Quinolinecarboxylic Acids. 3. Synthesis and Antibacterial Evaluation of 2-Substituted-7-0x0-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acids Related to Rufloxacin

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A series of 2-substituted-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylic acids has been prepared and evaluated for in vitro antibacterial activity. These derivatives were less active than corresponding desmethylated analogues. Among these derivatives, the most active compound **22a** was selected for preliminary pharmacokinetics in rats. The pharmacokinetic data indicated that **22a** was rapidly absorbed and induced lasting plasma and urinary levels. In comparison with rufloxacin, it was excreted in low quantity in urine; a significant amount of desmethylated piperazinyl urinary metabolite was observed.

Among the host fluoroquinolone antibacterials, flumequine,¹ ibafloxacin,² and ofloxacin³ typify *ortho-peri* annellated compounds, characterized by a three-atom bridge between N-1 and C-8 position supporting a methyl group on the α -carbon at N-1.

We recently reported the synthesis and antibacterial activity of a series of 3-desmethylated pyridobenzothiazine quinolones, among these the 9-fluoro-10-(4-methyl-1piperazinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acid (rufloxacin) (1)⁴ was found to show in vivo potency upon oral administration on the same order as ciprofloxacin and ofloxacin. Rufloxacin demonstrates high bioavailability and long half-life (30-35 h).⁵ Rufloxacin has been launched as a once-daily antibacterial quinolone.

As a continuation of our research program on benzothiazine quinolones,^{4,6} we have now synthesized a novel series of 9-fluoro-10-substituted-7-oxo-2,3-dihydro-7*H*-pyrido-[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acids 8**a**-**d**, 21**a**-**34a**, 21**b**, and 22**b** in which a methyl or fluorophenyl group is linked at the 2 position of the 1,8 three-atom bridge, a position that has been rarely studied in this type of tricyclic quinolones.⁷

Chemistry

The synthesis of the target compounds first involved the preparation of the framework pyridobenzothiazines 7a-d which were obtained by a known route, by reaction of the suitable 1,4-benzothiazine 6a-d with diethyl ethoxymethylenemalonate (EMME) followed by polyphosphoric acid (PPA) cyclization (Scheme II).

Among the precursor 1,4-benzothiazines, 6a was synthesized by the reaction of 3-chloro-2,4-difluoronitrobenzene with thiolactic acid, reductive cyclization of intermediate 2 to lactam 5a, and successive reduction by LiAlH₄. On the other hand, 1,4-benzothiazine 6b-d were obtained in similar fashion starting from 2-chloro-3-fluoro-5-nitrothiophenol by reaction with α -bromofluorobenzeneacetic acids 3b-d (Scheme I).

Since we had previously found that the desired nucleophilic substitution of chlorine at C-10 in pyrido[1,2,3de][1,4]benzothiazine nucleus could only be obtained after oxidation of thiazinic sulfur to sulfoxide,⁴ compounds 7a-d were first oxidated with *m*-chloroperbenzoic acid (MCP-BA) and then submitted to regiospecific substitution with heterocyclic bases. The target compounds were finally obtained by successive deoxygenation with PBr₃ and hydrolysis (Scheme II).

N-Substituted piperazine derivatives **32a** and **33a** (Table III) were synthesized starting from **21a** by alkylation with suitable fluorobenzyl chlorides, while **34a** was synthesized by sulfonylation with 4-fluorobenzenesulfonyl chloride.

