Synthesis and Antibacterial Activities of Novel Oxazine and Thiazine Ring-Fused Tricyclic Quinolonecarboxylic Acids: 10-(Alicyclic Amino)-9-fluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acids and the Corresponding 1-Thia Congeners

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Received January 7, 1991

Several analogs **4** and **5** of Ofloxacin (**1**) which contain the oxazine and thiazine rings fused with a quinolone carboxylic acid moiety, respectively, were prepared and their *in vitro* and *in vivo* antibacterial activities were compared with those of **1** and its previously prepared 3-exo-methylene analogs **2** and **3**. Unlike **1**, **2**, and **3**, analogs **4** and **5** possess an antiaromatic oxazine and thiazine moiety and show markedly lower antibacterial activities. Alteration of their C-10 amino-substituent groups from piperazine to azetidine significantly improved the *in vitro* antibacterial activities, particularly in the case of the thiazine derivative **5**, but not the *in vivo* ones. The antibacterial activities of these three types of tricyclic quinolonecarboxylic acids are briefly discussed on the basis of the molecular properties revealed by molecular orbital calculation. The molecular dipole moment was suggested to be one possible factor controlling the binding affinity of these compounds with DNA gyrase.

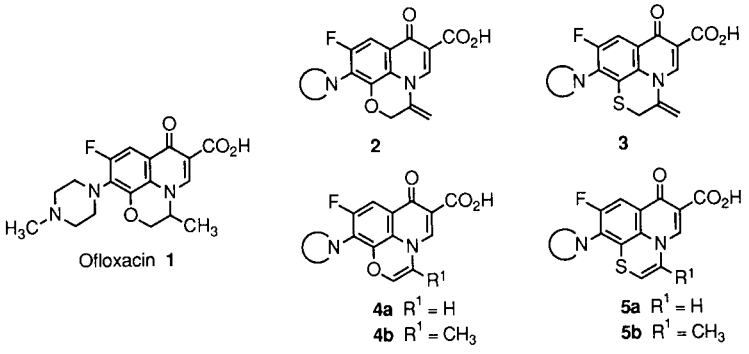
J. Heterocyclic Chem., **28**, 1067 (1991).

Our preceding paper reported the synthesis and antibacterial activities of the 3-exo-methylene analog **2** of the unique tricyclic quinolonecarboxylic acid Ofloxacin (**1**) and its thia-congener **3** [1]. Interestingly, these compounds showed almost the same or slightly decreased *in vitro* antibacterial activities as well as distinctly improved *in vivo* antibacterial activities compared to Ofloxacin, suggesting that their bioavailability might be superior to that of Ofloxacin. These results seem to support the idea that a partial chemical modification of the [2,3]dihydrooxazine ring of Ofloxacin may improve its antibacterial activity while maintaining its excellent pharmacodynamic characteristics. Thus, we were interested in examining the effects of the endo-cyclic double bond introduced into the dihydrooxazine and dihydrothiazine ring upon the antibacterial activity and pharmacological properties of Ofloxacin. As these analogs were predicted to show antiaromaticity at the newly formed oxazine or thiazine ring moiety unlike the previous **1**, **2**, and **3**, comparing the electronic effects on antibacterial activity should be of significant interest. This paper describes the convenient synthesis of 10-(alicyclic amino)-9-fluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acids **4** and its 1-thia congeners **5** as well as the evaluation of their antibacterial activities. The structure-activity relationships of all the oxazine and thiazine compounds having the exo-cyclic and the endo-cyclic double bond will also be discussed in terms of their structural features revealed by the molecular orbital calculation.

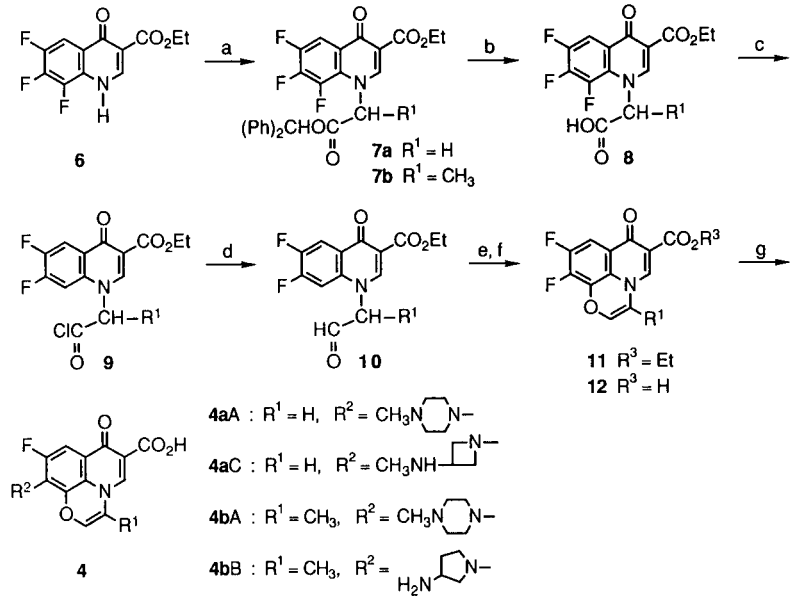
Results and Discussion.

Chemistry.

