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

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Received December 30, 1992•

A series of 2-substituted-7-oxo-2,3-dihydro-7*H*-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 ofloxacin[3] 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-1-piperazinyl)-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 **8a–d**, **21a–34a**, **21b**, and **22b** 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 $LiAlH_4$. 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[1,2,3-*de*][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 (MCPBA) and then submitted to regiospecific substitution with heterocyclic bases. The target compounds were finally obtained by successive deoxygenation with PBr_3 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 Gram-positive (*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 μg/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 Gram-positive bacteria; in particular **29a** showed the best activity against *Streptococcus* and *Staphylococcus* (MIC = 2 and 0.5 μg/mL, respectively) better than the control drug 1 (MIC = 16 and 1 μg/mL).

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

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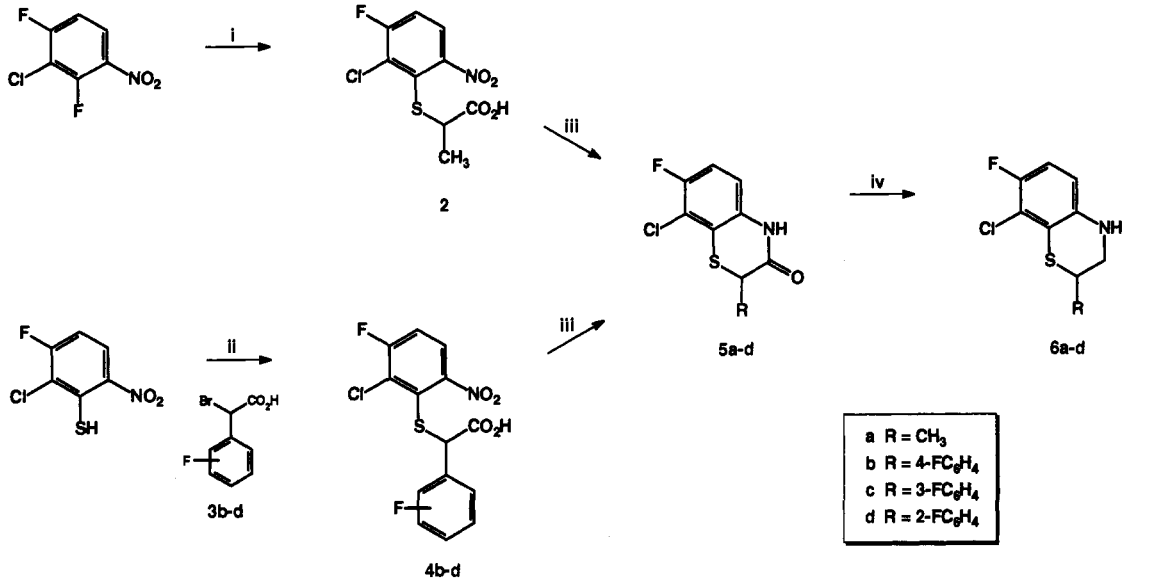
• Abstract published in *Advance ACS Abstracts*, September 15, 1993.

