Synthesis and Antibacterial Activity of a New Series of Tetracyclic Pyridone Carboxylic Acids. 2[1a]

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Received April 26, 1993®

A novel tetracyclic pyridone carboxylic acid with a thiazolidine ring, 1,2-dihydro-9,1-(epoxymethano)-7-fluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid (**4a**), and variants with a nitrogen atom (**4b**) or carbonyl group (**4c**) in the place of the 10-position oxygen atom of **4a** were prepared and tested for antibacterial activity and inhibitory activity on DNA gyrase from *Escherichia coli* KL-16. The in vitro antibacterial potency with regard to the 10-position atom was found to be of the following order; O > NCH_3 = C=O. The IC_{50} values for DNA gyrase inhibition activity for the **4a**, **4b**, and **4c** compounds were 0.33, 0.53, and 0.67 g/mL, respectively. The activity of **4a**, in which the C-3 methyl group and C-5 of ofloxacin (**2a**) were connected with a sulfur atom to restrict the conformation of **2a**, was more potent than that of **2a** against both Gram-positive and -negative bacteria, except for *Pseudomonas aeruginosa*. Compared to the tetracyclic pyridone carboxylic acid **1a**, which has a flat thiazole ring, compound **4a** showed comparable or slightly more potent activity against both Gram-positive and -negative bacteria, except for *P. aeruginosa*.

Since the development of norfloxacin[2] as a useful therapeutic agent, a large number of analogues in which a fluorine atom is introduced at the C-6 position of the quinolone ring system have been synthesized. We recently reported the synthesis and antibacterial activity of a series of tetracyclic pyridone carboxylic acids with a flat thiazole ring (**1a-d**).[1,3] The antibacterial activity of **1b** was comparable to or more potent than that of **1a**. The structure-activity relationship of these tetracyclic pyridone carboxylic acids (**1a-d**) is different from that of the tricyclic ofloxacin analogues (**2a-d**),[4-6] and the dicyclic ciprofloxacin analogues (**3a,b**).[7] In compounds **2a,b** and **3a,b**, the introduction of a nitrogen atom into the C-8 position of the quinolone rings resulted in an overall reduction in antibacterial activity.

In the course of our study of tetracyclic quinolone antibacterials, we focused on new tetracyclic pyridone carboxylic acids (**4a-c**) that have a thiazolidine ring that is not flat (Chart I).

Kondo et al.[8] and Segawa et al.[9a] have reported that tricyclic pyridone carboxylic acids (**5a,b**) with a non-flat thiazolidine ring exhibited potent antibacterial activity.

Ohta et al.[10] reported that ofloxacin (**2a**) had two stable conformations without significant energy difference. One of the stable conformers is that where the C-3 methyl moiety is perpendicular to the plane of the quinolone ring (dihedral angle 5-4-3-11 = 80°) and the other is that where it is rather inclined to the plane of the ring (dihedral angle 5-4-3-11 = 32°). They speculated that the latter was the active conformer, contributing to the antibacterial activity. Compound **4a** is a conformationally restricted analogue of **2a**, and its dihedral angle (3a-1a-1-2) would be close to that (5-4-3-11) of the active conformer of **2a**. This compound **4a** could therefore be expected to have potent antibacterial activity.

Here, we describe the synthesis, antibacterial activity, and inhibition of DNA gyrase supercoiling activity of a new series of tetracyclic pyridone carboxylic acids, **4a-c**.

Chemistry

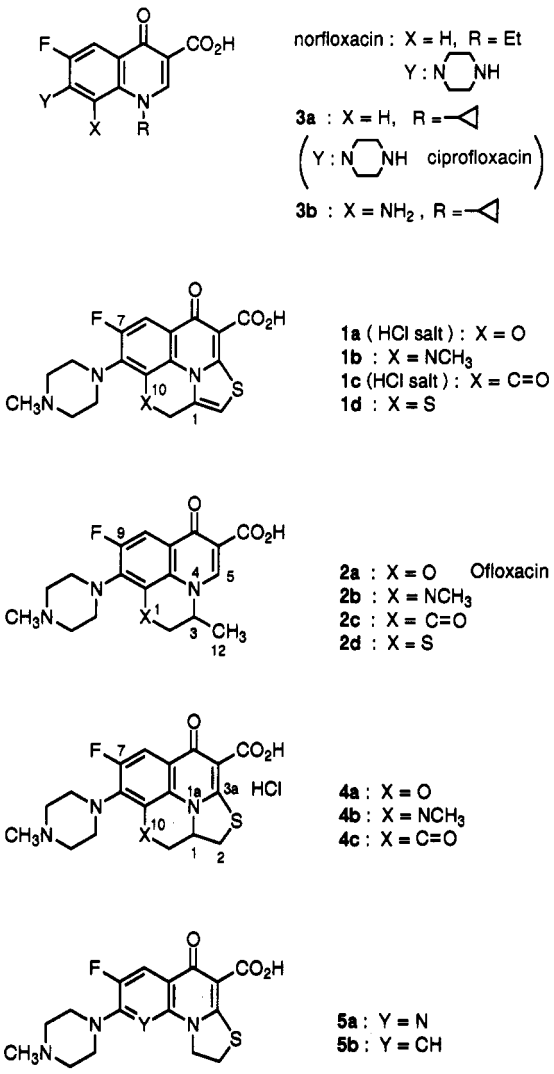
The route of synthesis of the target compound **4a** is summarized in Scheme I. The starting material[1a] **6** was reacted with 2,3-dibromopropanol in the presence of K_2CO_3 in DMF to give **7**. Treatment of **7** with K_2CO_3 in DMF gave the mixture of **8** and its rearranged isomer **9**, the product ratio being 1:9, on the basis of the ^1H NMR spectrum. These compounds could not be separated, and their structures were deduced from the structures of the final products **4a** and **10**. The construction of **9** is considered to have arisen as follows: the hydroxyl group of **7** attacked at the C-3a position to produce thiolate **11**, and this was followed by cyclization to afford the tetracyclic compound **9** (Scheme II). Attempts to convert **7** to **8** selectively, under various kinds of basic conditions (NaH/DMF, LDA/THF, potassium *tert*-butoxide/DMF, and Mg, CCl_4/DMF), were unsuccessful. The mixture of **8** and **9** was reacted with triacetoxyborane in acetic anhydride to isolate **13** and to afford a crude mixture containing **12**. Compound **13** was reacted with 1-methylpiperazine and was then hydrolyzed under acidic conditions to give **10**. Similar treatment of the crude mixture containing **12** gave **4a** (contaminated with a small amount of **10**). The structures of **4a** and **10** were determined on the basis of the ^{13}C NMR spectra (Table I). The signals attributed to C-9 of **4a** and **10** in D_2O were observed at 140.1 and 122.0 ppm (or 127.2 ppm), respectively. The higher shift of the signal of the former compared with that of the latter definitely supports the suggested structures of **4a** and **10**.

