Potential DNA bis-Intercalating Agents: Synthesis and Antitumor Activity of Novel, Conformationally Restricted bis(9-Aminoacridines)

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A series of bis(9-aminoacridines) bridged by conformationally restricted tethers was synthesized and evaluated against L1210 *in vitro*. Several of these compounds were found to be highly active in this test system, with ID_{50} values below 10^{-7} *M*. CPK molecular models suggest that this antitumor activity can be correlated to the ability of these bis(9-aminoacridines) to form bis-intercalative complexes with DNA.

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Planar polycyclic aromatic molecules show a strong propensity to bind to DNA by intercalation. In recent years efforts to identify molecules with a greater affinity and selectivity for DNA have resulted in the development of bifunctional intercalating agents in which two intercalating ligands are bridged by a central linking chain. Generally, enhanced DNA binding has been observed with many molecules of this type [1-4].

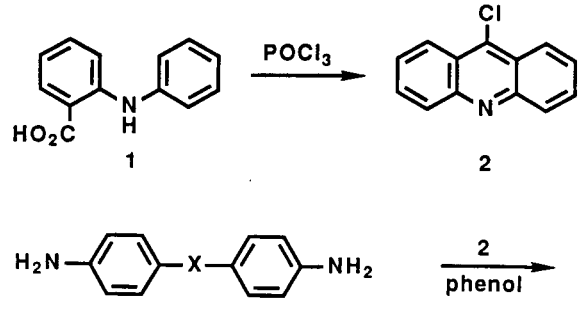
Not unexpectedly, both the chemical and physical nature of the linking moiety have been found to play a major role in the binding process. The influence of tether length and composition have been extensively studied in this regard [5-7].

The inherent flexibility of the tether is a more recent concern. bis-Intercalators bridged by flexible chains generally exhibit reduced affinities for DNA, in part because self-stacking interactions effectively compete with the binding process [8,9]. Moreover, bis-intercalation can introduce unfavorable entropic effects when a floppy tether is forced into an extended chain conformation. Indeed, bis-intercalators linked in such a fashion generally have association constants far below the theoretical maxima predicted for true bis-intercalative binding (square of the DNA mono-intercalator binding constant). This decrease in DNA binding is most pronounced for compounds tethered by simple polymethylene bridges (hydrophobic) which are incapable of associating favorably with the DNA backbone (hydrophilic) through hydrogen-bonding and other interactions [9,10,11].

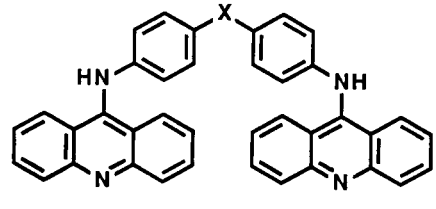
Another concern has been that flexible bis-intercalators can creep in stepwise ("inchworm") fashion along the DNA macromolecule, drastically lowering ligand residence lifetimes at any one site [11,12]. Such a process would have important biological implications as residence lifetimes have been correlated with *in vivo* antitumor activity for a large number of DNA intercalators [13].

In complete contrast to their synthetic analogues, naturally derived bis-intercalating antibiotics like echinomycin and triostin-A possess rigid tethers. These compounds function as true "molecular staples", their

Scheme I



3; X = O
4; X = CH_2
5; X = CH_2CH_2
6; X = CH=CH



7; X = O
8; X = CH_2
9; X = CH_2CH_2
10; X = CH=CH

quinoxaline ligands fixed in space with the requisite geometry to bis-intercalate DNA [14,15]. It is reasonable to assume that the activity exhibited by these compounds stems, in part, from their rigid tether framework.

In the interest of developing ligands with an enhanced affinity for DNA, we have prepared a series of bis(9-aminoacridines) bridged by conformationally restricted, diphenyl tethers (Scheme I). Corey-Pauling-Kolton (CPK) space filling models reveal that these bis(aminoacridines) are considerably more rigid than their polymethylene or polyamine tethered counterparts. Moreover, each is fully capable of spanning a requisite two-base pair distance along the DNA duplex in full accord with the principle of neighbor exclusion [16]. The 9-aminoacridine chromophore was selected for this study because of its well known propensity to intercalate DNA [17]. Herein we report the synthesis and preliminary *in vitro* L1210 activity of our conformationally restricted bis(9-aminoacridines).

Results and Discussion.

The bis(9-aminoacridines) were synthesized in two steps as outlined in Scheme I.

