0.15 mL of acetic anhydride yielded, after the usual aqueous workup, 0.402 g of **43**; mp 129–130 °C.

17α-[(Triethylsilyl)ethynyl]estra-1,3,5(10)-triene-3,17β-diol 3-Butyrate (44). To a solution of 0.50 g of **5** in 50 mL of pyridine was added 3.5 mL of butyryl chloride. After the reaction mixture was stirred at room temperature under nitrogen for 1 h, the resulting suspension was diluted with $CHCl_3$, washed with water, and dried ($MgSO_4$). The solvent was removed under vacuum to yield 0.419 g of crude material. Separation on a silica gel column with chloroform followed by crystallization from ligroin afforded 0.138 g of **44**; mp 86–89 °C.

17α-[(Triethylsilyl)ethynyl]estra-1,3,5(10)-triene-3,17β-diol 3,17-Dibutyrate (45). To a solution of 2.0 g of **5** in 250 mL of dry THF was added 3.0 mL of 1.6 M butyllithium. The reaction mixture was stirred for 1 h at room temperature under nitrogen, and then 0.52 mL of butyryl chloride was added. After the reaction mixture was stirred for an additional 1.0 h, the solution was diluted with $CHCl_3$, washed with water, saturated $NaHCO_3$, and water, and then dried ($MgSO_4$). The solvent was evaporated to yield 2.10 g of crude product. Purification on preparative silica gel plates with 5% MeOH/$CHCl_3$ afforded 0.869 g of **45**, which would not crystallize. Lyophilization from benzene gave an analytical sample.

17α-[(Triethylsilyl)ethynyl]estra-1,3,5(10)-triene-3,17β-diol 3-Methyl Ether 17-Carbonylimidazole (46). To a solution of 0.556 g of [(triethylsilyl)ethynyl]estradiol 3-methyl ether in 30 mL of dry THF was added 0.8 mL of 1.6 M butyllithium in hexane. After the mixture was stirred at room temperature for 2 h, 1.65 g of carbonyldiimidazole was added and stirring was continued for 1 h. The reaction mixture was then diluted with $CHCl_3$, washed with water, and dried ($MgSO_4$). The solvents were removed under reduced pressure to yield 0.98 g of crude product. Separation on silica gel with chloroform as eluant gave 0.568 g of **46**. Crystallization from petroleum ether (bp 30–60 °C) gave 0.385 g of pure **46**; mp 112–114 °C.

17α-[(Triethylsilyl)ethynyl]estra-1,3,5(10)-triene-3,17β-diol 3-Cyclopentyl Ether (47). To a solution of 0.8 g of **5** in 15 mL of anhydrous EtOH were added 2.1 g of anhydrous K_2CO_3 and 1.7 mL of cyclopentyl bromide. The mixture was stirred under nitrogen for 7 days. The EtOH was evaporated and the residue dissolved in $CHCl_3$ and water. The organic phase was washed with 1 N HCl, water, and saturated NaCl and then dried (Na_2SO_4). The solvent was removed at reduced pressure. The crude product was purified on silica gel preparative plates developed with 7% EtOAc/$CHCl_3$, which led to the isolation of **47** as a pale-yellow oil (0.847 g), which did not crystallize.

Method X: 17α-[*trans*-(2-Triethylsilyl)vinyl]estradiol (52) and 17α-[(1-Triethylsilyl)vinyl]estradiol (54). To a solution of 8.5 g of EE in 150 mL of diglyme in a stainless steel bomb were added 0.2 g of 5% Pt/C and 4.56 mL of triethylsilane. The reaction mixture was heated to 160 °C for 16 h and then cooled to room temperature. The suspension was filtered through Celite, and the solvent was removed to afford 11.0 g of crude product. The mixture was purified on 250 g of neutral silica gel and eluted with a benzene/5% ether mixture to afford 5.0 g of **54**. An analytical sample was prepared by crystallization from ether/hexane; mp 122–126 °C. Further elution with benzene/5% ether afforded 4.45 g of **52**. An analytical sample was prepared by crystallization from ether; mp 175–176 °C.

Method Y: 17α-[*cis*-(2-Triethylsilyl)vinyl]estradiol (53). A 0.4-g sample of 17α-[(triethylsilyl)ethynyl]estradiol (**5**) in 20 mL of 95% ethanol with 0.025 mg of 5% Pd on $BaSO_4$ was hydrogenated with stirring at atmospheric pressure for 25 h. Filtration through Celite and evaporated of the solvent gave 0.4 g of residue. The product was separated by silica gel preparative TLC, eluting with benzene/15% ether, to afford 0.08 g of pure **53**. An analytical sample of **53** was prepared by crystallization from hexane: mp 127–129 °C.

Biology. Oral Estrogenic Activity. The estrogenic activity was determined by using immature female rats ovariectomized at 21 days of age. Ten rats were used per dose. Treatment was by oral administration of test compound for 4 days, beginning on the day of ovariectomy. The test compounds were diluted with a 0.5% (carboxymethyl)cellulose suspension. Animals were autopsied on the day following the last administration of test compound. Vaginal smears were obtained from animals that had open vaginas at the time of autopsy. The end points for comparison with a standard estrogen were an increase in uterine weight and cornification of vaginal smears.

Oral Antifertility Activity. Oral antifertility activity was determined by using rats. Adult cycling female rats selected were in the proestrous phase of the cycle. Treatment with test compound using 10 animals/dose began on the day of proestrus. Each female was caged overnight with two adult males. The findings of sperm in a vaginal smear obtained the following morning was used as evidence of insemination. Compounds were given once daily for a total of 8 days. The rats were sacrificed on the day following the last treatment, and the implantation sites and corpora lutea were counted.

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Potential Antitumor Agents. 48. 3′-Dimethylamino Derivatives of Amsacrine: Redox Chemistry and in Vivo Solid Tumor Activity

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Structure–activity relationships for a series of acridine-substituted 3′-$N(CH_3)_2$ derivatives of the clinical antileukemic drug amsacrine (**1**) are reported. The parent (unsubstituted) compound **3** has activity against the Lewis lung solid tumor that is superior to amsacrine (**1**), the new clinical amsacrine analogue **4**, and the recently developed 3′-$NHCH_3$ derivative **2**. Although the compounds generally bind less well to DNA and are less dose potent in vivo than either their amsacrine (3′-OCH_3) or 3′-$NHCH_3$ analogues, they show very high levels of antitumor activity, with the 4-OCH_3 derivative capable of effecting 100% cures of the Lewis lung solid tumor. The broad structure–activity relationships for acridine substitution more closely resemble those of the amsacrine than the 3′-$NHCH_3$ series, with 4-substituted and 4,5-disubstituted compounds showing the highest activity.

