2-[(Arylmethyl)amino]-2-methyl-1,3-propanediol DNA Intercalators. An Examination of the Effects of Aromatic Ring Variation on Antitumor Activity and DNA Binding

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The effects of variation of aromatic ring size, shape, and side-chain position on antitumor activity and DNA binding in a series of carbocyclic 2-[(arylmethyl)amino]-2-methyl-1,3-propanediols (AMAPs) were examined. In general, the interaction of AMAPs with DNA increases as the intercalating ring system grows in area, with three distinct binding levels evident. Isomers from a specific ring system appear to bind DNA similarly. DNA binding is not the sole criterion for antitumor activity for the AMAPs studied; the magnitude of the ΔT_m does not correlate with the antitumor activity observed. Significant in vivo P388 activity was seen for AMAP congeners from several tetracyclic ring systems. However, isomers from each of the specific ring systems produced a wide range of in vivo P388 activity. Thus, AMAP antitumor activity is not a function of the ring system per se, but rather appears to be related to the shape of the specific molecule. Three AMAP congeners (crisnatol (770U82, 773U82, and 502U83) are currently in clinical trials.

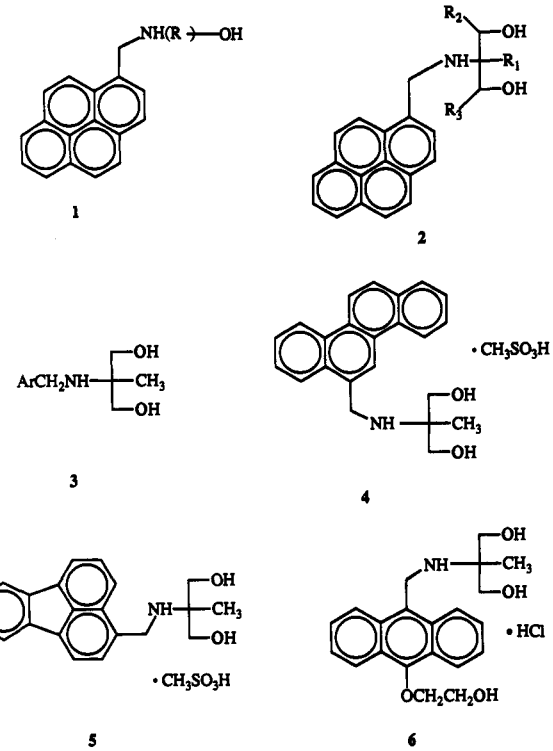
In the preceeding paper,[1] the effect of side-chain variation on antitumor activity in a series of [(1-pyrenylmethyl)amino]alcohol derivatives (1) was examined: optimal antitumor activity was seen for 2-[(1-pyrenylmethyl)amino]propanediols of general structure 2.

In order to evaluate the effects of aromatic ring size, shape, and side-chain position in the AMAP series on antitumor activity and DNA binding, a number of carbocyclic aromatic derivatives containing the same optimized side chain have been synthesized. In this second paper, the murine P388 lymphocytic leukemia activity and DNA binding of these compounds (general structure 3, 2-[(arylmethyl)amino]-2-methyl-1,3-propanediols, AMAPs) are examined. The names, structures, and numbering of the ring systems studied in this work are shown in Scheme I.

Following initial evaluation in the P388 screen, a number of these derivatives and additional in vivo and in vitro studies, three of the congeners were selected for further development. These compounds, 4 (crisnatol, 770U82), 5 (773U82), and 6 (502U83), are currently in clinical trials.

Chemistry

The target AMAP derivatives were prepared by reductive amination of ArCHOs with commercially available $NH_2C(CH_3)(CH_2OH)_2$ using $NaBH_4$[2] or $NaBH_3CN$[3] (Scheme II, reactions m or n, respectively) or by alkylation of $ArCH_2X$ derivatives with $NH_2C(CH_3)(CH_2OH)_2$ (Scheme II, reaction p). In turn, a number of ArCHO intermediates were prepared by formylation (Scheme II, reactions k[4] or l[5]) of ArHs or by refunctionalization of $ArCOCH_3$, ArCOOH, ArCOOR, ArCOCl, or $ArCH_2OH$ derivatives (Scheme II, reactions a,[6] b,[7] c,[8] d,[9] e,[10] f,[11] h,[12] i,[13] or j[14]). The reaction sequences used to prepare each of the ArCHOs are listed in Table I. $ArCH_2X$ derivatives were prepared from the corresponding $ArCH_3$ or $ArCH_2OH$ derivatives (Scheme II, reactions o or g, respectively). The synthesis of the target compounds was facilitated by the large number of commercially available ArH, ArCHO, $ArCOCH_3$, ArCOOH, ArCOOR, ArCOCl, $ArCH_3$, or $ArCH_2OH$ intermediates. The sources of these compounds are listed in the Experimental Section. In addition, formylation of some of the ArH intermediates produced several ArCHO isomers which could be separated by chromatography and/or crystallization and used to produce isomeric target compounds. Formylation of partially reduced ring



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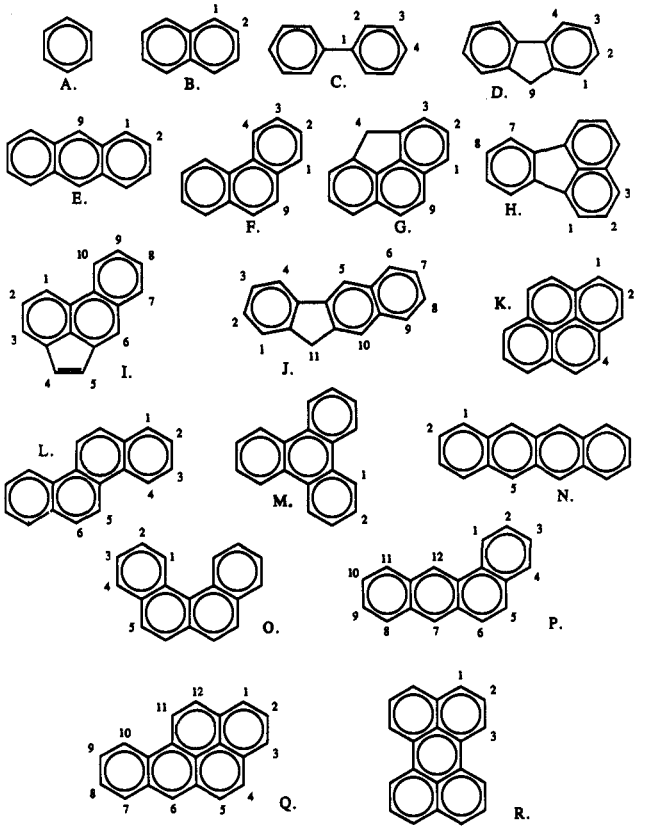
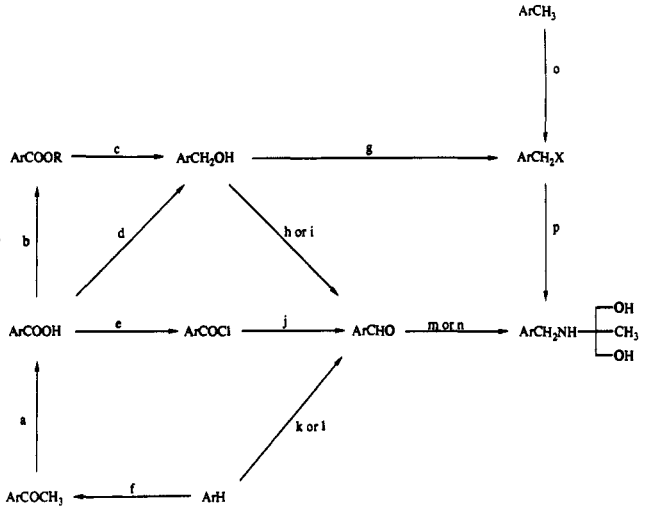
Scheme I