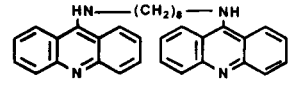
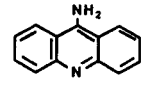
Treatment of *N*-phenylanthranilic acid **1** with refluxing phosphorus oxychloride afforded 9-chloroacridine **2** in good yield. The reaction of four molar equivalents of **2** with the appropriate diamine **3-6** in the presence of phenol gave **7-10** in 45-55% yield after flash chromatography. A stoichiometric excess of **2** was employed to drive each reaction toward completion [18]. The products were isolated as extremely hydroscopic orange or red solids and exhibited ^{1}H nmr, ir, uv-vis, and mass spectral data consistent with their proposed structures. Elemental analyses and nmr data reveal that each bis(9-aminoacridine) exists in hydrated form even after drying *in vacuo* at 100° for five days [19].

The cytotoxicity of the bis(9-aminoacridines) was evaluated by measuring the growth inhibition of L1210 murine leukemia cells in culture. ID_{50} values (calculated drug concentrations required to inhibit cell growth by 50% of control) for **7-10** are summarized in Table 1 along with those of several reference intercalating compounds. As the data indicate, **7** and **8** are potent cytotoxic agents, both having ID_{50} values below 10^{-7} *M*. In contrast, the *trans*-stilbene tethered **10** was only slightly more active than 9-aminoacridine.

We believe that these results are consistent with a DNA bis-intercalative binding process. CPK models reveal that, of the four bis(9-aminoacridines) in Scheme I, **7** and **8** are most capable of intimate association with the B-form of DNA. Complex formation is facilitated by the presence of oxygen and methylene groups in their linking moieties. These groups serve as pivot points, allowing the conformationally restricted bis(9-aminoacridines) to bend *and* twist along the helical contours of DNA. Similar findings have been reported recently by Cory [20] for a diphenyl ether bridged bis-phenanthridine. This compound was found to bis-intercalate DNA much more efficiently than a related polymethylene-linked analogue.

TABLE 1

In Vitro L1210 Cell Growth Inhibition

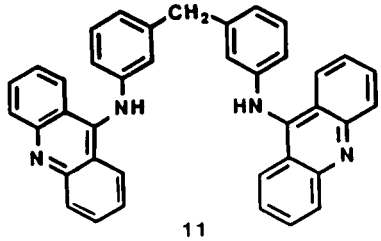
Compound	ID_{50} (μM)
7	0.090
8	0.081
9	0.42
10	1.0
11	0.27
12	0.60
	0.40
	2.3

Our models also show that the CH_2CH_2 unit in **9** functions less effectively as a pivot point. This pivoting or hinging action is further hindered in **10**. Indeed, for **10**, CPK models indicate that simultaneous intercalation of both acridine ligands can only occur if considerable strain is imparted to DNA. These observations are consistent with the L1210 data in Table 1.

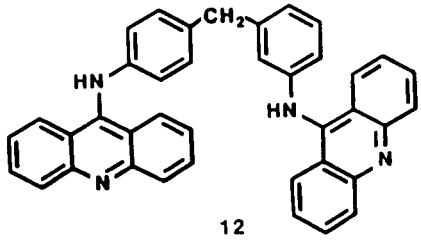
Additional evidence in support of our hypothesis can be gained by comparing the L1210 activity of **8** with the activities of the isomeric bis(9-aminoacridines) **11** and **12**.

Compounds **11** and **12** were synthesized from 3,3′- and 3,4′-diaminodiphenylmethane, respectively, in a manner analogous to the preparation of compound **8**. Each would be expected to share similar physical properties (aqueous solubility, membrane lypophilicity) with its more linear, 4,4′-isomer. Yet, as the data in Table 1 indicate, **11** and **12** are only moderately active in the L1210 screen, both con-

Scheme II



11



12

siderably less potent than **8**. Analysis of CPK models shows that the relative ability to bis-intercalate DNA follows the order **8** > > **11** > **12**. Again, this is consistent with the cell culture results and strongly suggests that DNA is serving as a substrate or target for our bis(9-aminoacridines).

Recent preliminary studies (unpublished) in our laboratory have shown that the dicyclohexyl-tethered analogue of **8** is also highly effective in the L1210 screen, with an ID_{50} value near 10^{-7} *M*. Apparently, it is the rigid tether framework and not the aromatic character of the linking chain that is responsible for the cytotoxic action of these compounds.

We are presently exploring the extent and mode(s) of DNA binding for **7-12** and other closely related bis(9-aminoacridines). The results of these studies will be communicated shortly.

