H_2O–CH_3COCH_3 (1:1) to give 87 mg (55%) of 11 (Table II).

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Potential Antitumor Agents. 21. Dialkanolamine Dialkanesulfonic Esters

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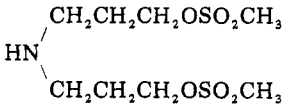
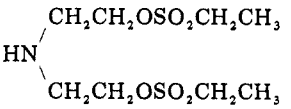
Homologous dialkanesulfonic esters of 2,2′-iminodiethanol, 3,3′-iminodi-1-propanol, *N*-(2-hydroxyethyl)-6-amino-1-hexanol, and *N*-(3-hydroxypropyl)-6-amino-1-hexanol were prepared via the *N*-trityl derivatives and screened for L1210 activity. For each active agent significant increases in life-span in L1210 tests (ILS) were correlated by linear least-squares regression with the corresponding log doses. The maximum ILS, taken from this regression line at the determined LD_{10}, was used as a quantitative measure of antileukemic effectiveness. Within each homologous series log ILS_{max} could be correlated with a binomial expression in lipophilic–hydrophilic balance, as measured by R_m values, but all active members from the four series could not be successfully included in a single correlation. By modification of R_m values with an ionization factor, log $(H^+/H^+ + K_a)$, all active compounds could then be included in a significant correlation equation.

A structurally simple antitumor agent containing an acylable amine function and having relatively high activity was required as the core molecule for projected latentiation studies.[1] From published data[2] 3,3′-iminodi-1-propanol dimethanesulfonate (1) appeared a suitable candidate. The recorded[2] antitumor activity (L1210) for this compound (1) is high in comparison with that found with nitrogen mustard, *N*-methyl-2,2′-dichlorodiethylamine (see Table I for comparative L1210 test data for these two agents). Our past experience has been that in many cases such differences in biologic activity result not from differing chemical structures per se but rather from the divergent physicochemical properties of the agents.[1,3,4] Usually drug lipophilic–hydrophilic balance has been found to be the dominant physicochemical parameter affecting biologic activity. To more rigorously assess the contribution of drug structural features to biologic activity, we have employed the simplifying device of conducting all agent comparisons at the equivalent of equilipophilicity.[1,3] Adopting this strategy, the biologic activity of molecules such as 1 and nitrogen mustard, these having different lipophilic characters (e.g., $\Sigma\pi$ values), would not be compared but rather, for example, those of 1 and the



1

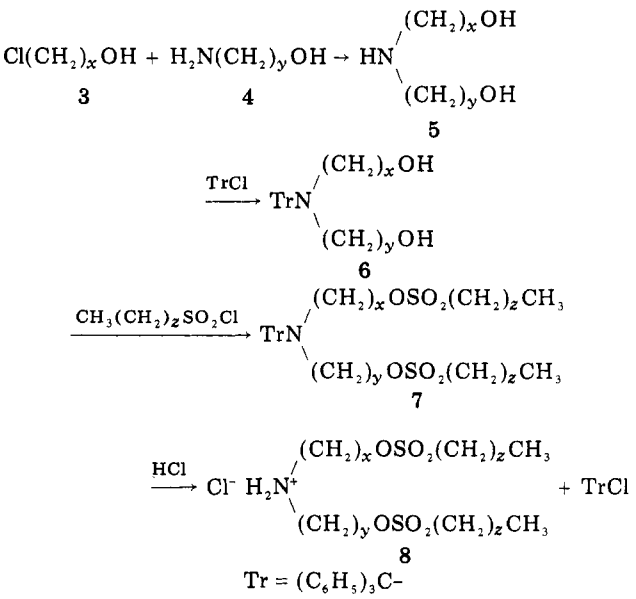


2

diethanesulfonic ester of 2,2′-iminodiethanol 2—these two molecules having comparable $\Sigma\pi$ values. Additionally, assessment of the maximum level of activity attainable in an agent series requires a knowledge of the optimum drug lipophilic–hydrophilic balance. Screening data from lower members of the homologous alkanesulfonic esters of 2,2′-iminodiethanol might then assist in answering the queries: (a) are there specific structural features of 1 which provide increased biologic selectivity, and (b) what agent lipophilic–hydrophilic balance is necessary for maximum experimental antileukemic activity?

Synthetic methods allowing convenient preparation of homologous series of alkanolamine alkanesulfonic esters,

Scheme I



antitumor (L1210) screening data, and quantitative structure–biologic activity relationships for these compounds form the basis of this publication.

