1-[(ω-Aminoalkyl)amino]-4-[*N*-(ω-aminoalkyl)carbamoyl]-9-oxo-9,10-dihydroacridines as Intercalating Cytotoxic Agents: Synthesis, DNA Binding, and Biological Evaluation

Ippolito Antonini,*[,⊥] Paolo Polucci,[⊥] Terence C. Jenkins,[†] Lloyd R. Kelland,[‡] Ernesto Menta,[§] Nicoletta Pescalli,[§] Barbara Stefanska,[∥] Jan Mazerski,[∥] and Sante Martelli[⊥]

Department of Chemical Sciences, University of Camerino, Via S. Agostino 1, 62032 Camerino, Italy, Cancer Research Campaign Biomolecular Structure Unit and Cancer Research Campaign Cancer Therapeutics Centre, The Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG, U.K., Boehringer Mannheim Italia, Research Center, 20052 Monza, Italy, and Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, 80952 Gdansk, Poland

Received February 25, 1997[⊗]

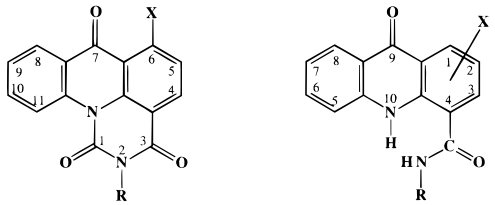
A series of DNA-intercalating potential antitumor agents, 1-[(ω-aminoalkyl)amino]-4-[*N*-(ω-aminoalkyl)carbamoyl]-9-oxo-9,10-dihydroacridines, has been prepared by aminolysis of the corresponding 4-[*N*-(ω-aminoalkyl)carbamoyl]-1-chloro derivative with a suitable ω-aminoalkylamine. The noncovalent DNA-binding properties of these bis-functionalized compounds have been examined using a combination of fluorometric and thermal denaturation techniques and are compared with the behaviors for established DNA intercalants and cationic minor groove ligands. The results indicate that (i) the agents are considerably more DNA-affinic than less functionalized acridinones, with 'apparent' binding constants of (0.1−2.1) × 10^7 and (0.3−7.5) × 10^7 M^{-1} at pH 5 and 7, respectively, (ii) overall affinity is sensitive to both the length of the flexible side chain and the complexity of the attached amine substituents, and (iii) the pendant side chains effect a switch to moderate AT-preferential binding. *In vitro* cytotoxic potencies toward six tumor cell lines broadly parallel the observed DNA affinities, although poor correlation is evident for certain compounds. The octanol/water partition coefficients have been also calculated, but there is no correlation with cytotoxicity values. Two highly DNA-affinic analogs, **10** and **13**, have been identified with a useful broad spectrum of cytotoxic activity.

Introduction

Antitumor cytotoxic agents with DNA-intercalative properties are characterized by the presence of a planar chromophore, generally a tri- or tetracyclic ring system, and one or two flexible basic side chains. Some meaningful examples include anthrapyrazoles,[1] pyrazoloacridines,[2] acridine-4-carboxamides,[3] mitoxantrone,[4] and several of its aza analogs.[5]

We recently described the synthesis and the antitumor properties of some tetracyclic pyrimido[5,6,1-*de*]acridines (**1**; Figure 1) with one or two basic side chains.[6,7] This family of drugs belongs to the group in which two side chains are essential for anticancer activity. In fact, while the derivatives with only one side chain [**1**: R = (alkylamino)alkyl; X = H, Cl, or NO_2] possess marginal or no antineoplastic activity, the derivatives with a second side chain in position 6 [**1**: R = (alkylamino)alkyl; X = [(alkylamino)alkyl]amino] comprise a novel group of potential intercalating agents endowed with remarkable anticancer properties both *in vitro* and *in vivo*.[6]

Certain 9-oxo-9,10-dihydroacridine-4-carboxamides ('acridone 4-carboxamides', **2**: X = H or NO_2; Figure 1), which can be considered as ring-opened models or precursors of tetracyclic structure **1**, have been investigated previously.[8] While the corresponding acridine-4-carboxamides are promising antitumor agents,[3] the 9(10*H*)-acridones **2** appear to intercalate DNA but are devoid of significant anticancer activity.[8]



1 **2**

Figure 1. Structures and ring numbering for the tetracyclic pyrimido[5,6,1-*de*]acridines **1** and the tricyclic 4-carbamoyl-9(10*H*)-acridinones **2**.

In order to establish if the introduction of a second basic side chain in tricyclic compounds **2** could effect a marked increase of antitumor activity, as seen for the tetracyclic derivatives **1**, we have synthesized and evaluated a series of bis-functionalized 1-[(ω-aminoalkyl)amino]-4-[*N*-(ω-aminoalkyl)carbamoyl]-9(10*H*)-acridinones **10**−**20**. The second substituent at ring position 1 of the 9-oxo-9,10-dihydroacridine chromophore here corresponds with that in position 6 of the pyrimido[5,6,1-*de*]acridine system (cf. **2** and **1**, respectively, in Figure 1).

DNA-binding results from thermal denaturation and fluorescence-based studies are reported for these compounds with calf thymus DNA and two polyoligonucleotide duplexes. *In vitro* cytotoxicity data against six tumor cell lines are described.

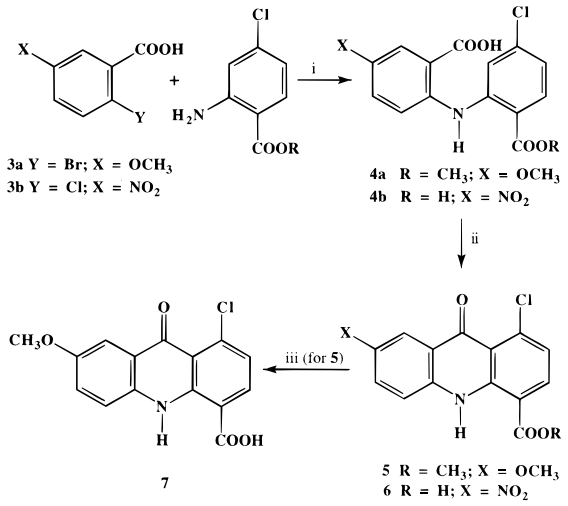
Chemistry

Schemes 1 and 2 show the synthetic pathway used, and the physical data for compounds **9**−**20** are given in

* Address correspondence to this author.
[⊥] University of Camerino.
[†] Cancer Research Campaign Biomolecular Structure Unit.
[‡] Cancer Research Campaign Cancer Therapeutic Centre.
[§] Boehringer Mannheim Italia.
[∥] Technical University of Gdansk.
[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1997.

