Antiproliferative activity of ruthenium(II) arene complexes with mono- and bidentate pyridine-based ligands

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Experimental and Spectroscopic Details for Ligands B-D

Synthesis of ethyl 4-(pyridin 2-ylamino)benzoate **(B).** The synthesis of ligand **B** was carried out according to the literature.[1] Yield 1.6 g (41%). Properties: light yellow powder; soluble in chloroform, ethyl acetate, THF; moderately soluble in methanol; insoluble in diethyl ether. ^{1}H NMR[2] ^{1}H NMR (300 MHz, $CDCl_3$): δ 1,45 (t, $^3J_{H,H}$ = 7,1 Hz, 3H, CH_3^a), 4,42 (q, $^3J_{H,H}$ = 7,1 Hz, 2H, CH_2^b), 6,72 ('t', 1H, CH^d), 6,83 ('d', 1H, CH^c), 7,32 (d, $^3J_{H,H}$ = 8,7 Hz, 2H, CH^e), 7,44 ('t', 1H, CH^h), 8,06 (d, $^3J_{H,H}$ = 8,7 Hz, 2H, CH^g), 8,34 ('d', 1H, CH^k).^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 14.4 (s, CH_3^a), 60.7 (s, CH_2^b), 110.2 (s, CH^c), 116.4 (s, CH^d), 117.4 (s, CH^e), 123.4 (s, C^f), 131.2 (s, CH^g), 137.9 (s, CH^h), 145.2 (s, C^j), 148.4 (s, CH^k), 154.8 (s, C^l), 166.5 (s, CO). IR: ν(cm^{-1}) 3339 (s), 3225 (w), 3134 (w), 2982 (w), 2363 (w), 1684 (s), 1623 (s), 1610 (s), 1587 (s), 1531 (s), 1482 (s), 1427 (s), 1353 (s), 1311 (s), 1285 (s), 1244 (m), 1176 (s), 1151 (s), 1109 (m), 1023 (m), 852 (w), 768 (s).

Synthesis of 4-(pyridin-2-ylamino)benzoic acid **(C).** The synthesis of ligand **C** was carried out according to the literature.1 Yield of 0.8 g (91%). Properties: white powder; soluble in chloroform, DMSO, DMF; moderately soluble in water; insoluble in *n*-hexane, diethyl ether. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.83 ('t', 1H, CH^d), 6.93 ('d', 1H, CH^c), 7.62 ('t', 1H, CH^h), 7.79–7.86 (m, 4H, CH^e, CH^g), 8.22 ('d', 1H, CH^k), 9.51 (s, 1H, N*H*), 12.44 (s, 1H, O*H*). ^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6): δ 111.8 (s, CH^c), 115.4 (s, CH^d), 116.5 (s, CH^e), 121.7 (s, C^f), 130.5 (s, CH^g), 137.6 (s, CH^h), 146.0 (s, C^j), 147.2 (s, CH^k), 155.2 (s, C^l), 167.2 (*C*O). IR: ν (cm^{-1}) 3332 (s), 3212 (m), 3137 (m), 2951 (w), 2364 (w), 1927 (w), 1687 (s), 1628 (s), 1611 (s), 1587 (s), 1528 (s), 1511 (s), 1483 (s), 1427 (s), 1410 (m), 1357 (s), 1312 (s), 1289 (s), 1245 (m), 1180 (m), 1171 (m), 1156 (m), 1118 (s), 1021 (w), 985 (m), 957 (m), 851 (m), 767 (s).

Synthesis of pyridine-2-ylmethyl)glycine·HCl **(D·HCl)** The synthesis of ligand **D** was carried out according to the literature.[3] Yield: 5.4 g (67%). Properties: yellow, microcrystalline powder; soluble in water, DMSO, DMF; moderately soluble in methanol; insoluble in *n*-hexane, diethyl ether. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.95 (s, 2H, CH_2^a), 4.44 (s, 2H, CH_2^b), 7.61 ('t', 1H, CH^c), 7.77 ('d', 1H, CH^d), 8.08 ('t', 1H, CH^e), 8.71 ('d', 1H, CH^f). ^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6): δ 47.6 (s, CH_2^a), 50.1 (s, CH_2^b), 123.2 (s, CH^c), 123.5 (s, CH^d), 137.3 (s, CH^e), 149.0 (s, CH^f), 152.3 (s, C^g), 168.1 (s, *C*O). IR: ν(cm^{-1}) 3422 (s, br), 2956 (s, br), 2713 (s, br), 2563 (s, br), 2363 (m), 1751 (s), 1635 (s), 1621 (s), 1569 (w), 1558 (w), 1541 (m), 1481 (m), 1457 (s), 1404 (s), 1261 (m), 1218 (m), 1188 (s), 1101 (m), 1163 (s), 924 (w), 850 (m), 778 (s).

