

Supporting Information

Synthesis and biological evaluation of novel chloroethylaminoanthraquinones with potent cytotoxic activity against cisplatin resistant tumour cells

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Experimental Section

The UV/Vis absorbance of the compounds investigated biologically was recorded on a Beckman DU70 UV/Vis Spectrophotometer fitted with deuterium and tungsten lamps. The infra-red absorbance was recorded on a Nicolet 205 FT-IR Spectrometer. NMR spectra were obtained on a Bruker AC-250 spectrometer. Fast Atom Bombardment (FAB+) Mass-Spectra sample identification was obtained on a V.G 70 SEQ Mass Spectrometer. Micro-elemental analysis was obtained using a Carlo-Erba EA 1108 instrument with a PC-based data system, Eager 200 for Windows and Sartorius ultra micro-balance 4504 MP8. Thin-layer chromatography was carried out on aluminium-backed silica plates (Merck, 60 F₂₅₄), and column chromatography on silica gel (particle size 20-35 μ m).

1,4-Bis-[[2-[bis(2-hydroxyethyl)amino]ethyl]amino]anthracene-9,10-dione (1)

Reaction procedure and conditions follow that for the synthesis of **3**, using *N,N*-bis(2-hydroxyethyl)ethylenediamine (3.7 g, 20.0 mmol) and leucoquinizarin (0.3 g, 1.26 mmol). Purification by flash column chromatography (CH₃OH:CH₂Cl₂, 1:1) afforded the product as dark blue crystals (132.3 mg, 22% yield). m.p. 177.5 - 178.9°C; ¹H NMR (CDCl₃/CD₃OD) peaks: δ 3.4 (t, 8H, 4 \times CH₂N), 3.5 (t, 4H, 2 \times CH₂N), 3.85 (q, 4H, 2 \times CH₂NHAr), 4.0 (t, 8H, 4 \times CH₂OH), 7.7 (s, 2H, ArH), 7.85 (m, 2H, ArH), 8.3 (m, 2H, ArH), 10.4 (t, 2H, 2 \times NHAr); IR ν_{\max} (KBr) cm⁻¹: 3600-3300 (OH), 1590 (C=O), 1230 (N-H); E _{λ} (dH₂O) = 8060 cm⁻¹; λ_{\max} (624 nm); FAB-MS, m/z (M+H)⁺ 501; Anal. (C₂₆H₃₆N₄O₆) C, H, N.

1,4-Bis-[[3-[bis(2-hydroxyethyl)amino]propyl]amino]anthracene-9,10-dione (2)

Reaction conditions and procedure follow that for the synthesis of **3**, using *N,N*-bis(2-hydroxyethyl)-1,3-propanediamine (3.64 g, 20.0 mmol) and leucoquinizarin (0.3 g, 1.26 mmol). Purification by flash column chromatography (CH₃OH:CH₂Cl₂, 3:7 increasing to 1:1) afforded the product as dark blue crystals (113.0 mg, 18% yield). m.p. 137.6-138.6°C; ¹H NMR (CDCl₃/CD₃OD) peaks: δ 1.8 (m, 4H, 2 \times CH₂CH₂CH₂), 2.3 (t, 8H, 4 \times CH₂N), 2.4 (t, 4H, 2 \times CH₂N), 3.4 (q, 4H, 2 \times CH₂NHAr), 3.7 (t, 8H, 4 \times CH₂OH), 7.7 (s, 2H, ArH), 7.85 (m, 2H, ArH), 8.3 (m, 2H, ArH), 10.7 (t, 2H, 2 \times NHAr); IR ν_{\max} (KBr) cm⁻¹: 3600-3300 (OH), 1590 (C=O), 1230 (N-H); E _{λ} (dH₂O) = 9964 cm⁻¹; λ_{\max} (630 nm); FAB-MS, m/z (M+H)⁺ 529; Anal. (C₂₈H₄₀N₄O₆) C, H, N.

