(all of which had $R_f$ values >0.9, compared with $R_f$ values for the complexes of 0.4–0.7).

HPLC studies were performed with a Waters Associates 600 multisolvent delivery system and 712 WISP automatic sample injector, with a Hewlett-Packard 1040A diode-array detector (wavelength range 190–600 nm) directly connected in line. The column was a Waters Associates Novapak $C_{18}$ reverse-phase bonded silica cartridge, and the detector response was monitored with Hewlett-Packard Chemstation software. The mobile phase was saturated aqueous NaCl/glycerol/$H_2O$/MeOH (1:1:1:2) adjusted to pH 4.00 with 1 N HCl.

The platinum content of collected column fractions was determined by flameless atomic absorption spectroscopy, using a Varian SpectrAA 20 spectrometer fitted with graphite furnace and autosampler.

**Formation of Compounds for Biological Testing: Example.** The free base of **9b** (23 mg) was suspended in dimethylacetamide (0.5 mL), and 0.5 mL of glycerol was added to give a homogeneous orange solution. Water (1–2 mL) was added last to make up the required concentration.

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# DNA-Directed Alkylating Agents. 3. Structure–Activity Relationships for Acridine-Linked Aniline Mustards: Consequences of Varying the Length of the Linker Chain

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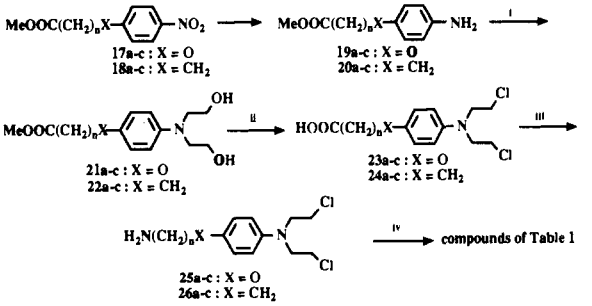
Four series of acridine-linked aniline mustards have been prepared and evaluated for in vitro cytotoxicity, in vivo antitumor activity, and DNA cross-linking ability. The anilines were attached to the DNA-intercalating acridine chromophores by link groups (-O-, $-CH_2-$, -S-, and $-SO_2-$) of widely varying electronic properties, providing four series of widely differing mustard reactivity where the alkyl chain linking the acridine and mustard moieties was varied from two to five carbons. Relationships were sought between chain length and biological properties. Within each series, increasing the chain length did not alter the reactivity of the alkylating moiety but did appear to position it differently on the DNA, since cross-linking ability (measured by agarose gel assay) altered with chain length, being maximal with the $C_4$ analogue. The in vivo antitumor activities of the compounds depended to some extent on the reactivity of the mustard, with the least reactive $SO_2$ compounds being inactive. However, DNA-targeting did appear to allow the use of less reactive mustards, since the S-linked acridine mustards showed significant activity whereas the parent S-mustard did not. Within each active series, the most active compound was the $C_4$ homologue, suggesting some relationship between activity and extent of DNA alkylation.

Several recent papers[1-3] have focused on the concept[4,5] of targeting alkylating agents to DNA by attaching them to DNA-intercalating ligands as DNA-affinic carriers. The aims of such an approach include increasing intrinsic drug potency,[1,6] avoiding some of the common mechanisms of cellular resistance to alkylating agents,[7] and altering the pattern of DNA lesions formed[8,9] and their repair.

We have recently shown[1] that the intrinsic cytotoxicities of simple aniline mustards can be drastically increased (up to 100-fold) by attaching them to the classic DNA-affinic intercalator 9-aminoacridine. The resulting compounds varied primarily in the reactivity of the mustard group (controlled by varying the electronic nature of the link group X), and the results showed that DNA-targeting decreased the usual tight dependence of cytotoxicity on mustard reactivity. While the untargeted aniline mustards showed a variation in cytotoxicity of about 50-fold between the most reactive compound (1a) and the least reactive one (13a), those of the corresponding targeted mustards 4 and 16 varied by less than 3-fold.[1] Most of the DNA-targeted mustards showed in vivo antitumor activity, being both more dose potent and more active than the clinically used

**Scheme I**[a]




[a] (i) oxirane/$H^+$; (ii) MsCl, LiCl/DMF/$\Delta$ $H^+$; (iii) EtOCOCl, $NaN_3$, $H^+$; (iv) 9-methoxyacridine.

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[∥] Peter MacCallum Cancer Institute.

(1) Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, B. C.; Wakelin, L. P. G.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 1177.
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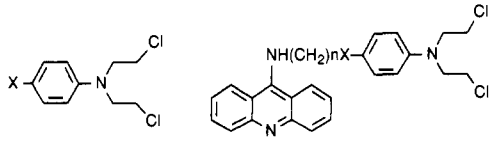
**Table I.** Physicochemical and Biological Properties of Acridine Mustards




