Synthesis and Antibacterial Activity of New Tetracyclic Quinolone Antibacterials[1]

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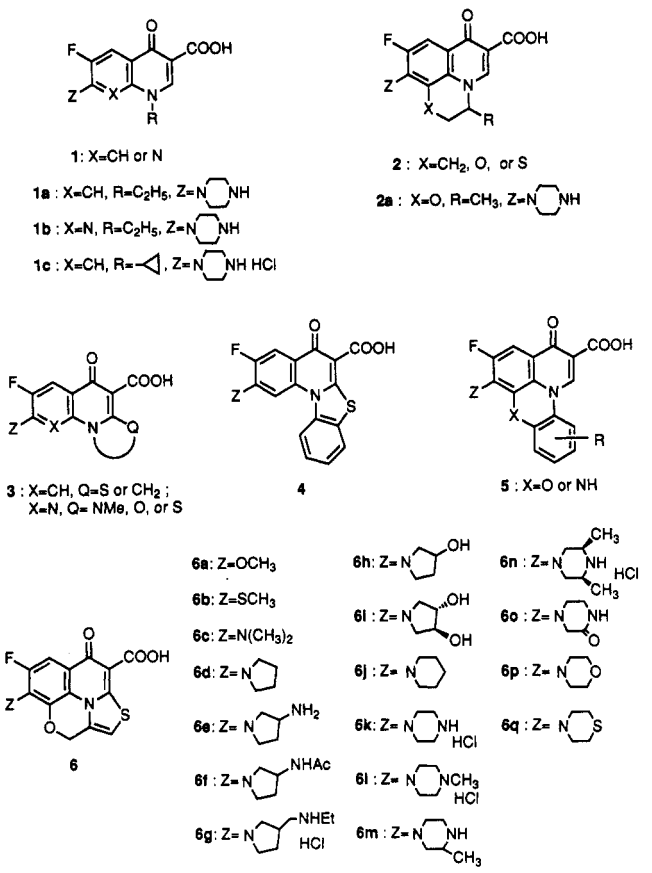
A series of 8-substituted-9,1-(epoxymethano)-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acids having a novel tetracyclic structure was synthesized and tested for antibacterial activity. The nature of the heteroatom (N, O, or S) substituted at the 8-position had little influence on the antibacterial activity. Among the six pyrrolidinyl derivatives and the five piperazinyl derivatives, the 8-(3-hydroxy-1-pyrrolidinyl) derivative **6h** and the hydrochloride of the 8-(4-methyl-1-piperazinyl) derivative **6l** showed the most potent activity against both Gram-positive and Gram-negative bacteria. Against nalidixic acid resistant strains, isolated from *Escherichia coli* KC-14, compound **6h** was less potent than **6l**. Replacement of the piperazinyl nitrogen atom by a carbon atom, an oxygen atom, or a sulfur atom (corresponding to the piperidino, morpholino, or thiomorpholino group, respectively) enhanced the activity against Gram-positive bacteria, but reduced the activity against Gram-negative bacteria. Compound **6l** also showed potent in vivo antibacterial activity against Gram-positive and Gram-negative bacteria, and did not cause convulsions in mice with the concomitant administration of fenbufen. Replacement of the carboxy group by a sulfonic acid group in **6l** resulted in a complete loss of antibacterial activity.

Since norfloxacin[2] (**1a**, Chart I) was developed as an antibacterial agent in 1980, many fluorinated pyridone carboxylic acids have been synthesized. These compounds can be classified structurally into three groups: the first, bicyclic pyridone carboxylic acids **1**, such as **1a**, enoxacin[3] (**1b**), and ciprofloxacin[4] (**1c**); the second, tricyclic pyridone carboxylic acids[5,6] **2**, such as ofloxacin[5a] (**2a**) and **3**; the third, tetracyclic pyridone carboxylic acids,[7,8] such as **4**, and **5**. We have previously reported that the sulfur atom plays an important role in the enhancement of antibacterial activity of tricyclic pyridone carboxylic acids **3**.[6c] This result led us to incorporate a sulfur atom into the tricyclic pyridone carboxylic acids **2** (X = O) to elaborate the novel tetracyclic pyridone carboxylic acids **6**. This

Chart I



(1) This work has been presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy [October 23-26, Los Angeles, CA, 1988; Abstract No. 1491] and at the 109th Annual Meeting of the Pharmaceutical Society of Japan [April 4-6, Nagoya in Japan, 1989; Abstract No. 6B 10-5].

