Quinolonecarboxylic Acids. 2. Synthesis and Antibacterial Evaluation of 7-Oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acids[1]

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A series of pyridobenzothiazine acid derivatives was synthesized and their in vitro antibacterial activity was evaluated. The 1,4-benzothiazine intermediates, which by Gould–Jacobs quinoline synthesis produced pyridobenzothiazine acids, were prepared by hydrolytic basic cleavage of substituted 2-aminobenzothiazoles and successive cyclocondensation with 1-bromo-2-chloroethane or alternatively with monochloroacetic acid, hence reduction by $LiAlH_4$. The pyridobenzothiazine acids **10c**, **30**, and **31** show potent antibacterial activities against Gram-positive and Gram-negative pathogens. Structure–activity relationships are discussed. The compound 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 (**31**) (MF-934) has been found to possess, together with the antibacterial activity, a weak acute toxicity and interesting pharmacokinetic characteristics in several animal species (rat, dog, monkey, man).

The introduction of fluorine atoms together with piperazinyl or pyrrolidinyl groups at a quinoline or naphthyridine ring and, in some instances, the replacement of the *N*-ethyl group with a group with larger steric bulk represented the structural modifications of the last generation of quinolone antibacterial agents, which include norfloxacin,[2] pefloxacin,[3] enoxacin,[4] ciprofloxacin,[5] amifloxacin,[6] ofloxacin,[7] A-56619,[8] A-56620,[8] AM-833,[9] and CI-934[10] (Figure 1). They possess a stronger antimicrobial potency and a broader spectrum compared with previous analogues of nalidixic acid. The increased potency coupled with sufficiently high tissue and plasma concentrations has broadened the therapeutic potential of quinolones for treatment of both systemic and urinary infections.[11]

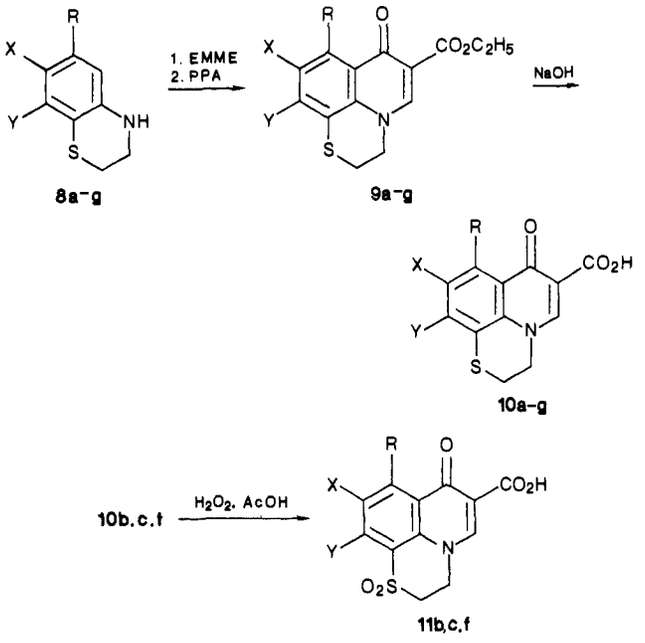
Growing interest in quinolone analogues led us to synthesize pyrido[1,2-*de*][1,4]benzothiazinecarboxylic acids,[12,13] which resulted to be isosteres of pyridobenzoxazinecarboxylic acids,[7] quinolones of the latest generation. These pyridobenzothiazine acids, obtained by peri-annelation of a 4-pyridone-3-carboxylated moiety on 1,4-benzothiazines, represented a structural modification of 1,4-thiazinoquinoline acids that we had previously prepared[14] and that showed an activity comparable to that of nalidixic acid.

Chemistry

The synthesis of the ortho-peri condensed pyridobenzothiazine acids **10a–g** was achieved by reaction of 1,4-benzothiazine intermediates **8a–g** with diethyl (ethoxymethylene)malonate (EMME) followed by polyphosphoric acid (PPA) cyclization and successive hydrolysis of the obtained esters **9a–g** (Scheme I).

The 7,8-dichloro-3,4-dihydro-2*H*-1,4-benzothiazine (**8a**) was obtained by reaction of 2,3,4-trichloronitrobenzene with thioglycolic acid and then reductive cyclization in order to obtain the 7,8-dichloro-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine (**2**),[15] which was successively reduced by $LiAlH_4$. On the other hand, the dihydro-1,4-benzothiazines **8b–e** were prepared by (a) thiocyanation of substituted anilines, (b) hydrolytic basic cleavage of obtained 2-aminobenzothiazoles, and (c) subsequent cyclocondensation with 1-bromo-2-chloroethane or, alternatively, with monochloroacetic acid and, in the latter case, reduction of dihydro-1,4-benzothiazinones by $LiAlH_4$. As the 3-chloro-4-fluoroaniline by thiocyanation furnished a mixture of the two 2-aminobenzothiazole isomers **4** and **5**, the next step was carried out on this mixture by using

Scheme I[a]



[a] **a**, R = H, X = Cl, Y = Cl; **b**, R = H, X = F, Y = H; **c**, R = H, X = F, Y = Cl; **d**, R = Cl, X = F, Y = H; **e**, R = H, X = Cl, Y = H; **f**, R = H, X = H, Y = H; **g**, R = CF_3, X = H, Y = H.

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(1) Preliminary account of this work was presented at the 15th National Congress of Italian Chemical Society, Grado, Italy, September 1984; Abstract FARM R7.
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(7) (a) Hayakawa, I.; Tanaka, Y.; Hiramitsu, T. Eur. Pat. Appl. 47 005; *Chem. Abstr.* **1982**, *97*, 55 821. (b) Hayakawa, I.; Hiramitsu, T.; Tanaka, Y. *Chem. Pharm. Bull.* **1984**, *32*, 4907.

