(1-Pyrenylmethyl)amino Alcohols, a New Class of Antitumor DNA Intercalators. Discovery and Initial Amine Side Chain Structure–Activity Studies

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In the series of 1-pyrenylmethylamines studied in this work the relationships among structure, interaction with DNA, and murine antitumor activity were examined. Binding studies show that all of these 1-pyrenylmethylamine derivatives bind to some extent to DNA by intercalation. The presence of additional basic amine groups in the side chain enhances DNA binding due to electrostatic interactions. Those compounds containing only a single basic benzylic amine bind similarly to DNA. Only the presence of bulky side chains appears to decrease the DNA interactions in the compounds examined. Although antitumor activity is seen for (1-pyrenylmethyl)amino alcohols, useful antitumor activity in the series is limited to those congeners bearing the 2-amino-1,3-propanediol-type side chain. These derivatives bind moderately to DNA. DNA binding is a necessary but not sufficient criterion for antitumor activity in the series. In addition, the strength of DNA binding does not correlate with the antitumor activity in the group of active compounds. Three related 2-[(arylmethyl)amino]-1,3-propanediol derivatives (AMAPs) [crisnatol (770U82), 773U82, and 502U83] are currently in clinical trials as potential antitumor agents.

Many drugs that possess chemotherapeutic activity intercalate with DNA.[1] The orientation and geometry of a limited number of drug–DNA complexes have been studied by using X-ray diffraction, NMR spectroscopy, and traditional solution methods. It has also been shown that a wide variety of planar ring systems can intercalate with DNA. This research has provided some tantalizing clues about the intercalation phenomenon, but a clear picture of the relationship among structure, DNA interaction, and chemotherapeutic activity does not exist.[1]

To design new types of antitumor DNA intercalators we began a systematic study of the interactions between DNA and polycyclic aromatic derivatives containing polar side chains. Compounds synthesized for this research were also evaluated in a number of chemotherapeutic screens. Simple carbocyclic aromatic ring systems were examined first in order to eliminate possible effects due to heteroatoms.[2] Antitumor activity was discovered in a series of (1-pyrenylmethyl)amino alcohol derivatives (1) by using a standard murine lymphocytic leukemia screen. Further examination of this series has shown that optimal antitumor activity is seen for 2-[(arylmethyl)amino]propanediols (AMAPs) of general structure 2.

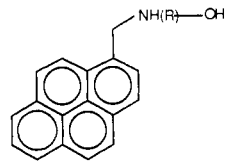
These compounds possess a broad spectrum of activity against both murine and human tumors in various in vitro and in vivo systems. From the large number of AMAPs synthesized, three congeners were chosen for development. Compounds 3 [crisnatol (770U82)], 4 (773U82), and 5 (502U83) are currently in clinical trials.

In this paper we report on the effect of variation of the amine side chain structure on antitumor activity and DNA binding in a series of pyrene derivatives. Although the level of antitumor activity in the pyrene series did not warrant further development, the relative effects of structural variation on DNA binding and antitumor activity are the same as that of the series chosen for clinical evaluation. In subsequent papers the effect of variation of size, shape, and substitution of the aromatic ring system in a series of derivatives (containing the optimized side chain elucidated in this work) on antitumor activity will be examined. Further studies of the effect of side-chain structure on antitumor activity in the more active ring systems will also be presented.

Chemistry

Most compounds of general structure 7 were synthesized from 1-pyrenecarbaldehyde (6) and a variety of primary amines (most commercially available) by reductive amination using either $NaBH_3CN$ under equilibrium conditions (method A)[3] or, preferably, by reduction of the preformed imine (produced by azeotropic removal of H_2O) with $NaBH_4$ (method B) (Scheme I). For compounds 8–12, a 5-fold excess of the symmetrical primary diamine was used to minimize production of the bis-derivative. The preparation of the amines used to produce compounds 13–17 (Table I) will be presented in a separate paper.[2]

Reduction of 6 with $NaBH_4$ in THF gave 1-pyrenylmethanol (18).[4] Reaction of 18 with $SOCl_2$/pyridine/Et_2O gave 1-(chloromethyl)pyrene (19),[4] which was in turn



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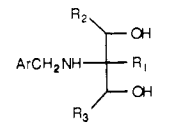

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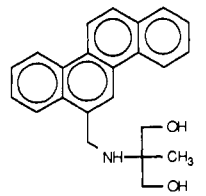

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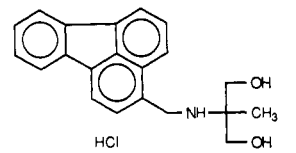

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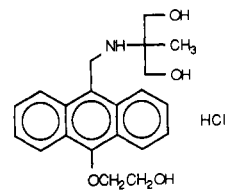

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(1) For a basic description of the intercalation process as well as a review of the structures of chemotherapeutic importance which intercalate with DNA, see: Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. *The Molecular Basis of Antibiotic Action, Second Edition*; John Wiley and Sons: London, 1981; p 273–370.
(2) Bair, K. W. Unpublished results.
(3) Borch, R. F.; Durst, H. D. *J. Am. Chem. Soc.* **1969**, *91*, 3996.
(4) Bachmann, W. E.; Carmack, M. *J. Am. Chem. Soc.* **1941**, *63*, 2494.

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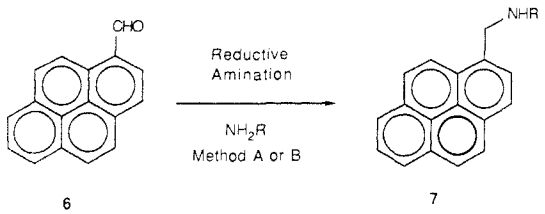
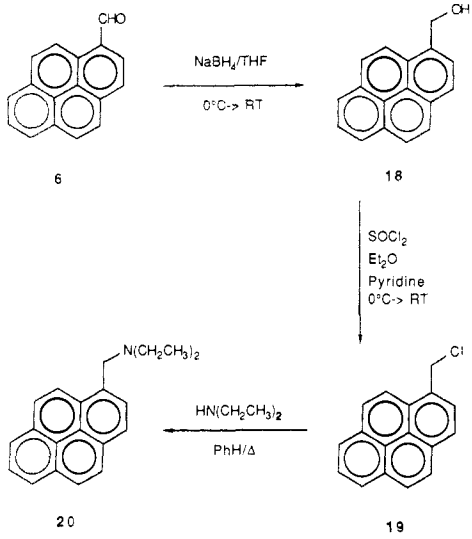
Scheme I

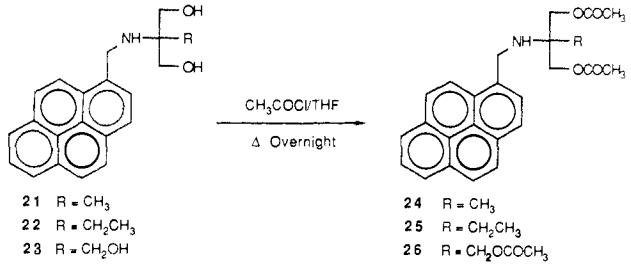


Method A $NaBH_3CN/EtOH/PhCH_3/g.$ HCl in EtOH at pH = 6-8, RT
Method B 1) $TosOH/PhCH_3/\Delta$ ($-H_2O$) 2) abs. $EtOH/NaBH_4$ (0°C-> RT)

Scheme II



Scheme III



converted to **20**[5] ($HNEt_2/PhH/\Delta$) (Scheme II).

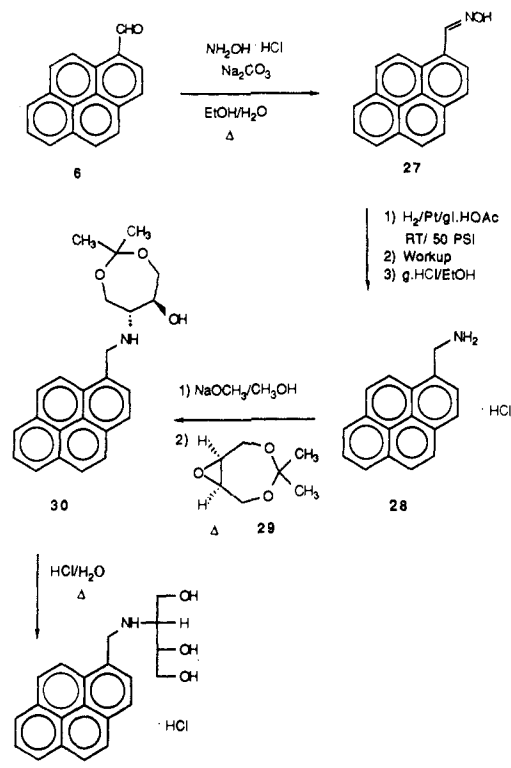
The acetoxy derivatives **24**, **25**, and **26** were prepared by reaction of the HCl salts **21**, **22**, and **23**, respectively, with AcCl in THF at reflux (Scheme III).

The oxime **27**[5] was also prepared from aldehyde **6** and reduced (H_2/PtO_2/glacial HOAc) to give 1-pyrenylmethylamine (**28**).[6] Further reaction of **28** with the epoxide **29** (prepared by the procedure of Elliot and Fried)[7] gave **30**, which in turn was hydrolyzed (HCl/H_2O/RT) to give the triol **31** (Scheme IV).

