Synthesis and Antitumor Evaluation of Symmetrical 1,5-Diamidoanthraquinone Derivatives as Compared to Their Disubstituted Homologues

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A series of symmetrical 1,5-diamidoanthraquinone derivatives with potentially bioreducible groups has been synthesized and their cytostatic activity against the panel of various cancer cell lines *in vitro* has been studied. Preliminary structure–activity relationships were established. The results indicated that compounds 5 and 18 exhibited significant potent cytotoxicity at 1.24—1.75 μM for Hepa G2 cell line; compounds 5, 16, and 18 exhibited cytotoxicity at 0.14—1.82 μM for 2.2.15 cell line as determined by XTT colorimetric assay. Two structurally related compounds, mitoxantrone and adriamycin, were tested in parallel as positive controls. In addition, it was found that compounds 5 and 18 were a more potent and specific human hepatoma cell line than mitoxantrone and showed comparable activity to adriamycin. Among them, compound 18 was the most potent for 2.2.15 cells. We have demonstrated that the anthraquinone moiety is essential for activity and that less sterically hindered substituents contribute to enhanced *in vitro* efficacy. Implications for amidoanthraquinone cytotoxicity as potential anticancer agents are discussed. We further delineate the nature of the pharmacophore for this class of compounds, which provides a rational basis for the structure–activity relationships.

Key words amidoanthraquinone; mitoxantrone; adriamycin; cytotoxicity; XTT colorimetric assay; structure–activity relationship

In a continuation of our work on anthraquinones that widely occur in the plant kingdom and that may have biological activities, we have developed and synthesized a series of small-molecule tricyclic anthraquinone structural motifs which represents an attractive target for the rational design of new anticancer agents.[1—8] Antineoplastic drugs generally have a narrow therapeutic index and are delivered at doses close to toxicity. Despite extensive and long-standing clinical utilization, the mechanisms responsible for the antiproliferative and cytotoxic effects of anthracycline antibiotic doxorubicin are still uncertain and have been the subject of considerable controversy.[9] Several naturally occurring compounds having substituted anthraquinone moieties are widely used in cancer chemotherapy and constitute some of the most powerful cytostatics.[10—12] In previous papers,[1—6,13,14] we reported a series of anthraquinone derivatives (Chart 1) that showed *in vitro* anticancer activity, together with some of human telomerase evaluation. The structure–activity relationships indicated amido substitution may lead to a different mechanism of cytotoxicity.[5] Although an explanation for the mechanism has not yet emerged, the cytotoxic activity in cultured tumor cell lines is well understood. We still envisioned a new approach to the anthraquinone, taking advantage of the amido side arms at different positions and pharmacophore moiety to the pharmacophore that, if it is not active in the anticancer, can develop activity to produce a synergistic effect. The primary aim for developing anticancer drugs has been for their cytotoxic and cytostatic properties and for their binding capacity to a recombinant fragment of the multi-drug-resistance transporter.[12] These hypotheses suggest that the three-ring aromatic moiety gives DNA-intercalating ability to cross-linkable side arms of substituents, and the anthraquinone analog is considered as a possible antitumor lead pharmacophore. We established a novel strategy to obtain new derivatives of anthraquinone and screened against the *in vitro* cytotoxicity in C6, Hepa G2 and 2.2.15 cell lines; the results are presented in Table 1.

We recently described the human telomerase inhibition and cytotoxicity properties of a series of regioisomeric difunctionalized anthraquinones at the 1,4-, 1,5- and 1,8-positions, respectively.[3—6,14] Their *in vitro* cytotoxicity was reported and compared with those of their isomers, and it was proposed that their activity may be due to their ability to bind to and stabilize G-quadruplex structures.[8,15] The positional attachment and character of the diacyloxy-, dithio-, diamino-, and diamido side chains have been shown to profoundly influence their mode of DNA binding and cytotoxicity. For example, 1,4-diamidoanthraquinones have been shown to bind to duplex DNA by classical intercalation,[16] whereas their cytotoxicity and human telomerase inhibition,[5,14] in which the different functionalized side chains may simultaneously occupy both the DNA major and minor grooves, with intercalation of the planar chromophore.[17] Biological results supporting these structural motif and side arms have shown structure–activity relationships. However, 1,4-diamido-isomers were, in general (approximately 6 of 38 test compounds), more cytotoxic than 1,5-diamido-, 1,5-diamino-, 1,5-diacyloxy-, or 1,5-dithio-substituted regioisomers in these three cultured tumor cell lines. This has enabled us to address the issue of how, and to what extent, the position and their longer side chains and better intercalation with DNA of side chain substituents affects cytotoxic and telomerase activity. Their *in vitro* cytotoxicity and some human telomerase inhibition properties are reported and compared with those of their 1,4- and 1,5-regioisomers in Table 2.

In the present paper we describe the study of a further series of regioisomeric 1,5-diamidoanthraquinones. Hence, amido substitution at anthraquinone may lead to a different mechanism of cytotoxicity, suppressing tumor growth mainly by intercalation into DNA and inhibition of topoisomerase II,

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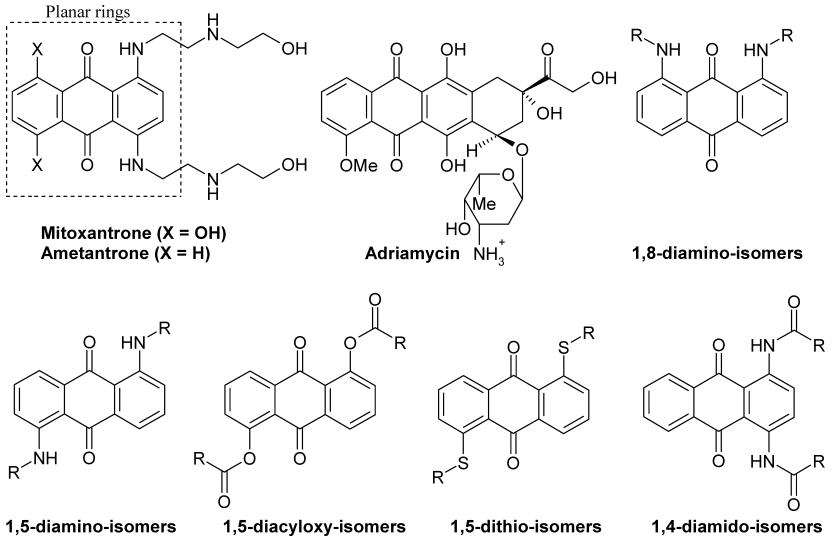