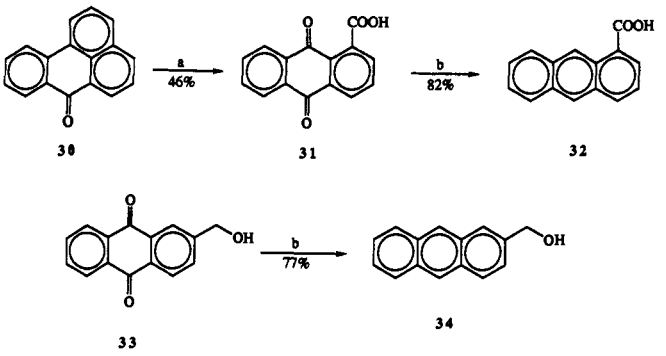
Scheme II[a]



[a] Reagents and conditions: (a) NaOCl, NaOH, H_2O, Δ; (b) R = CH_3, Et; CH_3OH or EtOH, cat. H_2SO_4, Δ (−H_2O); (c) $LiBH_4$, THF, Δ; (d) BH_3, THF, 0 °C, Δ; (e) $SOCl_2$, Δ; (f) $AlCl_3$, CH_3COCl, $PhNO_2$, 0 °C to room temperature; (g) $SOCl_2$, $PhCH_3$, Δ; (h) PCC, CH_2Cl_2, Δ; (i) $BaMnO_4$, CH_2Cl_2, Δ; (j) $Cu(PPh_3)_2BH_4$, acetone, room temperature; (k) $SnCl_4$, Cl_2CHOCH_3, CH_2Cl_2 or *o*-DCB, 0 °C to room temperature or 0 °C, Δ; (l) $POCl_3$, $PhN(CH_3)CHO$, CH_2Cl_2 or *o*-DCB, 0 °C to room temperature or 0 °C, Δ; (m) (1) $NH_2C(CH_3)(CH_2OH)_2$, TosOH, $PhCH_3$, Δ (−H_2O); (2) $NaBH_4$, EtOH, 0 °C to room temperature; (n) $NH_2C(CH_3)(CH_2OH)_2$, $NaBH_3CN$, HCl(g)/EtOH, $PhCH_3$, EtOH; (o) NBS, CCl_4, cat. AIBN or $(PhCOO)_2$, Δ; (p) X = Cl or Br; $NH_2C(CH_3)(CH_2OH)_2$, K_2CO_3, cat. KI, EtOH, Δ.

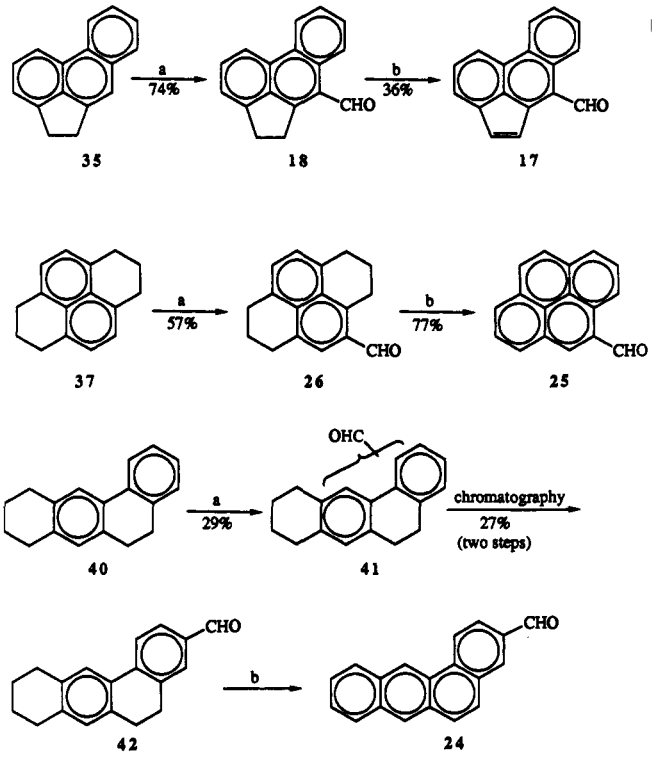
systems followed by dehydrogenation produced further isomeric ArCHOs not available from the other reactions. $ArCH_3$s were synthesized from smaller ring systems using

Scheme III[a]



[a] Reagents and conditions: (a) CrO_3, HOAc, Δ; (b) (1) Zn, NH_4OH, Δ; (2) *i*-PrOH, concentrated HCl, Δ.

Scheme IV[a]



[a] Reagents and conditions: (a) $SnCl_4$, Cl_2CHOCH_3, CH_2Cl_2 or *o*-DCB, 0 °C to room temperature or 0 °C, Δ; (b) DDQ, $PhCH_3$, Δ.

annellation methods in order to produce additional isomers not available from electrophilic reactions of the above ring systems.

Some additional reactions used to synthesize intermediates that were converted to target ArCHOs directly or by further employing the reactions shown in Scheme II are shown in Schemes III and IV. Both 1- and 2-anthracenecarbaldehyde (**8** and **9**, respectively) were prepared via the corresponding anthraquinone derivatives (**31** and **32**, respectively, Scheme III). Benzanthrone (**30**) was oxidized (CrO_3/HOAc/Δ) to 9,10-dihydro-9,10-dioxo-1-anthracenecarboxylic acid (**31**).[15] Reduction of **31** [(1) Zn/NH_4OH/Δ; (2) concentrated HCl/Δ] gave the acid **32**,[16] which was then converted to the aldehyde **8** (Scheme II, reactions d, h). Reduction of **33** using the same con-

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Table I. Physical Properties of ArCHO Intermediates

no.	Ar	ring system (Scheme I)	mp,[a] °C	recryst solvent[b]	method[c]	% yield	formula	analysis[d]
7	4-phenyl-1-naphthalenyl	B	60–75	e	Sch II-k	63	$C_{17}H_{12}O$	C, H
8	1-anthracenyl	E	130–131.5[f]	T/H	Sch III; Sch II-d,h	67	$C_{15}H_{10}O$	C, H
9	2-anthracenyl	E	201–202.5[g]	T/M	Sch III; Sch II-h	51	$C_{15}H_{10}$	C, H
10	2-phenanthrenyl	F	58–60[h]	e	Sch II-b,e,j	25	$C_{15}H_{10}O$	C, H
11	3-phenanthrenyl	F	78–80[i]	e	Sch II-b,e,j	58	$C_{15}H_{10}O$	C, H
12	4-phenanthrenyl	F	82.5–84.5[j]	M/T	Sch II-h	71	$C_{15}H_{10}O$	C, H
13	1-cyclopenta[*def*]phenanthrenyl	G	112–113.5	H	Sch II-k	54	$C_{16}H_{10}O$	C, H
14	3-fluoranthenyl	H	103.5–104.5[k]	DCM/H	Sch II-k, exp[l]	61	$C_{17}H_{10}O$	C, H
15	7-fluoranthenyl	H	139–141	DCM/H	Sch II-k, exp	2	$C_{17}H_{10}O$	C, H
16	8-fluoranthenyl	H	91.5-93	DCM/H	Sch II-k, exp	22	$C_{17}H_{10}O$	C, H
17	6-acephenanthrenyl	I	161–163	T/H	Sch IV, exp	36	$C_{17}H_{10}O$	C, H
18	4,5-dihydro-6-acephenanthrenyl	I	157–158[m]	T	Sch IV	95	$C_{17}H_{12}O$	C, H
19	5-(11*H*-benzo[*b*]fluorenyl)	J	104.5–106.5	DCM/H	Sch II-k	86	$C_{18}H_{12}O$	C, H
20	2-chrysenyl	L	215–216.5	T	Sch II-f,b,a,c,i, exp	88	$C_{19}H_{12}O$	C, H
21	3-chrysenyl	L	177–177.5	T	Sch II-f,b,a,c,i, exp	89	$C_{19}H_{12}O$	C, H
22	6-chrysenyl	L	167–169[n]	T	Sch II-k	80	$C_{19}H_{12}O$	C, H
23	2-triphenylenyl	M	160–161.5	DCM/M	Sch II-k	25	$C_{19}H_{12}O$	C, H
24	3-benz[*a*]anthracenyl	P	144–145	T/H	Sch IV, exp	29	$C_{19}H_{12}O$	C, H
25	4-pyrenyl	K	172–174[o]	DCM/H	Sch IV	77	$C_{17}H_{10}O$	C, H
26	1,2,3,6,7,8-hexahydro-4-pyrenyl	K	102.5–105[p]	T/H	Sch IV	57	$C_{17}H_{16}O$	C, H
27	5-naphthacenyl	N	163–164[q]	DCM/EA	Sch II-k	31	$C_{19}H_{12}O$	C, H
28	5-benzo[*c*]phenanthrenyl	O	133–134	DCM/H	Sch II-k	74	$C_{19}H_{12}O$	C, H
29	3-perylenyl	R	232–234[r]	M	Sch II-k	83	$C_{21}H_{12}O$	C, H