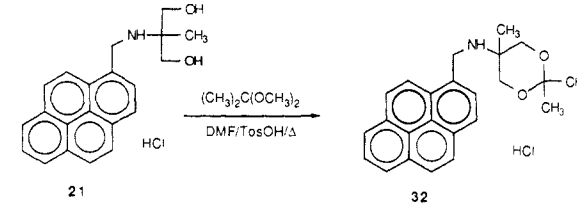
The isopropylidene derivative **32** was prepared from **21** with use of standard conditions [$(CH_3)_2C(OCH_3)_2$/DMF/TosOH/Δ] (Scheme V).

The route employed to synthesize the homologue **39** is shown in Scheme VI. The aldehyde **6** was condensed with $CH_2(COOEt)_2$ with pyridinium acetate as the catalyst to give **33**.[8] This aldehyde was then reduced by using PtO_2/H_2 in EtOAc to give **34**. Hydrolysis gave the malonic

Scheme IV



Scheme V



acid derivative **35**,[8] which was decarboxylated neat at 200 °C to give 3-(1-pyrenyl)propionic acid (**36**).[4,9] This acid was reduced with B_2H_6 in THF to the crude alcohol **37**, which in turn was oxidized ($PCC/CH_2Cl_2/\Delta$) to the aldehyde **38** in poor yield. Reductive amination of **38** using Method A produced the target compound **39**.

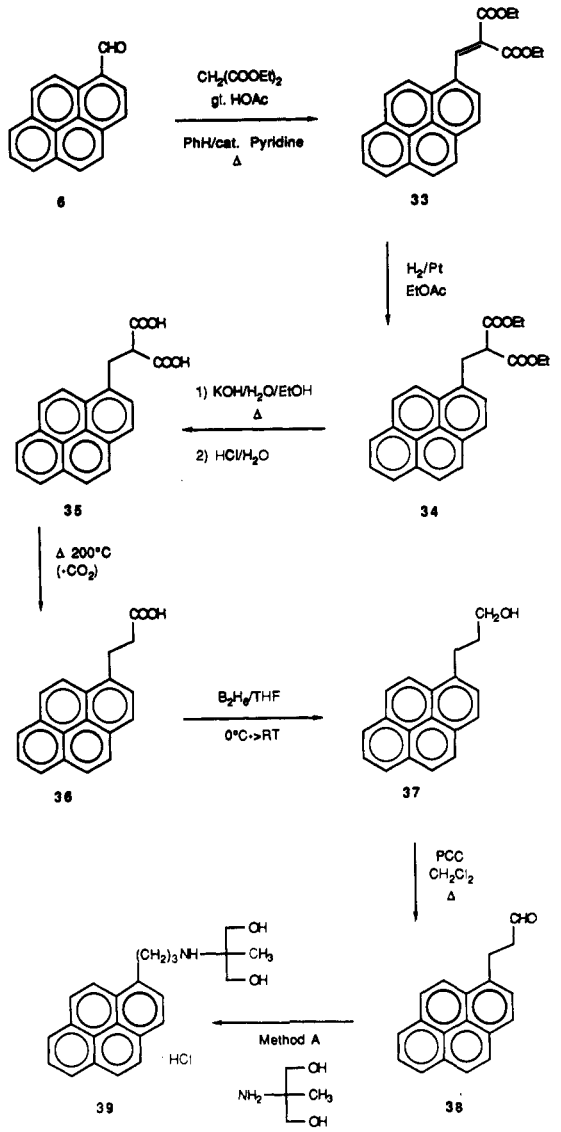
DNA Binding Studies

A detailed examination of the interaction between polycyclic aromatic compounds and DNA, which includes various binding studies as well as modeling work, is beyond the scope of this paper and will be reported separately.[2] However, some basic observations must be mentioned in order to discuss the trends observed in the active antitumor series.

Biophysical studies on a large variety of carbocyclic polycyclic aromatic derivatives have shown that two structural elements contribute to the interaction of the molecule with DNA. The ring system that intercalates[1] with DNA must contain at least three fused planar rings, although fused tetracyclic and pentacyclic ring systems are preferred. The second structural component required for good DNA binding with these compounds is a pendant side chain containing an amine group. The amine binds electrostatically to DNA and, in general, is required to solubilize these hydrophobic ring systems. For the carbocyclic

(5) Clarke, R. L.; Buck, J. S. US Patent 3,198,835.
(6) Marcus, E.; Fitzpatrick, J. T. *J. Org. Chem.* **1960**, *25*, 199.
(7) Elliot, W. J.; Fried, J. *J. Org. Chem.* **1976**, *41*, 2469.
(8) Vollman, H.; Becker, H.; Corell, M.; Streech, H. *Ann.* **1937**, *531*, 1.
(9) Gerasimenko, Y. E.; Shevchuk, I. N. *Zh. Org. Khim.* **1968**, *4*(12), 2198.

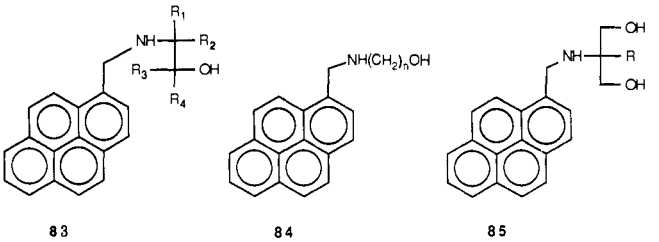
Scheme VI



ring systems containing amine side chains, optimal DNA binding occurs when the first amine group is one carbon atom removed (i.e. benzylic) from the aromatic ring. With these criteria, a series of readily available 1-pyrenylmethylamines was selected for evaluation (Table I). The simplest member of the series, 1-pyrenylmethylamine (**28**), intercalates with DNA as shown by viscometric studies on calf thymus DNA.[2,10] In thermal denaturation studies (as outlined in the Experimental Section) a ΔT_m of 11 °C is observed for **28**. The binding of the congeners to DNA is determined both by electronic and steric factors. In the limited series shown in Table I, the presence of CN, OCH_3, or OH groups (**40**, **41**, and **60**, respectively)—each two carbon atoms removed from the benzylic amine—did not decrease DNA binding to any extent. However, the addition of 1–3 more basic amine groups to the side chain produced compounds with substantially stronger binding to DNA as seen by the ΔT_m values observed for **8–12**, **52–54**, and **56–59**. This is primarily due to strong electrostatic interactions between the additional side-chain amine groups and the negatively charged phosphodiester groups on the DNA. The actual extent of the contribution of these forces to DNA binding varies considerably and is determined by several factors.[2]

Several substituents, if present in the side chain, can decrease the interaction of the molecule with DNA for steric reasons. Although congeners containing simple secondary amines usually bind to DNA as well as **28**, a definite decrease in binding is seen for tertiary amines (e.g. **20**, **42**, and **66**). Although the presence of a benzyl substituent did not seem to affect DNA binding in the series, a 3,5-dichlorobenzyl substituent decreased the binding substantially (e.g. **28** vs **43** vs **47**). Additional electronic factors may be responsible for this effect.

An examination of the large number of secondary (1-pyrenylmethyl)amino alcohols prepared as a result of the antitumor activity in the series shows that few steric or electronic factors substantially decrease DNA binding. For comparison, this set of compounds can be considered to be derivatives of 2-[(1-pyrenylmethyl)amino]ethanol (**60**). The presence of simple alkyl, hydroxylalkyl, or alkoxyalkyl substituents at either the α- or β-position of the ethanol group (i.e. **83** at R_1, R_2, R_3, and R_4) does not affect DNA binding appreciably (e.g. **13–15**, **21–23**, **31**, **64–72**, **74**, **75**, and **79–82**). However, compounds bearing a bulky phenyl substituent in either of these two positions (e.g. **76–78** and **81**) bind poorly. Compounds that have amine substituents that form a cyclic system (e.g. **30**, **32**, and **82**) or those in which all of the OH groups have been acetylated (e.g. **24–26**) bind very poorly to DNA.



83 84 85

Hydroxy groups in these side chains do not appreciably increase binding due to electrostatic interaction with various groups on the DNA. However, steric and electronic effects produced by the side chains substantially affect the conformations of the molecule and the molecule/DNA complex.[2]

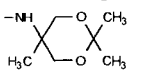
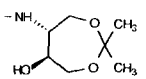
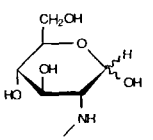
P388 Screening Results

Preliminary antitumor evaluation of the series was done by using murine lymphocytic leukemia P388 with slight modifications as outlined in the Experimental Section. An examination of Table I shows that derivatives of the secondary amine 1-pyrenylmethylamine (**28**) containing simple alkyl groups or more than one amine the side chain show little antitumor activity in the P388 screen. The first traces of activity are seen with congeners possessing an OH group in the side chain. The simplest member of this group is 2-[(1-pyrenylmethyl)amino]ethanol (**60**). Comparison of simple unbranched congeners containing one OH group (**84**, n = 2, 3, 4, and 5; compounds **60**, **61**, **62**, and **63**, respectively) shows that the optimal arrangement of the OH group is two carbon atoms from the NH group. Substitution at either the α- or β-side-chain position of **60** with small alkyl groups (i.e. **83** at R_1, R_2, R_3, and R_4) (e.g. compounds **64**, **65**, **67–71**, **74**, and **75**) produces compounds with similar, relatively low levels of antitumor activity unless the compounds have poor solubility. Congeners of **60** with bulky, lipophilic side chains (e.g. containing phenyl rings) (**76–78**) are inactive. Congeners of **60** with a single OH group not β to the NH group linked by a branched

(10) With use of the viscometric procedure described by Cory et al. (Cory, M.; McKee, D. D.; Kagan, J.; Henry, D. W.; Miller, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 2528) compound **28** gave a slope of 1.35 in 1 × 10^{-3} M NH_4F buffer (using a final drug/DNA ratio = 0.1). Under these conditions, both ethidium bromide and quinacrine gave slopes of 1.33.