To synthesize the desired oxazine compounds **4** fused with the quinolone skeleton, we adopted the method shown in Scheme I, which involves the cyclization of enolates derived from ethyl *N*-(2-substituted-acetaldehyde-2-yl)quinolonecarboxylate **10** to the oxazine ring. During our work, a similar method was reported [2]. It differs from



Scheme I

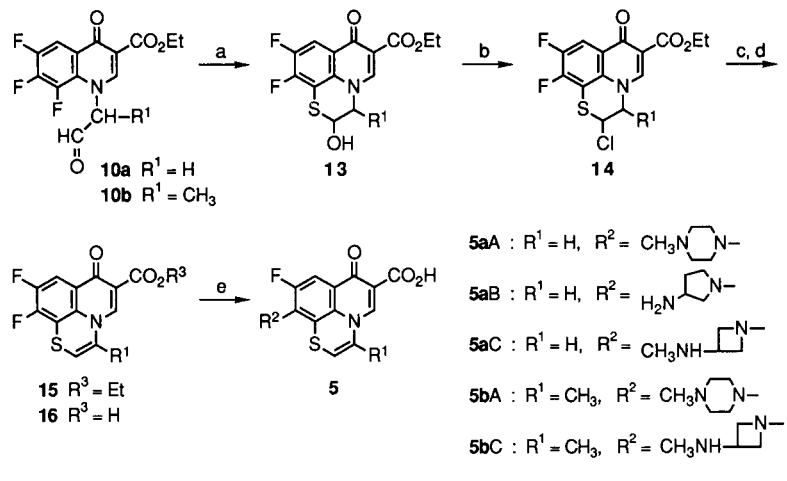


a) 1. NaH / DMF 2. $R^1CH(Br)CO_2CH(Ph)_2$ b) CF_3CO_2H c) $(COCl)_2$ d) Bu_3SnH
e) K_2CO_3 / DMF or NaH / DMF f) Dil. HCl (reflux) g) Cyclic amine-DBU

ours in that we introduced the aldehyde functions to the N-1 position after the quinolone skeleton had been constructed, thus making it easier to synthesize various 3-substituted analogs. The details of our method are as follows.

The starting material, ethyl 6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylate **6**, was easily prepared from 2,3,4-trifluoroaniline using a method similar to an earlier reported one [3]. It was converted into the *N*-alkylated products **7a** and **7b** in 86 and 57% yields, respectively, by treatment with benzhydryl bromoacetate or 2-bromo propionate in the presence of sodium hydride in *N*,*N*-dimethylformamide. In this reaction, a significant reactivity difference was observed between the two reagents, the former being more reactive than the latter and giving a higher yield of the desired product. The removal of the benzhydryl group by treatment with trifluoroacetic acid gave the free acids **8a** and **8b**, which were converted into acid chlorides **9a** and **9b** by treatment with oxalyl chloride in a usual way. This conversion, particularly for the former, required prior activation of the acid into a more reactive trimethylsilylester by treatment with bis-(trimeth-

Scheme II



a) NaSH / DMF b) $SOCl_2$ c) LiCl / DMF d) Dil. HCl e) Cyclic amine-DBU

ylsilyl)acetamide. The acid chlorides obtained were smoothly reduced to aldehydes **10a** and **10b** with tributyltin hydride in high yields [4]. Upon treatment with powdered potassium carbonate in dimethylformamide, intramolecular cyclization of the aldehyde **10b** proceeded smoothly *via* its enolate anion, producing the oxazine ring-fused tricyclic quinolone derivative **11b** in a yield of 85%. However, the same reaction with the unsubstituted derivative **10a** was very difficult, resulting in the formation of a complex mixture of products which could hardly be separated. Much effort was made to find the suitable conditions. The treatment of **10a** with sodium hydride at around 0° gave rise to the desired product **11a** but in a very poor yield of 10%. Finally, as described in our previous paper, the hydrolysis of esters **11a** and **11b** under acidic conditions, followed by incorporation of various cyclic amine substituents, such as *N*-methylpiperazine, 3-aminopyrrolidine, and 3-methylaminoazetidine, into C-10 gave the analogs **4aA** and **4bA**, **4bB**, and **4aC**, respectively. We confirmed that **4aA** was identical to that previously reported by the Bayer group [2].

Next, to prepare the hitherto unknown benzothiazine derivative **5** as shown in Scheme II, aldehydes **10a** and **10b** were treated with sodium hydrosulfide in dimethylformamide to produce the cyclized dihydrothiazine derivatives **13a** and **13b** in high yields. However, no dehydration of **13a** or **13b** occurred on treatment with concentrated sulfuric acid or *p*-toluenesulfonyl chloride in pyridine. The latter reaction did not even afford the tosylate. Thus, **13a** and **13b** were converted into the chlorides **14a** and **14b** by treatment with thionyl chloride and subjected to base-catalyzed E2-type dehydrochlorination using pyridine or DBU as a base. Again these attempts failed to give the desired products **15a** and **15b**. Consequently, the well known *cis*-elimination method using lithium chloride [5] was applied. When the reaction was done at between 110-140° for appropriate time intervals, it afforded the desired products **15a** and **15b** in good yields. The prolonged reaction caused cleavage of the ester function. Finally, after hydrolysis of the esters under acidic conditions, incorporation of the same three cyclic amino substituents into C-10 as done in the oxazine case gave compounds **5aA**, **5aB**, **5aC** and **5bC**. However, their yields were significantly lower than the oxazine cases, possibly because there was greater steric repulsion between the bulky sulfur atom and the C-10 substituents. Unfortunately, compound **5bA** desired for comparison of its antibacterial activity with those of Ofloxacin **1** and the exomethylene analogs **2** and **3** could not be isolated because of its poor yield, if any.

All the compounds thus far prepared were subjected to biological assays for their antibacterial activity.

Biological Results.

