[N-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamate (5e). A mixture of 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide[17] (1 mol equiv), $CaCO_3$ (1 mol equiv), and the appropriate propargylamine **4b,c,e** (1 mol equiv) in DMA (5 mL/mmol) was stirred at room temperature for 48 h (**5e** for 6 days). The mixture was filtered, the solid washed with DMA, and the solvent removed in vacuo. The resulting gum was purified by column chromatography (Table II).

Diethyl N-[3-Chloro-4-[N-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamate (5d). To a stirred suspension of **7**[9] (1.00 g, 1.88 mmol) in $CHCl_3$ (100 mL) was added SO_2Cl_2 (0.64 g, 4.74 mmol), and the mixture was stirred for 40 min at room temperature. H_2O (50 mL) was added, the organic phase separated, washed twice with H_2O, dried ($MgSO_4$), and the solvent removed in vacuo. The resulting gum was purified by chromatography (Table II).

N-[2-Chloro-4-[N-[(2-amino-4-hydroxy-6-quinazolinyl)-methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1b), N-[2-Methyl-4-[N-[(2-amino-4-hydroxy-6-quinazolinyl)-methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1c), N-[3-Chloro-4-[N-[(2-amino-4-hydroxy-6-quinazolinyl)-methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1d), N-[3,5-Dichloro-4-[N-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1e). The appropriate diester 4 (1 mol equiv) was suspended in 50% EtOH–H_2O (40 mL/mmol) and treated with 1.0 N NaOH [3 mol equiv (in the case of **1d** and 1e, 6 mol equiv was used with an initial compound concentration of 1 mmol/60 mL)] and the mixture stirred at room temperature for 18 h. Addition of 3 mol equiv of 0.1 N HCl (6 mol equiv for **1d** and 1e) to pH 4.0 gave a gelatinous precipitate, which was purified by six cycles of centrifugation–decantation and resuspension in H_2O. The final aqueous suspension was freeze-dried to give an amorphous white solid. Details of the individual preparations are collected in Table III; spectroscopic data are in Tables IV and V.

N-[4-(Prop-2-ynylamino)benzoyl]-L-glutamic Acid (9). NaOH (1 N; 38 mL, 38 mmol) was added to a stirred suspension of **6**[9] (3.44 g, 9.55 mmol) in 33% EtOH–H_2O (75 mL) and the mixture stirred for 18 h at room temperature. Some unreacted ester was removed by filtration and the basic solution extracted twice with ether, acidified to pH 4 with 2 N HCl, and extracted four times with EtOAc. The combined organic extracts were washed with brine and dried ($MgSO_4$), and the solvent was removed in vacuo to give a yellow oil, which solidified on standing (1.80 g, 58%): mp 90–95 °C dec; NMR satisfactory. Anal. ($C_{15}H_{16}N_2O_5 \cdot 0.25$ EtOAc) C, H, N. The EtOAc could not be removed by drying and it was confirmed by NMR spectroscopy.

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Registry No. 1a, 76849-19-9; **1b**, 80014-98-8; **1c**, 80014-99-9; **1d**, 100020-40-4; 1e, 100020-41-5; **2b**, 80014-91-1; **2c**, 80015-10-7; **3b**, 100020-42-6; **3c**, 80014-85-3; **4b**, 80014-87-5; **4c**, 80014-86-4; 4e, 100020-43-7; **5b**, 80014-79-5; **5c**, 80014-80-8; **5d**, 100020-44-8; **5e**, 100020-45-9; **6**, 76858-72-5; **7**, 76858-74-7; **9**, 100020-46-0; TS, 9031-61-2; DHFR, 9002-03-3; diethyl L-glutamate, 16450-41-2; 2-chloro-4-nitrobenzoyl chloride, 7073-36-1; 2-methyl-4-nitrobenzoyl chloride, 30459-70-2; propargyl bromide, 106-96-7; 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide, 77766-62-2.

Supplementary Material Available: Figure 1, UV spectra of the analogues **1b–e** and the quinazoline **8**. All solutions were 20 μM in 0.1 N NaOH. Figure 2, UV spectra of N^{10}-propargyl-5,8-dideazafolic acid (1a) and compounds **8** and **9**. All solutions were 20 μM in 0.1 N NaOH (2 pages). Ordering information is given on any current masthead page.

Potential Antitumor Agents. 46. Structure–Activity Relationships for Acridine Monosubstituted Derivatives of the Antitumor Agent N-[2-(Dimethylamino)ethyl]-9-aminoacridine-4-carboxamide

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A series of monosubstituted derivatives of the new antitumor agent N-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide has been prepared, bearing methyl, methoxy, and chloro groups at available acridine positions. The physicochemical properties and antitumor activity of these compounds varied more with the position than with the nature of the substituent groups. The highest levels of both in vitro and in vivo antileukemic activity were shown by 5-substituted derivatives, while 7- and 8-substituted derivatives possessed the highest selectivity toward the HCT-8 human colon carcinoma line compared to the L1210 mouse leukemia line in vitro.

