Synthesis and Antitumor Activities of Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones and Related Systems

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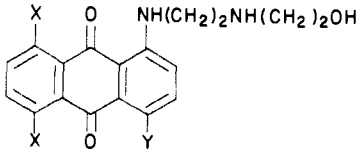
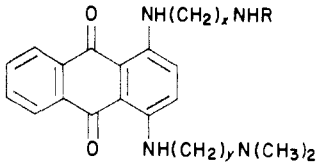
A number of unsymmetrically substituted 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones have been synthesized and evaluated for their antitumor activity against L1210 in vitro and in vivo. The high activity of several compounds observed in vitro was not paralleled by comparable activity in vivo. The activities of the substituted 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones as inhibitors of cell growth were generally much higher than those of the related 1-[(aminoalkyl)amino]-4-methoxyanthracene-9,10-diones, and this correlated with the relative abilities of compounds of the two types to interact with calf thymus DNA.

The synthesis and antineoplastic evaluation of a number of symmetrically substituted 1,4-bis[(aminoalkyl)amino]-anthracene-9,10-diones have been recently reported.[1] Compounds **1a** (ametantrone, AQ) and **1b** (mitoxantrone, DHAD, DHAQ) have shown outstanding antineoplastic activities, and both are currently undergoing clinical evaluations.[2]

Several unsymmetrically substituted preclinical analogues that hold a "mitoxantrone side-arm" at position 1 and a hydroxyl (**1c**)[1d,e,3] or amino substituent at position 4 (**1d**)[4] showed high antitumor activity in mice. Other highly active analogues of 1 with unsymmetrical 1,4-disubstitution and two anthraquinone units have been described.[5]



1a, X = H, Y = $NH(CH_2)_2NH(CH_2)_2OH$
b, X = OH, Y = $NH(CH_2)_2NH(CH_2)_2OH$
c, X = H, Y = OH
d, X = OH, Y = NH_2



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During the past year, we have reported a route that is adaptable to the synthesis of symmetrically or unsymmetrically substituted 1,4-bis[(aminoalkyl)amino]-anthracene-9,10-diones.[6] In this paper we report the antineoplastic activities of unsymmetrical compounds related to **2** and several other similar systems.

Mitoxantrone (**1b**) is a broad-spectrum oncolytic agent whose activity is similar to that of the anthracycline antibiotic doxorubicin. Preliminary studies suggested that DHAQ was less cardiotoxic than doxorubicin.[7] Preclinical screening in dogs did not demonstrate cardiac problems.[8] Some clinical cardiotoxicity with mitoxantrone has recently been reported, mostly in patients treated earlier with doxorubicin.[9]

Compound **1a** is reported to be 10-fold less cardiotoxic than **1b** in a rat cardiotoxicity model system.[10] However, since **1b** exhibits a greater antitumor potency (10-fold) than **1a**,[1f,g] the cardiotoxicities would probably be comparable at the optimal antitumor dosages for these compounds. A delayed lethality was exhibited when **1b** was administered intraperitoneally to non-tumor-bearing mice. Since **1a** did not exhibit this delayed toxicity, the presence of the 5,8-dihydroxy substitution in **1b** is implicated in the delayed deaths.[11] The myocardial effects of **1b** and doxorubicin have been found to be similar in the mouse and guinea pig.[12] This report describes studies of unsymmetrically substituted 1,4-bis[(aminoalkyl)amino]-anthracene-9,10-diones related to **2** that have been synthesized to provide guidance in the design of new drugs

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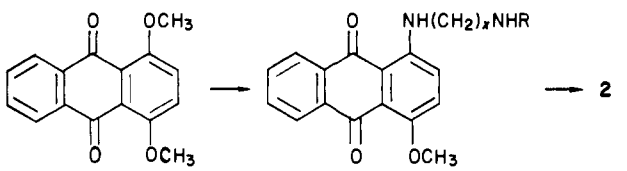
Table I. Activities of Analogues Related to 4 against L1210 Cells in Vitro

compd	x	R	ID_{50} (L1210), μg/mL
4a	2	H	2.5
4b	3	H	2.8
4c	4	H	1.8
4d	2	$COCH_3$	>10
4e	3	$COCH_3$	>10
4f	4	$COCH_3$	>10
4g	4	CO_2CH_2Ph	>10
4h	5	$COCH_3$	9.0