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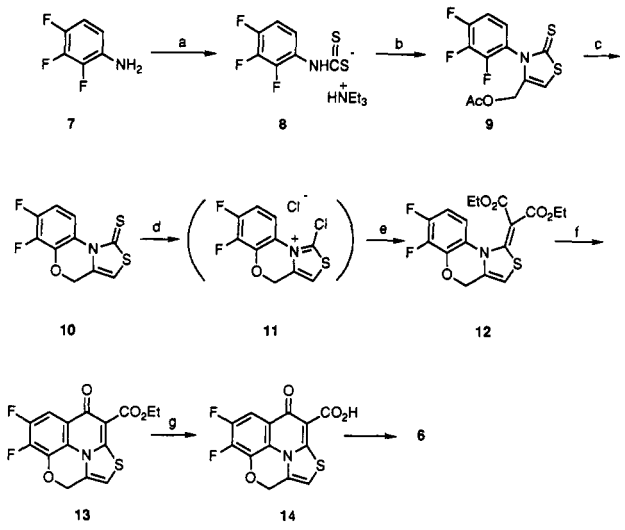
Scheme I[a]

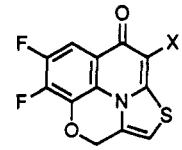


[a] (a) CS_2, NEt_3; (b) (i) $AcOCH_2COCH_2Cl/AcOEt$, (ii) HCl/AcOEt; (c) aqueous KOH/EtOH; (d) $ClCO_2CCl_3$/toluene; (e) $CH_2(CO_2Et)_2/NEt_3/CH_3CN$; (f) PPA; (g) fuming H_2SO_4.

paper describes the synthesis of the novel tetracyclic pyridone carboxylic acids **6**, and the related compounds **18** and **19** and the antibacterial activity of these compounds.[9]

Chemistry

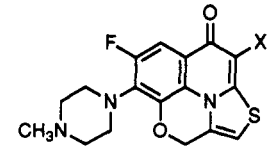
The synthetic routes leading to the novel tetracyclic pyridone carboxylic acids are summarized in Scheme I. The important intermediate **14** was synthesized from 2,3,4-trifluoroaniline (**7**). The reaction of **7** with carbon disulfide in triethylamine gave dithiocarbamate **8**. Compound **8** was allowed to react with 1-acetoxy-3-chloro-2-propanone in ethyl acetate, following by cyclization under acidic conditions, to give **9**, which, without purification, was treated with aqueous potassium hydroxide in ethanol to give tricyclic compound **10**. Treatment of compound **10** with trichloromethyl chloroformate in toluene yielded the iminium chloride **11** as a moisture-sensitive precipitate, which was allowed to react with diethyl malonate/triethylamine in acetonitrile to give **12**. Compound **12** was cyclized in polyphosphoric acid to give the pyridone carboxylic acid ester **13**. Finally, this ester was hydrolyzed in fuming sulfuric acid to produce **14**. Hydrolysis of **13** in a mixture of 1/4 concentrated hydrochloric acid and acetic acid resulted in decarboxylation, affording **15**. This decarboxylation is an interesting reaction, because the



15: X=H

16: $X=CO_2B(OAc)_2$

17: $X=SO_3H$



18: X=H

19: $X=SO_3H$

same conditions were used for the successful hydrolysis of the ethyl ester of **2** (X = 0).[10] The sulfur atom of **13** might contribute to the decarboxylation by making the 4-position more electron-rich and better able to accept a proton. Replacement of the 8-fluorine atom of **14** by the appropriate nucleophiles yields the desired 9,1-(epoxymethano)-7-fluoro-8-substituted-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acids **6** (Table I). Compound **6e** was obtained by hydrolysis of **6f** under alkaline conditions. The hydrochlorides **6g**, **6k**, **6l**, and **6n** were obtained by treatment of the corresponding free bases with hydrochloric acid.

Borate complex **16**, which was prepared by the reaction of **13** with boron triacetate in acetic anhydride,[11] could be another useful intermediate which can be prepared safely in large scale for the synthesis of compounds in series **6**. Thus, compound **6l** was obtained by the reaction of **16** with 1-methylpiperazine following by hydrolysis with hydrochloric acid in acetone.

Compound **18** was obtained by decarboxylation of **6l** under the same conditions described above for decarboxylation of **13**. Sulfonation of **15** in fuming sulfuric acid yielded **17**, which gave **19** on reaction with 1-methylpiperazine.

Biological Results and Discussion

The in vitro antibacterial activity of **6a–q**, **18**, and **19** against Gram-positive and Gram-negative bacteria is summarized in Table I. Data for **1a** (norfloxacin), **1b** (enoxacin), **1c** (ciprofloxacin), and **2a** (ofloxacin) are included for comparison purposes. From the data for the first three entries (compounds **6a–c**), the antibacterial activity of the tetracyclic pyridone carboxylic acids **6** seems to be hardly affected by the nature of the heteroatom substituted in the 8-position. The activity of **6a–c** against Gram-positive bacteria, except for *Enterococcus faecalis*, is more potent than that of the reference compounds **1a–c** and **2a**, but against Gram-negative bacteria, **6a–c** were less potent than the reference compounds. The data for pyrrolidinyl derivatives **6d**, **6e**, and **6h** indicate that the introduction of an amino group into the pyrrolidinyl moiety reduced the activity against Gram-positive bacteria, and the introduction of a hydroxyl group into the pyrrolidinyl moiety enhanced activity against Gram-negative bacteria. The introduction of one more hydroxyl group into the pyrrolidinyl moiety (compound **6i**) resulted in a considerable loss of activity against both Gram-positive and Gram-negative bacteria. The modification of the amino group on the pyrrolidinyl moiety (compounds **6f** and **6g**) reduced the activity against Gram-negative bacteria compared to that of **6e**. Among the pyrrolidinyl derivatives