Table SI-1. Selected Crystallographic Data for Complexes **2–6**.

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[1] K. Hino, H. Nakamura, Y. Nagai, H. Uno, H. Nishimura, *J. Med. Chem.*, 1983, **26**, 222.

[2] For numbering scheme please refer to Scheme 1 in the main text.

[3] X. Wang, J. J. Vittal, *Inorg. Chem.*, 2003, **42**, 5135.

	2	**3**	**4**	**5**	**6**
Formula	$[RuCl_2(p\text{-cym})(\mathbf{B})]$	$[RuCl_2(p\text{-cym})(\mathbf{C})]\cdot 2CHCl_3$	$[RuCl(p\text{-cym})\{\mathbf{D}(Me)\}][PF_6]$	$[Ru_2Cl_3(p\text{-cym})_2][RuCl(p\text{-cym})(\mathbf{E})]_2[PF_6]_3\cdot MeOH$	$[RuCl(p\text{-cym})(\mathbf{E}\text{-2H})][PF_6]\cdot MeOH$
Crystal system	Monoclinic	Triclinic	Orthorhombic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P\bar{1}$	$Pna2_1$	$P2_1/c$	$P2_1/n$
a/pm	2444.94(7)	1063.12(4)	1441.04(2)	1827.38(3)	1107.44(2)
b/pm	1299.40(3)	1124.65(4)	1712.26(2)	1800.87(3)	1329.12(2)
c/pm	726.84(1)	1376.26(4)	919.520(10)	2443.57(4)	1856.18(3)
α/(°)	90	89.789(3)	90	90	90
β/(°)	90.813(2)	78.641(3)	90	102.830(1)	95.464(1)
γ/(°)	90	71.348(3)	90	90	90
V/pm^3	2308.91(9)	1525.4(1)	2268.86(5)	7840.7(2)	2719.74(8)
Crystal size (mm^3)	0.35x0.2x0.01	0.1x0.05x0.03	0.3x0.2x0.05	0.2x0.15x0.02	0.4x0.3x0.1
Z	4	2	4	4	4
D_{calcd}/ g·cm^{-3}	1.578	1.653	1.745	1.730	1.646
F(000)	1120	760	1200	4088	1360
Θ range	2.951°–26.368°	2.802°–26.372°	2.379°–30.507°	2.050°–30.508°	2.204°–30.507°
Data/restraints/ parameters	4718/84/266	6231/7/360	6889/36/364	23939/210/991	8305/27/510
R_1, wR_2 [I > 2σ(I)]	0.0529, 0.1133	0.0431, 0.0848	0.0309, 0.0668	0.0573, 0.1281	0.0307, 0.0673
R_1, wR_2 (all Data)	0.0746, 0.1230	0.0617, 0.0914	0.0351, 0.0689	0.1050, 0.1511	0.0410, 0.0724
Residual electron density (e Å^{-3})	1.178/–0.991	1.259/–0.627	0.701/–0.427	1.630/–1.133	0.755/–0.417
Absolute structure parameter	-	-	–0.03(1)	-	-