The DNA-intercalating 9-anilinoacridine derivative amsacrine (**1**) is a useful clinical antileukemic drug,[1,2] but has been shown to have only a narrow spectrum of clinical antitumor activity. Following extensive studies of structure–activity relationships for acridine-substituted analogues,[3-6] a "second-generation" compound (**4**; CI-921; NSC

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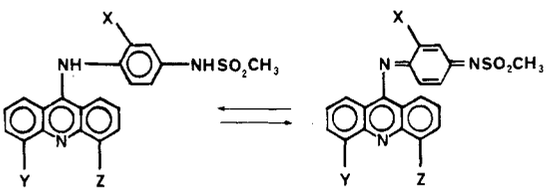
343499) with activity against a broader spectrum of experimental tumors (including solid tumors) has now begun phase I clinical trials.[7] Subsequent work in our laboratory on the development of other broad-spectrum antitumor agents of the 9-anilinoacridine class has concentrated on 3′-alkylamino derivatives, since both qualitative[8] and quantitative[9] structure–activity relationships suggested a requirement for electron-donating groups in the anilino ring. The 3′-$NHCH_3$ derivative **2** showed higher in vivo activity than amsacrine against both the P388 leukemia and the Lewis lung solid tumor (LL) used as front-line screens,[10] and the structure–activity relationships for a large number of acridine-substituted derivatives of **2** have been reported.[11] In contrast to the amsacrine (3′-OCH_3) series, where 4,5-disubstitution of the acridine was the preferred pattern (e.g., **4**), in the 3′-$NHCH_3$ series 3,5-disubstitution is superior. Several 3-halo-5-methyl and 3-halo-5-methoxy derivatives of **2** show about the same order of activity against the advanced LL tumor (about 50% cures) as the second-generation amsacrine analogue **4**.



1 X = OCH_3, Y = Z = H — **5**

2 X = $NHCH_3$, Y = Z = H — **6**

3 X = $N(CH_3)_2$, Y = Z = H — **7**

4 X = OCH_3, Y = CH_3, Z = $CONHCH_3$ — **8**

During the screening that detected the superior activity of the 3′-$NHCH_3$ derivative **2**, the exemplary antileukemic activity of the 3′-$N(CH_3)_2$ derivative **3** was also noted.[10] Although showing poor dose potency, the very high levels of P388 activity (ILS of 210%) of **3**, together with later determination of its high activity in the LL solid tumor screen, warranted further development of this class of compound. This paper details the synthesis, physicochemical properties, and structure–activity relationships for acridine-substituted derivatives of **3**, discusses the important changes in key physicochemical properties shown by the three parent molecules **1–3**, and attempts to relate the different patterns of structure–activity relationships seen among the three series to these changes.

Chemistry

The compounds of Table I were prepared by acid-catalyzed coupling of the unstable 4′-amino-3′-(dimethylamino)methanesulfonanilide with the appropriate 9-

Table I. Comparison of 3′-Substituted 9-Anilinoacridines

no.	solubility[a]	R_m[b]	pK_a[c]	E[d]	redox reversibility	log K[e] AT	log K[e] GC	in vitro IC_{50}[f] L1210	in vitro IC_{50}[f] HCT8	ratio	P388 in vivo D_{50}[g]	P388 in vivo OD[h]	P388 in vivo ILS_{max}[i]	LL in vivo D_{50}	LL in vivo OD	LL in vivo ILS_{max}
1	0.12	0.18	7.43	280	yes	5.57	5.65	33	120	3.6	3	13.3	78	*j*	13.3	42
2	0.49	0.17	7.47	145	no	6.42	6.51	70	178	2.5	1.5	13.3	152 (1)[k]	11	30	80
3	0.42	0.41	7.25	195	yes	5.34	5.68	520	268	0.52	25	166	210 (2)	35	100	192 (4)
4	0.72	0.34	6.40	240	yes	6.06	6.64	38	49	1.3	3.5	20	200 (3)	9	30	167 (3)

[a] Solubility of hydrochloride salts in water at 20 °C, in mg/mL. [b] R_m values were determined by liquid–liquid chromatography by the methods described in ref 8, with 4′-(9-acridinylamino)methanesulfonanilide as an internal reference. [c] pK_a values for the acridine nitrogen were determined spectrophotometrically in 20% DMF as detailed in ref 29. [d] Value in millivolts (relative to SCE) for the potential of the reversible two-electron oxidation to the corresponding quinone diimines, measured by cyclic voltammetry as detailed in ref 22. [e] log K: binding constant to poly-[d(A-T)] and poly-[d(G-C)], determined by ethidium bromide displacement; see ref 23. [f] IC_{50}: concentration of drug in nM to inhibit growth of murine leukemia (L1210) or human colon tumor (HCT-8) cells in culture by 50%, following a 40-h exposure; see ref 24. [g] D_{50}: dose of drug in mg/kg per day to provide an average % ILS of 50%; determined from dose–response graph, as in ref 27. [h] OD: optimal dose of drug in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol–water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 leukemia cells, or on days 5, 9, and 13 after intravenous inoculation of 10^6 Lewis lung carcinoma cells. See ref 15. [i] ILS_{max}: the percentage increase in lifespan of drug-treated, tumor-bearing animals compared to nontreated, tumor-bearing controls when treated at the optimal dose; values above 20% for P388 and above 40% for Lewis lung are considered statistically significant. [j] An ILS of 50% could not be reached at any dose level. [k] Numbers in parentheses indicate the number of animals in a group of six that were long-term survivors (50 days for P388, 60 days for Lewis lung).