Chart 1

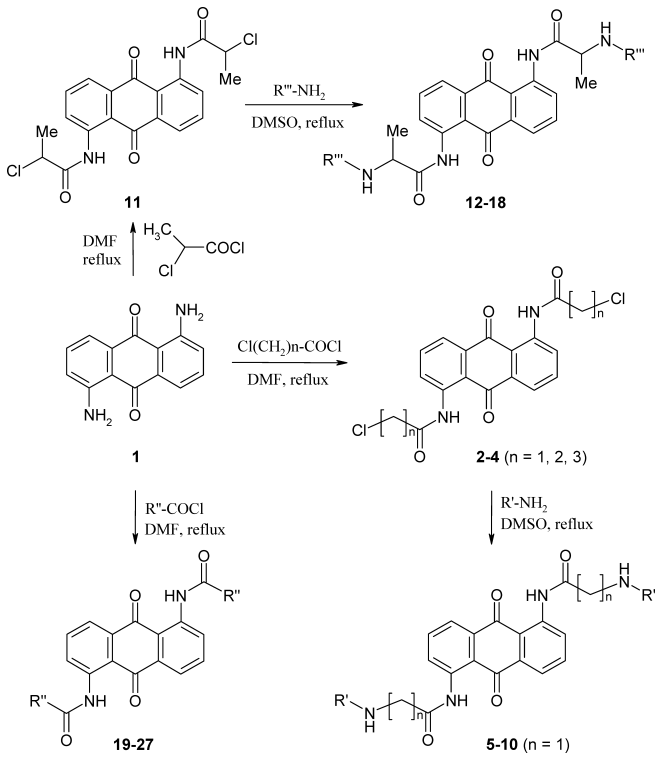

Chart 2

and is suspected of generating free radicals leading to DNA strand scission.[8,18—20] In addition, biophysical studies on a range of side arms and structure–activity relationships have been conducted in order to examine binding to the proposed target structure formed by the cytotoxicity and telomerase inhibition.

Chemistry

There has been continuing interest in the synthesis of naturally occurring anthraquinones largely on account of their biological activities. In this paper we report a very convenient methodology for the synthesis of 1,5-difunctionalized amidoanthraquinones with side chains having basic nitrogen atoms. We have prepared two intermediates which are useful for the synthesis of target compounds. Compounds **2**—**27** were synthesized using a novel synthetic strategy (Chart 2). Compounds **5**—**10** were synthesized by a two-stage reaction. The first stage involved acylation of the 1,5-diaminoanthraquinone with chloroacyl chloride in DMF as a solvent, with a catalytic quantity of pyridine, producing the 1,5-bis-(ω-chloroalkaneamido) side chain compounds **2**—**4** in essentially quantitative yield. Further amination of the intermediate **2** with the appropriate primary and secondary amines in DMSO gave the desired disubstituted anthraquinones **5**—**10**. Depending on the structure of the amine used further reactions occurred, broadening the range of structures. Reaction of the starting material with 2-chloropropionyl chloride under similar conditions afforded compound **11** which was used for the synthesis of compounds **12**—**18**. Compounds **19**—**27** were synthesized by direct one-stage acylation reaction. Structures of these compounds were obtained in good yield and determined to be pure by ^{1}H- and ^{13}C-NMR. We report here a simple and general methodology for the synthesis; all compounds were used for the cytotoxicity studies and telomerase inhibition which the results of which will be reported elsewhere.

Biological Activity and Discussion

In previous papers, we reported a series of 1,4-, 1,5- and 1,8-difunctionalized substituted anthraquinone derivatives that inhibit human telomerase and their cytotoxicity.[1—6,14] The present study details the preparation of 1,5-diamidoanthraquinones and their *in vitro* cytotoxicity compared with those of other types of regioisomeric difunctionalized anthraquinones substituted at the 1,4-, 1,5- and 1,8-positions. These compounds with diamido side arms are assumed to provide better intercalation with DNA but the mechanisms are still uncertain. Synthesized 1,5-diamidoanthraquinones were tested against a panel of cancer cells (G2, 2.2.15. and C6 cells) using XTT assay as described previously.[5] Two structurally related compounds, anthraquinone-based antitumor agents mitoxantrone and adriamycin, were tested in parallel as positive controls. Both the control agents are highly cytotoxic in the three cell lines used in this study. The results showed that some tested compounds inhibited the growth of

cancer cells at micromolar concentrations, and presented in Table 1 are the concentrations required to inhibit cell growth by 50% (IC_{50} values). The 1,5-diamidoanthraquinones described here exhibit no cytotoxicity except for **5**, **16**, and **18**, which have IC_{50} values in the low-micromolar range compared with mitoxantrone and adriamycin, against a panel of three cell lines (Murine C6, human Hepa G2, and 2.2.15). However, the difference between the 1,4-isomer,[5] 1,5-isomer[1,2,4] and 1,8-isomers[6] has a significant effect on the shape of chromophore. Keppler *et al.*[21] have indicated that 1,5-disubstituted anthraquinones interacted with duplex DNA in molecular modeling studies, which provide a rational basis for the SARs. By comparison, the amidoanthraquinones reported here are, in general, less effective as cytotoxic agents, although two of the most active compounds, **5**, and **18**, are better than mitoxantrone in the Hepa G2 cell line; compound **18** is better than mitoxantrone and adriamycin in the 2.2.15 cell line. Compounds **18** and 1,5-diaminoanthraquinone with $(CH_2)_2N(CH_3)_2$ substitutents were found to be the most potent of the compounds studied in the 2.2.15 cell line (Table 2). Members of the 1,5-difunctionalized series (compounds **2**—**4**, **6**—**15**, **17**, **19**—**27**) are generally less cytotoxic than their 1,4-isomers depending on the character of side chains. Prior to the evaluation of types of test compounds in the XTT assay, the derivatives were tested for their ability to inhibit telomerase and cytotoxicity to examine the selectivity of cytotoxic *versus* telomerase inhibition. In addition, two of the most active compounds of 1,5-diacyloxy isomers (compounds with $CH_2CH_2CH_3$ disubstituted and 2-ClC_6H_4 disubstituted) are better than mitoxantrone and adriamycin in the Hepa G2 cell line. We were unable to determine mechanisms from IC_{50} values for any type of 1,4-diamido-isomers, 1,5-diamido-isomers, 1,5-diamino-isomers, 1,5-diacyloxy-isomers, 1,5-dithio-isomers, or 1,8-diamido-isomers due to inhibition of tumor cell proliferation at concentrations above those of mitoxantrone and adriamycin. However, both drugs of 1,5-diacyloxy-isomers with $CH_2CH_2CH_3$ disubstituted and 2-ClC_6H_4 disubstituted showed cytotoxic activity against Hepa G2 cell lines at the lowest concentrations.