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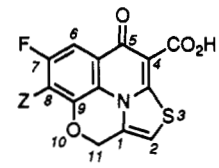
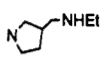
Table I. Tetracyclic Pyridone Carboxylic Acids **6a–q**, Related Compounds **18** and **19**, and Their in Vitro Antibacterial Activity (Minimum Inhibitory Concentration, μg/mL)



6a-q

					microorganism[a]								
					Gram-positive				Gram-positive				
compd	Z	method[b]	mp, °C	formula[c]	Sa(F)	Sa(I)	Se	Ef	Ec(N)	Ec(K)	Kp	Pa(I)	Pa(E)
6a	OCH_3	A	>280	$C_{14}H_8NO_5SF$	0.20	0.20	0.39	6.25	0.39	0.78	0.20	3.13	6.25
6b	SCH_3	A	>280	$C_{14}H_8NO_4S_2F \cdot 0.25H_2O$	0.20	0.10	0.20	3.13	0.39	0.20	0.39	1.56	3.13
6c	$N(CH_3)_2$	A	>280	$C_{15}H_{11}N_2O_4SF$	0.20	0.10	0.10	0.78	0.39	0.39	0.20	1.56	6.25
6d		A	>280	$C_{17}H_{13}N_2O_4SF$	0.025	0.05	0.05	0.20	0.20	0.20	0.20	1.56	1.56
6e		B	>280	$C_{17}H_{14}N_3O_4SF \cdot 0.75H_2O$	0.20	0.39	0.78	0.78	0.20	0.10	0.10	0.78	0.78
6f		A	>280	$C_{19}H_{16}N_3O_5SF$	0.20	0.10	0.78	0.78	1.56	1.56	1.56	6.25	6.25
6g		C	278 dec	$C_{20}H_{20}N_3O_4SF \cdot HCl \cdot 0.25H_2O$	0.20	0.20	0.10	0.39	0.39	0.39	0.39	25	3.13
6h		A	>280	$C_{17}H_{13}N_2O_5SF$	0.025	0.025	0.20	0.20	0.05	0.05	0.05	0.39	0.39
6i		A	>280	$C_{17}H_{13}N_2O_6SF \cdot 0.25H_2O$	1.56	1.56	>25	>25	3.13	3.13	1.56	>25	>25
6j		A	>280	$C_{18}H_{15}N_2O_4SF$	0.10	0.10	0.20	1.56	0.78	3.13	1.56	12.5	6.25
6k		C	>280	$C_{17}H_{14}N_3O_4SF \cdot HCl \cdot 0.5H_2O$	0.39	0.39	1.56	0.78	0.10	0.10	0.05	0.78	0.78
6l		D	>280	$C_{18}H_{16}N_3O_4SF \cdot HCl$	0.10	0.10	0.20	0.39	0.10	0.10	0.10	0.78	0.78
6m		C	240 dec	$C_{18}H_{16}N_3O_4SF \cdot 0.25H_2O$	0.20	0.20	0.78	0.78	0.20	0.10	0.10	1.56	0.78
6n		C	>280	$C_{19}H_{18}N_3O_4SF \cdot HCl \cdot H_2O$	0.20	0.20	0.39	0.78	0.20	0.10	0.39	3.13	3.13
6o		A	>280	$C_{17}H_{12}N_3O_5SF \cdot 0.25H_2O$	3.13	1.56	12.5	3.13	1.56	1.56	1.56	6.25	6.25
6p		A	>280	$C_{17}H_{13}N_2O_5SF$	0.05	0.05	0.10	0.39	0.39	0.39	0.20	1.56	0.39
6q		A	>280	$C_{17}H_{13}N_2O_4S_2F \cdot 0.5H_2O$	0.05	0.05	0.10	3.13	0.39	0.39	0.39	6.25	3.13
18	(the decarboxylated derivative)				>100	>100	>100	>100	>100	>100	>100	>100	>100
19	(the sulfonic acid derivative)				>100	>100	>100	>100	>100	>100	>100	>100	>100
1a	(norfloxacin)				0.39	1.56	3.13	1.56	0.20	0.20	0.10	1.56	1.56
1b	(enoxacin)				0.78	0.78	3.13	3.13	0.20	0.20	0.10	1.56	1.56
1c	(ciprofloxacin)				0.20	0.78	1.56	0.78	0.05	0.05	0.05	0.78	0.78
2a	(ofloxacin)				0.39	0.39	0.78	1.56	0.10	0.10	0.10	1.56	1.56

[a] 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; Pa(I), *Pseudomonas aeruginosa* IFO 3445; Pa(E), *P. aeruginosa* E-2. [b] See the Experimental Section. [c] The analyses for C, H, and N were within ±0.4% of the theoretical values.

