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# Design, synthesis and cytotoxic effect of hydroxy- and 3-alkylaminopropoxy-9,10-anthraquinone derivatives

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**Abstract**—In previous paper, we have reported the synthesis and the cytotoxic effect of 1,3-dihydroxy-9,10-anthraquinone derivatives. For further design of more potent compounds, a new series of 1-hydroxy-3-(3-alkylaminopropoxy)-9,10-anthraquinones and 3-(3-alkylaminopropoxy)-9,10-anthraquinones have been synthesized. The cytotoxicity of synthetic compounds were evaluated against human Hep G2, Hep 3B and HT-29 cells. Almost all compounds indicated significant inhibitory activity against Hep G2, Hep 3B and HT-29 cell lines in vitro. Compound **5** exhibited selective cytotoxicity against Hep G2 in a concentration-dependent manner with $ED_{50}$ value of $1.23 \pm 0.05$ μM. Structure–activity analysis revealed that most of the 1-hydroxy-3-(3-alkylamino-2-hydroxypropoxy)-9,10-anthraquinone showed stronger cytotoxic effects than those of 1-hydroxy-3- or 3-(3-alkylaminopropoxy)-9,10-anthraquinones against Hep 3B cell line in vitro. A sub-$G_1$ cell stage and DNA fragmentation in MCF-7 cells were significantly observed after 72 h incubation with selective compound **16**. The results show that **16** causes cell death by apoptosis.


## 1. Introduction

Apoptosis is considered to be the major process responsible for cell death in various physiological events. It acts as a regulating mechanism of tissue growth, where it balances cell proliferation.[1] Recently, apoptosis has become a focus of interest in oncology because a disregulation of the apoptosis process can prompt malignancy of tumours.[2,3] Previously we have reported the synthesis and cytotoxic effect of a series of 1-hydroxy-3-(ω-alkylamino-propoxy)-9,10-anthraquinone derivatives.[4] As part of our efforts to continually develop potent anti-tumour agents, which may lead to tumour cell apoptosis, we have further designed and synthesized a new series of 1,3-dihydroxy-9,10-anthraquinone (DHA), 1-hydroxy-3-(3-alkylaminopropoxy)-9,10-anthraquinone (MHA) and 3-(3-alkylaminopropoxy)-9,10-anthraquinone (NHA) derivatives. In the present paper, we report the synthesis, cytotoxic effects against several different cancer cell lines in vitro and cytotoxicity through apoptosis of various DHA, MHA and NHA derivatives and discuss their structure–activity relationships.

*Keywords*: Synthesis; Cytotoxic; Anthraquinone derivatives.
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## 2. Chemistry

As shown in Figure 1, a series of new DHA, MHA and NHA derivatives were synthesized by a method described elsewhere.[4] Briefly, potassium salt of **1** or **3** was allowed to react with 1,3-dibromoalkane in appropriate solvent and then aminated with appropriate amines to give various 1-hydroxy-3-(3-alkylamino-propoxy)-9,10-anthraquinones and 3-(3-alkyl aminopropoxy)-9,10-anthraquinones. Compound **1** in MeOH reacted with prenylbromide in the presence of NaOMe to give **5**.

## 3. Biological results and discussion

Cytotoxic activities of a series of DHA (**5**), MHA (**6**–**8**) and NHA (**9**–**16**) derivatives were studied against a number of cancer cell types. The results are listed in Table 1. A number of compounds indicated significant inhibitory activities against several cancer cell types. As shown in Figure 1, compound **1** did not show cytotoxic effects against several cancer cell lines in vitro (data not shown) while prenylation at C-4 of **1** (i.e., **5**) indicated potent cytotoxicity against human Hep G2 cells in vitro. It indicated that a prenyl group substituted at C-4 of **1** selectively enhanced the cytotoxic effects