The synthesis of **4b** was achieved according to the route of synthesis shown in Scheme III. The starting material[1a] **6** was reacted with *N*-(benzyloxycarbonyl)-*N*-methyl-2,3-dibromopropylamine[11] in the presence of K_2CO_3 in DMF to afford **14**. Deprotection of the benzyloxycarbonyl group of **14** with HBr/HOAc gave the crude **15**, followed by the cyclization of **15** without purification to give the tetracyclic compound **16**. In the ^1H NMR of **16**, the doublet signal at 3.19 ppm (3 H, d, J = 3 Hz), based on the coupling of N-CH_3 and the fluorine atom at the C-8 position, was observed, and this suggested the structure of N-CH_3 at

® Abstract published in *Advance ACS Abstracts*, September 15, 1993.

0022-2623/93/1836-3148$04.00/0 © 1993 American Chemical Society

Chart I



the 10-position. On cyclization of **15**, a rearranged isomer similar to **9** was not given; the reason for this was thought to be that the steric hindrance of the methylamino group prevented an attack on the C-3a position. The reaction of **16** with triacetoxyborane in acetic anhydride afforded **17**. Replacement of the C-8 fluorine atom of **17** by 1-methylpiperazine, followed by hydrolysis under acidic conditions, gave **4b**.

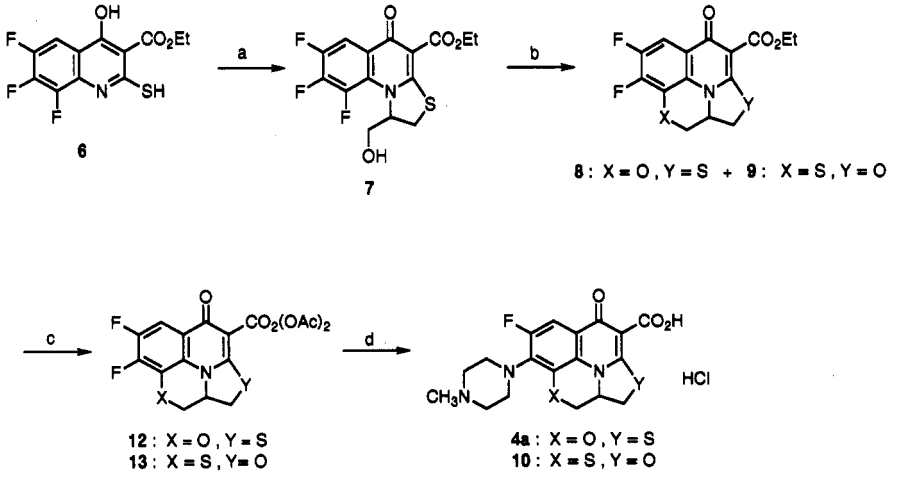
The synthesis of **4c** was achieved according to the route of synthesis shown in Scheme IV. The starting material[8] **18** was reacted with methyl 3,4-dibromobutyrate[12] in the presence of K_2CO_3 to give **19**, followed by the reaction with triacetoxyborane in acetic anhydride to afford **20**. Compound **20** was reacted with 1-methylpiperazine, and, when hydrolyzed under acidic conditions, gave **21**. Finally, cyclization of **21** in polyphosphoric acid afforded **4c**.