Results and Discussion

2-Substituted pyridobenzothiazine acids 8a-d, 21a-34a, and 21b-22b and rufloxacin (1), included for comparison, were tested for in vitro antibacterial activity against Grampositive (Streptococcus faecalis LEP Br and Staphylococcus aureus ATCC 6536) and Gram-negative bacteria (Proteus vulgaris CNUR 6, Enterobacter cloacae OMNFI 174, Shigella enteritidis, Klebsiella pneumoniae ATCC 10031, Pseudomonas aeruginosa ATCC 9027, and Escherichia coli ISF 432) by conventional agar dilution procedure. The minimal inhibitory concentrations (MICs), reported in Table IV, in general show that a methyl or a fluorophenyl group at C-2 in pyridobenzothiazine quinolones had an unfavorable influence on the in vitro activity; these derivatives were generally less effective than 2-unsubstituted analogues.⁴ For all tested compounds the decreased activity against Proteus and Pseudomonas bacteria is clear, while good activity was observed on E. coli which is particularly significant for 8a and 27a (MIC = 0.12 μ g/mL). The introduction of groups bulkier than methyl at N-4 piperazine of 21a, as 2-pyridyl (29a), 4-fluorophenyl (30a), 2-(trifluoromethyl)benzyl (31a), 3-fluorobenzyl (32a), 2.4-difluorobenzyl (33a), and 4-fluorobenzenesulfonyl (34a) resulted in substantial activity loss. Only 29a and 30a showed good activity against Grampositive bacteria; in particular 29a showed the best activity against Streptococcus and Staphylococcus (MIC = 2 and $0.5 \ \mu g/mL$, respectively) better than the control drug 1 (MIC = 16 and 1 μ g/mL).

Only compound 22a showed an activity which was on the same order as rufloxacin (1). Therefore, considering

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Scheme I^a



^a Reagents: (i) CH₃CH(SH)CO₂H, NaOH, DMSO, 80 °C; (ii) NaOH, EtOH, reflux; (iii) FeSO₄·7H₂O, NH₄OH; (iv) LiAlH₄, THF.

Scheme II^a



^a Reagents: (i) EMME, 150 °C; (ii) PPA, 160 °C; (iii) 10% NaOH, reflux; (iv) 55% MCPBA, EtOH; (v) cyclic amine, DMF, 100 °C; (vi) PBr₃, DMF.

the excellent pharmacokinetic characteristics of rufloxacin, preliminary pharmacokinetics for **22a** in rat were carried out.

The pharmacokinetic data concerning plasma levels and urinary excretion in rats of compound 22a and rufloxacin were evaluated after oral administration at 50 mg/kg (Table V). The data indicated that 22a was rapidly adsorbed and induced long-lasting plasma and urinary levels. Moreover 22a was actively metabolized as shown by the comparison with rufloxacin (1) regarding urinary recoveries of unmodified compounds and metabolites. In fact urinary excretion of unmodified 22a was significantly lower than the one of rufloxacin being 5.2% and 15.6% of the administered dose, respectively. In urine of rats treated with 22a, a greater amount of the metabolite, identified as desmethylated piperazinyl derivative 21a, was observed.

Experimental Section

Melting points were determined in capillary tubes (Buchi

 Table I.
 8-Chloro-7-fluoro-2-substituted-3-oxo-3,4-dihydro-2H-1,4-benzothiazine and 8-Chloro-7-fluoro-2-substituted-3,4-dihydro-2H-1,4-benzothiazine Derivatives

compd ^a	mp, °C	purification method ^b	% yield	formulac
5a	198-200	A	61	C ₉ H ₇ ClFNOS
5b	193-195	Α	36	C14H8CIF2NOS
5c	148-150	Α	76	C14H8ClF2NOS
5 d	234-235	Α	83	C14H8CIF2NOS
6 a	oil	В	46	C ₂ H ₂ ClFNS
6b	88-90	В	36	C14H10ClF2NS
6c	84-86	В	38	C14H10ClF2NS
6 d	82-84	В	40	C14H10ClF2NS

^a See Scheme I. ^b Solvent used for recrystallization or for silica gel column chromatography purification as follows: (A) recrystallization from EtOH and (B) elution with gradient of cyclohexane to 20% CHCl₃/cyclohexane. ^cC, H, N analyses were within $\pm 0.4\%$ of theoretical values.

melting point apparatus) and are uncorrected. Elemental analyses were performed on a Carlo Erba Model 1106 elemental