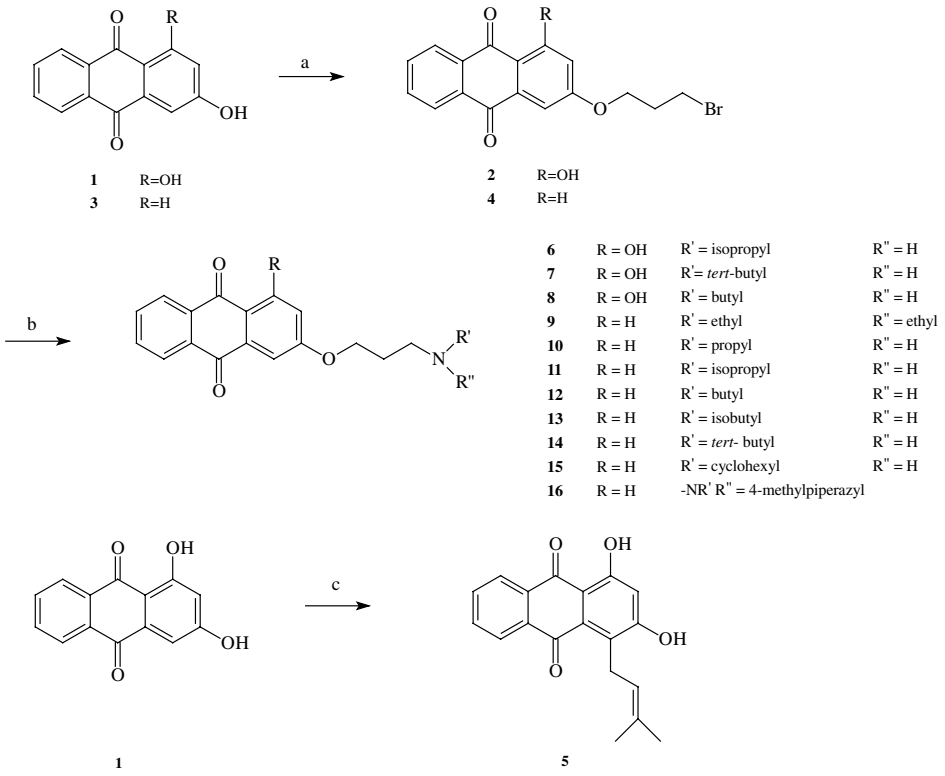


**Figure 1.** Reagents: (a) 1,3-dibromopropane, KOH, MeOH; (b) various amines; (c) prenyl bromide, sodium methoxide, MeOH.

**Table 1.** Cytotoxicity of **3**, **5**–**16** ($ED_{50}$ values in μM)

| Compound | Cell line | | | |
|---|---|---|---|---|
| | Hep G2 | Hep 3B | HT-29 | MCF-7 |
| **5** | 1.23 | 9.09 | 56.17 | 171.95 |
| **6** | 5.52 | 5.97 | 4.72 | n.d. |
| **7** | 5.30 | 6.80 | 7.93 | n.d. |
| **8** | 4.42 | 7.93 | 6.52 | n.d. |
| **9** | 17.21 | 12.76 | 11.87 | 16.07 |
| **10** | 8.92 | 12.38 | 6.19 | 8.36 |
| **11** | 5.39 | 11.46 | 7.12 | 11.46 |
| **12** | 7.12 | 14.24 | 14.54 | 10.39 |
| **13** | 10.39 | 9.26 | 7.72 | 14.24 |
| **14** | 17.51 | 23.15 | 12.88 | 20.77 |
| **15** | 15.98 | 4.96 | 3.86 | 5.79 |
| **16** | 8.41. | 13.74 | 4.67 | 3.02 |
| 5-Fu | 0.25 | 0.55 | 0.57 | |

For significant activity of **5**–**16**, an $ED_{50} \leqslant 4.0$ μg/mL is required; positive control: 5-fluorouracil (5-Fu); n.d.: not determined.

against Hep G2 and Hep 3B cells in vitro. Compounds **2**, **3** and **4** showed no significant cytotoxic activity (data not shown) against human Hep G2, Hep 3B and HT-29 cells while the bromo atom of **2** or **4** replaced by an alkylamino group enhanced the cytotoxic effects against several different human cancer cell line in vitro. Previous report indicated that the MHA derivative, 1-hydroxy-3-(dimethylamino-propoxy)-9,10-anthraquinone (**17**) revealed significant cytotoxic activities against Hep G2 and Hep 3B cell lines in a concentration-dependent manner with $ED_{50}$ values of about 2.6 and 7.1 μM, respectively.[4] As shown in Table 1 and the above result indicated that the increasing carbon number of N-substituted alkyl side chain of **6** enhanced the cytotoxic activity against human Hep G2 while the increasing carbon number of N-substituted alkyl side chain of **17** did not enhance the cytotoxic activities against human Hep G2 cells. On the contrary, the increasing carbon number of N-substituted alkyl side chain of **6** did not enhance the cytotoxic effect against Hep 3B cells but the increasing carbon number of N-substituted alkyl side chain of **17** enhanced the cytotoxic effect against Hep 3B cells. Compound with an isopropyl group substituted at N-atom of the NHA derivatives (i.e., **11**) showed potent cytotoxic activity against human Hep G2 cells. The increasing or decreasing carbon number of N-substituted alkyl side chain of **11** did not enhance the cytotoxic activity against human Hep G2 cells. Compound with a 4-methylpiperazyl group substituted at N-atom of the NHA derivatives (i.e., **16**) showed potent cytotoxic activities against HT-29 and MCF-7 cells but did not show potent cytotoxic activity against human Hep G2 and Hep 3B cells. As shown in Table 1, it clearly indicated that an N-substituted heterocyclic side chain in the NHA derivatives significantly enhanced the cytotoxic activity against HT-29 and MCF-7 cells but did not enhance the cytotoxic activity against Hep G2 and Hep 3B cells. Compound with a cyclohexyl N-substituted side chain of the NHA derivatives (i.e., **15**) showed stronger cytotoxic activities against Hep 3B and HT-29 cells than those of the same series of compounds. It also indicated that compound with a cyclohexyl N-substituted side chain in the NHA derivatives enhanced the cytotoxic activities against the Hep 3B and HT-29 cells.