Table I. Physicochemical and Biophysical Properties of (1-Pyrenyl)CH_2Z Derivatives

no.	Z[a]	mp, °C[b]	recryst solvent[c]	method[d]	yield[e]	formula	analysis[f]	ΔT_m (± st. dev.), °C[g]	P388 leukemia in vivo[g] LD_{20}	opt dose, mg/kg	% ILS, mg/kg
28	$-NH_2$	261–263[h]	M	SCH IV	45	$C_{17}H_{13}N\cdot HCl$	C, H, Cl, N	11	130	(194)[i]	I[j]
40	$-NHCH_2CH_2CN$	182–206 dec	E	A	13	$C_{20}H_{16}N_2\cdot HCl$	C, H, Cl, N	6	300	(300)	I
41	$-NHCH_2CH_2OCH_3$	200–202 dec	E-EE	A	43	$C_{20}H_{19}NO\cdot HCl$	C, H, Cl, N	10	200	(133)	I
20	$-N(CH_2CH_3)_2$	252–254 dec[k]	E	SCH II	65	$C_{21}H_{21}N\cdot HCl$	C, H, Cl, N	9.7	452	(452)	I
42	$-N(CH_2CH_2OH)_2$	207–209 dec[k]	E-H	A	11	$C_{21}H_{21}NO_2\cdot HCl$	C, H, Cl, N	7	225	(225)	I
43	$-NHCH_2Ph$	257–259	95% E	A	34	$C_{24}H_{19}N\cdot HCl$	C, H, Cl, N	9	600	[900][l]	I
44	$-NHCH_2CH_2Ph$	256–260 dec	96% E	A	36	$C_{25}H_{21}N\cdot HCl$	C, H, Cl, N	10	335	(335)	I
45	$-NH(CH_2)_3Ph$	205–207	E-H_2O	A	5	$C_{26}H_{23}N\cdot HCl$	C, H, Cl, N	9	300	(300)	I
46	$-NH(CH_2)_4Ph$	225–227 dec	95% E	A	57	$C_{27}H_{25}N\cdot HCl\cdot 0.5H_2O$	C, H, Cl, N	9	130	(88)	I
47	$-NHCH_2$(3,4-di-Cl-Ph)	235–238	95% E	A	24	$C_{24}H_{17}NCl_2\cdot HCl$	C, H, Cl, N	2	400	[900]	I
48	$-NH(CH_2)_3O$-*i*-Pr	192–195	I-EE	A	20	$C_{23}H_{25}NO\cdot HCl$	C, H, Cl, N	5	180	(120)	I
49	$-NHCH_2CH{=}CH_2$	220–221	95% E	A	38	$C_{20}H_{17}N\cdot HCl$	C, H, Cl, N	10 (± 0)	369	(550)	I
50	-NH-*n*-Pr	215–217.5	E-EE	A	31	$C_{20}H_{19}N\cdot HCl$	C, H, C, N	12	150	(225)	I
8	$-NH(CH_2)_4NH_2$	284–289 dec	E-H_2O	A	18	$C_{21}H_{22}N_2\cdot 2HCl$	C, H, Cl, N	28. (± 0)	28	(19)	I
9	$-NH(CH_2)_5NH_2$	290–292	E-EE	A	18	$C_{22}H_{24}N_2\cdot 2HCl$	C, H, Cl, N	29	20	(116)	I
10	$-NH(CH_2)_6NH_2$	181–183	E	A	15	$C_{23}H_{26}N_2\cdot 2TosOH$	C, H, N, S	29.8 (± 0.9)	75	(75)	I
51	$-NH(CH_2)_2NHPh$	133–134	95% E	A	16	$C_{25}H_{22}N_2\cdot HCl$	C, H, C, N	10	[300]	(200)	I
52	$-NH(CH_2)_3N(CH_3)_2$	237–240 dec	95% E	A	13	$C_{22}H_{24}N_2\cdot 2HCl$	C, H, Cl, N	24.5 (± 2.1)	85	(127)	I
53	$-NH(CH_2)_3N(CH_2CH_2)_2O$	264–267 dec	95% E	A	25	$C_{24}H_{26}N_2O\cdot 2HCl$	C, H, Cl, N	19	150	150	+20
54	$NHCH(CH_3)(CH_2)_3N(CH_2CH_3)_2$	140–143 dec	E	A	51	$C_{26}H_{32}N_2\cdot 2HCl\cdot 2H_2O$	C, H, Cl, N	31 (± 3.)	100	(150)	I
55	$-NHCH_2CH_2N(CH_2CH_3)_2$	225.5–227 dec	E	A	30	$C_{23}H_{26}N\cdot 2HCl$	C, H, Cl, N	14 (± 0)	120	(180)	I
56	$-NH(CH_2)_3NHCH(CH_2CH_2)_2CH_2$	256–260 dec	95% E	A	16	$C_{26}H_{30}N_2\cdot HCl\cdot H_2O$	C, H, Cl, N	25	134	(200)	I
57	$-NHCH_2CH_2NHCH_2CH_2OH$	274–282 dec	E-H_2O	A	23	$C_{21}H_{22}N_2O\cdot 2HCl$	C, H, Cl, N	16 (± 1.4)	200	(134)	I
58	$-NHCH_2CH_2NHCH_2CH(CH_3)OH$	261.5–262 dec	E-H_2O	A	25	$C_{22}H_{24}N_2O\cdot 2HCl$	C, H, Cl, N	15.5 (± 2.1)	150	(150)	I
59	$-NH(CH_2)_3N(CH_2CH_2OH)_2$	176–180 dec	E	A	23	$C_{24}H_{28}N_2O_2\cdot 2HCl$	C, H, Cl, N	21 (± 0)	96	(64)	I
11	$-NH(CH_2)_3N(CH_3)(CH_2)_3NH_2$	233–238	95% E	A	29	$C_{24}H_{29}N_3\cdot 3HCl$	C, H, Cl, N	31 (± 0)	23	23	+20
12	$-NH(CH_2)_3N(CH_2CH_2)_2N(CH_2)_3NH_2$	284–286 dec	E-H_2O	A	21	$C_{27}H_{34}N_4\cdot 4HCl$	C, H, Cl, N	32.5 (± 1.3)	37	(37)	I
60	$-NHCH_2CH_2OH$	202–203 dec	95% E	A	41	$C_{19}H_{17}NO\cdot HCl$	C, H, Cl, N	11.9 (± 2.1)	220	221	+31
61	$-NH(CH_2)_3OH$	194–195 dec	E-EE	A	29	$C_{20}H_{19}NO\cdot HCl$	C, H, Cl, N	11	120	[120]	+20
62	$-NH(CH_2)_4OH$	166–170 dec	E-EA	A	63	$C_{21}H_{21}NO\cdot HCl\cdot 0.5H_2O$	C, H, Cl, N	11	208	(139)	I
63	$-NH(CH_2)_5OH$	215–217	E-EE	A	36	$C_{22}H_{23}NO\cdot HCl$	C, H, Cl, N	11	196	(131)	I
64	$-NHCH(CH_3)CH_2OH$	230–232 dec	E	A	26	$C_{20}H_{19}NO\cdot HCl$	C, H, Cl, N	10.5 (± 0.7)	600	220	+25
65	$-NHCH_2CH(CH_3)OH$	217–218 dec	E	A	28	$C_{20}H_{19}NO\cdot HCl$	C, H, Cl, N	10.0 (± 0)	600	300	+28
66	$-N(CH_3)CH_2CH_2OH$	244–246 dec	95% E	A	40	$C_{20}H_{19}NO\cdot HCl$	C, H, Cl, N	8.6 (± 0.6)	366	[365]	I
67	$-NHC(CH_3)_2CH_2OH$	253.5–254	E-EE	A	35	$C_{21}H_{21}NO\cdot HCl$	C, H, Cl, N	11.3 (± 0.6)	450	250	+45
68	$-NHCH_2C(CH_3)_2OH$	225–226.5 dec	E	A	19	$C_{21}H_{21}NO\cdot HCl$	C, H, Cl, N	10	220	330	I
69	$-NHCH(i\text{-}Pr)CH_2OH$[m]	246–248 dec	95% E	A	15	$C_{22}H_{23}NO\cdot HCl$	C, H, Cl, N	10.3 (± 0.6)	450	200	+27
70	$-NHCH(CH_2\text{-}i\text{-}Pr)CH_2OH$	252–254 dec	E-EE	A	41	$C_{23}H_{25}NO\cdot HCl$	C, H, Cl, N	11	[675]	[1012]	I
71	$-NHCH(CH(CH_3)CH_2CH_3)CH_2OH$[m]	253–254 dec	E	A	46	$C_{23}H_{25}NO\cdot HCl$	C, H, Cl, N	9 (± 0)	[675]	[675]	I
72	$-NHCH_2CH_2CH(CH_3)OH$	204–206 dec	E-EE	A	41	$C_{21}H_{21}NO\cdot HCl$	C, H, Cl, N	11.6 (± 1.9)	168	(168)	I
73	$-NH(CH_2)_3C(CH_3)_2CH_2OH$	184–186	E-EE	A	40	$C_{24}H_{27}NO\cdot HCl$	C, H, Cl, N	10	215	(215)	I
74	$-NHCH_2CH(CH_2CH_3)OH$	237–238 dec	E	A	44	$C_{21}H_{21}NO\cdot HCl$	C, H, Cl, N	11	225	(225)	I
75	$-NHCH(CH_2CH_3)CH_2OH$[n]	220.5–222 dec	E	A	47	$C_{21}H_{21}NO\cdot HCl$	C, H, Cl, N	12 (± 0)	450	366	+25
76	$-NHCH(Ph)CH_2OH$	238–244 dec	E	A	31	$C_{25}H_{21}NO\cdot HCl$	C, H, Cl, N	8 (± 0.7)	[675]	[675]	I
77	$-NHCH_2CH(Ph)OH$	247–248 dec	E	A	28	$C_{25}H_{21}NO\cdot HCl$	C, H, Cl, N	11	[450]	[450]	I
78	$-NHCH(CH_3)(CH(Ph)OH)$	240–241 dec	E	A	6	$C_{26}H_{23}NO\cdot HCl$	C, H, Cl, N	7	[450]	[675]	I
79	$-NHCH_2CH(OH)CH_2OH$	208–210 dec	E	A	26	$C_{20}H_{19}NO_2\cdot HCl$	C, H, Cl, N	11 (± 0)	206	250	+25