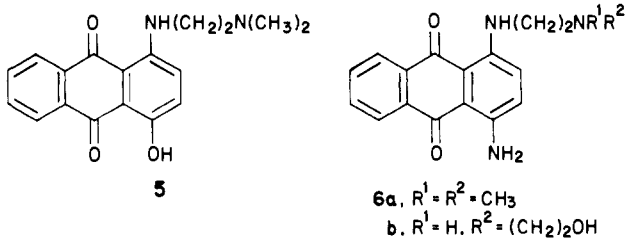
with good antitumor efficacy and reduced cardiotoxic potential.

Chemistry. Most of the compounds for biological evaluation were synthesized by the procedure recently published by us.[6] Irradiation of a methylene chloride solution of **3** in the presence of an α,ω-diamine leads to compounds of general structure **4** in good yields. In some cases purification of the amine **4** (R = H) was difficult, and treatment of the base with acetic anhydride or carbobenzoxy chloride led to the acetamido derivative **4** (R = $COCH_3$) or the carbobenzyloxy derivative **4** (R = CO_2CH_2Ph), respectively.



Difficulty was encountered in the deacylation of several acetamido derivatives **4** (R = $COCH_3$) as acidic hydrolysis also resulted in partial hydrolysis of the vinylogous amide functionality at position 1. The carbobenzoxy group could be removed in a reasonable yield from **4** (R = CO_2CH_2Ph) by treatment with Pd on C in cyclohexene to liberate the free amine.

The monohydroxy (aminoalkyl)aminoanthracene-9,10-dione **5** was prepared by reaction of quinizarin (1,4-dihydroxyanthracene-9,10-dione with 2-(dimethylamino)-ethylamine.[1d,e]



The amino-substituted analogues **6a** and **6b** were prepared by heating solutions of 1-amino-4-methoxyanthracene-9,10-dione in *o*-dichlorobenzene with excess 2-(dimethylamino)ethylamine or 2-[(2-aminoethyl)-amino]ethanol, respectively.

Biological Activities and Discussion. The analogues related to **4** were evaluated as inhibitors of the growth of L1210 cells in vitro, and the results of these studies are summarized in Table I. The activities observed for these compounds in vitro were generally quite low, and none of the compounds were deemed of sufficient potency to warrant in vivo evaluation.

The mechanism of action of **1a** and **1b** has not been clearly defined. Intercalative interaction with DNA has been proposed as a common mechanism for comparable antineoplastic drugs,[13] but other effects, such as those exerted at the level of the cell membrane,[14] may play an important role in the cytotoxic action of these drugs. It is pertinent to note that recent investigations have demonstrated that alterations in supercoiling of DNA, characteristic of intercalation, have not been observed with **1b**, even though this drug binds to DNA.[15] Except for compound **4a**, none of the compounds in Table I appeared to interact significantly with calf thymus DNA as determined by perturbation of the visible spectrum (bathochromic shift of the long-wavelength maximum) in the 600-nm range. Compounds **5**, **6a**, and **6b** exhibit ID_{50} values against L1210 leukemia cells in vitro of 0.68, 1.4, and 1.8 μg/mL, respectively. The higher in vitro activity of compound **5** compared with compounds bearing methoxy or amino substituents at position 4 is consistent with previous results.[1d,e,3,4]

Table II. Activities of **2** against L1210 in Vitro

compd	x	y	R	ID_{50} (L1210), μg/mL
2a	2	2	$COCH_3$	2.05
2b	3	2	$COCH_3$	0.40
2c	4	2	$COCH_3$	0.03
2d	5	2	$COCH_3$	2.3
2e	4	2	CO_2CH_2Ph	0.88
2f	3	2	H	0.05
2g	4	2	H	0.0045
2h	4	3	CO_2CH_2Ph	0.29
2i	4	3	H	0.18

The growth inhibitory activites of unsymmetrical 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones **2** are summarized in Table II. We found a striking increase in cytotoxicity in the series of compounds with a 2-(dimethylamino)ethylamine function at position 4, as the number of methylene groups in the side chain bearing the acetamido group increased from two to four (compounds **2a–2c** in Table II). An abrupt decrease (nearly 2 orders of magnitude) in cytotoxicity was observed when the number of methylene groups was increased from four to five (compound **2c** compared with **2d**).