Table I summarizes the *in vitro* antibacterial activities of Ofloxacin **1** and its structural analogs, **4** and **5**, respectively, against four Gram-positive and six Gram-negative bacteria. Table II summarizes their values of the median effective doses (ED_{50}) by the oral route, which were determined for acute lethal infection in mice. Surprisingly,

Table I

In Vitro Antibacterial Activity: MIC μg/ml[a]

Compound	Organism Sa(S)[b]	Sa(R)[c]	Sp[d]	Sn[e]	Ec(S)[f]	Ec(R)[g]	Kp[h]	Pv[i]	Ecl[j]	Pa[k]
Ofloxacin 1	0.4	0.4	1.6	1.6	0.1	0.8	0.1	0.1	0.2	1.6
4aA	1.6	3.1	12.5	25	0.2	6.3	0.2	0.4	0.4	6.3
4aC	0.8	1.6	3.1	6.3	0.2	3.1	0.2	0.2	0.4	3.1
4bA	6.3	6.3	12.5	12.5	0.4	12.5	0.8	0.4	0.8	25
4bB	0.8	3.1	3.1	6.3	0.2	6.3	0.2	0.2	0.4	3.1
5aA	50	100	25	50	50	100	50	50	100	>100
5aB	3.1	12.5	12.5	12.5	1.6	6.3	0.8	1.6	1.6	12.5
5aC	0.2	0.4	3.1	3.1	0.05	1.6	0.05	0.1	0.1	1.6
5bC	0.2	0.2	3.1	3.1	0.1	0.8	0.1	0.1	0.1	1.6

[a] MIC (minimum inhibitory concentrations) were determined by the agar dilution method. Inoculation was performed with one loopful 10^6 cells per ml. [b] *Staphylococcus aureus* Smith. [c] *Staphylococcus aureus* C-14. [d] *Streptococcus pyogenes* C-203. [e] *Streptococcus pneumoniae* Type 1. [f] *Escherichia coli* EC-14. [g] *Escherichia coli* SR73. [h] *Klebsiella pneumoniae* SR1. [i] *Proteus vulgaris* CN-329. [j] *Enterobacter cloacae* SR233. [k] *Pseudomonas aeruginosa* SR24.

Table II

In vivo Antibacterial Activity: ED_{50} mg/kg [a]

	S. aureus Smith		*E. coli* EC-14	
Ofloxacin 1	8.68	(0.4) [b]	1.74	(0.1)
4aA	12.5	(1.6)	1.16	(0.2)
4aC	30	(0.8)	3.84	(0.2)
4bA	[c]	(6.3)	2.17	(0.4)
4bB	30	(0.8)	10	(0.2)
5aA	[c]	(50)	[c]	(50)
5aB	30	(3.1)	30	(1.6)
5aC	23.7	(0.2)	3.87	(0.05)
5bC	27.6	(0.2)	3.61	(0.1)

[a] Median effective dose. Compounds were administered orally 1 hour after intraperitoneal infection in mice. [b] MIC from Table I. [c] The Ed_{50} values of these compounds were not measured because MIC values were weak.

the oxazine analog **4bA** which most resembles Ofloxacin showed significantly lower *in vitro* antibacterial activities than Ofloxacin. Its *in vivo* activity against the Gram-negative microorganism *E. coli* EC-14 was also distinctly lower. Undoubtedly, introduction of an endocyclic double bond into the dihydrooxazine ring of Ofloxacin decreased its antibacterial activity. Furthermore, as shown by the activities of **4aA** and **4bA**, introduction of the methyl group at the C-3 position of the oxazine ring decreased both the *in vitro* and *in vivo* antibacterial activities. Given this observation and the assay data of **5aA**, the thiazine analog

5bA, though not prepared, was expected to have by far lesser activity than Ofloxacin and even the oxazine analog **4bA**. What was noteworthy were the effects of the C-10 substituents on the *in vitro* antibacterial activity. The replacement of *N*-methylpiperazine with sterically less hindered substituents, 3-aminopyrrolidine and 3-methylaminoazetidine, somewhat improved the MIC values of oxazine derivatives (See compounds **4aC** and **4bB**). This tendency was more striking with the thiazine compounds **5aA**, **5aB**, and **5aC**. The activities of **5aC** and **5bC** were even higher than Ofloxacin. However, the *in vivo* antibacterial activities were quite different and significantly lower than that of Ofloxacin. As the *in vivo* antibacterial activity closely reflects the bioavailability of the tested compounds in the body, these thiazine derivatives may have exhibited lower bioavailability than the corresponding oxazine derivative but nothing was clarified for the observed discrepancy between the *in vitro* and *in vivo* antibacterial activities. One possible reason is that these antiaromatic thiazine derivatives might be metabolically more succeptible to enzymatic oxidation or other types of degradation.

Structure-Activity Relationships.

Here, we will briefly discuss the structure-activity relationships of these three different types of compounds, namely Ofloxacin **1**, the previously described 3-methylene-[2,3]dihydrooxazine **2** and its thia congener **3**, and their counterparts **4** and **5** with the endo-cyclic double bond, on the basis of their structural characteristics revealed by molecular orbital calculation.

Molecular Modeling.

Modelings of tricyclic quinolone derivatives were performed using the SYBYL program package [6]. The initial structures for calculation were constructed by adding requisite substituents to the quinolone skeleton which was determined by our own X-ray crystal analysis [7]. To simplify calculations, all the C-10 substituents were replaced with the *N,N*-dimethylamino group. The conformations of these model compounds were optimized by using the molecular mechanics based on MAXIMIN II, and then the molecular orbital calculations were carried out by AM1 program with geometry optimization [8].

Table IV HOMO and LUMO Energy Levels and Dipole Moments of Ofloxacin Analogs

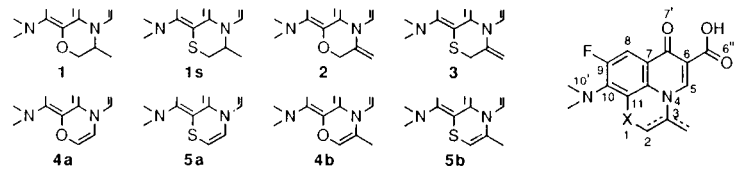
Compound Type	HOMO ev	LUMO ev	Dipole Moment (D)
1	-9.04583	-0.90903	8.437
1s	-9.00050	-1.04565	7.523
2	-9.04882	-1.07131	8.156
3	-9.05722	-1.12257	7.468
4a	-8.76422	-1.03887	8.294
5a	-8.63015	-1.20547	6.963
4b	-8.68878	-1.00052	8.429
5b	-8.55148	-1.14442	7.161