1,4-Bis-[[2-[(2-hydroxyethyl)(methyl)amino]ethyl]amino]anthracene-9,10-dione (3)

Leucoquinizarin (0.3 g, 1.26 mmol) was added to *N*-2-hydroxyethyl-*N*-methylethylenediamine (2.44 g, 20.0 mmol) at 60 °C under nitrogen and heated for 4 hours. The reaction mixture was cooled to room temperature, aqueous NaOH (15%, 0.2 cm³) was added and the reaction was left to stir overnight exposed to air. The reaction mixture was diluted with CH₂Cl₂ (80 cm³) and washed with water (3 \times 80 cm³). The organic layer was dried with MgSO₄ and the solvent was concentrated *in vacuo* to yield a waxy solid. This crude solid was purified by flash column chromatography (CH₃OH:CH₂Cl₂, 1:1 then CH₃OH) and further by re-dissolving in CH₃OH followed by precipitation with dry diethyl ether to give dark blue crystals (105.7 mg, 19% yield). m.p. 181.2-182.3°C; ¹H NMR (CDCl₃/CD₃OD) peaks: δ 2.5 (br s, 6H, 2 \times CH₃N), 2.8 (t, 2H, CH₂CH₂N), 3.0 (t, 2H, CH₂CH₂N), 3.4 (q, 2H, CH₂NHAr), 3.7 (t, 2H, CH₂OH), 7.5 (s, 2H, Ar-H), 7.7 (m, 2H, Ar-H), 8.2 (m, 2H, Ar-NH), 10.7 (t, 2H, 2 \times NHAr); IR ν_{\max} (KBr) cm⁻¹: 3600-3300 (OH), 1590 (C=O), 1230 (N-H); E _{λ} (dH₂O) = 7128 cm⁻¹; λ_{\max} (626 nm); FAB-MS, m/z (M+H)⁺ 441; Anal. (C₂₄H₃₂N₄O₄) C, H, N.

1,4-Bis[[3-[bis(2-hydroxyethyl)amino]propyl]amino]-5,8-dihydroxyanthracene-9,10-dione (4)

Reaction procedure and conditions follow that for the synthesis of **6**, using *N,N*-bis(2-hydroxyethyl)-1,3-propanediamine (2.16 g, 10.0 mmol) and 5,8-dihydroxy-leucoquinizarin (0.2 g, 0.75 mmol). Purification by flash column chromatography (CH₃OH:CH₂Cl₂:NH₃, 4.5:95:0.5) afforded the product as a dark blue solid (128.1 mg, 31% yield). m.p. 243.4 - 245.2°C; ¹H NMR (CDCl₃/CD₃OD) δ: 1.75 (t, 4H, 2× CH₂CH₂CH₂), 2.5 (t, 8H, 4×CH₂N), 2.6 (t, 4H, 2×CH₂N), 3.45 (q, 4H, 2×CH₂NAr), 3.6 (t, 8H, 4×CH₂OH), 7.2 (s, 2H, ArH), 7.6 (s, 2H, ArH), 10.6 (t, 2H, 2×NHAr); IR ν_{max} (KBr) cm⁻¹ 3600-3300 (OH), 1580 (C=O), 1230 (N-H); E_λ (dH₂O) = 9844 cm⁻¹; λ_{max} (612 nm); FAB-MS, m/z (M+H)⁺ 561; Anal. (C₂₈H₄₀N₄O₈) C, H, N.

1-[[2-[Dimethylamino]ethyl]amino]-4-[[2-(2-(hydroxyethyl)(methyl)amino)ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione (5)

5,8-Dihydroxy-leucoquinizarin (0.2 g, 0.75 mmol) was added to *N*-2-hydroxyethyl-*N*-methylethylenediamine (0.71 g, 6.0 mmol) at 60 °C under nitrogen and heated for 30 min. *N,N*-dimethylethylenediamine (0.194 g, 2.2 mmol) was added and the reaction mixture was stirred at 80°C for a further 5 hours. After cooling to room temperature, aqueous NaOH (15%, 0.2 cm³) was added and the reaction mixture was exposed to air and stirred overnight. The reaction mixture was diluted with CH₂Cl₂ (80 cm³) and washed with water (3×80 cm³). The organic layer was dried with MgSO₄ and the solvent was concentrated *in vacuo* to yield a waxy solid. This crude solid was purified by flash column chromatography (CH₃OH:CH₂Cl₂:NH₃, 4.5:94:0.5). The resulting solid was further purified by re-dissolving in CH₃OH and precipitation with dry diethyl ether to give the product as dark blue crystals (34.8 mg, 11% yield). m.p. 207.5 - 209.5°C; ¹H NMR (CDCl₃/CD₃OD) δ: 2.7 (s, 9H, 3×NCH₃), 3.1 (t, 4H, 2×CH₂N), 3.3 (t, 2H, CH₂N), 3.9 (q, 4H, 2×CH₂NHAr), 4.1 (t, 2H, CH₂OH), 7.1 (s, 2H, ArH), 7.3 (s, 2H, ArH), 10.8 (t, 2×H, NHAr); IR ν_{max} (KBr) cm⁻¹ 3600-3300 (OH), 1580 (C=O), 1230 (N-H); E_λ = 7128 cm⁻¹; λ_{max} (608 nm); FAB-MS, m/z (M+H)⁺ 443; Anal. (C₂₃H₃₀N₄O₅) C, H, N.