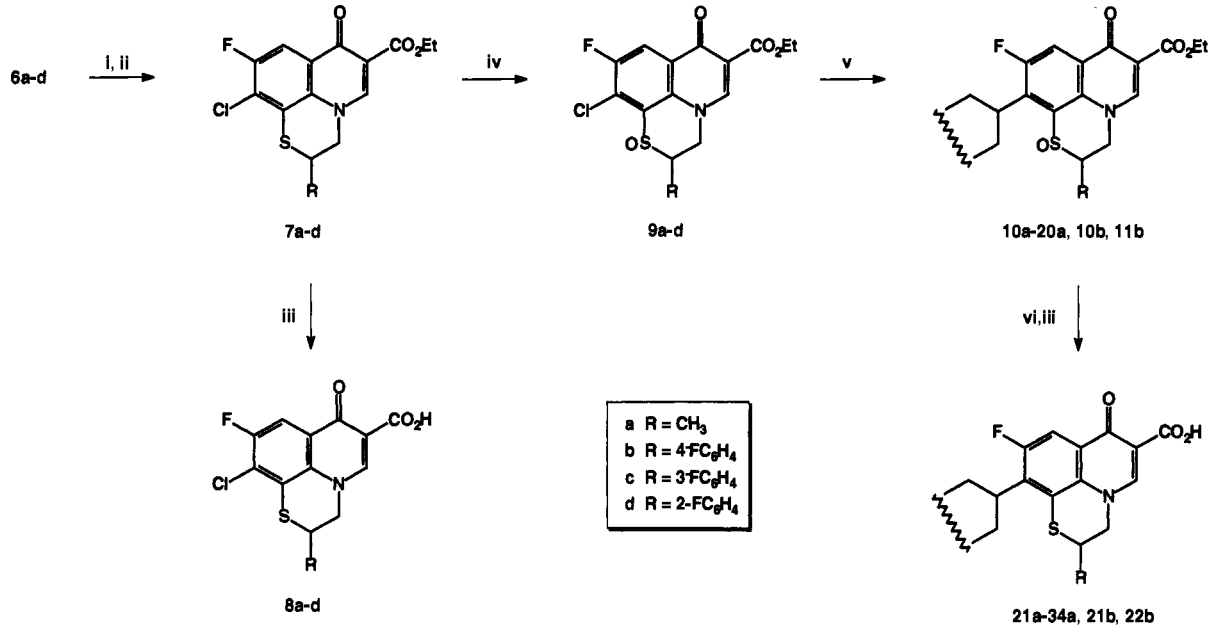
0022-2623/93/1836-3449$04.00/0

Scheme I[a]



[a] Reagents: (i) $CH_3CH(SH)CO_2H$, NaOH, DMSO, 80 °C; (ii) NaOH, EtOH, reflux; (iii) $FeSO_4 \cdot 7H_2O$, NH_4OH; (iv) $LiAlH_4$, 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) PBr_3, 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.

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

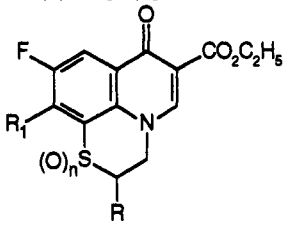
compd[a]	mp, °C	purification method[b]	% yield	formula[c]
5a	198–200	A	61	$C_9H_7ClFNOS$
5b	193–195	A	36	$C_{14}H_8ClF_2NOS$
5c	148–150	A	76	$C_{14}H_8ClF_2NOS$
5d	234–235	A	83	$C_{14}H_8ClF_2NOS$
6a	oil	B	46	C_9H_9ClFNS
6b	88–90	B	36	$C_{14}H_{10}ClF_2NS$
6c	84–86	B	38	$C_{14}H_{10}ClF_2NS$
6d	82–84	B	40	$C_{14}H_{10}ClF_2NS$

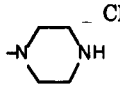
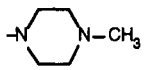
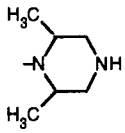
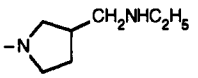
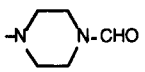
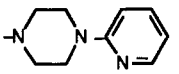
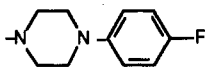
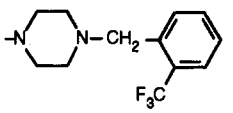
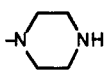
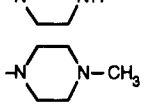
[a] See Scheme I. [b] Solvent used for recrystallization or for silica gel column chromatography purification as follows: (A) recrystallization from EtOH and (B) elution with gradient of cyclohexane to 20% $CHCl_3$/cyclohexane. [c] C, H, N analyses were within ±0.4% of theoretical values.

