CHAPTER III

MATERIALS AND METHODS

3.1 Chemical reagent

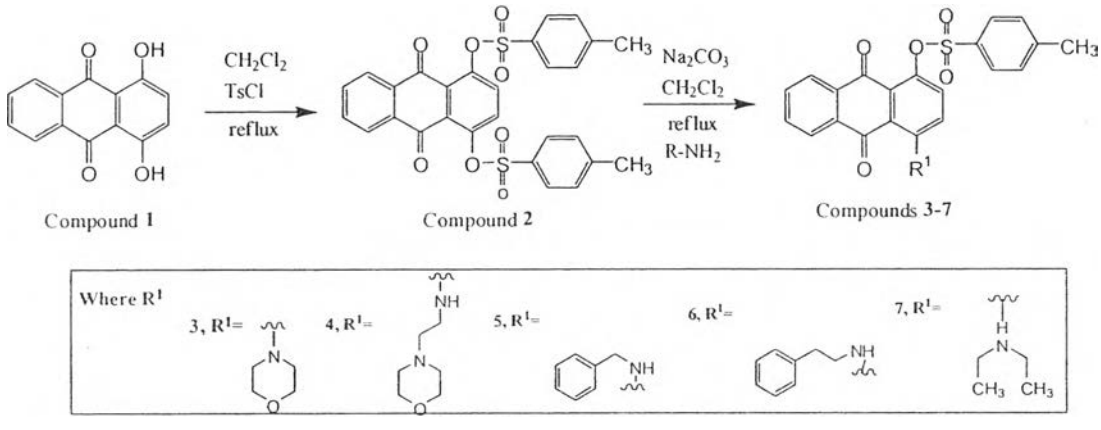
^{1}H-NMR and ^{13}C-NMR spectra were measured on a Varian Mercury plus spectrometer in $CDCl_3$ with TMS as the internal standard, where *J* (coupling constant) values are estimated Hertz (Hz). Mass spectra (MS) were recorded on a Water's Micromass Quattamicro API ESCi mass spectrometer using the electrospray (ES) ionization mode and matrix-assisted laser desorption ionization (MALDI-MS). Silica gel column chromatography was performed using Merck silica gel 60 ASTM (0.040-0.063). All other reagents, purchased from the Aldrich Chemical Company and Amersham (UK), were used without further purification. All solvents (Labscan, Poland) used were purified according to standard procedures.

General procedure for the synthesis of anthraquinone derivatives

Synthesis of 9,10-dioxo-4a,9,9a,10-tetrahydroanthracene-1,4-diyl bis(4-methylbenzenesulfonate) (2)

1, 4-Dihydroxy anthraquinone (2.41 g, 10.00 mmol) was dissolved in 10 mL dry dichloromethane under nitrogen and then triethylamine (2.05 g, 23.0 mmol) was added. The reaction mixture was refluxed, 2,4-diamino toluene hydrochloride (3.83 g, 20.1 mmol) was added and stirred for 6 h. After cooling down to room temperature (RT), the excess *p*-tosyl chloride was successfully removed by sequentially washing with 2 volumes of 3 N HCl and water and then extracted 3 times each with 2 volumes of dichloromethane (CH_2Cl_2). The combined organic layers were dried over anhydrous sodium sulfate and the solvent evaporated to dryness. The residue was dissolved in a minimum amount of CH_2Cl_2 and methanol was added to give compound 2 as a yellow

solid as described in the previous report (Ossowski and Aguirre-Ghiso, 2000), ^{1}H-NMR of 1,4-bis(tosyl)anthraquinone were recorded in $CDCl_3$ solution on a Varian Mercury Plus 400 spectrometer. ^{1}H NMR spectrum (δ, ppm): 7.94-8.02 (2H, m, Ar-H); 7.69-7.91 (6H, m, Ar-H); 7.45 (2H, s, Ar-H); 7.24-7.33 (4H, m, Ar-H); 2.35 (6H, s, CH_3).



Scheme 1. Synthesis pathway for of 1,4-dihydroxyanthraquinone derivatives (Compounds 3-7)

Synthesis of 1,4-dihydroxyanthraquinone derivatives (3-7)

To the solution of 2 in dry acetonitrile (10 ml) was added K_2CO_3 under a nitrogen atmosphere and refluxed for 30 minutes. After cooling down to room temperature, the reaction mixture was evaporated under vacuum and dissolved in 3N HCl and water. After extraction with dichloromethane the combined organic layers were dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness. The residue was filtered off and then washed with water to provide the solid products 3-7.

Synthesis of 4-morpholino-9,10-dioxo-4a,9,9a,10-tetrahydroanthracen-1-yl 4-methylbenzenesulfonate (3)

Compound 3 was prepared from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 ml), K_2CO_3 (0.34 g, 2.3 mmol) and morpholine (0.30 ml, 0.32 mmol) under a nitrogen atmospheres give solid 3 at 80% yield. ^{1}H-NMR (400 Hz, $CDCl_3$): 2.20 (s, 3H, Ar-CH_3), 3.21 (s, 4H, 2 x heterocyclic-CH_2), 4.01 (s, 4H, 2 x heterocyclic-CH_2) 7.19 (s, 1H, Ar-CH), 7.21 (s, 1H, Ar-CH), 7.30 (d, *J* = 12.0 Hz, 1H, Ar-CH), 7.46 (d, *J* = 8.0 Hz, 1H, Ar-CH), 7.64-7.71 (m, 2H, 2 x Ar-CH), 7.75 (d, *J* = 1.2 Hz, 2H, 2 x Ar-CH), 7.91 (d, *J* = 8.0 Hz, 1H, Ar-CH) and 8.14 (d, *J* = 4.00 Hz, 1H, Ar-CH). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.6 (Ar-C), 53.1 (2 x heterocyclic-C), 66.9 (2 x heterocyclic-C), 124.4 (Ar-C), 126.2 (Ar-C), 126.2 (Ar-C), 126.9 (Ar-C), 128.9 (Ar-C), 129.6 (3 x Ar-C), 129.7 (4 x Ar-C), 131.2 (Ar-C), 133.0 (Ar-C), 133.8 (Ar-C), 141.0 (Ar-C), 145.4 (Ar-C), 151. 9 (Ar-C) and 180.4 (2 x C=O). MS (*m/z*) calcd. for $[C_{25}H_{21}NO_6S+H]^+$465.50, found 465.425.