The calculated net charge on some representative atoms of the molecule as well as the energy levels of the HOMO and LUMO electrons and the molecular dipole moment were summarized in Table III and IV, respectively. Interestingly, in accordance with our prediction of the antiaromaticity with the oxazine and thiazine derivatives **4** and **5**, their HOMO and LUMO energy levels were specifically different from others. The HOMO energy levels were significantly higher than those of the other cases. Furthermore, their negative net charges on the N-4, O-1, and S-1 atoms were markedly lower than those of **1**, **2** and **3**, strongly suggesting a significant charge transfer from these atoms to the quinolone rings in order to decrease their antiaromaticity. As a result of this electron transfer, the calculated dipole moments of these antiaromatic molecules, for example, seem to be significantly lower than those of the other compounds, particularly with the thiazine compound **5**. The results of Table IV suggest that the

Table III Net Atomic Charges of Some Representative Atoms of Ofloxacin Analogs

Compound Type	Position						
	N-4	11	2	1	7'	6"	10'
1	-0.1793	0.0094	-0.0353	-0.1889	-0.3493	-0.3534	-0.2470
1s	-0.1852	-0.2423	-0.3200	0.2846	-0.3470	-0.3507	-0.2420
2	-0.1313	0.0061	0.0016	-0.1784	-0.3435	-0.3502	-0.2511
3	-0.1437	-0.2562	-0.2751	0.2811	-0.3442	-0.3488	-0.2519
4a	-0.1256	0.0095	-0.0261	-0.1289	-0.3451	-0.3517	-0.2516
5a	-0.1367	-0.2405	-0.3501	0.3939	-0.3407	-0.3451	-0.2432
4b	-0.1208	0.1909	-0.0316	-0.1265	-0.3479	-0.3533	-0.2480
5b	-0.1308	-0.2501	-0.3588	0.3919	-0.3441	-0.3499	-0.2482

Compound Types :



in vitro antibacterial activities show approximate correlation with the calculated dipole moments of these molecules, but not a good one with the oxazine case.

Here, we would like to briefly discuss how these electronic properties can affect the antibacterial activities of these molecules. For this purpose, we employ the attractive binding model of quinolone antibacterials with DNA gyrase proposed by Shen and his collaborators [9]. According to the model, the quinolone carboxylic acid molecules were divided into three different functional domains, those of hydrogen-bonding, drug-enzyme interaction, and drug-drug self-association as shown in Figure 1. As can be

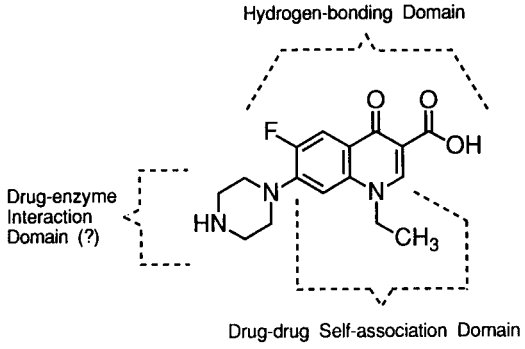


Figure 1

seen from Table III, our calculations revealed that the net electric charges of the atoms in these domains were mainly affected in the drug-drug association domain but not much in the hydrogen-bonding domain. Thus, the observed *in vitro* antibacterial activity may significantly reflect the degree of molecular association of each compound. If this assumption holds, the following explanation may be deduced for the observation that the molecules with the larger molecular dipole moment tend to show higher *in vitro* antibacterial activities. Namely. a significant electrostatic attraction force exists between the two counter-oriented dipoles and it may play an important role in stabilizing the drug association at the domain. Of course, other factors such as π-π stacking or tail-to-tail hydrophobic association forces, and steric repulsion may be very important for controlling the drug association. Particularly, as the HOMO energy levels of these compounds appear to approximately correlate with the *in vitro* antibacterial activities, π-π stacking of these drugs themselves or intermolecular charge transfer interactions between the drug and the enzyme may be more responsible for the changes of the *in vitro* antibacterial activity. Unfortunately, however, we can not properly estimate these factors at present. Also our calculations have not dealt with the effects of C-10 substituents despite their importance for the antibacterial activity. Thus, our present discussion on the structure-activity relationships may be oversimplified but our experimental data provide some useful information for estimating the validity of the fascinating binding models of Shen *et al.* and indicate a certain direction for studies on the modification of quinolone molecules to obtain better ones.

EXPERIMENTAL

Melting points were determined on a Yanagimoto hot-stage apparatus and were not corrected. Unless otherwise stated, the 90 MHz and 200 MHz ^{1}H nmr spectra were recorded for deuteriochloroform solutions on a Varian EM-390 and VXR-200, respectively, using tetramethylsilane (TMS) as an internal standard. For the solutions in deuterium oxide (D_2O), external TMS was used. Only representative proton signals were assigned. Column chromatography was performed on Merck Silica gel 60 (230-400 mesh or 70-230 mesh). Medium pressure liquid chromatographies were done with Merck 'Lobar' prepacked columns packed with Lichroprep SI 60. Organic extracts of reaction products were dried over anhydrous magnesium sulfate and solvents were evaporated under reduced pressure using a rotary evaporator.

Ethyl 1-(Benzhydryloxy carbonyl)methyl-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**7a**).