Chemistry. Required alkanolamines **5** were prepared from chlorohydrin (**3**) and amine precursors **4** in alkaline media (Scheme I). Masking of alkanolamine nitrogen by tritylation, as in **6**, provided virtually nonbasic, crystalline, lipophilic derivatives—contrasting with the basic, viscous, hydrophilic precursors. Reaction of the *N*-tritylalkanolamines **6** with alkanesulfonyl chlorides in pyridine provided high yields (>90%) of the sulfonate esters **7**. Most lower members of the homologous *N*-tritylalkanesulfonic esters **7** were crystalline solids but the higher homologues were not obtained crystalline. The lipophilic nature conferred by the trityl function permits solutions of these noncrystalline materials in nonpolar solvents to be freed of excess reagents that could interfere in the final demasking step (**7** → **8**). Treatment of dioxane solutions of the sulfonate esters **7** with dry HCl precipitated the agent

Table I

No.	8 x	8 y	8 z	Mp, °C	Formula[a]	Recrystn solvent	R_m[b]	LD_{10},[c] mg/kg	L1210 ILS_{max} at LD_{10}[d]
Nitrogen mustard (for comparison purposes)								2.6	43
9	2	2	0	111-112[e]	$C_6H_{15}NO_6S_2 \cdot HCl$	Me_2CO-*i*-Pr_2O	-1.13	25	–[f]
2	2	2	1	116-117	$C_8H_{19}NO_6S_2 \cdot HCl$	Me_2CO-C_6H_6	-0.64	35	26
10	2	2	2	95-95.5	$C_{10}H_{23}NO_6S_2 \cdot HCl$	Me_2CO-Et_2O	-0.10	210	57
11	2	2	3	87-88	$C_{12}H_{27}NO_6S_2 \cdot HCl$[g]	MEK[h]	0.37	750	105 (1-3)
12	2	2	4	114-115	$C_{14}H_{31}NO_6S_2 \cdot HCl$	Me_2CO	0.76	520	78
1	3	3	0	95-96	$C_8H_{19}NO_6S_2 \cdot HCl$	Me_2CO-*i*-Pr_2O	-0.88	170	84 (1)
13	3	3	1	81-82	$C_{10}H_{23}NO_6S_2 \cdot HCl$	Me_2CO-Et_2O	-0.47	410	100 (1-3)
14	3	3	2	91-92	$C_{12}H_{27}NO_6S_2 \cdot HCl$	Me_2CO-*i*-Pr_2O	0.09	285	75
15	3	3	3	98-99	$C_{14}H_{31}NO_6S_2 \cdot HCl$	MEK-Me_2CO	0.45	183	27
16	2	6	0	97-98	$C_{10}H_{23}NO_6S_2 \cdot HCl$	Me_2CO-*i*-Pr_2O	-0.71	185	91 (1)
17	2	6	1	52-53	$C_{12}H_{27}NO_6S_2 \cdot HCl$	Me_2CO-*i*-Pr_2O	-0.19	285	83
18	2	6	2	86-87	$C_{14}H_{31}NO_6S_2 \cdot HCl$	Me_2CO	0.27	225	59
19	2	6	3	76-77	$C_{16}H_{35}NO_6S_2 \cdot HCl$[i]	MEK	0.51	200	35
20	3	6	0	80.5-81	$C_{11}H_{25}NO_6S_2 \cdot HCl$	Me_2CO-MEK	-0.65	33	107 (1-3)
21	3	6	1	71.5-72	$C_{13}H_{29}NO_6S_2 \cdot HCl$	Me_2CO-MEK	-0.05	61	78
22	3	6	2	80-82	$C_{15}H_{33}NO_6S_2 \cdot HCl$[j]	MEK	0.32	137	45

[a] All agents were analyzed for C, H, N, S, and Cl; analytical figures were within ±0.4% of theoretical values except where noted. [b] R_m values were measured chromatographically as detailed in the Experimental Section. [c] Single dose LD_{10}; derived from plots of mortality on a probit scale vs. dose on a logarithmic scale. [d] Increase in life-span at the LD_{10}; taken from the log dose-ILS correlation. See section on biologic testing. The numbers of animals, from a group of six, surviving 50 days after receiving doses bracketing the determined LD_{10} are provided in parentheses. [e] Lit.[5] mp 112 °C. [f] –, significant (>25%) ILS was not observed. [g] Cl: calcd, 9.3; found, 9.8. [h] Methyl ethyl ketone. [i] S: calcd, 14.6; found, 14.1. [j] Cl: calcd, 8.4; found, 8.9.

hydrochlorides 8 which were often found to be of analytical purity. Even in cases where the intermediary trityl derivatives 7 had not been obtained crystalline, agent hydrochlorides 8 were obtained as crystalline solids and, after purification by recrystallization, in yields of greater than 80%.

Biologic Testing. In all L1210 tests agents, as the hydrochlorides, were dissolved in ice–water and immediately administered to tumored animals; non-drug-treated controls received ice–water alone. Drugs were injected intraperitoneally (ip) as a single dose 24 h after implantation of 10^5 leukemic cells ip into 18.5–22.5 g F_1 hybrid mice from C_3H/HeJ female × DBA_2/J males. There were six animals per group at each dose level and for every six test groups there was one control group. Dose levels were arranged at logarithmic intervals and ranged from greater than the LD_{100} to below that providing significant life extension (T/C < 125%). When preliminary experiments had delineated an approximate maximum tolerated dose, a series of doses at close logarithmic intervals (0.09), selected to span the region between the maximum tolerated and the LD_{100} dose, was then screened. Animals dying in such tests at high dose levels, before day 8, were considered to die from drug toxicity. The percentage of toxic animal deaths at a given dose was converted to probit values and these were plotted against the corresponding doses on semilogarithmic paper and the best fit straight line was added. From this mortality–log dose relationship an LD_{10} value was derived.

