Synthesis and Antibacterial Activity of a New Series of Tetracyclic Pyridone Carboxylic Acids[1]

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Received March 29, 1993

A series of novel tetracyclic pyridone carboxylic acids replacing the 10-position oxygen atom of 9,1-(epoxymethano)-7-fluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid by imino groups (NR; R = Me, Et, c-Pr, allyl, Ph, benzyl), a sulfur atom, or a carbonyl group was prepared and evaluated for antibacterial activity and inhibitory activity on DNA gyrase isolated from *E. coli* KL-16. The in vitro antibacterial potency and DNA gyrase inhibitory activity were found to be in the following order: NMe ≥ O > S ≫ C=O. Moreover, a methyl group was the optimal alkyl substituent at the 10-position nitrogen atom for antibacterial activity and for DNA gyrase inhibitory activity. 7-Fluoro-9,1-[(*N*-methylimino)methano]-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid (10-NCH_3) showed potent in vivo antibacterial activity.

Since nalidixic acid was reported by Lesher et al. in 1962,[2] pyridone carboxylic acid antibacterial agents have become an important class of therapeutically useful compounds. Ofloxacin[3] (2), a tricyclic pyridone carboxylic acid having an oxazine ring, is one of such agents, and the synthesis and the antibacterial activity of 2 and its variants[4-6] 3-7 have been reported. The oxygen atom of the oxazine ring of 2 was found to give optimal antibacterial activity vs a nitrogen, a sulfur, or a carbon atom.[7]

Recently, we reported[8] the synthesis and the antibacterial activity of the tetracyclic pyridone carboxylic acids having a thiazolooxazine ring and that the 8-(4-methyl-1-piperazinyl) derivative 1 showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria. In the course of the study, replacement of an oxygen atom of the thiazolooxazine ring of 1 by a nitrogen atom, a sulfur atom, or a carbonyl group was accomplished. It was found that the replacement of an oxygen atom of a thiazolooxazine ring of 1 by a nitrogen atom resulted in enhancement of both in vitro and in vivo antibacterial activity compared to those of 1. This paper describes the synthesis of tetracyclic pyridone carboxylic acids 8a[9]-f, 9, and 10 and their antibacterial activity and inhibition of DNA gyrase supercoiling activity.

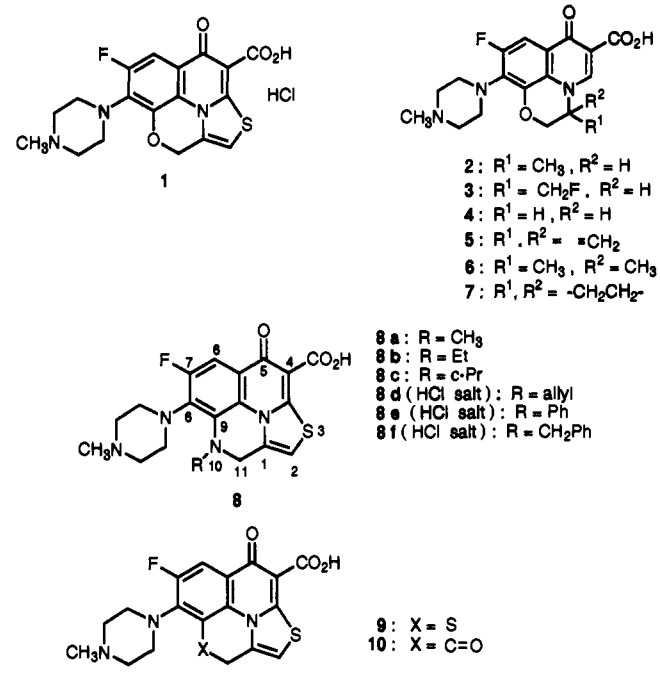

Figure 1.

Chemistry

The synthetic routes leading to the target compounds 8a-f and 9 are summarized in Scheme I. The key intermediate 18 was prepared from dithiocarbamate[8a] 11 by modification of the procedure[10] described by Matsumura et al.; dithiocarbamate 11 was reacted with ethyl chloroformate in the presence of triethylamine to afford isothiocyanate 12, which was sequentially treated with diethyl malonate, S-alkylated with *p*-methoxybenzyl chloride, and thermally cyclized and deprotected to give the quinoline derivative 16. Compound 16 was reacted with 1,3-dichloroacetone in the presence of triethylamine in dichloromethane, followed by cyclization in concentrated H_2SO_4, to afford 18. The formation of the tetracyclic intermediates 21a-f and 22 was achieved by the reaction of 18 with the appropriate primary amines or Na_2S followed by hydrolysis in concentrated H_2SO_4 or reaction with triacetoxyborane in acetic anhydride. Replacement of the 8-fluorine atom of 21a-c and 22 with 1-methylpiperazine afforded 8a-c and 9, respectively. Compounds 8d-f were obtained by the reaction of borate complexes 21d-f with 1-methylpiperazine, respectively, followed by hydrolysis under acidic conditions.