80	$-NHCH(CH_2OH)_2$	211–213 dec	E	A	52	$C_{20}H_{19}NO_2 \cdot HCl$	C, H, Cl, N	10 (± 0.1)	450	300	+63
21	$-NHC(CH_3)(CH_2OH)_2$	221.5–222	E-EE	A	49	$C_{21}H_{21}NO_2 \cdot HCl$	C, H, Cl, N	11.5 (± 0.8)	300	300	+125
22	$-NHC(CH_2CH_3)(CH_2OH)_2$	219–221 dec	E-EE	A	23	$C_{22}H_{23}NO_2 \cdot HCl$	C, H, Cl, N	12.7 (± 0.5)	[340]	303	+140
23	$-NHC(CH_2OH)_3$	232–234 dec	E-EE	A	42	$C_{21}H_{21}NO_3 \cdot HCl$	C, H, Cl, N	11.0 (± 0)	215	198	+90
13	$-NHC(n\text{-pentyl})(CH_2OH)_2$	192–193 dec	E-EE	A	35	$C_{25}H_{29}NO_2 \cdot HCl \cdot 0.25H_2O$	C, H, Cl, N	9.6 (± 0.4)	[675]	[675]	I
14	$-NHC(CH_2OCH_2CH_3)(CH_2OH)_2$	189–190 dec	E-EE	B	44	$C_{23}H_{25}NO_3 \cdot HCl \cdot 0.33H_2O$	C, H, Cl, N	9	225	168	+30
15	$-NHC(i\text{-Pr})(CH_2OH)_2$	244–245 dec	M-EE	B	13	$C_{23}H_{25}NO_2 \cdot HCl$	C, H, Cl, N	9.8 (± 1.7)	300	450	+55
81	$-NHCH(CH_2OH)(CH(Ph)OH)$[a]	217–220	E	A	23	$C_{26}H_{23}NO_2 \cdot HCl$	C, H, Cl, N	8.3 (± 0.5)	[675]	[1013]	I
31	$-NHCH(CH_2OH)(CH(OH)CH_2OH)$	166–167	E-EE	SCH IV	58	$C_{21}H_{21}NO_3 \cdot HCl$	C, H, Cl, N	9.4 (± 0.9)	140	125	+25
16	$-NHC(CH_3)(CH(CH_3)OH)(CH_2OH)$[p]	221–222 dec	E-EE	A	17	$C_{22}H_{23}NO_2 \cdot HCl$	C, H, Cl, N	11.6 (± 1.1)	125	125	+77
17	$-NHC(CH_3)(CH_2OCH_3)(CH_2OH)$	158–160	E-EE	B	51	$C_{23}H_{23}NO_2 \cdot CH_3SO_3H \cdot 0.33H_2O$	C, H, N, S	10.2	135	180	+30
24	$-NHC(CH_3)(CH_2OAc)_2$	91–92	H	SCH III	48	$C_{25}H_{25}NO_4$	C, H, N	3	[300]	(300)	+65
25	$-NHC(CH_2CH_3)(CH_2OAc)_2$	106–107	H	SCH III	71	$C_{26}H_{27}NO_4$	C, H, N	2.4	[675]	(303)	+40
26	$-NHC(CH_2OAc)_3$	150–150.5	EtOAc–H	SCH III	88	$C_{27}H_{27}NO_6$	C, H, N	2.3 (± 0.8)	[500]	315	+30
32		123–124 dec	E-EE	SCH V	49	$C_{24}H_{25}NO_2 \cdot HCl$	C, H, Cl, N	6.8 (± 0.3)	675	[675]	I
30		166–167	EtOAc	SCH IV	76	$C_{24}H_{25}NO_3$	C, H, N	5.3	[675]	[1013]	I
82		181–182 dec	M	A[q]	20	$C_{23}H_{23}NO_5$	C, H, N	6.4	400	450	+20
39	$-CH_2CH_2NHC(CH_3)(CH_2OH)_2$	~220 dec[r]	E-EE	A, SCH VI	51	$C_{23}H_{25}NO_2 \cdot HCl$	C, H, Cl, N	7.5 (± 0.4)	67.5	(100)	I

[a] All amine starting materials are *dl* (where possible) unless otherwise stated. [b] Melting points are uncorrected. Most compounds darkened when heated and decomposed (dec) at the melting point. [c] Abbreviations for recrystallization solvents are as follows: M = methanol, E = ethanol, EE = diethyl ether, H = hexane, I = 2-propanol, EtOAc = ethyl acetate. Where two solvents are shown, the compound was dissolved in the first solvent and diluted with the second solvent. [d] Preparations are indicated by General Methods A, B, or the scheme indicated in the text. [e] Yields indicated are for the reductive amination step or, if part of a scheme, the last step. [f] Elements analyzing within ± 0.4% of calculated value. [g] See Experimental Section for a description of the procedure. No standard deviation is shown if only one experiment has been done. [h] Lit.[6] mp 244–250 °C. [i] Values in parentheses represent the highest nontoxic doses used in the assay. [j] I = inactive. %ILS < 20. [k] The synthesis of the free base has been reported (ref 5). [l] Values in brackets represent the highest dose tested; no toxicity was observed in the test animals. [m] Starting amine had the *l* configuration. [n] Starting amine had the *d* configuration. [o] Starting amine had the 1-*S*, 2-*S* configuration. [p] Starting material had the 2-*R**,3-*S** relative configuration. [q] Glacial HOAc was used instead of HCl (g) in absolute EtOH in order to decrease the possibility of over reduction of the glucosamine. [r] Compound melts at 190–192 °C, resolidifies, and then gradually decomposes up to 220 °C.

chain also are inactive (e.g. **72** and **73**).

Optimal antitumor activity is seen for those congeners of general structure **2**. The side chain on these secondary amines (**13–17**, **21–23**, **30–32**, and **80–82**) contains two OH groups each of which is two carbon atoms away from the NH group. These compounds are derivatives of 2-amino-1,3-propanediol. The arrangement of the atoms in the side chain is critical as seen by the low activity of **79** (a 1-amino-2,3-propanediol) compared to **80**. Protection of these OH groups (e.g. by acetylation) leads to compounds with low solubility and little antitumor activity (**24**, **25**, and **26**). Similarly, those 2-amino-1,3-propanediol derivatives containing lipophilic groups (**13–15**, **30**, **32**, and **81**) have low solubility and are relatively nontoxic and inactive.

As seen for 2-amino-1,3-propanediol congeners of general structure **85**, the presence of small R groups (e.g. H (**80**), CH_3 (**21**), CH_2CH_3 (**22**), CH_2OH (**23**)) leads to optimal antitumor activity. This trend is observed for all of the other [(arylmethyl)amino]propanediols that have been synthesized and evaluated to date. Although a significant increase in life span was observed for animals treated with these compounds, long term survivors (≥30-day survivors) were infrequent. However, congeners bearing other aromatic ring systems with the optimized side chain have produced significant proportions of long term survivors. In addition, some of the lipophilic side chains have shown much better antitumor activity if present in a derivative containing a different ring system that has better water solubility.[2]

The absolute requirement that the side chain contain a benzylic secondary amine is seen by the lack of activity of tertiary amine derivatives (e.g. **66** and **42**) as well as the homologue **39**. In addition, in other ring systems where the 2-amino-1,3-propanediol side chain contains a NCH_3 group, no antitumor activity has been seen. A more detailed examination of the antitumor activity seen for the best side chains in a variety of additional ring systems will be presented in the next paper.[2]

Conclusions

In the series of 1-pyrenylmethylamines examined in this work we have explored the relationships among structure, interaction with DNA, and murine antitumor activity.

