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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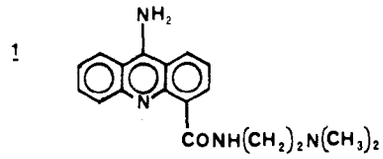
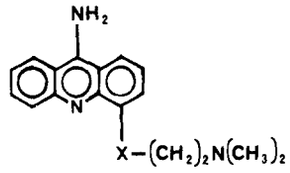
ture–activity relationships for these compounds showed the necessity for a cationic side chain in a fixed disposition with respect to the acridine chromophore.[1] Later work showed that, even if this geometrical relationship was retained, the group linking the side chain to the chromophore was also critical; thus the *N*-methyl carboxamide and sulfonamide derivatives **2** and **3** are inactive.[3] This requirement for a correctly positioned, strongly basic side chain linked to the acridine by a carboxamide function appears to be related to the dissociation kinetics of the compounds from DNA.[3] Only those derivatives bearing a side chain capable of providing two H-bond donors to a putative drug/DNA complex show a unique slow dissociation from DNA and have biological activity in vivo.



1 $CONH(CH_2)_2N(CH_3)_2$



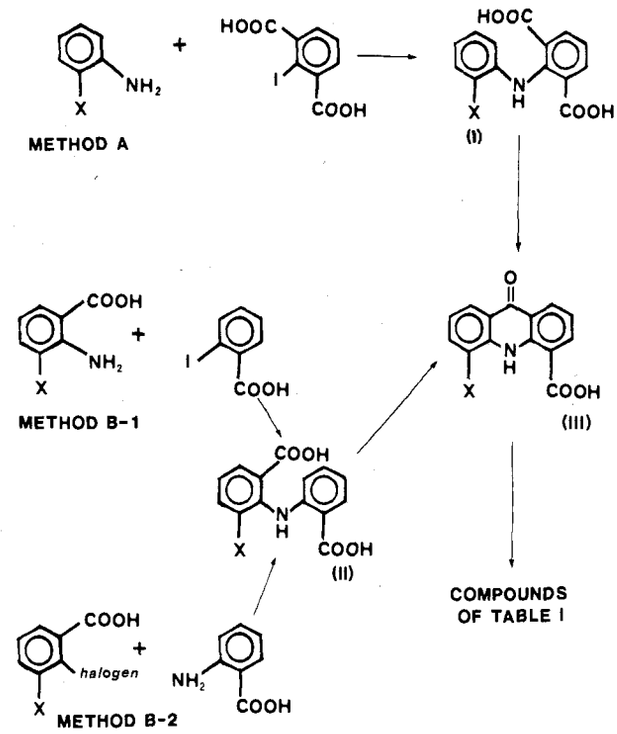
2 $X = -CON(CH_3)-$

3 $X = -SO_2NH-$

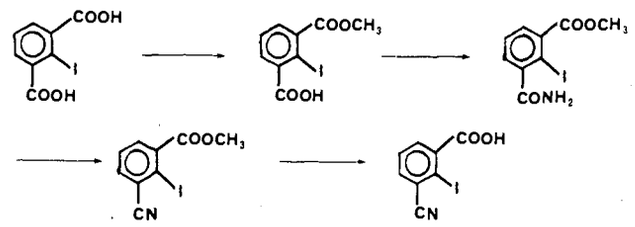
The parent compound **1** and a number of analogues bearing various (dialkylamino)ethyl side chains showed good in vivo activity (ILS values around 80%) and high potency against the P388 leukemia, but were inactive against the Lewis lung (LL) carcinoma.[1] This mouse solid tumor forms lung foci when inoculated intravenously, providing significant transport barriers to intraperitoneally inoculated drug.[4,5] In a search for analogues of **1** with a broader spectrum of activity (especially against solid tumors), a number of acridine-substituted derivatives were then made and similarly evaluated.[2] Methyl-, methoxy-, and chloro-substituted derivatives were studied, and marked effects were noted on both the absolute levels of in vitro cytotoxicity and on the ratios of cytotoxic potencies against leukemia (L1210) and solid-tumor (HCT-8 human colon carcinoma) cell lines. Compounds substituted in the 7- and 8-positions generally showed improved selectivity for the solid tumor in vitro but were inactive in vivo against both P388 leukemia and LL. Substitution in the 5-position led to highly dose-potent compounds that showed good in vivo antileukemic activity, but again the compounds were not active against the remotely implanted LL solid tumor.[2]