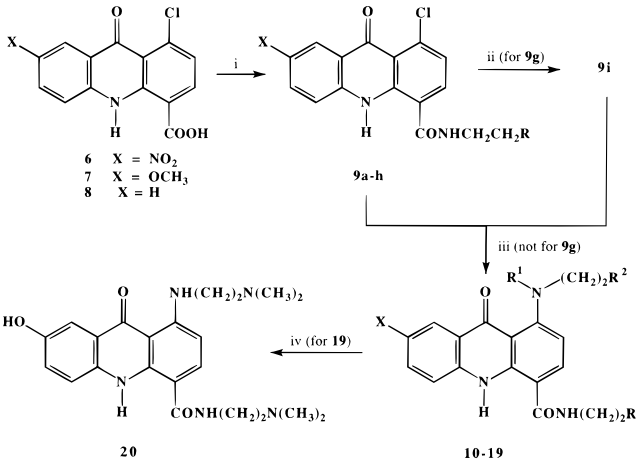
S0022-2623(97)00114-3 CCC: $14.00

Scheme 1[a]



[a] Reagents: (i) for **3a** $(MeCOO)_2Cu\cdot H_2O$/$[(Me)_2CH]_2NEt$/1-methyl-2-pyrrolidinone, for **3b** Cu/K_2CO_3/isopentyl alcohol; (ii) for **4a** PPE, for **4b** $POCl_3$/xylene; (iii) NaOH/EtOH.

Scheme 2[a]



[a] Reagents: (i) for **6** $ClCOOEt/H_2NCH_2CH_2R$, for **7** and **8** 1,1′-carbonyldiimidazole/$H_2NCH_2CH_2R$; (ii) HCl/dioxane; (iii) $HN(R^1)CH_2CH_2R^2$; (iv) HBr. Substituents for compounds **9** and **10**−**20** are shown in Tables 1 and 2, respectively.

Tables 1 and 2. Condensation of 2-chloro-5-nitrobenzoic acid (**3b**) with 2-amino-4-chlorobenzoic acid afforded diphenylamine **4b**; cyclization by reflux treatment with $POCl_3$ gave an isomeric mixture of the 1-chloro derivative **6** and 6-chloro-2-nitro-9-oxo-9,10-dihydroacridine-4-carboxylic acid in a ∼2:1 ratio, as judged from ^{1}H NMR analysis. This mixture proved difficult to separate by usual methods but could be used for subsequent steps without purification. Analogous reaction of 2-bromo-5-methoxybenzoic acid (**3a**) with methyl 2-amino-4-chlorobenzoate[9] afforded **4a**, which was cyclized with PPE (polyphosphoric acid ethyl ester) to the ester **5**; subsequent hydrolysis with aqueous NaOH provided the carboxylic acid **7**.

Treatment of either 1-chloro-7-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**7**) or 1-chloro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**8**)[9] with the required amine, after prior reaction of the acid with 1,1′-carbonyldiimidazole at room temperature in DMF, gave the 1-chloro carboxamides **9a**−**g**. The amide **9h** was prepared from carboxylic acid **6** by the "mixed anhydride" method,[10] while **9i** was obtained by deprotection of **9g** with HCl in dioxane solution at room temperature. Reaction of the appropriate amine with carboxamide **9** afforded the 1-[(ω-aminoalkyl)amino]-4-[*N*-(ω-aminoalkyl)carbamoyl]-9(10*H*)-acridinones **10**−**19**; cleavage of **19** with HBr gave the hydroxy derivative **20**.

In order to examine the DNA-binding properties and the *in vitro* antineoplastic activity of these agents, the free base forms of **10**−**15** and **17**−**20** were converted into their water-soluble dihydrochloride or maleate addition salts by the usual methods. This was not necessary for **16** as the free base is appreciably water-soluble.

Results and Discussion

DNA-Binding Properties. Thermal denaturation data for interaction of the acridinones **10**−**20**, simpler acridinone derivatives **21** and **22**,[11] acridines, and selected DNA-binding ligands with calf thymus DNA (CT-DNA) at three [ligand]:[DNAp] molar ratios are shown in Table 3. The results show that these agents strongly stabilize the thermal helix → coil transition (positive T_m increase) for the DNA duplex, although none is as effective as mitoxantrone. However, all derivatives are considerably more DNA-affinic than acridine, proflavine (an established DNA intercalant), or acridinones **21** and **22**, suggesting that the functionalized side chains are necessary for effective interaction. Melting profiles were monophasic at low [ligand]:[DNAp] molar ratios although biphasic behavior was found for **10**, **16**, and **18**−**20** at higher ratios, indicating possible secondary modes of interaction for these drugs. Examination of the ligand-induced effects upon the low- and high-temperature portions (AT and GC segments, respectively) of the melting curves[11] revealed no clear evidence for either base site- or sequence-preferential binding.

The ΔT_m data in Table 3 reveal a clear **18** > **10** ∼ **16** > **12** ∼ **13** ∼ **20** > **15** ∼ **19** > **17** > **11** ∼ **14** rank order for differential thermal stabilization of the DNA duplex that can be interpreted in terms of the extent and strength of ligand binding. The poor effect of **14** suggests that the protonation status of the pendant side chain(s) influences overall DNA binding, since the morpholine residue provides the least basic amine residue within this series and would be only ∼10−20% protonated at pH 7. Comparison of the relative effects induced by homologs **10** ($n = 2$) and **12** ($n = 3$) indicates that homologous extension of the side chains results in poorer stabilization. In general, side chain elaboration in terms of steric load appears to effect a decrease in relative binding strength, such that less crowded molecules provide superior DNA affinity. Similarly, the results determined for **10** and **17** show that *N*-methylation of the 1-amino group markedly weakens the binding efficiency, presumably as a result of reduced overall chromophore planarity due to elimination of the favorable intramolecular hydrogen-bonded interaction with the 9-carbonyl residue in **10**. The introduction of electron-releasing ring substituents (e.g., X = OMe or OH, **19** and **20**) leads to weaker overall binding, whereas the electron-withdrawing nitro group in **18** provides a small enhancement of stabilization.

Competitive displacement (C_{50}) and quenching (Q) fluorometric assays with DNA-bound ethidium can be used[11] to (i) determine 'apparent' equilibrium constants (K_{app}) for drug binding, as the C_{50} value is approximately inversely proportional to the binding constant,[12] (ii)

Table 1. Substituents, Melting Point, Yield, and Formula of Compounds **9** (Scheme 2)

no.	X	R	mp, °C	yield, %	formula
9a[a]	H	$N(Me)_2$	185−187	87	$C_{18}H_{18}ClN_3O_2$
9b	H	$N(Et)_2$	157−158	74	$C_{20}H_{22}ClN_3O_2$
9c	H	$CH_2N(Me)_2$	150−151	66	$C_{19}H_{20}ClN_3O_2$
9d	H	4-morpholinyl	188−190	83	$C_{20}H_{20}ClN_3O_3$
9e	H	1-piperidyl	158−161	54	$C_{21}H_{22}ClN_3O_2$
9f	OMe	$N(Me)_2$	170−172	66	$C_{19}H_{20}ClN_3O_3$
9g	H	$N(Boc)(CH_2)_2OZ$[b]	oil	85	$C_{28}H_{34}ClN_3O_6$
9h·HCl	NO_2	$N(Me)_2$	295−297	65	$C_{18}H_{18}Cl_2N_4O_4$
9i	H	$NH(CH_2)_2OH$	145−146	73	$C_{18}H_{18}ClN_3O_3$

[a] Data taken from ref 6. [b] Boc = *tert*-butyloxycarbonyl; Z = 2-tetrahydropyranyl.

