Synthesis and Antitumor Activity of 1,8-Diaminoanthraquinone Derivatives

Hsu-Shan HUANG,*[,a] Hui-Fen CHIU,[b] Wei-Chih LU,[c] and Chun-Lung YUAN[a]

[a] *School of Pharmacy, National Defense Medical Center; No. 161, Minquan E. Rd., Neihu, Taipei 11490, Taiwan, R.O.C.:* [b] *Department of Pharmacology, Kaohsiung Medical University; Kaohsiung: and* [c] *Cheng-Hsin Medical Center; Taipei, Taiwan, R.O.C.* Received April 6, 2005; accepted June 17, 2005

Continuing our ongoing studies on cytotoxic substances, a series of regioisomeric disubstituted aminoanthraquinone (DAAQ) derivatives have been synthesized as cytotoxic activity based on a proposed bioactive amino conformation. To assess the biological activity of amino-substitution in the side-chains of anthraquinone located at positions 1 and 8 of the anthraquinone ring system. The aim of the study was to determine if members of the anthraquinone family could be used as adjuncts to increase the growth inhibiting effect of anticancer agents in rat glioma C6 cells, human hepatoma G2 cells and 2.2.15 cells. *In vitro* cytotoxicity data is reported for the compounds and some indications of structure–activity relationships have been discerned. A number of compounds were found to have good cytotoxicity against proliferation in these three cell lines. This has led to the discovery some of the DAAQ as a conformationally constrained structure possessing anticancer properties that displays cytotoxicity for these above cell lines and is being investigated further.

Key words aminoanthraquinone; anthraquinone; cytotoxicity; rat glioma C6 cell; human hepatoma G2 cell; 2.2.15 cell

The aminoalkyl-functionalized anthraquinone series of synthetic compounds has been the subject of much study in the quest for more active and less toxic analogs of the anthracycline antitumor antibiotics, daunomycin and adriamycin.[1—4] In an attempt to increase therapeutic effectiveness and reduce toxicity by the anticancer drugs anthracyclines and anthraquinone during chemotherapy, we have developed a series of regioisomeric disubstituted aminoanthraquinone (DAAQ) derivatives by modifying the chromophore and the side arms on their anthraquinone skeleton.[5—11] The quinoid anthracycline-related anti-cancer agents represent an important group of antitumour drugs with a wide spectrum of activity.[12] On the basis of previously established structure–activity relationships (SARs) for compounds that are able to inhibit some of the tumor cell lines proliferation and dual modulation of the telomerase to varying degrees. A series of diaminoanthraquinones were discovered initially as protein kinase C inhibitors, and they also exhibited potent tumor cell growth inhibitory activity *in vitro* without cross resistance to adriamycin.[13] Recent reports suggest that triplex stabilization could be achieved with a series of DAAQs, which are able to discriminate between duplex and triplex DNA by virtue of their structural characteristics.[14,15] Mitoxantrone, a synthetic aminoanthraquinone belongs to chemical class of agents known as the anthraquinones which antitumor activity is attributed to its interaction with DNA topoisomerase II, and its interaction with human cells may also involve nonintercalary, electrostatic interactions.[16] The mitoxantrone-derived free radicals or their further oxidative activation of mitoxantrone to a DNA-damaging species may contribute to the mechanism of action of this antitumour agent.[17] Aminoanthraquinones may represent a class of polyamine binding site ligands with pharmacophore and may facilitate the rational design of *N*-methyl-D-aspartate (NMDA)-receptor modulators.[18] Aliphatic amine *N*-oxides have long been identified as non-toxic metabolites of a large number of tertiary amines drugs. Bioreduction of such *N*-oxides will generate the active parent amine. This principle has been adopted to develop AQ4N, a di-*N*-oxide anticancer prodrug with little intrinsic cytotoxicity.[19]