To a suspension of **6** (30.0 g, 110 mmoles) in DMF (400 ml) was added 60% sodium hydride oil dispersion (5.6 g, 140 mmoles) at room temperature and the mixture was stirred for 30 minutes. Benzhydryl bromacetate (60.0 g, 200 mmoles) was added to the reaction mixture and stirred for 3.0 hours. The reaction mixture was poured into water and extracted with dichloromethane. The dichloromethane layer was washed with water, dried, and concentrated. The resulting residue crystallized from dichloromethane-ether gave 47.0 g (86%) of **2a**, mp 173-174°; 90 MHz ^{1}H nmr: δ 1.32 (t, 3H, J = 7 Hz, CH_2CH_3), 4.33 (q, 2H, J = 7 Hz, CH_2CH_3), 5.03 (d, 2H, J = 6 Hz, N-CH_2), 6.92 (s, 1H, benzhydryl), 7.10-7.50 (m, 10H, aromatic *H*), 8.06 (ddd, 1H, J = 12, 10, 3 Hz, C_5-*H*), 8.23 (s, 1H, C_2-*H*).

Ethyl 1-[1-(Benzhydryloxy Carbonyl)ethyl]-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline Carboxylate (**7b**).

To a suspension of **6** (20.0 g, 74 mmoles) in DMF (200 ml) was added 60% sodium hydride oil dispersion (3.80 g, 95 mmoles) and this was stirred for 30 minutes at room temperature. Benzhydryl 2-brompropionate (40.0 g, 125 mmoles) was added to the reaction mixture and stirred for 5.0 hours at 90°. The reaction mixture was treated as described above. The resulting residue was crystallized from ether and collected to give 21.8 g (57%) of **2b**, mp 142-144°; 90 MHz ^{1}H nmr: δ 1.38 (t, 3H, J = 7 Hz), 1.95 (d, 3H, J = 7 Hz, $CHCH_3$), 4.37 (q, 3H, J = 7 Hz), 5.55 (m, 1H, $CHCH_3$), 6.93 (s, 1H), 7.10-7.45 (m, 10H), 8.15 (ddd, 1H, J = 13, 10, 3 Hz), 8.45 (s, 1H).

Ethyl 1-Carboxymethyl-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**8a**).

To a solution of **7a** (36.0 g, 73 mmoles) in dichloromethane (200 ml) was added trifluoroacetic acid (30 ml) and anisole (30 ml) and this was stirred for 1.0 hour at room temperature. The reaction mixture was concentrated and the residue after washing with ether gave 19.0 g (79%) of **8a**, mp 264-265°; 90 MHz ^{1}H nmr (DMSO-d_6): δ 1.29 (t, 3H, J = 7 Hz), 4.24 (q, 2H, J = 7 Hz), 5.26 (d, 2H, J = 6 Hz), 8.00 (ddd, 1H, J = 12, 10, 3 Hz), 8.71 (s, 1H).

Ethyl 1-[1-Carboxyethan-1-yl]-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**8b**).

The substrate **7b** was treated with trifluoroacetic acid as described above to obtain **8b** in quantitative yield, mp 170-172°; 90 MHz ^{1}H nmr (DMSO-d_6): δ 1.31 (t, 3H, J = 7 Hz), 1.78 (d, 3H, J = 7 Hz), 4.24 (q, 2H, J = 7 Hz), 5.68 (q, 1H, J = 7 Hz), 8.03 (ddd, 1H, J = 13, 10, 3 Hz), 8.70 (s, 1H).

Ethyl 1-Formylmethyl-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**10a**).

To a suspension of **8a** (10.0 g, 30 mmoles) in dichloromethane (100 ml) was added bis(trimethylsilyl)acetamide (15.0 g, 73 mmoles) and this was stirred for 10 minutes at room temperature. The reaction mixture was concentrated under a reduced pressure of 5 mm Hg at 80° and the resulting silyl ester was dissolved in dichloromethane (100 ml). To this solution oxalyl chloride (10.0 ml, 114 mmoles) was added dropwise. After stirring for 30 minutes at room temperature, the reaction mixture was concentrated and the residue washed with ether. The resulting crude acid chloride **9a** was dissolved in tetrahydrofuran (150 ml) and tributhyltin hydride (15 ml, 56 mmoles) was added at room temperature. After refluxing for 20 minutes, the reaction mixture was concentrated and the residue after washing with *n*-hexane gave 8.01 g (84%) of **10a** as a powder; 90 MHz ^{1}H nmr (DMSO-d_6): δ 1.27 (t, 3H, J = 7 Hz), 4.32 (q, 2H, J = 7 Hz), 5.49 (d, 2H, J = 7 Hz), 8.00 (ddd, 1H, J = 12, 10, 3 Hz), 8.57 (s, 1H), 9.77 (s, 1H, C*H*O).

Ethyl 1-[1-Formylethan-1-yl]-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**10b**).

To a suspension of **8b** (5.00 g, 15 mmoles) in dichloromethane (50 ml) oxalyl chloride (2.0 ml, 23 mmoles) was slowly added at room temperature. After stirring for 30 minutes, the reaction mixture was concentrated, tetrahydrofuran (50 ml) was added and the mixture was again concentrated to completely remove oxalyl chloride which was present in excess. The resulting crude acid chloride **9b** was dissolved in tetrahydrofuran (50 ml) and treated with tributhyltin hydride (10.0 g, 37 mmoles) at room temperature under stirring for 10 minutes. The reaction mixture was concentrated and the residue after washing with *n*-hexane gave 4.20 g (88%) of **10b** as a powder; 90 MHz ^{1}H nmr (DMSO-d_6): δ 1.30 (t, 3H, J = 7 Hz), 1.88 (dd, 3H, J = 7, 2 Hz, CHCH_3), 4.25 (q, 2H, J = 7 Hz), 5.61 (m, 1H), 8.00 (m, 1H), 8.60 (s, 1H), 9.75 (br s, 1H, C*H*O).

Ethyl 9,10-Difluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (**11a**).