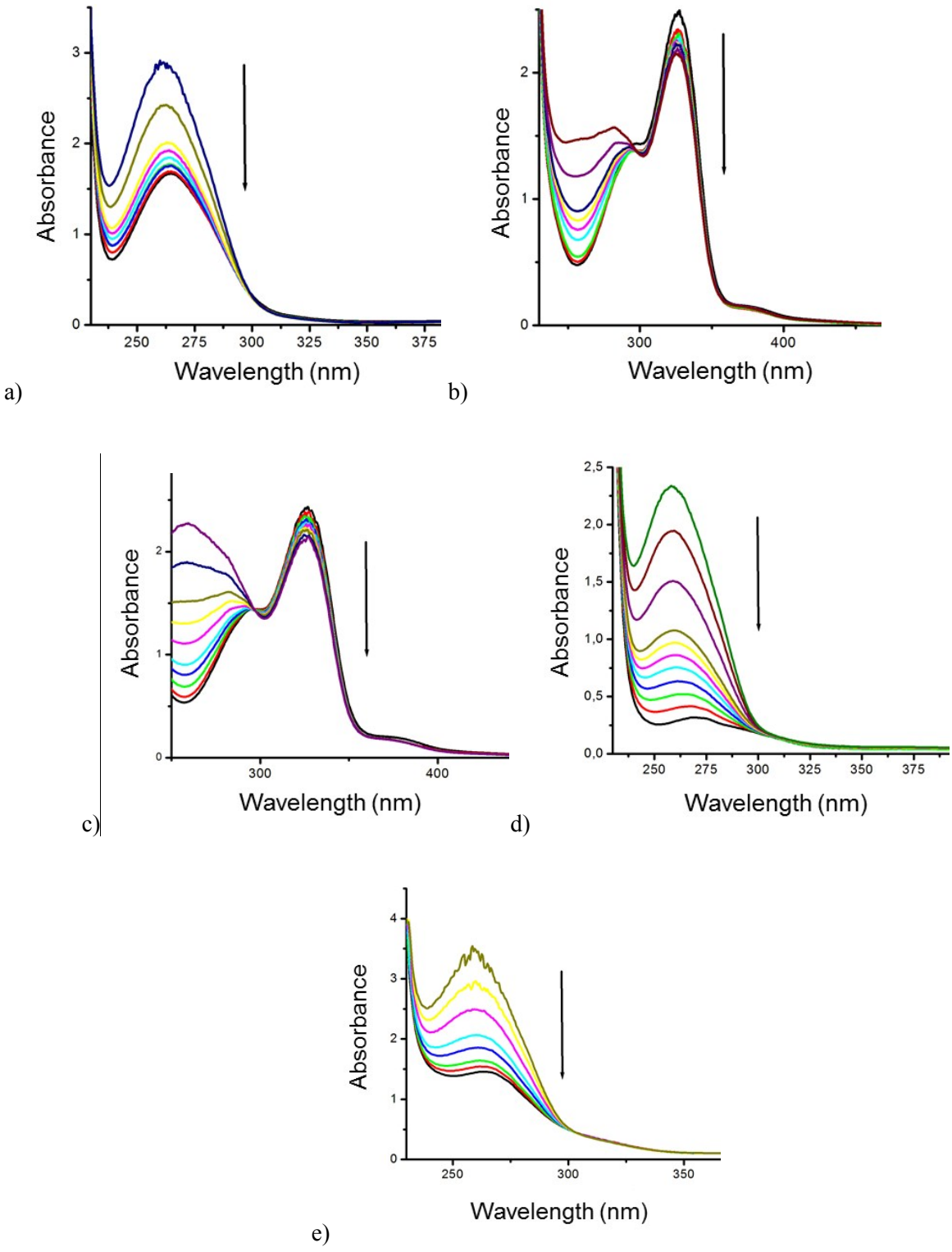

Figure SI-1. Absorption spectra of **1**–**4** and **6** (100 μM) with increasing concentration of CT-DNA (0–200 μM) in phosphate buffer (pH = 7.2).

