Supporting Information for:

Accumulation of an anthraquinone and its platinum complexes in cancer cell spheroids: the effect of charge on drug distribution in solid tumours

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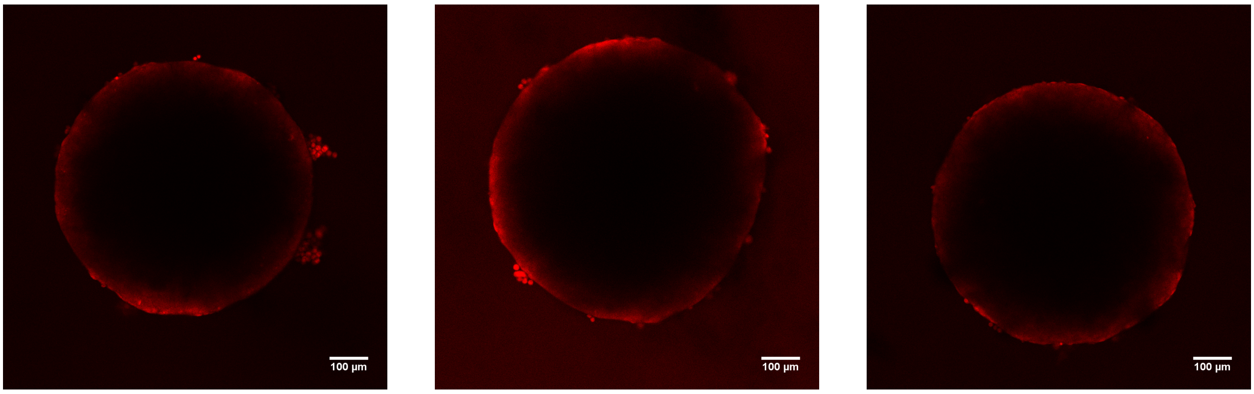

Figure S1: Fluorescence images of DLD-1 spheroids following treatment with doxorubicin. 2.5×10^5 cells/ml were plated onto agarose coated 96 well imaging plates (BD Biosciences) and allowed to aggregate for 96 hrs resulting in the formation of single spheroid per well. The spheroids were dosed with compound for a further 24 hrs and then imaged using a HC PL FLUOTAR 10X/0.30 PH1 objective on a Leica SP2 confocal microscope (ex 488nm, em 500-650nm). Image planes were selected approximately 175µm inside the spheroid to eliminate edge effects and reflection imaging was used to ensure 100% laser penetration and emission at that depth within the spheroid.

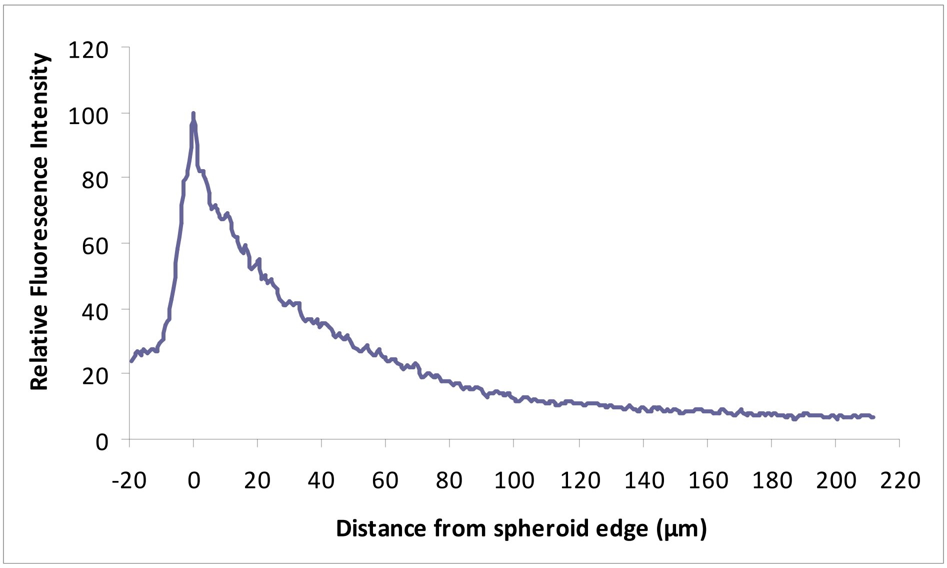

Figure S2: Fluorescence profile for DLD-1 spheroids following treatment with doxorubicin. Images were analysed using ImageJ software (NIH). A one pixel wide line was drawn through the centre of the spheroid and the fluorescence intensity was measured. The values for 15 spheroids were then normalised and the average intensity profile was calculated.