6d–i, compound **6h** showed very potent activity against both Gram-positive and Gram-negative bacteria; its activity was more potent than that of reference compounds **1a–c** and **2a**. The data for the piperazinyl derivatives **6k–n** indicate that the introduction of one or two methyl groups into the piperazinyl moiety enhanced activity against Gram-positive bacteria. The N-methylated derivative **6l** showed the most potent activity against Gram-positive bacteria. Against Gram-negative bacteria, **6m** and **6n** have reduced activity compared to that of **6k**, while **6l** showed similar activity to that of **6k**. Reducing the basicity of the piperazinyl nitrogen atom (compound **6o**) resulted in a considerable loss of activity against both Gram-positive and Gram-negative bacteria, compared to that of **6k**. Replacement of the piperazinyl nitrogen atom by a carbon atom, an oxygen atom, or a sulfur atom (compounds **6j**, **6p**, and **6q**) enhanced the activity against Gram-positive bacteria, but reduced the activity against Gram-negative bacteria. Among the piperazinyl derivatives **6k–o** and their related compounds **6j**, **6p**, and **6q**, compound **6l** showed very potent activity against both Gram-positive and Gram-negative bacteria; its activity against Gram-positive bacteria was more potent than those of reference compounds **1a–c** and **2a**. Against Gram-negative bacteria,

Table II. Inhibitory Effect (IC_{50}) of **6** on DNA Gyrase Supercoiling Activity from *E. coli* K-12 C600 and in Vitro Antibacterial Activity (MIC) against the Same Strain

compd	IC_{50}, μg/mL	MIC, μg/mL	compd	IC_{50}, μg/mL	MIC, μg/mL
6d	0.93	0.20	**6l**	0.31	0.10
6e	0.25	0.20	**6m**	0.28	0.20
6h	0.35	0.10	**1c**	0.20	0.10
6k	0.17	0.20	**2a**	0.59	0.20

Table III. In Vitro Antibacterial Activity of **6h** and **6l** against Nalidixic Acid (NA) Resistant Strains from *E. coli* KC-14

	MIC, μg/mL				
strain	**6h**	**6l**	**1c**	**2a**	NA
1	>3.13	1.56	1.56	6.25	>1600
2	>3.13	3.13	3.13	6.25	>1600
3	0.78	0.39	0.20	0.78	200
4	0.78	0.20	0.20	0.39	100
5	0.78	0.39	0.20	0.78	100
6	0.39	0.39	0.39	0.78	100
7	0.20	0.20	0.10	0.39	100

compound **6l** was comparable to, or more active than, the reference compounds.

Decarboxylation of **6l** (affording compound **18**) resulted in a complete loss of activity, as was found to be the case with **1a**.[2] Replacement of the carboxy group by a sulfonic acid group (forming compound **19**) also resulted in a complete loss of activity.

The inhibition of the supercoiling activity of DNA gyrase isolated from *E. coli* K-12 C600, along with in vitro activity against the same organism, was evaluated for several compounds in the series **6a–q**; the results are shown in Table II. The data for reference compounds **1c** and **2a** are also included for comparison purposes. The comparison of the IC_{50} values between **6k** and **1c** with the same substituent, or between **6l** and **2a** with the same substituent, indicates that the tetracyclic system enhanced the inhibitory effects on DNA gyrase compared to those of the bicyclic and tricyclic systems. The IC_{50} values were not directly proportional to the MIC values; this discrepancy could be due to differing cell permeability.[12]

Among the tetracyclic pyridone carboxylic acids **6a–q**, the two derivatives **6h** and **6l** with very potent antibacterial activity against both Gram-positive and Gram-negative bacteria were selected for further study, and their in vitro antibacterial activity against nalidixic acid resistant strains isolated from *E. coli* KC-14 was evaluated. The results are shown in Table III; the data for reference compounds **1c**, **2a**, and nalidixic acid are also included to facilitate comparison. The results show that **6h** was less potent than **6l**, and the activity of **6l** was comparable to that of **1c** and greater than that of **2a**. This result led us to select **6l** for further in vivo evaluations.