To provide an objective measure of therapeutic effectiveness, significant increases in life-span (ILS = T/C% – 100), recorded in the therapeutic dose range below the LD_{10}, were correlated with the logarithms of the corresponding doses by linear regression using the method of least squares. For the agents examined the correlation coefficient (r) in these regressions was in all cases greater than 0.88. The maximum life extensions quoted (Table I) are those specified by the life extension–log dose correlation at the LD_{10}'s. The measure of antitumor effectiveness provided by this method of data analysis incorporates all significant life extensions, recorded over the full therapeutic dose profile, and obviates the need to accept the "best ever" observed life extension. The latter often results from chance events and in many cases provides an unrealistic appraisal of antitumor efficacy.

Structure–Activity Relationships. Using a single dose schedule the known[5] dimethanesulfonic ester of 2,2′-iminodiethanol (**9**, Table I) proved L1210 inactive. The targeted diethanesulfonate **2** provided marginal activity. However, the dipropanesulfonate **10** was appreciably active and the next higher homologue **11** was markedly active. Antileukemic effectiveness then declined on ascending to the dipentanesulfonate **12**. Since other physicochemical properties, in particular, base strength and sulfonate ester reactivity, should remain essentially constant through this homologous series, the observed changes in biologic activity can be reasonably ascribed to altering lipophilic–hydrophilic balance.

Since the peak dibutanesulfonate **11** has a considerably greater $\Sigma\pi$ value than the 3,3′-iminodi-1-propanol dimethanesulfonate **1**, inversion of our original argument would suggest that higher alkanesulfonate homologues of this latter compound might prove more active. Within the qualifications imposed by the screening system and the reproducibility thereof, the diethanesulfonate analogue **13** did provide a slightly greater percentage increase in life-span in L1210 tests than did the corresponding dimethanesulfonate **1**. The next two higher homologues in the series proved moderately (**14**) and weakly active (**15**). Comparison of the activities of peak members of the 2,2′-iminodiethanol (**11**) and 3,3′-iminodi-1-propanol series (**13**) shows that no particular advantage acrues from the blend of higher pK_a, greater separation of the alkylating termini, and, presumably, lessened reactivity in the latter compound. However, there is one particular example in the literature where there might be a biologic advantage associated with greater separation of the sulfonyloxy functions. The agent involved, nonane-1,9-diol dimethanesulfonate, is significantly L1210 active while variants not markedly different in $\Sigma\pi$ values, but having different chain lengths, are inactive.[2]

Symmetric dialkanolamine disulfonic esters having the same separation of the sulfonyloxy functions as in the nonane-1,9-diol system (**8**, $x = y = 4$) would be predicted

to expend themselves by intramolecular reaction to provide pyrrolidine derivatives.[6] Such internal reaction drops to minimal levels when there is a linear six carbon atom chain between the basic nitrogen function and the leaving group. Ring closure of C_6-alkanolamine derivatives is quoted to be as difficult a process as it is in the C_3 case,[6] the latter chain length providing convincingly active agents (**1**, **13–15**). In an asymmetric dialkanolamine variant with carbon chains of two and six atoms, diversionary self-reaction should be minimal but there would be the same overall separation of the sulfonyloxy groups as in the nonane-1,9-diol example. Homologous alkanesulfonic esters of this asymmetric congener did display excellent L1210 activity (**16–19**); the highest activity was displayed by the dimethanesulfonate **16**. There was no activity enhancement associated with the increased separation of the leaving groups in these variants.

The most active members of the 3,3′-iminodi-1-propanol series (**13**; LD_{10} = 410 mg/kg) and that of the asymmetric series (**16**; LD_{10} = 185 mg/kg) are significantly more dosage potent than the most active 2,2′-iminodiethanol analogue (**11**; LD_{10} = 750 mg/kg). Hypotheses based on variations in base strength or sulfonic ester reactivity could be tendered to explain these potency differences. Regardless of which hypothesis is considered, it can be predicted that an unsymmetric analogue having carbon chains of three and six atoms on either side of the amine function should be even more dose potent. Homologues in this further series (**20–22**) displayed convincing L1210 activity with the optimum member **20** being considerably more dose potent (LD_{10} = 33 mg/kg) than former examples.

