Thymosin fraction V was used as a positive control and was prepared according to Goldstein.[5]

2. In Vivo Induction of Thy1$^+$ Antigen Expression in nu/nu Mice Spleen Cells. nu/nu female mice, 6-9 weeks old, were treated by gastric tubing with the test compounds by using 0.2 mL of 2% (w/v) arabic gum containing 0.1% (v/v) Tween-80 as vehicle, and 24 h later the spleens were removed and spleen cells were prepared as already described. The percentage of Thy1$^+$ positive cells was evaluated as already described in treated and control (vehicle only) animals.

Acknowledgment. We are indebted to Claire Chardon, Anne-Marie Dhennequin, and Gilles Martin-Gousset for their assistance in recording NMR spectra and carrying out elemental analysis. We thank Francoise Cermelj for preparation of the manuscript.

Registry No. 1, 86188-04-7; 1 (nitro derivative), 113456-79-4; 2, 86187-96-4; 2 (nitro derivative), 113456-74-9; 3, 86187-98-6; 3 (nitro derivative), 132439-22-6; 4, 86188-07-0; 4 (nitro derivative), 113456-81-8; 5, 86187-86-2; 6, 86188-02-5; 6 (nitro derivative), 113456-78-3; 7, 86187-97-5; 7 (nitro derivative), 113456-75-0; 8, 86187-95-3; 8 (nitro derivative), 132439-23-7; 9, 86188-05-8; 9 (nitro derivative), 113456-80-7; 10, 86188-14-9; 10 (nitro derivative), 113456-84-1; 11, 86188-06-9; 11 (nitro derivative), 132439-24-8; 12, 86187-94-2; 13, 132439-15-7; 14, 132439-16-8; 15, 132439-17-9; 16, 86187-85-1; 17, 86187-90-8; 18, 86188-01-4; 19, 86188-03-6; 20, 86187-88-4; 21, 86187-87-3; 22, 86187-91-9; 23, 86187-92-0; 24, 86187-89-5; 25, 132439-18-0; 26, 86202-31-5; 27, 86250-95-5; 28, 86188-00-3; 29, 132439-19-1; 30, 86188-08-1; 31, 86188-09-2; 32, 86188-10-5; 33, 86188-11-6; 34, 86188-12-7; 35, 86188-13-8; 36, 2516-96-3; 37, 25784-91-2; 38 (R^1 = 4-Cl, R^2 = R^3 = H), 108-90-7; 38 (R^1 = R^2 = R^3 = H), 71-43-2; 38 (R^1 = 4-CH_3, R^2 = R^3 = H), 108-88-3; 38 (R^1 = 4-F, R^2 = R^3 = H), 462-06-6; 38 (R^1 = 4-Br, R^2 = R^3 = H), 108-86-1; 38 (R^1 = 4-OCH_3, R^2 = R^3 = H), 100-66-3; 38 (R^1 = 2-CH_3, R^2 = 4-CH_3, R^3 = H), 108-38-3; 38 (R^1 = 3-OCH_3, R^2 = 4-OCH_3, R^3 = H), 91-16-7; 38 (R^1 = 3-Cl, R^2 = 4-Cl, R^3 = H), 95-50-1; 38 (R^1 = 2-Cl, R^2 = 4-Cl, R^3 = H), 541-73-1; 38 (R^1 = 3-CH_3, R^2 = 4-CH_3, R^3 = H), 95-47-6; 39 (R^1 = 4-Cl, R^2 = R^3 = H), 70132-91-1; 40, 626-58-4; 42, 55501-45-6; 43, 609-65-4; 44, 2011-66-7; 45, 54534-72-4; 46, 113456-71-6; 47, 78243-27-3; 48, 132439-20-4; 50 (R = Cl), 113456-93-2; 51, 66938-29-2; 52 (R = C_2H_5), 3230-23-7; 53 (R = *t*-Bu), 1882-42-4; 54, 3612-20-2; 55 (R = *n*-propyl), 24152-39-4; ClC_6H_4-3-Br, 108-37-2; $CH_3C_6H_4$-3-Br, 591-17-3; $CF_3C_6H_4$-3-Br, 401-78-5; *N*-benzyl-4-butylidenepiperidine, 132439-21-5; benzyl chloroformate, 501-53-1; 4-*tert*-butylpyridine, 3978-81-2; butyltriphenylphosphonium bromide, 1779-51-7; 2-chloro-5-nitrobenzophenone, 34052-37-4; 4-ethylpyridine, 536-75-4; formaldehyde, 50-00-0.

DNA-Directed Alkylating Agents. 4. 4-Anilinoquinoline-Based Minor Groove Directed Aniline Mustards

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A series of 4-anilinoquinoline-linked aniline mustards of widely varying mustard reactivity were prepared and evaluated for their antitumor activity. The compounds were designed as minor groove binding agents, where the aniline mustard ring is itself part of the DNA-binding ligand. While there was a general trend for cytotoxicity to correlate with mustard reactivity, this was much less pronounced than with untargeted mustards. The compounds were much more cytotoxic than the parent diols, and were also at least 10-fold more cytotoxic than the corresponding aniline mustards themselves. Comparative cell line studies suggested that the mechanism of cytotoxicity varied with mustard reactivity. The most reactive mustards cross-linked DNA, while cell killing by the less reactive compounds appeared to be by the formation of bulky monoadducts. The compounds were active but not particularly dose-potent against P388 leukemia in vivo. The modest potency may be related to their poor aqueous solubility, since the more soluble methyl quaternary salts were equally active at much lower doses.

The majority of alkylating antitumor drugs, including the clinically used compounds chlorambucil, melphalan, and cyclophosphamide, alkylate DNA primarily at the N-7 position of guanine, in a reaction dominated by the molecular electrostatic potential of the DNA site.[1] Thus, reaction occurs on DNA preferentially in the middle of runs of guanines,[2] where this potential is at its most negative.[3] In terms of chemotherapy, there are major drawbacks with such a "bonding-dominated"[4] mechanism of alkylation. The sequence specificity of these compounds is limited, and the electrophilicity of the drug needs to be high, leading to rapid loss by reaction with other (non-DNA) cell nucleophiles[5] and thus low potency.

There has been recent interest in overcoming these deficiencies by the use of DNA-targeted alkylating agents,[6,7] using DNA-intercalating carriers to increase the "binding component" of the DNA interaction. We have recently shown[5] that a series of acridine-carried aniline mustards of carefully varied reactivity (1) are both more potent in vitro and more active in vivo than the untargeted mustards themselves. In the latter study there was also some evidence that DNA targeting permitted the use of less reactive alkylating moieties. However, the binding selectivity of DNA intercalators is low,[6] and a more promising approach to highly potent sequence-selective alkylators appears to be the use of minor groove binding structures as carriers. Compounds such as the tris(pyrrolecarboxamide) (2) show highly AT-specific reversible

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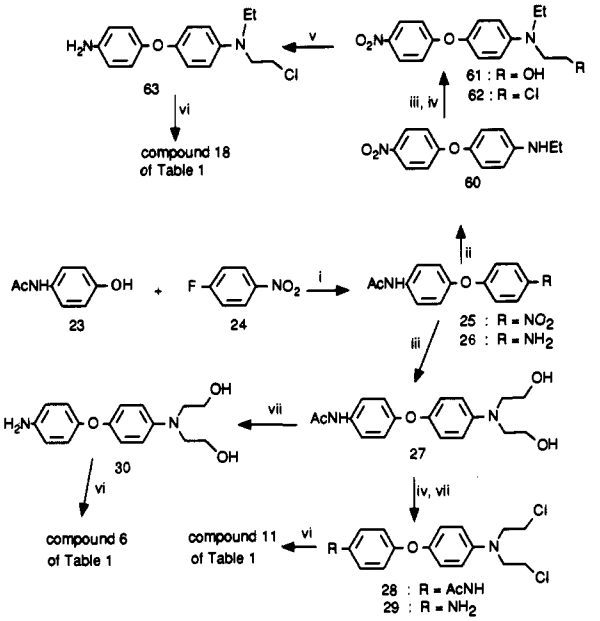
[‡] Section of Oncology.

(1) Mattes, W. B.; Hartley, J. A.; Kohn, K. W. *Nucleic Acids Res.* **1986**, *14*, 2971.
(2) Kohn, K. W.; Hartley, J. A.; Mattes, W. B. *Nucleic Acids Res.* **1987**, *15*, 10531.
(3) Pullman, A.; Pullman, B. *Q. Rev. Biophys.* **1981**, *14*, 289.
(4) Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315.
(5) 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.
(6) Denny, W. A. *Anti-Cancer Drug Des.* **1989**, *4*, 241.
(7) Koyama, M.; Takahashi, K.; Chou, T.-C.; Darzynkiewicz, Z.; Kapuscinski, J.; Kelly, T. R.; Wanatabe, K. A. *J. Med. Chem.* **1989**, *32*, 638.

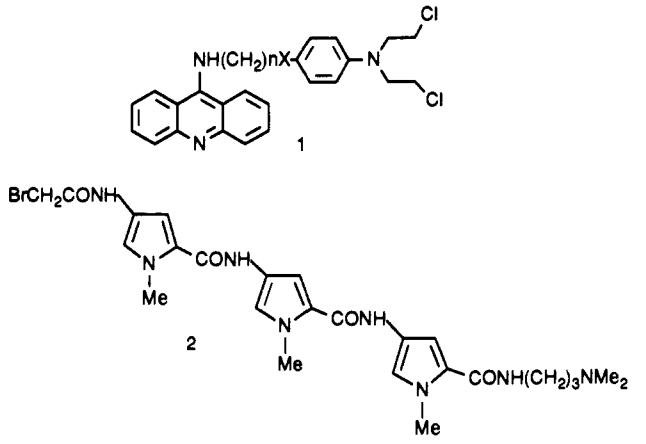
0022-2623/91/1834-1552$02.50/0

Scheme I[a]



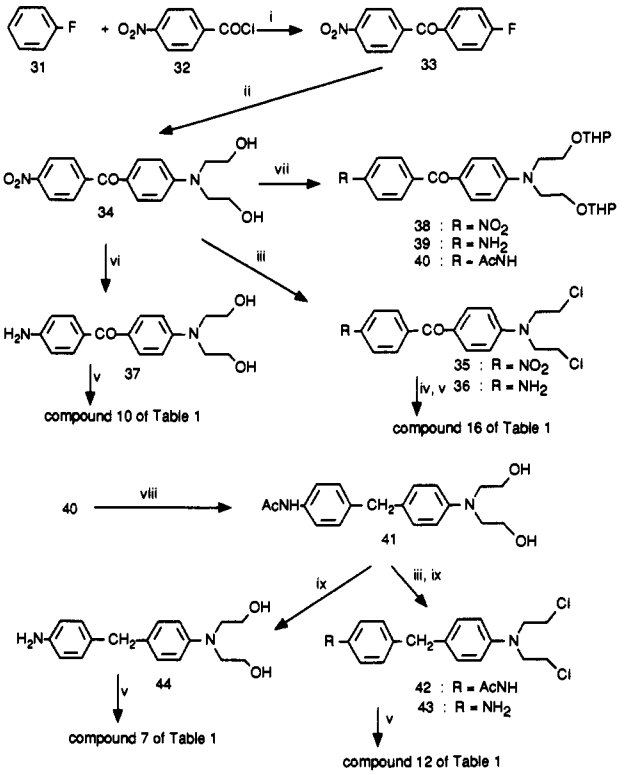
[a] (i) NaOH/EtOH/Δ; (ii) BH_3:dms/THF/Δ; (iii) oxirane/2 N AcOH/THF; (iv) MsCl/Et_3N; NaCl/DMF/140 °C; (v) H_2NNH_2·H_2O/Pd–C; (vi) 4-chloroquinoline/HCl/MeOH; (vii) H^+ (aq)/MeOH/Δ.