 Table II. Ethyl 2,10-Disubstituted-9-fluoro-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylate and Ethyl 2,10-Disubstituted-9-fluoro-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylate 1-Oxide Derivatives



compd	R	R1	n	mp, °C	purification method ^a	% yield	formula ^b
7a	CH ₃	Cl	0	1 99- 201	Α	74	C ₁₅ H ₁₃ ClFNO ₃ S
7b	4-FC ₆ H₄	Cl	0	240-242	В	91	C20H14ClF2NO3S
70	3-FC-H	Cl	Ó	260-262	B	90	Co-HuClFoNOoS
74	2.FC.H.	Či	ň	200 202 202 doc	B	00	C.H. CIF.NO.S
14	CU		1	292 000	БС	50	
98			L	202-204	č	60	C15H13CIFNO4S
9D	4-FC6H4		1	227-230	C	62	$C_{20}H_{14}CIF_2NO_4S$
10a	CH_3	-м мн	1	oil	D	72	C ₁₉ H ₂₂ FN ₃ O ₄ S
11 a	CH3	-N_N_СН ₃	1	235-237	D	64	C ₂₀ H ₂₄ FN ₃ O ₄ S
1 2a	CH3	нас	1	oil	D	41	C ₂₁ H ₂₈ FN ₃ O ₄ S
		н₃с					
13 a	CH3	-N	1	230–233	С	70	$C_{19}H_{21}FN_2O_4S$
14a	CH3	- N NH	1	oil	E	42	$C_{20}H_{24}FN_{3}O_{4}S$
15 a	CH ₈	-N_CH2NHC2H5	1	wax	D	35	$C_{22}H_{28}FN_3O_4S$
1 6a	CH₃		1	oil	D	24	C ₁₈ H ₁₆ FN ₃ O ₄ S
17a	CH3	-Л Л-СНО	1	Wax	F	54	$C_{20}H_{22}FN_3O_5S$
18a	CH3	-N_N_{N_=}	1	241-244	D	70	$C_{24}H_{25}FN_4O_4S$
19a	CH3		1	oil	Ε	63	$C_{25}H_{25}F_2N_3O_4S$
20a	CH₃		1	Wax	F	83	C ₂₇ H ₂₇ F ₄ N ₈ O ₄ S
		FC					
		۲ ₃ ۰					
10b	4-FC ₆ H ₄	-N_NH	1	wax	F	91	$C_{24}H_{23}F_2N_3O_4S$
11 b	4-FC ₆ H ₄		1	168-170	F	53	$C_{25}H_{25}F_2N_3O_4S$
		\smile					

^a Solvent used for recrystallization or for silica gel column chromatography purification as follows: (A) recrystallization from MeOH, (B) recrystallization from EtOAc, (C) recrystallization from EtOH, (D) isocratic elution with CHCl₃, (E) elution with cyclohexane/CHCl₃ 1:1, and (F) elution with gradient of CHCl₃ to 5% MeOH/CHCl₃. ^bC, H, N analyses were within $\pm 0.4\%$ of theoretical values.

analyzer, and the data for C, H, and N are within $\pm 0.4\%$ of the theoretical values. ¹H NMR spectra were recorded at 90 MHz (Varian EM 390) with Me₄Si as internal standard. Chemical shifts are given in ppm (δ), and the spectral data are consistent with the assigned structures. Reagents and solvents were purchased from common commerical suppliers and were used as received. Column chromatography separations were carried out on Merck silica gel 40 (mesh 70–230). Organic solutions were dried over anhydrous Na₂SO₄ and concentrated with a Buchi rotary evaporator at low pressure. Yields are of purified product and were not optimized. The physical properties of the synthesized compounds are summarized in Tables I–III.

