Potential Antitumor Agents. 43. Synthesis and Biological Activity of Dibasic 9-Aminoacridine-4-carboxamides, a New Class of Antitumor Agent

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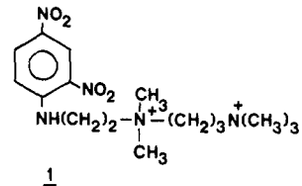
The synthesis and biological activities of representatives of a new class of antitumor agent, the *N*-[2-(dialkylamino)ethyl]-9-aminoacridine-4-carboxamides, are reported. Members of this class are stable and very water soluble with high levels of in vitro and in vivo antitumor activity. The compounds bind tightly to double-stranded DNA by intercalation, but the requirements for antitumor activity are more restrictive. They depend critically on the separation distance, positioning, and pK_a values of the two cationic centers. For in vivo activity, significant bulk tolerance exists for lipophilic but not hydrophilic groups about the C-9 acridine position and for both lipophilic and hydrophilic groups on the side-chain cationic moiety. Significant attenuation of the pK_a of the side-chain cationic center abolishes activity, as does alteration of either the disposition or separation distance of the side-chain charge with respect to the chromophore.

There has been increasing interest recently in the development of synthetic antitumor drugs belonging to the broad class of "DNA-binding agents", compounds that bind tightly but reversibly to double-stranded DNA. The most well-known subclass of these compounds are the DNA-intercalating agents, where much of the binding energy is obtained by the insertion of a flat, aromatic portion of the molecule between the base pairs of the DNA double helix.[1] Recent examples of new synthetic agents of this class now in clinical trial include amsacrine,[2] mitoxantrone,[3] ametantrone,[4] bisantrene,[5] and hycanthone.[6]

All of these compounds contain a fused linear tricyclic nucleus that posseses the necessary area widely accepted[7] to be needed for efficient intercalative binding. However, this in itself is not sufficient for effective antitumor activity, for the chromophores by themselves (e.g., 9-aminoacridine, anthracenedicarboxaldehyde, 1,4-diaminoanthraquinone) are inactive, even though they show intercalative binding. An absolute requirement for antitumor activity appears to be a suitably placed side chain. For cationic chromophores a neutral but sterically demanding side chain appears to be sufficient (ethidium bromide, amsacrine), but for the neutral chromophores either one[8] or two[9,10] cationic side chains are necessary. These side chains presumably have the function of additionally stabilizing the drug/DNA complex and influencing the kinetics of the binding process.[11]

Some empirical work has been done with analogues of the above series of compounds, particularly the anthracenediones, exploring the structure–activity relationships for alterations in the side chains[8-10] (although not in relation to the effects on DNA binding), but less attention has been paid to determining the optimal characteristics of the chromophore in this general class of tricyclic compounds. One general property that could assist DNA-binding affinity is the capacity of the binding chromophore to act as a charge-transfer acceptor from the nucleic acid bases. The importance of charge-transfer interactions in the binding of intercalating ligands to DNA has been shown by Gabbay and co-workers,[12,13] who employed electron-deficient nitrobenzenoid reporter molecules such as **1** as charge-transfer acceptors in an extensive study of ligand/DNA interactions. In later work with a series of tricyclic ligands of varying structure, Harples and Brown[14] demonstrated a positive correlation between DNA-binding ability and the relative charge-transfer affinities of the ligands.

The recent development[16] of derivatives of benz[*de*]-isoquinoline-1,3-dione as potential antitumor agents is of interest in this regard. The 5-nitro derivative **2**, known as mitonafide,[15] is an effective antitumor agent both in vivo and in vitro and binds to DNA by intercalation with an association constant of 1.5×10^5 M^{-1} at 0.01 M ionic strength,[15] although it possesses only two fused rings. However, the chromophore is very electron deficient and, in fact, readily forms an internal charge-transfer complex from the lone pair of the dimethylamino nitrogen, as evinced by the bright yellow color of the free base compared to the colorless salt form.



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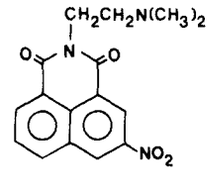

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These observations imply that new types of DNA-intercalating antitumor agents could be sought from the general class of fused, tricyclic chromophores bearing cationic side chains. A desirable property of the chromophore would be its ability to act as a charge-transfer acceptor, and this property could be enhanced by attaching the side chain via electron-withdrawing groups. As part of a general study based on the above observations, we have prepared and examined a series of 9-aminoacridine-carboxamides. The acridines are archetypical intercalating

† Deceased, January 1981.

