the clear hydrolysate was acidified with citric acid to pH 4.0. Upon chilling in the refrigerator for 4 h, cream-colored **4** separated and was collected by filtration, washed with water, and dried: yield 120 mg; UV (0.1 N NaOH) λ_{max} 325, 275, and 245 nm; NMR (TFA) δ 7.98 and 7.6 (2 d, 4 H, aromatic), 5.5 (s, 2 H, ring methylene), 4.41 (s, 2 H, bridge methylene), 3.9–2.2 (c, 4 H, glutamate). Anal. ($C_{19}H_{19}N_5O_7$) C, H, O.

The filtrate from which **4** was separated was deep yellow in color. This solution was adjusted to pH 7.0 with Na_2CO_3 and evaporated to ~20 mL; the pH was readjusted to 3.5 with citric acid, and the solution was refrigerated for 3 days. A bright yellow solid separated, which was washed quickly (moderately water soluble) with ice-cold water and dried: UV (0.1 N NaOH) λ_{max} 400, 250 nm; NMR (TFA) δ 7.9 and 7.75 (c, 5 H, 4 aromatic and 1 vinyl), 5.5 (s, 2 H, ring methylene), 4.9 (t, α proton of glutamate), 2.8–2.2 (c, 4 H, glutamate). This compound was found to be identical in all respects with the product **19** obtained by the glutamate coupling of **24**. Anal. ($C_{19}H_{19}N_5O_7$) C, H, N, O.

2-Amino-4-hydroxy-6-(*p*-carbomethoxybenzyl)-7,8-dihydro-8-oxapteridine (22). The synthesis of bromomethyl ketone **21** has been described previously from this laboratory.[8] In a three-necked round-bottomed flask, a mixture of 1.78 g (10 mmol) of **2**, 2.7 g (10 mmol) of bromo ketone **21**, and 400 mg of MgO was refluxed with 500 mL of MeOH in N_2 for 4 h. The reaction mixture was filtered hot, evaporated to ~100 mL, and chilled. Brown crystals of **22** were formed overnight; these were filtered, washed with water followed by minimum MeOH, and dried: yield 1.5 g (50%); NMR (TFA) δ 7.71 and 7.0 (2 d, 4 H, aromatic), 5.0 (s, 2 H, ring methylene), 4.25 (s, 3 H, carbomethoxy), 3.98 (s, 2 H, bridge methylene). Me_4Si was used as an external standard. Anal. ($C_{15}H_{14}N_4O_4 \cdot 0.5H_2O$) C, H, N.

2-Amino-4-hydroxy-6-(*p*-carboxybenzyl)-7,8-dihydro-8-oxapteridine (23). One gram of **22** was stirred with 300 mL of 0.1 N NaOH and 75 mL of MeCN in a 500-mL round-bottomed flask under nitrogen for 6 h. The clear solution was concentrated on a rotary evaporator to ~200 mL and filtered. The clear filtrate was acidified to pH 3.4 with glacial HOAc. A thick cream-colored precipitate that formed was filtered, washed with water, and dried: yield 750 mg; mp >300 °C; UV (0.1 N NaOH) λ_{max} 320, 270 nm; NMR (TFA) δ 7.76 and 7.70 (2 d, 4 H, aromatic), 5.02 (s, 2 H, ring methylene), 3.95 (s, 2 H, bridge methylene). Anal. ($C_{14}H_{12}N_4O_4 \cdot 0.5H_2O$) C, H, N.

***N*-[[(2-Amino-4-hydroxy-7,8-dihydro-8-oxa-6-pteridinyl)methenyl]benzoyl]-L-glutamate (19).** This reaction was done by dissolving 600 mg (2 mmol) of **23** in 50 mL of dry Me_2SO and activating the carboxyl group by the addition of 0.28 mL (2.5 mmol) of *N*-methylmorpholine, followed by 0.226 mL of freshly distilled isobutyl chloroformate. After 15 min, this solution was mixed with a solution of 960 mg (4 mmol) of diethyl glutamate hydrochloride in 20 mL of Me_2SO containing 0.45 mL (4 mmol) of *N*-methylmorpholine and stirred overnight. The solvent Me_2SO was distilled off in a vacuum at 80 °C and the bright-yellow residue was triturated with water and filtered. This crude product was hydrolyzed with 0.1 N NaOH (250 mL) and MeCN (30 mL) as described for **22**. Upon acidification of the hydrolysate, no precipitate was formed. The solution was adjusted to pH 7.5, diluted to 1 L, and chromatographed on DEAE-cellulose. Two bright-yellow compounds were eluted; the less polar was the isomer **25** of starting pteroic acid **23**, and the more polar glutamate conjugate was identified as the product **19**.

