Synthesis and Antibacterial Activity of Thiazolopyrazine-Incorporated Tetracyclic Quinolone Antibacterial Agents. 2[1]

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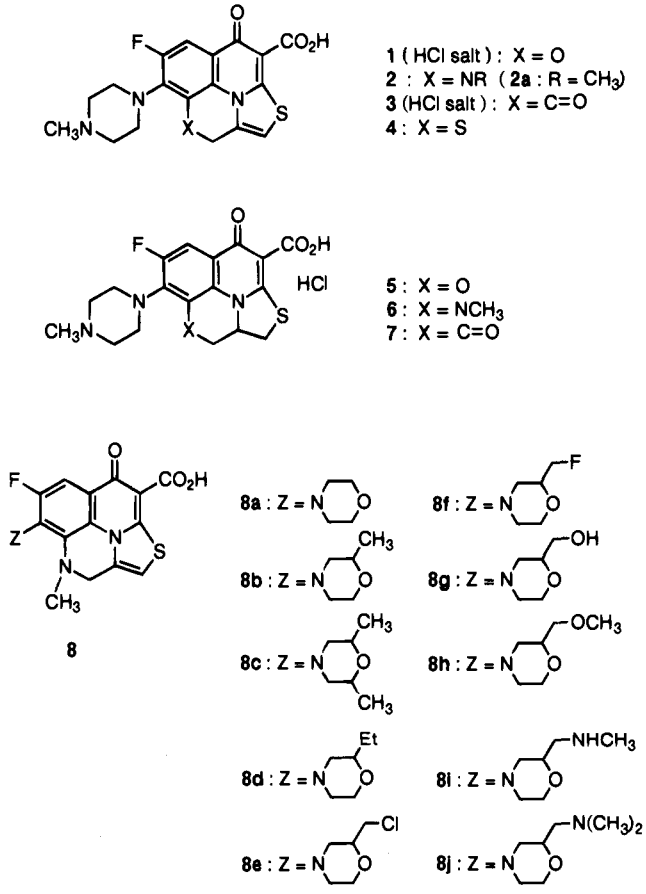
A novel series of 8-(2-substituted morpholino)-9,1-[(*N*-methylimino)methano]-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acids, designated **8a–j**, with a unique tetracyclic structure were synthesized, and the *in vitro* and *in vivo* antibacterial activities against Gram-positive strains, including methicillin-resistant *Staphylococcus aureus* isolates (MRSA), and Gram-negative strains were evaluated. These morpholino derivatives, **8a–j**, showed excellent *in vitro* antibacterial activities against Gram-positive bacteria. The substitutions at the C-2 position of the 8-morpholino moiety of compound **8** play an important role in the enhancement of *in vivo* antibacterial activity. The unsubstituted morpholino derivative **8a**, the 2,6-dimethyl derivative **8c**, and the 2-ethylmorpholino derivative **8d** showed poor *in vivo* antibacterial activity, while **8b**, **8f–h**, and **8j** exhibited good activities. The 2-(methoxymethyl)morpholino derivative, **8h**, showed the most potent activity *in vivo*. The therapeutic effects of **8h** on systemic infection against *S. aureus* IID 803 were over 10-fold more potent than that of ofloxacin. Compound **8h**, which showed superior oral bioavailability, has a chiral center. The enantiomers of **8h** were synthesized, and the *in vitro* and *in vivo* antibacterial activities were evaluated. Both enantiomers, (***S***)-**8h** and (***R***)-**8h**, and the racemic compound **8** exhibited similar activities *in vitro* and *in vivo*. Compounds **8b** and **8f–h** also showed good levels of antibacterial activity against MRSA strains. The morpholino derivatives with unique tetracyclic structures are characterized by strong antibacterial activities against MRSA strains.

Quinolone antibacterial agents, such as norfloxacin (NFLX),[2] enoxacin (ENX),[3] ofloxacin (OFLX),[4] ciprofloxacin (CPFX),[5] tosfloxacin (TFLX),[6] and lomefloxacin (LMLX),[7] show broad antibacterial spectra against Gram-positive and Gram-negative bacteria and are used clinically. One of the most significant shortcomings of these quinolone antibacterial agents is their inefficacy against some clinically important pathogenic bacteria, i.e., methicillin-resistant *Staphylococcus aureus* (MRSA), and resistance to NFLX, CPFX, and OFLX has already been observed clinically.[8] During our study of quinolone antibacterial agents, we were interested in the construction of a clinically useful quinolone for the therapy of infections caused by MRSA.

We recently reported the synthesis and the antibacterial activities of tetracyclic pyridonecarboxylic acids with a flat thiazole ring (**1–4**[9,10]) and with a nonflat thiazolidine ring (**5–7**[11]). Compounds **1**, **2** (X = NMe; **2a**), and **5** showed potent antibacterial activity. Moreover, with regard to the structure–activity relationship of cycloamino groups at the C-8 position in **2a**, we reported[1] that compound **8a**, with a morpholino group, showed the most potent antibacterial activity against Gram-positive bacteria and good activity against MRSA isolates, but had insufficient *in vivo* activity because of low oral absorption. We studied several morpholino derivatives for the purpose of improving their *in vivo* antibacterial activities.

In the present study, we describe the synthesis and antibacterial activity of a new series of tetracyclic pyridonecarboxylic acid compounds, **8a–j**, with several different morpholino groups at the C-8 position. In addition, we characterized the potency of these compounds against MRSA and report the antibacterial activities of the enantiomers, (***R***)-**8h** and (***S***)-**8h**, of the 2-methoxymethylmorpholino derivative **8h**, which showed superior oral bioavailability.