As part of our efforts to discover new chemotherapeutic agents, we have synthesized a series of 1,8-diaminoanthraquinones that have different profiles with cytotoxicity and other biological evaluation. Cytotoxicity and SARs studies demonstrated that 1,8-disubstituted aminoanthraquinones and not their 1,5-isomers can selectively inhibit some of the tumor cell lines. Previously, we have developed a series of structurally related symmetrical and asymmetrical substituted or disubstituted anthraquinones as potential chemotherapeutic agents.[5—11] The positional attachment of the amide and amino side chains have been shown to profoundly influence their cytotoxicity. For example, 1,4-diamidoanthraquinones have been shown more efficiently inhibited Hepa G2 cells, whereas their 1,5- and 1,8-disubstituted regioisomer, in which the functionalized side chains may simultaneously occupy both the DNA major and minor grooves, with intercalation of the planar chromophore.[2,20,21] Evidence supporting these distinct binding mechanisms and activity has been provided by their *in vitro* cytotoxicity and structure–activity relationships for the anthraquinone skeleton through spacer side chains at these different positions and results are compared with experimental data with these agents.

Chemistry

As a part of our program aimed at exploring the biological activity of symmetrical substitution of side chains into the anthraquinone chromophore, we have synthesized a series of diaminoanthraquinones that are related to the antitumor agent mitoxantrone. Based upon the anthraquinone skeleton, the symmetrical diamino derivatives can be readily prepared using the method of nucleophilic substitution reaction including substitution of 1,8-dichloroanthraquinone. It offers a number of possibilities as a starting material for the synthesis of more complex molecules because of the juxtaposition of the chlorine atoms and the carbonyl groups of the central ring. A key to developing such synthesis lies in differential nucleophilic substitution of the chlorine atoms in starting

* To whom correspondence should be addressed. e-mail: huanghs@ndmctsgh.edu.tw

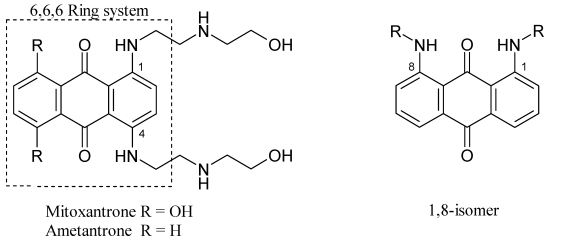

Chart 1

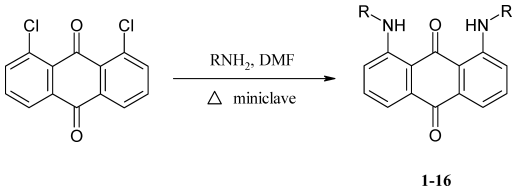

Chart 2

material. The anthraquinones were synthesized by condensing an excess of the appropriate amine with commercially available 1,8-dichloroanthraquinone and gave rise to the desired diaminoanthraquinones **1**—**16** in good overall yield. Such a reaction is not likely to be general for simpler substituted anthraquinones except for heating in the miniclave. More commonly, disubstitution are noted in reactions of starting material (1,8-dichloroanthraquinone) with nitrogen nucleophiles, and the problem becomes one of separation and purification of products.

Biological Activity and Discussion

Several classes of planar aromatic compounds have been shown to act as telomerase inhibitors or activators.[8,22] In connection with our interest in the development of new chromophoric anthraquinone isomers, we investigated the preparation of well defined anthraquinone structural motif that could be represent an attractive target for the rational design of new anticancer agents. The factors that control small molecule intercalation in DNA continue to be the focus of study, which the hydrophobicity of the small molecule and net charge are usually the most significant.[23] Additional factor specific to the side chains include their steric bulk, isohelicity of the side chains with the minor groove, and phasing of the ligand subunits with the edges of the base pairs.[24—26] We have recently shown that activation of human telomerase can be achieved with appropriately disubstituted anthraquinones.[8,11]

Comparisons with analogs anthraquinone-based compounds reveal a general reduction in the level of cellular cytotoxicity. Growth inhibition was determined in rat glioma C6 cells, human hepatoma G2 cells, and 2.2.15 cells using XTT assay. *In vitro* cytotoxicity and cell growth inhibitory data for the functionalized anthraquinones are collected in Table 1 and are compared with data for the corresponding mitoxantrone, adriamycin and cisplatin where commercial available. All compounds with the exception of compounds **2**, **7**, **9**, **10**, and **13** are remarkably non-toxic in these selected cell lines used. One of the most active *in vitro* compounds **7** displayed better cytotoxicity comparable with that of both mitoxantrone and adriamycin in C6 cells. Three of the most

