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Cytotoxicity of Fluorine-Containing Alkyl Alkanesulfonates to Cultured Leukemia L1210 Cells

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Ethyl esters of alkanesulfonic acids, isethionic acid, trifluoromethanesulfonic acid, and 2,2,2-trifluoroethanesulfonic acid were synthesized and tested for inhibitory activity toward the growth of cultured leukemia L1210 cells. A quantitative correlation with the rate of hydrolysis and the capacity factor (partition property) was demonstrated by a multiple linear regression analysis; the more reactive and more lipophilic, the more cytotoxic they are. Interestingly, fluorine substitution on the alcoholic moiety resulted in remarkable deviations of the toxicity from those expected from the correlation found for the ethyl esters without fluorine substitution. Then, some fluorine derivatives of busulfan, a clinically used bifunctional sulfonate, were synthesized and tested. 2,2-Difluoroethyl 2,2,2-trifluoroethanesulfonate was several times more cytotoxic than busulfan.

Keywords——alkanesulfonate; fluorine derivative; fluorinated; busulfan; cytotoxicity; L1210

Interest in fluorine substitution for hydrogen (CH→CF) in bioactive compounds has stemmed principally from the physicochemical characteristics of the fluorine (F) atom: 1) F mimics hydrogen with respect to steric requirements at enzyme receptor sites; 2) F causes polarization of the electronic structure of a molecule due to its high electronegativity, resulting in alterations of chemical reactivity, including complex formation with the surroundings, *i.e.*, solvent molecules, enzymes, *etc.*; 3) a C–F bond shows high resistance to oxidatively or thermally induced homolysis; and 4) F bestows increased hydrophobicity on the molecule.

Many alkyl alkanesulfonates are known to be bioactive alkylating agents which alkylate biological materials such as nucleic acids and proteins, leading to cell death, mutation, *etc.*[1] This paper is concerned with the effect of F-substitution in ethyl alkanesulfonates on the cytotoxicity to cultured leukemia L1210 cells. Since all these derivatives are direct-acting ethylating agents, the cytotoxicity might simply be correlated with the alkylating ability (rate) and partition property of a molecule and in addition, when a F atom(s) is located in the alcoholic ethyl moiety, the cytotoxicity might also depend upon the efficiency of the enzymic repair system in releasing the cell from the damage of fluoroethylations. Cytocidal activity of 2-haloethyl sulfonates including some fluoro derivatives has been thoroughly studied by Bowdon *et al.*[2] and several reports on other fluoroalkylating agents have appeared in the literature.[3] However, the role of fluorine substitution in cytotoxicity is not yet fully understood. In the present study, sets of mono-, di-, and trifluoroethyl derivatives of ethyl trifluoromethanesulfonate and ethyl 2,2,2-trifluoroethanesulfonate were synthesized and their cytotoxicities were subjected to regression analysis, together with those of some sulfonates without a F atom, in terms of rate of hydrolysis and partition property. Based on the results obtained along this line, some F-substituted busulfan derivatives were synthesized and their cytotoxicity was compared with that of busulfan, which is clinically used against chronic myeloid leukemia.

Results and Discussion

The compounds examined include 14 kinds of ethyl esters of alkanesulfonic acids, isethionic acid, trifluoromethanesulfonic acid, and trifluoroethanesulfonic acid. Six of the esters are substituted with fluorine atom(s) in the alcoholic ethyl moiety (**8**—**10** and **12**—**14**). Pseudo-first order rate constants and half-lives for the hydrolysis of these esters in 0.25 M phosphate buffer (pH 7.4) at 37 °C are shown in Table I. They may be a measure of the alkylating ability of the compounds. The log k' values (logarithmic capacity factors), which are a measure of the partition property obtained from the retention time on high performance liquid chromatography (HPLC), are also shown in the same table. The larger the value, the more lipophilic the compound. The concentration required to give a 50% reduction of the cell concentration (IC_{50}) after a 48 h incubation was read from the semi-log dose–response plot, as exemplified in Fig. 1. The IC_{50} values are listed in Table I. Preliminary data are also reported in Table II on the cytotoxicity of fluoro derivatives of busulfan, an anticancer alkylating agent.