Synthesis of 4-(2-morpholinoethylamino)-9,10-dioxo-4a,9,9a,10-tetrahydroanthracen-1-yl 4-methylbenzenesulfonate (4)

Compound 4 was prepared from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 ml), K_2CO_3 (0.34 g, 2.3 mmol) and amino-morpholine (0.42 ml, 0.32 mmol) under a nitrogen atmosphere to give solid 4 at 70%yield. ^{1}H-NMR (400 Hz, $CDCl_3$): 2.34 (s, 3H, Ar-CH_3), 2.57 (m, 4H, 2 x heterocyclic-CH_2), 2.74 (dd, *J* = 6.4, 6.0 Hz, 2H, aliphatic-CH2), 3.39-3.42 (m, 2H, aliphatic-CH_2), 3.80 (dd, *J* = 4.0, 4.4 Hz, 4H, heterocyclic-CH_2), 6.97 (s, 1H, Ar-CH), 6.99 (s, 1H, Ar-CH), 7.24 (s, 1H, Ar-CH), 7.33 (d, *J* = 9.2 Hz, 1H, Ar-CH), 7.64-7.73 (m, 2H, 2 x Ar-CH), 7.80 (s, 1H, Ar-CH), 7.81 (s, 1H, Ar-CH), 7.97 (d, *J* = 7.6, 1H, Ar-CH), 8.19 (d, *J* = 7.6, Ar-CH) and 10.12 (s, 1H, NH). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.62 (CH_3), 40.0 (aliphatic-CH_2), 53.3 (aliphatic-C), 53.5 (heterocyclic-C), 53.7 (heterocyclic-C), 66.9 (heterocyclic-C), 67.0 (heterocyclic-C), 118.2 (Ar-C), 126.2 (Ar-C), 126.3 (Ar-C), 128.9 (Ar-C), 129.5 (3 x Ar-C), 129.8 (2 x Ar-C), 132.4 (Ar-C), 132.5 (Ar-C), 132.7 (Ar-C), 132.9 (Ar-C), 133.4 (Ar-C), 133.8 (Ar-C), 137.2 (Ar-C), 145.2 (Ar-C), 150.3 (Ar-C) and 185.1 (2 x C=O). MS (*m/z*) calcd. for $[C_{27}H_{26}N_2O_6S+H]^+$506.57, found 507.469.

Synthesis of 4-(benzylamino)-9,10-dioxo-4a,9,9a,10-tetrahydroanthracen-1-yl 4-methylbenzenesulfonate (5)

Compound 5 was prepared from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 ml), K_2CO_3 (0.34 g, 2.3 mmol) and benzylamine (0.34 ml, 0.32 mmol) under a nitrogen atmosphere to give a red solid (5) at 65% yield. ^{1}H-NMR (400 Hz, $CDCl_3$): 2.34 (s, 3H, Ar-CH_3), 4.57 (d, *J* = 6.8 Hz, 2H, aliphatic-CH_2), 6.94 (d, *J* = 8.0 Hz, 1H, Ar-CH), 7.24 (s, 1H, Ar-CH), 7.26 (s, 2H, 2 x Ar-CH), 7.31-7.33 (m, 2H, 2 x Ar-CH), 7.34 (s, 2H, 2 x Ar-CH), 7.38 (s, 1H, Ar-CH), 7.65-7.72 (m, 2H, 2 x Ar-CH), 7.8 (d, *J* = 8.0 Hz, 2H, 2 x Ar-CH), 7.99 (d, *J* = 8.0 Hz, 1H, Ar-CH), 8.18 (d, *J* = 8.0 Hz, 1H Ar-CH) and10.35 (s, 1H, NH). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.6 (CH_3), 47.1 (2 x aliphatic-CH_2), 118.6 (2 x Ar-C), 126.3 (Ar-C), 127.1 (Ar-C), 127.2 (Ar-C), 127.7 (Ar-C), 128.9 (Ar-C), 129.0 (4 x Ar-C), 129.5 (4 x Ar-C), 132.5 (Ar-C), 132.7 (Ar-C), 133.2 (Ar-C), 133.6 (Ar-C), 137.6 (Ar-C), 137.8 (Ar-C), 145.2 (Ar-C), 150.4 (Ar-C), 182.0 (C=O) and 185.1 (C=O). MS (*m/z*) calcd. for $[C_{28}H_{21}NO_5 + H]^+$ 483.11, found 488.323

Synthesis of 9,10-dioxo-4-(phenethylamino)-4a,9,9a,10-tetrahydroanthracen-1-yl 4-methylbenzenesulfonate (6)