Table 2. Substituents, Melting Point, log *P*, Yield, and Formula of Compounds **10**−**20** (Scheme 2)

no.	X	R^1	R^2	R	mp, °C	log *P*[a]	yield, %	formula[b]
10	H	H	$N(Me)_2$	$N(Me)_2$	151−152 (259−260)[c]	5.12 ± 0.65	72	$C_{22}H_{29}N_5O_2$
11	H	H	$N(Et)_2$	$N(Et)_2$	96−97 (>350)[d]	7.25 ± 0.65	63	$C_{26}H_{37}N_5O_2$
12	H	H	$CH_2N(Me)_2$	$CH_2N(Me)_2$	108−110 (272−273)[c]	5.76 ± 0.63	67	$C_{24}H_{33}N_5O_2$
13	H	H	$NH(CH_2)_2OH$	$N(Me)_2$	120−121 (218−221)[c]	4.09 ± 0.66	57	$C_{22}H_{29}N_5O_3$
14	H	H	4-morpholinyl	4-morpholinyl	193−195 (124−126)[d]	4.42 ± 0.73	51	$C_{26}H_{33}N_5O_4$
15	H	H	1-piperidyl	1-piperidyl	128−130 (210−212 d)[d]	7.73 ± 0.68	33	$C_{28}H_{37}N_5O_2$
16	H	H	$NH(CH_2)_2OH$	$NH(CH_2)_2OH$	160−161	3.15 ± 0.68	39	$C_{22}H_{29}N_5O_4$
17[e]	H	Me	$N(Me)_2$	$N(Me)_2$	oil (159−160)[d]	4.21 ± 0.62	66	$C_{27}H_{35}N_5O_6$
18	NO_2	H	$N(Me)_2$	$N(Me)_2$	207−208 (187−188 d)[d]	5.68 ± 0.65	87	$C_{22}H_{28}N_6O_4$
19	OMe	H	$N(Me)_2$	$N(Me)_2$	167−168 (151−152 d)[d]	5.32 ± 0.66	82	$C_{23}H_{31}N_5O_3$
20	OH	H	$N(Me)_2$	$N(Me)_2$	236−237 (204−206 d)[d]	4.77 ± 0.66	80	$C_{22}H_{29}N_5O_3$

[a] log *P* is the calculated value ($\log_{10}$) of the octanol/water partition coefficient. [b] Analyses for C, H, and N. [c] In parentheses is the melting point of the dihydrochloride. [d] In parentheses is the melting point of the maleate; d = decomposition. [e] Isolated and characterized as the maleate.

distinguish intercalating agents from nonintercalative ligands,[13] and (iii) establish possible base- or sequence-preferential binding.[14]

In the present study, fluorescence displacement assays were performed at pH 5, to ensure that the added compounds were present as the fully amine-protonated cationic species, as in previous studies,[11,12b,14] and at pH 7 to enable comparison in more biologically relevant systems. The C_{50} values (Table 3) determined for ethidium displacement from CT-DNA by acridinones **10**−**20** indicate that these molecules, with the exception of **11** and **14**, are strong DNA-binding ligands at both pH 5 and 7, with greater affinity than simpler acridines or acridinones (e.g., **22**), *m*-AMSA[12b,14] or established minor groove-binding cationic ligands, including berenil and distamycin.[11,13,15,16] However, no compound is as efficient as mitoxantrone. It is noteworthy that 3−9-fold poorer binding of **10**−**20** is evident at the lower pH as compared to pH 7, presumably due to protonation of the host DNA bases and/or pH-dependent conformational effects. Opposite behavior is seen for mitoxantrone and acridine (Table 3), although in the latter case this reflects the poor basicity of the chromophore. On this basis, reported competitive C_{50} data determined for ligands at pH 5 (e.g., refs 11, 12b) should be treated with caution as this may not correctly predict the binding affinity at physiological pH.

The relative K_{app} values of $(0.1-2.1) \times 10^7$ M^{-1} at pH 5 and $(0.3-7.5) \times 10^7$ M^{-1} at pH 7 determined for binding of **10**−**20** with CT-DNA compare with 3.4×10^8 M^{-1} (pH 7) for mitoxantrone and $(2-4) \times 10^4$ M^{-1} for the unsubstituted acridine and 3,6-diaminoacridinone **22**, again indicating that the functionalized side chains are required for effective duplex binding. The **18** > **15** > **10** ∼ **12** ∼ **13** ∼ **16** ∼ **20** > **19** > **17** > **11** ∼ **14** rank order determined for binding is closely similar to that predicted from the DNA melting experiments, although the piperidine derivative **15** has greater affinity than expected from its induced ΔT_m (Table 3). The order **18** > **10** ∼ **20** > **19** > **17** reinforces conclusions for this subset of related compounds with regard to the effects of 7-substitution and *N*-methylation.

The fluorescence quenching (*Q*) values for **10**−**20** with $[poly(dA-dT)]_2$ at pH 5 are much smaller than expected for 'classical' DNA intercalants, including acridine and proflavine, and more closely resemble values for minor groove-binding ligands (e.g., berenil and distamycin in Table 3).[11,12b,14] This distinction arises from the larger DNA binding site sizes associated with nonintercalative drugs compared to planar intercalators,[12b,14] suggesting that the flexible protonated side chains in acridinones **10**−**20** are accommodated within the groove conduits of the host DNA duplex upon binding. Similar behavior is evident for mitoxantrone, where the small *Q* values indicate more efficient quenching of the bound ethidium fluorophore (under conditions of minimum displacement) than could be achieved by a strict intercalation process. However, such data do not preclude involvement of an intercalative binding mode. Compounds **10**−**20** show significantly different binding behavior to polyamines, which bind to DNA in a nonspecific manner by bridging the minor groove and by inter- and intrastrand electrostatic contacts to the phosphate residues.[17] Thus, for example, all derivatives are both considerably more affinic than spermidine (C_{50} = 37.5 μM at pH 7) and more efficient quenching ligands, suggesting deep penetration of the groove tracts. On this basis, we infer that DNA binding with these molecules involves *both* intercalative and groove-binding modes. Equivalent dual binding behavior has been established for hybrid ligands and combilexins.[11,14,18,19]