We recently reported the preparation and evaluation of the first examples of a new class of antitumor agent, the dibasic 9-aminoacridine-4-carboxamides.[1] These compounds fall into the general group of DNA-binding agents, binding tightly to double-stranded DNA by intercalation of the acridine chromophore between the base pairs.[1,2] The 9-aminoacridine-4-carboxamides (e.g., 1) show some selectivity for GC base pairs, suggesting that the cationic side chain makes additional binding contacts to accessible guanosine and/or cytosine residues.

Initial structure–activity relationships for this class of compound showed that a side chain that contained a cationic center positioned at a fixed distance (about 8 Å) from the acridine chromophore was essential. Significant attenuation of the pK_a of the side-chain nitrogen (e.g., to give 2 from 1) or alteration of its position relative to the chromophore (e.g., compounds 3 and 4) abolished cytotoxic activity, while compounds with the correct charge dispo-

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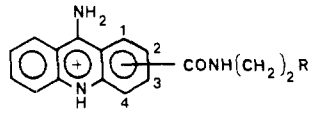
sition and similar lipophilicity remained highly active.



	SIDECHAIN POSITION	R
1	4	$N(CH_3)_2$
2	4	
3	3	$N(CH_3)_2$
4	2	$N(CH_3)_2$

The most active members of the series (e.g., 1) show promise as antitumor agents. They are stable, water-soluble compounds with high cytotoxicity against the L1210 mouse leukemia and HCT-8 human colon adenocarcinoma cell lines in vitro and good in vivo activity against the P388 leukemia.[1]

DNA-binding antitumor agents have generally been considered to exert their biological activity through their ability to interact tightly but reversibly with DNA,[3] and structure–activity relationship studies for several different classes of such compounds have shown a positive correlation between drug/DNA binding constants and cytotoxic activity.[4-6] Since substituent groups attached to the DNA-binding chromophore would be expected to alter binding properties, they may have a considerable effect on biological activity. A recent study[7] of analogues of bisantrene showed that small substituents on the anthracene chromophore did not enhance activity, but larger groups diminished it. This was interpreted as resulting from disadvantageous steric effects on intercalation, although no binding data was presented. In contrast, substitution of the acridine ring of amsacrine (a 9-anilinoacridine derivative) has been shown to have positive as well as negative effects on antitumor activity; while injudicious choice of substituent can abolish activity, certain combinations enormously enhance it. A comprehensive quantitative structure–activity relationship (QSAR) study[8] of 9-anilinoacridine derivatives showed that a 25-fold increase in the in vivo potency of amsacrine could be obtained by certain acridine substitution patterns (see compounds **162**, D_{50} = 3.3 mg/kg, and **379**, D_{50} = 0.14 mg/kg, of ref 8). Thus, a logical step in the development of the 9-aminoacridine-4-carboxamide class of antitumor agents was an examination of the effects of substituents at various positions of the acridine chromophore. We report here the synthesis and evaluation of monosubstituted derivatives of 1 bearing methyl, methoxy, and chloro groups.

Chemistry

Since a suitable preparation of 1 from 9-oxoacridan-4-carboxylic acid is available,[1] the chemistry reduced to the problem of preparing the appropriately substituted 9-oxoacridan-4-carboxylic acids (II).[9] The most widely employed route to 9-oxoacridans is acid-catalyzed ring closure of a diphenylamine-2-carboxylic acid, obtained in turn by Jourdan–Ullmann copper-catalyzed condensation of an aromatic halide and amine.[10,11] Logical extension of this scheme to the preparation of substituted 9-oxoacridan-4-carboxylic acids (II) would employ substituted 2-halobenzoic and anthranilic acids to provide the substituted *N*-(2-carboxyphenyl)anthranilic acids (I) (Scheme I, method A). Although the reactions to form I are virtually quantitative,[1] the usefulness of this route is compromised by the fact that the cyclization to II is unequivocal only for 3-substituted diacids I, which in turn require 3-substituted halobenzoic or anthranilic acids, some of which are difficult to obtain.

Ring closure of 4-, 5-, and 6-substituted diacids I gives a mixture of products, difficult to separate because of their insoluble nature. A detailed study[12] of the course of the reaction showed the direction of ring closure to be dictated predictably by the electronic and (for 6-substituted diacids) steric effects of the substitutent groups. While judicious choice of substituent and cyclizing reagent can lead to essentially one product in certain cases (and has been used previously[13] to prepare the substituted 9-oxoacridan-4-carboxylic acid precursors for the preparation of compounds **14–16** of Table I), the reaction is only of limited use as a general synthetic method.