In conclusion, we previously developed a series of isomeric symmetrical substituted anthraquinone derivatives as duplex DNA-interactive agents, inhibiting telomerase as well as being known for their antitumor activities.[1—6,13,14] The antitumor activity of anthraquinone derivatives on a panel of cancer cell lines during the last 30 years led investigators to synthesize thousands of anthracycline analogs and test their cytotoxicity to identify compounds superior to the parent drugs in terms of increased therapeutic effectiveness, reduced toxicity or both. Perry and Neidle[8] have shown that amidoanthraquinones favoring duplex binding are related to the inhibition of telomerase activity and assumed to be nonselective. This may be the necessary initial event that results in telomerase inhibition and cytotoxicity. From a structure-activity relationship point of view cytotoxicity is evident from the remarkable results presented here and previously reported. Compounds containing $-(CH_2)_n-$ side chains terminating in basic groups such as aminoalkyl-substituted, showed cytotoxic activity in several cell lines.[5] For example, the most active compound from the XTT assay in hepatitis B virus transfected hepatoma cell lines (HepG 2.2.15) displayed remarkable results; this is the 2-isopropylaminopropionamido-substituted derivative **18** which had an IC_{50} of 0.14 μM. Compounds **5** and **18** also showed good cytotoxicity (with IC_{50} values of 1.24 and 1.75 μM, respectively). Of note, by locating the disubstituents at the anthraquinone skeleton, the activities of the 1,5-diamino isomers with disubstituted of $(CH_2)_2N(CH_3)_2$ are significantly lower than other isomers, with IC_{50} values of 0.09 μM in Hepa G2 cell lines, 0.12 μM in C6 cell lines and 0.13 μM in 2.2.15 cell lines, respectively. Comparison of Tables 1 and 2 shows that the most two active compounds in Hepa G cell lines, with an IC_{50} of 0.02 and 0.04 μM, are two of the 1,5-diacyloxy-isomers.[1] This suggests that for these derivatives, simple butyryloxy and 2-chlorobenzoyl side chains were essentially found to be the most active, with 1,5-bis(butyryloxy)anthraquinone indicating about 100 times the potency of the positive control of mitoxantrone and 45 times that for adriamycin. If such compounds are to have application as antitumor agents, with cytotoxicity in tumor cell lines leading to the attrition of tumor cell growth and consequent apoptosis, then conventional cytotoxicity should be required. By contrast, the established anthraquinone-based anticancer drugs mitoxantrone and adriamycin, even though they show cytotoxicity at levels similar to those exhibited by some compounds presented here (Table 1). These represent some of the most potent non-nucleoside small molecule anthraquinones for Hepa G2 cell lines reported to date further investigation is worthwhile. Furthermore, there does not appear to be any correlation between telomerase inhibition and cytotoxicity.[3] The precise role of cytotoxicity has yet to be fully established, and thus its relevance as a selective target for chemotherapy remains to be proven. However, apart from the well-documented large differences in length between murine and human cytotoxicity, it is apparent there are a minority of mechanisms, and it is conceivable that some of the selective murine and human tumor cell lines operate by an analogous control process. In addition to this favorable feature, some compounds revealed interesting binding properties and detail SARs, and reversed the anthraquinone-based phenotype of cells, thus making them promising potential anti-tumor drugs. The activity of telomerase in human cells has not been clarified and the results of such studies will be reported elsewhere.

Experimental

Melting points were determined with a Büchi 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 (EI, 70 eV, unless otherwise stated): Finnigan MAT TSQ-46, Finnigan MAT TSQ-700 (Universität 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 1,5-Diamidoanthraquinones Method A (Compounds **2**—**10**): Chloroacylchloride (12 mmol) was added dropwise at 0 °C under N_2 to a solution of 1,5-diaminoanthraquinone (1 mmol) and pyridine (0.5 ml) in *N*,*N*-dimethylformamide (20 ml). The reaction mixture was stirred for 24 h at room temperature. The resulting precipitate was collected by filtration, washed with diethyl ether and purified by crystallization from ethyl acetate to afford desired compound **2**—**4**. A solution of appropriate amine (20 mmol) in DMF was added dropwise under N_2 to a suspended solution of **2** (1 mmol) in 40 ml of DMF and triethylamine (0.5 ml). The reaction mixture was heated and refluxed at

Table 1. Structures and Cytotoxic Activities of 1,5-Diamidoanthraquinones (**2**—**27**) in Suspended Murine and Human Tumor Cell Lines

Compd. No.	R	IC_{50} (μM)[a]		
		C6[b]	Hepa G2[c]	2.2.15[d]
2	CH_2Cl	143.4±10.8	134.3±11.3	229.2±21.2
3	$(CH_2)_2Cl$	37.1±2.3	50.0±5.7	243.0±10.7
4	$(CH_2)_3Cl$	208.6±10.7	131.3±10.4	190.8±14.2
5	$CH_2NHCH_2CH_3$	13.4±1.5	1.24±0.24	1.75±0.21
6	$CH_2NH(CH_2)_2CH_3$	11.5±1.2	11.9±1.1	12.1±1.4
7	$CH_2NH(CH_2)_3CH_3$	17.7±0.5	54.4±2.3	132.7±7.6
8	$CH_2NH(CH_2)_4CH_3$	167.3±5.2	17.3±0.5	15.1±0.7
9	$CH_2NH(CH_2)_5CH_3$	>200	39.0±4.1	65.0±5.9
10	$CH_2N(CH_2CH_3)_2$	208.6±7.8	27.2±3.2	17.7±3.4
11	$(CH)CH_3Cl$	86.5±5.3	119.0±7.1	>200
12	$(CH)CH_3NHCH_2CH_3$	52.5±1.8	15.1±1.7	21.2±1.1
13	$(CH)CH_3NH(CH_2)_2CH_3$	36.6±2.1	14.4±1.7	8.4±1.2
14	$(CH)CH_3NH(CH_2)_3CH_3$	63.6±1.9	110.8±9.6	109.9±2.4
15	$(CH)CH_3NH(CH_2)_4CH_3$	32.9±4.7	27.7±2.3	185.4±9.5
16	$(CH)CH_3NH(CH_2)_5CH_3$	>200	6.8±1.3	1.82±0.17
17	$(CH)CH_3NH(CH_2)_3N(CH_3)_2$	2.51±0.23	7.46±0.32	11.00±0.26
18	$(CH)CH_3NHCH(CH_3)_2$	86.5±1.2	1.75±0.17	0.14±0.02
19	CH_3	351.8±10.7	222.6±11.7	350.6±6.7
20	CH_2CH_3	163.4±9.7	135.6±5.7	56.3±4.1
21	3-$C_6H_4CH_3$	273.3±7.6	354.3±12.7	278.4±9.7
22	C_6H_{10} (cyclohexane)	124.6±10.3	139.4±7.2	201.0±6.1
23	$(CH_2)_2C_5H_9$	54.9±4.2	54.9±1.7	212.6±3.6
24	C_5H_9	88.1±1.5	98.5±3.2	231.0±8.4
25	C_3H_5	132.6±9.2	123.9±8.4	213.9±9.2
26	*trans*-$CH(CH_2)CHC_6H_5$	41.4±2.1	125.3±6.2	207.4±10.7
27	$CH_2SC_6H_5$	191.4±6.2	182.1±3.1	219.1±9.1
	Mitoxantrone	0.07±0.01	2.00±0.50	0.40±0.02
	Adriamycin	1.00±0.16	0.90±0.01	1.60±0.04