[a] Melting points are uncorrected. [b] Abbreviations for recrystallization solvents are M = CH_3OH, H = hexane, EA = EtOAc, T = $PhCH_3$, DCM = CH_2Cl_2. Where two solvents are shown, the compound was dissolved in the first solvent and diluted with the second solvent. [c] Methods of preparation are indicated by the schemes and reaction steps indicated in the text. [d] Elements analyzing within ±0.4% of calculated value. [e] Isolated directly after HPLC. [f] Lit.[21] mp 126.5–127.5 °C. [g] Lit.[21] mp 202–203 °C. [h] Lit.[10] mp 59–59.5 °C. [i] Lit.[10] mp 79.5–80 °C. [j] Lit.[22] mp 82–84 °C. [k] Lit.[23] mp 98–99 °C. [l] Procedure or sequence described in the Experimental Section. [m] Lit.[24] mp 161.5 °C. [n] Lit.[25] mp 168 °C. [o] Lit.[26] mp 177–179 °C. [p] Lit.[27] mp 116 °C. [q] Lit.[28] mp 164 °C. [r] Lit.[29] mp 236 °C.

ditions gave **34** (Scheme III). Oxidation of **34** gave the aldehyde **9** (Scheme II, reaction h).

The aldehydes **17**, **24**, and **25** were prepared by formylation of the corresponding reduced hydrocarbons followed by dehydrogenation (Scheme IV). Formylation of 4,5-dihydroacephenanthrene (**35**) using $SnCl_4$/Cl_2CHOCH_3/CH_2Cl_2/0 °C to room temperature (the same reagents used in Scheme II, reaction k) gave the aldehyde **18**. Dehydrogenation of **18** (DDQ/$PhCH_3$/Δ) gave the aldehyde **17**.[17] Formylation of acephenanthrene (**36**) itself with the same reagents gives a complex mixture of aldehydes.[18] Similarly, 1,2,3,6,7,8-hexahydropyrene (**37**) gave the aldehyde **26**, which upon dehydration gave **25**. Formylation of pyrene (**38**) with Vilsmeier reagent gives only 1-pyrenecarbaldehyde (**39**).[19] Formylation of 5,6,8,9,10,11-hexahydrobenz[*a*]anthracene (**40**) gave a complex mixture of 5,6,8,9,10,11-hexahydrobenz[*a*]anthracene aldehydes (**41**). Chromatography of this mixture provided a fraction containing 5,6,8,9,10,11-hexahydrobenz[*a*]anthracene-3-carbaldehyde (**42**), which was dehydrogenated to give the aldehyde **24**. Formylation of benz[*a*]anthracene (**43**) using Vilsmeier reagent gives only benz[*a*]anthracene-7-carbaldehyde (**44**).[20]

In order to produce significant amounts of several isomers, chrysene (**45**) was acetylated using $AlCl_3$/CH_3COCl in $PhNO_2$ (0 °C to room temperature).[11] The crude acetylated mixture (**46**) contained mainly the 2-, 3-, and 6-isomers (**47**, **48**, and **49**, respectively). These were separated by chromatography and crystallization and the crude isomerically pure 2- and 3-isomers (**47** and **48**, respectively) converted to the corresponding aldehydes **20** and **21** (Scheme II, reactions b, a, c, i).

DNA Binding Studies

As discussed in the previous paper,[1] (1-pyrenylmethyl)amino alcohol derivatives (**1**) including 2-[(1-pyrenylmethyl)amino]-2-methyl-1,3-propanediol (**50**) intercalate DNA. Examination of the ΔT_m values observed for the 2-[(arylmethyl)amino]-2-methyl-1,3-propanediol derivatives listed in Table II and additional biophysical data[18] on the AMAP series and a more limited number of $ArCH_2NHCH(CH_3)(CH_2)_3N(CH_2CH_3)_2$ congeners shows that the size and shape of the aromatic ring system are critical determinants of the magnitude of DNA interaction in either series.

Although only limited comparisons can be made, it appears that the interaction between the molecules in Table II and DNA increases as the number of atoms in the ring system (and as a result, the number of rings and surface area) increases. Using a shorthand notation where a benzene ring is represented by C_6, a five-membered ring, as contained in the fluorene, acephenanthrene, cyclopenta[*def*]phenanthrene, fluoranthene, or 11*H*-benzophenanthrene ring systems, is represented by C_5, assuming

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Table II. Physicochemical and Biophysical Properties and Antitumor Activity of $ArCH_2NHC(CH_3)(CH_2OH)_2$ Derivatives

