Synthesis and Antibacterial Activity of Thiazolopyrazine-Incorporated Tetracyclic Quinolone Antibacterials

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A novel series of 8-substituted-9,1-[(*N*-methylimino)methano]-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]-quinoline-4-carboxylic acids **5a-q** having a unique thiazolopyrazine-incorporated tetracyclic structure were synthesized, and the *in vitro* and *in vivo* activities were determined against Gram-positive and Gram-negative bacteria. All compounds **5a-q** had more potent activity than ofloxacin (**6**), which is one of the most popular quinolones, against Gram-positive and Gram-negative bacteria. The 8-pyrrolidinyl, **5a-e**, and 8-morpholino, **5p**, derivatives showed the most potent activity against Gram-positive bacteria. It is also significant that these compounds, **5a-q**, showed more potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* isolates (MRSA) than ofloxacin (**6**). The combination of the morpholino group and this unique tetracyclic thiazolopyrazine skeleton contributes to the enhancement of the antibacterial activity against MRSA isolates. The *in vivo* antibacterial activities of these compounds, **5a-q**, were limited and depended on the structure of the 8-substituent. The 8-(4-alkyl-1-piperazinyl) derivatives **5g**, **5h**, **5j**, and **5n** provided good oral efficacy and exhibited more potent activity than ofloxacin (**6**) against the systematic infection with *S. aureus* IID 803 in mice.

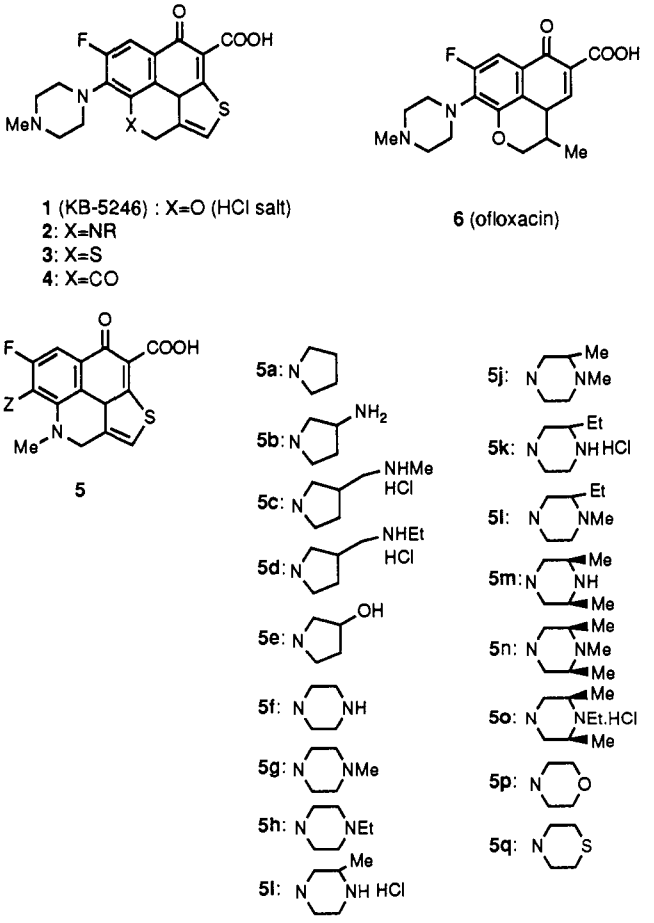
Introduction

Quinolone antibacterial agents such as norfloxacin, ofloxacin, and ciprofloxacin have been clinically used as effective drugs around the world because of their broad spectrum of activity against Gram-positive and Gram-negative bacteria. One of the most significant shortcomings of the quinolone antibacterial agents is a resistance against some clinically important pathogenic bacteria, i.e., methicillin-resistant *Staphylococcus aureus* (MRSA). It has been reported that norfloxacin, ciprofloxacin, and ofloxacin have already developed resistance to MRSA.[1] The need for a useful agent for the treatment of bacterial infections such as MRSA has become extremely important. During our study of quinolones, we were interested in the construction of a clinically useful compound for the treatment of these types of infections.

Recently, we reported the synthesis and the antibacterial activity of a series of thiazolooxazine-incorporated tetracyclic pyridonecarboxylic acids, in which the 8-(4-methyl-1-piperazinyl) derivative **1** (KB-5246) showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria.[2] Along with this study, and in regard to the structure-activity relationship of the tetracyclic quinolones, we have recently reported that the replacement of an oxygen atom of **1** by a nitrogen atom in **2**, a sulfur atom in **3**, or a carbonyl group in **4** and compound **2** (X = NMe; **5g**) provided compounds with the most potent antibacterial activity against both Gram-positive and Gram-negative bacteria.[3,4] We next focused on the structure of compound **2** and synthesized a series of 8-substituted-9,1-[(*N*-methylimino)methano]-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acids **5**. This paper describes the synthesis and the antibacterial activity of a new type of tetracyclic quinolone, **5**, including its potency against MRSA.

