*N*4-(*ω*-Aminoalkyl)-1-[(*ω*-aminoalkyl)amino]-4-acridinecarboxamides: Novel, Potent, Cytotoxic, and DNA-Binding Agents

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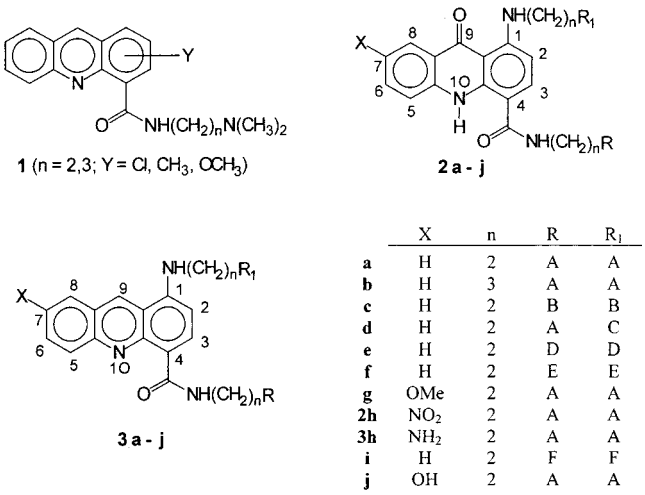
A series of DNA-binding potential antitumor agents, (*ω*-aminoalkyl)-4-acridinecarboxamides, has been prepared either by reduction of the corresponding (*ω*-aminoalkyl)-9-oxo-9,10-dihydro-4-acridinecarboxamides with aluminum amalgam or by aminolysis of the corresponding (*ω*-aminoalkyl)-1-chloro-4-acridinecarboxamides with the suitable amine. The noncovalent DNA-binding properties of these compounds have been examined using a fluorometric technique. In vitro cytotoxic potencies of these derivatives toward six tumor cell lines, including human colon adenocarcinoma (HT29) and human ovarian carcinoma (A2780-sensitive, A2780cisR cisplatin-resistant, CH1-sensitive, CH1cisR cisplatin-resistant, and SKOV-3) cells, are described and compared to that of reference drugs. One highly DNA affinic analogue (**3a**) has been identified with a useful broad spectrum of cytotoxic activity in the 4−7 nM range (mean IC_{50} of 6 nM).

Introduction

Among the different classes of antitumor DNA-binding agents, the acridine derivatives are an important group with many exhibiting interesting anticancer properties. The acridine ring system may constitute either part of the chromophore (as in the case of imidazo[4,5,1-*de*]acridines,[1] triazolo[4,5,1-*de*]acridines,[2] pyrimido[5,6,1-*de*]acridines,[3] pyrimido[4,5,6-*kl*]acridines,[4] pyrazolo[3,4,5-*kl*]acridine-5-carboxamides,[5] pyrazolo-[3,4,5-*mn*]pyrimido[5,6,1-*de*]acridines,[5] pyrazolo[4,5,1-*de*]acridines,[6] and 5-nitropyrazolo[3,4,5-*kl*]acridines[7]) or the whole chromophore (as in the case of amsacrine and nitracrine,[8] acridine-4-carboxamides **1**,[9] and acridone-4-carboxamides **2**[10]) (Figure 1). To confer antitumor activity, generally the chromophore should carry at least one basic side chain. However we have found that the addition of a second basic side chain is also important for biological activity in pyrimido[5,6,1-*de*]acridines,[3] pyrazolo[3,4,5-*kl*]acridines-5-carboxamides,[5] pyrazolo-[3,4,5-*mn*]pyrimido[5,6,1-*de*]acridines,[5] and acridone-4-carboxamides.[10]