Biological Evaluation. The antimicrobial activities of these compounds were evaluated by procedures that have been published previously.[9,10] All assays with thymidylate synthase were performed according to the procedure of Friedkin.[11]

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Registry No. **2**, 40769-69-5; **2**·HCl, 56830-58-1; **3**, 85828-43-9; **4**, 85828-44-0; **6**, 1679-64-7; **7**, 56893-25-5; **8**, 85828-45-1; **9**, 85828-46-2; **10**, 85828-47-3; **11**, 1075-49-6; **12**, 85828-48-4; **13**, 85828-49-5; **14**, 85828-50-8; **15**, 85828-51-9; **16**, 85828-52-0; **17**, 85828-53-1; **18**, 85828-54-2; **19**, 85828-55-3; **21**, 85828-56-4; **22**, 85828-57-5; **23**, 85828-58-6; **25**, 85828-59-7; diethyl L-glutamate, 16450-41-2; dihydrofolate reductase, 9002-03-3.

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2-Haloethylating Agents for Cancer Chemotherapy. 2-Haloethyl Sulfonates

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Because certain (2-chloroethyl)triazenes and (2-haloethyl)nitrosoureas have high antineoplastic activity, 2-chloroethyl and 2-fluoroethyl sulfonates were prepared to try to develop additional types of 2-haloethylating agents. In this initial study, it was demonstrated that antineoplastic activity much superior to that of the prototype, 2-chloroethyl methanesulfonate, could be found among 2-chloroethyl sulfonates. Among a variety of 2-chloroethyl alkane- and arenesulfonates, several substituted methanesulfonates displayed significant activity against P388 leukemia in mice; the chloromethanesulfonate showed high activity (T/C = 218%). None of the arenesulfonates were active in this test.

In tests against transplanted leukemia L1210 in mice, 5-[3,3-bis(2-chloroethyl)-1-triazenyl]-1*H*-imidazole-4-carboxamide (BIC), the chloroethyl analogue of the clinical anticancer drug DTIC[1-3] (dacarbazine), effects many cures[4,5] and is very much superior to DTIC and to other imidazole and benzenoid triazenes lacking a chloroethyl group.[6,7] By analogy to the metabolism[8-11] of DTIC,

(1) DTIC = 5-(3,3-dimethyl-1-triazenyl)-1*H*-imidazole-4-carboxamide; MCIC = 5-[3-(2-chloroethyl)-1-triazenyl]-1*H*-imidazole-4-carboxamide; BFIC = 5-[3,3-bis(2-fluoroethyl)-1-triazenyl]-1*H*-imidazole-4-carboxamide; MFIC = 5-[3-(2-fluoroethyl)-1-triazenyl]-1*H*-imidazole-4-carboxamide.
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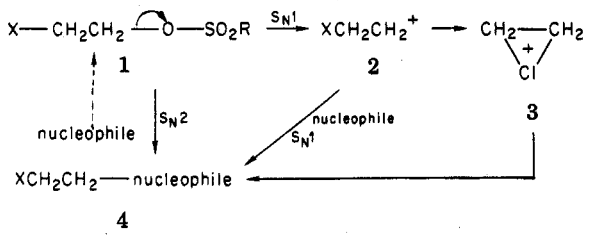
Scheme I

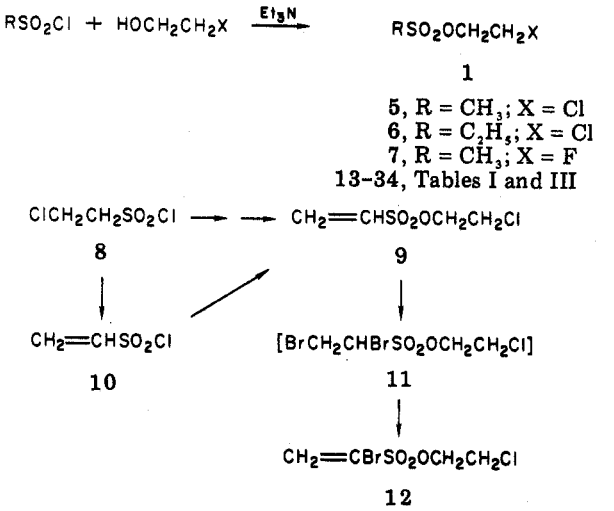


Shealy, O'Dell, and Krauth[12] postulated metabolic activation of BIC by dealkylation to the (chloroethyl)triazene (MCIC),[1] prepared the latter compound, found it to be at least as active in vivo as BIC, and showed that it dissociates in aqueous media to 5-amino-1*H*-imidazole-4-carboxamide (AIC), chloroethanol, and minor products, including a 2-chloroethyl derivative of AIC. Hill[13] confirmed that AIC is indeed formed by liver microsomal oxidases. These chemical, biochemical, and biological results indicate that BIC, after metabolic activation, and MCIC owe their high activity to the formation of chloroethylating species. The analogous fluoroethyltriazenes (BFIC[1] and MFIC[1]) also have antineoplastic activity, but they are more toxic than the 2-chloroethyl compounds.[14]

