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Synthesis and structure–activity correlations of the cytotoxic bifunctional 1,4-diamidoanthraquinone derivatives

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Abstract—Anthraquinone-based compounds are attractive target for the design of new anticancer drugs. We have previously described a series of 1,5- and 1,4-difunctionalized anthraquinones, which exhibit different spectra of potency, together with human telomerase evaluation. The present study details the preparation of further, distinct series of regioisomeric difunctionalized amidoanthraquinone and examines their in vitro cytotoxicity in C6, Hepa G2, and 2.2.15 cell lines. Two structurally related compounds, mitoxantrone and adriamycin, were tested in parallel as positive controls. The structure–activity relationships indicated amido substitution may lead to a different mechanism of cytotoxicity. Compounds, which have $-(CH_2)_n-$ side chains terminating in basic groups such as aminoalkyl-substituted, showed cytotoxic activity in several cell lines. The exact mode of intercalative binding may be dictated by the positional placement of substituent side chains. Implications for amidoanthraquinone cytotoxicity as potential anticancer agents are discussed. In addition, we further delineate the nature of the pharmacophore for this class of compounds, which provides a rational basis for the structure–activity relationships.

1. Introduction

There is continuing interest in the development of new agents in which the small-molecule tricyclic anthraquinone structural motif represents an attractive target for the rational design of new anticancer agents due to its central role in the control of cellular proliferation.[1–6] An area of particular activity and promise is concerned with aminoalkyl-substituted anthraquinone and the demonstration of clinical activity for the two structurally related 1,4-disubstituted compounds mitoxantrone and ametantrone.[7–10] Moreover, a reversal in the direction of mitoxantrone dipole moment occurs in the amido congeners. This may explain the lack of recognition of cleavable topoisomerase II-DNA complex and the loss of cleavage stimulation.[11] However, a more urgent need is apparent from the common experience of clinically limiting toxicities of most anticancer drugs, that is, the necessity to develop less toxic clinical drug candidates. Thus, medicinal chemists have turned toward analog development involving certain anthraquinones.[12]

We recently described the human telomerase activation properties of a series of disubstituted anthraquinones (1,4- and 1,5-isomers)[3] and proposed that their activity may be due to their ability to bind to and stabilize G-quadruplex structures.[5,13] The inhibition of telomerase by molecules such as disubstituted amidoanthraquinones is believed to be due to their stabilization of guanine-quadruplex complexes.[14] Computer modeling and energy calculations have shown that some amidoanthraquinones can bind intercalatively to DNA and that there are significant differences in the additional non-bonded and electrostatic interactions possible at the DNA binding site. Solution DNA binding and closed-circular DNA unwinding studies confirmed intercalative interactions, and the predicted differences in strength of interactions between mono- and disubstituted compounds have been found.[8] This may be due to marked differences in the nature of chromophore-base stacking and groove accessibility for these series compounds. The experimental model results are in accord with the predicted behavior and confirm that the 1,4-series bind preferentially to double- rather than triple-stranded

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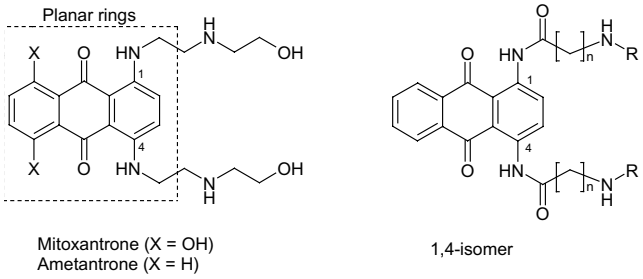


Chart 1.

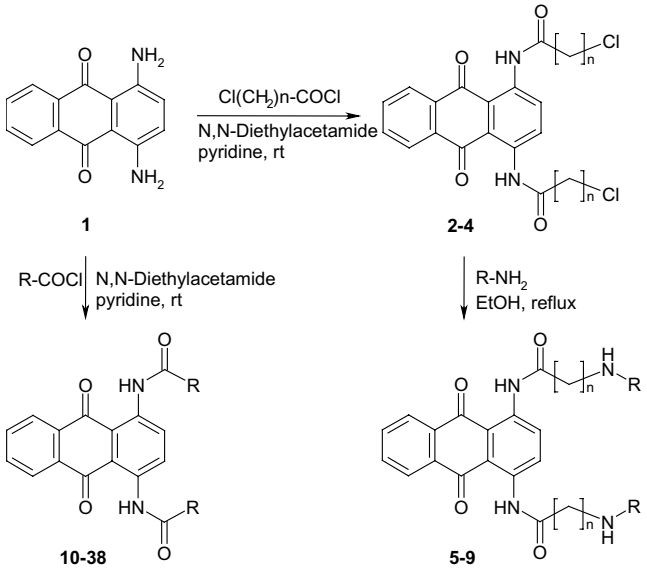
DNA.[15] Although the precise mode of action in vitro or in vivo for these compounds has not been fully elucidated, a number of studies have shown that DNA is a major biological target for mitoxantrone.[6,8,16–19] Triplex stabilization could be achieved with a series of disubstituted amidoanthraquinones, which are able to discriminate between duplex and triplex DNA by virtue of their structural characteristics.[15] Earlier studies by Palumbo et al.,[20,21] Keppler et al.,[19,22] and Collier and Neidle[8] have shown direct and indirect relationships between DNA-binding parameters and at least some biological effects for a series of mono- and disubstituted amidoanthraquinones. Hence, amido substitution may lead to a different mechanism of cytotoxicity, not related to classical protein or free radical mediated DNA damage, which points to a novel type of anticancer pharmacophore (Chart 1).