1a, 5a, 9a, 13a 1 - 16

| no. | X | n | mp, °C | formula | analyses | relative[a] lipophilicity | DNA[b] cross-linking | cytotoxicity; $IC_{50}$,[c] μM P388 | AA8 | HF[d] | in vivo activity (P388) OD[e] | ILS[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a[g] | MeO | | | | | | + | 0.63 ± 0.12 | 2.9 | 42 | 65 | 36 |
| 1 | O | 2 | >60 dec | $C_{25}H_{25}Cl_2N_3O$·1HCl | C,H,N | -0.02 | | 0.072 ± 0.006 | 0.27 ± 0.03 | 54 ± 24 | 30 | 42 |
| 2 | O | 3 | >110 dec | $C_{26}H_{27}Cl_2N_3O$·2HCl | C,[h]H,N | 0.26 | | 0.047 ± 0.001 | 0.38 ± 0.05 | 54 ± 12 | 8.9 | 27 |
| 3 | O | 4 | 170–200 d | $C_{27}H_{29}Cl_2N_3O$ | C,H,N,Cl | 0.64 | | 0.063 ± 0.008 | 0.28 ± 0.06 | 55 ± 16 | 45 | 47 |
| 4[g] | O | 5 | | | | 1.18 | + | 0.050 ± 0.006 | 0.21 ± 0.02 | 46 ± 26 | 20 | 28 |
| 5a[g] | Me | | | | | | + | 0.41 ± 0.004 | 3.2 | 46 | 65 | 22 |
| 5 | $CH_2$ | 2 | 175–185 | $C_{26}H_{27}Cl_2N_3$·2HCl | C,H,N,Cl | 0.67 | + | 0.061 ± 0.004 | 0.31 ± 0.06 | 43 ± 9 | 45 | 25 |
| 6 | $CH_2$ | 3 | 175–200 | $C_{27}H_{29}Cl_2N_3$·2HCl | C,H,N,Cl | 1.21 | + | 0.086 ± 0.001 | 0.44 ± 0.15 | 36 ± 4 | 45 | 52 |
| 7 | $CH_2$ | 4 | >50 dec | $C_{28}H_{31}Cl_2N_3$·2HCl·$3H_2O$ | C,H,N,Cl | 1.75 | ++ | 0.082 ± 0.005 | 0.31 ± 0.17 | 59 ± 25 | 45 | 58 |
| 8[g] | $CH_2$ | 5 | | | | 2.29 | + | 0.136 ± 0.006 | 0.53 ± 0.03 | 36 ± 15 | 13.3 | 22 |
| 9a[g] | MeS | | | | | | - | 1.38 ± 0.008 | 5.3 ± 0.6 | 33 ± 4 | 65 | NA[j] |
| 9 | S | 2 | 104–105 | $C_{25}H_{25}Cl_2N_3S$·2HCl | C,H,N | 0.42 | | 0.372 ± 0.028 | 1.2 ± 0.2 | 24 ± 6 | 65 | 37 |
| 10 | S | 3 | 75–76 | $C_{26}H_{27}Cl_2N_3S$·HCl·$0.5H_2O$ | C,H,N,Cl | 0.79 | | 0.36 ± 0.02 | 1.1 ± 0.3 | 18 ± 2 | 13.3 | 38 |
| 11 | S | 4 | 90–95 | $C_{27}H_{29}Cl_2N_3S$·HCl·$H_2O$ | C,H,N | 1.22 | | 0.49 ± 0.02 | 1.37 ± 0.05 | 21 ± 5 | 26 | 48 |
| 12[g] | S | 5 | | | | 1.76 | - | 0.38 ± 0.01 | 0.71 ± 0.04 | 19 ± 6 | 45 | 23 |
| 13a[g] | $MeSO_2$ | | | | | | - | 39.0 ± 0.7 | 145 | 3.5 | 100 | NA |
| 13 | $SO_2$ | 2 | 136–137 | $C_{25}H_{25}Cl_2N_3O_2S$·HCl·$0.5H_2O$ | C,H,N | -1.17 | | 2.3 ± 0.1 | 18.3 ± 2.7 | 3.3 ± 0.3 | 65 | NA |
| 14 | $SO_2$ | 3 | 175–176 | $C_{26}H_{27}Cl_2N_3O_2S$·HCl | C,H,N[i] | -1.14 | | 2.6 ± 0.35 | 9.8 ± 0.8 | 5.1 ± 0.4 | 45 | NA |
| 15 | $SO_2$ | 4 | 93–95 | $C_{27}H_{29}Cl_2N_3O_2S$·HCl | C,H,N,Cl | -0.92 | | 1.9 ± 0.1 | 1.7 ± 0.3 | 1.4 ± 0.2 | 30 | NA |
| 16[g] | $SO_2$ | 5 | | | | -0.38 | - | 0.75 ± 0.07 | 0.57 ± 0.02 | 1.0 ± 0.2 | 45 | 33 |
| chlorambucil | | | | | | | | 7.5 ± 0.5 | 26 ± 3 | 58 ± 19 | 225 | 37 |

[a] Relative lipophilicity of the (common) side chain fragment $-NH(CH_2)_nX-$, calculated from substituent fragment values (see the text). [b] Cross-linking determined by agarose gel electrophoresis of pBR322/DHFR26 DNA after exposure to drug at 0.2 μM for 4 h; see the text. [c] $IC_{50}$ values determined against either P388 or AA8 cells, as described in ref 22 and outlined in the text, and the standard error of the mean. [d] HF = hypersensitivity factor = $IC_{50}(AA8)/IC_{50}(UV4)$, as outlined in the text. [e] OD = optimal dose of drug in milligrams/kilogram, administered as a single intraperitoneal dose in 0.1 or 0.2 mL at 30% v/v ethanol–water on day 1 after intraperitoneal inoculation of $10^6$ P388 leukemia cells. [f] ILS = the percentage increase in lifespan of drug-treated tumor-bearing animals when treated at the optimal dose (determined by spanning a range of doses from inactive to toxic at 1.5-fold intervals). Values above 20% are considered statisically significant. [g] Reference 1. [h] C off by 0.7%. [i] N off by 0.5%. [j] No activity seen at all dose levels, including toxic ones.

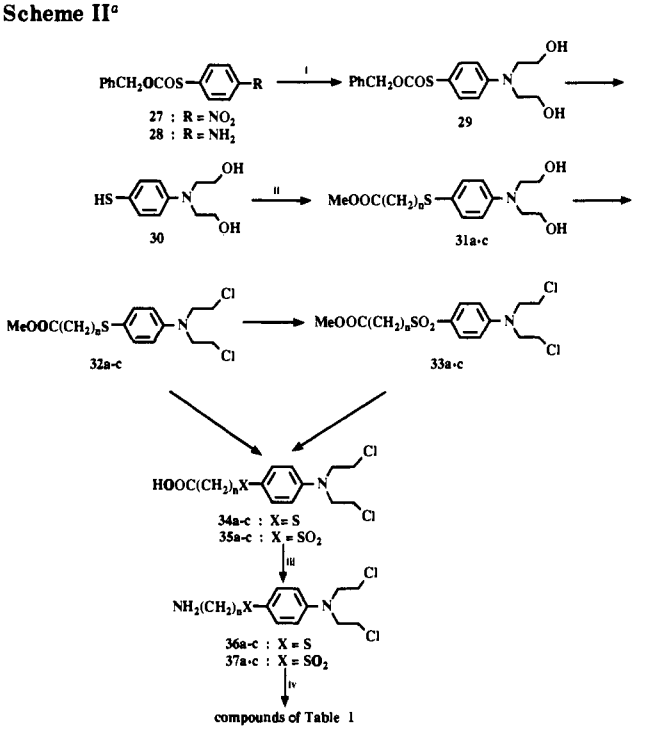
aniline mustard derivative chlorambucil.[1]

In the present paper we select four of the previous compounds, chosen to provide the widest range of reactivity of the aniline mustard, and study the consequences of varying the distance between the DNA-intercalating chromophore and the alkylating moiety by changing the length of the polymethylene linker chain.

