Structure−Activity Relationships for Substituted Bis(acridine-4-carboxamides): A New Class of Anticancer Agents

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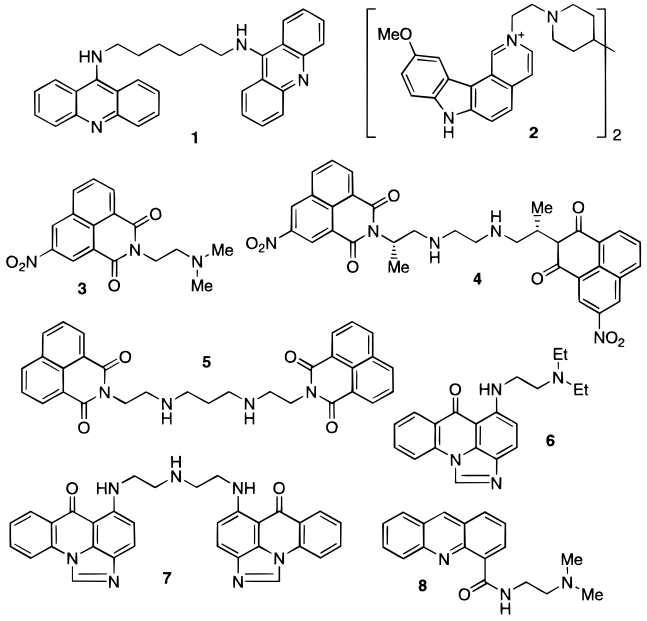
A series of acridine-substituted bis(acridine-4-carboxamides) linked by a $(CH_2)_3N(Me)(CH_2)_3$ chain have been prepared by reaction of the isolated imidazolides of the substituted acridine-4-carboxylic acids with *N*,*N*-bis(3-aminopropyl)methylamine. These dimeric analogues of the mixed topoisomerase I/II inhibitor *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA), currently in clinical trial, show superior potencies to the corresponding monomeric DACA analogues in a panel of cell lines, including wild-type (JL_C) and mutant (JL_A and JL_D) forms of human Jurkat leukemia. The latter mutant lines are resistant to topoisomerase II targeted agents because of lower levels of the enzyme. Analogues with small substituents (e.g., Me, Cl) at the acridine 5-position were clearly superior, with IC_{50}'s as low as 2 nM against the Lewis lung carcinoma and 11 nM against JL_C. Larger substituents at any position caused a steady decrease in potency, likely due to lowering of DNA binding affinity. A small series of analogues of the most potent bis(5-methylDACA) compound, with second substituents (Me and Cl) in the 1- or 8- position had broadly similar potencies to the 5-Me compound, indicating that, while the 1- and 8-substituents are acceptable, they add little to the enhancing effect of the 5-methyl group. All of the compounds were at least equitoxic (some up to 4-fold more cytotoxic) against the mutant Jurkat lines than in the wild-type, consistent with a relatively greater effect on topoisomerase I compared with topoisomerase II. The bis(5-methylDACA) compound was found to inhibit the action of purified topoisomerase I in a cell-free assay. Compounds were on average 10-fold less cytotoxic in an MCF7 breast cancer line overexpressing P-glycoprotein than in the wild-type line and showed some selectivity for colon tumor lines in the NCI human tumor cell line panel. Several analogues produced significant growth delays in the relatively refractory subcutaneous colon 38 tumor model in vivo at substantially lower doses than DACA. The bis-(acridine-4-carboxamides) represent a new and interesting class of potent topoisomerase inhibitors.

Introduction

Early structure−activity relationship (SAR) studies with DNA-intercalating topoisomerase (topo) II inhibitors suggested a positive correlation between cytotoxic potency and the strength of reversible DNA binding.[1−4] Because bis-intercalation would theoretically greatly increase DNA binding, a number of reports have discussed various dimeric compounds, designed as potential bis-intercalating agents.[5−7] While many of these compounds did show increased affinity for DNA, their biological activities were generally disappointing. The bis(acridine) (**1**) was considered for clinical trial[8] but had significant central nervous system (CNS) toxicity,[9] while the bis(ellipticine) analogue ditercalinium (**2**) had unacceptable mitochondrial toxicity.[10] Until recently the only dimeric, bis-intercalating agent to receive clinical evaluation was the bis(quinoxaline) natural product echinomycin.[11,12]



However, a number of recent papers have reported that lipophilic dimeric naphthalimides and imidazoacridinones show broad-spectrum activity against a variety of human solid tumor cell lines, both in culture and as xenografts in nude mice. The monomeric naphthalimide mitonafide (**3**) itself has undergone extensive clinical trials, where it has shown activity but also severe CNS toxicity.[13] The bis(naphthalimide) analogue DMP 840

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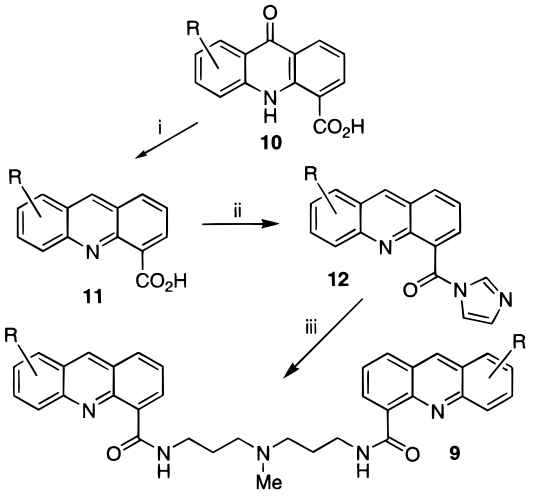
Scheme 1[a]



[a] (i) Al/Hg/NaOH/H_2O/EtOH/reflux, then $FeCl_3/H^+$/40 °C/2−20 h; (ii) CDI/DMF/12 h/50 °C; (iii) $[H_2N(CH_2)_3]_2NMe$/THF/20 °C/12 h.

(**4**) shows curative activity against a variety of human solid tumor xenografts in nude mice[14,15] and is presently in clinical trial.[16] The related compound LU 79553 (**5**) is also reported to be under clinical development.[17] The monomeric imidazoacridinone C-1311 (**6**) is also a candidate for clinical trial,[18] and a series of bis-analogues (e.g., WMC-26, **7**) show highly selective cytotoxicity toward human colon carcinoma cells both in culture and in xenografts, although they appear not to be bis-intercalating agents.[19]

The acridinecarboxamide derivative *N*-[(2-dimethylamino)ethyl]acridine-4-carboxamide (DACA, **8**) is another lipophilic mono-intercalator which entered phase I clinical trial[20] on the basis of its mixed inhibition of both topo I and topo II[21,22] and excellent activity in experimental solid tumor models.[20,23] DACA has been shown to retain activity in cell lines resistant due to alterations in topo I, topo II, and P-glycoprotein expression.[24,25] In a recent study[26] of a series of dimeric tricyclic aromatic carboxamides, the bis(DACA) analogue **9a** showed a 5-fold increase in cytotoxic potency over the monomer **8** and also showed significant growth delay (ca. 6 days) in the colon 38 tumor model.

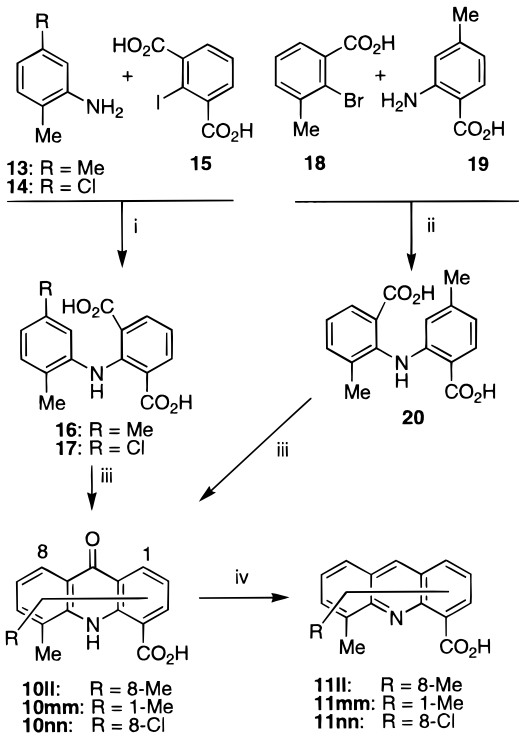
The activity profiles of **8** and **9a**, and the work reported above on the high solid tumor activity of dimeric analogues of other lipophilic mono-intercalators, prompted us to prepare and evaluate a series of bis(DACA) compounds (**9a**−**9oo**, Table 1), where the acridine substituents were varied.

Chemistry

The bis(acridines) **9** were prepared as shown in Scheme 1. The acids **11** (prepared via the acridones **10** or by direct ring closure) were activated with 1,1′-carbonyldiimidazole, and after isolation, the intermediate *N*-imidazolides **12** were reacted with a stoichiometric amount of the appropriate diamine. The bis(dimethylamino) analogues **9q**, **9x**, and **9hh** were prepared from the corresponding fluoro analogues **9m**, **9t**, and **9ee** by prolonged treatment with excess dimethylamine in aqueous MeOH under pressure at 100 °C.

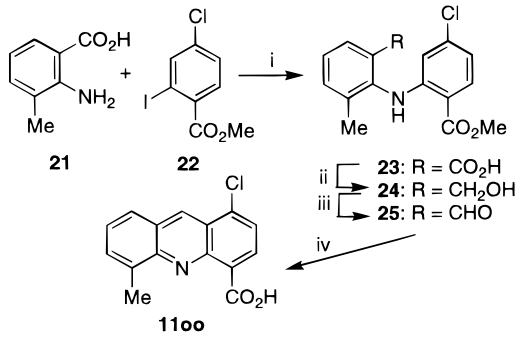
Most of the ring-substituted acridine-4-carboxylic acids **11** required were known, and these (and some new examples) were prepared by reduction of the corresponding acridone-4-carboxylic acids **10** with aluminum/mercury amalgam, followed by $FeCl_3$ reoxidation of the resulting acridans[23] (Scheme 2). Ullmann reaction of anilines **13** and **14** with 2-iodoisophthalic acid (**15**), or 2-bromo-3-methylbenzoic acid (**18**) with anthranilic acid (**19**) followed by cyclization of the resulting diphenylamine diacids **16**, **17**, and **20** in PPA, gave the required acridone-4-carboxylic acids **10ll**, **10nn**, and **10mm**.

Scheme 2[a]



[a] (i) Cu/CuI/*N*-ethylmorpholine/butane-2,3-diol; (ii) Cu/CuI/K_2CO_3/DMSO; (iii) PPA/100 °C; (iv) Al/Hg/NaOH/H_2O/EtOH/reflux, then $FeCl_3/H^+$.

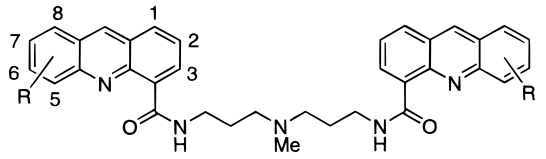
Scheme 3[a]



[a] (i) Cu/CuI/*N*-ethylmorpholine/butane-2,3-diol; (ii) CDI/THF/20 °C/18 h, then $NaBH_4$/20 °C/0.5 h; (iii) MnO_2/EtOAc/reflux/7 h; (iv) TFA/40 °C/4 h.