Table 1. Cytotoxic Activity of 1,8-Diaminoanthraquinone Derivatives

Compd No.	R	IC_{50} (μM)[a]		
		C6 cells[c]	Hep G2[b]	2.2.15[d]
1	CH_2CH_3	25.6±0.5	41.82±1.0	59.47±0.9
2	$CH_2CH_2CH_3$	0.61±0.01	0.19±0.01	1.06±0.03
3	$CH_2CH_2CH_2CH_3$	11.4±0.4	25.6±0.6	97.9±1.5
4	$CH_2CH(CH_3)_2$	14.57±1.1	34.0±0.9	115.27±1.8
5	$(CH_2)_5CH_3$	47.5±0.8	102.9±1.0	107.5±1.3
6	$CH(CH_3)_2$	1.24±0.01	24.25±0.7	92.30±0.9
7	CH_2CH_2OH	0.02±0.01	16.0±0.1	91.25±0.8
8	$CH_2CH_2CH_2OH$	1.00±0.01	102.67±0.9	99.94±0.9
9	$(C_2H_5)CH(CH_2OH)$	0.41±0.02	1.65±0.13	13.2±0.7
10	$CH_2CH_2N(CH_3)_2$	0.15±0.04	0.16±0.04	8.55±0.09
11	$CH_2CH_2CH_2NH_2$	5.4±0.1	11.43±0.17	57.0±0.6
12	$CH_2CH_2CH_2CH_2NH_2$	80.0±0.9	12.47±0.34	81.53±0.8
13	$CH_2CH_2CH_2CH_2CH_2NH_2$	0.11±0.01	0.09±0.01	1.29±0.06
14	Cyclopentane	88.75±0.9	126.0±1.5	131.0±1.3
15	$CH_2C_6H_5$	1.75±0.09	46.45±0.5	41.48±0.5
16	$CH_2CH_2C_6H_5$	70.9±0.7	133.08±1.6	115.33±1.5
	Mitoxantrone	0.07±0.01	2.0±0.50	0.40±0.02
	Adriamycin	1.00±0.16	0.90±0.01	1.60±0.04
	Cisplatin	>1.0	1.48±0.62	2.0±0.54

a) IC_{50}, drug concentration inhibiting 50% of cellular growth following 48 h of drug exposure. Values are in μM and represent an average of 3 experiments. The variance for the IC_{50} values was less than ±20%. Inhibition of cell growth was significantly different with respect to that of the control; $n=3$ or more, $p<0.01$. Inhibition was compared with that of the control (mitoxantrone–HCl and adriamycin; μM), and standard errors. *b*) Hep G2, human hepatoma G2 cells. *c*) C6 cells, rat glioma C6 cells. *d*) 2.2.15 cells, hepatitis B virus transfected hepatoma cell lines, HepG 2.2.15 cells.

active *in vitro* compounds **2**, **10**, and **13** displayed cytotoxicity comparable with that of both mitoxantrone and adriamycin in HepG2 cells and 2.2.15 cells. In addition, compounds bearing alkyl substituents and terminal amine moieties **11** and **12** were found to be inactive, but **13** was found superior activity in these three cell lines. Among these compounds, compound **13** displayed the most potent inhibitory activity in Hep G2 cell line. This also correlates with an observed **13** had IC_{50} values of 0.09 μM, 22-fold reduction in cytotoxicity for HepG2 cells compared to the mitoxantrone and 10-fold to adriamycin. Compound **13** with 5-aminopentylamino substituent, which were chosen as typical alkylamino group, had IC_{50} values of 0.11 μM in C6 cells and IC_{50} values of 1.29 μM in 2.2.15 cells, respectively. This result prompted us to introduce this kind of simple long side chain on other regioisomers. In conclusion, within a series of disubstituted amidoanthraquinones, Neidle *et al*. had found that the 2,7-regioisomer afforded the best stabilization of the TG triplex, though the 1,8-isomers also stabilized the interaction to some extent.[27] Expanding or contracting the ring puts the binding groups in different positions relative to each other and may lead to better interactions with the binding site. 6,6,6 Ring system has a good interaction with both hydrophobic regions.[28] With the aim to provide second-generation anthraquinone analogs endowed with reduced side effects and a wider spectrum of action than mitoxantrone and doxorubicin, a large number of new molecules bearing nitrogen atoms in the chromophore were synthesized and screened *in vitro*. All analogs showed *in vitro* cytotoxic activity against rat glioma C6 cells, human hepatoma G2 cells and 2.2.15 cell lines. From this screening, compounds **2**, **7**, **9**, **10**, and **13** emerged as the most interesting analogs which were tested *in vitro* on several murine and human tumor cell lines

