (po), and Physicochemical Property of Quinolones Prepared in This Study

| | *S. aureus* Smith | | *E. coli* KC14 | | partition coefficient[c] | solubility in water[d] |
|---|---|---|---|---|---|---|
| | MIC (μg/mL) | $ED_{50}$[a] (mg/mouse) | MIC (μg/mL) | $ED_{50}$[b] (mg/mouse) | | |
| 28 | 0.012 | 0.067 (0.047–0.098)[e] | 0.05 | 0.017 (0.012–0.024) | 0.57 | 580 |
| 29 | 0.012 | 0.044 (0.032–0.060) | 0.05 | 0.024 (0.017–0.034) | 1.40 | 630 |
| 31 | 0.012 | 0.035 (0.024–0.049) | 0.05 | 0.009 (0.005–0.012) | 20.1 | >1000 |
| 37 | 0.05 | 0.835 (0.600–1.161) | 0.10 | 0.139 (0.085–0.208) | 0.36 | 390 |
| 38 | 0.10 | 0.512 (0.356–0.728) | 0.39 | 0.232 (0.159–0.336) | 0.39 | 2900 |
| NFLX | 0.20 | 1.000 (0.688–1.452) | 0.05 | 0.054 (0.037–0.077) | 0.32 | 290 |
| CPFX | 0.10 | 0.354 (0.260–0.482) | ≤0.006 | 0.007 (0.005–0.009) | 0.78 | 70 |
| OFLX | 0.20 | 0.270 (0.186–0.392) | 0.05 | 0.009 (0.007–0.013) | 5.12 | 2300 |
| 4 | 0.05 | 1.304 (0.911–1.860) | 0.10 | 0.056 (0.030–0.094) | 305 | 9 |

[a] Inoculum size: $3.5 \times 10^6$ [$120LD_{50}$]. [b] Inoculum size: $1.0 \times 10^4$ [$40LD_{50}$]. [c] $CHCl_3$/0.1 M phosphate buffer (pH 7.4). [d] Solubility in water (μg/mL, at 25 °C). [e] 95% confidence limit.

and NFLX, which had lower partition coefficients, were not sufficient compared with those of other quinolones as shown in Table V.

Compound 4 is exceptional because of the lack of zwitterionic structure. Pharmacokinetic profile of an acidic quinolone like 4 may differ from that of a zwitterionic quinolone. The discrepancy in $ED_{50}$ values of 4 against *S. aureus* and *E. coli* infections seems to be derived from the difference in the modes of infection between both pathogens. In combination with BPAA, compound 28, 29, and 31 (as well as 4) did not reduce the GABA-induced chloride currents ($I_{Cl}$) and did not evoke any convulsive activities. The concentration of 31 in mouse brain after oral administration was much higher than those of 28 and 29 due to its increased partition coefficient.[25] The antibacterial activity of 28 against *Pseudomonas aeruginosa*, which is often resistant to various antibacterial agents, was more potent than that of 29. From these results, 28 (Y-26611) was selected as the candidate for clinical trial.

## Experimental Section

**Chemistry.** Melting points and boiling points are uncorrected. NMR spectra were recorded on a JEOL PS-100 or a JEOL GSX-400 spectrometer, and chemical shifts were reported in parts per million from internal tetramethylsilane. Mass spectra were obtained on a JMS-OISG instrument. The elemental analyses were performed by the Instrumental Analysis Section in the Research Laboratory of Yoshitomi Pharmaceutical Industries Ltd., Fukuoka, Japan, and were within ±0.3% of the theoretical values.