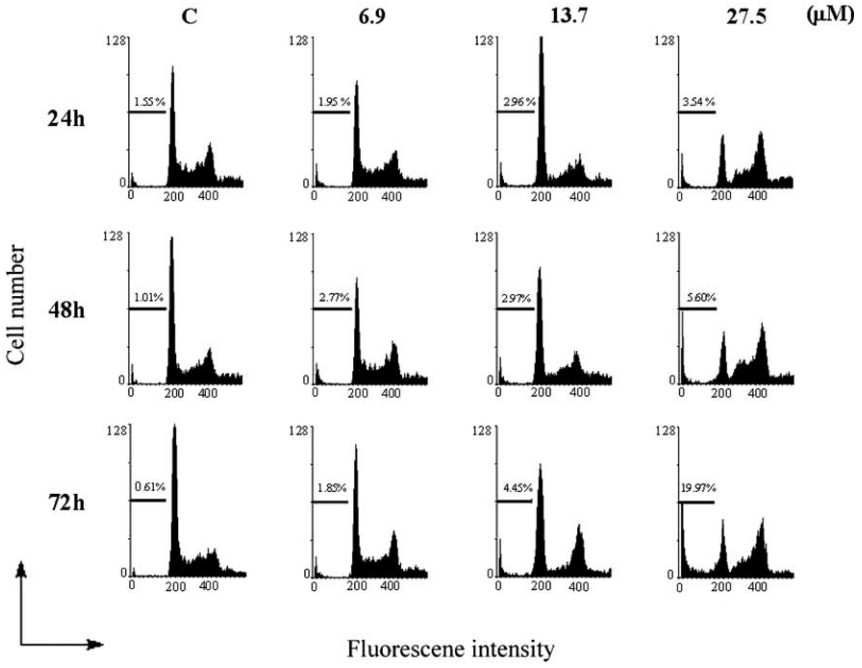


**Figure 2.** Flow cytometry analysis of **16** treated MCF-7 cells. MCF-7 ($1 \times 10^4$ cells/mL) were treated with various concentrations of **16** for different time periods. At the times indicated, cells were stained with propidium iodide (PI), and DNA contents were analyzed by flow cytometry, apoptosis was measured by the accumulation of sub-$G_1$ DNA contents in cells. The control cells were treated with medium. Results were representative of three independent experiments.

It is needed to further synthesize compounds with different N-substituted side chains for study the structure–activity relationship of this series of compound. It has been recognized that apoptotic cells have reduced DNA stainability with a variety of fluorochromes.[5,6] The appearance of cells with low DNA stainability forms a 'sub-G1 peak', which has been considered to the hallmark of cell death by apoptosis.[7] In the previous paper we indicate the MHA derivatives induce cell death by apoptosis.[4] In this study, we consider the NHA derivatives may have induced cell death by the same way. MCF-7 cells were treated with different concentrations of selective compound **16** for different time periods. As shown in Figure 2, a sub-G1 peak was detected in the DNA histograms of **16** at various concentrations for different time periods. The shift of $G_0/G_1$ cell cycles to the $G_2$/M phase is increased dose dependently in the MCF-7 cells treated by **16** for different time periods. However, a maximum 19.97% apoptosis cells were detected at 72 h. The flow cytometry analysis revealed that **16** could

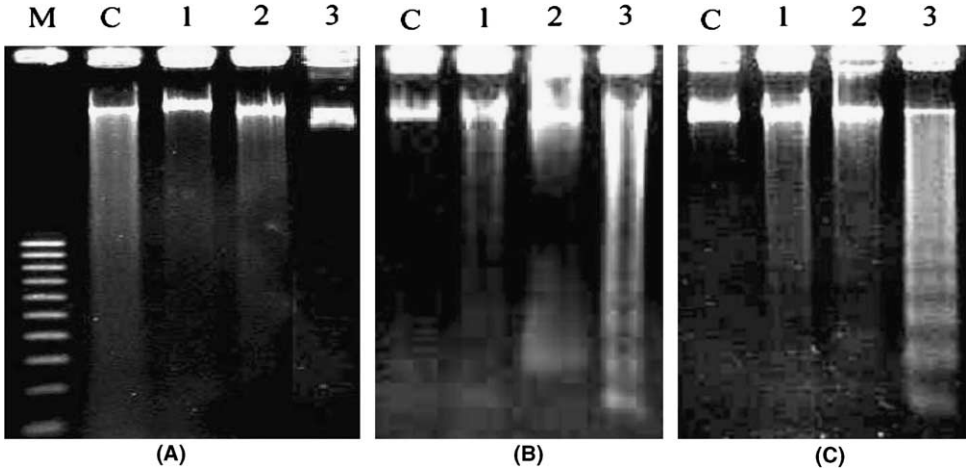


**Figure 3.** Compound **16** induces DNA fragmentation in MCF-7 cells. MCF-7 ($1 \times 10^4$ cells/mL) were treated with various concentrations of **16** for different time periods. At the times indicated, the cells were lyzed and DNA was prepared. DNA fragmentation was analyzed by 1% agarose gel electrophoresis. The compound **16** was diluted with culture medium. The control cells was treated with medium. M: 100 bp marker; C: control; lane 1: cells with **16** (6.9 μM); lane 2: cells with **16** (13.7 μM); lane 3: cells with **16** (27.5 μM). (A) Cells cultured in culture medium were treated **16** for 24 h; (B) cells cultured in culture medium were treated **16** for 48 h; (C) cells cultured in culture medium were treated **16** for 72 h. Result were representative of three independent experiments.

arrest $G_2$/M and S phases. In addition, DNA fragmentation in general used to characterize cell death by apoptosis.[8,9] Apoptosis of the MCF-7 cells after **16** treatment was also studied by DNA fragmentation. DNA fragmentation in MCF-7 cells was significantly observed after 48 and 72 h incubation with **16** (6.9, 13.7 and 27.5 μM) (Fig. 3). This study suggested that **16** may induce the shift of $G_0/G_1$ phase to $G_2$/M and S phases and causes cell death by apoptosis.