The oral antibacterial activity in mice of **6l** is shown in Table IV; data for reference compound **2a** are included for comparison. The data show that compound **6l** was effective against the experimental infections; its effects were superior to or comparable with those of **2a**. The acute toxicity (LD_{50}) of **6l** in mice was as follows: 1231 mg/kg po, 237 mg/kg iv. These data indicate that the acute toxicity of **6l** was comparable to or less than that of other pyridone carboxylic acids substituted with the 4-methyl-1-piperazinyl group.[3,5b]

Table IV. Therapeutic Effect of **6l** 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
6l	8.10 (6.53–10.1)	1.01 (0.59–1.73)	17.7 (11.9–26.2)
2a	11.5 (7.48–17.6)	1.10 (0.75–1.63)	22.9 (15.0–35.1)

Recently, it has been reported that some pyridone carboxylic acids cause convulsions as a side effect when administered concomitantly with fenbufen.[13] Therefore, the adverse interaction of **6l** with fenbufen was evaluated. Compound **6l** caused no convulsions at a dose of 200 mg/kg on concomitant oral administration with 200 mg/kg of fenbufen in mice.

In summary, the new tetracyclic pyridone carboxylic acid **6l** exhibited potent and broad in vitro antibacterial activity against both Gram-positive and Gram-negative bacteria, and was effective in the treatment of experimental systemic infections in mice. Moreover, the acute toxicity of **6l** was low, and concomitant oral administration of **6l** with fenbufen in mice did not cause convulsions. These findings suggest that the novel tetracyclic structure **6** is a useful structural variation of the pyridone carboxylic acids for use as an antibacterial.

Experimental Section

Melting points were determined on 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. *trans*-3,4-Dihydroxypyrrolidine,[14a] 1-acetoxy-3-chloro-2-propanone,[14b] 2-oxopiperazine,[14c] and triacetoxyborane[14d] were prepared according to previously reported methods.

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 dilution technique using Mueller–Hinton agar.[15] The inoculum size was approximately 10^6 colony forming units/mL. The MIC

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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* K-12 C600. This assay was carried out according to the method reported previously.[16]

In Vivo Efficacy on Systemic Infections. The in vivo assay was carried out according to the following general method.[17] Groups of five male ddY mice (20–22 g) were infected with bacteria. A 0.5-mL volume of a bacterial dilution, corresponding to 35 or 500 times greater than the 50% lethal dose, was inoculated intraperitoneally. The test compound, **2a** or **6l**, was suspended in 0.5% sodium (carboxymethyl)cellulose (**2a**) or dissolved in sterilized distilled water (**6l**) and administered orally at 1 h postinfection. Survival rates were evaluated after 1 week.

Acute Toxicity. A solution of **6l** in sterilized distilled water was administered orally to or injected intravenously into groups of five male ddY mice (20–25 g). Survival rates were evaluated after 1 week.

Effect of Concomitant Oral Administration with Fenbufen. Compound **6l** dissolved in sterilized distilled water was orally administered at a dose of 200 mg/kg to five male ddY mice (20–24 g) after 5 min or oral administration of fenbufen suspended in 0.5% sodium (carboxymethyl)cellulose at a dose of 200 mg/kg; then the behavior of the mice was observed for 4 h.

Triethylammonium *N*-(2,3,4-Trifluorophenyl)dithiocarbamate (8). A mixture of 2,3,4-trifluoroaniline (**7**) (221 g, 1.5 mmol), carbon disulfide (137 g, 1.8 mol), and triethylamine (456 g, 4.5 mol) was stirred at room temperature for 80 h. The precipitated crystals were collected and washed with ether to give **8** (427 g, 87%) as slightly pale yellow crystals: mp 87 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7 Hz, 9 H), 3.29 (q, J = 7 Hz, 6 H), 6.90 (m, 1 H), 7.95 (m, 1 H), 8.89 (br, 2 H). Anal. ($C_{13}H_{19}N_2S_2F_3$) C, H, N.

4-(Acetoxymethyl)-3-(2,3,4-trifluorophenyl)-2(3*H*)-thiazolethione (9) and 6,7-Difluoro-1*H*,4*H*-thiazolo[4,3-*c*][1,4]-benzoxazine-1-thione (10). Dithiocarbamate **8** (6.0 g, 18.5 mmol) was added to an ice-cooled solution of 1-acetoxy-3-chloro-2-propanone (3.0 g, 19.9 mmol) in ethyl acetate (20 mL) and the mixture was stirred at 5–18 °C for 3 h; then, a 4 N solution of hydrogen chloride in ethyl acetate (12 mL, 48 mmol) was added. The mixture was refluxed for 1 h and washed with brine and then evaporated in vacuo, affording a residue containing 4-(acetoxymethyl)-3-(2,3,4-trifluorophenyl)-2(3*H*)-thiazolethione (**9**). This residue was used for the following procedure without further purification. An analytical sample of **9** was similarly prepared in another experiment followed by recrystallization from isopropyl ether. To the residue were added ethanol (30 mL) and a solution of KOH (3.0 g, 53.5 mmol) in water (10 mL); the solution was refluxed for 40 min. The precipitated crystals were collected and washed with ethanol/water (2/1 v/v) to give **10** (3.9 g, 81%) as pale yellow crystals. **9**: mp 105–107 °C; ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3 H), 4.65 (d, J = 13.5 Hz, 1 H), 4.74 (d, J = 13.5 Hz, 1 H), 6.80 (s, 1 H), 7.15 (m, 2 H). Anal. ($C_{12}H_8NO_2S_2F_3$) C, H, N. **10** (recrystallized from hexane/ethyl acetate): mp 163–167 °C; ^{1}H NMR ($CDCl_3$) δ 4.97 (d, J = 1 Hz, 2 H), 6.56 (t, J = 1 Hz, 1 H), 6.97 (dt, J = 8, 9.5 Hz, 1 H), 9.50 (ddd, J = 2.5, 4.5, 9.5 Hz, 1 H). Anal. ($C_{10}H_5NOS_2F_2$) C, H, N.