To a solution of aldehyde **10a** (1.00 g, 3.2 mmoles) in DMF (30 ml) was added 60% sodium hydride in oil dispersion (144 mg, 3.6 mmoles) at 0° and this was stirred for 10 minutes at room temperature. The reaction mixture was poured into water and extracted with dichloromethane. The organic layer was washed with water, dried, and concentrated. The residue was chromatographed on silica gel and the fractions of the eluent chloroform-methanol (30:1) were collected and concentrated to give 95 mg (10%) of **11a**, mp 263-265°; 90 MHz ^{1}H nmr (DMSO-d_6): δ 1.37 (t, 3H, J = 7 Hz), 4.34 (q, 2H, J = 7 Hz), 6.15 (d, 1H, J = 5 Hz, C_3-*H*), 6.33 (d, 1H, J = 7 Hz, C_2-*H*), 7.66 (dd, 1H, J = 12, 9 Hz), 8.10 (s, 1H).

Ethyl 3-Methyl-9,10-difluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (**11b**).

To a solution of aldehyde **10b** (2.00 g, 6.1 mmoles) in DMF (30 ml) was added finely powdered potassium carbonate (600 mg, 4.3 mmoles) under vigorous stirring at room temperature. After 10 minutes, the reaction mixture was poured into water and extracted with dichloromethane. The organic layer was washed with water, dried, and concentrated. The residue was crystallized from ether and gave 1.62 g (85%) of **11b**, mp 267-269°; 90 MHz ^{1}H nmr (deuteriochloroform-tetradeuteriomethanol): δ 1.39 (t, 3H, J = 7 Hz), 2.11 (s, 3H, C_3-CH_3), 4.36 (q, 2H, J = 7 Hz), 6.38 (s, 1H, C_2-*H*), 7.67 (dd, 1H, J = 10, 8 Hz), 8.30 (s, 1H).

9,10-Difluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (**12a**).

A suspension of **11a** (240 mg, 0.82 mmole) in 6 *N* hydrochloric acid (3.0 ml) and ethanol (3.0 ml) was refluxed for 2.0 hours. The resulting crystals were collected and recrystallized from ether to give 220 mg of **12a** in a quantitative yield, mp 267-268°; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 6.99 (d, 1H, J = 5 Hz), 7.06 (d, 1H, J = 5 Hz), 7.94 (dd, J = 13, 10 Hz), 8.98 (s, 1H).

3-Methyl-9,10-difluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (**12b**).

Oxazine **11b** was treated as described above to obtain **12b** (97%), mp 269-273°; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 2.15 (s, 3H), 7.00 (s, 1H), 7.69 (dd, 1H, J = 11, 8 Hz), 8.39 (s, 1H).

10-[4-Methylpiperazin-1-yl]-9-fluoro-7-oxo-7*H*-pyrido[1,2,3-*de*]-[1,4]benzoxazine-6-carboxylic Acid Hydrochloride (**4aA**).

A mixed solution of **12a** (97 mg, 0.37 mmole) and *N*-methylpiperazine (82 μl, 0.74 mmoles) in DMSO (1.5 ml) was heated for 1.0 hour at 80°. The mixture was then concentrated and washed with methanol. The residue was dissolved in 1 *N* hydrochloric acid (0.4 ml)-methanol (1.0 ml) and heated at 60° for 10 minutes. After cooling, the precipitate was collected and gave 61 mg (43%) of **4aA** as yellow crystals, mp 290-295° dec; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 3.17 (s, 3H, N-CH_3), 3.3-4.1 (m, 8H, piperazine group), 7.00 (s, 2H, C_2-*H*, C_3-*H*), 7.87 (d, 1H, J = 14 Hz), 8.84 (s, 1H).

Anal. Calcd. for $C_{17}H_{17}N_3O_4FCl\cdot1.5H_2O$: C, 49.95; H, 4.93; N, 10.28; F, 4.65. Found: C, 49.64; H, 4.96; N, 10.26; F, 5.28.

10-[3-(Methylamino)azetidin-1-yl]-9-fluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (**4aC**).

A solution of **12a** (120 mg, 0.45 mmole), DBU (200 μl, 1.34 mmoles) and 3-methylaminoazetidine dihydrochloride (120 mg, 10.75 mmoles) in acetonitrile (0.7 ml) was heated in a sealed tube for 20 minutes at 80°. The precipitated crystals were collected by filtration and successively washed with acetonitrile, water, and methanol to give 140 mg (93%) of **4aC**, mp 260-265° dec as yellow crystals; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 3.01 (br s, 3H, NHCH_3), 4.3-5.2 (m, 5H, azetidine group), 6.70 (s, 2H, C_2-*H*, C_3-*H*), 7.68 (d, 1H, J = 14 Hz, C_8-*H*), 8.60 (s, 1H, C_5-*H*).

Anal. Calcd. for $C_{16}H_{14}N_3O_4F\cdot0.5H_2O$: C, 56.47; H, 4.44; N, 12.35; F, 5.58. Found: C, 56.80; H, 4.43; N, 12.42; F, 6.30.

10-[4-Methylpiperazin-1-yl]-9-fluoro-3-methyl-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid Hydrochloride (**4bA**).

Carboxylic acid **12b** was made to react with *N*-methylpiperazine in a usual manner to give **4bA** (57%), mp 290-293° dec as yellow crystals; 90 MHz ^{1}H nmr (deuterium oxide): δ 2.37 (s, 3H, C_3-C*H*$_3$), 3.50 (s, 3H, N-C*H*$_3$), 3.5-4.2 (m, 8H, piperazine group), 7.16 (s, 1H, C_2-*H*), 7.44 (d, 1H, J = 14 Hz, C_8-*H*), 8.17 (s, 1H, C_5-*H*).

Anal. Calcd. for $C_{18}H_{19}N_3O_4FCl\cdot H_2O$: C, 52.37; H, 4.88; N, 10.18; F, 4.60. Found: C, 52.59; H, 4.87; N, 10.27; F, 4.77.

10-(3-Aminopyrrolidin-1-yl)-9-fluoro-3-methyl-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid Hydrochloride (**4bB**).