Experimental Section

Melting points were determined in capillary tubes (Buchi 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-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate and Ethyl 2,10-Disubstituted-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate 1-Oxide Derivatives



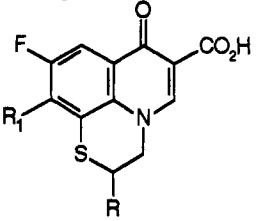
compd	R	R_1	*n*	mp, °C	purification method[a]	% yield	formula[b]
7a	CH_3	Cl	0	199–201	A	74	$C_{16}H_{13}ClFNO_3S$
7b	4-FC_6H_4	Cl	0	240–242	B	91	$C_{20}H_{14}ClF_2NO_3S$
7c	3-FC_6H_4	Cl	0	260–262	B	90	$C_{20}H_{14}ClF_2NO_3S$
7d	2-FC_6H_4	Cl	0	292 dec	B	90	$C_{20}H_{14}ClF_2NO_3S$
9a	CH_3	Cl	1	202–204	C	60	$C_{15}H_{13}ClFNO_4S$
9b	4-FC_6H_4	Cl	1	227–230	C	62	$C_{20}H_{14}ClF_2NO_4S$
10a	CH_3		1	oil	D	72	$C_{19}H_{22}FN_3O_4S$
11a	CH_3		1	235–237	D	64	$C_{20}H_{24}FN_3O_4S$
12a	CH_3		1	oil	D	41	$C_{21}H_{26}FN_3O_4S$
13a	CH_3		1	230–233	C	70	$C_{19}H_{21}FN_2O_4S$
14a	CH_3		1	oil	E	42	$C_{20}H_{24}FN_3O_4S$
15a	CH_3		1	wax	D	35	$C_{22}H_{28}FN_3O_4S$
16a	CH_3		1	oil	D	24	$C_{18}H_{16}FN_3O_4S$
17a	CH_3		1	wax	F	54	$C_{20}H_{22}FN_3O_5S$
18a	CH_3		1	241–244	D	70	$C_{24}H_{25}FN_4O_4S$
19a	CH_3		1	oil	E	63	$C_{25}H_{25}F_2N_3O_4S$
20a	CH_3		1	wax	F	83	$C_{27}H_{27}F_4N_3O_4S$
10b	4-FC_6H_4		1	wax	F	91	$C_{24}H_{23}F_2N_3O_4S$
11b	4-FC_6H_4		1	168–170	F	53	$C_{25}H_{25}F_2N_3O_4S$

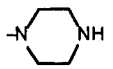
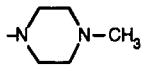
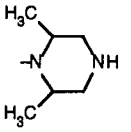
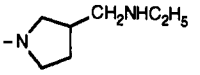
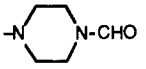
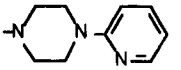
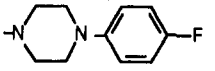
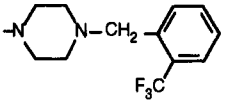
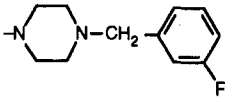
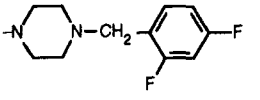
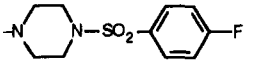
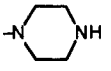
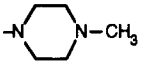
[a] Solvent used for recrystallization or for silica gel column chromatography purification as follows: (A) recrystallization from MeOH, (B) recrystallization from EtOAc, (C) recrystallization from EtOH, (D) isocratic elution with $CHCl_3$, (E) elution with cyclohexane/$CHCl_3$ 1:1, and (F) elution with gradient of $CHCl_3$ to 5% MeOH/$CHCl_3$. [b] C, H, N analyses were within ±0.4% of theoretical values.