binding to DNA, which serves to direct alkylation to a single adenine on a 167-base-pair fragment.[8]



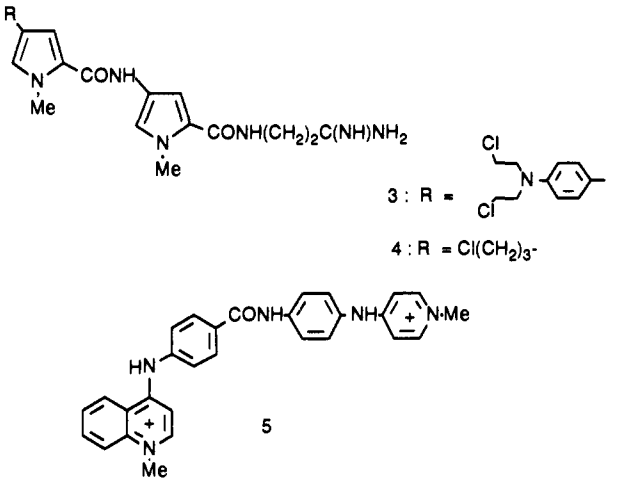
Related compounds (e.g. **3** and **4**) have been shown to be potent cytotoxic agents with good in vivo activity.[9,10] Following our work on DNA targeting of a series of aniline mustards of variable reactivity using an intercalating chromophore,[5,11] we wished to evaluate a comparable series of minor groove targeted compounds. In this case the aniline mustard ring could itself be part of the DNA-binding ligand. A suitable strategy was suggested by the family of 4-anilinoquinoline bisquaternary salts (e.g. **5**),[12] which are known from a series of studies[13] including NMR

Scheme II[a]



[a] (i) $AlCl_3/EtNO_2$; (ii) $HN(CH_2CH_2OH)_2$/DMSO/140 °C; (iii) MsCl/Et_3N; NaCl/DMF/140 °C; (iv) $SnCl_2$/concentrated HCl/Δ; (v) 4-chloroquinoline/HCl/MeOH; (vi) H_2/Pd–C/MeOH; (vii) dihydropyran/H^+; H_2/Pd–C/MeOH:MeCOCl/Et_3N; (viii) W-7 Raney nickel/EtOH/Δ; H^+(aq); (ix) H^+(aq)/MeOH/Δ.

DNA proton shift analysis[14] and 2D NMR of oligonucleotide complexes[15] to be minor groove binding ligands. In this paper we report the synthesis and biological evaluation of a series of aniline mustards, based on this structure and designed as minor groove targeted alkylating agents.

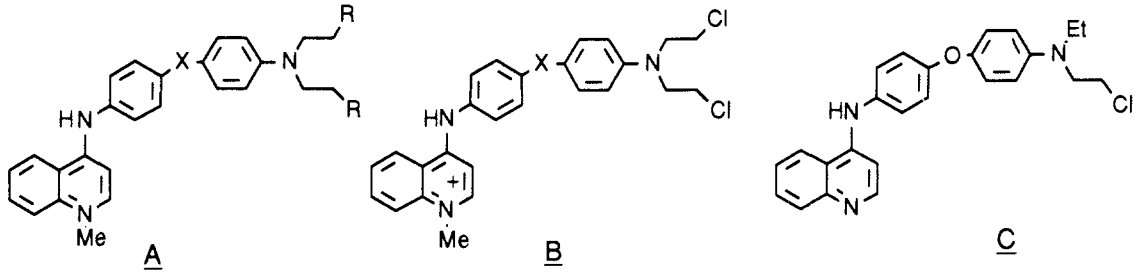


Chemistry

Most of the anilinoquinolines of Table I were prepared by acid-catalyzed coupling of the appropriate aniline with 4-chloroquinoline. The various substituted anilines were

(8) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700.
(9) Krowicki, K.; Balzarini, J.; De Clercq, E.; Newman, R. A.; Lown, J. W. *J. Med. Chem.* **1988**, *31*, 341.
(10) Arcamone, F. M.; Animati, F.; Barbieri, B.; Configliacchi, E.; D'Alessio, R.; Geroni, C.; Giuliani, F. C.; Lazzari, E.; Menozzi, M.; Mongelli, N.; Penco, S.; Verini, M. A. *J. Med. Chem.* **1989**, *32*, 774.
(11) Valu, K. K.; Gourdie, T. A.; Boritzki, T. J.; Gravatt, G. L.; Baguley, B. C.; Wilson, W. R.; Wakelin, L. P. G.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 3014.
(12) Denny, W. A.; Atwell, G. J.; Baguley, B. C.; Cain, B. F. *J. Med. Chem.* **1979**, *22*, 134.
(13) Braithwaite, A. W.; Baguley, B. C. *Biochemistry* **1980**, *19*, 1101.
(14) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.
(15) Leupin, W.; Chazin, W.; Hyberts, S.; Denny, W. A.; Wuthrich, K. *Biochemistry* **1986**, *25*, 5902.

Table I. Physicochemical and Biological Properties of 4-Anilinoquinolines



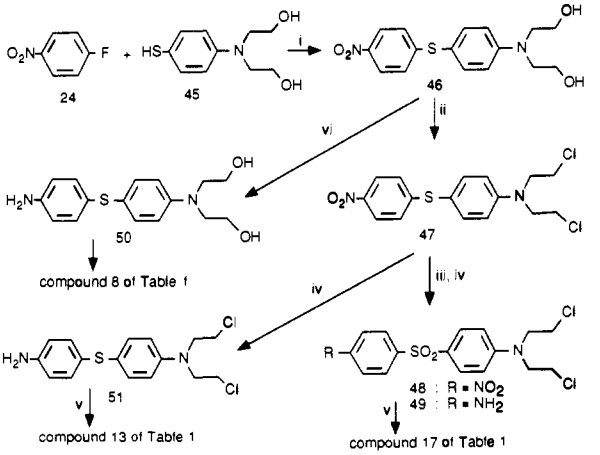
no.	type	R	X	mp,[a] °C	formula	analyses	10^5K_{obs}[b]	growth inhibition data: IC_{50} P388,[c] μM	AA8,[c] μM	HF[d]	P388 in vivo OD[e]	ILS[f]
6	A	OH	O	125–128	$C_{25}H_{25}N_3O_2 \cdot 2HCl$	C,H,N,Cl		0.80	29.0	1.6	ND[g]	
7	A	OH	CH_2	85–90 dec	$C_{26}H_{27}N_3O_2 \cdot 2HCl \cdot 2.5H_2O$	C,H,N		7.9	34.8	1.2	ND	
8	A	OH	S	53–59	$C_{25}H_{25}N_3O_2S \cdot 2HCl \cdot 2.5H_2O$	C,H,N,Cl		14	24.0	1.4	ND	
9	A	OH	CONH	160–170	$C_{26}H_{26}N_4O_3 \cdot 2HCl$	C,H,N,Cl		0.85	55	1.7	ND	
10	A	OH	CO	145–150 dec	$C_{26}H_{25}N_3O_2 \cdot 2HCl \cdot 0.5H_2O$	C,H,N		0.95	>40	>1.3	ND	
11	A	Cl	O	137–139	$C_{25}H_{23}Cl_2N_3O \cdot HCl$	C,H,N	24.7	0.48	0.60	39	150	57
12	A	Cl	CH_2	125–129	$C_{26}H_{25}Cl_2N_3 \cdot HCl \cdot 0.5H_2O$	C,H,N	22.7	0.19	1.40	18	150	43
13	A	Cl	S	>140 dec	$C_{25}H_{23}Cl_2N_3S \cdot HCl$	C,H,N,Cl	5.6	3.3	3.50	13	100	NA[h]
14	A	Cl	CONH	>250 dec	$C_{26}H_{24}Cl_2N_4O \cdot HCl$	C,H,N,Cl	23.0	0.054	0.48	40	225	46
15	A	Cl	NHCO	290 dec	$C_{26}H_{24}Cl_2N_4O \cdot HCl$	C,H,N,Cl		0.54	0.92	1.4	45	NA
16	A	Cl	CO	>170 dec	$C_{26}H_{23}Cl_2N_3O \cdot HCl \cdot 0.5H_2O$	C,H,N	0.26	3.8	6.90	3.6	100	NA
17	A	Cl	SO_2	198–201	$C_{25}H_{23}Cl_2N_3O_2S \cdot HCl$	C,H,N,Cl	0.07	>20	>3	100	29	
18	C			170 dec	$C_{25}H_{24}ClN_3O \cdot 2HCl \cdot H_2O$	C,H,N,Cl		0.50	7.0	11.3	30	NA
19	B		O	52–54	$C_{27}H_{26}Cl_2F_3N_3O_4S$	C,H,N,Cl,S		0.043	0.65	23	30	20
20	B		CH_2	70	$C_{28}H_{28}Cl_2F_3N_3O_3S \cdot 1.5H_2O$	C,H,N		0.072	0.60	20	45	46
21	B		CONH	115	$C_{28}H_{27}Cl_2F_3N_4O_4S$	C,H,N		0.49	0.34	17	30	28
22	B		CO	206–207	$C_{28}H_{26}Cl_2F_3N_3O_4S$	C,H,N		1.21	2.85	6.4	100	NA
chlorambucil								6.75	26	58	225	37

[a] Melting point for the form (free base or salt) indicated by the formula. [b] K_{obs}: pseudo-first-order rate constants for alkylation of 4-(4-nitrobenzyl)pyridine (NBP) in 50% aqueous Me_2CO at 66 °C, using HPLC analysis. See ref 18 for further details. [c] IC_{50}: concentration of drug in μM to inhibit cell growth in culture to 50% of controls, using the protocol detailed in ref 29; values are means of three determinations. P388(W): wild-type P388 murine leukemia. AA8: Chinese hamster ovary derived cell line. [d] HF: hypersensitivity factor = IC_{50}(AA8)/IC_{50}(UV4), where UV4 is a mutant AA8 line lacking the ability to perform incision repair; see text. [e] OD: optimal dose of drug, in milligrams/kilogram, administered as a single dose intraperitoneally as a solution in 0.1 mL of 30% v/v dimethylacetamide/water on day 1, 24 h after intraperitoneal inoculation of 10^6 tumor cells. [f] ILS: percentage increase in lifespan of drug-treated tumor-bearing animals when treated at the optimal dose. Values of ILS >20% are considered statistically significant. [g] ND: test not done. [h] NA: compound inactive at all nontoxic doses.

synthesized by the routes shown in Schemes I–IV. Anilines **28** and **30** for the O-linked compounds (**11** and **6**) were prepared (Scheme I) by condensation of 4-acetamidophenol and 4-fluoronitrobenzene, followed by reduction and elaboration to the bis(2-hydroxyethyl) compound **27** with oxirane. The half-mustard analogue (**18**) of **11** was also prepared from **25**, but in this case the *N*-acetyl group was reduced with diborane, and the resulting NEt compound (**60**) was treated with oxirane to give the 2-hydroxyethyl derivative (**61**), which was converted to the half mustard (**62**). Reduction of this proved difficult, with the usual Sn/HCl method cleaving the ether and neutral Sn treatment reducing the chloride. Conversion to the amine (**63**) was eventually achieved with hydrazine over Pd/C.