Chart 1



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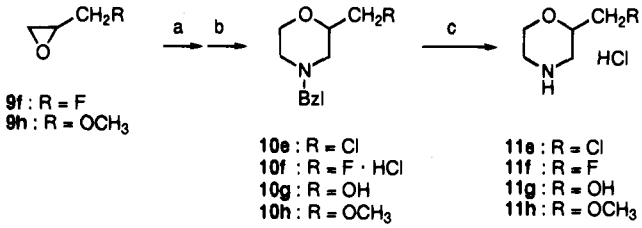
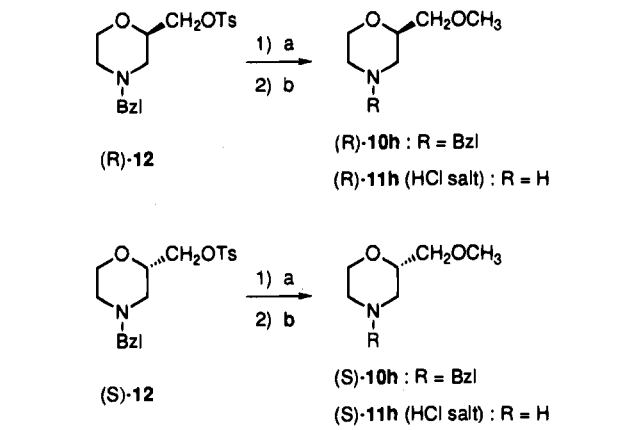
Scheme 1[a]



[a] (a) *N*-Benzylethanolamine; (b) *p*-TsCl, KOH, tris(3,6-dioxaheptyl)amine or (i) H_2SO_4, (ii) HCl; (c) (i) H_2/Pd–C, (ii) HCl.

Scheme 2[a]



[a] (a) CH_3ONa; (b) H_2/Pd–C, HCl.

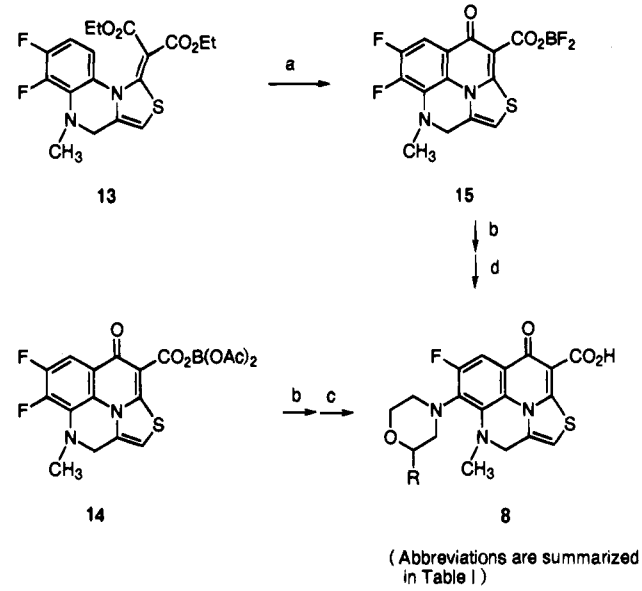
Chemistry

The 2-(substituted methyl)morpholine compounds, **11e–h**, were prepared according to Scheme 1. The substituted propylene oxides, **9f** and **9h**, were reacted with *N*-benzylethanolamine in ethyl acetate, followed by heating in H_2SO_4 or by treatment with *p*-TsCl, KOH, and tris(3,6-dioxaheptyl)amine to yield 1-benzyl-2-(fluoromethyl)morpholine (**10f**) and 1-benzyl-2-(methoxymethyl)morpholine (**10h**), respectively. Hydrogenation of **10f**, **10h**, 1-benzyl-2-(chloromethyl)morpholine (**10e**),[12] and 1-benzyl-2-(hydroxymethyl)morpholine (**10g**)[12] gave 2-(fluoromethyl)- (**11f**), 2-(methoxymethyl)- (**11h**), 2-(chloromethyl)- (**11e**), and 2-(hydroxymethyl)morpholine (**11f**), respectively. 2-Methyl- (**11b**),[13] 2-ethyl- (**11d**),[13] 2-[(methylamino)methyl]- (**11i**),[14] and 2-[(dimethylamino)methyl]-morpholine (**11j**)[14] were prepared according to the previously reported procedures.

The optically pure enantiomers, (*R*)-2-(methoxymethyl)morpholine [**(*R*)-11h**] and (*S*)-2-(methoxymethyl)-morpholine [**(*S*)-11h**], were prepared from (*R*)-1-benzyl-2-[[(*p*-tolylsulfonyl)oxy]methyl]morpholine (**(*R*)-12**)[15] and (*S*)-1-benzyl-2-[[(*p*-tolylsulfonyl)oxy]methyl]morpholine [**(*S*)-12**],[15] respectively (Scheme 2). Reaction of **(*R*)-12** and **(*S*)-12** with sodium methoxide, followed by hydrogenation, yielded **(*R*)-11h** and **(*S*)-11h**, respectively.

The desired quinolones, **8a–j**, were prepared according to Scheme 3 (R is summarized in Table 1). The quinolone intermediate **14** was prepared according to the previously reported procedure.[1] The quinolone intermediate **15** was prepared by cyclization and boration of the ester **13**[1] with borane trifluoride etherate in acetic anhydride. Compounds **14** and **15** were reacted with the corresponding 2-substituted morpholine in the presence of triethylamine in dimethyl sulfoxide and were hydrolyzed to yield the desired **8** series quinolones.

Scheme 3[a]



[a] (a) BF_3 etherate; (b) 2-substituted morpholine 11, Et_3N; (c) HCl; (d) NaOH.

The structures of compounds **8a–j**, listed in Table 1, were confirmed by their NMR spectra and elemental analysis.