no.	Ar	ring system (Scheme I)	mp,[a] °C	recryst solvent[b]	method[c]	% yield	formula	analysis[d]	ΔT_m,[e] °C	opt dose, mg/kg	LD_{20},[f] mg/kg	% ILS[g]	30 day surv	60 day surv
51	phenyl[h]	A	157–159	E/EE	Sch II-n	60	$C_{11}H_{17}NO_2$·HCl	C, H, Cl, N	0.7	350	(350)	I	–	–
52	1-naphthyl	B	190.5–191.5	E/EE	Sch II-n	59	$C_{15}H_{19}NO_2$·HCl	C, H, Cl, N	1.0 ± 0.2	180	(180)	I	–	–
53	2-naphthyl	B	226–227	E/EE	Sch II-n	48	$C_{15}H_{19}NO_2$·HCl	C, H, Cl, N	0.8 ± 0.2	240	(240)	I	–	–
54	*n*-dodecyl	–	87–88	EA	Sch II-n	67	$C_{16}H_{35}NO_2$·HCl	C, H, Cl, N	0.5 ± 0.1	75	(75)	I	–	–
55	4-biphenylyl	C	171–173	E/EE	Sch II-n	57	$C_{17}H_{21}NO_2$·HCl	C, H, Cl, N	0.8 ± 0.6	283	(283)	I	–	–
56	2-fluorenyl	D	241–243	E/EE	Sch II-n	28	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	1.4 ± 0.1	225	(225)	I	–	–
57	4-phenyl-1-naphthyl	B	240–241 (D)	E/EE	Sch II-n	28	$C_{21}H_{23}NO_2$·HCl	C, H, Cl, N	1.4 ± 0.7	175	(175)	I	–	–
58	1-anthracenyl	E	189–190 (D)	E/EE	Sch II-m	38	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	7.3	120	110	+125	0/6	–
59	2-anthracenyl	E	224–226	E/EE	Sch II-m	63	$C_{19}H_{21}NO_2$·HCl·0.45H_2O	C, H, Cl, N	4.6 ± 0.7	250	(250)	I	–	–
60	9-anthracenyl	E	139–140 (D)	E/EE	Sch II-n	40	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	6.2 ± 0.7	150	150	+30	0/6	–
61	1-phenanthrenyl	F	210–211	E/EE	Sch II-p	24	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	4.9 ± 0.6	230	225	+65	0/6	–
62	2-phenanthrenyl	F	243–245	E/EE	Sch II-n	55	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	4.1 ± 0.3	200	175	+33	0/6	–
63	3-phenanthrenyl	F	209–211	E/EE	Sch II-n	66	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	6.1 ± 0.2	225	250	+20	0/6	–
64	4-phenanthrenyl	F	198–200 (D)	E/EE	Sch II-n	70	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	4 ± 0.4	269	(269)	I	–	–
65	9-phenanthrenyl	F	145–147	E/EE	Sch II-n	36	$C_{19}H_{21}NO_2$·HCl	C, H, Cl, N	7.2 ± 0.2	150	125	+60	0/6	–
66	1-cyclopenta-[*def*]phenanthrenyl	G	160–162	IP/EE	Sch II-m	10	$C_{20}H_{21}NO_2$·MS·0.1IP	C, H, N, S	7.1 ± 0.3	270	300	+45	0/6	–
67	1-fluoranthenyl	H	185–188 (D)	E/EE	Sch II-p	9	$C_{21}H_{21}NO_2$·HCl·0.3H_2O	C, H, Cl, N	8.5 ± 1.0	140	140	+100	0/6	–
68	2-fluoranthenyl	H	193–194	IP/EE	Sch II-p	16	$C_{21}H_{21}NO_2$·HCl·0.2H_2O	C, H, Cl, N	9.6 ± 0.1	160	160	+105	0/6	–
5	3-fluoranthenyl[i]	H	265–266 (D)	M/EE	Sch II-m	66	$C_{21}H_{21}NO_2$·HCl	C, H, Cl, N	9.3 ± 0.7	105	110	+190	5/6	3/6
69	7-fluoranthenyl	H	204–206 (D)	M/EE	Sch II-n	30	$C_{21}H_{21}NO_2$·HCl	C, H, Cl, N	8.9 ± 0.6	100	123	+142	0/6	–
70	8-fluoranthenyl	H	233–234.5 (D)	M/E	Sch II-n	52	$C_{21}H_{21}NO_2$·HCl	C, H, Cl, N	8.0 ± 0.8	150	(166)	I	–	–
71	6-acephenanthrenyl	I	232–233 (D)	E/EE	Sch II-m	62	$C_{21}H_{21}NO_2$·MS	C, H, N, S	8.4 ± 0.7	110	125	+225	4/6	0/6
72	4,5-dihydro-6-acephenanthrenyl	I	240–242 (D)	E/H	Sch II-m	74	$C_{21}H_{23}NO_2$·MS	C, H, N, S	7.9 ± 0.3	60	70	+200	3/6	0/6
50	1-pyrenyl[j]	K	231–233	E	Sch II-n	49	$C_{21}H_{21}NO_2$·HCl	C, H, Cl, N	11.5 ± 0.8	260	275	+120	0/6	–
73	4-pyrenyl	K	225–227 (D)	E/EE	Sch II-n	37	$C_{21}H_{21}NO_2$·HCl·0.25H_2O	C, H, Cl, N	12.1 ± 0.5	40	40	+45	0/6	–
74	1,2,3,6,7,8-hexahydro-4-pyrenyl	K	202–205 (D)	E/EE	Sch II-n	37	$C_{21}H_{27}NO_2$·HCl·0.25H_2O	C, H, Cl, N	1.3 ± 0.8	151	(151)	I	–	–
75	5-(11*H*-benzo[*b*]fluorenyl)	J	211–213	E/EE	Sch II-m	50	$C_{22}H_{23}NO_2$·MS	C, H, N, S	6.6 ± 1.	250	250	+210	3/6	0/6
76	2-chrysenyl	L	225–227 (D)	E	Sch II-m	43	$C_{23}H_{23}NO_2$·MS	C, H, N, S	7.0 ± 1.1	450	(450)	I	–	–
77	3-chrysenyl	L	180–182 (D)	E/EE	Sch II-m	63	$C_{23}H_{23}NO_2$·MS	C, H, N, S	11.1 ± 0.8	110	110	+20	0/6	–
78	4-chrysenyl	L	192–193.5	IP/EE	Sch II-p	28	$C_{22}H_{23}NO_2$·MS·0.1IP·0.4H_2O	C, H, N, S	7.6 ± 0.7	225	225	+120	1/6	0/6
4	6-chrysenyl[k]	L	239–240 (D)	M/T	Sch II-m	93	$C_{23}H_{23}NO_2$·MS	C, H, N, S	10.5 ± 0.8	120	120	+215	6/8	3/6
79	1-triphenylenyl	M	200–203	E/EE	Sch II-p	11	$C_{23}H_{23}NO_2$·HCl	C, H, Cl, N	6.8 ± 0.5	155	165	+25	0/6	–
80	2-triphenylenyl[l]	M	207–208.5 (D)	E/EE	Sch II-n	43	$C_{23}H_{23}NO_2$·HCl·0.5H_2O	C, H, Cl, N	13.1 ± 0.7	85	85	+100	0/6	–
81	3-benz[*a*]anthracenyl	P	189–191.5	I/EE	Sch II-m	53	$C_{22}H_{23}NO_2$·MS	C, H, N, S	7.0 ± 0.7	200	200	+20	0/6	–
82	7-benz[*a*]anthracenyl	P	233–234 (D)	E/EE	Sch II-m	85	$C_{22}H_{23}NO_2$·MS	C, H, N, S	10.7 ± 0.8	180	220	+180	2/6	0/6
83	5-naphthacenyl	N	197.5–199	E/EE	Sch II-m	50	$C_{22}H_{23}NO_2$·MS	C, H, N, S	7.4 ± 0.5	60	60	+165	2/6	0/6
84	5-benzo[*c*]phenanthrenyl	O	205–207 (D)	E/EE	Sch II-m	58	$C_{22}H_{23}NO_2$·MS	C, H, N, S	8.6 ± 0.4	7.5	15	+42	0/6	–
85	6-benzo[*a*]pyrenyl	Q	275 (D)[m]	M/EE	Sch II-m	53	$C_{22}H_{23}NO_2$·MS·0.25H_2O	C, H, N, S	15.7 ± 0.1	100	123	+100	0/6	–
86	3-perylenyl[n]	R	225–227 (D)	M/EE	Sch II-n	31	$C_{25}H_{23}NO_2$·HCl	C, H, Cl, N	15.5 ± 0.5	350	425	+100	0/6	–

[a] Melting points are uncorrected. Most compounds darkened when heated and decomposed (D) at the melting point. [b] Abbreviations for recrystallization solvents are M = MeOH, E = EtOH, EE = Et_2O, H = hexane, IP = 2-propanol, EA = EtOAc, T = $PhCH_3$. Where two solvents are shown, the compound was dissolved in the first solvent and diluted with the second solvent. [c] Methods of preparation are indicated by General Methods m, n, or p. [d] Elements analyzing within ±0.4% of calculated value. [e] See the Experimental Section for a description of the procedure. [f] Values in parentheses represent the highest nontoxic doses used in the assay. [g] I = inactive. % ILS < 20. [h] The free base has been reported; lit.[30] 83–85 °C. [i] The MS·0.35H_2O·0.25EtOH salt had mp 165–166 °C (E/EE). [j] The MS salt had mp 184–186 °C (M/EE). [k] The HCl salt had mp 247–248 °C (D) (M/EE). [l] The MS salt had mp 259–261 °C (D) (M/EE). [m] Slow decomposition. [n] The MS 1.85 H_2O salt had mp 211–213 °C (D) (M/EE).

that the ring systems being considered are fused, and that the actual relationships of the individual rings are not indicated in the representations, some observations can be made. Examination of the maximum ΔT_m values observed for isomers within a ring system containing a C_6 (**51**, 0.7 °C) ring system or $C_{6,6}$ (**52**, 1.0 °C), $C_{5,6,6}$ (**56**, 1.4 °C), $C_{6,6,6}$ (**58**, 7.3 °C), $C_{5,6,6,6}$ (**68**, 9.6 °C), $C_{6,6,6,6}$ (**80**, 13.1 °C), or $C_{6,6,6,6,6}$ (**85**, 15.7 °C) fused ring systems shows that on average, for the $(C_6)_n$ series (for n = 2, 3, 4, 5) an increase in ΔT_m of approximately 5 °C is seen per C_6 ring added.

Structural features which reduce the planar area of a particular ring system can decrease DNA binding. Compare ΔT_m values for isomers of fused $C_{6,6,6}$ ring systems (anthracenes **58–60**, 4.55–7.3 °C, and phenanthrenes **61–65**, 4–7.02 °C) are all greater than that of the partially fused 4-phenyl-1-naphthyl derivative **57** (1.4 °C).