and showed cytotoxic potency lower than that of mitoxantrone and adriamycin. In particular, those compounds with methylene links in each side chain separating the amine and terminal amine moieties have superior cytotoxicity and, in general, enhanced anticancer characteristics. Based on this correlation, the most likely models of cytotoxicity complexes were postulated: (i) mitoxantrone and one derivative of bis(aminopentylamino)-substituted anthraquinone **13** were found superior activity in these three cell lines, both with the same distance of two long side chains, maybe intercalate from the minor groove of DNA and bind with both chains in this groove; (ii) propylamino disubstituted **2** and 2-dimethylaminoethylamino disubstituted **10** both with two methylene links in each side chain also have superior cytotoxicity.

Experimental

Melting points were determined with a Buchi B-545 melting point apparatus and are uncorrected. All reactions were monitored by TLC (silica gel 60 F_{254}). ^{1}H-NMR: Varian GEMINI-300 (300 MHz) and Brucker AM-500 (500 MHz); δ values are in ppm relative to TMS as an internal standard. Fourier-transform IR spectra (KBr): Perkin-Elmer 983G spectrometer. The UV spectra were recorded on a Shimadzu UV-160A. Mass spectra (PI-EI-MS, 70 eV, unless otherwise stated): Finnigan MAT TSQ-46, Finnigan MAT TSQ-700 (Universitat Regensburg, Germany) and Finnigan MAT LCQ-MS (National Research Institute of Chinese Medicine, Taipei, Taiwan). Typical experiments illustrating the general procedures for the preparation of the anthraquinones are described below.

General Procedure for the Preparation of the 1,8-Diaminoanthraquinones A mixture of 1,8-dichloroanthraquinone (1.0 g, 3.6 mmol) and DMF (20 ml) containing an appropriate amine (8.0 mmol) was heated in a miniclave (Büchi®) for 30 min. After cooling and the reaction mixture was treated with crushed ice. The resulting precipitate was collected by filtration, washed well with water and further purified by recrystallization from ethylacetate (EA)/*n*-hexane afforded the final product as red needles.

1,8-Bis(ethylamino)anthraquinone (**1**): 46% yield. mp 152—154 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 522 (0.78). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (6H, t, J=7.2 Hz), 3.39—3.48 (4H, m), 7.12 (2H, d, J=5.1 Hz), 7.48—7.62 (4H, m), 9.60 (2H, br). ^{13}C-NMR ($CDCl_3$) δ: 14.50 (CH_3), 37.60 (CH_2), 114.93 (CH), 118.16 (CH), 126.32 (C), 134.41 (CH), 137.89 (C), 151.35 (C), 182.96 (C), 188.91 (C). PI-EI-MS *m*/*z*: 294 (M^+), 285, 270.

1,8-Bis(propylamino)anthraquinone (**2**): 83% yield. mp 158—160 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 554 (0.80), 282 (0.87). ^{1}H-NMR (DMSO) δ: 1.10 (6H, t, J=7.4 Hz), 1.78—1.90 (4H, m), 3.29—3.36 (4H, m), 7.04 (2H, d, J=8.1 Hz), 7.47—7.58 (4H, m), 9.67 (2H, br). ^{13}C-NMR (DMSO-*d*) δ: 11.76 (CH_3), 22.43 (CH_2), 44.85 (CH_2), 114.34 (CH), 114.74 (CH), 117.65 (C), 134.04 (CH), 134.37 (C), 151.24 (C), 184.75 (C), 189.03 (C). EI-MS *m*/*z*: 322 (M^+), 279.