The *Q* values determined for **10**−**20** using CT-DNA, $[poly(dA-dT)]_2$, and $[poly(dG-dC)]_2$ indicate a moderate preference for binding to AT-rich duplexes. This AT > GC behavior contrasts with the GC > AT preference shown by acridine, proflavine, and *m*-AMSA (Table 3), where DNA binding is likely to involve strict 'classical' intercalation. Acridinones **21** and **22** also appear to

Table 3. DNA Binding[a] and *in Vitro* Cytotoxicity Data for the 9(10*H*)-Acridinone Derivatives and Related Ligands

	Q (μM)[b]			C_{50} (μM)[c]		induced ΔT_m shift (°C)[d]			inhibitory IC_{50} concn (μM)[e]					
compd	AT	CT-DNA	GC	pH 5	pH 7	1:20	1:10	1:5	L1210	A2780	CH1	G-361	SKOV-3	HT29
10[f]	3.1	3.3	3.5	1.1	0.34	11.8	21.3	≥27	0.044	0.015	0.02	0.015	0.4	0.094
11[g]	19	27	36	20	2.3	2.4	4.4	8.6	3.3	0.76	1.25	6.2	2.4	5.7
12[f]	3.2	4.8	6.6	3.2	0.38	10.9	19.2	≥25	0.52	0.11	0.335	3.6	0.4	0.58
13[f]	3.8	4.2	4.7	2.0	0.35	10.2	17.6	≥25	0.035	0.017	0.016	0.28	0.17	0.14
14[g]	3.7	4.8	6.0	16	3.7	3.8	5.8	7.8	0.44	1.5	1.25	7.2	7.4	4.5
15[g]	3.4	4.4	5.6	0.83	0.28	8.1	15.7	21.9	1.2	0.024	1.2	0.67	0.62	0.45
16	2.6	3.2	4.1	1.7	0.36	11.2	20.5	≥25	4.0	4.8	0.165	15.5	21.5	>23
17[g]	13	14	15	4.8	0.94	9.2	12.3	16.0	1.65	0.41	3.4	1.5	1.0	3.0
18[g]	1.8	2.1	2.4	0.61	0.17	12.6	22.8	≥28	0.46	0.042	0.175	1.8	0.21	0.19
19[g]	5.3	5.8	6.5	3.1	0.69	8.6	16.1	22.4	0.41	0.057	0.49	1.25	0.42	0.50
20[g]	4.4	4.8	5.4	2.2	0.37	10.9	17.9	≥25	0.14	0.051	0.64	0.3	0.17	2.0
mitoxantrone	0.32	0.34	0.37	0.021	0.037	19.2	≥25	≥32	0.00004	0.00055	0.00265	0.00065	0.0053	0.010
acridine[h]	26	24	22	142	320[i]	0.3	0.5	0.7	45	>100				
21[j]	>500[i]	≥400[i]	340[i]	*k*	*k*	0.2	0.3	0.4	>100[l]	>100	77	>100	>100	
22[m]	>400[i]	340[i]	280[i]	660[i]	630[i]	0.3	0.4	0.6	35[n]	37[n]	>100		>100	
2a[o]									1.7					
proflavine[h]	24	20	14	*k*	*k*	5.0	9.1	11.5	2.7	0.47				
m-AMSA[p]	25	3.8	4.5	8.9[q]			1		0.05[r]	0.1				
berenil	5[h]	9[h]	19[h]	4.4[h]	1.5[s]	9.6	14.1	18.9	32[h]					
distamycin A	1.6[p]	39.5[p]	130[p]	5.0	1.3[t]	8.7	11.7	14.5						

[a] AT, CT-DNA, and GC refer to $[poly(dA-dT)]_2$, calf thymus DNA, and $[poly(dG-dC)]_2$, respectively. [b] Drug concentration to give 50% fluorescence quenching of bound ethidium at pH 5 for an [ethidium]:[DNA] molar ratio of 0.1:1 (see text); mean value (±1−5%) from three determinations. [c] Drug concentration to effect 50% drop in fluorescence of bound ethidium at an [ethidium]:[CT-DNA] molar ratio of 1.26:1 and the pH shown (see text); mean value (±1−5%) from three experiments. [d] Mean value (±0.1−0.2 °C) from three determinations at pH 7 for the [drug]:[DNAp] ratio shown. [e] Drug concentration required to inhibit cell growth by 50% after cell−drug contact for 48 h (L1210), 96 h (A2780), 144 h (HT29). [f] Tested as the dihydrochloride. [g] Tested as the dimaleate. [h] Data taken from ref 11. [i] Extrapolated value due to precipitation at high ligand concentrations. [j] **21** = 9(10*H*)-Acridinone. [k] Measurement not possible due to ligand fluorescence. [l] 100 μM gave 40% inhibition. [m] **22** = 3,6-diamino-9(10*H*)-acridinone. [n] cf. IC_{50} values of 67 and >100 μM for L1210 and A2780 cells, respectively (from ref 11). [o] **2a**: X = H, R = $(CH_2)_2N(Me)_2$, Figure 1; data from reference 8a. [p] Data from ref 14. [q] Data from ref 12b. [r] Following drug exposure for 24 h (from ref 14). [s] C_{50} = 1.4 μM at pH 7.4 in TES buffer (from ref 21). [t] C_{50} = 1.3 μM (TES, pH 7.4) and 1.6 μM (TRIS, pH 8), from refs 21 and 24, respectively.

favor GC sequences although binding is considerably weaker. These data indicate that the binding behavior of **10**−**20** is largely dictated by the functionalized side chains, since AT-preferential binding is characteristic for most cationic minor groove-binding ligands,[15,16] including berenil and distamycin (Table 3). In contrast, the binding behavior of mitoxantrone appears to be base- or sequence-neutral.

Cytotoxic Activity. *In vitro* cytotoxic potency was determined for acridinones **10**−**20** and related DNA-binding compounds using six cell lines, including murine leukemia (L1210), human ovarian carcinoma (A2780, CH1, and SKOV-3), human melanoma (G-361), and human colon adenocarcinoma (HT29) cells. Table 3 reveals that all compounds show excellent or good cytotoxic activity, but no compound approaches the potency of mitoxantrone. In qualitative agreement with both our spectroscopic and binding data, the cytotoxic potencies generally parallel the experimental DNA affinity, although there are clear discrepancies and differences among the cell lines examined. Thus, for example, derivatives **11**, **14**, and **17** are uniformly poorly cytotoxic, as would be expected from their high C_{50} (i.e., low K_{app}) and low ΔT_m values, whereas the highly affinic compound **18** is of only average potency within this series. This behavior suggests that other factors (e.g., cellular uptake) must also influence the cytotoxic efficiency. The octanol/water partition coefficients for compounds **10**−**20** have also been calculated (Table 2) using ACD/Labs software (Advanced Chemistry Development, Inc., Toronto, Ontario M5H 2L3, Canada). However, attempts to correlate $\log_{10}$ of partition coefficients with *in vitro* cytotoxic activity failed. Two DNA-affinic compounds from this series, **10** and **13**, provide a useful degree of biological potency toward a range of tumor cell lines.