(1) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18.
(2) Cain, B. F.; Atwell, G. J. *Eur. J. Cancer* **1974**, *10*, 539.
(3) Griffen, J. P.; Newman, R. A.; McCormack, J. J.; Krakoff, I. H. *Cancer Treat. Rep.* **1982**, *66*, 1323.
(4) Piccart, M. *Eur. J. Cancer* **1981**, *17*, 775.
(5) Spiegel, R. J. *Cancer Res.* **1982**, *42*, 354.
(6) Haq, M. M.; Leha, S. S.; Wiseman, C. W.; Bodey, G. P. *Cancer Treat. Rep.* **1980**, *64*, 929.
(7) Albert, A. "The Acridines", 2nd ed.; Edward Arnold: London, 1966.
(8) Zee-Cheng, R. K-Y.; Podrebarac, E. G.; Menon, C. S.; Cheng, C. C. *J. Med. Chem.* **1979**, *22*, 501.
(9) Zee-Cheng, R. K-Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291.
(10) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024.
(11) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.
(12) Gabbay, E. J. *J. Am. Chem. Soc.* **1968**, *90*, 6574.
(13) Gabbay, E. J.; DePaolis, A. *J. Am. Chem. Soc.* **1971**, *93*, 562.
(14) Sharples, D.; Brown, J. R. *FEBS Lett.* **1976**, *69*, 37.
(15) Brana, M. F.; Sanz, A. M.; Castellano, J. M.; Roldan, M.; Roldan, C. *Eur. J. Med. Chem.* **1981**, *16*, 207.
(16) Cain, B. F.; Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1976**, *19*, 772.

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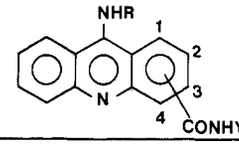
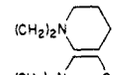
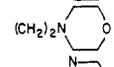
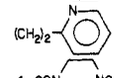
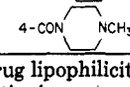
Table I. Physicochemical and Biological Data for 9-Aminoacridine-4-carboxamides



no.	side-chain position	R	Y	R_m[a]	pK_a[b]	log K[c] AT	log K[c] GC	ID_{50}[d] L1210	ID_{50}[d] HCT-8	P388 OD[e]	P388 ILS[f]
3	2	H	$(CH_2)_2N(CH_3)_2$	−1.00	8.6	7.08	7.55	>2000	>5000	12.5	*g*
4	3	H	$(CH_2)_2N(CH_3)_2$	−1.06		7.18	7.66	>2000	>5000	250	
5	4	H	$(CH_2)_2N(CH_3)_2$	−1.11		7.35	7.70	15	55	4.5	98 (1)[h]
6	4	CH_3	$(CH_2)_2N(CH_3)_2$	−1.11		7.17	7.65	15	125	5.9	53
7	4	CH_2CH_3	$(CH_2)_2N(CH_3)_2$	−0.88		7.14	7.69	94	188	8.9	30
8	4	$(CH_2)_2CH_3$	$(CH_2)_2N(CH_3)_2$	−0.57		7.23	7.79	46	203	13.3	64
9	4	$(CH_2)_3CH_3$	$(CH_2)_2N(CH_3)_2$	−0.34		7.10	7.69	41	150	20	70
10	4	$(CH_2)_4CH_3$	$(CH_2)_2N(CH_3)_2$	−0.23		6.91	7.58	43	160	13.3	64
11	4	$(CH_2)_5CH_3$	$(CH_2)_2N(CH_3)_2$	−0.04		7.00	7.79	65	210	30	88
12	4	$(CH_2)_2OH$	$(CH_2)_2N(CH_3)_2$	−1.19		6.82	7.61	484	540	8.9	*g*
13	4	$(CH_2)_2OCH_3$	$(CH_2)_2N(CH_3)_2$	−0.76		6.73	7.35	85	400	13.3	47
14	4	$(CH_2)_2N(CH_3)_2$	$(CH_2)_2N(CH_3)_2$	−1.72		7.83	8.20	460	1300	8.9	*g*
15	4	H	$(CH_2)_3N(CH_3)_2$	−0.93		7.51	7.60	157	520	50	*g*
16	4	H	$(CH_2)_4N(CH_3)_2$	−0.89		7.49	7.43	430	1000	NT[i]	
17	4	H	$(CH_2)_5N(CH_3)_2$	−0.73		7.45	7.45	1370	1800	NT	
18	4	H	$(CH_2)_6N(CH_3)_2$	−0.58		7.41	7.40	1540	1750	NT	
19	4	H	$(CH_2)_7N(CH_3)_2$	−0.44		7.43	7.30	1180	2000	NT	
20	4	H	$(CH_2)_2NH_2$	−1.18	9.1	7.36	7.67	414	1500	20	71
21	4	H	$(CH_2)_2N(Et)_2$	−0.67	9.2	7.26	7.59	5.5	83	5.9	70
22	4	H	$(CH_2)_2NH(CH_2)_2OH$	−1.06	8.5	7.34	7.73	77	1600	20	73
23	4	H		−0.70	8.9	7.12	7.10	102	380	2.6	33
24	4	H		−1.00	6.7	7.29	7.58	1025	2000	30	*g*
25	4	H		−0.57	5.9	6.94	7.03	1900	3200	40	*g*
26	4	H		−1.29		6.58	6.45	>2000	>1500	NT	