Synthesis of *cis*-1-[[(3-aminopropyl)amino]- anthracene-9,10-dione]aminedichloroplatinum(II), Pt1C3

The following synthetic procedure is based upon that outlined by Gibson *et al.*[88] 1C3 (0.226 g, 0.807 mmol) was dissolved in methanol (~ 500 mL) and was added slowly to an aqueous solution of $K[PtCl_3(NH_3)].½H_2O$ (0.300 g, 0.816 mmol) over a period of approximately one hour. The solution was stirred at room temperature in the absence of light for 2 days, during which time a dark red precipitate formed. The solid was filtered and washed with methanol. The resulting red solid was ground to a fine powder, resuspended in methanol (500 mL), stirred for 2 hours, then filtered and collected as a red solid (0.149 g, 0.265 mmol, 32 % yield).

^{1}H NMR (400 MHz, DMF) δ 9.79 (s, 1H), 8.28 (d, 1H), 8.19 (d, 1H), 7.94 (t, 1H), 7.88 (t, 1H), 7.70 (t, 1H), 7.52 (d, 1H), 7.39 (d, 1H), 5.12 (s, 2H), 4.26 (s, 3H), 2.98 (m, 4H), 2.23 (m, 2H). ^{195}Pt NMR (DMF/HCl): δ -2165 ppm. MS (ESI, MeOH/DMF): m/z calc for $C_{17}H_{19}Cl_2N_3O_2Pt$ (M-Cl$^-$) = 527.89; found: 527.69; Elemental analysis calculated for $PtC_{17}H_{19}N_3O_2Cl_2$: C, 36.25; H, 3.40; N, 7.46. Found: C, 35.10; H, 3.39; N, 6.90.

Synthesis of 1-[[[(3-aminopropyl)amino](diethylenetriamine) platinum(II)]-anthracene-9,10-dione]dichloride, $[Pt(1C3)dien]Cl_2.2H_2O$

The following synthetic procedure was adapted from that reported by Kalayda et al.[233] [PtCl(dien)].Cl (835 mg, 2.26 mmol) was dissolved in water (20 mL) and combined with a solution of $AgNO_3$ (761 mg, 4.48 mmol). The mixture was stirred, in the absence of light, in room temperature for 24 hours. The white precipitate was removed by filtration and the resulting filtrate was added to a solution of 1C3 (633 mg, 2.26 mmol) in ethanol (80 mL). The mixture was stirred, in the absence of light, at room temperature for 24 hours. The solution was then evaporated to dryness under reduced pressure, then redissolved in water (80mL), and the starting material was extracted using chloroform (3 x 80 mL). The aqueous solution was loaded onto a Dowex 2 x 8 50/100 strongly basic anion exchange column (2 x 40 cm). The column was washed with water and the first major red band was collected. The eluted band was then evaporated to dryness under reduced pressure to give a dark red solid, which was dried in vacuo over P_2O_5 to give the final product (1.35 g, 0.788 mmol, 87% yield).

^{1}H NMR (400 MHz, D_2O) δ 7.54 (m, 3H), 7.36 (m, 1H), 7.00 (t, 1H), 6.76 (d, 1H), 6.45 (d, 1H), 3.34-2.80 (m, 16H), 1.90 (m, 2H); ^{13}C NMR (300 MHz, D_2O) δ 183.97, 183.45, 150.71, 135.58, 134.83, 133.50, 132.40, 131.48, 126.31, 126.13, 119.22, 116.43, 110.69, 53.85, 50.98, 45.17, 39.71, 30.46; ^{195}Pt NMR (DMF): δ 2937.2 ppm; MS (ESI, MeOH): m/z calc for $C_{21}H_{29}N_5O_2Pt(M^{2+}+Cl^{-})$ = 614.0; found: 614.1 Elemental analysis calculated for $PtC_{21}H_{33}N_5O_4Cl_2$: C, 36.79; H, 4.85; N, 10.22. Found: C, 36.97; H, 4.82; N, 10.02.