DACA (**1**, Y = H, n = 2), an anticancer drug in clinical trials,[9,11] exemplifies the class of acridine-4-carboxamides **1** that possess only one basic side chain. Since the chromophore of this compound is closely related to that of bifunctional acridone-4-carboxamides **2**, in which two side chains are necessary for the activity, we have synthesized and evaluated a series of *N*4-(*ω*-aminoalkyl)-1-[(*ω*-aminoalkyl)amino]-4-acridinecarboxamides **3a**−**j** to establish if the second basic side chain in position 1 of **1** could improve the promising antitumor characteristics of these compounds as observed in the case of derivatives **2**. In this paper we describe the synthesis of this new class of compounds and we report on their in vitro cytotoxicity against six tumor cell lines and on their DNA binding from fluorescence-based studies with calf thymus DNA and two polyoligonucleotide duplexes.



	X	n	R	R_1
a	H	2	A	A
b	H	3	A	A
c	H	2	B	B
d	H	2	A	C
e	H	2	D	D
f	H	2	E	E
g	OMe	2	A	A
2h	NO_2	2	A	A
3h	NH_2	2	A	A
i	H	2	F	F
j	OH	2	A	A

Figure 1. Structures of monofunctionalized acridine-4-carboxamides **1**, bisfunctionalized 9(10*H*)-acridone-4-carboxamides **2**, and bisfunctionalized acridine-4-carboxamides **3**: A = $N(CH_3)_2$; B = $N(C_2H_5)_2$; C = $NHCH_2CH_2OH$; D = 1-piperidyl; E = 4-morpholinyl; F = NH_2.

Chemistry

Scheme 1 shows the synthetic pathways used. According to pathway A, compounds **2a**−**h**[10] and the new derivative **2i** were reduced to the corresponding target compounds **3a**−**i**, by using aluminum amalgam in aqueous ethanol. To prepare **2i**, the 1-chloroacridone-4-carboxylic acid (**4**)[12] was reacted with 1,1′-carbonyldiimidazole and *tert*-butyl *N*-(2-aminoethyl)carbamate in chloroform to afford the intermediate **5**. The reaction of **5** with *tert*-butyl *N*-(2-aminoethyl)carbamate at 120 °C in dimethylformamide (DMF) followed by hydrolysis with aqueous HCl in dioxane yielded **2i**.

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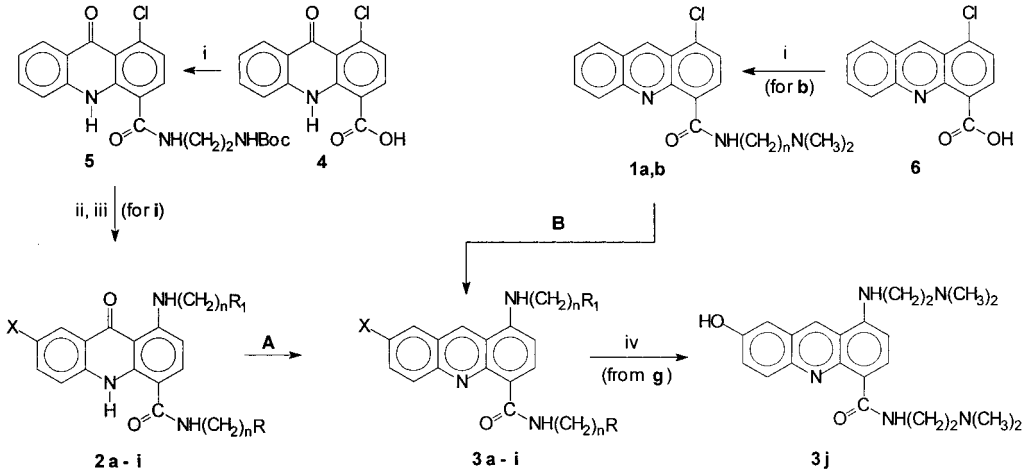
Scheme 1[a]



[a] Reagents: (i) 1,1′-carbonyldiimidazole and either $H_2NCH_2CH_2NHBoc$ for **5** or $H_2N(CH_2)_3N(CH_3)_2$,for **1b**; (ii) $H_2NCH_2CH_2NHBoc$; (iii) HCl/dioxane; (iv) HBr; (method A) Al/$HgCl_2$; (method B) $H_2N(CH_2)_nR$. For X, *n*, R, and R_1, see Figure 1; Boc = $COOC(CH_3)_3$.