Diethyl (6,7-Difluoro-1*H*,4*H*-thiazolo[4,3-*c*][1,4]benzoxazin-1-ylidene)malonate (12). Trichloromethyl chloroformate (11.0 mL, 91.7 mmol) was added to a suspension of compound **10** (21.3 g, 82.8 mmol) in dry toluene (200 mL) and the mixture was stirred at 80 °C for 46 h to yield compound **11**, which was not isolated because of its moisture-sensitivity. The supernatant solution was removed by decantation and the resulting solid was washed with dry toluene by decantation. A solution of diethyl malonate (14.5 g, 90.5 mmol) in dry acetonitrile (200 mL) was added to the solid, chilled in ice. Triethylamine (18.4 g, 182 mmol) was added gradually to the mixture. After being stirred at room temperature for 1 h, the mixture was evaporated in vacuo, water was added, and the products were extracted with $CHCl_3$. The extract was washed with water, dried over magnesium sulfate, and evaporated in vacuo, and the residue was crystallized from ethyl acetate to afford **12** (24.9 g, 78%) as yellow crystals. **12** (recrystallized from hexane/ethyl acetate): mp 161–164 °C; ^{1}H NMR ($CDCl_3$) δ 1.17 (t, J = 7 Hz, 6 H), 3.98 (q, J = 7 Hz, 4 H), 5.01 (d, J = 1 Hz, 2 H), 6.61 (t, J = 1 Hz, 1 H), 6.89 (dt, J = 7.5, 9.5 Hz, 1 H), 7.33 (ddd, J = 2.5, 4.5, 9.5 Hz, 1 H). Anal. ($C_{17}H_{15}NO_5SF_2$) C, H, N.

Ethyl 9,1-(Epoxymethano)-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (13). A mixture of compound **12** (67.0 g, 175 mmol) and polyphosphoric acid (460 g) was stirred at 115 °C for 4 h and poured onto ice. The precipitates were collected and washed with water to afford **13** (57.5 g, 97%) as colorless crystals. **13** (recrystallized from DMF): mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7 Hz, 3 H), 4.30 (q, J = 7 Hz, 2 H), 5.60 (d, J = 1 Hz, 2 H), 7.47 (t, J = 1 Hz, 1 H), 7.64 (dd, J = 11, 8 Hz). Anal. ($C_{15}H_9NO_4SF_2$) C, H, N.

9,1-(Epoxymethano)-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (14). Compound **13** (7.4 g, 21.9 mmol) was added to ice-cooled fuming sulfuric acid (35 mL), stirred at room temperature for 5 h, and then poured onto ice. The precipitate was collected and washed with water to afford **14** (6.7 g, 98%) as colorless crystals. **14** (recrystallized from DMSO): mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 5.70 (d, J = 1 Hz, 2 H), 7.72 (t, J = 1 Hz, 1 H), 7.86 (dd, J = 11, 7 Hz, 1 H), 15.33 (br, 1 H). Anal. ($C_{13}H_5NO_4SF_2$) C, H, N.

Method A. 9,1-(Epoxymethano)-7-fluoro-5-oxo-8-(1-pyrrolidinyl)-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (6d). A mixture of compound **14** (300 mg, 0.97 mmol) and pyrrolidine (350 mg, 4.9 mmol) in DMSO (10 mL) was stirred at 90 °C for 43 h and then evaporated in vacuo. Dilute HCl was added to the residue, and the precipitates were collected, washed with water, and recrystallized from DMSO to afford **6d** (258 mg, 73%) as yellow crystals.

By similar procedures, using triethylamine as an acid scavenger if necessary, compounds **6a** (51%), **6b** (66%), **6c** (77%), **6f** (79%), **6h** (81%), **6i** (63%), **6j** (80%), **6o** (60%), **6p** (76%), and **6q** (79%) were prepared.

Method B. 8-(3-Amino-1-pyrrolidinyl)-9,1-(epoxymethano)-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (6e). A suspension of compound **6f** (1.5 g, 3.6 mmol) in 10% aqueous NaOH solution (400 mL) was refluxed for 15 h, and the mixture was adjusted to pH 7.0 with dilute HCl. The precipitates were collected, washed with water, and recrystallized from DMF to afford **6e** (0.82 g, 58%) as green-yellow crystals.