## Chemistry

The O- and $CH_2$-linked compounds were prepared essentially by the methods described previously[1] and outlined in Scheme I. This method was not as suitable for the S-linked compounds, since reaction of the amines corresponding to **19** and **20** with oxirane was very slow and led to the formation of impurities which were difficult to remove. The S-linked compounds were therefore prepared by a modified method, involving initial synthesis of ben-

**Scheme II**[a]




[a] (i) oxirane/$H^+$; (ii) $MeOOC(CH_2)_nBr$; (iii) EtOCOCl, $NaN_3$, $H^+$; (iv) 9-methoxyacridine.

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Table II. Reactivity and DNA Cross-Linking Data for the Polymethylene-Linked Mustards

| no. | $K_H$,[a] $s^{-1} \times 10^4$ | $K_{NBP}$,[b] $s^{-1} \times 10^4$ | cross-linking[c] 0.2 μM | 0.02 μM | 0.002 μM |
|---|---|---|---|---|---|
| 5a | 4.71 | 111 | + | ND[d] | ND |
| 5 | 1.45 | 14.6 | + | ± | - |
| 6 | 2.06 | 22.5 | + | ± | - |
| 7 | 3.20 | 14.4 | ++ | + | - |
| 8 | 3.39 | 15.7 | + | + | - |

[a] $K_H$: rate constant for hydrolysis of the mustard in aqueous acetone; see ref 1. [b] $K_{NBP}$: rate constant for alkylation of 4-(4-nitrobenzyl)pyridine; see ref 1. [c] Cross-linking determined by agarose gel electrophoresis of pBR322/DHFR26 DNA after exposure to varying concentrations of drug for 4h (++, extensive cross-linking; +, significant cross-linking; ±, slight cross-linking; -, no cross-linking detected. [d] ND: not done.

zenethiol **30** and subsequent reaction of this with methyl ω-bromoalkanoates as outlined in Scheme II. The alkylamines were then generated by the Curtius reaction and coupled with 9-methoxyacridine to give the compounds of Table I.

## DNA Cross-Linking

This was assayed by incubation of the compounds for various times with a linear DNA fragment, followed by denaturation with methylmercury hydroxide. After allowing samples to stand under renaturing conditions for 1 h, the extent of renaturation was estimated with agarose gel electrophoresis. The usual denaturation procedure (heating to 95 °C) could not be used, since the alkylated DNA underwent extensive breakage under these conditions.

## Biological Evaluation

In vitro cytotoxicities were determined in 96-well cultures as described previously,[10,11] with murine leukemia P388 and Chinese hamster ovary derived AA8 and UV4 cell lines. The UV4 cell line is deficient in the normal excision repair of DNA adducts and is thus hypersensitive to DNA-alkylating agents.[10,12] The hypersensitivity ratio (HF = $IC_{50}$(AA8)/$IC_{50}$(UV4)) is an indication of the mode of cytotoxicity, with bifunctional DNA cross-linking agents usually showing HFs of 20–60. The compounds were evaluated for in vivo activity against P388 leukemia, with a single-dose protocol and chlorambucil as the standard.

## Results and Discussion

**Physicochemical Properties.** Tables I and II provide physicochemical and biological data for four homologous series of acridine-targeted aniline mustards, together with data for the corresponding untargeted compound in each case. As noted above, the four series were selected to provide the widest possible range in mustard reactivity. Previous studies[1] on the reactivity of these compounds (as measured by rates of hydrolysis and alkylation of NBP) have shown that this is controlled, as expected, by the electronic properties of the link group. The rates of hydrolysis ($k_H$ values) for parent mustards (**1a** and **13a**) from the most reactive (O-linked) and least reactive ($SO_2$-linked) series differ by 225-fold, and those for comparable acridine-linked compounds (**4** and **16**) differ by 36-fold (Table I). However, within a series the difference in mustard reactivity is much smaller, with only a 2-fold change in $k_H$ values and no significant change at all in the rates of alkylation of 4-(4-nitrobenzyl)pyridine ($k_{NBP}$ values) across the $CH_2$ series from **5** ($n$ = 2) to **8** ($n$ = 5) which was studied as a typical example (Table II).

The series also vary widely in lipophilicity. The relative lipophilicity of the variable side chain $-NH(CH_2)_nX-$ can be estimated from Hansch–Leo fragment constants[13] ($f$ and $f^{\phi}$). Summing these parameters and taking into account flexibility and polar proximity effects[13] gives the values listed in Table I. It can be seen that relative to the most lipophilic $CH_2$ series, use of the S link group is equivalent to one less methylene in the chain (in terms of lipophilicity), the O link to two less, and the $SO_2$ link to approximately four less methylenes.

The relative cross-linking abilities of the $C_5$ compounds in each series were determined by agarose gel electrophoresis, using plasmid DNA and a constant drug concentration (0.2 μM) and exposure time (4 h) (Table I). This allowed an estimation of the absolute cross-linking abilities of the compounds as the reactivity of the mustard varied. For the $CH_2$ series (**5–8**) the assay was also carried out with a series of different drug concentrations to evaluate in more detail the effect of chain length on this property when mustard reactivity is relatively constant. Although the data (Table II) are only semiquantitative, they suggest that (in the more reactive series at least) the compounds with longer chain lengths are more efficient DNA cross-linkers. No evidence of cross-linking was seen for the $SO_2$-linked compounds in this assay. With these compounds, the rate of the second reaction under these conditions must be so slow that degradation of the DNA monoadduct occurs first.

More detailed DNA alkylation studies[14] have shown that, particularly in the $C_nO$ and $C_nS$ series, there is an increased sequence preference for alkylation by the DNA-targeted compounds compared with the parent mustards, especially at guanines in 5′-GT sequences. As the chain length increases, this preference declines at the expense of reaction at adenines in AC sequences.

**Biological Properties.** As shown previously,[1] DNA-targeting of the mustard by the acridine provides compounds of much higher in vitro cytotoxicity than the corresponding untargeted parent in all four series. The data in Table I show that, across the four series, there is less difference in cytotoxicities among the DNA-targeted than the untargeted compounds, reflecting an apparently lessened importance of mustard reactivity in the former. Within each series there is generally little change in cytotoxicity against either P388 or AA8 cells with chain length. There is also little change in HFs for UV4 versus AA8 within each series, suggesting no alterations in the mode of cytotoxicity. Compounds in the O and $CH_2$ series show HFs of 40–55-fold, similar to those for their parent compounds **1a** and **5a** and indicative of DNA cross-linking. The S series shows slightly lower HFs (ca. 20-fold, slightly less than their parent compound **9a**) but still within the range indicative of action by cross-linking DNA.

The $SO_2$ series is the exception. These compounds are the weakest alkylating agents, and there is a distinct variation in cytotoxicity across the series, particularly evident in the AA8 data. The HF values also vary, from 3–5 for the parent **13a** and the early members of the series (consistent with monoalkylation events) to a value of ca.

