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Electronic Supplementary Information

Hypoxia Sensitive Metal β -Ketoiminato Complexes Showing Induced Single Strand DNA Breaks and Cancer Cell Death by Apoptosis

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X-ray Crystallography Data

Table S1 X-ray crystallographic data for L3-6, 8 and 11

	L3	L4	L5	L6	L8	L11
formula	C ₁₆ H ₁₄ ClNO	C ₁₆ H ₁₃ Cl ₂ NO	C ₁₆ H ₁₃ Cl ₂ NO	C ₁₆ H ₁₂ Cl ₃ NO	C ₁₆ H ₁₄ BrNO	C ₁₇ H ₁₇ NO
formula wt	271.73	306.17	306.17	340.62	316.19	251.32
cryst syst	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Monoclinic
space group	<i>P2₁/c</i>	<i>P2₁/c</i>	<i>P2₁/n</i>	<i>P2₁/c</i>	<i>P2₁/c</i>	<i>P2₁/n</i>
a (Å)	7.5074(8)	10.9556(15)	12.3180(4)	11.4886(4)	7.4911(4)	7.3817(3)
b (Å)	12.0260(13)	9.8430(15)	7.4714(2)	9.5459(3)	11.9162(7)	12.0369(4)
c (Å)	0.901(3)	13.3164(18)	16.4245(5)	13.6456(4)	30.9906(19)	29.9551(11)
α (°)	90.00	90.00	90.00	90.00	90.00	90.00
β (°)	102.524(5)	97.711(5)	108.3020(10)	98.829(3)	101.624(3)	90.0110(10)
γ (°)	90.00	90.00	90.00	90.00	90.00	90.00
V (Å³)	2723.5(5)	1423.0(3)	1435.13(7)	1478.77(8)	2709.7(3)	2661.59(17)
Z	8	4	4	4	8	8
density (mg/m³)	1.325	1.429	1.417	1.530	1.550	1.254
absorp coeff (mm⁻¹)	0.271	0.450	0.446	0.616	3.025	0.078
λ[Mo-Kα] (Å)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
T (K)	150.15	150.15	150.15	173(2)	150.15	150.15
reflns collected	20137	11908	12165	6404	44035	40768
independent reflns	5970	3269	3264	3469	6156	10589
R₁	0.0395	0.0367	0.0343	0.0285	0.0256	0.0517
wR₂	0.0934	0.078	0.0933	0.0455	0.0606	0.1322
GOOF	1.025	1.047	1.030	1.054	1.024	1.020

Table S2 a) Selected bond lengths and D...A distances, and b) Selected bond angles for ligands L3-6, 8 and 11

a)	N(1)-C(8)	C(8)-C(9)	C(9)-C(10)	C(10)-O(1)	N(1)-H(1N)...O(1)	b)	N(1)-C(8)-C(9)	C(8)-C(9)-C(10)	C(9)-C(10)-O(1)
L3	1.3627(19)/ 1.3620(19)	1.396(2)/ 1.397(2)	1.442(2)/ 1.439(2)	1.2683(18)/ 1.2709(18)	2.6620(16)/ 2.6340(17)	L3	119.32(13)/ 119.4(13)	123.06(14)/ 123.65(14)	123.06(14)/ 122.73(14)
L4	1.4243(16)	1.3893(18)	1.421(2)	1.2565(17)	2.6672(16)	L4	119.96(13)	123.73(13)	124.67(12)
L5	1.3451(16)	1.3884(16)	1.4160(17)	1.2478(16)	2.6799(14)	L5	119.92(11)	123.79(11)	125.42(11)
L6	1.346(2)	1.380(2)	1.418(2)	1.245(2)	2.6792(19)	L6	120.35(16)	123.85(16)	125.19(15)
L8	1.355(2)/ 1.350(2)	1.387(3)/ 1.383(2)	1.432(2)/ 1.433(2)	1.259(2)/ 1.258(2)	2.6505(19)/ 2.619(2)	L8	119.64(16)/ 119.96(16)	124.31(16)/ 123.32(16)	123.02(16)/ 122.93(17)
L11	1.3546(13)/ 1.3508(13)	1.3797(14)/ 1.3788(14)	1.4206(13)/ 1.4275(13)	1.2548(12)/ 1.2606(12)	2.6278(12)/ 2.6103(12)	L11	119.61(9)/ 119.81(9)	124.13(9)/ 123.64(9)	122.66(9)/ 122.54(10)

Table S3 a) X-ray crystallographic data for complexes **2**, **4-9**

	2	4	5	6	7	8	9
Formula	C ₂₆ H ₂₇ ClFNORu	C ₂₆ H ₂₆ Cl ₃ NORu	C ₂₆ H ₂₆ Cl ₃ NORu	C _{26.35} H _{26.4} Cl ₄ N O _{1.35} Ru	C ₂₆ H ₂₇ BrClNORu	C ₂₆ H ₂₇ BrClNORu	C ₂₆ H ₂₇ ClINORu
formula wt	525.01	575.90	575.90	621.55	585.92	585.92	632.91
cryst syst	Triclinic	Monoclinic	Triclinic	Triclinic	Monoclinic	Triclinic	Triclinic
space group	<i>P</i> $\bar{1}$	<i>Cc</i>	<i>P</i> $\bar{1}$	<i>P</i> $\bar{1}$	<i>Cc</i>	<i>P</i> $\bar{1}$	<i>P</i> $\bar{1}$
a (Å)	7.5924(8)	14.2172(6)	11.3992(12)	11.4864(13)	14.8307(4)	7.7084(3)	7.7700(7)
b (Å)	10.2922(11)	14.3632(6)	11.6491(11)	13.9138(16)	13.6346(4)	11.3524(5)	11.5552(10)
c (Å)	15.9991(15)	11.7497(4)	19.5678(19)	18.324(2)	11.8013(4)	15.1513(7)	15.2078(15)
α (°)	101.176(8)	90	88.692(4)	76.377(6)	90.00	68.311(4)	106.660(9)
β (°)	94.516(8)	90.373(4)	87.058(4)	86.339(6)	90.573(3)	82.804(4)	97.552(8)
γ (°)	106.506(9)	90	80.287(4)	75.571(6)	90.00	70.580(4)	109.544(8)
V (Å³)	1164.0(2)	2557.5(4)	2557.5(4)	2756.4(5)	2386.24(13)	1161.91(9)	1193.01(19)
Z	2	4	4	4	4	2	2
density (mg/m³)	1.498	1.594	1.496	1.498	1.631	1.675	1.762
absorp coeff (mm⁻¹)	0.814	1.007	0.945	0.977	2.462	2.528	2.081
λ[Mo–Kα] (Å)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
T (K)	128(11)	100.01(10)	150.15	150.15	100.00(10)	100.01(10)	100.01(10)
reflns collected	8512	6051	47038	135883	17206	10992	10248
independent reflns	5315	3800	12361	16216	4849	4770	5583
R₁	0.0555	0.0382	0.0254	0.0365	0.0237	0.0305	0.0429
wR₂	0.1024	0.0699	0.0658	0.0828	0.0483	0.0613	0.0841
GOOF	1.037	1.047	1.043	1.019	1.034	1.052	1.020