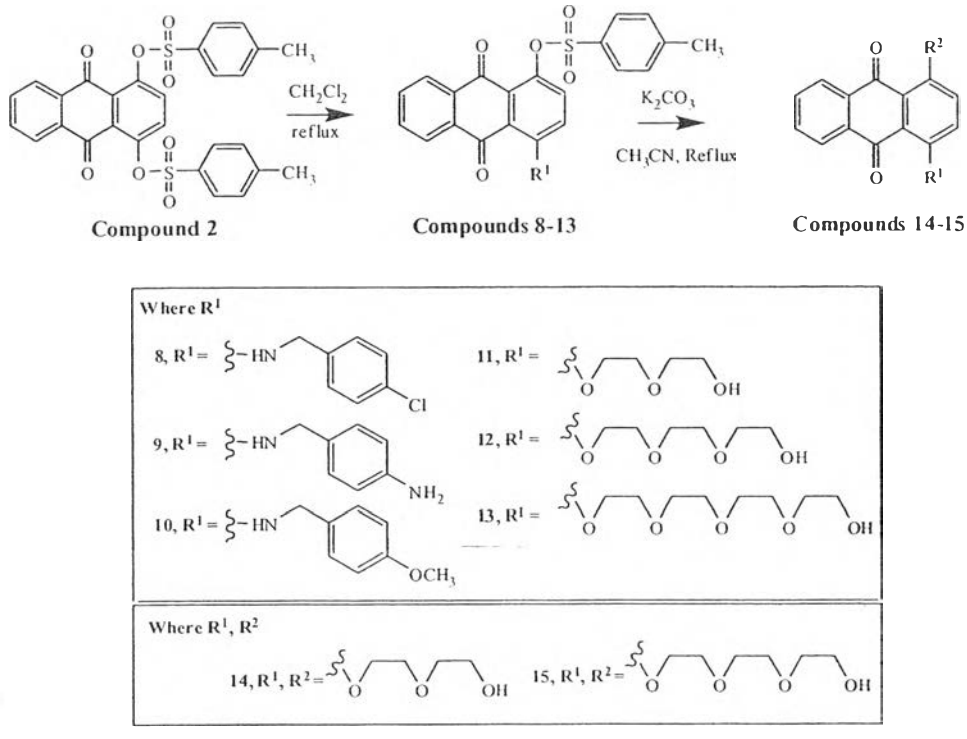
Compound 6 was prepared from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 ml), K_2CO_3 (0.34 g, 2.3 mmol) and phenylamine (0.39 ml, 0.32 mmol) under a nitrogen atmosphere to give solid 6 at 65% yield. ^{1}H-NMR (400 Hz, $CDCl_3$): 2.33 (s, 3H, Ar- CH_3), 3.06 (dd, *J* = 7.2 , 6.4 Hz, 2H, aliphatic-CH_2), 3.58 (tri, 2H, aliphatic-CH_2), 6.97 (d, *J* = 8.0 Hz, 1H, Ar-CH), 7.23 (s, 1H, Ar-CH), 7.20 (s, 1H, Ar-CH), 7.31 (s, 3H, 3 x Ar-CH),7.34 (s, 2H, 2 x Ar-CH), 7.35 (s, 1H, Ar-CH), 7.64-7.73 (m, 2H, 2 x Ar-CH), 7.78 (s, 1H, Ar-CH), 7.80 (s, 1H, Ar-CH), 7.95 (s, 1H, Ar-CH), 8.16 (s, 1H, Ar-CH) and 10.04 (s, 1H, NH). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.6 (CH_3-C), 35.6 (aliphatic-C), 44.7 (aliphatic-C), 118.1 (Ar-C), 125.2 (Ar-C), 125.4 (Ar-C), 126.3 (Ar-C), 126.57 (Ar-C), 127.6 (Ar-C), 126.8 (Ar-C), 127.1 (Ar-C), 127.2 (Ar-C), 128.8 (Ar-C), 128.8 (Ar-C), 128.9 (Ar-C),

129.5 (Ar-C), 129.7 (Ar-C), 132.5 (Ar-C), 132.7 (Ar-C), 133.0 (Ar-C), 133.5 (Ar-C), 133.8 (Ar-C), 134.0 (Ar-C), 134.9 (Ar-C), 138.5 (Ar-C), 145.2 (Ar-C), 150.4 (Ar-C) and 184.8 (2 x C=O). MS (*m/z*) calcd. for $[C_{29}H_{23}NO_5S+H]^+$ 497.13, found 497.492

Synthesis of 4-(diethylamino)-9,10-dioxo-4a,9,9a,10-tetrahydroanthracen-1-yl 4-methylbenzenesulfonate (7)

Compound 7 was prepared from compound 2 (0.30 mg, 0.16 mmol) in dry acetonenitrile (10 ml), K_2CO_3 (0.34 g, 2.3 mmol) and diethylamine (0.23 ml, 0.32 mmol) under a nitrogen atmosphere to give solid 7 at 65% yield. ^{1}H-NMR (400 Hz, $CDCl_3$): 1,14 (s, 3H, Ar- CH_3), 1,18 (s, 3H, aliphatic-CH_3), 1.61 (s, 3H, Ar-CH_3), 3.37 (m, 4H, 2 x aliphatic-CH_2), 7.19 (s, 1H, Ar-CH), 7.21 (s, 1H, Ar-CH), 7.31 (s, 1H, Ar-CH), 7.48 (d, *J* = 9.2 Hz, 1H, Ar-CH), 7.62-7.71 (m, 2H, 2 x Ar-CH), 7.76 (s, 1H, Ar-CH), 7.83 (s, 1H, Ar-CH), 7. 93 (d, *J* = 7.6, 1H, Ar-CH) and 8.13 (d, *J* = 7.6, Ar-CH). ^{13}C-NMR (100 Hz, $CDCl_3$): 12.1 (2 x Ar-C), 21.3 (Ar-C), 42.3 (Ar-C), 43.9 (Ar-C), 47.1 (2 x CH_2-C), 118.1 (2 x Ar-C), 124.0 (4 x Ar-C), 129.1 (4 x Ar-C), 130.5 (2 x Ar-C), 137.1 (Ar-C), 134.1 (Ar-C), 145.1 (Ar-C), 150.6 (Ar-C), 180.4 (C=O), 184.8 (C=O). MS (*m/z*) calcd. for $[C_{29}H_{23}NO_5S+H]^+$ 446.13, found 448.461