It is obvious that all of these 1-pyrenylmethylamine derivatives (**7**) bind to some extent to DNA as evidenced by the ΔT_m values observed (3–32 °C). The presence of additional basic amine groups in the side chain enhances DNA binding due to electrostatic interactions. Those compounds containing only a single basic benzylic amine bind similarly to DNA. Only the presence of bulky side chains appears to decrease the DNA interactions in the compounds examined.

Although antitumor activity is seen for (1-pyrenylmethyl)amino alcohols (**1**), useful antitumor activity in the series is limited to those congeners bearing the 2-amino-1,3-propanediol-type side chain (**2**). These derivatives bind moderately to DNA (ΔT_m = 9–12 °C). Thus, DNA binding is a necessary but not sufficient criterion for antitumor activity in the series. In addition, the strength of DNA binding does not correlate with the antitumor activity in the group of active compounds. The same observations have been made for the large number of other ring systems studied.[2]

Experimental Section

General Comments. All solvents were reagent grade and used without further purification with the following exceptions. THF was dried by distillation from Na/K alloy under N_2 and used immediately. $PhCH_3$ was distilled from CaH_2 under N_2 and stored over 3-Å molecular sieves. Chemicals used were reagent grade and used without further purification unless noted. The amines used to produce compounds via Methods A and B and Scheme II were purchased from Aldrich Chemical Co., Inc. with the following exceptions; the amines for **20** and **43** from Eastman; for **48**, **51**, and **56** from Fluka; for **52** and **12** from Jefferson Chemicals, Inc.; for **70** and **77** from ROC/RIC; for **58** from P&B; for **62** from K&K Chemicals; for **73** from CPL; for **74** from TWO Chemical Co.; for **80** from Sigma Chemical Co.[11] The preparation of the amines used to produce **13–17** will be discussed in a separate paper.[2]

Silica gel used for flash chromatography was Merck & Co., Inc. silica gel 60, 230–400 mesh. An appropriate volume sintered glass funnel was filled approximately $^3/_4$ full with the silica gel and packed evenly by tapping the outside of the funnel. A piece of filter paper was then placed on top of the silica gel and a solution of the material to be purified was applied evenly to the top of the plug. Gentle suction through a filter flask moved the eluting solvent through the plug rapidly. The appropriate size fractions were combined as needed and further manipulated.

NMR (^1H, ^{13}C), IR, and MS data of all products were consistent with the expected and proposed structures. Elemental analyses were ±0.4% of the calculated value for all compounds except where noted. All final products were dried in a vacuum oven at 15–20 mmHg pressure at 80–90 °C overnight.

1-Pyrenylmethanol (18). To a RB flask equipped with magnetic stirring bar, condenser, and N_2 inlet tube were added **6** (Aldrich, 115.14 g, 0.5 mol) and dry THF (500 mL). To the flask solid $NaBH_4$ (Aldrich, 9.46 g, 0.25 mol) was added in portions over a 10-min period. The reaction was stirred overnight at room temperature. The reaction mixture was then poured into H_2O (4 L) and the resulting white solid was filtered and washed with H_2O (4 × 300 mL). The damp solid was dried in a vacuum oven until the H_2O was removed. The crude material was crystallized from EtOAc, filtered, and dried to give 101.3 g (87%) of pure **18**, mp 123–124 °C (lit.[4] mp 121.5–122.5 °C). Anal. ($C_{17}H_{12}O$) C, H.

1-(Chloromethyl)pyrene (19). To a RB flask equipped with magnetic stirring bar, condenser, pressure equalizing dropping funnel, and N_2 inlet tube were added **18** (23.2 g, 0.1 mol), pyridine (Mallinckrodt, 11.87 g, 0.15 mol, 11.89 mL), and CH_2Cl_2 (500 mL). The mixture was cooled to 0 °C. $SOCl_2$ (MC&B, 17.85 g, 0.15 mol, 10.94 mL) was added to the dropping funnel by syringe and then added dropwise to the flask over 15 min. The mixture was stirred overnight at room temperature and poured into H_2O (1 L), and then further CH_2Cl_2 (500 mL) was added to the flask. The CH_2Cl_2 layer was washed sequentially with 5% $NaHCO_3$ solution (500 mL), saturated NaCl solution (500 mL), dried (Na_2SO_4), and filtered to give a clear, light-green solution. The solvent was removed to give 23.4 g (93%) of crude material, which was used without further purification. A small sample was crystallized from $PhCH_3$–petroleum ether to give pure **19**, mp 147–149 °C (lit.[4] mp 144–145 °C). Anal. ($C_{17}H_{11}Cl$) C, H, Cl.

***N*,*N*-Diethyl-1-pyrenylmethylamine Hydrochloride (20).** To a RB flask equipped with stirring bar, condenser, and N_2 inlet tube were added **19** (10.0 g, 0.04 mol), $HN(CH_2CH_3)_2$ (Eastman, 11.7 g, 0.16 mol, 16.55 mL), and PhH (200 mL). The mixture was refluxed overnight. The white solid that formed was filtered and washed with PhH (200 mL). A solution of 2.5 M (HCl (g) in EtOH was added to the PhH solution until no more precipitate formed. After 15 min the solid was filtered and washed with Et_2O (500 mL). The solid was then crystallized from absolute EtOH, filtered, and dried to give 8.38 g (64%) of **20**, mp 252–254 °C dec (lit.[5] reports the free base). Anal. ($C_{21}H_{22}ClN$) C, H, N, Cl.

1-Pyrenecarbaldehyde Oxime (27). To a RB flask equipped with magnetic stirring bar, pressure equalizing dropping funnel, and condenser were added **6** (Aldrich, 11.51 g, 0.05 mol), a 2 M solution of $NH_2OH·HCl$ in H_2O (50 mL), and 95% EtOH. A 2 M aqueous solution of Na_2CO_3 (50 mL) was placed in the addition funnel and added to the flask over 5 min. The reaction mixture was refluxed for 2 h, poured into H_2O (2 L), and then stirred for

(11) The addresses of the suppliers of these amines as well as other reagent chemicals used in this work can be found in *Chem. Sources-U.S.A.*; Directories Publishing Company, Inc.: Ormand Beach, FL, 1988.

10 min. The light-green solid that formed was filtered, washed with additional H_2O (500 mL), and dried to give 12.01 g of crude solid. This solid was crystallized from 95% EtOH, filtered, and dried to give 8.32 g (68%) of pure **27**, mp 191–192 °C (lit.[5] mp 192.2–194 °C (corrected)). Anal. ($C_{17}H_{11}NO$) C, H, N.

1-Pyrenylmethylamine Hydrochloride (28). To a pressure bottle were added 27 (24.5 g, 0.1 mol), PtO_2 (1 g), and glacial HOAc (350 mL). The bottle was placed in a Parr hydrogenator, pressurized to 55 psi with H_2, and shaken overnight at room temperature. The reaction mixture was filtered through a pad of Celite to remove the catalyst. The clear, pale-green solution was diluted with H_2O and neutralized with solid KOH. The resulting solid was filtered, washed with H_2O, and dried overnight. The HCl salt was made by suspending the crude free base in CH_3OH (1 L) and adding excess 2.5 M HCl (g) in EtOH. The mixture was heated to reflux and filtered to give a clear, slightly yellow solution that was concentrated to volume of 500 mL. The solution was refrigerated overnight. The solid that formed was filtered and crystallized in the same fashion from CH_3OH to give 6.45 g of **28**, mp 261–263 °C dec (lit.[6] reports the HOAc salt, mp 241–250 °C). Anal. ($C_{17}H_{14}ClN$) C, H, N, Cl. Two additional crops (13.49 g and 4.24 g) of **28** were obtained upon concentration of the filtrate to give a combined yield of 90%.

***trans*-2,2-Dimethyl-6-[(1-pyrenylmethyl)amino]-1,3-dioxepan-5-ol (30).** To a RB flask equipped with magnetic stirring bar, condenser, and N_2 inlet tube were added **28** (5.34 g, 0.02 mol), $NaOCH_3$ (MC&B, 1.08 g, 0.02 mol), and CH_3OH (250 mL). To the resulting free base was added 4,4-dimethyl-3,5,8-trioxabicyclo[5.1.0]octane (**29**, 3.60 g, 0.025 mol) prepared by the method of Elliot et al.[7] The mixture was refluxed overnight. The solvent was removed from the mixture to give a solid that was extracted with warm EtOAc (4 × 250 mL). The EtOAc fractions were combined and filtered through a pad of Celite. The clear solution was diluted to 2 L with hexane and the solid that formed was removed by filtration. The solid was crystallized from EtOAc, filtered, and dried to give 5.74 g (77%) of **30**, mp 166–167 °C. Anal. ($C_{24}H_{25}NO_3$) C, H, N.