EXPERIMENTAL

Melting points were determined in open capillary tubes with a Laboratory Devices Mel-Temp unit and are uncorrected. The ^{1}H nmr (300 MHz) spectra were obtained on a Varian XL300 spectrometer at room temperature. The ftir spectra were recorded with a Bio-Rad Digilab FTS-40 spectrometer. The uv-vis absorbance spectra were obtained at room temperature on a Cary 219 double beam spectrophotometer. Mass spectra (ei) were measured on a Finnegan 4023 gc/ms system typically at 70 eV. Mass spectra (fab) were provided by the NIH Regional Facility at the Massachusetts Institute of Technology, Cambridge, MA. Elemental analyses were performed by Atlantic Microlab Inc. Flash chromatography was carried out with silica gel (Kieselgel 60, 230-400 mesh). Commercially available reagents were obtained from Aldrich Chemical Company, Milwaukee, WI and Fluka Chemical Corporation, Ronkonkoma, NY.

9-Chloroacridine (**2**).

A magnetically stirred solution of *N*-phenylanthranilic acid **1** (5.45 g, 0.026 mole) in phosphorus oxychloride (100 ml, 1.18 moles) was treated with concentrated sulfuric acid (0.5 ml), then brought to reflux for 12 hours. The solution was cooled to room temperature, added slowly to cracked ice (650 g), and made slightly alkaline with concentrated ammonium hydroxide. A light tan solid was collected by filtration, washed with water, and air dried for 24 hours. Flash chromatography (silica gel/75% hexane, 20% THF, 5% triethylamine) afforded 4.67 g of **2** (84% yield) as fine yellow needles, mp 117-119° (lit mp 119-120° [21]); ^{1}H nmr (deuteriochloroform): δ 8.50-8.10 (m, 4H), 7.95-7.50 (m, 4H); ftir (THF): 3075, 1622, 1562, 1438, 1404, 1324, 1287, 1014, 831, 767, 647, 608 cm^{-1}; uv-vis (chloroform): λ max 360.5 nm (ε = 10,000); ms: (ei) m/e (relative intensity) 213 (100, M$^+$), 178 (21), 177 (16).

Anal. Calcd. for $C_{13}H_8ClN$: C, 73.07; H, 3.77; N, 6.55; Cl, 16.59. Found: C, 72.99; H, 3.80; N, 6.55; Cl, 16.58.

N,N'-(Oxydi-4,1-phenylene)bis[9-acridinamine] (**7**).

A magnetically stirred solution of **2** (0.51 g, 2.39 mmoles) and 4,4'-diaminodiphenyl ether **3** (0.12 g, 0.59 mmole) was warmed at 85° in phenol (20 g, 0.21 mole) for 4 hours. After being cooled to room temperature, the red solution was treated with 1.0 *N* sodium hydroxide (100 ml) and extracted with chloroform (3 x 100 ml). The chloroform extract was washed with water (5 x 100 ml) and brine (1 x 75 ml), then dried over sodium sulfate. The solution was concentrated *in vacuo* to afford after flash chromatography (silica gel/95% ethyl ether, 5% triethyl amine) a bright orange, hydroscopic solid. The solid was recrystallized from THF/hexane and dried *in vacuo* at 100° for 5 days to give 0.16 g (49%) of **7** as an orange powder; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 8.50-7.95 (m, 12 H), 7.80-7.25 (m, 12 H); ftir (THF): 3302, 1633, 1607, 1536, 1507, 1423, 1360, 1240, 1165, 797, 765 cm^{-1}; uv-vis (chloroform): λ max 411 nm (ε = 16,600); ms: (fab) m/e 555 (MH$^+$).

Anal. Calcd. for $C_{38}H_{26}N_4O \cdot 3/4\ H_2O$: C, 80.33; H, 4.89; N, 9.86. Found: C, 80.38; H, 5.30; N, 10.10.

N,N'-(Methylenedi-4,1-phenylene)bis[9-acridinamine] (**8**).

Compound **8** was prepared from the reaction of **2** with 4,4'-diaminodiphenylmethane **4** in a manner similar to the synthesis of **7**. Compound **8** was obtained in 56% yield as an orange powder, ^{1}H nmr (deuteriotrifluoroacetic acid): δ 8.50-7.95 (m, 12 H), 7.80-7.30 (m, 12 H), 3.75 (s, 2 H); ftir (THF): 3301, 1635, 1602, 1536, 1430, 1366, 1161, 1080, 794, 769 cm^{-1} uv-vis (chloroform): λ max 412 nm (ε = 16,600); ms: (fab) m/e 553 (MH$^+$).

Anal. Calcd. for $C_{39}H_{28}N_4 \cdot 3/4\ H_2O$: C, 82.73; H, 5.26; N, 9.89. Found: C, 82.62; H, 5.57; N, 10.12.

N,N'-(1,2-Ethylenedi-4,1-phenylene)bis[9-acridinamine] (**9**).