General procedure for 1,4-dihydroxy anthraquinone derivatives (8-15)



Scheme 2. Synthesis pathway for anthraquinone derivatives (Compounds 8-15)

Synthesis of 1,4-anthraquinone derivatives (8-10)

To the solution of 2 in dry acetonitrile (10 mL) was added K_2CO_3 under nitrogen. The reaction mixture was refluxed for 30 min, cooled to RT and evaporated to dry residue under vacuum. The residue was dissolved in 3 N HCl and water, and the organic layers were extracted 3 times each with 2 volumes of CH_2Cl_2. The combined layers were dried over anhydrous sodium sulfate, the solvent evaporated to dryness and the residue washed with water to provide the solid products.

Synthesis of 4-(4-chlorobenzylamino)-9,10-dioxo-9,10-dihydroanthracen-1-yl 4-methylbenzenesulfonate (8)

Compound 8 was prepared as described in section above from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 mL), K_2CO_3 (0.34 g, 2.3 mmol) and N-(4-chlorophenyl)-acetamide (0.22 ml, 2.32 mmol) under nitrogen to provide yellow solid 8 at

56% yield: ^{1}H-NMR ($CDCl_3$): δ: 2.35 (3H, s, Ar-CH_3), 4.38 (2H, d, J = 8.0 Hz, CH_2-Ar), 6.85 (1H, d, J = 7.6 Hz, Ar-H), 7.20 (1H, d, J = 9.2 Hz, Ar-H), 7.28 (2H, s, Ar-H), 7.45 (2H, d, J = 10.0 Hz, Ar-H), 7.64 (2H, t, J = 7.2 Hz, Ar-H), 7.82 (4H, d, J = 7.2 Hz, Ar-H), 8.00 (1H, d, J = 9.2 Hz, Ar-H), 8.18 (1H, d, J = 7.2 Hz, Ar-H), 10.25 (1H, Ar-NH-CH_2). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.6 (Ar-C), 61.7 (aliphatic-C), 69.0 (aliphatic-CH), 96.5 (aliphatic-CH), 72.6 (aliphatic-CH), 77.3 (aliphatic-CH), 119.7 (Ar-C), 126.2 (2 x Ar-C), 126.4 (2 x Ar-C), 128.5 (4 x Ar-C), 128.9 (4 x Ar-C), 131.6 (2 x Ar-C), 133.3 (2 x Ar-C), 134.0 (2 x Ar-C), 140.9 (Ar-C), 145.5 (Ar-C), 158.2 (Ar-C), 181.5 (C=O) and 182.03 (C=O). MS (ESI *m/z*) calcd. for $[C_{28}H_{20}ClNO_5S+H]^+$ 517.08, found 517.302.

Synthesis of 4-(4-aminobenzylamino)-9,10-dioxo-9,10-dihydroanthracen-1-yl 4-methylbenzenesulfonate (9)

Compound 9 was prepared as described in section above from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 mL), K_2CO_3 (0.34 g, 2.3 mmol) and 4-(aminomethyl)aniline (0.44 mL, 2.32 mmol) under nitrogen to provide yellow solid 9 at 78% yield. ^{1}H-NMR ($CDCl_3$): δ: 2.25 (3H, s, Ar-CH_3), 3.67 (2H, s, Ar-NH_2), 4.42 (2H, d, J = 7.2, CH_2-Ar), 6.65 (2H, d, J = 8.0 Hz, Ar-H), 6.95 (1H, d, J = 9.2 Hz, Ar-H), 7.18 (2H, d, J = 10.0 Hz, Ar-H), 7.20 (2H, d, J = 10.0 Hz, J = 10.8 Hz, Ar-H), 7.25 (2H, s, Ar-H), 7.52 (2H, m, Ar-H), 7.8 (2H, d, J = 9.2 Hz, Ar-H), 8.18 (1H, dd, J = 7.2 Hz, J = 7.6 Hz, Ar-H), 10.22 (1H, Ar-NH). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.3 (Ar-C), 61.7 (aliphatic-CH), 69.0 (aliphatic-CH), 69.5 (aliphatic-C), 72.6 (aliphatic-C), 76.7 (aliphatic-C), 77.4 (aliphatic-C), 119.7 (Ar-C), 126.2 (2 x Ar-C), 126.4 (2 x Ar-C), 129.0 (4 x Ar-C), 129.6 (4 x Ar-C), 131.5 (2 x Ar-C), 133.3 (2 x Ar-C), 133.8 (2 x Ar-C), 140.5 (Ar-C), 145.6 (Ar-C), 157.9 (Ar-C), 181.2 (C=O) and 182.1 (C=O). MS (ESI *m/z*) calcd. for $[C_{28}H_{22}N_2O_5S+H]^+$ 498.12, found 498.535.