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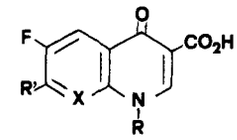
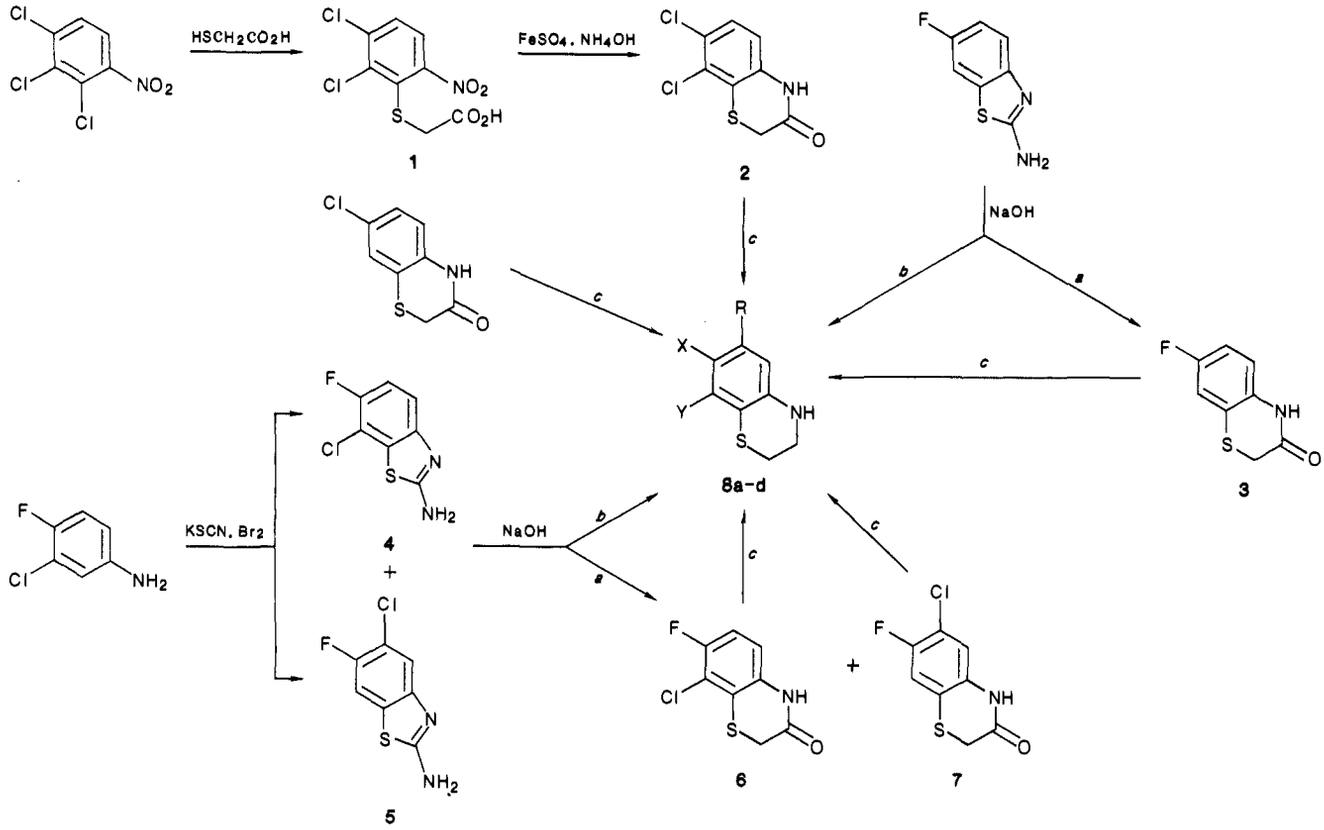


norfloxacin	R = C_2H_5 ; R' = 1-piperazinyl ; X = CH
pefloxacin	R = C_2H_5 ; R' = 4-methyl-1-piperazinyl ; X = CH
enoxacin	R = C_2H_5 ; R' = 1-piperazinyl ; X = N
ciprofloxacin	R = cyclopropyl ; R' = 1-piperazinyl ; X = CH
amifloxacin	R = $NHCH_3$; R' = 4-methyl-1-piperazinyl ; X = CH
ofloxacin	R = $CH(CH_3)CH_2OX$; R' = 4-methyl-1-piperazinyl ; X = C
A-56619	R = 4-FC_6H_5 ; R' = 4-methyl-1-piperazinyl ; X = CH
A-56620	R = 4-FC_6H_5 ; R' = 1-piperazinyl ; X = CH
AM-833	R = CH_2CH_2F ; R' = 4-methyl-1-piperazinyl ; X = CF
CI-934	R = C_2H_5 ; R' = 3-methylaminoethyl-1-pyrrolidinyl ; X = CF

Figure 1.

Scheme II[a]



[a] $ClCH_2CO_2H$; see Experimental Section, method Aa. [b] $BrCH_2CH_2Cl$; see Experimental Section, method B. [c] $LiAlH_4$; see Experimental Section, method Ab.

either monochloroacetic acid or 1-bromo-2-chloroethane. Consequently, in the first case a mixture was obtained of dihydro-1,4-benzothiazinones **6** and **7**, which was then separated by flash chromatography and subsequently re-

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(12) (a) During the course of this work, a related patent appeared: Daiichi Seiyaku Co., Ltd. Jpn. Kokai Tokkyo Koho 57203085; *Chem. Abstr.* **1983**, *98*, 198294. (b) Following completion of this work, another patent appeared: Otsuka Pharmaceutical Co. Ltd. Jpn. Kokai Tokkyo Koho 5976091; *Chem. Abstr.* **1984**, *101*, 151868.

Table I. 3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazine Derivatives and 3,4-Dihydro-2*H*-1,4-benzothiazine Derivatives

compd[a]	meth[b]	mp, °C	crystn solvent	yield, %	formula[c]	^{1}H NMR[d] spectral data
3		215–217	EtOH	77.6	C_8H_6FNOS	
6		211–213	EtOH	17.8	$C_8H_5ClFNOS$	
7		220–222	EtOH	20.0	$C_8H_5ClFNOS$	
8a		*e*		71.8	$C_8H_7Cl_2NS$	2.90–3.10 (2 H, m, SCH_2), 3.38–3.57 (2 H, m, NCH_2), 5.30 (1 H, br s, NH), 6.45 and 6.90 (each 1 H, d, J = 9 Hz, H-5 and H-6)
8b	A	*e*		75.0[f]	C_8H_8FNS	2.90–3.08 (2 H, m, SCH_2), 3.38–3.56 (2 H, m, NCH_2), 5.55 (1 H, br s, NH), 6.48–6.80 (3 H, m, aromatic protons)
	B			65.0		
8c	A	*e*		15.9[f]	C_8H_7ClFNS	3.00–3.20 (2 H, m, SCH_2), 3.42–3.62 (2 H, m, NCH_2), 6.18 (1 H, br s, NH), 6.52 (1 H, dd, J = 9 Hz and J_{H-F} = 6 Hz, H-5), 6.90 (1 H, t, J = 9 Hz and J_{H-F} = 9 Hz, H-6)
	B			19.2		
8d	A	88–92	EtOAc	18.1[f]	C_8H_7ClFNS	2.93–3.13 (2 H, m, SCH_2), 3.42–3.62 (2 H, m, NCH_2), 6.15 (1 H, br s, NH), 6.65 (1 H, d, J_{H-F} = 6 Hz, H-5), 6.95 (1 H, d, J_{H-F} = 9 Hz, H-8)
	B			28.8		
8e	A	*e*		71.8	C_8H_8ClNS	2.85–3.03 (2 H, m, SCH_2), 3.38–3.55 (2 H, m, NCH_2), 5.26 (1 H, br s, NH), 6.52 (1 H, d, J = 9 Hz, H-5), 6.65–6.85 (2 H, m, H-6 and H-7)

[a] See Schemes I and II. [b] See Experimental Section. [c] C, H, N analyses were within ±0.4% of theoretical values. [d] Solvent: Me_2SO-d_6. [e] Oil. [f] Yield calculated from the corresponding aminobenzothiazole.

duced with $LiAlH_4$ to dihydro-1,4-benzothiazines **8c** and **8d**, respectively. However, with 1-bromo-2-chloroethane, a mixture of **8c** and **8d** isomers was directly obtained, which was separated by column chromatography (Scheme II).

The halogenated pyridobenzothiazines **10a–d** were finally submitted to nucleophilic substitution with heterocyclic bases. This nucleophilic substitution gave different results. From 9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido-[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acid (**10b**), **12–17** were easily obtained. On the contrary, the nucleophilic reaction with 9,10-dichloro-7-oxo-2,3-dihydro-7*H*-pyrido-[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acid (**10a**) under different conditions (solvent, temperature, autoclaved reaction) failed. The 8-chloro-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acid (**10d**) gave a regiospecific displacement; indeed, with apolar solvents (benzene, toluene), chlorine substitution at C-8 (**19**, **20**) was observed. With aprotic polar solvents (Me_2SO, DMF, HMPA), the substitution occurred on carbon bearing fluorine at C-9 (**21**).[16] The 10-chloro-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acid (**10c**), irrespective of the solvent employed, merely produced fluorine substitution (**23–26**). On the other hand, chlorine substitution at C-10 occurred in both polar and apolar solvents only after enhancement of the aromatic nucleus reactivity by reversible oxidation of thiazinic sulfur to sulfoxide **22** by KBr and $Pb(OAc)_4$.[17] After the nucleophilic substitution was carried out, the crude intermediate sulfoxides (only **28** was purified and characterized) thus formed were then easily reduced with PBr_3[18] or PCl_3 in DMF to give **27**, **30–33**, **35**, and **36** (Scheme III).