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1 for the longer chain lengths. These last two compounds thus appear not to alkylate DNA at all by this criterion. $C_5$ compound **16** also showed no evidence of DNA cross-linking in the gel assays (see above). The relatively high cytotoxicity of this compound may be due to the fact that it acts via a topo II mediated mechanism. Preliminary studies[15] show that the ADR-1 cell line, which overexpresses topo II,[16] shows hypersensitivity to **16** but not to other compounds in the series.

Compounds in the first three series all showed significant in vivo antileukemic activity (ILS 50–60%) at a single dose of 20–40 mg/kg (Table I). The compounds are considerably more dose potent than the clinically used aniline mustard chlorambucil (ILS of 37% at a single dose of 225 mg/kg). In each series, the most active compound was the $C_4$ homologue. Since the relative lipophilicity varies markedly between each series, the superior activity of the $C_4$ homologues may be due to this configuration being optimal for DNA alkylation. In the $CH_2$ series (the only one studied in detail), the $C_4$ analogue did appear to be the most efficient DNA cross-linker.

## Conclusions

Following on from our earlier paper[1] looking at the effect of varying the reactivity of the mustard group in this class of DNA-targeted alkylating agents, this study focused on the consequences of chain-length variation. Increasing the chain length does not alter the reactivity of the alkylating moiety but does appear to position it differently on the DNA. Cross-linking ability (measured by agarose gel assay) did appear to alter with chain length, being maximal with the $C_4$ analogue.

However, in the O, $CH_2$, and S series, there is little change in in vitro cytotoxicity with chain length, with all compounds appearing to act by cross-linking DNA. In the least reactive ($SO_2$) series, there may be a change in mechanism, with the earlier members forming monoadducts and the compounds of longer chain length not reacting covalently with DNA at all. The in vivo antitumor activities of the compounds depended to some extent on the reactivity of the mustard, with the least reactive $SO_2$ compounds being inactive. However, DNA-targeting does appear to allow the use of less reactive mustards, since the S-linked series (**9–12**) showed significant activity whereas the parent (**9a**) did not. Within each active series, the most active compound was the $C_4$ homologue, suggesting some relationship between activity and extent of DNA alkylation. However, the levels of in vivo activity seen in the standard P388 screen do not seem sufficient (even allowing for the single-dose protocol) to warrant further development of the series.

## Experimental Section

Elemental analyses were carried out by the Microchemical Laboratory, University of Otago, and are indicated by the symbols of the elements when they are within ±0.4% of theoretical values. Melting points were determined on a Reichardt-Kofler block and are uncorrected. High-resolution mass spectra were recorded on an AEI MS-30 spectrometer at nominal 3000 resolution. NMR spectra were recorded on Bruker WP-60 or AM-400 spectrometers (400 MHz unless noted otherwise) and are reported as chemical shifts in ppm downfield from $Me_4Si$.

**Preparation of Compounds 1–3 of Table I by the Method of Scheme I.** A solution of methyl 3-(4-nitrophenoxy)propionate[17] (**17a**) in EtOAc was hydrogenated over Pd/C to give crude amine **19a** as an oil: $^1$H NMR ($CDCl_3$) δ 6.64 (m, 4 H, H-2′,3′), 4.15 (t, $J$ = 8 Hz, 2 H, $OCH_2$), 3.68 (s, 3 H, COOMe), 3.39 (br s, 2 H, $NH_2$), 2.71 (t, $J$ = 8 Hz, 2 H, $CH_2$COOMe). Similar treatment of methyl 4-(4-nitrophenoxy)butyrate (**17b**) [Prepared from the corresponding acid by Fischer esterification: mp (benzene/hexanes) 56–57 °C. Anal. ($C_{11}H_{13}NO_5$) C, H, N.] and 5-(4-nitrophenoxy)valerate (**17c**) [Prepared from the corresponding acid by Fischer esterification: mp (benzene/hexanes) 78–80 °C. Anal. ($C_{12}H_{15}NO_5$) C, H, N.] gave the corresponding amines **19b** and **19c**.

The above amine **19a** (2.0 g, 10.3 mmol) was dissolved in a mixture of THF (20 mL), AcOH (3 drops), and oxirane (1.0 mL) and stirred at 110–120 °C for 48 h in a pressure vessel. Solvents were removed under reduced pressure, and the residue was chromatographed on $SiO_2$. EtOAc/petroleum ether (2:3) eluted methyl 3-[4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenoxy]propionate (**21a**; 1.01 g, 35% yield) as an oil (lit.[18] oil): $^1$H NMR ($CDCl_3$) δ 6.74 (d, $J$ = 10 Hz, 2 H, H-2′), 6.54 (d, $J$ = 10 Hz, H-3′), 4.15 (t, $J$ = 8 Hz, 2 H, PhO$CH_2$), 3.72 (t, $J$ = 7 Hz, 4 H, $CH_2$OH), 3.68 (s, 3 H, COOMe), 3.16 (t, $J$ = 7 Hz, 4 H, $NCH_2$), 2.73 (t, $J$ = 7 Hz, 2 H, $CH_2$COOMe). Similar reactions with amines **19b** and **19c** gave, respectively, diols **21b** [in 41% yield as a solid, mp (benzene) 63–65 °C (lit.[18] mp 37 °C] and **21c** [in 46% yield as an oil (lit.[18] oil)].

A solution of diol **21a** (1.0 g, 3.53 mmol) in benzene (12 mL) was treated with $POCl_3$ (0.65 mL, 7 mmol) under reflux for 1 h. Removal of the volatiles under reduced pressure gave a residue which was dissolved in concentrated HCl (20 mL), warmed for 30 min at 60 °C and finally heated under reflux for 10 min. Evaporation of volatiles under reduced pressure gave a residue which was diluted with ice water, neutralized with concentrated ammonia, and extracted with EtOAc. Workup of the organic layer gave 3-[4-[*N*,*N*-bis(2-chloroethyl)amino]phenoxy]propionic acid (**23a**; 0.73 g, 68% yield), which was crystallized from benzene/hexane as needles: mp 92–95 °C (lit.[18] mp 93 °C); $^1$H NMR ($CDCl_3$) δ 6.85 (d, $J$ = 9.07 Hz, 2 H, H-2′), 6.70 (d, $J$ = 9.09 Hz, 2 H, H-3′), 4.18 (t, $J$ = 6.23 Hz, 2 H, $OCH_2$), 3.64 (m, 4 H, $NCH_2$), 3.58 (m, 4 H, $CH_2Cl$), 2.81 (t, $J$ = 6.24 Hz, 2 H, H-2). Similar reactions with diol esters **21b** and **21c** gave, respectively, mustards **23b** [in 72% yield, mp (benzene) 83–84 °C (lit.[18] mp 85.5 °C)] and **23c** [in 89% yield, mp (benzene/hexane) 87–89 °C (lit.[18] mp 87.5 °C)].