## 4. Conclusions

This study further verifies that some NHA derivatives show significant cytotoxicity against several human cancer lines used in this study, although they did not exert stronger cytotoxicity than those of MHA derivatives. Compound **5** demonstrated selective cytotoxic activity against Hep G2. It may be as a lead structure for design and synthesis of this series of compound. These compounds may induce cell death by apoptosis and they might be developed as anti-cancer agents. Further experiments are needed to elucidate the mechanism of action of **5** and **16**.

## 5. Experimental

Tumour cell growth inhibition assays: The cytotoxicity was determined by colorimetric MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-1*H*-tetrazolium bromide) assay as described previously. Briefly, cells ($5 \times 10^3$/well) were plated in the 96-well plates and incubated in medium for 6 h. Serial tested drug dilutions (50 μL) were added. The cells were incubated for 5 or 6 days at 37 °C and then pulsed with 10 μL of MTT (5 mg/mL; Sigma, St. Louis, MO) and incubated for an additional 4 h at 37 °C. Reduced MTT was measured spectrophotometrically by a Dynatech MR 5000 microplate reader (Dynatech Laboratories, VA) at 550 nm after lysis of cells with 100 μL of 10% SDS in 0.01 M HCl. Control wells contained medium plus cells (total absorbance) or medium alone (background absorbance) cell death was calculated as the percentage of MTT inhibition:

$$\%\ \text{Inhibition} = \frac{1 - \text{mean experimental absorbance}}{\text{mean control absorbance}} \times 100.$$

Hep 3B cells, human hepatitis B surface antigen (HBs Ag) contains an integrated hepatitis B virus genome. Human hepatoma, Hep 3B and Hep G2, human colerectal adenocarcinoma HT-29, and human breast adenocarcinoma, MCF-7 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD) and grown in Dulbecco's modified Eagle medium (DMEM; Gibco BRL, Grand Island, NY),[10,11] containing 10% foetal bovine serum (FBS; Gibco BRL), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. For the microassay, the growth medium was supplemented with 10 mM HEPES buffer pH 7.3 and incubated at 37 °C in a $CO_2$ incubator.

## 6. Flow cytometry

Compounds were added to cells ($1 \times 10^7$). At various time interval, the reactions were terminated by washing with PBS. The cells were fixed with 4% paraformaldehyde/PBS (pH 7.4) at room temperature for 30 min. After centrifugation at 1000 rpm for 10 min, the cells were permeabilized with 0.1% Triton-X-100/0.1% sodium citrate at 4 °C for 2 min. Propidium iodide (Sigma) in PBS (10 μg/mL) was added to stain the cells at 37 °C for 30 min. The intensity of fluorescence was measured with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). A minimum of 5000 cell counts were collected for the analysis by LYSIS II Software.

## 7. DNA fragmentation assay

After testing the drug (8 μg/mL) treatment for 48 h, cells in 150-mm plates were harvested and washed with PBS. After the addition of 100 μL lysis buffer [1% of NP-40 (Sigma) in 20 mM EDTA, 50 mM Tris–HCl, pH 7.5] and mixing, the cell lysates were centrifuged at 14,000 rpm for 5 min and the supernatants were collected. The supernatants were incubated with 50 μL of RNase A (20 mg/mL) and 20 μL of SDS (10%) at 56 °C for 2 h. Then, 35 μL of proteinase K (20 mg/mL) was added and incubated at 37 °C overnight. DNA fragments were precipitated after the addition of 150 μL of 10 M $NH_4OAc$ and 1.2 mL of 100% ethanol at −20 °C overnight. After centrifuging and drying, the DNA pellets were resuspended in 15 μL Tris–EDTA buffer and electrophoresed on a 1% agarose gel in TBE buffer at 30 V for 8 h, DNA ladder was observed after staining with ethidium bromide solution and exposing to the UV light.[12]

## 8. Chemistry

Melting points (uncorrected) were determined with a Yanco Micro-Melting point apparatus. IR spectra were determined with a Perkin–Elmer system 2000 FTIR spectrophotometer. $^1H$ (400 MHz) and $^{13}C$ (100 MHz) NMR were recorded on a Varian UNITY-400 spectrometer, and mass were obtained on a JMX-HX 100 mass spectrometer. Elemental analyses were within ±0.4% of the theoretical values, unless otherwise noted. Chromatography was performed using a flash-column technique on silica gel 60 supplied by E. Merck.

### 8.1. 1,3-Dihydroxy-4-prenyl-9,10-anthraquinone (5)

Compound **1** (0.482 g, 2 mmol) in anhydrous methanol was added to methanolic solution of sodium methoxide (9 mL). Prenyl bromide (2 mL) was added to the mixture under ice bath and then refluxed for 3 h. After removal of the solvent, water was added to the mixture and acidified with concentrated HCl. The precipitated solid was collected and purified by chromatography (silica gel and *n*-hexane–EtOAc (4:1)) to give **5**, as orange needles ($CHCl_3$) (0.027 g, 0.08 mmol, 4.0%). Mp 198 °C. IR (KBr) 3391, 1665, 1624, 1590 $cm^{-1}$. $^1H$ NMR ($(CD_3)_2CO$): $\delta$ 1.67

(3H, s, Me), 1.80 (3H, s, Me), 3.45 (2H, d, $J$ = 7.3 Hz, –C$H_2$CH=), 5.28 (1H, m, –C$H$=C<), 7.35 (1H, s, H-2), 7.86–7.92 (2H, m, H-6 and H-7), 8.17–8.30 (2H, m, H-5 and H-8), 13.23 (1H, s, OH-1). $^{13}$C NMR ((CD$_3$)$_2$CO): $\delta$ 18.7 (Me), 23.6 (Me), 26.6 (–$C$H$_2$CH=), 108.4 (C-9a), 109.1 (C-2), 122.6 (–$C$H=C<), 123.2 (C-4), 128.1 (C-8), 128.3 (C-5), 133.5 (C-4a), 134.2 (C-10a), 135.1 (C-8a), 135.2 (–CH=$C$<), 135.8 (C-6 and C-7), 163.7 (C-3), 164.6 (C-1), 183.3 (C-10), 188.6 (C-9). EIMS (70 eV) $m/z$ (% rel int.): 308 (56) [M]$^+$. Anal Calcd for $C_{17}H_{16}O_4$: C, 74.00; H, 5.20. Found: C, 73.50; H, 5.26.