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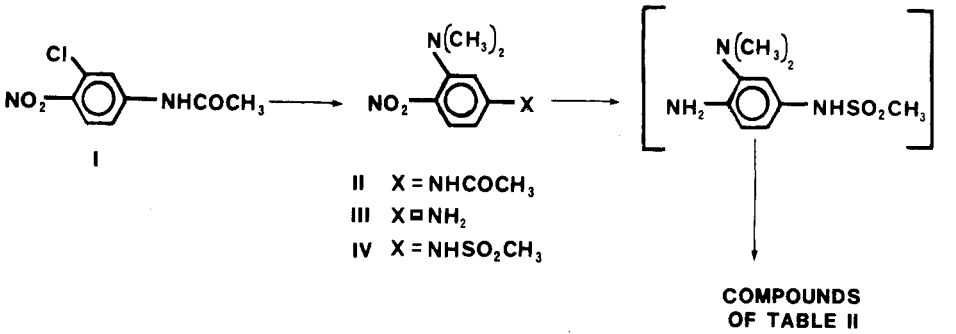
Scheme I



chloroacridine (Scheme I). Preparation of the 9-chloroacridines has been reported previously.[9,12] Synthesis of the precursor 3′-(dimethylamino)-4′-nitromethanesulfonanilide (IV) has been reported[10] from 3′-chloro-4′-nitromethanesulfonanilide. An alternative preparation that does not require high-pressure conditions is outlined in Scheme I. Reaction of 3′-chloro-4′-nitroacetanilide (I) with aqueous dimethylamine proceeds smoothly at 100 °C, and the resulting 3′-(dimethylamino)-4′-nitroacetanilide (II) is readily converted to the desired precursor IV in high yield. This is reduced with $Pd/C/H_2$ in methanol, and the resulting solution of unstable diamine is reacted immediately with the appropriate 9-chloroacridine. Oxidation of compounds 1–4 to the respective quinone imines 5–8 was carried out with activated MnO_2 as published.[13]

Intercomparison of the Parent Compounds 1–3

Although the structural differences between the three parent compounds 1–3 are small, they result in significant alterations in a number of physicochemical properties previously shown to be of critical importance for the biological activity of 9-anilinoacridine derivatives (Table I). While replacement of the 3′-OCH_3 group (π −0.02) of amsacrine (1) with the more hydrophilic 3′-$NHCH_3$ group (π −0.47) to give 2 leads to no significant alteration in lipophilicity as determined by chromatographic R_m values, it does result in a 4-fold increase in water solubility of the corresponding HCl salts (Table I). Thus it is to be expected that replacement by the more lipophilic 3′-$N(CH_3)_2$ group (π 0.18) to give 3 also leads to significantly increased lipophilicity (R_m 0.41) and a decrease in water solubility (0.42 mg/mL for the HCl salt of 3 compared to 0.12 mg/mL for amsacrine hydrochloride). This loss of solubility coupled with the lower dose potency (the increase in optimal dose is 12-fold) means that water solubility is a significant drug design problem for the 3′-$N(CH_3)_2$ series.

Detailed studies[4,14,15] of the amsacrine analogue 4 have suggested that its enhanced spectrum of activity compared to that of amsacrine (especially against solid tumors) is a result of favorable physicochemical properties such as tighter DNA binding (allowing increased cellular uptake and longer pharmacokinetic halflife) and lower pK_a (allowing better distribution to remote sites due to a greater proportion of neutral form), together with an improved selectivity (of unknown origin) for solid tumor cells compared to leukemia cell lines. This particular combination of tighter DNA binding together with lower pK_a is not easy to achieve, since the two properties are usually collinear among 9-anilinoacridine derivatives.[3]

It can be seen from Table I that the 3′-$NHCH_3$ derivative 2 possesses an almost equally favorable set of physicochemical properties, combining much greater DNA binding with a pK_a little different from that of amsacrine, despite the greatly increased electron-donor properties of the $NHCH_3$ group. However, the much larger 3′-$N(CH_3)_2$ group of 3 results in a dramatic decrease in DNA binding, making the compound a weaker binder than amsacrine itself. As expected from earlier quantitative structure–activity relationship (QSAR) results,[9] this leads to a significant loss of dose potency, and the optimal dose (approximately the LD_{10}) of 3 is 1 order of magnitude greater than those of amsacrine and the 3′-$NHCH_3$ derivative 2. However, the compound does show a remarkable selectivity in culture for the HCT-8 human colon tumor line compared to the L1210 leukemia (ratio of IC_{50} values 0.52 compared to 3.6 for amsacrine and 2.5 for the 3′-$NHCH_3$ derivative). For derivatives of amsacrine, this ratio is found to correlate with in vivo solid tumor activity.[16] In agreement with this, 3 shows activity against the LL carcinoma that is clearly superior to that of the other two compounds and comparable to that of the new clinical analogue CI-921 (4) (Table I). Although the 3′-$N(CH_3)_2$ derivative is considerably less dose potent than either amsacrine or the 3′-$NHCH_3$ compound, its very high level of activity (as % ILS) and shallow dose–response curve (higher OD/D_{50} ratio) means that it still shows good activity at doses comparable to the optimal doses of the latter two compounds.

Early preclinical studies[13,17] with amsacrine established that an important route of metabolism was two-electron oxidation of the anilino ring to give the reactive quinone diimine 5, which could rapidly undergo 1,4-addition with low molecular weight thiols (in particular glutathione (GSH)). In some studies, such GSH conjugates were the major metabolic products.[13] Later work[18,19] has favored inhibition of the DNA replication enzyme topoisomerase II and the concomitant formation of DNA strand breaks as the major mechanism of cytotoxicity, but the role of this facile redox pathway in determining the biological activity of amsacrine derivatives has not been clarified. Oxidation to the quinone imine may result in detoxification, but the importance of redox cycling in the mode of action of other