1,4-Bis[[2-[2-(hydroxyethyl)(methyl)amino]ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione (6)

5,8-Dihydroxy-leucoquinizarin (0.2 g, 0.75 mmol) was added to *N*-2-hydroxyethyl-*N*-methylethylenediamine (1.3 g, 12.0 mmol) at 60 °C under nitrogen and heated for 5 hours. The reaction mixture was cooled to room temperature, aqueous NaOH (2 M, 0.2 cm³) was added and the reaction was exposed to air and stirred overnight. The reaction mixture was diluted with CH₂Cl₂ (80 cm³) and washed with water (3×80 cm³). The organic layer was dried with MgSO₄ and the solvent was concentrated *in vacuo* to yield a waxy solid. This crude solid was purified by flash column chromatography (CH₃OH:CH₂Cl₂:NH₃, 5:94:1). The resulting solid was further purified by re-dissolving in CH₃OH and precipitation with dry diethyl ether to give the product as dark blue crystals (87.1 mg, 25% yield). m.p. 211.3 - 213.5°C; ¹H NMR (CDCl₃/CD₃OD) δ: 2.3 (s, 6H, 2×CH₃N), 2.7 (t, 4H, 2×CH₂N), 2.8 (t, 4H, 2×CH₂N), 3.6 (q, 4H, 2×CH₂NAr), 3.7 (t, 4H, 2×CH₂OH), 7.2 (s, 2H, ArH), 7.4 (s, 2H, ArH), 10.6 (t, 2H, 2×NHArH); IR ν_{max} (KBr) cm⁻¹ 3600-3300 (OH), 1580 (C=O), 1230 (N-H); E_λ (dH₂O) = 14604 cm⁻¹; λ_{max} (608 nm); FAB-MS, m/z (M+H)⁺ 473; Anal. (C₂₄H₃₂N₄O₆) C, H, N.

1,4-Bis[[2-[bis(2-hydroxyethyl)amino]ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione (8)

Reaction procedure and conditions follow that for the synthesis of **6**, using *N,N*-bis(2-hydroxyethyl)ethylenediamine (2.0 g, 10 mmol) and 5,8-dihydroxy-leucoquinizarin (0.2 g, 0.75 mmol). Purification by flash column chromatography (CH₃OH:NH₃, 99.5:0.5) afforded the product as a dark blue solid (94.2 mg, 24% yield); m.p. 218.5 - 219.5°C; ¹H NMR (CDCl₃/CD₃OD) δ: 2.7 (t, 8H, 4×CH₂N), 2.9 (t, 4H, 2×CH₂N), 3.5 (q, 4H, 2×CH₂NAr), 3.7 (t, 8H, 4×CH₂OH), 7.1 (s, 2H, ArH), 7.3 (s, 2H, ArH), 10.6 (t, 2H, 2×NHAr); IR ν_{max} (KBr) cm⁻¹ 3600-3300 (OH), 1580 (C=O), 1230 (N-H); E_λ (dH₂O) = 8876 cm⁻¹; λ_{max} (606 nm); FAB-MS, m/z (M+H)⁺ 533; Anal. (C₂₆H₃₆N₄O₈) C, H, N.

1-[[2-[2-(Hydroxyethyl)(methyl)amino]ethyl]amino]anthracene-9,10-dione (9)