The most active of the *N*-nitrosoureas against experimental cancers are *N*-(2-chloroethyl)-*N*-nitrosoureas, $ClCH_2CH_2N(NO)CONHR$.[15,16] Studies of the mode of aqueous decomposition[17-21] of these compounds showed that at least one of the major pathways must proceed through species (e.g., 2-chloroethyl diazohydroxide or diazotate, 2-chloroethyldiazonium ion, or 2-chloroethylcarbonium ion) that can attach a chloroethyl group to biological macromolecules by reaction at nucleophilic centers. Subsequent studies indicate that haloethylnitrosoureas do, indeed, react with DNA and that the attached chloroethyl group can then react further and cross-link DNA.[22,23]

Thus, evidence from two sources (triazenylimidazoles and nitrosoureas) show that chloroethylating agents are exceptionally effective in manifesting antineoplastic activity. This evidence provides the rationale for investigations of other structures that might function as haloethylating agents.

Scheme II



A potential way of chloroethylating (or haloethylating) biological macromolecules is through the use of 2-chloroethyl (or other 2-haloethyl) sulfonates (1). In fact, 2-chloroethyl *p*-toluenesulfonate and the benzenesulfonate are well-known chemical chloroethylating agents.[24] In aqueous media, 2-haloethyl sulfonates may react by either an S_N2 or an S_N1 mechanism (Scheme I). Although there are two potential leaving groups in this type of structure, it is anticipated that chloroethylation of biological macromolecules by an S_N2 mechanism will be predominant, if not exclusive, and that cross-linking of DNA may subsequently occur by displacement of the chloro group. The fact that 1,2-migration of the chloro group (via 3) may occur in some degree, as during the solvolysis of 2-chloroethyl 4-nitrobenzenesulfonate,[25,26] does not appear to alter this possibility. In addition to the S_N1 and S_N2 reactions of 1, another potential type of reaction is the expulsion of the chloroethyl sulfonate group by attack of a nucleophile at the Ar–S bond when Ar of 1 is a negatively substituted aryl or heteroaryl group.[27,28]

Although a considerable number of 2-haloethyl sulfonates have been synthesized for various purposes, a systematic correlation of the effect on biological activity of varying the R group of 1 has not been carried out. However, several 2-haloethyl sulfonates have been tested for antitumor activity,[29-34] along with other sulfonates. 2-

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Table I. Tests of 2-Haloethyl Sulfonates and Related Compounds against P388 Leukemia in Mice[a]