Acid **23a** (0.7 g, 2.29 mmol) was dissolved in $Me_2CO$ (2 mL), and the solution was cooled to 0 °C and treated successively with $Et_3N$ (0.38 mL, 2.7 mmol), ethyl chloroformate (0.26 mL, 2.7 mmol), and (after a further 15 min) a solution of $NaN_3$ (0.3 g, 4.7 mmol) in water (2 mL). The mixture was stirred for a further 30 min, diluted with ice water, and extracted with benzene (3 × 50 mL). The combined, dried organic layers were heated under reflux for 1 h, and solvent was removed under reduced pressure. The residue was heated under reflux in 8 N HCl (20 mL) for 10 min, and the solution was concentrated under reduced pressure. The pH of the concentration was adjusted to 12 with concentrated ammonia, and the concentrate was then extracted with EtOAc (3 × 100 mL). Workup of the organic layer gave amine **25a** (0.57 g, 95% yield) as an oil: $^1$H NMR ($CDCl_3$) δ 6.79 (m, 4 H, H-2′,3′), 4.14 (t, $J$ = 8 Hz, 2 H, $OCH_2$), 3.62 (m, 8 H, $NCH_2Cl$), 2.68 (br s, 2 H, $NH_2$). The crude amine was coupled directly with 9-methoxyacridine in MeOH at 20 °C for 24 h, and the mixture was concentrated to dryness under reduced pressure. Chromatography of the residue on $SiO_2$ and elution with EtOAc gave the free base of compound 1 as a yellow gum (0.82 g, 84% yield). This was dissolved in EtOAc and treated with HCl-saturated EtOAc to give the dihydrochloride salt as a yellow powder: mp <60 °C dec; $^1$H NMR ($CD_3SOCD_3$) δ 14.30 (s, 1 H, HCl), 10.11 (d, $J$ = 7.96 Hz, 2 H, acridine H-1,8), 8.03 (d, $J$ = 8.61 Hz, 2 H, acridine H-4,5), 7.98 (d, $J$ = 6.61 Hz, 2 H, acridine H-3,6), 7.54 (t, $J$ = 7.08 Hz, 2 H, acridine H-2,7), 6.79 (d, $J$ = 9.11 Hz, 2 H, H-2′), 6.68 (t, $J$ = 8.83 Hz, 2 H, H-3′,5′), 4.45 (s, 4 H, $NHCH_2CH_2O$), 3.64 (m, 8 H, $NCH_2CH_2Cl$). Anal. ($C_{25}H_{25}Cl_2N_3O$·HCl) in Table I. Similar treatment of acids **23b** and **23c** gave compounds **2** and **3** of Table I.

(15) Unpublished data, this laboratory.
(16) Robson, C. N.; Hoban, P.; Harris, A. L.; Hickson, I. D. *Cancer Res.* **1987**, *47*, 1560.
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(18) Davis, W.; Roberts, J. J.; Ross, W. C. J. *J. Chem. Soc.* **1955**, 890.

**Preparation of Compounds 5–7 of Table I by the Method of Scheme I.** Catalytic hydrogenation of methyl 3-(4-nitrophenyl)butyrate[19] (**18a**) and treatment of the resulting amine **20a** with oxirane as detailed above gave methyl 3-[4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenyl]butyrate (**22a**) as an oil in 46% overall yield: $^1$H NMR ($CDCl_3$) $\delta$ 7.03 (d, $J$ = 8.60 Hz, 2 H, H-2′,6′), 6.63 (d, $J$ = 8.64 Hz, 2 H, H-3′,5′), 3.80 (t, $J$ = 4.8 Hz, 4 H, $NCH_2$), 3.65 (s, 3 H, Me), 3.52 (t, $J$ = 4.7 Hz, 4 H, $CH_2OH$), 2.50 (t, $J$ = 7.64 Hz, 2 H, H-4), 2.20 (t, $J$ = 7.20 Hz, 2 H, H-2), 1.72 (quintet, $J$ = 7.38 Hz, 2 H, H-3). Similar treatment of amines **20b** and **20c** gave the corresponding diols **22b** [as a solid, mp (benzene/hexane) 29–31 °C] and **22c** (as an oil).

Treatment of diol **22a** with $POCl_3$ followed by acid hydrolysis as detailed above gave 3-[4-[*N*,*N*-bis(2-chloroethyl)amino]-phenyl]butyric acid (**24a**, chlorambucil) in 71% yield: mp 63–65 °C (lit.[19] mp 64–66 °C). $^1$H NMR ($CD_3SOCD_3$) $\delta$ 7.02 (d, $J$ = 8.5 Hz, 2 H, H-2′), 6.66 (d, $J$ = 8.5 Hz, 2 H, H-3′), 3.69 (s, 8 H, $NCH_2CH_2Cl$), 2.46 (t, $J$ = 7.64 Hz, 2 H, H-4), 2.19 (t, $J$ = 7.19 Hz, 2 H, H-2), 1.73 (quintet, $J$ = 7.37 Hz, 2 H, H-3). Similar reaction of diols **22b** and **22c** gave the corresponding mustards **24b** [as a solid, mp (toluene/hexane) 87–88 °C (lit.[19] mp 87 °C)] and **24c** (as an oil).

Acid **24a** was then transformed via the modified Curtius reaction described above to give amine **26a** in 84% yield: $^1$H NMR ($CDCl_3$) $\delta$ 7.07 (d, $J$ = 10 Hz, 2 H, H-2′), 6.60 (d, $J$ = 10 Hz, 2 H, H-3′), 3.63 (s, 8 H, $NCH_2CH_2Cl$), 2.66 (m, 4 H, H-1,3), 1.75 (m, 2 H, H-2), 1.36 (br s, 2 H, $NH_2$). This was coupled directly with 9-methoxyacridine as described above to give compound **5** in 76% yield. This was crystallized from EtOAc/HCl as the dihydrochloride salt: mp 175–185 °C; $^1$H NMR ($CD_3SOCD_3$) $\delta$ 14.10 (s, 1 H, HCl), 10.02 (t, $J$ = 5.72 Hz, 1 H, NH), 8.45 (br s, 2 H, acridine H-1,8), 7.92 (m, 4 H, acridine H-3,6,4,5), 7.39 (br s, 2 H, acridine H-2,7), 6.92 (d, $J$ = 8.54 Hz, 2 H, H-2′), 6.57 (d, $J$ = 8.63 Hz, 2 H, H-3M), 4.01 (q, $J$ = 6.06 Hz, 2 H, $NHCH_2$), 3.63 (br s, 8 H, $NCH_2CH_2Cl$), 2.55 (t, $J$ = 7.09 Hz, 2 H, H-3), 2.13 (quintet, $J$ = 7.1 hz, 2 H, H-2). Anal. in Table I. Similar treatment of acids **24b** and **24c** gave compounds **6** and **7** of Table I via the corresponding amines **26b** and **26c**.