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 three 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 and standard errors. *b*) Hepa G2, human hepatoma G2 cells. *c*) C6 cells, rat glioma C6 cells. *d*) 2.2.15 cells, hepatitis B virus transfected hepatoma cell lines, Hepa G 2.2.15 cells.

Table 2. *In Vitro* Cytotoxicity Data for Isomeric Anthraquinone Derivatives

Isomers	R	IC_{50} (μM)[a]		
		C6[b]	Hepa G2[c]	2.2.15[d]
1,5-diamido	$CH_2NHCH_2CH_3$ (**5**)	13.43±0.10	1.24±0.24	1.75±0.01
1,5-Diamido	$(CH)CH_3NH(CH_2)_5CH_3$ (**16**)	>200	6.76±1.36	1.82±0.17
1,5-Diamido	$(CH)CH_3NHCH(CH_3)_2$ (**18**)	86.48±1.21	1.75±2.17	0.14±1.92
1,5-Diacyloxy[1]	$CH_2CH_2CH_3$	38.5±2.8	0.02±0.01	ND
1,5-Diacyloxy[1]	2-ClC_6H_4	40.7±4.7	0.04±0.01	ND
1,5-Diacyloxy[1]	$CH_2CH_2C_6H_5$	40.1±5.5	0.4±0.1	ND
1,5-Dithio[2]	CH_2CH_3	0.02±0.01	12.2±1.1	ND
1,5-Dithio[2]	4-$NH_2C_6H_4$	0.05±0.01	17.4±1.5	ND
1,5-Diamino[4]	$CH_2CH_2N(CH_3)_2$	0.12±0.01	0.09±0.01	0.13±0.01
1,5-Diamino[4]	$CH_2CH_2NH(CH_2)_2OH$	1.17±0.03	1.20±0.02	7.01±0.11
1,5-Diamino[4]	$CH_2CH_2CH_3$	16.03±0.68	1.74±0.14	1.94±0.02
1,4-Diamido[5]	$CH_2N(CH_2CH_3)_2$	0.60±0.10	1.43±0.24	1.98±0.01
1,4-Diamido[5]	$CH_2CH_2N(CH_2CH_3)_2$	1.06±0.12	0.89±0.02	1.15±0.04
1,4-Diamido[5]	$CH_2CH_2NHCH_2CH(CH_2)_2$	0.40±0.09	1.01±0.01	6.04±0.59
1,4-Diamido[5]	Cyclopropane	13.25±0.03	0.53±0.01	2.61±0.03
1,4-Diamido[5]	2,5-$(CF_3)_2C_6H_3$	0.49±0.05	6.51±0.07	6.57±0.06
1,8-Diamino[6]	$CH_2CH_2CH_3$	0.61±0.01	0.19±0.01	1.06±0.03
1,8-Diamino[6]	CH_2CH_2OH	0.02±0.01	16.0±0.1	91.25±0.8
1,8-Diamino[6]	$CH_2CH_2N(CH_3)_2$	0.15±0.04	0.16±0.04	8.55±0.09
1,8-Diamino[6]	$CH_2CH_2CH_2CH_2CH_2NH_2$	0.11±0.01	0.09±0.01	1.29±0.06
	Mitoxantrone	0.07±0.01	2.00±0.50	0.40±0.02
	Adriamycin	1.00±0.16	0.90±0.01	1.60±0.04

For significant selective cytotoxicity against Hepa G2, human hepatoma G2 cells; C6 cells, rat glioma C6 cells; 2.2.15 cells, hepatitis B virus transfected hepatoma cell lines, Hepa G 2.2.15 cells. ND: not determined. *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 three 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 and standard errors. *b*) Hepa G2, human hepatoma G2 cells. *c*) C6 cells, rat glioma C6 cells. *d*) 2.2.15 cells, hepatitis B virus transfected hepatoma cell lines, Hepa G 2.2.15 cells.

130 °C in a miniclave (Büchi®) for 1 h. After cooling the reaction mixture was treated with crushed ice and the resulting precipitate was collected by filtration, washed and purified by crystallization from ethyl acetate to afford desired compounds **5**—**10**.

Method B (Compounds **11**—**18**): 2-Chloropropionyl chloride (12 mmol) was added dropwise at 0 °C under N_2 to a solution of 1,5-diaminoanthraquinone (1 mmol) and pyridine (0.5 ml) in *N*,*N*-dimethylformamide (20 ml). The reaction mixture was stirred for 24 h at room temperature. The resulting precipitate was collected by filtration, washed with diethyl ether and purified by crystallization from ethyl acetate to afford desired compound **11**. A solution of an appropriate amine (20 mmol) in DMF was added dropwise under N_2 to a suspended solution of **11** (1 mmol) in 40 ml of DMF and triethylamine (0.5 ml). The reaction mixture was heated and refluxed at 130 °C in a miniclave (Büchi®) for 1 h. After cooling the reaction mixture was treated with crushed ice and the resulting precipitate was collected by filtration, washed and purified by crystallization from ethyl acetate to afford desired compounds **12**—**18**.

Method C (Compounds **19**—**27**): Acyl chloride (12 mmol) was added dropwise at 0 °C under N_2 into a solution of 1,5-diaminoanthraquinone (1 mmol) and pyridine (0.5 ml) in *N*,*N*-dimethylformamide (20 ml). The reaction mixture was stirred for 24 h at room temperature. The resulting precipitate was collected by filtration, washed with diethyl ether and purified by crystallization from ethyl acetate to afford desired compounds **19**—**27**.

1,5-Bis(2-chloroacetamido)anthraquinone (2) 93% yield. mp 370 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 4.33 (4H, s, CH_2), 7.80 (2H, t, J=8.1 Hz, Ar-H), 8.15 (2H, d, J=6.9 Hz, Ar-H), 9.12 (2H, d, J=8.7 Hz, Ar-H), 11.70 (2H, s, NH). IR (KBr): 1684, 3200 cm^{-1}. EI-MS *m*/*z*: 392 (M^+), 341, 264.9.