An obvious extension of method A is to employ substituted diacids V, where one of the acid groups is suitably protected (e.g., as a methyl ester). Cyclization with polyphosphate ester then proceeds cleanly to the 9-oxoacridans VI, which give the desired compounds II on hydrolysis (Scheme I). Preparation of the half esters V by the usual base-catalyzed Jourdan–Ullman reaction using substituted methyl anthranilates IV is compromised by significant amounts of hydrolysis during the reaction, leading to undesired diacid I.[13] This can be avoided by the use of diphenyliodonium-2-carboxylate (III) as the acid component[14] (Scheme I, method B). The reaction then proceeds cleanly in the absence of base to give the half-esters V. The substituted 9-oxoacridan-4-carboxylic acids needed for the preparation of compounds **5–10** of Table I were prepared by this route. In an extension of this method, the precursors for compounds **20** and **22** were similarly prepared from substituted diphenyliodonium-2-carboxylates.

For the 5- and 7-substituted 9-oxoacridan-4-carboxylic acids (precursors for compounds **11–13** and **17–19** of Table I), the method of choice is usually to employ 2-haloisophthalic acid in the Jourdan–Ullmann reaction, providing diacids VII (Scheme I, method C). The use of *N*-ethylmorpholine as an anhydrous base to avoid hydrolytic side-reactions and employment of secondary diols such as butane-2,3-diol as solvent to give adequate solubility of all components result in high yields of diacids VII from this

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(9) For clarity, all substituted derivatives have been named from 9-oxoacridan-4-carboxylic acid, although it is recognized that for some compounds (e.g., 7-methoxy-9-oxoacridan-4-carboxylic acid) the alternative nomenclature (2-methoxy-9-oxoacridan-5-carboxylic acid) is correct.
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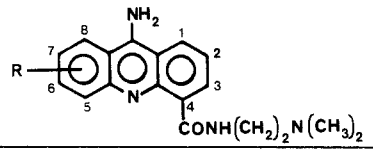
Table I. Physiochemical and Biological Data for Substituted 9-Aminoacridinecarboxamides



			chromatographic data			DNA binding			in vitro cytotoxicity (ID_{50})			in vivo act. (P388)	
no.	R	method[a]	ΔR_m[b]	MPH[c] (× 10^{-7})	retention time, min	unwinding angle,[d] deg	log K(AT)[e]	log K(GC)[f]	L1210[g]	HCT-8[h]	ratio	OD[i]	ILS[j]
1	H	A	−1.11	8.62	4.67	15	7.08	7.55	15	66	4.4	4.5	98
5	1-OCH_3	B	−1.12	4.30	6.00	13	7.31	7.52	0.55	19	34	8.9	27
6	1-Cl	B	−1.07	5.02	5.33		7.13	7.30	4.8	360	75	8.9	29
7	2-CH_3	B	−0.93	5.64	5.67		7.11	7.07	590	425	0.72	30	k
8	2-OCH_3	B	−0.95	5.62	5.87	17	7.68	8.44	2.9	32	11.0	5.9	72
9	2-Cl	B	−0.99	6.26	5.47		7.30	7.47	300	325	1.1	20	k
10	3-OCH_3	B	−1.21	8.10	5.67	17	7.21	7.12	300	780	2.2	20	k
11	5-CH_3	C	−1.02	5.16	6.07		7.55	7.86	0.47	11	23	2.6	107
12	5-OCH_3	C	−1.06	4.20	5.80	18	7.62	7.83	4.3	89	21	3.9	81
13	5-Cl	C	−1.03	4.80	6.00		7.48	7.23	2.9	33	11	2.6	81
14	6-CH_3	A	−0.82	4.76	6.13		7.26	7.99	55	120	2.2	2.6	20
15	6-OCH_3	A	−0.88	6.14	6.00	12	7.33	7.71	15	113	0.75	8.9	k
16	6-Cl	A	−0.81	5.04	6.53		7.32	7.87	105	245	2.3	8.9	k
17	7-CH_3	C	−0.87	5.02	6.27		7.35	7.30	605	485	0.80	13.3	k
18	7-OCH_3	C	−0.90	3.78	6.20	13	7.64	7.66	670	600	0.89	13.3	k
19	7-Cl	C	−0.89	4.78	6.40		7.67	7.85	722	485	0.67	13.3	k
20	8-CH_3	B	−1.10	4.16	5.67		7.21	7.12	340	250	0.73	8.9	k
21	8-OCH_3	C	−1.07	2.90	6.07		7.68	7.55	220	486	2.2	45	k
22	8-Cl	B	−1.11	4.38	5.67	14	7.23	7.39	250	360	0.47	20	k