Since the 9-aminoacridine-4-carboxamides are very hydrophilic, dicationic species, it is possible that poor distribution limits their biological activity against remote targets. Studies with analogues of the clinical antileukemic agent amsacrine have shown that more weakly basic derivatives have a broader spectrum of activity, and this has been attributed partly to the fact that such compounds, with a greater proportion of neutral form at physiological pH, will distribute more effectively.[6,7] A logical extension of our development of the 9-aminoacridine-4-carboxamides was then to seek more weakly basic derivatives. Earlier work[1] had shown that attenuation of the pK_a of the cationic side chain led to complete loss of activity, but the limited range of acridine substituents so far examined had provided little information about the pK_a requirements for the chromophore. Since previous work had also shown that only substitution at the 5-position is generally compatible with high dose potency and in vivo activity (albeit antileukemic), this paper presents the synthesis, physicochemical properties, and biological evaluation of a number of new 9-aminoacridine-4-carboxamides with an extended range of substituents (especially electron-withdrawing ones) at the 5-position.

Scheme I



Scheme II



Chemistry

Basic routes to the substituted 9-oxoacridan-4-carboxylic acids (III) needed for the preparation of the compounds of Table I have been reported.[8,9] Many of the new 5-

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(5) Atwell, G. J.; Baguley, B. C.; Finlay, G. J.; Rewcastle, G. W.; Denny, W. A. *J. Med. Chem.* **1986**, *29*, 1769.
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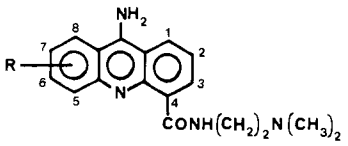
Table I. Physiochemical and Biological Properties of Substituted 9-Aminoacridinecarboxamides



					log K^c		in vitro $IC_{50}{}^d$			P388 in vivo		LL in vivo	
no.	R	method or ref	$R_m{}^a$	$pK_a{}^b$	AT	GC	L1210	HCT-8	ratio	OD[e]	$ILS_{max}{}^f$	OD	ILS_{max}
1	H	1	−1.11	8.30	7.08	7.55	15	66	4.4	4.5	98	4.5	NA[g]
4	5-CH_3	2	−1.02	8.01	7.55	7.86	0.47	11	23	2.6	107	1.8	NA
5	5-Ph	A	−0.57	7.50	6.92	7.41	1.1			2.3	54		
6	5-Ph-*p*-NO_2	A	−0.74		7.05	7.35	3.6			5.9	65		
7	5-Ph-*p*-NH_2	(A)	−1.16		7.46	7.64	5.5			5.9	68		
8	5-OCH_3	2	−1.06	7.80	7.62	7.83	4.3	89	21	3.9	81	3.9	NA
9	5-OPr	A	−0.85		8.00	7.92	3.3			5.9	47		
10	5-F	A	−1.11	7.11	7.90	8.18	1.4	73	52	3.9	90		
11	5-Cl	2	−1.03	6.87	7.48	7.23	2.9	33	11	2.6	81	13.3	NA
12	5-Br	A	−0.78	6.56	7.93	8.00	2.5			3.9	82	5.9	NA
13	5-NO_2	B-2	−1.18	6.59	8.41	8.41	1.6			0.8	39	0.8	NA
14	5-NH_2	(B-2)	−1.10	7.41	8.14	8.10	18	120		5.9	32	13.3	NA
15	5-CF_3	B-1	−0.55	5.89	7.85	8.03	5.7			13.3	115	13.3	40
16	5-SO_2CH_3	A	−1.35	5.15	7.32	8.30	2.8	451		30	138 (5)[h]	65	106 (1)
17	5-CN	B-2	−0.86	5.00	7.74	8.04	0.9			20	NA	20	NA
18	7-Ph	A	−0.61	7.27	7.96	7.96	78			13.3	NA		
19	7-OCH_3	2	−0.90	7.74	7.64	7.66	670	600	0.89	13.3	NA		
20	7-F	A	−0.98	7.76	7.76	8.03	33			20	37		
21	7-Cl	2	−0.89	7.48	7.67	7.85	722	485	0.67	13.3	NA		
22	7-SO_2CH_3	A	−1.24	6.93	7.71	8.00	3090			100	NA		NA

[a] R_m values were determined as detailed in ref 17, using 4′-(9-acridinylamino)methanesulfonanilide (AMSA) as a standard. [b] pK_a values were determined in aqueous solution spectrophotometrically, as detailed in ref 18. [c] log K = binding constant to poly[d(A-T)] or poly[d-(G-C)], determined by ethidium bromide displacement; see ref 19. [d] 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 20, 21. [e] OD = optimal dose of drug in mg kg^{-1} day^{-1}, 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 4. [f] ILS_{max} = the percentage increase in lifespan of drug-treated tumor-bearing (animals compared to that of untreated tumor-bearing) controls when treated at the optimal dose; values above 20% for P388 and above 40% for Lewis lung are considered statistically significant. [g] Compound inactive at all dose levels. [h] 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 LL).