$RSO_2OCH_2CH_2X$

no.	X	R	schedule[b]	dose,[c] mg/kg	mortality[d]	wt change diff,[e] g: T − C	survival time, days: T/C	T/C ratio,[f] %
5	Cl	CH_3 (cf. Table II)	D1	400[g]	6/6			t
			D1	200[g]	1/6	−1.6	13.1/11.9	110
			1-9	200[g]	5/6	−1.2		t
			1-9	100[g]	0/6	−1.2	15.3/11.4	134
			1-9	50[g]	0/6	+0.4	12.8/11.4	112
6	Cl	C_2H_5	1-9	200[g]	0/6	−2.5	14.3/12.3	116
			1-9	100[g]	0/6	0	12.9/12.3	104
7	F	CH_3	1-9	50[g]	6/6			t
			1-9	12.5[g]	0/6	−6.0	10.0/11.2	89, t
			1-9	6.3[g]	1/6	−5.7	13.0/11.7	111, t
			1-9	3.1[g]	0/6	−4.3	12.1/11.7	103, t
			1-9	1.6[g]	0/6	−1.5	11.8/11.7	100
9	Cl	CH_2=CH	1-9	50	6/6			t
			1-9	25	3/6	+0.5		t
			1-9	25[g]	0/6	−0.7	15.1/12.0	125
			1-9	12.5	0/6	−0.7	13.8/11.0	125
			1-9	6.3	0/6	−0.1	12.3/11.0	111
12	Cl	CH_2=C(Br)	1-5	25	6/6			t
			1-5	12.5	0/6	−2.8	12.8/12.3	104
			1-5	6.3	0/6	−1.4	13.0/12.3	105
13	Cl	C_6H_5CH=CH	1-9	200[h]	0/6	−0.1	12.8/11.9	107
14	Cl	4-$O_2NC_6H_4CH$=CH	1-5	100	3/6	−2.4		t
			1-5	50	0/6	−1.1	12.0/11.9	100
			1-5	25	0/6	−0.3	13.4/12.8	104
15	Cl	$ClCH_2$ (cf. Table II)	1-9	150[h]	6/6			t
			1-9	100[g]	1/6	−3.8	26.0/12.1	214
			1-9	100[h]	3/6	−4.8		t
			1-9	75[h]	0/6	−4.3	23.0/11.4	201, t
			1-9	50[h]	0/6	−3.6	20.0/11.4	175
			D1	100	1/6	−0.7	14.3/10.9	131
16	Cl	2,3,4,5,6-Me_5Ph	1-5	200	0/6	−0.2	10.7/11.2	95
17	Cl	2,4,6-Me_3Ph	1-5	200	6/6			t
			1-5	100	0/6	−2.2	12.3/12.9	95
			1-5	50	0/6	−0.7	13.8/12.9	106
18	Cl	4-MeOPh	1-9	200	0/6	0.0	11.3/11.3	100
19	Cl	4-FPh	1-9	200[h]	0/5	−1.5	12.0/11.9	100
			1-9	100[h]	0/6	−0.1	12.1/11.9	101
20	Cl	2,4,5-Cl_3Ph	1-5	200	0/6	−6.2	10.1/11.1	90, t
			1-5	100	0/6	−3.5	12.7/11.1	114
			1-5	50	0/6	−1.0	11.7/11.1	105
21	Cl	2,3,4,5,6-Cl_5Ph	1-5	200	5/5			t
			1-5	100	0/5	−3.2	11.0/11.4	96
			1-5	50	0/5	−4.3	10.5/11.4	92, t
			1-5	25	0/6	−1.6	10.9/11.8	92
22	Cl	2,3,4,5,6-F_5Ph	1-9	50	4/6	−3.8		t
			1-9	25	2/6	−4.4		t
			1-9	12.5	0/6	−1.6	12.1/11.0	110
			1-9	6.3	0/6	−1.0	12.0/11.0	109
23	Br	CH_3	D1	200[g]	3/6	−5.4		t
			1-9	100[g]	0/6	−3.0	13.3/11.4	116
			1-9	50[g]	0/6	−1.5	13.1/11.4	114
24	F	C_2H_5	1-9	6.3[g]	6/6			t
			1-9	3.1[g]	0/6	−0.7	11.3/10.6	106
			1-9	1.6[g]	0/6	−0.6	11.8/10.6	111
25	F	2,4,5-Cl_3Ph	1-5	100	3/6	−4.2		t
			1-5	50	0/6	−4.9	14.0/12.3	113, t
			1-5	25	0/6	−1.2	11.3/11.5	98
26	F	$ClCH_2$	1-9	12.5[h]	6/6			t
			1-9	6.3[h]	1/6	−2.1	14.0/11.9	117
			1-9	3.1[h]	0/6	−0.8	12.2/11.9	102
27	H	$ClCH_2$	1-9	100[h]	0/5	−4.6	6.8/11.3	60, t
			1-9	50[h]	0/6	−3.0	12.3/11.4	107
			1-9	25[h]	0/6	0.0	11.4/11.4	100
28	Cl	$BrCH_2$	1-5	200	2/6	−5.2	20.3/12.3	165, t
			1-5	200	0/6	−3.6	23.0/11.5	200
			1-5	150	0/6	−1.8	18.3/11.5	159
			1-5	100	0/6	−0.7	14.8/11.5	128
			1-5	50	0/4	−0.2	13.8/11.5	120
29	Cl	ICH_2	1-5	200	6/6			t
			1-5	100	0/6	−0.1	14.3/12.9	110
			1-5	50	0/6	−0.1	12.0/12.9	93
30	Cl	$CNCH_2$	1-5	50	5/6			t

Table I (*Continued*)

no.	X	R	schedule[b]	dose,[c] mg/kg	mortality[d]	wt change diff, g: T − C	survival time, days: T/C	T/C ratio,[f] %
			1-5	37.5	0/5	−1.5	16.8/11.1	151
			1-5	25	0/5	−2.1	16.0/11.1	144
			1-5	11	0/5	−1.1	14.0/11.1	126
31	Cl	4-$O_2NC_6H_4CH_2$	1-5	200	1/5	−1.1	11.0/11.7	94
			1-5	100	0/5	−0.3	11.5/11.7	98
32	$ClCH_2SO_2NHCH_2CH_2Cl$		1-9	200	0/6	−2.9	11.7/11.0	106
			1-9	100	0/6	−0.9	11.7/11.0	106
33	$ClCH_2CONHCH_2CH_2Cl$		1-9	200	3/6	−3.3		t
			1-9	100	0/6	−2.1	11.4/11.3	100
			1-9	50	0/6	−0.8	11.0/11.3	97