Extension of the above hypotheses suggests that an alkanolamine analogue with two chains of six carbon atoms should be even more dose potent but it is clear from the examples presented that the dimethanesulfonic ester of such an alkanolamine would be supralipophilic and therefore of low activity. Successful employment of such a di-C_6-alkanolamine would require the preparation of a diester of an acceptable sulfonic acid that was itself more hydrophilic than methanesulfonic acid.

Quantitative Structure–Activity Relationships. The reactivity of these agents presents considerable difficulties when attempting to accurately measure the partition properties of neutral drug species. The agent cations are considerably more stable and we have employed an acidic reversed-phase chromatographic system and obtained R_m values of the cations as a measure of agent lipophilic–hydrophilic balance.[7] The chromatographic system employed was that earlier used with a series of acridine antitumor agents.[7] Experience with this system has shown R_m values for agent cations to be reproducible to ±0.03 and there is a good correlation between these values and measured partition coefficients in isobutyl alcohol–0.1 N HCl ($P_{i\text{-BuOH}}$) (eq 1).

$$-\log P_{i\text{-BuOH}} = 1.987\ (\pm 0.193)\ R_m + 0.746\ (\pm 0.036) \quad (1)$$

$$s = 0.095,\ r = 0.964,\ n = 32$$

In this equation, n is the number of data values, r is the correlation coefficient, and s the standard deviation of the regression.

Since L1210 activity had been observed to drop from that of a peak member of a series (cf. **11**) when either a more (**12**) or less (**10**) lipophilic congener was examined, it was expected that drug effectiveness would correlate with a binomial expression in R_m. However, such attempted correlation, using the data for all active agents, was not statistically significant (eq 2).

$$\log \mathrm{ILS}_{max} = -0.018\ (\pm 0.56)\ R_m^2 - 0.12\ (\pm 0.26)\ R_m + 1.80 \quad (2)$$

$$n = 15,\ r = 0.29,\ s = 0.21$$

Determination of agent pK_a values is also made difficult by drug instability although that of the 3,3′-iminodi-1-propanol dimethanesulfonic ester (**1**) has been measured (pK_a = 8.77).[8] Since the common sulfonyloxy group most influences agent pK_a's, the method of Perrin[9] was applied to gain the ΔpK_a value for this group and, using this figure, pK_a values for the various series were then computed. Assuming that homologation of the alkanesulfonate residue has negligible effect on base strength, computed pK_a values for sulfonate esters of the various alkanolamine series are 2,2′-iminodiethanol, 6.39; *N*-(2-hydroxyethyl)-6-amino-1-hexanol, 8.62; 3,3′-iminodi-1-propanol, 8.77; *N*-(3-hydroxypropyl)-6-amino-1-hexanol, 9.81.

The failure of our original argument that the diethanesulfonic ester of 2,2′-iminodiethanol (**2**) might have similar antitumor activity to the 3,3′-iminodi-1-propanol dimethanesulfonate (**1**) demonstrates that, if these agent are congeneric, simple π additivity does not operate satisfactorily in this series. ILS_{max} and either R_m or $\Sigma\pi$ values for members of each homologous series can be related via parabolic expressions but, because of the limited number of data values, these are of low significance. However, if the parabolic curves so generated from data from the four series are plotted on an R_m or π scale, it is found that the peaks of the parabolas are differently positioned. To merge the peak positions of these curves, so that an overall improved correlation might result, can only be achieved within a two-dimensional framework by modification of the measured R_m values. On the graphical representation the peak of the parabola for the *N*-(3-hydroxypropyl)-6-amino-1-hexanol series is at lowest R_m ($\Sigma\pi$) with those of the *N*-(2-hydroxyethyl)-6-amino-1-hexanol and 3,3′-iminodi-1-propanol series close together and placed centrally, while that of the 2,2′-iminodiethanol homologues is at a higher value; i.e., order of the peak positions on an R_m ($\Sigma\pi$) scale corresponds to order of pK_a values. Modification of R_m values by an ionization factor, as in eq 3, furnishes corrected (R_c) values which afford an excellent correlation with the biological data for all active agents (eq 4).

$$R_c = R_m + \log (H^+/K_a + H^+) \quad (3)$$

$$-\log \mathrm{ILS}_{max} = -0.46\ (\pm 0.07)\ R_c^2 - 0.51\ (\pm 0.09)\ R_c + 1.86 \quad (4)$$

$$n = 15,\ r = 0.97,\ s = 0.05,\ F_{2,12} = 94,\ p < 0.000001$$

Similarly, modification of $\Sigma\pi$ values by a pK_a factor (as in eq 5) furnishes π_c values which provide a significantly improved correlation (eq 6) over that obtained using $\Sigma\pi$ values alone (eq 7).