The synthesis of 10 was achieved according to the synthetic route shown in Scheme II. The quinoline derivative[10] 23 was reacted with ethyl chloroacetoacetate, followed by treatment with concentrated H_2SO_4, to yield the diethyl ester 24, which afforded borate complex 25 by the reaction with triacetoxyborane in acetic anhydride. Borate complex 25 was then reacted with 1-methylpiperazine, followed by acidic hydrolysis, to give 26. Finally, cyclization of 26 in polyphosphoric acid (PPA) afforded 10. The ^1H NMR spectrum of 10 in D_2O showed a singlet signal at δ 6.65, and the integration of the signal (0.6 protons) indicated that the proton corresponding to the signal was partially exchanged with deuterium. This signal

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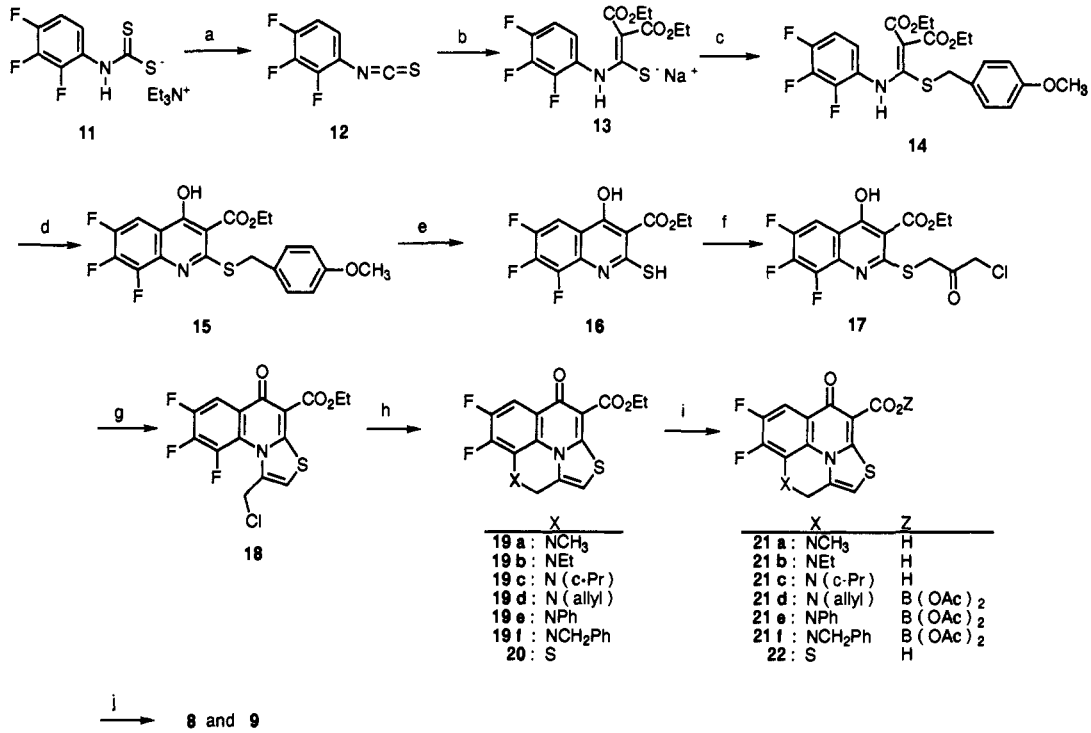
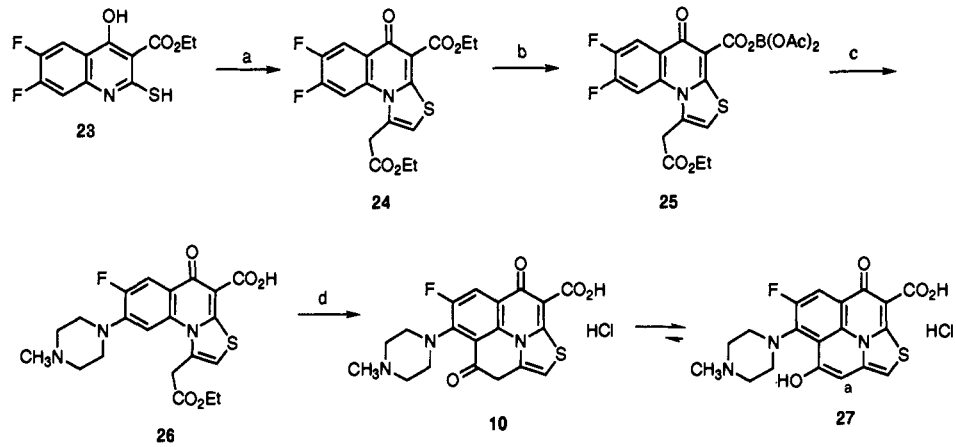
Scheme I[a]



[a] Key: (a) $ClCO_2Et$, Et_3N; (b) diethyl malonate; NaH; (c) *p*-methoxybenzyl chloride; (d) 250–260 °C, diphenyl ether; (e) CF_3CO_2H, CF_3SO_3H; (f) $ClCH_2COCH_2Cl$, Et_3N; (g) H_2SO_4; (h) RNH_2, Et_3N, or Na_2S; (i) H_2SO_4 or $B(OAc)_3$; (j) 1-methylpiperazine or (i) 1-methylpiperazine, (ii) HCl.

Scheme II[a]



[a] Key: (a) (i) $ClCH_2COCH_2CO_2Et$, Et_3N; (ii) H_2SO_4; (b) $B(OAc)_3$; (c) (i) 1-methylpiperazine, (ii) HCl, (iii) NaOH; (d) PPA.

should be assigned to the proton of the a-position of **27**, and no signal due to the keto form **10** was observed. This indicates that the enol form **27**, rather than the keto form **10**, predominates in water.

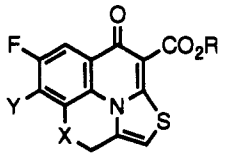
Synthetic methods and physical data (melting points and analytical data) for all tetracyclic pyridone carboxylic acids prepared in this paper are summarized in Table I.