Biological Results and Discussion

Table II shows the in vitro antibacterial activity of a series of tetracyclic pyridone carboxylic acids (**4a-c**), **10**, **1a**, **1b**, and **2a** (ofloxacin) against four Gram-positive bacteria (*Staphylococcus aureus* FDA 209P JC-1, *Staphylococcus aureus* IID 803, *Staphylococcus epidermidis* IAM 1296, and *Enterococcus faecalis* IID 682) and five Gram-negative bacteria (*E. coli* NIHJ JC-2, *E. coli* KC-14, *Klebsiella pneumoniae* B54, *Pseudomonas aeruginosa* IFO 3445, and *Pseudomonas aeruginosa* E-2). Compound **4a** exhibited 2–16 times more potent antibacterial activity than **4b** and **4c** against both Gram-positive and -negative bacteria. The activity of **4b** was comparable to that of **4c**. This result clearly indicated that an oxygen atom in the 10-position of a tetracyclic pyridone carboxylic acid with a non-flat thiazolidine ring was optimal relative to a nitrogen atom or a carbonyl group. This structure–activity relationship is similar to that[4-6] noted for a series of tricyclic ofloxacin analogues and is different from that[1a] of tetracyclic quinolone analogues that have a flat thiazole ring (**1a-c**). The antibacterial activity of **4a** was compared to that of the reference compounds **1a**, **1b**, and **2a**. The activity of **4a** against Gram-positive bacteria was more potent than that of **2a**, comparable to that of **1a**, and less potent than that of **1b**. Against Gram-negative bacteria, except for *P. aeruginosa*, **4a** was slightly more potent than **1a** and **2a** and comparable to **1b**.

Figure 1 and Table III show the inhibition of the supercoiling activity of DNA gyrase isolated from *E. coli* KL-16, along with the in vitro activity against the same organism, of **4a-c**, **10**, **1a**, **1b**, and **2a**. When the IC_{50} values of the tetracyclic quinolone series that have a thiazoline ring (**4a-c**) are compared, it can be seen that replacement of the 10-position oxygen atom of **4a** by a nitrogen atom or a carbonyl group somewhat reduces the inhibition of DNA gyrase. The MIC value of **4b** was 16-fold greater than that of **4a**, representing a much wider potency difference than the 1.6-fold differential in their IC_{50}. This

Scheme I[a]



[a] (a) 2,3-Dibromopropanol, K_2CO_3 in DMF; (b) K_2CO_3 in DMF; (c) $B(OAc)_3$ in Ac_2O; (d) (i) 1-methylpiperazine, (ii) HCl.

Scheme II

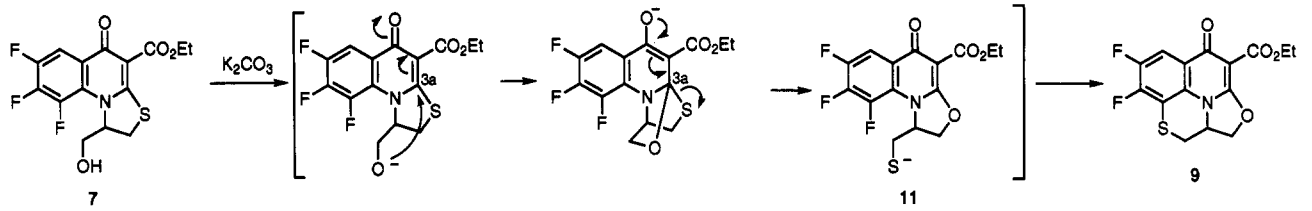
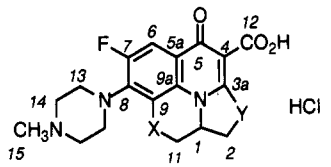


Table I. ^{13}C NMR Chemical Shifts (ppm) of **4a** and **10** in D_2O and Their Assignment



4a X = O, Y = S
10 X = S, Y = O

assignment	4a	10
1	63.8	59.8
2	34.1	78.7
3a[a]	165.1	168.9
4	103.7	93.8
5	177.8 (d, J = 3 Hz)	180.9 (d, J = 3 Hz)
5a	118.6 (d, J = 10 Hz)	127.2[b] (br)
6	106.2 (d, J = 25 Hz)	110.3 (d, J = 24 Hz)
7	157.6 (d, J = 248 Hz)	159.9 (d, J = 253 Hz)
8	133.8 (d, J = 15 Hz)	141.2 (d, J = 16 Hz)
9	140.1 (d, J = 7 Hz)	122.0[b] (d, J = 7 Hz)
9a	126.7	129.4
11	70.2	28.6
12[a]	170.6	164.0
13	56.8	56.9 (d, J = 1 Hz)
14	49.9	49.9
15	45.9	45.8

[a,b] These assignments may be interchanged.

finding indicates that replacement of the 10-position oxygen atom of **4a** by a nitrogen atom reduced cell permeability[13] along with producing a decrease in inherent activity. The IC_{50} data of **4a** and **1a** and **4b** and **1b** show that a replacement of the flat thiazole ring of tetracyclic pyridone carboxylic acid with non-flat thiazolidine ring reduces the inhibitory effect on DNA gyrase. It appears that a modification of a flat thiazole ring with a non-flat thiazolidine ring had slightly a unfavorable effect on DNA gyrase inhibitory activity. When the IC_{50} value of **4a** was compared with that of **2a**, we found that the DNA gyrase inhibitory activity of **4a** was 1.6-fold more potent than that of **2a**. Compound **4a** exhibited more potent antibacterial activity and was a more potent inhibitor of DNA gyrase than **2a**. This result might be interpreted in terms of the effects of conformational restrictions; a stable conformer of **4a** fixed by connection of the 3-methyl group and the C-5 of **2a** with a sulfur atom fitted the active conformer of **2a**. This speculation was supported by performing conformational analysis of the model compound **22** by molecular mechanics (Chart II). The compound **22** has only one minimum-energy conformer; this has a dihedral angle (3a–1a–1–2) of 24°, and its conformer resembles the active conformer of **2a** (corresponding dihedral angle (5–4–3–11) is 32°).[10]