Chemistry The synthetic routes leading to the 8-substituted tetracyclic pyridonecarboxylic acids **5a-q** are summarized in Scheme 1. The important intermediates for **5a-q** are the borate complex **14** or the acid **15**. Namely, dithiocarbamate **7** was reacted with 1,3-dichloroacetone in ethyl acetate followed by cyclization under acidic conditions to give **9**, which was next converted to the tricyclic compound **10** by reaction with aqueous methy-

Chart 1



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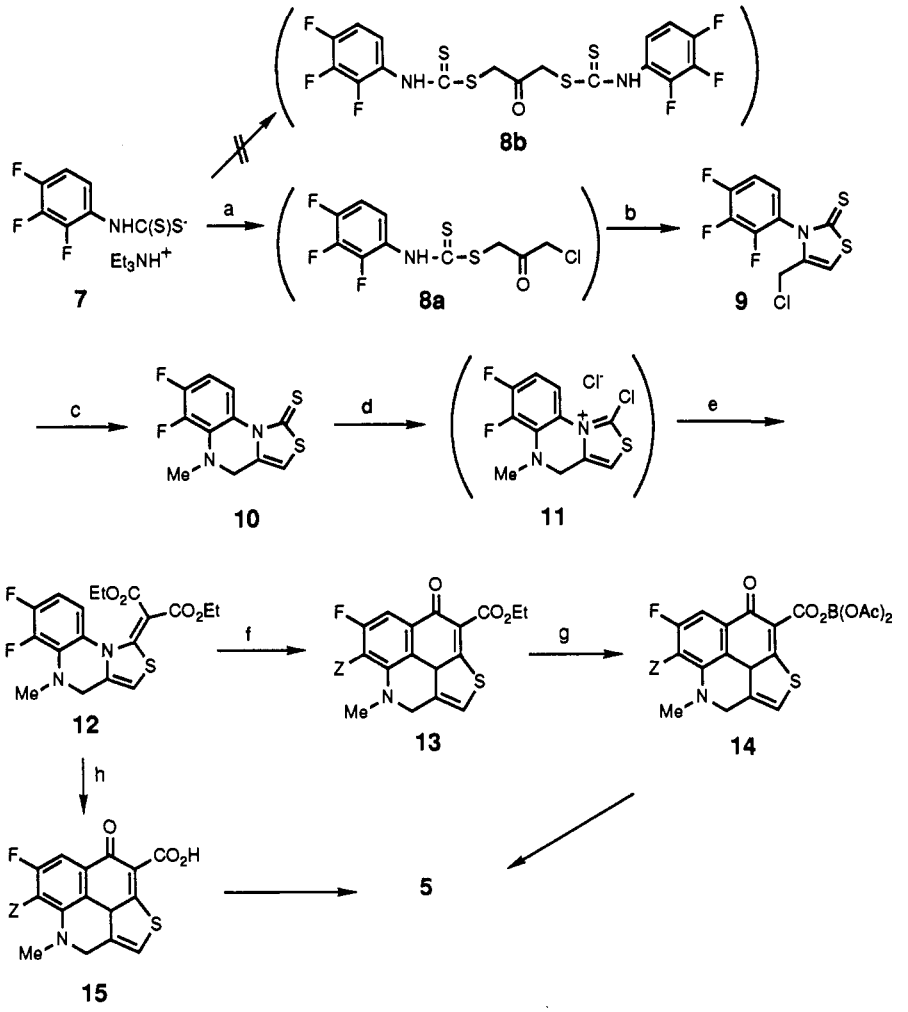
Scheme 1[a]



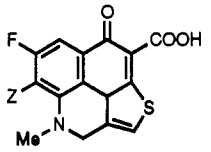
[a] (a) $ClCH_2COCH_2Cl$/AcOEt; (b) HCl/AcOEt; (c) aqueous CH_3NH_2/CH_3CN; (d) $ClCO_2CCl_3$/toluene; (e) $CH_2(CO_2Et)_2/NEt_3/CH_3CN$; (f) PPA; (g) $B(OAc)_3/Ac_2O$; (h) fuming H_2SO_4.