$$\pi_c = \Sigma\pi + \log (H^+/K_a + H^+) \quad (5)$$

$$\log \mathrm{ILS}_{max} = -0.080\ (\pm 0.031)\ \pi_c^2 + 0.11\ (\pm 0.07)\ \pi_c + 1.92 \quad (6)$$

$$n = 15,\ r = 0.85,\ s = 0.12,\ F_{2,12} = 15.8,\ p = 0.0044$$

$$\log \mathrm{ILS}_{max} = -0.088\ (\pm 0.065)\ (\Sigma\pi)^2 + 0.17\ (\pm 0.18)\ \Sigma\pi + 1.85 \quad (7)$$

$$n = 15,\ r = 0.67,\ s = 0.17,\ F_{2,12} = 4.9,\ p = 0.028$$

R_m and $\Sigma\pi$ values are clearly related (eq 8).

$$R_m = 0.36\ (\pm 0.10)\ \Sigma\pi - 0.535\ (\pm 0.18) \quad (8)$$

$$n = 16,\ r = 0.90,\ s = 0.26,\ F_{1,14} = 58,\ p < 0.0005$$

If R_m values provided a measure of modified $\Sigma\pi$ values, then it could be expected that the pK_a modified term π_c (eq 5) might provide an improved correlation with R_m but, in fact, a less well fit equation results (eq 9).

$$R_m = 0.26\ (\pm 0.13)\ \pi_c - 0.33\ (\pm 0.22) \quad (9)$$

$$n = 16,\ r = 0.76,\ s = 0.38,\ F_{1,14} = 19.7,\ p < 0.005$$

Discussion

Firstly, it must be decided if the method of measuring relative lipophilic–hydrophilic balance of agents has introduced the need for an ionization factor in order to obtain significant correlations. Several points suggest that is not so. (a) Chromatographic phases used in R_m determinations were 0.05 M in methanesulfonic acid, certainly sufficiently acidic that the present agents should have migrated as the cations.[10] It is unlikely that varying degrees of agent ionization during chromatography have produced the need for pK_a modulation. (b) $\Sigma\pi$ values are independent of the R_m measurement method, and of ionization, and significant improvements in correlation result when ionization corrected $\Sigma\pi$ values are used (cf. eq 6 and 7). (c) $\Sigma\pi$ and R_m values are clearly linearly related (eq 8). (d) This interrelationship is not improved by inclusion of a pK_a correction term (eq 9).

The best correlation (eq 4) embraces 94% (r^2) of the variance in the biologic data. Provided there is little covariance between pK_a, reactivities, and molecular dimensions, these latter two factors can then be of little importance in the antitumor action of the agents examined; agent lipophilic–hydrophilic balance is the prime determinant of attainable biologic selectivity. The results provide no clear answer as to how a more active agent could be obtained; rather they suggest that maximum activity has been reached and additional changes could merely further increase dose potency.

Modification of measures of log P according to agent ionization is novel but inescapable with the approach method used. The concept of an optimum log P (log P_0) for a congeneric drug series was introduced by Hansch.[21] The present results show that if drug variants have differing degrees of ionization, the optimum log P, within a single drug series, can alter. Our earlier simplifying expedient of comparing the biologic activity of analogues at the equivalent of equilipophilicity[1,3,4] (e.g., **1** and **2**) could lead to erroneous conclusions with varying ionized congeners. There are numerous literature examples of ionization corrections used in multiple regression analysis. Reexamination may show that equivalent correlations can be reached by using pK_a modified measures of log P.

The variation of optimum log P with pK_a provides further scope to the medicinal chemist. If, for pharmacologic or other reasons, a more lipophilic agent was required, then attenuation of agent pK_a would permit this. However, if the present agents are representative, this may be at the price of dose potency.

In the present work measures of lipophilic–hydrophilic balance for cationic drug species (DH^+) have been utilized. If usual partition coefficient (P) measures of such balance are employed, then

$$P_{DH^+} = [DH^+]_L/[DH^+]_A \quad (10)$$

in which expression subscripts denote the drug species found in the lipoidal (L) and aqueous (A) phases of the partition system, respectively.

When drug partition is examined at physiologic pH, the concentration of cation and neutral drug (D) in the aqueous phase will be interrelated by the expression

$$[DH^+]_A/[D]_A = [H^+/(H^+ + K_a)]/[1 - H^+/(H^+ + K_a)] \quad (11)$$

Rearrangement then provides

$$H^+/(H^+ + K_a) = [DH^+]_A/([DH^+]_A + [D]_A) \quad (12)$$

The pK_a corrected measures of lipophilic–hydrophilic balance (P_c), earlier employed in successful correlations, eq 4 and 6, were related to those of the ions by the relationship

$$\log P_c = \log P_{DH^+} + \log (H^+/H^+ + K_a) \quad (13)$$

Substitution of the latter term in this expression by eq 12, and following simplification, provides the relationship

$$\log P_c = \log [DH^+]_L/([DH^+]_A + [D]_A) \quad (14)$$

The operational P_c can then be considered to provide a partition coefficient describing the lipoidal concentration of ion, at physiologic pH, in relation to the total drug concentration (neutral plus ionized) in the aqueous phase. Such a conclusion runs counter to usually accepted views of the role of partition-dependent drug movement to the ultimate site of action. Normally the concentration of the more readily penetrating neutral drug species is considered all important in drug migration and the lesser amounts of ionic species in lipoidal phases are usually ignored.