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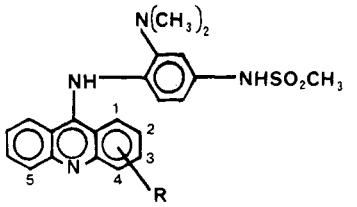
Table II. Physicochemical and Biological Data for 3′-Dimethylamino Analogues of Amsacrine



				in vitro IC_{50} values[c]			P388 leukemia in vivo			Lewis lung carcinoma in vivo		
no.	R	R_m[a]	log K[b]	L1210	HCT-8	ratio	D_{50}[d]	OD[e]	ILS_{max}[f]	D_{50}	OD	ILS_{max}
3	H	0.41	5.34	520	270	0.52	25	250	210 (2)[g]	35	100	192 (4)
9	3-CH_3	0.56	5.63	450	185	0.41	35	65	155 (3)	45	100	154 (2)
10	3-OCH_3	0.44	5.45	480	210	0.44	20	45	127	20	45	127 (4)
11	3-F	0.33	5.50	77	770	10.0	*h*	65	42	*h*	45	*i*
12	3-Cl	0.45	5.65	460	200	0.43	55	150	99	80	150	122
13	3-Br	0.42	6.18	20	170	8.5	150	150	48	*h*	150	*i*
14	3-NO_2	0.19	5.24	2100	430	0.20	50	100	62	100	100	46
15	4-CH_3	0.36	5.40	90	70	0.78	15	45	141	25	45	235 (3)
16	4-OCH_3	0.33	5.58	320	250	0.78	50	100	91		100	(6)[j]
17	4-F	0.26	5.27	560	790	1.41	55	150	98	50	100	88
18	4-Cl	0.29	5.58	860	1050	1.22	90	150	60	*h*	100	*i*
19	4-$CONHCH_3$	0.24	5.40	1200	1170	0.98	65	150	108 (2)	70	150	68 (3)
20	4-$CONHCH_2CONH_2$	−0.41	5.32	3700	9400	2.54	60	150	116	*h*	150	*i*
21	3,4-$(CH_3)_2$	0.41	5.82	8	160	20.0	20	65	115	40	100	97 (1)
22	3.4-benz	0.47	6.27	560	2600	4.64	100	100	46	*h*	150	*i*
23	3,5-$(CH_3)_2$	0.50	5.81	140	46	0.32	10	45	89	*h*	45	*i*
24	3-OCH_3, 5-CH_3	0.44	5.75	80	30	0.34	7	20	79 (1)	15	30	128 (1)
25	3-F, 5-CH_3	0.46	5.60	490	420	0.85	60	100	122	15	100	115
26	3-Cl, 5-CH_3	0.53	6.37	150	240	1.33	50	100	157 (1)	40	150	148 (4)
27	3-Br, 5-CH_3	0.51	6.80	120	140		30	65	135	*h*	65	*i*
28	3-CH_3, 5-OCH_3	0.46	5.57	200	96	0.48	13	45	195 (3)	20	45	113 (2)
29	3-F, 5-OCH_3	0.38	5.75	670	690	1.03	50	100	128	*h*	150	*i*
30	3-Cl, 5-OCH_3	0.50	5.76	200	260	1.30	60	150	77	50	100	69
31	3-Br, 5-OCH_3	0.53	5.99	250	200	0.80	25	30	62	150	150	43
32	3-Cl, 5-$CONHCH_3$	0.41	6.43	1800	2670	1.48	100	100	50	*h*	100	*i*
33	4,5-$(CH_3)_2$	0.37	6.60	290	800	2.75	65	150	98	*h*	150	*i*
34	4,5-$(OCH_3)_2$	0.36	6.73	340	250	0.73	80	150	62	*h*	100	*i*
35	4-CH_3, 5-$CONHCH_3$	0.40	7.03	240	270	1.12	35	100	139 (4)	55	100	168 (3)
36	4-OCH_3, 5-$CONHCH_3$	0.27	7.20	690	770	1.11	25	100	180 (1)	65	150	123 (3)

[a] Footnote *b*, Table I. [b–f] Footnotes *e–i*, respectively, of Table I. [g] Footnote *k*, Table I. [h] Footnote *j* of Table I. [i] Compound inactive at all dose levels up to toxic ones. [j] All animals long-term survivors.

DNA-intercalating agents has been noted.[20,21] The ease of this oxidation in the case of amsacrine is shown by its low oxidation potential (280 mV referenced to SCE; determined by cyclic voltammetry[22]) and its rapid oxidation by mild chemical oxidants such as MnO_2.[13] The reaction is completely reversible, as shown by the closed cyclic voltammetry traces (Figure 1a) and the fact that treatment of the quinone diimine **5** with mild reductants such as ascorbic acid results in its quantitative reduction to amsacrine.[22]

As expected, the more powerful electron-donating properties of the 3′-$NHCH_3$ group make the oxidation of **2** even more facile, and this compound has an oxidation potential of 145 mV. However, the cyclic voltammogram (Figure 1b) shows that the reaction is not chemically reversible, and oxidation of **2** with MnO_2 gave a variety of products that could not be rereduced to the parent compound. This irreversible oxidation is presumably due to the 3′-hydrogen, which allows prototropic rearrangement of the initially formed 1,4-diimine **6** (see ref 22). In contrast, the 3′-$N(CH_3)_2$ compound **3** behaves similarly to amsacrine, undergoing a reversible two-electron oxidation to the reactive but isolable diimine **7**. However, this reaction is much more facile than that of amsacrine itself, as shown (Table I) by the lower oxidation potential of 195 mV.

The 3′-Dimethylamino Series

For both amsacrine and the 3′-$NHCH_3$ derivative **2**, detailed structure–activity relationships for acridine substitution have been determined.[3-6,11] The high in vivo biological activity (high ILS value and shallow dose–response curve) and favorable in vitro selectivity of the 3′-$N(CH_3)_2$ derivative **3** and its significantly different pattern of physicochemical properties made it of interest to similarly determine the structure–activity relationships for acridine substitution among this series and compare them. Accordingly, a series of 12 monosubstituted derivatives (**9–20**) and 16 disubstituted derivatives (**21–36**) were synthesized, and data on their physicochemical properties and biological activity are presented in Tables II and III. Lipophilicity was determined chromatographically for the drug cations[8] at pH 1–2, and DNA association constants were determined by the ethidium displacement method.[23] IC_{50} values are the nanomolar concentration of drug required to reduce cell growth to 50% of control values, and