Compounds **2f** and **2g** (free amines) show a 10-fold increase in activity in comparison to **2b** and **2c** (acetylated analogues). Compound **2g** is somewhat more active than a comparable symmetrical analogue, 1,4-bis[(aminoethyl)amino]anthracene-9,10-dione, which has an ID_{50} of about 0.01 μg/mL against L1210. It is important to note that the number of methylene groups in the side chain bearing the tertiary nitrogen functionality is also a critical determinant of cytotoxicity as evidenced by the large decrease in inhibitory activity found in the comparison of **2g** and **2i**, which differ by one methylene unit. The replacement of an acetamido function (**2c**) by a carbobenzyloxy function (**2e**) results in a 30-fold increase in cytotoxic activity. On the other hand, the free amine **2i** shows comparable cytotoxicity in comparison with **2h** (carbobenzyloxy analogue).

All of the compounds in Table II were capable of rapid interaction with calf thymus DNA as measured by essentially instantaneous perturbation of the visible absorption spectrum by addition of DNA. For selected compounds the ability to interact with DNA was also assessed by use of a method which involves fluorometric measurement of the displacement of ethidium bromide from ethidium

(13) Reference 1a, p 104.
(14) Tritton, T. R.; Yee, G.; Wingard, L. B., Jr. *Fed. Proc.* 1983, *42*, 284.
(15) Bowden, G. T.; Roberts, R.; Alberts, D. S.; Peng, Y.-M.; Garcia D. *Cancer Res.* 1985, *45*, 4915.

bromide–DNA complexes by a given compound. It is of interest that a relatively potent compound in terms of cytotoxicity, such as **2f** showed an ability to displace ethidium bromide that was similar to that observed for **1b**. Both **1b** and **2f** required concentrations of approximately 1×10^{-7} M to displace 50% of ethidium bromide from an ethidium bromide–DNA complex. It should also be noted that compounds with lower cytotoxic potential (such as **2a**) required much higher concentrations (at least 10-fold) to produce equivalent displacement of ethidium bromide as observed with compounds such as **2f**.

Several of the compounds listed in Table II were selected for evaluations as inhibitors of the growth of L1210 inoculated mice. No significant antineoplastic activity was observed in the dosage range used (25–100 mg/kg) for **2a**, **2b**, **2d**, **2e**, **2g**, and **2h**. To ascertain whether the poor in vivo activity was associated with poor solubility of parent compounds, the dihydrochloride salt of compound **2g** was prepared. This salt, which was administered on a schedule (days 1, 5, and 9 following tumor inoculation) that produces excellent activity for **1b**, gave an insignificant (10%) prolongation of lifespan. The data obtained in vivo for compound **2g** are particularly disappointing because this particular compound is so active in suppressing cell growth in vitro. We plan to carry out exploratory pharmacokinetic and pharmacodynamic studies in an effort to try to understand why there is such a large difference between the in vitro and in vivo activities of **2g** and related analogues, since understanding the basis for such differences may permit the more rational design of new drugs of this important class.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were run on a Bruker WM-250 pulsed Fourier transform NMR spectrometer. TLC precoated silica gel plates (Eastman chromagram sheets with fluorescent indicator) were used to monitor reactions. For column chromatography Baker analyzed 80–200-mesh silica gel was utilized. Microanalyses were performed by Robertson Laboratories, Florham Park, NJ. Mass spectra were run on a Finnigan MAT 4610 mass spectrometer.

Synthesis. The experimental procedures for the preparation of those compounds that have not previously been reported in ref 6 will be detailed here.

1-[(4-Aminobutyl)amino]-4-methoxyanthracene-9,10-dione (4c). 1,4-Diaminobutane (1.65 g, 3.7 mmol) was added to a solution of 1,4-dimethoxyanthracene-9,10-dione (1.0 g, 3.7 mmol) in CH_2Cl_2 (700 mL). After 7 h of irradiation, the solvent was removed and the residue chromatographed on silica gel. The starting material was eluted with $CHCl_3$ and the product with 5% CH_3OH in $CHCl_3$. A purple solid (0.61 g, 50%) was obtained which was converted to the benzyloxycarbonyl derivative.