S-(2-Chloro-3-fluoro-6-nitrophenyl)thiolactic Acid (2). A solution of thiolactic acid (13.4 g, 126 mmol) and NaOH (10.13 g, 250 mmol) in water (20 mL) was added portionwise to a stirred solution of 3-chloro-2,4-difluoronitrobenzene (25 g, 126 mmol) in DMSO (50 mL). The resulting mixture was stirred at 80 °C for 2 h, then poured into ice-water, acidified with concentrated HCl, and extracted with EtOAc. The organic phases were reextracted with 2 N Na₂CO₃, and the alkaline solution was acidified with dilute HCl. The separated oil was extracted with CHCl₃. The organic phases were washed with water, dried, and evaporated to dryness to give 2 (30 g, 98%) as a dark red oil which was used in the next step without purification: ¹H NMR

 Table III.
 9-Fluoro-2,10-disubstituted-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylic Acid Derivatives



			R			
compd	R	R ₁	mp, °C	crystn solvent	% yield	formulaª
8a	CH ₃	Cl	311-312	AcOH	95	C ₁₃ H ₉ ClFNO ₃ S
80	$4 - F C_6 H_4$	CI	310-312	D .	04 CE	$C_{18}H_{10}CIF_2NO_3S$
26	3-FU6H4		320-328	0	60 70	$C_{18}\Pi_{10}CIF_{2}NO_{3}S$
80. 91.0	2-FU6H4		291-299		10	C. H. FNLOS 0 25 H.O
218	CH3	-м мн	331-332	Acon-n ₂ o	49	C17H18F N3O3S 0.25 H2O
22a	CH3	-м_м-сн,	328-330	DMF	76	$\mathrm{C_{18}H_{20}FN_{3}O_{3}S}$
23a	CH₃		27 9– 282	DMF-H ₂ O	67	C ₁₉ H ₂₂ FN ₃ O ₃ S
24a	CH3	-N	267-268	AcOH	71	$C_{17}H_{17}FN_2O_3S$
25 a	CH3	-N NH	320-321	AcOH-H ₂ O	18	$C_{18}H_{20}FN_3O_3S 0.5 H_2O$
26a	CH ₃	-N	245-246	b	34	$C_{20}H_{24}FN_3O_3S$
27a	CH3		211-216	DMF	24	C ₁₆ H ₁₂ FN ₃ O ₃ S 1.25 H ₂ O
28a	CH3	-N_N-СНО	305-307	Ь	77	C ₁₈ H ₁₈ FN ₃ O ₄ S 0.75 H ₂ O
29a	CH_3	-1 1 -	304-308	DMF	46	$C_{22}H_{21}FN_4O_3S$
30 a	CH3		313-315	DMF	44	$C_{23}H_{21}F_2N_3O_3S$
31a	CH3		281–283	EtOH	78	$C_{25}H_{23}F_4N_3O_3S$
32a	CH ₃		285-286	DMF	52	$C_{24}H_{23}F_2N_3O_3S$
33a	CH3	-N_N-CH ₂ -	298-300	DMF	43	$C_{24}H_{22}F_3N_3O_3S$
34a	CH3	-N_N-SO ₂ -	260-262	AcOH	52	$C_{23}H_{21}F_2N_3O_5S_2$
21b	4-FC ₆ H ₄		315-317	b	64	$C_{22}H_{19}F_2N_3O_3S$
22b	4-FC ₆ H ₄	-N_N-CH3	312-315	b	54	$C_{23}H_{21}F_2N_3O_3S$

^a C, H, N analyses were within $\pm 0.4\%$ of theoretical values. ^b Purified by reprecipitation on treatment with the base and subsequently with the acid.

 $(\text{CDCl}_3) \delta 1.55 (3 \text{ H}, \text{d}, J = 7.5 \text{ Hz}, \text{CH}_3), 3.95 (1 \text{ H}, \text{q}, J = 7.5 \text{ Hz}, \text{CHCH}_3), 7.10 (1 \text{ H}, \text{t}, J = 9 \text{ Hz}, \text{H-4}), 7.50 (1 \text{ H}, \text{dd}, J = 4.5 \text{ and } 9 \text{ Hz}, \text{H-5}).$

 α -Bromo-4-fluorobenzeneacetic Acid (3b). Br₂ (6 mL, 11.7 mmol) was added to a solution of 4-fluorobenzeneacetic acid (15 g, 97.4 mmol) in CCl₄ (180 mL), and the mixture was refluxed and irradiated with a 300-W tungsten lamp for 2 h. The solvent

was evaporated to dryness to give 3b (22.5 g, 99%) as a yellow solid which was used in the next step without futher purification: mp 53-55 °C.