Conclusions

The introduction of a second basic side chain in position 1 of tricyclic compounds **2** results in an excellent increase of cytotoxicity, as predicted. In fact, derivative **10** is about 40 times more cytotoxic against the L1210 cell line (Table 3) than the corresponding unsubstituted acridonecarboxamide **2a**. Acridinones **10**−**20** show a usefully wide spectrum of cytotoxic activity toward a variety of tumor cell lines, although no compound approaches the potency of mitoxantrone. Generally, there is qualitative agreement between cytotoxic potency and the DNA affinity although there are some discrepancies among the various cell lines examined. Two compounds, **10** and **13**, have been identified as worthwhile leads for the development of more potent cytotoxins in this family of bis(amine-functionalized) acridinone carboxamides.

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). Quartets that are transformed into triplets by addition of D_2O are labeled with an asterisk. Elemental analyses were performed on a Model 1106 elemental analyzer (Carlo Erba Strumentazione); all analytical values for C, H, and N were within ±0.4% of the theoretical values.

2-[[5-Chloro-2-(methoxycarbonyl)phenyl]amino]-5-methoxybenzoic Acid (4a). A suspension of methyl 2-amino-4-chlorobenzoate[9] (0.8 g, 4.3 mmol), commercially available 2-bromo-5-methoxybenzoic acid (**3a**; 1 g, 4.3 mmol), and $Cu(OAc)_2 \cdot H_2O$ (0.86 g, 4.3 mmol) in 1-methyl-2-pyrrolidinone (5 mL) and *N*,*N*-diisopropylethylamine (10 mL) was stirred for 6 h at 160 °C. After cooling, dilution with water, and acidification to pH 2, the dried collected precipitate was stirred with Et_2O and then filtered to give **4a** (0.75 g, 52%): mp 176−177 °C (MeOH).

Methyl 1-Chloro-7-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylate (5). Compound **4a** (1 g, 3 mmol) was refluxed with PPE (40 g) in $CHCl_3$ (50 mL) until all solid was dissolved. The reflux condenser was removed, and the $CHCl_3$ was allowed to evaporate, to give an oil which was heated for 1 h at 100 °C. The mixture was diluted cautiously with MeOH (5 mL) and water (10 mL) and then extracted with $CHCl_3$ (3 × 20 mL). The concentrated chloroform extracts were flash-chromatographed on silica gel using $CHCl_3/C_6H_6$ (3:2, v/v) to yield the ester **5** (0.58 g, 61%): mp 219−220 °C.

1-Chloro-7-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylic Acid (7). The ester **5** (1 g, 3.1 mmol) was suspended in EtOH (100 mL) and 2 M NaOH (100 mL) and heated under reflux for 30 min. The resulting mixture was acidified with 4 M HCl and stirred at room temperature for 20 min; the precipitate was collected and washed with water, MeOH, and Et_2O to give pure **7** (0.72 g, 75%): mp 340−341 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 3H, CH_3), 7.28 (d, 1H, 2-H, ar), 7.43 (m, 1H, 6-H, ar), 7.59 (d, 1H, 8-H, ar), 7.75 (d, 1H, 5-H, ar), 8.30 (d, 1H, 3-H, ar), 12.35 (s, 1H, NH, ex).

1-Chloro-7-nitro-9-oxo-9,10-dihydroacridine-4-carboxylic Acid (6). 2-Amino-4-chlorobenzoic acid (7.0 g, 40.8 mmol), 2-chloro-5-nitrobenzoic acid (**3b**; 7.0 g, 34.7 mmol), K_2CO_3 (7 g, 50.6 mmol), and Cu powder (0.25 g, 3.9 mmol) were suspended in isopentyl alcohol (150 mL). After refluxing and stirring for approximately 20 min, a red-orange mass precipitated; reaction was continued for 4−5 h, after which time 1 M aqueous K_2CO_3 (150 mL) was added and insolubles were removed from the hot mixture by filtration. The aqueous layer was separated and acidified to pH 5 with 2 M HCl to precipitate the product, which was filtered, suspended in boiling MeOH (100 mL), suspended in boiling water (200 mL), filtered again, and washed with EtOH to give adduct **4b**. The crude diacid was treated with $POCl_3$ (55 mL) in xylene (55 mL) under reflux for 2 h. The precipitate that appeared upon cooling was collected, boiled with MeOH, filtered, washed with Et_2O, and dried to give a mixture (5.8 g, 52%) of **6** and 6-chloro-2-nitro-9-oxo-9,10-dihydroacridine-4-carboxylic acid, in an approximate 2:1 ratio by NMR. The isomeric mixture was used for the next step without further purification.

4-[*N*-[2-(Diethylamino)ethyl]carbamoyl]-1-chloro-9-oxo-9,10-dihydroacridine (9b). Example of the General Method. The carboxylic acid **8** (1 g, 3.65 mmol) and 1,1′-carbonyldiimidazole (1.1 g, 6.8 mmol) in DMF (10 mL) were stirred, warming if necessary, until homogeneous. The mixture was cooled to 10 °C, and *N*,*N*-diethylethylenediamine (1.16 g, 10 mmol) was added. After 15 min at room temperature, the mixture was partitioned between $CHCl_3$ and aqueous 1 M Na_2CO_3. The organic layer was worked up to give the crude carboxamide **9b**, which solidified on washing with Et_2O and was used for the next step: ^{1}H NMR ($CDCl_3$) δ 1.08 (t, 6H, 2 × CH_3), 2.60 (q, 4H, 2 × CO-N-C-C-N-CH_2), 2.72 (t, 2H, CO-N-C-CH_2), 3.55 (q*, 2H, CO-N-CH_2), 7.20 (d, 1H, 2-H, ar), 7.23−7.40 (m, 3H, 2 ar + CO-NH ex), 7.60−7.72 (m, 2H, ar), 8.42 (dd, 1H, 8-H or 5-H, ar), 12.77 (br s, 1H, 10-H, ex).

The 4-[*N*-(*ω*-aminoalkyl)carbamoyl]-9(10*H*)-acridinones **9c**−**g** were prepared in a similar manner by analogous treatment of an appropriate acid with the corresponding amine. All amine reagents were commercially available, with the exception of *O*-tetrahydropyranyl-2-[[(2-aminoethyl)-*N*-*tert*-butoxycarbonyl]amino]ethanol.[20] Amides **9b**−**f** were solidified by washing with Et_2O, whereas **9g** was chromatographed on a

silica gel column eluted with $CHCl_3/C_6H_6$/MeOH (10:10:1, v/v) to obtain a dense oil. All the carboxamide products were used for subsequent steps without further purification.