### 8.2. 1-Hydroxy-3-[3-(isopropylamino)-propoxy]-9,10-anthraquinone (6)

Compound **2** (0.12 g, 0.33 mmol) in EtOH (40 mL) was added propylamine (1.37 g, 23.10 mmol) and then reflux for 1 h. The product was purified by column chromatography (silica gel and MeOH) and crystallized from MeOH to give **6**, green needles (0.018 g, 0.05 mmol, 18.7%). Mp 212 °C. IR (KBr) 3421, 1670, 1635, 1592 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.28 (6H, d, $J$ = 6.8 Hz, 2 × Me), 2.18 (2H, m, –OCH$_2$C$H_2$–), 3.06 (2H, m, –C$H_2$NH–), 3.25 (1H, m, –C$H$(CH$_3$)$_2$), 4.28 (2H, t, $J$ = 6 Hz, –OCH$_2$–), 6.88 (1H, d, $J$ = 2.4 Hz, H-2), 7.19 (1H, d, $J$ = 2.4 Hz, H-4), 7.92 (2H, m, H-6 and H-7), 8.18 (2H, m, H-5 and H-8), 9.11 (2H, br s, –N$^{\oplus}H_2$–), 12.76 (1H, s, OH-1). $^{13}$C NMR (CDCl$_3$): $\delta$ 18.5 (2 × Me), 25.3 (–OCH$_2$$C$H$_2$–), 40.9 (–CH$_2$NH–), 49.6 (–NHCH<), 66.0 (–OCH$_2$–), 106.7 (C-2), 107.6 (C-4), 110.3 (C-9a), 126.4 (C-8), 126.8 (C-5), 132.9 (C-8a and C-10a), 134.7 (C-6 and C-7), 134.9 (C-4a), 164.6 (C-1), 164.9 (C-3), 181.6 (C-10), 186.3 (C-9). EIMS (70 eV) $m/z$ (% rel int.): 339 (4) [M]$^+$. HREIMS $m/z$ [M]$^+$ 339.1468 (calcd for $C_{20}H_{21}NO_4$, 339.1470).

### 8.3. 3-[3-(*tert*-Butylamino)-propoxy]-1-hydroxy-9,10-anthraquinone (7)

Compound **2** (0.12 g, 0.33 mmol) in EtOH (40 mL) was added *tert*-butylamine (1.78 g, 24.30 mmol). The mixture was treated and purified as **6** to yield **7**, as yellow needles (0.016 mg, 0.045 mmol, 16.2%). Mp 205 °C. IR(KBr) 3447, 1670, 1634, 1593 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.32 (9H, s, Me), 2.17 (2H, m, –OCH$_2$C$H_2$–), 3.04 (2H, br s, –C$H_2$NH–), 4.31 (2H, t, $J$ = 6.2 Hz, –OCH$_2$–), 6.92 (1H, d, $J$ = 2.4 Hz, H-2), 7.24 (1H, d, $J$ = 2.4 Hz, H-4), 7.94 (2H, m, H-6 and H-7), 8.21 (2H, m, H-5 and H-8), 9.09 (2H, br s, –N$^{\oplus}H_2$–), 12.76 (1H, s, OH-1). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.1 (Me), 25.8 (–OCH$_2$$C$H$_2$–), 37.7 (–NHC–), 55.9 (–CH$_2$NH–), 66.1 (–OCH$_2$–), 106.8 (C-2), 107.6 (C-4), 110.3 (C-9a), 126.4 (C-8), 126.9 (C-5), 132.9 (C-8a and C-10a), 134.7 (C-6, C-7 and C-4a), 164.5 (C-1), 165.0 (C-3), 181.6 (C-10), 186.3 (C-9). EIMS (70 eV) $m/z$ (% rel int.): 353 (1) [M]$^+$. HREIMS $m/z$ [M]$^+$ 353.1630 (calcd for $C_{21}H_{23}NO_4$, 353.1627).