Biological Results and Discussion

Table II shows the in vitro antibacterial activity of a series of the tetracyclic pyridone carboxylic acids **8a–f**, **9**, and **10** against four Gram-positive bacteria (*Staphylococcus aureus* FDA 209P JC-1, *Staphylococcus aureus* IID 803, *Staphylococcus epidermidis* IAM 1296, *E. faecalis* IID 682), and six Gram-negative bacteria (*E. coli* NIHJ JC-2, *E. coli* KC-14, *Klebsiella pneumoniae* B54, *Salmonella typhimurium* IID 971, *Pseudomonas aeruginosa* IFO 3445, *Pseudomonas aeruginosa* E-2). Data for **1** and **2** (ofloxacin) are included for comparison. From the data for **8a** and **1**, replacement of the 10-position oxygen atom of **1** by a *N*-methylimino group (compound **8a**) resulted in 2–4-fold enhancement of the activity against Gram-positive bacteria as compared to that of **1**; against Gram-negative bacteria, such a modification had no effect or somewhat favorable effect on the activity, with **8a** being at most 2-fold more potent than **1**. When the 10-position oxygen atom of **1** was replaced by a sulfur atom (compound **9**) or a carbonyl group (compound **10**), there was a general decrease in activity. The activity of **9** was decreased by a factor of 2-4 times against both Gram-positive and Gram-negative bacteria compared to that of **1**. Compound **10** exhibited only poor activity against both Gram-positive and Gram-negative bacteria.

Next, we investigated the effect of the substituent at the 10-position nitrogen atom of **8a** on antibacterial activity. Among compounds **8a–f**, compound **8a** showed

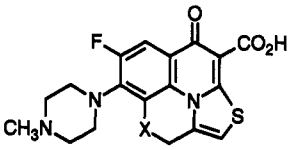
Table I. Tetracyclic Pyridone Carboxylic Acids and Derivatives



compd	X	Y[a]	R	synth[b] method	% yield	mp, °C	recryst solvent	formula[c]
19a	NCH_3	F	Et	A	61	>280 dec	$CHCl_3$/EtOH	$C_{18}H_{12}F_2N_2O_3S \cdot 0.25H_2O$
19b	NEt	F	Et	A	84	257 dec	$CHCl_3$/EtOH	$C_{17}H_{14}F_2N_2O_3S \cdot 0.5H_2O$
19c	N(c-Pr)	F	Et	A	46[f]	267 dec	DMSO	$C_{18}H_{14}F_2N_2O_3S$
19d	N(allyl)	F	Et	A	22	252 dec	$CHCl_3$/EtOH	$C_{18}H_{14}F_2N_2O_3S$
19e	NPh	F	Et	A	34	>280	$CHCl_3$/EtOH	$C_{21}H_{14}F_2N_2O_3S$
19f	NCH_2Ph	F	Et	A	80	243 dec	*d*	$C_{22}H_{16}F_2N_2O_3S$
20	S	F	Et	A	47[f]	>280	DMF	$C_{15}H_6F_2NO_3S_2$
21a	NCH_3	F	H	B	86[f]	>280	DMSO	$C_{14}H_8F_2N_2O_3S$
21b	NEt	F	H	B	79	256 dec	DMSO	$C_{15}H_{10}F_2N_2O_3S$
21c	N(c-Pr)	F	H	B	93	265 dec	DMSO	$C_{18}H_{10}F_2N_2O_3S$
21d	N(allyl)	F	$B(OAc)_2$	C	77	260 dec	*e*	$C_{20}H_{15}BF_2N_2O_7S$
21e	NPh	F	$B(OAc)_2$	C	39	>280	*e*	$C_{23}H_{15}BF_2N_2O_7S$
21f	NCH_2Ph	F	$B(OAc)_2$	C	90	245 dec	*e*	$C_{24}H_{17}BF_2N_2O_7S \cdot 0.25H_2O$
22	S	F	H	B	68[f]	>280	DMSO	$C_{18}H_5F_2NO_3S_2$
8a	NCH_3	MP	H	D	56	257 dec	$CHCl_3$/EtOH	$C_{18}H_{16}FN_4O_3S$
8b	NEt	MP	H	D	36	238 dec	EtOH	$C_{20}H_{21}FN_4O_3S \cdot 0.5H_2O$
8c	N(c-Pr)	MP	H	D	6	250 dec	$CHCl_3$/EtOH	$C_{21}H_{21}FN_4O_3S$
8d	N(allyl) (HCl salt)	MP	H	E	25	>280	dil HCl	$C_{21}H_{21}FN_4O_3S \cdot HCl \cdot 1.5H_2O$
8e	NPh (HCl salt)	MP	H	E	8	>280	$CHCl_3$/EtOH	$C_{24}H_{21}FN_4O_3S \cdot 0.25H_2O$
8f	NCH_2Ph (HCl salt)	MP	H	E	29	275 dec	H_2O	$C_{25}H_{23}FN_4O_3S \cdot HCl \cdot 1.5H_2O$
9	S	MP	H	D	12	262 dec	DMSO	$C_{18}H_{16}FN_3O_3S_2$
10	C=O (HCl salt)	MP	H	F	10	>280	1N HCl	$C_{19}H_{16}FN_3O_4S \cdot HCl \cdot 1.5H_2O$

[a] MP is a 4-methylpiperazinyl group. [b] See the Experimental Section. [c] Analyses for C, H, and N were within ±0.4% of the theoretical values. [d] Purified by washing with CH_3CN. [e] Purified by washing with Ac_2O. [f] Yield before recrystallization.

Table II. In Vitro Antibacterial Activity (Minimum Inhibitory Concentration,[a] μg/mL) of Tetracyclic Pyridone Carboxlic Acids 1, 8a-f, 9, and 10



		microorganism[b]									
		Gram-positive				Gram-negative					
compd	X	Sa(F)	Sa(I)	Se	Ef	Ec(N)	Ec(K)	Kp	St	Pa(I)	Pa(E)
8a	NCH_3	0.05	0.05	0.05	0.10	0.05	0.05	0.05	0.05	0.39	0.78
8b	NEt	0.20	0.20	0.20	0.78	0.39	0.20	0.20	0.20	3.13	3.13
8c	N(c-Pr)	0.20	0.20	0.39	0.78	0.20	0.10	0.20	0.39	1.56	3.13
8d	N(allyl)	0.39	0.39	0.78	3.13	0.78	0.39	12.5	0.78	1.56	6.25
8e	NPh	1.56	0.78	1.56	25.0	3.13	3.13	3.13	3.13	100	100
8f	NCH_2Ph	6.25	3.13	12.5	25.0	25.0	12.5	50.0	50.0	100	100
9	S	0.20	0.20	0.39	0.78	0.20	0.20	0.20	0.10	3.13	3.13
10	C=O	6.25	6.25	12.5	50.0	12.5	12.5	25.0	12.5	100	100
1	O	0.10	0.10	0.20	0.39	0.10	0.10	0.10	0.05	0.78	0.78
2 (OFX)		0.39	0.39	0.78	1.56	0.10	0.10	0.10	0.05	1.56	1.56