[a] R_m: chromatographic measure of drug lipophilicity determined by liquid–liquid chromatography on a cellulose support, as detailed in ref 16. [b] pK_a values for the side-chain cationic center from ref 27. [c] Log K: logarithmic association constant determined by ethidium displacement. Since these compounds did not quench ethidium fluorescence, correction for quenching was unnecessary. [d] ID_{50}: the nanomolar concentration of drug that when added to cultures of L1210 or HCT-8 cells for a period of 70 h reduces the counted cells to 50% of controls (ref 24). [e] OD: optimal drug dose in mg/(kg day), administered intraperitoneally as a solution in 0–.1 mL of 30% v/v ethanol/water on days 1, 5, and 9 after inoculation of 10^6 P388 leukemia cells. [f] ILS: the percentage increase in lifespan of treated animals over that of control groups of animals injected with tumor alone. The average survival of control mice was 11 days. The average survival of control mice was 11 days. Values of ILS greater than 20% are considered statistically significant. [g] No activity (ILS < 20%) at all dose levels. [h] Numbers in parentheses are the number of animals (out of a group of six) that survived indefinitely. [i] NT: compound not tested in vivo.

Table II. Physiochemical Properties for the Compounds of Table I

no.	mp, °C	formula	anal.
3	212–214	$C_{18}H_{20}N_4O\cdot 2HCl\cdot {}^1/_4H_2O$	C, H, N, Cl
4	293 dec	$C_{18}H_{20}N_4O\cdot 2HCl\cdot {}^1/_4H_2O$	C, H, N, Cl
5	292–293 dec	$C_{18}H_{20}N_4O\cdot 2HCl\cdot {}^1/_2H_2O$	C, H, N, Cl
6	231–323	$C_{19}H_{22}N_4O\cdot 2HCl$	C, H, N, Cl[a]
7	174–176	$C_{20}H_{24}N_4O\cdot 2HCl$	C, H, N, Cl
8	152–155	$C_{21}H_{26}N_4O\cdot 2HCl$	C, H, N, Cl
9	136–138	$C_{22}H_{28}N_4O\cdot 2HCl$	C, H, N, Cl
10	172–174	$C_{23}H_{30}N_4O\cdot 2HCl\cdot H_2O$	C, H, N, Cl
11	207–210	$C_{24}H_{32}N_4O\cdot 2HCl\cdot {}^1/_2H_2O$	C, H, N
12	225–226	$C_{20}H_{24}N_4O_2\cdot 2HCl$	C, H, N, Cl
13	69–72	$C_{21}H_{26}N_4O_2\cdot 2HCl$	C, H, N
14	244–246	$C_{22}H_{29}N_5O\cdot 3HCl\cdot {}^1/_4H_2O$	C, H, N, Cl
15	290–292	$C_{19}H_{22}N_4O\cdot 2HCl$	C, H, N
16	323–324	$C_{20}H_{24}N_4O\cdot 2HCl$	C, H, N, Cl
17	291–293	$C_{21}H_{26}N_4O\cdot 2HCl$	C, H, N, Cl
18	295–297	$C_{22}H_{28}N_4O\cdot 2HCl$	C, H,[b] N, Cl
19	287–289	$C_{23}H_{30}N_4O\cdot 2HCl$	C, H, N, Cl
20	344–345	$C_{16}H_{16}N_4O\cdot 2HCl$	C, H, N, Cl
21	283–285	$C_{20}H_{24}N_4O\cdot 2HCl$	C, H, N, Cl
22	292–293	$C_{18}N_{20}N_4O_2\cdot 2HCl$	C, H, N, Cl
23	220–223	$C_{21}H_{24}N_4O\cdot 2HCl$	C,[c] H, N, Cl
24	282–284	$C_{20}H_{22}N_4O_2\cdot 2HCl$	C, H, N, Cl
25	280–282	$C_{21}H_{18}N_4O\cdot 2HCl$	C, H, N, Cl
26	352–353	$C_{19}H_{20}N_4O\cdot 2HCl$	C, H, N, Cl

[a] Cl out by 0.6%. [b] H out by 0.5%. [c] C out by 0.5%.