To *N*-2-hydroxyethyl-*N*-methylethylenediamine (2.4 g, 0.02 mol) in 2-methoxyethanol (10 cm³) was added 1-chloroanthraquinone (0.5 g, 2.05 mmol) and the mixture was refluxed for 6 hours. After evaporation, the resulting waxy solid was diluted with CH₂Cl₂ (20 cm³) and washed with water (3×20 cm³) to remove any unreacted amine. Following evaporation, the crude product was lyophilised and subsequently purified by flash column chromatography (CH₂Cl₂: EtOH, 9:1). The product was further purified by re-dissolving the product in EtOH and precipitation with dry diethyl ether to give the target compound as a red/orange solid. (100 mg, 15% yield). m.p. 105.9 - 106.8°C; ¹H NMR (CDCl₃/CD₃OD) δ: 2.3 (s, 3H, CH₃N), 2.7 (t, 2H, CH₂CH₂N), 2.8 (t, 2H, CH₂CH₂N), 3.4 (q, 2H, CH₂NHAr), 3.7 (t, 2H, CH₂OH), 7.0 (d, 1H, Ar-H), 7.5 (m, 2H, Ar-H), 7.7 (m, 2H, Ar-H), 8.2 (d, 1H, Ar-H), 8.3 (d, 1H, Ar-H), 10.1 (t, 1H, NHAr); IR ν_{max} (KBr) cm⁻¹: 3600-3300 (OH), 1650 (C=O), 1040 (N-H); E_λ (CH₃OH/DMSO) = 4761 cm⁻¹; λ_{max} (521 nm); FAB-MS m/z (M+H)⁺ 325; Anal. (C₁₉H₂₀N₂O₃) C, H, N.

1-[[3-[2-(Hydroxyethyl)(methyl)amino]propyl]amino]anthracene-9,10-dione (10)

Reaction procedure and conditions follow the method for the synthesis of **9**, using 1-chloroanthraquinone (0.2 g, 0.82 mmol) and *N*-2-hydroxyethyl-*N*-methyl-1,3-propanediamine (1.1 g, 8.2 mmol). Purification by flash column chromatography (CH₂Cl₂:EtOH, 9:1) yielded the product as a red solid (47.2 mg, 18% yield). m.p. 98.5 - 99.2°C; ¹H NMR (CDCl₃/CD₃OD) δ: 1.8 (m, 2H, CH₂CH₂CH₂), 2.3 (s, 3H, CH₃N), 2.7 (t, 2H, CH₂N), 2.8 (t, 2H, HNCH₂CH₂N), 3.4 (q, 2H, CH₂NHAr), 3.7 (t, 2H, CH₂OH), 7.0 (d, 1H, Ar-H), 7.5 (m, 2H, Ar-H), 7.7 (m, 2H, Ar-H), 8.2 (d, 1H, Ar-H), 8.3 (d, 1H, Ar-H), 10.1 (t, 1H, NHAr); IR ν_{max} (KBr) cm⁻¹: 3600-3300 (OH), 1650 (C=O), 1040 (N-H); E_λ (CH₃OH/DMSO) = 3514 cm⁻¹; λ_{max} (504 nm); FAB-MS, m/z (M+H)⁺ 339; Anal. (C₂₀H₂₂N₂O₃) C, H, N.

1,4-Bis[[2-[bis(2-chloroethyl)amino]ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione dihydrochloride (11)

Reaction conditions and procedure follow that for the synthesis of **8** (0.05 g, 0.09 mmol), triphenylphosphine (0.147 g, 0.56 mmol) and carbon tetrachloride (0.34 g, 2.25 mmol). The product was isolated as a dark blue solid (45.6 mg, 75% yield). m.p. 191.6 - 192°C; ¹H NMR (CDCl₃/CD₃OD) δ 3.0 (m, 12H, 6×CH₂N), 3.5 (q, 4H, 2×CH₂NHAr), 3.7 (t, 8H, 2×CH₂Cl), 7.1 (s, 2H, ArH), 7.3 (s, 2H, ArH), 10.4 (t, 2H, 2×NHAr); IR ν_{max} (KBr) cm⁻¹ 3600-3300 (OH), 1580 (C=O), 1230 (N-H); E_λ (CH₃OH/DMSO) = 8950 cm⁻¹; λ_{max} (673 nm); FAB-MS, m/z (M+H)⁺ 607; Anal. (C₂₆H₃₂N₄O₄Cl₄ · 2HCl) C, H, N.