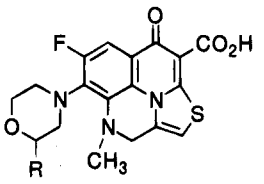
Biological Results and Discussion

Table 2 shows the *in vitro* antibacterial activity of the tetracyclic pyridonecarboxylic acids, **8a–j**, against five Gram-positive bacteria (*Staphylococcus aureus* FDA 209P JC-1, *Staphylococcus aureus* IID 803, *Staphylococcus epidermidis* IAM 1296, *E. faecalis* IID 682, and *Micrococcus luteus* ATCC 9341), and five Gram-negative bacteria (*E. coli* NIHJ JC-2, *E. coli* KC-14, *Klebsiella pneumoniae* B54, *Salmonella typhimurium* IID 971, *Pseudomonas aeruginosa* IFO 3445, and *Pseudomonas aeruginosa* E-2). Data for OFLX are included for comparison. Against Gram-positive bacteria, compounds **8b** and **8e–g**, with methyl, chloromethyl, fluoromethyl, or hydroxymethyl functional groups at the C-2 position of the morpholino moiety, exhibited antibacterial activity equivalent to that of the unsubstituted morpholino compound **8a**, and compounds **8d** and **8h**, with an ethyl or methoxymethyl group, and the 2,6-dimethylmorpholino derivative **8c** were slightly less potent than compound **8a**. Compounds **8i** and **8j**, with an aminomethyl group substituted at the C-2 position of the morpholino moiety, were 4–8-fold less potent than compound **8a** against Gram-positive bacteria. The introduction of aminomethyl groups resulted in a reduction in the *in vitro* antibacterial activity. This result was different from that reported in the 1-cyclopropyl-8-fluoroquinolonecarboxylic acid system,[14] in which this modification allowed the maintenance of moderate antibacterial activity.

On the other hand, against Gram-negative bacteria, the introduction of functional groups on the morpholino moiety tended to weaken the antibacterial activity, as compared to the unsubstituted compound **8a**. Especially against *K. pneumoniae*, the substituted morpholino compounds showed 4–32-fold less potency than **8a**.

The oral efficacy of several **8** series morpholino derivatives against *S. aureus* IID 803 on systemic

Table 1. Tetracyclic Pyridonecarboxylic Acids **8**



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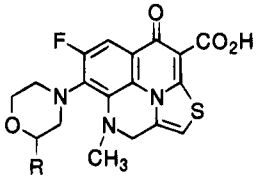
compd	R	method[a]	yield, %[b]	mp, °C	recrst solvent	formula[c]
8b	CH_3	A	62	256 dec	CH_3CN/EtOH	$C_{19}H_{18}FN_3O_4S$
8c[d]	$(CH_3)_2$	A	69	>280	CH_3CN/EtOH	$C_{20}H_{20}FN_3O_4S$
8d	Et	A	71	242–243	CH_3CN/EtOH	$C_{20}H_{20}FN_3O_4S$
8e	CH_2Cl	A	65	250 dec	CH_3Cl/MeOH	$C_{19}H_{17}ClFN_3O_4S$
8f	CH_2F	A	46	245 dec	CH_3CN/EtOH	$C_{19}H_{17}F_2N_3O_4S$
8g	CH_2OH	A	42	257 dec	DMSO	$C_{19}H_{18}FN_3O_5S$
8h	CH_2OCH_3	A	62	220 dec	DMSO/EtOH	$C_{20}H_{20}FN_3O_5S$
8i	CH_2NHCH_3	B	28	220 dec	EtOH/$CHCl_3$	$C_{20}H_{21}FN_4O_4S$
8j	$CH_2N(CH_3)_2$	B	12	195–199	EtOH	$C_{21}H_{23}FN_4O_4S$
(***S***)-**8h**	CH_2OCH_3	C	70	220 dec	DMSO/EtOH	$C_{20}H_{20}FN_3O_5S$
(***R***)-**8h**	CH_2OCH_3	C	68	220 dec	DMSO/EtOH	$C_{20}H_{20}FN_3O_5S$

[a] See the Experimental Section. [b] Yields are those obtained from the replacement step to the final product isolation including hydrolysis. [c] The analyses for C, H, N were within ±0.4% of the theoretical values. [d] 2,6-Dimethylmorpholino compound and the mixture of cis and trans isomer (about 10:1 from NMR).

Table 2. *In Vitro* Antibacterial Activity[a] (Minimum Inhibitory Concentration, μg/mL) of Tetracyclic Pyridonecarboxylic Acids **8**



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		Gram-positive microorganism[b]					Gram-negative microorganism[b]				
compd	R	Sa(F)	Sa(I)	Se	Ef	MI	Ec(N)	Ec(K)	Kp	Pa(I)	Pa(E)
8a	H	0.006	0.006	0.025	0.05	0.10	0.10	0.10	0.05	0.39	0.39
8b	CH_3	0.0125	0.006	0.0125	0.05	0.10	0.10	0.10	0.39	0.78	0.78
8c[c]	$(CH_3)_2$	0.0125	0.0125	0.10	0.20	0.20	0.39	0.39	0.78	3.13	3.13
8d	Et	0.025	0.025	0.05	0.10	0.20	0.39	0.39	0.39	3.13	1.56
8e	CH_2Cl	0.0125	0.006	0.0125	0.05	0.05	0.20	0.20	0.39	1.56	1.56
8f	CH_2F	0.0125	0.006	0.025	0.05	0.10	0.10	0.10	0.20	0.78	0.78
8g	CH_2OH	0.0125	0.006	0.05	0.10	0.20	0.10	0.10	0.39	1.56	1.56
8h	CH_2OCH_3	0.025	0.0125	0.05	0.20	0.20	0.20	0.39	0.78	3.13	3.13
8i	CH_2NHCH_3	0.025	0.05	0.10	0.20	0.78	0.20	0.20	1.56	3.13	3.13
8j	$CH_2N(CH_3)_2$	0.025	0.025	0.05	0.10	0.20	0.39	0.20	0.39	6.25	6.25
(***R***)-**8h**	CH_2OCH_3	0.025	0.006	0.025	0.20	0.20	0.39	0.39	0.39	3.13	3.13
(***S***)-**8h**	CH_2OCH_3	0.025	0.0125	0.05	0.20	0.20	0.20	0.39	0.78	3.13	3.13
ofloxacin		0.39	0.39	0.78	1.56	3.13	0.10	0.10	0.10	1.56	1.56

[a] All values are the mean from duplicate or triplicate experiments. [b] Microorganism: Sa(F), *S. aureus* FDA 209P JC-1; Sa(I), *S. aureus* IID 803; Se, *S. epidermidis* IAM 1296; Ef, *E. faecalis* IID 682; MI, *M. luteus* ATCC 9341; Ec(N), *E. coli* NIHJ JC-2; Ec(K), *E. coli* KC-14; Kp, *K. pneumoniae* B54; Pa(I), *P. aeruginosa* IFO 3445; Pa(E), *P. aeruginosa* E-2. [c] 2,6-Dimethylmorpholino compound and the mixture of cis and trans isomer (about 10:1 from NMR).