substituted derivatives were conveniently prepared by condensation of the appropriate 2-substituted aniline and 2-iodoisophthalic acid[8] (method A; Scheme I). However this reaction fails if the substituent on the aniline is too deactivating; thus the 5-CF_3, 5-CN, and 5-NO_2 compounds were prepared from condensation of appropriately substituted 2-halo- and 2-aminobenzoic acids (methods B-1 and B-2, Scheme I), taking advantage of the fact that, for this substitution pattern only, the ring closure of the resulting diphenylamine diacids (II) is unequivocal.[10] 2-Iodo-3-cyanobenzoic acid for preparation of the 5-CN derivative was prepared from 2-iodoisophthalic acid by the method of Scheme II.

To circumvent this problem of deactivated anilines, the 5-SO_2CH_3 derivative was prepared by method A with 2-(methylthio)aniline, followed by cyclization and subsequent oxidation of the methylthio group; the corresponding 7-SO_2CH_3 derivative was similarly prepared. 5-Cyano-9-oxoacridan-4-carboxylic acid (III, X = CN) was also prepared from 5-(methoxycarbonyl)-9-oxoacridan-4-carboxylic acid (III, X = $COOCH_3$) by formation of the corresponding 9-chloroacridine-4-carbonyl chloride with $SOCl_2$, treatment of this with NH_4OH to give the amide, and subsequent dehydration with $POCl_3$ to the cyanide. Mild acid hydrolysis of the 9-chloro group gave methyl 5-cyano-9-oxoacridan-4-carboxylate, which on basic hydrolysis gave the corresponding acid (III, X = CN). However, although of fewer steps from the starting 2-iodoisophthalic acid than the route described above via 2-iodo-3-cyanobenzoic acid, the overall yield was inferior. Conversion of the 9-oxoacridan-4-carboxylic acids to the compounds of Table I followed established procedures, which involve conversion to the 9-chloroacridine-4-carbonyl chloride followed by selective sequential reaction with the appropriate amines.[1,2] While this procedure is usually efficient, conversion to the 9-Cl group is slow for acridines bearing electron-withdrawing substituents. To force this conversion to completion in such cases required long reaction times, and the 5-CF_3, 5-CN, and 5-SO_2CH_3 derivatives had to be heated under reflux in $SOCl_2$ for 4–6 h with occasional addition of catalytic amounts of DMF. The yield of product was

Table II. Dose–Response Relationships for Compounds 1 and 16 of Table I

	P388 in vivo		LL in vivo	
no.	dose[a]	ILS[b]	dose	ILS
1	6.7	77 (1)[c]	inact	inact
	4.5	93 (1)		
	3.0	98		
	1.2	67		
16	65	toxic	65	106 (1)
	45	38 (4)	45	76
	30	138 (5)	30	52
	20	217 (3)		
	8.9	133 (1)		
	5.9	152 (1)		
	2.6	76		
	1.2	38		

[a] Dose in mg kg^{-1} day^{-1}, given by the schedules noted in footnote *e*, Table I. [b] Percent ILS at the given dose. [c] See footnote *h*, Table I.

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(10) Stewart, G. M.; Rewcastle, G. W.; Denny, W. A. *Aust. J. Chem.* **1984**, *37*, 1939.

Table III. Analytical Data for the New Compounds of Table I

no.	mp, °C	formula	anal.
5	322–325	$C_{24}H_{24}N_4O\cdot 2HCl$	C, H, N, Cl
6	301–304	$C_{24}H_{23}N_4O_3\cdot 2HCl$	C, H, N, Cl
7	240–243	$C_{24}H_{25}N_5O\cdot 3HCl$	C, H[a], N, Cl
9	221–272	$C_{21}H_{26}N_4O_2$	C, H, N
10	294–296	$C_{18}H_{19}FN_4O\cdot 2HCl$	C[b], H, N, Cl
12	295–297	$C_{18}H_{19}BrN_4O\cdot 2HCl\cdot H_2O$	C, H, N, Cl
13	>360	$C_{18}H_{19}N_5O_3\cdot 2HCl$	C, H, N, Cl
14	326–329	$C_{18}H_{21}N_5O\cdot 2HCl\cdot H_2O$	C, H, N
15	301–304	$C_{19}H_{19}F_3N_4O\cdot 2HCl$	C, H, N, Cl
16	295–296	$C_{19}H_{22}N_4O_3S\cdot 2HCl$	C, H, N, Cl, S
17	305–307	$C_{19}H_{19}N_5O\cdot 2HCl\cdot H_2O$	C, H, N
18	300–302	$C_{24}H_{24}N_4O\cdot 2HCl\cdot H_2O$	C, H, N, Cl
20	320–323	$C_{18}H_{19}FN_4O\cdot 2HCl$	C, H, N, Cl
22	304–306	$C_{19}H_{22}N_4O_3S\cdot 2HCl$	C, H, N, Cl

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

reduced (in the case of the CN derivative to 20%) by the long reaction times, and extensive subsequent purification was required.