1,5-Bis(2-chloropropionamido)anthraquinone (3) 48% yield. mp 275 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 3.01 (4H, t, J=6.3 Hz, CH_2), 3.92 (4H, t, J=6.6 Hz, CH_2), 7.80 (2H, t, J=8.1 Hz, Ar-H), 8.06 (2H, d, J=7.5 Hz, Ar-H), 9.15 (2H, d, J=8.4 Hz, Ar-H), 12.39 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ 186.64 (C-9,10), 169.24 (NCO-1′,5′), 141.62 (C-1,5), 136.05 (C-4a,8a), 134.54 (C-3,7), 126.40 (C-5a,9a), 122.96 (C-4,8), 117.19 (C-2,6), 41.53 (CH_2), 39.29 (CH_2). IR (KBr): 1681, 3205 cm^{-1}. EI-MS *m*/*z*: 418 (M^+), 382, 355, 328, 237.9.

1,5-Bis(4-chlorobutylamido)anthraquinone (4) 90% yield. mp 206 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 2.28—2.37 (4H, m, CH_2), 2.82 (4H, t, J=7.2 Hz, CH_2), 3.75 (4H, t, J=6.3 Hz, CH_2), 7.84 (2H, t, J=8.1 Hz, Ar-H), 8.11 (2H, dd, J=7.6, 1.1 Hz, Ar-H), 9.18 (2H, dd, J=8.7, 1.1 Hz, Ar-H), 12.39 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.62 (C-9,10), 171.65 (NCO-1′,5′), 141,90 (C-1,5), 135.95 (C-4a,8a), 134.59 (C-3,7), 126.24 (C-5a,9a), 122.66 (C-4,8), 117.06 (C-2,6), 44.13 (CH_2), 35.40 (CH_2), 27.93 (CH_2). IR (KBr): 1680, 3215 cm^{-1}. EI-MS *m*/*z*: 446 (M^+), 342, 265, 238.

1,5-Bis(2-ethylaminoethylamido)anthraquinone (5) 77% yield, mp 163 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (6H, t, J=7.2 Hz, CH_3), 2.79 (4H, q, J=7.2 Hz, CH_2), 3.53 (4H, s, CH_2), 7.76 (2H, t, J=8.4 Hz, Ar-H), 8.09 (2H, d, J=6.3 Hz, Ar-H), 9.21 (2H, d, J=7.2 Hz, Ar-H), 13.08 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.96 (C-9,10), 172.87 (NCO), 141.09 (C-1,5), 135.46 (C-4a,8a), 134.90 (C-3,7), 126.14 (C-5a,9a), 122.66 (C-4,8), 117.93 (C-2,6), 53.97 (CH_2), 44.73 (CH_2), 15.25 (CH_3). IR (KBr): 1685, 3330 cm^{-1}. EI-MS *m*/*z*: 408 (M^+), 351, 266.

1,5-Bis(2-propylaminoethylamido)anthraquinone (6) 80% yield. mp 172 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.99 (6H, t, J=7.5 Hz, CH_3), 1.64—1.74 (4H, m, CH_2), 2.71 (4H, t, J=6.9 Hz, CH_2), 3.52 (4H, s, CH_2), 7.75 (2H, t, J=8.1 Hz, Ar-H), 8.06 (2H, dd, J=7.8, 1.2 Hz, Ar-H), 9.20 (2H, d, J=7.5 Hz, Ar-H), 13.04 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.92 (C-9,10), 172.91 (NCO), 141.04 (C-1,5), 135.46 (C-4a,8a), 134.86 (C-3,7), 126.13 (C-5a,9a), 122.64 (C-4,8), 117.90 (C-2,6), 53.13 (CH_2), 52.32 (CH_2), 23.19 (CH_2), 11.65 (CH_2). IR (KBr): 1647, 3339 cm^{-1}. PI-EI-MS *m*/*z*: 437 (M^+), 436, 365, 337, 266, 238, 72.

1,5-Bis(2-butylaminoethylamido)anthraquinone (7) 77% yield. mp 170 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.93 (6H, t, J=7.5 Hz, CH_3), 1.39—1.46 (4H, m, CH_2), 1.60—1.69 (4H, m, CH_2), 2.74 (4H, t, J=7.2 Hz, CH_2), 3.53 (4H, s, CH_2), 7.75 (2H, t, J=8.1 Hz, Ar-H), 8.07 (2H, d, J=7.5 Hz, Ar-H), 9.20 (2H, d, J=8.7 Hz, Ar-H), 13.02 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.51 (C-9,10), 172.47 (NCO), 140.62 (C-1,5), 135.05 (C-4a,8a), 134.46 (C-3,7), 125.72 (C-5a,9a), 122.20 (C-4,8), 117.50 (C-2,6), 53.77 (CH_2), 49.73 (CH_2), 31.76 (CH_2), 19.90 (CH_2), 13.60 (CH_3). IR (KBr): 1647, 2354, 2928, 3339 cm^{-1}. PI-EI-MS *m*/*z*: 464 (M^+), 379, 351, 266.

1,5-Bis(2-pentylaminoethylamido)anthraquinone (8) 78% yield. mp 190 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, t, J=7.2 Hz, CH_3), 1.23—1.36 (8H, m, CH_2), 1.64—1.68 (4H, m, CH_2), 2.72 (4H, t, J=6.9 Hz, CH_2), 3.52 (4H, s, CH_2), 7.75 (2H, t, J=8.1 Hz, Ar-H), 8.08 (2H, d, J=7.8 Hz, Ar-H), 9.21 (2H, d, J=8.7 Hz, Ar-H), 13.05 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.51 (C-9,10), 172.47 (NCO), 140.62 (C-1,5), 135.05 (C-4a,8a), 134.46 (C-3,7), 125.72 (C-5a,9a), 122.20 (C-4,8), 117.50 (C-2,6), 53.79 (CH_2), 50.05 (CH_2), 29.33 (CH_2), 28.97 (CH_2), 22.20 (CH_2), 13.62 (CH_3). IR (KBr): 1495, 1647, 2918, 3339 cm^{-1}. PI-EI-MS *m*/*z*: 492 (M^+), 393, 365, 266.