Table 3. Oral Efficacy of **8** on Systemic Infections in Mice

compd	ED_{50}[a] mg/kg po *S. aureus* IID 803	compd	ED_{50}[a] mg/kg po *S. aureus* IID 803
8a	>12	**8g**	5.1
8b	3.5	**8h**	1.7
8c	>12	**8j**	4.8
8d	>12	ofloxacin	11.4
8f	1.9		

infections in mice are compared with that of OFLX in Table 3. Morpholino derivatives **8b**, **8f–h**, and **8j** exhibited good oral efficacy, but compounds **8a**, **8c**, and **8d** were ineffective.

With regard to the structure–activity relationship of cycloamino groups at the C-8 position of the thiazolopyrazine-incorporated tetracyclic pyridone carboxylic acid framework, we recently reported[1] that there were structural restrictions for producing oral antibacterial activity; only piperazinyl derivatives with an alkyl group at the N-4 position of the 8-piperazinyl moiety showed oral efficacy. Interestingly, we also found that the introduction of functional groups at the C-2 position of the 8-morpholino moiety enhanced the oral antibacterial activity.

The serum concentrations following a single oral administration of **8b** and **8f–h** to mice are shown in Figure 1. The peak serum concentrations of **8b** and **8f–h** were 0.05, 0.63, 0.25, and 2.63 μg/mL, respectively. The serum concentration of **8h** was 4–50-fold higher than those of the other three compounds. The introduction of a methoxymethyl group at the C-2 position of the 8-morpholino moiety resulted in excellent bioavailability after oral administration.

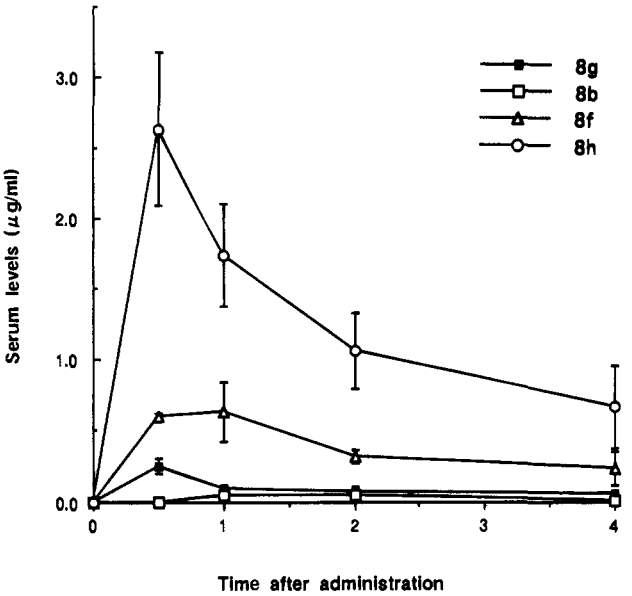

Figure 1. Serum concentrations of **8b** and **8f–h** following single oral administration of 20 mg/kg in mice.

Table 4. Therapeutic Effects of **8h** and its enantiomers (***R***)-**8h**, (***S***)-**8h** on Systemic Infections in Mice

compd	ED_{50}, mg/kg po (95% CL) *S. aureus* IID 803
8h	1.09 (0.73–1.61)
(***R***)-**8h**	1.25 (0.94–1.64)
(***S***)-**8h**	0.82 (0.53–1.28)
ofloxacin	14.6 (4.11–51.8)

Compound **8h** has an asymmetric center on the morpholino moiety. Therefore, we were interested in the enantiomers of **8h** and examined their relative *in vitro* and *in vivo* antibacterial activities. Compound **8h** and both its *R* and *S* enantiomers ((***R***)-**8h** and (***S***)-**8h**, respectively) showed essentially identical antibacterial potencies *in vitro* (Table 2). The oral efficacies of **8h** and its enantiomers, (***R***)-**8h** and (***S***)-**8h**, against *S. aureus* IID803 on systemic infections in mice are compared with that of OFLX in Table 4. The *in vivo* activities of **8h** and its enantiomers, (***R***)-**8h** and (***S***)-**8h**, were over 10-fold more potent than that of OFLX. This indicates that the introduction of a methoxymethyl group at the C-2 position of the 8-morpholino moiety enhanced the *in vivo* antibacterial activity and that the stereochemical properties have no influence on either *in vitro* or *in vivo* antibacterial activity.

Pathogenic infections caused by MRSA strains are a serious medical problem. The *in vitro* antibacterial activities of compounds **8a**, **8b**, and **8e–h** against MRSA strains isolated clinically in 1988 and 1989 in Japan are shown in Table 5. Data for OFLX are included for comparison, showing that OFLX-resistant MRSA strains have emerged. All compounds showed excellent activities against MRSA isolates, including the OFLX-resistant strains. It is notable that these MIC values were lower than 1 μg/mL, and that the activities of the **8** series of quinolones were 64–250-fold more potent than that of OFLX.