Synthesis of 4-(4-methoxybenzylamino)-9,10-dioxo-9,10-dihydroanthracen-1-yl 4-methylbenzenesulfonate (10):

Compound **10** was prepared as described in section above from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 mL), K_2CO_3 (0.34 g, 2.3 mmol) and (4-methoxyphenyl)methanamine (0.44 mL, 2.32 mmole) under nitrogen to provide yellow solid **10** at 74% yield. ^{1}H-NMR ($CDCl_3$):): δ: 2.25 (3H, s, Ar-CH_3), 3.82 (3H, s, O-CH_3), 4.65 (2H, d, *J* = 7.2, CH_2-Ar), 6.25 (1H, d, *J* = 4.8 Hz, Ar-H), 6.89 (2H, d, *J* = 4.8 Hz, Ar-H), 7.22 (2H, m, Ar-H), 7.65 (2H, m, Ar-H), 7.80 (2H, s, Ar-H), 7.98 (2H, d, *J* = 11.6 Hz, Ar-H), 8.18 (1H, dd, *J* = 6.8 Hz, *J* = 6.8 Hz, Ar-H), 10.22 (1H, s, Ar-NH-CH_2). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.3 (Ar-C), 61.7 (aliphatic-C), 69.0 (aliphatic-C), 69.5 (aliphatic-C), 72.6 (aliphatic-C), 76.7 (aliphatic-C), 77.4 (aliphatic-C), 119.7 (Ar-C), 126.2 (2 x Ar-C), 126.4 (2 x Ar-C), 129.0 (4 x Ar-C), 129.6 (4 x Ar-C), 131.5 (2 x Ar-C), 133.3 (2 x Ar-C), 133.8 (2 x Ar-C), 140.5 (Ar-C), 145.6 (Ar-C), 157.9 (Ar-C), 181.2 (C=O) and 182.1 (C=O). MS (ESI *m/z*) calcd. for $[C_{29}H_{23}NO_6S+H]^+$ 513.12, found 513.287.

Synthesis of toluene-4-sulfonic acid 4-[2-(2-hydroxy-ethoxy)-ethoxy]-9,10-dioxo-9,10-dihydro-anthracen-1-yl ester (**11**)

Compound **11** was prepared as described in section above from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 mL), K_2CO_3 (0.34 g, 2.3 mmol) and diethylene glycol (0.22 mL, 2.32 mmol) under nitrogen to provide yellow solid **11** at 66% yield: ^{1}H-NMR ($CDCl_3$): δ: 2.35 (3H, s, Ar-CH_3), 3.78-3.80 (2H, m, Aliphatic-H), 3.83 (2H, s, 2H, Aliphatic-H), 4.02-4.04 (2H, m, 2H, Aliphatic-H), 4.25 (2H, s, Aliphatic-H), 7.40 (1H, d, *J* = 9.2 Hz, Ar-H), 7.51 (1H, d, *J* = 9.2 Hz, Ar-H), 7. 60 (2H, s, Ar-H), 7.69 (2H, t, *J* = 8.4 Hz, Ar-H), 7.85 (2H, d, *J* = 8.0 Hz, Ar-H), 8.05 (1H, dd, *J* = 6.8 Hz, *J* = 6.2 Hz, Hz, Ar-H), 8.22 (1H, d, *J* = 9.2 Hz, Ar-H) and 13.0 (1H, s, aliphatic-OH). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.6 (Ar-C), 61.7 (aliphatic-C), 68.9 (aliphatic-C), 96.5 (aliphatic-C), 72.6 (aliphatic-C), 77.3 (aliphatic-C), 119.7 (Ar-C), 126.2 (2 x Ar-C), 126.4 (2 x Ar-C), 128.5 (4 x Ar-C), 128.9 (4 x Ar-C), 131.5 (2 x Ar-C), 133.3 (2 x Ar-C), 134.0 (2 x Ar-C), 140.8 (Ar-

C), 145.5 (Ar-C), 158.2 (Ar-C), 181.5 (C=O) and 182.0 (C=O). MS (ESI *m/z*) calcd. for $[C_{25}H_{22}O_8S+H]^+$ 482.10, found 481.531.

Synthesis of toluene-4-sulfonic acid 4-{2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy}-9,10-dioxo-9,10-dihydro-anthracen-1-yl ester (12)

Compound **12** was prepared as described in section above from compound **2** (0.30 mg, 0.16 mmol) in dry acetonitrile (10 mL), K_2CO_3 (0.34 g, 2.3 mmol) and tri-ethylene glycol (0.44 mL, 2.32 mmol) under nitrogen to provide yellow solid **12** at 74% yield. ^{1}H-NMR ($CDCl_3$): δ: 2.28 (3H, s, Ar-CH_3), 3.8 (2H, dd, *J* = 7.2, 7.6 Hz, Aliphatic-H), 3.85 (2H, dd, *J* = 5.6, 6.4 Hz, Aliphatic-H), 4.01 (2H, dd, *J* = 4.4, 4.4 Hz, Aliphatic-H), 4.30 (2H, dd, *J* = 4.4, 4.8 Hz, Aliphatic-H), 7.20 (1H, s, Ar-H), 7.25 (1H, d, *J* = 7.6 Hz, Ar-H), 7.43 (1H, d, *J* = 9.2 Hz, Ar-H), 7.66 (2H, t, *J* = 7.2 Hz, Ar-H), 7.80 (1H, d, *J* = 8.0 Hz, Ar-H), 7.88 (2H, dd, *J* = 6.8 Hz, *J* = 6.8 Hz, Ar-H), 8.17 (1H, d, *J* = 7.6 Hz, Ar-H). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.3 (Ar-C), 61.7 (aliphatic-C), 68.9 (aliphatic-C), 69.5 (aliphatic-C), 72.6 (aliphatic-C), 76.7 (aliphatic-C), 77.4 (aliphatic-C), 119.7 (Ar-C), 126.2 (2 x Ar-C), 126.4 (2 x Ar-C), 129.0 (4 x Ar-C), 129.6 (4 x Ar-C), 131.5 (2 x Ar-C), 133.3 (2 x Ar-C), 133.8 (2 x Ar-C), 140.5 (Ar-C), 145.6 (Ar-C), 157.9 (Ar-C), 181.2 (C=O) and 182.1 (C=O). MS (ESI *m/z*) calcd. for $[C_{27}H_{26}O_9S+H]^+$ 526.55, found 526.338.