Recently it has been demonstrated that nitrogen mustard enters examined, sensitive tumor cells by an active, carrier-mediated process.[11–20] The carrier implicated is that normally involved in cellular uptake of choline.[11–14] Certain structurally similar cationic substrates, ethanolamine, diethanolamine, and hemicholinium-3, can also compete for the same transport system.[11] Drugs which stimulate choline uptake also augment uptake of nitrogen mustard.[20] The examined compounds accepted by the choline transport system all have in common a cationic charge. There is an obvious structural relationship between nitrogen mustard and the dialkanolamine dialkanesulfonates described (Table I), particularly the diethanolamine congeners **9–12**. If drug cations of all the listed alkanesulfonates (Table I) were accepted by such a transport system, the pK_a corrected measures of lipophilic–hydrophilic balance, required in regression eq 4 and 6, would then have direct physiologic significance in quantifying drug cation available for acceptance by a carrier embedded in a lipoidal environment.

The very similar antileukemic efficacy of peak members of the four series of alkylating agents generated (**11**, **13**, **16**, **20**), these having varying separation of the alkanesulfonyloxy leaving groups, suggests that such separation is not of real significance in the antileukemic activity of these agents. As examples which are too hydrophilic are tumor inactive (e.g., **9**), but still host toxic, and the trend with increasing supralipophilic character is also toward inactivity (cf. **13–15**), it could be conjectured that the observed selectivity toward tumor actually resides in the lipophilic–hydrophilic balance of these agents. From the above hypothesis on the physiologic significance of pK_a modified measures of lipophilic–hydrophilic balance, it could be further conjectured that agent acceptance by a cell membrane carrier was a crucial factor in selective toxicity. If host–tumor selectivity was determined in such a fashion then, to obtain observable antitumor activity, it would only be necessary for the carrier introduced intracellular agent to provide a dose-related lethal event. As a corollary, for observable antitumor selectivity, drug structural features not influencing carrier acceptance (e.g.,

leaving group separation) could be unimportant as would the nature of the target molecule(s) involved in the lethal events. It would merely be necessary that drug structural features were such that adequate intracellular levels would provide lethal derangement of some critical cellular component(s).

In usual nitrogen mustard type alkylating agents the need for sufficient reactivity of the halogen atom leaving groups dictates quite strict adherence to the 2-haloethylamine structural unit.[22] Normal sulfonate esters are of sufficient reactivity[23] that there is considerably greater flexibility in the structural units to which they can be acceptably attached.[2] Further, the sulfonyloxy function is strongly hydrophilic ($\pi_{CH_3SO_2O-} = -1.77$), particularly in comparison with the lipophilic halogen atoms ($\pi_{Cl} = 0.39$; $\pi_{Br} = 0.60$), but the use of higher alkane- or arenesulfonates permits synthesis of more lipophilic congeners at will. It is clear that there is considerable latitude available to the medicinal chemist through the use of sulfonate esters as leaving groups in alkylating agents; the ready synthesis of lipophilic series increases the chance of spanning the optimum lipophilic–hydrophilic balance and, therefore, of observing high biologic activity.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal melting point apparatus with the makers' supplied stem corrected thermometer; melting points are as read.

Chromatography. Reversed-phase chromatography used the top phase of a mixture of glycerol (250 ml), *i*-BuOH (250 ml), and 1-pentanol (250 ml) plus methanesulfonic acid (2.7 ml) as developing solvent. The mixture of solvents was shaken thoroughly for 30 min and then allowed to settle for 48 h before phase separation. Prelegended Reidel de Hahn cellulose DC cards were impregnated by immersion in a 30% methanolic solution of the bottom phase of the solvent mixture. After rapid draining, excess solvent was removed from the sheets by blotting between filter paper and they were then dried at room temperature in a fume hood for 1 h. Alkanolamine alkanesulfonic esters were applied as solutions in 0.1 N $MeSO_3H$. Ascending development was for 24 h. 4′-(9-Acridinylamino)methanesulfonanilide[4] was included as standard on all sheets.

Alkanesulfonic esters were visualized by spraying developed sheets with a 2% solution of 4-(4-nitrobenzyl)pyridine in EtOH and then a 4% solution of K_2CO_3 in EtOH–H_2O (1:1). On warming the sprayed sheets to 60 °C the agents were located as blue spots and their position was immediately marked.