Preparation of the CO-linked anilines **36** and **37** began with Friedel–Crafts acylation of fluorobenzene with 4-nitrobenzoyl chloride, followed by direct displacement of the fluorine with diethanolamine (Scheme II). To generate the CH_2-linked anilines **43** and **44**, the key nitro diol **34** was reduced and protected as the N-acetylated bis(OTHP ether) **40**, and the ketone was subsequently reduced with W-7 Raney nickel to give the protected diphenylmethane **41** (Scheme II). Selective deprotection then gave the desired anilines **43** and **44**. Reaction of 4-fluoronitrobenzene with the substituted thiol **45**[5] gave the sulfide **46** (Scheme III), which in turn yielded the S-linked anilines **50** and **51**. The corresponding SO_2-linked aniline **49** was then elaborated by oxidation of the nitro mustard **47** with 3-chloroperbenzoic acid.

Scheme III[a]

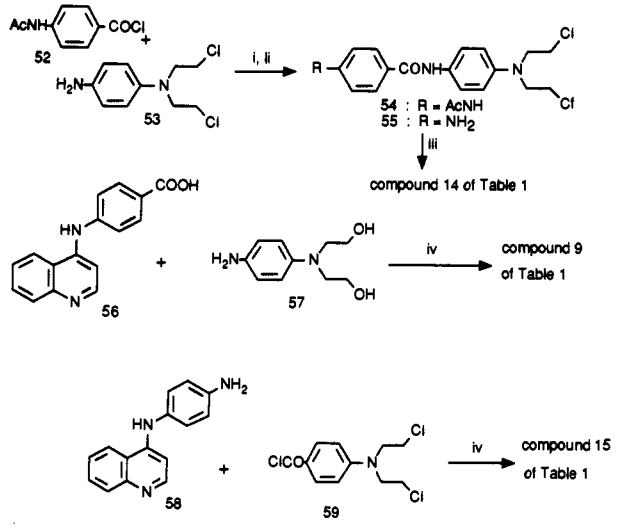


[a] (i) NaOH/MeOH/Δ (under N_2); (ii) MsCl/Et_3N; NaCl/DMF/140 °C; (iii) *m*-cpba/CH_2Cl_2; (iv) 4-chloroquinoline/HCl/MeOH; (v) $SnCl_2$/EtOAc/Δ.

The amide **55** for synthesis of **14** was prepared by condensation of 4-acetamidobenzoyl chloride (**52**) and the known[16] 4-[*N*,*N*-bis(2-chloroethyl)amino]aniline (**53**) (Scheme IV), while the isomeric mustard **15** was prepared directly from 4-(4-aminoquinolinyl)aniline **58** and the

(16) Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 112.

Scheme IV[a]



[a] (i) ClCOOEt/Et_3N/Me_2CO/0 °C; (ii) H^+(aq)/MeOH/Δ; (iii) 4-chloroquinoline/HCl/MeOH; (iv) DMF/CH_2Cl_2/20 °C.

known[17] 4-[bis(2-chloroethyl)amino]benzoyl chloride (**59**) (Scheme IV). The corresponding diol **9** was prepared directly from 4-(4-aminoquinolinyl)benzoic acid (**56**) and the known[5] 4-[*N*,*N*-bis(2-hydroxyethyl)amino]aniline (**57**). The quaternary quinolinium salts (**19–22**) were prepared from the appropriate representative mustards by direct quaternization with methyl triflate.

Results and Discussion

Table I gives physicochemical and biological data for a series of seven mustards (**11–18**), and for the corresponding methyl quaternary salts (**19–22**) of four of these. As in our earlier studies[5,11] of intercalator-targeted aniline mustards, a central strategy of this work was to explore the consequences of varying the reactivity of the alkylating unit while minimizing changes in other physicochemical properties. This was achieved by using different link groups of widely varying electronic properties. In the present series the reactivity of the mustard varies over a 190-fold range in terms of rates of hydrolysis in 50% aqueous acetone at pH 7,[18] and over a 350-fold range in terms of alkylation of 4-(4-nitrobenzyl)pyridine[18] (Table I). The dependence of these rates on the electronic properties of the substituents is in accord with those seen in studies with simple mustards,[5,19] with the O-substituted compound **11** being the most reactive and the SO_2-substituted compound **17** being the least.

The DNA-binding properties of the diols were studied by the ethidium displacement assay,[20] using the homopolymers poly(dA-dT) and poly(dG-dC). One characteristic of minor groove binding ligands is their preference for binding to AT-rich sequences.[21] The results (Table II) show the compounds do not bind particularly strongly, with C_{50} values in the range 80–200 μM, and also do not (in this assay) show any sequence selectivity. However, more detailed studies[22] on the quaternary analogues **19–22** are consistent with a minor groove binding mode for these compounds.

Table II. DNA Binding Properties of Selected 4-Anilinoquinolines

compd	X	ethidium displacement: C_{50}, μM poly(dA-dT)	ethidium displacement: C_{50}, μM poly(dG-dC)	spectrophotometry:[b] log K, M^{-1}
6	O	87 ± 1	74 ± 2	
7	CH_2	77 ± 1	86 ± 1	
9	CONH	34 ± 5	22.9 ± 1	
10	CO	179 ± 17	206 ± 6	
19	O			6.45
20	CH_2			6.58
21	CONH			6.48
22	CO			6.61

[a] In 0.01 M SHE buffer (pH 7.0) at 25 °C; see ref 20; value ± SE. [b] For binding to calf thymus DNA in 0.01 M HEPES buffer (pH 7.0) at 25 °C, analyzed by the Scatchard model; data taken from ref 22.

All compounds were evaluated in vitro for antiproliferative activity against three cell lines; the murine P388 leukemia, the Chinese hamster fibroblast AA8, and the AA8-derived mutant line UV4. The latter is deficient in performing certain steps in the excision repair of DNA adducts,[23] and is thus hypersensitive to DNA interstrand cross-linking agents. We have previously[5,16,24] used the ratio of IC_{50}s determined in the wild-type AA8 and mutant UV4 lines (the hypersensitivity factor HF) as a determinant of the mode of cell killing by various alkylating agents. With agents of known modes of action, this assay shows HFs of ca. 1 for nonalkylating agents, HF factors of ca. 10–15 for agents which form bulky monoadducts, and HF factors of ca. >25 for DNA cross-linking agents.[24] Although clearly not a definitive test, within series it provides useful information about trends in the mechanism of cytotoxicity.

As expected, the parent diols (**6–10**) showed very low cytotoxicity (1–14 μM in P388, 25–55 μM in AA8), and had HF factors of close to unity, indicating that the cytotoxic events were not due to DNA adducts. The use of nonactive carrier moieties assists in evaluating the effects of the alkylating species.

The cytotoxicities of simple substituted aniline mustards themselves against a number of different cell lines have been shown to be dominated by the substituent electronic parameter σ, which controls the reactivity of the mustard.[5,16] This is summarized[5] by eq 1 for the P388 cytotoxicity (IC_{50} values) of these compounds against P388 cells (data of ref 16). However, as found previously[5] with a similar series of 9-aminoacridine-linked mustards, the cytotoxic potency of the anilinoquinoline mustards (**11–18**) is not dominated so completely by mustard reactivity. While there is a general trend for the less reactive mustards to be less cytotoxic in both cell lines, there are many exceptions for which no explanation is currently available (Table I).

$$\log IC_{50}(\text{molar}) = 2.01\sigma - 5.66 \quad (1)$$

The targeted alkylating agents were much more cytotoxic than the parent diols (**6–10**) (Table I), and also showed at least a 10-fold increase in cytotoxic potency over the correspondingly substituted aniline mustards themselves (see data of ref 16). Surprisingly, the monofunctional alkylator (**18**) showed similar cytotoxicity to the

(17) Ross, W. C. J. *J. Chem. Soc.* **1949**, 183.
(18) O'Connor, C. J.; Denny, W. A.; Fan, J.-Y.; Gravatt, G. L.; Grigor, B. A.; McClennan, D. J. Manuscript submitted to *J. Phys. Org. Chem.*
(19) Panthananickal, A.; Hansch, C.; Leo, A.; Quinn, F. R. *J. Med. Chem.* **1978**, *21*, 16.
(20) Baguley, B. C.; Falkenhaug, E.-M. *Nucleic Acids Res.* **1978**, *5*, 161.
(21) Braithwaite, A. W.; Baguley, B. C. *Biochemistry* **1980**, *19*, 1101.
(22) O'Connor, C. J.; Denny, W. A.; Fan, J.-Y. *Chem.-Biol. Int.* **1991**, *77*, 223.
(23) Hoy, C. A.; Thompson, L. H.; Mooney, C. L.; Salazar, E. P. *Cancer Res.* **1985**, *45*, 1737.
(24) Wilson, W. R.; Thompson, L. H.; Anderson, R. F.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 31.

analogous full mustard (11) in P388 cells (although there was a 10-fold difference in the AA8 cell line). However, the HF values seen between AA8 and UV4 cells for 11 and 18 (39 and 11, respectively) are consistent with the full mustard killing via a DNA cross-linking mechanism and the monofunctional mustard by monoadduct formation. The HF values for the other mustards suggest that 12 and 14 also cross-link DNA, but (and again similar to the results with the 9-aminoacridine-linked mustards[5]) the less reactive NHCO-, CO-, and SO_2-linked compounds (15–17) show much lower HF values (and lower cytotoxicity), suggesting that cell killing by these compounds might be by the formation of bulky monoadducts.

Conversion of the compounds 11, 12, 14, and 16 to the corresponding methyl quaternary salts 19–22 did provide more soluble compounds. These showed somewhat better cytotoxicities, but slightly lower HF values.

Antitumor activity in vivo was measured against P388 leukemia, using a series of doses spaced 1.5-fold apart for each compound, covering the range from inactive to toxic. With use of this protocol, the optimal dose (OD) is defined as that providing the maximal life extension, and is quite close to the LD_{10}. The mustards (11–18) proved quite insoluble, and were formulated in aqueous dimethylacetamide to ensure solubility for injection, using a single drug dose on day 1. This is a stringent protocol, and under these conditions the clinical agent chlorambucil gives an ILS of 37% at a dose of 225 mg/kg (Table I). The more reactive O, CH_2, and CONH mustards (11, 12, and 14) did show significant in vivo activity, but only at relatively high doses (100–200 mg/kg). This low potency may be related to the poor aqueous solubility of the compounds, since the more soluble methyl quaternary salts (19–22) were equally active at much lower doses (30–45 mg/kg).

Conclusions

Previous work has shown that DNA-targeting of aniline mustards by use of either DNA-intercalating[5,11] or minor groove binding[9,10] carrier molecules serves to provide compounds of greatly increased cytotoxic and in vivo potency. In the present case, use of the putative DNA minor groove binding 4-anilinoquinoline moiety has resulted in aniline mustards of enhanced cytotoxic potency. The modest in vivo dose potency and activity of these compounds may be related to their poor aqueous solubility.