Both the substitution of a five-membered ring for a six-membered ring where the overall ring shape does not change drastically and the partial reduction of the ring system produce a decrease in DNA binding in the series. The ΔT_m value for the $C_{5,6,6}$ derivative **56** (1.45 °C) is less than that of the $C_{6,6,6}$ derivative **62** (4.1 °C) and the ΔT_ms of both the $C_{5,6,6,6}$ derivative **66** (7.07 °C) and hexahydro-$C_{6,6,6,6}$ derivative **74** (1.26 °C) with the $C_{6,6,6,6}$ derivative **50** (11.5 °C).

In addition to the increased interaction between the molecules in these series and DNA that is observed as the size of the aromatic ring system grows, there appear to be three distinct levels of molecule/DNA interaction that are associated with the size of the intercalating ring system. An examination of the compounds in Table II shows that derivatives containing no rings (**54**), a C_6 (**51** and **55**), a fused $C_{6,6}$ (**52** and **53**), or a fused $C_{5,6,6}$ (**56**) ring system bind DNA weakly. Compounds bearing a fused $C_{6,6,6}$ ring system (**58–65**) bind DNA at an intermediate level while compounds with fused $C_{5,6,6,6}$ (**5**, **66–72**), $C_{6,6,6,6}$ (**4**, **50**, and **73–84**) and $C_{6,6,6,6,6}$ (**85** and **86**) ring systems bind DNA strongly. These three levels of molecule/DNA interaction were also observed in viscometric studies.[18]

Comparisions between ring systems of different size or shape or between isomeric derivatives are difficult since the orientation of each ring system in the intercalation site may be different. We are currently examining these structural effects in greater detail.

P388 Screening Results

Preliminary antitumor evaluation of the carbocyclic AMAPs was done with murine lymphocytic leukemia P388 employing methods used in the Tumor Panel by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, with slight modifications as outlined in the Experimental Section of the previous paper.[1] A number of conclusions regarding the effects of ring size and shape can be made from examination of the data in Table II.

The data in Table II shows that the ring system must be of a minimal size before antitumor activity is seen. At least three fused aromatic rings ($C_{6,6,6}$) are required for antitumor activity in the AMAP series. All congeners derived from smaller ring systems (i.e. **51–57**) were inactive in the P388 screen. High antitumor activity was seen for compounds derived from a number of tetracyclic ring systems ($C_{5,6,6,6}$ and $C_{6,6,6,6}$). At least one isomer from each of the fully aromatic tetracyclic ring systems produced ≥100% ILS in the P388 assay. Although only two pentacyclic derivatives have been made, it is apparent that the level of antitumor activity observed has decreased in these larger ring systems. The lower activity observed for the pentacyclic AMAPs may be due to their higher lipophilicity. Thus, for unsubstituted AMAPs, optimal antitumor activity in the P388 assay is produced by tetracyclic derivatives.

From Table II, it is also apparent that antitumor activity varies within the isomers of a given ring system. Compare the data for isomeric fluoranthene (**5** and **67–70**) and chrysene (**4** and **76–78**) congeners. For each of these tetracyclic AMAP series, the P388 activity ranges from inactive to very active with long-term survivors produced. Within each of the ring systems, the congeners appear to interact similarly with DNA. The observed ΔT_ms, unpublished DNA viscometric slopes, unwinding angles, and binding constants are comparable within each series of isomers. Examination of a larger group of AMAP congeners including heterocyclic ring systems[18] shows that the shape of the AMAP, not the particular ring system, is the key to activity in the tetracyclic series. We are currently evaluating the effects of the AMAPs on a number of target enzymes (including topoisomerases I and II) and investigating the effects of ring type and shape on metabolism.

The most active of the congeners examined produced a significant number of long-term survivors in the assay. On the basis of the P388 and other in vivo assays compounds **4** and **5** and the substituted anthracene derivative **6** were chosen for development. Further antitumor studies on the carbocyclic AMAP congeners and a detailed examination of the three compounds ultimately chosen for clinical development will be presented separately.

Conclusions

In general, the interaction of the AMAPs with DNA increases as the intercalating ring system grows in area, with three distinct binding levels evident. Also, the isomers from a specific ring system appear to bind DNA similarly. DNA binding is not the sole criterion for antitumor activity for the AMAPs studied; the magnitude of the ΔT_m does not correlate with the antitumor activity observed.

Significant in vivo P388 activity was seen for AMAP congeners from several tetracyclic ring systems. However, isomers from each of the specific ring systems produced a wide range of in vivo P388 activity. Thus, AMAP antitumor activity is not a function of the ring system per se but rather appears to be related to the shape of the specific molecule.

Experimental Section

General Comments. All solvents were of 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. Silica gel (SiO_2) used for flash chromatography was Merck & Co. silica gel 60, 230–400 mesh. Chemicals used were reagent grade and used without further purification unless noted.

9-Anthracenecarbaldehyde, 2-fluorenecarbaldehyde, 4-biphenylcarbaldehyde, benzaldehyde, 1-pyrenecarbaldehyde (**39**), 1-naphthalenecarbaldehyde, 2-naphthalenecarbaldehyde, and 9-phenanthrenecarbaldehyde were purchased from Aldrich Chemical Co. (Aldrich). 4,5-Dihydroacephenanthrene (**35**), 5,6,8,9,10,11-hexahydrobenz[*a*]anthracene (**40**), benzo[*c*]-phenanthrene, 1,2,3,10b-tetrahydro-1-fluoranthenecarboxylate (**105**), 11*H*-benzo[*b*]fluorene, 1-methylphenanthrene (**98**), 2-methylfluoranthene (**99**), 4-methylchrysene (**100**), 1-methyltriphenylene (**101**), benz[*a*]anthracene-7-carbaldehyde (**43**), chrysene (**45**), and benz[*a*]pyrene-6-carbaldehyde, were purchased from Cambridge Chemicals, Inc. (CCI), 202 E. Smith Street, Milwaukee, WI 53207.[31]

(31) A number of these intermediates are now available from ChemSyn Science Laboratories, 13605 W. 96th Terrace, Lenexa, KS 66215-1297.

NMR (^1H, ^{13}C), IR, and MS data of all products were consistent with the expected and proposed structures. Many of the aromatic aldehydes used to produce the target AMAPs were first synthesized before modern analytical techniques were available.[32] Assignment of the aldehyde aromatic ring position has been confirmed for all intermediates listed in Table I. Routinely, proton spin systems were identified by 2D-COSY studies and the aldehyde position located by NOE difference experiments. Yields, melting points, and analyses are reported for all aldehydes in Table I. 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.

Scheme II, General Method b.[7] **Ethyl 3-Chrysenecarboxylate (87).** To a round-bottom flask equipped with magnetic stirring bar and condenser were added 3-chrysenecarboxylic acid (88,[33] 18.3 g, 0.067 mol), absolute EtOH (800 mL), and concentrated H_2SO_4 (1 mL). The mixture was refluxed for 4 days. The solvent was removed from the resulting solution by rotary evaporation. The crude solid was dissolved in $PhCH_3$ (500 mL) and passed through a plug of SiO_2 using $PhCH_3$ as the eluting solvent. The appropriate fractions were combined, and the solvent was removed to give crude product. After crystallization from $PhCH_3$–hexane and drying, 18.09 (90%) of 87 was obtained, mp 123–124 °C. Anal. ($C_{21}H_{16}O_2$) C, H.

Ethyl 2-Chrysenecarboxylate (89). Using the above procedure, 2-chrysenecarboxylic acid (90[33]) gave after crystallization from $PhCH_3$–hexane, 89 (92%), mp 205–207 °C. Anal. ($C_{21}H_{16}O_2$) C, H.