Table S3 b) X-ray crystallographic data for complexes 10-14, 17 and 18

	10	11	12	13	14	17	18
Formula	C ₂₈ H ₃₂ ClNO ₂ Ru	C ₂₇ H ₃₀ ClNORu	C ₆₀ H ₆₀ Cl ₂ N ₂ O ₂ Ru ₂	C ₂₆ H ₂₅ ClF ₃ NORu	C ₂₅ H ₂₆ ClNORu	C ₂₀ H ₂₅ ClIrNO	C ₂₀ H ₂₃ ClIrO ₂
formula wt	551.07	521.04	1114.14	560.99	492.99	523.06	542.03
cryst syst	Triclinic	Triclinic	Triclinic	Monoclinic	Monoclinic	Triclinic	Orthorhombic
space group	<i>P</i> $\bar{1}$	<i>P</i> $\bar{1}$	<i>P</i> $\bar{1}$	<i>Cc</i>	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> $\bar{1}$	<i>P</i> 2 ₁ 2 ₁
a (Å)	9.5876(5)	7.7691(10)	13.7807(7)	14.0726(14)	9.4633(9)	7.76702(19)	8.3447(9)
b (Å)	11.5769(5)	11.4508(14)	14.3381(6)	14.2648(14)	25.1628(17)	10.7935(3)	15.2720(18)
c (Å)	13.3575(6)	15.4307(18)	14.7586(6)	12.2405(14)	9.9589(8)	11.4075(2)	15.4729(18)
α (°)	106.127(4)	68.399(6)	90.868(4)	90.00	90.00	101.4001(19)	90.00
β (°)	102.743(4)	83.185(6)	114.234(5)	90.643(5)	113.399(10)	91.3221(17)	90.00
γ (°)	110.150(5)	71.461(6)	104.725(4)	90.00	90.00	103.213(2)	90.00
V (Å³)	1252.18(11)	1210.1(3)	2548.1(2)	2457.0(4)	2176.4(3)	910.30(4)	1971.9(4)
Z	2	2	2	4	4	2	4
density (Mg/m³)	1.462	1.430	1.452	1.517	1.505	1.908	1.826
absorp coeff (mm⁻¹)	0.758	0.777	0.743	0.788	0.859	7.487	6.925
λ[Mo-Kα] (Å)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
T (K)	100.01(10)	173.15	100.01(10)	150.15	100.01(10)	100.01(10)	150.15
reflns collected	22866	76554	19223	39809	10507	20472	109495
independent reflns	5114	8288	9023	7151	4459	3736	6682
R₁	0.0262	0.0321	0.0714	0.0272	0.0594	0.0610	0.0144
wR₂	0.0611	0.0840	0.1589	0.0826	0.0795	0.0420	0.0323
GOOF	1.063	1.077	1.151	1.085	1.085	1.038	1.038

Table S4 a) Selected bond lengths and angles, and **b)** Selected bond angles for complexes **2, 4-14, 17** and **18**