Table II. Ethyl 7-Oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]-benzothiazine-6-carboxylate Derivatives

compd[a]	mp, °C	crystn solvent	yield, %	formula[b]
9a	215–217	EtOH	46.7	$C_{14}H_{11}Cl_2NO_3S$
9b	226–229	EtOH	36.8	$C_{14}H_{12}FNO_3S$
9c	254–256	EtOH	55.0	$C_{14}H_{11}ClFNO_3S$
9d	245–248	EtOH	62.4	$C_{14}H_{11}ClFNO_3S$
9e	238–240	pyridine	48.2	$C_{14}H_{12}ClNO_3S$
9f	184–187	EtOH	67.7	$C_{14}H_{13}NO_3S$
9g	248–250	EtOH	49.9	$C_{15}H_{12}F_3NO_3S$

[a] See Scheme I. [b] See Table I, footnote *c*.

Sulfones **11b**, **11c**, and **11f** were prepared by peracetic acid oxidation of **10b**, **10c**, and **10f** (Scheme I), while **29** was obtained from sulfone **11c** by using pyrrolidine in Me_2SO (Scheme III).

Biological Results and Discussion

The in vitro antibacterial activity of pyridobenzothiazine acids against recent clinical isolates of Gram-positive (*Staphylococcus aureus* 18773) and Gram-negative bacteria (*Escherichia coli* 15, *Pseudomonas aeruginosa* 2437, *Proteus morganii* 27, *Klebsiella pneumoniae* 4, and *Enterobacter cloacae* 041) is shown in Table IV. The antibacterial activity of norfloxacin, ofloxacin, and pipemidic acid are included for comparison. The structure–activity relationships (SARs) of pyridobenzothiazine compounds indicate that the presence of a fluorine atom at C-9 (**10b**) increases activity against both Gram-positive and Gram-negative bacteria (compare with **10f**). The substitution of fluorine with chlorine (**10e**) or cyclic bases (**12–18**) results in a reduction or in a complete loss of activity (compare with **10b**). The simultaneous presence of fluorine at C-9 and chlorine (**10c**) or cyclic amines at C-10 (**27**, **30–36**) generally gives the most active compounds. Among these, **10c** and the piperazinyl derivatives **30** and **31** show the best and most balanced activity. Antibacterial activity decreases in the piperazinyl derivatives as the size of the 4-substituent increases. The pyrrolidine derivative **27** exhibits potent activity against *S. aureus*. The introduction of substituents at C-8 (**10g**, **10d**, **19–21**) results in a complete loss of activity and thus confirms that such a position should not be substituted.[19] When a base is substituted at C-9 and chlorine is present at C-10 (**23–26**),

(13) Note added in proof: Further examples of antibacterial compounds containing the pyridobenzothiazine nucleus have been reported by Chu, D. T. (a) U.S. Patent 4 528 285; *Chem. Abstr.* **1985**, *103*, 178 270. (b) U.S. Patent 4 529 725; *Chem. Abstr.* **1986**, *104*, 5885. (c) U.S. Patent 4 533 663; *Chem. Abstr.* **1986**, *104*, 19 598.

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(15) Coutts, R. T.; Pound, N. J. *Can. J. Chem.* **1970**, *48*, 1859.

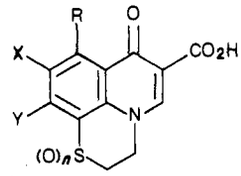
(16) An interpretation of this interesting regiospecific change, conditioned by the solvent, should require a detailed mechanistic investigation, which is beyond the scope of the present work.

(17) Casalini, C.; Cesarano, G.; Mascellani, G. *Anal. Chem.* **1977**, *49*, 1002.

(18) Kukolja, S.; Lammert, S. R.; Gleissner, M. R. B.; Ellis, A. I. *J. Am. Chem. Soc.* **1976**, *98*, 5040.

(19) (a) Minami, S.; Shono, T.; Matsumoto, J. *Chem. Pharm. Bull.* **1971**, *19*, 1482. (b) Mitscher, L. A.; Gracey, H. E.; Clark, G. W., III; Suzuki, T. *J. Med. Chem.* **1978**, *21*, 485.

Table III. 7-Oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid Derivatives