Scheme II, General Method c.[8] **1-Fluoranthenemethanol (91).** To a round-bottom flask equipped with magnetic stirrer, condenser, and N_2 inlet line with bubbler were added methyl 1-fluoranthenecarboxylate (92, 71.54 g, 0.275 mol), $LiBH_4$ (Aldrich, 10.0 g, 0.459 mol), and dry THF (1 L). The mixture was refluxed overnight, cooled, and poured into cold H_2O (2 L). The mixture was cautiously acidified with 1 N HCl. The resulting solid was removed by filtration, washed with H_2O (2 × 300 mL), and dried in a vacuum oven overnight. The crude product was recrystallized from CH_2Cl_2–hexane and dried to give 54.74 g (86%) of 91, mp 147–148.5 °C. Anal. ($C_{17}H_{12}O$) C, H.

3-Chrysenemethanol (93). Using the above procedure, ethyl 3-chrysenecarboxylate (87) gave after crystallization from THF–hexane, 93 (98%), mp 187–189 °C. Anal. ($C_{19}H_{14}O$) C, H.

2-Chrysenemethanol (94). Using the above procedure, ethyl 2-chrysenecarboxylate (89) gave after crystallization from THF–hexane, 94 (95%), mp 261–262 °C. Anal. ($C_{19}H_{14}O$) C, H.

Scheme II, General Method g. 1-(Chloromethyl)-fluoranthene (95). To a round-bottom flask equipped with magnetic stirring bar, condenser, addition funnel, thermometer, and N_2 inlet line with bubbler were added 1-fluoranthenemethanol (91, 12.0 g, 0.052 mol) and dry $PhCH_3$ (500 mL). To the mixture was added $SOCl_2$ (Aldrich, 15.73 g, 0.132 mol, 9.5 mL) dropwise over 15 min. The mixture was then heated at 80 °C overnight and then refluxed for 1 h. The solvent was then removed by rotary evaporation to give a crude off-white solid. The material was redissolved in $PhCH_3$ (300 mL), and the solvent was removed again by rotary evaporation. The process was repeated twice to give crude 95, which was used without further purification.

Scheme II, General Method i.[13] **3-Chrysenecarbaldehyde (21).** To a round-bottom flask equipped with magnetic stirring bar and condenser were added 3-chrysenemethanol (93, 14.6 g, 0.057 mol), CH_2Cl_2 (2 L), and $BaMnO_4$ (Aldrich, 29 g, 0.113 mol). The mixture was refluxed for 15 h, cooled, and filtered to remove the inorganic solid, and the solvent was removed to give a crude solid. This material was chromatographed on SiO_2 using $PhCH_3$ as the eluting solvent. The appropriate fractions were combined, and the solution was concentrated to a volume of 75 mL. The solid that formed was filtered and dried to give 12.88 g of 21: ^1H NMR ($CDCl_3$) δ 10.28 (s, 1 H, CHO), 8.85 (s, 1 H, H_4), 8.82–8.73 (m, 3 H, $H_{5,10,11}$), 8.08–8.00 (m, 5 H, $H_{1,2,6,7,12}$), 7.77–7.68 (m, 2 H, $H_{8,9}$).

2-Chrysenecarbaldehyde (20). Using the above procedure, 2-chrysenemethanol (94) gave, after crystallization from $PhCH_3$ and drying, 20: ^1H NMR ($CDCl_3$) δ 10.24 (s, 1 H, CHO), 8.88–8.68 (m, 4 H, $H_{4,5,10,11}$), 8.45 (s, 1 H, H_1), 8.18–8.00 (m, 4 H, $H_{3,6,7,12}$), 7.77–7.67 (m, 2 H, $H_{8,9}$).

Scheme II, General Method k.[4] **3-Fluoranthenecarbaldehyde (14), 7-Fluoranthenecarbaldehyde (15), 8-Fluoranthenecarbaldehyde (16).** To a round-bottom flask equipped with overhead stirrer, thermometer, addition funnel, condenser, and N_2 inlet line with bubbler were added fluoranthene (96, Aldrich, 100 g, 0.49 mol) and CH_2Cl_2 (700 mL). After the mixture was cooled to 5 °C with a salt–ice bath, $SnCl_4$ (Aldrich, 98%, 250 g, 0.96 mol, 112 mL) was added to the solution in one portion. No temperature change occurred. Cl_2CHOCH_3 (Aldrich, 67.6 g, 0.59 mol, 53 mL) was then added dropwise to the mixture, keeping the temperature at ≤5 °C (≈1 h). The resulting suspension was warmed slowly to reflux over 2 h and further stirred for 16 h. Considerable HCl gas was evolved during the warming and early part of the reaction. The reaction mixture was cooled to 10 °C and hydrolyzed by careful addition of cold H_2O (1 L). After 4 h the layers were separated and the organic layer was filtered, dried with Na_2SO_4, and filtered again. The clear yellow solution was concentrated by rotary evaporation to give a yellow oil. The crude material was chromatographed on SiO_2 (1 kg) using $PhCH_3$ as the eluting solvent (3 L). The fractions containing the aldehyde isomers were combined, and the solvent was removed to give 115 g of yellow oil. This material was dissolved in CH_2Cl_2 (500 mL) and diluted with hexane (500 mL). A yellow solid formed and was removed by filtration. This material was recrystallized from CH_2Cl_2–hexane and dried to give 45.7 g of 14. The two filtrates were combined, and the solvent was removed to give a yellow oil, which was again chromatographed on SiO_2 (1 kg) using $PhCH_3$ as the eluting solvent. Appropriate fractions were combined and further purified by HPLC, again using $PhCH_3$ as the eluting solvent. Additional 14 as well as two other aldehydes were obtained isomerically pure. After crystallization from CH_2Cl_2–hexane and drying, the three aldehyde isomers were obtained in the following yields. 14: 68.73 g; R_f = 0.27 (SiO_2, $PhCH_3$); ^1H NMR (DMSO-d_6) δ 10.24 (s, 1 H, CHO), 8.73 (d, 1 H, $J_{4,5}$ = 8.55 Hz, H_4), 7.85 (d, 1 H, $J_{1,2}$ = 7.08 Hz, H_2), 7.72–7.69 (m, 4 H, $H_{1,6,7,10}$), 7.58 (dd, 1 H, $J_{4,5}$ = 8.30 Hz, $J_{5,6}$ = 8.55 Hz, H_5), 7.38–7.30 (m, 2 H, $H_{8,9}$). 15: 2.10 g; R_f = 0.38 (SiO_2, $PhCH_3$); ^1H NMR ($CDCl_3$) δ 10.54 (s, 1 H, CHO), 8.91 (d, 1 H, $J_{5,6}$ = 7.32 Hz, H_6), 8.14 (dd, 1 H, $J_{8,9}$ = 7.26 Hz, $J_{8,10}$ = 1.22 Hz, H_8), 8.01–7,86 (m, 4 H, $H_{1,3,4,10}$), 7.75–7.65 (m, 2 H, $H_{2,5}$), 7.52 (m, 1 H, H_9). 16: 24.8 g; R_f = 0.19 (SiO_2, $PhCH_3$); ^1H NMR (DMSO-d_6) δ 10.12 (s, 1 H, CHO), 8.60 (s, 1 H, H_7), 8.60–8.29 (m, 3 H, $H_{3,4,9}$), 8.12–8.00 (m, 3 H, $H_{1,6,10}$), 7.83–7.78 (m, 2 H, $H_{2,5}$).[34]

The ^1H NMR spectra of additional novel aromatic aldehydes appearing in Table I prepared by the above method are as follows.

7: ($CDCl_3$) δ 10.41 (s, 1 H, CHO), 9.45 (d, 1 H, $J_{7,8}$ = 8.60 Hz, H_8), 8.20 (d, 1 H, $J_{2,3}$ = 7.32 Hz, H_2), 7.94 (d, 1 H, $J_{5,6}$ = 8.51 Hz, H_5), 7.72–7.66 (m, 1 H, H_6), 7.59–7.42 (m, 6 H, H_7 and Ph).

13: ($CDCl_3$) δ 10.42 (s, 1 H, CHO), 8.90 (d, 1 H, $J_{8,9}$ = 7.1 Hz, H_9), 8.03 (d, 1 H, $J_{2,3}$ = 3.91 Hz, H_2), 8.02 (d, 1 H, $J_{8,9}$ = 7.1 Hz, H_8), 7.90–7.82 (dd, 1 H, $J_{6,7}$ = 6.3 Hz, $J_{5,7}$ = 2.3 Hz, H_7), 7.80–7.68 (m, 3 H, $H_{3,5,6}$), 4.35 (s, 2 H, CH_2).