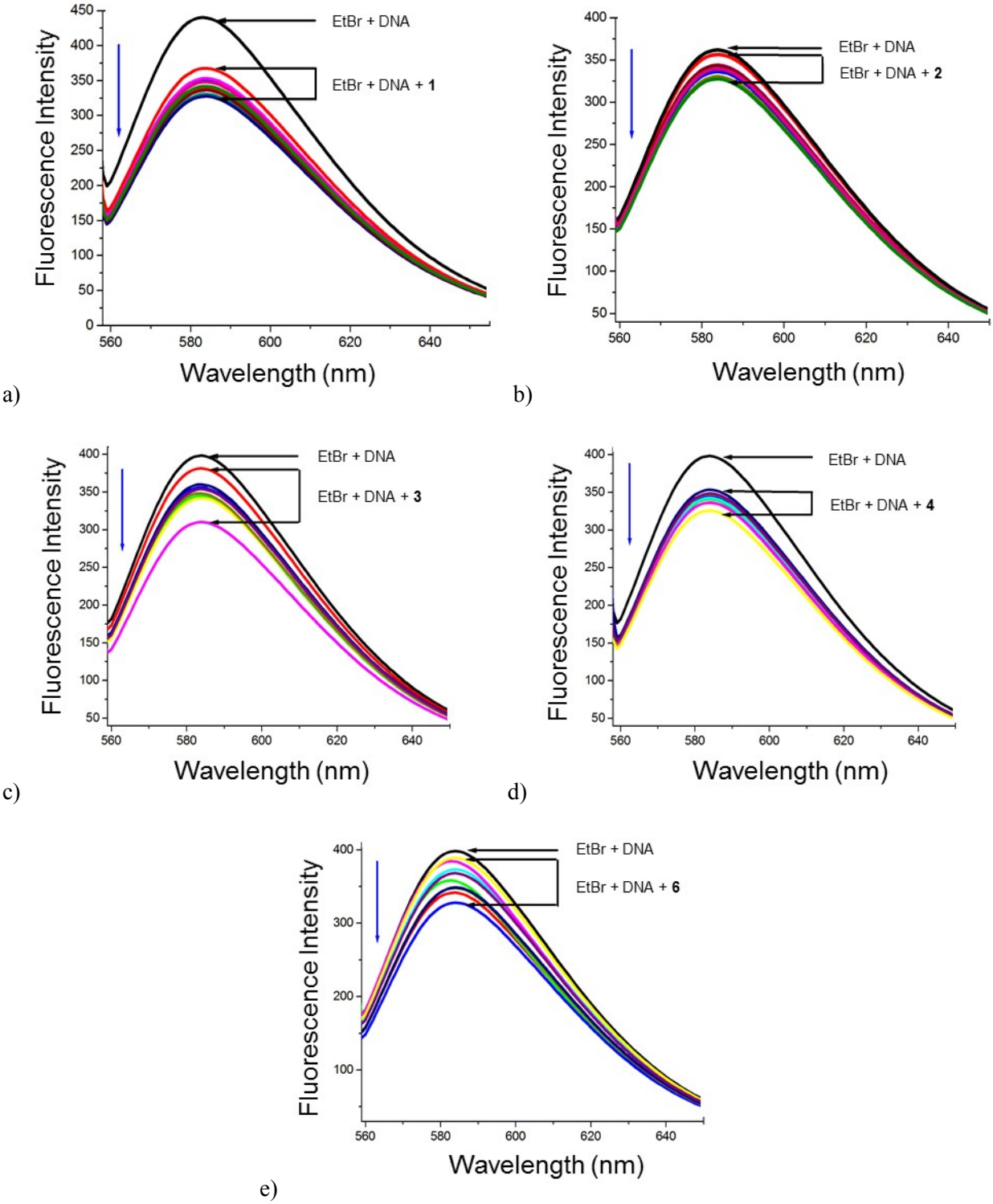

Figure SI-2. Effect of increasing concentration of **1–4** and **6** on the emission intensity of CT-DNA bound ethidium bromide (20 μM)

Table SI-2. Thermal melting data of complexes **1–4** and **6**.