The in vitro cytotoxicity of these compounds have been reported and compared with mitoxantrone and adriamycin, which were tested in parallel as positive controls. We used XTT method to measure the relative cytotoxicity of 1,4-disubstituted amidoanthraquinone compounds, and have examined how the attached base-functionalized substituents affects their cytotoxicity to several tumor cell lines. Of the compounds studied, the 1,4-diaminoalkyl-substituted amidoanthraquinone displayed better in vitro cytotoxicity. These results have been confirmed by previous molecular modeling studies,[19] which provide a rational basis for the structure–activity relationships. These suggest that, although all of the compounds bind through an intercalative mode, the 1,5-disubstituted isomers bind with their two side groups occupying adjacent triplex grooves, in contrast with the other isomer, which is positioned with both side groups in the same triplex groove. This inhibitory ability also provides an indication of selective tumor cell lines inhibition. A range of side chains has been examined to establish SAR as a basis for subsequent rational drug design.

2. Results and discussion

2.1. Synthetic approach

A series of 1,4-difunctionalized amidoanthraquinones have been prepared, with side chains having basic nitrogen atoms. Compounds **5**–**9** were synthesized by a two-stage reaction. The first stage involved acylation of the



Scheme 1.

1,4-diaminoanthraquinone with chloro-acyl chloride, in an inert *N*,*N*-diethylacetamide as a solvent, with a catalytic quantity of pyridine, producing the 1,4-bis-(ω-chloroalkaneamido) side chain compounds **2**–**4** in essentially quantitative yield. Further condensation of these intermediates with the appropriate amines in ethanol gave the desired disubstituted anthraquinones **5**–**9**. Compounds **10**–**38** were synthesized by direct one-stage acylation reaction. Müller and Prinz[23] have shown that electrophilic additions or substitutions and nucleophilic additions occur at different oxidation states of anthraquinones and the reactions are complementary from a preparative viewpoint. These compounds were obtained in good yield and determined to be pure by mass spectrometry, ^{1}H NMR, and ^{13}C NMR. The amide group is coplanar with the anthraquinone ring, and there is an intramolecular hydrogen bond between the amide hydrogen atom and the quinoid oxygen atom on the ring.[8] These heteroatoms have a significant effect on the anthraquinone moiety, influencing the electron density and thus affecting π-electron interaction with DNA bases and also electron-accepting ability of the quinonoid system, essential for the metabolic activation of the molecule. This amidation causes the strong diminishment of π-electron density in the quinone moiety[24] (Scheme 1).

2.2. Biological activity tests

The cytotoxic behavior of the compounds were evaluated against a panel of murine tumor cell line and human hepatoma cell lines (heap G2 and 2.2.15) using the XTT assay, as described previously.[25,26] Results are presented in Table 1 as the concentrations required to inhibit cell growth by 50% (IC_{50} values). Prior to the evaluation of 1,5-isomers in XTT assay and telomerase assay,[3,25,26] the agents were tested for their cytotoxicity and dual telomerase activity. As part of work in our laboratories on the use of anthraquinones as anticancer agents, a number of bis-(amino)-substituted[27]

Table 1. Chemical yields of 1,4-diamidoanthraquinones (**2**–**38**), and their cytotoxicity against suspended murine and human tumor cell lines

Compound	R	Yield (%)	IC_{50} (μM)[a]		
			C6[b]	Hepa G2[c]	2.2.15[d]
2	CH_2Cl	77	8.51 ± 0.08	27.41 ± 1.32	51.51 ± 2.21
3	CH_2CH_2Cl	78	20.62 ± 0.96	32.27 ± 0.74	31.36 ± 0.87
4	$CHClCH_3$	76	3.81 ± 0.07	35.37 ± 0.54	71.17 ± 1.92
5	$CH_2N(CH_2CH_3)_2$	50	0.60 ± 0.10	1.43 ± 0.24	1.98 ± 0.01
6	$CH_2CH_2N(CH_2CH_3)_2$	40	1.06 ± 0.12	0.89 ± 0.02	1.15 ± 0.04
7	$CHCH_3N(CH_2CH_3)_2$	75	1.42 ± 0.33	13.49 ± 0.39	16.74 ± 1.63
8	$CHCH_3NHCH_2CH(CH_2)_2$	63	5.24 ± 0.12	10.62 ± 0.30	15.42 ± 0.28
9	CH_2CH_2 $NHCH_2CH(CH_2)_2$	63	0.40 ± 0.09	1.01 ± 0.01	6.04 ± 0.59
10	CH_3	92	19.12 ± 0.84	57.36 ± 3.24	108.3 ± 3.74
11	Cyclopropane	76	13.25 ± 0.03	0.53 ± 0.01	2.61 ± 0.03
12	Cyclopentane	80	22.82 ± 1.82	46.62 ± 1.07	43.57 ± 1.14
13	Cyclohexane	82	29.51 ± 2.17	44.57 ± 1.78	47.25 ± 1.24
14	$CH_2CH_2C_5H_9$	87	24.41 ± 1.94	57.05 ± 1.96	64.28 ± 2.14
15	2-Thiophenyl	79	17.39 ± 0.07	3.24 ± 0.03	9.33 ± 0.05
16	2-Furyl	63	11.70 ± 0.03	18.44 ± 1.36	23.51 ± 0.17
17	2-Thiopheneacetyl	75	8.26 ± 0.03	13.33 ± 0.92	13.21 ± 0.06
18	C_6H_5	83	10.15 ± 1.21	53.12 ± 2.17	39.54 ± 1.92
19	3-$CH_3C_6H_4$	82	25.52 ± 1.07	103.84 ± 3.17	86.17 ± 4.67
20	2-FC_6H_4	83	23.08 ± 0.97	44.42 ± 0.57	100.26 ± 4.13
21	3-FC_6H_4	75	13.12 ± 0.76	54.34 ± 1.27	94.20 ± 3.47
22	4-FC_6H_4	88	9.48 ± 0.03	10.42 ± 0.72	12.31 ± 0.61
23	2-ClC_6H_4	82	25.16 ± 0.12	27.23 ± 1.78	93.10 ± 3.62
24	3-ClC_6H_4	85	21.24 ± 1.56	29.75 ± 0.32	21.56 ± 0.84
25	4-ClC_6H_4	84	24.02 ± 1.02	31.33 ± 1.04	18.86 ± 0.92
26	2-$NO_2C_6H_4$	70	73.68 ± 2.12	40.88 ± 0.62	48.31 ± 1.07
27	4-$CF_3C_6H_4$	78	24.51 ± 0.62	58.11 ± 3.01	36.57 ± 2.91
28	2,5-$(CF_3)_2C_6H_3$	87	0.49 ± 0.05	6.51 ± 0.07	6.57 ± 0.06
29	2,4-$F_2C_6H_3$	66	22.33 ± 1.67	15.23 ± 0.92	18.62 ± 0.92
30	2,4-$Cl_2C_6H_3$	80	22.16 ± 0.86	32.93 ± 0.32	34.82 ± 0.62
31	2,4,6-$Cl_3C_6H_2$	60	0.71 ± 0.21	10.26 ± 0.13	10.95 ± 0.06
32	2,3,6-$F_3C_6H_2$	84	20.62 ± 0.68	25.92 ± 0.84	25.82 ± 0.66
33	2,4,5-$F_3C_6H_2$	70	17.91 ± 1.13	17.41 ± 0.37	19.43 ± 1.02
34	2,3-Cl_2-5-FC_6H_2	77	103.58 ± 0.04	10.71 ± 0.11	13.53 ± 0.14
35	*trans*-$CH(CH_2)CHC_6H_5$	68	11.31 ± 0.06	12.86 ± 0.46	21.10 ± 1.67
36	$CH_2SC_6H_5$	84	5.82 ± 0.05	23.46 ± 1.21	13.32 ± 0.05
37	$CH_2C_6H_4F(p)$	85	14.14 ± 0.72	25.88 ± 0.72	16.43 ± 0.04
38	2,5-Dimethylfuryl	79	11.83 ± 0.07	8.12 ± 0.67	21.17 ± 0.21
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 micromolar 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] 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.