Compound **10**, in which the 3-sulfur atom and 10-oxygen atom of **4a** were interchanged, exhibited poor antibacterial activity and inhibitory effect against DNA gyrase.

These findings suggests that a tetracyclic quinolone framework that has a non-flat thiazolidine ring, i.e., the framework of **4a**, is a structural variation of the pyridone carboxylic acids that will be of value as an antibacterial agent.

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus, Model 535; all melting points are uncorrected. ^1H 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.

Ethyl 1,2-Dihydro-1-(hydroxymethyl)-5-oxo-7,8,9-trifluoro-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (7). A mixture of ethyl 4-hydroxy-6,7,8-trifluoro-2-mercaptoquinoline-3-carboxylate (**6**) (3.80 g, 12.5 mmol), 2,3-dibromopropanol (3.29 g, 15.1 mmol), and K_2CO_3 (6.08 g, 44.0 mmol) in DMF (38 mL) was stirred at 50–55 °C for 4 h. The resulting precipitate was collected by filtration and washed with DMF, water, and acetone to afford **7** (2.96 g, 65%) as colorless crystals. **7** (recrystallized from DMSO): mp 266 °C dec; ^1H NMR (DMSO-d_6) δ 1.27 (3 H, t, J = 7 Hz), 3.36 (1 H, d, J = 12 Hz), 3.5–3.7 (3 H, m), 4.25 (2 H, q, J = 7 Hz), 5.32 (1 H, t, J = 6 Hz), 5.61 (1 H, q, J = 6.5 Hz), 7.90 (1 H, ddd, J = 2, 8.5, 10.5 Hz). Anal. ($C_{15}H_{12}F_3NO_4S$) C, H, N.

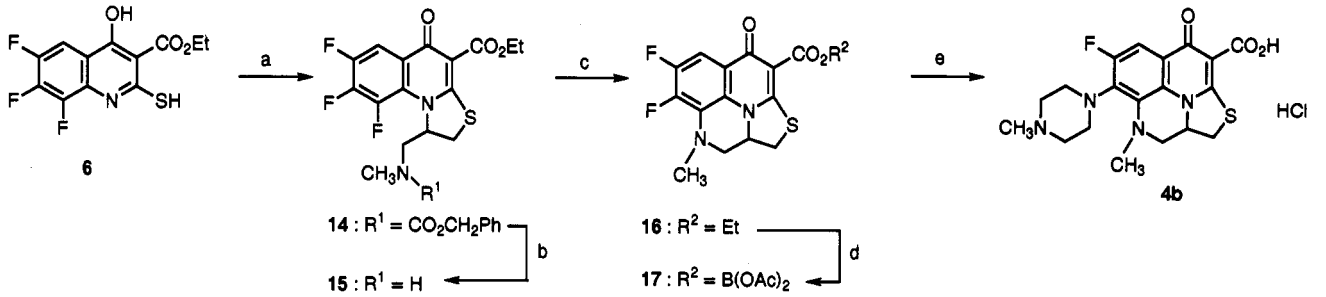
Mixture of Ethyl 1,2-Dihydro-9,1-(epoxymethano)-7,8-difluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (8) and Ethyl 1,2-Dihydro-9,1-(epithiomethano)-7,8-difluoro-5-oxo-5*H*-oxazolo[3,2-*a*]quinoline-4-carboxylate (9). A mixture of **7** (5.42 g, 15.1 mmol) and K_2CO_3 (10.13 g, 73.3 mmol) in DMF (50 mL) was stirred at 45–50 °C for 17 h. The resulting precipitate was collected by filtration washed with DMF, water, and acetone, and recrystallized from DMSO to afford a mixture of **8** and **9** (**8**:**9** = 1:9, determined by the integration of the signal at δ 7.70 and 7.51) (1.16 g, 22%) as colorless crystals: mp >280 °C. Anal. ($C_{15}H_{11}F_2NO_4S$·0.5H_2O) C, H, N.

8: ^1H NMR (DMSO-d_6) δ 1.28 (3 H, t, J = 7 Hz), 3.27 (1 H, dd, J = 6, 11 Hz), 3.60 (1 H, dd, J = 7, 11 Hz), 4.24 (2 H, q, J = 7 Hz), 4.36 (1 H, dd, J = 9.5, 10.5 Hz), 4.8–5.0 (1 H, m), 5.02 (1 H, dd, J = 4, 10.5 Hz), 7.51 (1 H, dd, J = 7.5, 11 Hz).