1-[[4-[(Benzyloxy)carbamoyl]butyl]amino]-4-methoxyanthracene-9,10-dione (4g). To a stirred solution of 1-[(4-aminobutyl)amino]-4-methoxyanthracene-9,10-dione (0.68 g, 2.1 mmol) and triethylamine (0.23 g, 2.3 mmol) in $CHCl_3$ (30 mL) was added, in one portion, benzyl chloroformate (0.393 g, 2.3 mmol). The reaction mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure, and the residue was chromatographed (silica gel, $CHCl_3$) to yield 0.82 g (85%) of a dark purple solid. Recrystallization from toluene gave purple needles melting at 147–149 °C. An ^{1}H NMR ($CDCl_3$) δ 9.80 (br t, 1 H), 8.15 (m, 2 H), 7.62 (m, 2 H), 7.16–7.35 (m, 7 H), 5.19 (br t, 1 H), 5.09 (s, 2 H), 3.90 (s, 3 H), 3.21 (m, 4 H); MS, m/e (relative intensity) 81.2 (100.0), 85.0 (73.0). Anal. Calcd for $C_{27}H_{26}N_2O_5$: C, 70.72; H, 5.71; N, 6.10. Found: C, 70.74; H, 5.93; N, 6.01.

1-[[4-[(Benzyloxy)carbamoyl]butyl]amino]-4-[[2-(dimethylamino)ethyl]amino]anthracene-9,10-dione (2e). Compound **4g** (0.28 g, 0.62 mmol) and *N,N*-dimethylethylenediamine (3 mL) were stirred under reflux for 5 h. The hot reaction mixture was poured into saturated brine. The resultant blue gummy material was collected by filtration and chromatographed (silica gel, 2% CH_3OH in $CHCl_3$) to give 0.17 g (53%) of a blue solid, which was crystallized from toluene to give blue needles; mp 146.5–146.8 °C; ^{1}H NMR ($CDCl_3$) δ 10.72 (m, 2 H), 8.31 (m, 2 H), 7.65 (m, 2 H), 7.38–7.24 (m, 5 H), 7.13 (m, 2 H), 5.10 (s, 2 H), 5.06 (br s, 1 H), 3.51–3.19 (m, 6 H), 2.66 (t, 2 H), 2.33 (s, 6 H), 1.81–1.61 (m, 4 H); MS, m/e (relative intensity) 514.2 (27, M^+), 58.1 (100). Anal. Calcd for $C_{30}H_{34}N_4O_4$: C, 70.01; H, 6.65; N, 10.88. Found: C, 70.23; H, 6.63; N, 10.69.

1-[(4-Aminobutyl)amino]-4-[[2-(dimethylamino)ethyl]amino]anthracene-9,10-dione (2g). Palladium on carbon (10%, 100 mg) is added to a solution of **2e** (0.0500 g), toluene (4 mL), ethanol (4 mL), and cyclohexene (2 mL). The resultant mixture was stirred under reflux for 1 h. The catalyst was filtered from the hot solution. Concentration under vacuum gave 0.0156 g (37%) as an amorphous blue solid: mp 88–90 °C; ^{1}H NMR ($CDCl_3$) δ 10.80 (br s, 2 H), 8.35 (m, 2 H), 7.69 (m, 2 H), 7.26 (s, 2 H), 3.48 (m, 4 H), 2.80 (br s, 2 H), 2.69 (t, 2 H), 2.36 (s, 6 H), 1.83 (m, 4 H), 1.68 (m, 2 H); CI-MS, m/e (relative intensity) 380 (94, M^+), 58 (100). Analyzed as the benzyl carbamate.