1,4-Bis[[3-[bis(2-chloroethyl)amino]propyl]amino]-5,8-dihydroxyanthracene-9,10-dione dihydrochloride (13)

Reaction conditions and procedure follow that for the synthesis of **16**, using **4** (0.05 g, 0.79 mmol), triphenylphosphine (0.124 g, 0.47 mmol) and carbon tetrachloride (0.288 g, 1.89 mmol). The product was isolated as a dark blue solid (44.7 mg, 81% yield). m.p. 205.1 - 206.5°C; ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ : 2.2 (t, 4H, $2\times\text{CH}_2\text{CH}_2\text{CH}_2$), 3.5 (t, 8H, $4\times\text{CH}_2\text{N}$), 3.6 (t, 4H, $2\times\text{CH}_2\text{N}$), 3.7 (q, 4H, $2\times\text{CH}_2\text{NHAr}$), 3.9 (t, 8H, $4\times\text{CH}_2\text{Cl}$), 7.1 (s, 2H, ArH), 7.3 (s, 2H, ArH), 10.6 (t, 2H, $2\times\text{NHAr}$); IR ν_{max} (KBr) cm^{-1} 3600-3300 (OH), 1580 (C=O), 1230 (N-H); E_{λ} ($\text{CH}_3\text{OH}/\text{DMSO}$) = 9.125 cm^{-1} ; λ_{max} (675 nm); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 635; Anal. ($\text{C}_{28}\text{H}_{36}\text{N}_4\text{O}_4\text{Cl}_4 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$) C, H, N.

1-[[3-[Bis(2-chloroethyl)amino]propyl]amino]anthracene-9,10-dione hydrochloride (14)

Reaction procedure and conditions follow that for the synthesis of **12**, using **14a** (0.05 g, 0.136 mmol); triphenylphosphine (0.071 g, 0.27 mmol) and carbon tetrachloride (0.246 g, 1.62 mmol). The product was isolated as a red solid (48.0 mg, 80% yield). m.p. 194.5 - 196.5°C; ^1H NMR (CD_3OD) δ : 2.2 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.5 (t, 2H, $\text{CH}_2\text{CH}_2\text{N}$), 3.54 (t, 2H, $\text{CH}_2\text{CH}_2\text{N}$), 3.65 (t, 2H, $\text{CH}_2\text{CH}_2\text{N}$), 3.7 (q, 2H, CH_2NHAr), 4.0 (t, 4H, $2\times\text{CH}_2\text{Cl}$), 7.3 (d, 1H, Ar-H), 7.7 (m, 2H, Ar-H), 7.8 (m, 2H, Ar-H), 8.2 (d, 1H, Ar-H); 8.3 (d, 1H, Ar-H); IR ν_{max} (KBr) cm^{-1} 3550-3310 (NRH), 1600 (C=O), 1230 (N-H); E_{λ} ($\text{CH}_3\text{OH}/\text{DMSO}$) = 7948 cm^{-1} ; λ_{max} (503 nm); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 405; Anal. ($\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2\text{Cl}_2 \cdot 2\text{HCl}$) C, H, N.

1-[[3-[Bis(2-hydroxyethyl)amino]propyl]amino]anthracene-9,10-dione (14a)

To *N,N*-bis(2-hydroxyethyl)propanediamine (3.3 g, 20.0 mmol) in 2-methoxyethanol (10 cm^3) was added 1-chloroanthraquinone (0.5 g, 2.05 mmol) and the mixture was refluxed for 6h. On cooling, the reaction mixture was diluted with CH_2Cl_2 (20 cm^3) and washed with water ($3\times 20\text{ cm}^3$) to remove any unreacted amine. Following evaporation, the crude product was further purified by precipitation in CH_3OH with dry diethyl ether to give the target compound as a red powder (75.3 mg, 10.2% yield). m.p. 91.2 - 92.2 °C. ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) peaks: δ 1.8 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.3 (t, 6H, $3\times\text{CH}_2\text{N}$), 3.4 (q, 2H, CH_2NHAr), 3.7 (t, 4H, $2\times\text{CH}_2\text{OH}$), 7.0 (d, 1H, ArH), 7.5 (m, 2H, ArH), 7.7 (m, 2H, ArH), 8.2 (d, 1H, ArH), 8.3 (d, 1H, ArH), 10.1 (t, 2H, NHAr); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 369; IR ν_{max} (KBr) cm^{-1} 3600-3300 (OH), 1650 (C=O), 1040 (N-H); E_{λ} (MeOH/DMSO) = 1961 cm^{-1} ; λ_{max} (510 nm).