9: ^1H NMR (DMSO-d_6) δ 1.24 (3 H, t, J = 7 Hz), 3.37 (1 H, dd, J = 10.5, 12.5 Hz), 3.73 (1 H, dd, J = 2.5, 12.5 Hz), 4.19 (2 H, q, J = 7 Hz), 4.55 (1 H, dd, J = 8.5, 11 Hz), 4.8–5.0 (1 H, m), 5.16 (1 H, t, J = 8.5 Hz), 7.70 (1 H, dd, J = 8.5, 10.5 Hz).

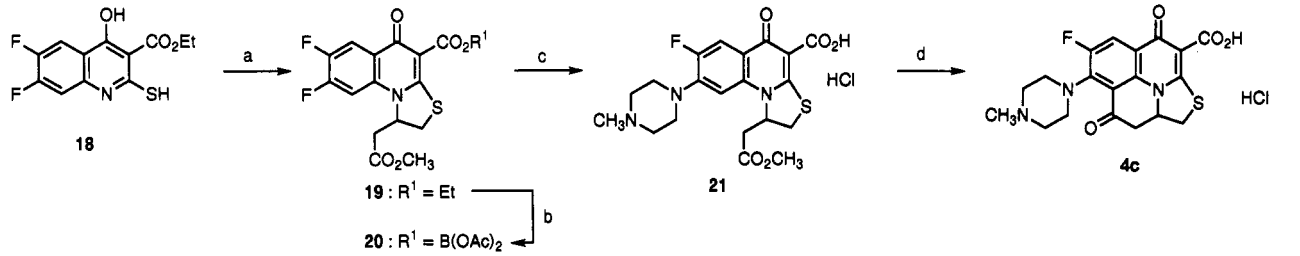
1,2-Dihydro-9,1-(epoxymethano)-7-fluoro-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid Hydrochloride (4a) and Diacetoxy [[[1,2-dihydro-9,1-(epithiomethano)-7-fluoro-5-oxo-5*H*-oxazolo[3,2-*a*]quinolin-4-yl]carbonyl]oxy]borane (13). The mixture described above (**8** and **9**) (500 mg, 1.47 mmol) was added to a suspension of triacetoxyborane (420 mg, 2.23 mmol) in acetic anhydride (6 mL), and the mixture was stirred at room temperature for 68 h. After addition of isopropyl ether (6 mL) to the mixture, the resulting precipitate was collected by filtration and washed with isopropyl ether to give the crude crystals of **13**. The filtrate was concentrated in vacuo, and the residue was washed with isopropyl ether to give the precipitate (189 mg). The mixture of this precipitate and 1-methylpiperazine (0.15 mL, 1.35 mmol) in DMSO (1.9 mL) was stirred at room temperature for 67 h, and then the solvent was evaporated in vacuo. HCl (2 N, 2 mL) was added to the residue, and the mixture was stirred at room temperature for 1 h. The solvent was

Scheme III[a]



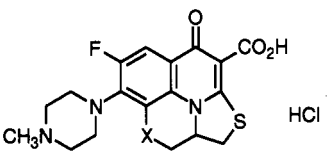
[a] (a) *N*-(Benzyloxycarbonyl)-*N*-methyl-2,3-dibromopropylamine, K_2CO_3 in DMSO; (b) HBr/AcOH; (c) Et_3N; (d) $B(OAc)_3$ in Ac_2O; (e) (i) 1-methylpiperazine, (ii) HCl.

Scheme IV[a]



[a] (a) Methyl 3,4-dibromobutylate, K_2CO_3 in DMF; (b) $B(OAc)_3$ in Ac_2O; (c) (i) 1-methylpiperazine, (ii) HCl; (d) (i) PPA, (ii) HCl.

Table II. In Vitro Antibacterial Activity[a] (Minimum Inhibitory Concentration, μg/mL) of Tetracyclic Pyridone Carboxylic Acids **4a–c**, **10**, **1a**, **1b**, and **2a**



4a-c

		microorganism[b]								
		Gram-positive				Gram-negative				
compd	X	Sa(F)	Sa(I)	Se	Ef	Ec(N)	Ec(K)	Kp	Pa(I)	Pa(E)
4a[c]	0	0.10	0.10	0.20	0.78	0.05	0.025	0.05	1.56	1.56
4b	NCH_3	0.78	0.39	0.78	1.56	0.78	0.78	0.39	6.25	6.25
4c	C=O	0.78	0.78	3.13	3.13	0.78	0.39	0.78	6.25	3.13
10	S(3-O)	1.56	3.13	6.25	6.25	1.56	1.56	1.56	50	60
1a		0.10	0.10	0.20	0.39	0.10	0.10	0.10	0.78	0.78
1b		0.05	0.05	0.05	0.10	0.05	0.05	0.05	0.39	0.78
2a (OFL)		0.39	0.39	0.78	1.56	0.10	0.10	0.10	1.56	1.56

[a] All values were 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; Pa(I), *Pseudomonas aeruginosa* IFO 3445; Pa(E), *Pseudomonas aeruginosa* E-2. [c] Contaminated with a small amount (8% from NMR) of **10**.

evaporated in vacuo, and the residue was recrystallized from methanol to afford **4a** (7 mg, 11%) as colorless crystals. The above crude crystals of **13** were recrystallized twice from acetonitrile to give **13** (209 mg, 35%) as colorless crystals.