Synthesis of toluene-4-sulfonic acid 4-(2-{2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy}-ethoxy)-9,10-dioxo-9,10-dihydro-anthracen-1-yl ester (13)

Compound **13** was prepared as described in section above from compound **2** (0.30 mg, 0.16 mmol) in dry ACN (10 mL), K_2CO_3 (0.34 g, 2.3 mmol) and benzylamine (0.40 mL, 2.32 mmol) under nitrogen to provide yellow solid **13** at 85% yield. ^{1}H-NMR ($CDCl_3$): δ: 2.29 (3H, s, Ar-CH_3), 3.48 (2H, dd, *J* = 4.4, 4.8 Hz, aliphatic-H), 3.65 (4H, dd, *J* = 5.6, 6.4 Hz, aliphatic-H), 4.68 (4H, dd, *J* = 4.4, 4.4 Hz, aliphatic-H), 3.82 (2H, dd, *J* = 4.4, 4.8 Hz, aliphatic-H), 4.0 (2H, dd, *J* = 4.4, 4.8 Hz, aliphatic-H), 4.25 (2H, dd, *J* = 4.4, 4.8 Hz, aliphatic-H), 7.23 (2H, s, Ar-H), 7.28 (1H, d, *J* = 9.2 Hz, Ar-H), 7.45 (1H, d, *J* = 9.2 Hz, Ar-H), 7.65 (2H, t, *J* = 7.2 Hz, Ar-H), 7.80 (2H, s, *J* = 8.0 Hz, Ar-H), 8.17 (2H, d, *J*

= 7.6 Hz, *J* = 6.8 Hz, Ar-H). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.2 (Ar-C), 58.6 (Aliphatic-C), 61.5 (Aliphatic-C), 69.5 (Aliphatic-C), 70.4 (Aliphatic-C), 72.5 (2 x Aliphatic-C), 76.9 (Aliphatic-C), 77.5 (Aliphatic-C), 120.3 (Ar-C), 126.3 (2 x Ar-C), 127.4 (2 x Ar-C), 128.8 (4 x Ar-C), 129.8 (4 x Ar-C), 131.6 (2 x Ar-C), 133.4 (2 x Ar-C), 133.8 (2 x Ar-C), 140.7 (Ar-C), 145.6 (Ar-C), 158.2 (Ar-C), 181.6 (C=O) and 181.8 (C=O). MS (ESI *m/z*) calcd. for $[C_{29}H_{30}O_{10}S+Na]^+$ 570.16, found 592.330.

Synthesis of 1,4-bis(2-(2-hydroxyethoxy)ethoxy)anthracene-9,10-dione (**14**)

Compound **14** was prepared as described in section above from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 mL), K_2CO_3 (3.45 g, 2.3 mmol) and di-ethylene glycol (0.22 mL, 2.32 mmol) under nitrogen to provide yellow solid **14** at 66% yield: ^{1}H-NMR ($CDCl_3$): δ: 3.65 (2H, s, Aliphatic-H), 3.68 (2H, s, Aliphatic-H), 3.88 (2H, s, Aliphatic-H), 4.20 (2H, s, Aliphatic-H), 7.18 (1H, d, *J* = 12 Hz, Ar-H), 7.22 (1H, d, *J* = 8.0 Hz, Ar-H), t (2H, *J* = 7.7 Hz, Ar-H), 7.28 (2H, *J* = 7.2 Hz, Ar-H), 7.67-7.80 (2H, m, Ar-H), 8.18-8.25 (2H, m, Ar-H) and 13.00 (2H, s, aliphatic-OH). ^{13}C-NMR (100 Hz, $CDCl_3$): 61.9 (2 x aliphatic-C), 69.7 (2 x aliphatic-C), 72.5 (2 x aliphatic-C), 119.8 (2 x Ar), 126.4 (2 x Ar), 127.9 (2 x Ar), 132.3 (2 x Ar-C), 134.7 (2 x Ar-C), 157.9 (C-aliphatic), 181.7 (C=O and 188.6 (C=O). MS (ESI *m/z*) calcd. for $[C_{22}H_{24}O_8+H]^+$ 416.15, found 416.276.