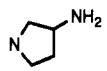
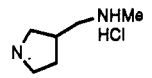
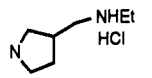
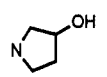
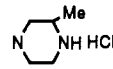
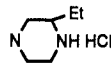
lamine in acetonitrile. The reaction of **7** with 1,3-dichloroacetone leading to the intermediate **8a** seemed to proceed via a 1:1 charge-transfer complex of **7** and 1,3-dichloroacetone. The color of the reaction solution became dark at the moment of the addition of **7** to the solution of 1,3-dichloroacetone in ethyl acetate and finally changed to a pale yellow solution. The formation of the 1:1 charge-transfer complex might contribute to the exclusive production of the intermediate **8a** without the dimer **8b**. The treatment of compound **10** with trichloromethyl chloroformate in toluene yielded the iminium chloride **11** as a moisture-sensitive precipitate, which afforded **12** by the reaction with diethyl malonate/triethylamine in acetonitrile. Compound **12** was obtained by cyclization in polyphosphoric acid to afford the pyridonecarboxylic acid ester **13**, which reacted with triacetoxyborane in acetic anhydride to produce the borate complex **14**. Compound **15** was obtained directly from **12** by the treatment of **12** with fuming sulfuric acid. The desired 9,1-[(*N*-methylimino)methano]-7-fluoro-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic acids **5** were synthesized by the replacement of the 8-fluorine atom of **14** or **15** with the appropriate nucleophiles. The *N*-methyl derivatives **5j** and **5l** were prepared by the reductive methylation of the corresponding **5i** and **5k**, respectively, using formalin and formic acid. The *N*-alkyl derivatives **5n** and **5o** were prepared by the *N*-alkylation of compound **5m** with the alkyl halide in the presence of K_2CO_3 in DMF followed by the alkaline hydrolysis of the corresponding esters. Compound **5b** was prepared by the alkaline hydrolysis of its *N*-acetyl derivative. All compounds **5a–q** are listed in Table 1.

Results and Discussion

The *in vitro* antibacterial activity of **5a–q** against five Gram-positive bacteria and five Gram-negative bacteria is shown in Table 2. Data for **1** (KB-5246) and **6** (ofloxacin) are included for comparison purposes. The introduction of functional groups such as an amino or hydroxyl group on the pyrrolidinyl moiety enhanced the antibacterial activity against Gram-negative bacteria (except for *Pseudomonas aeruginosa*) compared to the unsubstituted pyrrolidinyl compound **5a**. Especially, compound **5b** having an amino group in the pyrrolidinyl ring showed (4–16)-fold more potent activity than compound **5a** against Gram-negative bacteria, except for *P. aeruginosa*. The effect of the introduction of an amino group into the pyrrolidinyl ring for antibacterial activity was quite different from that realized in the case of the thiazolooxazine-incorporated tetracyclic pyridonecarboxylic acid system such as **1**. In the latter case, such a modification significantly affected the activity against Gram-positive bacteria, being (4–16)-fold less potent than that of the unsubstituted pyrrolidinyl derivative, while against Gram-negative bacteria, no change or only a slight increase in activity was realized.[2] All pyrrolidinyl derivatives **5a–e**

Table 1. Tetracyclic Pyridonecarboxylic Acids 5a–q



compd	Z	method[a]	yield, %[b]	mp, °C	recrst solvent	formula[c]
5a		A	54	275 dec	DMSO	$C_{18}H_{16}N_3O_3SF$
5b		B	55[d]	230 dec	none[e]	$C_{18}H_{17}N_4O_3SF \cdot 0.5H_2O$[f]
5c		C	57	250 dec	DMF	$C_{20}H_{21}N_4O_3SF \cdot HCl \cdot 1.5H_2O$
5d		C	44	254 dec	DMF	$C_{21}H_{23}N_4O_3SF \cdot HCl \cdot H_2O$
5e		A	50	265 dec	DMSO	$C_{18}H_{16}N_3O_4SF$
5f		A	78	260 dec	$CHCl_3$/EtOH	$C_{18}H_{17}N_4O_3SF \cdot 0.25H_2O$
5g		D	56	257 dec	$CHCl_3$/EtOH	$C_{19}H_{19}N_4O_3SF$
5h		A	75	276 dec	$CHCl_3$/EtOH	$C_{20}H_{21}N_4O_3SF$
5i		E	63	>280	dilute HCl	$C_{19}H_{19}N_4O_3SF \cdot HCl$
5j		F	32[g]	244 dec	EtOH	$C_{20}H_{21}N_4O_3SF$
5k		E	34	>280	dilute HCl	$C_{20}H_{21}N_4O_3SF \cdot HCl \cdot 0.5H_2O$
5l		F	63[g]	240 dec	CH_3CN/EtOH	$C_{21}H_{23}N_4O_3SF$
5m		D	23	254 dec	$CHCl_3$/EtOH	$C_{20}H_{21}N_4O_3SF$
5n		G	15[h]	250 dec	CH_3CN/EtOH	$C_{21}H_{23}N_4O_3SF$
5o		G	58[h]	>280	dilute HCl	$C_{22}H_{25}N_4O_3SF \cdot HCl \cdot 1.75H_2O$
5p		H	56	265 dec	CH_3CN/EtOH	$C_{18}H_{16}N_3O_4SF$
5q		H	55	270 dec	$CHCl_3/CH_3OH$	$C_{18}H_{16}N_3O_3S_2F \cdot 0.75H_2O$

[a] See the Experimental Section. [b] Yields are those obtained from the replacement step to the final product isolation, including hydrolysis and the salt formation if applicable. [c] The analyses for C, H, and N were within ±0.4% of the theoretical values. [d] From the *N*-acetyl derivative of **5b**. [e] Purified by washing with water and ethanol. [f] N: calcd, 14.10; found, 13.61. [g] From compound **5i**. [h] From compound **5m**.

were much more active than reference compounds **1** and **6** against Gram-positive bacteria. They were comparable to compounds **1** and **6** against Gram-negative bacteria, except for *Klebsiella pneumoniae*, while compound **5b** was more active than the reference compounds **1** and **6**. It was found that the amino pyrrolidyl group enhanced the activity of the tetracyclic thiazolopyrazine skeleton to provide the most potent activity against Gram-positive and Gram-negative bacteria.