[a] All values have been obtained from duplicate or triplicate experiments. [b] Microorganism: Sa(F), *Staphylococcus aureus* FDA 209P JC-1; Sa(I), *Staphylococcus aureus* IID 803; Se, *Staphylococcus epidermidis* IAM 1296; Ef, *E. Faecalis* IID 682; Ec(n), *E. coli* NIHJ JC-2; Ec(K), *E. coli* KC-14; Kp, *Klebsiella pneumoniae* B54; St, *Salmonella typhimurium* IID 971; Pa(I), *Pseudomonas aeruginosa* IFO 3445; Pa(E), *Pseudomonas aeruginosa* E-2.

the most potent activity against both Gram-positive and Gram-negative bacteria, and it was found that an increase in size of the substituent (**8b–f** vs **8a**) caused a decrease in activity.

To clarify the reason for these results of in vitro antibacterial activity, the inhibition of the supercoiling activity of DNA gyrase isolated from *E. coli* KL-16, along with in vitro activity against the same organism, was evaluated for **8a–f**, **9**, and **10**; the results are shown in Table III. The data for reference compound **1** are also included for comparison purposes. Compound **8a** exhibited the most potent inhibitory effect against DNA gyrase supercoiling activity; the inhibitory potency was found to be in the following order: **8a** = **1** > **9** ≫ **10**. The order of **8a–f** was as follows: **a** > **b** > **c** > **d** > **e** > **f**, changing with the substituent at the 10-position nitrogen atom, and their inhibitory effects on DNA gyrase correlated with their in vitro antibacterial activity, indicating that the decrease of antibacterial activity was caused by the decrease of their inhibitory activity on DNA gyrase.

Table III. Inhibitory Effect (IC_{50})[a] of 8a-f, 9 and 10 on DNA Gyrase Supercoiling Activity from *E. coli* KL-16 and in Vitro Antibacterial Activity (MIC)[b] against the Same Strain

compd	IC_{50}, μg/mL	MIC, μg/mL
8a	0.16	0.05
8b	0.25	0.20
8c	0.34	0.20
8d	0.45	0.78
8e	1.76	3.13
8f	>10	25.0
9	0.24	0.20
10	>10	12.5
1	0.14	0.05

[a] Calculated by the quantitative measurement of the supercoiled DNA peak in an agarose gel by densitometric assay. [b] See footnote *a* of Table II.

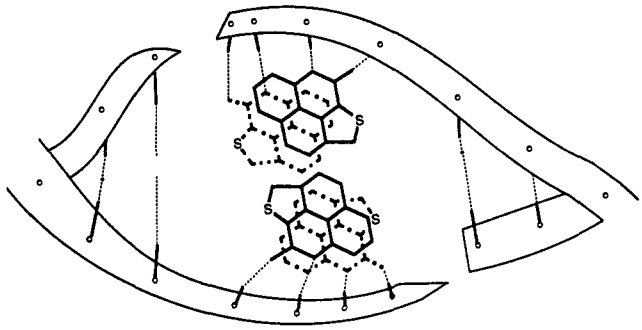


Figure 2. The proposed quinolone-DNA cooperative binding in the ternary complex model.

Sanchez et al.[11] reported that incorporation of an amino group into the 8-position of 7-substituted 1-cyclopropyl-6-fluoroquinoline-3-carboxylic acids resulted in a general decrease in in vitro and in vivo potency and in gyrase activity. Hayakawa et al.[7a] also indicated that replacement of the 10-position oxygen atom of ofloxacin by a nitrogen atom (NMe) resulted in an overall decrease in activity. However, compound 8a exhibited potent in vitro antibacterial activity against both Gram-positive and Gram-negative bacteria as described above and showed an inhibitory effect on DNA gyrase similar to that of 1. This might be interpreted in terms of the ternary complex model (Figure 2) for gyrase inhibition by quinolones and the drug–drug self-association mechanism proposed by Shen et al.;[12] the planar structure and the higher hydrophobicity[9] of 8a having the flat thiazole ring might be favorable for the π–π ring stackings of the quinolone ring to compensate for the disadvantageous effect of the 10-position nitrogen atom on the antibacterial activity. The inhibitory effect on the DNA gyrase of 8f and 10 was poor as shown in Table III. This might also be interpreted by the model of Shen et al.; the decrease of the tail to tail hydrophobic interaction (in the case of 8f, due to the steric hindrance of the large benzyl group at the 10-position nitrogen atom, and in the case of 10, due to the hydrophilic hydroxyl group at the 4-position of the enol structure 27) resulted in the decrease of the interaction of the drug–drug self association.

The oral antibacterial activity in mice of 8a is shown in Table IV; data for reference compound 1 are included for comparison. The data show that compound 8a was effective against the experimental infections; it was four times more active than 1 against *S. aureus* and similar to 1 against *E. coli* and *P. aeruginosa*.

These findings suggest that the replacement of the 10-position oxygen atom of the tetracyclic pyridone carboxylic acid system 1 by a methyl-substituted nitrogen atom is a useful structural variation for the enhancement of antibacterial activity.