**2-(Acetamidomethyl)morpholine (7) (Method A).** A mixture of compound 5 (400 g, 1.77 mol), potassium phthalimide (394 g, 2.13 mol), and 1.2 L of DMF was refluxed for 5 h. To the solution was added 2 L of water, and the precipitates were collected by filtration to give 518 g (87.0%) of 4-benzyl-2-(phthalimidomethyl)morpholine: mp 136 °C; $^1$H NMR ($CDCl_3$) δ 1.92–2.36 (m, 2 H), 2.5–2.9 (m, 2 H), 3.4–4.0 (m, 7 H), 7.29 (s, 5 H), 7.6–7.9 (m, 4 H). A solution of 4-benzyl-2-(phthalimidomethyl)morpholine (336 g, 1 mol) and hydrazine monohydrate (100 g, 2 mol) in 3.36 L of EtOH was refluxed for 4 h. The precipitates were filtered off, and the filtrate was concentrated to give an oily product, which was then distilled to afford 172 g (83.4%) of 6: bp 128–133 °C (0.5 mm); $^1$H NMR ($CDCl_3$) δ 1.48 (s, 2 H), 1.76–2.84 (m, 4 H), 2.70 (d, 2 H, $J$ = 6 Hz), 3.38–3.50 (s, 2 H), 7.30 (s, 5 H), 4.10 (m, 3 H). Acetic anhydride (39.2 g, 0.384 mol) was added dropwise to a solution of 6 (66 g, 0.32 mol) in 330 mL of toluene at ice-cooled temperature, stirred for 3 h at room temperature, and then extracted with water. The aqueous layer was neutralized with $NaHCO_3$, and the resulting precipitates were collected by filtration to give 78.5 g (82.3%) of 2-(acetamidomethyl)-4-benzylmorpholine: mp 113 °C; $^1$H NMR ($CDCl_3$) δ 1.78–2.28 (m, 2 H), 1.98 (s, 3 H), 2.56–2.80 (m, 2 H), 3.0–3.9 (m, 5 H), 3.48 (s, 2 H), 5.7–6.0 (bs, 1 H), 7.28 (s, 5 H). To a mixture of 2-(acetamidomethyl)-4-benzylmorpholine (60 g, 0.242 mol), 10% Pd–C (15 g), and 500 mL of i-PrOH was added hydrazine monohydrate (12 g) and refluxed for 1.5 h. After removing the catalyst, the filtrate was concentrated in vacuo to give 35.1 g (91.9%) of 7 as an oil, which was crystallized by standing: $^1$H NMR ($CDCl_3$) δ 1.89 (s, 1 H), 1.98 (s, 3 H), 2.3–3.2 (m, 5 H), 3.3–3.9 (m, 4 H), 5.9–6.2 (bs, 1 H); MS (EI) $m/z$ 158 ($M^+$).

**2-(((Ethoxycarbonyl)amino)methyl)morpholine (11) (Method B).** Sulfuric acid (11 mL) was added to compound 8 (8.65 g, 40 mmol) at ice-cooled temperature. After being stirred at 40 °C for 4 h, the mixture was poured onto ice, made alkaline with NaOH (16.5 g), and then extracted with a mixture of AcOEt–THF (1:1, 50 mL × 2). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to leave an oily product which was crystallized from *n*-hexane to give 8.0 g (85%) of 9: mp 97–100 °C; $^1$H NMR ($CDCl_3$) δ 1.8–2.4 (m, 4 H), 2.4–2.8 (m, 2 H), 3.48 (s, 2 H), 3.5–4.0 (m, 3 H), 5.6–5.8 (bs, 1 H), 7.28 (s, 5 H). An aqueous solution of sodium hypochlorite (12%, 13.1 mL, 21 mmol) was added to a solution of 9 (2.34 g, 10 mmol) in 120 mL of EtOH at 6–9 °C and stirred for 1 h at ice-cooled temperature and then for an additional 1 h at 72 °C. The solution was concentrated to leave the residue, which was partitioned between water and $CHCl_3$. The separated organic layer was dried ($MgSO_4$) and evaporated at reduced pressure to leave the residue, which was chromatographed, eluting with $CHCl_3$–MeOH to give 1.7 g (61.1%) of 10: NMR ($CDCl_3$) δ 1.23 (t, 3 H, $J$ = 7 Hz), 1.80–2.28 (m, 2 H), 2.5–2.8 (m, 2 H), 2.9–3.8 (m, 5 H), 3.5 (s, 2 H), 4.09 (q, 2 H, $J$ = 7 Hz), 4.5–5.1 (bs, 1 H), 7.30 (s, 5 H). Compound 10 was converted to 11, mp 87–93 °C, in 87% of yield by the procedure used to prepare 7: $^1$H NMR ($CDCl_3$) δ 1.24 (t, 3 H, $J$ = 7 Hz), 2.0–2.4 (bs, 1 H), 2.4–3.9 (m, 9 H), 4.12 (q, 2 H, $J$ = 7 Hz), 5.0–5.2 (bs, 1 H).