and bis-(amido)-substituted derivatives have been synthesized and also fully characterized as potential telomerase inhibitors.

The amidoanthraquinones described here exhibit no cytotoxicity except for **5**–**9**, **11**, **28**, and **31**, which 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). The regioisomeric alkylamino-amido derivatives **5**–**9** were found to be the most potent of the amidoanthraquinone compounds studied. The most potent compounds described here show levels of cytotoxicity that compare favorably with mitoxantrone and adriamycin. Where direct comparisons with analogous 1,4-isomer anthraquinones are possible (Table 1), the levels of cytotoxic activity shown by the substituents of tricyclic derivatives is at first surprising given their structural similarities. However, compounds **5**–**7**, **9**, **28**, and **31** exhibited potent cytotoxic activities on C6 cell lines; **5**, **6**, **9**, and **11** exhibited potent cytotoxic activities on hepaG2 cell lines; and **5**, **6**, and **11** exhibited potent cytotoxic activities on 2.2.15 cell lines.

3. Conclusions

This study has shown that other tricyclic systems in addition to anthraquinone-based molecules can selectively inhibit the growth of tumor cell lines. Furthermore, we also have demonstrated that conventional cytotoxicity may be moderated through rational design

according to the substituent on the small-molecule tricyclic anthraquinone structural motif. However, the difference between the 1,4-isomer and 1,5-isomer[27] has a significant effect on the shape of the chromophore. The 1,5-disubstituted derivative also shows some interaction with duplex DNA, and these results have been confirmed by molecular modeling studies, which provide a rational basis for the structure–activity relationships.[19] This, in turn, affects the angular disposition of the substituents compared to the mitoxantrone anthraquinone, so that a good fit of the disubstituted substituents into the putative binding site can only be achieved with a degree of distortion of the site and a resultant energy cost. The exploitation of rationally derived structure–activity relationships, such as have been found in this study, is likely to be a key to future success.

The aim of this study was to elucidate the structure–activity relationships of simple disubstituted amidoanthraquinones and aminoanthraquinones to further delineate the nature of the requirements of the pharmacophore. A striking feature of the results given in Table 1 is the marked cytotoxicity between the three different tumor cell lines. Anthraquinone derivatives are known for their antitumor activities. Also, these effects are very sensitive to slight modifications of anthraquinone substituents. In light of these findings it is suggested that cytotoxicity in cell lines is not sufficient for potent antiproliferative action. Finally, the effects of anthraquinones on the cytotoxicity in cancer cells are very diverse, so a simple correlation between the modifications of anthraquinones with their biological effects only can be achieved when concerned with aminoalkyl-substituted anthraquinone. Thus, it appears that the anthraquinone chromophore itself causes the nature of minimum requirements of the pharmacophore of cytotoxicity. Whatever be the molecular mechanism of the cytotoxicity of anthraquinone derivatives and wherever be its locus, the results described herein indicate that it is sensitive to the slightest modification in the structure of anthraquinones. However, the molecular mechanisms behind these diversified biological effects are unclear. Thus, a early decision in our attempts to develop new therapeutically effective anticancer drugs was to keep the anthraquinone unchanged[3,25,26] because these structural portions may be important for the pharmacophore. It now appears that the proposed structural pattern hypothesis has been substantiated by the experimental results obtained from the present paper. This unique property should definitely be noted in future drug design; and the potential application of these compounds in tumor biology, stem cell research, and tissue engineering has made them a group of compounds that are worth extensive study.

4. Experimental

4.1. Materials

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 parts per million 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.

4.2. General procedure for the preparation of 1,4-diamidoanthraquinones

4.2.1. Method A (compounds 2–9). Chloroacetylchloride (12 mmol) was added dropwise at 0 °C under N_2 to a solution of 1,4-diaminoanthraquinone (4 mmol) and pyridine (0.5 mL) in *N*,*N*-diethylacetamide (70 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/*n*-hexane. A solution of an appropriate amines (20 mmol) in ethanol was added dropwise under N_2 to a suspended solution of **2**–**4** (1 mmol) in 70 mL of ethanol. The reaction mixture was refluxed for 18 h. The solvent was removed under reduced pressure to yield dark brown solids and the resulting precipitate was collected by filtration, washed with diethyl ether, and purified by crystallization from ethyl acetate/*n*-hexane.