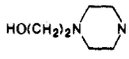
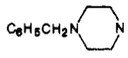
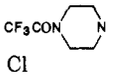
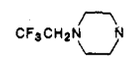
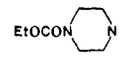
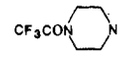
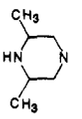
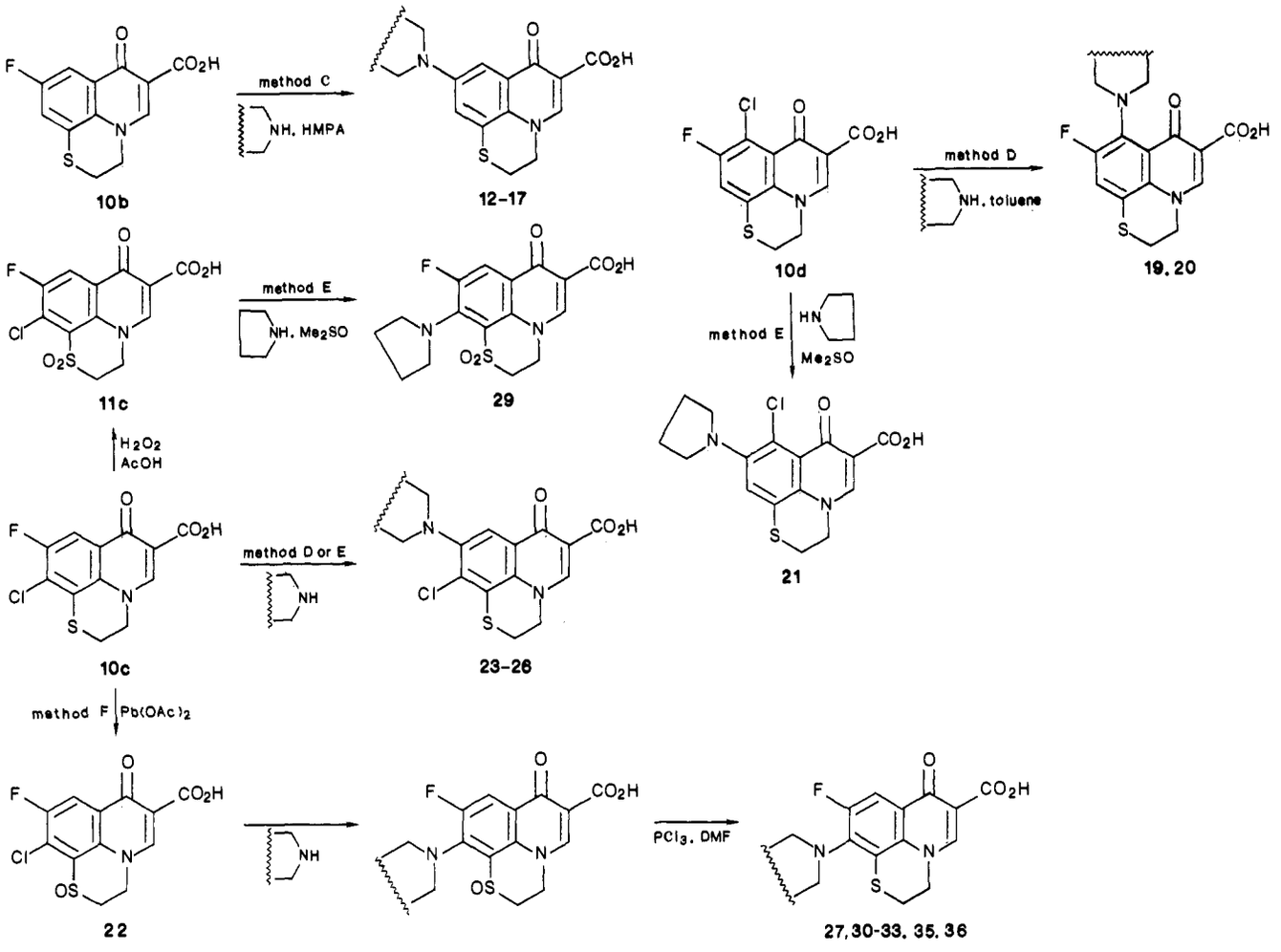
no.	R	X	Y	*n*	meth[a]	mp, °C	crystn solvent	yield, %	formula[b]
10f	H	H	H	0		291–293	AcOH	59.9	$C_{12}H_9NO_3S$
11f	H	H	H	2		322	AcOH	70.8	$C_{12}H_9NO_5S$
10g	CF_3	H	H	0		318–319	DMF	80.2	$C_{13}H_8F_3NO_3S$
10e	H	Cl	H	0		297–300	DMF	70.0	$C_{12}H_8ClNO_3S$
10b	H	F	H	0		304–306	AcOH	92.0	$C_{12}H_8FNO_3S$
11b	H	F	H	2		323–324	AcOH	35.9	$C_{12}H_8FNO_5S$
12	H		H	0	C	313–314	DMF	85.7	$C_{16}H_{16}N_2O_3S$
13	H		H	0	C	322–324	DMF	70.5	$C_{16}H_{16}N_2O_4S$
14	H		H	0	C	>340	AcOH–H_2O	73.0	$C_{16}H_{17}N_3O_3S$·HCl
15	H		H	0	C	275–276	DMF	76.9	$C_{17}H_{19}N_3O_3S$
16	H		H	0	C	263–265	DMF–EtOH	76.5	$C_{18}H_{21}N_3O_4S$
17	H		H	0	C	276–278	pyridine	52.3	$C_{23}H_{23}N_3O_3S$
18	H		H	0		>340	pyridine	83.3	$C_{18}H_{16}F_3N_3O_4S$
10a	H	Cl	Cl	0		320–322	DMF	80.2	$C_{12}H_7Cl_2NO_3S$
10d	Cl	F	H	0		325–327	pyridine	76.0	$C_{12}H_7ClFNO_3S$
19		F	H	0	D	298–301	DMF	81.0	$C_{16}H_{15}FN_2O_3S$
20		F	H	0	D	312–314	pyridine	65.3	$C_{16}H_{16}FNO_3S$
21	Cl		H	0	E	287–290	AcOH	63.0	$C_{16}H_{15}ClN_2O_3S$
10c	H	F	Cl	0		311–313	AcOH	78.7	$C_{12}H_7ClFNO_3S$
22	H	F	Cl	1	Fa	295–298	AcOH	53.8	$C_{12}H_7ClFNO_4S$
11c	H	F	Cl	2		321–323	DMF	45.3	$C_{12}H_7ClFNO_5S$
23	H		Cl	0	D, E	288–291	DMF	50.0	$C_{16}H_{15}ClN_2O_3S$
24	H		Cl	0	D, E	332–334	DMF	68.2	$C_{16}H_{15}ClN_2O_4S$
25	H		Cl	0	D, E	>340	AcOH	61.0	$C_{16}H_{16}ClN_3O_3S$
26	H		Cl	0	D, E	310–312	DMF	63.0	$C_{17}H_{18}ClN_3O_3S$
27	H	F		0	F	290–292	DMF	74.5	$C_{16}H_{15}FN_2O_3S$
28	H	F		1	Fb	253–255	AcOH	65.7	$C_{16}H_{15}FN_2O_4S$
29	H	F		2	E	286–289	EtOH–H_2O	41.0	$C_{16}H_{15}FN_2O_5S$·C_4H_9N[c]
30	H	F		0	E	335–336	EtOH–H_2O	89.0	$C_{16}H_{16}FN_3O_3S$·HCl
31	H	F		0	E	322–324	EtOH–H_2O	59.0	$C_{17}H_{18}FN_3O_3S$·HCl
32[d]	H	F		0	E	291–293	DMF	43.0	$C_{18}H_{17}F_4N_3O_3S$
33	H	F		0	E	313–315	DMF	86.2	$C_{19}H_{20}FN_3O_5S$
34	H	F		0		309–312	DMF	79.4	$C_{18}H_{15}F_4N_3O_4S$

Table III (Continued)

no.	R	X	Y	*n*	meth[a]	mp, °C	crystn solvent	yield, %	formula[b]
35	H	F		0	E	339–341	DMF	51.3	$C_{18}H_{20}FN_3O_3S$
36	H	F		0	E	329–332	DMF	86.0	$C_{16}H_{15}FN_2O_3S_2$

[a] See Experimental Section. [b] See Table I, footnote *c*. [c] Pyrrolidine. [d] The trifluoroethylpiperazine intermediate of **32** was prepared according to ref 27.

Scheme III



activity is markedly reduced. It seems that sulfur should not be oxidated as in such a case sulfones **11b**, **11c**, and **11f** became inactive while sulfone **29** and sulfoxide **28** show a reduced activity. This loss of activity could depend on a presumed steric hindrance. None of the ethyl esters corresponding to the carboxylic acids prepared showed in vitro antibacterial activity. Compounds **10c**, **30**, and **31** possess a more potent activity than pipemidic acid, and the last two show a potency and a broad spectrum of antibacterial activity that nearly match those of norfloxacin and ofloxacin.

Preliminary pharmacokinetic studies were carried out on compounds **10c**, **30**, and **31**. The pharmacokinetic data after a single dose of compound **31** (MF-934) are available for some animal species and for humans (Tables V and VI). The drug was administered by oral and by ip route in rat and by oral route in dog and monkey, suspended in carboxymethyl cellulose (CMC). In humans, capsules were administered at three doses. Blood and tissues were sampled in rats after killing them; in dogs, monkeys, and humans, venous blood was withdrawn into heparinized test tubes and plasma was obtained by centrifugation at low speed. Urine was collected by placing rats, dogs, and monkeys in metabolic cages.

It is interesting to point out that the drug shows a long half-life in all the species so far studied, that in rat the tissue levels are higher than the plasma levels,[20] that the bioavailability (in rat) is very high, and that the urinary excretion is about 25% of the dose in rat and about 50% of the dose in humans. Most of the drug should undergo extensive biotransformation; in rat, the urinary recovery determined by microbiological assay is higher than that of the unmodified drug, thus indicating the presence of

(20) Fravolini, A.; Terni, P.; Mascellani, G.; Pagella, P. G.; Segre, G. *Recent Advances in Chemotherapy*, Proceedings of the 14th International Congress of Chemotherapy, Kyoto, 1985; pp 640–641.