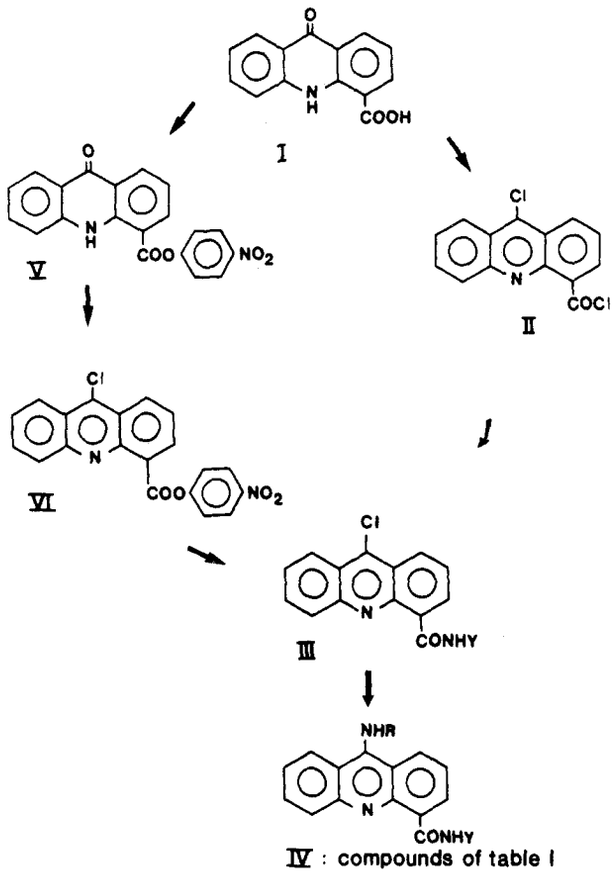
agents,[1] and both the simple acridines and derivatives with cationic side chains have a number of interesting biological properties.

We have now found that 9-aminoacridines bearing a cationic group at the 4-position attached by an electron-withdrawing alkylcarbamoyl side chain (Tables I and II) are potent antitumor agents both in vitro and in vivo, and this paper describes the synthesis and biological evaluation of some examples of this new class.

Chemistry. The 9-aminoacridinecarboxamides listed in Table I were prepared from 9-oxoacridan-4-carboxylic acid (I) by one of the routes shown in Scheme I. For most of the compounds reported, the route via 9-chloroacridine-4-carbonyl chloride (II) was the more convenient. Reaction of this compound with a primary alkylamine in an anhydrous solvent such as CH_2Cl_2 or DMF buffered with Et_3N gave excellent yields of the carboxamide derivatives III, with no concomitant replacement of the nuclear chlorine. These intermediates could be conveniently isolated from excess water-soluble amines as their free bases by extraction with CH_2Cl_2 and were purified by crystallization from nonpolar solvents.

Subsequent treatment of III with excess phenol at 50 °C gave the 9-phenoxy compounds, which were reacted, without isolation, with dry ammonia or primary aliphatic

Scheme I



amines at 110–115 °C to give the desired products IV. These were again isolated and purified as the free bases, before being converted to the very water-soluble disalt forms recorded in Table I.

This procedure was not suitable for compounds such as **22**, where the primary amine to be used in the initial reaction with the 4-carbonyl chloride also contained hydroxyl and/or secondary amine groups. These amines were used with the 9-chloro *p*-nitrophenyl ester VI, when displacement of the *p*-nitrophenoxy group is effected only by the primary amine to provide the pure chloro compounds III, which are elaborated by the initial route to the desired products (e.g., compound **22** of Table I).

Results and Discussion

Physicochemical and biological data for 24 examples of the new 9-aminoacridinecarboxamide class of antitumor agents are recorded in Table I. As expected of dicationic compounds, the 9-aminoacridinecarboxamides are very hydrophilic, with R_m values[16] comparable to those determined for mitoxantrone (R_m = −1.53), ametantrone (R_m = −1.25), and bisantrene (R_m = −0.9) and much lower than those determined for monocationic drugs of proven clinical usefulness such as amsacrine (R_m = 0.18) and daunomycin (R_m = −0.47).[17] The first three compounds in Table I explore the placement of the side chain at different positions on the acridine ring. The 1-substituted compound could not be prepared, but the other three positional isomers were readily synthesized from the corresponding acridone carboxylic acids. All three compounds (**3–5**) are of approximately the same lipophilicity. They also show similar levels of DNA binding, as determined by their association constants (determined by displacement of ethidium from synthetic DNA copolymers and assuming competitive binding[18]), with a preference for binding to poly(dG-dC). In the helix unwinding assay using closed circular supercoiled DNA, they all have similar unwinding angles of about 16° (Figure 1). This compares well to the value reported[19] (17.4°) and that determined by us (15°) for 9-aminoacridine itself, indicating that the compounds bind primarily by intercalation.