In cases where this was not compatible with the ring substituent, the recently reported[27,28] synthesis via ring closure of a precursor aldehyde was employed (Scheme 3). Thus condensation of 3-methylanthranilic acid (**21**) and methyl 4-chloro-2-iodobenzoate (**22**) gave acid **23**, which was reduced to **24** and reoxidized with MnO_2 to the aldehyde **25**. Cyclization of this in TFA followed by hydrolysis gave the required acridine-4-carboxylic acid **11oo**.

Table 1. Cytotoxicity Data for Substituted Bis(acridine-4-carboxamides)



			IC_{50} (nM)[a]			ratios[b]	
no.	R	mp (°C)	P388[c]	LL[d]	JL_C[e]	JL_A/JL_C[f]	JL_D/JL_C[g]
8		ref 27	98	189	580	1.9	2.3
9a[h]	H	168−170[i]	130	30	110	0.7	0.8
9b	1-Me	nc[j]	170	14	41	0.6	1.0
9c	1-Cl	94−96	400	42	127	0.6	1.0
9d	2-Me	nc	220	67	255	0.7	1.1
9e	2-Cl	175.5−176.5	113	5.9	46	0.7	0.9
9f	3-Me	nc	3500	2400	1295	0.9	1.0
9g	3-Cl	117 dec	$>2 \times 10^5$	2980	900	0.9	1.2
9h	5-Me	118−120	23	1.8	11	0.4	0.7
9i	5-Et	70−73	170	27	113	0.7	0.9
9j	5-^{i}Pr	nc	2760	1050	2080	0.9	1.1
9k	5-Ph	nc	1230	1085	2540	0.5	0.8
9l	5-OMe	nc	430	170	345	0.5	0.8
9m	5-F	134−136	16	35	49	0.6	0.9
9n	5-Cl	89−93	46	8	33	0.55	1.0
9o	5-Br	172−174.5	15	6	24	0.4	0.9
9p	5-CF_3	231−233[i]	240	39	115	0.85	1.1
9q	5-NMe_2	nc	2130	670	180	0.7	0.9
9r	6-Me	nc	345	56	177	0.7	0.9
9s	6-OMe	180−181	76	20	51	0.5	0.8
9t	6-F	165 dec[i]	24	20	52	0.6	0.9
9u	6-Cl	149−150	66	13	51	0.5	0.9
9v	6-Br	171−172	49	18	58	0.6	0.9
9w	6-CF_3	169−171	9600	160	355	0.6	1.1
9x	6-NMe_2	nc	760	320	225	0.3	0.6
9y	7-Me	nc	270	73	275	0.9	1.1
9z	7-Et	nc	1080	770	1640	0.9	1.4
9aa	7-^{i}Pr	nc	1450	2080	2080	1.1	1.1
9bb	7-tBu	nc	1760	1400	1070	1.1	1.1
9cc	7-Ph	162−163	710	610	1020	1.0	1.1
9dd	7-OMe	nc	132	20	147	0.70	1.1
9ee	7-F	128−133	140	32	96	0.6	0.8
9ff	7-Cl	183−185	170	46	136	0.8	0.9
9gg	7-Br	201−202	225	35	226	0.7	0.9
9hh	7-NMe_2	nc	970	183	760	1.0	0.9
9ii	8-Me	nc	180	16	71	0.7	1.0
9jj	8-Cl	nc	750	123	238	0.8	1.2
9kk	5,7-diMe	nc	210	20	105	0.5	0.8
9ll	5,8-diMe	119−124	24	3.2	11	0.4	0.7
9mm	1,5-diMe	110−116	21	1.4	3.9	0.3	0.7
9nn	5-Me, 8-Cl	212−215	41	8.8	20	0.5	0.7
9oo	1-Cl, 5-Me	156−158.5	48	6.0	12	0.3	0.6
amsacrine			20	12	37	85	74
doxorubicin			31	22	9.6	4.4	13

[a] IC_{50}, concentration of drug (nM) to reduce cell number to 50% of control cultures (see text). The value is the average of at least two independent determinations; the coefficient of variation was 12−18%. [b] Ratios of IC_{50}'s in the cell lines shown. [c] Murine P388 leukemia. [d] Murine Lewis lung carcinoma. [e] JL_C, wild-type human Jurkat leukemia. [f] JL_A, amsacrine-resistant Jurkat. [g] JL_D, doxorubicin-resistant Jurkat. [h] Data from ref 26. [i] HCl salt. [j] Noncrystalline.

Results and Discussion

IC_{50} values were determined for all the compounds in a panel of cell lines in culture, and the results are given in Table 1. The murine P388 leukemia was used to provide comparison with previous data on related compounds.[23] The three human leukemia (Jurkat) lines have been described in detail previously.[25,29] JL_C is the wild-type (sensitive) line, while JL_A is resistant to the DNA intercalator amsacrine and similar agents (85-fold resistant to amsacrine) by virtue of a reduced level of topo II enzyme. JL_D is a doxorubicin-resistant line, primarily by virtue of altered levels of topo II but probably also by additional mechanisms. LLTC is a murine Lewis lung carcinoma line,[30] included as a solid tumor model. Absolute IC_{50} values are given for the P388, LLTC, and JL_C lines, together with ratios of IC_{50} values against JL_C and the other two Jurkat lines (ratios JL_A/JL_C and JL_D/JL_C). Values of these ratios of less than about 2-fold suggest a novel, non-topo II-mediated mechanism of action. DACA itself (**8**), which is a mixed topo I/II inhibitor,[21] has ratios of 2.3 and 2.5, respectively, while the 6-chloro derivative, which has been shown to be a preferential topo I inhibitor,[21] has ratios of 1.2 and 1.3. The panel was designed to provide an initial screen for selecting analogues of DACA with greater absolute potency but similar or lower JL_A/JL_C and JL_D/JL_C ratios, for advanced evaluation against subcutaneous colon 38 tumors and human tumor xe-

nografts in vivo. For quantitative structure−activity relationships (QSAR), MR (molar refractivity) was used as a measure of steric bulk because values were available for all substituents, and it correlated well ($r = 0.96$) with the Taft steric parameter E_s in the cases where these values were also available. Lipophilicity was measured by π values, and electronic properties by σ_p values for 5- and 7-substituents and σ_m values for 6-and 8-substituents (reflecting contributions to acridine pK_a).[31]

Previous work[26] showed the utility of the $(CH_2)_3N(Me)(CH_2)_3$ linker chain, and this was used for studying the SAR of the acridine-substituted bis(DACA) analogues. The parent $(CH_2)_3N(Me)(CH_2)_3$-linked compound **9a** was 5-fold more cytotoxic than DACA in the JL_C line (IC_{50} 110 versus 580 nM). A series of compounds (**9a**−**9oo**) was prepared and evaluated in the cell line panel. For the whole set, IC_{50} values against the different lines correlated reasonably well (eqs 1 and 2), although not as tightly as for the substituted monoDACA analogues.[28]

$$\log(IC_{50})_{LLTC} = 0.98(\pm0.08)\log(IC_{50})_{P388} - 0.52(\pm0.21) \quad (1)$$

$$n = 40,\ r = 0.88,\ F = 135$$

$$\log(IC_{50})_{JLC} = 0.77(\pm0.07)\log(IC_{50})_{P388} + 0.38(\pm0.17) \quad (2)$$

$$n = 40,\ r = 0.87,\ F = 120$$

The dataset included two substituents (Me and Cl) in all seven available positions. A comparative analysis of the IC_{50}'s of these compounds showed that the positional effects fell into three categories. Substitution at the 3-position dramatically decreased potency over the parent unsubstituted compound **9a** in all cell lines (for example, 9-fold for the 3-Cl analogue **9g**, 12-fold for the 3-Me analogue **9f** in the JL_C line). Substitution at this position, *ortho* to the CONH, is likely to interfere with the conformation of the side chain. In the monoDACA series, 3-substitution led to a marked drop in DNA binding (and cytotoxicity).[23] In contrast, substitution at the 5-position markedly increased potency (for example, 3-fold for the 5-Cl compound **9n**, 10-fold for the 5-Me analogue **9h** in the JL_C line). For the remaining positions, there were no marked effects in potency compared to the parent compound.

The 5-substituted analogues **9h**−**9q** contained substituents possessing a wider range of electronic, steric, and hydrophobic properties. For the 5-substituted compounds **9h**−**9q** (and **9a**), regression equations correlating IC_{50} values with electronic, hydrophobic, and steric properties were developed, but only those with MR alone (for example, eq 3) were significant.

$$\log(IC_{50})_{JLC} = 0.78(\pm0.24)[0.1\ MR] + 1.51(\pm0.28) \quad (3)$$

$$n = 11,\ r = 0.74,\ F = 11$$

This overall equation suggests that larger groups lead to a loss of activity (probably by lowering DNA binding ability) but does not highlight the significant increase in potency of analogues substituted with small groups over the unsubstituted derivative **9a**. For example, **9h** (5-Me) and **9n** (5-Cl) have IC_{50}'s of 11 and 33 nM, respectively, against JL_C, compared with 110 nM for **9a**. Importantly, these increases in potency do not appear to be the result of a change in mechanism (as in the monoDACA series).[28] The compounds show even better activity against the resistant Jurkat lines (JL_A/JL_C and JL_D/JL_C ratios < 1), suggesting retention of the desired mixed topo I/II profile.

Most of the 6-substituted derivatives **9r**−**9x** were also slightly more potent than the parent compound, and all retained ratios of <1, but there were insufficient numbers of compounds to carry out QSAR studies. The 7-substituted analogues **9y**−**9hh** behaved broadly as the 5-substituted compounds but were less potent. The cytotoxicity data were again fitted best by the steric parameter MR alone (eq 4).

$$\log(IC_{50})_{JLC} = 0.50(\pm0.13)[0.1\ MR] + 2.05(\pm0.16) \quad (4)$$

$$n = 11,\ r = 0.79,\ F = 15$$

Multiple linear regressions with MR and σ for both the 5- and 7-substituted compounds provided negative coefficients for the σ parameter, suggestive of electron-withdrawing groups increasing cytotoxicity, but these did not quite reach the 5% level.

Because the 5-methyl derivative **9h** showed the highest cytotoxicity, a small series of 5,*x*-disubstituted compounds (**9kk**−**9oo**) was also evaluated. The 5,7-dimethyl compound **9kk** was prepared because studies with 5,7-disubstituted monomeric compounds[32] had shown that such compounds retained both the high potency of 5-substituted analogues and the mixed topo I/II-type activity pattern of the 5-substituted compounds. However, **9kk** was less cytotoxic than the corresponding 5-Me derivative **9h**.

The other disubstituted analogues focused on Cl or Me groups at the 1- or 8-position. These substituents by themselves had resulted in compounds of enhanced or equal potency to that of the parent compound **9a**, and the possible influence of steric bulk close to C-9, the site of the major route of cellular metabolism of DACA by aldehyde oxidase,[33] was of interest.

The results for these compounds are listed in Table 1, together with background data for the respective monosubstituted analogues. The 1,5- and 5,8-dimethyl analogues **9mm** and **9ll** had similar potencies to the 5-Me derivative **9h** (in fact **9mm** was about 2-fold more cytotoxic and was the most potent analogue studied). The 5-Me, 8-Cl and 1-Cl, 5-Me, derivatives **9nn** and **9oo** were slightly less effective but still had IC_{50}'s broadly similar to that of **9h** (and were much superior to the corresponding 1- and 8-Cl analogues **9c** and **9jj**). Thus, while the above 1- and 8-substituents are acceptable, it is the 5-methyl group that is largely responsible for the greatly enhanced potencies of these compounds compared to the unsubstituted parent **9a**.