[a] A, B, C: method of preparation of corresponding 9-oxoacridan-4-carboxylic acid; see Scheme I. [b] R_m values determined by liquid–liquid chromatography as in ref 16, using 4′-(9-acridinylamino)methanesulfonanilide (AMSA) as standard. [c] MPH: molar peak height in OD units/mole determined at 254 nM. [d] Unwinding angles for closed circular supercoiled DNA from *E. coli* plasmid PML-21, relative to ethidium bromide as 26°, determined as described in ref 1. [e,f] Log K: association constants for drug binding to poly·d(AT) and poly·d(GC) determined by ethidium bromide displacement according to ref 5; no quenching correction was found necessary. [g,h] ID_{50}: the concentration of drug (in nM) needed to reduce the number of cells in L1210 leukemia or HCT-8 human colon carcinoma cell cultures to 50% of controls over a 70-h incubation. [i] OD: optimal dose of drug in milligrams/kilogram 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. [j] ILS: the average percentage increase in lifespan of treated animals over that of control groups of tumor-bearing untreated animals. Values of ILS greater than 20% (for P388) or greater than 40% (for Lewis lung) are considered statistically significant. [k] –: no activity at all dose levels up to acutely toxic ones.

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

no.	mp, °C	formula	anal.
5	182–183	$C_{19}H_{22}N_4O_2$	C, H, N
6	169–170	$C_{18}H_{19}ClN_4O{\cdot}2HCl$	C, H, N, Cl
7	308–311	$C_{19}H_{22}N_4O{\cdot}2HCl$	C, H, N, Cl
8	309–310	$C_{19}H_{22}N_4O_2{\cdot}2HCl$	C, H, N, Cl
9	319–321	$C_{18}H_{19}ClN_4O{\cdot}2HCl$	C, H, N, Cl
10	270–272	$C_{19}H_{22}N_4O_2{\cdot}2HCl$	C, H, N, Cl
11	321–323	$C_{19}H_{22}N_4O{\cdot}2HCl$	C,[a] H, N, Cl
12	>360	$C_{19}H_{22}N_4O_2{\cdot}2HCl$	C, H, N
13	311–312	$C_{18}H_{19}ClN_4O{\cdot}2HCl$	C, H, N, Cl
14	326–328	$C_{19}H_{22}N_4O{\cdot}2HCl$	C, H, N, Cl
15	256–258	$C_{19}H_{22}N_4O{\cdot}2HCl$	C, H, N, Cl
16	285–286	$C_{18}H_{19}ClN_4O{\cdot}2HCl$	C, H, N, Cl
17	316–319	$C_{19}H_{22}N_4O{\cdot}2HCl$	C, H, N, Cl
18	290–292	$C_{19}H_{22}N_4O{\cdot}2HCl{\cdot}{}^1/_2H_2O$	C, H, N, Cl
19	310–311	$C_{18}H_{19}ClN_4O{\cdot}2HCl$	C,[a] H, N, Cl
20	244–246	$C_{19}H_{22}N_4O{\cdot}2HCl$	C, H, N, Cl
21	243–246	$C_{19}H_{22}N_4O_2{\cdot}2HCl{\cdot}{}^1/_2H_2O$	C, H, N, Cl[b]
22	241–243	$C_{18}H_{19}ClN_4O{\cdot}2HCl$	C, H, N, Cl

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

reaction.[15] 8-Methoxy-9-oxoacridan-4-carboxylic acid for compound **21** was prepared by hydrogenolysis of the corresponding 5-chloro-8-methoxy isomer,[15] obtained by method C.

Elaboration of the acids to the compounds of Table I followed the previously described method[1] (Scheme I). Treatment with $SOCl_2$ gave the 9-chloroacridine-4-carbonyl chlorides VIII, which were treated sequentially with *N,N*-dimethylethylenediamine to give the 9-chloro 4-amides IX and then by dry NH_3 in phenol to replace the 9-chloro group. The compounds were obtained as dihydrochloride salts (from MeOH–EtOAc–HCl) and were monitored for purity by HPLC. Some difficulties were encountered with 1- and 3-substituted 9-oxoacridan-4-carboxylic acids, where the presence of the electron-withdrawing 4-substituent activated substituents in the same ring. This had previously been noted[13] and in the present case precluded the preparation of the 1- and 3-methyl isomers, where all attempts at activation of the precursor 1- or 3-methyl-9-oxoacridan-4-carboxylic acids gave intractable mixtures. Treatment of the 3-chloro derivative of IX under the usual conditions (excess phenol at 110 °C, followed by treatment with NH_3) resulted in reaction of the 3-chloro group with phenol; the structure of the resulting 9-amino-3-phenoxy derivative was confirmed by ^1H NMR spectroscopy and elemental analysis and by HPLC.

Results and Discussion

Physicochemical and biological data for the parent unsubstituted compound **1** and 18 monosubstituted derivatives are recorded in Table I. As part of a systematic exploration of this new class of antitumor agent, the methyl, methoxy, and chloro substituent groups were chosen for evaluation. These provide a reasonable variation of electronic and hydrophobic properties, and our aim was to prepare all seven possible monosubstituted derivatives of **1** bearing each substituent. Evaluation of the resulting structure–activity relationships should then provide information about the electronic, hydrophobic, and steric influence of substituent groups at all available acridine positions.