4.2.2. Method B. Acyl chlorides (12 mmol) were added dropwise at 0 °C under N_2 blanketing to a solution of 1,4-diaminoanthraquinone (4 mmol) and pyridine (0.5 mL) in *N*,*N*-diethylacetamide (70 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/*n*-hexane.

4.2.3. 1,4-Bis(chloroacetamido)anthraquinone (2). Yield: 77%; mp 285–287 °C (chloroform); ^{1}H NMR (DMSO): δ 4.60 (4H, s, CH_2), 7.97–8.00 (2H, m, H-6,7), 8.24–8.27 (2H, m, H-5,8), 8.99 (2H, s, H-2,3), 12.80 (2H, s, NH); IR (KBr): 1595, 1650 cm^{-1}; UV λ_{max} (MeOH): 486.0 (1.52); MS *m*/*z*: 390 (100%), 341 (95%), 237 (61%).

4.2.4. 1,4-Bis(3-chloropropionamido)anthraquinone (3).[8] Yield: 78%; mp 230–231 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 3.04–3.08 (4H, t, J = 6.6 Hz, CH_2), 3.96–4.00 (4H, t, J = 6.5 Hz, CH_2), 7.87–7.97 (H, m, H-6,7), 8.32–8.35 (2H, m, H-5,8), 9.23 (2H, s, H-2,3), 12.72 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 39.3 (CH_2), 41.52 (CH_2), 116.95 (CH), 127.17 (C), 129.12 (CH), 133.23 (CH), 134.56 (C), 138.12 (C), 169.08 (C), 186.98 (C); IR (KBr): 1600, 1650, 1710 cm^{-1}; UV λ_{max} (MeOH): 465.0 (1.04); MS *m*/*z*: 418 (68%), 328 (92%), 238 (100%).

4.2.5. 1,4-Bis(2-chloropropionamido)anthraquinone (4). Yield: 76%; mp 233–235 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.91–1.94 (6H, d, J = 7.2 Hz, CH_3), 4.62–

4.69 (2H, q, $J = 7.7$ Hz, CH), 7.86–7.89 (2H, m, H-6,7), 8.36–8.39 (2H, m, H-5,8), 9.21 (2H, s, H-2,3), 13.24 (2H, s); ^{13}C NMR ($CDCl_3$): δ 22.44 (CH_3), 55.09 (CH), 118.10 (CH), 127.30 (C), 128.71 (CH), 133.05 (CH), 134.55 (C), 137.83 (C), 169.67 (C), 186.76 (C); IR (KBr): 3155, 1648 cm^{-1}; UV λ_{max} (MeOH): 459.0 (0.51); MS *m*/*z*: 418 (63%), 355 (100%), 292 (36%).

4.2.6. 1,4-Bis[2-(diethylamino)acetamido]anthraquinone (5). Yield: 50%; mp 85–86 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.50–1.55 (12H, t, $J = 8.4$ Hz, CH_3), 3.05–3.08 (8H, s, CH_2), 4.29–4.32 (4H, s, $COCH_2$), 7.87–7.90 (2H, m, H-6,7), 8.36–8.39 (4H, m, H-5,8), 9.22 (2H, s, H-2,3), 13.28 (2H, s, NH); IR (KBr): 3365, 1688 cm^{-1}; UV λ_{max} (MeOH): 525.0 (0.50); MS *m*/*z*: 463 (55%), 237 (31%).

4.2.7. 1,4-Bis[3-(diethylamino)propionamido]anthraquinone (6).[27] Yield: 40%; mp 169–171 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 2.76–2.80 (12H, t, $J = 7.6$ Hz, CH_3), 3.04–3.12 (8H, m, CH_2), 3.21–3.25 (8H, m, CH_2), 7.80–7.86 (2H, m, H-6,7), 8.23–8.31 (2H, m, H-5,8), 9.01 (2H, s, H-2,3), 12.68 (2H, br, NH); IR (KBr): 3160, 1636 cm^{-1}; UV (MeOH): 485.0 (0.57); MS *m*/*z*: 491 (45%), 237 (58%).

4.2.8. 1,4-Bis[2-(diethylamino)propionamido]anthraquinone (7). Yield: 75%; mp 175–177 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.49–1.53 (12H, t, $J = 7.5$ Hz, CH_3), 1.90–1.94 (8H, m, CH_2), 3.06–3.08 (6H, d, $J = 8.0$ Hz, CH_3), 4.59–4.66 (2H, m, CH), 7.76–7.86 (2H, m, H-6,7), 8.32–8.37 (2H, m, H-5,8), 8.93–8.96 (2H, d, $J = 8.0$ Hz, H-2,3), 13.23 (2H, br, NH); IR (KBr): 3390, 1645 cm^{-1}; UV (MeOH): 546 (0.9); MS *m*/*z*: 491 (48%), 237 (65%).

4.2.9. 1,4-Bis[2-(cyclopropylmethylamino)propionamido]-anthraquinone (8). Yield: 63%; mp 172–174 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.90–1.94 (10H, t, $J = 6.6$ Hz), 4.61–4.66 (2H, m, CH), 7.78–7.88 (2H, m, H-6,7), 8.32–8.38 (2H, m, H-5,8), 8.93–8.96 (2H, d, $J = 8.0$ Hz, H-2,3), 13.24 (2H, br, NH); IR (KBr): 3170, 1660 cm^{-1}; UV (MeOH): 489.0 (0.72); MS *m*/*z*: 487 (58%), 341 (45%), 307 (38%), 237 (55%).

4.2.10. 1,4-Bis[3-(cyclopropylmethylamino)propionamido]-anthraquinone (9). Yield: 63%; mp 208–210 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 0.90–0.92 (10H, t, $J = 7.5$ Hz, CH_2), 1.90–1.94 (4H, t, $J = 7.5$ Hz, CH_2), 4.59–4.66 (10H, m), 7.77–7.86 (2H, m, H-6,7), 8.33–8.38 (2H, m, H-5,8), 8.94–8.97 (2H, d, $J = 9.6$ Hz, H-2,3), 13.23 (2H, br, NH); IR (KBr): 3325, 1650 cm^{-1}; UV (MeOH): 525.0 (0.29); MS *m*/*z*: 487 (41%), 237 (75%).