Method C. 9,1-(Epoxymethano)-8-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]-quinoline-4-carboxylic Acid Hydrochloride (6g). A mixture of compound **14** (3.50 g, 11.3 mmol), 3-[(ethylamino)methyl]-pyrrolidine dihydrochloride (3.10 g, 15.4 mmol), and triethylamine (6.80 g, 67.2 mmol) in DMSO (120 mL) was stirred at 90 °C for 43 h and then evaporated in vacuo. Water was added to the residue and the mixture was adjusted to pH 8.0 with dilute aqueous NaOH solution. The insoluble materials were collected, washed with water, and recrystallized from DMSO to afford the free base of **6g** (3.81 g, 80%) as yellow crystals; mp 247 °C dec. Anal. ($C_{20}H_{20}N_3O_4SF\cdot0.75H_2O$) C, H,N. The free base of **6g** (3.00 g, 7.18 mmol) was dissolved in water (80 mL) by the addition of 1 N HCl (7.2 mL) and the solution was filtered. The pH of the filtrate was lowered by addition of concentrated HCl (5 mL) to afford **6g** (2.78 g, 85%) as yellow crystals.

By a similar procedure, the free base of **6k** (77%) and **6n** (46%), compound **6m** (52%; in this case, the filtrate was adjusted to pH 7.0), and hydrochlorides **6k** (81%) and **6n** (88%) were prepared. The free base of **6k** (recrystallized from DMF): mp 255 °C dec. Anal. ($C_{17}H_{14}N_3O_4SF$) C, H, N. The free base of **6n**: mp 259 °C dec. Anal. ($C_{19}H_{18}N_3O_4SF\cdot0.5H_2O$) C, H, N.

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Method D. 9,1-(Epoxymethano)-7-fluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid Hydrochloride (6l). A mixture of compound **14** (15.0 g, 48.5 mmol) and 1-methylpiperazine (15.0 g, 150 mmol) in DMSO (350 mL) was stirred at 85 °C for 3.5 h and then evaporated in vacuo. Water was added to the residue and then acetic acid (20 mL) was added. The resultant solution was filtered, and the filtrate was adjusted to pH 7.0 with dilute aqueous NaOH solution and then extracted with $CHCl_3/CH_3OH$ (10/1 v/v). The extract was washed with water and was evaporated in vacuo. The residue was recrystallized from $CHCl_3/CH_3CH_2OH$ to afford the free base of **6l** (16.3 g, 86%) as pale yellow crystals; mp 255 °C dec. Anal. ($C_{18}H_{16}N_3O_4SF$) C, H, N. The free base of **6l** (1.50 g, 3.85 mmol) was dissolved in hot water (100 mL, 45 °C) by addition of concentrated HCl (4 mL) and the solution was filtered. The filtrate was cooled with ice/water to afford **6l** (1.60 g, 97%) as pale yellow crystals.

Compound **6l** can also be obtained from compound **16** by the following procedure. A mixture of compound **16** (3.65 g, 8.55 mmol) and 1-methylpiperazine (2.23 g, 22.3 mmol) in DMSO (10 mL) was stirred at 80 °C for 1 h and then evaporated in vacuo. Acetone (20 mL) and 6 N HCl (10 mL) were added to the residue, and the mixture was stirred at room temperature for 2.5 h. The insoluble materials were collected and dissolved in hot water (40 mL, 40 °C), and then the resultant solution was filtered. The pH of the filtrate was lowered by the addition of concentrated HCl (6 mL), and **6l** (2.84 g, 75%) separated as pale yellow crystals.

9,1-(Epoxymethano)-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline (15). A solution of compound **14** (500 mg, 1.62 mmol) in a mixture (10 mL) of 1/4 concentrated HCl/acetic acid was refluxed for 11 h and cooled to room temperature. The resultant precipitates were collected and recrystallized from $DMSO/H_2O$ to afford **15** (174 mg, 39%) as slightly dark yellow crystals: mp 259–262 °C; ^1H NMR (DMSO-d_6) δ 5.53 (d, J = 1 Hz, 2 H), 6.62 (s, 1 H), 7.23 (t, J = 1 Hz, 1 H), 7.58 (dd, J = 7.5, 11 Hz, 1 H). Anal. ($C_{12}H_5NO_2SF_2 \cdot 0.25H_2O$) C, H, N.

Diacetoxy[[[9,1-(epoxymethano)-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl]carbonyl]oxy]borane (16). A mixture of compound **13** (500 mg, 1.48 mmol) and triacetoxyborane (420 mg, 2.21 mmol) in acetic anhydride (5 mL) was stirred at 80 °C for 3.5 h. The precipitates were collected and washed with acetic anhydride and hexane to afford **16** (578 mg, 89%) as colorless crystals: mp 269 °C dec; ^1H NMR (DMSO-d_6) δ 1.91 (s, 6 H), 5.83 (d, J = 1 Hz, 2 H), 8.03 (dd, J = 7, 10 Hz, 1 H), 8.11 (t, J = 1 Hz, 1 H). Anal. ($C_{17}H_{10}NO_8SF_2B$) C, H, N.