1,8-Bis(butylamino)anthraquinone (**3**): 71% yield. mp 133—134 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 565 (1.19), 282 (1.27). ^{1}H-NMR ($CDCl_3$) δ: 1.03 (6H, t, J=7.2 Hz), 1.50—1.59 (4H, m), 1.63—1.81 (4H, m), 3.32—3.39 (4H, m), 7.04 (2H, d, J=8.1 Hz), 7.47—7.58 (4H, m), 9.64 (2H, br). ^{13}C-NMR ($CDCl_3$) δ: 13.88 (CH_3), 20.41 (CH_2), 31.25 (CH_2), 42.80 (CH_2), 114.34 (CH), 114.74 (CH), 117.64 (C), 134.04 (CH), 134.38 (C), 151.23 (C), 184.76 (C), 189.03 (C). EI-MS *m*/*z*: 350 (M^+), 307, 293, 251.

1,8-Bis(isobutylamino)anthraquinone (**4**): 70% yield. mp 166—168 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 556 (0.52), 283 (0.68). ^{1}H-NMR ($CDCl_3$) δ: 1.11 (12H, d, J=6.6 Hz), 2.06—2.15 (2H, m), 3.18 (4H, t, J=6.3 Hz), 7.04 (2H, d, J=4.2 Hz,), 7.46—7.57 (4H, m), 9.80 (2H, br). ^{13}C-NMR (DMSO-*d*) δ: 20.56 (CH_3), 28.05 (CH), 50.87 (CH_2), 114.33 (CH), 114.71 (CH), 117.68 (C), 134.00 (CH), 134.38 (C), 151.37 (C), 184.70 (C), 189.00 (C). APCI-MS: 351 (M^+), 352, (24, $[M+1]^+$).

1,8-Bis(hexylamino)anthraquinone (**5**): 52% yield. mp 80—82 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 556 (0.53), 283 (0.56). ^{1}H-NMR ($CDCl_3$) δ: 0.90—0.99 (6H, m), 1.29—1.39 (8H, m), 1.41—1.59 (4H, m), 1.76—1.84 (4H, m), 3.31—3.37 (4H, m), 7.04 (2H, d, J=8.1 Hz), 7.47—7.58 (4H, m), 9.66 (2H, br). ^{13}C-NMR ($CDCl_3$) δ: 14.01 (CH_3), 22.57 (CH_2), 26.87 (CH_2), 29.11 (CH_2), 31.55 (CH_2), 43.11 (CH_2), 114.34 (CH), 114.74 (CH), 117.65 (C), 134.05 (CH), 134.39 (C), 151.23 (C), 184.77 (C), 189.02 (C). EI-MS *m*/*z*: 406 (M^+), 335, 321, 251.

1,8-Bis(isopropylamino)anthraquinone (**6**): 74% yield. mp 198—200 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 554 (1.13). ^{1}H-NMR ($CDCl_3$) δ: 0.87—0.93 (6H, m), 1.30—1.43 (6H, m), 3.86—3.92 (2H, m), 7.06 (2H, d, J=8.4 Hz), 7.47—7.57 (4H, m), 9.64 (2H, br). ^{13}C-NMR ($CDCl_3$) δ: 22.86 (CH_3), 43.68 (CH), 114.33 (CH), 114.62 (CH), 118.08 (C), 133.97 (CH), 134.55 (C), 150.34 (C), 184.70 (C), 189.00 (C). APCI-MS: 323 (M^+), 324 (24, $[M+1]^+$).