A subset of the compounds (the parent, all the methyl- and chloro-substituted analogues, the 5-substituted compounds, and two 5,8-disubstituted derivatives) was evaluated in the NCI cell human line panel,[34] and results for six of those lines are presented in Table 2 as GI_{50} values (the concentration of drug resulting in inhibition of cell growth to 50% of controls; equivalent to IC_{50}), together with the mid-value (the average GI_{50} value for the drug over the whole cell line panel). A comparison of the GI_{50}(mid) value for each compound

Table 2. Cytotoxicity of Selected Bis(acridine-4-carboxamides) in the NCI Cell Line Panel

		GI_{50} (nM)[a]						
no.	substituent	MCF7	NCI-ADR	CAK-1	SKOV3	HT29	HCT-116	mid[b]
9a	H	24	408	14	8570	<10[c]	<10	130
9b	1-Me	54	77	42	1170	<10	<10	130
9d	2-Me	546	657	247	460	265	<10	470
9f	3-Me	3030	1.6×10^4	6360	1.3×10^4	1430	446	3800
9g	3-Cl	488	3.7×10^4	4580	8380	727	171	2240
9h	5-Me	<10	116	<10	87	<10	<10	17
9i	5-Et	265	1340	104	1.0×10^4	1390	221	910
9k	5-Ph	1020	1790	1.2×10^4	1.2×10^4	1620	5060	4070
9l	5-OMe	394	1390	1490	1.2×10^4	103	330	600
9m	5-F	<10	93	34	845	<10	<10	40
9n	5-Cl	167	1200	95.9	1.2×10^4	31	14	290
9o	5-Br	13	110	<10	166	<10	<10	26
9p	5-CF_3	79	1290	600	1260	33	32	175
9r	6-Me	530	407	669	3040	62	130	930
9u	6-Cl	13	274	<10	1.1×10^4	<10	<10	80
9y	7-Me	190	480	268	1180	34	12	220
9ii	8-Me	174	1040	58	594	60	13	165
9jj	8-Cl	614	624	450	1070	120	216	480
9ll	5,8-diMe	<10	172	<10	320	<10	<10	32

[a] GI_{50}, concentration of drug (nM) resulting in inhibition of cell growth to 50% of controls. [b] Mid: the average GI_{50} value for the drug over the whole cell line panel. [c] Lowest dose tested.

with IC_{50} values in the JL_C line shows a good correlation (eq 5), indicating that the latter is a useful model for broadly ranking the cytotoxicity of the compounds (in this and other calculations, values of <10 nM in Table 2 were taken as 10 nM).

$$\log(GI_{50})_{mid} = 0.98(\pm 0.10) \log(IC_{50})_{JLC} + 0.35(\pm 0.22) \quad (5)$$

$$n = 19,\ r = 0.92,\ F = 89$$

The MCF7 and NCI/ADR lines were selected to measure the degree to which the compounds are affected by P-glycoprotein-mediated multidrug resistance (the NCI/ADR line overexpresses P-glycoprotein). The degree of resistance shown by the P-glycoprotein overproducing line [measured as GI_{50}(NCI-ADR)/GI_{50}(MCF7)] varied from 0.75 (for **9r**) to 75 (for **9g**), with an average value over all the compounds of 10-fold. This suggests a moderate but significant degree of resistance for the class, but no SARs were obvious.

The CAK-1 renal tumor line and the SKOV3 ovarian carcinoma were selected as representative refractory solid tumor lines. Overall, the compounds displayed some selectivity toward colon cell lines, and HT29 and HCT-116 were selected as representative of the colon line subpanel. Selectivity for HT-116 [measured as GI_{50}(mid)/GI_{50}(HCT-116)] varied from 0.8 (for **9k**) to 47 (for **9d**), with an average of 11-fold, again with no obvious SARs. Results for HT29 were broadly similar.

The 5-Me, 5-Br, and 6-Cl derivatives **9h**, **9o**, and **9u** were evaluated in vivo against subcutaneous colon 38 tumors. The unsubstituted bis(DACA) **9a** provides a 5.5-day growth delay in this assay when given on a 4d×3 schedule at its MTD of 65 mg/kg/day but only a 2-day growth delay using a single-dose protocol (MTD of 90 mg/kg).[26] The analogues were therefore evaluated using the more stringent single-dose protocol (administered in two halves 1 h apart, commencing when the tumors were ca. 4 mm in diameter). The 6-Cl derivative **9u** showed modest growth delay (≈2 days), while the 5-bromo and 5-methyl compounds **9o** and **9h** were more effective, with growth delays of 5 and 6 days, respectively (Figure 1).

Conclusions

The parent bis(DACA) analogues **9**, where the two acridine-4-carboxamide chromophores are linked by a $(CH_2)_3N(Me)(CH_2)_3$ chain, are significantly more cytotoxic than the corresponding monomeric analogues. This phenomenon has been demonstrated previously with other neutral DNA-binding chromophores such as the naphthalimides (e.g., **3**)[14−17] and the imidazoacridinones (e.g., **6**).[18,19]

SARs for ring-substituted bis(DACA)s were broadly similar to those of the monomeric analogues,[28] with small substituents (e.g., Me, Cl) at the 5-position providing compounds of highest potency (IC_{50}'s as low as 2 nM against the Lewis lung carcinoma and 11 nM against the wild-type human Jurkat leukemia). Large substituents at any position caused a decrease in potency, likely due to lowering of DNA binding affinity. An important difference between the substituted mono-(DACA) and bis(DACA) series is that the JL_A and JL_D lines, which express low amounts of topo II as the cause of resistance to doxorubicin and amsacrine, are cross-resistant to some DACA derivatives but to none of the bis(DACA) derivatives. In the latter series, some members are up to 4-fold cytotoxic toward the JL_A and JL_D lines as compared to the wild-type JL_C (IC_{50} ratios ≤ 1, Table 1). The bis(5-methylDACA) compound **9h** was found to inhibit the action of purified topo I in a cell-free assay system (D. J. A. Bridewell, personal communication) in a manner similar to that shown by aclarubicin,[35] consistent with the hypothesis that topo I is involved in its cytotoxicity. The compounds were on average about 10-fold less potent toward a cell line that overexpresses P-glycoprotein and had some selectivity toward colon lines in the NCI human tumor panel. Of the four compounds evaluated in vivo, the two 5-substituted analogues **9o** and **9h** were the most effective, giving growth delays in the subcutaneous colon 38 tumor model of 5−6 days, the latter at 30 mg/kg. These results show that substituted bis(DACA)s are an interesting new class of topoisomerase inhibitors, worthy of further development.

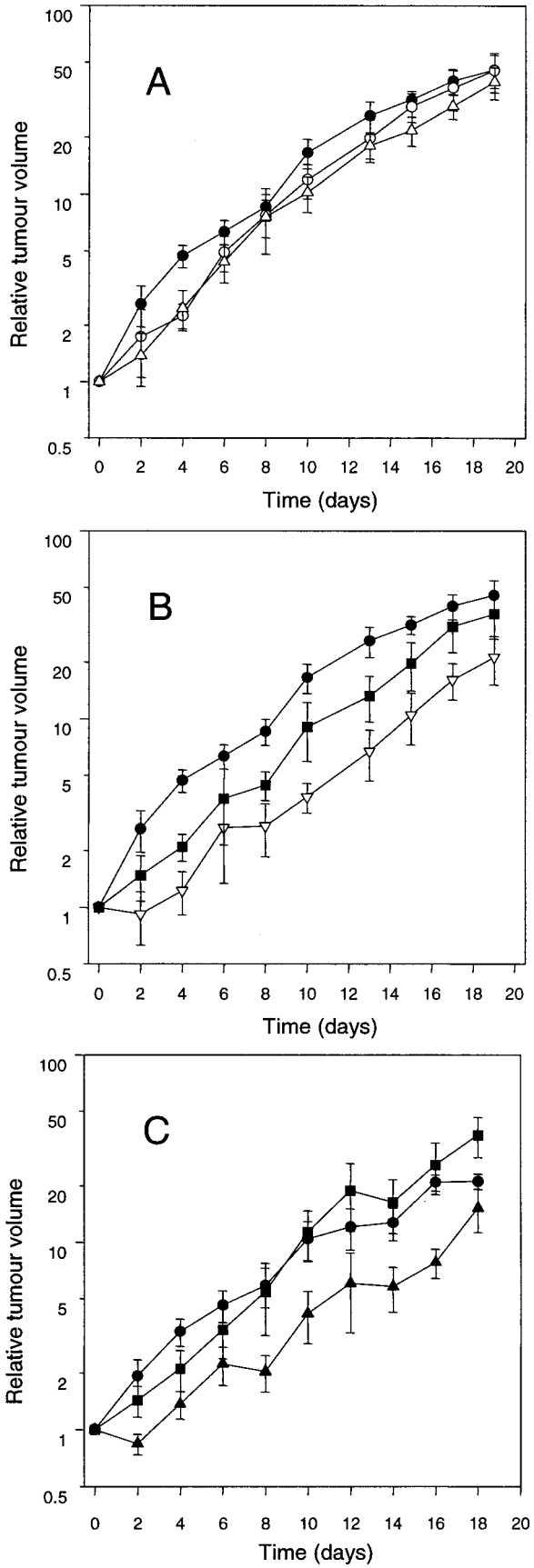


Figure 1. Averaged growth delay data for control (●) and treatment groups of five mice bearing advanced subcutaneous colon 38 tumors (see text), using a single drug dose (given in halves, 1 h apart): A, **9u** at 60 (△) and 90 (○) mg/kg; B, **9o** at 40 (■) and 60 (▽) mg/kg; C, **9h** at 20 (■) and 30 (▲) mg/kg. Higher doses than those indicated were toxic.

Experimental Section

Chemistry. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal 2300 melting point apparatus. NMR spectra were obtained on a Bruker DRX-400 spectrometer and are referenced to Me_4Si for organic solutions and 3-(trimethylsilyl)propanesulfonic acid, sodium salt for D_2O solutions. Thin-layer chromatography was carried out on aluminum-backed silica gel (Merck 60 F_{254}) or alumina plates. Flash column chromatography was carried out on Merck silica gel (230−400 mesh) or alumina. Petroleum ether refers to the fraction boiling at 40−60 °C. Satisfactory high-resolution mass spectral data were obtained for these compounds, using a VG 7070 spectrometer at nominal 5000 resolution. All of the bis(acridinecarboxamides) **9** were judged to be >98% pure by reverse-phase HPLC analysis with diode array detection. While the free bases of these compounds were stable, the hydrochloride salts were difficult to obtain free of water, and these hygroscopic forms decomposed slowly to give a mixture of compounds, primarily acridones.