Table I. Rate Constants for Hydrolysis and Capacity Factors of Alkyl Alkanesulfonates and Their 50% Growth-Inhibitory Concentrations toward Cultured Leukemia L1210

Compd. No.		R_1–SO_2–O–R_2 R_1	R_2	Rate constant[a] $t_{1/2}$ (h)	k_{obs} (1/h)	Capacity factor[b] log k'	IC_{50} (mM)[c]
1	EMS	CH_3–	–CH_2CH_3	6.63	0.105	−0.439	2.5
2	EES	C_2H_5–	–CH_2CH_3	6.35	0.109	−0.241	2.2
3	EPS	C_3H_7–	–CH_2CH_3	7.16	0.097	−0.086	1.8
4	EBS	C_4H_9–	–CH_2CH_3	7.34	0.094	0.116	1.5
5	EPeS	C_5H_{11}–	–CH_2CH_3	7.93	0.087	0.361	1.2
6	EIse	$HOCH_2CH_2$–	–CH_2CH_3	2.45	0.283	−0.685	1.7
7	EMS (3-0)	CF_3–	–CH_2CH_3	(Too fast)		—[d]	—[d]
8	EMS (3-1)	CF_3–	–CH_2CH_2F	(Too fast)		0.234	10
9	EMS (3-2)	CF_3–	–CH_2CHF_2	1.15	0.603	0.321	1.5
10	EMS (3-3)	CF_3–	–CH_2CF_3	—[d]	—[d]	—[d]	0.1
11	EES (3-0)	CF_3CH_2–	–CH_2CH_3	0.12	5.78	−0.091	0.25
12	EES (3-1)	CF_3CH_2–	–CH_2CH_2F	0.33	2.10	−0.191	0.040
13	EES (3-2)	CF_3CH_2–	–CH_2CHF_2	0.25	2.77	−0.103	0.040
14	EES (3-3)	CF_3CH_2–	–CH_2CF_3	—[d]	—[d]	0.065	0.060

a) Pseudo-first order rate constant was measured in 0.25 M phosphate buffer (pH 7.4) at 37 °C. *b*) Capacity factor (k') was measured by HPLC using a column of Finepack SIL C-18 eluted with 60% MeOH–H_2O: $k' = (t - t_0)/t_0$, where t and t_0 are the retention times of the test compound and solvent, respectively. *c*) IC_{50} was the value after incubation for 48 h. *d*) Not available. Abbreviation: EMS, ethyl methanesulfonate; EES, ethyl ethanesulfonate; EPS, ethyl propanesulfonate; EBS, ethyl butanesulfonate; EPeS, ethyl pentanesulfonate; EIse, ethyl isethionate.

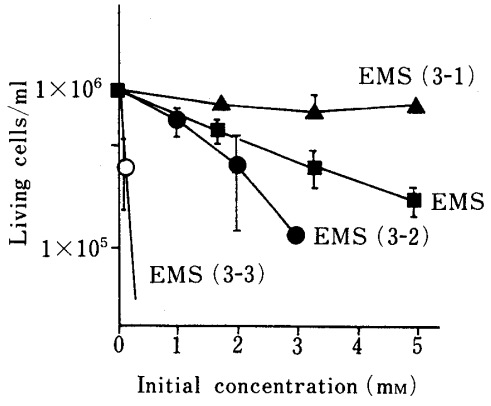

Fig. 1. Growth-Inhibitory Effects of Fluorinated Ethyl Ethanesulfonates on Cultured L1210 after Incubation for 48 h

Abbreviations are given in Table I.

TABLE II. The 50% Growth-Inhibitory Concentrations of Bifunctional Alkanesulfonates toward Cultured Leukemia L1210 after Co-incubation in Culture Medium at 37 °C for 48 h

No.	Chemical	IC_{50} (mM)[a]
15	$(CH_3SO_2\text{–}O\text{–}CH_2CF_2\text{–})_2$	0.93 (±0.10)
16	$(CF_3CH_2SO_2\text{–}O\text{–}CH_2CH_2\text{–})_2$	0.05 (±0.01)
17	$(CF_3CH_2SO_2\text{–}O\text{–}CH_2CF_2\text{–})_2$	0.03 (±0.01)
18[b]	$(CH_3SO_2\text{–}O\text{–}CH_2CH_2\text{–})_2$	0.13 (±0.03)

a) Mean of three measurements (±S.D.). *b*) Busulfan.