4-[*N*-[2-(Dimethylamino)ethyl]carbamoyl]-1-chloro-7-nitro-9-oxo-9,10-dihydroacridine Hydrochloride (9h). To a stirred and cooled (ice−water, 0 °C) isomeric mixture of **6** and 6-chloro-2-nitro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (1 g, 3.14 mmol of isomeric mixture, ∼2.1 mmol of **6**) and anhydrous Et_3N (0.314 g, 3.14 mmol) in $CHCl_3$ (30 mL) was added a solution of EtOCOCl (0.34 g, 3.14 mmol) in $CHCl_3$ (20 mL) dropwise. After stirring at room temperature for 1 h and cooling to 0 °C, the mixture was treated with *N*,*N*-dimethylethylenediamine (0.32 g, 3.14 mmol) and then stirred overnight at room temperature. The solid precipitate was filtered and washed sequentially with MeOH, $CHCl_3$, and then Et_2O to give **9h** (0.58 g, ∼65% based upon **6**): ^{1}H NMR (DMSO-d_6) δ 2.88 (s, 6H, 2 × CH_3), 3.34 (t, 2H, CO-N-C-CH_2), 3.75 (q*, 2H, CO-N-CH_2), 7.33 (d, 1H, 2-H, ar), 7.90 (d, 1H, 5-H, ar), 8.30 (d, 1H, 3-H, ar), 8.49 (dd, 1H, 6-H, ar), 8.93 (d, 1H, 8-H, ar), 9.47 (t, 1H, CO-NH, ex), 10.03 (br s, 1H, 10-H, ex), 11.03 (br m, 1H, N^+-H, ex).

4-[*N*-[2-[(2-Hydroxyethyl)amino]ethyl]carbamoyl]-1-chloro-9-oxo-9,10-dihydroacridine (9i). A mixture of **9g** (0.45 g, 0.83 mmol) in dioxane (30 mL) and aqueous HCl (3 mL of 37%, w/w) was stirred for 30 min at room temperature. The reaction mixture was partitioned between $CHCl_3$ and an excess of 1 M aqueous Na_2CO_3. The organic layer was worked up to give the crude carboxamide **9i**, which solidified upon washing with Et_2O: ^{1}H NMR (DMSO-d_6) δ 2.70 (t, 2H, CH_2), 2.85 (t, 2H, CH_2), 3.42−3.57 (m, 4H, CO-N-CH_2 + O-CH_2), 4.65 (br m, 1 H, ex), 7.27−7.38 (m, 2H, ar), 7.62−7.80 (m, 2H, ar), 8.12−8.23 (m, 2H, ar), 9.31 (br s, 1H, 10-H, ex). This material was used for the next step without further purification.

1-[[2-(Dimethylamino)ethyl]amino]-4-[*N*-[2-(dimethylamino)ethyl]carbamoyl]-9-oxo-9,10-dihydroacridine (10). Example of General Procedure for the Preparation of 10−19. A suspension of the carboxamide **9a** (0.48 g, 1.4 mmol) in DMF (10 mL) and the 2,2′-dimethylethylenediamine (0.53 g, 6 mmol) were 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 chromatographed on a silica gel column eluted with $CHCl_3$/MeOH (7:3, v/v) and 32% aqueous NH_3 (10 mL for 1 L of eluent) to afford pure **10**: ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 6H, 2 × CH_3), 2.36 (s, 6H, 2 × CH_3), 2.60 (t, 2H, C-CH_2), 2.70 (t, 2H, C-CH_2), 3.44 (q*, 2H, 1-N-CH_2), 3.55 (q*, 2H, CO-N-CH_2), 6.22 (d, 1H, 2-H, ar), 6.92 (t, 1H, CO-NH, ex), 7.23 (t, 1H, 7-H or 6-H, ar), 7.36 (d, 1H, 5-H or 8-H, ar), 7.62 (t, 1H, 6-H or 7-H, ar), 7.70 (d, 1H, 3-H, ar), 8.38 (d, 1H, 8-H or 5-H, ar), 11.02 (t, 1H, 1-NH, slow ex), 13.42 (s, 1H, 10-H, ex).

The 1-[(ω-aminoalkyl)amino]-4-[*N*-(ω-aminoalkyl)carbamoyl]-9(10*H*)-acridinones **10−19** were prepared in a similar manner by analogous treatment of the appropriate compound **9** with the suitable amine. Compound **17**, obtained as a dense oil, was converted into a maleate, crystallized from EtOH−Et_2O, and then characterized.

1-[[2-(Dimethylamino)ethyl]amino]-4-[*N*-[2-(dimethylamino)ethyl]carbamoyl]-7-hydroxy-9-oxo-9,10-dihydroacridine (20). Compound **19** (0.26 g, 0.61 mmol) in 48% HBr (10 mL) was refluxed for 1 h. The reaction mixture was partitioned between $CHCl_3$ and 0.5 M aqueous Na_2CO_3. Workup of the organic phase and column chromatography on silica gel eluted with $CHCl_3$/MeOH (4:1, v/v) and 32% aqueous NH_3 (10 mL for 1 L of eluent) afforded **20**: ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H, 2 × CH_3), 2.52 (s, 6H, 2 × CH_3), 2.68 (t, 2H, C-CH_2), 2.90 (t, 2H, C-CH_2), 3.40 (q*, 2H, 1-N-CH_2), 3.66 (q*, 2H, CO-N-CH_2), 6.03 (d, 1H, 2-H, ar), 6.72 (dd, 1H, 6-H, ar), 6.82 (d, 1H, 5-H, ar), 6.86 (m, 1H, CO-NH, ex), 7.35 (d, 1H, 8-H, ar), 7.54 (d, 1H, 3-H, ar), 10.90 (t, 1H, 1-NH, slow ex), 12.58 (s, 1H, 10-H, ex).

Biophysical Evaluation. 1. Thermal DNA Denaturation Studies. The experimental protocol used has been outlined previously.[11] Briefly, melting studies were performed in stoppered quartz cuvettes using a Shimadzu UV-2101PC spectrophotometer fitted with a SPR-8 heating controller and an Omega DP4-TC high-performance temperature probe. Heating was applied at a rate of 1 °C min^{-1} in the 45−98 °C range, with absorbance (260 nm) and temperature data sampling at 8-s intervals. DNA helix → coil transition temperatures (T_m) were determined at the midpoint of the normalized melting profiles. Drug-induced alterations in DNA melting behavior are given by: $\Delta T_m = T_m$(DNA + drug) − T_m(DNA), where the T_m for the drug-free DNA control is 67.8 ± 0.1 °C; results are given as the mean from three determinations.