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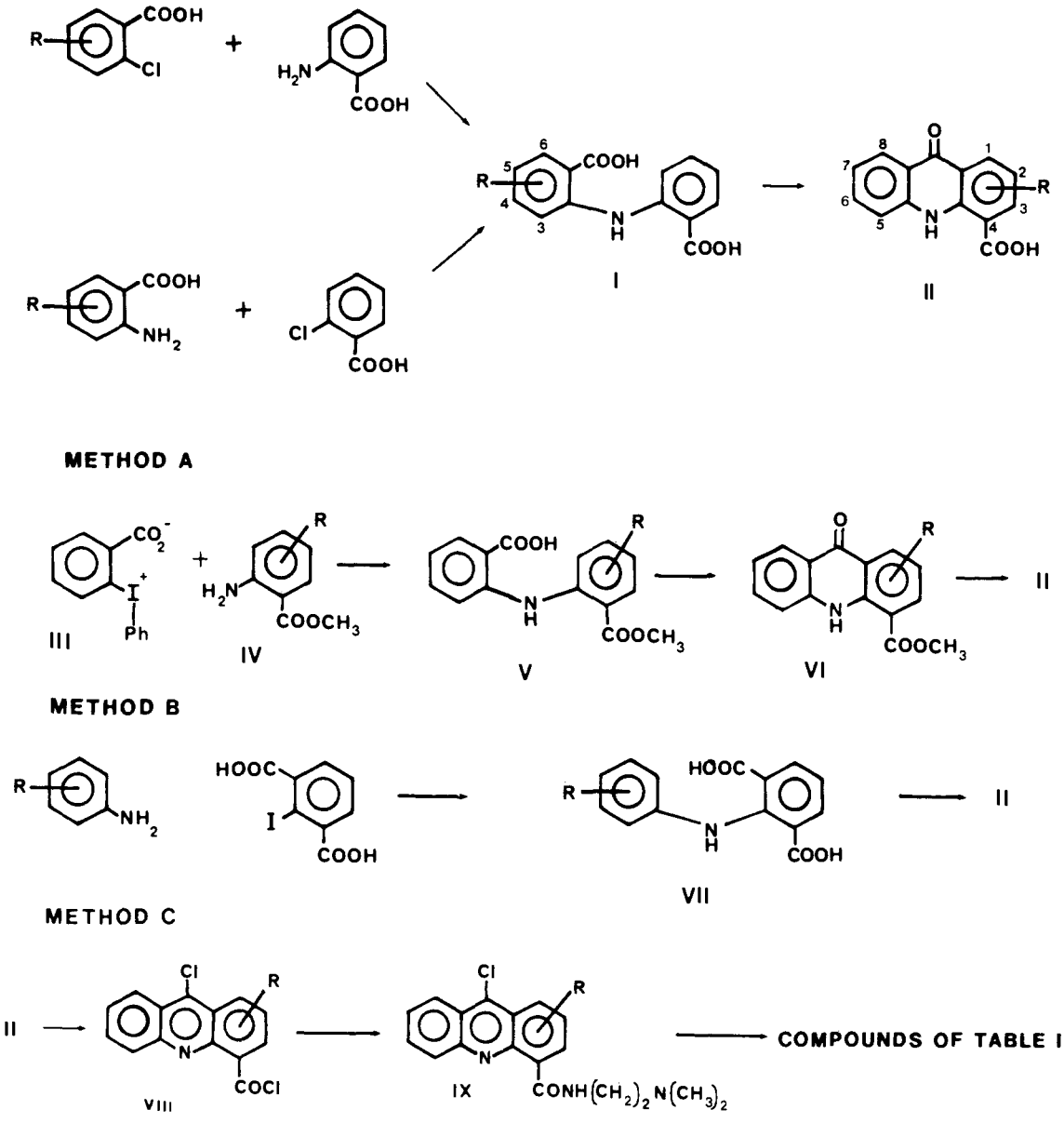
Scheme I. Synthesis of Substituted 9-Aminoacridine-4-carboxamides



As discussed above, we were unable to prepare the 1- and 3-methyl and 3-chloro 4-carboxamide derivatives. However, the remaining 18 compounds allow a clear picture to be drawn of substituent effects on antitumor activity in this series of compounds.

The substituted 9-aminoacridine-4-carboxamides are very polar, as determined by liquid–liquid chromatography (ΔR_m values[16]). The three substituents chosen range in lipophilicity over 0.73 log P unit[17] (π_{CH_3} 0.56, π_{OCH_3} −0.02, π_{Cl} 0.71), which corresponds to a range of 0.2 R_m unit.[16] However, for substitution at any particular position these substituent group differences appear to be masked, with variations of only 0.03–0.07 R_m unit observed (Table I). In contrast, variation of lipophilicity depending on *position* of substitution is much greater (nearly 0.3 R_m unit difference between the average values for 8-substituted compounds **20–22** and the corresponding 6-substituted derivatives **14–16**). Lipophilic substituents such as methyl and chloro placed at positions on the short axis of the acridine nucleus have a smaller effect on overall drug lipophilicity than when placed off the long axis (e.g., methyl derivatives **14** and **20**, chloro derivatives **6** and **19**, compared to **1**). However, on the basis of previous relationships among 9-anilinoacridine derivatives,[8] these differences in overall lipophilicity are not large enough to account for the observed variation in biological activity.