[a] Mice were inoculated intraperitoneally with 10^6 P388 leukemia cells on day 0. C = control group of animals; T = treated animals. Not all of the results of tests vs. P388 leukemia are listed. The results listed are those necessary to indicate that a compound was not active, was toxic at a certain dose, or was active at certain doses; e.g., if a compound was inactive and nontoxic at three doses, only the highest dose is listed. Higher toxic doses, lower nonactive doses, or additional tests at the same dose may not be listed. [b] D1 = a single dose administered on day 1; 1-9 = administration q.d. 1-9; 1-5 = administration q.d. 1-5. [c] Unless indicated otherwise, all compounds were administered within about 5 min of the preparation of solutions or suspensions (5-min unstable compound). [d] Number of treated mice dead on or before day 5/number of treated mice. [e] Average change in weight on day 5 of treated mice minus average change in weight of control mice. [f] A dose is considered toxic (t) if mortality by day 5 ≥ 3 of 6, T/C < 85%, or the weight-change difference (T − C) is greater in magnitude than −4 g. According to protocols of the National Cancer Institute, a compound is considered to be active in preliminary testing if it produces a value of T/C ≥ 120%. [g] Administered as a stable compound. [h] Administered within 15 min of the preparation of solutions or suspensions (15-min unstable compound).

Table II. Comparison of the 2-Chloroethyl Esters of Methanesulfonic Acid and Chloromethanesulfonic Acid against P388 Leukemia in Mice[a,b]

dose, mg/(kg day) q.d. 1-9	2-chloroethyl methanesulfonate (5)				2-chloroethyl chloromethanesulfonate (15)			
	mortality	wt change diff, g: T − C	survival time, days: T/C	T/C ratio, %	mortality	wt change diff, g: T − C	survival time, days: T/C	T/C ratio, %
150	0/6	−2.2	14.7/11.0	133	6/6			Toxic
100	0/6	−0.9	12.3/11.0	111	0/6	−3.0	24.0/11.0	218
75	0/6	−0.9	11.3/11.0	102	0/6	−2.4	18.8/11.0	170
50	0/6	−0.1	11.8/11.0	107	0/6	−1.8	15.8/11.0	143
25	0/6	−0.8	11.9/11.0	108	0/6	−1.4	13.3/11.0	120

[a] Tested simultaneously. Both compounds were administered as 5-min unstable compounds at all doses. [b] See footnotes to Table I for definitions.

Chloroethyl methanesulfonate (**5**, Scheme II), 2-chloroethyl ethanesulfonate (**6**), and 2-fluoroethyl methanesulfonate (**7**) were among various sulfonates prepared by Ross and Davis[29] as potential biological alkylating agents. These three compounds, as well as simple alkyl sulfonates, inhibited the growth of Walker 256 carcinosarcoma. 2-Chloroethyl methanesulfonate was the most effective of several types of alkylating agents against Lymphoma 8 in the rat.[30] It was also reported that 2-fluoroethyl methanesulfonate, as well as 2-chloroethyl methanesulfonate, inhibits the growth of Walker 256 carcinosarcoma, a myeloma, and Yoshida sarcoma in the rat and sarcoma 180 and sarcoma 37 in the mouse.[32-34] Although these compounds have received considerable study, they are not promising anticancer agents. Because of our interest in haloethylating agents, we chose to try to improve the activity of 2-haloethyl sulfonates by varying the sulfonate moiety.

Chemistry. The 2-haloethyl sulfonates (**1**) were prepared by treating the appropriate alkane- or arenesulfonyl chloride with the 2-haloethanol and triethylamine in ethyl acetate or diethyl ether. The complete list of 2-haloethyl sulfonates and related compounds (**5–7, 9,** and **12–33**) that were prepared and tested biologically is shown in Table I. The preparation and some of the properties of these compounds are summarized in Table III; spectroscopic data are summarized in the Experimental Section. Some of the sulfonyl chlorides or their precursor sulfonic acids were available from commercial sources; others were prepared by methods reported in the literature. 2-Chloroethyl ethenesulfonate (**9**) and the 1-bromoethenesulfonate (**12**) were prepared from 2-chloroethanesulfonyl chloride as outlined in Scheme II.

Biological Evaluation. The results of tests of the 2-haloethyl sulfonates against P388 leukemia in mice are tabulated in Tables I and II. In tests against L1210 leukemia cells in culture, 2-chloroethyl ethenesulfonate (**9**) was quite active;[35] in addition, this derivative showed borderline activity in initial tests against P388 leukemia in vivo. These observations motivated the preparation of other ethenesulfonates (**12–14**), none of which were active. The most interesting finding, however, was that 2-chloroethyl chloromethanesulfonate (**15**) has high activity in vitro[35] and in vivo. The highest increases in life span (percent T/C) in tests vs. P388 leukemia in vivo (Tables I and II) were 214–218 and 201% at doses of 100 and 75 mg/(kg day), respectively. In comparison, in tests of the extensively studied prototype, 2-chloroethyl methanesulfonate (**5**), against P388 leukemia in vivo only modest increases in life span (T/C = 133–134%) were observed. Simultaneous tests (Table II) of these two compounds