1,4-Bis[[2-[2-(chloroethyl)(methyl)amino]ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione dihydrochloride (15)

Reaction procedure and conditions follow that for the synthesis of **16**, using **6** (0.05 g, 0.11 mmol), triphenylphosphine (0.08 g, 0.32 mmol) and carbon tetrachloride (0.19 g, 1.2 mmol). The product was isolated as a dark blue solid (52.5 mg, 85% yield). m.p. 201.2 - 203.1°C; ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ : 3.0 (s, 6H, $2\times\text{CH}_3\text{N}$), 3.5 (t, 4H, $2\times\text{CH}_2\text{NCH}_2$), 3.6 (t, 4H, $2\times\text{CH}_2\text{NCH}_2$), 3.9 (q, 4H, $2\times\text{CH}_2\text{NHAr}$), 3.9 (t, 4H, $2\times\text{CH}_2\text{Cl}$), 7.2 (s, 2H, ArH), 7.4 (s, 2H, ArH), 10.6 (t, 2H, $2\times\text{NHAr}$); IR ν_{max} (KBr) cm^{-1} 3600-3300 (OH), 1580 (C=O), 1230 (N-H); E_{λ} ($\text{CH}_3\text{OH}/\text{DMSO}$) = 27026 cm^{-1} ; λ_{max} (666 nm); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 509; Anal. ($\text{C}_{24}\text{H}_{30}\text{N}_4\text{O}_4\text{Cl}_2 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$) C, H, N.

1,4-Bis[[2-[2-(chloroethyl)(methyl)amino]ethyl]amino]anthracene-9,10-dione dihydrochloride (16)

Triphenylphosphine (0.086 g, 0.33 mmol), then carbon tetrachloride (0.15 g, 1.32 mmol) were added to a stirred solution of **3** (0.05 g, 0.11 mmol) in a mixture of CH_2Cl_2 (4.0 cm^3) and CH_3CN (1.0 cm^3) under nitrogen. The resulting suspension was allowed to stir at room temperature for 48 hours. The crude product was precipitated by

the addition of dry ethereal hydrogen chloride, isolated by filtration and dried under vacuum. The solid residue was dissolved in a minimum quantity of $\text{CH}_2\text{Cl}_2/\text{EtOH}$ (1:1) at 60°C and isolated from the triphenylphosphine oxide by-product and excess triphenylphosphine by precipitation with EtOH/EtOAc (1:1). The product was isolated as a dark blue solid (47.4 mg, 79% yield). m.p. $185.4 - 186^\circ\text{C}$; ^1H NMR ($\text{CD}_3\text{OD}/\text{CDCl}_3$) δ : 3.1 (s, 6H, $2\times\text{CH}_3\text{N}$), 3.6 (t, 4H, $2\times\text{CH}_2\text{CH}_2\text{N}$), 3.7 (t, 4H, $2\times\text{CH}_2\text{CH}_2\text{N}$), 3.9 (q, 4H, $2\times\text{CH}_2\text{NHAr}$), 3.95 (t, 4H, $2\times\text{CH}_2\text{Cl}$), 7.6 (s, 2H, Ar-H), 7.8 (m, 2H, Ar-H), 8.3 (m, 2H, Ar-H), 10.1 (t, 1H, NHAr); IR ν_{max} (KBr) cm^{-1} 3550-3310 (NRH), 1600 (C=O), 1230 (N-H); E_λ ($\text{CH}_3\text{OH}/\text{DMSO}$) = 12027 cm^{-1} ; λ_{max} (638 nm); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 477; Anal. ($\text{C}_{24}\text{H}_{30}\text{N}_4\text{O}_2\text{Cl}_2 \cdot 2\text{HCl} \cdot \text{H}_2\text{O}$) C, H, N.

1-[[3-[2-(Chloroethyl)(methyl)amino]propyl]amino]anthracene-9,10-dione hydrochloride (17)

Reaction procedure and conditions follow that for the synthesis of **12**, using **10** (0.05 g, 0.15 mmol), triphenylphosphine (0.078 g, 0.3 mmol) and carbon tetrachloride (0.137 g, 0.9 mmol). The product was isolated as a red solid (35.5 mg, 60%). m.p. $205 - 207^\circ\text{C}$; ^1H NMR (CD_3OD) δ : 2.25 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.1 (s, 3H, CH_3N), 3.4 (t, 2H, $\text{CH}_2\text{CH}_2\text{N}$), 3.54 (t, 2H, $\text{CH}_2\text{CH}_2\text{N}$), 3.7 (q, 2H, CH_2NHAr), 4.0 (t, 2H, CH_2Cl), 7.3 (d, 1H, Ar-H), 7.7 (m, 2H, Ar-H), 7.8 (m, 2H, Ar-H), 8.2 (d, 1H, Ar-H), 8.3 (d, 1H, Ar-H), 10.1 (t, 1H, NHAr); IR ν_{max} (KBr) cm^{-1} 3550-3310 (NRH), 1600 (C=O), 1230 (N-H); E_λ ($\text{CH}_3\text{OH}/\text{DMSO}$) = 1297.5 cm^{-1} ; λ_{max} (498 nm); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 357; Anal. ($\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}_2\text{Cl} \cdot \text{HCl}$) C, H, N.