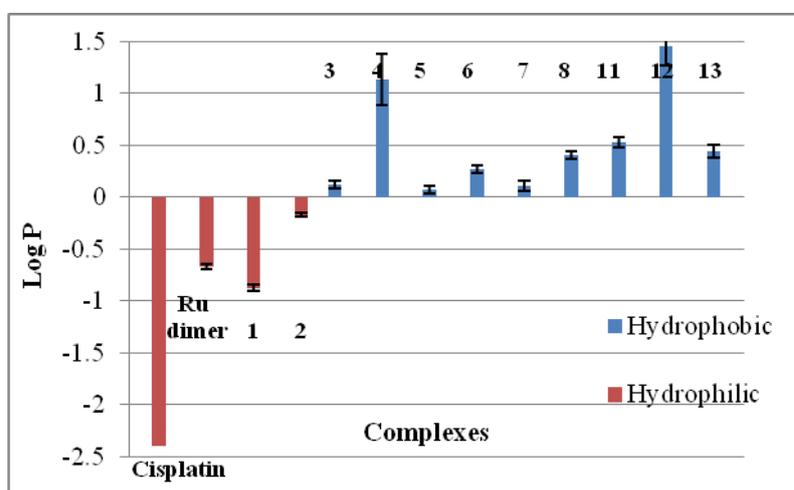
a)	M-Cl	M-N(1)	M-O(1)	M-Cg	b)	N(1)-M-Cl	O(1)-M-Cl	N(1)-M-O(1)	Cg-M-Cl	Cg-M-N(1)	Cg-M-O(2)
2	2.4362(12)	2.088(3)	2.058(3)	1.6683(19)	2	83.86(10)	85.01(9)	89.08(12)	128.44(8)	130.90(12)	124.72(11)
4	2.4318(12)	2.096(5)	2.076(3)	1.670(2)	4	85.70(13)	82.82(10)	89.58(16)	129.04(8)	129.27(14)	125.57(12)
5	2.4587(5)/ 2.4477(5)	2.1223(15)/ 2.1198(14)	2.0762(12)/ 2.0874(12)	1.6836(8)/ 1.6790(7)	5	84.56(4)/ 88.09(4)	85.35(4)/ 84.91(4)	88.06(5)/ 88.25(5)	126.88(3)/ 126.02(3)	132.30(5)/ 130.12(5)	124.74(4)/ 125.49(4)
6	2.4617(6)/ 2.4706(8)	2.1281(18)/ 2.1271(18)	2.0851(16)/ 2.1005(17)	1.6841(11)/ 1.6916(11)	6	84.17(5)/ 84.60(5)	84.45(5)/ 85.45(5)	88.51(7)/ 88.31(7)	128.65(4)/ 127.49(5)	131.37(7)/ 131.64(6)	124.55(6)/ 124.58(7)
7	2.4272(7)	2.101(2)	2.0509(19)	1.6656(12)	7	85.03(7)	83.22(6)	89.09(9)	129.44(4)	129.71(8)	125.31(7)
8	2.4378(7)	2.086(2)	2.0517(18)	1.6663(11)	8	83.68(6)	84.85(5)	89.39(8)	128.80(5)	130.74(8)	124.54(7)
9	2.4421(11)	2.088(3)	2.063(3)	1.6663(19)	9	84.06(10)	84.75(9)	89.47(13)	128.81(8)	130.49(13)	124.54(11)
10	2.4269(5)	2.0885(17)	2.0674(14)	1.6646(10)	10	83.60(5)	86.96(4)	88.31(6)	126.20(4)	132.55(6)	124.64(6)
11	2.4624(5)	2.1065(13)	2.0710(12)	1.6828(7)	11	83.89(4)	84.84(4)	89.41(5)	128.90(3)	130.71(5)	124.31(5)
12	2.4343(18)/ 2.4363(18)	2.095(5)/ 2.101(5)	2.069(4)/ 2.070(4)	1.674(3)/ 1.673(3)	12	85.73(16)/ 85.80(16)	83.36(13)/ 84.28(14)	88.46(19)/ 88.75(18)	128.04(13)/ 128.57(13)	129.9(2)/ 129.75(18)	126.29(17)/ 125.06(16)
13	2.4660(6)	2.131(2)	2.0931(17)	1.6861(10)	13	86.24(6)	83.86(5)	89.32(8)	128.98(4)	129.46(7)	124.53(6)
14	2.4417(10)	2.051(3)	2.058(3)	1.6481(17)	14	86.29(13)	85.83(8)	87.46(11)	127.21(6)	129.39(11)	126.39(10)
17	2.4232(8)	2.047(3)	2.107(2)	1.7665(16)	17	87.00(8)	86.57(6)	86.43(10)	129.10(5)	128.35(10)	125.23(8)
	M-Cl	M-O(1)	M-O(2)	M-Cg		O(1)-M-Cl	O(2)-M-Cl	O(1)-M-O(2)	Cg-M-Cl	Cg-M-O(1)	Cg-M-O(2)
18	2.4412(5)	2.1178(15)	2.1269(12)	1.7711(10)	18	86.10(5)	87.33(4)	85.94(5)	128.69(3)	125.31(5)	129.07(5)

Partition Coefficients

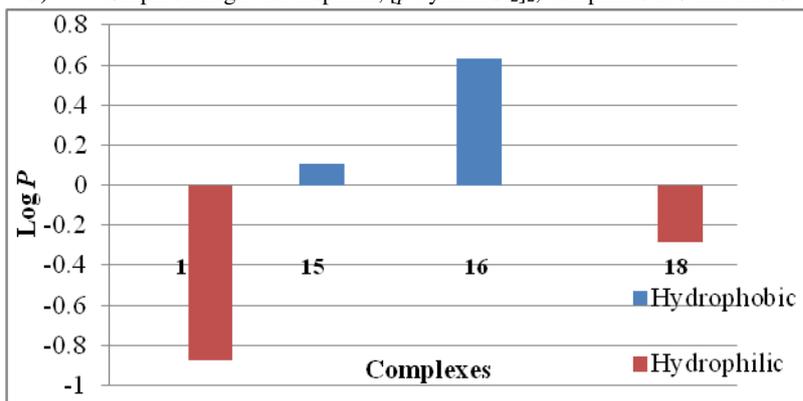
Experiments were carried out to assess the partition coefficients (Log P) for complexes **1-13** and furthermore the adapted complexes **15, 16** and **18** (Table S5 and Figures S1a and b), showing a range of values between -2.36-1.5. Cytotoxic studies show complex **1** to be the most promising drug candidate and it is in fact also hydrophilic with a Log P of -0.87. These results show that the hydrophilic nature of the compound means it is unlikely to cross the cell membrane by passive transport and the idea of a receptor-mediated endocytosis is more plausible. An unusual result was seen for the iridium complexes **16** and **18**, where the Log P values are the reverse of their ruthenium analogues, and in fact the most cytotoxic compound **16** is in fact hydrophobic. This result could mean the iridium undergoes a different uptake mechanism than the ruthenium and further work in protein interactions are needed to understand these results.

Table S5 Partition coefficients from complexes **1-8, 11-13, 15-16** and **18**

Complex	Log P	± SD	Complex	Log P	± SD
Cisplatin	-2.36	0.01	8	0.41	0.03
[<i>p</i> -cymRuCl ₂] ₂	-0.67	0.03	11	0.53	0.05
1	-0.87	0.04	12	1.5	0.2
2	-0.17	0.02	13	0.46	0.06
3	0.12	0.04	15	0.11	0.06
4	1.1	0.2	16	0.63	0.06
7	0.11	0.05	18	-0.28	0.09



a) Graph of Log P for cisplatin, [*p*-cymRuCl₂]₂, complexes **1-8** and **11-13**



b) Graph of Log P for complexes **1, 15, 16** and **18**

Figure S1 Log P for complexes a) **1-8, 11-13** and b) complexes **1, 15, 16** and **18**

Hydrolysis

Experiments have been carried out using either one equivalent or an excess of water. ^1H NMR spectra were recorded over a 5 day period as to mimic the 5-day MTT assay. To date, results have been inconclusive and no changes have been observed in the NMR spectra after a period of 5-days, suggesting that these complexes do not undergo hydrolysis. Attempts have been made to increase the concentration of water added to the samples, however this causes them to precipitate and neither NMR nor UV-*vis* spectrometry could be conducted.