Table 1. Physicochemical and DNA-Binding Properties[a] of Target Derivatives **3a**−**j** and Reference Compounds DACA and **2a**

				K_{app}[e] × 10^{-7} M^{-1}			
no.	mp, °C[b]	yield, %[c]	formula[d]	CT-DNA	AT	GC[f]	binding site preference[g]
3a	135−137 (150−152 dec)	52 (56)	$C_{22}H_{29}N_5O$	150	2.8	23 (8.2)	G−C
3b·maleate	137−139 dec	60 (20)	$C_{28}H_{37}N_5O_5$	NT[h]	NT[h]	NT[h]	
3c	143−144 (208−210 dec)	56	$C_{26}H_{37}N_5O$	15	1.7	9.1 (5.4)	G−C
3d	118−119 (154−156 dec)	69	$C_{22}H_{29}N_5O_2$	9.9	4.5	30 (6.7)	G−C
3e	151−152 (217−218 dec)	58	$C_{28}H_{37}N_5O$	3.4	2.7	6.3 (2.3)	G−C
3f	181−182 (210−211 dec)	54	$C_{26}H_{33}N_5O_3$	0.44	0.5	0.3 (0.6)	A−T
3g	158−159 (244−246 dec)	47	$C_{23}H_{31}N_5O_2$	1.9	1.4	5.9 (4.2)	G−C
3h·3HCl	250−252 dec	46	$C_{22}H_{33}Cl_3N_6O$	140	28	41 (1.5)	G−C
3i	>300 (>300)	34	$C_{18}H_{21}N_5O$	8.5	2.2	5.6 (2.5)	G−C
3j	221−222 (258−260 dec)	54	$C_{22}H_{29}N_5O_2$	29	3.1	10 (3.2)	G−C
2a				3.7[i]			
DACA				0.11	0.14	0.10 (0.71)	A−T

[a] CT-DNA, AT, and GC refer to calf thymus DNA, $[poly(dA\text{-}dT)]_2$, and $[poly(dG\text{-}dC)]_2$, respectively. [b] In parentheses is the melting point of the dihydrochlorides; dec, decomposition. [c] In parentheses is the yield according to method B. [d] Analyses for C, H, and N; all analytical values for C, H, and N were within ±0.4% of the theoretical values. [e] $K_{app} = 1.26/C_{50} \times 10^7$, in which 1.26 is the concentration (μM) of ethidium in ethidium−DNA complex, C_{50} is drug concentration (μM) to effect 50% drop in fluorescence of bound ethidium, and 10^7 is the value of K_{app} assumed for ethidium in the complex. [f] In parentheses is the [GC]/[AT] ratio. [g] The binding site preference is considered to be significant only for [GC]/[AT] ratio differing by >30% from the sequence-neutral unity value (i.e. <0.7 or >1.3). [h] NT = not tested due to the noticeable fluorescence of **3b**. [i] Data from ref 10.

Alternatively, as illustrated for **3a**,**b**, target compounds can be prepared according to route B by refluxing **1a**[9] or **1b** in the suitable amine. **1b** was obtained by reaction of 1-chloroacridine-4-carboxylic acid (**6**)[9] with 1,1′-carbonyldiimidazole and *N*1,*N*1-dimethyl-1,3-propanediamine in chloroform. Finally, the 7-hydroxy derivative **3j** was obtained by refluxing the corresponding 7-methoxy derivative **3g** in aqueous HBr.