4.2.11. 1,4-Bis(acetamido)anthraquinone (10). Yield: 92%; mp 278–280 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 2.38 (6H, s, CH_3), 7.85–7.88 (2H, m, H-6,7), 8.31–8.34 (2H, m, H-5,8), 9.20 (2H, s, H-2,3), 12.56 (2H, s); ^{13}C NMR ($CDCl_3$): δ 25.70 (CH_3), 116.05 (CH), 127.02 (C), 129.05 (CH), 133.33 (CH), 134.36 (C), 138.49 (C), 169.71 (C), 186.88 (C); IR (KBr): 3370, 1610 cm^{-1}; UV (MeOH): 465.0 (0.25); MS *m*/*z*: 321 (92%), 280 (66%), 237 (70%).

4.2.12. 1,4-Bis(cyclopropylamido)anthraquinone (11). Yield: 76%; mp 281–282 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 0.98–1.02 (8H, m), 1.79–1.84 (2H, m), 7.85–7.88 (2H, m, H-6,7), 8.33–8.36 (2H, m, H-5,8), 9.19 (2H, s, H-2,3), 12.86 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 8.51 (CH_2), 17.00 (CH_2), 116.26 (CH), 127.01 (C), 129.22 (CH), 133.48 (CH), 134.22 (C), 138.63 (C), 173.40 (C), 186.88 (C); IR (KBr): 3315, 1680 cm^{-1}; UV (MeOH): 490.0 (0.64); MS *m*/*z*: 374 (87%), 306 (51%), 238 (100%).

4.2.13. 1,4-Bis(cyclopentylamido)anthraquinone (12). Yield: 80%; mp 243–244 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.90–1.99 (8H, m), 2.01–2.17 (8H, m), 2.90–3.01 (2H, d, $J = 8.1$ Hz), 7.82–7.88 (2H, m, H-6,7), 8.29–8.34 (2H, m, H-5,8), 9.22 (2H, s, H-2,3), 12.63 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 25.88 (CH_2), 29.62 (CH_2), 30.37 (CH_2), 48.13 (CH), 116.52 (CH), 127.00 (C), 129.18 (CH), 133.42 (CH), 134.17 (C), 138.77 (C), 176.09 (C), 186.85 (C); IR (KBr): 3375, 1645 cm^{-1}; UV (MeOH): 474.0 (0.50); MS *m*/*z*: 430 (36%), 334 (28%), 238 (100%).

4.2.14. 1,4-Bis(cyclohexylamido)anthraquinone (13). Yield: 82%; mp 309–310 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.91–1.95 (12H, m), 2.11–2.15 (8H, d, $J = 8.0$ Hz), 2.42–2.52 (2H, m), 7.83–7.89 (2H, m, H-6,7), 8.33–8.36 (2H, m, H-5,8), 9.24 (2H, s, H-2,3), 12.61 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 25.72 (CH_2), 29.64 (CH_2), 47.45 (C), 116.66 (CH), 127.04 (C), 129.27 (CH), 133.39 (CH), 134.22 (C), 138.79 (C), 176.08 (C), 186.89 (C); IR (KBr): 3365, 1649 cm^{-1}; UV (MeOH): 470.0 (0.70); MS *m*/*z*: 458 (96%), 348 (47%), 238 (100%).

4.2.15. 1,4-Bis(3-cyclopentanepropylamido)anthraquinone (14). Yield: 87%; mp 166–167 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 1.62–1.72 (4H,d, $J = 5.1$ Hz), 1.86–1.91 (10H, m), 2.58–2.63 (2H, t, $J = 7.5$ Hz, CH_2), 7.85–7.88 (2H, m, H-6,7), 8.33–8.36 (2H, m, H-5,8), 9.24 (2H, s, H-2,3), 12.60 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 25.14 (CH_2), 31.68 (CH_2), 32.50 (CH_2), 38.21 (CH_2), 39.69 (CH_2), 114.98 (CH), 127.03 (CH), 129.15 (CH), 133.38 (CH), 134.26 (CH), 138.60 (C), 173.14 (CH), 186.87 (C); IR (KBr): 3360, 1625 cm^{-1}; UV (MeOH): 469.0 (038); MS *m*/*z*: 486 (47%), 362 (33%), 238 (100%).

4.2.16. 1,4-Bis(2-thiophenylamido)anthraquinone (15). Yield: 79%; mp 321–322 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.26–7.29 (2H, t, $J = 3.1$ Hz), 7.67–7.69 (2H, d, $J = 5.1$ Hz), 7.88–7.91 (2H, m, H-6,7), 8.02–8.03 (2H, t, $J = 2.4$ Hz), 8.39–8.42 (2H, m, H-5,8), 9.38 (2H, s, H-2,3), 13.61 (2H, s, NH); IR (KBr): 3380, 1605 cm^{-1}; UV (MeOH): 514.0 (0.49); MS *m*/*z*: 458 (60%), 111 (100%).

4.2.17. 1,4-Bis(2-furylamido)anthraquinone (16). Yield: 63%; mp 365–367 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$):

δ 6.66–6.67 (2H, d, $J = 3.6$ Hz), 7.39–7.40 (2H, d, $J = 3.6$ Hz), 7.76 (2H, s), 7.87–7.90 (2H, m, H-6,7), 8.41–8.43 (2H, m, H-5,8), 9.42 (2H, s, H-2,3), 13.55 (2H, s, NH); IR (KBr): 3366, 1658 cm^{-1}; UV (MeOH): 578.0 (0.39); MS *m/z*: 427 (100%), 343 (12%), 166 (11%).