Results and Discussion

Tables I–III present physicochemical and biological data for 14 5-substituted derivatives of *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (**1**). The 5-CH_3 (**4**), 5-OCH_3 (**8**), and 5-Cl (**14**) derivatives have been reported previously[2] and provided a small set of substituents with a reasonable range of physicochemical (particularly electronic and lipophilic) properties. The remaining 11 compounds now bear substituents with a much greater range of electronic (NO_2 σ_p 0.78, SO_2CH_3 σ_p 0.72 to OPr σ_p −0.25) and lipophilic (OPr π 1.05 to SO_2CH_3 π −1.63) properties, as well as significant differences in steric bulk (F, E_s −0.46 to Ph, E_s −3.82). To provide a limited amount of pairwise comparison with substituents at different ring positions, several substituents of widely differing electronic and steric properties were also evaluated at position 7 on the acridine ring. The 7-substituted compounds were intended to serve as an indication of electronic effects, since both 5- and 7-substituents have been shown to have a similar electronic influence on acridine pK_a; any additional steric effects of the 5-substituents could thus be evaluated. Structure–activity relationships of substituents at the 7-position (in the direction of the long axis of the chromophore) have been shown to be very different from those at the 5-position (in the direction of the short axis);[2] all substituents so far examined (CH_3, OCH_3, and Cl) provided active derivatives at position 5 and inactive derivatives at position 7.

Drug lipophilicity was measured as previously[2] by liquid–liquid chromatography of the dications, and the results are as expected. For the 5-substituted derivatives, there is a reasonable relationship between substituent π values and the measured R_m values (eq 1; values from ref 11 and Table I).

$$R_m = -1.05\pi + 0.18 \quad (1)$$

$$n = 14,\ r = 0.80$$

The pK_a of the acridine nitrogen was considered to be an important parameter (see above). 9-Aminoacridine itself has a pK_a in water at 25 °C of 9.99, due to a large resonance stabilization of the charged form.[12] The pK_a value for the parent compound **1** determined spectroscopically is 8.30. This large decrease is due entirely to the carboxamide group and not to the second cationic function in the side chain, since the pK_a of the model compound *N*-methyl-9-aminoacridine-4-carboxamide was also 8.30. However, the pK_a of **1** is still high enough to ensure that the compound exists essentially as the dication at physiological pH (93% at pH 7.2). The 7-methoxy derivative **19** has a slightly lower pK_a (7.74) as expected from previous results with both 9-aminoacridines[12] and 9-anilinoacridines,[13] where substituents at both positions 2 and 7 exercise electronic influences on the acridine nitrogen according to their σ_m values. The 7-chloro derivative **21** has a lower pK_a again (7.48), but even this compound will exist mainly as the dication at physiological pH.

While both methyl and methoxy groups at the 5-position provided compounds of relatively high pK_a, the combination of electron-withdrawing and steric properties of the halogens have a considerable effect (compounds **10**–**12**). The steric effects on pK_a are clearly seen with the 5-Cl and 5-Br compounds (**11** and **12**) where, despite identical electronic effects, the greater size of the Br groups in limiting proton approach lowers pK_a by 0.31 unit over that of the 5-Cl analogue. The more powerful electron-withdrawing CF_3 group lowers pK_a to 5.89 (compound **15**), and even greater effects are seen with the very powerful electron-withdrawing SO_2CH_3 and CN groups. Thus, compound **16** has a pK_a of only 5.15, ensuring that the acridine is ionized to the extent of only 0.9% at pH 7.2. The significance of the steric effects of 5-substituents on acridine pK_a can also be seen by comparing the pK_a values of the 5-Cl and 5-SO_2CH_3 derivatives (**11** and **16**) with those of the 7-substituted analogues (**21** and **22**).

We have previously shown that in vitro activity of the substituted 9-aminoacridine-4-carboxamides depends more on the position of the substituent group than its nature.[2] Whereas methyl, methoxy, and chloro groups at the 5-position increase IC_{50} about 3–5-fold over that of the unsubstituted parent compound, the same groups at the 7-position greatly lower in vitro cytotoxicity (by about 50-fold). This pattern is maintained among the much greater range of substituted derivatives studied here. Although the groups at the 5-position in compounds **4**–**17** vary widely in electronic, hydrophobic, and steric properties, in vitro cytoxicity is remarkably constant. As an example, IC_{50} values for the 5-Ph, 5-Cl, 5-OCH_3, and 5-SO_2CH_3 compounds (**5**, **11**, **8**, and **16**) vary from 1.1 to 2.9 nM, while IC_{50} values for compounds bearing the same substituents at the 7-position (**18**, **19**, **21**, and **22**) vary between 670 and 720 nM. Representative compounds were also evaluated against the HCT-8 human colon line, and the same pattern of cell-line selectivity observed earlier[2] was seen; the 5-substituted compounds generally show marked selectivity for the leukemia cell line (HCT-8/L1210 ratios of 11 to 451), whereas the 7-substituted derivatives show ratios at or below unity.