According to this procedure, compound 3c and 3d were prepared from 3-fluorobenzeneacetic acid and 2-fluorobenzeneacetic acid, respectively: 3c, mp 50-52 °C (95%); 3d, mp 50-53 °C (97%).

Table IV. In Vitro Antibacterial Activity (MIC, $\mu g/mL$)^a

compd	S. fa.	S. au.	P. vu.	E. cl.	S. en.	K. pn.	P. ae.	E. co.
1	16	1	4	2	2	0.12	8	0.25
8a	32	2	>64	2	4	8	32	0.12
8b	8	4	>64	8	>64	0.25	>64	0.25
8c	32	2	>64	>64	>64	2	>64	2
8 d	4	8	>64	>64	>64	0.5	>64	0.5
21a	>64	8	>64	16	8	2	64	0.25
22a	32	1	32	4	2	0.25	16	0.25
23a	64	4	>64	4	4	0.25	32	0.25
24a	32	2	>64	16	16	2	>64	0.25
25a	32	2	64	8	32	1	>64	4
26a	16	4	>64	8	8	0.25	>64	0.25
27a	32	16	>64	16	4	0.25	64	0.12
28a	64	16	64	1	1	0.5	32	0.5
29a	2	0.5	>64	>64	>64	2	>64	1
30a	4	8	>64	>64	>64	>64	>64	8
31a	>64	>64	>64	>64	>64	8	>64	8
32a	>64	32	>64	>64	>64	>64	>64	16
33a	64	32	>64	>64	>64	>64	>64	8
34a	16	4	>64	8	8	1	>64	2
21b	16	16	>64	32	32	16	>64	2
22b	64	16	>64	32	64	>64	>64	8

^a Organisms selected for the table are as follows: S. fa., Streptococcus faecalis LEP Br; S. au., Staphylococcus aureus ATCC 6538; P. vu., Proteus vulgaris CNUR6; E. cl., Enterobacter cloacae OMNFI 174; S. en., Shigella enteritidis; K. pn., Klebsiella pneumoniae ATCC 10031; P. ae., Pseudomonas aeruginosa ATCC 9027; E. co., Escherichia coli ISF 432.

S-(2-Chloro-3-fluoro-6-nitrophenyl)- α -mercapto-4-fluorobenzeneacetic Acid (4b). A solution of NaOH (0.4 g, 10 mmol) in water (10 mL) was added to a solution of 2-chloro-3fluoro-6-nitrothiophenol⁸ (1 g, 5 mmol) in EtOH (50 mL) cooled in ice-water. After 10 min, a solution of α -bromo-4-fluorobenzeneacetic acid (1.1 g, 5 mmol) in EtOH (10 mL) was added. The reaction mixture was refluxed for 2 h. The solvent was evaporated to dryness, and ice-water was added to the residue. The precipitate obtained by addition of dilute HCl was filtered off to give 4b (1.3 g, 77%) as light brown solid: mp 150 °C. This compound was used in the next step without further purification.

In the same manner compounds 4c and 4d were prepared from 3c and 3d, respectively: 4c, mp 155–158 °C (98%); 4d, mp 165–168 °C (79%).

8-Chloro-7-fluoro-2-methyl-3-oxo-3,4-dihydro-2H-1,4-benzothiazine (5a). An aqueous solution of FeSO₄.7 H₂O (208 g dissolved in 500 mL of hot water) was added to a solution of nitro acid 2 (30 g, 107 mmol) in NH₄OH (200 mL). The mixture was stirred for 2 h at room temperature and then for 15 min at 50 °C. The hot mixture was filtered, and the collected precipitate was washed on the filter with dilute NH₄OH. The filtrate was acidified with dilute HCl, and the precipitated solid was filtered off, washed with water, and recrystallized from EtOH to give 5a (15 g, 61%) as a pink solid: mp 198-200 °C; ¹H NMR (DMSOde) δ 1.38 (3 H, d, J = 6 Hz, CH₃), 3.75 (1 H, q, J = 6 Hz, CHCH₃), 6.80 (1 H, dd, J = 6 and 9 Hz, H-5), 7.10 (1 H, t, J = 9 Hz, H-6). Anal. (C₉H₇CIFNOS) C, H, N.