Quantitative Structure–Cytotoxicity Relationship among Monofunctional Alkyl Alkanesulfonates

The quantitative correlation of $1/IC_{50}$ values with the rate of hydrolysis and the capacity factor was tested for statistical significance by a multiple linear regression analysis using SALS (statistical analysis of least square fitting) program.[4] It should be noted that compounds **7**, **8**, **10**, and **14** are excluded from the present regression analysis because **7** and **8** were hydrolyzed too fast to be assayed and **10** and **14** were difficult to analyze by gas chromatography (GC) for rate measurement probably due to their low solubility and high volatility, and the low analytical sensitivity of the refractive index (RI) detector for them. Judging from the correlation Eq. 1, among all seven ethylating agents, a good correlation was obtained regardless of the structure of the leaving group: methane-, ethane-, propane-, butane-, pentane-, 2-hydroxyethane-, or 2,2,2-trifluoroethanesulfoxy.

$$\log(1/IC_{50}) = 0.487(\pm 0.056)\log k_{obs} + 0.392(\pm 0.103)\log k' + 0.271(\pm 0.051) \quad (1)$$

$$r = 0.997; \quad r^2 = 0.994; \quad n = 7$$

(samples of ethyl esters alone)

The 95% confidence limit is given in parentheses for each term of the correlation equation, along with the correlation coefficient (r) and sample number (n). When fluoroethylating esters, **9**, **12**, and **13**, are included in the population for regression analysis, the correlation becomes much poorer as indicated by Eq. 2.

$$\log(1/IC_{50}) = 0.823(\pm 0.501)\log k_{obs} + 0.111(\pm 1.08)\log k' + 0.538(\pm 0.416) \quad (2)$$

$$r = 0.827; \quad r^2 = 0.684; \quad n = 10$$

(samples including fluoroethyl esters)

It is, therefore, suggested that F-substitution on the alcoholic ethyl moiety might confer certain additional characteristics on the parent compounds in the cell inactivation besides the effects on alkylating ability and partition property of the compound. For the ethyl esters examined, plots of the found values of $1/IC_{50}$ *versus* the values calculated by Eq. 1 fit a straight line, as shown in Fig. 2. However, for two fluoroethyl esters, **12** and **13**, the found values are much larger than those calculated by Eq. 1, and for the other fluoroethyl ester **9**, the found value is less than the calculated value. Although one of the possible mechanisms involved in the F-substitution effect may indeed be related to the ease of enzymic repair of alkylated deoxyribonucleic acid (DNA) produced by the ethylating and fluoroethylating agents, an additional mechanism might possibly be operating since the cytocidal potencies of two 2,2-difluoroethylating agents, **13** and **9**, deviate from the linear correlation in different directions, as seen in Fig. 2.

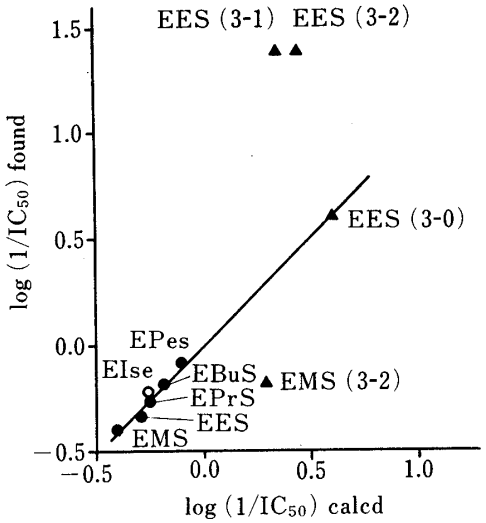

Fig. 2. Plots of $1/IC_{50}$ of the Sulfonates Including Fluorinated Esters *versus* Values Calculated by Using Eq. 1 Regressed with 7 Ethyl Esters without F Atom(s)