1,5-Bis(2-hexylaminoethylamido)anthraquinone (9) 76% yield. mp 204 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (6H, t, J=7.2 Hz, CH_3), 1.23—1.41 (12H, m, CH_2), 1.63—1.68 (4H, m, CH_2), 2.72 (4H, t, J=7.2 Hz, CH_2), 3.52 (4H, s, CH_2), 7.76 (2H, t, J=8.1 Hz, Ar-H), 8.08 (2H, dd, J=7.8, 1.2 Hz, Ar-H), 9.21 (2H, dd, J=7.2, 1.2 Hz, Ar-H), 13.06 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.52 (C-9,10), 172.50 (NCO), 140.67 (C-1,5), 135.03 (C-4a,8a), 134.48 (C-3,7), 125.75 (C-5a,9a), 122.22 (C-4,8), 117.52 (C-2,6), 53.80 (CH_2), 50.10 (CH_2), 31.38 (CH_2), 29.64 (CH_2), 26.46 (CH_2), 22.23 (CH_2), 13.61 (CH_3). IR (KBr): 1695, 2937, 3186 cm^{-1}. PI-EI-MS *m*/*z*: 520 (M^+), 407, 292, 266.

1,5-Bis(N,N-diethylaminoethylamido)anthraquinone (10) 68% yield. mp 224 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.16 (6H, t, J=6.9 Hz, CH_3), 2.72 (8H, q, J=7.2 Hz, CH_2), 3.26 (4H, s, CH_2), 7.74 (2H, t, J=8.7 Hz, Ar-H), 8.07 (2H, d, J=7.8 Hz, Ar-H), 9.20 (2H, d, J=8.7 Hz, Ar-H), 13.13 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.55 (C-9,10), 173.48 (NCO), 140.87 (C-1,5), 135.21 (C-4a,8a), 135.02 (C-3,7), 125.97 (C-5a,9a), 122.49 (C-4,8), 118.06 (C-2,6), 59.16 (CH_2), 48.70 (CH_2), 12.17 (CH_3). IR (KBr): 1695 cm^{-1}. PI-EI-MS *m*/*z*: 464 (M^+), 393, 86.

1,5-Bis(2-chloropropionylamido)anthraquinone (11) 70% yield. mp 288 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.87 (3H, d, CH_3), 4.60 (1H, q, J=6.9 Hz, CH), 7.81 (2H, t, J=8.1 Hz, Ar-H), 8.14 (2H, d, J=6.9 Hz, Ar-H), 9.12 (2H, d, J=8.7 Hz, Ar-H), 12.94 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.90 (C-9,10), 169.40 (NCO-1′,5′), 140.70 (C-1,5), 135.45 (C-4a,8a), 134.21 (C-3,7), 125.86 (C-5a,9a), 123.08 (C-4,8), 114.66 (C-2,6), 55.54 (CH), 22.05 (CH_3). IR (KBr): 1680, 1705, 3192 cm^{-1}. EI-MS *m*/*z*: 418 (M^+), 355, 264.7.

1,5-Bis(2-ethylaminopropionamido)anthraquinone (12) 86% yield. mp 217 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (6H, t, J=6.9, CH_3), 1.47 (6H, d, J=5.7, CH_3), 2.74 (4H, q, J=5.7, 1.5 Hz, CH_2), 3.40 (2H, q, J=6.9 Hz, CH), 7.76 (2H, t, J=8.1 Hz, Ar-H), 8.10 (2H, dd, J=6.3, 1.2 Hz, Ar-H), 9.21 (2H, dd, J=7.2, 1.2 Hz, Ar-H), 13.08 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.01 (C-9,10), 176.35 (NCO), 141.36 (C-1,5), 135.44 (C-4a,8a), 134.98 (C-3,7), 126.14 (C-5a,9a), 122.60 (C-4,8), 118.06 (C-2,6), 59.92 (CH), 43.32 (CH_2), 20.12 (CH_3), 15.31 (CH_3). IR (KBr): 1666, 2947, 3339 cm^{-1}. PI-EI-MS *m*/*z*: 436 (M^+), 365, 266.

1,5-Bis(2-propylaminopropionamido)anthraquinone (13) 70% yield. mp 203 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (6H, t, J=7.8, CH_3), 1.33—1.40 (4H, m, CH_2), 1.44 (4H, d, J=6.9 Hz, CH_2), 1.58—1.69 (4H, m, CH_2), 2.66—2.71 (4H, t, J=6.9 Hz, CH_2), 3.38 (2H, q, J=6.9 Hz, CH), 7.76 (2H, t, J=8.1 Hz, Ar-H), 8.10 (2H, dd, J=6.3, 1.2 Hz, Ar-H), 9.21 (2H, dd, J=7.5, 1.2 Hz, Ar-H), 13.05 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.97 (C-9,10), 176.38 (NCO), 141.35 (C-1,5), 135.42 (C-4a,8a), 134.97 (C-3,7), 126.14 (C-5a,9a), 122.56 (C-4,8), 118.04 (C-2,6), 60.02 (CH), 50.95 (CH_2), 23.29 (CH_2), 20.09 (CH_3), 11.71 (CH_3). IR (KBr): 1671, 2851, 3157, 3320 cm^{-1}. PI-EI-MS *m*/*z*: 464 (M^+), 463, 435, 379, 266.

1,5-Bis(2-butylaminopropionamido)anthraquinone (14) 70% yield. mp 196 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (6H, t, J=7.8 Hz, CH_3), 1.44 (6H, d, J=6.9 Hz, CH_3), 1.54—1.69 (6H, m, CH_2), 1.63—1.68 (4H, m, CH_2), 2.66 (4H, t, J=6.9 Hz, CH_2), 3.38 (2H, q, J=6.9 Hz, CH), 7.76 (2H, t, J=8.1 Hz, Ar-H), 8.10 (2H, dd, J=6.3, 1.2 Hz, Ar-H), 9.21 (2H, dd, J=7.5, 1.2 Hz, Ar-H), 13.05 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.42 (C-9,10), 176.78 (NCO), 141.80 (C-1,5), 135.88 (C-4a,8a), 135.42 (C-3,7), 126.59 (C-5a,9a), 122.99 (C-4,8), 118.50 (C-2,6), 60.53 (CH), 49.18 (CH_2), 32.72 (CH_2), 20.82 (CH_2), 20.53 (CH_3), 14.42 (CH_3). IR (KBr): 1642, 3444 cm^{-1}. EI-MS (APCI) *m*/*z*: 493.1 (M^+), 491.4, 267.