Calf thymus (CT-DNA) [Sigma; 42% G + C content, ϵ_{260} = 6600 (M phosphate)$^{-1}$ cm^{-1}] was dialyzed extensively against water before use. Solutions were prepared in aqueous phosphate buffer (10 mM Na_2HPO_4/NaH_2PO_4, 1 mM EDTA, pH 7.00 ± 0.01); working solutions (100 μM) were prepared by dilution of a stock solution. DNA−drug solutions were prepared by addition of the compound in DMSO to give a final drug concentration of 5, 10, or 20 μM. It was established that the highest [drug]:[DNAp] molar ratio used did not effect saturation of the DNA duplex by any candidate ligand. Linear correction factors were applied to correct for the effects of DMSO cosolvent (max 2.5%, v/v) used in the DNA−drug studies.[11]

2. Fluorescence Binding Studies. The fluorometric assays have been described previously.[11] Quenching *Q* values were determined for CT-DNA, [poly(dA−dT)]$_2$, and [poly(dG−dC)]$_2$ for solutions (20 μM DNAp) in 0.01 M ionic strength aqueous buffer (9.3 mM NaCl, 2 mM NaOAc, 0.1 mM EDTA, pH 5.0) containing 2 μM ethidium bromide.[14] These concentrations effect minimal displacement of the ethidium fluorophore and maximum drug-induced quenching of the fluorescence due to the DNA-bound ethidium intercalant.[12] Molar extinction values of ϵ_{260} = 6550 and 8400 (M phosphate)$^{-1}$ cm^{-1}, respectively, were used for the AT and GC oligonucleotides (Sigma). The C_{50} values for ethidium displacement were determined using solutions in either the same buffer (for pH 5.0 studies) or aqueous TES buffer (10 mM TES, 0.1 mM EDTA, pH 7.0) containing 1.26 μM ethidium bromide and 1 μM CT-DNA.[11,12,14,21]

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 *Q* and 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.[12a]

3. *In Vitro* Cytotoxicity. A. L1210 Experimental Protocol. Drug solutions of appropriate concentration were added to a culture containing mouse L1210 leukemic cells at 5 × 10^4 cells/mL of medium.[11] Stock aqueous drug solutions (≤0.5%, v/v, DMSO) were used, and it was separately established that this level of DMSO was tolerated. Cells were exposed to the compounds for 48 h at 37 °C, and the IC_{50} values were calculated by counting (Coulter counter) the number of remaining living cells and comparison with drug-free controls. All assays were performed in duplicate.

B. Human Ovarian Carcinoma and Melanoma Cell Lines. Human melanoma cell line G-361 was obtained from the European Collection of Animal Cell Cultures, Porton Down, Salisbury, U.K., via the American Type Culture Collection. Establishment details and biological properties of human ovarian carcinoma cell lines (A2780, CH1, and SKOV-3) have been described previously.[22] The sulforhodamine B (SRB) experimental protocol used has been described previously.[11,22] 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 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.

C. HT29 Human Colon Adenocarcinoma Experimental Protocol. Drug solutions of appropriate concentration were added to a culture containing HT29 cells (American Type Culture Collection, Maryland) at 2.5×10^4 cells/mL of medium.[23] All assays were performed in duplicate.

Acknowledgment. Financial support from CNR, Rome, Italy, and from the Cancer Research Campaign (T.C.J. and L.R.K.) of the U.K. is gratefully acknowledged.

References

(1) (a) Showalter, H. D. H.; Johnson, J. L.; Werbel, L. M.; Leopold, W. R.; Jackson, R. C.; Elslager, E. F. 5-[(Aminoalkyl)amino]-substituted Anthra[1,9-*cd*]pyrazol-6(2*H*)-ones as Novel Anticancer Agents. Synthesis and Biological Evaluation. *J. Med. Chem.* **1984**, *27*, 253−255. (b) Leopold, W. R.; Nelson, J. M.; Plowman, J.; Jackson, R. C. Anthrapyrazoles. A New Class of Intercalating Agents with High Level, Broad Spectrum Activity Against Murine Tumors. *Cancer Res.* **1985**, *45*, 5532−5539.

(2) (a) Capps, D. B.; Kesten, S. R.; Shillis, J.; Plowman, J. 2-Aminoalkyl-5-nitropyrazolo[3,4,5-*kl*]acridines, a New Class of Anticancer Agents. *Proc. Am. Assoc. Cancer Res.* **1986**, *27*, 277. (b) LoRusso, P.; Wozniak, J.; Polin, L.; Capps, D.; Leopold, W. R.; Werbel, L. M.; Biernat, L.; Dan, M. E.; Corbett, T. H. Antitumor Efficacy of PD 115934 (NSC 366140) Against Solid Tumors of Mice. *Cancer Res.* **1990**, *60*, 4900−4905. (c) Capps, D. B.; Dunbar, J.; Kesten, S. R.; Shillis, J.; Werbel, L. M.; Plowman, J.; Ward, D. L. 2-(Aminoalkyl)-5-nitropyrazolo[3,4,5-*kl*]acridines, a New Class of Anticancer Agents. *J. Med. Chem.* **1992**, *35*, 4770−4778.

(3) (a) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. Potential Antitumor Agents. 50. In Vivo Solid Tumor Activity of *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide. *J. Med. Chem.* **1987**, *30*, 664−669. (b) Haldane, A.; Finlay, G. J.; Baguley, B. C. Unusual Dynamics of Killing of Cultured Lewis Lung Cells by the DNA-intercalating Antitumor Agent *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide. *Cancer Chemother. Pharmacol.* **1992**, *29*, 474−479.

(4) (a) Zee-Cheng, R. K. Y.; Cheng, C. C. Antineoplastic Agents. Structure−Activity Relationship Study of Bis(substituted aminoalkylamino)anthraquinones. *J. Med. Chem.* **1978**, *21*, 291−294. (b) Zee-Cheng, R. K. Y.; Podrebarac, E. G.; Menon, C. S.; Cheng, C. C. Structural Modification Study of Bis(substituted aminoalkylamino)anthraquinones. An Evaluation of the Relationship of the [2-[(2-Hydroxyethyl)amino]ethyl]amino Side Chain with Antineoplastic Activity. *J. Med. Chem.* **1979**, *22*, 501−505. (c) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. Antitumor Agents. 1. 1,4-Bis[(aminoalkyl)amino]-9,10-anthracenediones. *J. Med. Chem.* **1979**, *22*, 1024−1030.

(5) Krapcho, A. P.; Petry, M. E.; Getahun, Z.; Landi, J. J.; Stallman, J.; Polsenberg, J. F.; Gallagher, C. E.; Maresch, M. J.; Hacker, M. P.; Giuliani, F. G.; Beggolin, G.; Pezzoni, G.; Menta, E.; Manzotti, C.; Oliva, A.; Spinelli, S.; Tognella, S. 6,9-Bis[(aminoalkyl)amino]benzo[*g*]isoquinoline-5,10-diones. A Novel Class of Chromophore-modified Antitumor Anthracene-9,10-diones: Synthesis and Antitumor Evaluation. *J. Med. Chem.* **1994**, *37*, 828−837.