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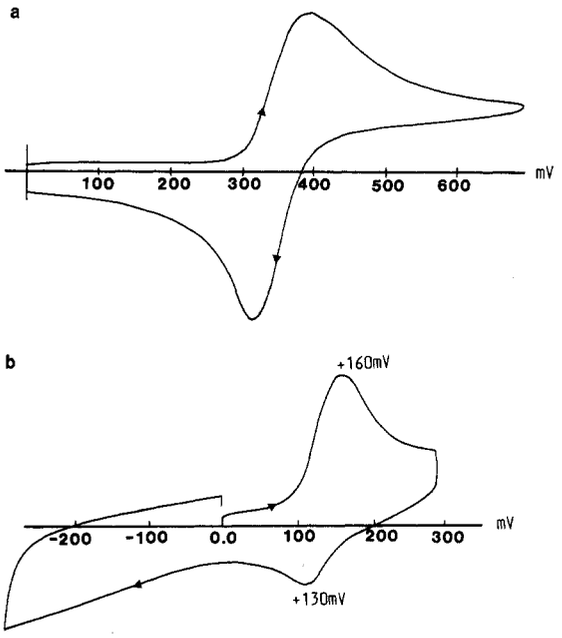

Figure 1. Cyclic voltammograms of amsacrine derivatives as 0.1 mM solutions in 40% acetonitrile/acetate buffer at pH 4.5: (a) compound 1 (amsacrine), (b) compound **2**.

Table III. Physical Data for the New Compounds of Table II

no.	mp, °C	formula	analysis
9	208–212	$C_{23}H_{24}N_4O_2S$·HCl	C, H, N[a], Cl
10	220–225	$C_{23}H_{24}N_4O_3S$·HCl	C, H, N, Cl
11	190–194	$C_{22}H_{21}FN_4O_2S$·HCl	C, H, N, Cl
12	267–270	$C_{22}H_{21}ClN_4O_{2s}$·HCl	C, H, N, Cl
13	257–260	$C_{22}H_{21}BrN_4O_2S$·HCl	C, H, N, Cl
14	210–215	$C_{22}H_{21}N_5O_4S_2$HCl	C, H, N, Cl
15	198–199	$C_{23}H_{24}N_4O_2S$·HCl·H_2O	C, H, N, Cl
16	205–209	$C_{23}H_{24}N_4O_3S$·HCl·H_2O	C, H, N, Cl
17	239–242	$C_{22}H_{21}FN_4O_2S$·HCl	C[b], H, N, Cl
18	196–200	$C_{22}H_{21}ClN_4O_2S$·HCl·H_2O	C, H, N, Cl
19	202–205	$C_{24}H_{25}N_5O_3S$·2HCl	C, H, N[a], Cl
20	205–209	$C_{25}H_{26}N_6O_4S$·2HCl	C, H, N, Cl
21	205–210	$C_{24}H_{26}N_4O_2S$·2HCl	C, H, N, Cl
22	227–231	$C_{26}H_{24}N_4O_2S$·2HCl	C, H, N, Cl
23	279–282	$C_{24}H_{26}N_4O_2S$·HCl·H_2O	C, H, N, Cl
24	255–256	$C_{24}H_{26}N_4O_3S$·HCl	C, H, N, Cl
25	205–210	$C_{23}H_{23}FN_4O_2S$·HCl·H_2O	C, H, N[a], Cl
26	225–230	$C_{23}H_{23}ClN_4O_2S$·2HCl	C, H, N, Cl
27	251–254	$C_{23}H_{23}BrN_4O_2S$·HCl	C, H, N, Cl
28	210–213	$C_{24}H_{26}N_4O_3S$·HCl	C, H, N, Cl
29	204–207	$C_{23}H_{23}FN_4O_3S$·HCl·H_2O	C, H, N, Cl
30	205–210	$C_23H_{23}ClN_4O_3S$·2HCl	C[a], H, N, Cl
31	238–240	$C_{23}H_{23}BrN_4O_3S$·HCl	C, H, N
32	256–258	$C_{24}H_{24}ClN_5O_3S$·HCl	C, H, N, Cl
33	255–257	$C_{24}H_{26}N_4O_2S$·HCl	C, H, N, Cl
34	210–213	$C_{24}H_{26}N_4O_4S$·HCl	C[b], H, N, Cl
35	273–275	$C_{25}H_{27}N_5O_3S$·HCl	C, H, N, Cl
36	234–236	$C_{25}H_{27}N_5O_4S$·HCl·H_2O	C, H, N

[a] N out by 0.5%. [b] C out by 0.5%.

protocols for both L1210 leukemia[24] and HCT-8 human colon carcinoma cells[25] have been published. In vivo testing against the P388 leukemia and LL carcinoma followed established protocols,[11,26] with the drug being given intraperitoneally as a solution in 30% aqueous EtOH 1 day after intraperitoneal inoculation of P388 leukemia or 5 days after intravenous inoculation of LL carcinoma cells. Drugs were given at 1.5 × dose intervals, up to and exceeding an acutely toxic dose, and the optimal dose (OD) quoted is the highest nontoxic dose (approximately the LD_{10}). Quoted ILS_{max} values are those determined at the optimal dose.

No 1- or 2-substituted compounds were studied, since well-established general structure–activity relationships for both the amsacrine and 3′-$NHCH_3$ series show that groups at these positions are always dystherapeutic.[9,11] While the use of 3-NHR substituents has been able to provide leukemia-active compounds of high potency in the amsacrine[9] and 3′-$NHCH_3$ series,[11] these compounds do not show LL activity (possibly due to concomitant high pK_a values) and thus similar derivatives were not evaluated in the current series. With these restrictions, the substituents were chosen to have a wide variation of electronic, lipophilic, and steric properties.

Lipophilicity. Lipophilicity has been shown to be a significant determinant of the in vivo activity of 9-anilinoacridines.[9] As noted above, the parent compound **3** is considerably more lipophilic than either amsacrine or the 3′-$NHCH_3$ derivative **2**. Pairwise comparison of the lipophilicities of the acridine-substituted compounds of all three series show this to be general as expected, with the compounds **9–36** of Table II being on average 0.19 R_m unit more lipophilic than their 3′-$NHCH_3$ counterparts (data from ref 11), and 0.10 R_m unit more lipophilic than the corresponding amsacrine analogues (data from ref 3 and 5).