Compound **9** was prepared from the reaction of **2** with 4,4'-ethylenedianiline **5** in a manner similar to the synthesis of **7**. Compound **9** was obtained in 51% yield as an orange powder; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 8.45-7.90 (m, 12 H), 7.75-7.25 (m, 12 H), 3.15 (s, 4 H); ftir (THF): 3300, 1635, 1601, 1540, 1429, 1365, 1155, 796, 764 cm^{-1}; uv-vis (chloroform): λ max 412 nm (ε = 16,600); ms: (fab) m/e 567 (MH$^+$).

Anal. Calcd. for $C_{40}H_{30}N_4 \cdot 1/2\ H_2O$: C, 83.45; H, 5.43; N, 9.73. Found: C, 83.33; H, 5.79; N, 9.74.

N,N'-(1,2-Ethenediyldi-4,1-phenylene)bis[9-acridinamine] (**10**).

Compound **10** was prepared from the reaction of **2** with *trans*-4,4'-diaminostilbene **6** in a manner similar to the synthesis of **7**. Compound **10** was obtained in 45% yield as a dark red powder; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 8.51-7.95 (m, 12 H), 7.85-7.33 (m, 14 H); ftir (THF): 3297, 1633, 1587, 1537, 1524, 1491, 1428, 1364, 1272, 1160, 975, 766 cm^{-1}; uv-vis (chloroform): λ max 441.5 nm (ε = 26,000): ms: (fab) m/e 565 (MH$^+$).

Anal. Calcd. for $C_{40}H_{28}N_4 \cdot 1/4\ H_2O$: C, 84.41; H, 5.05, N, 9.84. Found: C, 84.49; H, 5.22; N, 9.80.

N,N'-(Methylenedi-3,1-phenylene)bis[9-Acridinamine] (**11**).

Compound **11** was prepared from the reaction of **2** with 3,3'-diaminodiphenylmethane in a manner similar to the synthesis of **7**. Compound **11** was obtained in 60% yield as an orange powder; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 7.50-7.10 (m, 12 H), 6.90-6.40 (m, 12 H), 3.31 (s, 2 H); ftir (THF): 3300, 1635, 1600, 1534, 1430, 1368, 1161, 1090, 795, 760 cm^{-1}; uv-vis (chloroform): λ max 405 nm (ε = 16,100); ms: (fab) m/e 553 (MH^+).

Anal. Calcd. for $C_{39}H_{28}N_4 \cdot 3/4\ H_2O$: C, 82.73; H, 5.26; N, 9.89. Found: C, 82.46; H, 5.71; N, 10.19.

N,N'-(Methylene-3,1-phenylene-4,1-phenylene)bis[9-Acridinamine] (**12**).

Compound **12** was prepared from the reaction of **2** with 3,4'-diaminodiphenylmethane in a manner similar to the synthesis of **7**. Compound **12** was obtained in 53% yield as an orange powder; ^{1}H nmr (deuteriotrifluoroacetic acid): δ 8.35-7.88 (m, 12 H), 7.70-7.20 (m, 12 H), 4.20 (s, 2 H); ftir (THF): 3300, 1630, 1600, 1532, 1431, 1367, 1160, 1090, 796, 760 cm^{-1}; uv-vis (chloroform): λ max 409 nm (ε = 15,700); ms: (fab) m/e 553 (MH^+).

Anal. Calcd. for $C_{39}H_{28}N_4 \cdot 1/2\ H_2O$: C, 83.39; H, 5.20; N, 9.97. Found: C, 83.39; H, 5.48; N, 9.71.

Biological Studies. *In Vitro* Cytotoxicity Evaluation.

L1210 murine leukemia cells were routinely maintained as suspension cultures in McCoy's 5A medium supplemented with 10% horse serum, glutamine, penicillin, and streptomycin and grown in a humidified environment of 10% carbon dioxide and 90% air at 37°. To assess the *in vitro* toxicity, each compound was dissolved in dimethyl sulfoxide (DMSO) and 40 μg was added to 4 ml of L1210 cells (10^5 cells/tube) to attain final drug concentrations of 0.01, 0.1, and 10 μg/ml of culture. After 72 hours of continuous exposure to the drug, the cell concentration was determined with a Coulter counter (Model ZBF, Hialeah, FL). Growth inhibition was calculated for each drug concentration using the following formula:

$$\% \text{ growth inhibition} = 1 - \frac{\text{cell no. treated}}{\text{cell no. DMSO alone}} \times 100\%$$

The growth inhibition data were then used to calculate the ID_{50} value (the calculated drug concentration required to inhibit cell growth by 50% of control).

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[19] Other bifunctional derivatives have also been reported to exist in a hydrated form. See, for example, K. F. Kuhlmann, N. J. Charbeneau, and C. W. Mosher, *Nuc. Acids Res.*, **5**, 2629 (1978) and ref [20].

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