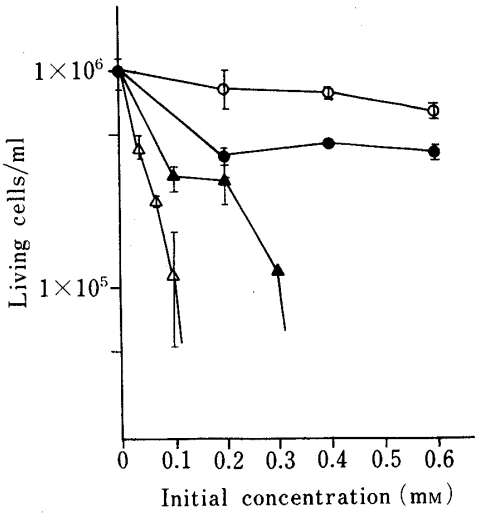

Fig. 3. Growth-Inhibitory Effects of Fluorinated Busulfan Derivatives on Cultured L1210 after Incubation for 48 h

○, $(CH_3SO_2OCH_2CF_2-)$; ●, $(CH_3SO_2OCH_2CH_2-)_2$; △, $(CF_3CH_2SO_2OCH_2CF_2-)_2$; ▲, $(CF_3CH_2SO_2OCH_2CH_2-)_2$.

Cytotoxicity of Fluorinated Derivatives Related to Busulfan

The regression analysis of cytotoxicity for monofunctional sulfonates suggests that F-substitution may have certain unexpected effect(s) on the cytotoxicity of chemicals. Fluorinated derivatives of busulfan were then synthesized and tested for cytocidal activity toward cultured L1210 cells. The dose–response plots of three fluorinated busulfans, **15**—**17**, are shown in Fig. 3 together with that of busulfan (**18**). The IC_{50} values that were read from the plots are shown in Table II. Two of the fluorinated derivatives (**16** and **17**) were more effective than the parent busulfan, whereas **15** was almost inactive compound. **17** was about 4 times more toxic than busulfan.

Conclusion

Biological activity of ethylating agents such as ethyl methanesulfonate is thoroughly documented in connection with DNA-ethylation leading to genotoxic events in both bacterial and mammalian assay systems.[1] Although the present study is only concerned with the cytotoxicity of a limited number of compounds toward a mammalian cultured cell line, the toxicity seemed to be quantitatively correlated simply with alkylating ability and partition property; the more reactive and more lipophilic, the more toxic the compounds are. It was further demonstrated that F-substitution on the alcoholic ethyl moiety of EMS and EES induced remarkable deviations of cytotoxicity from those expected from the regression analysis of ethyl esters without F atom(s). The deviation might be mainly due to a difference in the biological effect of ethylation and fluoroethylation of the target cell-constituent(s), but an additional mechanism might possibly be involved, since two difluoroethylating agents, **9** and **13**, were endowed with increased and decreased capacities for cell inactivation, respectively, depending on the structure of the leaving group.

Expecting that F-substitution on the alcoholic and/or acid moieties of carcinostatic bifunctional sulfonates might also modify the cytocidal capacity, we synthesized some F-substituted busulfan derivatives and tested them for cytocidal capacity. The result revealed

that **17** was about 4 times more toxic and **16** slightly more toxic than busulfan. Their tumor-selective toxicity *i.e.*, therapeutic efficacy toward tumor-bearing animals, is now being investigated. As for **15**, lack of cytotoxicity might be due to too low an alkylating ability to produce a significant alkylating modification in the target(s) within the exposure duration, although no experimental evidence is yet available.

Experimental

All ethyl alkanesulfonates having no fluorine atom in the molecule were synthesized according to reported methods (abbreviations are given in Table I): EMS, bp 86 °C/10 mmHg[5]; EES, bp 76 °C/7 mmHg[5]; EPS, bp 86 °C/8 mmHg[5]; EBS, bp 98 °C/8 mmHg[5]; EPeS, bp 102 °C/6 mmHg[5]; ethyl isethionate, colorless oil[6]; These compounds were proved to be pure by thin layer chromatography (TLC), HPLC, and nuclear magnetic resonance (NMR) spectroscopy.