19: (DMSO-d_6) δ 11.24 (s, 1 H, CHO), 8.83 (d, 1 H, $J_{3,4}$ = 8.37, H_4), 8.29 (s, 1 H, H_{10}), 8.10–8.07 (m, 1 H, H_6), 8.10–8.07 (m, 1 H, H_6), 8.02–7.99 (m, 1 H, H_6), 7.70 (m, 1 H, H_1), 7.66–7.46 (m, 4 H, $H_{2,3,7,8}$), 4.18 (s, 2 H, CH_2).

(32) Carruthers, W. *J. Chem. Soc.* **1953**, 3486.

(33) The original syntheses of many of the aromatic aldehydes were done on a small scale. Since these intermediates were used to produce several target AMAPs modifications of the original procedures were required. Additionally, in a number of cases, alternative synthetic routes were used to produce larger amounts of these aldehydes. Full syntheses of aldehydes not described in the Experimental Section, as well as the corresponding AMAP preparations, are in the following patents. **8, 9**: Bair, K. W. U.S. Patent 4,803,221, 1989. **10, 11, 12**: Bair, K. W. U.S. Patent 4,719,055, 1988. **13, 18, 19, 27, 28**: Bair, K. W. U.S. Patent 4,791,23, 1988. **20, 21, 22**: Bair, K. W. U.S. Patent 4,810,727, 1988. **23**: Bair, K. W. U.S. Patent 4,551,282, 1985. **29**: Bair, K. W. U.S. Patent 4,719,055, 1988.

(34) Tucker, S. H.; Whalley, M. *J. Chem. Soc.* **1949**, 3213 report a crude mixture containing 16, but give no properties. The 2,4-DNPH derivative had mp 296–298 °C and upon reduction gave the corresponding 8-CH_3 compound, mp 88–90 °C.

23: ($CDCl_3$) δ 10.22 (s, 1 H, CHO), 9.08 (d, 1 H, $J_{1,3}$ = 1.71 Hz, H_1), 8.72–8.61 (m, 5 H, $H_{4,5,8,9,12}$), 7.94 (dd, 1 H, $J_{3,4}$ = 8.55 Hz, $J_{1,3}$ = 1.71 Hz, H_3), 7.76–7.64 (m, 6 H, $H_{6,7,10,11}$).

28: ($CDCl_3$) δ 10.45 (s, 1 H, CHO), 9.37–9.33 (m, 1 H, H_4), 9.05–8.99 (m, 2 H, $H_{1,12}$), 8.65 (s, 1 H, H_6), 8.20–8.16 (m, 1 H, H_9), 8.13, 8.09 (2 d, 2 H, $J_{7,8}$ = 8.34 Hz, $H_{7,8}$), 7.85–7.76 (m, 4 H, $H_{2,3,10,11}$).

Scheme II, General Method m.[2] To a round-bottom flask equipped with magnetic stirring bar, condenser, and Dean–Stark trap were added the aldehyde (0.05 mol), the amine (0.1 mol), *p*-toluenesulfonic acid hydrate (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 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. 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 was 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 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 recrystallized twice using the solvent(s) indicated in Table I, and, after filtration, washed with the less polar solvent (500 mL). The pure product was then dried in a vacuum oven (80 °C) overnight. In other preparations where the crude free base 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 of the crude free base was made and purified as described above. 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%.

Scheme II, General Method n.[3] To a Erlenmeyer flask equipped with a magnetic stirring bar were added the aldehyde (0.1 mol), the amine (0.105 mol), *p*-toluenesulfonic acid hydrate (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 absolute EtOH (10–20 mL) and 2–4 L of Et_2O were added to the flask. The mixture was filtered through a medium-porosity sintered-glass funnel and pressed dry. The crude salt was then recrystallized using 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 crude solid was then dried followed by salt formation and purified as in Scheme II, general method m.

Scheme II, General Method o. 1-(Bromomethyl)-phenanthrene (97). To a round-bottom flask equipped with stirring bar and N_2 inlet line with bubbler were added 1-methylphenanthrene (**98**, CCI, 25.0 g, 0.130 mol), *N*-bromosuccinimide (Aldrich (recrystallized from H_2O and dried overnight under high vaccum), 25.45 g, 0.143 mol), benzoyl peroxide (Aldrich, 1000 mg), and CCl_4 (500 mL). The mixture was stirred at reflux for 2.5 h and cooled, and the succinimide formed in the reaction was removed by filtration. The solvent was removed from the filtrate by rotary evaporation, and the residue was dissolved in EtOAc (650 mL), washed with H_2O (3 × 150 mL), and dried (Na_2SO_4). The solvent was then removed by rotary evaporation, and the crude product was recrystallized from hexane–EtOAc (10:1), filtered, and dried to give 25.3 g (73% yield) of **97**, mp 90–91 °C. Anal. ($C_{15}H_{11}Br$) C, H, Br.

Using this procedure, 2-methylfluoranthene (**99**, CCI), 4-methylchrysene **100**, CCI), and 1-methyltriphenylene (**101**, CCI) gave nearly quantitative crude yields of 2-(bromomethyl)-fluoranthene (**102**), 4-(bromomethyl)chrysene (**103**), and 1-(bromomethyl)triphenylene (**104**), respectively, which were used without further purification.

Scheme II, General Method p. 2-[(1-Fluoranthenylmethyl)amino]-2-methyl-1,3-propanediol·HCl·0.3H_2O (67). To a round-bottom flask equipped with magnetic stirring bar, condenser, and N_2 inlet line with bubbler were added crude 1-(chloromethyl)fluoranthene (**95**, 13.01 g, 52 mmol), 2-amino-2-methyl-1,3-propanediol (Aldrich, 5.46 g, 52 mmol), K_2CO_3 (MC&B, 14.37 g, 0.104 mol), and absolute EtOH (300 mL). The reaction was stirred at reflux overnight and filtered hot, and the solvent was removed by rotary evaporation to give a crude dark oil. This was acidified with 1 N HCl, dissolved in H_2O, filtered, basified with 5 N NaOH solution, and filtered to give a crude white solid. The material was dissolved in EtOH containing HCl(g), filtered, and precipitated with Et_2O. Filtration followed by crystallization (absolute EtOH–Et_2O, 1:4) gave after filtration and drying 1.6 g (9%) yield of **67**, mp 185–188 °C (dec). Anal. ($C_{21}H_{21}NO_2$·HCl·0.3H_2O) C, H, Cl, N.

Using the same procedure **97** gave **61** (24%), **102** gave **68** (16%), **103** gave **78** (28%), and **104** gave **79** (11%).

5,6,8,9,10,11-Hexahydrobenz[*a*]anthracene-3-carbaldehyde (42). 5,6,8,9,10,11-Hexahydrobenz[*a*]anthracene (**40**, CCI) was formylated using the procedure described in Scheme II, general method k.[4] The resulting crude aldehyde mixture **41** was passed through a 40 × 10 cm plug of SiO_2 using $PhCH_3$ as the eluting solvent. Three aldehyde fractions were isolated. The least mobile of the fractions by TLC contained crude **42** (27%) (identified by ^1H NMR), which was used directly without further purification.

Methyl 1-Fluoranthenecarboxylate (94).[17] To a round-bottom flask equipped with magnetic stirrer, condenser, and N_2 inlet line with bubbler were added methyl 1,2,3,10b-tetrahydro-1-fluoranthenecarboxylate (**105**, CCI, 98.0 g, 0.0372 mol), DDQ (Aldrich, 177.0 g, 0.781 mol), and dry $PhCH_3$ (2 L). The reaction mixture was heated at 90 °C for 10 h, cooled, filtered, and concentrated to a 500-mL volume. The crude material was purified on a plug of SiO_2 (500 g) using $PhCH_3$ as the eluting solvent. The appropriate fractions were combined and concentrated by rotary evaporation to give a crude pale yellow solid, which was recrystallized (CH_2Cl_2–hexane), filtered, and dried to give 94.5 g (96%) of **92**, mp 70–71 °C. Anal. ($C_{18}H_{12}O_2$) C, H.