In general, substitution increased the binding affinity to DNA. The marked GC selectivity shown by the parent compound (Δ log K = 0.47) was generally reduced by substitution. As a class, only the 6-substituted derivatives **14–16** showed increased GC selectivity, but this was not marked (average Δ log K = 0.55). Absolute binding levels to poly·d(AT) generally increased about twofold (average log K_{AT} for all substituted compounds of 7.36, cf. 7.08 for **1**), with the 5- and 7-substituted compounds showing the greatest effect while absolute binding to poly·d(GC) increased less.

To determine whether substituents had any significant influence on the mode of binding, unwinding angles for binding to closed circular supercoiled DNA were measured for all the methoxy-substituted analogues (Table I). The values obtained (12–18°) indicate that all the compounds

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bind by intercalation, by comparison with unwinding angles of 15° for the unsubstituted parent compound (1, Table I) and 16° for 9-aminoacridine.[1] However, with binding constants for all derivatives of greater than 10^7 M^{-1}, and thus little correction for unbound drug necessary, the variation in unwinding angles with position of substitution is significant, suggesting better intercalation geometry for the 5-methoxy derivative (12; unwinding angle 18°) than for the corresponding 6- and 7-substituted analogues 15 and 18 (unwinding angles 12° and 13°, respectively). We have previously noted the importance of apparently minor changes in intercalation geometry on biological activity for intercalating agents.[18]

The compounds were evaluated in vitro against both the murine leukemia L1210 and the human colon carcinoma line HCT-8. Among the substituted compounds, in vitro cytotoxicity against L1210 leukemia varied enormously (total range of 1540-fold). For substitution at any one position, the methoxy derivatives were usually the most cytotoxic. However, ID_{50} values were overall more sensitive to the position rather than the nature of the substitution. Whereas the range of ID_{50} values for substitution at any one position varied from 1.4- to 14-fold (except for the 2-substituted derivatives 7 and 8), the difference in *average* ID_{50} values for the 7-substituted carboxamides 17–19 and the 5-substituted analogues 11–13 is 260-fold. As a subclass, only the latter 5-substituted compounds showed higher cytotoxicity than the parent compound, with the 1-methoxy derivative 5 and the 5-methyl derivative 11 possessing remarkably low ID_{50} values. The only other derivatives to have lower ID_{50} values than the parent were the 2- and 5-methoxy derivatives (8 and 12) and the 1- and 5-chloro derivatives (6 and 13). A broadly similar picture is seen for the HCT-8 results, except that the total range is much smaller (71-fold). However, the most striking results are the ratio of L1210 and HCT-8 ID_{50} values. For the unsubstituted parent compound 1, a ratio of 4.4 shows that the human colon line is more than fourfold more resistant than the mouse leukemia, but this ratio varies markedly with substitution pattern. Thus while the 5-substituted derivatives as a class show greatly enhanced L1210 cytotoxicity, their HCT-8 cytotoxicity changes little, resulting in much larger ratios in favor of the leukemia. In contrast, all the other substituted compounds (5–10, 14–22), while generally less cytotoxic than the parent, have much lower ratios and in some cases are more toxic to HCT-8 than L1210 cells (ratio below 1.0).

Compounds were also tested in vivo against P388 leukemia. Generally, only those compounds that possessed higher in vitro cytotoxicity than the parent against the L1210 leukemia showed in vivo activity. The 1-methoxy and 1-chloro compounds 5 and 6 had low but reproducible activity, while the 2-methoxy derivative 8 and all of the 5-substituted compounds 11–13 had activity comparable to that of the parent 1. The only other compound with even marginal activity was the 6-methyl analogue 14.

Other work[19] with derivatives of amsacrine shows a clear relationship between the ratio of ID_{50} values for L1210 and HCT-8 cells and the in vivo activity of the compounds against a solid tumor and the Lewis lung carcinoma (LL). Amsacrine itself, with an ID_{50} ratio of 1.6 in favor of the leukemia, has only marginal activity in the in vivo LL assay, while several acridine-substituted derivatives with markedly lower ratios show excellent LL activity.[19,20] The parent 9-aminoacridine-4-carboxamide (1), with an ID_{50} ratio of 4.4 in favor of the leukemia, was found to be inactive in the LL screen (results not shown). In view of the altered ID_{50} ratio of some of the substituted derivatives in favor of the human colon line, compounds 7, 15, 19, and 22 were also evaluated in the LL but found to be inactive.