(2-*RS*,3-*SR*)-3-[(1-Pyrenylmethyl)amino]-1,2,4-butanetriol Hydrochloride (31). To a RB flask were added **30** (9.70 g, 0.0258 mol) and 10% HCl (250 mL). The mixture was stirred for 2 h at room temperature and filtered through a medium-porosity sintered-glass funnel. The solid in the frit was washed with an additional 250 mL of 10% HCl. The solution was neutralized with solid KOH, and the resulting solid was filtered, washed with H_2O (3 × 100 mL), and dried. The free base was dissolved in absolute EtOH and treated with 2.5 M HCl (g) in EtOH until no more precipitate formed. The mixture was diluted with Et_2O to 1500 mL, and the resulting solid was filtered. This solid was crystallized from absolute EtOH–Et_2O, filtered, and dried to give 5.52 g (58%) of **31**, mp 166–167 °C. Anal. ($C_{21}H_{22}ClNO_3$) C, H, N, Cl.

1,3-*O*-Isopropylidene-2-methyl-2-[(1-pyrenylmethyl)amino]-1,3-propanediol Hydrochloride (32). To a RB flask equipped with magnetic stirring bar, condenser, and N_2 inlet tube were added **21** (2.0 g, 0.00562 mol), 2,2-dimethoxypropane (Eastman, 30 mL), dry DMF (10 mL), and *p*-toluenesulfonic acid·H_2O (0.05 g). The mixture was refluxed for 1 h, diluted to 1 L with Et_2O, and the resulting solid was filtered to give the crude product. This material was crystallized from absolute EtOH–Et_2O, filtered, and dried to give 1.08 g (49%) of **32**, mp 123–124 °C. Anal. ($C_{24}H_{26}ClNO_2$) C, H, N, Cl.

Diethyl 2-(1-Pyrenylmethylene)malonate (33). To a RB flask equipped with stirring bar, N_2 inlet tube, Dean–Stark trap, and condenser were added 6 (Aldrich, 50.0 g, 0.217 mol), $CH_2(COOEt)_2$ (Baker, 34.76 g, 0.217 mol), piperidine (MC&B, 1.0 mL), glacial HOAc (MC&B, 0.5 mL), and PhH (1500 mL). The mixture was refluxed overnight with a total of 5.7 mL of H_2O collecting in the trap. A yellow solid formed. The mixture was cooled to 10 °C, and the solvent was removed by rotary evaporation. The solid was resuspended in hexane (2 L), filtered, and washed with warm H_2O (3 × 500 mL), sucked semidry on the frit, and placed in a vacuum oven at 80 °C overnight. A total of 68.41 g (ca. 85%) of yellow solid was obtained, mp 112–114 °C, which was one spot by TLC (SiO_2, CH_2Cl_2). A portion of the material was crystallized from CCl_4–hexane to furnish an analytical sample of **33**, mp 114–115 °C (lit.[8] mp 113–114 °C). Anal. ($C_{24}H_{20}O_4$) C, H. The remainder of **33** was used without further purification.

Diethyl 2-(1-Pyrenylmethyl)malonate (34). A solution of **33** (20.0 g, 0.053 mol) in EtOAc (350 mL) was reduced with PtO_2 (0.1 g) using a Parr Hydrogenator at 50 psi overnight. The catalyst was removed by filtration through a Celite pad, and the solvent was removed by rotary evaporation to give 19.49 g (96.9%) of crude product. This and two other batches run on the same scale were combined and crystallized from CCl_4–hexane to give 59.43 g (98%) of pure **34**, mp 78–79 °C. Anal. ($C_{24}H_{22}O_4$) C, H.

2-(1-Pyrenylmethyl)malonic Acid (35). To a suspension of **34** (56.61 g, 0.152 mol) in EtOH (250 mL) was added a solution of 85% KOH (Fisher, 50 g, 0.75 mol) in H_2O (250 mL). The mixture was refluxed overnight (becoming homogeneous after 4 h). The solution was cooled to room temperature and acidified with concentrated HCl to pH = 1. Cold H_2O (1 L) was added to the resulting suspension, and after stirring for 10 min, the solid was filtered and washed with H_2O (3 × 500 mL). The damp solid was dissolved in EtOAc (2 L), dried (Na_2SO_4), filtered, and decolorized with Norit and filtered through a Celite pad, and the solvent was removed to give, after drying 40.99 g (99%) of crude **35** which was used without further purification. An analytical sample was prepared by crystallization from EtOAc–hexane, mp 190–191.5 °C dec (lit.[8] mp 189–190 °C). Anal. ($C_{20}H_{14}O_4$) C, H.

3-(1-Pyrenyl)propionic Acid (36). A RB flask containing **35** (20.40 g, 0.064 mol) was heated under N_2 in an oil bath maintained at 210 °C for 35 min. The material melted and turned black with loss of CO_2. Upon cooling the material solidified. The solid was dissolved in EtOAc (1.5 L) and decolorized with Norit, filtered through Celite, and concentrated to 300 mL by rotary evaporation. The yellow solution was diluted with hexane (200 mL). The crystals that formed were filtered and crystallized from $PhCH_3$. After filtration and drying, 9.30 g (53%) of **36** was obtained, mp 180–181 °C (lit.[4,9] mp 178–179 °C). Anal. ($C_{19}H_{14}O_2$) C, H. A second crop of **36** (4.36 g, 24%) was obtained upon refrigerating the filtrate.

3-(1-Pyrenyl)propanol (37). A RB flask equipped with magnetic stirring bar, pressure equalizing dropping funnel, condenser, and N_2 inlet tube was flame dried and allowed to cool under N_2. A solution of **36** (Aldrich, 29.0 g, 0.106 mol) in dry THF (300 mL) was added to the flask by cannula. A solution of B_2H_6 in THF (1.0 M, 159 mL) was also added by cannula to the dropping funnel. The flask was cooled to 0 °C with an ice bath and the B_2H_6–THF solution was added dropwise at a rate that did not elevate the reaction mixture temperature above 20 °C. The mixture was then stirred at room temperature overnight. The reaction was incomplete after 20 h, so an additional portion of B_2H_6 (20 mL) was added by syringe to the flask, and the mixture was stirred an additional 6 h. The unreacted B_2H_6 was quenched with CH_3OH (20 mL), and the mixture was allowed to stir overnight. The solvent was then removed by rotary evaporation and the crude material was stirred with CH_3OH (300 mL) to give a yellow solid. This solid was purified by flash chromatography with EtOAc as the eluting solvent to give 20.6 g (75%) of a pale yellow solid. The crude **37** gave the expected NMR, but attempts to crystallize it failed due to decomposition, and it was used without further purification.

3-(1-Pyrenyl)propionaldehyde (38). To an Erlenmeyer flask were added crude **37** (29.93 g, 0.115 mol), PCC (Aldrich, 37.17 g, 0.172 mol), and CH_2Cl_2 (1 L). The mixture was stirred at room temperature for 3 h and filtered through a pad of Celite, and the resulting clear, orange solution was purified by flash chromatography. TLC analysis of the material, a yellow oil that solidified upon standing (12.33 g, ca. 41%), showed mainly the desired aldehyde accompanied by a close running impurity. This aldehyde was used without further purification. A portion of the impure aldehyde was purified by preparative HPLC (Waters Prep 500) by using the recycling technique and after crystallization from CH_2Cl_2–hexane gave pure **38**, mp 72–73.5 °C. Anal. ($C_{19}H_{14}O$) C, H.

2-Methyl-2-[(1-pyrenylmethyl)amino]-1,3-propanediyl Diacetate (24). To a RB flask equipped with stirring bar, condenser, and N_2 inlet tube was added **21** (3.6 g, 0.001 mol), CH_3COCl (Mallinckrodt, 3 mL), and THF (100 mL). The mixture was refluxed overnight. An additional portion of CH_3COCl (7 mL) was added. A solution formed momentarily followed by the formation of a white solid. The mixture was again refluxed

overnight. After cooling, the mixture was poured into a saturated $NaHCO_3$ solution (300 mL) and extracted with EtOAc (2 × 300 mL). The organic layers were combined and washed with additional saturated $NaHCO_3$ (300 mL), saturated (2 × 300 mL), dried (K_2CO_3), and filtered, and the solvent was removed to give the crude product. After crystallization from hexane, 1.95 g (48%) of **24** was obtained, mp 91–92 °C. Anal. ($C_{25}H_{25}NO_4$) C, H, N.

By using the above procedure, **22** gave **25** (hexane), mp 106–107 °C (71%). Anal. ($C_{26}H_{27}NO_4$) C, H, N. Compound **23** gave **26** (EtOAc–hexane), mp 150–150.5 °C (88%). Anal. ($C_{27}H_{27}NO_6$) C, H, N.