In the same manner compounds **5b-d** were prepared from **4b-d**, respectively.

8-Chloro-7-fluoro-2-methyl-3,4-dihydro-2H-1,4-benzothiazine (6a). A solution of 5a (7 g, 30 mmol) in dry THF (130 mL) was added dropwise to a suspension of LiAlH₄ (2.3 g, 60 mmol) in THF (50 mL). After the addition was complete, the mixture was left at room temperature for 30 min; dilute H₂SO₄ was then added to destroy the excess of LiAlH₄. The mixture was filtered, and the solution was alkalinized with 10% NaOH and then extracted with CHCl₃. The organic extract was washed with water, dried, and evaporated to dryness yielding a dark oil which was purified by silica gel column chromatography eluting with cyclohexane/CHCl₃ 8:2 to give 6a (3 g, 46%) as a yellow oil: ¹H NMR (CDCl₉) δ 1.35 (3 H, d, J = 7.5 Hz, CH₃), 2.85–3.55 (3 H, m, CHCH₃ and CH₂), 3.95 (1 H, m, NH), 6.15 (1 H, dd, J = 4.5 and 9 Hz, H-5), 6.52 (1 H, t, J = 9 Hz, H-6). Anal. (C₉H₉ClFNS) C, H, N.

Compounds 6b-d were obtained from 5b-d by this procedure. Ethyl 10-Chloro-9-fluoro-2-methyl-7-oxo-2,3-dihydro-7*H*pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate (7a). A mixture of 6a (3 g, 14 mmol) and EMME (4.47 g, 20 mmol) was heated fro 2 h at 150 °C. PPA (15 g) was added, and the mixture was heated at 160 °C for 1 h. After cooling, the reaction mixture was poured into ice-water, and the precipitate was collected by filtration, washed with 10% Na₂CO₃ and water, and then recrystallized from MeOH to give 7a (3.5 g, 74%) as an off-white solid: mp 199-201 °C; ¹H NMR (TFA) δ 1.30-1.75 (6 H, m, CHCH₃ and CH₂CH₃), 3.68-4.03 (1 H, m, CHCH₃), 4.50-5.20 (4 H, m, CH₂CH₃ and NCH₂), 8.05 (1 H, d, J = 7.5 Hz, H-8), 9.13 (1 H, s, H-5). Anal. (C₁₅H₁₃ClFNO₃S) C, H, N.

According to this procedure, compounds 7b-d were prepared from 6b-d.

10-Chloro-9-fluoro-2-methyl-7-oxo-2,3-dihydro-7*H*-pyrido-[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (8a). A suspension of 7a (3.41 g, 10 mmol) in 10% NaOH (30 mL) was refluxed for 30 min, then poured into ice-water, and acidified with dilute HCl. The precipitate solid was filtered off, washed with water, and recrystallized from AcOH to give 8a (2.98 g, 95%) as an off-white solid: mp 311-312 °C; ¹H NMR (TFA) δ 1.62 (3 H, d, J = 6 Hz, CHCH₃), 3.75-4.05 (1 H, m, CHCH₃), 4.50-5.26 (2 H, m, NCH₂), 8.10 (1 H, d, J = 7.5 Hz, H-8), 9.30 (1 H, s, H-5). Anal. (C₁₃H₉CIFNO₃S) C, H, N.

Carboxylic acid derivatives 8b-d were made in a similar fashion from 7b-d.