4a: mp >280 °C; ^{1}H NMR (D_2O) δ 2.99 (3 H, s), 3.2–3.3 (3 H, m), 3.4–3.8 (7 H, m), 4.15 (1 H, t, J = 11 Hz), 4.6–4.8 (1 H, m), 4.98 (1 H, dd, J = 3.5, 11 Hz), 7.01 (1 H, d, J = 12 Hz); IR (KBr): 1698, 1482 cm^{-1}. Anal. ($C_{18}H_{19}ClFN_3O_4S\cdot1.5H_2O$) C, H, N.

13: mp 253 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.90 (3 H, s), 1.91 (3 H, s), 3.59 (1 H, dd, J = 10.5, 13 Hz), 3.88 (1 H, dd, J = 2.5, 13 Hz), 4.94 (1 H, dd, J = 9, 11.5 Hz), 5.32 (1 H, m), 5.52 (1 H, t, J = 9 Hz), 8.04 (1 H, dd, J = 8, 10 Hz). Anal. ($C_{17}H_{12}BF_2NO_8S\cdot0.5H_2O$) C, H, N.

1,2-Dihydro-9,1-(epithiomethano)-7-fluoro-5-oxo-5*H*-oxazolo[3,2-*a*]quinoline-4-carboxylic Acid Hydrochloride (10). A mixture of **13** (300 mg, 0.68 mmol) and 1-methylpiperazine (0.25 mL, 2.25 mmol) in DMSO (3 mL) was stirred at room temperature for 2 h, and then the solvent was evaporated in vacuo. HCl 2 N, 4 mL) was added to the residue, and the mixture was stirred at room temperature for 1 h. The resulting precipitate was collected by filtration and recrystallized from 1 N HCl to afford **10** (72 mg, 24%) as colorless crystals: mp >280 °C; ^{1}H NMR (D_2O) δ 3.00 (3 H, s), 3.2–3.7 (7 H, m), 4.66 (1 H, dd, J = 8.5, 11.5 Hz), 4.8–5.0 (1 H, m), 5.34 (1H, t, J = 8.5 Hz), 7.26 (1 H, d, J = 11.5 Hz); IR (KBr) 1714, 1511 cm^{-1}. Anal. ($C_{18}H_{19}ClFN_3O_4S\cdot1.5H_2O$) C, H, N.

Ethyl 1-[[*N*-(Benzyloxycarbonyl)-*N*-methylamino]-methyl]-1,2-dihydro-5-oxo-7,8,9-trifluoro-5*H*-thiazolo[3,2-*a*]-quinoline-4-carboxylate (14). A mixture of ethyl 4-hydroxy-6,7,8-trifluoro-2-mercaptoquinoline-3-carboxylate (**6**) (1.20 g, 3.96 mmol), *N*-(benzyloxycarbonyl)-*N*-methyl-2,3-dibromopropylamine (1.80 g, 4.93 mmol), and K_2CO_3 (2.70 g, 19.5 mmol) in DMF (15 mL) was stirred at 50 °C for 3 h, and then the solvent was evaporated in vacuo. The residue was added to water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water and dried over Na_2SO_4. Evaporation of solvents in vacuo, purification of the residue by silica gel column chromatography (silica gel 60, 230–400 mesh, Merck; $CHCl_3$ as an eluent), and recrystallization from EtOAc/cyclohexane gave **14** (1.10 g, 55%) as colorless crystals: mp 152.5–153.0 °C; ^{1}H NMR ($CDCl_3$) δ 1.43

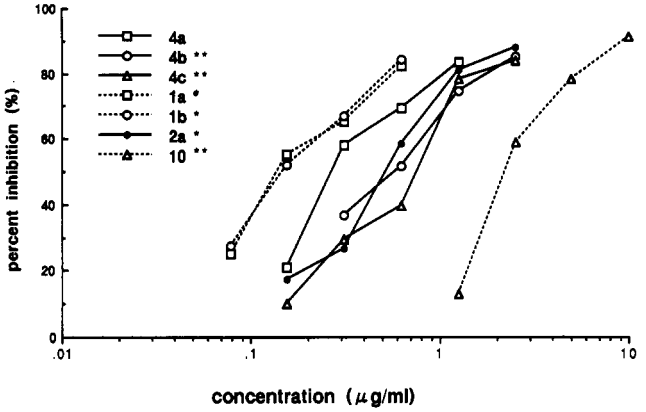


Figure 1. Inhibitory patterns of **4a–c**, **10**, **1a**, **1b**, and **2a** against the supercoiling activity of *E. coli* KL-16 DNA gyrase: differences between **4a** and other compounds. * $p < 0.05$, ** $P < 0.01$ vs **4a**.