General Method A.[3] To a 2-L Erlenmeyer flask were added **6** (Aldrich, 0.1 mol), the amine (0.105 mol), *p*-toluenesulfonic acid·H_2O (0.5 g), and $PhCH_3$ (500 mL). The mixture was heated to reflux until H_2O (2–3 mL) was driven off. In most cases the mixture became homogeneous at this point. After the solution cooled to room temperature, absolute EtOH (500 mL) and $NaBH_3CN$ (Aldrich, 0.05 mol) were added. After the $NaBH_3CN$ dissolved, an indicator (bromocresol green) (0.005 g) was added to the mixture. To the resulting blue solution was added 1 M HCl (g) in absolute EtOH at a rate such that the pH of the solution was in the range of 6–7. After 2–3 days of acid addition the indicator remained yellow in color, and a voluminous precipitate was present in the flask. An additional amount of the HCl (g) in EtOH (10–20 mL) and 2–4 L of Et_2O was added to the flask. The mixture was filtered through a medium-porosity sintered-glass funnel and pressed dry. The crude salt was then recrystallized with use of the solvent(s) indicated in Table I. If the salt was of poor quality it was neutralized in solution and precipitated by further dilution with H_2O. The damp solid was placed in a vacuum oven (80–90 °C) until all the H_2O was removed. The dry solid was suspended in absolute EtOH, CH_3OH, or *i*-PrOH (200 mL), and a solution of 2.5 M HCl (g) in absolute EtOH (10 mL) or CH_3SO_3H (Alfa-Ventron, 99.5%) was added to form the salt. If necessary the mixture was warmed until all of the solid dissolved. The solution was filtered through a medium-porosity sintered-glass funnel and then if necessary diluted with Et_2O, hexane, or $PhCH_3$ to a final volume of ~2 L. The resulting solid was filtered and crystallized further (usually twice) with use of the solvent(s) indicated in Table I and, after filtration, washed with solvent (500 mL). The pure product was then dried in a vacuum oven (80 °C) overnight. In other preparations where the crude free base, which formed upon addition of H_2O, was an oil or a gum, the material was partitioned between EtOAc and H_2O (500 mL of each). The EtOAc layer was washed sequentially with H_2O (2 × 500 mL) and saturated. NaCl (2 × 500 mL), dried (K_2CO_3), and filtered through a medium-porosity sintered-glass funnel. The resulting solution was concentrated by rotary evaporation to give the dry free base. The salt was produced and purified as above. The low yields seen for a number of the examples are due to the poor solubility of **6** in the EtOH–$PhCH_3$ mixture and the fact that the target salts were usually crystallized three times. The yields for the reductive amination reaction are much higher when General Method B is used.

General Method B. To a RB flask equipped with overhead stirrer, condenser, thermometer, and Dean–Stark trap were added **6** (Aldrich, 0.05 mol), the amine (0.1 mol), *p*-toluenesulfonic acid·H_2O (Aldrich, 0.05 g), and $PhCH_3$ (300 mL). The mixture was stirred at reflux with removal of H_2O for 2 h (or until H_2O no longer distills into the trap) and about 200 mL of the $PhCH_3$ was removed by distillation. The mixture was cooled and diluted with absolute EtOH (200 mL) and further cooled to 0 °C with an ice bath. Solid $NaBH_4$ (MC&B, 0.05 mol) was added in portions to the stirred mixture with the temperature kept below 20 °C by external cooling. After the addition was completed, the reaction was stirred overnight at room temperature. In the morning the solvent was removed by rotary evaporation and the crude reaction mixture was shaken with warm H_2O (500 mL). The mixture was allowed to stand for about 1 h and the resulting solid filtered and washed further with H_2O (2 × 500 mL). The H_2O washes serve to remove the excess starting amine as well as any inorganic material. The damp solid was placed in a vacuum oven (80 °C) until all the H_2O was removed. The salt of the crude free base was made and purified as described in Method A. In general the crude yields of the free bases were greater than 80%. After salt formation and crystallization, the isolated yields of the target AMAPs were 25–90% with an average yield of greater than 50%.

Thermal Denaturation Studies. Thermal denaturation experiments were done by the method of Cory et al.[10] with use of a Varian Cary 2290 UV–vis recording spectrophotometer with a five-cell temperature-controlled turret. The solution used contains 5.0 mM Tris·HCl buffer at pH 7.55, 50 μM calf thymus DNA phosphate (Worthington, average molecular weight 1.2 × 10^6 D), 5 μM of drug, and 5% DMSO. Previous studies have shown that this concentration of DMSO does not appreciably affect the binding or ΔT_m values of drugs, and only a slight decrease in T_m is seen for calf thymus DNA alone. Control experiments performed in the absence of drug were used for all ΔT_m values reported. Absorbance and temperature readings were recorded once every 2 min while the temperature in the cells was increased at a constant rate of 18 °C/h. Under these conditions the average T_m value for calf thymus DNA was 58.1 °C (n = 40, SD = 1.5 °C). The ΔT_m of control drugs [quinacrine (24.9 °C) and ethidium bromide (13.0 °C)] and the T_m of calf thymus DNA were used to monitor the quality of these experiments.

DNA. Calf thymus DNA (Worthington) used for T_m studies was dissolved in buffer containing 0.15 M NaCl and 0.015 M sodium citrate at pH 7.0 to a final concentration of 3 mg/mL and sonicated as described by Davidson et al.[12] Residual protein and peptides were removed by extraction first with a mixture of $CHCl_3$ and amyl alcohol (24:1), followed by phenol. The aqueous DNA solution was then passed through a Dowex/Sephadex column with use of the same buffer solution as eluent. The center portions of the eluted solution were pooled and used for subsequent studies. An average molecular weight of 1.2 × 10^6 Da, determined by viscometric analysis as described by Godfrey[13] and verified by agarose gel electrophoresis, was obtained for DNA processed in this manner. Calf thymus DNA concentrations were determined by using an extinction coefficient of 6600 M at 260 nm and are expressed as nucleotide equivalents/liter. The purified, sonicated DNA samples displayed a A_{260}/A_{280} ratio of 1.85–1.90 and a total hyperchromicity of 30%. The DNA contained less than 0.5% residual RNA as determined by the method of Savitsky and Stand.[14]

Murine Lymphocytic Leukemia P388 Screen. Preliminary antitumor evaluation of the compounds was done with use of murine lymphocytic leukemia P388 and methods used in the Tumor Panel by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.[15] Modifications in the selection and number of doses used have been made to increase test efficiency.

Male CD2-F_1 mice weighing 20 ± 3 g were used for the test. Control and test animals were injected intraperitoneally with a suspension of 1 × 10^6 viable P388 tumor cells on day 0. In each test, several dose levels that bracketed the LD_{20} of the compound were evaluated with use of six animals per dose level.[16] The test compounds were prepared as aqueous suspensions or solutions containing 0.9% NaCl or 5% dextrose and administered intraperitoneally on days 1, 5, and 9, following tumor implant. The doses were administered to each animal on a milligram/kilogram basis individually, according to body weight. The day of death for each animal in the treated and control groups was recorded and the median day of death (MDD) determined for each group. The effect of the drug at each dose was measured as %ILS (% Increase in Life Span), which was calculated by using the following equation:

(12) Davidson, M. W.; Griggs, B. G.; Boykin, D. W.; Wilson, D. W. *J. Med. Chem.* **1977**, *20*, 1117.
(13) Godfrey, J. E. *Biophys. Chem.* **1976**, *5*, 285.
(14) Savitsky, J. P.; Stand, F. *Nature (London)* **1965**, *207*, 758.
(15) Goldin, A. et al. *Methods in Cancer Research*; Academic Press: New York, 1979; Vol. XVI, p 165.
(16) After the LD_{20} was determined, antitumor screening was performed under contract by Southern Research Institute, 2000 Ninth Avenue South, P.O. Box 55305, Birmingham, AL 35255-5305. We acknowledge the consultation of the late Frank Schabel Jr. and the assistance of W. Russell Laster, Jr., the late Mary Trader, and Daniel P. Griswold in the testing of these compounds.

$$\%\,\mathrm{ILS} = \frac{\mathrm{MDD\ (treated\ animals)} - \mathrm{MDD\ (control\ animals)}}{\mathrm{MDD\ (control\ animals)}} \times 100\%$$

The results indicated in Table I are optimal values for at least two separate experiments. Animal deaths occuring before the median day of death of the control group animals were considered to be due to drug toxicity.