Complex	ΔT_m/°C
1	1.0
2	3.1
3	3.2
4	3.2
6	2.5

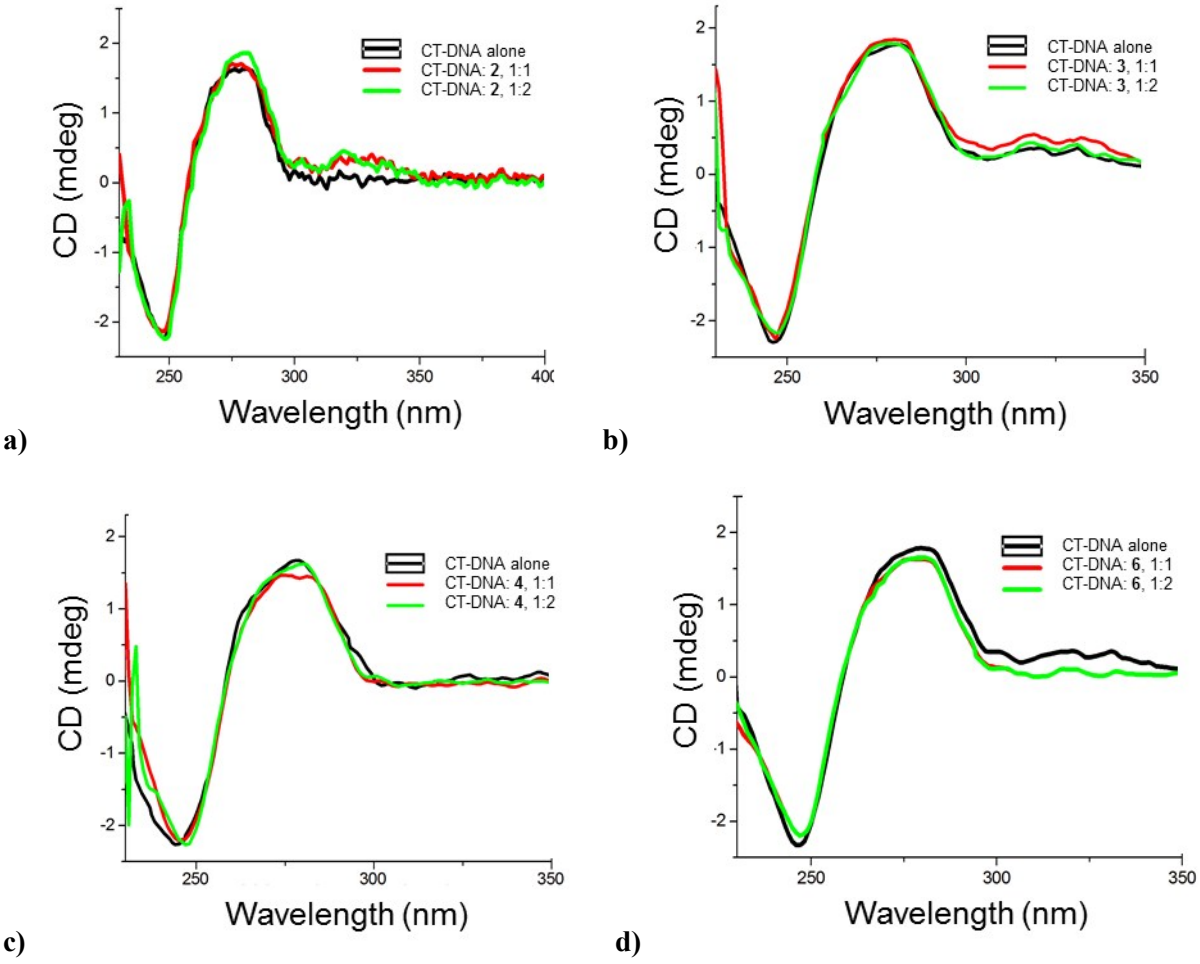

Figure SI-3. CD spectra of CT-DNA in the absence and presence of a) **2**, b) **3**, c) **4** and d) **6** in 10 mM phosphate buffer, pH = 7.2, [DNA] = 20 μM.

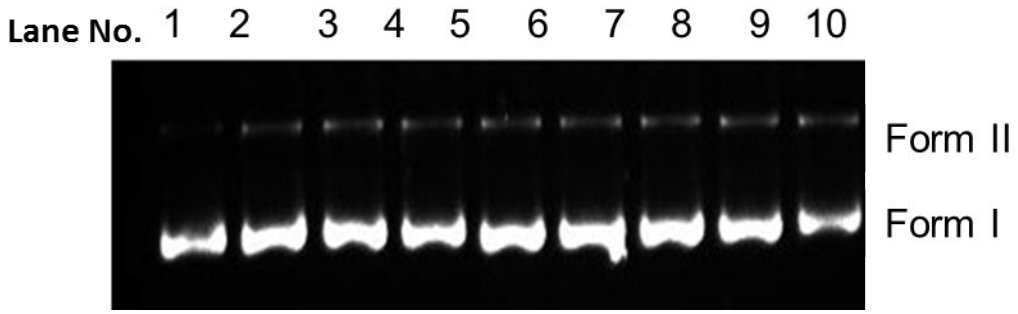

Figure SI-4. Agarose (1%) gel of plasmid pBR 322 DNA in the presence of complex **4**, incubated for 30 min at 37 °C followed by irradiation at 365 nm for 60 min. TBE buffer, pH =8.2. Form I – supercoiled DNA, Form II – nicked circular plasmid DNA. Lane 1: DNA control, Lanes 2–9: DNA + **4** (20, 60, 80, 100, 120, 140, 160, 200 μM).