2-Fluoroethyl Trifluoromethanesulfonate (8)——A solution of 2-fluoroethanol (3.56 g, 55.6 mmol) in 20 ml of CH_2Cl_2 was slowly added to a CH_2Cl_2 solution (40 ml) of trifluoromethanesulfonic anhydride (15.7 g, 55.6 mmol) at room temperature with stirring. The reaction mixture was maintained at room temperature for 1 h, then refluxed for 30 min. The mixture was washed with 50 ml of 5% $NaHCO_3$ and the aqueous washing was extracted twice with 100 ml of ether. The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was distilled off, and the residue was distilled under reduced pressure to give 6.10 g of the product (56% yield), bp 49—50 °C/13 mmHg, n_D^{20} 1.3452. *Anal.* Calcd for $C_3H_4F_4O_3S$: C, 18.37; H, 2.06. Found: C, 18.51; H, 2.25. ^{1}H-NMR (in $CDCl_3$) δ: 4.4—5.1 (4H, A_2B_2X). ^{19}F-NMR (in $CDCl_3$) ppm from CF_3COOH: 4.0 (3F, d, J=2 Hz), −148.8 (1F, ttq, J=49, 27, 2 Hz).

2,2-Difluoroethyl Trifluoromethanesulfonate (9)——This ester was prepared according to the reported procedure[7]; bp 114—118 °C, n_D^{20}, 1.3303.

2,2,2-Trifluoroethyl Trifluoromethanesulfonate (10)——This ester was prepared according to the reported procedure[8]; bp 90.5—91 °C, n_D^{25} 1.3037.

Ethyl 2,2,2-Trifluoroethanesulfonate (11)——A solution of 2,2,2-trifluoroethanesulfonyl chloride[9] (5.0 g, 27.4 mmol) in 20 ml of CH_2Cl_2 was slowly added to 50 ml of CH_2Cl_2 containing ethanol (1.26 g, 27.4 mmol) and triethylamine (4.15 g, 41.0 mmol). The reaction mixture was stirred at room temperature for 30 min and then washed successively with 50 ml each of chilled water, 10% HCl, 5% $NaHCO_3$ and then saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4. After the solvent was distilled off, the residue was distilled under reduced pressure to give 3.35 g of the product (64% yield), bp 74—76 °C/10 mmHg, n_D^{20} 1.3757. *Anal.* Calcd for $C_4H_7F_3O_3S$: C, 25.00; H, 3.67. Found: C, 24.52; H, 3.49. ^{1}H-NMR (in $CDCl_3$) δ: 1.40 (3H, t, J=7 Hz) 3.90 (2H, q, J=9 Hz), 4.40 (2H, q, J=7 Hz). ^{19}F-NMR in $CDCl_3$ ppm from CF_3COOH: 16.0 (3F, t, J=9 Hz).

2-Fluoroethyl 2,2,2-Trifluoroethanesulfonate (12)——A solution of 2,2,2-trifluoroethanesulfonyl chloride (9.13 g, 50 mmol) in 20 ml of CH_2Cl_2 was slowly added at room temperature with stirring to 50 ml of CH_2Cl_2 containing 2-fluoroethanol (3.20 g 50 mmol) and triethylamine (7.08 g, 70 mmol). The reaction mixture was stirred at room temperature for 30 min and washed successively with 50 ml each of chilled water, 10% HCl, 5% $NaHCO_3$, and then saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4. After the solvent was distilled off, the residue was distilled under reduced pressure to give 5.41 g of the product (52% yield), bp 90—92 °C/5 mmHg, n_D^{20} 1.3791. *Anal.* Calcd for $C_4H_6F_4O_3S$: C, 22.86; H, 2.86. Found: C, 22.92; H, 2.94. ^{1}H-NMR (in $CDCl_3$) δ: 3.98 (2H, q, J=9 Hz), 4.6—5.0 (4H, A_2B_2X). MS m/z: 210 (M^+), 177 ($M^+ - CH_2F$), 147 ($M^+ - OCH_2CH_2F$).