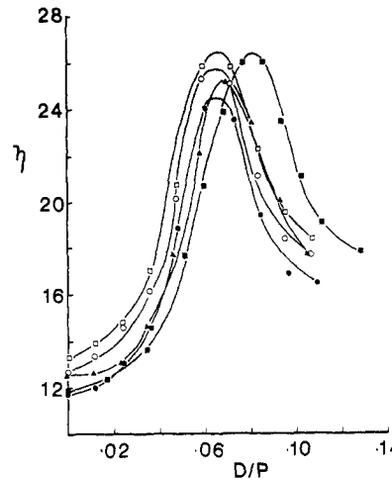


Figure 1. Effect of acridinecarboxamides **3** (○), **4** (□), **5** (●), **15** (▲), and 9-aminoacridine (■) on the reduced viscosity (η) of covalently closed circular duplex DNA from *E. coli* plasmid PML-21. The abscissa shows the molar ratios of drug to DNA phosphate (D/P). The peaks of the curves (representing fully relaxed DNA circles) correspond to unwinding angles for compounds **3**, **4**, **5**, and **15** of respectively 16°, 16°, 16°, and 17°, referenced to 9-aminoacridine as 15° and ethidium as 26° (ethidium data not shown). The determinations followed the procedures of ref 26.

Despite this similarity in lipophilic character and DNA binding mode and affinity, the three positional isomers **3–5** show strikingly different biological activity profiles (Table I). The compounds were evaluated against both murine leukemia L1210 and a cultured human colon carcinoma (HCT-8)[20,21] in vitro and the P388 leukemia in vivo. The HCT-8 colon carcinoma line is being used as part of our primary screening to detect compounds with selective activity toward solid tumors. The 2- and 3-substituted derivatives **3** and **4** show low levels of in vitro cytotoxicity and are inactive as antitumor agents in vivo, although with quite different levels of mammalian toxicity (an approximate maximum tolerated dose of 250 mg/kg of **3** compared with 12.5 mg/kg for **4**, when given on a q4d×3 schedule). However, the 4-substituted derivative **5** is a very active antitumor agent, with an ID_{50} against L1210 cells in culture of 15 nM and against HCT-8 human colon adenocarcinoma cells in culture of 55 nM. These values are lower than those obtained in this laboratory[21] for DNA-intercalating agents of clinical utility such as amsacrine (ID_{50} values of 35 and 70 nM, respectively) and adriamycin (30 and 60 nM, respectively). The 4-carboxamide **5** also proved highly active against the P388 leukemia in vivo, giving ILS values of around 100% at the optimal dose of 4.5 mg/kg and providing a proportion of long-term survivors. The compound also showed signifi-

(17) Ferguson, L. R.; Denny, W. A. *J. Med. Chem.* **1979**, *22*, 251.

(18) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170.

(19) Wright, R. D. McR.; Wakelin, L. P. G.; Fields, A.; Acheson, R. M.; Waring, M. J. *Biochemistry* **1980**, *19*, 5825.

(20) Tompkins, W. A. F.; Watrach, A. M.; Schrocale, J. D.; Schultz, R. M.; Harris, J. A. *J. Natl. Cancer Inst.* **1974**, *52*, 1101.

(21) Finlay, G. F.; Baguley, B. C. *Eur. J. Cancer Clin. Oncol.* **1984**, *20*, 947.

cant activity against the same tumor (ILS of 60%) when given orally, albeit at higher dose levels (33 mg/kg).

Encouraged by the high levels of activity shown by **5**, we set out to explore the structure–activity relationships for this class of DNA-binding antitumor agents. Compounds **6–11** were made to evaluate the effect of increasing lipophilicity and also to probe the bulk tolerance about the 9-position. As shown by the results in Table I, both in vitro and in vivo activity decrease steadily but not precipitously as the alkyl chain at position 9 is lengthened. Such a steady decline suggests that the loss is due largely to nonspecific drug lipophilicity rather than by the alkyl chain being placed in a position of bulk intolerance. This view is supported by the essentially constant levels of DNA binding through the homologous series and by the fact that in vivo activity declined much more rapidly than the in vitro potency.

Replacement of the lipophilic alkyl chain with either polar (compounds **12**, **13**) or charged (compound **14**) functionality greatly decreased or abolished in vivo activity and also greatly decreased in vitro potency. DNA binding for the hydroxy compound **12** was similar to that for the alkyl derivatives and increased as expected for the tris-cationic compound **14**. Since the previous data had established bulk tolerance around the 9-position, the inactivity of **12** in particular is unexpected. The compound is of similar overall lipophilicity to **5** (R_m = 1.19), but has lost the significant GC selectivity shown by **5** and the alkyl analogues.