***N*-(2-Hydroxyethyl)-6-amino-1-hexanol.** To a stirred solution of 6-amino-1-hexanol (117 g, 1 mol) in warm (60 °C) H_2O (100 ml) 2-chloroethanol (13.9 ml, 0.25 mol) was added in dropwise fashion and then the clear solution boiled under reflux conditions for 15 min. After cooling to 60 °C solid NaOH (11 g) was stirred in and, when dissolved, further 2-chloroethanol (13.9 ml, 0.25 mol) was added and the solution boiled for 15 min. This cycle of events was repeated using a total of four additions of both 2-chloroethanol and NaOH. Finally the solution was boiled for 1 h, toluene (100 ml) was added, a Dean and Stark water separating head was fitted, and then all water was removed by azeotropic distillation. Stirring must be sufficiently vigorous to maintain formed NaCl in suspension to avoid explosive boiling. When all H_2O had been removed NaCl was filtered off and the flask and crystals were washed well with propan-2-ol. A preliminary vacuum distillation provided an enriched fraction of bp 185–210 °C (18 mm). Fractional distillation in vacuo then provided a clean product of bp 190–192 °C (18 mm) (57.3 g, 37% yield). Anal. ($C_8H_{19}NO_2$) C, H, N.

***N*-(3-Hydroxypropyl)-6-amino-1-hexanol** was prepared by the same method, substituting 3-chloro-1-propanol for the 2-chloroethanol. Work-up, as above, and fractional distillation in vacuo provided pure product as an extremely viscous liquid of bp 162–164 °C (2 mm) (47.4 g). Careful refractionation of mixed fractions provided a further quantity (5.3 g): total yield 30%. Anal. ($C_9H_{12}NO_2$) C, H, N.

Table II. *N*-Trityldialkanolamines 6

x	y	Mp, °C	Formula	Recrystn solvent	Analyses
2	2	160–161[a]	$C_{23}H_{25}NO_2$	C_6H_6-MeOH	C, H, N
3	3	132–133	$C_{25}H_{29}NO_2$	C_6H_6	C, H, N
2	6	97.5–98	$C_{27}H_{33}NO_2$	C_6H_6-petr ether	C, H, N
3	6	90–91	$C_{28}H_{35}NO_2$	C_6H_6-petr ether	C, H, N

[a] Lit.[5] mp 160 °C.

***N*-Trityldialkanolamine Derivatives 6.** Directions provided use 2,2′-iminodiethanol as an example; essentially similar methods were used with all alkanolamines. Redistilled 2,2′-iminodiethanol (21 g, 0.2 mol) was dissolved in pyridine (125 ml), Et_3N (25 ml, 0.185 mol) added and the solution cooled to −5 °C. Freshly prepared trityl chloride (50 g, 0.18 mol) was added portionwise to the well-stirred solution so that the temperature remained below −5 °C. The mixture was stirred until all trityl chloride had dissolved and then allowed to warm to room temperature. After 24 h at this temperature the mixture was heated on a steam bath for 1 h and then as much of the solvent as possible removed in vacuo. After addition of H_2O, product was removed in C_6H_6. The organic layer was washed with H_2O (twice); evaporation of the dried (Na_2SO_4) solution then provided crude product. With the 2,2′-iminodiethanol derivative **6** ($x = y = 2$) it is necessary to use hot C_6H_6 in the extraction to prevent premature crystallization of product. Two crystallizations (C_6H_6–MeOH) provided homogeneous product (51.7 g, 83% yield). To monitor purity during work-up, TLC on Al_2O_3 (Merck, DC Al_2O_3 F_{254}, neutral type E; Me_2CO–C_6H_6) was used. The *N*-trityl derivatives were partially cleaved when solutions were applied and then dried on SiO_2 TLC sheets (Merck DC SiO_2 F_{254}); the liberated triphenylcarbinol then makes even homogeneous samples appear impure. The cleavage of *O*-trityl ethers on columns of SiO_2 has been previously recorded and used for deprotection.[24]

Once seed crystals of the 3,3′-iminodi-1-propanol derivative **6** ($x = y = 3$) were obtained, purification by crystallization presented no problems. Before attempted crystallization of the *N*-trityl derivative of *N*-(2-hydroxyethyl)-6-amino-1-hexanol **6** ($x = 2$; $y = 6$), filtration of an Me_2CO solution of crude product through a short column of Al_2O_3 (Type H) removed by-products inhibiting crystallization. To isolate the further asymmetric derivative **6** ($x = 3$; $y = 6$) it was necessary to chromatograph the entire crude product. A C_6H_6 solution of crude product was applied to an Al_2O_3 column (10 g of Type H/g product). The column was washed in succession with two column volumes of C_6H_6 and then like volumes of C_6H_6 containing 1, 2, 4%, etc., of MeOH. TLC (Al_2O_3, C_6H_6–Me_2CO, 4:1, R_f ca. 0.4) showed that the required product emerged in fractions containing 4 and 8% of MeOH. Acquisition of seed crystals required several weeks of occasional trituration and storage of C_6H_6–petroleum ether solution at −15 °C. Even when this latter product had been obtained crystalline, and shown homogeneous to TLC, recrystallization (C_6H_6–petroleum ether) required considerable attention; very slow cooling rates were necessary to prevent deposition of product as an extremely viscous gum (Table II).