9,1-(Epoxymethano)-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-sulfonic Acid (17). Compound **15** (1.00 g, 3.77 mmol) was added to ice-cooled fuming sulfuric acid (12.3 g), and the mixture was stirred at room temperature for 4 h, and then poured onto ice (200 g). The resultant precipitate was collected and dissolved in water (500 mL), and the solution was filtered. The pH of the filtrate was lowered by addition of concentrated H_2SO_4 (10 mL) to afford **17** (1.11 g, 85%) as colorless crystals; mp >280 °C; ^1H NMR (D_2O) δ 5.46 (d, J = 1 Hz, 2 H), 7.27 (t, J = 1 Hz, 1 H), 7.71 (dd, J = 7 Hz, 10.5 Hz, 1 H). Anal. ($C_{12}H_5NO_5S_2F_2$) C, H, N.

9,1-(Epoxymethano)-7-fluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline (18). A solution of compound **6l** (2.00 g, 4.70 mmol) in a mixture (50 mL) of 1/4 concentrated HCl/AcOH was refluxed for 13 h and evaporated in vacuo. The residue was dissolved in water, and the solution was basified with dilute NaOH. The resultant precipitates were collected, washed with water, and recrystallized from CH_3OH to afford **18** (900 mg, 55%) as colorless crystals: mp 260–265 °C; ^1H NMR ($CDCl_3$) δ 2.37 (s, 3 H), 2.56 (m, 4 H), 3.36 (m, 4 H), 5.24 (d, J = 1 Hz, 2 H), 6.50 (s, 1 H), 6.54 (t, J = 1 H), 7.64 (d, J = 12 Hz, 1 H). Anal. ($C_{17}H_{16}N_3O_2SF$) C, H, N.

9,1-(Epoxymethano)-7-fluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-sulfonic Acid (19). A mixture of compound **17** (300 mg, 8.69 mmol) and 1-methylpiperazine (557 mg, 5.56 mmol) in DMSO (5 mL) was stirred at 90 °C for 40 h and then evaporated in vacuo. Water was added to the residue, and the mixture was adjusted to pH 5 with dilute HCl. The insoluble materials were collected, washed with water, and dissolved in dilute aqueous NaOH solution, and the solution was filtered. The filtrate was adjusted to pH 5 with dilute hydrochloric acid to afford **19** (285 mg, 83%) as colorless crystals: mp >280 °C; ^1H NMR (D_2O + NaOD) δ 2.30 (s, 3 H), 2.55 (br, 4 H), 3.18 (br, 4 H), 5.15 (s, 2 H), 7.38 (d, J = 13 Hz); the signal due to H-2 was not observed, probably due to exchange with deuterium. Anal. ($C_{17}H_{16}N_3O_5S_2F \cdot 0.75H_2O$) C, H, N.

Registry No. **6a**, 136708-87-7; **6b**, 136708-88-8; **6c**, 136736-95-3; **6d**, 119474-52-1; **6e**, 119474-54-3; **6f**, 119474-32-7; **6g**, 136708-96-8; **6g**·HCl, 136708-97-9; **6h**, 119474-53-2; **6i**, 136708-98-0; **6j**, 136708-99-1; **6k**, 119474-49-6; **6k**·HCl, 119474-56-5; **6l**, 119474-47-4; **6l**·HCl, 119474-55-4; **6m**, 119474-50-9; **6n**, 136709-00-7; **6p**, 136709-01-8; **6q**, 136709-02-9; **7**, 3862-73-5; **8**, 119474-39-4; **9**, 136708-89-9; **10**, 119474-31-6; **11**, 136708-90-2; **12**, 119474-42-9; **13**, 119474-43-0; **14**, 119474-45-2; **15**, 136708-91-3; **16**, 136708-92-4; **17**, 136708-93-5; **18**, 136708-94-6; **19**, 136708-95-7; methanol, 67-56-1; methanethiol, 74-93-1; dimethylamine, 124-40-3; pyrrolidine, 123-75-1; 3-acetamidopyrrolidine, 79286-74-1; 3-[(ethylamino)-methyl]pyrrolidine, 132306-05-9; 3-pyrrolidinone, 96-42-4; *trans*-3,4-dihydroxypyrrolidine, 136779-52-7; piperidine, 110-89-4; *N*-methylpiperazine, 109-01-3; 2-methylpiperazine, 109-07-9; piperazine dihydrochloride, 142-64-3; *cis*-2,6-dimethylpiperazine dihydrochloride, 19146-08-8; 2-piperazinone, 5625-67-2; morpholine, 110-91-8; thiomorpholine, 123-90-0.

Supplementary Material Available: Spectral data of tetracyclic pyridone carboxylic acids **6a–q** (2 pages). Ordering information is given on any current masthead page.