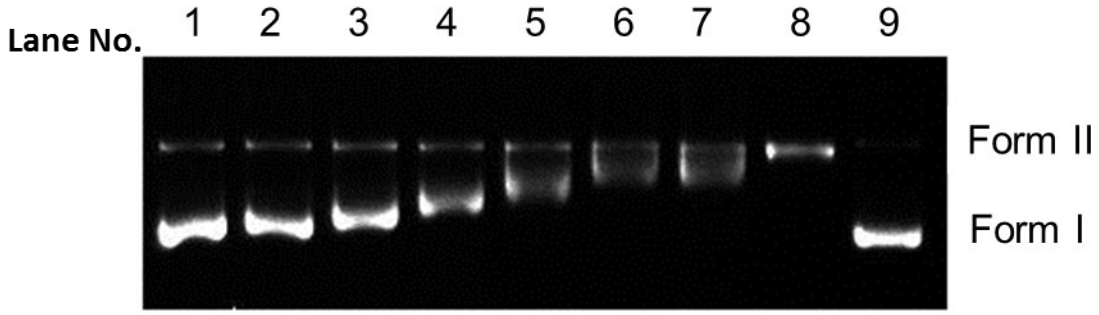

Figure SI-5. Agarose (1%) gel electrophoresis of plasmid pBR322 DNA, complex **6**, incubation time = 30 min, at 37 °C on irradiation. TBE buffer, pH = 8.2. Form I – supercoiled DNA, Form II – nicked circular plasmid DNA. Lanes 1 to 8: DNA + **6** (5, 10, 15, 20, 30, 35, 40, 50 μM), Lane 9: DNA control.