DNA Binding. The data listed in Table I show that the 3′-$N(CH_3)_2$ group significantly lowers DNA binding. The decrease in binding constant for **3** over the 3′-$NHCH_3$ analogue **2** is 1.08 log units. This trend is closely followed by the acridine- monosubstituted and 3,5-disubstituted compounds of Table II (compounds **9–32**), which have association constants for binding to poly·[d(A-T)] on average 1.02 units lower than for the corresponding 3′-$NHCH_3$ derivatives (data from ref 11). The same set of compounds have association constants lower on average by 0.28 log unit than those of their amsacrine counterparts (data from ref 3 and 5), which is in exact agreement with the differential shown by the parent compounds **1** and **3** (Table I). For reasons that are not clear, the 4,5-disubstituted derivatives **33–36** do not follow this pattern and have binding constants little different from those of their amsacrine and 3′-$NHCH_3$ counterparts.

Within the present series itself, the pattern of acridine substituent effects upon DNA binding is similar to that observed previously for the 3′-$NHCH_3$ series,[11] with the 3,5-disubstitution pattern providing compounds of greater DNA affinity than would be expected from the effects of the individual substituents.

In Vitro Cytotoxicity. The 3′-$N(CH_3)_2$ derivatives generally show much lower potency against both the L1210 and HCT-8 cell lines than do the corresponding amsacrine and 3′-$NHCH_3$ derivatives. The parent compound **3** is 7.4-fold less potent against the L1210 than the corresponding 3′-$NHCH_3$ compound **2**, and this is also true of the substituted derivatives. Although there are individual variations, with the ratios varying from 23.7 to 1.3, all the acridine-substituted 3′-$N(CH_3)_2$ derivatives in Table II are less potent against the L1210 leukemia line than the corresponding 3′-$NHCH_3$ compounds (data from Table I and ref 11), and the average differential is 7.4-fold.

For the limited set of acridine-substituted compounds studied here, the structure–activity relationships for P388 cytotoxicity were broadly similar to those shown in the amsacrine and 3′-$NHCH_3$ series. Halogen, CH_3, and OCH_3

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substitution at the 3-position had little effect on cytotoxicity. The 4-CH_3 and 4-OCH_3 derivatives **15** and **16** did show improved potency, but no further enhancement was seen for the 3,5-disubstituted compounds **23–32**. The most striking feature of the in vitro data is the marked selectivity of the 3′-$N(CH_3)_2$ derivatives for the HCT-8 solid tumor line compared to the L1210 leukemia. The parent compound **3** has a ratio of 0.52 in favor of the HCT-8, and the monosubstituted derivatives **9–16**, especially the 4-substituted compounds, generally have similarly low ratios.

In Vivo Antitumor Activity. Complete dose–response curves were obtained for all compounds against both the P388 leukemia and LL carcinoma systems in vivo, and these data are summarized in Table II. ILS_{max} values are the %ILS (increase in lifespan of tumor-bearing, treated animals over tumor-bearing, untreated controls) determined at the OD for each compound and are a measure of tumor cell selectivity.[27] The D_{50} values (dose of drug needed to give an ILS of 50%; computed from the dose–response curves; see ref 27) are a measure of the dose needed to elicit a constant response and can also be used to determine therapeutic ratios (OD/D_{50}).

As noted above, the parent compound **3** shows very high P388 activity. Cures are seen at the OD of 250 mg/kg and for each lower dose level below that down to 66 mg/kg. The therapeutic ratio (OD/IC_{50}) is 10, and the compound still shows significant activity at a dose of 20 mg/kg. It also shows excellent activity against LL, with a proportion of cures with doses from 100 mg/kg down to 44 mg/kg. The 3-CH_3 and 3-OCH_3 derivatives **9** and **10** also showed good P388 and LL activity, but had smaller therapeutic ratios. However, the 3-halogen compounds **11–13** were significantly less active than the parent, in contrast to the results in the 3′-$NHCH_3$ series.[11] The 4-CH_3 and 4-OCH_3 derivatives **15** and **16** also show excellent activity, particularly against the LL. Indeed the 4-OCH_3 derivative showed the best LL activity of any 9-anilinoacridine to date, effecting 100% cures in repeated experiments at the OD of 100 mg/kg and at the next lowest dose of 67 mg/kg. The good activity of the 4-$CONHCH_3$ compound **19** is interesting, as the amsacrine analogue has similar activity[4] while the 3′-$NHCH_3$ analogue has very low activity.[11] The 3,4-disubstituted compounds **21** and **22** were only moderately active, and it was the 3,5-disubstituted derivatives **23–32** that generally showed the best activity. Of the two main sets of 3-halo-5-methyl (**25–27**) and 3-halo-5-methoxy (**29–31**) compounds, the former appeared to be the more active with the 3-Cl-5-CH_3 compound **26** providing some cures in both the P388 and LL systems. The 3-F-5-CH_3 derivative **25** was markedly inferior, in sharp contrast to the 3′-$NHCH_3$ series, where this latter substitution pattern clearly provides the best activity.[11] Of the other 3,5-disubstituted derivatives, the 3-OCH_3-5-CH_3 compound **24** is the most dose potent of all the 3′-$N(CH_3)_2$ analogues (as is also the case in the 3′-$NHCH_3$ series), but the isomeric 3-CH_3-5-OCH_3 compound **28** is the more active.

The 4,5-disubstituted compounds **33–36** offer an interesting contrast with the 3′-$NHCH_3$ series. In that series, the $(CH_3)_2$ and $(OCH_3)_2$ compounds have high activity in both tumor systems, while the 5-$CONHCH_3$ analogues are much less active.[11] In the present series the opposite is true, with the $(CH_3)_2$ and $(OCH_3)_2$ compounds **33** and **34** showing very poor activity compared to that of **35** and **36**. In fact, the 4-CH_3-5-$CONHCH_3$ compound **35** (the 3′-$N(CH_3)_2$ analogue of the clinical candidate **4**) is among the most active of the series, showing 50% cures in both tumor systems, a level of activity similar to that of **4** itself.

(27) Denny, W. A.; Cain, B. F. *J. Med. Chem.* **1978**, *21*, 430.