When the length of the side chain joining the cationic center to the chromophore is similarly increased to give compounds **15–19**, a quite different pattern is seen. Extension of the chain of **5** by only one methylene unit to give **15** is sufficient to reduce in vitro potency against L1210 leukemia 10-fold and abolish in vivo activity. This is despite the fact that **15** still binds tightly to DNA by intercalation, as evidenced by an unwinding angle of 17° (Figure 1). In comparison to the 9-*N*-alkyl series **6–11**, the compound is also sufficiently hydrophilic, and some other aspect of DNA-binding or metabolism must be responsible for the loss of activity. It is interesting to note that among other series of tricyclic compounds bearing cationic side chains, such as the anthracenediones, a similar precise requirement on the distance between the chromophore and the cationic center is seen.

The remainder of the compounds in Table I (**20–26**) were prepared to explore the nature of the cationic center required. Removal of the methyl groups of **5** to give **20** provided a more hydrophilic compound, with much lower potency both in vitro and in vivo but still with good in vivo activity. Replacement of the methyl groups by the bulkier ethyl groups in **21** restored potency while retaining high in vivo activity, in spite of higher lipophilicity. The requirement for a tertiary amine on the side chain for high potency may be related to easier metabolic oxidation of the primary amine. The more hydrophilic secondary amine **22** also had relatively low-dose potency compared to that of **5**, especially against the HCT-8 human colon line. The much greater loss in potency against the human-derived line than the L1210 leukemia is similar to the pattern of activity seen with 4-carboxamide derivatives of amsacrine which bear complex polar side chains.[21] Compounds **20–23**, with varying degrees of amine substitution but with uniformly high pK_a values (Table I), thus had varying levels of potency (related to the degree of amine substitution) but retained in vivo activity. However, the attachment of more weakly basic groups to the side chain gave compounds (e.g., **24** and **25**) of very low in vitro potency which also proved inactive in vivo. It is not completely clear if the morpholino and 2-pyridyl groups of compounds **24** and **25** render these compounds inactive because of their low pK_a values or because of their significant steric bulk, but the latter seems unlikely in view of the activity of the diethyl and piperidine derivatives (**21** and **23**) and the fact that they retain high DNA-binding capability. Finally, compound **26**, possessing a tertiary amine of high pK_a, is nevertheless inactive both in vitro and in vivo. This cationic center would be expected to have much less conformational flexibility than that of **5** and possibly cannot make the same DNA-binding contacts. This is borne out by its measured log K values, which are nearly 10-fold higher than those of **5** and similar analogues and which show a preference for binding to poly(dA-dT).

Several conclusions can be drawn from this initial structure–activity study of the new 9-aminoacridinecarboxamide class of antitumor agents. The most active members of the class (e.g., **5**, **21**, **8**, **22**) show great promise as antitumor agents. They are stable and water soluble and possess high levels of activity against the L1210 leukemia and HCT-8 human colon adenocarcinoma in vitro and good in vivo activity against the P388 leukemia. Although none of the compounds show selective activity toward the HCT-8 colon carcinoma, the ratios (3–4-fold) are comparable to those found for clinical drugs useful in solid tumor therapy, e.g., methotrexate and 5-fluorouracil. For in vivo activity, significant bulk tolerance exists for lipophilic (**9**) but not hydrophilic (**12**) or charged (**14**) groups off the 9-position and for both lipophilic (**21**) and hydrophilic (**22**) groups off the side-chain cationic moiety. The requirement for two cationic centers of high pK_a separated by a fixed distance (approximately 8 Å) appears absolute. Significant attenuation of the pK_a of the side-chain nitrogen (**24**, **25**) abolishes activity, as does alteration of the disposition of the side-chain charge with respect to the chromophore (**3** and **4**).

These restrictions provide a rather narrower structure–activity spectrum for the 9-aminoacridinecarboxamides than that found for the 9-anilinoacridine class of antitumor agents.[22] While the biological activity of the 9-aminoacridinecarboxamides is undoubtably dependent on their ability to interact with cellular DNA, the correlation is not a simple one solely concerned with the tightness of this binding. Recent work[23] suggests that drug/DNA binding kinetics may be important. As the next step in the development of this new class of antitumor agents, we are currently studying the effects of acridine ring substitution on the biological activity of the 9-aminoacridinecarboxamides.

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, N.Z., 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. NMR spectra were obtained on a Varian 360-L spectrometer (Me_4Si).