### 8.4. 3-[(3-Butylamino)-propoxy]-1-hydroxy-9,10-anthraquinone (8)

Compound **2** (0.12 g, 0.33 mmol) in EtOH (40 mL) was added *n*-butylamine (1.61 g, 22.0 mmol). The mixture was treated and purified as **6** to yield **8**, as yellow needles (0.030 g, 0.084 mmol, 30.3%). Mp 220 °C. IR (KBr) 3402, 1670, 1636, 1592 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 0.90 (3H, t, $J$ = 2.6, Me), 1.34 (2H, m, –C$H_2$CH$_3$), 1.62 (2H, m, –C$H_2$CH$_2$CH$_3$), 2.14 (2H, m, –OCH$_2$C$H_2$–), 2.51 (2H, br s, –CH$_2$NHC$H_2$–), 3.08 (2H, br s, –C$H_2$NHCH$_2$–), 4.28 (2H, t, $J$ = 6.0 Hz, –OCH$_2$–), 6.89 (1H, d, $J$ = 2.6 Hz, H-2), 7.21 (1H, d, $J$ = 2.4 Hz, H-4), 7.99 (2H, m, H-6 and H-7), 8.18 (2H, m, H-5 and H-8), 8.93 (2H, br s, –N$^{\oplus}H_2$–), 12.76 (1H, s, OH-1). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 13.4 (Me), 19.3 (–$C$H$_2$CH$_3$), 25.1 (–$C$H$_2$CH$_2$CH$_3$), 27.5 (–OCH$_2$$C$H$_2$–), 43.9 (–CH$_2$NH–), 46.6 (–NHCH$_2$–), 66.0 (–OCH$_2$–), 106.7 (C-2), 107.6 (C-4), 110.3 (C-9a), 126.4 (C-8), 126.9 (C-5), 132.9 (C-8a and 10a), 134.7 (C-6, C-7 and C-4a), 164.6 (C-1), 164.9 (C-3), 181.6 (C-10), 186.3 (C-9). EIMS (70 eV) $m/z$ (% rel int.): 353 (1) [M]$^+$. HREIMS $m/z$ [M]$^+$ 353.1618 (calcd for $C_{21}H_{23}NO_4$, 353.1627).

### 8.5. 3-[(3-Diethylamino)-propoxy]-9,10-anthraquinone (9)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added diethylamine (1.42 g, 19.39 mmol) and then refluxed for 1 h. The mixture was treated and purified as **6** to yield **9**, as a yellow powder (0.031 g, 0.09 mmol, 32.1%). IR (KBr) 1670, 1590 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.23 (6H, t, $J$ = 7.4 Hz, 2 × Me), 2.19 (2H, m, –OCH$_2$C$H_2$–), 3.21 (6H, m, –C$H_2$N(C$H_2$)$_2$–), 4.32 (2H, t, $J$ = 5.6 Hz, –OCH$_2$–), 7.47 (1H, dd, $J$ = 7.8, 2.8 Hz, H-2), 7.64 (1H, d, $J$ = 2.4 Hz, H-4), 7.94 (2H, m, H-6 and H-7), 8.20 (3H, m, H-1, H-5 and H-8), 9.80 (1H, br s, ≥N$^{\oplus}H$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 8.5 (Me), 23.0 (–OCH$_2$$C$H$_2$–), 46.3 (–N(CH$_2$)$_2$–), 47.7 (–CH$_2$NH–), 65.7 (–OCH$_2$–), 110.8 (C-4), 121.0 (C-2), 126.5 (C-9a), 126.6 (C-8), 126.7 (C-5), 129.5 (C-1), 133.0 (C-10a), 133.0 (C-8a), 134.2 (C-7), 134.6 (C-6), 135.0 (C-4a), 162.9 (C-3), 181.3 (C-9), 182.3 (C-10). EIMS (70 eV) $m/z$ (% rel int.): 337 (1) [M]$^+$. HREIMS $m/z$ [M]$^+$ 337.1679 (calcd for $C_{21}H_{23}NO_3$, 337.1678).

### 8.6. 3-[(3-Propylamino)-propoxy]-9,10-anthraquinone (10)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added propylamine (0.79 g, 19.39 mmol). The mixture was treated and purified as **6** to yield **10**, as a purplish powder (0.028 g, 0.088 mmol, 30.2%). IR (KBr) 1667, 1589 cm$^{-1}$. $^1$H NMR (CD$_3$OD): $\delta$ 1.05 (3H, t, $J$ = 7.6 Hz, Me), 1.75 (2H, m, –C$H_2$CH$_3$), 2.27 (2H, m, –OCH$_2$C$H_2$–), 3.03 (2H, m, –CH$_2$N–), 3.27 (2H, t, $J$ = 6 Hz, –NCH$_2$–), 4.34 (2H, t, $J$ = 5.6 Hz, –OCH$_2$–), 7.41 (1H, dd, $J$ = 8.8, 2.8 Hz, H-2), 7.76 (1H, d, $J$ = 2.4 Hz, H-4), 7.87 (2H, m, H-6 and H-7), 8.27 (3H, m, H-1, H-5 and H-8). $^{13}$C NMR (CD$_3$OD): $\delta$ 11.2 (Me), 20.8 (–NCH$_2$$C$H$_2$–), 27.2 (–OCH$_2$$C$H$_2$–), 46.5 (–NHCH$_2$–), 50.8 (–CH$_2$NH–), 66.9 (–OCH$_2$–), 112.0 (C-4), 122.1 (C-2), 128.0 (C-5 and C-8), 128.6 (C-9a), 130.8 (C-1), 134.9 (C-8a and C-10a), 135.1 (C-7), 135.6 (C-6), 136.9 (C-4a), 164.8 (C-3), 183.3 (C-9), 184.2 (C-10). EIMS (70 eV) $m/z$ (% rel int.): 323 (5) [M]$^+$. HREIMS $m/z$ [M]$^+$ 323.1517 (calcd for $C_{20}H_{21}NO_3$, 323.1521).