To examine the DNA-binding properties and the in vitro antineoplastic activity of these agents, the free base forms of **3** were converted into their water-soluble hydrochloride or maleate salts by the usual methods. The chemical structures of the synthesized compounds were determined by ^{1}H NMR spectroscopy. In Figure 1 are reported structures and in Table 1 melting points, yields, and formula of target derivatives **3a**−**j**.

Results and Discussion

DNA-Binding Properties. Competitive displacement (C_{50}) fluorometric assays with DNA-bound ethidium can be used[13] to (a) determine 'apparent' equilibrium constants (K_{app}) for drug binding, as the C_{50} value is approximately inversely proportional to the binding constant,[14] and (b) establish possible base- or sequence-preferential binding.[15]

In the present study, fluorescence displacement assays were performed at pH 7 to enable comparison in biological conditions. The K_{app} values of bis(amine-functionalized) acridines **3a**−**j**, calculated for CT-DNA, AT, and GC, are reported in Table 1. The results indicate that: (i) the target compounds are DNA-binding ligands with greater affinity than ethidium with the exception of **3f**, in which the 4-morpholinyl group on side chains seems to affect unfavorably the binding as previously we found also for the corresponding acridone derivative **2f**;[10] (ii) generally compounds **3** bind DNA more strongly than the corresponding compounds **2**,[10] in particular **3a** binds CT-DNA with a K_{app} which is 40 times that of **2a**; (iii) the second side chain in position 1 is very important for DNA binding, in fact the dibranched **3a** binds CT-DNA 1400 times more strongly than monobranched DACA; (iv) moreover, while DACA seems to be slightly AT-selective, the second side chain in position 1 makes the compounds **3** GC-selective, again with the exception of **3f**.

Table 2. Cytotoxic Activity of **3a**−**j** in Comparison to **2a**, DACA, **1a**, Doxorubicin, and Cisplatin versus Human Colon Adenocarcinoma Cell Line[a] and Ovarian Carcinoma Cell Line Panels[b]

	IC_{50}, μM[c]					
compd	HT29	A2780	A2780/Cs[d]	CH1	CH1/Cs[d]	SKOV-3
3a	0.007 (0.002)	0.0043	0.007 (1.6)	0.007	0.005 (0.7)	0.11
3b	0.71 (0.056)	1.95	1.25 (0.6)	0.86	0.53 (0.6)	0.66
3c	0.58 (0.045)	0.415	0.475 (1.1)	0.185	0.375 (2.1)	0.5
3d	0.10 (0.011)	0.085	0.091 (1.1)	0.052	0.046 (0.9)	0.24
3e	0.44 (0.036)	0.1	0.12 (1.2)	0.135	0.11 (0.8)	0.14
3f	2.70 (0.23)	2.3	2.5 (1.1)	1.85	2.15 (1.2)	3.2
3g	0.56 (0.041)	0.096	0.125 (1.3)	0.05	0.044 (0.9)	0.06
3h	0.70 (0.057)	0.32	0.365 (1.1)	0.089	0.1 (1.1)	0.155
3i	6.94 (0.57)	12	3.15 (0.3)	1.2	1.55 (1.3)	12
3j	0.85 (0.061)	0.11	0.19 (1.7)	0.15	0.20 (1.3)	0.9
2a[e]	0.094	0.015		0.02		0.4
DACA	0.99 (0.063)	0.51	0.57 (1.1)	0.48	0.53 (1.1)	0.98
1a	0.79 (0.058)	2.05	2.04 (1)	1.75	1.9 (1.1)	3.3
Cs		0.89	3.4 (3.8)	0.15	2.4 (16)	3.4
Dx	0.026 (0.012)	0.0096	0.017 (1.8)	0.0063	0.45 (71)	0.078

[a] All assays were performed in triplicate (in parentheses is the standard deviation). [b] Values represent the mean of a representative head-to-head experiment using 4 replicate wells per drug concentration and 8 control wells. Dx = doxorubicin; Cs = cisplatin; colon adenocarcinoma cell line = HT29; ovarian carcinoma cell lines = A2780, A2780/Cs cisplatin-resistant, CH1, CH1/Cs cisplatin-resistant, and SKOV-3. [c] Drug concentration required to inhibit cell growth by 50%. [d] In parentheses is RF (resistance factor), the ratio of IC_{50} values of cisplatin-resistant cell line to the sensitive cell line. [e] Data from ref 10.