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Table III. Preparation of 2-Haloethyl Sulfonates

			$RSO_2OCH_2CH_2X$					
no.	X	R	reaction solvent[a]	recrystn solvent[b]	yield, %	mp or bp (mmHg), °C	formula	anal.
9	Cl	CH_2=CH	EA		80[c]	79-81 (0.1)	$C_4H_7ClO_3S$	C, H
			E		31[d]	80 (0.1)		
12	Cl	CH_2=CBr	*e*		59	66-66.5 (0.15)	$C_4H_6BrClO_3S$	C, H
13	Cl	C_6H_5CH=CH	E	E-H	45	66-68	$C_{10}H_{11}ClO_3S$	C, H
14	Cl	4-$O_2NC_6H_4CH$=CH	D	D-C	55	121-122.5	$C_{10}H_{10}ClNO_5S$	C, H, N
15	Cl	$ClCH_2$	E		51	87-88 (0.1)[f]	$C_3H_6Cl_2O_3S$	C, H
16	Cl	2,3,4,5,6-Me_5Ph	EA	H	48	103-104	$C_{13}H_{19}ClO_3S$	C, H
17	Cl	2,4,6-Me_3Ph	EA	H	73	56-57	$C_{11}H_{15}ClO_3S$	C, H
18	Cl	4-MeOPh	E		52	133 (0.015)	$C_9H_{11}ClO_4S$	C, H
19	Cl	4-FPh	E		33	99-99.5 (0.01)[g]	$C_8H_8ClFO_3S$	C, H
20	Cl	2,4,5-Cl_3Ph	EA	H	76	99-100	$C_8H_6Cl_4O_3S$	C, H
21	Cl	2,3,4,5,6-Cl_5Ph	EA	*h*		126-128	$C_8H_4Cl_6O_3S$	C, H
22	Cl	2,3,4,5,6-F_5Ph	E		20	86-87 (0.02)	$C_8H_4ClF_5O_3S$	C, H
24	F	C_2H_5	E		62	76 (0.5)	$C_4H_9FO_3S$	C, H
25	F	2,4,5-Cl_3Ph	EA	B-H	78	99-101	$C_8H_6Cl_3FO_3S$	C, H
26	F	$ClCH_2$	E		51	79-81 (0.1)	$C_3H_6ClFO_3S$	C, H
27	H	$ClCH_2$	E		22	59-60 (0.5)[i]	$C_3H_7ClO_3S$	C, H
28	Cl	$BrCH_2$	EA		41	105-106 (0.1)	$C_3H_6BrClO_3S$	C, H
29	Cl	ICH_2	EA	B-C	14	51-52	$C_3H_6ClIO_3S$	C, H
30	Cl	$CNCH_2$	EA		25	102-103 (0.02)	$C_4H_6ClNO_3S$	C, H, N
31	Cl	4-$O_2NC_6H_4CH_2$	EA	D-P	20	84	$C_9H_{10}ClNO_5S$	C, H, N
34	Cl	4-$ClC_6H_4CH_2$	EA	B-H	53	70-71	$C_9H_{10}Cl_2O_3S$	C, H

[a] E = diethyl ether, EA = ethyl acetate, D = dichloromethane. [b] C = cyclohexane, E = diethyl ether, H = hexane, D = dichloromethane, B = benzene, P = petroleum ether. [c] Prepared from 2-chloroethanesulfonyl chloride (**8**) by the general procedure with ethyl acetate as solvent; dehydrochlorination occurred during this reaction. Kostsova et al.[44] prepared **9** by this method in chloroform-pyridine, bp 119-120 °C (7 mm). [d] Prepared from ethenesulfonyl chloride by the general procedure with ether as the solvent; the yield probably would have been higher if ethyl acetate had been the solvent. [e] Prepared by bromination of **9** followed by dehydrobromination of the dibromoethanesulfonate. [f] Prepared from chloromethanesulfonyl chloride by the general procedure. Compound **15** was prepared previously from chloromethanesulfonic acid and 2-chloroethyl chlorosulfite by Etienne et al.,[45] bp 125-130 °C (3 mm). [g] Prepared from 4-fluorobenzenesulfonyl chloride by the general procedure. Compound **19** was prepared previously by refluxing either 4-fluorobenzenesulfonyl chloride[46] or 4-fluorobenzenesulfonic acid[47] with 2-chloroethanol, bp 115-120 °C (0.3 mm),[46] bp 162 °C (2.2 mm).[47] [h] The crude product was recrystallized from benzene-petroleum ether. An ethyl acetate solution of the recrystallized material was washed several times with water, dried, and concentrated to dryness in vacuo. The residue was pure **21**. [i] Prepared by Étienne et al.[45] from chloromethanesulfonic acid and ethyl chlorosulfite, bp 80-82 °C (1.5 mm).