1,4-Bis[[3-[bis(2-chloroethyl)amino]propyl]amino]anthracene-9,10-dione dihydrochloride (18)

Reaction procedure and conditions follow that for the synthesis of **16**, using **2** (0.05 g, 0.095 mmol), triphenylphosphine (0.15 g, 0.57 mmol) and carbon tetrachloride (0.346 g, 2.28 mmol). The product was isolated as a dark blue solid (44.9 mg, 70% yield). m.p. $187.2 - 188.9^\circ\text{C}$; ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ : 2.2 (m, 4H, $2\times\text{CH}_2\text{CH}_2\text{CH}_2$), 3.5 (t, 4H, $2\times\text{CH}_2\text{N}$), 3.6 (t, 8H, $4\times\text{CH}_2\text{N}$), 3.7 (q, 4H, CH_2NHAr), 3.9 (t, 8H, $4\times\text{CH}_2\text{Cl}$), 7.4 (s, 2H, Ar-H), 7.7 (m, 2H, Ar-H), 8.2 (m, 2H, Ar-H); IR ν_{max} (KBr) cm^{-1} 3550-3310 (NRH), 1600 (C=O), 1230 (N-H); E_λ ($\text{CH}_3\text{OH}/\text{DMSO}$) = 2171 cm^{-1} ; λ_{max} (642 nm); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 603; Anal. ($\text{C}_{28}\text{H}_{36}\text{N}_4\text{O}_2\text{Cl}_4 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$) C, H, N: C, 47.23, H, 5.95, N, 7.87. Found: C, 47.89, 6.35, 7.39.

1,4-Bis[[2-[bis(2-chloroethyl)amino]ethyl]amino]anthracene-9,10-dione dihydrochloride (19)

Reaction procedure and conditions follow that for the synthesis of **16**, using **1** (0.05 g, 0.09 mmol), triphenylphosphine (0.144 g, 0.5 mmol) and carbon tetrachloride (0.32 g, 2.1 mmol). The product was isolated as a dark blue solid (35.4 mg, 64% yield). m.p. $194 - 196^\circ\text{C}$; ^1H NMR ($\text{CD}_3\text{OD}/\text{CDCl}_3$) δ : 3.6 (t, 4H, $2\times\text{CH}_2\text{CH}_2\text{N}$), 3.64 (t, 8H, $4\times\text{CH}_2\text{CH}_2\text{N}$), 3.9 (q, 4H, $2\times\text{CH}_2\text{NHAr}$), 4.0 (t, 8H, $4\times\text{CH}_2\text{Cl}$), 7.3 (d, 1H, Ar-H), 7.7 (m, 2H, Ar-H), 7.8 (m, 2H, Ar-H), 8.2 (d, 1H, Ar-H), 8.3 (d, 1H, Ar-H); IR ν_{max} (KBr) cm^{-1} 3550-3310 (NRH), 1600 (C=O), 1230 (N-H); E_λ ($\text{CH}_3\text{OH}/\text{DMSO}$) = 8570 cm^{-1} ; λ_{max} (638 nm); FAB-MS, m/z ($\text{M}+\text{H}$) $^+$ 575; Anal. ($\text{C}_{26}\text{H}_{32}\text{N}_4\text{O}_2\text{Cl}_4 \cdot 2\text{HCl}$) C, H, N.