Table IV. In Vitro Antibacterial Activity (MIC, μg/mL)[a]

compd	*S. aureus* 18773	*E. coli* 15	*P. aeruginosa* 2437	*P. morganii* 27	*K. pneumoniae* 4	*E. cloacae* 041
10f	25	6.25	50	12.5	25	25
11f	>100	100	100	>100	>100	>100
10g	>50	>50	100	>50	>50	>50
10e	25	3.12	>100	6.25	6.25	12.5
10b	6.25	0.78	50	3.12	3.12	6.25
11b	>50	100	>50	NT[b]	NT	NT
12	50	50	100	NT	NT	NT
13	>50	>50	6.25	>50	>50	>50
14	>50	>50	50	50	50	50
15	100	100	>100	100	NT	NT
16	100	100	>100	>50	NT	NT
17	50	50	>50	>50	NT	NT
18	50	>50	100	>50	NT	NT
10a	0.78	>50	>50	1.56	12.5	>50
10d	>50	>50	>50	>50	>50	>50
19	>100	>100	NT	NT	>100	NT
20	>100	>100	>100	>100	>100	>100
21	100	>100	>100	>100	>100	NT
10c	0.39	0.39	>25	6.25	0.78	0.39
22	>100	>100	>100	>100	>100	>100
11c	NT	>50	NT	NT	NT	NT
23	>50	>50	>50	>50	>50	>50
24	>50	6.25	6.25	>50	>50	>50
25	>50	>50	>50	>50	>50	>50
26	>50	>50	6.25	>50	>50	>50
27	<0.39	1.56	3.12	3.12	1.56	1.56
28	12.5	12.5	>50	>50	25	50
29	3.12	3.12	>50	>50	25	25
30	0.78	0.39	12.5	1.56	<0.39	<0.39
31	0.78	0.78	12.5	1.56	<0.39	<0.39
32	0.39	>50	>50	>50	>50	>50
33	0.39	6.25	>50	>50	6.25	6.25
34	1.56	<0.39	12.5	1.56	<0.39	<0.39
35	3.12	6.25	100	25	6.25	3.12
36	0.78	3.12	>50	6.25	1.56	1.56
NOR[c]	0.19	0.19	1.56	0.78	0.19	0.39
OFL[d]	0.19	0.19	1.56	0.78	0.39	0.19
PPA[e]	3.12	0.39	>25	3.12	0.78	0.78

[a] See Experimental Section. [b] NT, not tested. [c] Norfloxacin. [d] Ofloxacin. [e] Pipemidic acid.

Table V. Pharmacokinetic Parameters of a Single Dose of MF-934 in Rat

dose, mg/kg	plasma peak, μg/mL	plasma t_{max}, h	plasma $t_{1/2}$, h	plasma V_d,[a] L/kg	urinary excretn, % of dose	clearance, mL h^{-1} kg^{-1} Cl_u[b]	clearance, mL h^{-1} kg^{-1} Cl_t[c]
10 ip			9.9	3	26.0	45.0	253
25 ip			9.9	3	22.2	47.4	251
25 os	6	1	8.7		24.5	72.6	
50 os	8	1	8.7		16.7		
100 os	21	1	8.7				

Tissue and Plasma Levels (μg/mL) after 50 mg/kg po

			tissue/plasma ratio	
	at 1 h	at 15 h	at 1 h	at 15 h
liver	80	11	8	3.7
kidney	61	8	6	2.7
heart	31	5	3	1.7
brain	0	0	0	0
plasma	10	3		

Bioavailability at 25 mg/kg

from AUC^d_{os}/AUC_{ip}	0.85
from urinary excretion os/ip	0.91

[a] Apparent volume of distribution. [b] Urinary clearance. [c] Total clearance. [d] Area under the curve.

active metabolite(s). In humans, a secondary plasma peak points to a noteworthy enterohepatic recirculation.

After a single oral administration, the urinary excretion (24 h) of compound **31** in rat was 63% and 74% of the dose as microbiologically active compounds in two separate experiments.

Compounds **10c** and **30** were either sparingly absorbed or inactivated by metabolic pathways, their urinary recovery being less than 15% as active compounds.[21]

Plasma protein binding of compound **31** is equal to 79.6% at 5 μg/mL and to 58.1% at 30 μg/mL.

Previous studies of ofloxacin in rats indicate a biological half-life of 1.1 h and a cumulative urinary recovery of

(21) Cecchetti, V.; Fravolini, A.; Mattina, R.; Pagella, P. G.; Palmioli, M.; Rugarli, P. L.; Terni, P. 5th National Meeting of Pharmaceutical Chemistry of Italian Chemical Society, Rimini, Italy, May 1985; Abstract S 34.

Table VI. Pharmacokinetic Parameters after Single Dose of MF-934 in Dog, Monkey, and Human

species	oral dose, mg/kg	plasma			urine	
		peak, μg/mL	t_{max}, h	$t_{1/2}$, h	Cl_u,[a] mL h^{-1} kg^{-1}	urinary excretn, % of dose
dog (beagle)	20	5.7	4	12		8–10
monkey (*Macaca fascicularis*)	20	5.5–6	2	50		
human	100	1.87	4	30	5.6	45
	200	1.92	4	35	14.3	55
	400	2.73	2	32	13.6	33

[a] See Table V, footnote *b*.

39.4% of the dose within 24 h after oral dosing.[22]

Compound **31** possesses a low toxicity after single administration to the rat, the median lethal dose (LD_{50}) values being as follows: rat, 285 mg/kg iv, 631 mg/kg po (male), and 501 mg/kg po (female); mouse, 224 mg/kg iv; rabbit, 660 mg/kg po.

As a result of these studies, compound **31** was found to possess broad and potent in vitro antibacterial activity against Gram-positive and Gram-negative bacteria, a low acute toxicity, and a favorable kinetic behavior in the experimental animal. These findings suggest the possible use of compound **31** as an oral antibacterial agent useful not only as a urinary antiseptic but also in the treatment of systemic infections.

An extensive description of the antibacterial and pharmacokinetic profiles of **31** will be reported in subsequent papers.

Experimental Section

Melting points were determined in capillary tubes (Büchi melting point apparatus) and are uncorrected. All compounds were analyzed for C, H, and N, and the analytical values are within ±0.4% of the theoretical values. ^1H NMR spectra were recorded on a 90-MHz Varian EM 390 spectrometer using Me_4Si as internal standard, and chemical shifts are given in ppm (δ). ^1H NMR spectra of all compounds obtained were consistent with assigned structures. IR spectra were recorded on a Perkin-Elmer Model 247 instrument. HPLC analyses were carried out with a Gilson liquid chromatograph. Column chromatography separations were carried out on Merck silica gel 40 (mesh 70–230) and flash chromatography was carried out on Merck silica gel 60 (mesh 230–400). Yields are of purified products and are not optimized. The characteristics of synthesized compounds are summarized in Tables I, II, and III. The oils **8a–c,e** are characterized by ^1H NMR data.

***S*-(2,3-Dichloro-6-nitrophenyl)thioglycolic Acid (1).** To a solution of 1.76 g (0.044 mol) of NaOH in 15 mL of H_2O cooled to 10 °C was added 2 g (0.022 mol) of thioglycolic acid. The resulting solution was then treated portionwise with 5 g (0.022 mol) of 2,3,4-trichloronitrobenzene dissolved in 40 mL of EtOH, while the temperature was maintained below 15–20 °C. Successively, the mixture was refluxed for 40 min and then poured into ice–water, acidified with HCl, and extracted with EtOAc. The organic phases were combined, washed with water, and dried over sodium sulfate. The solvent was evaporated to give 4.7 g (75.6%) of a yellow oil, which then solidified. Crude **1** was used in the successive reaction without purification.