Experimental Section

Where analyses are indicated by symbols of the elements, results were within ±0.4 of theoretical, and were performed by the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal apparatus using the supplied, stem-corrected thermometer, and are as read. NMR spectra were measured on a Bruker AM-400 or WP60 spectrometers (Me_4Si).

Preparation of Compounds 6, 11, and 18 by the Method of Scheme I. 4-Nitrophenyl 4'-Acetamidophenyl Ether (25). Solid NaOH (1.46 g, 36.5 mmol) was added to a stirred solution of 4-acetamidophenol (23) (5.02 g, 33.2 mmol) in EtOH (80 mL). The solution was heated under reflux for 30 min before a solution of 4-nitrofluorobenzene (24) (5.6 g, 39.7 mmol) was added. The mixture was heated under reflux for a further 1 h and then evaporated under reduced pressure at 20 °C. The residue was partitioned between water and EtOAc, and the organic phase was chromatographed on SiO_2. Elution with CH_2Cl_2/EtOAc (4:1) gave solid 4-nitrophenyl 4'-acetamidophenyl ether (25)[25] (5.6 g, 62%), sufficiently pure to use directly: ^1H NMR ($CDCl_3$) δ 8:10 (d, $J_{2,3}$ = 9.0 Hz, 2 H, H3 and H5), 7.65–6.62 (m, 7 H, H2, H6, H2', H6', H3', H5' and N*H*Ac), 2.12 (s, 3 H, NHCOCH_3).

(25) Rarick, M. J.; Brewster, R. Q.; Pains, F. B. *J. Am. Chem. Soc.* **1933**, *55*, 1289.

4'-Acetamidophenyl 4-[Bis(2-hydroxyethyl)amino]phenyl Ether (27). The above compound (25) (4.3 g, 15.8 mmol) was hydrogenated over 10% Pd/C in MeOH, filtered, and evaporated to dryness. The resulting crude amide (26) was suspended in 1 N aqueous AcOH (30 mL), and the stirred suspension was treated with 2 × 1 mL portions of oxirane over a 24-h period. The mixture was then concentrated, and the resulting gum was partitioned between saturated $NaHCO_3$ solution and CH_2Cl_2. The aqueous phase was extracted with two further portions of CH_2Cl_2, and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a brown oil. Chromatography on SiO_2 and elution with EtOAc gave 4'-acetamidophenyl 4-[bis(2-hydroxyethyl)amino]phenyl ether (27) (5.6 g, 95%) as a white solid: mp 159.5–160.5 °C; ^1H NMR (CD_3SOCD_3) δ 9.85 (s, 1 H, N*H*Ac), 7.50 (d, $J_{2',3'}$ = 9.0 Hz, 2 H, H3' and H5'), 6.85 9d, $J_{2,3}$ = 9.1 Hz, 2 H, H2 and H6), 6.83 (d, $J_{2',3'}$ = 9.0 Hz, 2 H, H2 and H6), 6.68 (d, $J_{2,3}$ = 9.1 Hz, 2 H, H3 and H5), 4.76 (t, J = 5.4 Hz, 2 H, OH), 3.54 (dt, J = 6.5 Hz and J = 5.4 Hz, 4 H, NCH_2CH_2OH), 3.39 (t, J = 6.5 Hz, 4 H, NCH_2CH_2OH), 2.02 (s, 3 H, NHCOCH_3); ^{13}C NMR δ 167.77, 153.98, 145.84, 144.68, 133.77, 120.49, 120.37, 116.96, 112.23, 58.14, 53.49, 23.71. Anal. ($C_{18}H_{22}N_2O_4$) C, H, N.

Compound 11 of Table I. The above diol (27) (1.31 g, 3.95 mmol) was converted to the corresponding mustard, 4'-acetamidophenyl 4-[bis(2-chloroethyl)amino]phenyl ether (28) by conversion to the dimesylate followed by treatment with NaCl,[5] and the crude mustard was heated under reflux for 1 h in a mixture of MeOH/water/concentrated HCl (1:1:1, 100 mL). The solution was concentrated under reduced pressure, basified with concentrated ammonia, and partitioned between brine and EtOAc, the organic phase yielding crude 4'-aminophenyl 4-[bis(2-chloroethyl)amino]phenyl ether (29) as a brown oil. This amine (0.38 g, 1.16 mmol) was dissolved in MeOH and added to a stirred solution of 4-chloroquinoline (1 molar equiv) and concentrated HCl (1 molar equiv) in MeOH, and the mixture was stirred at 20 °C for 18 h before being basified with concentrated ammonia and partitioned between water and EtOAc. The residue from the organic phase was chromatographed on SiO_2, and elution with CH_2Cl_2 and CH_2Cl_2/EtOAc (1:4) gave a pale yellow solid, which was recrystallized from EtOAc/petroleum ether to give the pure free base of 6 (0.35 g, 67%) as pale yellow needles. This was converted to the hydrochloride salt: mp 137–139 °C; ^1H NMR (CD_3SOCD_3) δ 15.01 (br d, J = 5.8 Hz, 1 H, HCl), 11.22 (s, 1 H, NH), 8.96 (d, $J_{7,8}$ = 8.5 Hz, 1 H, H8), 8.49 (t, J = 6.6 Hz, 1 H, H2), 8.16 (dd, $J_{5,6}$ = 8.5 Hz and $J_{5,7}$ = 0.7 Hz, 1 H, H5), 8.01 (dt, J_{ortho} = 7.1 Hz and J_{meta} = 0.7 Hz, 1 H, H7), 7.77 (dt, J_{ortho} = 7.8 Hz and J_{meta} = 1.0 Hz, 1 H, H6), 7.45 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H3' and H5'), 7.07 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H2' and H6'), 7.04 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H2'' and H6''), 6.85 (d, $J_{2'',3''}$ = 9.1 Hz, H3'' and H5''), 6.71 (d, $J_{2,3}$ = 6.9 Hz, 1 H, H3), 3.76 (s, 8 H, NCH_2CH_2Cl); ^{13}C NMR δ 157.61, 155.32, 146.35, 143.41, 142.37, 138.21, 133.77, 131.19, 127.44, 126.87, 123.99, 121.39, 120.09, 117.81, 117.02, 113.41, 99.56, 52.46, 41.12. Anal. ($C_{25}H_{23}Cl_2N_3O$·HCl) Table I.

Compound 6 of Table I. A solution of 4'-acetamidophenyl 4-[bis(2-hydroxyethyl)amino]phenyl ether (27) (1.5 g, 4.7 mmol) in MeOH/water/concentrated HCl (1:1:1, 100 mL) was heated under reflux for 2 h and then concentrated under reduced pressure. The resulting oil was basified with concentrated ammonia and extracted with EtOAc, and the resulting crude amine (30) was condensed with 4-chloroquinoline according to the standard procedure above. Chromatography of the product on SiO_2, and elution with EtOAc/MeOH (9:1) gave the free base of 6 as a yellow-green foam (1.2 g, 61%). Conversion to the dihydrochloride salt afforded a pale green solid: mp 125–128 °C; ^1H NMR (CD_3SOCD_3) δ 15.0 (br d, J = 5.2 Hz, 1 H, HCl), 11.2 (s, 1 H, NH), 8.95 (d, $J_{7,8}$ = 8.5 Hz, 1 H, H8), 8.51 (t, J = 6.2 Hz, 1 H, H2), 8.16 (d, $J_{5,6}$ = 8.4 Hz, 1 H, H5), 8.03 (dt, J_{ortho} = 7.6 Hz and J_{meta} = 1.0 Hz, 1 H, H7), 7.79 (t, J_{ortho} = 8.2 Hz, 1 H, H6), 7.52 (d, J = 8.8 Hz, 4 H, H3', H5', H2'', and H6''), 7.19 (d, J = 8.8 Hz, 2 H) and 7.17 (d, J = 8.8 Hz, 2 H, H2', H6', H3'', and H5''), 6.78 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H3), 4.6 (br t, 3 H, OH and HCl), 3.61 (br t, J = 5.1 Hz, 4 H, NCH_2CH_2OH), 3.56 (br t, J = 5.1 Hz, 4 H, NCH_2CH_2OH). Anal. ($C_{25}H_{25}N_3O_3$·2HCl) Table I.

Compound 18 of Table I. A stirred solution of 4-nitrophenyl 4'-acetamidophenyl ether (25) (5.0 g, 18.4 mmol) in THF (40 mL) was treated with diborane–dimethyl sulfide complex (5.5 mL of

a 10.0 M solution in THF, 55 mmol) and then heated under reflux in N_2 for 2 h, cooled, and quenched with MeOH. Evaporation of volatiles and percolation of the residue through SiO_2 in CH_2Cl_2 gave 4-nitrophenyl 4'-(ethylamino)phenyl ether (**60**) (4.5 g, 96%) as a bright yellow solid. This was immediately dissolved in a mixture of 2 N aqueous AcOH (30 mL) and THF (30 mL) and treated portionwise with oxirane over 1 week. Solvents were removed under reduced pressure, and the residue was partitioned between saturated aqueous $NaHCO_3$ and CH_2Cl_2. The aqueous phase was extracted twice with CH_2Cl_2, and the combined organic layers were worked up to give crude 4-nitrophenyl 4'-[*N*-ethyl-*N*-(2-hydroxyethyl)amino]phenyl ether (**61**) as a yellow oil, which was converted (without further purification) to the mustard by the method described above. The crude product was chromatographed on SiO_2, and elution with CH_2Cl_2/petroleum ether (1:2) gave 4-nitrophenyl 4'-[*N*-ethyl-*N*-(2-chloroethyl)amino]phenyl ether (**62**) as an orange oil (4.2 g, 71% overall): ^{1}H NMR ($CDCl_3$) δ 8.16 (d, $J_{2,3}$ = 9.3 Hz, 2 H, H3 and H5), 6.97 ($J_{2',3'}$ = 9.1 Hz, 2 H, H2' and H6'), 6.96 (d, $J_{2,3}$ = 9.3 Hz, 2 H, H2 and H6), 6.71 (d, $J_{2',3'}$ = 9.1 Hz, 2 H, H3' and H5'), 3.61 (s, 4 H, NCH_2CH_2Cl), 3.44 (q, J = 7.1 Hz, 2 H, NCH_2CH_3), 1.21 (t, J = 7.1 Hz, 3 H, NCH_2CH_3); ^{13}C NMR δ 164.62, 144.99, 144.80, 142.05, 125.87, 122.00, 116.15, 113.03, 52.65, 45.69, 40.47, 12.42. Anal. ($C_{16}H_{17}ClN_2O_3$) C, H, N, Cl.