6-Acephenanthrenecarbaldehyde (17). Using procedure outlined above, 4,5-dihydro-6-acephenanthrenecarbaldehyde (**18**) gave, after crystallization from $PhCH_3$–hexane and drying, **17**: ^1H NMR ($CDCl_3$) δ 10.92 (s, 1 H, CHO), 9.27–9.23 (m, 1 H, H_7), 8.59–8.55 (m, 1 H, H_{10}), 8.53 (d, 1 H, $J_{1,2}$ = 8.06 Hz, H_1), 7.71–7.57 (m, 4 H, $H_{2,3,8,9}$), 7.42 (d, 1 H, $J_{4,5}$ = 5.47 Hz, H_5), 7.27 (d, 1 H, $J_{4,5}$ = 5.47 Hz, H_4).

Benz[*a*]anthracene-3-carbaldehyde (24). Using procedure outlined above, crude 5,6,8,9,10,11-hexahydrobenz[*a*]-anthracene-3-carbaldehyde (**42**) gave, after crystallization from $PhCH_3$–hexane and drying, **24**: ^1H NMR ($CDCl_3$) δ 10.16 (s, 1 H, CHO), 9.13 (s, 1 H, H_{12}), 8.86 (d, 1 H, $J_{1,2}$ = 7.51 Hz, H_1), 8.35 (s, 1 H, H_7), 8.26 (d, 1 H, $J_{2,4}$ = 1.30 Hz, H_4), 8.14–8.08 (m, 2 H, $H_{2,11}$), 8.08–8.01 (m, 1 H, H_8), 7.83 (d, 1 H, $J_{5,6}$ = 7.8 Hz, H_6), 7.65 (d, 1 H, $J_{5,6}$ = 7.8 Hz, H_5), 7.63–7.55 (m, 2 H, $H_{8,9}$).

Thermal Denaturation Studies. Thermal denaturation (ΔT_m) experiments were done using the method of Cory et al.[35]

Murine Lymphocytic Leukemia P388 Screen. Preliminary antitumor evaluation of the compounds was done using murine lymphocytic leukemia P388 and methods used in the Tumor Panel

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by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.[36] Modifications in the selection and number of doses used have been made to increase test efficiency.[37]

Registry No. 4, 96389-69-4; 5, 96404-51-2; 7, 133550-74-0; 8, 1140-79-0; 9, 2143-81-9; 10, 26842-00-2; 11, 7466-50-4; 12, 41498-43-5; 13, 104500-25-6; 14, 28440-63-3; 15, 96403-40-6; 16, 96403-41-7; 17, 104500-30-3; 18, 26947-45-5; 19, 104500-12-1; 20, 96403-57-5; 21, 96403-23-5; 22, 22138-85-8; 23, 96404-79-4; 24, 104500-22-3; 25, 22245-51-8; 26, 24295-83-8; 27, 14214-57-4; 28, 4466-76-6; 29, 133550-75-1; 40, 67064-61-3; 41, 133550-76-2; 42, 104500-21-2; 50, 96403-91-7; 51, 133550-77-3; 52, 133550-78-4; 53, 133550-79-5; 54, 133550-80-8; 55, 133550-81-9; 56, 133550-82-0; 57, 133550-83-1; 58, 96404-62-5; 59, 96403-65-5; 60, 96538-95-3; 61, 133550-84-2; 62, 96404-35-2; 63, 96403-63-3; 64, 96403-62-2; 65, 96404-20-5; 66, 104500-26-7; 67, 133550-85-3; 68, 133550-86-4; 69, 96389-48-9; 70, 96403-44-0; 71, 104500-31-4; 72, 104500-28-9; 73, 96403-30-4; 74, 133550-87-5; 75, 104500-13-2; 76, 96403-59-7; 77, 96403-25-7; 78, 133550-88-6; 79, 133550-89-7; 80, 96422-39-8; 81, 104500-23-4; 82, 104500-07-4; 83, 104500-10-9; 84, 104500-15-4; 85, 104525-00-0; 86, 133550-90-0; 87, 96403-22-4; 88, 96403-20-2; 89, 96403-55-3; 90, 96403-26-8; 91, 133550-91-1; 92, 133550-92-2; 93, 96403-21-3; 94, 96403-56-4; 95, 103395-25-1; 96, 206-44-0; 97, 42050-05-5; 98, 832-69-9; 99, 33543-31-6; 100, 3351-30-2; 101, 2871-91-2; 102, 88746-58-1; 103, 133550-93-3; 104, 133550-94-4; 105, 133550-95-5; $SnCl_4$, 7646-78-8; Cl_2CHOCH_3, 4885-02-3; 2-amino-2-methyl-1,3-propanediol, 115-69-5.

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(37) After the LD_{20} was determined, antitumor screening was performed under contact 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.

Computer Simulation of the Binding of Saframycin A to d(GATGCATC)$_2$

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The binding of Saframycin A to the octanucleotide duplex d(GATGCATC)$_2$ was investigated using molecular dynamics. For covalent binding at N2 of the central guanine, only the *R* configuration at the alkylating carbon (C7) was permitted for B DNA and the 3′ direction in the minor groove was preferred by 50.6 kcal/mol. The dihydroquinone form of saframycin A gave stronger binding than the quinone, in agreement with the literature. Addition of solvent and counterions made no significant change in the geometry model. The proposed mechanism of DNA alkylation, involving iminium ion intermediates from the dihydroquinone or quinone, was investigated by modeling these species. They gave models with good net binding enthalpies, and C7 was in close proximity to N2 of guanine. The noncovalent binding of saframycin A and its dihydroquinone in the vicinity of guanine also was favorable in the 3′ direction.

The saframycins were discovered in a thorough investigation of satellite antibiotics produced by *Streptomyces lavendulae* No. 314.[1] Saframycin A (Figure 1, IUPAC numbering) had the best antibacterial and antitumor activity among the earlier compounds isolated, and it has been the one most thoroughly studied. Structures of the saframycins are based mainly on the X-ray diffraction of saframycin C.[2] Saframycin A and otherr saframycins were related to it by ^{1}H and ^{13}C NMR spectrometry.[3-5] Absolute stereochemistry of the saframycins follows from that of a closely related compound, the 15-bromo derivative of safracin A (Figure 1, 4-bromo in IUPAC numbering).[6]

The saframycins have pentacyclic, dimeric structures containing two units of 7-methoxy-6-methyl-1,2,3,4-isoquinoline-5,8-dione joined through the fifth ring and bearing a methylene group substituted with a pyruvamide moiety.[7] Certain saframycins have labile leaving groups at C7, including cyano (saframycin A) and hydroxyl (saframycin S). Thus, saframycin S is converted into saframycin A on treatment with sodium cyanide.[8] Furthermore, acid hydrolysis of saframycin A results in the release of 1 equiv of HCN (presumably with formation of saframycin S).[3] Saframycins have two tertiary nitrogens, but only one of them, N16, is protonated in dilute acid.[9] The two quinone rings of safracins or saframycins are at 75° angles to each other (Figure 2).[6] Solution conformations of saframycins A and C, determined by ^{1}H NMR studies, show that ring B deforms from half-boat to half-chair and the chair conformation of ring C is slightly twisted, when compared with their crystal structures.[4,5] The pyruvamide side chain has a slightly skewed gauche conformation with a dihedral angle of about 90°, and the orientation of this chain with respect to the C9 methylene depends on the solvent. The 7-cyano group of saframycin A has the axial conformation.[9]

A mode of action has been proposed for the antitumor activity of saframycins. It incorporates the chemical evidence described above, plus the following biochemical studies. (1) Treatment of [^{14}C]saframycin A (derived from [^{14}C]tyrosine) with dithiothreitol in the presence of calf thymus DNA resulted in incorporation of radioactivity into the DNA; however, excess sodium cyanide inhibited this reaction.[10] There was no radioactivity incorporated when saframycin A with ^{14}CN at C7 was used. Reduction of the

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