**4-Benzyl-2-(carbamoylmethyl)morpholine (9) (Method C).** To a solution of 12 (1.32 g, 5.0 mmol) in 15 mL of 70% aqueous MeOH was added KOH (0.5 g), and the mixture was stirred overnight. The mixture was concentrated and partitioned between water and ether. The separated aqueous layer was taken at pH 6.8 with 3 N HCl, concentrated to 2 mL, and treated with 10 mL of EtOH. After removing the precipitates, the filtrate was concentrated at reduced pressure to give 1.1 g (93.3%) of 13 as a solid: $^1$H NMR ($CDCl_3$) δ 1.8–2.9 (m, 6 H), 3.3–4.0 (m, 5 H), 7.26 (s, 5 H), 9.9–10.1 (bs, 1 H). To a mixture of $PCl_5$ (0.96 g, 4.6 mmol) and 40 mL of $CH_2Cl_2$ was added 13 (1 g, 4.3 mmol) over 15 min at 12–15 °C under ice-cooling, and the mixture was stirred for an additional 3 h at 12 °C to give a clear solution. After the solution was concentrated, aqueous $NH_4OH$ was added to the residue, and the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried ($MgSO_4$), and concentrated at reduced pressure to give an oily residue, which was chromatographed eluting with $CHCl_3$–MeOH and crystallized from *n*-hexane to afford 0.43 g (43%) of 9.

**2-((Methylamino)methyl)morpholine, Dihydrochloride, Monohydrate (16a).** A mixture of compound 5 (58 g, 0.257 mol), *N*-benzyl-*N*-methylamine (62.3 g, 0.514 mol), NaI (38.6 g, 0.257 mol), and 220 mL of DMF was stirred for 6 h at 140 °C. The reaction mixture was concentrated and partitioned between water and AcOEt. The organic layer was washed with water, dried ($MgSO_4$), and evaporated. Treatment of the residue with 37% HCl–EtOH gave 67.0 g (68.0%) of 15a·2HCl: mp 122–124 °C dec; $^1$H NMR ($D_2O$) δ 2.8–3.6 (m, 6 H), 2.9 (s, 3 H), 3.7–4.5

(m, 3 H), 4.39 (s, 4 H), 7.51 (s, 10 H). Anal. ($C_{20}H_{26}N_2O{\cdot}2HCl$) C, H, N. A solution of **15a** (29.7 g, 0.077 mol) in 50 mL of EtOH and 80 mL of water was hydrogenated over 10% Pd-C (3 g) at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated and crystallized from EtOH to afford 16.6 g (97.0%) of **16a**: mp 122-124 °C; $^1$H NMR ($D_2O$) δ 2.76 (s, 3 H), 2.85-3.52 (m, 6 H), 3.70-4.30 (m, 3 H); MS (EI) $m/z$ 138 ($M^+$). Anal. ($C_6H_{14}N_2O{\cdot}2HCl{\cdot}H_2O$) C, H, N.

**2-((Ethylamino)methyl)morpholine, Dihydrochloride (16b).** Compound **16b**, mp 222-223 °C, was prepared starting from **5** and *N*-benzyl-*N*-ethylamine in 38.5% yield by the procedure used to prepare **16a**: $^1$H NMR ($D_2O$) δ 1.48 (t, 3 H, $J$ = 7 Hz), 2.9-3.5 (m, 8 H), 3.7-4.4 (m, 3 H).

**2-((*N,N*-Dimethylamino)methyl)morpholine (16c).** A solution of **5** (113 g, 0.50 mol) in 200 mL of 40% aqueous dimethylamine solution and 200 mL of EtOH was heated for 4 h at 150 °C in an autoclave. After concentration of the reaction solution, the residue was partitioned between water and toluene. The organic layer was washed with water, dried ($MgSO_4$), concentrated, and treated with 23% HCl-EtOH to give 123 g (79.5%) of **15c**·2HCl: mp 271-273 °C dec; $^1$H NMR ($D_2O$) δ 2.96 (s, 6 H), 3.08-4.40 (m, 9 H), 4.45 (s, 2 H). A solution of **15c** (122.5 g, 0.397 mol) in a mixture of 400 mL of EtOH and 400 mL of water was hydrogenated over 10% Pd-C (15 g) at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated. To a solution of the residue in 600 mL of MeOH was added a solution of sodium methoxide prepared from sodium (18.3 g, 0.80 mol) and 200 mL of MeOH. The resulting precipitates were removed by filtration, and the filtrate was concentrated and distilled to give 41.6 g (73%) of **16c**: bp 70-74 °C (6 mm); $^1$H NMR ($CDCl_3$) δ 1.7 (s, 1 H), 2.25 (s, 6 H), 2.0-3.0 (m, 6 H), 3.4-5.0 (m, 3 H).