### 8.7. 3-[(3-Isopropylamino)-propoxy]-9,10-anthraquinone (11)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added isopropylamine (0.79 g, 19.39 mmol). The mixture was treated and purified as **6** to yield **11**, as a pink powder (0.027 g, 0.083 mmol, 28.7%). IR (KBr) 1672, 1590 $cm^{-1}$. $^1H$ NMR ($CD_3OD$): $\delta$ 1.38 (6H, d, $J$ = 6.8 Hz, 2 × Me), 2.27 (2H, m, –$OCH_2CH_2$–), 3.29 (2H, m, –$CH_2N$–), 3.45 (1H, m, –NC*H*–), 4.34 (2H, t, $J$ = 5.6 Hz, –$OCH_2$–), 7.40 (1H, dd, $J$ = 8.8, 2.8 Hz, H-2), 7.73 (1H, d, $J$ = 2.4 Hz, H-4), 7.86 (2H, m, H-6 and H-7), 8.25 (3H, m, H-1, H-5 and H-8). $^{13}C$ NMR ($CD_3OD$): $\delta$ 19.3 (Me), 27.3 (–$OCH_2CH_2$–), 43.6 (–$CH_2NH$–), 52.1 (–NHCH–), 66.8 (–$OCH_2$–), 112.0 (C-4), 122.1 (C-2), 128.0 (C-5 and C-8), 128.6 (C-9a), 130.8 (C-1), 134.9 (C-8a and C-10a), 135.1 (C-7), 135.6 (C-6), 136.9 (C-4a), 164.8 (C-3), 183.2 (C-9), 184.1 (C-10). EIMS (70 eV) *m/z* (% rel int.): 323 (7) $[M]^+$. HREIMS *m/z* $[M]^+$ 323.1526 (calcd for $C_{20}H_{21}NO_3$, 323.1521).

### 8.8. 3-[(3-Butylamino)-propoxy]-9,10-anthraquinone (12)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added *n*-butylamine (0.98 g, 19.39 mmol). The mixture was treated and purified as **6** to yield **12**, as a yellowish powder (0.030 g, 0.088 mmol, 30.4%). IR (KBr) 1671, 1591 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 0.90 (3H, t, $J$ = 7.2 Hz, Me), 1.35 (2H, m, –$CH_2CH_3$), 1.62 (2H, m, –$CH_2CH_2CH_3$), 2.20 (2H, m, –$OCH_2CH_2$–), 2.90 (2H, br s, –$CH_2NH$–), 3.09 (2H, br s, –NHC$H_2$–), 4.32 (2H, m, –$OCH_2$–), 7.45 (1H, dd, $J$ = 8.8, 2.8 Hz, H-2), 7.61 (1H, d, $J$ = 2.4 Hz, H-4), 7.92 (2H, m, H-6 and H-7), 8.19 (m, 3H, H-1, H-5 and H-8), 9.05 (2H, br s, $\geq N^{\oplus}H_2$). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 13.0 (Me), 18.8 (–$CH_2CH_3$), 24.7 (–$NCH_2CH_2$–), 26.9 (–$OCH_2$-$CH_2$–), 43.3 (–$CH_2NH$–), 46.0 (–$NHCH_2$–), 65.2 (–$OCH_2$–), 110.3 (C-4), 120.6 (C-2), 126.0 (C-8), 126.1 (C-5), 126.2 (C-9a), 129.0 (C-1), 132.5 (C-10a), 132.5 (C-8a), 133.7 (C-7), 134.1 (C-6), 134.5 (C-4a), 162.6 (C-3), 180.8 (C-9), 181.9 (C-10). EIMS (70 eV) *m/z* (% rel int.): 337 (1) $[M]^+$. HREIMS *m/z* $[M]^+$ 337.1678 (calcd for $C_{21}H_{23}NO_3$, 337.1678).

### 8.9. 3-[(3-Isobutylamino)-propoxy]-9,10-anthraquinone (13)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added isobutylamine (0.98 g, 19.39 mmol). The mixture was treated and purified as **6** to yield **13**, as a pink powder (0.030 g, 0.09 mmol, 31.1%). IR (KBr) 1673, 1593 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.07 (6H, d, $J$ = 8 Hz, 2 × $CH_3$), 2.05 (1H, m, –$CH(CH_3)_2$), 2.30 (2H, m, –$OCH_2CH_2$–), 2.93 (2H, d, $J$ = 7.2 Hz, –NHC$H_2$–), 3.26 (2H, m, –NHC$H_2$–), 4.34 (2H, t, $J$ = 5.6 Hz, –$OCH_2$–), 7.40 (1H, dd, $J$ = 8.8, 2.8 Hz, H-2), 7.75 (1H, d, $J$ = 2.4 Hz, H-4), 7.87 (2H, m, H-6 and H-7), 8.26 (3H, m, H-1, H-5 and H-8). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 20.3 (Me), 26.9 (–$OCH_2CH_2$–), 27.4 (–$CH(CH_3)_2$), 47.0 (–$CH_2NH$–), 56.3 (–$NHCH_2$–), 66.9 (–$OCH_2$–), 112.0 (C-4), 122.0 (C-2), 128.0 (C-5 and C-8), 128.6 (C-9a), 130.8 (C-1), 135.0 (C-8a and C-10a), 135.1 (C-7), 135.6 (C-6), 136.9 (C-4a), 164.8 (C-3), 183.3 (C-9), 184.2 (C-10). EIMS (70 eV) *m/z* (% rel int.): 337 (1) $[M]^+$. HREIMS *m/z* $[M]^+$ 337.1677 (calcd for $C_{21}H_{23}NO_3$, 337.1678).