***S*-[(Benzyloxy)carbonyl]-4-[*N*,*N*-bis(2-hydroxyethyl)-amino]benzenethiol (29).** Carbobenzyloxy chloride (50.9 g, 0.36 mol) was added dropwise to a stirred, cooled (ice bath) solution of sodium 4-nitrothiophenate (52.6 g, 0.30 mol) in EtOH (500 mL). The resulting yellow suspension was stirred for a further 2 h, the solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and water. The organic phase was washed and worked up to give *S*-[(benzyloxy)carbonyl]-4-nitrobenzenethiol (**27**), which crystallized from aqueous EtOH as yellow plates (79.7 g, 92%): mp 82–83 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.23 (d, $J$ = 8.92 Hz, 2 H, H-3′), 7.71 (d, $J$ = 8.92 Hz, 2 H, H-2′), 7.38 (br s, 5 H, phenyl), 5.30 (s, 2 H, $CH_2$). Anal. ($C_{14}H_{11}NO_4S$) C, H. N.

A suspension of the above compond **27** (45.9 g, 0.16 mol) and $SnCl_2 \cdot 2H_2O$ (183.9 g, 0.82 mol) in EtOAc (300 mL) was heated under gentle reflux for 4 h in an atmosphere of $N_2$. The cooled mixture was basified with ammonia and filtered. The filtrate was washed with water and worked up to give *S*-[(benzyloxy)-carbonyl]-4-aminobenzenethiol (**28**), which crystallized from EtOH as an off-white solid (37.2 g, 88%): mp 85.5–86.5 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.35 (m, 5 H, phenyl), 7.28 (d, $J$ = 8.57 Hz, 2 H, H-2′), 6.64 (d, $J$ = 8.57 Hz, 2 H, H-3′), 5.22 (s, 2 H, $CH_2$), 3.84 (br s, 1 H,NH). Anal. ($C_{14}H_{13}NO_2S$) C, H, N.

This amine (**28**; 25.5 g, 0.10 mol) was dissolved in a mixture of THF (250 mL), AcOH (250 mL), and oxirane (5.0 mL, 0.10 mol). The reaction was stirred at 20 °C for 2 weeks, during which time further additions (10 × 0.5 mL) of oxirane were made. The mixture was then neutralized with $Na_2CO_3$, and the solvents were removed under reduced pressure. The residue was partitioned between EtOAc and water, and the organic layer was washed with water and worked up to give an oil. This was chromatographed on $SiO_2$. Elution with EtOAc/petroleum ether (3:7) gave *S*-[(benzyloxy)carbonyl]-4-[*N*-(2-hydroxyethyl)amino]benzenethiol (**29a**; 4.5 g, 12%), which was crystallized from EtOAc/hexane: mp 91–92 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.38 (m, 5 H, phenyl), 7.32 (d, $J$ = 8.79 Hz, 2 H, H-2′), 6.63 (d, $J$ = 8.80 Hz, 2 H, H-3′), 5.23 (s, 2 H, $CH_2OCO$), 4.28 (s, 1 H, NH), 3.84 (q, $J$ = 5.29 Hz, 2 H, $CH_2OH$), 3.31 (t, $J$ = 5.29 Hz, 2 H, $CH_2OH$). Anal. ($C_{16}H_{17}NO_3S$) C, H, N. Elution with EtOAc gave *S*-[(benzyloxy)carbonyl]-4-[*N*,*N*-bis(2-hydroxyethyl)amino]benzenethiol (**29**; 28.4 g, 79%), which was crystallized from EtOAc/hexane: mp 81–82 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.35 (m, 5 H, phenyl), 7.32 (d, $J$ = 8.94 Hz, 2 H, H-2′), 6.64 (d, $J$ = 9.01 Hz, 2 H, H-3′), 5.21 (s, 2 H, $CH_2OCO$), 4.04 (br s, 2 H, OH), 3.70 (t, $J$ = 4.43 Hz, 4 H, $CH_2OH$), 3.55 (t, $J$ = 4.88 Hz, 4 H, $NCH_2$). Anal. ($C_{18}H_{21}NO_4S$) C, H, N.

The above diol **29** (28.3 g, 0.08 mol) was dissolved in EtOH (200 mL) containing NaOH (16.0 g, 0.4 mol), and the mixture was heated under reflux for 2 h. Solvent was removed under reduced pressure, and the residue was diluted with water and neutralized with AcOH. The mixture was then saturated with NaCl and extracted with EtOAc (3×). The combined extracts were washed with brine and worked up to give a yellow oil, which was chromatographed on $SiO_2$. Elution with EtOAc/petroleum ether (2:3) gave 4-[*N*,*N*-bis(2-hydroxyethyl)amino]benzenethiol (**30**; 10.2 g, 56%), which crsytallized from EtOAc/hexane as yellow plates: mp 136 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.20 (d, $J$ = 8.75 Hz, 2 H, H-2), 6.55 (d, $J$ = 8.92 Hz, 2 H, H-3), 3.75 (m, 4 H, $CH_2OH$), 3.49 (t, $J$ = 4.91 Hz, 4 H, $NCH_2$).