***N,N*-Bis[3-(5-methylacridine-4-carboxamido)propyl]-methylamine (9h) by the Method of Scheme 3: General Example**. A suspension of 5-methylacridine-4-carboxylic acid[23] (**11h**) (5.30 g, 22.4 mmol) in DMF (60 mL) was treated with CDI (7.25 g, 44.7 mmol) and stirred at 40 °C until a clear solution was obtained. After cooling, the mixture was diluted with CH_2Cl_2 (50 mL) followed by petroleum ether (200 mL) to complete precipitation of product, which was collected, washed with petroleum ether/CH_2Cl_2 (4:1), and dried to give the moisture-sensitive imidazolide **12h**. This was dissolved in dry THF (100 mL), and the solution was cooled to 0 °C and treated with *N,N*-bis(3-aminopropyl)methylamine (1. 80 mL, 0.5 equiv). The mixture was stirred at 20 °C for 8 h, the THF was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (250 mL). The organic layer was washed with 1 M Na_2CO_3 (250 mL) and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The product was purified by chromatography on alumina-90, eluting with CH_2Cl_2/MeOH (200:1) to give **9h** (4.64 g, 66%): mp (CH_2Cl_2/hexane) 118−120 °C; ^{1}H NMR ($CDCl_3$) δ 1.97−2.00 (m, 4 H, 2×CH_2CH_2-CH_2), 2.30 (s, 3 H, NCH_3), 2.58 (t, J = 7.5 Hz, 4 H, $CH_2N(CH_3)CH_2$), 2.79 (s, 6 H, 2×CH_3), 3.70 (q, J = 6.7 Hz, 4 H, 2×CH_2NH), 7.37 (dd, J = 8.5, 6.8 Hz, 2 H, H-7), 7.57 (br d, J = 6.7 Hz, 2 H, H-8), 7.61 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 7.74 (br d, J = 8.6 Hz, 2 H, H-6), 8.03 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.69 (s, 2 H, H-9), 8.94 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.79 (br t, J = 5.1 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) *m/z* calcd for $C_{37}H_{38}N_5O_2$ 584.3026 (MH^+), found 584.3044. Anal. ($C_{37}H_{37}N_5O_2 \cdot 1.5H_2O$) C, H, N.

***N,N*-Bis[3-(1-methylacridine-4-carboxamido)propyl]-methylamine (9b).** From similar reaction of 1-methylacridine-4-carboxylic acid[23] (**11b**) (91% yield): oil; ^{1}H NMR ($CDCl_3$) δ 2.02−2.09 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.75 (t, J = 7.5 Hz, 4 H, $CH_2N(CH_3)CH_2$), 2.80 (d, J = 0.7 Hz, 6 H, 2×CH_3), 3.73 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.36 (ddd, J = 8.2, 6.7, 0.9 Hz, 2 H, H-6 or H-7), 7.43 (dd, J = 7.6, 0.8 Hz, 2 H, H-2), 7.66 (ddd, J = 8.7, 6.7, 1.4 Hz, 2 H, H-7 or H-6), 7.80 (d, J = 8.0 Hz, 2 H, H-5 or H-8), 7.99 (dd, J = 8.7, 0.8 Hz, 2 H, H-8 or H-5), 8.78 (s, 2 H, H-9), 8.80 (d, J = 7.3 Hz, 2 H, H-3), 11.77 (br t, J = 5.1 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) *m/z* calcd for $C_{37}H_{38}N_5O_2$ 584.3026 (MH^+), found 584.3041. Anal. ($C_{37}H_{37}N_5O_2$) C, H, N.

***N,N*-Bis[(1-chloroacridine-4-carboxamido)propyl]-methylamine (9c).** From similar reaction of 1-chloroacridine-4-carboxylic acid[23] (**11c**) (83% yield): mp (CH_2Cl_2/*n*-hexane) 94−96 °C; ^{1}H NMR ($CDCl_3$) δ 2.02−2.09 (m, 4 H, 2×CH_2CH_2-CH_2), 2.39 (s, 3 H, NCH_3), 2.79 (t, J = 7.4 Hz, 4 H, CH_2N-$(CH_3)CH_2$), 3.72 (q, J = 6.2 Hz, 4 H, 2×CH_2NH), 7.31 (ddd, J = 8.2, 6.7, 0.8 Hz, 2 H, H-6 or H-7), 7.63 (ddd, J = 8.7, 6.7, 1.3 Hz, 2 H, H-7 or H-6), 7.67 (d, J = 7.9 Hz, 2 H, H-2), 7.74 (br d, J = 8.0 Hz, 2 H, H-5 or H-8), 7.91 (br d, J = 8.7 Hz, 2 H, H-8 or H-5), 8.75 (d, J = 8.0 Hz, 2 H, H-3), 8.98 (s, 2 H, H-9), 11.45 (br t, J = 4.8 Hz, 2 H, 2×CONH). Anal. ($C_{35}H_{31}$-$Cl_2N_5O_2 \cdot 0.5H_2O$) C, N; H: calcd, 5.8; found, 5.1.

***N,N*-Bis[3-(2-methylacridine-4-carboxamido)propyl]-methylamine (9d).** From similar reaction of 2-methylacridine-4-carboxylic acid[23] (**11d**) (90% yield): foam; ^{1}H NMR ($CDCl_3$) δ 2.01−2.08 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.60 (s, 6 H, 2×CH_3), 2.73 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.74 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.38 (ddd, J = 8.6, 6.7, 0.9 Hz, 2 H, H-6 or H-7) 7.64 (ddd, J = 8.7, 6.7, 1.4 Hz, 2 H, H-7 or H-6), 7.76 (br s, 2 H, H-1), 7.79 (d, J = 8.8 Hz, 2H, H-5 or H-8), 8.00 (dd, J = 8.7, 0.7 Hz, 2 H, H-8 or H-5), 8.55 (s, 2 H, H-9), 8.76 (d, J = 2.1 Hz, 2 H, H-3), 11.79 (br t, J = 5.0 Hz, 2 H, 2×CONH). Anal. ($C_{37}H_{37}N_5O_2$) C, H, N.

***N,N*-Bis[(2-chloroacridine-4-carboxamido)propyl]-methylamine (9e).** From similar reaction of 2-chloroacridine-4-carboxylic acid[23] (**11e**) (54% yield): mp (CH_2Cl_2/hexane) 175.5−176.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.00−2.07 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.75 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.71 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.42 (ddd, J = 8.3, 6.6, 0.9 Hz, 2 H, H-6 or H-7), 7.68 (ddd, J = 8.7, 6.6, 1.3 Hz, 2 H, H-7 or H-6), 7.74 (br d, J = 7.9 Hz, 2 H, H-5 or H-8), 7.93−7.96 (m, 4 H, H-1 and H-8 or H-5), 8.49 (s, 2 H, H-9), 8.74 (d, J = 2.5 Hz, 2 H, H-3), 11.52 (br t, J = 5.0 Hz, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{35}H_{32}35Cl_2N_5O_2$ 624.1933 (MH^+), found 624.1900. Anal. ($C_{35}H_{21}Cl_2N_5O_2$) C, H, N.

***N,N*-Bis[3-(3-methylacridine-4-carboxamido)propyl]-methylamine (9f).** From similar reaction of 3-methylacridine-4-carboxylic acid[23] (**11f**), followed by purification using sequential chromatography on alumina followed by silica gel (15% yield): gum; ^{1}H NMR ($CDCl_3$) δ 1.78−1.84 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.18 (s, 3 H, NCH_3), 2.48 (s, 6 H, 2×CH_3), 2.67 (t, J = 6.5 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.56 (q, J = 6.1 Hz, 4 H, 2×CH_2NH), 7.19 (d, J = 8.6 Hz, 2 H, H-1 or H-2), 7.33 (ddd, J = 8.4, 6.6, 1.0 Hz, 2 H, H-6 or H-7), 7.39 (br t, J = 5.3 Hz, 2 H, 2×CONH), 7.58 (ddd, J = 8.8, 6.6, 1.4 Hz, 2 H, H-7 or H-6), 7.66 (d, J = 8.7 Hz, H-2 or H-1), 7.70 (br d, J = 7.9 Hz, 2 H, H-5 or H-8), 7.94 (d, J = 8.6 Hz, 2 H, H-8 or H-5), 8.37 (s, 2 H, H-9); HRMS (FAB$^+$) m/z calcd for $C_{37}H_{38}N_5O_2$ 584.3026, found 584.3016.

***N,N*-Bis[3-(3-chloroacridine-4-carboxamido)propyl]-methylamine (9g).** A suspension of 3-chloroacridine-4-carboxylic acid[23] (**11g**) (540 mg, 2.10 mmol) in $SOCl_2$ (30 mL) was heated under reflux for 15 min, and excess reagent was then evaporated under reduced pressure. The residue was azeotroped with dry benzene (30 mL) to give crude 3-chloroacridine-4-carbonyl chloride. This was cooled, dissolved in THF (50 mL) containing triethylamine (5% v/v), and then treated with bis-(3-aminopropyl)methylamine (150 mg, 1.05 mmol). The mixture was stirred at room temperature overnight, the THF was removed under reduced pressure, and the residue was partitioned between aqueous Na_2CO_3/CH_2Cl_2. Evaporation of the organic layer and chromatography of the residue on alumina, eluting with 0.5% MeOH/CH_2Cl_2, gave **9g** (73%): mp (CH_2Cl_2/hexane) 117 °C dec; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74−1.83 (m, 4 H, 2$CH_2CH_2CH_2CH_2$), 2.16 (s, 3 H, NCH_3), 2.65 (t, J = 6.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.57 (q, J = 6.1 Hz, 4 H, 2CH_2NH), 6.90 (br t, J = 5.4 Hz, 2 H, 2xCONH), 7.32 (d, J = 8.9 Hz, 2 H, ArH), 7.40 (ddd, J = 8.3, 6.6, 0.9 Hz, 2 H, ArH), 7.63 (ddd, J = 8.9, 6.6, 1.3 Hz, 2 H, ArH), 7.71 (d, J = 9.0 Hz, 2 H, ArH), 7.75 (d, J = 8.9 Hz, 2 H, ArH), 8.00 (d, J = 9.0 Hz, 2H, ArH), 8.44 (s, 2H, H-9); HRMS (FAB$^+$) m/z calcd for $C_{35}H_{32}$-$^{35}Cl_2N_5O_2$ 624.1933 (MH^+), found 624.1945. Anal. ($C_{35}H_{31}Cl_2N_5O_2\cdot 3H_2O$) C, N; H: found, 4.7; calcd 5.5.

***N,N*-Bis[3-(5-ethylacridine-4-carboxamido)propyl]-methylamine (9i).** From similar reaction of 5-ethylacridine-4-carboxylic acid[28] (**11i**) (57% yield): mp (CH_2Cl_2/hexane) 70−73 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J = 7.5 Hz, 6 H, 2×CH_3), 1.97−2.04 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.32 (s, 3 H, NCH_3), 2.61 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.24 (q, J = 7.5 Hz, 4 H, 2×CH_2CH_3), 3.69 (q, J = 6.2 Hz, 4 H, 2×CH_2NH), 7.40 (dd, J = 8.4, 6.8 Hz, 2 H, H-7), 7.53 (dd, J = 6.7 Hz, 1.0, 2 H, H-6), 7.60 (dd, J = 8.3, 7.1 Hz, 2 H, H-2), 7.74 (dd, J = 8.2, 1.0 Hz, 2 H, H-8), 8.02 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.69 (s, 2 H, H-9), 8.93 (dd, J = 7.1, 1.6 Hz, 2 H, H-3), 11.77 (br t, J = 5.5 Hz, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{39}H_{42}N_5O_2$ 612.3339 (MH^+), found 612.3343. Anal. ($C_{39}H_{41}N_5O_2\cdot H_2O$) C, H, N.

***N,N*-Bis[3-(5-isopropylacridine-4-carboxamido)propyl]-methylamine (9j).** From similar reaction of 5-isopropylacridine-4-carboxylic acid[28] (**11j**) (70% yield): oil; ^{1}H NMR ($CDCl_3$) δ 1.47 (d, J = 7.0 Hz, 12 H, 4CH_3), 1.96−2.03 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.32 (s, 3 H, NCH_3), 2.59 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.70 (q, J = 6.8 Hz, 4 H, 2×CH_2NH), 4.15−4.18 (m, 2 H, 2×CH), 7.53 (dd, J = 8.4, 6.9 Hz, 2 H, H-7), 7.63 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 7.67 (br d, J = 6.6 Hz, 2 H, H-6), 7.83 (dd, J = 8.4, 1.1 Hz, 2 H, H-8), 8.07 (dd, J = 8.3, 1.5 Hz, 2 H, H-1), 8.80 (s, 2 H, H-9), 8.95 (dd, J = 7.1, 1.6 Hz, 2 H, H-3), 11.80 (br t, J = 5.6 Hz, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{41}H_{46}N_5O_2$ 640.3652 (MH^+), found 640.3655.