Cytotoxic Activity. In vitro cytotoxic potencies of target acridines **3a**−**j**, of parent compounds **2a**, DACA, and **1a**, and of reference compounds cisplatin (Cs) and doxorubicin (Dx) against human colon adenocarcinoma cell line (HT29) and five human ovarian carcinoma (A2780-sensitive, A2780cisR cisplatin-resistant, CH1-sensitive, CH1cisR cisplatin-resistant, and SKOV-3) cell lines are reported in Table 2.

The results indicate that: (i) **3a** emerges as the most active among the new derivatives with IC_{50} values in the 4−7 nM range in five out of six of the tumor cell lines tested; (ii) all compounds **3** possess a good antiproliferative activity in the submicromolar range, with the exception of **3f**,**i** that are the least effective in the series, with values in the micromolar range; (iii) apart from **3a**, noticeable cytotoxic activity is also shown by **3d**,**g** − many of their IC_{50} values are <0.1 μM, with **3g** being more potent than **3a** with respect to the SKOV-3 cell line; (iv) generally, the SKOV-3 cell line seems to be less sensitive than the others to killing by these derivatives − acridines **3** have interesting cytotoxic properties and, moreover, exhibit a lack of cross-resistance to cisplatin in two cisplatin-resistant cell lines (with RFs often close to 1); (v) regarding all cell lines, **3a** is 10−140-fold more potent than DACA, 4−10 times more potent than **2a**, 30−500 times more potent than Cs, and comparable to or better than Dx itself.

Regarding the side chains, it was observed that: (i) the optimal distance between the two nitrogen atoms is two methylenes, as seen from the remarkable decrease in activity between **3a,b**; (ii) two bulky substituents at the terminal nitrogen atoms (compounds **3c**,**e**,**f**) result in a decrease in cytotoxicity; (iii) a unique substituent on the distal nitrogen of the side chain in position 1 (compound **3d**), which parallels the side chain substitution in mitoxantrone, preserves good antiproliferative action; (iv) the complete absence of substituents on the terminal nitrogen atoms (compound **3i**), which provides side chains similar to that of the anticancer derivative BBR 2778,[16] leads to marginal cytotoxic potency; (v) the observations at points i−iii are confirmed by the parallel results obtained with the corresponding derivatives **2**;[10] (vi) the second side chain in position 1 is very important for cytotoxicity as can be seen from the in vitro activity of monobranched DACA in comparison with that of dibranched **3a**, with the latter being more potent than the former by 2 orders of magnitude.

Concerning the chromophore and the substituents in position 7 it was observed that: (i) from cytotoxicity comparisons of classes **3** and **2**,[10] usually there are no pronounced differences in activity between class **3** (chromophore constituted by acridine) and class **2** (chromophore constituted by 9(10*H*)-acridone), but in the case of **3a** and **2a**, the lead compounds in the series, there is a difference of 1 order of magnitude for **3a**; (ii) the methoxy substituent in position 7 leads to contrasting results − compound **3g** shows the highest activity in the series against the SKOV-3 cell line and a dramatic drop in activity, with respect to unsubstituted **3a**, against the HT29 cell line; (iii) polar substituents, able to form hydrogen bonds, in position 7, such as amino and hydroxy groups (compounds **3h**,**j**), in general lead to a large reduction in cytotoxicity.