**2-((*N,N*-Diethylamino)methyl)morpholine (16d).** Compound **16d**, bp 100-101 °C (13 mm), was prepared in 18.1% yield starting from **5** and *N,N*-diethylamine by the procedure used to prepare **16c**: $^1$H NMR ($CDCl_3$) δ 1.0 (t, 3 H), 1.76 (s, 1 H), 2.10-3.05 (m, 10 H), 3.4-4.0 (m, 3 H).

**2-((*N*-Benzyl-*N*-methylamino)methyl)morpholine, Dihydrochloride (16e).** A solution of **5** (13.5 g, 0.060 mol) in 210 mL of EtOH was hydrogenated over 10% Pd-C (2 g). The catalyst was filtered off, and the filtrate was concentrated to give 8.33 g (quant) of **17**. A solution of **17** (8.33 g, 0.060 mol) and ethyl formate (44 g, 0.6 mol) in 50 mL of $CHCl_3$ was refluxed for 3 h and concentrated at reduced pressure, and then the residue was chromatographed eluting with $CHCl_3$-MeOH to give 9.9 g (quant) of **18** as an oil: $^1$H NMR ($CDCl_3$) δ 2.5-4.5 (m, 9 H), 8.08 (s, 1 H). A mixture of **18** (9.9 g, 0.060 mol), NaI (9 g, 0.060 mol), *N*-benzyl-*N*-methylamine (7.63 g, 0.063 mol), and $K_2CO_3$ (8.29 g, 0.060 mol) in 50 mL of DMF was stirred at 150-160 °C for 4 h. The volatile was evaporated at reduced pressure, and the residue was partitioned between water and $CHCl_3$. The separated organic layer was dried ($MgSO_4$), concentrated, and chromatographed eluting with $CHCl_3$-MeOH to give 10.1 g (54.2%) of **19** as an oil: $^1$H NMR ($CDCl_3$) δ 2.30 (s, 3 H), 2.5-4.5 (m, 9 H), 3.53 (s, 2 H), 7.27 (s, 5 H), 8.03 (s, 1 H). A solution of **19** (2.49 g, 8.0 mmol) in 14.4 mL of 4 N HCl and 45 mL of MeOH was refluxed for 3 h, concentrated, and then treated with 15 mL of EtOH to give precipitates which were collected by filtration to give 3.15 g (68.0%) of **16e**: mp 207-209 °C; $^1$H NMR ($D_2O$) δ 2.93 (s, 3 H), 2.7-3.5 (m, 6 H), 3.7-4.6 (m, 5 H), 7.51 (s, 5 H).

**2-(Piperidinomethyl)morpholine, Dihydrochloride (16f).** Compound **16f**, mp 172-174 °C, was prepared in 49% yield starting from **18** and piperidine by the procedure used to prepare **16e**: $^1$H NMR ($D_2O$) δ 1.4-2.0 (m, 6 H), 2.8-3.7 (m, 10 H), 3.8-4.4 (m, 3 H); MS (EI) $m/z$ 184 ($M^+$).

**4-Benzyl-2-(2-chloroethyl)morpholine (21).** To a solution of **20** (6.16 g, 0.028 mol) in 70 mL of $CHCl_3$ was added thionyl chloride (4.0 mL, 0.055 mol), and the mixture was refluxed for 2.5 h. After concentration of the reaction mixture, water and ether were added to the residue, and then neutralized with $K_2CO_3$. The separated ether layer was dried ($MgSO_4$), evaporated, and chromatographed, eluting with $CHCl_3$-MeOH to give 2.8 g (42%) of **21** as an oil: $^1$H NMR ($CDCl_3$) δ 1.5-2.3 (m, 4 H), 2.5-2.8 (m, 2 H), 3.48 (s, 2 H), 3.5-3.9 (m, 5 H), 7.27 (s, 5 H).