Table IV. Therapeutic Effect of 8a on Systemic Infections in Mice

	ED_{50}, mg/kg po (95% confidence limits)		
compd	*S. aureus* IID 803	*E. coli* KC-14	*P. aeruginosa* E-2
8a	2.80 (1.50–4.10)	0.90 (0.50–1.30)	10.2 (6.55–15.7)
1	12.3 (8.80–18.1)	1.40 (0.80–2.20)	11.7 (7.52–18.1)

Experimental Section

Melting points were determined with a Yamato capillary melting point apparatus, Model MP-21; 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.

2,3,4-Trifluorophenyl Isothiocyanate (12). To a mixture of triethylammonium *N*-(2,3,4-trifluorophenyl)dithiocarbamate (11) (92.0 g, 0.284 mol) and triethylamine (31.5 g, 0.311 mol) in chloroform (360 mL) at 2–6 °C was added dropwise ethyl chloroformate (33.8 g, 0.311 mol) over a period of 1 h. The solution was stirred for 10 min, washed with 3 N hydrochloric acid and water, and dried over $MgSO_4$. Evaporation of solvents in vacuo, purification of the residue by silica gel column chromatography (silica gel 60, 230–400 mesh, Merck; hexane as an eluent), and vacuum distillation in vacuo (96–97 °C/17 mmHg) gave 12 (42.0 g, 78%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 6.9–7.0 (2 H, m); IR (neat) 2018 cm^{-1}.

Sodium Diethyl [(2,3,4-Trifluoroanilino)sulfidomethylene]malonate (13). To a suspension of sodium hydride (8.6 g, 60 w/w % in oil, 0.215 mol) in THF (250 mL) at 5–10 °C was added dropwise diethyl malonate (34.4 g, 0.226 mol) over a period of 40 min. The mixture was stirred at 5–10 °C for 10 min, and 12 (40.5 g, 0.214 mol) was added dropwise at 5–10 °C over 40 min. The reaction mixture was stirred at room temperature for 105 min, and then the solvent was evaporated. The residue was washed with ether and dried to give 13 (78.5 g, 98%) as colorless crystals: ^{1}H NMR (DMSO-d_6) δ 1.13 (6 H, t, J = 7 Hz), 3.95 (4 H, q, J = 7 Hz), 7.12 (1 H, dq, J = 2, 10.5 Hz), 8.5–8.6 (1 H, m), 11.79 (1 H, s).

Diethyl[(2,3,4-Trifluoroanilino)[(4-methoxybenzyl)thio]methylene]malonate (14). To a solution of 13 (68.5 g, 0.184 mol) in DMF (200 mL) at 3–5 °C was added dropwise 4-methoxybenzyl chloride (28.9 g, 0.184 mol). The mixture was stirred at room temperature for 1 h, and then the solvent was evaporated in vacuo. Water was added to the residue, and the mixture was extracted with chloroform. The extract was washed with brine, dried over $MgSO_4$, and evaporated in vacuo to give 14 (82.9 g, 95%) as colorless crystals. 14 (recrystallized from hexane/benzene): mp 64–151 °C (the crystals gradually melted; began to melt at 64 °C and completely melted at 150 °C). Anal. ($C_{22}H_{22}F_3NO_5S$) C, H, N.

Ethyl 4-Hydroxy-2-[(4-methoxybenzyl)thio]-6,7,8-trifluoroquinoline-3-carboxylate (15). To diphenyl ether (210 mL) at 250–260 °C was added dropwise under nitrogen stream a solution of 14 (80.9 g, 0.172 mol) in hot diphenyl ether (60 mL, 110 °C) over a period of 15 min. The mixture was stirred for 5 min, cooled to room temperature, and diluted with hexane (3 L). The precipitate was collected by filtration, washed with hexane, and dried to give 15 (59.9 g, 82%) as yellow crystals. 15 (recrystallized from ethyl acetate): mp 174–176 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (3 H, t, J = 7 Hz), 3.78 (3 H, s), 4.47 (2 H, s), 4.53 (2 H, q, J = 7 Hz), 6.83 (2 H, m), 7.44 (2 H, m), 7.73 (1 H, ddd, J = 2.5, 8, 10 Hz), 13.38 (1 H, s). Anal. ($C_{20}H_{16}F_3NO_4S$) C, H, N.

Ethyl 4-Hydroxy-2-mercapto-6,7,8-trifluoroquinoline-3-carboxylate (16). To a stirred mixture of trifluoroacetic acid (500 g), trifluoromethanesulfonic acid (100 g), and anisole (92.5 g) at −20 °C was added 15 (59.9 g 0.141 mol) over a period of 15 min. The solution was stirred at −15 °C for 30 min and then at

room temperature for 1.5 h. The solution was evaporated in vacuo, poured into ice/water, basified with 10% sodium hydroxide, and filtered. The filtrate was washed with ether and acidified with hydrochloric acid. The precipitate was collected and washed with water to afford **16** (33.0 g, 77%) as yellow crystals. **16** (recrystallized from ethanol): mp 190 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.51 (3 H, t, J = 7 Hz), 4.55 (2 H, q, J = 7 Hz), 7.72 (1 H, ddd, J = 2.5, 7.5, 9.5 Hz). Anal. ($C_{12}H_8F_3NO_3S$) C, H, N.

Ethyl 2-[(3-Chloro-2-oxopropyl)thio]-4-hydroxy-6,7,8-trifluoroquinoline-3-carboxylate (17). To a stirred solution of 1,3-dichloroacetone (6.72 g, 73.4 mmol) in CH_2Cl_2 (200 mL) at 0 °C was added **16** (16.0 g, 52.8 mmol) and triethylamine (8.0 g, 79.1 mmol). The solution was stirred at 0 °C for 1 h and then at room temperature for 1 h. The mixture was diluted with CH_2Cl_2, washed with 0.1 N HCl, H_2O, and brine, dried over $MgSO_4$, and then evaporated. The residue was recrystallized from $CHCl_3$/isopropyl ether to give **17** (17.5 g, 84%) as colorless crystals: mp 175–181 °C. Anal. ($C_{15}H_{11}ClF_3NO_4S$) C, H, N.