A mixed solution of **12b** (240 mg, 0.85 mmole) and 3-(tritylamino)pyrrolidine (363 mg, 1.11 mmoles) in DMSO (4.0 ml) was heated at 80° for 1.0 hour. The reaction mixture was concentrated. The resulting residue was suspended in a mixture of 2 *N* hydrochloric acid (3.0 ml) and ethanol (3.0 ml) and heated at 70° for 30 minutes. After evaporation of the solvent, the residue was crystallized from methanol to give 110 mg (34%) of **4bB**, mp 280-283° dec as yellow crystals; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 2.27 (s, 3H, C*H*$_3$), 2.50 (m, 2H, pyrrolidine C_4-*H*$_2$), 4.0-4.5 (m, 5H, pyrrolidine group), 6.85 (s, 1H, C_2-*H*), 7.78 (d, 1H, J = 14 Hz, C_8-*H*), 8.60 (s, 1H, C_5-*H*).

Anal. Calcd. for $C_{17}H_{17}N_3O_4FCl\cdot 0.5H_2O$: C, 52.25; H, 4.64; N, 10.75; F, 4.86; Cl, 9.07. Found: C, 51.91; H, 4.51; N, 10.40; F, 5.06; Cl, 9.11.

Ethyl 9,10-Difluoro-7-oxo-2,3-dihydro-2-hydroxy-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate (**13a**).

To a solution of aldehyde **10a** (1.00 g, 3.2 mmoles) in DMF (10 ml) was added a solution of sodium hydrosulfide hydrate (400 mg) in DMF (2 ml) and the mixture was stirred at room temperature for 20 minutes. It was then poured into water and the crystalline precipitates were collected and washed with water to give 570 mg (55%) of **13a**, mp 265-267° dec; 90 MHz ^{1}H nmr (DMSO-d_6): δ 1.27 (t, 3H, J = 7 Hz), 4.24 (q, 2H, J = 7 Hz), 4.39 (d, 1H, J = 14 Hz, C_3-*H*), 4.79 (dd, 1H, J = 14, 4 Hz, C_3-*H*), 5.90 (m, 1H, C_2-*H*), 7.26 (d, 1H, J = 5 Hz, O*H*), 7.86 (dd, 1H, J = 13, 10 Hz), 8.66 (s, 1H).

Ethyl 9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-3-hydroxy-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate (**13b**).

Aldehyde **10b** was treated as described above to give (81%), mp 290-295° dec; 200 MHz ^{1}H nmr (DMSO-d_6): δ 1.30 (t, 3H, J = 7 Hz), 1.42 (d, 3H, J = 7 Hz, C_3-C*H*$_3$), 4.27 (q, 2H, J = 7 Hz), 5.21 (m, 1H, C_3-*H*), 6.50 (d, 1H, J = 3 Hz, C_2-*H*), 8.02 (dd, 1H, J = 11, 9 Hz), 8.85 (s, 1H).

Anal. Calcd. for $C_{15}H_{13}NO_4F_2S$: C, 52.78; H, 3.84; N, 4.10; S, 9.39; F, 11.13. Found: C, 52.54; H, 3.92; N, 4.02; S, 9.11; F, 10.92.

Ethyl 9,10-Difluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate (**15a**).

To a suspension of **13a** (1.30 g, 4.0 mmoles) in dichloromethane was added thionyl chloride (1.0 ml). After stirring at room temperature for 10 minutes, the reaction mixture was concentrated to obtain crude chlorothiazine **14a**. To a solution of the crude **14a** in DMF (25 ml) was added lithium chloride (1.5 g) and the mixture was heated at 110° for 3.0 hours under stirring. Next, the reaction mixture was poured into water. The precipitates were collected and chromatographed on a Lobar column (size B). The fractions eluted with chloroform-methanol (50:1) were collected and concentrated to obtain 440 mg (36%) of **15a**, mp 223-225°; 200 MHz ^{1}H nmr: δ 1.40 (t, 3H, J = 7 Hz), 4.38 (q, 2H, J = 7 Hz), 5.54 (d, 1H, J = 8 Hz, C_3-*H*), 6.51 (d, 1H, J = 8 Hz, C_2-*H*), 7.85 (dd, 1H, J = 10, 9 Hz), 8.15 (s, 1H).

Anal. Calcd. for $C_{14}H_9NO_3F_2S$: C, 54.37; H, 2.93; N, 4.54; S, 10.37; F, 12.29. Found: C, 54.09; H, 3.11; N, 4.55; S, 10.50; F, 12.72.

In this reaction, the thiazine carboxylic acid **16a** was obtained at the same time.

9,10-Difluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (**16a**).

A suspension of **15a** (320 mg, 1.14 mmoles) in a mixture of 6 *N* hydrochloric acid (3.0 ml) and ethanol (3.0 ml) was refluxed for 3.5 hours. The resulting crystals were collected to give 304 mg (100%) of **16a**, mp 285-287°; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 6.57 (d, 1H, J = 8 Hz, C_3-*H*), 7.29 (d, 1H, J = 8 Hz, C_2-*H*), 8.03 (dd, 1H, J = 9, 9 Hz), 9.01 (s, 1H).

Ethyl 9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-3-chloro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate (**14b**).

To a suspension of **13b** (380 mg, 1.10 mmoles) in dichloromethane (3.0 ml) was added thionyl chloride (0.4 ml) under ice cooling and this was stirred at room temperature for 20 minutes. The reaction mixture was concentrated to give crude chlorothiazine **14b**; 90 MHz ^{1}H nmr (DMSO-d_6): δ 1.30 (t, 3H, J = 7 Hz), 1.38 (d, 3H, J = 7 Hz, C_3-C*H*$_3$), 4.25 (q, 2H, J = 7 Hz), 5.20 (m, 1H, C_3-H), 6.47 (d, 1H, J = 4 Hz, C_2-*H*), 8.00 (dd, 1H, J = 11, 9 Hz), 8.85 (s, 1H).