2,2-Difluoroethyl 2,2,2-Trifluoroethanesulfonate (13)——A solution of 2,2,2-trifluoroethanesulfonyl chloride (7.3 g, 40 mmol) in 10 ml of CH_2Cl_2 was slowly added to 40 ml of CH_2Cl_2 containing 2,2-difluoroethanol (3.28 g, 40 mmol) and triethylamine (5.67 g, 56 mmol). By the same treatment as aforementioned, 2.82 g of the product was obtained (yield, 31%), bp 80—82 °C/8 mmHg, n_D^{20}, 1.3656. *Anal.* Calcd for $C_4H_5F_5O_3S$: C, 21.05; H, 2.19. Found: C, 21.34; H, 2.34. ^{1}H-NMR (in $CDCl_3$) δ: 4.00 (2H, q, J=9 Hz), 4.45 (2H, td, J=3, 13 Hz), 5.98 (1H, tt, J=54, 3 Hz). ^{19}F-NMR (in $CDCl_3$) ppm from CF_3COOH: 16.1 (3F, t, J=9 Hz); −48.3 (2F, dt, J=54, 13 Hz). MS m/z: 228 (M^+), 177 ($M^+ - CHF_2$), 147 ($M^+ - OCH_2CHF_2$).

2,2,2-Trifluoroethyl 2,2,2-Trifluoroethanesulfonate (14)——A solution of 2,2,2-trifluoroethanesulfonyl chloride (5.0 g, 27.4 mmol) in 10 ml of CH_2Cl_2 was added to 40 ml of CH_2Cl_2 containing 2,2,2-trifluoroethanol (2.74 g, 27.4 mmol) and triethylamine (3.89 g, 38.4 mmol). By the same treatment as aforementioned, 2.15 g of the product was obtained (yield, 32%), bp 64—66 °C/12 mmHg, mp 15 °C, n_D^{20} 1.3480. *Anal.* Calcd for $C_4H_4F_6O_3S$: C, 19.51; H, 1.63. Found: C, 19.66; H, 1.79. ^{1}H-NMR (in $CDCl_3$) δ: 4.03 (2H, q, J=9 Hz), 4.58 (2H, q, J=8 Hz). ^{19}F-NMR (in $CDCl_3$) ppm from CF_3COOH: 16.0 (3F, t, J=9 Hz); 4.1 (3F, t, J=8 Hz). MS m/z: 246 (M^+), 227 ($M^+ - F$), 177 ($M^+ - CF_3$), 147 ($M^+ - OCH_2CF_3$).

2,2,3,3-Tetrafluorobutane-1,4-diol Bis(methanesulfonate) (15)——A solution of 2,2,3,3-tetrafluorobutane-1,4-diol[10] (1.00 g, 6.17 mmol) and triethylamine (1.25 g, 12.4 mmol) in 10 ml of $CHCl_3$ was added to a $CHCl_3$ solution (10 ml) of methanesulfonyl chloride (2.04 g, 17.8 mmol) at −25 °C over a period of 2 h with stirring. The reaction mixture was stirred at −25 to −30 °C for another hour and then poured into 200 ml of ice-water. The resulting

slurry was collected on a glass filter and dried. The solid thus obtained was recrystallized from benzene to give 920 mg of the product (yield, 47%), mp 104—104.5 °C. *Anal.* Calcd for $C_6H_{10}F_4O_6S_2$: C, 22.64; H, 3.17. Found: C, 22.80; H, 2.93. ^{1}H-NMR (in acetone-d_6) δ: 4.79 (2H, t, J=14, 2 Hz), 3.27 (3H, s). ^{19}F-NMR (in acetone-d_6) ppm from CF_3COOH: −44.7 (4F, t, J=14 Hz). MS (CI, isobutane) m/z: 319 (M^++H), 223 ($CH_3SO_3CH_2CF_2CF_2^+$).

1,4-Butanediol Bis(2,2,2-trifluoroethanesulfonate) (16)——A solution of 1,4-butanediol (0.99 g, 11 mmol) and triethylamine (2.42 g, 24 mmol) in 20 ml of $CHCl_3$ was slowly added to a $CHCl_3$ solution (15 ml) of trifluoroethanesulfonyl chloride (4.29 g, 23.5 mmol) over a period of 2 h at −25 °C with stirring. The reaction mixture was stirred at −25 to −30 °C for another hour and poured into 200 ml of ice-water. The resulting slurry was collected on a glass filter and dried. Recrystallization from benzene gave 2.91 g (69% yield) of the product, mp 95—97 °C. *Anal.* Calcd for $C_8H_{12}F_6O_6S_2$: C, 25.13; H, 3.16. Found: C, 24.78; H, 2.91. ^{1}H-NMR (in $CDCl_3$) δ: 4.40 (2H, t, J=2.5 Hz), 3.93 (2H, q, J=9 Hz), 1.89 (2H, m). ^{19}F-NMR (in $CDCl_3$) ppm from CF_3COOH: 16.8 (6F, t, J=9 Hz). MS (CI, isobutane) m/z: 383 (M^++H), 219 ($CF_3CH_2SO_3C_4H_8^+$); 147 ($CF_3CH_2SO_2^+$).