against P388 leukemia under comparable conditions confirmed the significantly higher activity of the chloromethanesulfonate (**15**). Because of these findings, several congeners of **15** were prepared for evaluation. These include the 2-fluoroethyl (**26**) and ethyl (**27**) chloromethanesulfonates and the isosteres **32** and **33**. None of these compounds showed significant activity in vivo (Table I). In addition, the 2-chloroethyl bromomethanesulfonate (**28**), iodomethanesulfonate (**29**), and cyanomethanesulfonate (**30**) analogues were synthesized. All three of these analogues of **15** are cytotoxic to L1210 cells,[35] and **28** and **30** significantly increased life span in tests vs. P388 leukemia in vivo. In contrast to the chloro-, bromo-, and cyanomethanesulfonates, the iodomethanesulfonate (**29**) was not active in a test vs. P388 leukemia at three doses [200, 100, 50 mg/(kg day)]; the second test at the same doses confirmed the results of the first test.

The 2-haloethyl arenesulfonates synthesized (**16–22** and **25**) ranged in expected reactivity from the relatively unreactive pentamethylbenzenesulfonate[36] (**16**) to the highly reactive pentafluorobenzenesulfonate (**22**) and included aryl groups with intermediate electronic properties (**17–21**). The high cytotoxicity of **22** to L1210 cells in vitro[35] was the only activity observed in vitro or in vivo among the arenesulfonates synthesized.

Experimental Section

General Methods. Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Infrared spectra were recorded with a Nicolet MX-1E or a Perkin-Elmer Model 621 spectrometer from samples in KBr disks. Mass spectral data were taken from low-resolution, electron-impact spectra determined at 70 eV with a Varian/MAT Model 311A spectrometer. Nuclear magnetic resonance spectra were determined with a Varian Model XL-100-15 spectrometer operating at 100.1 MHz for proton NMR spectra; the internal reference was $(CH_3)_4Si$, and the solvent was $CDCl_3$.

Intermediates. Ethenesulfonyl chloride (**10**; bp 61.5–62.5 °C at 9.5 mm) was prepared from 2-chloroethanesulfonyl chloride (**8**) by the procedure of Rondestvedt.[37] 2-(4-Nitrophenyl)-ethenesulfonyl chloride (mp 173–175 °C) was prepared from 2-phenylethenesulfonyl chloride (commerically available) by the method of Bordwell et al.[38] Pentachlorobenzenesulfonyl chloride was obtained in low yield from pentachloroaniline by the sequence of Farrar[39] and was used without purification for the preparation of **21**. Bromomethanesulfonyl chloride,[40] iodomethanesulfonyl chloride,[41] cyanomethanesulfonyl chloride,[42] 4-nitrobenzenemethanesulfonyl chloride,[43] and 4-chlorobenzenemethanesulfonyl

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chloride[43] required for **28**, **29**, **30**, **31**, and **34**, respectively, were prepared by methods described in the cited literature.

2-Haloethyl Sulfonates (Table III). General Procedure. A solution of the sulfonyl chloride in anhydrous ethyl acetate (5–10 mL/g), protected from atmospheric moisture, was placed in an ice bath, and a mixture of the 2-haloethanol (1.1 equiv) and triethylamine (1.2 equiv) was added dropwise (about 10–20 min) to the stirred mixture. The 2-haloethanol–triethylamine mixture was sometimes diluted with an equal volume of dry ethyl acetate to facilitate slow addition. The mixture was then stirred for 2 h and stored in a refrigerator overnight. Triethylamine hydrochloride was removed by filtration and washed with dry ethyl acetate. The filtrate (plus washings) was washed quickly with three to four portions of dilute NaCl solution, dried ($MgSO_4$), and concentrated to a solid or an oil in vacuo. The crude product was purified further by distillation or recrystallization (Table III). The procedure was the same when ether was the reaction solvent.

2-Chloroethyl 1-Bromoethenesulfonate (12). Bromine (1.28 mL, 24.9 mmol) was added in small portions during 2 h to a gently refluxing solution of 4.25 g (24.9 mmol) of 2-chloroethyl ethenesulfonate (**9**) in carbon tetrachloride (8 mL). Additional bromine (10 drops) was added, and the solution was heated under gentle reflux for 6 h and then concentrated in vacuo to a light yellow oil (**11**). A solution of the crude 2-chloroethyl 1,2-dibromoethanesulfonate (**11**) in 30 mL of ethyl acetate was cooled to −30 °C, and 2,6-dimethylpyridine (about 1.6 g) was added dropwise. The solution was allowed to warm slowly to −10 °C, additional 2,6-dimethylpyridine (total, 3.2 g, 29.9 mmol) was added dropwise, and the mixture was stirred in an ice bath for 2 h. A white precipitate was separated by filtration and washed with ethyl acetate, and the filtrate (including washings) was concentrated in vacuo. A solution of the residual oil in ethyl acetate was washed successively with two portions of water, dilute sulfuric acid, and NaCl solution. Ethyl acetate was evaporated in vacuo from the dried ($MgSO_4$) solution, and two fractions were collected by distilling the residual oil (5.38 g) through a Vigreaux column: fraction 1, 1.43 g, bp 65–66 °C (0.015 mm); fraction 2, 2.26 g (36%), bp 66–66.5 °C (0.015 mm). Fraction 2 gave satisfactory ^1H NMR, IR, MS, and elemental analysis.