A suspension of the above compound (**62**) (0.90 g, 2.81 mmol) and a trace of Pd/C in hydrazine hydrate (1.0 mL) was heated under reflux for 4 h. The catalyst was removed by filtration, the filtrate was partitioned between EtOAc and water, and workup of the organic phase gave the crude amine (**63**) as a colorless oil (0.85 g, 100%). This was dissolved in MeOH (10 mL) and treated with 1 equiv of 4-chloroquinoline as described above. Chromatography of the product on SiO_2 and elution with CH_2Cl_2 followed by EtOAc gave the free base of compound (**18**) (0.84 g, 72% overall). The dihydrochloride salt crystallized from EtOAc/MeOH as a yellow solid: mp 60–65 °C; ^{1}H NMR (CD_3SOCD_3/D_2O) δ 8.62 (d, $J_{7,8}$ = 8.5 Hz, 1 H, H8), 8.43 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H2), 8.06 (t, J = 7.2 Hz, H7), 7.98 (d, $J_{5,8}$ = 8.2 Hz, 1 H, H5), 7.84 (d, J = 7.6 Hz, 1 H, H6), 7.54 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H3' and H5'), 7.46 (d, $J_{2'',3''}$ = 8.6 Hz, 2 H, H2'' and H6''), 7.26 (d, J = 8.6 Hz, 2 H) and 7.25 (d, J = 8.6 Hz, 2 H, H2', H6', H3', H5''), 6.90 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H3), 3.91 (br t, J = 5.6 Hz, 2 H, NCH_2CH_2Cl), 3.68 (br t, J = 5.6 Hz, 2 H, CH_2CH_2Cl), 3.61 (q, J = 7.0 Hz, 2 H, NCH_2CH_3), 1.12 (t, J = 7.0 Hz, 3 H, NCH_2CH_3); ^{13}C NMR 156.40, 156.15, 155.02, 143.23, 138.71, 135.21, 133.16, 128.49, 128.30, 123.91, 122.09, 121.22, 120.97, 120.89, 117.74, 100.62, 56.91, 52.53, 40.02, 11.31. Anal. Table I.

Preparation of Compounds 7, 10, 12, and 16 by the Method of Scheme II. 4-Fluoro-4'-nitrobenzophenone (33). Fluorobenzene (**31**) (5.9 mL, 62.9 mmol) was slowly added to a stirred solution of 4-nitrobenzoyl chloride (**32**) (11.6 g, 62.5 mmol) and anhydrous $AlCl_3$ (42 g, 0.315 mol) in dry nitroethane (100 mL). The resulting mixture was stirred at 20 °C for 4 h, before being slowly quenched with 6 N HCl and extracted with EtOAc. The organic phase was sequentially washed with dilute aqueous NaOH solution and saturated brine and then evaporated under reduced pressure. Crystallization of the residue from petroleum ether gave 4-fluoro-4'-nitrobenzophenone (**33**) (7.7 g, 60%) as pale yellow needles: mp 81 °C; ^{1}H NMR ($CDCl_3$) δ 8.62 (d, $J_{2,3}$ = 9.0 Hz, 2 H, H2 and H6), 8.15 (d, $J_{2,3}$ = 9.0 Hz, 2 H, H3 and H5), 8.07 (d, $J_{2',3'}$ = 9.0 Hz, 2 H, H3' and H5'), 7.47 (d, $J_{2',3'}$ = 9.0 Hz, 2 H, H2' and H6'). Anal. ($C_{13}H_8FNO_3$) C, H.

4-[Bis(2-hydroxyethyl)amino]-4'-nitrobenzophenone (34). A solution of the above fluoro compound (**33**) (6.3 g, 27.7 mmol) in DMSO (30 mL) and diethanolamine (6.3 g, 69.2 mmol) was heated to 140 °C for 5 h. The cooled reaction mixture was then partitioned between water and EtOAc, and the aqueous phase was extracted with a second portion of EtOAc. The combined organic phases were then washed with water (2×), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography on SiO_2 and elution with EtOAc afforded 4-[bis(2-hydroxyethyl)amino]-4'-nitrobenzophenone (**34**) (5.2 g, 60%) as an orange oil, which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 8.31 (d, $J_{2,3}$ = 9.0 Hz, 2 H, H2 and H6), 7.78 (d, $J_{2',3'}$ = 8.4 Hz, 2 H, H2' and H6'), 7.68 (d, $J_{2,3}$ = 9.0 Hz, 2 H, H3 and H5), 6.61 (d, $J_{2',3'}$ = 8.4 Hz, 2 H, H3' and H5'), 3.47 (br m, 10 H, NCH_2CH_2OH).

4-[Bis(2-chloroethyl)amino]-4'-nitrobenzophenone (35). This was prepared from diol **34** by treatment with MsCl/NaCl as above. Chromatography of the crude material on SiO_2 and elution with CH_2Cl_2 afforded 4-[bis(2-chloroethyl)amino]-4'-nitrobenzophenone (**35**) (80%) as a yellow solid, which was crystallized from CH_2Cl_2 as fine yellow needles: mp 128 °C; ^{1}H NMR ($CDCl_3$) δ 8.32 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H2' and H6'), 7.85 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H3' and H5'), 7.78 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H2'' and H6''), 6.73 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H3'' and H5''), 3.86 (t, J = 6.8 Hz, 4 H, NCH_2CH_2Cl), 3.70 (t, J = 6.8 Hz, 4 N, NCH_2CH_2Cl); ^{13}C NMR δ 192.76, 150.43, 149.29, 144.39, 133.10, 130.12, 125.29, 123.43, 111.03, 53.25, 40.05. Anal. ($C_{17}H_{16}N_2O_3Cl_2$) C, H, N, Cl.

Compound 16 of Table I. Tin(II) chloride dihydrate (4.5 g, 20 mmol) was added to a stirred suspension of the above nitro compound (**35**) (1.4 g, 4.01 mmol) in concentrated HCl (100 mL). The mixture was heated under reflux for 1 h, cooled to room temperature, basified with concentrated ammonia, and extracted with EtOAc (3×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated to give crude amine **36**, which was immediately dissolved in MeOH (10 mL) and coupled with 4-chloroquinoline as described above. Chromatography of the crude product on SiO_2 and elution with CH_2Cl_2/EtOAc gave the pure free base of **16** as a pale yellow solid (1.3 g, 73%). This was converted to the hydrochloride salt: mp >170 °C dec; ^{1}H NMR (CD_3SOCD_3) δ 15.10 (s, 1 H, HCl), 11.30 (s, 1 H, NH), 8.98 (dd, $J_{7,6}$ = 8.7 Hz, and $J_{6,8}$ = 1.2 Hz, 1 H, H8), 8.60 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H2), 8.18 (dd, $J_{5,6}$ = 8.5 Hz and $J_{5,7}$ = 1.2 Hz, 1 H, H5), 8.06 (ddd, $J_{7,8}$ = 8.7 Hz, $J_{6,7}$ = 7.0 Hz and $J_{5,7}$ = 1.2 Hz, 1 H, H7), 7.84 (d, J = 8.5 Hz, 2 H, H3' and H5'), 7.83 (dt, J_{ortho} = 8.5 Hz and $J_{6,8}$ = 1.2 Hz, 1 H, H6), 7.73 (d, J = 9.1 Hz, 2 H, H2'' and H6''), 7.69 (d, J = 8.5 Hz, 2 H, H2' and H6'), 7.08 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H3), 6.92 (d, J = 9.1 Hz, 2 H, H3'', H5''), 3.88 (br t, J = 5.5 Hz, 4 H, NCH_2CH_2Cl), 3.82 (br t, J = 5.5 Hz, 4 H, NCH_2CH_2Cl); ^{13}C NMR δ 192.42, 154.39, 150.33, 142.73, 140.22, 138.21, 136.57, 133.87, 132.29, 130.59, 127.07, 124.65, 124.39, 123.95, 120.13, 117.47, 111.06, 100.40, 51.63, 40.82. Anal. ($C_{26}H_{25}N_3OCl_4 \cdot HCl \cdot {}^1/_2H_2O$) Table I.

Compound 10 of Table I. 4-Nitro-4'-[bis(2-hydroxyethyl)-amino]benzophenone (**34**) (1.4 g, 4.49 mmol) was hydrogenated over 10% Pd/C in MeOH (30 mL) dissolved in MeOH (30 mL). The resulting solution of crude amine **37** was added to a stirred solution of 4-chloroquinoline (0.73 g, 4.49 mmol) and concentrated HCl (0.37 mL, 4.49 mmol) in MeOH (50 mL) according to the general procedure described above. Chromatography on SiO_2 and elution with EtOAc/MeOH (9:1) afforded the free base of **10** as an orange solid (1.0 g, 54%). This was converted to the bright orange hydrochloride salt: mp 145–150 °C dec; ^{1}H NMR (CD_3SOCD_3) δ 15.4 (br s, 1 H, HCl), 12.4 (br s, 1 H, NH), 8.78 (d, J = 6.7 Hz, 1 H, H2), 8.19 (br d, J = 7.9 Hz, 1 H, H8), 7.96 (ddd, $J_{7,8}$ = 8.5 Hz, $J_{6,7}$ = 7.1 Hz and $J_{5,7}$ = 1.2 Hz, 1 H, H7), 7.89 (dd, $J_{5,6}$ = 8.8 Hz and $J_{5,7}$ = 1.2 Hz, 1 H, H5), 7.75 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H3' and H5'), 7.64 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H2'' and H6''), 7.60 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H2' and H6'), 7.47 (d, $J_{2,3}$ = 6.7 Hz, 1 H, H3), 7.61–7.57 (m, 1 H, H6), 6.82 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H3'' and H5''), 4.5 (br s, 3 H, OH and HCl), 3.59 (br t, J = 4.6 Hz, 4 H, NCH_2CH_2OH), 3.55 (br t, J = 4.6 Hz, 4 H, NCH_2CH_2OH); ^{13}C NMR δ 191.95, 155.18, 151.84, 146.51, 142.36, 142.31, 138.75, 138.20, 133.42, 132.20, 130.44, 126.59, 126.13, 124.21, 122.75, 120.40, 116.70, 110.52, 104.61, 57.84, 52.95. Anal. ($C_{26}H_{25}N_3O_3 \cdot 2HCl \cdot {}^1/_2H_2O$) Table I.

1-(4-Acetamidophenyl)-1-[4'-[bis(2-hydroxyethyl)-amino]phenyl]methane (41). Dihydropyran (2.2 mL, 2.4 mmol) was added to stirred solution of 4-[bis(2-hydroxyethyl)amino]-4'-nitrobenzophenone (**34**) (2.5 g, 8.01 mmol) and D-camphorsulfonic acid (0.1 g) in CH_2Cl_2 (100 mL). The solution was stirred at 20 °C for 10 h before being washed with saturated $NaHCO_3$ solution. The organic phase was dried (Na_2SO_4), filtered, and concentrated, and the crude product (**38**) was dissolved in MeOH and hydrogenated over Pd/C for 5 h to give the crude amine **39**. A solution of this amine in CH_2Cl_2 (50 mL) was sequentially treated with Et_3N (3.34 mL, 24 mmol) and acetyl chloride (0.85 mL, 12.0 mmol) at 0 °C, and this mixture was stirred at 0 °C for 20 min before being washed with saturated $NaHCO_3$ solution. The organic phase was separated, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the crude amide **40** as

a brown oil. This was dissolved in 50% aqueous EtOH (200 mL), W-7 Raney nickel (13 g) was added, and the resulting slurry was heated under reflux for 12 h. The catalyst was removed by filtration and the filtrate was acidified with 2 N HCl and left to stand for 5 h before being partitioned between water and EtOAc. The aqueous layer was extracted with a second portion of EtOAc, and the combined organic phases were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography of the crude product on SiO_2 and elution with EtOAc/MeOH (19:1) yielded the amide **41** as a pale yellow solid (1.7 g, 46%): ^{1}H NMR (CD_3SOCD_3) δ 9.0 (s, 1 H, NHAc), 7.3 (d, J = 7.0 Hz, 2 H, H2′ and H6′), 7.0 (d, J = 7.0 Hz, 2 H, H3′ and H5′), 6.8 (d, J = 7.8 Hz, 2 H, H2″ and H6″), 6.5 (d, J = 7.8 Hz, 2 H, H3″ and H5″), 4.3 (s, 2 H, Ar–CH_2–Ar), 3.9 (s, 2 H, OH), 3.77 (br t, J = 6.0 Hz, 4 H, NCH_2CH_2OH), 3.60 (br t, J = 6.0 Hz, 4 H, NCH_2CH_2OH), 2.27 (s, 3 H, $NHCOCH_3$). This compound was used directly without further purification.