The 5-substituted compounds (**4**, **8**, and **11**) had been previously shown to have good in vivo activity against the P388 leukemia (ILS values of 80–100%) at low dose levels (of 3–5 mg/kg), whereas the 7-substituted derivatives were inactive.[2] This pattern is again extended to the greater range of substituents examined here. Most of the 5-substituted derivatives (**4**–**17**) have ILS values from 60% to 90%, whereas all the 7-substituted compounds (**18**–**22**) are inactive. However, the weakly basic 5-substituted compounds (**15** and **16**) show the best activity. In particular,

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the 5-SO_2CH_3 compound **16** has greatly superior activity, with five/six animals cured at the optimal dose and a therapeutic ratio (optimal dose over minimum effective dose) of over 40 compared to a ratio of 5 for the parent compound (Table II). Although the weakly basic 5-CN derivative **17** was inactive in vivo, it showed very potent in vitro cytotoxicity.

None of the 9-aminoacridine-4-carboxamides previously evaluated[1,2] showed activity against the Lewis lung carcinoma. This tumor forms lung foci on intravenous injection and provides significant transport barriers to intraperitoneally administered drugs.[4,5] Although the majority of the 5-substituted derivatives studied here were also inactive against the solid tumor, moderate activity levels was shown by two of the least basic analogues (**15** and **16**).

Conclusions

A series of 9-aminoacridine-4-carboxamides bearing a wide range of substituents at the 5-position have been evaluated for antitumor activity. The weakly basic compounds **15** and **16** show the highest in vivo antileukemic activity and in addition are the first 9-aminoacridine-4-carboxamides to show in vivo activity against the remotely implanted LL solid tumor. Particularly for the 5-SO_2CH_3 derivative **16**, this activity cannot be due to any extraordinary level of intrinsic cytotoxicity or solid-tumor selectivity, since it has IC_{50} values against the L1210 leukemia similar to those of other derivatives and a ratio (L1210/HCT-8) poorer than any. Thus it is reasonable to attribute the superior level and broader spectrum of activity of this compound to its very weakly basic chromophore (pK_a = 5.15), which permits it to distribute almost entirely as a monocation, although metabolic redox modification of the methylsulfonyl group cannot be ruled out.

Experimental Section

Where elemental analyses are indicated only by the symbols of the element, results obtained were within ±0.4% of the theoretical value. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ, under direction of Professor A. D. Campbell. Melting points were determined on an Electrothermal apparatus using the supplied stem-corrected thermometer and are as read. ^1H NMR spectra were obtained on a Bruker WP-60 spectrometer (Me_4Si).

Synthesis of 5-Substituted 9-Oxoacridan-4-carboxylic Acids. Method A. 2-[*N*-[2-(Propyloxy)phenyl]amino]isophthalic Acid (I, X = OPr). A mixture of 2-iodoisophthalic acid (5.84 g, 20 mmol) and 2-(propyloxy)aniline (4.2 g, 28 mmol) in butane-2,3-diol (20 mL) and benzene (10 mL) were heated until the internal temperature reached 100 °C and most of the benzene had distilled. The mixture was cooled, and CuCl (1 g) and *N*-ethylmorpholine (7 mL) were added. The mixture was stirred at 120 °C for 2 h, cooled, and diluted with 2 N aqueous Na_2CO_3 (100 mL). The solution was treated with charcoal, filtered, acidified with 2 N HCl, and extracted into EtOAc. The organic layer was then extracted with 2 N aqueous Na_2CO_3, and this was acidified to give the crude product. Crystallization from EtOAc/petrol gave yellow needles (4.3 g, 68%), mp 222–224 °C. Anal. ($C_{17}H_{17}NO_5$) C, H, N.