Thus, favorable ID_{50} ratios in themselves are clearly not sufficient to indicate a broader spectrum of activity for the substituted 9-aminoacridine-4-carboxamides. However, compounds with similar favorable ratios but also lower absolute ID_{50} values would be attractive candidates for LL-active derivatives. The present results emphasize that substituent effects on biological activity depend more on the position rather than the intrinsic properties (e.g., lipophilicity, electronic character) of the substituent groups. This suggests important steric constraints at all positions except 2 and 5 and indicates that further development of this series should concentrate on extending the range of substituent groups placed at these positions.

Experimental Section

Where analyses are indicated only by the symbols of the elements, results obtained were within ±0.4% of the theoretical value. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ, under the 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).

Preparation of 1-Methoxy-9-oxoacridan-4-carboxylic Acid: Example of Method B. Methyl 4-methoxyanthranilate (3.62 g, 20 mmol), diphenyliodonium-2-carboxylate (6.5 g, 20 mmol), and cupric acetate (100 mg) were dissolved in dry DMF (20 mL) and heated at 90 °C for 12 h. The solvent was removed under vacuum and the residue dissolved in EtOAc. After washing with 0.01 N HCl, the organic layer was extracted with 0.1 N ammonia, and the aqueous layer was poured into excess 0.1 N HCl to give *N*-[5-methoxy-2-(methoxycarbonyl)phenyl]anthranilic acid, mp 196–198 °C (benzene–acetone) (5 g, 84%), identical with an authentic sample.[14]

The above product (4 g, 13 mmol) was suspended in a solution of polyphosphate ester (PPE) (10 mL; prepared by the method of ref 21) and heated at 100 °C for 1 h. The cooled mixture was diluted with methanol and then with water, and the solid was collected and heated under reflux in 2 N ethanolic NaOH for 30 min. The cooled solution was decolorized with charcoal and acidified with 2 N HCl to provide crude 1-methoxy-9-oxoacridan-4-carboxylic acid (3.3 g, 92% overall). Crystallization from aqueous ethanol gave needles, mp 266–268 °C, identical with an authentic sample.[14]

Preparation of 7-Methyl-9-oxoacridan-4-carboxylic Acid: Example of Method C. A mixture of 2-iodoisophthalic acid (5.84 g, 20 mmol), 4-methylaniline (3.21 g, 30 mmol), and cuprous chloride (1.5 g) in 2,3-butanediol (20 mL) and benzene (15 mL) was stirred at 120 °C until the internal temperature reached 100 °C. After addition of *N*-ethylmorpholine (10 mL), the mixture was stirred at 120 °C for a further 1 h, then cooled, and diluted with 0.5 M NH_4OH (150 mL). After clarification with charcoal and acidification with 2 N HCl, the product was extracted into EtOAc. The organic layer was separated and extracted with 1 N NH_4OH, followed by acidification of the aqueous layer to give crude product (12 g, 81%). Crystallization from aqueous MeOH gave 2-[*N*-(4-methylphenyl)amino]isophthalic acid as needles, mp 207–208 °C (lit.[22] mp 214–215 °C; corrected).

Cyclization of the above product (2.71 g, 10 mmol) in polyphosphoric acid (50 g) at 120 °C for 1 h gave 7-methyl-9-oxoacridan-4-carboxylic acid. Purification was effected by dissolving the sodium salt in aqueous MeOH and adding AcOH to the boiling

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solution to provide needles (2.2 g, 91%), mp 313–315 °C (lit.[22] mp 325–327 °C, corrected).

Preparation of Compound 9 of Table I: General Example. A suspension of 2-chloro-9-oxoacridan-4-carboxylic acid (5 g) in $SOCl_2$ (10 mL) containing a trace of DMF was heated under gentle reflux until homogeneous (1 h). The $SOCl_2$ was removed under vacuum at 30 °C, and the residue was azeotroped twice with dry benzene at 30 °C to give the crude carbonyl chloride as a yellow powder (VIII, R = 2-Cl).

To the above product (5 g, 15 mmol) was added all at once an ice-cold solution of *N,N*-dimethylethylenediamine (4 g, 45 mmol) in dry CH_2Cl_2 (150 mL). The homogeneous solution was kept at 20 °C for 30 min and then washed successively with 2 N aqueous Na_2CO_3 (3 × 100 mL) and saturated aqueous NaCl. Evaporation of solvent gave a yellow solid (IX, R = 2-Cl), which was crystallized from benzene–petroleum ether (with charcoal decolorization). (For less crystalline intermediates, filtration of the product through a column of alumina in CH_2Cl_2 was also an effective method for removing colored impurities.)