analyzer, and the data for C, H, and N are within ±0.4% of the theoretical values. ^1H NMR spectra were recorded at 90 MHz (Varian EM 390) with Me_4Si 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 Na_2SO_4 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 Na_2CO_3, and the alkaline solution was acidified with dilute HCl. The separated oil was extracted with $CHCl_3$. 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-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid Derivatives



compd	R	R_1	mp, °C	crystn solvent	% yield	formula[a]
8a	CH_3	Cl	311–312	AcOH	95	$C_{13}H_9ClFNO_3S$
8b	4-FC_6H_4	Cl	310–312	*b*	64	$C_{18}H_{10}ClF_2NO_3S$
8c	3-FC_6H_4	Cl	325–328	*b*	65	$C_{18}H_{10}ClF_2NO_3S$
8d	2-FC_6H_4	Cl	>350	*b*	70	$C_{18}H_{10}ClF_2NO_3S$
21a	CH_3		331–332	AcOH-H_2O	49	$C_{17}H_{16}FN_3O_3S$ 0.25 H_2O
22a	CH_3		328–330	DMF	76	$C_{18}H_{20}FN_3O_3S$
23a	CH_3		279–282	DMF-H_2O	67	$C_{19}H_{22}FN_3O_3S$
24a	CH_3		267–268	AcOH	71	$C_{17}H_{17}FN_2O_3S$
25a	CH_3		320–321	AcOH-H_2O	18	$C_{18}H_{20}FN_3O_3S$ 0.5 H_2O
26a	CH_3		245–246	*b*	34	$C_{20}H_{24}FN_3O_3S$
27a	CH_3		211–216	DMF	24	$C_{16}H_{12}FN_3O_3S$ 1.25 H_2O
28a	CH_3		305–307	*b*	77	$C_{18}H_{16}FN_3O_4S$ 0.75 H_2O
29a	CH_3		304–308	DMF	46	$C_{22}H_{21}FN_4O_3S$
30a	CH_3		313–315	DMF	44	$C_{23}H_{21}F_2N_3O_3S$
31a	CH_3		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	CH_3		298–300	DMF	43	$C_{24}H_{22}F_3N_3O_3S$
34a	CH_3		260–262	AcOH	52	$C_{23}H_{21}F_2N_3O_5S_2$
21b	4-FC_6H_4		315–317	*b*	64	$C_{22}H_{19}F_2N_3O_3S$
22b	4-FC_6H_4		312–315	*b*	54	$C_{23}H_{21}F_2N_3O_3S$

[a] C, H, N analyses were within ±0.4% of theoretical values. [b] Purified by reprecipitation on treatment with the base and subsequently with the acid.

($CDCl_3$) δ 1.55 (3 H, d, *J* = 7.5 Hz, CH_3), 3.95 (1 H, q, *J* = 7.5 Hz, C*H*CH_3), 7.10 (1 H, t, *J* = 9 Hz, H-4), 7.50 (1 H, dd, *J* = 4.5 and 9 Hz, H-5).