Similar reactions using other anilines gave the following compounds: **2-[*N*-[2-(Methylthio)phenyl]amino]isophthalic acid (I, X = SCH_3)**, 69%, mp 211–213 °C (aqueous MeOH). Anal. ($C_{15}H_{13}NO_4S$) C, H, N, S. 2-[*N*-[2-(4-Nitrophenyl)phenyl]-amino]isophthalic acid (I, X = 4-NO_2Ph), 71%, mp 267–269 °C (EtOH). Anal. ($C_{20}H_{14}N_2O_6$) C, H, N. Also obtained from the appropriate 4-substituted anilines were the following compounds: 2-(*N*-4-Biphenylylamino)isophthalic acid, 82%, mp 260–262 °C (EtOH). Anal. ($C_{20}H_{15}NO_4$) C, H, N. 2-[*N*-(4-Fluorophenyl)-amino]isophthalic acid, 86%, mp 225–227 °C (EtOH). Anal. ($C_{14}H_{10}FNO_4$) C, H, N. 2-[*N*-[4-(Methylthio)phenyl]amino]isophthalic acid, 76%, mp 247–248 °C (aqueous EtOH). Anal. ($C_{15}H_{13}NO_4S$) C, H, N.

9-Oxo-5-(propyloxy)acridan-4-carboxylic Acid (III, X = OPr). The propyloxy diacid I (X = OPr) (3.3 g, 10.4 mmol) and polyphosphate ester (20 mL of the solution prepared by the method of Fieser and Fieser[14]) were heated to 100 °C for 1 h (allowing volatile solvents to evaporate). The cooled mixture was diluted with water, made basic with Na_2CO_3, treated with charcoal, filtered, and acidified (2 N HCl) to give the crude product (2.3 g, 74%), homogeneous on TLC. A sample crystallized from EtOH as yellow needles, mp 330–332 °C. Anal. ($C_{17}H_{15}NO_4$) C, H, N.

9-Oxo-5-(methylthio)acridan-4-carboxylic Acid (III, X = SCH_3). The methylthio diacid I (X = SCH_3) (3.0 g, 9.9 mmol) and polyphosphoric acid (50 g) were heated together with stirring at 120 °C for 1 h. The clear melt was poured slowly into hot water, and the precipitate was collected and washed well with water to give 9-oxo-5-(methylthio)acridan-4-carboxylic acid (III, X = SCH_3) (2.6 g, 91%), suitable for use in the next step. A sample was recrystallized from EtOH as yellow microcrystals, mp 297–299 °C (EtOH). Anal. ($C_{15}H_{11}NO_3S$) C, H, N, S.

Similar reactions with other diacids gave the following compounds: 9-Oxo-5-(4-nitrophenyl)acridan-4-carboxylic acid (III, X = 4-NO_2Ph) 87%, mp >360 °C (EtOH). Anal. ($C_{20}H_{12}N_2O_5$) C, H, N. 9-Oxo-5-(phenylthio)acridan-4-carboxylic acid (III, X = SPh), 86%, mp 204–206 °C (EtOH). Anal. ($C_{20}H_{13}NO_3S$) C, H, N. 9-Oxo-7-(methylthio)acridan-4-carboxylic acid, 92%, mp 343–344 °C (EtOH). Anal. ($C_{15}H_{11}NO_3S$) C, H, N. 9-Oxo-7-phenylacridan-4-carboxylic acid, 91%, mp >350 °C (EtOH). Anal. ($C_{20}H_{13}NO_3 \cdot 0.5H_2O$) C, H, N. 9-Oxo-7-fluoroacridan-4-carboxylic acid, 87%, mp >350 °C (EtOH). Anal. ($C_{14}H_8FNO_3$) C, H, N.

9-Oxo-5-(methylsulfonyl)acridan-4-carboxylic Acid (III, X = SO_2CH_3). A solution of 9-oxo-5-(methylthio)acridan-4-carboxylic acid (7 g, 24.5 mmol) in 1500 mL of glacial AcOH at 65 °C was treated with 150 mL of 30% H_2O_2. The mixture was maintained at 65–70 °C for 8 h and allowed to cool overnight. The crystalline product was collected and washed successively with glacial AcOH, MeOH, and water (6.13 g, 79%). A sample was crystallized from MeOH as needles, mp 310 °C dec. Anal. ($C_{15}H_{11}NO_5S \cdot H_2O$) C, H, N, S.

9-Oxo-7-(methylsulfonyl)acridan-4-carboxylic Acid. A solution of 9-oxo-7-(methylthio)acridan-4-carboxylic acid (2.6 g, 9.1 mmol) in aqueous KOH (1 equiv) was stirred at 70 °C and treated with 30% H_2O_2 (40 mL in two equal lots at an interval of 6 h). After a total of 12 h at 70 °C, the mixture was acidified with HCl and the precipitated product was collected and washed well with water (2.6 g, 90%). A sample was crystallized from EtOH as yellow plates, mp 350–352 °C. Anal. ($C_{15}H_{11}NO_5S$) C, H, N, S.