2,2,3,3-Tetrafluorobutane-1,4-diol Bis(2,2,2-trifluoroethanesulfonate) (17)——A solution of 2,2,3,3-tetrafluorobutanediol (300 mg, 1.85 mmol) and triethylamine (490 mg, 4.85 mmol) in $CHCl_3$ (10 ml) was added to a $CHCl_3$ solution (3 ml) of trifluoroethanesulfonyl chloride (790 mg, 4.3 mmol). By the same treatment as aforementioned 270 mg of the product was obtained (32% yield), mp 105—107 °C. *Anal.* Calcd for $C_8H_8F_{10}O_6S_2 \cdot 0.1\ C_6H_6$:[11] C, 22.35; H, 1.88. Found: C, 22.68; H, 1.92. ^{1}H-NMR (in acetone-d_6) δ :5.00 (2H, t, J=15 Hz), 4.79 (2H, q, J=9 Hz). ^{19}F-NMR (in acetone-d_6) ppm from CF_3COOH: 15.2 (6F, t, J=9 Hz), −44.3 (4F, t, J=15 Hz). MS (CI, isobutane) m/z: 455 (M^++H), 291 ($CF_3CH_2SO_3CH_2CF_2CF_2CH_2^+$), 147 ($CF_3CH_2SO_2^+$).

Busulfan (18)——Busulfan was prepared by the reported method[12] with a slight modification. Methanesulfonyl chloride (5.18 g, 45.2 mmol) was added dropwise to a CH_2Cl_2 solution (40 ml) of 1,4-butanediol (1.87 g, 20.8 mmol) and triethylamine (6 ml, *ca.* 43 mmol) over a period of 1 h under ice-methanol cooling with vigorous stirring. The reaction mixture was maintained at *ca.* 10 °C and stirred at room temperature for 1 d, then evaporated to dryness. Chilled water (100 ml) was poured onto the residue. The resulting slurry was collected by filtration and dried. Recrystallization from ethanol gave 4.19 g (86% yield) of the product, mp 121—121.5 °C.

Rate for Hydrolysis——An appropriate amount of the sulfonate to be examined was dissolved in 0.25 ml of dimethyl sulfoxide (DMSO) and diluted to 50 ml with 0.25 M phosphate buffer (pH 7.4). The concentration of the sulfonate solutions thus prepared ranged from 5 to 20 mM. The test solution was placed in samll sealed glass tubes (2.0 ml each) and these tubes were kept at 37±0.2 °C in a water bath incubator. The tubes were unsealed one by one after an appropriate period. An aliquot of the test solution was taken out of each tube (exactly 0.8 ml) and combined with 0.8 ml of $CHCl_3$ containing an appropriate amount of the internal standard for quantitative analysis by GC. The internal standard used for the quantitative analysis was either mesitylene, cymene, cumene or tetralin. The $CHCl_3$ extract thus obtained was dried over anhydrous $MgSO_4$. Quantitative analysis of the extracted sulfonate was performed on a Shimadzu GC-8APF gas chromatograph equipped with an FID detector and a 3.2 mm×2.0 m 5% SE30 column (column temperature between 55 and 110 °C) or Porapack P column (column temperature between 155 and 170 °C). The quantification was carried out by measuring the peak area relative to that of the internal standard with the help of the working curve previously prepared. All the data listed in Table I are the averages of duplicate or triplicate separate experiments; the deviations fell within ±5% in all cases.