Cell Line Chemosensitivity Studies

Cell survival was determined as the absorbance of treated cells divided by the absorbance of controls and expressed as a percentage. The IC_{50} values were determined from plots of % survival against drug concentration. Each experiment was repeated three times and a mean value obtained and stated as IC_{50} (μM) \pm SD. To quantify the response of tumour cells compared to normal cells, IC_{50} values were expressed as the ratio of IC_{50} in ARPE-19 cells divided by the IC_{50} for individual tumour cells evaluated. A ratio of greater than 1 indicates selectivity towards cancer cells. IC_{50} values (μM) and the standard deviations (SD) after a minimum of three repeats. The data is presented as a bar-chart of IC_{50} values against HT-29, MCF-7, A2780 and A2780*cis* cancer cell lines (**Figure S2**). Data for ARPE-19 cells has been omitted from this figure in the interest of clarity.

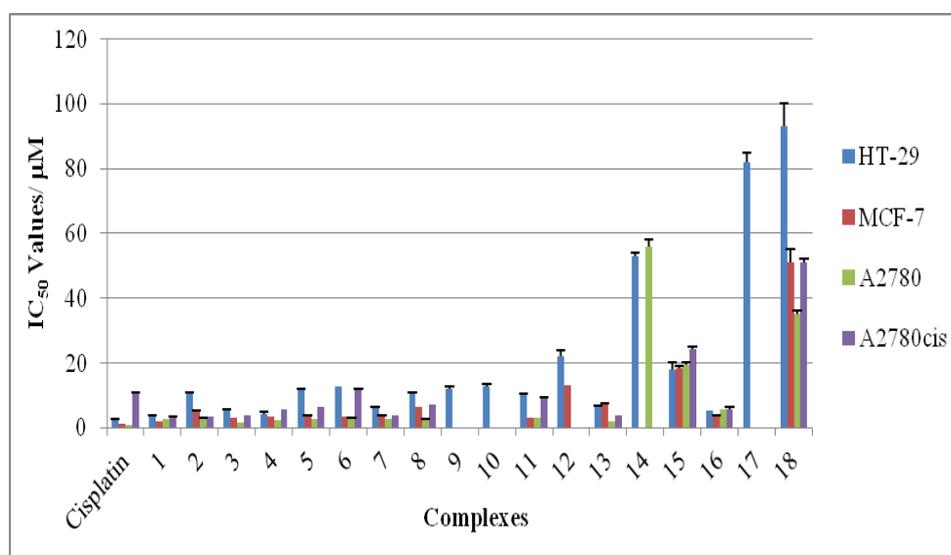


Figure S2 Bar-chart of IC_{50} values against HT-29, MCF-7, A2780 and A2780*cis* cancer cell lines. Data for ARPE-19 cells has been omitted from this figure in the interest of clarity.

Influence of Hypoxia

The hypoxia assay was conducted according to the protocol stated previously for normoxic conditions. However, the incubation period, the addition of the drug dilutions and the addition of the MTT solution were carried out inside a Don Whitley Scientific H35 Hypoxystation which was set at 1.0 or 0.1% O_2 . Cisplatin was tested as a comparison and a well-known hypoxic sensitive compound tirapazimine (TPZ) was tested as a positive control. **Table S6a** states the IC_{50} values for complexes **1**, **15**, **16** and **18**, whilst **Table S6b** states the IC_{50} values for complexes **2**, **3**, **8**, **9** and **11**.

Table S6 HT-29 IC₅₀ values under hypoxic conditions for **a)** complexes **1, 15, 16** and **18**, and **b)** complexes **2, 3, 8, 9** and **11**

Complexes	21.0% O ₂		1.0% O ₂		0.1% O ₂	
	IC ₅₀ (μM)	± SD	IC ₅₀ (μM)	± SD	IC ₅₀ (μM)	± SD
TPZ	31	3	3.2	0.5	2.4	0.4
Cisplatin	2.4	0.1	3.5	0.2	2.8	0.4
1	3.5	0.3	6.4	0.2	5.7	0.2
15	18	2	62.9	0.3	95	4
16	5.1	0.3	10.6	0.2	20	2
18	93	7	109	2	121	3

Complexes	21% O ₂		0.1% O ₂	
	IC ₅₀ (μM)	± SD	IC ₅₀ (μM)	± SD
TPZ	31	3	2.8	0.4
Cisplatin	2.4	0.1	2.4	0.4
2	10.5	0.4	5.7	0.2
3	5.40	0.09	4.6	0.3
8	10.2	0.6	5.76	0.09
9	11.8	0.8	7.0	0.1
11	10.21	0.09	6.3	0.3

Inhibition of thioredoxin reductase activity

Thioredoxin reductase sourced from rat liver was obtained from Sigma Aldrich. It is a buffered aqueous glycerol solution, ≥ 100 units/ mg protein. Solution in 50 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM EDTA, and 10% glycerol.