Alkanesulfonic esters were prepared by solution of the requisite *N*-tritylalkanolamine in pyridine (4 ml/g), cooling while stirring to −5 °C and adding alkanesulfonyl chloride (1.3 × theory) in dropwise fashion so that the temperature remained below −5 °C. The mixture was stirred in a refrigerator for 24 h and then excess crushed ice was added, followed by H_2O. After short standing the bulk of the aqueous mother liquors could be decanted from the viscous crude product. The remaining gum was dissolved in cold C_6H_6 by stirring and the organic layer washed well in succession with 10% aqueous citric acid, 10% $KHCO_3$, and H_2O, and then dried (Na_2SO_4). Removal of solvent in vacuo below 20 °C provided crude alkanesulfonic esters (Table III).

Dialkanolamine Dialkanesulfonic Ester Hydrochlorides 8. The foregoing *N*-trityl dialkanesulfonates **7** were dissolved in anhydrous dioxane (3 ml/g), the solution was cooled in an ice–water mixture, and a moderate stream of dry HCl was bubbled through the solution. Less lipophilic members crystallized directly

Table III. *N*-Trityldialkanolamine Dialkanesulfonates 7

x	y	z	Mp, °C	Formula	Recrystn solvent	Analyses
2	2	0	143-144[a]	$C_{25}H_{29}NO_6S_2$	C_6H_6	C, H, N, S
2	2	1	103-104	$C_{27}H_{33}NO_6S_2$	*i*-Pr_2O	C, H, N, S
2	2	2	121-122	$C_{29}H_{37}NO_6S_2$	C_6H_6-petr ether	C, H, N, S
3	3	0	51-51.5	$C_{27}H_{33}NO_6S_2$	C_6H_6-petr ether	C, H, N, S
2	6	0	41-41.5	$C_{29}H_{37}NO_6S_2$	C_6H_6-petr ether	C, H, N, S

[a] Lit.[5] mp 144 °C.

from the medium; with more lipophilic members it was necessary to add small volumes of C_6H_6 to initiate crystallization. The crystalline products were collected and washed free of trityl chloride with C_6H_6, Et_2O, or *i*-Pr_2O, taking care to minimize exposure of the crystals to the air. Products were dried in an evacuated desiccator over P_2O_5. In cases where the precursor *N*-trityl derivative had been obtained crystalline, the melting point of product obtained directly from the reaction mixture could not be improved by recrystallization. When the precursor had not been obtained crystalline, one recrystallization was necessary to obtain product hydrochloride of maximum melting point. Agents were stored at all times in an evacuated desiccator over P_2O_5.

The 3,3′-iminodi-1-propanol dimethanesulfonate 8 ($x = y = 3$; $z = 0$) prepared by the above route had the same melting point as an authentic sample which had been prepared by the interaction of methanesulfonic anhydride with 3,3′-imino-di-1-propanol in acetonitrile solution.[25] There was no melting point depression on admixture of the samples and these could not be distinguished or separated using the described reversed-phase TLC system.

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Potential Antitumor Agents. 22. Latentiated Congeners of the 4′-(9-Acridinylamino)methanesulfonanilides

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N^1-Acyl derivatives of the tumor inhibitory 4′-(9-acridinylamino)methanesulfonanilide agents act as prodrugs undergoing deacylation to liberate the core agents on incubation with pH 7.5 buffer or mouse blood. Against L1210 tumor implanted remotely from the drug administration site, lower acyl derivatives often provide enhanced effects over that obtained with nonacylated precursor alone. In certain homologous series of acyl derivatives, toxicity first increased with increasing lipophilic character, until greater than that of the core agent alone, and then at higher lipophilic levels decreased. Tumor inhibitory properties of the acyl derivatives in such series appeared inversely related to their toxicity. Several 3-(3,3-dialkyl-1-triazeno)acridine-substituted congeners provided excellent L1210 activity. Contrasting with most other tumor-active triazenes, one alkyl group need not be a methyl group for antileukemic activity to be observed. 3-Methyl-3-propyl-1-triazene and 3,3-diethyl-1-triazene analogues had comparable lipophilic–hydrophilic balance, toxicity, and antileukemic effectiveness; usual metabolic activation of the triazene *N*-methyl group may make little contribution to antitumor properties in the examples presented. Prepared as a nonalkylated triazene analogue, a 3-azidoacridine congener had high L1210 activity.

Earlier, active members of the 4′-(9-acridinylamino)-alkanesulfonanilide antitumor agents were screened using early, limited period, intraperitoneal (ip) dosing of animals implanted with L1210 leukemia at different anatomic sites.[1] It was found that the drug congeners most active against the leukemia implanted at sites remote from the point of drug administration were more lipophilic than considered optimal for ip implanted L1210.

If drug selectivity between leukemic cells and those of host tissues providing dose limitation could be effectively assayed in vitro, an optimum agent lipophilic–hydrophilic balance (log P_0) for selectivity would likely be found.