Ethyl 1-(Chloromethyl)-5-oxo-7,8,9-trifluoro-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (18). A solution of **17** (17.5 g, 44.4 mmol) in concentrated H_2SO_4 (80 mL) was stirred at room temperature for 20 h. The mixture was then poured into ice/water, and the resultant precipitate was collected, washed with water, and recrystallized from $CHCl_3$/isopropyl ether to give **18** (14.2 g, 85%) as pale yellow crystals: mp 158–160 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (3 H, t, J = 7 Hz), 4.48 (2 H, q, J = 7 Hz), 4.93 (2 H, d, J = 4 Hz), 7.22 (1 H, s), 8.19 (1 H, ddd, J = 2.5, 8, 10 Hz). Anal. ($C_{15}H_9ClF_3NO_3S$) C, H, N.

Method A. Ethyl 7,8-Difluoro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (19a). A solution of **18** (14.0 g, 37.3 mmol), methylamine (40% methanol solution, 3.5 g, 45.1 mmol), and triethylamine (4.5 g, 44.5 mmol) in CH_3CN was stirred at room temperature for 23 h. The precipitates were collected by filtration, washed with CH_3CN, water, and ethanol, and recrystallized from $CHCl_3$/ethanol to give **19a** (8.0 g, 61%) as pale yellow crystals: ^{1}H NMR (DMSO-d_6) δ 1.28 (3 H, t, J = 7 Hz), 3.22 (3 H, d, J = 5.5 Hz), 4.29 (2 H, q, J = 7 Hz), 4.55 (2 H, d, J = 1 Hz), 7.31 (1H, t, J = 1 Hz), 7.40 (1 H, dd, J = 7.5, 10.5 Hz).

By similar procedures, compounds **19b–f** were prepared. **19b**: ^{1}H NMR (DMSO-d_6) δ 1.20 (3 H, t, J = 7 Hz), 1.30 (3 H, t, J = 7 Hz), 3.49 (2 H, q, J = 7 Hz), 4.29 (2 H, q, J = 7 Hz), 4.59 (2 H, s), 7.30 (1 H, s), 7.47 (1 H, dd, J = 7.5, 10.5 Hz). **19c**: ^{1}H NMR (DMSO-d_6) δ 0.7–0.9 (4 H, m), 1.30 (3 H, t, J = 7 Hz), 3.0–3.2 (1 H, m), 4.29 (2 H, q, J = 7 Hz), 4.63 (2 H, s), 7.30 (1 H, s), 7.45 (1 H, dd, J = 7, 10 Hz). **19d**: ^{1}H NMR (DMSO-d_6) δ 1.31 (3 H, t, J = 7 Hz), 4.05 (2 H, d, J = 5 Hz), 4.28 (2 H, q, J = 7 Hz), 4.55 (2 H, s), 5.23 (1 H, d, J = 10 Hz), 5.32 (1 H, d, J = 17 Hz), 5.8–6.0 (1 H, m), 7.29 (1 H, s), 7.39 (1 H, dd, J = 8, 10.5 Hz). **19e**: ^{1}H NMR ($CDCl_3$) δ 1.49 (3 H, t, J = 7 Hz), 4.50 (2 H, q, J = 7 Hz), 5.01 (2 H, d, J = 1 Hz), 6.76 (1 H, t, J = 1 Hz), 7.0–7.4 (5 H, m), 7.93 (1 H, dd, J = 7.5, 10 Hz). **19f**: ^{1}H NMR (DMSO-d_6) δ 1.30 (3 H, t, J = 7 Hz), 4.28 (2 H, q, J = 7 Hz), 4.55 (2 H, s), 4.60 (2 H, s), 7.22 (1 H, s), 7.2–7.4 (5 H, m), 7.48 (1 H, dd, J = 8, 10.5 Hz).

Ethyl 9,1-(Epithiomethano)-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (20). A mixture of **18** (10.0 g, 26.6 mmol), sodium sulfide (12.86 g 0.234 mol), and tetrabutylammonium bromide (0.1 g, 0.31 mmol) in a mixture of $CHCl_3$ (200 mL), ethanol (40 mL), and water (100 mL) was stirred at room temperature for 19 h. The precipitates were collected by filtration, washed with water, ethanol, and ether, and recrystallized from DMSO to give **20** (4.4 g, 47%) as colorless crystals: ^{1}H NMR (DMSO-d_6) δ 1.31 (3 H, t, J = 7 Hz), 4.30 (2H, q, J = 7 Hz), 4.48 (2 H, d, J = 1 Hz), 7.47 (1 H, t, J = 1 Hz), 7.93 (1 H, dd, J = 8.5, 10.5 Hz).

Method B. 7,8-Difluoro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (21a). A solution of **19a** (16.0 g, 0.051 mol) in concentrated H_2SO_4 (160 mL) was heated at 90 °C for 7 h. The mixture was then poured into ice/water, and the resultant precipitate was collected by filtration and washed with water to afford **21a** (12.8 g, 86%) as pale yellow crystals. **21a**: (recrystallized from DMSO): ^{1}H NMR (DMSO-d_6) δ 3.28 (3 H, d, J = 6 Hz), 4.65 (2 H, s), 7.50 (1 H, dd, J = 7.5, 10 Hz), 7.56 (1 H, s), 15.61 (1 H, s).