On the other hand, compounds **5f–o**, which have the piperazinyl group at the 8-position of compound **5**, indicate that alkylation or polyalkylation of the piperazine ring has no effect or a somewhat favorable effect on the activity against Gram-positive *S. aureus* compared to the unsubstituted piperazinyl derivative **5f**. This result was similar to that of the N1-cyclopropyl quinolonecarboxylic acid system.[5] Of the series of piperzinyl derivatives **5f–o**, the dimethyl derivative **5j** and the trimethyl derivative **5n** showed the best activity against *S. aureus*. Their activity was comparable to that of the pyrrolidinyl derivatives **5a–e**. On the activity against Gram-positive *Micrococcus luteus*, the same modifications of the piperazine ring had no effect or a somewhat unfavorable effect. Against Gram-positive *Staphylococcus epidermidis* and *Enterococcus faecalis* and Gram-negative bacteria, such modifications of the piperazinyl ring caused a rather deleterious influence

Table 2. *In Vitro* Antibacterial Activity (minimum inhibitory concentration, g/mL) of Tetracyclic Pyridonecarboxylic Acids **5a–q**

	microorganism[a]									
	Gram-positive					Gram-negative				
compd	Sa(F)	Sa(I)	Se	Ef	Ml	Ec(N)	Ec(K)	Kp	Pa(I)	Pa(E)
5a	0.0125	0.0125	0.025	0.10	0.39	0.20	0.20	0.20	0.78	0.39
5b	0.025	0.006	0.05	0.05	0.20	0.05	0.0125	0.025	0.20	0.39
5c	0.025	0.0125	0.05	0.05	0.20	0.10	0.05	0.025	0.78	0.78
5d	0.025	0.0125	0.05	0.05	0.20	0.10	0.10	0.05	1.56	1.56
5e	0.0125	0.0125	0.0125	0.05	0.39	0.10	0.10	0.10	0.78	0.78
5f	0.05	0.05	0.05	0.05	0.39	0.05	0.05	0.025	0.39	0.39
5g	0.05	0.05	0.05	0.10	0.39	0.05	0.05	0.05	0.39	0.78
5h	0.05	0.05	0.10	0.10	0.39	0.10	0.05	0.05	0.78	0.78
5i	0.05	0.05	0.10	0.10	0.39	0.05	0.05	0.10	0.78	0.39
5j	0.025	0.025	0.05	0.10	0.39	0.05	0.05	0.10	1.56	0.78
5k	0.05	0.05	0.10	0.20	0.39	0.10	0.10	0.20	1.56	1.56
5l	0.05	0.05	0.10	0.20	0.78	0.20	0.10	0.20	3.13	1.56
5m	0.05	0.05	0.10	0.20	0.39	0.10	0.05	0.10	1.56	1.56
5n	0.025	0.025	0.10	0.20	0.39	0.10	0.10	0.20	3.13	1.56
5o	0.05	0.05	0.20	0.39	0.78	0.20	0.20	0.39	3.13	3.13
5p	0.006	0.006	0.025	0.05	0.10	0.10	0.10	0.05	0.39	0.39
5q	0.025	0.0125	0.025	0.10	0.20	0.10	0.10	0.39	0.78	0.78
1 (KB-5246)	0.10	0.10	0.20	0.39	1.56	0.10	0.10	0.10	0.78	0.78
6 (ofloxacin)	0.39	0.39	0.78	1.56	3.13	0.10	0.10	0.10	1.56	1.56

[a] Microorganisms: Sa(F), *Staphylococcus aureus* FDA 209P JC-1; Sa(I), *Staphylococcus aureus* IID 803; Se, *Staphylococcus epidermidis* IAM 1296; Ef, *Enterococcus faecalis* IID 682; Ml, *Micrococcus luteus* ATCC 9341; Ec(N), *Escherichia coli* NIHJ JC-2; Ec(K), *Escherichia coli* KC-14; Kp, *Klebsiella pneumoniae* B54; Pa(I), *Pseudomonas aeruginosa* IFO 3445; Pa(E), *Pseudomonas aeruginosa* E-2.