1,8-Bis(ethanolamino)anthraquinone (**7**): 80% yield. mp 214—215 °C (EA/*n*-hexane); lit.[29]: 260—265 °C, EtOH). UV λ_{max} (MeOH) nm (log ε): 546 (0.70), 282 (0.83). ^{1}H-NMR ($CDCl_3$) δ: 3.21 (4H, m, CH_2), 3.66 (4H, m, CH_2), 4.93 (2H, t, J=2.7 Hz), 7.20 (2H, d, J=8.7 Hz), 7.35 (2H, t, J=3.9 Hz), 7.52 (2H, t, J=8.2 Hz), 9.67 (2H, br, NH). ^{13}C-NMR ($CDCl_3$) δ: 45.13 (CH_2), 59.54 (CH_2), 113.66 (CH), 114.33 (CH), 118.63 (C), 133.75 (CH), 134.53 (C), 151.15 (C), 183.67 (C), 187.95 (C). PI-EI-MS *m*/*z*: 326 (M^+), 295.

1,8-Bis(propanolamino)anthraquinone (**8**): 71% yield. mp 211—212 °C (EA/*n*-hexane); lit.[29]: 215—220 °C, EtOH). UV λ_{max} (MeOH) nm (log ε): 552 (2.77), 282 (2.31). ^{1}H-NMR (DMSO) δ: 1.79 (4H, quint, CH_2), 3.21 (4H, m, CH_2), 3.55 (4H, m, CH_2), 4.62 (4H, t, J=3.0 Hz, OH), 7.19 (2H, d, J=8.7 Hz), 7.34 (2H, d, J=3.9 Hz), 7.53 (2H, t, J=8.2 Hz). 9.59 (2H, br). PI-EI-MS *m*/*z*: 354 (M^+), 309, 277.

1,8-Bis(2-amino-1-butanol)anthraquinone (**9**): 68% yield. mp 180—181 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 552 (1.38), 283 (1.51). ^{1}H-NMR (DMSO) δ: 0.92—0.97 (6H, m), 1.54—1.59 (4H, m), 1.70—1.73 (4H, m), 3.32—3.59 (2H, m), 4.90 (2H, s), 7.23 (2H, d, J=9.3 Hz), 7.32—7.35 (2H, m), 7.49 (2H, t, J=7.9 Hz), 9.67 (2H, d, J=7.2 Hz). EI-MS *m*/*z*: 383 (M^+), 291, 253.

1,8-Bis{[2-(dimethylamino)ethyl]amino}anthraquinone (**10**)[30]: 68% yield. mp 118—120 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 513 (0.61), 256 (3.31). ^{1}H-NMR ($CDCl_3$) δ: 0.87—1.02 (6H, m), 1.02—1.34 (6H, m), 2.28—2.40 (4H, m), 3.44—3.52 (4H, m), 7.12—7.15 (2H, m), 7.57—7.62 (2H, m), 7.64—7.86 (2H, m), 9.70 (2H, br). ^{13}C-NMR ($CDCl_3$) δ: 41.33, 45.60, 58.11, 114.99, 118.10, 126.24, 134.88, 137.61, 151.37, 182.99, 183.92. EI-MS *m*/*z*: 381 (M^+), 329.

1,8-Bis[(3-aminopropyl)amino]anthraquinone (**11**): 80% yield. mp 162—163 °C (EA/*n*-hexane); lit.[30]: 165 °C). UV λ_{max} (MeOH) nm (log ε): 544 (0.49), 281 (0.56). ^{1}H-NMR ($CDCl_3$) δ: 1.94—1.99 (4H, m), 2.82—2.84 (4H, m), 2.97—2.99 (4H, m), 3.42—3.46 (4H, m), 7.07—7.10 (2H, m), 7.48—7.51 (2H, m), 7.57—7.60 (2H, m), 9.67 (2H, br). APCI-MS: 353 (M^+), 354 (20, $[M+1]^+$).

1,8-Bis[(4-aminobutyl)amino]anthraquinone (**12**)[30]: Yield 43%; mp 154—155 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 551 (0.21), 282 (0.25). ^{1}H-NMR ($CDCl_3$) δ: 1.45—1.48 (4H, m), 1.57—1.60 (4H, m), 1.81—1.90 (4H, m), 2.71—2.81 (4H, m), 3.27—3.42 (4H, m), 7.03 (2H, d, J=8.4 Hz), 7.48—7.58 (4H, m), 9.64 (2H, br). APCI-MS: 381.1 (M^+), 382.2 (20, $[M+1]^+$).