Compound 12 of Table I. The above compound (**41**) was converted to the corresponding mustard (**42**) as above. Chromatography of the crude product on SiO_2 and elution with CH_2Cl_2/EtOAc (4:1) gave pure **42** as a pale yellow oil in 60% yield: ^{1}H NMR ($CDCl_3$) δ 7.39 (d, $J_{2',3'}$ = 8.5 Hz, 2 H, H2′ and H6′), 7.20 (br s, 1 H, NHAc), 7.12 (d, $J_{2',3'}$ = 8.5 Hz, 2 H, H3′ and H5′), 7.05 (d, $J_{2'',3''}$ = 8.7 Hz, 2 H, H2″ and H6″), 6.61 (d, $J_{2'',3''}$ = 8.7 Hz, 2 H, H3″ and H5″), 3.84 (s, 2 H, Ar–CH_2–Ar), 3.69 (br t, J = 7.6 Hz, 4 H, NCH_2CH_2Cl), 3.61 (br t, J = 7.6 Hz, 4 H, NCH_2CH_2Cl), 2.08 (s, 3 H, $NHCOCH_3$); ^{13}C NMR δ 168.20, 144.30, 137.76, 135.79, 130.39, 130.05, 129.28, 120.10, 112.21, 53.60, 40.46, 40.20, 24.56.

A solution of the above acetamide mustard (**42**) (0.75 g, 2.16 mmol) in MeOH/water/concentrated HCl (1:1:1, 100 mL) was heated under reflux for 1 h, and the cooled solution was then basified with concentrated ammonia and extracted with EtOAc (2×). The combined organic phases were washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the crude amine (**43**), which was immediately condensed with 4-chloroquinoline as described above. Chromatography on SiO_2 and elution with CH_2Cl_2/EtOAc (1:1) yielded the pure free base of **12** as an oil (0.70 g, 75%). Conversion to the hydrochloride salt afforded a yellow solid: mp 125–129 °C; ^{1}H NMR (CD_3SOCD_3) δ 14.89 (br d, J = 6.2 Hz, 1 H, HCl), 11.12 (s, 1 H, NH), 8.90 (d, $J_{7,8}$ = 8.3 Hz, 1 H, H8), 8.46 (dd, $J_{2,3}$ = 7.0 Hz and $J_{2,HCl}$ = 6.2 Hz, 1 H, H2), 8.13 (dd, $J_{5,6}$ = 8.5 Hz and $J_{5,7}$ = 1.1 Hz, 1 H, H5), 8.02 (ddd, $J_{7,8}$ = 8.3 Hz, $J_{6,7}$ = 7.0 Hz and $J_{5,7}$ = 1.1 Hz, 1 H, H7), 7.78 (ddd, $J_{5,6}$ = 8.5 Hz, $J_{6,7}$ = 7.0 Hz and $J_{8,8}$ = 1.2 Hz, 1 H, H6), 7.40 (s, 4 H, H2′, H3′, H5′, and H6′), 7.12 (d, $J_{2'',3''}$ = 8.9 Hz, 2 H, H2″ and H6″), 6.76 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H3), 6.76 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H3), 6.71 (d, $J_{2'',3''}$ = 8.9 Hz, 2 H, H3″ and H5″), 3.90 (s, 2 H, Ar–CH_2–Ar), 3.71 (br s, 8 H, NCH_2CH_2Cl); ^{13}C NMR δ 154.91, 144.50, 142.33, 141.42, 138.08, 134.77, 133.70, 129.79, 129.66, 128.98, 126.80, 125.42, 123.73, 120.01, 116.94, 111.89, 99.54, 52.03, 41.01, 39.51. Anal. ($C_{26}H_{25}N_3Cl_2$·HCl·$^1/_2H_2O$) Table I.

Compound 7 of Table I. A solution of 1-(4-acetamidophenyl)-1-[4′-[bis(2-hydroxyethyl)amino]phenyl]methane (**41**) (0.55 g, 1.77 mmol) in MeOH/H_2O/concentrated HCl (1:1:1, 50 mL) was heated under reflux for 2 h, after which time the solution was concentrated under reduced pressure. The resulting oil was basified with concentrated ammonia and extracted with EtOAc to give the crude amine **44**, which was condensed with 4-chloroquinoline according to the general procedure above. Chromatography of the crude product on SiO_2 and elution with EtOAc/MeOH (9:1) yielded the free base of **7** as a pale yellow foam (0.40 g, 57%). Conversion to the dihydrochloride salt gave a yellow solid: mp 85–90 °C dec; ^{1}H NMR (CD_3SOCD_3) δ 14.8 (br d, J = 4.7 Hz, 1 H, HCl), 11.1 (s, 1 H, NH), 8.91 (d, $J_{7,8}$ = 8.4 Hz, 1 H, H8), 8.48 (t, J = 6.4 Hz, 1 H, H2), 8.12 (dd, $J_{5,6}$ = 8.5 Hz, $J_{5,7}$ = 0.9 Hz, 1 H, H5), 8.02 (dt, J_{ortho} = 7.1 Hz and J_{meta} = 1.1 Hz, 1 H, H7), 7.78 (dt, J_{ortho} = 7.8 Hz and J_{meta} = 1.1 Hz, 1 H, H6), 7.43 (s, 4 H, H3′, H5′, H2″, and H6″), 7.34 (br s, 4 H, H2′, H6′, H3″, and H5″), 6.76 (d, $J_{2,3}$ = 7.0 Hz, 1 H, H3), 4.1 (br s, 3 H, OH and HCl), 4.01 (s, 2 H, Ar–CH_2–Ar), 3.57 (br t, J = 5.1 Hz, 4 H, NCH_2CH_2OH), 3.50 (br t, J = 5.1 Hz, 4 H, NCH_2CH_2OH); ^{13}C NMR δ 154.9, 142.3, 140.5, 138.1, 135.0, 133.7, 129.9 (2 peaks superimposed), 129.8 (2 peaks superimposed), 126.8, 125.5 (2 peaks superimposed), 123.7, 120.0, 117.0, 99.6, 59.6, 56.2, 39.7. Anal. ($C_{26}H_{27}N_3O_2$·2HCl·2$^1/_2H_2O$) Table I.

Preparation of Compounds 8, 13, and 17 of Table I by the Method of Scheme III. 4-Nitrophenyl 4′-[Bis(2-chloroethyl)amino]phenyl Sulfide (46). Solid NaOH (1.8 g, 45.0 mmol) was added to a stirred solution of 4-[bis(2-hydroxyethyl)amino]thiophenol[11] (**45**) (8.7 g, 40.8 mmol) in dry EtOH (150 mL) under N_2. The resulting solution was heated under reflux for 25 min, and a solution of 4-fluoronitrobenzene (**24**) (6.3 g, 44.7 mmol) in dry EtOH (50 mL) was then added. The mixture was heated under reflux for a further 1 h before being cooled to room temperature and concentrated under reduced pressure. The resulting solid was partitioned between EtOAc and water, and the separated organic phase gave a red oil which was percolated through a pad of SiO_2 in EtOAc to give crude 4-nitrophenyl 4′-[bis(2-hydroxyethyl)amino]phenyl sulfide (**46**), which was used without further purification.

Compound 13 of Table I. The above sulfide (**46**) was converted into the corresponding mustard as above. Chromatography of the crude product on SiO_2 and elution with CH_2Cl_2/petroleum ether (1:1) gave 4-nitrophenyl 4′-[bis(2-chloroethyl)amino]phenyl sulfide (**47**) as a yellow-orange solid in 50% overall yield. Recrystallization from petroleum ether gave needles: mp 110.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, $J_{2,3}$ = 9.1 Hz, 2 H, H3 and H5), 7.43 (d, $J_{2',3'}$ = 9.0 Hz, 2 H, H2′ and H6′), 7.10 (d, $J_{2,3}$ = 9.1 Hz, 2 H, H2 and H6), 6.75 (d, $J_{2',3'}$ = 9.0 Hz, 2 H, H3′ and H5′), 3.81 (t, J = 7.4 Hz, 4 H, NCH_2CH_2Cl), 3.69 (t, J = 7.4 Hz, 4 H, NCH_2CH_2Cl); ^{13}C NMR δ 150.65, 147.51, 144.89, 137.44, 135.33, 123.94, 115.82, 113.01, 55.33, 40.15. Anal. ($C_{16}H_{16}N_2O_2SCl_2$) C, H, N.

Tin(II) chloride dihydrate (4.5 g, 20.0 mmol) was added to a stirred mixture of the above mustard (**47**) (2.1 g, 5.66 mmol) in concentrated HCl (100 mL). The mixture was heated under reflux for 3 h and then cooled and basified with concentrated ammonia and extracted with EtOAc (3×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated to give crude amine **51**, which was dissolved in MeOH (30 mL) and coupled with 4-chloroquinoline as described above. Chromatography on SiO_2 and elution with EtOAc gave the free base of **13** as a yellow solid (1.3 g, 50%). This material was further purified by recrystallization from EtOAc/petroleum ether, and the pure free base was then converted to the hydrochloride salt: mp >140 °C dec; ^{1}H NMR (CD_3SOCD_3) δ 14.80 (br s, 1 H, HCl), 11.10 (s, 1 H, NH), 8.85 (d, $J_{7,8}$ = 8.5 Hz, 1 H, H8), 8.48 (br d, $J_{2,3}$ = 6.0 Hz, 1 H, H2), 8.11 (d, $J_{5,6}$ = 8.1 Hz, 1 H, H5), 8.02 (dt, J_{ortho} = 7.5 Hz and J_{meta} = 0.6 Hz, 1 H, H7), 7.79 (dt, J_{ortho} = 7.7 Hz and J_{meta} = 1.0 Hz, 1 H, H6), 7.42 (d, $J_{2'',3''}$ = 8.9 Hz, 2 H, H2″ and H6″), 7.41 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H3′ and H5′), 7.23 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H2′ and H6′), 6.88 (d, $J_{2'',3''}$ = 8.9 Hz, 2 H, H3″ and H5″), 6.79 (d, $J_{2,3}$ = 6.0 Hz, 1 H, H3), 3.78 (br s, 8 H, NCH_2CH_2Cl); ^{13}C NMR δ 154.91, 147.35, 142.54, 138.56, 138.17, 136.56, 136.42, 134.51, 133.86, 127.58, 127.06, 126.98, 126.22, 123.76, 120.16, 117.11, 166.34, 99.83, 51.84, 40.92. Anal. ($C_{25}H_{23}N_3SCl_2$·HCl) Table I.