***N,N*-Bis[3-(5-phenylacridine-4-carboxamido)propyl]-methylamine (9k).** From similar reaction of 5-phenylacridine-4-carboxylic acid (**11k**)[23] (64% yield): mp (CH_2Cl_2/hexane) 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 1.24−1.26 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.04 (s, 3 H, NCH_3), 2.06−2.10 (br t, J = 7.7 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.13 (q, J = 6.9 Hz, 4 H, 2×CH_2NH), 7.43−7.45 (m, 2 H, H-4'), 7.49−7.53 (m, 4 H, H-3',5'), 7.59−7.67 (m, 8 H, H-2,2',6',7), 7.75 (dd, J = 6.7, 1.4 Hz, 2 H, H-6), 7.99 (dd, J = 8.5, 1.3 Hz, 2 H, H-8), 8.09 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.88 (s, 2 H, H-9), 8.94 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.06 (br t, J = 6.0 Hz, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{47}H_{42}N_5O_2$ 708.3339 (MH^+), found 708.3345. Anal. ($C_{47}H_{41}N_5O_2\cdot 0.5H_2O$) C, H, N.

***N,N*-Bis[3-(5-methoxyacridine-4-carboxamido)propyl]-methylamine (9l).** From similar reaction of 5-methoxyacridine-4-carboxylic acid[23] (**11l**) (71% yield): oil; ^{1}H NMR ($CDCl_3$) δ 1.99−2.06 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.72 (t, J = 7.6 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.65 (q, J = 6.4 Hz, 4 H, 2×CH_2NH), 3.87 (s, 6 H, 2×OCH_3), 6.67 (dd, J = 6.5, 2.0 Hz, 2 H, H-6), 7.10−7.16 (m, 4 H, H-7,8), 7.54 (dd, J = 8.2, 7.2 Hz, 2 H, H-2), 7.86 (dd, J = 8.4, 1.3 Hz, 2 H, H-1), 8.36 (s, 2 H, H-9), 8.82 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 12.04 (br t, J = 4.6 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{37}H_{38}N_5O_4$ 616.2924 (MH^+), found 616.2943.

***N,N*-Bis[3-(5-fluoroacridine-4-carboxamido)propyl]-methylamine (9m).** From similar reaction of 5-fluoroacridine-4-carboxylic acid[28] (**11m**) (96% yield): mp (CH_2Cl_2/hexane) 134−136 °C; ^{1}H NMR (free base in $CDCl_3$) δ 2.06 (quin, J = 7.2 Hz, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.73 (t, J = 7.6 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.72 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.26 (m, 4 H, ArH), 7.56 (m, 2 H, ArH), 7.59 (dd, J = 8.4, 7.2 Hz, 2 H, H-2), 7.99 (dd, J = 8.5, 1.4 Hz, 2 H, ArH), 8.64 (d, J = 0.6 Hz, 2 H, H-9), 8.93 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.61 (t, J = 4.6 Hz, 2 H, CONH). Anal. ($C_{35}H_{31}F_2N_5O_2\cdot H_2O$) C, H, N, F. Trihydrochloride salt: mp (MeOH/EtOAc) 188 °C dec. Anal. ($C_{35}H_{31}F_2N_5O_2\cdot 3HCl$) C, H, N.

***N,N*-Bis[3-(5-chloroacridine-4-carboxamido)propyl]-methylamine (9n).** From similar reaction of 5-chloroacridine-4-carboxylic acid[23] (**11n**) (62% yield): mp (CH_2Cl_2/hexane) 89−93 °C; ^{1}H NMR ($CDCl_3$) δ 2.01−2.05 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.33 (s, 3 H, NCH_3), 2.64 (t, J = 7.5 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.72 (q, J = 6.6 Hz, 4 H, 2×CH_2NH), 7.33 (dd, J = 8.4, 7.3 Hz, 2 H, H-7), 7.58 (dd, J = 8.2, 7.2 Hz, 2 H, H-2), 7.74−7.77 (m, 4 H, H-6 and H-8), 7.96 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.64 (s, 2 H, H-9), 8.91 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.74 (br t, J = 5.3 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{35}H_{32}$-$^{35}Cl_2N_5O_2$ 624.1933 (MH^+), found 624.1940. Anal. ($C_{35}H_{31}Cl_2N_5O_2\cdot 1.5H_2O$) C, H, N.

***N,N*-Bis[3-(5-bromoacridine-4-carboxamido)propyl]-methylamine (9o).** From similar reaction of 5-bromoacridine-4-carboxylic acid[28] (**11o**) (80% yield): mp (CH_2Cl_2/hexane) 172−174.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.05 (quin, J = 7.3 Hz, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.33 (s, 3 H, NCH_3), 2.63 (t, J = 7.6 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.73 (q, J = 6.7 Hz, 4 H, 2×CH_2NH), 7.28 (dd, J = 8.2, 7.2 Hz, 2 H, H-2), 7.57 (dd, J = 8.4, 7.2 Hz, 2 H, H-7), 7.81 (dd, J = 8.5, 0.9 Hz, 2 H, H-1), 7.91 (m, 4 H, ArH), 8.64 (s, 2 H, H-9), 8.90 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.72 (t, J = 5.6 Hz, 2 H, CONH). Anal. ($C_{35}H_{31}Br_2N_5O_2\cdot H_2O$) C, H, N, Br.

***N,N*-Bis[3-(5-(trifluoromethyl)acridine-4-carboxamido)propyl]methylamine (9p).** From similar reaction of 5-(trifluoromethyl)acridine-4-carboxylic acid[28] (**11p**) (52% yield): oil; ^{1}H NMR [free base in $(CD_3)_2SO$] δ 1.81 (quin, J = 7.1 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.42 (s, 3 H, NCH_3), 2.44 (t, J = 7.1 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.51 (q, J = 6.8 Hz, 4 H, 2×CH_2NH), 7.73 (q, J = 7.4 Hz, 4 H, ArH), 8.24−8.29 (m, 4 H, ArH), 8.42 (d, J = 8.1 Hz, 2 H, ArH), 8.78 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 9.30 (s, 2 H, 2×H-9), 10.92 (t, J = 5.8 Hz, 2 H, 2×CONH). Anal. ($C_{37}H_{31}F_6N_5O_2$·3HCl·2H_2O) C, H, N.

***N,N*-Bis[3-(6-methylacridine-4-carboxamido)propyl]methylamine (9r).** From similar reaction of 6-methylacridine-4-carboxylic acid[23] (**11r**) (89% yield): foam; ^{1}H NMR ($CDCl_3$) δ 2.08 (quin, J = 7.0 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.42 (s, 3 H, NCH_3), 2.44 (d, J = 0.7 Hz, 6 H, 2×CH_3), 2.82 (t, J = 7.5 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.75 (q, J = 6.2 Hz, 4 H, 2×CH_2NH), 7.07 (dd, J = 8.6, 1.5 Hz, 2 H, H-7), 7.54−7.59 (m, 4 H, H-2,8), 7.70 (br s, 2 H, H-5), 7.99 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.52 (s, 2 H, H-9), 8.89 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.83 (t, J = 5.0 Hz, 2 H, 2×CONH). Anal. ($C_{37}H_{37}N_5O_2$) C, H, N.

***N,N*-Bis[3-(6-methoxyacridine-4-carboxamido)propyl]methylamine (9s).** From similar reaction of 6-methoxyacridine-4-carboxylic acid[23] (**11s**) (68% yield): mp (CH_2Cl_2/hexane) 180−181 °C dec; ^{1}H NMR [free base in $(CD_3)_2SO$] δ 2.07 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.41 (s, 3 H, NCH_3), 2.78 (m, 4 H, $CH_2N(CH_3)CH_2$), 3.74 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 3.89 (s, 6 H, 2×OCH_3), 6.98 (d, J = 9.0 Hz, 2 H, H-7), 7.08 (br s, 2 H, H-5), 7.54 (dd, J = 8.1, 7.3 Hz, 2 H, H-2), 7.60 (d, J = 9.1 Hz, 2 H, H-8), 7.96 (dd, J = 8.3, 1.3 Hz, 2 H, H-1), 8.50 (s, 2 H, H-9), 8.88 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.77 (br s, 2 H, 2×CONH). Anal. ($C_{37}H_{37}N_5O_4$) C, H, N. Dihydrochloride salt: mp (MeOH/EtOAc) mp 204−206 °C.

***N,N*-Bis[3-(6-fluoroacridine-4-carboxamido)propyl]methylamine (9t).** From similar reaction of 6-fluoroacridine-4-carboxylic acid[28] (**11t**) (57% yield): oil; ^{1}H NMR (free base in $CDCl_3$) δ 2.04 (quin, J = 7.1 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.39 (s, 3 H, NCH_3) 2.72 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.45 (q, J = 6.4 Hz, 4 H, 2×CH_2NH), 7.28 (ddd, J = 9.2, 8.0, 2.4 Hz, 2 H, H-7), 7.62 (dd, J = 8.4, 7.2 Hz, 2 H, H-2), 7.69 (dd, J = 7.6, 2.4 Hz, 2 H, H-1), 7.89 (dd, J = 9.2, 6.1 Hz, 2 H, H-8), 8.05 (dd, J = 8.3, 1.5 Hz, 2 H, H-5), 8.74 (s, 2 H, H-9), 8.95 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.57 (t, J = 4.9 Hz, 2 H, CONH). Hydrochloride salt: mp (MeOH/EtOAc) 165.5 °C dec; Anal. ($C_{35}H_{31}F_2N_5O_2$·HCl·4H_2O) C, H, N.

***N,N*-Bis[3-(6-chloroacridine-4-carboxamido)propyl]methylamine (9u).** From similar reaction of 6-chloroacridine-4-carboxylic acid[23] (**11u**) (76% yield): mp (MeOH/EtOAc) 149−150 °C; ^{1}H NMR ($CDCl_3$) δ 2.06 (quin, J = 7.1 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.40 (s, 3 H, NCH_3), 2.75 (t, J = 7.2 Hz, 4 H, CH_2NCH_3), 3.74 (q, J = 6.3 Hz, 4 H, $CH_2N(CH_3)CH_2$), 7.32 (dd, J = 8.9, 2.0 Hz, 2 H, H-7), 7.64 (dd, J = 8.2, 7.2 Hz, 2 H, H-2), 7.75 (d, J = 8.9 Hz, 2 H, H-8), 8.03 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.05 (d, J = 2.0 Hz, 2 H, H-5), 8.67 (s, 2 H, H-9), 8.95 (dd, J = 7.1, 1.6 Hz, 2 H, H-3), 11.50 (t, J = 5.1 Hz, 2 H, 2×CONH). Anal. ($C_{35}H_{31}Cl_2N_5O_2$) C, H, N, Cl.

***N,N*-Bis[3-(6-bromoacridine-4-carboxamido)propyl]methylamine (9v).** From similar reaction of 6-bromoacridine-4-carboxylic acid[28] (**11v**) (91% yield): mp (CH_2Cl_2/hexane) 171−172 °C; ^{1}H NMR ($CDCl_3$) δ 2.07 (quin, J = 7.0 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.41 (s, 3 H, CH_3), 2.76 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.75 (q, J = 6.4 Hz, 4 H, 2×CH_2NH), 7.42 (dd, J = 9.0, 1.8 Hz, 2 H, H-7), 7.65 (m, 4 H, H-2,8), 8.03 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.25 (d, J = 0.9 Hz, 2 H, H-5), 8.67 (s, 2 H, H-9), 8.95 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.45 (t, J = 5.0 Hz, 2 H, CONH). Anal. ($C_{35}H_{31}Br_2N_5O_2$) C, H, N, Br.