In some cases there is a notable correspondence between binding with CT-DNA and in vitro activity, while in others, a big discrepancy: **3a** is the most cytotoxic derivative and possesses the highest K_{app}, while **3h** with a similar K_{app} is one of the least potent in the series. This fact clearly indicates that DNA binding is not the only relevant factor for the antiproliferative action of these derivatives. Factors such as cellular uptake may also contribute.

Conclusions

The present study allows us to conclude the following: (i) The *N*4-(*ω*-aminoalkyl)-1-[(*ω*-(aminoalkyl)amino]-4-acridinecarboxamides **3a**−**j** constitute a class of derivatives which possess potent cytotoxic activity and relevant DNA-binding properties. (ii) In comparison to DACA, the introduction of a second side chain in position 1 of the chromophore leads in many cases to an increase of in vitro activity and DNA-binding ability. (iii) Compound **3a** shows a very marked difference of

in vitro activity and DNA-binding capacity in comparison with DACA and also with the parent acridone derivative **2a**.[10] (iv) Substitution of DACA with a second basic side chain in position 1 led to *N*4-[2-(dimethylamino)ethyl]-1-[2-(dimethylamino)ethyl]amino-4-acridinecarboxamide (**3a**), a new potential anticancer lead possessing useful broad-spectrum cytotoxic activity in the 4−7 nM range.

Experimental Section

Synthetic Chemistry. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Thin-layer chromatography (TLC) was accomplished using plates precoated with silica gel 60 F-254 (Merck). All ^{1}H NMR spectra were recorded on a Varian VXR 300 instrument. Chemical shifts are reported as δ values (ppm) downfield from internal Me_4Si in the solvent shown. The following NMR abbreviations are used: br (broad), s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet), ar (aromatic proton), ex (exchangeable with D_2O). Elemental analyses were performed on a model 1106 elemental analyzer (Carlo Erba Strumentazione).

***N*4-[3-(Dimethylamino)propyl]-1-chloro-4-acridinecarboxamide (1b). 6**[9] (1 g, 3.65 mmol) and 1,1′-carbonyldiimidazole (1.1 g, 6.8 mmol) in DMF (10 mL) were stirred until it became homogeneous. The mixture was cooled to 10 °C and a solution of *N*1,*N*1-dimethyl-1,3-propanediamine (1.8 g, 17.5 mmol) in $CHCl_3$ (10 mL) was added. After 30 min at room temperature, the reaction mixture was partitioned between $CHCl_3$ (70 mL) and an excess of 1 M aqueous Na_2CO_3 (50 mL). The organic layer was worked up to give a residue which was flash-chromatographed on silica gel column eluted with $CHCl_3$/MeOH (9:1 v/v) to give crude **1b** (0.96 g, 74%), which was used for next step.

***tert*-Butyl *N*-(2-[(1-Chloro-9-oxo-9,10-dihydro-4-acridinyl)carbonyl]aminoethyl)carbamate (5).** The carboxylic acid **4**[12] (1 g, 3.65 mmol) and 1,1′-carbonyldiimidazole (1.1 g, 6.8 mmol) in DMF (10 mL) were stirred until it became homogeneous. The mixture was cooled to 10 °C and a solution of *tert*-butyl *N*-(2-aminoethyl)carbamate (1.6 g, 10 mmol) in $CHCl_3$ (10 mL) was added. After 30 min at room temperature, the reaction mixture was partitioned between $CHCl_3$ (70 mL) and an excess of 1 M aqueous Na_2CO_3 (50 mL). The organic layer was worked up to give a residue which was washed with Et_2O to give crude **5** (0.91 g, 60%), which was used for next step.