1-[[2-[2-(Chloroethyl)(methyl)amino]ethyl]amino]anthracene-9,10-dione hydrochloride (20)

Procedure as **16** using triphenylphosphine (0.16 g, 0.6 mmol), carbon tetrachloride (0.28 g, 1.8 mmol), **9** (0.1 g, 0.31 mmol) in CH_2Cl_2 (5.0 cm^3). The product was isolated as a red solid (93.5 mg, 80% yield). m.p. $207.1 - 209.2^\circ\text{C}$; ^1H NMR

(CD₃OD/CDCl₃) δ : 3.1 (s, 3H, CH₃N), 3.5 (t, 2H, CH₂CH₂N), 3.7 (t, 2H, CH₂CH₂N), 3.9 (q, 2H, CH₂NHAr), 3.95 (t, 2H, CH₂Cl), 7.3 (d, 1H, Ar-*H*), 7.7 (m, 2H, Ar-*H*), 7.8 (m, 2H, Ar-*H*), 8.2 (d, 1H, Ar-*H*), 8.3 (d, 1H, Ar-*H*), 10.1 (t, 1H, NHAr); IR ν_{\max} (KBr) cm⁻¹ 3550-3310 (NRH), 1600 (C=O), 1230 (N-H); E _{λ} (CH₃OH/DMSO) = 6963 cm⁻¹; λ_{\max} (488 nm); FAB-MS, m/z (M+H)⁺ 343; Anal. (C₁₉H₁₉N₂O₂Cl·HCl) C, H, N.

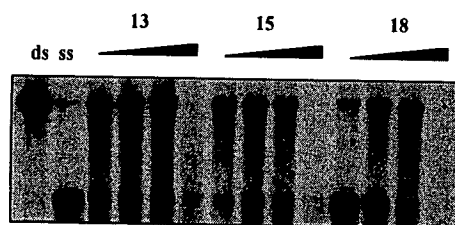
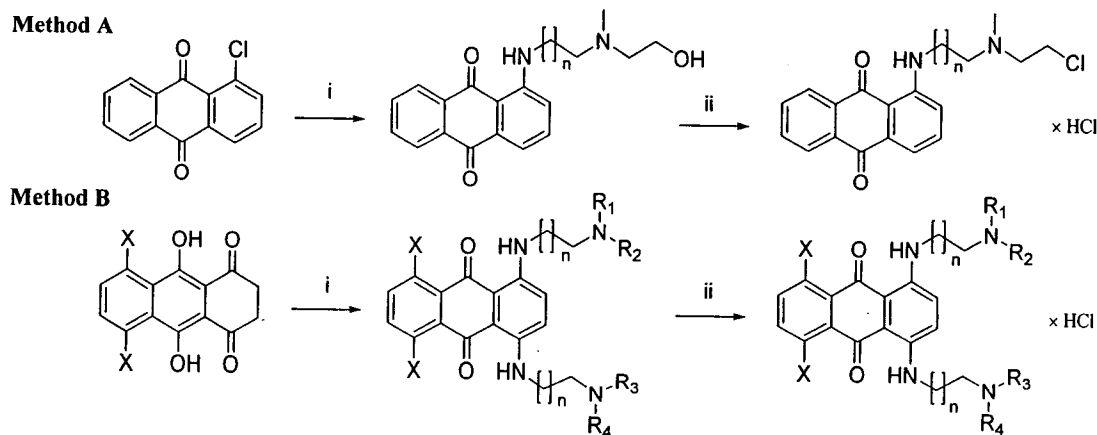


Figure 1 Effect of **13**, **15** and **18** on supercoiled double stranded plasmid DNA strand separation. Concentration of chloroethylaminoanthraquinones (from left to right): 0.1, 1, 10, and 100 nM); ds = double stranded DNA, no drug; ss = single stranded DNA, no drug. See methods for full details.



Scheme 1 *Method A:* (i) *N*-alkyl-*N*-hydroxyalkylaminoalkylamine, reflux in 2-methoxyethanol, 6 h; (ii) $\text{Ph}_3\text{P}-\text{CCl}_4$ in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (4:1), rt, 48 h, hydrochloride salt made by the addition of ethereal hydrogen chloride. *Method B:* (i) leucoquinizarine ($\text{X} = \text{H}$) or 5,8-dihydroxyleucoquinizarine ($\text{X} = \text{OH}$) heated with appropriate *N*-alkyl-*N*-hydroxyalkylaminoalkylamine, 5h, R_1 , R_2 , R_3 , $\text{R}_4 = \text{CH}_3$ or $\text{CH}_2\text{CH}_2\text{OH}$, $n = 1$ or 2); (ii) carried out as under step ii in method A, R_1 , R_2 , R_3 , $\text{R}_4 = \text{CH}_3$ or $\text{CH}_2\text{CH}_2\text{Cl}$, $n = 1$ or 2). For specific structures, see Table 1.