4.2.18. 1,4-Bis(2-thiopheneacetamido)anthraquinone (17). Yield: 75%; mp 158–160 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 4.09 (4H, s, CH_2), 7.10–7.13 (2H, t, $J = 4.4$ Hz), 7.17–7.18 (2H, d, $J = 3.3$ Hz), 7.34–7.36 (2H, d, $J = 5.7$ Hz), 7.81–7.84 (2H, m, H-6,7), 8.22–8.25 (2H, m, H-5,8), 9.19 (2H, s, H-2,3), 12.58 (H, s, NH); ^{13}C NMR ($CDCl_3$): δ 39.71 (CH_2), 117.23 (CH), 125.60 (CH), 127.05 (C), 127.22 (CH), 127.68 (CH), 128.91 (CH), 133.14 (CH), 134.29 (C), 134.97 (C), 138.19 (C), 169.67 (C), 186.66 (C); IR (KBr): 3375, 1610 cm^{-1}; UV (MeOH): 486.56 (0.72); MS *m/z*: 486 (19%), 389 (33%), 292 (29%), 265 (40%), 97 (100%).

4.2.19. 1,4-Bis(benzamido)anthraquinone (18). Yield: 83%; mp 285–287 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.64–7.69 (6H, m), 7.89–7.92 (2H, m, H-6,7), 8.23–8.26 (4H, d, $J = 2.1$ Hz), 8.41–8.44 (2H, m, H-5,8), 9.52 (2H, s, H-2,3), 13.62 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 117.15 (CH), 127.22 (C), 127.66 (CH), 128.86 (CH), 129.37 (CH), 132.23 (CH), 133.36 (CH), 134.42 (C), 134.61 (C), 139.05 (C), 166.41 (C), 187.19 (C); IR (KBr): 3340, 1635 cm^{-1}; UV (MeOH): 486.0 (1.76); MS *m/z*: 446 (46%), 105 (100%).

4.2.20. 1,4-Bis(3-methylbenzamido)anthraquinone (19). Yield: 82%; mp 242–244 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 2.57 (6H, s, CH_3), 7.47–7.56 (6H, m), 7.87–7.90 (2H, m, H-6,7), 8.00–8.02 (2H, s), 8.39–8.42 (2H, m, H-5,8), 9.48 (2H, s, H-2,3), 13.54 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 21.09 (CH_3), 117.30 (CH), 124.20 (CH), 126.87 (CH), 128.18 (C), 128.38 (CH), 129.07 (CH), 132.67 (CH), 133.03 (CH), 134.02 (C), 134.27 (CH), 138.39 (C), 138.70 (C), 166.31 (C), 186.80 (C); IR (KBr): 3335, 1620 cm^{-1}; UV (MeOH): 352 (2.33); MS *m/z*: 474 (50%), 328 (12%), 119 (100%).

4.2.21. 1,4-Bis(2-fluorobenzamido)anthraquinone (20). Yield: 83%; mp 238–239 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.29–7.40 (4H, m), 7.58–7.65 (2H, m), 7.83–7.87 (2H, m, H-6,7), 8.12–8.18 (2H, t, $J = 3.5$ Hz), 8.34–8.37 (2H, m, H-5,8), 9.41 (2H, s, H-2,3), 13.31 (2H, d, $J = 6.9$ Hz, NH); ^{13}C NMR ($CDCl_3$): δ 116.35 (CH), 118.29 (CH), 122.85 (C), 124.64 (CH), 127.11 (C), 129.57 (CH), 131.70 (CH), 133.36 (CH), 133.59 (CH), 134.23 (C), 138.19 (C), 160.88 (C), 163.09 (C), 186.60 (C); IR (KBr): 3375, 1660 cm^{-1}; UV (MeOH): 466.0 (1.09); MS *m/z*: 482 (48%), 361 (12%), 123 (100%).

4.2.22. 1,4-Bis(3-fluorobenzamido)anthraquinone (21). Yield: 75%; mp 273–275 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.34–7.40 (2H, t, $J = 3.8$ Hz), 7.59–7.66 (4H, m), 7.89–7.92 (2H, m, H-6,7), 8.00–8.02 (2H, d, $J = 7.5$Hz), 8.40–8.43 (2H, m, H-5,8), 9.47 (2H, s, H-2,3), 13.61 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 114.87 (CH), 115.17 (CH), 119.15 (CH), 123.07 (CH), 127.31 (C), 129.30 (CH), 130.44 (CH), 133.27 (CH), 134.58 (C), 135.23 (C), 138.85 (C), 162.88 (C), 165.05 (C), 187.25 (C); IR (KBr): 3370, 1655 cm^{-1}; UV (MeOH): 521.0 (1.08); MS *m/z*: 482 (79%), 123 (100%).

4.2.23. 1,4-Bis(4-fluorobenzamido)anthraquinone (22). Yield: 88%; mp 309–311 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.28–7.34 (4H, m), 7.89–7.92 (2H, m, H-6,7), 8.22–8.26 (4H, m), 8.39–8.42 (2H, m, H-5,8), 9.47 (2H, s, H-2,3), 13.59 (2H, s, NH), ^{13}C NMR ($CDCl_3$): δ 116.35 (CH), 118.39 (CH), 122.85 (CH), 124.64 (CH), 127.11 (C), 129.57 (CH), 131.70 (CH), 133.36 (CH), 133.59 (CH), 134.23 (C), 138.19 (C), 160.88 (C), 163.09 (C), 186.60 (C); IR (KBr): 3390, 1680 cm^{-1}; UV (MeOH): 590.0 (0.09); MS *m/z*: 482 (100%), 343 (34%).

4.2.24. 1,4-Bis(2-chlorobenzamido)anthraquinone (23). Yield: 82%; mp 324–326 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.46–7.54 (4H, m), 7.55–7.60 (4H, m), 7.78–7.86 (2H, m, H-6,7), 8.28–8.31 (2H, m, H-5,8), 9.46 (2H, s, H-2,3), 13.05 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 117.17 (CH), 127.14 (C), 127.17 (CH), 129.27 (CH), 129.45 (CH), 131.65 (C), 131.72 (CH), 134.46 (C), 138.37 (C), 166.10 (C), 186.10 (C); IR (KBr): 3365, 1655 cm^{-1}; UV (MeOH): 365.0 (1.18); MS *m/z*: 514 (10%), 141 (31%), 139 (100%).