on the activity, resembling the case of the N1-ethyl quinolonecarboxylic acid system.[5] However, the *N*-methylated derivative **5g** maintained substantially the same activity as that of the unsubstituted piperazinyl derivative **5f**. All piperazinyl derivatives **5f–o** were more active than compounds **1** and **6** against Gram-positive bacteria, while they were comparable to **1** and **6** against the Gram-negative ones. Compounds **5l**, **5n**, and **5o**, which are piperazinyl derivatives having the trialkyl chains, tended to be less active than the reference compounds **1** and **6**. From the data for **5b** and **5f**, the 3-aminopyrrolidinyl derivative **5b** tended to be somewhat more active than the piperazinyl derivative **5f** against both Gram-positive and Gram-negative bacteria, resembling the case of other pyridonecarboxylic acid systems.[6] It was of interest that the replacement of the piperazinyl nitrogen atom by an oxygen atom or a sulfur atom (compounds **5p** and **5q**) enhanced the activity against Gram-positive bacteria compared to that of **5f**. The morpholino derivative **5p** showed the most potent antibacterial activity against Gram-positive bacteria in the series **5a–q**. Fortunately, we found that the morpholino group was the most efficient as the 8-substituent of the thiazolopyrazine-incorporated tetracyclic pyridonecarboxylic acid system for enhancing the activity against Gram-positive bacteria. This is consistent with the effect seen in the case of the 8-methylated N1-cyclopropyl quinolonecarboxylic acid system.[7] Against Gram-negative bacteria, **5p** and **5q** were comparable to **5f** and reference compounds **1** and **6**.

The tetracyclic compounds **5a–q** were all much more active than ofloxacin (**6**) against Gram-positive bacteria, which indicated that the thiazolopyrazine-incorporated tetracyclic pyridonecarboxylic acid structure is one of the favorable pyridonecarboxylic acid frameworks against Gram-positive bacteria. To clarify the reason for enhancement of the activity of **5** against Gram-positive bacteria, the inhibition of the supercoiling activity of DNA gyrase from Gram-positive *M. luteus* was evaluated for **5g** and reference compound **6** with the same substituent. The inhibitory activity (IC_{50}) on DNA gyrase was as follows: **5g**, 10.3 μg/mL; **6**, 21.6 μg/mL. The inhibitory activity of **5g** on DNA gyrase was about 2-fold more potent than that of **6**, indicating that the large discrepancy between the activity of **5g** and **6** against Gram-positive bacteria might be explained in part by the different inhibitory activity of **5g** and **6** on DNA gyrase.

Table 3. *In Vitro* Antibacterial Activity (MIC, μg/mL) of **5** against 16 Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*[a]

compd	MIC range[b]	MIC_{50}[c]	MIC_{90}[d]
5a	0.05–6.25	6.25	6.25
5b	0.05–3.13	0.39	1.56
5c	0.05–1.56	0.39	0.39
5d	0.025–0.78	0.20	0.39
5e	≤0.006–0.78	0.20	0.39
5j	0.025–3.13	0.78	3.13
5n	0.0125–3.13	0.78	1.56
5p	≤0.006–0.39	0.20	0.20
6 (ofloxacin)	0.39–50	6.25	50

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

Recently, infections caused by MRSA strains have become a serious medical problem.[8] *In vitro* antibacterial activity of compounds **5a–q** against MRSA strains isolated clinically in 1988 and 1989 in Japan is shown in Table 3. Data for **6** (ofloxacin) are included for comparison purposes, showing that ofloxacin-resistant MRSA strains have emerged. All tetracyclic derivatives showed excellent activity against MRSA isolates including ofloxacin-resistant strains. The morpholino derivative **5p** showed the most potent antibacterial activity against MRSA isolates in the tetracyclic derivative series, indicating that the morpholino group is also an efficient group for increasing the activity against clinical MRSA isolates.

Oral efficacy of several compounds in the series **5a–q** on systemic infections in mice is shown in Table 4. Only four compounds (**5g**, **5h**, **5j**, and **5n**), which include the piperazinyl nitrogen atoms, showed good oral efficacy, indicating that there were structural restrictions on the 8-substituent of the thiazolopyrazine-incorporated tetracyclic pyridonecarboxylic acid system for producing the oral antibacterial activity.

In conclusion, the thiazolopyrazine-incorporated tetracyclic pyridonecarboxylic acid structure was one of the

Table 4. Oral Efficacy of 5 on Systemic Infections in Mice

compd	ED_{50}, mg/kg	
	S. aureus IID 803	*E. coli* KC-14
5a		>2
5b		>2
5c	>12	
5d	>12	
5e	>12	
5f		>2
5g	3	1
5h	3	1
5i		>2
5j	3	2
5m		>2
5n	2	2
5p	>12	
5q	>12	
6 (ofloxacin)	11	1

favorable pyridonecarboxylic acid frameworks against Gram-positive bacteria including MRSA strains. However, oral efficacy against experimental infections was restricted by the structure of the 8-position substituent on the tetracyclic quinolones. Further modification of the 8-substituent may result in improved oral efficacy. We are now investigating other morpholino derivatives as 8-substituents in our continuing search for quinolone antibacterials with advantages over those currently available.

Experimental Section

Melting points were determined on a Yamato capillary melting point apparatus, Model MP-21, or a Buchi capillary melting point apparatus, Model 535; all melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer, with TMS as an internal reference in a solution of $CDCl_3$ or DMSO-d_6. IR spectra were recorded with a Hitachi IR 270–50 infrared spectrometer. Elemental analyses were performed with a Yanagimoto CHN-CORDER MT-3 instrument, and all analytical values are within ±0.4% of the calculated theoretical values.