9,10-Difluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (**16b**).

To a solution of **14b** in DMF (4.0 ml) was added lithium chloride (1.6 g) and this was stirred at 140° for 4.0 hours. After cooling, the crystalline precipitates were collected and washed with water to give 200 mg of **16b** (60% from **13b**), mp >310°; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 2.52 (s, 3H, C_3-C*H*$_3$), 6.57 (s, 1H, C_2-*H*), 8.11 (dd, 1H, J = 9, 9 Hz), 9.08 (s, 1H).

10-[4-Methylpiperazin-1-yl]-9-fluoro-7-oxo-7*H*-pyrido[1,2,3-*de*]-[1,4]benzothiazine-6-carboxylic Acid Hydrochloride (**5aA**).

A mixed solution of **16a** (140 mg, 0.50 mmole) and *N*-methylpiperazine (100 μl, 0.92 mmole) in acetonitrile (0.8 ml) was heated at 80° for 1.0 hour. The reaction mixture was concentrated and washed with ether. The residue was dissolved in 1 *N* hydrochloric acid (1.0 ml) and again concentrated. The obtained residue was crystallized from methanol to give 42 mg (20%) of **5aA**, mp 270-274° dec as yellow crystals; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 3.17 (br s, 3H, N-C*H*$_3$), 3.4-4.2 (m, 8H, piperazine group), 6.55 (d, 1H, J = 8 Hz, C_3-*H*), 7.24 (d, 1H, J = 8 Hz, C_2-*H*), 7.71 (d, 1H, J = 12 Hz), 8.88 (s, 1H).

Anal. Calcd. for $C_{17}H_{17}N_3O_3FCl\cdot H_2O$: C, 49.10; H, 4.61; N, 10.10; F, 4.57; S, 8.12. Found: C, 49.10; H, 4.41; N, 9.97; F, 4.51; S, 8.12.

10-[3-Aminopyrrolidin-1-yl]-9-fluoro-7-oxo-7*H*-pyrido[1,2,3-*de*]-[1,4]benzothiazine-6-carboxylic Acid Hydrochloride (**5aB**).

A mixed solution of **16a** (160 mg, 0.60 mmole) and 3-tritylaminopyrrolidine (242 mg, 0.75 mmole) in DMSO (3.0 ml) was heated at 80° for 2.0 hours. The reaction mixture was concentrated and the residue was washed with ether and methanol. The

residue was again dissolved in a mixture of 2 *N* hydrochloric acid (2.0 ml) and ethanol (2.0 ml) and heated at 70° for 1.0 hour. After the evaporation of the solvents, the residue was crystallized from methanol to give 113 mg (49%) of **5aB**, mp 290-293° dec as yellow crystals; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 2.60 (m, 2H, C_4-*H* of pyrrolidine), 4.0-4.6 (m, 5H, pyrrolidine group), 6.55 (d, 1H, J = 5 Hz, C_3-*H*), 6.60 (d, 1H, J = 5 Hz, C_2-*H*), 7.92 (d, 1H, J = 14 Hz), 8.66 (s, 1H).

Anal. Calcd. for $C_{16}H_{15}N_3O_3FClS\cdot0.7H_2O$: C, 48.48; H, 4.17; N, 10.60; F, 4.79; S, 8.57. Found: C, 48.60; H, 4.06; N, 10.38; F, 4.99; S, 8.57.

10-[3-Methylaminoazetidin-1-yl]-9-fluoro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (**5aC**).

A solution of **16a** (120 mg, 0.40 mmole), DBU (0.3 ml) and 3-methylaminoazetidine dihydrochloride (120 mg, 0.75 mmole) in acetonitrile (0.6 ml) was heated at 80° for 30 minutes in a sealed tube. The crystalline precipitates were collected by filtration and successively washed with acetonitrile, water and methanol to give 140 mg (94%) of **5aC**, mp 250-254° dec as yellow crystals; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 3.00 (br s, 3H, NH-CH_3), 4.3-5.2 (m, 5H, azetizine group), 6.34 (d, 1H, J = 8 Hz, C_3-H), 7.04 (d, 1H, J = 8 Hz, C_2-*H*), 7.77 (d, 1H, J = 13 Hz), 8.67 (s, 1H).

Anal. Calcd. for $C_{16}H_{14}N_3O_3FS\cdot0.5H_2O$: C, 53.93; H, 4.24; N, 11.79; F, 5.33; S, 9.00. Found: C, 54.14; H, 4.11; N, 11.68; F, 6.00; S, 9.01.

10-[3-Methylaminoazetidin-1-yl]-9-fluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (**5bC**).

A solution of **16b** (100 mg, 0.33 mmole) , DBU (0.2 ml) and 3-methylaminoazetidine dihydrochloride (100 mg, 0.62 mmole) in acetonitrile was heated at 90° for 1.5 hours in a sealed tube. The reaction mixture was concentrated and the residue washed with ether. The residue was crystallized from acetonitrile to give 65 mg (53%) of **5bC**, mp 210-213° dec as yellow crystals; 90 MHz ^{1}H nmr (trifluoroacetic acid): δ 2.40 (s, 3H, C_3-CH_3), 3.05 (br s, 3H, NH-CH_3), 4.3-5.2 (m, 5H, azetidine group), 6.40 (s, 1H, C_2-*H*), 7.89 (d, 1H, J = 14 Hz), 8.88 (s, 1H).

Anal. Calcd. for $C_{17}H_{16}N_3O_3FS\cdot H_2O$: C, 53.82; H, 4.78; N, 11.08; F, 5.01; S, 8.45. Found: C, 53.47; H, 4.63; N, 10.89; F, 5.76; S, 8.64.

Acknowledgments.

We thank Dr. H. Miwa and Messrs. Y. Kameda, and K. Motokawa for the microbiological testing and Dr. M. Yamakawa for his helpful suggestions on the analysis of the structure-activity relationships.

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