1,8-Bis[(5-aminopentyl)amino]anthraquinone (**13**): 35% yield. mp 115—116 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 545 (1.72), 281 (1.89). ^{1}H-NMR ($CDCl_3$) δ: 0.86—0.92 (4H, m), 1.48—1.52 (4H, m), 1.57—1.58 (4H, m), 1.71—1.84 (4H, m), 2.71—2.79 (4H, m), 3.33—3.39 (4H, m), 7.03 (2H, d, J=8.7 Hz), 7.47—7.58 (4H, m), 9.65 (2H, br). APCI-MS: 409.1 (M^+), 410.2 (20, $[M+1]^+$).

1,8-Bis(cyclopentylamino)anthraquinone (**14**): 52% yield. mp 196—198 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 561 (1.70), 284 (1.80). ^{1}H-NMR ($CDCl_3$) δ: 1.85—1.87 (8H, m), 2.14—2.20 (8H, m), 4.00—4.02 (2H, m), 7.07 (2H, d, J=7.8 Hz), 7.46—7.57 (4H, m), 9.86 (2H, d, J=6.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 24.2, 33.62, 53.96, 114.41, 114.68, 118.58, 133.85, 134.41, 150.73, 184.76, 188.88. APCI-MS: 375.1 (M^+), 376 (20, $[M+1]^+$).

1,8-Bis[(phenylmethyl)amino]anthraquinone (**15**): 63% yield. mp 188—190 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 525 (0.56), 278 (0.93). ^{1}H-NMR ($CDCl_3$) δ: 4.62 (4H, d, J=6.0 Hz), 6.99 (2H, d, J=8.4 Hz), 7.30—7.42 (10H, m), 7.47 (2H, d, J=7.8 Hz), 7.61 (2H, d, J=7.8 Hz). 10.11 (2H, br). ^{13}C-NMR ($CDCl_3$) δ: 47.05, 115.42, 118.06, 126.95, 127.26, 128.72, 134.20, 134.40, 138.36, 151.03, 184.48, 188.89. EI-MS: 418 (M^+), 328.

1,8-Bis[(phenylethyl)amino]anthraquinone (**16**): 54% yield. mp 162—164 °C (EA/*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 544 (0.55). ^{1}H-NMR ($CDCl_3$) δ: 3.09—3.14 (4H, m), 3.59—3.65 (4H, m), 7.07 (2H, d, J=8.7 Hz), 7.30—7.39 (10H, m), 7.51 (2H, d, J=7.8 Hz), 7.57 (2H, d, J=7.8 Hz), 9.72 (2H, br). ^{13}C-NMR ($CDCl_3$) δ: 35.70, 44.65, 114.58, 114.99, 117.46, 126.51, 128.73, 134.08, 134.40, 138.99, 150.90, 184.48, 188.89. EI-MS: 446 (M^+), 356.

Cell Culture Various cancer cell lines (G2, 2.2.15. cells and C6 cells) were cultured in minimum essential medium (MEM), supplemented with

10% fetal calf serum, 100 units/ml penicillin and 100 mg/ml streptomycin in a humidified atmosphere in 5% CO_2 at 37 °C. Cell culture media were renewed every three days, up to the confluence of the monolayer. Cell culture was passaged when they had formed confluent cultures, using trypsin-EDTA to detach the cells from their culture flasks or dishes. Test compounds were stored at −70 °C and solublized in 100% DMSO. All the drug solutions were prepared immediately before the experiments and were diluted into complete medium before addition to cell cultures. All data presented in this report are form at least three independent experiments showing the same pattern of expression.

XTT Method The tetrazolium reagent (XTT) was designed to yield a suitably colored, aqueous-soluble, non-toxic formazan upon metabolic reduction by viable cells. Approximately 2×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. Test compounds were then added to the culture medium for a designated various concentrations. After 72 h, fresh XTT 50 μl and electron coupling reagent (PMS) 1 μl were mixed together, and 50 μl of this mixture were added to each well. After an appropriate incubation at 37 °C for 6 h, the absorbency at 490 nm was measured with the ELISA reader.

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