Synthesis of 1,4-bis(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)anthracene-9,10-dione (15)

Compound **15** was prepared as described in section above from compound 2 (0.30 mg, 0.16 mmol) in dry acetonitrile (10 mL), K_2CO_3 (0.34 g, 2.3 mmol) and tri-ethylene glycol (0.44 mL, 2.32 mmol) under nitrogen to provide yellow solid **15** at 64% yield. ^{1}H-NMR ($CDCl_3$): δ: 3.60 (4H, dd, *J* = 5.6, 6.4 Hz, aliphatic-H), 3.65 (4H, dd, *J* = 4.0, 4.8 Hz, aliphatic-H), 3.80 (4H, dd, *J* = 4.0, 4.8 Hz, aliphatic-H), 4.01 (4H, dd, *J* = 4.4, 4.4 Hz, aliphatic-H), 4.30 (4H, dd, *J* = 4.4, 4.8 Hz, aliphatic-H), 7.23 (1H, d, *J* = 9.2 Hz, Ar-H), 7.25 (1H, d, *J* = 8.0 Hz, Ar-H), 7.80 (4H, m, Ar-H), 8.25 (1H, d, *J* = 7.6 Hz) and 13.00 (2H, d, *J* = 7.2 Hz, aliphatic-OH). ^{13}C-NMR (100 Hz, $CDCl_3$): 21.3 (Ar-C), 61.7 (aliphatic-C), 68.9 (aliphatic-C), 69.5 (aliphatic-C), 72.6 (aliphatic-C), 76.7 (aliphatic-C),

77.4 (aliphatic-C), 119.7 (Ar-C), 126.2 (2 x Ar-C), 126.4 (2 x Ar-C), 128.9 (4 x Ar-C), 129.6 (4 x Ar-C), 131.5 (2 x Ar-C), 133.3 (2 x Ar-C), 133.8 (2 x Ar-C), 140.5 (Ar-C), 145.6 (Ar-C), 157.9 (Ar-C), 181.5 (Ar-C) and 188.5 (Ar-C). MS (ESI *m/z*) calcd. for $[C_{26}H_{32}O_{10}+H]^+$ 504.20, found 505.437.

3.2 Cell lines and media

The cell lines, colorectal (HCT 116 and SW620), pancreatic (BxPC3, MiaPaCa-2), prostate (DU145, PC-3, LNCaP), ovarian (CAOV3, OVCAR-8, NCI/ADR-RES), osteosarcoma (U2OS), colorectal (HCT116), human large cell line carcinoma of the lung (H1299), gastric (KATO), lung (CHAGO), hepato (HEP-G2), cervical (Ca Ski) carcinomas) and normal lung (WI-38) (ATTC, USA) will be maintained in RPMI-1640 medium (Hyclone, England) containing 10% fetal bovine serum (v/v) (Hyclone, England), 100 U/ml penicillin (General Drugs House Co., Ltd., Thailand), 0.4 mg/mL streptomycin (M & H Manufacturing Co., Ltd., Thailand) and incubated in humidified 5% CO_2 incubator (Thermo Electron Corporation, USA). Confluent cell monolayer will be maintained and used for experiments by treating with 0.25% (w/v) trypsin-0.53 mM EDTA (Hyclone, England)

3.3 X-ray structure analysis

All crystals for x-ray analysis, recrystallized from MeOH:CH_2Cl_2 (3:1), were analyzed with the intensity data being collected for each crystal at up to 2 theta= 25°on a Bruker APEXII at 293 K using omega scans and Mo-Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods (SHELXS) and refined with the program SHELXL97. Anisotropic temperature factors were introduced for the non-hydrogen atoms and protons were refined at geometrically calculated positions as riding atoms.

3.4 Cytotoxic assay

The cytotoxic activity of each test compound was evaluated in terms of the *in vitro* inhibition of cell line growth in tissue culture using the MTT (USB, USA) assay to approximate cell viability and replication. Cancerous cells were cultured according to the supplier's instructions with initial seeding at 2-4 x 10^4 cells per well in RPMI 1640 medium supplemented with10% (v/v) Fetal Bovine Serum and incubated under 5% (v/v) CO_2 at 37 °C, overnight. Various concentrations of each test compound (range of 4.3 nM to 4.3 µM) were added to triplicate wells and then incubated at the same condition for three days, resulting in the exponential growth phase of the cells at the time of addition of each test compound. To each well was then added the MTT solution (final concentration 0.5 mg/mL), incubated for 4 hours under the same conditions and then the culture medium in each well was discarded and replaced with 150 µL of DMSO v/v (Sigma Aldrich, USA). After mixing to ensure cell lysis and solubilization of the formazan crystals, the absorbance of each well was determined by an Automatic Elisa Reader System at 540 nm (Bio-Tek instrument, Canada). Percentage of cell growth inhibition was expressed as: (1-A/C) x 100% (A and C were the absorbance values from experimental and control cells, respectively). Inhibitory concentration 50% (IC_{50}) values were determined for each drug from a plot of log (drug concentration) versus percentage of cells killed. Standard deviation was calculated based on the IC_{50} values obtained from at least three independent experiments.

3.5 Colony formation assay:

This technique was also performed to confirm the activity. Briefly, cells were plated in 24-well plates at a density of 100-200 cells/well and allowed to attach. The next day, serial dilutions of the corresponding compounds were added and allowed to

incubate for 24 h. After exposure, cells were washed in PBS and cultured in free media until colonies were formed (8-10 days). Cells were subsequently washed, fixed with a 1% glutaraldehyde solution (Sigma Aldrich, USA) for 30 min, and stained with a solution of crystal violet (Sigma Aldrich, USA) at 2% for 30 min. After staining, cells were thoroughly washed with water. Colonies were imaged on the inverted fluorescence microscope. The data reported represent means of at least two independent experiments.

3.6 Cell cycle:

Cell cycle perturbations were analyzed by propidium iodide (Sigma Aldrich, USA) DNA staining. Briefly, exponentially growing cells were treated with different doses of compounds for 24 h. At the end of each treatment time, cells were collected and washed with PBS after a gentle centrifugation at 3,000 rpm for 5 mins. Cells were thoroughly resuspended in 0.5 mL of PBS and fixed in 70%ethanol for at least 2 h at 4°C. Ethanol-resuspended cells were then centrigued at 3,000 rpm for 5 min and washed twice in PBS to remove residual ethanol. For cell cycle analysis, the pellets were resuspended in 1 mL of PBS containing 0.02 mg/mL of propidium idodide, 0.5 mg/mL of DNase-free RNase A (Sigma Aldrich, USA) and incubated for 2 h in the dark. The data was analyzed by FACS analysis. Acquired data were analyzed by Flow Jo flow cytometry analysis software (BD Biosciences, USA).