***N,N*-Bis[3-(6-(trifluoromethyl)acridine-4-carboxamido)propyl]methylamine (9w).** From similar reaction of 6-(trifluoromethyl)acridine-4-carboxylic acid[28] (**11w**) (60% yield): mp (CH_2Cl_2/hexane) 169−171 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 1.89 (quin, J = 6.6 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.28 (s, 3 H, NCH_3), 2.66 (t, J = 6.8 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.56 (q, J = 6.1 Hz, 4 H, 2×CH_2NH), 7.60 (dd, J = 8.8, 1.5 Hz, 2 H, H-7), 7.68 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 8.14 (d, J = 8.8 Hz, 2 H, H− 8), 8.23 (dd, J = 8.4, 1.4 Hz, 2 H, H-1), 8.38 (s, 2 H, H-5), 8.55 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 9.13 (s, 2 H, H-9), 10.78 (t, J = 5.5 Hz, 2 H, CONH). Anal. ($C_{37}H_{31}F_6N_5O_2$·0.5H_2O) C, H, N.

***N,N*-Bis[3-(7-methylacridine-4-carboxamido)propyl]methylamine (9y).** From similar reaction of 7-methylacridine-4-carboxylic acid[23] (**11y**) (73% yield): oil; ^{1}H NMR ($CDCl_3$) δ 2.03−2.10 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.34 (s, 6 H, 2×CH_3), 2.39 (s, 3 H, NCH_3), 2.80 (t, J = 7.6 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.73 (q, J = 6.2 Hz, 4 H, 2×CH_2NH), 7.30 (br s, 2 H, H-8), 7.35 (dd, J = 8.8, 1.9 Hz, 2 H, H-6), 7.55 (dd, J = 8.4, 7.1 Hz, 2 H, H-2), 7.77 (d, J = 8.9 Hz, 2 H, H-5), 7.92 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.36 (s, 2 H, H-9), 8.84 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.74 (br t, J = 5.0 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{37}H_{38}N_5O_2$ 584.3026 (MH^+), found 584.3043. Anal. ($C_{37}H_{37}N_5O_2$·0.5H_2O) C, H, N.

***N,N*-Bis[3-(7-ethylacridine-4-carboxamido)propyl]methylamine (9z).** From similar reaction of 7-ethylacridine-4-carboxylic acid[28] (**11z**) (47% yield): oil; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.5 Hz, 6 H, 2×CH_3), 2.03−2.11 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.40 (s, 3 H, NCH_3), 2.70 (q, J = 7.6 Hz, 4 H, 2×CH_2CH_3), 2.79 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.74 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.46 (br s, 2 H, H-8), 7.48 (dd, J = 8.9, 1.8 Hz, 2 H, H-6), 7.56 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 7.89 (d, J = 8.8 Hz, 2 H, H-5), 7.98 (dd, J = 8.3, 1.5 Hz, 2 H, H-1), 8.51 (s, 2 H, H-9), 8.86 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.80 (br t, J = 5.0 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{39}H_{42}N_5O_2$ 612.3339 (MH^+), found 612.3333.

***N,N*-Bis[3-(7-isopropylacridine-4-carboxamido)propyl]methylamine (9aa).** From similar reaction of 7-isopropylacridine-4-carboxylic acid[28] (**11aa**) (73% yield): oil; ^{1}H NMR ($CDCl_3$) δ 1.33 (d, J = 6.9 Hz, 12 H, 4CH_3), 2.04−2.08 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.74 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.03−3.06 (m, 2 H, 2×CH), 3.74 (q, J = 6.4 Hz, 4 H, 2×CH_2NH), 7.58 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 7.60−7.66 (m, 4 H, H-6 and H-8), 8.01 (d, J = 9.5 Hz, 2 H, H-5), 8.03 (dd, J = 8.3, 1.5 Hz, 2 H, H-1), 8.66 (s, 2 H, H-9), 8.88 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.85 (br t, J = 5.1 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{41}H_{46}N_5O_2$ 640.3652 (MH^+), found 640.3657.

***N,N*-Bis[3-(7-*tert*-butylacridine-4-carboxamido)propyl]methylamine (9bb).** From similar reaction of 7-*tert*-butylacridine-4-carboxylic acid[28] (**11bb**) (82% yield): oil; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 18 H, 6CH_3), 2.04−2.07 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.72 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.74 (q, J = 6.4 Hz, 4 H, 2×CH_2NH), 7.59 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 7.81 (d, J = 2.1 Hz, 2 H, H-8), 7.88 (dd, J = 9.2, 2.1 Hz, 2 H, H-6), 8.05 (dd, J = 8.3, 1.4 Hz, 2 H, H-1), 8.07 (d, J = 9.3 Hz, 2 H, H-5), 8.73 (s, 2 H, H-9), 8.89 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.87 (br t, J = 5.1 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{43}H_{50}N_5O_2$ 668.3965 (MH^+), found 668.3963.

***N,N*-Bis[3-(7-phenylacridine-4-carboxamido)propyl]methylamine (9cc).** From similar reaction of 7-phenylacridine-4-carboxylic acid[28] (**11cc**) (90% yield): mp (CH_2Cl_2/MeOH) 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 2.07−2.14 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.42 (s, 3 H, NCH_3), 2.82 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.77 (q, J = 6.2 Hz, 4 H, 2×CH_2NH), 7.40−7.52 (m, 4 H, ArH), 7.48−7.52 (m, 4 H, ArH), 7.63−7.65 (m, 4 H, ArH), 7.82−7.84 (m, 4 H, ArH), 7.87 (dd, J = 8.4, 1.4 Hz, 2 H, H-1), 7.97 (d, J = 9.5 Hz, 2 H, H-5), 8.54 (s, 2 H, H-9), 8.80 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.69 (br t, J = 5.2 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{47}H_{42}N_5O_2$ 708.3339 (MH^+), found 708.3351. Anal. ($C_{47}H_{41}N_5O_2$·0.5H_2O) C, H, N.

***N,N*-Bis[3-(7-methoxyacridine-4-carboxamido)propyl]methylamine (9dd).** From similar reaction of 7-methoxyacridine-4-carboxylic acid[23] (**11dd**) (93% yield): oil; ^{1}H NMR ($CDCl_3$) δ 2.02−2.06 (m, 4 H, 2×$CH_2CH_2CH_2$), 2.37 (s, 3 H, NCH_3), 2.74 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.72 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 3.88 (s, 6 H, 2×OCH_3), 6.89 (d, J = 2.7 Hz, 2 H, H-8), 7.32 (dd, J = 9.3, 2.7 Hz, 2 H, H-6), 7.54 (dd, J = 8.2, 7.2 Hz, 2 H, H-2), 7.85 (d, J = 9.3 Hz, 2 H, H-5), 7.94 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.44 (s, 2 H, H-9), 8.82 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.69 (br t, J = 5.1 Hz, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{37}H_{38}N_5O_4$ 616.2924 (MH^+), found 616.2927.

***N,N*-Bis[3-(7-fluoroacridine-4-carboxamido)propyl]-methylamine (9ee).** From similar reaction of 7-fluoroacridine-4-carboxylic acid[28] (**11ee**) (57% yield): mp (CH_2Cl_2/hexane) 128−133 °C; ^{1}H NMR ($CDCl_3$) δ 2.04 (quin, J = 7.17 Hz, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.73 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.73 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.32 (dd, J = 8.6, 2.7 Hz, 2 H, H-8), 7.42 (ddd, J = 9.4, 8.1, 2.8 Hz, 2 H, H-6), 7.63 (dd, J = 7.7, 7.2 Hz, 2 H, H-2), 7.96 (dd, J = 9.1, 4.9 Hz, 2 H, H-5), 7.99 (dd, J = 7.7, 1.5 Hz, 2 H, H-1), 8.56 (s, 2 H, H-9), 8.89 (dd, J = 7.0, 1.5 Hz, 2 H, H-3), 11.50 (t, J = 5.0 Hz, 2 H, 2×CONH). Anal. ($C_{35}H_{31}F_2N_5O_2$) C, H, N, F.

***N,N*-Bis[3-(7-chloroacridine-4-carboxamido)propyl]-methylamine (9ff).** From similar reaction of 7-chloroacridine-4-carboxylic acid[23] (**11ff**) (75% yield): mp (CH_2Cl_2/hexane) 183−185 °C; ^{1}H NMR ($CDCl_3$) δ 2.03−2.07 (m, 4 H, 2×$CH_2CH_2CH_2$-CH_2), 2.38 (s, 3 H, NCH_3), 2.75 (t, J = 7.4 Hz, 4 H, CH_2N-$(CH_3)CH_2$), 3.73 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.45 (dd, J = 9.2, 2.3 Hz, 2 H, H-6), 7.63−7.67 (m, 4 H, H-2 and H-8), 7.84 (d, J = 9.2 Hz, 2 H, H-5), 7.99 (dd, J = 8.4, 1.5 Hz, 2 H, H-1), 8.48 (s, 2 H, H-9), 8.93 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.42 (br t, J = 5.0 Hz, 2×CONH); HRMS (FAB+) *m/z* calcd for (MH^+) $C_{35}H_{32}{}^{35}Cl_2N_5O_2$ 624.1933, found 624.1923. Anal. ($C_{35}H_{31}$-$Cl_2N_5O_2$) C, H, N.

***N,N*-Bis[3-(7-bromoacridine-4-carboxamido)propyl]-methylamine (9gg).** From similar reaction of 7-bromoacridine-4-carboxylic acid[28] (**11gg**) (55% yield): mp (CH_2Cl_2/hexane) 201−202 °C; ^{1}H NMR ($CDCl_3$) δ 2.04 (quin, J = 7.0 Hz, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.75 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.73 (q, J = 6.3 Hz, 4 H, 2×CH_2NH), 7.55 (dd, J = 9.1, 2.1 Hz, 2 H, H-6), 7.66 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 7.76 (d, J = 9.2 Hz, 2 H, H-5), 7.83 (d, J = 2.1 Hz, 2 H, H-8), 7.99 (dd, J = 8.2, 1.4 Hz, 2 H, H-1), 8.46 (s, 2 H, H-9), 8.94 (dd, J = 7.0, 1.5 Hz, 2 H, H-3), 11.41 (t, J = 4.9 Hz, 2 H, 2×CONH). Anal. ($C_{35}H_{31}Br_2N_5O_2$) C, H, N, Br.

***N,N*-Bis[3-(8-methylacridine-4-carboxamido)propyl]-methylamine (9ii).** From similar reaction of 8-methylacridine-4-carboxylic acid[23] (**11ii**) (61% yield): oil; ^{1}H NMR ($CDCl_3$) δ 2.03−2.10 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.39 (s, 3 H, NCH_3), 2.66 (s, 6 H, 2×CH_3), 2.79 (t, J = 7.5 Hz, 4 H, $CH_2N(CH_3)$-CH_2), 3.74 (q, J = 6.2 Hz, 4 H, 2×CH_2NH), 7.09 (d, J = 6.9 Hz, 2 H, H-5 or H-7), 7.49 (dd, J = 8.8, 6.8 Hz, 2 H, H-6), 7.62 (dd, J = 8.4, 7.1 Hz, 2H, H-2), 7.83 (d, J = 8.7 Hz, 2 H, H-7 or H-5), 8.04 (dd, J = 8.3, 1.5 Hz, 2 H, H-1), 8.74 (s, 2 H, H-9), 8.91 (dd, J = 7.1, 1.5 Hz, 2H, H-3), 11.78 (br t, J = 4.8 Hz, 2 H, 2×CONH). Anal. ($C_{37}H_{37}N_5O_2$·0.5H_2O) C, H, N.