In conclusion, the morpholino derivatives with thiazolopyrazine-incorporated tetracyclic pyridonecarboxylic acid structures exhibited excellent activities against Gram-positive bacteria, including MRSA strains, and the introduction of several functional groups at the C-2 position of the 8-morpholino moiety resulted in good oral efficacy. Of these, compound **8h** showed superior oral activity and is expected to become a candidate for use as a therapeutic anti-MRSA bacterial agent.

Table 5. *In Vitro* Antibacterial Activity (MIC, μg/mL) of **8** against 16 Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*[a]

compd	MIC range[b]	MIC_{50}[c]	MIC_{90}[d]
8a	≤0.006–0.39	0.20	0.20
8b	≤0.006–0.20	0.10	0.20
8e	≤0.006–0.20	0.10	0.20
8f	≤0.006–0.20	0.20	0.20
8g	≤0.006–0.78	0.39	0.78
8h	≤0.006–0.78	0.39	0.78
ofloxacin	0.39–50	6.25	50

[a] Strains were isolated between 1988 and 1989 in Japan. [b] Range of MIC value for isolates. [c] MIC value for 50% of isolates. [d] MIC value for 90% of isolates.

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus, Model 535; all melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer, with TMS or 3-(trimethylsilyl)-3-propanesulfonic acid sodium salt as an internal reference in a solution of $CDCl_3$, DMSO-d_6, or D_2O. IR spectra were recorded with a Hitachi IR 270-50 infrared spectrometer. Elemental analyses were performed with a Yanagimoto CHN-CORDER MT-3, and all analytical values were within ±0.4% of the calculated theoretical values.

4-Benzyl-2-(fluoromethyl)morpholine Hydrochloride (10f). The mixture of *N*-benzylethanolamine (10.0 g, 0.066 mol) and epifluorohydrin (10.0 g, 0.131 mol) was stirred at 45 °C for 3 h and then evaporated the excess epifluorohydrin *in vacuo*. To the residue was added sulfuric acid (20 mL), and the mixture was stirred at 150 °C for 1 h. The reaction mixture was allowed to cool and then poured into ice water. The solution was made basic by adding aqueous NaOH and extracted with toluene. The toluene layer was washed with brine, dried over $MgSO_4$, and then evaporated. To the residue was added HCl/ethyl acetate (4 N), and the precipitates were collected by filtration, washed with ether to give **10f** (8.9 g, 54%) as colorless crystals: mp 160–163 °C; ^{1}H-NMR ($CDCl_3$) δ 3.1–3.3 (2 H, m), 3.5–3.6 (2 H, m), 3.9–4.2 (3 H, m), 4.4–4.7 (4 H, m), 7.54 (5 H, m). Anal. ($C_{12}H_{16}FNO$·HCl) C, H, N.

4-Benzyl-2-(methoxymethyl)morpholine (10h). The mixture of *N*-benzylethanolamine (459 g, 3.04 mol) and 2,3-epoxypropyl methyl ether (422 g, 4.79 mol) was stirred at 50 °C for 16 h and then evaporated the excess 2,3-epoxypropyl methyl ether *in vacuo*. The residue was dissolved in 1,4-dioxane (3 L), and powdery potassium hydroxide (692.5 g, 12.34 mol) and tris(3,6-dioxaheptyl)amine (11.4 g, 35.2 mmol) were added. To the mixture was added dropwise with stirring a solution of *p*-toluenesulfonyl chloride (809.4 g, 4.25 mol) in 1,4-dioxane (2 L) over a period of 1.5 h. The mixture was stirred for 2 h and filtered, and the filtrate was evaporated *in vacuo*. To the residue were added water (600 mL) and concentrated HCl (300 mL) to make an acidic solution. The aqueous solution was washed with ethyl acetate, made strongly basic by adding NaOH (160 g) under ice cooling, and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and then evaporated. The residue was distilled *in vacuo* (103 °C/0.25 mmHg) to give **10h** (391.3 g, 58%) as colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 1.97 (1 H, dd, J = 10.5, 11 Hz), 2.19 (1 H, dt, J = 3, 11 Hz), 2.66 (1 H, dq, J = 2, 11.5 Hz), 2.73 (1 H, dt, J = 2, 11 Hz), 3.3–4.0 (7 H, m), 3.36 (3 H, s), 7.2–7.4 (5 H, m). Anal. ($C_{13}H_{19}NO_2$) C, H, N.

2-(Fluoromethyl)morpholine Hydrochloride (11f). A solution of **10f** (1.46 g, 5.94 mmol) in ethanol (85 mL) was hydrogenated over 10% Pd–C (0.20 g) under maximum hydrogen pressure of 6 kg/cm^2 at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated. To the residue was added ether/ethanol (4:1, 2.5

mL), and the precipitated crystals were collected by filtration to give **11f** (0.60 g, 64%) as colorless crystals: ^{1}H-NMR (D_2O) δ 3.1–3.5 (4 H, m), 3.9–4.2 (3 H, m), 4.5–4.7 (2 H, m).

2-(Chloromethyl)morpholine Hydrochloride (11e). A solution of 1-benzyl-2-(chloromethyl)morpholine (**10e**) (5.50 g, 24.4 mmol) in acetic acid (55 mL) was hydrogenated over 10% Pd–C (1.65 g) under hydrogen pressure of 5 kg/cm^2 at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated. To the residue was added HCl (2 N, 19.5 mL) and ethanol (50 mL), the solvent was evaporated, and the residue was washed with acetone to give **11e** (3.76 g, 89%) as colorless crystals: ^{1}H-NMR (D_2O) δ 2.9–3.1 (4 H, m), 3.5–4.0 (5 H, m).

By use of this procedure, **11g** and **11h** were prepared from **10g** and **10h**, respectively. **11g**: ^{1}H-NMR (D_2O) δ 3.0–3.2 (4 H, m), 3.5–4.0 (5 H, m). **11h**: ^{1}H-NMR ($CDCl_3$) δ 3.0–3.2 (2 H, m), 3.38 (3 H, s), 3.3–3.6 (4 H, m), 4.0–4.2 (3 H, m), 10.03 (2 H, br).