Stock solutions:

1 M K₂HPO₄ - 17.42 g in 100 mL distilled water

1 M KH₂PO₄ - 13.6 g in 100 mL distilled water

100 mM EDTA - 3.72 g in 100 mL distilled water at pH 7.0

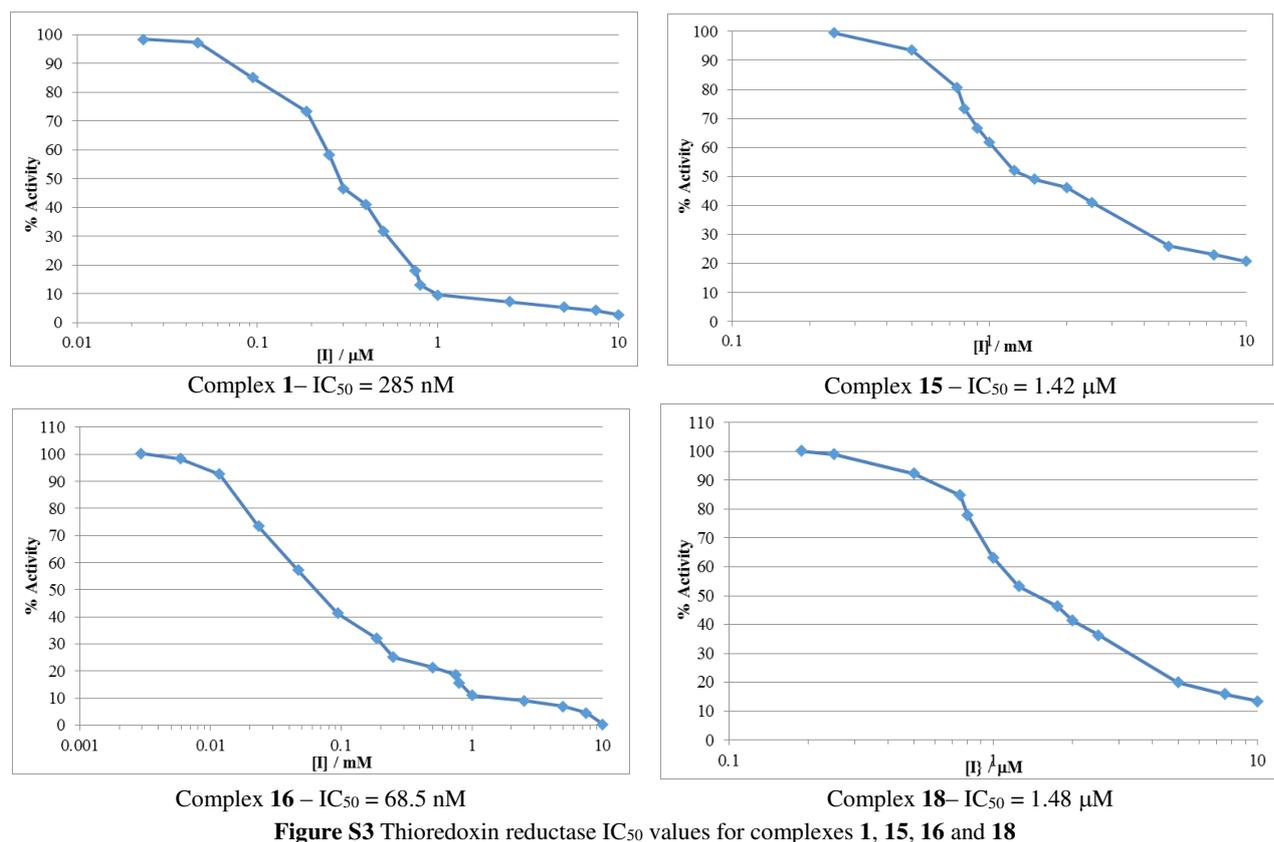
Potassium phosphate buffer (0.1 M at pH 7.0)

K₂HPO₄ (61.5 mL), KH₂PO₄ (38.5 mL), EDTA (10 mL) all made up to 1 L using distilled water and the pH was adjusted to 7.0.

Reaction Buffer A - Potassium phosphate buffer (50 mL), NADPH (8.3 mg) and BSA (5.0 mg)

Reaction Buffer B - Phosphate buffer (50 mL), NADPH (8.3 mg), BSA (5.0 mg) and DTNB (1.98 mg)

Buffer A (0.5 mL) was added to a 1 mL cuvette and to this Trx-R (2 μL) was added. The test compound or dimethylsulfoxide control (1 μL) was added and the reaction left for approximately 30 seconds. Buffer B (0.5 mL) was added to the cuvette and using a pipette mixed well. The rate of change of UV-*vis* absorbance was measured at 412 nm over 1 min to give the reaction velocity. The experiment was carried out using just the enzyme to get the control (no inhibitor) reaction velocity and then varying dilutions of the test compound were added up to a maximum of 10 μM. The reaction velocity in the presence of inhibitor was normalised relative to the control to generate % activity and plots of % activity versus concentration were constructed to obtain IC₅₀ values (concentration that inhibited 50% of enzyme activity) (**Figure S3**).



Induction of Cancer Cell Death by Apoptosis

Complexes 1, 15, 16 and 18 were incubated with either HT-29 or A2780 cells at concentrations of 0, 10 and 20 μM over a period of 48 hours. The amount of living, mechanical damage, early apoptotic and late apoptotic/necrotic cells was determined as a percentage, these are stated in **Table S7**.

Table S7 Percentages of living, early apoptotic and late apoptotic/necrotic cells on incubation of either HT-29 or A2780 cells with complexes 1, 15, 16 and 18 over 48 hours

Complexes	Concentration (μM)	HT-29			A2780		
		Live (%)	Early Apoptotic (%)	Late apoptotic/necrotic (%)	Live (%)	Early Apoptotic (%)	Late apoptotic/necrotic (%)
1	0	88.67	5.70	4.07	91.37	2.91	4.95
	10	9.03	47.19	43.45	49.31	5.76	43.28
	20	7.86	18.95	72.25	31.52	6.63	60.13
15	0	90.43	5.94	3.57	92.25	3.45	2.99
	10	90.54	5.21	2.88	90.54	3.48	5.21
	20	84.96	10.07	4.62	82.29	5.99	11.38
16	0	93.68	2.52	2.40	88.52	7.39	1.81
	10	67.18	27.49	5.01	45.55	10.92	41.83
	20	18.03	31.67	49.94	3.34	34.57	61.09
18	0	92.42	3.04	2.65	87.60	6.91	2.01
	10	88.49	4.73	3.74	86.25	7.60	3.75
	20	86.26	5.73	5.98	84.52	7.63	5.41

Analysis of cellular DNA damage by the comet assay**Reagents for double strand breakage:**

Neutral Lysing Solution (2% sarkosyl, 0.5 M Na₂EDTA, 0.5 mg/ mL proteinase K (pH 8.0))

Sarkosyl (2 g) and Na₂EDTA (18.61 g) were added to distilled water (80 mL) and the pH adjusted to 8.0 with 10M NaOH. Proteinase K (50 mg) was then added and made up to a final volume of 100 mL.