***In Vitro* Antibacterial Activity.** MICs were determined by the 2-fold agar dilution method recommended by the Japan Society of Chemotherapy using Sensitivity Disk Agar-N (SDA; Nissui Pharmaceutical, Tokyo, Japan). The overnight broth cultures of the bacterial strains were diluted with buffered saline containing 0.01% gelation to a final concentration of approximately 10^6 CFU/mL, and 1 loopful (5 μL/spot) of each bacterial suspension was inoculated onto an agar plate containing various concentrations of agents with an inoculator (Microplanter; Sakuma Seisakusho, Tokyo, Japan). The plates were incubated at 37 °C for 18 h, and the MIC was defined as the lowest concentration of agent at which visible bacterial growth was inhibited.[9]

Inhibitory Effect on DNA Gyrase Supercoiling Activity. DNA gyrase of *M. luteus* (8 U/μL) was obtained from a commercial source (Bethesda Research Laboratories, Gaitherberg, MD). The assay of inhibition of DNA supercoiling activity of DNA gyrase was performed as previously described.[10] Relaxed pBR322 was prepared by treatment with calf thymus topoisomerase I (Bethesda Research Laboratories, Gaitherberg, MD). Reactions of DNA gyrase and topoisomerase I were carried out as recommended by the manufacturer.

***In Vivo* Efficacy on Systemic Infections in Mice.** Mouse protection tests were performed against *S. aureus* IID 803 and *Escherichia coli* KC-14. Male ddY mice weighing 25 g (SLC Japan Inc., Sizuoka, Japan) were infected intraperitoneally with 0.5 mL of a bacterial suspension from an overnight culture (in brain heart infusion; Difco) diluted in 5% gastric mucin (Difco) to yield about from 35 to 500 times the 50% lethal dose; 1% aqueous gum arabic suspensions (**5a**, **5b**, **5e–h**, **5j**, **5m–q**, and **6**) or distilled water solutions (**5c**, **5d**, and **5i**) of test compounds were orally administrated 1 h after infection. Four groups of five mice each were treated with each test compound at different dose levels. The number of mice surviving at each dose was determined daily for 7 days, at which time the 50% effective dose (ED_{50}) was calculated by the Weil method.[11]

4-(Chloromethyl)-3-(2,3,4-trifluorophenyl)-2-(3*H*)-thiazolethione (9). Dithiocarbamate[2] **7** (176 g, 0.542 mol) was added gradually to an ice-cooled solution of 1,3-dichloro-2-propanone (68.6 g, 0.540 mol) in ethyl acetate (270 mL) at 10–20 °C, and the mixture was stirred at the same temperature for 0.5 h. Insoluble materials were filtered off, and the insoluble materials were washed with ethyl acetate (100 mL). The washings and a 4 N solution of hydrogen chloride in ethyl acetate (270 mL, 1.08 mol) were added to the filtrate containing compound **8**, and the mixture was stirred at room temperature for 3.5 h and then evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (1 L), washed with water and brine, dried over magnesium sulfate, and concentrated to the volume of about 0.3 L. Isopropyl ether (0.4 L) was added to the concentrated solution, and the precipitate was collected to give **9** (142 g, 92%) as colorless crystals. **9** (recrystallized from cyclohexane): mp 127–130 °C; ^{1}H NMR ($CDCl_3$) δ 4.10 (d, J = 13 Hz, 1 H), 4.25 (d, J = 13 Hz, 1 H), 6.80 (s, 1 H), 7.19 (m, 2 H). Anal. ($C_{10}H_5NS_2F_3Cl$) C, H, N.

6,7-Difluoro-5-methyl-1*H*,4*H*-thiazolo[3,4-*a*]quinoxaline-1-thione (10). A mixture of compound **9** (2.96 g, 10.0 mmol) and a 40% aqueous solution of methylamine (3.88 g, 50.0 mmol) in acetonitrile (9 mL) was refluxed for 18.5 h and then cooled with ice/water. The precipitates were collected and washed with acetonitrile/water (1/4 v/v) to give **10** (2.42 g, 89%) as yellow crystals. **10** (recrystallized from cyclohexane/ethyl acetate): mp 165–167 °C; ^{1}H NMR ($CDCl_3$) δ 2.96 (d, J = 2.5 Hz, 3 H), 3.96 (d, J = 1 Hz, 2 H), 6.42 (t, J = 1 Hz, 1 H), 6.94 (dt, J = 8, 9.5 Hz, 1 H), 9.34 (ddd, J = 2.5, 5, 9.5 Hz, 1 H). Anal. ($C_{11}H_8N_2S_2F_2$) C, H, N.