Method B-1. 2-[(2-Carboxyphenyl)amino]-3-(trifluoromethyl)benzoic Acid (II, X = CF_3). A suspension of 2-amino-3-(trifluoromethyl)benzoic acid[15] (2 g, 10 mmol), 2-iodobenzoic acid (2.4 g, 9 mmol), K_2CO_3 (2.1 g, 15 mmol), and Cu (0.01 g) in dry 2-ethoxyethanol (20 mL) was heated at 140 °C for 2 h with stirring. The cooled mixture was diluted with water, filtered, and acidified with 2 N HCl to give the crude diacid (1.9 g, 58%), homogeneous on TLC. A sample was crystallized from aqueous EtOH as plates, mp 243–246 °C. Anal. ($C_{15}H_{10}F_3NO_4$) C, H, N, F.

The crude compound was cyclized in polyphosphate ester by the method given above to give 9-oxo-5-(trifluoromethyl)-acridan-4-carboxylic acid (III, X = CF_3), 87%, mp >340 °C (EtOH). Anal. ($C_{15}H_8F_3NO_3$) C, H, N.

Method B-2. 2-[(2-Carboxyphenyl)amino]-3-nitrobenzoic Acid (II, X = NO_2). 2-Bromo-3-nitrobenzoic acid (7.5 g, 32 mmol), anthranilic acid (4.05 g, 30 mmol), and K_2CO_3 (6.2 g, 41 mmol) were stirred in *N*-methylpyrrolidone (20 mL) in an open beaker. When gas evaluation ceased, Cu (0.1 g) was added and the mixture was heated to 150 °C and held there for 30 min. The cooled mixture was diluted with water, filtered, and acidified with 2 N HCl. The resulting solid was dissolved in dilute aqueous K_2CO_3/EtOH (2:1, 150 mL), and the solution was added dropwise to rapidly stirred dilute HCl at 5 °C to give a yellow powder (8.06

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g, 89%), homogeneous by TLC. A sample crystallized from DMF had mp 270–272 °C (lit.[16] mp 283–285 °C).

9-Oxo-5-nitroacridan-4-carboxylic Acid (III, X = NO_2). The above diacid (8 g) was cyclized in concentrated H_2SO_4 (24 mL) at 100 °C for 3 h, to give a quantitative yield of the acridone, mp 347–350 °C (DMF). Anal. ($C_{14}H_8N_2O_5$) C, H, N.

Preparation of 3-Cyano-2-iodobenzoic Acid. 2-Iodoisophthalic acid (30 g, 0.103 mmol) was treated successively with $SOCl_2$ and anhydrous MeOH, and the product was dissolved in EtOAc and washed with 10% aqueous $KHCO_3$ to give dimethyl 2-iodoisophthalate (31.1 g, 95% yield). The crude diester was dissolved in MeOH (200 mL) and a solution of NaOH (4.26 g, 1.1 equiv) in water (50 mL) was added. The mixture was heated under reflux for 1 h, and the MeOH was removed under vacuum.

The aqueous solution was washed with EtOAc to remove unhydrolyzed starting material and acidified (2 N HCl), and the oily precipitate was extracted into EtOAc. Removal of solvent gave a solid, which was extracted with benzene at 20 °C. Concentration of the filtrate to small volume gave 2-iodo-3-(methoxycarbonyl)benzoic acid (23.8 g, 75% yield based on 2-iodoisophthalic acid), mp 118–119.5 °C. Anal. ($C_9H_7IO_4$) C, H, I.

A solution of 2-iodo-3-(methoxycarbonyl)benzoic acid (10 g, 33 mmol) in $SOCl_2$ (30 mL) was heated under reflux for 1 h. The residue after vacuum removal of volatiles was dissolved in CH_2Cl_2 and the solution was added to ice-cold aqueous NH_4OH. The organic layer was washed and concentrated to yield crude methyl 3-carbamoyl-2-iodobenzoate (95 g, 95%) suitable for use in the next step. A sample crystallized from water as needles, mp 174–176 °C. Anal. ($C_9H_8INO_3$) C, H, N.

The above crude product (9 g, 30 mmol) was heated at 100 °C for 1 h in $POCl_3$ (20 mL). After removal of volatiles, the residue was dissolved in CH_2Cl_2 and shaken with excess aqueous K_2CO_3 until gas evolution ceased. Removal of solvent gave a solid, which was filtered through SiO_2 in CH_2Cl_2 to give methyl 3-cyano-2-iodobenzoate (5 g, 59%), mp 94.5–95.5 °C (aqueous MeOH). Anal. ($C_9H_6NIO_2$) C, H, N.