9-Oxoacridan-4-carboxylic Acid (I). A mixture of *N*-(2-carboxyphenyl)anthranilic acid (80 g, 0.31 mol) and concentrated H_2SO_4 (250 mL) was heated at 100 °C for 4 h, cooled, and poured into ice–water and the precipitated solid collected and washed well with water. This was dissolved in dilute aqueous NaOH and following filtration was diluted with an equal volume of EtOH

(22) Denny, W. A.; Cain, B. F.; Atwell, G. J.; Hansch, C.; Panthananickal, A.; Leo, A. *J. Med. Chem.* **1982**, *25*, 276.
(23) Wakelin, L. P. G.; Denny, W. A., unpublished results.

and then acidified with glacial acetic acid (this left any sulfonated impurities in solution). The acridone acid that slowly crystallized from the hot solution was collected after thorough cooling, washed with aqueous EtOH, and dried, providing pure material in 83% yield, mp 342–343 °C dec.

9-Chloroacridine-4-carbonyl Chloride (II). A suspension of the preceding acridone acid (20 g, 84 mmol) in $SOCl_2$ (60 mL) containing DMF (2 drops) was heated gently under reflux with stirring until homogeneous and then for a further 45 min. The solution was evaporated to dryness in vacuo below 40 °C, and residual traces of $SOCl_2$ were removed by addition of dry benzene and complete reevaporation of all solvents to give the crude product as a yellow powder.

***N*-[2-(Dimethylamino)ethyl]-9-chloroacridine-4-carboxamide (III, Y = $(CH_2)_2N(CH_3)_2$).** The above carbonyl chloride was cooled to −5 °C and to this was added in one portion an ice-cold solution of *N,N*-dimethylethylenediamine (36.5 mL, 0.33 mol) in dry CH_2Cl_2 (200 mL). After being stirred at 30 °C until homogeneous, the reaction solution was left for a further 15 min and then shaken with 10% aqueous Na_2CO_3. The organic layer was washed twice with 10% aqueous Na_2CO_3 and once with saturated aqueous NaCl solution and then dried (Na_2SO_4). Evaporation of the solvent left an oil, which slowly solidified. This was extracted with hot dry benzene–petroleum ether (1:5), treated with charcoal–Celite, and filtered quickly through a hot Celite pad. Crystalline material rapidly separated, and addition of further petroleum ether completed precipitation of the product. The yellow solid was collected, washed with petroleum ether, and dried to give the 9-chloro carboxamide III (19.5 g, 71% yield). TLC indicated only trace quantities of the corresponding acridone, and this product was stored over KOH and used without further purification.

***N*-[2-(Dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (5).** The 9-chloro carboxamide III (4.0 g, 13 mmol) was dissolved in dry phenol (12.8 g, 130 mmol) and heated slowly to 50 °C to provide a solution of the 9-phenoxy compound in excess phenol. A stream of dry ammonia was passed into the solution while the temperature was raised from 50 to 115 °C. Addition of ammonia was continued for 15 min, after which the mixture was cooled and diluted with excess 40% aqueous NaOH. Prolonged cooling gave a solid that was crystallized from aqueous EtOH and then EtOAc. The resulting pure base was converted to the dihydrochloride salt by dissolving in MeOH, treating with 12 N HCl (2.2 equiv), and precipitating with EtOAc. Crystallization from MeOH/EtOAc gave hygroscopic yellow prisms of the pure dihydrochloride of compound **5**, mp 292–293 °C (72% yield).

The other compounds of Table I (except for **22**) were similarly prepared from the appropriate acridone acids and side chains.

***p*-Nitrophenyl Acridone-4-carboxylate (V).** Pure, finely powdered acridone-4-carboxylic acid (19.8 g, 83 mmol) and *p*-nitrophenol (22.2 g, 160 mmol) were suspended in pyridine (200 mL). The mixture was stirred vigorously at 60 °C while PCl_3 (4.4 mL, 53 mmol) was added dropwise. The mixture was immediately heated to 100 °C until homogeneous. The product separated on cooling, and after 1 h at 0 °C the precipitate was collected and washed well with acetone. Recrystallization from DMF gave pure compound **V** (74% yield), mp 280–281 °C.

***p*-Nitrophenyl 9-Chloroacridine-4-carboxylate (VI).** The above compound (2.02 g, 5.6 mmol) was refluxed gently in $SOCl_2$ (6 mL) and a drop of DMF for 1 h. The volatiles were evaporated, dry benzene was added, and the volatiles were evaporated again to remove all traces of HCl and $SOCl_2$. The residue was dissolved in CH_2Cl_2 and cooled to 0 °C, and ice-cold 10% $KHCO_3$, 20 mL) was added. The organic layer was separated, dried, and concentrated to a small volume to provide the product as yellow needles (80%), mp 194–196 °C.