Table III. Inhibitory Effects (IC_{50})[a] of **4a–c**, **10**, **1a**, **1b**, and **2a** on Supercoiling Activity of DNA Gyrase from *E. coli* KL-16 and in Vitro Antibacterial Activity (MIC)[b] against the Same Strain

compd	IC_{50}, μg/mL	MIC, μg/mL	compd	IC_{50}, μg/mL	MIC, μg/mL
4a	0.33	0.05	**1a**	0.14	0.05
4b	0.53	0.78	**1b**	0.16	0.05
4c	0.67	0.39	**2a**	0.53	0.10
10	2.61	1.56			

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

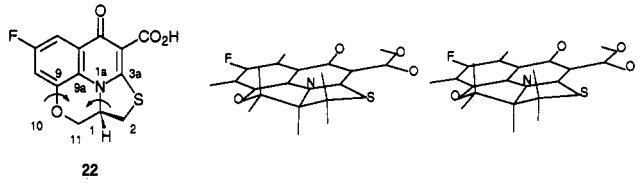
(3 H, t, J = 7 Hz), 3.7–3.9 (1 H, m), 4.43 (2 H, q, J = 7 Hz), 4.9–5.3 (2 H, m), 5.7–5.9 (1 H, m), 7.3–7.4 (5 H, m), 8.08 (1 H, ddd, J = 2, 9, 10 Hz). Anal. ($C_{24}H_{21}F_3N_2O_5S$) C, H, N.

Ethyl 7,8-Difluoro-1,2-dihydro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (16). A mixture of **14** (1.00 g, 1.98 mmol) and 30% HBr/AcOH (10 mL) was stirred at room temperature for 2 h. The solvent was evaporated in vacuo, and the residue was washed with methanol to give the crystals (450 mg). Triethylamine (0.55 mL, 3.95 mmol) was added to a solution of these crystals in CH_3CN (20 mL), and the mixture was stirred at 70 °C for 18 h. The mixture was diluted with $CHCl_3$, washed with dilute HCl and brine, and dried over $MgSO_4$. The solvent was evaporated in vacuo, and the residue was recrystallized from CH_3CN/CH_3OH to afford **16** (200 mg, 28%) as colorless crystals: mp >280 °C; ^1H NMR ($CDCl_3$) δ 1.43 (3 H, t, J = 7 Hz), 3.15 (1 H, dd, J = 11, 12.5 Hz), 3.19 (3 H, d, J = 3 Hz), 3.36 (1 H, dd, J = 7, 11 Hz), 3.44 (1 H, dd, J = 10, 13 Hz), 3.58 (1 H, dd, J = 3.5, 13 Hz), 4.40 (2 H, q, J = 7 Hz), 4.68 (1 H, m), 7.58 (1 H, dd, J = 8, 10.5 Hz). Anal. ($C_{16}H_{14}F_2N_2O_3S$) C, H, N.

Diacetoxy[[7,8-difluoro-1,2-dihydro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl]carbonyl]oxy]borane (17). A mixture of **16** (1.00 g, 2.84 mmol) and triacetoxyborane (0.80 g, 4.26 mmol) in acetic anhydride (20 mL) was stirred at 70 °C for 1 h. The solvent was evaporated in vacuo, and the residue was washed with isopropyl ether (50 mL) to give **17** (1.10 g, 85%) as colorless crystals: mp 269 °C dec; ^1H NMR (DMSO-d_6) δ 1.90 (6 H, s), 3.21 (3 H, d, J = 4 Hz), 3.54 (1 H, dd, J = 11.5, 13.5 Hz), 3.67 (1 H, dd, J = 10.5, 13 Hz), 3.8–3.9 (2 H, m), 5.2–5.4 (1 H, m), 7.59 (1 H, dd, J = 7.5, 10 Hz). Anal. ($C_{18}H_{15}BF_2N_2O_7S \cdot 1.5H_2O$) C, H, N.

1,2-Dihydro-7-fluoro-9,1-[(*N*-methylimino)methano]-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid Hydrochloride (4b). A mixture of **17** (230 mg, 0.509 mmol) and 1-methylpiperazine (0.23 mL, 2.07 mmol) in DMSO (2 mL) was stirred at 60 °C for 15 h. Ethanol (6 mL) was to the mixture, and the mixture was filtered. The filtrate was concentrated in vacuo, and 2 N HCl (1 mL) was added to the residue. The mixture was stirred at room temperature for 15 min. After addition of acetone (20 mL) to the mixture, the resulting precipitate was collected by filtration and recrystallized from methanol to afford **4b** (58 mg, 25%) as colorless crystals:

Chart II



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[a] The dihedral angles 1 (3a–1a–1–2) and 2 (11–10–9–9a) of the stable conformation were 24° and 21°, respectively.

mp >280 °C dec; ^1H NMR (D_2O) δ 2.89 (3 H, s), 3.02 (3 H, s), 2.9–3.1 (2 H, m), 3.2–3.4 (3 H, m), 3.4–3.8 (7 H, m), 4.4–4.6 (1 H, m), 6.74 (1 H, d, J = 12 Hz); IR (KBr) 1689, 1473 cm^{-1}. Anal. ($C_{19}H_{22}ClFN_4O_3S \cdot 1.25H_2O$) C, H, N.