4-Nitrophenyl 4′-[Bis(2-chloroethyl)amino]phenyl Sulfone (48). A stirred solution of 4-nitrophenyl 4′-[bis(2-chloroethyl)amino]phenyl sulfide (**47**) (0.45 g, 1.21 mmol) in CH_2Cl_2 (20 mL) was treated portionwise with 3-chloroperbenzoic acid (0.54 g, 3.13 mmol). The solution was stirred at 20 °C for 1 h and then diluted with saturated sodium metabisulfite solution. The phases were separated, and the organic layer was washed sequentially with saturated $NaHCO_3$ and brine and then dried (Na_2SO_4), filtered, and concentrated to give the crude product as a lemon yellow solid. Recrystallization from CH_2Cl_2/petroleum ether afforded 4-nitrophenyl 4′-[bis(2-chloroethyl)amino]phenyl sulfone (**48**) (0.51 g, 100%) as bright yellow needles: mp 133 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (d, $J_{2,3}$ = 8.7 Hz, 2 H, H2 and H6), 8.08 (d, $J_{2,3}$ = 8.7 Hz, 2 H, H3 and H5), 7.80 (d, $J_{2',3'}$ = 9.1 Hz, 2 H, H2′ and H6′), 6.73 (d, $J_{2',3'}$ = 9.1 Hz, 2 H, H3′ and H5′), 3.81 (6, J = 6.9 Hz, 4 H, NCH_2CH_2Cl), 3.64 (t, J = 6.9 Hz, 4 H, NCH_2CH_2Cl); ^{13}C NMR δ 150.48, 149.87, and 148.58 (C1, C4, and C1′). Anal. ($C_{16}H_{16}N_2O_4Cl_2S$) C, H, N.

Compound 17 of Table I. Tin(II) chloride dihydrate (4.9 g, 21.7 mmol) was added to a stirred suspension of the above sulfone (**48**) (2.5 g, 6.20 mmol) in concentrated HCl (80 mL). The mixture was heated under reflux for 1 h and then cooled, basified with concentrated ammonia, and extracted with EtOAc (3×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated to give the crude amine **49**, which was immediately

dissolved in MeOH and coupled with 4-chloroquinoline as described above. Chromatography of the product on SiO_2 and elution with CH_2Cl_2/EtOAc (1:1) yielded the free base of **17** as a pale yellow foam (2.8 g, 75%). This was converted to the hydrochloride salt: mp 198–201 °C; ^1H NMR (CD_3SOCD_3) δ 15.1 (br s, 1 H, HCl), 11.3 (s, 1 H, NH), 8.94 (d, $J_{7,8}$ = 8.4 Hz, 1 H, H8), 8.58 (d, $J_{2,3}$ = 6.9 Hz, 1 H, H2), 8.16 (d, $J_{5,6}$ = 8.1 Hz, 1 H, H5), 8.05 (dt, J_{ortho} = 6.9 Hz and J_{meta} = 0.7 Hz, 1 H, H7), 8.04 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H3′ and H5′), 7.82 (dt, J_{ortho} = 7.3 Hz and J_{meta} = 0.8 Hz, 1 H, H6), 7.77 (d, $J_{2'',3''}$ = 9.2 Hz, 2 H, H2″ and H6″), 7.75 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H2′ and H6′), 7.10 (d, $J_{2,3}$ = 6.9 Hz, 1 H, H3), 6.95 (d, $J_{2'',3''}$ = 9.2 Hz, 2 H, H3″ and H5″), 3.83 (br t, J = 5.5 Hz, 4 H, NCH_2CH_2Cl), 3.81 (br t, J = 5.5 Hz, 4 H, NCH_2CH_2Cl); ^{13}C NMR δ 154.15, 150.49, 143.04, 141.72, 140.24, 138.33, 134.06, 129.46, 128.49, 127.30, 126.99, 125.12, 124.10, 120.29, 117.77, 111.86, 100.96, 51.64, 40.76. Anal. ($C_{25}H_{23}N_3O_2SCl_2$·HCl) Table I.

Compound 8 of Table I. Tin(II) chloride dihydrate (3.2 g, 14.2 mmol) was added to a stirred solution of 4-nitrophenyl 4′-[bis(2-hydroxyethyl)amino]phenyl sulfide (**46**) (0.94 g, 2.81 mmol) in EtOAc (50 mL). The mixture was heated under reflux for 4 h, cooled, and basified with concentrated ammonia. The resulting slurry was filtered and the residue was washed with EtOAc (3×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated to give the crude amine **50**, which was dissolved in MeOH (20 mL) and coupled with 4-chloroquinoline according to the general procedure above. Chromatography of the product on SiO_2 and elution with EtOAc/MeOH (9:1) gave the free base of **8** as a yellow solid (0.5 g, 41%). This was converted to the hydrochloride salt: mp 53–57 °C; ^1H NMR (CD_3SOCD_3) δ 14.82 (br d, J = 5.2 Hz, 1 H, HCl), 11.08 (s, 1 H, NH), 8.86 (d, $J_{7,8}$ = 8.5 Hz, 1 H, H8), 8.48 (t, $J_{2,3}$ = 6.5 Hz, 1 H, H2), 8.12 (d, $J_{5,6}$ = 8.5 Hz, 1 H, H5), 8.01 (dt, J_{ortho} = 7.1 Hz and J_{meta} = 1.0 Hz, 1 H, H7), 7.78 (dt, J_{ortho} = 7.2 Hz and J_{meta} = 1.0 Hz, 1 H, H6), 7.41 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H3 and H5), 7.36 (d, $J_{2'',3''}$ = 8.9 Hz, 2 H, H2″ and H6″), 7.22 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H2 and H6), 6.88 (d, $J_{2'',3''}$ = 8.9 Hz, 2 H, H3″ and H5″), 6.78 (d, J = 6.5 Hz, 1 H, H3), 4.60 (br s, 2 H, OH), 3.57 (t, J = 5.5 Hz, 4 H, NCH_2CH_2OH), 3.49 (t, J = 5.5 Hz, 4 H, NCH_2CH_2OH); ^{13}C NMR δ (one quaternary aromatic signal not resolved) 154.82, 142.41, 138.75, 138.09, 135.95, 34.33, 133.73, 127.39, 126.87, 126.09 (two signals superimposed), 123.71, 120.05, 117.03, 113.19, 99.73, 57.67, 53.46. Anal. ($C_{25}H_{25}N_3O_2S$·2HCl·2.5H_2O) Table I.

Preparation of Compounds 9 and 14 of Table I by the Method of Scheme IV. *N*-[4′-[Bis(2-chloroethyl)amino]-phenyl]-1-acetamidobenzamide (54). Ethyl chloroformate (2.7 mL, 28.9 mmol) was added dropwise to a solution of 4-acetamidobenzoic acid (**52**) (4.7 g, 26.3 mmol) and Et_3N (13.2 mL, 94.6 mmol) in Me_2CO (100 mL) at 0 °C. The solution was stirred at 0 °C for 20 min before being added slowly to a stirred suspension of 4-[bis(2-chloroethyl)amino]aniline dihydrochloride (**53**) (8.0 g, 26.3 mmol) in Me_2CO (50 mL). The mixture was stirred at 20 °C overnight and subsequently concentrated under reduced pressure. The resulting solid was triturated with 1 N HCl and filtered, and the residue was sequentially washed with EtOAc and MeOH to yield **54** as a white solid (4.8 g, 47%): mp 202–205 °C; ^1H NMR ($CDCl_3$) δ 9.58 (s, 1 H), 9.34 (s, 1 H, CONH and NHAc), 7.46–8.10 (m, 6 H, H2′, H3′, H5′, H6′, H3″, and H5″), 6.62 (d, $J_{2'',3''}$ = 6.7 Hz, 2 H, H2″ and H6″), 3.64 (br s, 8 H, NCH_2CH_2Cl), 2.12 (s, 3 H, $NHCOCH_3$). Anal. ($C_{19}H_{21}N_3O_2Cl_2$) C, H, N.

A solution of **54** (1.0 g, 2.54 mmol) in MeOH/water/concentrated HCl (1:1:1) was heated under reflux for 1 h, and the cooled solution was basified with ammonia and extracted into EtOAc to give the crude amine **55**, which was immediately condensed with 4-chloroquinoline as described above.

Chromatography on SiO_2 and elution with EtOAc gave the free base of **14** as a pale yellow solid (1.1 g, 90%). The hydrochloride salt crystallized from EtOAc/MeOH as a yellow solid: mp >250 °C dec; ^1H NMR (CD_3SOCD_3) δ 15.0 (br s, 1 H, HCl), 11.28 (s, 1 H, NH), 10.22 (s, 1 H, CONH), 8.95 (d, $J_{7,8}$ = 8.6 Hz, 1 H, H8), 8.60 (d, $J_{2,3}$ = 6.9 Hz, 1 H, H2), 8.16 (br d, $J_{2',3'}$ = 8.4 Hz, 3 H, H5, H3′, H5′), 8.05 (dt, J_{ortho} = 8.0 Hz and J_{meta} = 0.7 Hz, 1 H, H7), 7.82 (t, J_{ortho} = 7.3 Hz, 1 H, H6), 7.67 (d, $J_{2',3'}$ = 8.4 Hz, 2 H, H2′, H6′), 7.65 (d, $J_{2'',3''}$ = 9.0 Hz, 2 H, H2″, H6″), 6.98 (d, $J_{2,3}$ = 6.9 Hz, 1 H, H3), 6.77 (d, $J_{2'',3''}$ = 9.0 Hz, 2 H, H3″, H5″), 3.74 (br s, 8 H, NCH_2CH_2Cl); ^{13}C NMR δ 163.77, 154.46, 142.82, 142.74, 139.91, 138.19, 133.86, 133.27, 129.11, 127.49, 127.05, 124.54, 123.87, 122.23, 120.14, 117.37, 111.82, 100.16, 52.17, 41.13. Anal. ($C_{26}H_{24}Cl_2N_4O$·HCl) Table I.

Compound 9 of Table I. A suspension of 4-(4-aminoquinolyl)benzoic acid (**56**) (0.96 g, 3.19 mmol) in Me_2CO (100 mL) was treated sequentially with Et_3N (0.93 mL, 6.68 mmol) and ethyl chloroformate (0.34 mL, 3.56 mmol). After the mixture was stirred for 20 min, a solution of 4-[bis(2-hydroxyethyl)amino]aniline (**57**) (0.63 g, 3.83 mmol) in Me_2CO (20 mL) was added. The reaction mixture was stirred for a further 18 h before being partitioned between water and EtOAc, and the organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography of the crude product on SiO_2 and elution with EtOAc/MeOH (9:1) afforded the free base of **9** as a pale yellow solid (0.27 g, 20%). This was converted to the bright yellow dihydrochloride salt: mp 160–170 °C; ^1H NMR (CD_3SOCD_3) δ 9.88 (s, 1 H, HCl), 9.25 (br s, 1 H, NH), 8.57 (d, $J_{2,3}$ = 5.2 Hz, 1 H, H2), 8.41 (br d, $J_{7,8}$ = 8.2 Hz, 1 H, H8), 8.01 (d, $J_{2',3'}$ = 8.6 Hz, 2 H, H3′ and H5′), 7.94 (br d, $J_{5,6}$ = 8.4 Hz, 1 H, H5), 7.74 (dt, J_{ortho} = 6.9 Hz and J_{meta} = 1.1 Hz, 1 H, H7), 7.58 (dt, J_{ortho} = 7.5 Hz and J_{meta} = 1.0 Hz, 1 H, H6), 7.54 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H2″ and H6″), 7.48 (d, $J_{2',6'}$ = 8.6 Hz, 1 H, H2′ and H6′), 7.19 (d, $J_{2,3}$ = 5.2 Hz, 1 H, H3), 6.68 (d, $J_{2'',3''}$ = 9.1 Hz, 2 H, H3″ and H5″), 4.78 (br s, 2 H, OH), 3.56 (t, J = 6.0 Hz, 4 H, NCH_2CH_2OH), 3.42 (t, J = 6.0 Hz, 5 H, NCH_2CH_2OH and HCl); ^{13}C NMR δ 163.96, 150.45, 148.73, 146.49, 144.52, 143.80, 129.42, 128.97, 128.82 (two signals superimposed), 127.79, 124.91, 122.23, 122.11, 120.23, 119.76, 111.04, 103.31, 58.17, 53.37. Anal. ($C_{26}H_{26}N_4O_3$·2HCl) Table I.