***N*-[2-[(2-Hydroxyethyl)amino]ethyl]-9-aminoacridine-4-carboxamide (22).** 4-Nitrophenyl 9-chloroacridine-4-carboxylate (3.5 g, 10 mmol) was added in one portion to an ice-cooled, stirred solution of 2-[(2-aminoethyl)amino]ethanol (1.26 mL, 0.12 mmol) and triethylamine (1.5 mL, 11 mmol) in anhydrous CH_2Cl_2 (20 mL). The mixture was stirred until homogeneous, and then for a further 10 min. Dry phenol (11 g) was then added to the solution and anhydrous ammonia was passed in while the temperature was raised to 115 °C. After contact with ammonia at this temperature for a further 10 min, the mixture was cooled and excess 5 N aqueous NaOH added. The resulting solid was dissolved in 1 N aqueous HCl and this solution was slowly neutralized with 1 N aqueous NH_4OH, precipitating a quantity of material that was removed by filtration and discarded. Treatment of the filtrate with excess aqueous NaOH gave crude material, which was recycled through the above purification process. The resulting free base was crystallized from MeOH–H_2O. Crystallization of the dihydrochloride salt from MeOH–EtOAc then provided pure product, mp 292–293 °C dec.

Biological Testing. Cell culture methods for determining ID_{50} values for L1210 leukemia are given in ref 24. HCT-8 human colon adenocarcinoma cells were grown in Alpha MEM medium supplemented with glutamine for 2 days prior to drug addition. After incubation for 4 days, trypsinized cells were counted electronically.[21]

P388 leukemia cells were obtained as frozen stock from Mason Research Inc. and passaged intraperitoneally according to standard methods[25] in DBA-2 mice of either sex. Groups of six hybrid mice (DBA-2 male × C_{57} B1 female), weight 20 ± 1 g) were injected intraperitoneally with 10^6 cells on day 0.

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Registry No. **3**, 91549-52-9; **3** (free base), 91549-70-1; **4**, 91549-53-0; **4** (free base), 91549-71-2; **5**, 89459-02-9; **5** (free base), 89459-43-8; **6**, 89459-01-8; **6** (free base), 89459-42-7; **7**, 91549-54-1; **7** (free base), 91585-91-0; **8**, 91549-55-2; **8** (free base), 91549-72-3; **9**, 91549-56-3; **9** (free base), 91549-73-4; **10**, 91549-57-4; **10** (free base), 91549-74-5; **11**, 91549-58-5; **11** (free base), 91549-75-6; **12**, 91549-59-6; **12** (free base), 91549-76-7; **13**, 91549-60-9; **13** (free base), 91549-77-8; **14**, 91549-61-0; **14** (free base), 91549-78-9; **15**, 89459-03-0; **15** (free base), 89459-44-9; **16**, 91549-62-1; **16** (free base), 91549-79-0; **17**, 91549-63-2; **17** (free base), 91549-80-3; **18**, 91549-64-3; **18** (free base), 91549-81-4; **19**, 91549-65-4; **19** (free base), 91549-82-5; **20**, 89459-06-3; **20** (free base), 89459-46-1; **21**, 89459-05-2; **21** (free base), 89459-45-0; **22**, 89459-04-1; **22** (free base), 89459-30-3; **25**, 91549-66-5; **25** (free base), 91549-83-6; **26**, 91549-67-6; **26** (free base), 91549-84-7; **27**, 91549-68-7; **27** (free base), 91549-85-8; **28**, 91549-69-8; **28** (free base), 91549-86-9; I, 24782-64-7; II, 89459-22-3; III (Y = $(CH_2)_2N(CH_3)_2$), 89459-23-4; V, 63178-94-9; VI, 63178-95-0; *N*-(2-carboxyphenyl)anthranilic acid, 579-92-0; *N,N*-dimethylethylenediamine, 108-00-9; phenol, 108-95-2; *N*-[2-(dimethylamino)ethyl]-9-phenoxyacridine-4-carboxamide, 89459-24-5; *p*-nitrophenol, 100-02-7; 2-[(2-aminoethyl)amino]ethanol, 111-41-1.

(24) Baguley, B. C.; Nash, R. *Eur. J. Cancer* **1981**, *17*, 671.
(25) Geran, R. I.; Greenburg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 9.
(26) Baguley, B. C.; Ferguson, L. R.; Denny, W. A. *Chem.-Biol. Interact.* **1982**, *42*, 97.
(27) Perrin, D. D.; Dempsey, D.; Serjeant, E. P. "pKa Prediction for Organic Acids and Bases"; Chapman and Hall: London, 1981.