(2*S*)-4-Benzyl-2-(methoxymethyl)morpholine ((*S*)-10h). To a solution of (2*S*)-4-benzyl-2-[[(*p*-tolylsulfonyl)oxy]methyl]-morpholine [(*S*)-**12**] (10.0 g, 0.028 mol) in methanol (10 mL) was added 28% CH_3ONa/methanol (53.0 g, 0.27 mol), and the mixture was refluxed for 18 h. The reaction mixture was adjusted to pH 8 with diluted HCl and extracted with ether. The extract was washed with water, dried over $MgSO_4$, and then evaporated. The residue was purified by silica gel column chromatography (silica gel 60, 230–400 mesh, Merck; chloroform as an eluent) and then distilled *in vacuo* (114 °C/0.25 mmHg) to give (*S*)-**10h** (3.5 g, 56%) as a colorless liquid: $[\alpha]^{21}_D$ = +28.1° (*c* = 1.0, CH_3OH); ^{1}H-NMR ($CDCl_3$) δ 1.97 (1 H, dd, *J* = 10.5, 11 Hz), 2.19 (1 H, dt, *J* = 3, 11 Hz), 2.66 (1 H, dq, *J* = 2, 11.5 Hz), 2.72 (1 H, dt, *J* = 2, 11 Hz), 3.3–4.0 (7 H, m), 3.35 (3 H, s), 7.2–7.4 (5 H, m). Anal. ($C_{13}H_{19}NO_2$) C, H, N.

By use of this procedure, (*R*)-**10h** was prepared from (*R*)-**12**: bp 120 °C/0.45 mmHg; $[\alpha]^{21}_D$ = −27.9° (*c* = 1.0, CH_3OH); ^{1}H-NMR ($CDCl_3$) δ 1.97 (1 H, dd, *J* = 10.5, 11 Hz), 2.19 (1 H, dt, *J* = 3, 11 Hz), 2.66 (1 H, dq, *J* = 2, 11.5 Hz), 2.72 (1 H, dt, *J* = 2, 11 Hz), 3.3–4.0 (7 H, m), 3.35 (3 H, s), 7.2–7.4 (5 H, m). Anal. ($C_{13}H_{19}NO_2$) C, H, N.

(2*S*)-2-(Methoxymethyl)morpholine Hydrochloride ((*S*)-11h). A solution of (*S*)-**10h** (3.00 g, 0.0136 mol) and HCl/dioxane (4 N, 3.4 mL) in ethanol (50 mL) was hydrogenated over 10% Pd–C (0.30 g) under hydrogen pressure of 5 kg/cm^2 at 40 °C for 6 h. The catalyst was removed by filtration, and the filtrate was evaporated. The residue was recrystallized from added ethyl acetate/methanol to give (*S*)-**11h** (1.80 g, 78%) as colorless crystals: mp 141–142 °C; $[\alpha]^{20}_D$ = −1.7° (*c* = 1.0, CH_3OH); ^{1}H-NMR ($CDCl_3$) δ 3.0–3.2 (2 H, m), 3.38 (3 H, s), 3.3–3.6 (4 H, m), 4.0–4.2 (3 H, m), 10.03 (2 H, bs). Anal. ($C_6H_{13}NO_2$·HCl) C, H, N.

By use of this procedure, (*R*)-**11h** was prepared from (*R*)-**10h**: mp 141–142 °C; $[\alpha]^{20}_D$ = +1.9° (*c* = 1.0, CH_3OH); ^{1}H-NMR ($CDCl_3$) δ 3.0–3.2 (2 H, m), 3.38 (3 H, s), 3.3–3.6 (4 H, m), 4.0–4.2 (3 H, m), 10.02 (2 H, bs). Anal. ($C_6H_{13}NO_2$·HCl) C, H, N.

Difluoro[[7,8-difluoro-9,1-[(methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl]carbonyl]borane (15). A mixture of diethyl (5-methyl-6,7-difluoro-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate **13** (40.0 g, 0.10 mol) and borane trifluoride etherate (15.0 mL, 0.12 mol) in acetic anhydride (100 mL) was heated at 100 °C for 10 h. The precipitates were collected by filtration and washed with acetic anhydride and isopropyl ether to give **15** (32.9 g, 88%) as yellow crystals: mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 3.35 (3 H, d, *J* = 6 Hz), 4.81 (2 H, d, *J* = 1 Hz), 7.66 (1 H, dd, *J* = 7, 10 Hz), 7.98 (1 H, t, *J* = 1 Hz). Anal. ($C_{14}H_7BF_4N_2O_3S$) C, H, N.

Method A. 7-Fluoro-9,1-[(methylimino)methano]-8-(2-methylmorpholino)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (8b). A mixture of diacetyl[[7,8-difluoro-9,1-[(methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl]carbonyl]borane **14** (750 mg, 1.67 mmol), 2-methylmorpholine hydrochloride (350 mg, 2.54 mmol), and triethylamine (0.80 g, 7.91 mol) in DMSO (30 mL) was stirred at 80 °C for 15 h. DMSO was removed under reduced pressure, and the residue was washed with isopropyl ether and acetonitrile. To the precipitate was added acetone (50 mL), concentrated HCl (5 mL), and water (10 mL), and the mixture was stirred for 2 h at room temperature. The resulting precipitates were collected by filtration, washed with water and ethanol, and recrystallized from acetonitrile/ethanol to give **8b** (0.42 g, 62%) as pale yellow crystals: ^{1}H NMR (DMSO-d_6) δ 1.13 (3 H, d, *J* = 6 Hz), 2.78 (3 H, s), 2.9–3.0 (1 H, m), 3.2–3.5 (3 H, m), 3.7–3.8 (2 H, m), 3.8–3.9 (1 H, m), 4.45 (2 H, s), 7.55 (1 H, s), 7.62 (1 H, d, *J* = 12.5 Hz), 15.82 (1 H, s).