1,5-Bis(2-pentylaminopropionamido)anthraquinone (15) 55% yield. mp 208 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, t, J=7.2 Hz, CH_3), 1.24—1.52 (18H, m, CH_2, CH_3), 2.67 (4H, t, J=7.2 Hz, CH_2), 3.38 (2H, q, J=6.9 Hz, CH), 7.76 (2H, t, J=8.4 Hz, Ar-H), 8.09 (2H, d, J=7.8 Hz, Ar-H), 9.22 (2H, d, J=8.4 Hz, Ar-H), 13.07 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.96 (C-9,10), 176.40 (NCO), 141.36 (C-1,5), 135.44 (C-4a,8a), 135.44 (C-3,7), 126.14 (C-5a,9a), 122.56 (C-4,8), 118.41 (C-2,6), 60.11 (CH), 49.08 (CH_2), 29.85 (CH_2), 29.47 (CH_2), 22.62 (CH_2), 20.14 (CH_3), 14.03 (CH_3). IR (KBr): 1647, 1680, 2918, 3425 cm^{-1}. PI-EI-MS *m*/*z*: 520 (M^+), 407, 266.

1,5-Bis(2-hexylaminopropionamido)anthraquinone (16) 70% yield. mp 184 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.84 (6H, t, J=6.6 Hz, CH_3), 1.27—1.68 (22H, m, CH_2, CH_3), 2.67 (t, 4H, J=7.2 Hz, CH_2), 3.38 (q, 2H, J=6.9, CH), 7.76 (t, 2H, J=8.1 Hz, Ar-H), 8.09 (d, 2H, J=7.5 Hz, Ar-H), 9.22 (d,

2H, J=8.4 Hz, Ar-H), 13.06 (s, 2H, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.36 (C-9,10), 176.83 (NCO), 141.75 (C-1,5), 135.86 (C-4a,8a), 135.34 (C-3,7), 126.55 (C-5a,9a), 122.99 (C-4,8), 118.41 (C-2,6), 60.52 (CH), 49.54 (CH_2), 32.20 (CH_2), 30.53 (CH_2), 27.36 (CH_2), 23.04 (CH_2), 20.57 (CH_3), 14.45 (CH_3). IR (KBr): 1680, 2918, 3435 cm^{-1}. APCI-EI-MS m/z: 549 (M^+), 281, 266.

1,5-Bis(*N*,*N*-dimethylaminopropylpropionamido)anthraquinone (17) 40% yield. mp 190 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (12H, t, J=7.8 Hz, CH_3), 1.33—1.40 (4H, m, CH_2), 1.44 (4H, d, J=6.9, CH_2), 1.58—1.69 (4H, m, CH_2), 2.74 (4H, t, J=6.9 Hz, CH_2), 3.38 (2H, q, J=6.9 Hz, CH), 7.75 (2H, t, J=8.4 Hz, Ar-H), 8.07 (2H, dd, J=7.8, 1.2 Hz, Ar-H), 9.21 (2H, d, J=7.5 Hz, Ar-H), 13.08 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.91 (C-9,10), 176.36 (NCO), 141.35 (C-1,5), 135.43 (C-4a,8a), 134.89 (C-3,7), 126.11 (C-5a,9a), 122.51 (C-4,8), 117.96 (C-2,6), 60.09 (CH), 58.09 (CH_2), 47.65 (CH2), 45.56 (CH_3), 28.00 (CH_3), 20.05 (CH_3). IR (KBr): 1661, 2938, 3430 cm^{-1}. PI-EI-MS m/z: 550 (M^+), 505, 492, 450, 129.

1,5-Bis(2-isopropylaminopropionamido)anthraquinone (18) 65% yield. mp 271—273 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.17 (6H, d, J=8.7 Hz), 1.34 (6H, d, J=6.6 Hz, CH_3), 1.43 (6H, d, J=7.2 Hz, CH_3), 2.85—2.93 (2H, m, CH), 3.46 (2H, q, J=7.2 Hz, CH), 7.75 (2H, t, J=8.1 Hz, Ar-H), 8.08 (2H, d, J=7.8 Hz, Ar-H), 9.21 (2H, d, J=8.4 Hz, Ar-H), 13.13 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 185.81 (C-9,10), 177.12 (NCO), 141.22 (C-1,5), 135.38 (C-4a,8a), 134.92 (C-3,7), 125.99 (C-5a,9a), 122.50 (C-4,8), 117.97 (C-2,6), 57.41 (CH), 48.40 (CH), 23.26 (CH_3), 22.87 (CH_3), 20.69 (CH_3). IR (KBr): 1662, 2940, 3420 cm^{-1}. PI-EI-MS m/z: 464 (M^+), 266.

1,5-Bis(methylacetamido)anthraquinone (19) 45% yield. mp 321 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 2.33 (6H, s, CH_3), 7.76 (2H, t, J=8.7 Hz, Ar-H), 8.03 (2H, d, J=7.8 Hz, Ar-H), 9.12 (2H, d, J=8.7 Hz, Ar-H), 12.25 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.61 (C-9,10), 169.89 (NCO-1′,5′), 142.04 (C-1,5), 135.90 (C-4a,8a), 134.57 (C-3,7), 126.15 (C-5a,9a), 122.48 (C-4,8), 116.96 (C-2,6), 25.70 (CH_3). IR (KBr): 1637, 1702, 3209 cm^{-1}. APCI-EI-MS m/z: 323 (M^+), 281, 238.

1,5-Bis(ethylacetamido)anthraquinone (20) 70% yield. mp 265 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (6H, t, J=7.5 Hz, CH_3), 2.57 (4H, q, J=7.5 Hz, CH_2), 7.75 (2H, t, J=8.1 Hz, Ar-H), 8.01 (2H, dd, J=6.6, 1.2 Hz, Ar-H), 9.14 (2H, dd, J=7.2, 1.2 Hz, Ar-H), 12.26 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.62 (C-9,10), 173.79 (NCO-1′,5′), 142.15 (C-1,5), 135.87 (C-4a,8a), 134.57 (C-3,7), 126.17 (C-5a,9a), 122.39 (C-4,8), 116.96 (C-2,6), 31.94 (CH_2), 9.52 (CH_3). IR (KBr): 1656 cm^{-1}. EI-MS m/z: 350 (M^+), 294, 265, 238.

1,5-Bis(3-methylbenzamido)anthraquinone (21) 63% yield. mp 290 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 2.55 (6H, s, CH_3), 7.48—7.52 (6H, m, Ar-H), 7.89 (2H, t, J=7.5 Hz, Ar-H), 7.99 (2H, d, J=6 Hz, Ar-H), 8.19 (2H, d, J=6.3 Hz, Ar-H), 9.40 (2H, d, J=8.7 Hz, Ar-H), 13.24 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.91 (C-9,10), 166.83 (NCO-1′,5′), 138.86 (C), 136.02 (C), 134.51 (C), 133.19 (C), 128.84 (C), 128.53 (CH), 126.44 (CH), 124.59 (CH), 122.84 (CH), 21.51 (CH_3). IR (KBr): 1637, 3245 cm^{-1}. EI-MS m/z: 474 (M^+), 356, 119.