Preparation of Compound 15 of Table I. A solution of 4-(4′-acetamidoanilino)quinoline (2.0 g, 7.22 mmol) in MeOH/water/concentrated HCl (1:1:1, 100 mL) was heated under reflux for 1 h to obtain the crude amine **58**. This was suspended in CH_2Cl_2 (50 mL) and DMF (10 mL), and the suspension was treated with a solution of freshly prepared 4-[bis(2-chloroethyl)amino]benzoyl chloride (**59**)[17] (2.0 g, 7.22 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at 20 °C for 15 h before being concentrated under reduced pressure. The residue was basified with concentrated ammonia, triturated with water, collected, and washed sequentially with water and CH_2Cl_2 to give the crude free base of **15** as a pale yellow solid (1.2 g, 35%). Conversion to the dihydrochloride salt and recrystallization of this from MeOH/EtOAc gave a yellow solid: mp 290 °C; ^1H NMR (solvent) δ 14.8 (br s, 1 H, HCl), 11.1 (s, 1 H, NH), 10.25 (s, 1 H, NH), 8.88 (d, $J_{7,8}$ = 8.5 Hz, 1 H, H8), 8.49 (br t, J = 6.4 Hz, 1 H, H7), 8.13 (br d, $J_{5,6}$ = 8.4 Hz, 1 H, H5), 8.03 (d, $J_{2,3}$ = 5.8 Hz, 1 H, H2), 8.02 (d, $J_{2',3'}$ = 8.8 Hz, 2 H, H3′ and H5′), 7.96 (d, $J_{2'',3''}$ = 9.0 Hz, 2 H, H2″ and H6″), 7.79 (dt, J_{ortho} = 8.1 Hz and J_{meta} = 0.8 Hz, 1 H, H6), 7.46 (d, $J_{2',3'}$ = 8.8 Hz, 2 H, H2′ and H6′), 6.88 (d, $J_{2'',3''}$ = 9.0 Hz, 2 H, H3″ and H5″), 6.77 (d, $J_{2,3}$ = 6.9 Hz, 1 H, H3), 3.8 (m, 8 H, NCH_2CH_2Cl); ^{13}C NMR δ 165.16, 155.17, 149.22, 142.4, 139.00, 133.80, 131.74, 129.70, 126.92, 125.89, 123.75, 122.05, 121.21, 120.14, 117.01, 110.95, 99.64, 51.78, 41.06. Anal. ($C_{25}H_{24}Cl_2N_4O$·HCl) Table I.

Compound 19 of Table I. A solution of the free base of **11** (0.25 g, 0.55 mmol) in dry benzene (2 mL) was treated with methyl triflate (0.7 mL, 0.6 mmol) at 20 °C for 24 h with stirring. The resulting yellow oil was triturated sequentially with dry benzene (3 × 10 mL) and water (3 × 5 mL) and dried under reduced pressure to give the quinolinium salt **19** as a yellow powder (0.82 g, 82%): mp 52–54 °C; ^1H NMR (CD_3OD) δ 8.60 (dd, $J_{7,8}$ = 8.4 Hz, $J_{6,8}$ = 1.5 Hz, 1 H, H8), 8.43 (d, $J_{2,3}$ = 7.3 Hz, 1 H, H2), 8.16–8.09 (m, 2 H, H5 and H7), 7.86 (ddt, $J_{8,5}$ = 8.4 Hz, $J_{6,7}$ = 6.6 Hz, $J_{6,8}$ = 1.5 Hz, 1 H, H6), 7.47 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H2′ and H6′), 7.31 (d, $J_{2',3'}$ = 8.9 Hz, 2 H, H3′ and H5′), 7.20 (d, J = 9.4 Hz, 2 H) and 7.18 (d, J = 9.4 Hz, 2 H), H2″, H3″, H5″, and H6″), 6.85 (d, $J_{2,3}$ = 7.3 Hz, 1 H, H3), 4.21 (s, 3 H, Me), 3.96 (t, J = 6.5 Hz, 4 H, NCH_2), 3.68 (t, J = 6.9 Hz, 4 H, CH_2Cl_2); ^{13}C NMR δ 157.25, 148.75, 140.68, 135.82, 133.52, 129.65, 128.95, 128.75, 128.68, 124.70, 123.40, 121.93, 121.04, 120.23, 119.61, 119.58, 101.17, 57.89, 43.18, 39.81. Anal. ($C_{27}H_{26}Cl_2F_3N_3O_4S$) Table I.

The other three quaternary salts (**20–22**) were prepared similarly.

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.[16,26] IC_{50} values were determined with 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 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 by using a minor modification of the MTT method of Mossman.[27] 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.[28]

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Registry No. 6·2HCl, 133041-48-2; 7·2HCl, 133041-49-3; 8·2HCl, 133041-50-6; 9·2HCl, 133041-51-7; 10·2HCl, 133041-52-8; 11·HCl, 133041-53-9; 12·HCl, 133041-54-0; 13·HCl, 133041-55-1; 14·HCl, 133041-56-2; 15·HCl, 133041-57-3; 16·HCl, 133041-58-4; 17·HCl, 133041-59-5; 18·2HCl, 133041-60-8; **19**, 133041-62-0; **20**, 133041-64-2; **21**, 133041-66-4; **22**, 133041-68-6; **23**, 103-90-2; **24**, 350-46-9; **25**, 2687-40-3; **26**, 2687-41-4; **27**, 133041-69-7; **28**, 133041-70-0; **29**, 92961-98-3; **30**, 93538-06-8; **31**, 462-06-6; **32**, 122-04-3; **33**, 2195-47-3; **34**, 133041-71-1; **35**, 133041-72-2; **36**, 133041-73-3; **37**, 133041-74-4; **38**, 133041-75-5; **39**, 133041-76-6; **40**, 133041-77-7; 41, 133041-78-8; **42**, 133041-79-9; **43**, 133041-80-2; **44**, 133041-81-3; **45**, 100132-31-8; **46**, 133041-82-4; **47**, 133041-83-5; **48**, 133041-84-6; **49**, 133041-85-7; **50**, 133041-86-8; **51**, 133041-87-9; **52**, 16331-48-9; **53**, 2067-58-5; **54**, 133041-88-0; **55**, 133041-89-1; **56**, 133041-90-4; **57**, 7575-35-1; **58**, 133041-91-5; **59**, 15944-88-4; **60**, 133041-92-6; **61**, 133041-93-7; **62**, 133041-94-8; **63**, 133041-95-9; 4-chloroquinoline, 611-35-8.

(26) Baguley, B. C.; Wilson, W. R. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 607.
(27) Mossman, T. J. *Immunol. Methods* **1983**, *65*, 55.
(28) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Anal. Biochem.* **1986**, *22*, 655.
(29) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Eur. J. Cancer Clin. Oncol.* **1986**, *22*, 655.

5-Lipoxygenase Inhibitors: The Synthesis and Structure–Activity Relationships of a Series of 1-Phenyl-3-pyrazolidinones[1]

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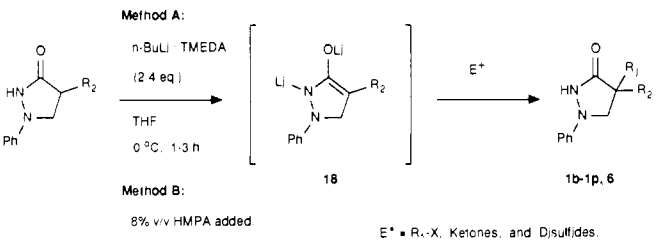
A series of analogues of the 5-lipoxygenase inhibitor 1-phenyl-3-pyrazolidinone (phenidone, **1a**) has been prepared via two complementary new synthetic methods. The reaction of various electrophiles with the dianion of **1a** or with an *N*-silylpyrazolidinone anion gave the desired 4-substituted pyrazolidinones (Scheme I and II). A new procedure was developed for the resolution of 4-substituted pyrazolidinones (Scheme V). A regression study on 21 compounds in this series showed a correlation of increased inhibitor potency (pIC_{50}) with increased compound lipophilicity (log *P*) and with an *N*-phenyl electronic effect as measured by the ^{13}C NMR chemical shift parameter CNMR1′ (R^2 = 0.79). The most potent 5-lipoxygenase inhibitor in this series was 4-(ethylthio)-1-phenyl-3-pyrazolidinone (**1n**) with an IC_{50} of 60 nM. Another member of this series, 4-(2-methoxyethyl)-1-phenyl-3-pyrazolidinone (**1f**, IC_{50} = 0.48 μM), although less potent than **1n**, was better tolerated in the whole animal relative to phenidone (**1a**) and also displayed good oral activity in two models of 5-lipoxygenase inhibition. On the basis of a structure–activity relationship study, a mechanism for the inhibition of 5-lipoxygenase by this class of inhibitors was proposed.

The identification of the leukotrienes (LTC_4, LTD_4, LTE_4) as mediators in the pathophysiology of allergic disease has attracted the interest of many laboratories to discover agents which either antagonize the leukotriene receptor(s)[2] or inhibit leukotriene biosynthesis.[3] Since 5-lipoxygenase (5-LO) oxidizes arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) in the first step of the leukotriene pathway in the arachidonic acid cascade, inhibitors of this enzyme should prevent leukotriene biosynthesis and therefore prove useful in the treatment of allergic asthma.

Phenidone (1a) and BW-755C were reported to inhibit 5-LO both in vitro[4,5] and orally ex vivo.[6] More recently the phenidone analogues A-53612 (**2a**)[7] and A-65260[8] have been reported to possess improved selectivity for 5-LO, compared to that of phenidone, as well as oral activity in the inhibition of leukotriene biosynthesis. We now report our findings in the chemistry and in the structure–activity

Scheme I. 4-Substituted Pyrazolidinones via the Dianion 18 (Methods A and B)



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(2) Musser, J. H.; Kubrak, D. M.; Chang, J.; DiZio, S. M.; Hite, M.; Hand, J. M.; Lewis, A. J. *J. Med. Chem.* **1987**, *30*, 400 and cited references.

0022-2623/91/1834-1560$02.50/0