**Preparation of Compounds 9–11 of Table I by the Method of Scheme II.** A mixture of the above thiol **30** (2.06 g, 0.01 mol), methyl 3-bromopropionate (1.78 g, 0.011 mol), and dry $K_2CO_3$ (1.47 g, 0.011 mol) in dry MeOH (80 mL) was heated under reflux in an atmosphere of $N_2$ for 5–10 min. The solvent was removed under reduced pressure, and the residue was partitioned between $CH_2Cl_2$ and water. The organic phase was worked up to give an oil which was chromatographed on $SiO_2$. Elution with EtOAc gave methyl 3-[[4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenyl]thio]-propionate (**31a**; 1.60 g, 57%), as a colorless oil which darkened rapidly on standing: $^1$H NMR ($CDCl_3$) $\delta$ 7.30 (d, $J$ = 9.03 Hz, 2 H, H-2′), 6.59 (d, $J$ = 9.03 Hz, 2 H, H-3′), 4.32 (br s, 2 H, OH), 3.78 (t, $J$ = 6.76 Hz, 4 H, $CH_2OH$), 3.47 (s, 3 H, COOMe), 3.53 (t, $J$ = 7.21 Hz, 4 H, $NCH_2$), 2.97 (t, $J$ = 3.74 Hz, 2 H, $SCH_2$), 2.55 (t, $J$ = 7.27 Hz, 2 H, $COCH_2$); mass spectrum, $m/z$ 268 (M - OMe, 100), 224, 213, 182, 164, 150, 136, 45. Similar reactions using the appropriate homologous bromo esters gave methyl 4-[[4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenyl]thio]butyrate (**31b**) and methyl 5-[[4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenyl]thio]-pentanoate (**31c**) as oils (yields 56% and 74%, respectively).

An ice-cold solution of diol **31a** (2.0 g, 6.69 mmol) in dry $CH_2Cl_2$ (200 mL) was treated sequentially with $Et_3N$ (2.37 mL, 17 mmol) and methanesulfonyl chloride (1.29 mL, 17 mmol), and the mixture was stirred at 0 °C for a further 45 min. The mixture was diluted with cold $CH_2Cl_2$, washed with ice-cold aqueous $NaHCO_3$ and brine, and solvent was then removed at room temperature under reduced pressure. The resulting crude dimesylate was dissolved in dry DMF (20 mL), treated with NaCl (0.8 g, 13 mmol), and heated to 120 °C for 5 min. Solvent was removed under reduced pressure, and the residue was dissolved in EtOAc. The organic layer was washed with water, treated with charcoal, and worked up to give methyl 3-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]-thio]propionate (**32a**) as a colorless oil (1.65 g, 74% yield from **31a**): $^1$H NMR ($CDCl_3$) $\delta$ 7.35 (d, $J$ = 8.16 Hz, 2 H, H-2′), 6.63 (d, $J$ = 9.03 Hz, 2 H, H-3′), 3.73 (t, $J$ = 6.42 Hz, 4 H, $NCH_2$), 3.68 (s, 3 H, COOMe), 3.63 (t, $J$ = 7.31 Hz, 4 H, $CH_2Cl$), 3.00 (t, $J$ = 7.25 Hz, 2 H, H-3), 2.57 (t, $J$ = 7.55 Hz, 2 H, H-2). Similar reaction of **31b** and **31c** gave methyl 4-[[4-[*N*,*N*-bis(2-chloroethyl)-amino]phenyl]thio]butyrate (**32b**) and methyl 5-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]thio]pentanoate (**32c**) as oils (yields 55% and 62%, respectively).

The above methyl ester **32a** (1.65 g, 4.93 mmol) was heated under reflux in concentrated HCl (80 mL) for 1.5 h, and the cooled mixture was neutralized with concentrated ammonia and extracted with EtOAc. The organic layer was dried, treated with charcoal, and evaporated to give 3-[[4-[*N*,*N*-bis(2-chloroethyl)amino]-phenyl]thio]propanoic acid (**34a**; 1.58 g, 73% yield) as a colorless oil; $^1$H NMR ($CDCl_3$) $\delta$ 7.36 (d, $J$ = 8.92 Hz, 2 H, H-2′), 6.62 (d, $J$ = 8.92 Hz, 2 H, H-3′), 3.73 (t, $J$ = 6.62 Hz, 4 H, $NCH_2$), 3.65 (t, $J$ = 6.71 Hz, 4 H, $CH_2Cl$), 3.00 (t, $J$ = 7.31 Hz, 2 H, H-3), 2.61 (t, $J$ = 7.31 Hz, 2 H, H-2. Similar reaction of **32b** and **32c** gave 4-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]thio]butyric acid (**34b**)

(19) Everett, J. L.; Roberts, J. J.; Ross, W. C. J. *J. Am. Chem. Soc.* **1953**, *75*, 2386.

and 5-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]thio]pentanoic acid (**34c**) as oils in 99% and 96% yields, respectively.

A solution of the acid mustard **34a** (0.95 g, 2.96 mmol) in $Me_2CO$ (12 mL) was cooled to −5 °C and $Et_3N$ (0.45 mL, 3.26 mmol) was added dropwise. After 5 min a solution of ethyl chloroformate (0.31 mL, 3.26 mmol) in $Me_2CO$ (2 mL) was added, followed after 10 min by a solution of $NaN_3$ (0.38 g, 5.92 mmol) in water (2 mL). The mixture was stirred at 0 °C for 30 min, poured into ice water, and extracted with toluene (2 × 150 mL). The combined organic fractions were dried ($Na_2CO_3$) and heated under reflux for 1.5 h. Solvent was removed under reduced pressure, and the residue was dissolved in 8 N HCl (12 mL) and heated under reflux for 10 min. The cooled mixture was diluted with ice water and extracted with $CH_2Cl_2$. Workup gave the crude amine **36a** (0.74 g, 83% yield) as an oil which was coupled immediately with 9-methoxyacridine (0.59 g, 2.58 mmol) in MeOH (60 mL) at 20 °C for 14 h. Solvent was removed under reduced pressure, and the residue was chromatographed on $SiO_2$, with EtOAc/petroleum ether (1:1) eluting **9** as a yellow oil (0.81 g, 76% yield). This was dissolved in EtOAc and treated with dry, HCl-saturated EtOAc to give the dihydrochloride of **9** as a yellow, hygroscopic powder: mp 104–105 °C; $^1H$ NMR ($CD_3OD$) $\delta$ 8.32 (br s, 2 H, acridine, H-1,8), 7.95 (t, $J$ = 8.18 Hz, 2 H, acridine H-3,6), 7.83 (m, 2 H, acridine H-4,5), 7.43 (br s, 2 H, acridine H-2,7), 7.10 (d, $J$ = 8.82 Hz, 2 H, H-2′), 6.55 (d, $J$ = 8.84 Hz, 2 H, H-3′), 4.28 (t, $J$ = 6.55 Hz, 2 H, $NHCH_2$), 3.73 (t, $J$ = 7.05 Hz, 4 H, $NCH_2$), 3.61 (t, $J$ = 6.5 Hz, 4 H, $CH_2Cl_2$). Anal. in Table I. Similar reaction of **34b** and **34c** gave the corresponding amines **36b** and **36c** in 80% and 64% crude yields, respectively, and these were similarly coupled with 9-methoxyacridine to give compounds **10** and **11** of Table I.