3.7 Western blot:

Cells were plated in 6 well plates, treated with CDDP at 3.0 μM and compounds for 24 h and lysed in 50 μL of RIPA lysis buffer. Equal amounts of protein were electrophoresed on 12% SDS-polyacrylamide gel and transferred to a PVDF membrane. The membrane was incubated for 1 h in blocking buffer (5% skim milk powder in TBS containing 0.1% Tween) and then incubated with the mouse antibody against Akt, p-Akt, p53, β-actin (Cell Signaling Technology, USA) and Bcl-2 and Caspase-3 AB15470; Millipore) at 4 °C overnight. Membranes were washed with TBST and incubated with

rabbit or mouse antibodies (C20; Santa Cruz Biotechnology Inc., USA) for 1 h prior to detection with enhanced chemiluminescence (ECL) system. Signals were detected by the chemiluminescent method (Amersham Biosciences, England).

3.8 Fluorescence recoding

Ca Ski cells were treated with compound 5 and cisplatin as reference, were fixed in 4% paraformaldehyde for 10 min at room temperature and permeabilized in PBS with 0.2% Triton X-100 for 2 min. After washing with PBS, cells were stained with DAPI in PBS (1:1000). The cells were observed under an inverted fluorescent microscope.

3.9 RNA extraction

Ca Ski cancer cells were seeded in 12 well-plate for 24 h. Then they were treated with compounds. The cells were removed by 1 mL of Trizol reagent (Invotrogen, England). The mixture solution was incubated for 5 min at room temperature, and 0.2 mL of chloroform (Lab-Scan, Ireland) was added. All tubes were vigorously mixed by hands for 15 sec and incubated at room temperature for 3 min. The samples were centrifuged using colorless aqueous phase and carefully transferred to fresh tubes. RNA was precipitated by gently mixing with 0.5 mL of isopropanol (Merck, Germany). The samples were incubated at room temperature for 10 min and centrifuged at 12,000xg for 10 min, 4 °C. The RNA pellets on the bottom side of each tube were visible at this stage. The supernatants were rinsed and the RNA pellets were washed once with 1 mL of ice cold 75% ethanol in 0.01%DEPC water. The samples were mixed by vortex mixer and centrifuged at 7,500xg for 5 min at 4 °C. RNA pellets were dried for 15 min, dissolved in 20 µL of 0.01%DEPC water, and incubated for 10 min at 55 °C. RNA samples were kept at -80 °C until used for further experiments.

3.10 Quantitation of RNA using spectrophotometer

RNA was diluted to 50 to 100-fold dilution in 0.01%DEPC water. The diluted RNA was subjected to absorbance measurement at 260 and 280 nm using the spectrophotometer. An A_{260} corresponds to a concentration of 40 µg/mL single stranded RNA. The concentration of RNA was calculated in µg/mL by using the following equation.

$$RNA\ \left(\frac{\mu g}{mL}\right) = A260\ \times 40\ \times dilution\ factor$$

The purity of RNA was evaluated from a ratio of A260/280. The ratio of appropriately purified RNA was in the range of 1.8-2.0.

3.11 cDNA synthesis by reverse transcriptase

Obtained RNA 1 µg was used for converting to cDNA. Total RNA was mixed with 0.2 1 µg of random hezamer (Qiagen, Germany), and the volumn was adjusted to 12.5 1 µL by 0.01%DEPC water. The RNA mixture was heated at 65 °C for 5 min and placed on ice for 5 min. Then 1x reverse transcriptase buffer (Fermentus, Canada), 1 mM dNTP mix (Fermentus, Canada) was added to final amount of 200 U per reaction, and the reaction was performed using Bioer Lifer Express (Bioer technology, China) at 25 °C for 10 min, 42 °C for 60 min, 70 °C for 10 min and 25 °C. The cDNA was stored at -20 °C until use.

3.12 Quantitative RT-PCR

Total RNA was isolated from Ca Ski using Trizol reagent (Invitrogen, USA). The RNA was converted to get cDNA using reverse transcriptase (Fermentas, USA) and random hexamers (Invitrogen, USA). PCR reactions were performed using specific primers for human *TP53* and *HPV16/E6*. β-Actin was used as a loading control. The forward and reverse primers used for PCR amplification are as follows: human *HPV16/E6* (5'-TCAAAAGCCACTGTGTCCTGA-3'), and (5'-CGTGTTCTTGATGATCTGCAA-3'); and *β-actin* (5'-ACCAACTGGGACGACATGGAGAA-3'), (5'-GTGGTGGTGAAGCTGTAGCC-3'). PCR reactions were carried out using Bioer Life Express by condition as follows; 95 °C for 5 min, 94 °C for 1 min, 55 °C (β-actin) or 57 °C (Human HPV16 E6) for 1 min, 72 °C for 1 min and 72 °C for 10 min. PCR were amplified for 35 cycles.

The qPCR implications were performed with the 1x Maxima^{TM} SYBR Green/ROX qPCR Master Mix (Fermentas, USA) according to the manufacturer's protocol. Quantitative RT-PCRs were carried out using the MJ Mini Peronal Thermal cycler (BioRad, USA). The relative expression levels were calculated and analyzed by $^{2\text{-}\Delta\Delta_{CP}}$.