***N*4-(2-Aminoethyl)-1-[(2-aminoethyl)amino]-9-oxo-9,-10-dihydro-4-acridinecarboxamide Dihydrochloride (2i·2HCl).** A suspension of **5** (0.58 g, 1.4 mmol) and *tert*-butyl *N*-(2-aminoethyl)carbamate (0.96 g, 6 mmol) in DMF (10 mL) was stirred for 2 h at 120 °C. The reaction mixture was partitioned between $CHCl_3$ (70 mL) and an excess of 1 M aqueous Na_2CO_3 (50 mL). The organic layer was worked up to give a residue which was flash-chromatographed on silica gel column eluted with $CHCl_3$/MeOH (49:1 v/v) to afford **2i** Boc protected, which was diluted in dioxane (20 mL) and 37% HCl (2 mL) and stirred for 2 h at room temperature. The reaction mixture was evaporated to give a residue which was flash-chromatographed on silica gel column eluted with $CHCl_3$/MeOH (1:1 v/v) and 32% aqueous NH_3 (10 mL for 1 L of eluent) to afford **2i**, which was converted directly to the hydrochloride (0.34 g, 58%) and as such used for next step: mp 250−252 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.97−3.15 (m, 4H, 2 × CH_2), 3.52−3.80 (m, 4H, 2 × CH_2), 6.54 (d, 1H, ar), 7.30 (t, 1H, ar), 7.53 (d, 1H, ar), 7.71 (t, 1H, ar), 8.13−8.40 (m, 8H, 2 ar + 6H ex), 8.92 (br t, 1H, CO-NH, ex), 10.91 (b t, 1H, NH, ex), 13.78 (br s, 1H, 10-H, ex).

***N*4-[2-(Dimethylamino)ethyl]-1-{[2-(dimethylamino)-ethyl]amino}-4-acridinecarboxamide (3a). Example of General Procedure for the Preparation of 3a−i. 2a** (0.8 g, 2.2 mmol) was refluxed in EtOH/H_2O (3:1, 100 mL). Portions of aluminum foil (0.4 g) were amalgamated in a solution of $HgCl_2$ (3.2 g) in EtOH (76 mL) and added to the above boiling solution over 30 min. The mixture was refluxed for other 30 min, then filtered and the solid collected were washed with hot EtOH. The filtrate was diluted with H_2O (100 mL) and $FeCl_3$ (2.5 g) was added. The resulting mixture was partitioned between $CHCl_3$ (3 × 50 mL) and an excess of 1 M aqueous Na_2CO_3 (50 mL). The organic layer was worked up to give a residue which was flash-chromatographed on silica gel column eluted with $CHCl_3$/MeOH (7:3 v/v) to afford pure **3a**: ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6H, 2 × CH_3), 2.47 (s, 6H, 2 × CH_3), 2.68−2.85 (m, 4H, 2 × CH_2), 3.30−3.45 (m, 2H, CH_2), 3.70−3.87 (m, 2H, CH_2), 6.07 (br s, 1H, CO-NH, ex), 6.50 (d, 1H, ar), 7.51 (t, 1H, ar), 7.80 (t, 1H, ar), 8.04 (d, 1H, ar), 8.17 (d, 1H, ar), 8.80−8.90 (m, 2H, ar), 11.90 (br t, 1H, NH, ex).

Derivatives **3b**−**i** were prepared in a similar manner by corresponding acridones **2b**−**i**.

***N*4-[2-(Dimethylamino)ethyl]-1-{[2-(dimethylamino)-ethyl]amino}-7-methoxy-4-acridinecarboxamide (3j). 3g** (0.25 g, 0.61 mmol) was suspended in 48% HBr (2 mL) and refluxed for 1 h. The reaction mixture, diluted with water (20 mL), was partitioned between $CHCl_3$ (3 × 50 mL) and an excess of 1 M aqueous Na_2CO_3 (100 mL). The organic layer was worked up to give a residue which was flash-chromatographed on silica gel column eluted with $CHCl_3$/MeOH (1:1 v/v) and 32% aqueous NH_3 (10 mL for 1 L of eluent) to afford **3j**, which was further purified by washing with ether: ^{1}H NMR ($CDCl_3$) δ 2.39−2.49 (m, 12H, 4 × CH_3), 2.75−2.86 (m, 4H, 2 × CH_2), 3.34−3.46 (m, 2H, CH_2), 3.71−3.83 (m, 2H, CH_2), 5.47 (br t, 1H, CO-NH, ex), 6.38 (d, 1H, ar), 6.76 (s, 1H, ar), 7.10 (d, 1H, ar), 7.49 (d, 1H, ar), 7.86 (s, 1H, ar), 8.68 (d, 1H, ar), 11.71 (br t, 1H, NH, ex).