Ethyl 7,8-Difluoro-1,2-dihydro-1-[(methoxycarbonyl)methyl]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (19). A mixture of ethyl 6,7-difluoro-4-hydroxy-2-mercaptoquinoline-3-carboxylate (**18**) (3.00 g, 10.5 mmol), methyl 3,4-dibromobutylate (3.00 g, 11.5 mmol), and K_2CO_3 (4.38 g, 31.7 mmol) in DMF (30 mL) was stirred at 50–55 °C for 3 h. The solvent was evaporated in vacuo, and the residue was washed with H_2O and acetone to give **19** (1.91 g, 47%) as colorless crystals. **19** (recrystallized from methanol): mp 183.0–184.4 °C; ^1H NMR (DMSO-d_6) δ 1.28 (3 H, t, J = 7 Hz), 2.69 (1 H, dd, J = 2.5, 16.5 Hz), 2.88 (1 H, dd, J = 10, 16.5 Hz), 3.42 (1 H, d, J = 12 Hz), 3.62 (3 H, s), 3.71 (1 H, dd, J = 7.5, 12 Hz), 4.24 (2 H, q, J = 7 Hz), 5.6–5.7 (1 H, m), 7.94 (1 H, dd, J = 6.5, 12 Hz), 7.99 (1 H, dd, J = 9, 10.5 Hz). Anal. ($C_{17}H_{15}F_2NO_5S$) C, H, N.

Diacetoxy [[(7,8-difluoro-1,2-dihydro-1-[(methoxycarbonyl)methyl]-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl)carbonyl]oxy]borane (20). A mixture of **19** (1.81 g, 4.72 mmol) and triacetoxyborane (1.34 g, 7.12 mmol) in acetic anhydride (9 mL) was stirred at room temperature for 25 min and then at 45–50 °C for 135 min. After addition of ether (100 mL) to the mixture, the resulting precipitate was collected by filtration, washed with ether, and recrystallized from $CHCl_3$/ether to give **20** (1.55 g, 67%) as colorless crystals: mp 215 °C dec; ^1H NMR (DMSO-d_6) δ 1.90 (3 H, s), 1.91 (3 H, s), 2.82 (1 H, dd, J = 2.5, 16.5 Hz), 3.07 (1 H, dd, J = 9.5, 16.5 Hz), 3.62 (3 H, s), 3.72 (1 H, d, J = 12 Hz), 4.02 (1 H, dd, J = 8.5, 12 Hz), 6.05 (1 H, m), 8.33 (1 H, dd, J = 8.5, 10 Hz), 8.52 (1 H, dd, J = 6.5, 12 Hz). Anal. ($C_{19}H_{16}BF_2NO_9S \cdot 2H_2O$) C, H, N.

1,2-Dihydro-7-fluoro-1-[(methoxycarbonyl)methyl]-8-(4-methyl-1-piperazinyl)-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acid Hydrochloride (21). A mixture of **20** (500 mg, 1.03 mmol) and 1-methylpiperazine (0.49 mL, 4.42 mmol) in DMSO (4.4 mL) was stirred at room temperature for 75 min. The solvent was evaporated in vacuo, 2 N HCl (10 mL) was added to the residue, and the mixture was stirred at room temperature for 1 h. The resulting precipitate was collected by filtration and washed with 2 N HCl, water, and acetone to afford the hydrochloride of **21** (310 mg, 63%) as colorless crystals. **21** (recrystallized from water): mp 235 °C dec; ^1H NMR (D_2O) δ 2.75 (1 H, d, J = 16 Hz), 3.01 (1 H, dd, J = 9, 16 Hz), 3.06 (3 H, s), 3.3–3.5 (4 H, m), 3.53 (1 H, d, J = 12 Hz), 3.73 (3 H, s), 3.7–4.0 (5 H, m), 5.81 (1 H, m), 6.88 (1 H, d, J = 7 Hz), 7.37 (1 H, d, J = 13 Hz). Anal. ($C_{20}H_{24}ClFN_3O_5S \cdot 1H_2O$) C, H, N.

6-Fluoro-5-(4-methyl-1-piperazinyl)-4,8-dioxo-2,2a,3,4-tetrahydro-8*H*-benzo[*ij*]thiazolo[2,3,4-*de*]quinolizine-9-carboxylic Acid Hydrochloride (4c). A mixture of **21** (350 mg, 0.740 mmol) and polyphosphoric acid (3.5 g) was heated at 130 °C for 18 h. Water (12 mL) and 2 N HCl (4 mL) were added to the mixture, and the mixture was stirred at room temperature for 30 min. The resulting precipitate was collected by filtration, washed with 2 N HCl and acetone, and recrystallized from dilute HCl to give **4c** (84 mg, 25%) as colorless crystals: mp >280 °C dec; ^1H NMR (DMSO-d_6) δ 2.85 (3 H, s), 3.1–3.6 (9 H, m), 3.7–3.9 (2 H, m), 3.82 (1 H, dd, J = 7.5, 11.5 Hz), 5.07 (1 H, m), 8.08 (1 H, d, J = 13.5 Hz), 10.92 (1 H, br), 15.66 (1 H, s); IR (KBr) 1693, 1574, 1487 cm^{-1}. Anal. ($C_{19}H_{19}ClFN_3O_4S \cdot 0.5H_2O$) C, H, N.

In Vitro Antibacterial Activity. The MIC (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 Disk agar (Nissui;

Tokyo, Japan).[14] 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 Supercoiling Activity of DNA Gyrase Isolated from *E. coli* KL-16. This assay was carried out according to a method reported previously.[15] Statistical analysis of data was performed by means of a simple linear regression analysis of the inhibitory patterns of DNA gyrase according to a logistic model, with a comparison of regression intercepts of **4a** and other compounds. Planned pairwise comparisons were made by Student's *t* test.

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