7,8-Dichloro-3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine (2). To a solution of the above acid (4.7 g) in NH_4OH (30 mL) was added an aqueous solution of $FeSO_4 \cdot 7H_2O$ (33 g dissolved in 100 mL of water) with stirring for 15 min. A black precipitate was separated during addition. The reaction mixture was heated on a water bath for 1 h and then cooled and filtered. The filtrate was acidified with dilute HCl and the resulting solid filtered off, washed with water, dried, and recrystallized from EtOH to give 2.6 g (66.7%) of **2**, mp 251–253 °C (lit.[15] mp 249–251 °C).

2-Amino-7-chloro-6-fluorobenzothiazole (4) and 2-Amino-5-chloro-6-fluorobenzothiazole (5). 3-Chloro-4-fluoroaniline (30 g, 0.200 mol), KSCN (40 g, 0.412 mol), and 500 mL of AcOH were combined in a flask with continuous mechanical stirring. Bromine (48 g, 0.300 mol) in 500 mL of AcOH was added dropwise to this mixture, the temperature being kept below 30–35 °C throughout the addition. Stirring was continued for an additional 1 h after the bromine addition. The filtered solution was basified with NH_4OH, and the precipitated solid was collected and washed with water to give a 21-g (50.3%) mixture of two benzothiazole isomers, **4** and **5**. The crude mixture was used in the next reaction.

The mixture of **4** and **5** was chromatographed by silica gel (eluent benzene–EtOAc, 1/1) to obtain analytical samples. Compound **4**: mp 189–192 °C; ^1H NMR (Me_2SO-d_6) δ 6.93–7.28 (2 H, m, aromatic protons), 7.60 (2 H, br s, NH_2). Anal. ($C_7H_4ClFN_2S$) C, H, N. Compound **5**: mp 217–220 °C; ^1H NMR (Me_2SO-d_6) δ 7.55 (1 H, d, $J_{H\text{-}F}$ = 6 Hz, H-4), 8.00 (1 H, d, $J_{H\text{-}F}$ = 9 Hz, H-7), and 9.75 (2 H, br s, NH_2). Anal. ($C_7H_4ClFN_2S$) C, H, N.

8-Chloro-7-fluoro-3,4-dihydro-2*H*-1,4-benzothiazine (8c) and 6-Chloro-7-fluoro-3,4-dihydro-2*H*-1,4-benzothiazine (8d). The crude mixture of **4** and **5** (21 g) was refluxed with 300 mL of 50% NaOH until the evolution of ammonia ceased (about 24 h). After cooling, the reaction mixture was filtered with charcoal and the resulting clear solution acidified with AcOH. The collected precipitate was washed with water, dried, and extracted with boiling EtOH. To the reduced ethanol extract (about 80 mL) were added 6 g of NaOH and 2 mL of water, and the resulting solution was refluxed for 20 min.

Method A. (a) The above solution was added to a solution of monochloroacetic acid (10 g) in 10 mL of water and then refluxed for 1 h. The reaction mixture was then poured into ice–water and acidified with HCl. The separated solid was collected, washed with water, and then dried to give 10.30 g of a mixture, which was separated by flash chromatography eluting with hexane–EtOAc, 7/3, yielding 4.0 g (17.8%) of 8-chloro-7-fluoro-3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine (**6**) and 4.5 g (20.0%) of 6-chloro-7-fluoro-3-oxo-3,4-dihydro-2*H*-1,4-benzothiazine (**7**). Compound **6**: crystallized from EtOH; mp 211–213 °C; ^1H NMR (Me_2SO-d_6) δ 3.60 (2 H, s, SCH_2), 6.85 (1 H, dd, J = 9 Hz and $J_{H\text{-}F}$ = 6 Hz, H-5), 7.15 (1 H, t, J = 9 Hz and $J_{H\text{-}F}$ = 9 Hz, H-6), and 10.58 (1 H, br s, NH). Anal. ($C_8H_5ClFNOS$) C, H, N. Compound **7**: crystallized from EtOH; mp 220–222 °C; ^1H NMR (Me_2SO-d_6) δ 3.48 (2 H, s, SCH_2), 7.00 (1 H, d, $J_{H\text{-}F}$ = 7.5 Hz, H-5), 7.38 (1 H, d, $J_{H\text{-}F}$ = 9 Hz, H-8), and 10.50 (1 H, br s, NH). Anal. ($C_8H_5ClFNOS$) C, H, N.

According to this procedure, 7-fluoro-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine (**3**) was obtained by starting from 2-amino-6-fluorobenzothiazole.[23]

(b) A solution of **6** (6.5 g, 0.030 mol) in dry THF (500 mL) was added dropwise to a suspension of $LiAlH_4$ (1.55 g, 0.040 mol) in dry THF (50 mL). After the addition was complete, the mixture was left at room temperature for 1 h; dilute HCl 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 chloroform extract was washed with water, dried, and evaporated to dryness, yielding a red oil, which was purified by silica gel column chromatography eluting with $CHCl_3$ to give **8c** (90.0%).

According to this procedure, derivatives **8a**, **8d**, and **8e** were prepared, respectively, by starting from **2**, **7**, and the known 7-chloro-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine.[24]

(22) Osada, Y.; Tsumura, M.; Tachizawa, H.; Uüe, T.; Sano, M. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 1981; Abstract N. 562.

(23) Jackson, F. H.; Peters, A. T. *J. Chem. Soc. C* **1969**, 268.

Method B. In a procedure similar to that described in the preliminary procedure to method A, 13.5 g of 1-bromo-2-chloroethane was added to the obtained alkaline solution and the mixture was heated to reflux for 2 h, poured into ice–water, and extracted with $CHCl_3$. The extract was washed with water and dried, and the solvent was evaporated in vacuo. The oil residue was chromatographed on silica gel with benzene–hexane, 7/3, as eluent to give **8c** (19.2%) as an oil and **8d** (28.8%), mp 88–92 °C. Compounds **8c** and **8d**: Anal. (C_8H_7ClFNS) C, H, N.

Compound **8b** was obtained following both methods A and B by starting from 2-amino-6-fluorobenzothiazole.[23]

Ethyl 10-Chloro-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylate (9c). A mixture of **8c** (7 g, 0.034 mol) and 10 g of diethyl (ethoxymethylene)-malonate (EMME) was heated for 2 h at 120 °C (bath temperature). Polyphosphoric acid (35 g) was added, and the mixture was then gradually heated to 160 °C and kept at that temperature for 1 h. After cooling, the reaction mixture was poured into ice–water, and the precipitate was separated by filtration, washed with 10% $NaHCO_3$ and water, and then recrystallized with EtOH to give 6.19 g (55.0%) of **9c**, mp 254–256 °C. Anal. ($C_{14}H_{11}ClFNO_3S$) C, H, N.

According to this procedure, compounds **9a,b,d–g** were prepared from **8a,b,d,e**, **8f**,[25,26] and **8g**.[25]

10-Chloro-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (10c). A stirred suspension of **9c** (1.5 g) in 50 mL of 15% NaOH was refluxed for 45 min. The cooled mixture was acidified with HCl, and the precipitate was filtered off and washed with water and EtOH. Recrystallization from AcOH yielded **10c** (78.7%): mp 311–313 °C; ^1H NMR (TFA) δ 3.58–3.78 (2 H, m, SCH_2), 5.05–5.25 (2 H, m, NCH_2), 8.23 (1 H, d, J_{H-F} = 9 Hz, H-8), and 9.40 (1 H, s, H-5). Anal. ($C_{12}H_7ClFNO_3S$) C, H, N.