***N*4-[2-(Dimethylamino)ethyl]-1-{[2-(dimethylamino)-ethyl]amino}-4-acridinecarboxamide (3a). Example of General Procedure for the Preparation of 3a,b. 1a** (0.2 g, 0.51 mmol) was refluxed in *N*1,*N*1-dimethyl-1,2-ethanediamine (2 mL) for 24 h. The reaction mixture was partitioned between $CHCl_3$ (3 × 20 mL) and 1 M aqueous Na_2CO_3 (20 mL). The organic layer was worked up to give a residue which was flash-chromatographed on silica gel column eluted with $CHCl_3$/MeOH (7:3 v/v) to afford pure **3a**.

Derivative **3b** was prepared in a similar manner by reaction of **1b** and *N*1,*N*1-dimethyl-1,3-propanediamine.

Biophysical Evaluation. 1. Fluorescence Binding Studies. The fluorometric assays have been described previously.[13] The C_{50} values for ethidium displacement from CT-DNA and from synthetic [poly(dA-dT)]$_2$ (AT) and [poly(dG-dC)]$_2$ (GC) oligonucleotides were determined using aqueous buffer (10 mM Na_2HPO_4, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.0) containing 1.26 μM ethidium bromide and 1 μM CT-DNA, AT, and GC, respectively.[13,14]

All measurements were made in 10-mm quartz cuvettes at 20 °C using a Perkin-Elmer LS5 instrument (excitation at 546 nm; emission at 595 nm) following serial addition of aliquots of a stock drug solution (~5 mM in DMSO). The C_{50} values are defined as the drug concentrations which reduce the fluorescence of the DNA-bound ethidium by 50% and are reported as the mean from three determinations. Apparent equilibrium binding constants were calculated from the C_{50} values (in μM) using: $K_{app} = (1.26/C_{50}) \times K_{ethidium}$, and with a value of $K_{ethidium} = 10^7$ M^{-1} for ethidium bromide.[14a]

2. In Vitro Cytotoxicity. A. Human Colon Adenocarcinoma Experimental Protocol. Establishment details of human colon adenocarcinoma carcinoma cell line (HT29) have been previously described.[4a] Drug solutions of appropriate concentration were added to a culture containing HT29 cells at 2.5 × 10^4 cells/mL of medium and the drug exposure was protracted for 144 h. All assays were performed in triplicate, as previously described.[4a]

B. Human Ovarian Carcinoma Experimental Protocol. Establishment details and biological properties of human ovarian carcinoma cell lines (A2780, A2780cisR, CH1, CH1cisR, and SKOV-3) have been described previously.[17] The sulforhodamine B (SRB) experimental protocol used has been described previously.[13a,17] Cells were plated (100−5000 cells) in 96-well microtiter plates and left overnight to adhere prior

to drug treatment. Aqueous drug solutions at pH 7.0 were then added to the cells at various concentrations following dilution of a stock DMSO solution. After 96 h of continuous drug exposure at 37 °C, growth inhibition was assessed using SRB protein staining. IC_{50} values (drug dose required for 50% growth inhibition compared to drug-free controls) were determined by comparing treated and untreated cells.

Supporting Information Available: Detailed information on target compounds (^{1}H NMR, purification procedure). This material is available free of charge via the Internet at http://pubs.acs.org.

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