By use of this procedure, **8c–h** were prepared from **14** and amines **11c–h**.

Method B. 7-Fluoro-9,1-[(methylimino)methano]-8-[2-(dimethylaminomethyl)morpholino]-5-oxo-5*H*-thiazolo-[3,2-*a*]quinoline-4-carboxylic Acid (8j). A mixture of **14** (700 mg, 1.55 mmol), 2-[(dimethylamino)methyl]morpholine dihydrochloride (**11j**) (406 mg, 1.87 mmol), and triethylamine (1.10 g, 10.9 mmol) in DMSO (20 mL) was stirred at 80 °C for 30 min. DMSO was removed under reduced pressure, and to the residue was added acetone (40 mL), concentrated HCl (5 mL), and water (10 mL), and the mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with water, washed with $CHCl_3$, adjusted to pH 9 with aqueous NaOH, and then extracted with a mixture of $CHCl_3$ and MeOH (4:1). The extract was dried over $MgSO_4$ and then evaporated. The residue was recrystallized from ethanol to give **8j** (85 mg, 12%) as pale yellow crystals: ^{1}H NMR (DMSO-d_6) δ 2.18 (6 H, s), 2.2–2.4 (2 H, m), 2.78 (3 H, s), 2.9–3.0 (1 H, m), 3.2–3.4 (1 H, m), 3.4–3.5 (1 H, m), 3.5–3.6 (1 H, m), 3.6–3.8 (2 H, m), 3.8–4.0 (1 H, m), 4.45 (2 H, s), 7.55 (1 H, s), 7.65 (1 H, d, *J* = 12.5 Hz), 15.84 (1 H, s).

By use of this procedure, **8i** was prepared from **14** and **11i**.

Method C. 7-Fluoro-9,1-[(methylimino)methano]-8-[(*S*)-2-(methoxymethyl)morpholino]-5-oxo-5*H*-thiazolo-[3,2-*a*]quinoline-4-carboxylic Acid [(*S*)-8h]. A mixture of **15** (2.50 g, 6.76 mmol), (*S*)-**11h** (1.70 g, 10.1 mmol), and triethylamine (3.44 g, 34.0 mmol) in DMSO (30 mL) was stirred at 55–60 °C for 18 h. DMSO was removed under reduced pressure, to the residue was added ethanol (60 mL), and the mixture was stirred at room temperature. The resulting precipitates were collected by filtration, washed with ethanol, suspended in ethanol (20 mL) and aqueous NaOH (2 N, 15 mL), and then stirred at 80 °C for 2 h. The insoluble materials was filtered off, and the filtrate was adjusted to about pH 3 with HCl (2 N). The resulting precipitates were collected by filtration and recrystallized from DMSO/ethanol to give (*S*)-**8h** (1.90 g, 70%) as pale yellow crystals: $[\alpha]^{20}_D$ = +39.5° (*c* = 0.5, 0.1 N NaOH); ^{1}H NMR (DMSO-d_6) δ 2.79 (3 H, s), 3.0–3.1 (1 H, m), 3.2–3.5 (5 H, m), 3.30 (3 H, s), 3.7–3.8 (2 H, m), 3.9–4.0 (1 H, m), 4.45 (2 H, s), 7.49 (1 H, d, *J* = 12.5 Hz), 7.54 (1 H, s), 15.69 (1 H, s).

By similar procedure, (*R*)-**8h** was also prepared: $[\alpha]^{20}_D$ = −39.9° (*c* = 0.5, 0.1 N NaOH).

***In Vitro* Antibacterial Activity.** The MICs (minimum inhibitory concentrations) of compounds tested in this study were determined according to the standard method by a serial 2-fold agar dilution technique using Sensitivity Test agar (Nissui; Tokyo, Japan).[16] The inoculum size was approximately 10^6 colony forming units/mL. The MIC of a compound was defined as the lowest concentration that prevented visible growth of bacteria after incubation at 37 °C for 18 h.

***In Vivo* Efficacy on Systemic Infections.** Mouse protection tests were performed against *S. aureus* IID 803. Groups of five male mice (ddY, 25–28 g, Japan SLC Inc., Shizuoka, Japan) were infected with bacteria. A 0.5-mL volume of a bacterial dilution, corresponding to 100 or 200 times greater than the 50% lethal dose, was inoculated intraperitoneally. The test compounds were suspended in 1% aqueous gum arabic and administered orally at 1 h postinfection. Survival rates were evaluated after 1 week, at which time the 50% effective dose (ED_{50}) and 95% confidence limits were calculated by the Weil method.[17] All values in Table 3 were the average of ED_{50} obtained from several experiments.

Serum Levels in Mice. **8b** and **8f–h** were administrated orally at a dose of 20 mg/kg of body weight in male ddY mice weighing 20–25 g (3 animals/group; SCL Japan Inc., Sizuoka, Japan), and blood samples were obtained at 0.5, 1, 2, and 4 h

after administration. These samples were allowed to clot for 30 min and then centrifuged to separate the serum.

Serum concentrations of these compounds were assayed by a reverse-phase high-performance liquid chromatography (HPLC) as follows. HPLC was performed by utilizing Waters model 600 multisolvent delivery system (Waters Associates, Milford, Wass.). The mobile phase for separation of **8b** and **8f–h** consisted of 0.1 M sodium phosphate (pH 5.0)–methanol (2:3 vol/vol). The mobile phase was passed through Inertsil ODS-2 colimn (4.6 by 150 mm; particle size, 5 μm; GL Sciences Inc. Tokyo, Japan) at flow rate 1.0 mL/min. The mobile phase was monitored by a Hitachi F-1050 fluorescence spectrophotometer (Hitachi, LTD., Tokyo, Japan) with an excitation wavelength of 305 nm and an emission wavelength of 525 nm.