### 8.10. 3-[(3-*tert*-Butylamino)-propoxy]-9,10-anthraquinone (14)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added *tert*-butylamine (0.98 g, 19.39 mmol). The mixture was treated and purified as **6** to yield **14**, as a light brown powder: (0.033 g, 0.097 mmol, 33.3%). IR (KBr) 1673, 1591 $cm^{-1}$. $^1H$ NMR ($CD_3OD$): $\delta$ 1.41 (9H, s, 3 × Me), 2.26 (2H, m, –$OCH_2CH_2$–), 3.24 (2H, m, –$CH_2N$–), 4.35 (2H, t, $J$ = 5.6 Hz, –$OCH_2$–), 7.41 (1H, dd, $J$ = 8.8, 2.8 Hz, H-2), 7.75 (1H, d, $J$ = 2.4 Hz, H-4), 7.86 (2H, m, H-6 and H-7), 8.23 (3H, m, H-1, H-5 and H-8). $^{13}C$ NMR ($CD_3OD$): $\delta$ 26.0 (Me), 27.9 (–$OCH_2CH_2$–), 40.1 (–$CH_2NH$–), 57.9 (–$NHC\leq$), 66.9 (–$OCH_2$–), 112.0 (C-4), 122.1 (C-2), 128.0 (C-5 and C-8), 128.6 (C-9a), 130.8 (C-1), 134.8 (C-8a), 134.9 (C-10a), 135.1 (C-7), 135.6 (C-6), 136.9 (C-4a), 164.8 (C-3), 183.3 (C-9), 184.2 (C-10). EIMS (70 eV) *m/z* (% rel int.): 337 (1) $[M]^+$. HREIMS *m/z* $[M]^+$ 337.1682 (calcd for $C_{21}H_{23}NO_3$, 337.1678).

### 8.11. 3-[(3-Cyclohexylamino)-propoxy]-9,10-anthraquinone (15)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added cyclohexylamine (1.33 g, 19.39 mmol). The mixture was treated and purified as **6** to yield **15**, as a pink powder (0.034 g, 0.092 mmol, 32.0%). IR (KBr): 1675, 1591 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.34 (6H, m, –$CH_2CH_2CH_2$–), 1.82 (2H, m, –$OCH_2CH_2$–), 2.15 (4H, m, –$CH_2CHCH_2$–), 2.82 (1H, m, –NHC*H*<), 3.08 (2H, m, –$CH_2NH$–), 4.29 (2H, t, $J$ = 5.6 Hz, –$OCH_2$–), 7.35 (1H, dd, $J$ = 8.8, 2.8 Hz, H-2), 7.67 (1H, d, $J$ = 2.4 Hz, H-4), 7.66 (2H, m, H-6 and H-7), 8.16 (3H, m, H-1, H-5 and H-8). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 25.7 (–$CH_2$–), 26.5 (–$CH_2$–), 27.9 (–$CH_2$–), 28.3 (–$OCH_2$-$CH_2$–), 43.6 (–$CH_2NH$–), 58.4 (–NHCH<), 67.4 (–$OCH_2$–), 112.0 (C-4), 122.1 (C-2), 126.5 (C-9a), 126.7 (C-8), 126.7 (C-5), 129.6 (C-1), 133.0 (C-8a), 133.1 (C-10a), 134.2 (C-7), 134.7 (C-6), 135.1 (C-4a), 163.9 (C-3), 181.3 (C-9), 182.4 (C-10). EIMS (70 eV) *m/z* (% rel int.): 363 (1) $[M]^+$. HREIMS *m/z* $[M]^+$ 363.1834 (calcd for $C_{23}H_{25}NO_3$, 337.1834)

### 8.12. 3-[3-(4-Methylpiperazinopropoxy)]-9,10-anthraquinone (16)

Compound **4** (0.1 g, 0.28 mmol) in EtOH (40 mL) was added 1-methylpiperazine (1.33 g, 19.39 mmol). The mixture was treated and purified as **6** to yield **10**, as a yellow powder (0.037 g, 0.103 mmol, 35.4%). IR (KBr) 1672, 1594 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.92 (2H, m, –$OCH_2CH_2$–), 2.13 (3H, s, Me), 2.33–2.44 (10H, m, –$CH_2N(CH_2CH_2)_2N$–), 4.31 (2H, t, $J$ = 5.6, –$OCH_2$–), 7.32 (1H, dd, $J$ = 8.8, 2.8 Hz, H-2), 7.57 (1H, d, $J$ = 2.4 Hz, H-4), 7.91 (2H, m, H-6 and H-7), 8.15 (3H, m, H-1, H-5 and H-8). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 26.4 (Me), 46.2 (–$OCH_2CH_2$–), 53.2 (–$N(CH_2CH_2)_2N$–),

54.6 (–$CH_2N$–), 55.2 (–$N(CH_2CH_2)_2N$–), 67.3 (–$OCH_2$–), 111.1 (C-4), 121.6 (C-2), 127.1 (C-9a), 127.2 (C-5 and C-8), 130.0 (C-1), 133.6 (C-8a and C-10a), 134.6 (C-6 and C-7), 135.1 (C-4a), 163.9 (C-3), 181.8 (C-9), 182.9 (C-10). EIMS (70 eV) *m/z* (% rel int.): 364 (2) $[M]^+$. HREIMS *m/z* $[M]^+$ 364.1792 (calcd for $C_{22}H_{24}N_2O_3$, 364.1788).

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