Conclusions

Structure–activity relationships for acridine substitution in the 3′-$N(CH_3)_2$ series of 9-anilinoacridines very broadly follow those observed previously in the amsacrine[3,5] and particularly the 3′-$NHCH_3$ series;[11] monosubstitution at the 3- and 4-positions and disubstitution at the 3,4- and 3,5-positions are compatible with activity in both the P388 and LL systems. When investigated in more detail, however, they lie closer to those for the amsacrine than the 3′-$NHCH_3$ series, particularly for the most weakly basic compounds. The 3′-$NHCH_3$ compounds also differ from those of the other two series in their irreversible redox chemistry,[22] but whether this is related to their different SAR is unclear. In the previous series, acridine substitution had a significant effect on biological activity, with certain compounds showing greatly superior activity to the parent (cf. amsacrine and **4**). In the present case, the very high activity of the parent compound **3** makes it difficult to improve upon, but the 4-OCH_3 and the 4-CH_3-5-$CONHCH_3$ derivatives (compounds **16** and **35**) may have superior ILS_{max} values. However, they have inferior therapeutic ratios compared to **3**.

The 3′-$N(CH_3)_2$ compounds do have much lower DNA binding levels and poorer in vitro cytotoxicity than the corresponding amsacrine and 3′-$NHCH_3$ compounds, and the much poorer in vivo potency of the series is probably related to these factors. Low potency, combined with the decreased water solubility, is a potentially serious problem, for a major clinical difficulty with amsacrine has been its low aqueous solubility.[28] An important goal of the current work was thus to identify acridine-substitution patterns that improved potency while retaining high, broad-spectrum activity. The best compounds in this regard are the isomeric 3,5 methyl/methoxy derivatives **24** and **28**. Although **24** is the more potent, with an OD of 20 mg/kg, the best compromise between potency and activity is the 3-Me-5-OMe derivative **28**, with an OD of 45 mg/kg.

With this detailed study of the 3′-$N(CH_3)_2$ series of amsacrine analogues, development of the 9-anilinoacridines has now progressed from amsacrine (**1**), which has only moderate P388 activity and is essentially inactive against the LL, to compounds such as **3** and its 4-OCH_3 analogue **16**, capable of effecting a majority of cures in both test systems. Selected members of the current series are now under advanced evaluation.

Experimental Section

Analyses within ±0.4% of theoretical values are indicated by symbols of the elements concerned. Analyses were carried out under the direction of Professor A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal apparatus using the manufacturers' thermometer and are as read. All intermediates had consistent NMR spectra, determined on a Varian 360-L spectrometer (Me_4Si).

Oxidation of Amsacrine Derivatives. A solution of the free base of **3** (1 g, 2.46 mmol) was suspended in dry EtOAc (150 mL). Activated MnO_2 (4 g) was added and the mixture was stirred for 1 h at 20 °C. The purple solution, which showed only one nonpolar spot on TLC, was filtered through Celite and evaporated at low temperature. The residue (1 g) was dissolved in EtOAc and crystallized by vapor diffusion of Et_2O to give black plates of N^1-(methylsulfonyl)-N^4-(9-acridinyl)-3-(dimethylamino)-2,5-cyclohexadiene-1,4-diimine (**7**), mp 260–280 °C dec. Anal. ($C_{22}H_{20}N_4O_2S$) C, H, N.

(28) Hodgson, H.; Kershaw, A. *J. Chem. Soc.* **1929**, 2917.
(29) Atwell, G. J.; Cain, B. F.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 1128.

Repeating the experiment with the free base of amsacrine (1) gave a good yield of the corresponding diimine **5**, mp 219–222 °C (lit.[14] mp 219–220 °C).

However, reaction of the free base of the 3-$NHCH_3$ derivative **2** gave a purple solution showing several products of varying polarity by TLC, and no pure compound corresponding to the above diimines could be isolated.

The free base of **4** was too insoluble in EtOAc, but a solution in Me_2CO oxidized smoothly to the corresponding diimine **8**, mp ($CHCl_3$/vapor diffusion with hexane) 225–228 °C. Anal. ($C_{24}H_{22}N_4O_4S$) C, H, N, S.

General Procedure: Preparation of Compound 4 of Table I. 3′-(Dimethylamino)-4′-nitroacetanilide (II). A suspension of 3′-chloro-4′-nitroacetanilide (I)[28] (6.8 g, 32.6 mmol) in 40% aqueous dimethylamine (45 mL) was heated at 80 °C with stirring for 3 h and then diluted with water (50 mL). The solid collected after 15 h at 0 °C (6.9 g, 98%) was homogeneous on TLC and could be used directly in the next step. A sample was recrystallized from aqueous EtOH, mp 107–107.5 °C. Anal. ($C_{10}H_{13}N_3O$) C, H, N.

3-(Dimethylamino)-4-nitroaniline (III). A solution of the above acetanilide (5 g, 23 mmol) in a mixture of 4 N aqueous HCl (25 mL) and EtOH (25 mL) was heated under reflux for 3 h. Evaporation of solvents followed by basification with NH_4OH gave the crude amine III in quantitative yield, sufficiently pure to use in the next step. A sample was recrystallized from aqueous EtOH, mp 114–115 °C. Anal. ($C_8H_{11}N_3O_2$) C, H, N.

3′-(Dimethylamino)-4′-nitromethanesulfonanilide (IV). A solution of the above amine (4.4 g, 24.3 mmol) in pyridine (20 mL) was treated at 0 °C with methanesulfonyl chloride (2.09 mL, 27.0 mmol). The mixture was kept at 20 °C for 4 h, and excess solvent was removed under vacuum. The residue was triturated with water to give a solid, which was extracted with 1 N aqueous NaOH. Neutralization of the filtered solution gave the desired sulfonamide (6.0 g, 96%), identical with an authentic sample.[10]

A suspension of the nitro compound IV in MeOH was hydrogenated over Pd/C at 2 atm until H_2 uptake ceased (15 min). The colorless solution was filtered to remove catalyst and immediately added to solid 9-chloroacridine (0.95 equiv). A trace of HCl was added to initiate the reaction, and the solution was then concentrated to small volume by boiling off the MeOH. EtOAc was then added dropwise to the hot solution until crystallization began. Recrystallization from MeOH/EtOAc gave red needles of the hydrochloride, mp 248–249 °C (ref 10) (Table II).