Ethyl 10-Chloro-9-fluoro-2-methyl-7-oxo-2,3-dihydro-7*H*pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate 1-Oxide (9a). A solution of 55% MCPBA (4.2 g, 13 mmol) in absolute EtOH (20 mL) was added portionwise to a stirred solution of 7a (3 g, 9 mmol) in absolute EtOH (300 mL). After the addition, the mixture was stirred at room temperature for 2 h. The separated solid was filtered off and combined with the other one obtained by concentrating the filtrate to half the initial volume. The combined precipitates were recrystallized from EtOH to give 9a (1.9 g, 60%) as a light yellow solid: mp 202-204 °C; ¹H NMR (DMSO- d_6) δ 0.98 (3 H, d, J = 6 Hz, CHCH₃), 1.30 (3 H, t, J = 6 Hz, CH₂CH₃), 3.58-3.90 (1 H, m, CHCH₃), 4.21 (2 H, q, J = 6 Hz, CH₂CH₃), 4.50-4.70 (2 H, m, NCH₂), 8.18 (1 H, d, J =9 Hz, H-8), 8.62 (1 H, s, H-5). Anal. (C₁₅H₁₃ClFNO₄S) C, H, N.

Compound 9b was prepared in an identical fashion from 7b. Ethyl 9-Fluoro-2-methyl-10-(1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylate 1-Oxide (10a). Piperazine (3.13 g, 36 mmol) was added to a suspension of sulfoxide 9a (4 g, 11 mmol) in DMF (15 mL). The mixture was allowed to react at 100 °C for 2 h. The solvent was distilled off, and the crude residue was purified by column chromatography eluting with CHCl₃ yielding yellow oil 10a (3.30 g, 72%) as an approximate 3:2 or 2:3 mixture of diastereoisomers: ¹H NMR (CDCl₃) δ 1.10 and 1.65 (3 H, each d, J = 6 Hz, CHCH₃), 1.40 (3 H, t, J = 6 Hz, CH₂CH₃), 3.00-3.55 (9 H, m, piperazine CH₂ and CHCH₃), 4.10-5.10 (4 H, m, NCH₂ and CH₂-CH₃), 8.10 and 8.30 (1 H, each d, J = 12 Hz, H-8), 8.50 and 8.60 (1 H, each s, H-5). Anal. (C₁₉H₂₂FN₃O₄S) C, H, N.

Compounds 11a-20a were prepared from 9a while 10b and 11b from 9b by reaction with the appropriate amine, according to this procedure.

9-Fluoro-2-methyl-10-(1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylic Acid (21a). A solution of 10a (1.5 g, 3.68 mmol) in DMF (10 mL) was cooled to 0 °C, and PBr₃ (1.14 g, 5.53 mmol) was added. The reaction mixture was stirred for 3 h at room temperature and then evaporated to dryness. The crude residue was triturated with EtOH to give ethyl 9-fluoro-2-methyl-10-(1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzothiazine-6-carboxylate as a solid which was filtered off (1.1 g, 76%) and then suspended in 10% NaOH (20 mL) and EtOH (3 mL). The suspension was refluxed for 40 min, then cooled, diluted with ice-water, and finally acidified (ca. pH 6) with dilute HCl. The precipitate was filtered off, dried, and recrystallized from aqueous AcOH yielding 21a (0.65 g, 49%): mp 331-332 °C; ¹H NMR (TFA) δ 1.58 (3 H, d, J = 6 Hz, CHCH₃), 3.42-3.86 (9 H, m, piperazine CH₂ and CHCH₃), 4.35-5.10 (2 H, m, NCH₂), 7.65 (1 H, br s, NH), 7.94 (1 H, d, J = 12 Hz, H-8), 9.10 (1 H, s, H-5). Anal. $(C_{17}H_{18}FN_3O_3S \cdot 0.25H_2O)$ C, H, N.

Compounds 22a-31a and 21b, 22b were prepared in the same manner.