**2-(2-Acetamidoethyl)morpholine (22).** Compound **22**, an oily product, was prepared in 31.3% yield according to the same procedure as for **7** starting from **21**: $^1$H NMR ($CDCl_3$) δ 1.6-2.0 (m, 2 H), 1.89 (s, 1 H), 1.98 (s, 3 H), 2.3-3.3 (m, 6 H), 3.5-3.9 (m, 3 H), 5.9-6.2 (bs, 1 H).

**2-(Aminomethyl)hexahydro-1,4-oxazepine (26).** A mixture of **23** (165 g, 1.0 mol) and epichlorohydrin (92 g, 1.0 mol) was stirred at 40 °C for 2.5 h and concentrated at reduced pressure. To the residue was added 300 mL of $H_2SO_4$, and the mixture was stirred at 150 °C for 0.5 h and then poured onto ice-water. The resulting solution was made alkaline with aqueous NaOH solution and extracted with toluene. The organic layer was washed with water, dried ($MgSO_4$), and concentrated to give an oil, which was distilled to give 110 g (45.9%) of **24**: bp 125-129 °C (0.4 mm); $^1$H NMR ($CDCl_3$) δ 1.7-2.0 (m, 2 H), 2.4-3.0 (m, 4 H), 3.3-3.5 (m, 2 H), 3.65 (s, 2 H), 3.7-4.1 (m, 3 H), 7.1-7.4 (m, 5 H). A mixture of **24** (47.9 g, 0.2 mol), potassium phthalimide (44.5 g, 0.24 mol), and 300 mL of DMF was refluxed for 3 h, poured onto ice-water, and then extracted with AcOEt. The organic layer was washed with water, dried ($MgSO_4$), and concentrated. Isopropyl ether was added to the residue, and the precipitates were collected by filtration and dried to give 54.1 g (77.2%) of 4-benzyl-2-(phthalimidomethyl)hexahydro-1,4-oxazepine: mp 83-87 °C; $^1$H NMR ($CDCl_3$) δ 1.7-2.1 (m, 2 H), 2.4-3.0 (m, 4 H), 3.3-4.2 (m, 7 H), 7.05-7.45 (m, 5 H), 7.5-8.0 (m, 4 H). A solution of hydrazine monohydrate (16.2 g, 0.324 mol) in 100 mL of EtOH was added to a solution of this compound (54.0 g, 0.154 mol) in 540 mL of EtOH while refluxing, and this was continued for an additional 1 h. After cooling, the precipitated solid was filtered off and the filtrate was concentrated at reduced pressure. The residue was partitioned between water and toluene, and the separated organic layer was washed with water, dried ($MgSO_4$), and evaporated in vacuo to give an oily product, which was distilled to give 27.3 g (80.4%) of **25**: bp 157-160 °C (9 mm); $^1$H NMR ($CDCl_3$) δ 1.3 (s, 2 H), 1.7-2.05 (m, 2 H), 2.2-3.0 (m, 6 H), 3.4-4.1 (m, 5 H), 7.1-7.5 (m, 5 H). To a solution of **25** (27.3 g, 0.124 mol) in 270 mL of EtOH was added 5% Pd-C (11 g) and hydrazine monohydrate (16.2 g, 0.324 mol), and the mixture was refluxed for 4 h. The catalyst was removed by filtration, and the filtrate was evaporated to give an oil, which was distilled to give 13.9 g (86.2%) of **26**: bp 88-89 °C (10 mm); $^1$H NMR ($CDCl_3$) δ 1.44 (s, 3 H), 1.7-2.0 (m, 2 H), 2.5-2.8 (m, 3 H), 2.8-3.1 (m, 3 H), 3.3-4.2 (m, 3 H).