1,5-Bis(cyclohexylamido)anthraquinone (22) 68% yield. mp 296 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.24—1.76 (12H, m, CH_2), 1.86—1.90 (4H, m, CH_2), 2.06—2.10 (4H, m, CH_2), 2.43 (2H, m, CH), 7.76 (2H, t, J=8.4 Hz, Ar-H), 8.04 (2H, d, J=7.8 Hz, Ar-H), 9.17 (2H, d, J=8.4 Hz, Ar-H), 12.29 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.73 (C-9,10), 176.28 (NCO), 142.40 (C-1,5), 135.82 (C-4a,8a), 134.67 (C-3,7), 126.34 (C-5a,9a), 122.39 (C-4,8), 117.14 (C-2,6), 47.47 (CH), 29.69 (CH_2), 25.82 (CH_2), 25.78 (CH_2). IR (KBr): 1640, 1690, 2856, 2931 cm^{-1}. APCI-EI-MS m/z: 459 (M^+), 441, 439, 252, 238.

1,5-Bis(cyclopentanepropylamido)anthraquinone (23) 72% yield. mp 221 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.18—1.23 (8H, m, CH_2), 1.54—1.63 (8H, m, CH_2), 1.54—1.63 (2H, m, CH), 1.81 (4H, q, J=5.7 Hz, CH_2), 2.55 (4H, t, J=8.1 Hz, CH_2), 7.76 (2H, t, J=8.4 Hz, Ar-H), 8.03 (2H, dd, J=1.2, 7.8 Hz, Ar-H), 9.15 (2H, d, J=8.1 Hz, Ar-H), 12.27 (s, 2H, NH). ^{13}C-NMR ($CDCl_3$) δ: 187.10 (C-9,10), 173.79 (NCO), 142.60 (C-1,5), 136.29 (C-4a,8a), 135.07 (C-3,7), 126.64 (C-5a,9a), 122.84 (C-4,8), 117.14 (C-2,6), 40.18 (CH_2), 38.69 (CH_2), 32.99 (CH_2), 32.15 (CH), 25.65 (CH_2). IR (KBr): 1698, 3190 cm^{-1}. APCI-EI-MS m/z: 487 (M^+), 453, 431, 363, 285.

1,5-Bis(cyclopentylamido)anthraquinone (24) 77% yield. mp 258 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.66—1.70 (4H, m, CH_2), 1.79—1.84 (4H, m, CH_2), 1.93—1.98 (4H, m, CH_2), 2.05—2.09 (4H, m, CH_2), 2.88—2.93 (4H, m, CH_2), 7.76 (2H, t, J=8.1 Hz, Ar-H), 8.02 (2H, dd, J=7.8, 1.2 Hz, Ar-H), 9.16 (2H, dd, J=8.7, 1.2 Hz, Ar-H), 12.32 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.67 (C-9,10), 176.35 (NCO), 142.37 (C-1,5), 135.82 (C-4a,8a), 134.63 (C-3,7), 126.20 (C-5a,9a), 122.31 (C-4,8), 116.99 (C-2,6), 48.15 (CH), 30.44 (CH_2), 25.97 (CH_2). IR (KBr): 1685, 3205 cm^{-1}. APCI-EI-MS m/z: 430.9 (M^+), 335, 317.

1,5-Bis(cyclopropylamido)anthraquinone (25) 75% yield. mp 328 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 0.92—0.98 (4H, m, CH_2), 1.12—1.17 (4H, m, CH_2), 1.75—1.79 (2H, m, CH), 7.75 (2H, t, J=8.1 Hz, Ar-H), 8.02 (2H, dd, J=6.6, 1.2 Hz, Ar-H), 9.11 (2H, d, J=9 Hz, Ar-H), 12.52 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.28 (C-9,10), 173.24 (NCO), 141.81 (C-1,5), 135.44 (C-4a,8a), 134.23 (C-3,7), 125.82 (C-5a,9a), 121.85 (C-4,8), 116.40 (C-2,6), 16.64 (CH), 8.29 (CH_2). IR (KBr): 1680, 2342, 2368, 3454 cm^{-1}. EI-MS m/z: 374.2 (M^+), 306.3, 238.4.

1,5-Bis(trans-2-pheny-1-cyclopropanecarboxamido)anthraquinone (26) 75% yield. mp 249 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 1.43—1.49 (4H, m, CH_2), 1.75—1.78 (2H, m, CH), 1.99—2.02 (2H, m, CH), 7.16—7.33 (10H, m, CH) 7.75 (2H, t, J=8.4 Hz, Ar-H), 8.01 (2H, d, J=7.8 Hz, Ar-H), 9.15 (2H, d, J=8.4 Hz, Ar-H), 12.55 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.64 (C-9,10), 171.97 (NCO), 142.09 (C-1,5), 140.17 (C), 135.44 (C-4a,8a), 134.60 (C-3,7), 128.60 (CH), 126.59 (CH), 126.24 (CH), 126.32 (C-5a,9a), 122.44 (C-4,8), 116.58 (C-2,6), 28.56 (CH), 26.74 (CH), 17.19 (CH_2). IR (KBr): 1633, 1676, 3224, 3435 cm^{-1}. APCI-EI-MS m/z: 527.1 (M^+), 509.5, 383.2, 365.4.

1,5-Bis(phenylthioacetamido)anthraquinone (27) 70% yield. mp 197 °C (EA). ^{1}H-NMR ($CDCl_3$) δ: 3.87 (4H, s, CH_2), 7.17 (2H, t, J=6.9 Hz, Ar-H), 7.26 (2H, t, J=7.2 Hz, Ar-H), 7.45 (2H, d, J=7.8 Hz, Ar-H), 7.75 (2H, t, J=5.1 Hz, Ar-H), 8.06 (2H, d, J=7.8 Hz, Ar-H), 9.09 (2H, d, J=8.4 Hz, Ar-H), 12.92 (2H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 186.16 (C-9,10), 168.80 (NCO), 141.27 (C-1,5), 135.68 (C-4a,8a, SC), 134.66 (C-3,7), 129.81 (C-5a,9a), 129.26 (C-4,8), 127.11 (CH), 126.25 (CH), 123.09 (CH,C-2,6), 40.27 (CH_2). IR (KBr): 1614, 3301, 3416 cm^{-1}. EI-MS m/z: 538 (M^+), 428.2, 415.2, 388.2, 305.4, 238.4.

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. The 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 in complete medium before addition to cell cultures. All data presented in this report are from 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 in 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 at 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 was 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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