Table V. Preliminary Pharmacokinetics in Rats: Comparison between 22a and Rufloxacin (1) Orally Administrated at 50 mg/kg^a

plasma, concn μ g/mL ^b				urinary excretion, % of administered dose ^b			
compd	1 h	4 h	15 h	compd	0-4 h	4-8 h	8-24 h
22a 1	14.06 ± 2.07 7.74 ± 1.94	7.61 ± 0.17 4.43 ± 0.47	0.27 ± 0.05 0.41 ± 0.03	22a metabolite ^c 1 metabolite ^b	$\begin{array}{c} 1.50 \pm 0.12 \\ 2.73 \pm 1.14 \\ 2.18 \pm 0.10 \\ 0.37 \pm 0.11 \end{array}$	$\begin{array}{c} 2.59 \pm 0.31 \\ 5.59 \pm 2.50 \\ 10.48 \pm 6.52 \\ 1.92 \pm 1.13 \end{array}$	5.17 ± 0.51 11.53 \pm 4.82 15.60 \pm 4.71 3.12 \pm 0.90

^a See Experimental Section. ^b Mean \pm SE of three rats. ^c Desmethylated piperazinyl metabolite of 22a corresponding to compound 21a. ^d Desmethylated metabolite of 1.

9-Fluoro-2-methyl-10-[4-(3-fluorobenzyl)-1-piperazinyl]-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6carboxylic Acid (32a). 3-Fluorobenzyl chloride (0.062 g, 0.43 mmol) was added to a solution of 21a (0.120 g, 0.33 mmol) and triethylamine (0.07 g, 0.7 mmol) in DMF (5 mL). The mixture was heated at 100 °C for 1 h. After cooling, the resulting precipitate was collected, washed with EtOH, and recrystallized from DMF to give 32a (0.08 g, 52%) as a light yellow solid: mp 285-286 °C. Anal. ($C_{24}H_{23}F_2N_3O_3S$) C, H, N.

Replacement of the 3-fluorobenzyl chloride in the above procedure by 2,4-difluorobenzyl bromide gave 9-fluoro-2-methyl-10-[4-(2,4-difluorobenzyl)-1-piperazinyl]-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acid (**33a**) in 43% yield: mp 298-300 °C. Anal. ($C_{24}H_{22}F_3N_3O_3S$) C, H, N.

9-Fluoro-2-methyl-10-[4-[(4-Fluorophenyl)sulfonyl]-1piperazinyl]-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (34a). Starting from 21a and using 4-fluorobenzenesulfonyl chloride, compound 34a was obtained by the above procedure, except for the workup of the reaction mixture: after cooling, it was poured into ice-water to give a precipitate which was filtered off and recrystallized from AcOH in 52% yield: mp 260-262 °C. Anal. $(C_{23}H_{21}F_2N_3O_5S_2)$ C, H, N.

In Vitro Antibacterial Activity. The in vitro antibacterial activity was determined by standard agar dilution procedure on TSA agar. Suspension of microorganisms were stored at -80 °C and diluted before using in peptonated water up to $1-2 \times 10^7$ colony-forming units (CFU). The compounds were incorporated by the 2-fold dilution method into a melted medium of 50 °C just prior to the pouring and use of the plates. Bacterial inocula were applied to the agar surface with a multipoint inoculator. Minimum inhibitory concentrations (MICs) were defined as the lowest concentration of the compounds that prevented visible growth of bacteria after incubation at 35 °C for 24 h.

Pharmacokinetic Tests. Plasma and urine were obtained from fasted male Wistar rats, using three rats per group. The compounds were orally administered to animals at dosage levels of 50 mg/kg.

Plasma and urine samples were assayed at various times after drug administrations by an HPLC method.

HPLC Assay in the Plasma and Urine. Plasma (0.2 mL) was added to water (0.2 mL) and 70% perchloric acid $(20 \mu \text{L})$. The samples were vortexed and centrifuged. The clear surnatant was used for HPLC determination. Urine samples were diluted

1:100 with ultrapure water and then processed as plasma samples for HPLC determinations. A Perkin-Elmer Series 410 LC was used with a column supplex pkb-100 (5 μ m). The phosphate buffer for the mobile phase was prepared by adding 0.17% phosphoric acid to a 0.01 M solution of KH₂PO₄ until pH 2.8 was reached. A spectrofluorimeter RF-551, exitation wavelength 294 nm and emission wavelength 521 nm, was used as detector.

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