(6) Antonini, I.; Cola, D.; Polucci, P.; Bontemps-Gracz, M.; Borowski, E.; Martelli, S. Synthesis of (Dialkylamino)alkyl-disubstituted Pyrimido[5,6,1-*de*]acridines, a Novel Group of Anticancer Agents Active on a Multidrug Resistant Cell Line. *J. Med. Chem.* **1995**, *38*, 3282−3286.

(7) Antonini, I.; Cola, D.; Martelli, S.; Cholody, W. M.; Konopa, J. Pyrimidoacridine Derivatives as Potential Antitumor Agents. *Farmaco* **1992**, *47*, 1035−1046.

(8) (a) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. Potential Antitumor Agents. 54. Chromophore Requirements for in Vivo Antitumor Activity among the General Class of Linear Tricyclic Carboxamides. *J. Med. Chem.* **1988**, *31*, 707−712. (b) Chen, Q.; Deady, L. W.; Baguley, B. C.; Denny, W. A. Electron-deficient DNA-intercalating Agents as Antitumor Drugs: Aza Analogues of the Experimental Clinical Agent N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide. *J. Med. Chem.* **1994**, *37*, 593−597.

(9) Rewcastle, G. W.; Denny, W. A. The Synthesis of Substituted 9-Oxoacridan-4-carboxylic Acids; Part 3. The Reaction of Methyl Anthranilates with Diphenyliodonium-2-carboxylates. *Synthesis* **1985**, 220−222.

(10) Antonini, I.; Polucci, P.; Cola, D.; Palmieri, G. F.; Martelli, S. Synthesis of 7-Oxo-7*H*-benzo[*e*]perimidine-4-carboxamides as Potential Antitumor Drugs. *Farmaco* **1992**, *47*, 1385−1393.

(11) McConnaughie, A. W.; Jenkins, T. C. Novel Acridine−Triazenes as Prototype Combilexins: Synthesis, DNA Binding, and Biological Activity. *J. Med. Chem.* **1995**, *38*, 3488−3501.

(12) (a) Morgan, A. R.; Lee, J. S.; Pulleyblank, D. E.; Murray, N. L.; Evans, D. H. Review: Ethidium Fluorescence Assays. Part 1. Physicochemical Studies. *Nucleic Acids Res.* **1979**, *7*, 547−569. (b) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. Potential Antitumor Agents. 34. Quantitative Relationships between DNA Binding and Molecular Structure for 9-Anilinoacridines Substituted in the Anilino Ring. *J. Med. Chem.* **1981**, *24*, 170−177.

(13) Baguley, B. C. Nonintercalative DNA-Binding Antitumour Compounds. *Mol. Cell Biochem.* **1982**, *43*, 167−181.

(14) Bailly, C.; Pommery, N.; Houssin, R.; Hénichart, J.-P. Design, Synthesis, DNA Binding, and Biological Activity of a Series of DNA Minor Groove-Binding Intercalating Drugs. *J. Pharm. Sci.* **1989**, *78*, 910−917.

(15) (a) Brown, D. G.; Sanderson, M. R.; Skelly, J. V.; Jenkins, T. C.; Brown, T.; Garman, E.; Stuart, D. I.; Neidle, S. Crystal Structure of a Berenil−Oligonucleotide Complex: The Role of Water in Sequence-Specific Ligand Binding. *EMBO J.* **1990**, *9*, 1329−1334. (b) Lane, A. N.; Jenkins, T. C.; Brown, T.; Neidle, S. Interaction of Berenil with the *Eco*RI Dodecamer d(CGCGAATTCGCG)$_2$ in Solution Studied by NMR. *Biochemistry* **1991**, *30*, 1372−1385. (c) Conte, M. R.; Jenkins, T. C.; Lane, A. N. Interaction of Minor Groove-Binding Diamidine Ligands with an Asymmetric DNA Duplex: NMR and Molecular Modelling Studies. *Eur. J. Biochem.* **1995**, *229*, 433−444.

(16) (a) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. The Molecular Origin of DNA−Drug Specificity in Netropsin and Distamycin. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376−1380. (b) Lown, J. W. Lexitropsins: Rational Design of DNA Sequence Reading Agents as Novel Anti-Cancer Agents and Potential Cellular Probes. *Anti-Cancer Drug Des.* **1988**, *3*, 25−40.

(17) Osland, A.; Kleppe, K. Polyamine Induced Aggregation of DNA. *Nucleic Acids Res.* **1977**, *4*, 685−695.

(18) See, for example: (a) Bailly, C.; Helbecque, N.; Hénichart, J. P.; Colson, P.; Houssier, C.; Rao, K. E.; Shea, R. G.; Lown, J. W. Molecular Recognition Between Oligopeptides and Nucleic Acids: DNA Sequence Specificity and Binding Properties of an Acridine-Linked Netropsin Hybrid Ligand. *J. Mol. Recognit.* **1990**, *3*, 26−35. (b) Bailly, C.; Hénichart, J.-P. Molecular Pharmacology of Intercalator−Groove Binder Hybrid Molecules. In *Molecular Aspects of Anticancer Drug−DNA Interactions*; Neidle, S., Waring, M., Eds.; Macmillan Press Ltd.: London, U.K., 1994; Vol. 2, pp 162−196.

(19) McConnaughie, A. W. Ph.D. Thesis, University of London, England, 1993.

(20) Dzieduszycka, M.; Martelli, S.; Borowski, E. Synthesis of N$^{\omega}$ and OH Protected 2-[(2-Aminoethyl)amino]ethanol. *Polish J. Chem.* **1995**, *69*, 632−634.

(21) Jenkins, T. C.; Parrick, J.; Porssa, M. DNA-Binding Properties of Nitroarene Oligopeptides Designed as Hypoxia-Selective Agents. *Anti-Cancer Drug Des.* **1994**, *9*, 477−493.

(22) Kelland, L. R.; Abel, G.; McKeage, M. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Murrer, B. A.; Harrap, K. R. Preclinical Antitumor Evaluation of Bis-acetato-ammine-dichlorocyclohexylamine Platinum(IV): an Orally Active Platinum Compound. *Cancer Res.* **1993**, *53*, 2581−2586.

(23) Antonini, I.; Polucci, P.; Cola, D.; Bontemps-Gracz, M.; Pescalli, N.; Menta, E.; Martelli, S. Pyrimido[4,5,6-*kl*]acridines, a New Class of Potential Anticancer Agents. Synthesis and Biological Evaluation. *Anti-Cancer Drug Des.* **1996**, *11*, 339−349.

(24) Lown, J. W.; Krowicki, K.; Balzarini, J.; Newman, R. A.; De Clercq, E. Novel Linked Antiviral and Antitumor Agents Related to Netropsin and Distamycin: Synthesis and Biological Evaluation. *J. Med. Chem.* **1989**, *32*, 2368−2375.

JM970114U