α-Bromo-4-fluorobenzeneacetic Acid (3b). Br_2 (6 mL, 11.7 mmol) was added to a solution of 4-fluorobenzeneacetic acid (15 g, 97.4 mmol) in CCl_4 (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, μg/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
8d	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* CNUR 6; 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-3-fluoro-6-nitrothiophenol[8] (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-2*H*-1,4-benzothiazine (5a). An aqueous solution of $FeSO_4 \cdot 7\ H_2O$ (208 g dissolved in 500 mL of hot water) was added to a solution of nitro acid **2** (30 g, 107 mmol) in NH_4OH (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 NH_4OH. 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; ^{1}H NMR (DMSO-d_6) δ 1.38 (3 H, d, J = 6 Hz, CH_3), 3.75 (1 H, q, J = 6 Hz, C*H*CH_3), 6.80 (1 H, dd, J = 6 and 9 Hz, H-5), 7.10 (1 H, t, J = 9 Hz, H-6). Anal. ($C_9H_7ClFNOS$) 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-2*H*-1,4-benzothiazine (6a). A solution of **5a** (7 g, 30 mmol) in dry THF (130 mL) was added dropwise to a suspension of $LiAlH_4$ (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 H_2SO_4 was then added to destroy the excess of $LiAlH_4$. The mixture was filtered, and the solution was alkalinized with 10% NaOH and then extracted with $CHCl_3$. 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/$CHCl_3$ 8:2 to give **6a** (3 g, 46%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.35 (3 H, d, J = 7.5 Hz, CH_3), 2.85–3.55 (3 H, m, C*H*CH_3 and CH_2), 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. (C_9H_9ClFNS) 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% Na_2CO_3 and water, and then recrystallized from MeOH to give **7a** (3.5 g, 74%) as an off-white solid: mp 199–201 °C; ^{1}H NMR (TFA) δ 1.30–1.75 (6 H, m, CHC*H*$_3$ and CH_2C*H*$_3$), 3.68–4.03 (1 H, m, C*H*CH_3), 4.50–5.20 (4 H, m, C*H*$_2$$CH_3$ and NCH_2), 8.05 (1 H, d, J = 7.5 Hz, H-8), 9.13 (1 H, s, H-5). Anal. ($C_{15}H_{13}ClFNO_3S$) 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; ^{1}H NMR (TFA) δ 1.62 (3 H, d, J = 6 Hz, CHC*H*$_3$), 3.75–4.05 (1 H, m, C*H*CH_3), 4.50–5.26 (2 H, m, NCH_2), 8.10 (1 H, d, J = 7.5 Hz, H-8), 9.30 (1 H, s, H-5). Anal. ($C_{13}H_9ClFNO_3S$) 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; ^{1}H NMR (DMSO-d_6) δ 0.98 (3 H, d, J = 6 Hz, CHC*H*$_3$), 1.30 (3 H, t, J = 6 Hz, CH_2C*H*$_3$), 3.58–3.90 (1 H, m, C*H*CH_3), 4.21 (2 H, q, J = 6 Hz, C*H*$_2$$CH_3$), 4.50–4.70 (2 H, m, NCH_2), 8.18 (1 H, d, J = 9 Hz, H-8), 8.62 (1 H, s, H-5). Anal. ($C_{15}H_{13}ClFNO_4S$) 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-7*H*-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 $CHCl_3$ yielding yellow oil **10a** (3.30 g, 72%) as an approximate 3:2 or 2:3 mixture of diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 1.10 and 1.65 (3 H, each d, J = 6 Hz, CHC*H*$_3$), 1.40 (3 H, t, J = 6 Hz, CH_2C*H*$_3$), 3.00–3.55 (9 H, m, piperazine CH_2 and C*H*CH_3), 4.10-5.10 (4 H, m, NCH_2 and C*H*$_2$$CH_3$), 8.10 and 8.30 (1 H, each d, J = 12 Hz, H-8), 8.50 and 8.60 (1 H, each s, H-5). Anal. ($C_{19}H_{22}FN_3O_4S$) 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-7*H*-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 PBr_3 (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-7*H*-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; ^{1}H NMR (TFA) δ 1.58 (3 H, d, J = 6 Hz, CHC*H*$_3$), 3.42–3.86 (9 H, m, piperazine CH_2 and C*H*CH_3), 4.35–5.10 (2 H, m, NCH_2), 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 μg/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	1.50 ± 0.12	2.59 ± 0.31	5.17 ± 0.51
22a	14.06 ± 2.07	7.61 ± 0.17	0.27 ± 0.05	metabolite[c]	2.73 ± 1.14	5.59 ± 2.50	11.53 ± 4.82
1	7.74 ± 1.94	4.43 ± 0.47	0.41 ± 0.03	1	2.18 ± 0.10	10.48 ± 6.52	15.60 ± 4.71
				metabolite[b]	0.37 ± 0.11	1.92 ± 1.13	3.12 ± 0.90

[a] See Experimental Section. [b] Mean ±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-6-carboxylic 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. ($C_{24}H_{22}F_2N_3O_3S$) 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]-1-piperazinyl]-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 × 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 μL). 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 μm). The phosphate buffer for the mobile phase was prepared by adding 0.17% phosphoric acid to a 0.01 M solution of KH_2PO_4 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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