Electrophoresis Buffer (90 mM Tris buffer, 90 mM boric acid, 2 mM Na₂EDTA (pH 8.0))

Tris base (32.707 g), boric acid (16.694 g) and Na₂EDTA (2.233 g) were added to distilled water (2.5 L). The pH was adjusted to 8.0 and the volume made up to 3 L with distilled water.

Conducting double strand breakage assay:

Preparation of sample slides (conducted under low light conditions):

0.5% low melting agarose (LMPA) was prepared (250 mg/ 50 mL PBS) and heated until fully dissolved; this was kept in a water bath at 37°C. The sample eppendorfs were defrosted and centrifuged at 16.1 rcf for 20 seconds. The supernatant was removed and the pellet re-suspended in LMPA (150-1000 µL, depending on pellet size). The cell suspension (150 µL) was added to a previously coated glass slide and a cover slip placed over, these were placed on a cool tray and allowed to set (3-5 minutes). Once set the slip was removed and LMPA (150 µL) added to the slide and another slip placed on top. These were again transferred to a cool tray to set. The slip was again removed and all slides placed into a tray where freshly prepared neutral lysing solution was added and the slides incubate for 1 hour at 37°C in dark conditions.

Electrophoresis Conditions

The lysing solution was gently poured off and the slides submerged in electrophoresis buffer for 30 minutes. The buffer was gently poured off and this step repeated two more times. The slides were then placed close together in an electrophoresis chamber and reservoirs were carefully filled with freshly prepared buffer (pH 8.0) and filled until the buffer just covered the slides. The chamber was adjusted to 24 V and the electrophoresis was run for 25 minutes. The slides were transferred to a clean tray and rinsed three times with distilled water and finally with 100% ice cold ethanol. They were then transferred to a slide rack and left to dry overnight before imaging could commence.

Reagents for crosslinking and single strand breakage:

Alkaline Lysing Solution - 2.5 M NaCl (146.1 g), 100 mM EDTA (37.2 g) and 10 mM Trizma base (1.2 g) were added to distilled water (700 mL). NaOH (8 g) was added and stirred until fully dissolved, once dissolved the pH was adjusted to pH 10.0 and made up to a final volume of 890 mL and stored at 4°C.

Final Lysing Solution - The previously made lysing solution (178 mL) was added to Triton-X-100 (2 mL) and DMSO (20 mL)

Electrophoresis Buffer (300 mM NaOH/ 1mM EDTA)

10 M NaOH - 200 g/ 500 mL distilled water

200 mM EDTA - 14.89 g/ 200 mL distilled water at pH 10.0

10 M NaOH (90 mL) was added to 200 mM EDTA (15 mL) and made up to a total volume of 3 L using distilled water and adjusted to a pH > 13.0

Neutralisation Buffer - Tris base (48.5 g) was dissolved in distilled water (800 mL) and adjusted to pH 7.5 using concentrated HCl. This was then made up to 1 L using distilled water and stored at room temperature.

Staining Solution - SYBRTMGold solution (molecular probes inc, S-11494) (1 µL) was added to PBS 1(10 mL); this was made on the day of imaging.

Imaging and Analysis - Staining solution (150 mL) was added to each slide and a cover slip placed on top. Excess solution was blotted away and the comets analysed using Comet assay III software. A minimum of 50 different comets were scored and the computer outputs an average on head and tail intensities and tail moments. Images were taken of the comets and the tail moments plotted against concentration of drug sample used.

Conduction crosslinking or single:

Preparation of sample slides (conducted under low light conditions):

0.5% low melting agarose (LMPA) was prepared (250 mg/ 50 mL PBS) and heated until fully dissolved; this was kept in a water bath at 37°C. The sample eppendorfs were defrosted and centrifuged at 16.1 rcf for 20 seconds. The supernatant was removed and the pellet re-suspended in LMPA (150-1000 µL, depending on pellet size). The cell suspension (150

μL) was added to a previously coated glass slide and a cover slip placed over, these were placed on a cool tray and allowed to set (3-5 minutes). Once set the slip was removed and LMPA (150 μL) added to the slide and another slip placed on top. These were again transferred to a cool tray to set. The slip was again removed and all slides placed into a tray where freshly prepared ice cold lysing solution was added, these were left to incubate for 1 hour at 4°C.

Electrophoresis Conditions

After the 1 hour incubation, the lysing solution was gently poured off and the slides placed close together in an electrophoresis chamber. The reservoirs were carefully filled with freshly prepared buffer (pH > 13.0) and filled until the buffer just covered the slides. The slides were incubated for 30 minutes and then the chamber adjusted to 24 V, the electrophoresis was run for 25 minutes and then the slides were removed. The slides were transferred to a clean tray and drop-wise neutralisation with the neutralisation buffer. The buffer was added to coat the slides and then these were incubated for 5 minutes. The buffer was removed and this step repeated twice more. Then slides were then rinsed three times with distilled water and finally with 100% ice cold ethanol. They were then transferred to a slide rack and left to dry overnight before imaging could commence.

Upon imaging of the slides, a minimum of 50 cells were scored and an average tail moment calculate, this is quantitative of the amount of DNA damage present. Complexes **1**, **15**, **16** and **18** were incubated with HT-29 cells and the following figures show plots of concentration (0-20 μM) versus tail moment for DSB (**Figure S4**), cross-linking (**Figure S5**) and SSB (**Figure S6**).