**Preparation of Compounds 13–15 of Table I by the Methods of Scheme II.** A solution of **32a** (0.80 g, 2.39 mmol) in dry $CH_2Cl_2$ (150 mL) was treated portionwise with 3-chloroperoxybenzoic acid (1.11 g, 6.45 mmol) at 20 °C for 20 h and then diluted with $CH_2Cl_2$. The organic layer was washed with aqueous $NaHCO_3$ and worked up, and the residue was filtered through a column of $SiO_2$ in EtOAc/petroleum ether (1:4) to give methyl 3-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]sufonyl]propionate (**33a**; 0.74 g, 84% yield), which was crystallized from EtOAc/hexane: mp 63–64 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.74 (d, $J$ = 9.07 Hz, 2 H, H-2′), 6.75 (d, $J$ = 9.10 Hz, 2 H, H-3′), 3.84 (t, $J$ = 6.93 Hz, 4 H, $NCH_2$), 3.68 (t, $J$ = 6.71 Hz, 4 H, $CH_2Cl$), 3.66 (s, 3 H, OMe), 3.39 (t, $J$ = 7.55 Hz, 2 H, H-3), 2.75 (t, $J$ = 7.96 Hz, 2 H, H-2); $C_{14}H_{19}Cl_2NO_4S$ requires 367.0412, found 367.04236. Similar treatment of **32b** and **32c** gave methyl 4-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]sulfonyl]butyrate (**33b**) in 88% yield as a white solid [Mp (benzene/hexane) 80 °C. Anal. ($C_{15}H_{21}Cl_2NO_4S$) C, H, N, Cl.] and methyl 5-[[4-[*N*,*N*-bis(2-chloroethyl)amino]-phenyl]sulfonyl]pentanoate (**33c**) as an oil in 78% yield. $C_{16}H_{23}Cl_2NO_4S$ requires 395.0718, found 395.0725.

A solution of **33a** (1.30 g, 3.54 mmol) in concentrated HCl (80 mL) was heated under reflux for 2 h. The cooled solution was just neutralized with concentrated ammonia and extracted with EtOAc. The organic layer was dried and treated with charcoal to give 3-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]sulfonyl]-propionic acid (**35a**; 1.12 g, 90% yield), which crystallized from MeOH/EtOAc as white needles: mp 171–172 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.75 (d, $J$ = 9.02 Hz, 2 H, H-2′), 6.76 (d, $J$ = 9.10 Hz, 2 H, H-3′), 3.84 (t, $J$ = 6.94 Hz, 4 H, $NCH_2$), 3.70 (t, $J$ = 6.95 Hz, 4 H, $CH_2Cl$), 3.38 (t, $J$ = 7.44 Hz, 2 H, H-3), 2.78 (t, $J$ = 7.71 Hz, 2 H, H-2). Anal. ($C_{13}H_{17}Cl_2NO_4S$) C, H, N, Cl. Similar reactions with **33b** and **33c** gave 4-[[4-[*N*,*N*-bis(2-chloroethyl)amino]-phenyl]sulfonyl]butyric acid (**35b**), which crystallized from EtOAc/hexane [Mp 102 °C (89% yield). Anal. ($C_{14}H_{19}Cl_2NO_4S$) C, H, N; required 367.0412, found 367.04387.] and 5-[[4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl]sulfonyl]pentanoic acid (**35c**) as an oil in 99% yield [$C_{15}H_{21}Cl_2NO_4S$ requires 381.0568, found 381.0564.].

Acid **35a** was then subjected to the modified Curtius reaction described above for **34a–c** to give crude amine **37a** in 75% yield. This was coupled immediately with 9-methoxyacridine as described above to give compound **13**. The hydrochloride salt crystallized from EtOAc/HCl as a yellow powder: mp 136–137 °C; $^1H$ NMR ($CD_3OD$) $\delta$ 8.34 (d, $J$ = 8.63, 2 H, acridine H-1,8), 7.97 (d, $J$ = 7.15 Hz, 2 H, acridine H-2,7), 7.85 (d, $J$ = 8.55 Hz, 2 H, acridine H-4,5), 7.51 (t, $J$ = 8.00 Hz, 2 H, acridine H-3,6), 7.50 (d, $J$ = 9.14 Hz, 2 H, H-2′), 6.68 (d, $J$ = 9.13 Hz, 2 H, H-3′), 4.55 (d, $J$ = 5.66 Hz, 2 H, $CH_2SO_2$), 3.85 (t, $J$ = 6.07 Hz, 2 H, $NHCH_2$), 3.78 (t, $J$ = 7.02 Hz, 4 H, $NCH_2$), 3.67 (t, $J$ = 6.38 Hz, 4 H, $CH_2Cl$). Anal. in Table I. Similar reactions on compounds **35b** and **35c** gave compounds **14** and **15**, via amines **37b** and **37c**, respectively.

**Assay for DNA Cross-Linking.** Linearized pBR322/DHFR26 DNA[20] (6 μL of a 1 mg/mL solution) was placed in 1.5-mL microfuge tubes together with 4 μL of drug solution in TE-80 buffer (10 mM Tris·HCl, 1 mM EDTA at pH 8). Samples were shielded from ambient light and incubated for various times at 20 °C and then denatured by the addition of 2 μL of 1% sodium dodecyl sulfate and 10 μL of 50 mM methylmercury hydroxide and incubated for 30 min at 20 °C in the dark. Renaturation of drug-treated DNA samples was carried out by incubation with 2.5 μL of 2-mercaptoethanol for 1 h. Samples were prepared for electrophoresis by the addition of 0.1 mg/mL bromophenol blue, 0.5 μg/mL ethidium bromide, and 5 μL of 40% sucrose. Electrophoresis was carried out at 80 V in 89 mM Tris/borate buffer at pH 8 containing 2 mM EDTA and 0.5 μg/mL ethidium bromide. DNA was visualized with 302-nm transillumination and was photographed with Polaroid type 55 film and a Wratten 3A filter.

**Growth Inhibition Assays.** Cell lines were maintained in exponential growth phase by subculturing in RPMI 1640 (P388) or Alpha MEM (AA8, UV4) containing 10% fetal calf serum as previously described.[21,22] $IC_{50}$ values were determined using log-phase cultures in 96-well microculture plates and are calculated as the nominal drug concentration required to reduce the cell density to 50% of that of control values, with eight control cultures on each microplate. For P388 cultures, drug was present throughout the growth period (72 h) and final cell densities were determined with a minor modification of the MTT method of Mossman.[23] For AA8 and UV4 cultures, drug exposure was terminated after 18 h by washing three times with fresh medium. Cultures were grown for a further 72 h before determining cell density by staining with methylene blue.[24]

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