The above ester (4 g, 14 mmol) was dissolved in MeOH (40 mL) and a solution of NaOH (1.1 equiv) in water (80 mL) was added. The resulting suspension was warmed until homogeneous, treated with charcoal, filtered, and acidified with HCl to give a quantitative yield of 3-cyano-2-iodobenzoic acid, mp 277–279 °C (aqueous EtOH). Anal. ($C_8H_4INO_3$) C, H, N.

2-[(2-Carboxyphenyl)amino]-3-cyanobenzoic Acid (II, X = CN). A mixture of 3-cyano-2-iodobenzoic acid (1.80 g, 6.6 mmol), 2-aminobenzoic acid (1.3 g, 9.9 mmol), CuCl (0.4 g), and *N*-ethylmorpholine (4 mL) in butane-2,3-diol (15 mL) was heated at 120 °C for 2 h. The cooled mixture was diluted with 1 N NH_4OH and worked up as above to give the cyano diacid (1.32 g, 71%), mp 339–341 °C (EtOH). Anal. ($C_{15}N_{10}N_2O_4$) C, H, N.

5-Cyano-9-oxoacridan-4-carboxylic Acid. The above diacid (II, X = CN) (1.2 g, 4.25 mmol) was cyclized in polyphosphate ester by the method given above, to yield 5-cyano-9-oxoacridan-4-carboxylic acid (0.95 g, 85%), mp >360 °C (EtOH). Anal. ($C_{15}H_8N_2O_3$) C, H, N.

Alternative Preparation of 5-Cyano-9-oxoacridan-4-carboxylic Acid. Reaction of 2-iodo-3-(methoxycarbonyl)benzoic acid (3.66 g, 0.01 mol) with 2-aminobenzoic acid as for method A above gave 2-[*N*-(2-carboxyphenyl)amino]-3-(methoxycarbonyl)benzoic acid (2.69 g, 85%), mp 192–195 °C ($CHCl_3$). Anal. ($C_{16}H_{13}NO_6$) C, H, N.

5-(Methoxycarbonyl)-9-oxoacridan-4-carboxylic Acid. The above monoester (2.0 g, 6.3 mmol) was heated with polyphosphate ester at 100 °C for 1 h. The cooled reaction mixture was diluted with water, and the resultant yellow solid that forms was collected and washed with MeOH/water (4:1). The crude product was dissolved in 50% aqueous MeOH containing 5% Et_3N at room temperature, filtered, and acidified with glacial AcOH. Concentration of the solution gave pure material (1.22 g, 65%), mp 334–336 °C. Anal. ($C_{16}H_{11}NO_5$) C, H, N.

5-(Methoxycarbonyl)-9-oxoacridan-4-carboxamide. A suspension of 5-(methoxycarbonyl)-9-oxoacridan-4-carboxylic acid (1.0 g, 3.36 mmol) in $SOCl_2$ (50 mL) containing DMF (1 drop) was heated and stirred under reflux until a clear solution was obtained and then for a further 1 h. After evaporation of volatiles under vacuum, the residue was azeotroped twice with dry benzene to remove traces of $SOCl_2$ and stirred in dry CH_2Cl_2, and an ice-cold mixture of CH_2Cl_2 (100 mL) and concentrated NH_4OH (50 mL) was added. The organic layer was washed once with water, dried, and evaported to give crude 9-chloro-5-(methoxycarbonyl)acridine-4-carbonyl chloride as a yellow solid. This was suspended in hot MeOH (100 mL) and 2 N HCl (10 mL) was added; initial dissolution of the 9-chloroacridine was followed by precipitation of 5-(methoxycarbonyl)-9-oxoacridan-4-carboxamide (0.60 g, 60%), mp 313–315 °C. Anal. ($C_{16}H_{12}N_2O_4$) C, H, N.

A suspension of the above ester (0.5 g) in hot MeOH (20 mL) was diluted with hot 1 N NaOH solution (30 mL) and resulting solution was filtered and acidified with glacial AcOH to give 5-carbamoyl-9-oxoacridan-4-carboxylic acid (0.42 g, 88%), mp 344–346 °C. Anal. ($C_{15}H_{10}N_2O_4$) C, H, N.

The above 5-(methoxycarbonyl)-9-oxoacridan-4-carboxamide (1.0 g, 3.38 mmol) was heated in $POCl_3$ at 100–110 °C for 1 h. Excess reagent was removed under vacuum, and the residue was dissolved in CH_2Cl_2 and treated as usual with ice-cold NH_4OH. Usual workup gave methyl 5-cyano-9-oxoacridan-4-carboxylate (0.87 g, 93%), mp 231–233 °C (EtOH). Anal. ($C_{16}H_{10}N_2O_3$) C, H, N.

Basic hydrolysis then gave 5-cyano-9-oxoacridan-4-carboxylic acid (III, X = CN), identical with the sample prepared above.

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