DSB Results

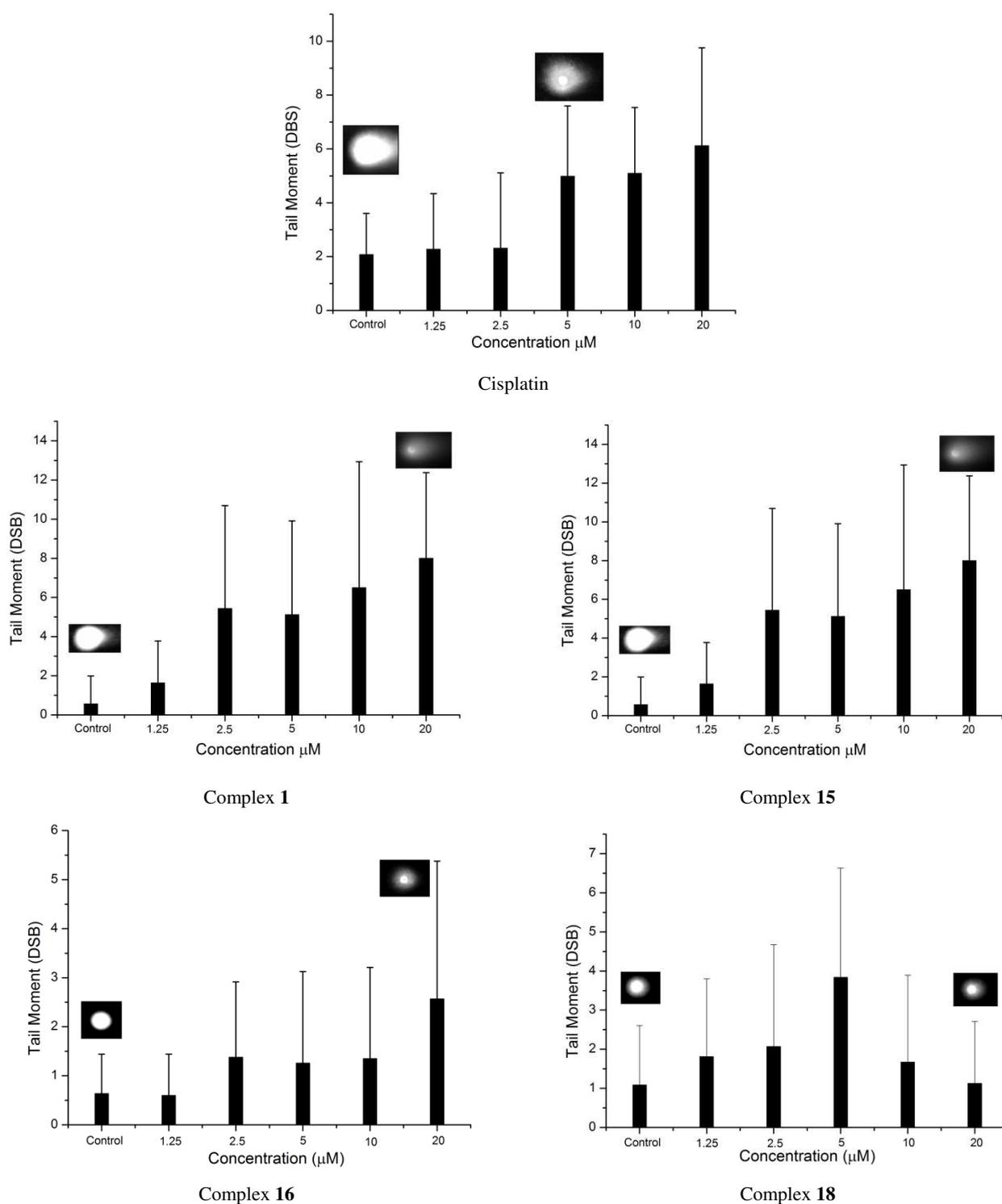


Figure S4 Double Strand Break (DSB) Comet assay results for complexes **1**, **15**, **16** and **18** incubated with HT-29 cells