**7-[2-(Aminomethyl)morpholino]-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid, Dihydrate (28).** A mixture of **27a** (24.45 g, 0.086 mol), **7** (16.4 g, 0.104 mol), $Et_3N$ (9.21 g, 0.091 mol), and 300 mL of $CH_3CN$ was refluxed for 8.5 h. After cooling, 150 mL of EtOH was added to the reaction mixture. The precipitates were collected by filtration, washed with EtOH (20 mL), and dried to give 35.0 g (96.2%) of **34**: mp 230-234 °C; $^1$H NMR (pyridine-$d_5$) δ 0.9-1.2 (m, 4 H), 2.1 (s, 3 H), 3.1-4.1 (m, 9 H), 4.7-5.1 (m, 1 H), 8.03 (dd, 1 H, $J$ = 13, 2 Hz), 8.85 (s, 1 H). A mixture of **34** (35.0 g, 0.083 mol), 105 mL of water, and 140 mL of 12 N HCl was refluxed for 2.5 h and concentrated to one-third of the volume at reduced pressure. To the residue was added 400 mL of acetone, and the resulting precipitates were collected and dried to give 21.0 g of **28** as hydrochloride: mp 277-279 °C. The hydrochloride of **28** (21.0 g) was dissolved in 400 mL of water, and the solution was adjusted to pH 6.8 with 10% aqueous NaOH solution. The precipitates were collected by filtration and washed with water to give 17.3 g (50.1% from **34**) of **28**: mp 183-185 °C; $^1$H NMR ($CD_3COOD$) δ 1.2-1.4 (m, 4 H), 3.0-3.7 (m, 6 H), 3.7-4.3 (m, 4 H), 7.90 (dd, $J$ = 12, 2 Hz, 1 H), 8.87 (s, 1 H); MS (EI) $m/z$ 379 ($M^+$). Anal. ($C_{18}H_{19}F_2N_3O_4{\cdot}2H_2O$) C, H, N.

Compound **28** was also prepared in 68.4% yield according to the same procedure described above starting from **27a** and **11**.

**1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[2-[(methylamino)methyl]morpholino]-4-oxo-3-quinolinecarboxylic Acid (29).** A mixture of **27a** (8.3 g, 0.029 mol), **16a** (7.1 g, 0.032 mol), DBU (15.2 g, 0.01 mol), and 166 mL of $CH_3CN$ was refluxed for 12 h. After cooling, the resulting precipitates were collected by filtration, washed with water, and recrystallized from DMF to give 4.3 g (37.3%) of **29**: mp 257-259 °C dec; $^1$H NMR ($CD_3COOD$) δ 1.2-1.4 (m, 4 H), 2.88 (s, 3 H), 3.0-3.7 (m, 6 H), 3.7-4.3 (m, 4 H), 7.90 (dd, $J$ = 12, 2 Hz, 1 H), 8.87 (s, 1 H); MS (EI) $m/z$ 393 ($M^+$). Anal. ($C_{19}H_{21}F_2N_3O_4$) C, H, N.

Compounds **30-42** were also prepared by the similar procedure.

**7-[2-(Aminomethyl)morpholino]-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid, Hydrochloride (45).** To a solution of 34 (4.21 g, 0.01 mol) in 80 mL of $CHCl_3$ was added a solution of sulfuryl chloride (3.38 g, 0.025 mol) in 20 mL of $CHCl_3$, and the mixture was stirred for 0.5 h at room temperature and washed with 50 mL of water. The separated aqueous layer was extracted with 50 mL of $CHCl_3$. The combined $CHCl_3$ layer was dried ($MgSO_4$) and evaporated, to which 10 mL of EtOH was added. The precipitates were collected and dried to give 3.74 g (81.8%) of 44: mp 220–222 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 0.9–1.3 (m, 4 H), 1.82 (s, 3 H), 2.8–4.05 (m, 9 H), 4.2–4.5 (m, 1 H), 7.94 (d, $J$ = 12 Hz, 1 H), 7.8–8.1 (b, 1 H), 8.8 (s, 1 H). A mixture of 44 (1.39 g, 3.2 mmol), 6 mL of 12 N HCl, and 6 mL of water was refluxed for 2 h and concentrated in vacuo. To this residue was added 30 mL of EtOH, and the resulting precipitates were collected and recrystallized from a mixed solvent of acetic acid and toluene to give 400 mg (31.8%) of 45: mp 265–266 °C dec; $^1H$ NMR ($CD_3COOD$) $\delta$ 0.9–1.5 (m, 4 H), 3.0–3.7 (m, 6 H), 3.7–4.6 (m, 4 H), 8.0 (d, $J$ = 12 Hz, 1 H), 9.04 (s, 1 H); MS (EI) $m/z$ 395 ($M^+$). Anal. ($C_{18}H_{19}ClFN_3O_4{\cdot}HCl{\cdot}{}^1/_2H_2O$) C, H, N.

**Acknowledgment.** We thank Dr. Yasunori Morio and Mr. Masamitsu Sakamori for expert technical assistance.

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