The same procedure was used to prepare the other compounds of Table I. The purity of these compounds was carefully monitored by TLC and by evaluation of the UV spectra for the characteristic 9-anilinoacridine absorption band around 434 nM (log E ca. 4.0).[30]

Registry No. **1**, 51264-14-3; **1**·HCl, 54301-15-4; **2**, 88412-78-6; **2**·HCl, 88412-53-7; **3**, 88412-94-6; **3**·HCl, 88412-72-0; **4**, 80841-47-0; **4**·HCl, 92138-16-4; **5**, 87764-57-6; **7**, 106063-43-8; **8**, 106063-47-2; **9**, 106521-45-3; **9**·HCl, 106521-30-6; **10**, 106521-46-4; **10**·HCl, 106521-31-7; **11**, 106521-47-5; **11**·HCl, 106521-32-8; **12**, 88914-34-5; **12**·HCl, 88913-76-2; **13**, 106521-48-6; **13**·HCl, 106521-33-9; **14**·2HCl, 106521-34-0; **15**, 88914-35-6; **15**·HCl, 88913-77-3; **16**, 88914-36-7; **16**·HCl, 88913-78-4; **17**, 106521-50-0; **17**·HCl, 106521-35-1; **18**, 106521-51-1; **18**·HCl, 106521-36-2; **19**, 88914-37-8; **19**·2HCl, 88913-79-5; **20**, 106521-52-2; **20**·2HCl, 106542-98-7; **21**, 106521-53-3; **21**·2HCl, 106521-37-3; **22**, 106543-00-4; **22**·2HCl, 106542-99-8; **23**, 106521-54-4; **23**·HCl, 106521-38-4; **24**, 88914-42-5; **24**·HCl, 88913-84-2; **25**, 88914-38-9; **25**·HCl, 88913-80-8; **26**, 88914-39-0; **26**·2HCl, 88913-81-9; **27**, 106521-55-5; **27**·HCl, 106521-39-5; **28**, 106521-56-6; **28**·HCl, 106521-40-8; **29**, 106521-57-7; **29**·HCl, 106521-41-9; **30**, 88914-40-3; **30**·2HCl, 106521-42-0; **31**, 106521-58-8; **31**·HCl, 106521-43-1; **32**, 88914-41-4; **32**·HCl, 88913-83-1; **33**, 88914-43-6; **33**·HCl, 88913-85-3; **34**, 88914-44-7; **34**·HCl, 88913-86-4; **35**, 88914-45-8; **35**·HCl, 88913-87-5; **36**, 106521-59-9; **36**·HCl, 106521-44-2; **I**, 712-33-4; **II**, 88914-67-4; **III**, 55851-38-2; **IV**, 88413-20-1; $NH(CH_3)_2$, 124-40-3; H_3CSO_2Cl, 124-63-0; 9-chloroacridine, 1207-69-8; 9-chloro-3-methylacridine, 16492-10-7; 9-chloro-3-methoxyacridine, 16492-14-1; 9-chloro-3-fluoroacridine, 2377-16-4; 3,9-dichloroacridine, 35547-70-7; 3-bromo-9-chloroacridine, 35547-72-9; 9-chloro-3-nitroacridine, 1744-91-8; 9-chloro-4-methylacridine, 16492-11-8; 9-chloro-4-methoxyacridine, 16492-15-2; 9-chloro-4-fluoroacridine, 3829-32-1; 4,9-dichloroacridine, 10166-44-6; 9-chloro-4-((*N*-methylamino)carbonyl)acridine, 63178-97-2; 9-chloro-4-((*N*-((carbamoyl)methyl)amino)carbonyl)acridine, 102940-90-9; 9-chloro-3,4-dimethylacridine, 6514-58-5; 3,4-benzo-9-chloroacridine, 102940-92-1; 9-chloro-3,5-dimethylacridine, 88914-93-6; 9-chloro-3-methoxy-5-methylacridine, 88914-94-7; 9-chloro-3-fluoro-5-methylacridine, 88914-95-8; 3,9-dichloro-5-methylacridine, 88914-96-9; 3-bromo-9-chloro-5-methylacridine, 88914-98-1; 9-chloro-3-methyl-5-methoxyacridine, 88914-99-2; 9-chloro-3-fluoro-5-methoxyacridine, 102940-93-2; 3,9-dichloro-5-methoxyacridine, 88914-97-0; 3-bromo-9-chloro-5-methoxyacridine, 6534-56-1; 3,9-dichloro-5-(*N*-methylcarbonyl)acridine, 86187-39-5; 9-chloro-4,5-dimethylacridine, 63345-58-4; 9-chloro-4,5-dimethoxyacridine, 89784-84-9; 9-chloro-4-methyl-5-(*N*-methylcarbonyl)acridine, 88915-00-8; 9-chloro-4-methoxy-5-(*N*-methylcarbonyl)acridine, 88377-34-8; 3-methoxy-4-nitro-*N*-(methylsulfonyl)benzenamine, 57165-05-6; 3-(methamine)-4-nitro-*N*-(methylsulfonyl)benzenamine, 88413-07-4.

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Potential Antitumor Agents. 49. 5-Substituted Derivatives of *N*-[2-(Dimethylamino)ethyl]-9-aminoacridine-4-carboxamide with in Vivo Solid-Tumor Activity

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Derivatives of *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide bearing a wide variety of different groups at the 5-position (and for comparative purposes at the 7-position) have been prepared, and their physicochemical properties and biological activities have been determined. Although both 5- and 7-substituted compounds bind equally well to DNA by intercalation, only the 5-substituted compounds have in vivo antitumor activity. All the 5-substituted compounds showed in vivo antileukemic activity, but only those bearing electron-withdrawing substituents sufficiently powerful to ensure the acridine chromophore was uncharged at physiological pH showed activity in vivo against the Lewis lung solid tumor. The weakly basic derivatives do not show greater intrinsic cytotoxicity or selectivity toward solid tumor cells, and their broader spectrum of in vivo antitumor activity is attributed to the fact that they exist predominantly as monocations, which can distribute more efficiently.

The DNA-intercalating agent *N*-[2-(dimethylamino)-ethyl]-9-aminoacridinecarboxamide (**1**) is the parent of a new class of antitumor drugs shown[1,2] to have good antileukemic activity both in vitro and in vivo. Initial struc-

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