1-Chloro-*N*-(2-Chloroethyl)methanesulfonamide (32). A solution of 3.90 g of 2-chloroethylamine hydrochloride and 100 mL of water cooled in an ice bath was made basic with aqueous NaOH and extracted quickly with ethyl acetate (3 × 150 mL). The extract was washed with saturated NaCl solution, dried (first with $MgSO_4$ and then with $CaSO_4$) at 0 °C, and concentrated in vacuo to about one-half of the original volume. Triethylamine (6 mL) was added to the chloroethylamine–ethyl acetate solution, and this solution was added slowly to a well-stirred solution of chloromethanesulfonyl chloride in ethyl acetate (10 mL) at 0–5 °C. The mixture was stirred for 2 h in the ice bath, the white precipitate was separated by filtration, and the filtrate (plus ethyl acetate washings) was washed twice with water and concentrated in vacuo. A solution of the residual oil in a small volume of ethyl acetate was diluted slowly with petroleum ether until cloudiness appeared and was then refrigerated. The white crystalline product was collected by filtration and dried in vacuo: mp 56–57 °C; MS (direct-probe temperature, 20 °C), *m/e* 191 (M), 156 (M − Cl), 142 (M − CH_2Cl), 113 (M − $NHCH_2CH_2Cl$); IR 1320 (s), 1145 (s) (SO_2NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.46–3.84 (m, CH_2CH_2Cl), 4.59 (s, $ClCH_2S$), 5.35 (NH). Anal. ($C_3H_7Cl_2NO_2S$) C, H, N.

Other Compounds. In addition to the compounds listed in Table III, the following 2-haloethyl sulfonates reported previously were prepared by the general procedure described above: 2-chloroethyl methanesulfonate (**5**), bp 60–65 °C (0.02 mm) [lit.[29] bp 130 °C (11 mm)]; 2-chloroethyl ethanesulfonate (**6**), bp 78 °C (0.4 mm) [lit.[48] bp 63–64 °C (0.09 mm); lit.[44] bp 122 °C (9 mm)]; 2-fluoroethyl methanesulfonate (**7**), bp 60 °C (0.1 mm) [lit.[29] bp 130 °C (19 mm)]; and 2-bromoethyl methanesulfonate (**23**), bp 74 °C (0.02 mm) [lit.[29] bp 126–128 °C (5 mm)]. 2-Chloro-*N*-(2-chloroethyl)acetamide (**33**) was prepared from chloroacetyl chloride and 2-chloroethylamine hydrochloride in aqueous sodium hydroxide solution and recrystallized from petroleum ether: mp 57–58 °C [lit.[49] 57–57.5 °C]; IR 3315 (s), 1650 (s) (CONH), 1540 (s), 760 cm^{-1}.

Spectroscopic Data. The IR spectra of the sulfonates of Tables I and III include the expected strong sulfonate bands in the regions 1345–1395 and 1155–1190 cm^{-1}. These bands often show multiple structure, the main peaks being split or having shoulders. The mass spectra of all of the arenesulfonates (**16–22** and **25**) and the arylmethanesulfonates (**31** and **34**) included strong molecular-ion peaks (M). Peaks corresponding to M or (M + H) appeared in the spectra of the alkanesulfonates, alkenesulfonates, and substituted methanesulfonates. The spectra of all of the sulfonates, $RSO_2OCH_2CH_2X$, of Tables I and III included a peak, usually intense, corresponding to R^+, and peaks resulting from RSO_2^+ and $(M - CH_2X)^+$ were strong in most of the spectra except those of **31** and **34**, in which these peaks were relatively minor. These observations are in accord with earlier mass spectral studies[50,51] of alkyl alkanesulfonates. Peaks corresponding to $(CH_2X)^+$, $(CH_2CH_2X)^+$, and $(OCH_2CH_2X)^+$ were prominent in most of the spectra. The ^1H NMR spectra of the 2-chloroethyl sulfonates include the chemical-shift pattern expected of the AA'BB' system $SO_2OCH_2CH_2Cl$. The spectra of the 2-fluoroethyl sulfonates include the AA'BB'X pattern arising from the SO_2O-CH_2CH_2F grouping.

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