1-[[4-[(Benzyloxy)carbamoyl]butyl]amino]-4-[[3-(dimethylamino)propyl]amino]anthracene-9,10-dione (2h). Compound **4g** (0.77 g, 1.7 mmol) was stirred under reflux with 3-(dimethylamino)propylamine (8 mL) for 4 h. The hot reaction mixture was poured into saturated brine (75 mL). The brine was extracted thoroughly with methylene chloride. The dried extracts afforded, upon evaporating, a blue solid, which was chromatographed (silica gel, $CHCl_3$, then 5% CH_3OH in $CHCl_3$) to give **2h** as a blue solid (0.54 g, 60%). An analytical sample was prepared by two crystallizations from toluene/heptane. The product is obtained as blue needles: mp 114 °C; ^{1}H NMR ($CDCl_3$) δ 10.81 (br t, 2 H), 8.32 (m, 2 H), 7.68 (m, 2 H), 7.40–7.10 (m, 7 H), 5.10 (s, 2 H), 4.83 (br s, 1 H), 3.45 (m, 4 H), 3.29 (q, 2 H), 2.45 (t, 2 H), 2.28 (s, 6 H), 1.92 (m, 2 H), 1.71 (m, 6 H); MS, m/e (relative intensity) 528.4 (1.78, M^+), 58.0 (100). Anal. Calcd for $C_{31}H_{36}N_4O_4$: C, 70.43; H, 6.86; N, 10.60. Found: C, 70.42; H, 6.78; N, 10.40.

1-[(4-Aminobutyl)amino]-4-[[3-(dimethylamino)propyl]amino]anthracene-9,10-dione (2i). Compound **2h** (100 mg, 0.19 mmol) and 10% Pd–C (200 mg) were heated with stirring in toluene (8 mL)8, ethanol (8 mL), and cyclohexene (4 mL) for 45 m. At this time, the reaction mixture was filtered hot and the filtrate concentrated under vacuum to give **2i** (20 mg, 27%) as a blue solid: mp 139–140 °C; ^{1}H NMR ($CDCl_3$) δ 10.76 (br s, 2 H), 8.28 (m, 2 H), 7.69 (m, 2 H), 7.29 (m, 2 H), 3.41 (m, 6 H), 2.44 (t, 2 H), 2.22 (s, 6 H), 1.52–2.08 (m, 8 H); MS m/e (relative intensity) 394 (3.9, M^+), 84 (3.9), 72 (5.6), 70 (4.5), 58 (100).

1-[[2-(Dimethylamino)ethyl]amino]-4-aminoanthracene-9,10-dione (6a). A solution of 1-amino-4-methoxyanthraquinone (1.0 g, 0.0040 mol) in 2-(dimethylamino)ethylamine (8.0 mL, 0.729 mol) was heated with stirring for 6 h at 75 °C. The reaction mixture was cooled and poured into an aqueous brine solution. The precipitate was filtered and dried in vacuo to afford a dark solid (1.09 g). Column chromatography (silica gel, CH_2Cl_2) of 1.0 g of crude product afforded a first fraction that was identified as 1-amino-4-methoxyanthraquinone by comparative TLC (0.14 g, 15%). The major blue zone was eluted first with $CHCl_3$, to remove any minor components, then methanol. A center cut was collected, and, after removal of the solvent, a blue crystalline solid remained (0.54 g, 47%). Recrystallization from petroleum ether (high boiling) afforded an analytical sample: mp 135–136 °C; ^{1}H NMR ($CDCl_3$) δ 10.67 (br t, 1 H), 8.30–8.38 (m, 2 H), 7.67–7.74 (m, 2 H), 7.16 (d, 1 H), 7.11 (br s, 2 H), 7.00 (d, 1 H), 3.45–3.53 (m, 2 H), 2.67 (t, 2 H), 2.35 (s, 6 H). An NMR was run with D_2O, and the peaks at 10.67 and 7.11 disappear leaving two clear doublets at 7.16 and 7.00 ppm: MS, m/e (relative intensity) 310 (1.5), 309 (6.9, M^+), 58 (100). Anal. Calcd for $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58. Found: C, 69.96; H, 6.32; N, 13.05.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-aminoanthracene-9,10-dione (6b). A solution of 1-amino-4-methoxyanthraquinone (1.50 g, 0.0059 mol) and 2-[(2-aminoethyl)amino]ethanol (3.0 mL, 0.0297 mol) was refluxed with stirring for 6 h in *o*-dichlorobenzene (25 mL). The reaction mixture was cooled and introduced directly onto a column of silica gel made up with CCl_4. The column was eluted initially with CCl_4, then changed to CH_2Cl_2 to elute off the solvent and excess amine. Eluting with methanol produced a minor fraction identified as