Diethyl (6,7-Difluoro-5-methyl-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (12). A mixture of compound **10** (18.0 g, 66.6 mmol) and trichloromethyl chloroformate (9.74 mL, 81.2 mmol) in dry toluene (110 mL) was stirred at 80 °C for 17 h to yield compound **11** as a precipitate, which was not isolated because of its moisture-sensitivity. The supernatant solution was removed by decantation, and the precipitate was washed with dry toluene by decantation. A solution of diethyl malonate (12.9 g, 80.5 mmol) in dry acetonitrile (60 mL) was added to the precipitate chilled in ice. Triethylamine (14.9 g, 147 mmol) was added gradually to the mixture. After being stirred at room temperature for 40 min, the mixture was evaported *in vacuo*. Water was added, and the products were extracted with $CHCl_3$. The extract was washed with water, dried over sodium sulfate, and evaporated *in vacuo*. The residue was washed with isopropyl ether to afford **12** (24.3 g, 92%) as yellow crystals. **12** (recrystallized from hexane/ethyl acetate): mp 146–148 °C; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 6 H), 3.09 (d, J = 4.5 Hz, 3 H), 3.95 (q, J = 7 Hz, 4 H), 3.99 (d, J = 1 Hz, 2 H), 6.50 (t, J = 1 Hz, 1 H), 6.78 (dt, J = 8, 9 Hz, 1 H), 7.32 (ddd, J = 2, 5, 9 Hz, 1 H). Anal. ($C_{18}H_{18}N_2O_4SF_2$) C, H, N.

Ethyl 7,8-Difluoro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylate (13). A mixture of compound **12** (222 g, 560 mmol) and polyphosphoric acid (1790 g) was stirred at 110–115 °C for 1 h and then poured onto ice. The precipitate was collected and washed with water to afford **13** (187 g, 95%) as pale yellow crystals. **13** (recrystallized from $CHCl_3$/ethanol): mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (t, J = 7 Hz, 3 H), 3.22 (d, J = 5.5 Hz, 3 H), 4.29 (q, J = 7 Hz, 2 H), 4.55 (d, J = 1 Hz, 2 H), 7.31 (t, J = 1 Hz, 1 H), 7.40 (dd, J = 7.5, 10.5 Hz, 1 H). Anal. ($C_{16}H_{12}N_2O_3SF_2 \cdot 0.25H_2O$) C, H, N.

Diacetoxy[[[7,8-difluoro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinolin-4-yl]carbonyl]oxy]borane (14). To a solution of triacetoxyborate in acetic anhydride, which was prepared by stirring the mixture of boric acid (49.7 g, 804 mmol) and acetic anhydride (555 g, 5.44 mol) at 80 °C for 1 h,[12] was added compound **12** (187 g, 534 mmol), and the mixture was stirred at 100 °C for 3 h and then left standing at room temperature overnight. The precipitates were collected and washed with acetic anhydride and isopropyl ether to afford **14** (234 g, 97%) as a pale yellow crystals. **14** (recrystallized from acetonitrile): mp >280 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.91 (s, 6 H), 3.32 (d, J = 6 Hz, 3 H), 4.82 (d, J = 1 Hz, 2 H), 7.63 (dd,

J = 7, 10 Hz, 1 H), 7.95 (t, J = 1 Hz, 1 H). Anal. ($C_{18}H_{13}N_2O_7SF_2B$) C, H, N.

7,8-Difluoro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (15). Compound 12 (20.0 g, 50.5 mmol) was added to ice-cooled fuming sulfuric acid (236 g), and the mixture was stirred at room temperature for 21 h and then poured onto ice. The precipitate was collected and washed with water to afford 15 (15.8 g, 97%) as crystals. 15 (recrystallized from DMSO): mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 3.28 (d, J = 6 Hz, 3 H), 4.65 (s, 2 H), 7.50 (dd, J = 7.5, 10 Hz, 1 H), 7.56 (s, 1 H), 15.61 (s, 1 H). Anal. ($C_{14}H_8N_2O_3SF_2$) C, H, N.

(Method A): 7-Fluoro-9,1-[(*N*-methylimino)methano]-5-oxo-8-(1-pyrrolidinyl)-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (5a). A mixture of compound 15 (300 mg, 0.93 mmol) and pyrrolidine (330 mg, 4.6 mmol) in DMSO (3 mL) was stirred at 90 °C for 8 h and then evaporated *in vacuo*. The residue was washed with DMSO, water, and ethanol and recrystallized from DMSO to afford 5a (190 mg, 54%) as pale yellow crystals.

By similar procedures, compounds 5e (50%), 5f (78%), and 5h (75%) were prepared.

(Method B): 8-(3-Amino-1-pyrrolidinyl)-7-fluoro-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (5b). A suspension of the *N*-acetyl derivative of 5b (200 mg, 0.46 mmol), which was prepared from compound 15 and 3-(acetylamino)pyrrolidine by procedures similar to that described in method A, in a 10% aqueous NaOH solution (10 mL) was refluxed for 12 h, and the mixture was adjusted to pH 8.0 with dilute HCl. The precipitate was collected and washed with water and ethanol to afford 5b (100 mg, 55%) as pale yellow crystals.