***N,N*-Bis[3-(8-chloroacridine-4-carboxamido)propyl]-methylamine (9jj).** From similar reaction of 8-chloroacridine-4-carboxylic acid[23] (**11jj**) (88% yield): oil; ^{1}H NMR ($CDCl_3$) δ 2.04−2.10 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.40 (s, 3 H, NCH_3), 2.84 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.74 (q, J = 6.1 Hz, 4 H, 2×CH_2NH), 7.10 (dd, J = 7.3, 0.8 Hz, 2 H, H-5 or H-7), 7.33 (dd, J = 8.8, 7.3 Hz, 2 H, H-2 or H-6), 7.65 (dd, J = 8.3, 7.2 Hz, 2H, H-6 or H-2), 7.73 (d, J = 8.7 Hz, 2H, H-7 or H-5), 8.06 (dd, J = 8.8, 1.5 Hz, 2 H, H-1), 8.86 (s, 2H, H-9), 8.90 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.36 (br t, J = 5.0 Hz, 2 H, 2×CONH); HRMS (FAB^+) *m/z* calcd for $C_{35}H_{32}{}^{35}Cl_2N_5O_2$ 624.1933 (MH^+), found 624.1939. Anal. ($C_{35}H_{31}Cl_2N_5O_2$) C, H, N.

***N,N*-Bis[3-(5,7-dimethylacridine-4-carboxamido)propyl]-methylamine (9kk).** From similar reaction of 5,7-dimethylacridine-4-carboxylic acid[32] (**11kk**) (56% yield): oil; ^{1}H NMR ($CDCl_3$) δ 1.94−2.05 (m, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.31 (s, 3 H, NCH_3), 2.45 (s, 6 H, 2×CH_3), 2.58 (t, J = 7.4 Hz, 4 H, CH_2N-$(CH_3)CH_2$), 2.70 (s, 6 H, 2CH_3), 3.68 (dd, J = 7.2, 5.7 Hz, 4 H, 2×CH_2NH),7.32 (br s, 2 H, H-6 or H-8) 7.41 (br s, 2 H, H-8 or H-6), 7.57 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 7.96 (dd, J = 8.4, 1.4 Hz, 2 H, H-1), 8.49 (s, 2 H, H-9), 8.89 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 11.75 (br t, J = 5.3 Hz, 2 H, 2×CONH); HRMS (FAB^+) *m/z* calcd for $C_{39}H_{42}N_5O_2$ 612.3339 (MH^+), found 612.3330. Anal. ($C_{39}H_{41}N_5O_2$·0.5H_2O) C, H, N.

***N,N*-Bis[3-(5,8-dimethylacridine-4-carboxamido)propyl]-methylamine (9ll).** Reaction of 2,5-dimethylaniline (**13**) and 2-iodobenzene-1,3-dicarboxylic acid (**15**) as reported[28] gave crude 2-[(2,5-dimethylphenyl)amino]benzene-1,3-dicarboxylic acid (**16**). This was cyclized directly with PPA to give 5,8-dimethylacridone-4-carboxylic acid (**10ll**) (46% overall): mp (MeOH/H_2O) 343−346 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.87 (s, 6 H, 2×CH_3), 6.98 (d, J = 7.3 Hz, 1 H, H-6), 7.33 (t, J = 7.7 Hz, 1 H, H− 2), 7.51 (d, J = 7.5 Hz, 1 H, H-7), 8.41 (dd, J = 7.6, 1.6 Hz, 1 H, H-1), 8.46 (dd, J = 7.9, 1.6 Hz, 1 H, H-3), 12.00 (br s, 1 H, NH), 13.93 (br s, 1 H, COOH). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

Reduction of **10ll** as above gave 5,8-dimethylacridine-4-carboxylic acid (**11ll**) (82%): mp (MeOH/H_2O) 239−241 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.78 (s, 3 H, CH_3), 2.83 (s, 3 H, CH_3), 7.50 (d, J = 6.7 Hz, 1 H, H-6), 7.81 (d, J = 7.0 Hz, 1 H, H-7), 7.88 (dd, J = 8.3, 7.2 Hz, 1 H, H-2), 8.62 (dd, J = 8.4, 1.4 Hz, 1 H, H-1), 8.76 (dd, J = 7.0, 1.4 Hz, 1 H, H-3), 8.61 (s, 1 H, H-9), 17.48 (s, 1 H, COOH). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

Reaction of **11ll** as above gave **9ll** (79%): mp (CH_2Cl_2/hexane) 119−124 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (quin, J = 7.3 Hz, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.31 (s, 3 H, NCH_3), 2.60 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 2.70 (s, 6 H, 2×CH_3), 2.73 (s, 6 H, 2×CH_3), 3.70 (q, J = 6.7 Hz, 4 H, 2×CH_2NH),7.16 (d, J = 7.1 Hz, 2 H, H-6), 7.40 (d, J = 6.8 Hz, 2 H, H-7), 7.61 (dd, J = 8.1, 7.3 Hz, 2 H, H-2), 8.06 (dd, J = 8.3, 1.4 Hz, 2 H, H-1), 8.81 (s, 2 H, H-9), 8.93 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.81 (br s, 2 H, 2×CONH). Anal. ($C_{39}H_{41}N_5O_2$) C, H, N.

***N,N*-Bis[3-(1,5-dimethylacridine-4-carboxamido)propyl]-methylamine (9mm).** Reaction of 3-methylanthranilic acid (**19**) and 2-bromo-3-methylbenzoic acid (**18**) as reported gave crude 5,6′-dimethyl-2,2′-iminodibenzoic acid (**20**), which was cyclized as above to give 1,5-dimethylacridone-4-carboxylic acid (**10mm**) (49% overall): mp (MeOH) 317−318 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.51 (s, 3 H, CH_3), 2.91 (s, 3 H, CH_3), 8.08 (d, J = 7.1 Hz, 1 H, H-2), 7.20 (t, J = 7.0 Hz, 1 H, H-7), 7.51 (d, J = 7.0 Hz, 1 H, H-6), 8.05 (d, J = 7.7 Hz, 1 H, H-3), 8.26 (d, J = 7.8 Hz, 1 H, H-8). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

Reduction of **10mm** as above gave 1,5-dimethylacridine-4-carboxylic acid (**11mm**) (98%): mp (MeOH) 267 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 2.83 (s, 3 H, CH_3), 2.93 (s, 3 H, CH_3), 7.70 (m, 2 H, H-2 and H-7), 7.95 (d, J = 6.7 Hz, 1 H, H-6), 8.25 (d, J = 8.4 Hz, 1 H, H-8), 8.67 (d, J = 7.3 Hz, 1-H, H-3), 9.63 (s, 1 H, H-9), 17.55 (s, 1 H, CO_2H). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

Reaction of **11mm** as above gave **9mm** (82%): mp (CH_2Cl_2/hexane) 110−116 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.01 (quin, J = 6.9 Hz, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.34 (s, 3 H, NCH_3), 2.64 (br t, 4 H, 2×CH_2NCH_3), 2.77 (s, 6 H, 2×CH_3), 3.69 (q, J = 6.7 Hz, 4 H, 2×CH_2NH), 7.36 (dd, J = 8.4, 6.9 Hz, 2 H, H-7), 7.42 (dd, J = 7.2, 0.8 Hz, 2 H, H-6), 7.52 (d, J = 6.8 Hz, 2 H, H-8), 7.75 (d, J = 8.4 Hz, 2 H, H-2), 8.80 (s, 2 H, H-9), 8.8 (d, J = 8.6 Hz, 2 H, H-3), 11.80 (br s, 2 H, 2×CONH). Anal. ($C_{39}H_{41}N_5O_2$·2H_2O) C, H, N.

***N,N*-Bis[3-(8-chloro-5-methylacridine-4-carboxamido)-propyl]methylamine (9nn).** Reaction of 2-methyl-5-chloroaniline (**14**) and 2-iodo-1,3-dibenzoic acid (**15**) by the reported method[28] gave crude 2-[(5-chloro-2-methylphenyl)amino]benzene-1,3-dicarboxylic acid (**17**). This was cyclized directly with PPA to give 8-chloro-5-methylacridone-4-carboxylic acid (**10nn**) (51% overall): mp (MeOH) 325−330 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.50 (s, 3 H, CH_3; overlapped with DMSO peak), 7.81 (d, J = 7.2 Hz, 1 H, H-6), 7.38 (t, J = 7.8 Hz, 1 H, H-2), 7.61 (d, J = 7.7 Hz, 1 H, H-7), 8.43−8.48 (m, 2 H, H-1,3). Anal. ($C_{15}H_{10}$-$ClNO_3$) C, H, N.

Reduction of **10nn** as above gave 8-chloro-5-methylacridine-4-carboxylic acid (**11nn**) (84%): mp (MeOH) 259−260 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.81 (s, 3 H, CH_3), 7.86−7.95 (m, 3 H, H-1,2,3), 8.74 (d, J = 8.4 Hz, 1 H, H-6), 8.80 (d, J = 7.0 Hz, 1 H, H-7), 9.70 (s, 1 H, H-9), 16.83 (br s, 1 H, CO_2H). Anal. ($C_{15}H_{10}ClNO_2$) C, H, N.

Reaction of **11nn** as above gave **9nn** (81%): mp (CH_2Cl_2/hexane) 212−215 °C; ^{1}H NMR ($CDCl_3$) δ 1.98 (quin, J = 7.3 Hz, 4 H, 2×$CH_2CH_2CH_2CH_2$), 2.02 (s, 3 H, NCH_3), 2.60 (t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$), 2.67 (s, 6 H, 2×CH_3), 3.70 (q, 4 H, 2×CH_2NH),7.28 (dd, J = 7.7, 0.9 Hz, 2 H, H-7), 7.32 (d, J = 7.4 Hz, 2 H, H-6), 7.65 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 8.07 (dd, J = 8.6, 1.5 Hz, 2 H, H-1), 8.96 (dd, J = 7.2, 1.5 Hz, 2 H, H-3), 9.01 (s, 2 H, H-9), 11.41 (t, J = 5.3 Hz, 2 H, 2×CONH). Anal. ($C_{37}H_{35}Cl_2N_2O_5$·0.5H_2O) C, H, N, Cl.

***N,N*-Bis[3-(1-chloro-5-methylacridine-4-carboxamido)-propyl]methylamine (9oo).** A mixture of 3-methylanthranilic acid (**21**) (7.6 g, 50 mmol), methyl 4-chloro-2-iodobenzoate (**22**) (19.2 g, 65 mmol), Cu, and CuI (catalytic) in 2,3-butanediol (20 mL) was heated with benzene (30 mL) in an oil bath. After the benzene had distilled off, *N*-ethylmorpholine (50 mL) was added, and the stirred mixture was heated at 110 °C for 18 h, then diluted with dilute HCl, extracted into EtOAc, and filtered to remove Cu salts. The organic layer was separated and extracted into dilute NH_4OH, when the ammonium salt of the product crystallized. This was collected and stirred in dilute HCl, and the mixture was filtered and washed with water to give 2-[[5-chloro-2-(methoxycarbonyl)phenyl]amino]-3-methylbenzoic acid (**23**) (6.6 g, 41%): mp (MeOH) 187−188.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.10 (s, 3 H, CH_3), 3.87 (s, 3 H, OCH_3), 6.12 (d, J = 2.0 Hz, 1 H, H-6′), 6.79 (dd, J = 8.6, 2.0 Hz, 1 H, H-4′), 7.29 (t, J = 7.6 Hz, 1 H, H-5), 7.52 (d, J = 7.4 Hz, 1 H, H-4), 7.74 (d, J = 7.4 Hz, 1 H, H-6), 7.89 (d, J = 8.5 Hz, 1 H, H-3′), 9.9 (br s, 1 H, NH). Anal. ($C_{16}H_{14}ClNO_4$) C, H, N, Cl.