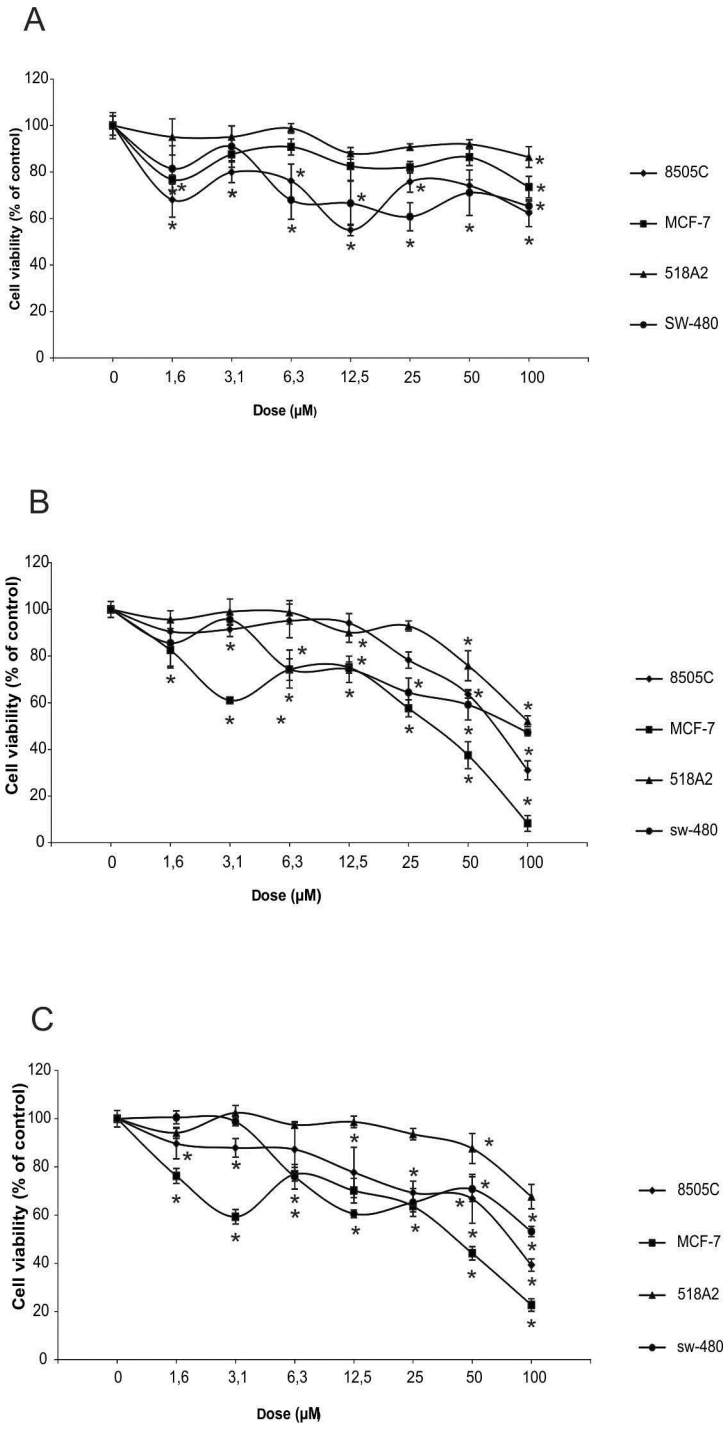

Figure SI-6. The influence of ruthenium(II) arene complexes **1**–**3** on tumour cell viability. 8505C (1.5 x 10^3), MCF-7 (2 x 10^3), 518A2 (1 x 10^3) and SW480 (1.5 x 10^3) were treated with various concentrations of complex **1** (A), **2** (B) and **3** (C) and viability was measured after 96 h. The data are presented as mean±SD from three independent experiments. *$p < 0.05$, refers to control, untreated cells.

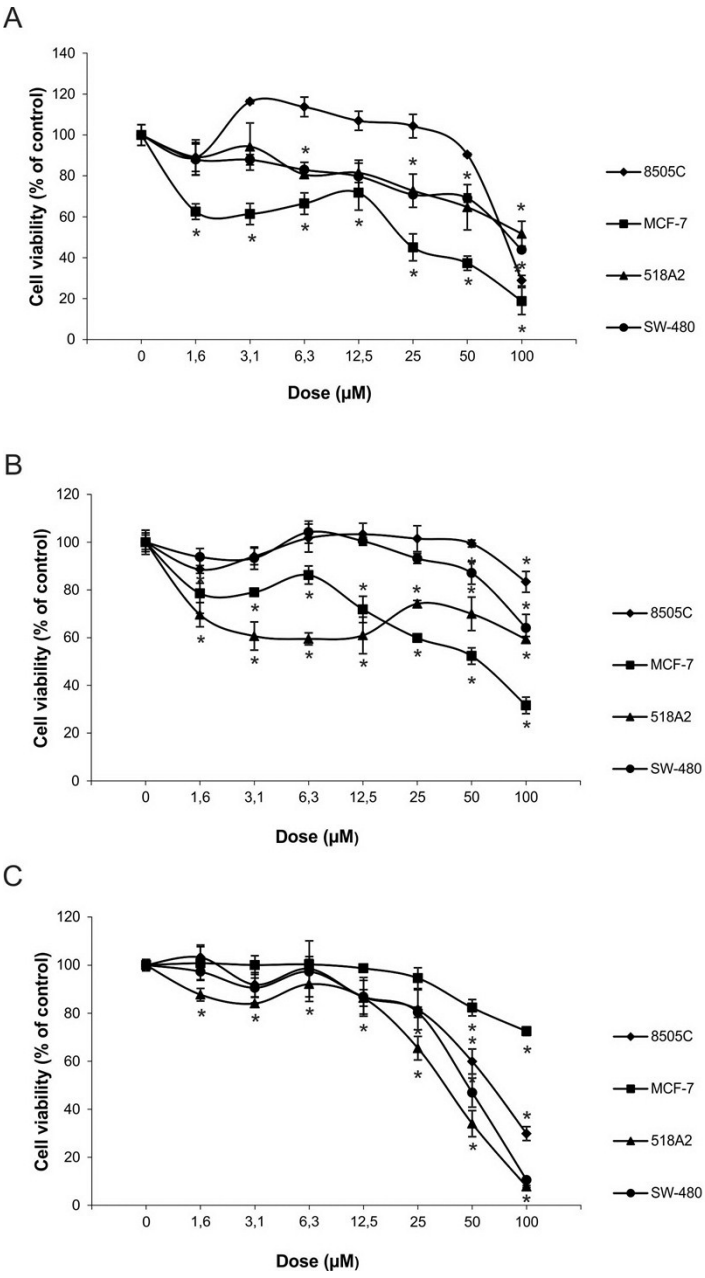

Figure SI-7. The influence of ligands **B**, **C**, and **E** on tumour cell viability. 8505C (1.5 x 10^3), MCF-7 (2 x 10^3), 518A2 (1 x 10^3) and SW480 (1.5 x 10^3) were treated with various concentrations of ligand **B** (A), **C** (B) and **E** (C), and viability was measured after 96 h. The data are presented as mean±SD from three independent experiments. *$p < 0.05$, refers to control, untreated cells.