By similar procedures, compounds **21b**, **21c**, and **22** were prepared. **21b**: ^{1}H NMR (DMSO-d_6) δ 1.24 (3 H, t, J = 7 Hz), 3.56 (2 H, dq, J = 2.5, 7 Hz), 4.71 (2 H, s), 7.56 (1 H, s), 7.60 (1 H, dd, J = 7.5, 10 Hz), 15.67 (1 H, s). **21c**: ^{1}H NMR (DMSO-d_6) δ 0.7–0.9 (4 H, m), 3.1–3.2 (1 H, m), 4.74 (2 H, s), 7.55 (1 H, s), 7.61 (1 H, dd, J = 7.5, 10 Hz), 15.66 (1 H, s). **22**: ^{1}H NMR (DMSO-d_6) δ 4.59 (2 H, s), 7.73 (1 H, s), 8.10 (1 H, dd, J = 7, 11 Hz), 15.42 (1 H, s).

Method C. Diacetoxy[[9,1-[(*N*-benzylimino)methano]-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl]carbonyl]oxy]borane (21f). A mixture of **19f** (600 mg, 1.41 mmol) and triacetoxyborane (397 mg, 2.12 mmol) in acetic anhydride (6 mL) was heated at 100 °C for 40 min. The precipitates were collected by filtration and washed with isopropyl ether to afford **21f** (673 mg, 90%) as colorless crystals: ^{1}H NMR (DMSO-d_6) δ 1.92 (6 H, s), 4.78 (2 H, s), 4.82 (2 H, d, J = 1 Hz), 7.2–7.4 (5 H, m), 7.73 (1 H, dd, J = 7.5, 10 Hz), 7.91 (1 H, t, J = 1 Hz).

By similar procedures, compounds **21d** and **21e** were prepared. **21d**: ^{1}H NMR (DMSO-d_6) δ 1.91 (6 H, s), 4.20 (2 H, d, J = 4.5 Hz), 4.83 (2 H, d, J = 1 Hz), 5.27 (1 H, dd, J = 1.5, 10.5 Hz), 5.38 (1 H, dd, J = 1.5, 17 Hz), 5.9–6.1 (1 H, m), 7.68 (1 H, dd, J = 7.5, 10 Hz), 7.96 (1 H, s). **21e**: ^{1}H NMR (DMSO-d_6) δ 1.93 (6 H, s), 5.38 (2 H, s), 7.1–7.5 (5 H, m), 7.91 (1 H, dd, J = 7.5, 10 Hz), 7.97 (1 H, s).

Method D. 7-Fluoro-9,1-[(*N*-methylimino)methano]-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (8a). A mixture of **21a** (0.3 g, 0.931 mmol) and 1-methylpiperazine (0.47 g, 4.69 mmol) in DMSO (3 mL) was heated at 100 °C for 10 h. The solvent was evaporated in vacuo, and the residue was washed with ethanol and dissolved with water/acetic acid (30 mL/5 mL). The solution was washed with $CHCl_3$ and adjusted to pH 7.5 with 1 N NaOH. The precipitates were collected by filtration, washed with water and ethanol, and recrystallized from $CHCl_3$/ethanol to give **8a** (0.21 g, 56%) as pale yellow crystals: ^{1}H NMR (DMSO-d_6) δ 2.29 (3 H, s), 2.4–2.5 (4 H, m), 2.77 (3 H, s), 3.40 (4 H, m), 4.45 (2 H, s), 7.55 (1 H, s), 7.63 (1 H, d, J = 13 Hz), 15.86 (1 H, br); IR (KBr) 1696, 1504 cm^{-1}.

By similar procedures, compounds **8b**, **8c**, and **9** were prepared. **8b**: ^{1}H NMR (DMSO-d_6) δ 0.96 (3 H, t, J = 7 Hz), 2.25 (3 H, s), 2.4–2.5 (4 H, m), 3.09 (2 H, q, J = 7 Hz), 3.3–3.4 (4 H, m), 4.51 (2 H, s), 7.53 (1 H, s), 7.67 (1 H, d, J = 13 Hz), 15.88 (1 H, bs); IR (KBr) 1696, 1490 cm^{-1}. **8c**: ^{1}H NMR (DMSO-d_6) δ 0.51 (4 H, m), 2.24 (3 H, s), 2.4–2.5 (4 H, m), 2.65 (1 H, m), 3.2–3.4 (4 H, m), 4.56 (2 H, s), 7.57 (1 H, s), 7.65 (1 H, d, J = 13 Hz), 15.9 (1 H, s); IR (KBr) 1706, 1496 cm^{-1}. **9**: ^{1}H NMR (DMSO-d_6) δ 2.26 (3 H, s), 2.4–2.6 (4 H, m), 3.18 (4 H, m), 4.46 (2 H, s), 7.63 (1 H, s), 7.88 (1 H, d, J = 12 Hz), 15.73 (1 H, br); IR (KBr) 1692, 1508 cm^{-1}.

Method E. 9,1-[(*N*-Benzylimino)methano]-7,8-difluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carbxylic Acid Hydrochloride (8f). A mixture of **21f** (500 mg, 0.950 mmol) and 1-methylpiperazine (289 mg, 2.88 mmol) in DMSO (5 mL) was stirred at room temperature for 16 h. The mixture was then poured into ice/water, and the resultant precipitates were collected and washed with water and isopropyl ether. HCl (2 N, 5 mL) was added to this precipitate, and the mixture was stirred at room temperature for 40 min. The precipitates were collected by filtration, washed with water and acetone, and recrystallized from dilute HCl to afford **8f** (143 mg, 38%) as pale yellow crystals: ^{1}H NMR (D_2O) δ 2.97 (3 H, s), 3.1–3.3 (2 H, m), 3.5–3.7 (4 H, m), 3.8–4.0 (2 H, m), 4.28 (2 H, s), 4.58 (2 H, s), 6.7–7.2 (7 H, m); IR (KBr) 1679, 1477 cm^{-1}.