1-amino-4-methoxyanthraquinone by comparative TLC. The major dark-blue band was collected, and, after removal of the solvent, a blue crystalline solid remained, 0.78 g, 40% yield. Recrystallization from acetonitrile afforded an analytical sample: mp 174–176 °C; ^{1}H NMR ($CDCl_3$) δ 10.88 (br t, 1 H), 8.30–8.37 (m, 2 H), 7.67–7.74 (m, 2 H), 7.14 (d, 1 H), 7.12 (br s, 2 H), 6.98 (d, 1 H), 3.67–3.72 (m, 2 H), 3.47–3.55 (m, 2 H), 3.03 (t, 2 H), 2.87–2.91 (m, 2 H). An NMR was run with D_2O, and the peaks at 10.88 and 7.12 disappear leaving two clear doublets at 7.14 and 6.98 ppm, and the multiplet structures upfield all became triplets (2.87–3972 ppm): MS, *m/e* (relative intensity) 325 (8.4, M^+), 252 (100), 251 (35.2). Anal. Calcd for $C_{18}H_{19}N_3O_3$: C, 66.47; H, 5.89; N, 12.91. Found: C, 66.49; H, 6.02; N, 12.84.

Biological Studies. In Vitro Cytotoxicity Evaluation. L1210 murine leukemia cells are 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% CO_2 and 90% air at 37 °C. To assess the in vitro toxicity, each compound was dissolved in dimethyl sulfoxide (Me_2SO), and 40 μL 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 7 h 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:

$$\% \text{ growth inhibn} = \left[1 - \frac{\text{cell no. treated}}{\text{cell no. } Me_2SO \text{ alone}}\right] \times 100$$

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).

In Vivo Efficacy Studies. L1210 murine leukemia cells are maintained in vivo by intraperitoneal injections of 10^6 cells to BFD_1 mice on a weekly basis. For test purposes, mice were inoculated ip with 10^6 cells, and drugs were administered as a single ip injection approximately 24 h later or as three ip injections on days 1, 5, and 9 following inoculation. Prior to injection, the drug suspended in 3% hydroxypropyl cellulose solution at a concentration such that 0.1 mL/10 g of body weight delivered the desired dose. Mice were observed daily for signs of toxicity and survival. When all mice had died the mean survival time (MST) for each treatment group (6 mice/group) was calculated and the percent T/C determined by using the following formula:

$$\% \text{ T/C} = \left[\frac{\text{MST treated}}{\text{MST control}}\right] \times 100$$

DNA Binding Studies. Studies of the intercalation of selected compounds with calf thymus DNA were done in two ways, essentially as described by Double and Brown.[16] The first approach involves examination of the perturbation of visible spectra at 37 °C and pH 7.0 (phosphate buffer) by addition of successive increments of DNA to the drug in solution. The second approach involves the measurement of the decrease in fluorescence of ethidium bromide–DNA complexes that results when a drug competes with ethidium bromide for DNA binding sites or otherwise alters the affinity of DNA for ethidium bromide. The positive control for these studies is compound **1b**, which causes 50% displacement of ethidium bromide in the range of 0.5 to 1 $\times 10^{-7}$ M.[17]

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Registry No. **2a**, 93184-45-3; **2b**, 93184-47-5; **2c**, 93184-48-6; **2d**, 93184-49-7; **2e**, 102650-21-5; **2f**, 93184-46-4; **2g**, 102650-22-6; **2h**, 102650-23-7; **2i**, 102682-10-0; **3**, 6119-74-0; **4a**, 93184-51-1; **4b**, 93184-52-2; **4c**, 102650-24-8; **4d**, 93184-53-3; **4e**, 93184-54-4; **4f**, 93184-55-5; **4g**, 102650-25-9; **4h**, 93184-56-6; **6a**, 102650-26-0; **6b**, 102650-27-1; $H_2N(CH_2)_2NH_2$, 107-15-3; $H_2N(CH_2)_3NH_2$, 109-76-2; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_5NH_2$, 462-94-2; $H_2N(CH_2)_2NMe_2$, 108-00-9; $H_2N(CH_2)_3NMe_2$, 109-55-7; $HO(CH_2)_2NH(CH_2)_2NH_2$, 111-41-1; 1-amino-4-methoxyanthraquinone, 116-83-6.

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