Carboxylic acid derivatives **10a,b,d–g** were made in similar fashion from **9a,b,d–g**.

Method C. 9-(1-Pyrrolidinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (12). Compound **10b** (0.8 g, 0.003 mol) was suspended in 12 mL of HMPA, and 2 g (0.028 mol) of pyrrolidine was added to the suspension. The mixture was then allowed to react at 150 °C for 3 h. After cooling, the precipitated solid was filtered, washed with MeOH, and recrystallized from DMF, giving 0.65 g (85.7%) of **12**: mp 313–314 °C; ^1H NMR (TFA) δ 2.32–2.68 (4 H, m, CH_2CH_2 pyrrolidine), 3.47–3.70 (2 H, m, SCH_2), 3.92–4.23 (4 H, m, CH_2NCH_2 pyrrolidine), 4.97–5.20 (2 H, m, NCH_2), 8.18 and 8.55 (each 1 H, d, J = 2.4 Hz, H-8 and H-10), and 9.38 (1 H, s, H-5). Anal. ($C_{16}H_{16}N_2O_3S$) C, H, N.

Compounds **13–17** were prepared from **10b** with an excess of the appropriate amines according to this procedure.

Method D. 9-Fluoro-8-(1-pyrrolidinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (19). A mixture of 0.6 g (0.002 mol) of **10d** and 2 g (0.028 mol) of pyrrolidine in 15 mL of toluene was refluxed for 40 h. The mixture was then cooled and filtered to remove insoluble material. The filtrate was evaporated to dryness in vacuo. EtOH was added to the residue, and the separated solid was collected by filtration, washed with EtOH, and crystallized from DMF to provide 0.54 g (81.0%) of **19**: mp 298–301 °C; ^1H NMR (TFA) δ 2.40–2.75 (4 H, m, CH_2CH_2 pyrrolidine), 3.50–3.70 (2 H, m, SCH_2), 3.75–3.98 (2 H, m, CH_2NCH_2 pyrrolidine), 4.08–4.33 (2 H, m, CH_2NCH_2 pyrrolidine), 4.87–5.07 (2 H, m, NCH_2), 7.87 (1 H, d, J_{H-F} = 12 Hz, H-10), and 9.20 (1 H, s, H-5). Anal. ($C_{16}H_{15}FN_2O_3S$) C, H, N.

In the same manner, compound **20** was prepared from **10d**.

Method E. 8-Chloro-9-(1-pyrrolidinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (21). A mixture of **10d** (1 g, 0.003 mol) and 2 g (0.028 mol) of pyrrolidine in 10 mL of Me_2SO was heated at 130 °C for 30 min and then allowed to cool. The precipitate was filtered, washed with EtOH, and recrystallized from AcOH to give 0.73 g (63.0%) of **21**: mp 287–290 °C; ^1H NMR (TFA) δ 2.43–2.68 (4 H, m, CH_2CH_2 pyrrolidine), 3.50–3.70 (2 H, m, SCH_2), 4.03–4.28 (4 H, m, CH_2NCH_2 pyrrolidine), 4.90–5.10 (2 H, m, NCH_2), 8.15 (1 H, s, H-10), and 9.25 (1 H, s, H-5). Anal. ($C_{16}H_{15}ClN_2O_3S$) C, H, N.

Via this procedure, compound **29** was obtained from **11c**.

10-Chloro-9-(1-pyrrolidinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic acid (23) was prepared from **10c** according to method D or method E.

Compounds **24–26** were prepared in the same manner.

Method F. (a) 10-Chloro-9-fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid 1-Oxide (22). To a suspension of **10c** (3 g, 0.010 mol) and KBr (0.7 g, 0.006 mol) in 300 mL of water and 1000 mL of AcOH was added dropwise at 30 °C under potentiometric control a 0.065 M acetic acid solution (170 mL) of $Pb(OAc)_4$. The solid, separated by solvent concentration, was crystallized from AcOH to give 1.7 g (53.8%) of sulfoxide **22**: mp 295–298 °C; IR (Nujol) 1049, 1029 cm^{-1} (SO); ^1H NMR (Me_2SO-d_6) δ 3.56–4.32 (2 H, m, SCH_2), 5.15–5.78 (2 H, m, NCH_2), 8.75 (1 H, d, J_{H-F} = 7.5 Hz, H-8), and 9.70 (1 H, s, H-5). Anal. ($C_{12}H_7ClFNO_4S$) C, H, N.

(b) 9-Fluoro-10-(1-pyrrolidinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid 1-Oxide (28). To a suspension of 0.66 g (0.002 mol) of **22** in toluene (10 mL) was added pyrrolidine (1.5 g, 0.020 mol), and the mixture was refluxed for 3 h and then cooled. The resulting precipitate was collected and crystallized from AcOH to yield 0.48 g (65.7%) of **28**: mp 253–255 °C; IR (Nujol) 1033 cm^{-1} (SO); ^1H NMR (TFA) δ 2.13–2.38 (4 H, m, CH_2CH_2 pyrrolidine), 3.48–4.58 (6 H, m, SCH_2 and CH_2NCH_2 pyrrolidine), 4.98–5.75 (2 H, m, NCH_2), 8.20 (1 H, d, J_{H-F} = 15 Hz, H-8), and 9.32 (1 H, s, H-5). Anal. ($C_{16}H_{15}FN_2O_4S$) C, H, N.

(c) 9-Fluoro-10-(1-pyrrolidinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (27). A solution of **28** (0.5 g, 0.0014 mol) in 75 mL of DMF was cooled to 0 °C, and 0.25 g (0.0018 mol) of PCl_3 was added. The reaction mixture was stirred for 2 h at room temperature and then diluted with water. The precipitate was collected, washed with water, dried, and crystallized from DMF, yielding 0.35 g (74.5%) of **27**: mp 290–292 °C; IR (Nujol) the SO group frequency disappears. Anal. ($C_{16}H_{15}FN_2O_3S$) C, H, N.

Compounds **30–33**, **35**, and **36** were prepared according to method F.

9-Fluoro-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid 1,1-Dioxide (11b). To a warm solution of **10b** (0.8 g, 0.003 mol) in 40 mL of AcOH was added dropwise 2 mL of 36% H_2O_2, and the reaction mixture was maintained in a water bath for 2 h and then allowed to stand at room temperature overnight. The precipitate was collected and recrystallized from AcOH to give 0.32 g (35.9%) of **11b**, mp 323–324 °C. Anal. ($C_{12}H_8FNO_5S$) C, H, N.

Compounds **11c** and **11f** were prepared from **10c** and **10f**, respectively, via this procedure.

9-[4-(Trifluoroacetyl)-1-piperazinyl]-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (18). Compound **14** (0.42 g) was added to an excess of trifluoroacetic anhydride, and the mixture was refluxed for 2 h. The reaction mixture was then concentrated to dryness in vacuo, and 40 mL of water was added to the oily residue. The solid precipitate was collected and crystallized from pyridine to give 0.45 g (83.3%) of **18**, mp 340 °C. Anal. ($C_{18}H_{16}F_3N_3O_4S$) C, H, N.

Compound **34** was prepared from **30** in the same manner.