Cross-link Results

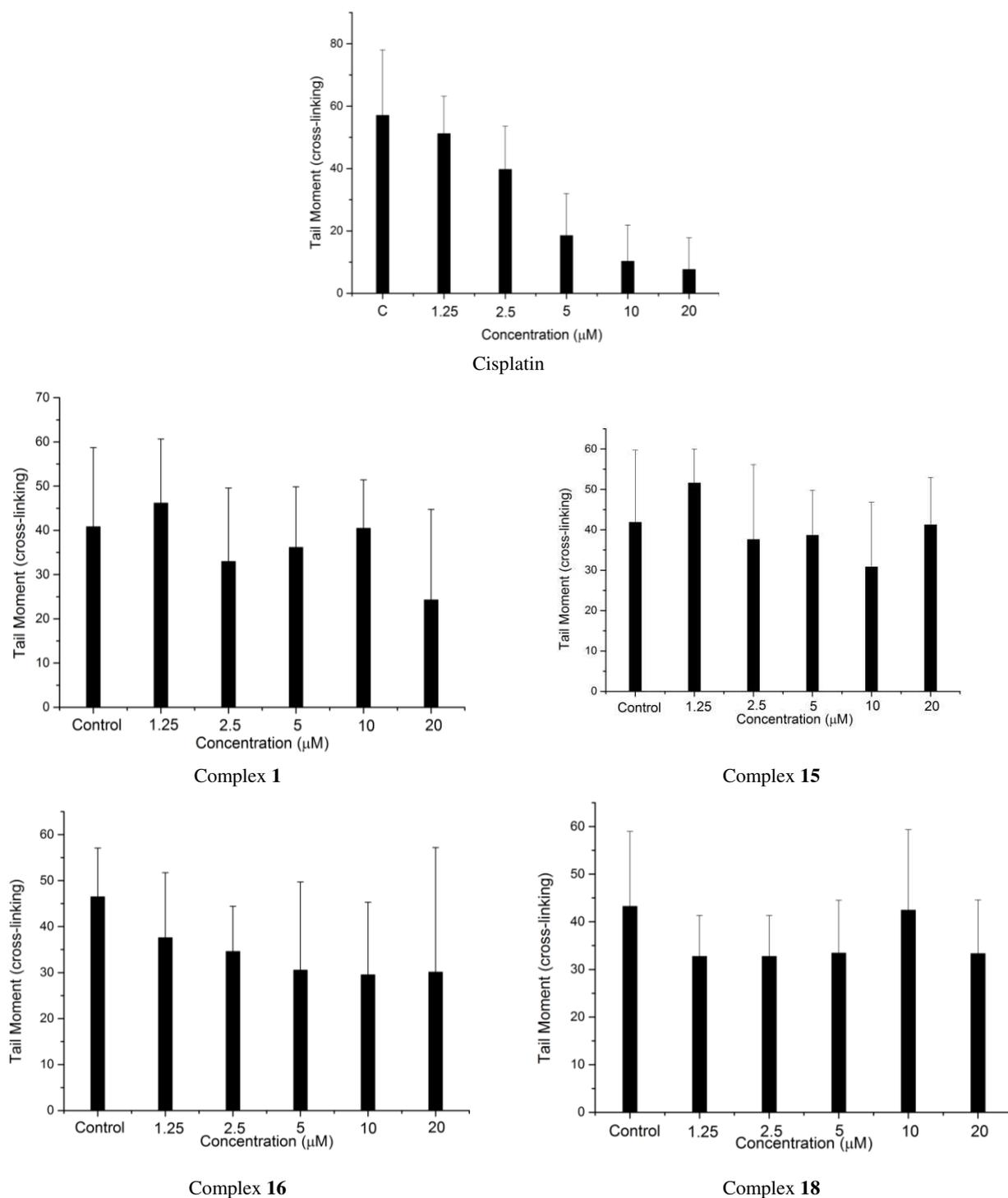


Figure S5 Cross-linking Comet assay results for complexes 1, 15, 16 and 18 incubated with HT-29 cells

SSB Results

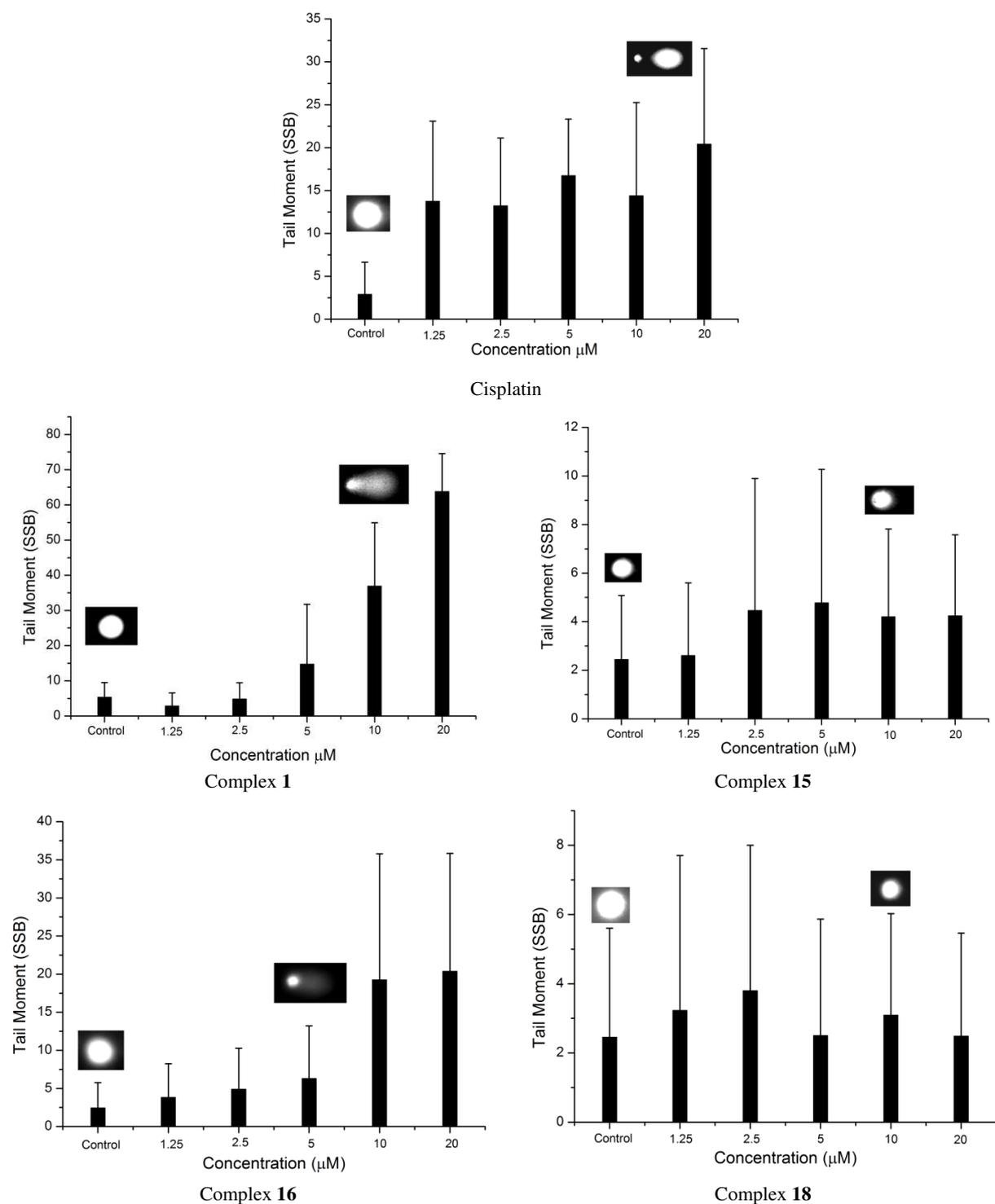


Figure S6 Single Strand Break (SSB) Comet assay results for complexes **1**, **15**, **16** and **18** incubated with HT-29 cells