Registry No. **6**, 3029-19-4; **8**, 127856-48-8; **8** (free base), 127856-49-9; **9**, 127856-50-2; **9** (free base), 127856-51-3; **10**, 127856-53-5; **10** (free base), 127856-52-4; **11**, 127856-54-6; **11** (free base), 127856-55-7; **12**, 127856-56-8; **12** (free base), 127856-57-9; **13**, 127856-58-0; **13** (free base), 127856-59-1; **14**, 96403-75-7; **14** (free base), 96403-76-8; **15**, 96403-17-7; **15** (free base), 127856-60-4; **16**, 96403-71-3; **16** (free base), 96403-72-4; **17**, 127856-62-6; **17** (free base), 127856-61-5; **18**, 24463-15-8; **19**, 1086-00-6; **20**, 95948-96-2; **20** (free base), 3712-78-5; **21**, 96403-91-7; **21** (free base), 96403-92-8; **22**, 96403-99-5; **22** (free base), 96404-00-1; **23**, 96404-10-3; **23** (free base), 96404-11-4; **24**, 96422-29-6; **25**, 96404-38-5; **26**, 96404-37-4; **27**, 3786-56-9; **28**, 93324-65-3; **28** (free base), 3786-54-7; **29**, 100572-41-6; **30**, 127856-63-7; **31**, 127856-64-8; **31** (free base), 127856-65-9; **32**, 127856-66-0; **32** (free base), 127856-67-1; **33**, 127856-68-2; **34**, 127856-69-3; **35**, 4643-67-8; **36**, 61098-93-9; **37**, 61098-94-0; **38**, 127880-45-9; **39**, 127856-70-6; **39** (free base), 127856-71-7; **40**, 127856-72-8; **40** (free base), 127856-73-9; **41**, 127856-74-0; **41** (free base), 127856-75-1; **42**, 127856-76-2; **42** (free base), 3712-79-6; **43**, 127856-77-3; **43** (free base), 127856-78-4; **44**, 127856-79-5; **44** (free base), 127856-80-8; **45**, 127856-81-9; **45** (free base), 127856-82-0; **46**, 127856-83-1; **46** (free base), 127856-84-2; **47**, 127856-85-3; **47** (free base), 127856-86-4; **48**, 127856-87-5; **48** (free base), 127856-88-6; **49**, 127856-89-7; **49** (free base), 127856-90-0; **50**, 127856-91-1; **50** (free base), 127856-92-2; **51**, 127856-93-3; **51** (free base), 127856-94-4; **52**, 127856-95-5; **52** (free base), 127856-96-6; **53**, 127856-97-7; **53** (free base), 127856-98-8; **54**, 127856-99-9; **54** (free base), 127857-00-5; **55**, 127857-01-6; **55** (free base), 127857-02-7; **56**, 127857-03-8; **56** (free base), 127857-04-9; **57**, 127857-05-0; **57** (free base), 127857-06-1; **58**, 127857-07-2; **58** (free base), 127857-08-3; **59**, 127857-09-4; **59** (free base), 127857-10-7; **60**, 96404-16-9; **60** (free base), 96404-17-0; **61**, 96403-97-3; **61** (free base), 96403-98-4; **62**, 127857-11-8; **62** (free base), 127857-12-9; **63**, 127857-13-0; **63** (free base), 127857-14-1; **64**, 127857-15-2; **64** (free base), 127857-16-3; **65**, 96404-05-6; **65** (free base), 96404-06-7; **66**, 96404-08-9; **66** (free base), 96404-09-0; **67**, 96422-34-3; **67** (free base), 96403-96-2; **68**, 96404-14-7; **68** (free base), 96404-15-8; **69**, 127857-17-4; **69** (free base), 127857-18-5; **70**, 127857-19-6; **70** (free base), 127857-20-9; **71**, 127857-21-0; **71** (free base), 127857-22-1; **72**, 127857-23-2; **72** (free base), 127857-24-3; **73**, 127857-25-4; **73** (free base), 127857-26-5; **74**, 127857-27-6; **74** (free base), 127857-28-7; **75**, 127857-29-8; **75** (free base), 127857-30-1; **76**, 127857-31-2; **76** (free base), 127857-32-3; **77**, 127857-33-4; **77** (free base), 127857-34-5; **78**, 127857-35-6; **78** (free base), 127857-36-7; **79**, 127857-37-8; **79** (free base), 127857-38-9; **80**, 96404-12-5; **80** (free base), 96404-13-6; **81**, 127857-39-0; **81** (free base), 127857-40-3; **82**, 127857-41-4; $CH_2(COOEt)_2$, 105-53-3; $H_2NC(CH_3)(CH_2OH)_2$, 115-69-5; $H_2N(CH_2)_2CN$, 151-18-8; $H_2N(CH_2)_2OMe$, 109-85-3; $HN(CH_2CH_2OH)_2$, 111-42-2; H_2NCH_2Ph, 100-46-9; $H_2N(CH_2)_2Ph$, 64-04-0; $H_2N(CH_2)_3Ph$, 2038-57-5; $H_2N(CH_2)_4Ph$, 13214-66-9; 3,4-$ClC_6H_4CH_2NH_2$, 102-49-8; $H_2N(CH_2)_3OPr$-*i*, 2906-12-9; $H_2NCH_2CH{=}CH_2$, 107-11-9; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_5NH_2$, 462-94-2; $H_2N(CH_2)_6NH_2$, 124-09-4; $H_2N(CH_2)_2NHPh$, 1664-40-0; $H_2N(CH_2)_3N(CH_3)_2$, 109-55-7; $H_2N(CH_2)_3N(CH_2CH_2)_2O$, 123-00-2; $H_2NCH(CH_3)(CH_2)_3N(CH_2CH_3)_2$, 67459-49-8; $H_2N(CH_2)_2N(CH_2CH_3)_2$, 100-36-7; $H_2N(CH_2)_3NHCH(CH_2CH_2)_2CH_2$, 3312-60-5; $H_2NCH_2CH_2NHCH_2CH_2OH$, 111-41-1; $H_2NCH_2CH_2NHCH_2CH(CH_3)OH$, 121565-46-6; $H_2N(CH_2)_3N(CH_2CH_2OH)_2$, 4985-85-7; $H_2N(CH_2)_3N(CH_3)(CH_2)_3NH_2$, 105-83-9; $H_2N(CH_2)_3N(CH_2CH_2)_2N(CH_2)_3NH_2$, 7209-38-3; $H_2NCH_2CH_2OH$, 141-43-5; $H_2N(CH_2)_3OH$, 156-87-6; $H_2N(CH_2)_4OH$, 13325-10-5; $H_2N(CH_2)_5OH$, 2508-29-4; $H_2NCH(CH_3)CH_2OH$, 6168-72-5; $H_2NCH_2CH(CH_3)OH$, 1674-56-2; $MeNHCH_2CH_2OH$, 109-83-1; $H_2NC(CH_3)_2CH_2OH$, 124-68-5; $H_2NCH_2C(CH_3)_2OH$, 2854-16-2; $H_2NCH(i\text{-}Pr)CH_2OH$, 2026-48-4; $H_2NCH(CH_2\text{-}i\text{-}Pr)CH_2OH$, 16369-17-8; $H_2NCH(CH(CH_3)CH_2CH_3)CH_2OH$, 24629-25-2; $H_2NCH_2CH_2CH(CH_3)OH$, 127912-49-6; $H_2N(CH_2)_3C(CH_3)_2CH_2OH$, 13532-77-9; $H_2NCH_2CH(CH_2CH_3)OH$, 13552-32-4; $H_2NCH(CH_2CH_3)CH_2OH$, 5856-63-3; $H_2NCH(Ph)CH_2OH$, 71006-16-1; $H_2NCH_2CH(Ph)OH$, 1936-63-6; $H_2NCH(CH_3)(CH(Ph)OH)$, 48115-38-4; $H_2NCH_2CH(OH)CH_2OH$, 13552-31-3; $H_2NCH(CH_2OH)_2$, 534-03-2; $H_2NC(CH_2CH_3)(CH_2OH)_2$, 115-70-8; $H_2NC(CH_2OH)_3$, 77-86-1; $H_2NC(n\text{-pentyl})(CH_2OH)_2$, 95051-23-3; $H_2NC(CH_2OCH_2CH_3)(CH_2OH)_2$, 96422-30-9; $H_2NC(i\text{-}Pr)(CH_2OH)_2$, 60204-51-5; $H_2NCH(CH_2OH)(CH(Ph)OH)$, 28143-91-1; $H_2NC(CH_3)(CH(CH_3)OH)(CH_2OH)$, 96403-70-2; $H_2NC(CH_3)(CH_2OCH_3)(CH_2OH)$, 127857-42-5; glucosamine, 3416-24-8.

Novel Agents Effective against Solid Tumors: The Diarylsulfonylureas. Synthesis, Activities, and Analysis of Quantitative Structure–Activity Relationships

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A series of diarylsulfonylureas with exceptionally broad-spectrum activity against syngeneic rodent solid tumors in vivo is described. Their discovery resulted from a program dedicated to in vivo screening for novel oncolytics in solid tumor models, rather than traditional ascites leukemia models. The structures, oral efficacy, side-effect profile, and mechanism of action of these sulfonylureas appear to be distinct from previously known classes of oncolytics. An extensive series of analogues was prepared to probe structure–activity relationships (SAR), with particular focus on the substituent patterns of each aryl domain. Quantitative analysis of these substituent SARs, using the method of cluster significance analysis, showed the lipophilicity of the substituents to be the dominant determinant of activity. One compound from the series, LY186641 (**104**, sulofenur), has progressed to Phase I clinical trials as an antitumor drug.

Introduction

The chemotherapy of neoplastic disease has made impressive advances since the first clinically active regimens were introduced in the 1940s,[1] so that today chemotherapy constitutes front-line therapy for approximately 25% of all cancer patients. The advent of truly curative chemotherapy in the mid-1960s[2] generated continuing hope that most, if not all, of the diverse spectrum of clinical tumors would eventually be curable. Unfortunately, in terms of

(1) Gilman, A.; Philips, F. S. *Science* **1946**, *103*, 409. Gilman, A. *Am. J. Surg.* **1963**, *105*, 574.

(2) Hertz, R. *Ann. Intern. Med.* **1963**, *59*, 931.

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