(Method C): 7-Fluoro-8-[3-[(methylamino)methyl]-1-pyrrolidinyl]-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid Hydrochloride (5c). A mixture of compound 15 (322 mg, 1.00 mmol), 3-[(methylamino)-methyl]pyrrolidine dihydrochloride (564 mg, 3.01 mmol), and triethylamine (810 mg, 8.00 mmol) in DMSO (3 mL) was stirred at 95 °C for 8 h and then cooled to room temperature. The insoluble material was collected, washed with DMSO, ethanol, and ether, and recrystallized from *N*,*N*-dimethylformamide to afford 5c (260 mg, 57%) as pale yellow crystals.

By similar procedures, compound 5d (44%) was prepared.

(Method D): 7-Fluoro-8-(4-methyl-1-piperazinyl)-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (5g). A mixture of compound 15 (300 mg, 0.93 mmol) and 1-methylpiperazine (470 mg, 4.69 mmol) in DMSO (3 mL) was stirred at 100 °C for 10 h and evaporated *in vacuo*, and the residue was washed with ethanol. Water (30 mL) and acetic acid (5 mL) were added to the residue, and the mixture was washed with $CHCl_3$ and then adjusted to pH 7.5 with a dilute aqueous NaOH solution. The precipitate was collected, washed with water and ethanol, and recrystallized from $CHCl_3$/ethanol to afford 5g (210 mg, 56%) as pale yellow crystals.

By similar procedures, compound 5m (23%; in this case, the pH value was adjusted to 9.0) was prepared.

(Method E): 7-Fluoro-8-(3-methyl-1-piperazinyl)-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid Hydrochloride (5i). A mixture of compound 14 (7.00 g, 15.5 mmol) and 2-methylpiperazine (3.70 g, 36.9 mmol) in DMSO (8 mL) was stirred at 80 °C for 4 h. The precipitates were collected and washed with acetonitrile and then added to ice/water (100 g). To the suspension was added concentrated HCl (8 mL), and the mixture was stirred at room temperature for 1.5 h. The insoluble material was collected and recrystallized from dilute HCl, affording 5i (4.31 g, 63%) as pale yellow crystals.

By a similar procedure, compound 5k (34%) was also prepared.

(Method F): 7-Fluoro-8-(3,4-dimethyl-1-piperazinyl)-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (5j). A mixture of compound 5i (3.00 g, 6.84 mmol), sodium formate (0.93 g, 13.7 mmol), formic acid (15 mL), and formalin (15 mL) was stirred for 80 °C for 14 h and then added to ice/water (500 g). The mixture was adjusted to pH 9.0 with dilute NaOH, and the precipitate was collected, washed with water, and dissolved in $CHCl_3$/methanol (4/1 v/v). The solution was washed with water and evaporated *in vacuo*, and the residue was recrystallized from ethanol to afford 5j (0.92 g, 32%) as pale yellow crystals.

By a similar procedure, compound 5l (63%) was also prepared from compound 5k.

(Method G): 7-Fluoro-8-(3,4,5-trimethyl-1-piperazinyl)-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (5n). A mixture of compound 5m (500 mg, 1.20 mmol), methyl iodide (430 mg, 3.03 mmol), and potassium carbonate (500 mg, 3.62 mmol) in *N*,*N*-dimethylformamide (20 mL) was stirred at room temperature for 23 h and then added to water (200 mL). The mixture was adjusted to pH 9.5 with dilute HCl and extracted with $CHCl_3$/methanol (10/1 v/v). The extract was washed with water and evaporated *in vacuo*, and to the residue was added a 5% aqueous KOH solution (6 mL), water (18 mL), and ethanol (12 mL). After being refluxed for 15 min with stirring, the mixture was adjusted to pH 9.5 with dilute HCl and extracted with $CHCl_3$/methanol (4/1 v/v). The extract was washed with water and evaporated *in vacuo*, and the residue was recrystallized from acetonitrile/ethanol to afford 5n (81 mg, 15%) as pale yellow crystals.

By similar procedures, the residue containing the free base of 5o was obtained from compound 5m and ethyl iodide, and then, compound 5o (58%) was prepared by recrystallization of the residue from dilute HCl.

(Method H): 7-Fluoro-8-morpholino-9,1-[(*N*-methylimino)methano]-5-oxo-5*H*-thiazolo[3,2-*a*]quinoline-4-carboxylic Acid (5p). A mixture of compound 14 (1.20 g, 2.67 mmol) and morpholine (0.70 g, 8.03 mmol) in DMSO (10 mL) was stirred at 90 °C for 2 h and then evaporated *in vacuo*. The residue was washed with acetone, and to the residue was added acetone (40 mL), 10% hydrochloric acid (10 mL), and acetonitrile (50 mL). After the mixture was stirred at room temperature for 30 min, water was added to the mixture. The insoluble material was collected, washed with water and ethanol, and recrystallized from acetonitrile/ethanol to afford 5p (582 mg, 56%) as yellow crystals.

By a similar procedure, compound 5a (55%) was also prepared.

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