By similar procedures, **8d** and **8e** were prepared. **8d**: ^{1}H NMR (DMSO-d_6) δ 2.85 (3 H, s), 3.2–3.8 (8 H, m), 3.67 (2 H, d, J = 6.5 Hz), 4.51 (2 H, s), 5.09 (1 H, d, J = 10 Hz), 5.13 (1 H, d, J = 15.5 Hz), 5.6–5.8 (1 H, m), 7.54 (1 H, s), 7.72 (1 H, d, J = 12.5 Hz), 10.86 (1 H, br), 15.78 (1 H, s); IR (KBr) 1682, 1488 cm^{-1}. **8e**: ^{1}H NMR ($CDCl_3$) δ 2.15 (7 H, s), 3.17 (3 H, s), 3.1–3.2 (1 H, m), 5.06 (2 H, s), 6.7–7.3 (5 H, m), 6.88 (1 H, s), 7.84 (1 H, d, J = 12.5 Hz), 15.4 (1 H, br); IR (KBr) 1692, 1502 cm^{-1}.

Ethyl 1-[(Ethoxycarbonyl)methyl]-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (24). To a solution of ethyl 4-chloroacetoacetate (2.32 g, 0.014 mol) in $CHCl_3$ at 0 °C was added ethyl 4-hydroxy-6,7-difluoro-2-mercaptoquinoline-3-carboxylate (**23**) (4.00 g, 0.014 mol) and triethylamine (2.83 g, 0.028 mol). The solution was stirred at 0 °C for 15 min and then at room temperature for 1 h. The mixture was diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and then evaporated in vacuo. The residue was washed with isopropyl ether and dissolved

in H_2SO_4 (26 mL). The solution was stirred at room temperature for 40 min. The mixture was then poured into ice/water, and the resultant precipitates were collected and washed with water to give **24** (4.35 g, 78%) as a colorless crystals: mp 240 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.02 (3 H, t, J = 7 Hz), 1.31 (3 H, t, J = 7 Hz), 4.03 (2 H, q, J = 7 Hz), 4.31 (2H, q, J = 7 Hz), 4.70 (2 H, s), 7.50 (1 H, s), 8.1–8.3 (2 H, m). Anal. ($C_{18}H_{15}F_2NO_5S$) C, H, N.

Diacetoxy[[[1-[(ethoxycarbonyl)methyl]-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl]carbonyl]oxy]borane (25). A mixture of **24** (3.32 g, 8.40 mmol) and triacetoxyborane (2.39 g, 12.6 mmol) in acetic anhydride (50 mL) was heated at 100 °C for 40 min. The precipitates were collected by filtration and washed with acetic anhydride and isopropyl ether to afford **25** (3.76 g, 90%) as colorless crystals: mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 1.09 (3 H, q, J = 7 Hz), 1.92 (6 H, s), 4.10 (2 H, q, J = 7 Hz), 5.01 (2 H, s), 8.19 (1 H, s), 8.50 (1 H, dd, J = 9, 10 Hz), 8.65 (1 H, dd, J = 6.5, 12.5 Hz). Anal. ($C_{20}H_{16}BF_2NO_9S$) C, H, N.

1-[(Ethoxycarbonyl)methyl]-7-fluoro-8-(4-methyl-1-piperazinyl)-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]qunoline-4-carboxylic Acid Hydrochloride (Hydrochloride of 26). A mixture of **25** (2.02 g, 4.44 mmol) and 1-methylpiperazine (1.33 g, 13.3 mmol) in DMSO (22 mL) was stirred at room temperature for 70 min. The reaction mixture was poured into ice/water, and the resultant precipitates were collected by filtration and washed with water. HCl (1 N) was added to the precipitates, and the mixture was stirred at room temperature for 20 min. The precipitates were collected by filtration and washed with 1 N HCl and acetone to afford the hydrochloride of **26** (1.62 g, 73%) as colorless crystals. A solution of the hydrochloride of **26** (1.40 g) in water was adjusted to pH 8 with 1 N NaOH, and the resulting precipitates were collected by filtration and washed with water and acetone to give the free base **26** (1.12 g), which was used for the preparation of **10**. The hydrochloride of **26** (recrystallized from dilute HCl): mp 267 °C dec; ^{1}H NMR (D_2O) δ 1.17 (3 H, t, J = 7 Hz), 3.07 (3 H, s), 3.2–4.0 (8 H, m), 4.22 (2 H, q, J = 7 Hz), 4.51 (2 H, s), 7.29 (1 H, d, J = 6.5 Hz), 7.37 (1 H, d, J = 12.5 Hz), 7.58 (1 H, s). Anal. ($C_{21}H_{23}ClFN_3O_5S\cdot0.75H_2O$) C, H, N.

Method F. 4-Hydroxy-6-fluoro-5-(4-methyl-1-Piperazinyl)-8-oxo-8*H*-benzo[*ij*]thiazolo[2,3,4-*de*]quinolizine-9-carboxylic Acid Hydrochloride (27). A mixture of **26** and polyphosphoric acid was heated at 130 °C for 5 h. The mixture was poured into ice/water and adjusted to pH 9 with 2 N NaOH. The precipitates were collected by filtration, washed with water, and recrystallized from dilute HCl to give **27** (20 mg, 10%) as pale brown crystals: ^{1}H NMR (D_2O) δ 3.11 (3 H, s), 3.5–4.0 (8 H, m), 6.65 (0.6 H, s; this proton should be partially exchanged with deuterium), 7.20 (1 H, s), 7.75 (2 H, d, J = 12 Hz); IR (KBr) 1683, 1639, 1491 cm^{-1}.

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).[13] 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.

Inhibitory Effect on DNA Gyrase Supercoiling Activity Isolated from *E. coli* KL-16. This assay was carried out according to the method reported previously.[14]

In Vivo Efficacy on Systemic Infections. The in vivo assay was carried out according to the following general method.[4b] 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 (**8a**) or dissolved in sterilized distilled water (**1**) and administered orally at 1 h post infection. Survival rates were evaluated after 1 week.

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