Partition Property Estimated by HPLC——HPLC was carried out with a JASCO TWINCLE HPLC apparatus equipped with a RI detector and a 4.0 mm×20 cm JASCO Finepak SIL C-18 column. The neat sample was injected and eluted with MeOH–H_2O (6 : 4, v/v) at room temperature and the flow rate was 0.7 ml/min. The capacity factor, k', which is a measure of the partition property,[13,14] was calculated as $(t-t_0)/t_0$, where t and t_0 are the retention times of the sulfonate tested and the solvent (estimated by injecting aqueous KI solution), respectively.

Assay for Cytotoxicity (Growth Inhibition) to Leukemia L1210——The cell line was a gift from Dr. T. Kato of Aichi Cancer Center Research Institute, Nagoya. The cells in an early log phase (1×10^5 cells) were seeded in a 16.5×105 mm test tube with a molton cap containing 2.0 ml of RPMI 1640 (Nissui Seiyaku Co., Ltd., Tokyo) medium. The medium was prepared by mixing the following solutions: 100 ml of distilled water containing 1.02 g of RPMI and 10 units kanamycin, 1 ml of water containing 19.2 mg glutamine, 3.3 ml of water containing 198 mg of $NaHCO_3$ and 10 ml of fetal calf serum (GIBCO, New York). After incubation for 14—16 h at 37 °C in a CO_2-incubator (5% CO_2 in air), 20 μl of DMSO containing an appropriate amount of a test chemical was added. After a 48 h incubation at 37 °C, the cells which were not stained with 0.5% trypan blue solution were counted as surviving cells.

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References and Notes

1) G. A. Sega, *Mutat. Res.*, **134**, 113 (1984); and references cited therein.
2) B. J. Bowdon, G. P. Wheeler, D. J. Adamson, and Y. F. Shealy, *Biochem. Pharmacol.*, **33**, 2951 (1984).

3) R. Preussmann, M. Habs, B. Pool, D. Stummeyer, W. Lijinsky, and M. D. Reuber, *Carcinogenesis*, **2**, 755 (1981); C. Janzowski, B. L. Pool, R. Preussmann, and G. Eisenbrand, *ibid.*, **3**, 155 (1982); C. Janzowski, J. Gottfried, G. Eisenbrand, and R. Preussmann, *ibid.*, **3**, 777 (1982); B. L. Pool, C. Janzowski, G. Eisenbrand, and R. Preussmann, *ibid.*, **3**, 781 (1982); R. Preussmann, M. Habs, H. Habs, and D. Stummeyer, *ibid.*, **3**, 1219 (1982); W. Lijinsky, J. E. Saavedra, M. D. Reuber, and S. S. Singer, *J. Natl. Cancer Inst.*, **68**, 681 (1982); T. P. Johnston, C. L. Kussner, R. L. Carter, J. L. Frye, N. R. Lomax, J. Plowman, and V. L. J. Narayanan, *Med. Chem.*, **27**, 1422 (1984).
4) T. Nakagawa and Y. Oyanagi, "Recent Developments in Statistical Inference and Data Analysis," ed. by K. Matushita, North Holland Publishing Co., 1980, pp. 221—225.
5) S. Ninomiya, K. Kohda, and Y. Kawazoe, *Chem. Pharm. Bull.*, **32**, 1326 (1984).
6) Y. Kawazoe and N. Tamura, *Gann*, **72**, 862 (1981).
7) W. G. Reifenrath, E. B. Roche, W. A. Al-Turk, and H. L. Johnson, *J. Med. Chem.*, **23**, 985 (1980).
8) J. Burdon and V. C. R. McLoughlin, *Tetrahedron*, **21**, 1 (1965).
9) C. Bunyagidj, H. Piotrowska, and M. H. Aldridge, *J. Org. Chem.*, **46**, 3335 (1981).
10) E. T. McBee, W. F. Marzluff, and O. R. Pierce, *J. Am. Chem. Soc.*, **74**, 444 (1952).
11) The preparation was not completely freed from benzene (used for crystallization) even on standing at 30°C *in vacuo* for 1 d.
12) R. Criegee and G. Müller, *Chem. Ber.*, **89**, 240 (1956).
13) R. J. Kaliszan, *Chromatogr.*, **220**, 71 (1981).
14) A. Hakura, S. Ninomiya, K. Kohda, and Y. Kawazoe, *Chem. Pharm. Bull.*, **32**, 3626 (1984).