The above compound (IX, R = 2-Cl) (3 g, 8 mmol) was dissolved in dry phenol (8 g) and heated to 100 °C for 15 min. The melt was cooled to 50 °C, and a stream of dry ammonia was passed through while the temperature was raised to 110–120 °C and held there for 15 min. The cooled mixture was then diluted with excess 5 N NaOH, and the resulting solid was collected and crystallized from aqueous EtOH. The free base was then dissolved in MeOH and treated with a slight excess of 12 N HCl. Addition of EtOAc precipitated the dihydrochloride salt, which was recrystallized from MeOH–EtOAc to give compound **9** of Table I (4.5 g, 79%), mp 319–321 °C.

In initial attempts to prepare compound II of Table I, treatment of the corresponding 9-chloro derivative IX (R = 3-Cl) with excess phenol as above led to replacement of both chloro groups and eventual isolation in good yield of *N*-[2-(dimethylamino)-ethyl]-9-amino-3-phenoxyacridine-4-carboxamide, mp 294–296 °C. Anal. ($C_{24}H_{24}N_4O_2 \cdot 2HCl$) C, H, N, Cl.

HPLC. Retention times and response factors for the compounds of Table I were obtained by reversed-phase chromatography on a Waters μ-Bondapak C-18 steel column. The mobile phase was 28% (v/v) acetonitrile in water and contained 0.005 M dibutylammonium phosphate and 0.0025 M heptanesulfonic acid, buffered to pH 3.5, at a flow rate of 1.0 mL min^{-1}.

For injection, a solid sample of each compound was accurately weighed and completely dissolved in mobile phase, to give a solution of known concentration. The retention time and peak height of a 5-nmol aliquot were then observed, and the response factor was determined as the molar peak height (MPH) in OD units/mole.

Chromatography. Reversed-phase chromatography used the top phase of a mixture of glycerol (250 mL), *i*-BuOH (250 mL), and 1-pentanol (250 mL) plus methanesulfonic acid (2.7 mL) as developing solvent. The mixture of solvents was shaken thoroughly for 0.5 h and then allowed to settle for 48 h before separation. Premarked Reidel de Hahn cellulose DC cards were impregnated by immersion in a 30% methanolic solution of the bottom phase of the solvent mixture. After rapid draining excess solvent was removed from the sheets by blotting between filter paper, and they were then dried at room temperature in a fume hood for 1 h. Agents were applied as solutions in 65% Me_2CO–H_2O and sheets were air-dried for a further 15 min. Ascending development was for 24 h. 4′-(Acridin-9-ylamino)methanesulfonanilide (R_m = –0.08) was included as standard on all sheets, and ΔR_m values are quoted in reference to this compound.

DNA Binding Assays. Log *K* values for the synthetic self complementary polymers poly[d(AT)] and poly[d(GC)] were determined by the ethidium displacement technique.[5] Unwinding angles using closed circular duplex DNA were determined by a viscometric method.[1]

Biological Testing. Cell culture methods for determination were carried out as described previously.[19] In vivo testing has also been described in detail in a previous publication.[20]

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Registry No. 1, 89459-43-8; 1·2HCl, 89459-02-9; **5**, 100113-16-4; **5**·2HCl, 100113-01-7; **6**, 100113-17-5; **6**·2HCl, 100113-02-8; **7**, 100113-18-6; **7**·2HCl, 100113-03-9; **8**, 100113-19-7; **8**·2HCl, 100113-04-0; **9**, 100113-20-0; **9**·2HCl, 100113-05-1; **10**, 100113-21-1; **10**·2HCl, 100113-06-2; 11, 89459-51-8; 11·2HCl, 89459-10-9; **12**, 89459-52-9; **12**·2HCl, 89459-11-0; **13**, 89459-53-0; **13**·2HCl, 89459-12-1; 14, 89459-56-3; 14·2HCl, 89459-15-4; **15**, 89459-57-4; **15**·2HCl, 89459-16-5; **16**, 100113-22-2; **16**·2HCl, 100113-07-3; **17**, 89459-60-9; **17**·2HCl, 100113-08-4; **18**, 89459-61-0; **18**·2HCl, 89459-20-1; **19**, 89459-62-1; **19**·2HCl, 89459-21-2; **20**, 100113-23-3; **20**·2HCl, 100113-09-5; **21**, 100113-24-4; **21**·2HCl, 100113-10-8; **22**, 100165-33-1; **22**·2HCl, 100113-11-9; II (R = 1-OCH_3), 94636-70-1; II (R = 7-CH_3), 24782-65-8; II (R = 2-Cl), 94654-58-7; III, 1488-42-2; IV (R = 4-OCH_3), 50413-30-4; V (R = 5-OCH_3), 100038-83-3; VII (R = 4-cH_3), 98370-37-7; VIII (R = 2-Cl), 100113-12-0; IX (R = 2-Cl), 100113-13-1; IX (R = 3-Cl), 100113-15-3; *N*-[2-(dimethylamino)ethyl]-9-amino-3-phenoxyacridine-4-carboxamide dihydrochloride, 100113-14-2; 2-iodoisophthalic acid, 2902-65-0; 4-methylaniline, 106-49-0; *N,N*-dimethylethylenediamine, 108-00-9.