4.2.25. 1,4-Bis(3-chlorobenzamido)anthraquinone (24). Yield: 85%; mp 244–246 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.54–7.64 (4H, m), 7.87–7.90 (2H, m, H-6,7), 8.16–8.17 (2H, s), 8.37–8.40 (2H, m, H-5,8), 9.41 (2H, s, H-2,3), 13.56 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 117.28 (CH), 125.45 (CH), 127.34 (C), 128.18 (CH), 129.30 (CH), 130.11 (CH), 132.30 (CH), 133.23 (CH), 134.60 (C), 135.23 (C), 136.35 (C), 138.82 (C), 164.96 (C), 187.22 (C); IR (KBr): 3380, 1665 cm^{-1}; UV (MeOH): 486.0 (1.60); MS *m/z*: 514 (49%), 139 (100%).

4.2.26. 1,4-Bis(4-chlorobenzamido)anthraquinone (25). Yield: 84%; mp 320–322 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.59–7.62 (4H, d, $J = 8.7$ Hz), 7.89–7.92 (2H, m, H-6,7), 8.15–8.17 (4H, d, $J = 8.1$ Hz), 8.39–8.42 (2H, m, H-5,8), 9.48 (2H, s, H-2,3), 13.61 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 116.35 (CH), 118.39 (CH), 122.85 (CH), 124.64 (CH), 127.11 (C), 129.57 (CH), 131.70 (CH), 133.36 (CH), 133.59 (C), 134.23 (C), 138.19 (C), 160.88 (C), 163.09 (C), 186.60 (C); IR (KBr): 3400, 1690 cm^{-1}; UV (MeOH): 517.0 (0.34); MS *m/z*: 514 (55%), 139 (100%).

4.2.27. 1,4-Bis(2-nitrobenzamido)anthraquinone (26). Yield: 70%; mp 349–351 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.87–7.93 (4H, m), 7.94–8.04 (4H, m), 8.06–8.24 (4H, m), 9.00 (2H, s, H-2,3), 12.65 (2H, s, NH); IR (KBr): 3355, 1645 cm^{-1}; UV (MeOH): 437.0 (1.42); MS *m/z*: 536 (6%), 386 (100%), 150 (95%).

4.2.28. 1,4-Bis(4-trifluorobenzamido)anthraquinone (27). Yield: 78%; mp 331–333 °C (EA/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ 7.60 (4H, s), 7.90–7.92 (2H, d, $J = 8.1$ Hz, H-6,7), 8.32–8.35 (2H, d, $J = 7.8$ Hz, H-5,8), 8.40–8.42 (4H, m), 9.51 (2H, s, H-2,3), 13.70 (2H, s, NH); ^{13}C

NMR ($CDCl_3$): δ 117.82 (CH), 120.08 (C), 125.57 (CH), 127.30 (C), 127.72 (CH), 129.25 (CH), 133.03 (CH), 134.29 (C), 134.79 (C), 136.88 (C), 138.12 (C), 165.26 (C), 187.01 (C); IR (KBr): 3335, 1645 cm^{-1}; UV (MeOH): 518.0 (0.35); MS *m*/*z*: 581 (11%), 359 (15%), 111 (100%).

4.2.29. 1,4-Bis(2,5-trifluorobenzamido)anthraquinone (28). Yield: 87%; mp 245–247 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 7.16–7.19 (2H, d, *J* = 9.6 Hz), 7.84–7.87 (2H, m, H-6,7), 7.96–8.01 (2H, t, *J* = 8.0 Hz), 8.04–8.07 (2H, d, *J* = 9.0 Hz), 8.25–8.28 (2H, d, *J* = 7.8 Hz, H-5,8), 9.38 (2H, s), 13.03 (2H, s); ^{13}C NMR ($CDCl_3$): δ 112.40 (C), 117.82 (CH), 120.64 (C), 124.86 (CH), 125.57 (CH), 127.30 (C), 127.72 (CH), 129.25 (CH), 130.44 (C), 133.03 (CH), 134.29 (CH), 134.79 (C), 136.88 (C), 138.12 (C), 165.26 (C), 187.01 (C); IR (KBr): 3355, 1655 cm^{-1} ; UV (MeOH): 524.0 (0.58); MS *m*/*z*: 717 (100%), 359 (13%), 211 (14%).

4.2.30. 1,4-Bis(2,4-difluorobenzamido)anthraquinone (29). Yield: 66%; mp 315–317 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 7.03–7.13 (2H, m), 7.85–7.88 (2H, m, H-6,7), 8.00–8.02 (2H, t, *J* = 6.3 Hz), 8.34–8.37 (2H, m, H-5,8), 9.38 (2H, s, H-2,3), 13.30 (2H, d, *J* = 7.0 Hz); IR (KBr): 3325, 1615 cm^{-1}; UV (MeOH): 469.0 (1.31); MS *m*/*z*: 518 (63%), 141 (100%).

4.2.31. 1,4-Bis(2,4-dichlorobenzamido)anthraquinone (30). Yield: 80%; mp 331–333 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 7.45–7.49 (2H, d, *J* = 9.3 Hz), 7.60 (2H, s), 7.75 (2H, d, *J* = 8.5 Hz), 7.85–7.87 (2H, m, H-6,7), 8.28–8.31 (2H, m, H-5,8), 9.42 (2H, s, H-2,3), 13.09 (2H, s, NH); IR (KBr): 3340, 1635 cm^{-1}; UV (MeOH): 519.0 (0.07); MS *m*/*z*: 582 (2%), 354 (100%), 298 (41%), 238 (30%).

4.2.32. 1,4-Bis(2,4,6-trichlorobenzamido)anthraquinone (31). Yield: 60%; mp 361–363 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 7.33–7.41 (2H, m), 7.85–7.91 (2H, m), 8.08 (2H, d, *J* = 7.8 Hz, H-6,7), 8.19 (2H, d, *J* = 7.5 Hz, H-5,8), 10.33 (2H, s, H-2,3), IR (KBr): 3355, 1675 cm^{-1}; UV (MeOH): 479.0 (0.43); MS *m*/*z*: 652 (2%), 482 (67%), 123 (100%).