A solution of **23** (6.0 g, 18.8 mmol) in dry THF (100 mL) was treated with CDI (6.0 g, 37.6 mmol) at 20 °C for 18 h, and the solution was then added dropwise to a suspension of $NaBH_4$ (0.69 g, 5 equiv) in H_2O (50 mL). When the reaction was complete (30 min, as monitored by TLC), the mixture was quenched with dilute HCl and extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried (Na_2SO_4) and evaporated to give a crude product that was chromatographed on silica gel, eluting with a gradient of MeOH in CH_2Cl_2, to give methyl 4-chloro-2-[[2-(hydroxymethyl)-6-methylphenyl]amino]benzoate (**24**) (1.0 g, 17%): mp (CH_2Cl_2/hexane) 114−115 °C; ^{1}H NMR ($CDCl_3$) δ 1.78 (br s, 1 H, OH), 2.18 (s, 3 H, CH_3), 3.92 (s, 3 H, CO_2CH_3), 4.54 (dd, J = 12.8, 4.3 Hz, 1 H, *CH*OH), 4.67 (dd, J = 12.8, 4.3 Hz, 1 H, *CH*OH), 6.01 (d, J = 2.0 Hz, 1 H, H-3), 6.63 (dd, J = 8.3, 2.0 Hz, 1 H, H-5), 7.24−7.29 (m, 2 H, 2×ArH), 7.35−7.39 (m, 1 H, ArH), 7.89 (d, J = 8.6 Hz, 1 H, H-6), 9.22 (s, 1 H, NH). Anal. ($C_{16}H_{16}ClNO_3$) C, H, N. Unreacted starting material (2 g) was also recovered.

A solution of **24** (0.72 g, 2.35 mmol) in EtOAc (100 mL) was heated under reflux for 7 h with MnO_2 (1 g). The mixture was filtered through Celite to remove Mn residues, and the solvent was evaporated and the residue filtered through a column of silica gel in CH_2Cl_2 to give methyl 4-chloro-2-[(2-formyl-6-methylphenyl)amino]benzoate (**25**) (0.7 g, 98%): mp (MeOH/H_2O) 81−82 °C; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3 H, CH_3), 3.95 (s, 1 H, CO_2CH_3), 6.27 (d, J = 2.0 Hz,1 H, H-3), 6.70 (dd, J = 8.7, 2.0 Hz, 1 H, H-5), 7.37 (t, J = 7.6 Hz, 1 H, H-4′), 7.58 (d, J = 7.9 Hz, 1 H, H-5′), 7.81(dd, J = 7.7, 1.3 Hz, 1 H, H-3′), 7.92 (d, J = 8.6 Hz, 1 H, H-6), 9.68 (br s, 1 H, NH), 10.15 (s, 1 H, CHO). Anal. ($C_{16}H_{14}ClNO_3$) C, H, N.

A solution of **25** (0.65 g, 2.1 mmol) in trifluoroacetic acid (8 mL) was stirred at 40 °C for 4 h under nitrogen. Excess reagent was removed under reduced pressure at 40 °C, and the residue was suspended in 2 N NaOH (25 mL) and EtOH (18 mL) and heated for 1 h until a clear solution was obtained. The cooled reaction mixture was neutralized with AcOH, and the resulting precipitate was collected, washed with water, and dried to give 1-chloro-5-methylacridine-4-carboxylic acid (**11oo**) (0.6 g, 100%): mp (MeOH/H_2O) 260 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 3 H, CH_3), 7.64 (dd, J = 8.4, 7.9 Hz, 1 H, H-7), 7.83−7.86 (m, 2 H, H-2 & H-6 or H-8), 8.08 (d, J = 8.6 Hz, 1 H, H-8 or H-6), 8.84 (d, J = 7.8 Hz, 1 H, H-3), 9.4 (s, 1 H, H-9), 17.26 (s, 1 H, CO_2H). Anal. ($C_{15}H_{10}ClNO_2$) C, H, N.

Reaction of **11oo** as above gave **9oo** (84%): mp (CH_2Cl_2/hexane) 156−158.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.85 (quin, J = 7.2 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.32 (s, 3 H, NCH_3), 2.60 (t, 4 H, 2×CH_2NCH_3), 2.74 (s, 6 H, 2×CH_3), 3.68 (q, J = 6.7 Hz, 4 H, 2×CH_2NH), 7.38 (dd, J = 8.3, 6.8 Hz, 2 H, H-7), 7.52 (d, J = 6.8 Hz, 2 H, H-6), 7.65 (d, J = 8.0 Hz, 2 H, H-2), 7.76 (d, J = 8.5 Hz, 2 H, H-8), 8.80 (d, J = 8.0 Hz, 2 H, H-3), 9.04 (s, 2 H, H-9), 11.50 (br s, 2 H, 2×CONH). Anal. ($C_{37}H_{35}Cl_2N_5O_2$) C, H, N, Cl.

***N,N*-Bis[3-(6-(dimethylamino)acridine-4-carboxamido)-propyl]methylamine (9x): General Example**. The bis(6-fluoroacridine) **9t** (0.52 g, 0.7 mmol) was heated with 40% aqueous dimethylamine (10 mL) in MeOH (10 mL) in a pressure vessel at 100 °C for 1 week. Solvent and excess reagent were evaporated under reduced pressure, ammonia was added, and the mixture was extracted with CH_2Cl_2. Evaporation and chromatography of the residue on alumina, eluting with a gradient of MeOH in CH_2Cl_2, gave **9**× (84% yield). Hydrochloride salt: mp (MeOH/EtOAc) 100 °C; ^{1}H NMR (free base in $CDCl_3$) δ 2.03 (quin, J = 7.0 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.37 (s, 3 H, NCH_3), 2.82 (t, J = 7.6 Hz, 4 H, $CH_2N(CH_3)CH_2$), 2.85 (s, 12 H, 2×$N(CH_3)_2$), 3.73 (q, J = 6.1 Hz, 4 H, 2×CH_2NH), 6.54 (d, J = 2.2 Hz, 2 H, H-5), 6.67 (dd, J = 9.2, 2.4 Hz, 2 H, H-7), 7.31 (d, J = 9.2 Hz, 2 H, H-8), 7.37 (t, J = 7.6 Hz, 2 H, H-2), 7.86 (dd, J = 8.2, 1.6 Hz, 2 H, H-1), 8.21 (s, 2 H, H-9), 8.81 (dd, J = 7.2, 1.6 Hz, 2 H, H-3), 12.15 (t, J = 5.0 Hz, 2 H, 2×CONH); HRMS (FAB$^+$) m/z calcd for $C_{39}H_{44}N_7O_2$ 642.3556 (MH^+), found 642.3557.

***N,N*-Bis[3-(5-(dimethylamino)acridine-4-carboxamido)-propyl]methylamine (9q).** From similar reaction of the bis-(5-fluoroacridine) analogue **9m** in excess 40% aqueous dimethylamine/MeOH for 8 weeks in a pressure vessel (60% yield): foam; ^{1}H NMR ($CDCl_3$) δ 1.97 (quin, J = 7.3 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.30 (s, 3 H, NCH_3), 2.59 (t, J = 7.3 Hz, 4 H, $CH_2N(CH_3)CH_2$), 3.01 (s, 12 H, 2×$N(CH_3)_2$), 3.68 (q, J = 6.7 Hz, 4 H, 2×CH_2NH), 7.12 (dd, J = 7.2, 0.9 Hz, 2 H, H-6), 7.39 (dd, J = 8.4, 7.3 Hz, 2 H, H-7), 7.51 (dd, J = 8.2, 0.8 Hz, 2 H, H-8), 7.62 (dd, J = 8.3, 7.2 Hz, 2 H, H-2), 8.04 (dd, J = 8.4, 1.4 Hz, 2 H, H-1), 8.70 (s, 2 H, H-9), 8.91 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.94 (br s, 2 H, 2×CONH). Anal. ($C_{39}H_{43}N_7O_2\cdot H_2O$) C, H.

Bis[3-(7-(dimethylamino)acridine-4-carboxamido)propyl]methylamine (9hh). From similar reaction of the bis(7-fluoroacridine) analogue **9ee** in excess 40% aqueous dimethylamine/MeOH for 6 weeks in a pressure vessel (89% yield): foam; ^{1}H NMR ($CDCl_3$) δ 2.08 (quin, J = 7.0 Hz, 4 H, 2×$CH_2CH_2CH_2$), 2.40 (s, 3 H, NCH_3), 2.86 (t, J = 7.6 Hz, 4 H, $CH_2N(CH_3)CH_2$), 2.99 (s, 12 H, 2×$N(CH_3)_2$), 3.75 (q, J = 6.1 Hz, 4 H, 2×CH_2NH), 6.30 (d, J = 2.8 Hz, 2 H, H-8), 7.18 (dd, J = 9.5, 2.8 Hz, 2 H, H-6), 7.44 (dd, J = 8.2, 7.2 Hz, 2 H, H-2), 7.67 (d, J = 9.5 Hz, 2 H, H-5), 7.82 (dd, J = 8.5, 1.4 Hz, 2 H, H-1), 8.13 (s, 2 H, H-9), 8.69 (dd, J = 7.1, 1.5 Hz, 2 H, H-3), 11.84 (t, J = 5.0 Hz, 2 H, 2×CONH). Anal. ($C_{39}H_{43}N_7O_2\cdot H_2O$) C, H, N.

In Vitro Growth Delay Assays. Murine P388 leukemia cells, Lewis lung carcinoma cells (LLTC), and human Jurkat leukemia cells (JL_C), together with their amsacrine- and doxorubicin-resistant derivatives (JL_A and JL_D, respectively), were obtained and cultured as described.[21,30] Growth inhibition assays were performed by culturing cells at 4.5×10^3 (P388), 10^3 (LLTC), and 3.75×10^3 (Jurkat lines) cells/well in microculture plates (150 mL/well) for 3 (P388) or 4 days in the presence of drug. Cell growth was determined by [^{3}H]TdR uptake (P388)[36] or the sulforhodamine assay.[37] Independent assays were performed in duplicate, and coefficients of variation were ca. 25%.

In Vivo Colon 38 Tumor Assay. Colon 38 tumors were grown subcutaneously from 1-mm^3 fragments implanted in one flank of mice (anesthetized with pentobarbitone 90 mg/kg). When tumors reached a diameter of approximately 4 mm (7−8 days), mice were divided into control and drug treatment groups (5 mice/group), with similar average tumor volumes in each group. Drugs were administered as solutions of the hydrochloride salts in distilled water and were injected in a volume of 0.01 mL/g of body weight in two equal injections administered 1 h apart. The mice were monitored closely, and tumor diameters were measured with callipers three times a week. Tumor volumes were calculated as $0.52\times a^2\times b$, where a and b are the minor and major tumor axes, and data plotted on a semilogarithmic plot as mean tumor volumes (±SEM) versus time after treatment. The growth delay was calculated as the time taken for tumors to reach a mean volume 4-fold higher than their pretreatment volume.

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