In Vitro Antibacterial Activity. Antibacterial activity was determined by agar dilution assay using a multipoint inoculator. Isosensitest agar (Oxoid) (20 mL in a Petri dish) was used. Test compounds were dissolved and incorporated by the twofold dilution method in the agar medium. Bacterial inocula, coming from overnight broth and containing 10^5 colony-forming units per point, were inoculated by multipoint inoculator. Bacterial growth was observed after 18 h incubation at 37 °C. The lowest concentration of test compounds that completely inhibited growth was considered to be the minimal inhibitory concentration (MIC).

Acute Toxicity Tests. Male Wistar rats weighing 130–150 g were orally or intravenously treated with at least three different

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doses with the test compound suspended in CMC (1%) or dissolved in water, respectively. Male Swiss mice weighing 30 g were treated intravenously, and male New Zealand rabbits weighing about 10 kg received the drug by oral administration. The number of dead animals was counted after 14 days, and the LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[28]

Pharmacokinetic Tests. Groups of fasted male Wistar rats weighing 180–230 g received the drug by oral and ip routes. Four male beagle dogs and two monkeys (*Macaca fascicularis*) received the drug by oral route. Four healthy volunteers of both sexes received the drug in capsules of 100 mg. Plasma and urine samples in the animals and in humans and tissue homogenates in rat were assayed by an HPLC method. Some sample of rat urine was also assayed by a microbiological method.

Microbiological Assay of the Urine. Compounds **10c**, **30**, and **31** were orally administered to rats (50 mg/kg), and urinary recoveries were evaluated within 24 h after dosing. Quantitative determinations of microbiologically active compounds in the urine were made according to Bennet,[29] with *K. pneumoniae* 4 as bioassay organism.[21]

HPLC Assay in the Plasma and Organs. Compound **31** was administered to rats by oral and ip routes at various doses, and the heparinized plasma was withdrawn at various times after drug administration. The unmodified compound was determined in plasma, urine, and homogenized organs according to the following procedure. *N*-Dimethyldiazepam (2 μg; internal standard), 1 mL of citrate buffer (0.1 M, pH 6.2), and 6 mL of $CHCl_3$ were added to 1 mL of plasma or homogenized organs. After mixing for 20 min and centrifugation at 3000 rpm for 5 min, the organic layer was separated and dried in a 70 °C water bath. The residue was recovered with 50 μL of MeOH, 20 μL of which were then injected into a HPLC μ-Bondapak C-18 (Waters) column: eluent AcOH (0.1%) (A)–MeOH (B); gradient outline 60%, 70%, and 60% of B at 1, 10, and 18 min, respectively; flow rate 0.7 mL/min; effluent monitored at 245 nm (UV detector). The retention times were 7.7 min for compound **31** and 13.8 min for the internal standard. The coefficients of interassay and intraassay variability were about 10%.

Registry No. **1**, 106016-79-9; **2**, 106016-80-2; **3**, 100638-20-8; **4**, 101337-93-3; **5**, 101337-92-2; **6**, 101337-95-5; **7**, 106016-81-3; **8a**, 106016-82-4; **8b**, 106016-85-7; **8c**, 101337-96-6; **8d**, 106016-83-5; **8e**, 106016-84-6; **8f**, 3080-99-7; **8g**, 6431-65-8; **9a**, 106016-86-8; **9b**, 106016-87-9; **9c**, 101337-97-7; **9d**, 106016-88-0; **9e**, 106039-46-7; **9f**, 106016-89-1; **9g**, 106016-90-4; **10a**, 106016-91-5; **10b**, 106016-92-6; **10c**, 101337-81-9; **10d**, 106016-93-7; **10e**, 106016-94-8; **10f**, 106016-95-9; **10g**, 106016-96-0; **11b**, 106017-12-3; **11c**, 101337-82-0; **11f**, 106017-13-4; **12**, 106016-97-1; **13**, 106016-98-2; **14**, 106016-99-3; **14** (free base), 106017-14-5; **15**, 106017-00-9; **16**, 106017-01-0; **17**, 106017-02-1; **18**, 106017-03-2; **19**, 106017-04-3; **20**, 106017-05-4; **21**, 106017-06-5; **22**, 101337-84-2; **23**, 106017-07-6; **24**, 106017-15-6; **25**, 106017-16-7; **26**, 106039-47-8; **27**, 85741-48-6; **28**, 101337-85-3; **29**, 101337-83-1; **29**·C_4H_9N, 102052-49-3; **30**, 102052-48-2; **30** (free base), 101337-87-5; **31**, 106017-08-7; **31** (free base), 101363-10-4; **32**, 106017-09-8; **33**, 101337-99-9; **34**, 101337-88-6; **35**, 106017-10-1; **36**, 106017-11-2; EMME, 87-13-8; 2,3,4-trichloronitrobenzene, 17700-09-3; thioglycolic acid, 68-11-1; 3-chloro-4-fluoroaniline, 367-21-5; 2-amino-6-fluorobenzothiazole, 348-40-3; 7-chloro-3,4-dihydro-3-oxo-2*H*-[1,4]benzothiazine, 5333-05-1; 1-bromo-2-chloroethane, 107-04-0; pyrrolidine, 123-75-1; morpholine, 110-91-8; piperazine, 110-85-0; 1-methylpiperazine, 109-01-3; 1-piperazineethanol, 103-76-4; 1-benzylpiperazine, 2759-28-6; *N*-(1,1,1-trifluoroethan-2-yl)piperazine, 13349-90-1; 1-(ethoxycarbonyl)piperazine, 120-43-4; 2,6-dimethylpiperazine, 108-49-6; tetrahydrothiazine, 123-90-0.

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Redox Chemistry of the 9-Anilinoacridine Class of Antitumor Agents

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9-Anilinoacridines bearing a 1′-NHR substituent on the anilino ring undergo facile, chemically reversible, two-electron oxidation to quinone diimines. The chemical and electrochemical oxidation of three groups of 9-anilinoacridines (1′-substituted derivatives, together with 3′-substituted analogues and acridine-substituted analogues of the clinical antileukemic drug amsacrine) have been studied and their redox potentials determined. For aniline-substituted derivatives, redox potentials ($E_{1/2}$) correlate well with substituent electronic properties, with electron-donating substituents facilitating oxidation. Substituents in the acridine ring have little effect on redox potentials, indicating minimal transmission of electronic effects from the acridine to the aniline rings. Although the broad class of 9-anilinoacridines show biological activity over a very wide range of structural variations, a 1′-NHR substituent is a common feature of the most active derivatives. Nevertheless, no clear quantitative relationships between redox potential and biological activity could be discerned, and the relevance of this redox chemistry to the mode of action of amsacrine and other 9-anilinoacridines remains unclear.

The 9-anilinoacridines have been extensively investigated as antitumor agents.[1-3] One derivative, amsacrine (**18**), has become a valuable clinical drug for the treatment of leukemia (reviewed in ref 4 and 5), and a second analogue (CI-921; **39**) has recently begun clinical trials.[6,7] The 9-anilinoacridines belong to the broad class of compounds known as DNA-intercalating agents, whose biological activity is probably due to their causation of double-strand DNA breaks;[8,9] amsacrine in particular is known to be a very potent inhibitor of the DNA nicking–closing enzyme topoisomerase II.[10,11] The aniline ring of amsacrine is readily and reversibly oxidized either chemically[12] or microsomally[13] to give the quinone diimine **40** (Scheme I), and this redox chemistry has been shown to play a major part in both its mammalian metabolism[12,13] and its ability

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