4.2.33. 1,4-Bis(2,3,6-trifluorobenzamido)anthraquinone (32). Yield: 84%; mp 345–347 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 7.08 (2H, t, *J* = 8.9 Hz), 7.32–7.42 (2H, m), 7.85–7.88 (2H, m, H-6,7), 8.29–8.32 (2H, m, H-5,8), 9.43 (2H, s, H-2,3), 13.18 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 109.11 (CH), 112.11 (CH), 117.73 (CH), 127.28 (C), 129.22 (CH), 133.09 (CH), 134.69 (C), 138.02 (C), 144.80 (C), 158.44 (C), 149.50 (C), 165.05 (C), 186.95 (C); IR (KBr): 3370, 1610 cm^{-1}; UV (MeOH): 463.0 (1.40); MS *m*/*z*: 554 (34%), 158 (100%).

4.2.34. 1,4-Bis(2,4,5-trifluorobenzamido)anthraquinone (33). Yield: 70%; mp 310–312 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 7.14–7.19 (2H, m), 7.86–7.89 (2H, m, H-6,7), 8.02–8.04 (2H, m), 8.33–8.36 (2H, m, H-5,8), 9.36 (2H, s, H-2,3), 13.32 (2H, s, NH); IR (KBr): 3340, 1620 cm^{-1}; UV (MeOH): 486.0 (0.84); MS *m*/*z*: 554 (71%), 159 (100%).

4.2.35. 1,4-Bis(2,3-dichloro-5-fluorobenzamido)anthraquinone (34). Yield: 77%; mp 321–322 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 7.62 (2H, d, *J* = 8.7 Hz), 7.67 (2H, d, *J* = 6.6 Hz), 7.87–7.89 (2H, m, H-6,7), 8.31–8.34 (2H, m, H-5,8), 9.38 (2H, s, H-2,3), 13.13 (2H, s, NH); IR (KBr): 3300, 1650 cm^{-1}; UV (MeOH): 487.0 (0.87); MS *m*/*z*: 620 (55%), 191 (100%).

4.2.36. 1,4–Bis(*trans*-2-phenyl-1-cyclopropanecarboxamido)anthraquinone (35). Yield: 68%; mp 272–274 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 1.79–1.86 (2H, m), 2.03–2.09 (2H, m), 2.69–2.76 (4H, m), 7.22–7.40 (10H, m), 7.83–7.86 (2H, m, H-6,7), 8.29–8.32 (2H, m, H-5,8), 9.26 (2H, s, H-2,3), 12.93 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 16.59 (CH_2), 17.07 (CH), 28.59 (CH), 116.34 (CH), 126.24 (CH), 126.46 (CH), 127.02 (C), 128.47 (CH), 129.14 (CH), 133.34 (CH), 134.32 (C), 138.51 (C), 140.21 (C), 171.76 (C), 186.85 (C); IR (KBr): 3320, 1635 cm^{-1}; UV (MeOH): 486.0 (0.74); MS *m*/*z*: 526 (100%), 365 (25%).

4.2.37. 1,4-Bis(phenylthioacetamido)anthraquinone (36). Yield: 84%; mp 137–139 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 3.91 (4H, s, CH_2), 7.19–7.33 (6H, t, *J* = 5.5 Hz), 7.48–7.51 (4H, d, *J* = 4.5 Hz), 7.84–7.87 (2H, m, H-6,7), 8.30–8.33 (2H, m, H-5,8), 9.13 (2H, s, H-2,3), 12.18 (2H, s, NH); ^{13}C NMR ($CDCl_3$): δ 40.16 (CH_2), 117.75 (CH), 126.98 (CH), 127.11 (C), 128.76 (CH), 129.12 (CH), 129.63 (CH), 133.22 (CH), 134.32 (C), 134.55 (C), 137.82 (C), 158.59 (C), 186.52 (C); IR (KBr): 3345, 1670 cm^{-1}; UV (MeOH): 485.0 (0.69); MS *m*/*z*: 538 (94%), 415 (59%), 388 (23%).

4.2.38. 1,4-Bis(4-fluorophenylacetamido)anthraquinone (37). Yield: 85%; mp 228–230 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 3.85 (4H, s, CH_2), 7.11–7.17 (4H, t, *J* = 4.0 Hz), 7.41–7.46 (4H, m), 7.82–7.85 (2H, m, H-6,7), 8.21–8.24 (2H, m, H-5,8), 9.16 (2H, s, H-2,3), 12.54 (2H, s); ^{13}C NMR ($CDCl_3$): δ 45.09 (CH_2), 115.62 (CH), 117.01 (CH), 127.04 (C), 128.88 (CH), 129.79 (CH), 131.09 (C), 133.13 (CH), 134.36 (C), 138.31 (C), 161.20 (C), 170.62 (C), 186.72 (C); IR (KBr): 3350, 1655 cm^{-1}; UV (MeOH): 485.0 (0.64); MS *m*/*z*: 511 (100%), 427 (8%), 333 (4%), 166 (6%).

4.2.39. 1,4-Bis(2,5-dimethylfuryl-3-carbonylamido)anthraquinone (38). Yield: 79%; mp 284–286 °C (EA/*n*-hexane); ^1H NMR ($CDCl_3$): δ 2.40 (6H, s, CH_3), 2.69 (6H, s, CH_3), 6.59 (2H, s), 7.84–7.87 (2H, m, H-6,7), 8.36–8.39 (2H, m, H-5,8), 9.37 (2H, s, H-2,3), 13.05 (2H, s, NH); IR (KBr): 310, 1645 cm^{-1}; UV (MeOH): 582.0 (0.31); MS *m*/*z*: 482 (100%), 343 (48%).

4.3. 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 from at least three independent experiments showing the same pattern of expression.

4.4. 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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