Fifty microliters of sera was taken, and 200 μL of methanol was added. The mixture was voltexed and then centrifuged at 18000*g* for 10 min. Two hundred microliters of the supernatant was mixed with 100 μL of 0.1 M sodium phosphate (pH 5.0) and 10 μL of mixture was injected onto column. Calibration standards were prepared by adding **8b** or **8f–h** to blank serum and assaying as described above. The concentrations of these compounds were calculated from linear regression analysis of the peak area.

Supplementary Material Available: ^{1}H NMR spectra of **8c–i** and (***R***)-**8h** and *in vitro* antibacterial activities of **8b** and **8e–h** against five Gram-positive bacteria (*Streptococcus pneumoniae* IID 552, *Streptococcus pneumoniae* IID 554, *Streptococcus pyogenes* Su, *Streptococcus pyogenes* Sv, and *Bacillus subtilis* ATCC 6633) (3 pages). Ordering information is given on any current masthead page.

References

(1) Inoue, Y.; Kondo, H.; Taguchi, M.; Jinbo, Y.; Sakamoto, F.; Tukamoto, G. Synthesis and Antibacterial Activity of Thiazolopyrazine-incorporated tetracyclic Quinolone Antibacterials. *J. Med. Chem.* **1994**, *37*, 586–592.

(2) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. Structure-Activity Relationships of Antibacterial 6,7- and 7,8-Disubstituted 1-Alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids. *J. Med. Chem.* **1980**, *23*, 1358–1363.

(3) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. Pyridonecarboxylic Acids as Antibacterial Agents. 2. Synthesis and Structure-Activity Relationships of 1,6,7-Trisubstituted 1,4-Dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids, Including Enoxacin, a New Antibacterial Agents. *J. Med. Chem.* **1984**, *27*, 292–301.

(4) (a) Hayakawa, I.; Hiramitsu, T.; Tanaka, Y. Synthesis and Antibacterial Activities of Substituted 7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acids. *Chem. Pharm. Bull.* **1984**, *32*, 4907–4913. (b) Sato, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhashi, S. *In Vitro* and *In Vivo* Activity of DL-8280, a New Oxazine Derivative. *Antimicrob. Agents Chemother.* **1982**, *22*, 548–553.

(5) Wise, R.; Andrews, J. M.; Edwards, L. *In Vitro* Activity of Bay 09867, a New Quinolone Derivative, Compared with Those of Other Antibacterial Agents. *Antimicrob. Agents Chemother.* **1983**, *23*, 559–564.

(6) Fujinami, K.; Noumi, T.; Sakawa, I.; Inoue, M.; Mitsuhashi, S. *In Vitro* and *In Vivo* Antibacterial Activities of T-3262, a New Fluoroquinolone. *Antimicrob. Agents Chmother.* **1988**, *32*, 827–833.

(7) Hirose, T.; Okezaki, E.; Kato, H.; Ito, Y.; Inoue, M.; Mitsuhashi, S. *In Vitro* and *In Vivo* Activity of NY-198, a New Difluorinated Quinolone. *Antimicrob. Agents Chemother.* **1987**, *31*, 854–859.

(8) Harnett, N.; Brown, S.; Krishnan, C. Emergence of Quinolone Resistance Among Clinical Isolates of Methicillin-Resistant Staphylococcus Aureus. *Antimicrob. Agents Chemother.* **1991**, *35*, 1911–1913.

(9) Taguchi, M.; Kondo, H.; Inoue, Y.; Kawahata, Y.; Jinbo, Y.; Sakamoto, F.; Tsukamoto, G. Synthesis and Antibacterial Activity of New Tetracyclic Quinolone Antibacterials. *J. Med. Chem.* **1992**, *35*, 94–99.

(10) Jinbo, Y.; Kondo, H.; Inoue, Y.; Taguchi, M.; Tsujishita, H.; Kotera, Y.; Sakamoto, F.; Tsukamoto, G. Synthesis and Antibacterial Activity of a New Series of Tetracyclic pyridonecarboxylic Acids. *J. Med. Chem.* **1993**, *36*, 2621–2626.

(11) Jinbo, Y.; Taguchi, M.; Inoue, Y.; Kondo, H.; Miyasaka, ka, T.; Tsujishita, H.; Sakamoto, F.; Tsukamoto, G. Synthesis and Antibacterial Activity of a New Series of Tetracyclic pyridonecarboxylic Acids. 2. *J. Med. Chem.* **1993**, *36*, 3148–3153.

(12) Loflus, F. The Synthesis of Some 2-substituted Morpholines. *Synth. Commun.* **1980**, *10*, 59–73.

(13) Cottle, D. L.; Jeitsch, A. E.; Stoudt, T. H.; Walters, D. R. The Preparation of Some C-Alkylmorpholines. *J. Org. Chem.* **1946**, *11*, 286–291.

(14) Araki, K.; Kuroda, T.; Uemori, S.; Moriguchi, A.; Ikeda, Y.; Hirayama, F.; Yokoyama, Y.; Iwao, E.; Yakushiji, T. Quinolone Antimicrobial Agents Substituted with Morpholines at the 7-Position. Syntheses and Structure-Activity Relationships. *J. Med. Chem.* **1993**, *36*, 1356–1363.

(15) Howe, R.; Leigh, T.; Rao, B. S.; Todd, A. H. Optical Isomers of 2-(2-Ethoxyphenoxymethyl)tetrahydro-1,4-oxazine (viloxazine) and Related Compounds. *J. Med. Chem.* **1976**, *19*, 1074–1076.

(16) Goto, S.; Jo, K.; Kawakita, T.; Kosakai, N.; Mitsuhashi, S.; Nishino, T.; Ohsawa, N.; Tanami, H. Saisho-hatsuiku-soshi-nodo (MIC) Sokutei-ho Sai-kaitei Ni Tsuite. (About the Re-revised Method of Determination of MIC.) *Chemotherapy* **1981**, *29*, 76–79.

(17) Miller, L.; Tainter, M. L. Estimation of the ED_{50} and its error by means of logarithmic probit graph paper. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261.