Cu(I) complexes as new antiproliferative agents against sensitive and doxorubicin resistant colorectal cancer cells: Synthesis, Characterization, and Mechanisms of Action

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Spectroscopic studies

Table S1. Electronic spectra data for complexes **2**, **3**, precursor and free ligands in dichloromethane solution.

Compound	**□max (nm) (□ $M^{-1}cm^{-1}$)**	**Compound**	**□max (nm) (□ $M^{-1}cm^{-1}$)**
$[Cu(PPh_3)_2(NCMe)_2][BF_4]$	259 (sh) 279 (sh)		
$[Cu(PPh_3)_2(Me2bpy)][BF_4]$ (**2**)	250 (147000) 288 (sh) 361 (7480)	Me_2bpy	249 (1295) 281(13849) –
$[Cu(PPh_3)_2(dpytz)][BF_4]$ (**3**)	242 (36200) 288 (34300) 342 (sh) 415 (6440)	Dpytz	241 (19540) 283 (22147) 320 (sh) 385 (sh)

Single Crystal X-Ray Diffraction studies

Table S2. Selected bonds (Å) and bond angles (°) of the cations $[Cu(PPh_3)_2(Me_2bpy)]^+$ (**2**) and $[Cu(PPh_3)_2(dpypz)]^+$ (**3**)

Compound	**2**	**3**
Bond lenghts (Å)		
Cu(1)-N(1)	2.0557(16)	2.0784(19)
Cu(1)-N(2)	2.0905(16)	2.0398(19)
Cu(1)-P(1)	2.2302(6)	2.2506(6)
Cu(1)-P(2)	2.2448(5)	2.2133(6)
Angles (°)		
N(1)-Cu(1)-N(2)	79.43(6)	79.90(8)
P(1)-Cu(1)-P(2)	126.18(2)	116.82(2)
N(1)-Cu(1)-P(1)	115.77(5)	113.33(6)
N(1)-Cu(1)-P(2)	112.60(5)	117.92(6)
N(2)-Cu(1)-P(1)	104.54(5)	104.47(6)
N(2)-Cu(1)-P(2)	106.12(5)	118.31(6)

Antiproliferative assays

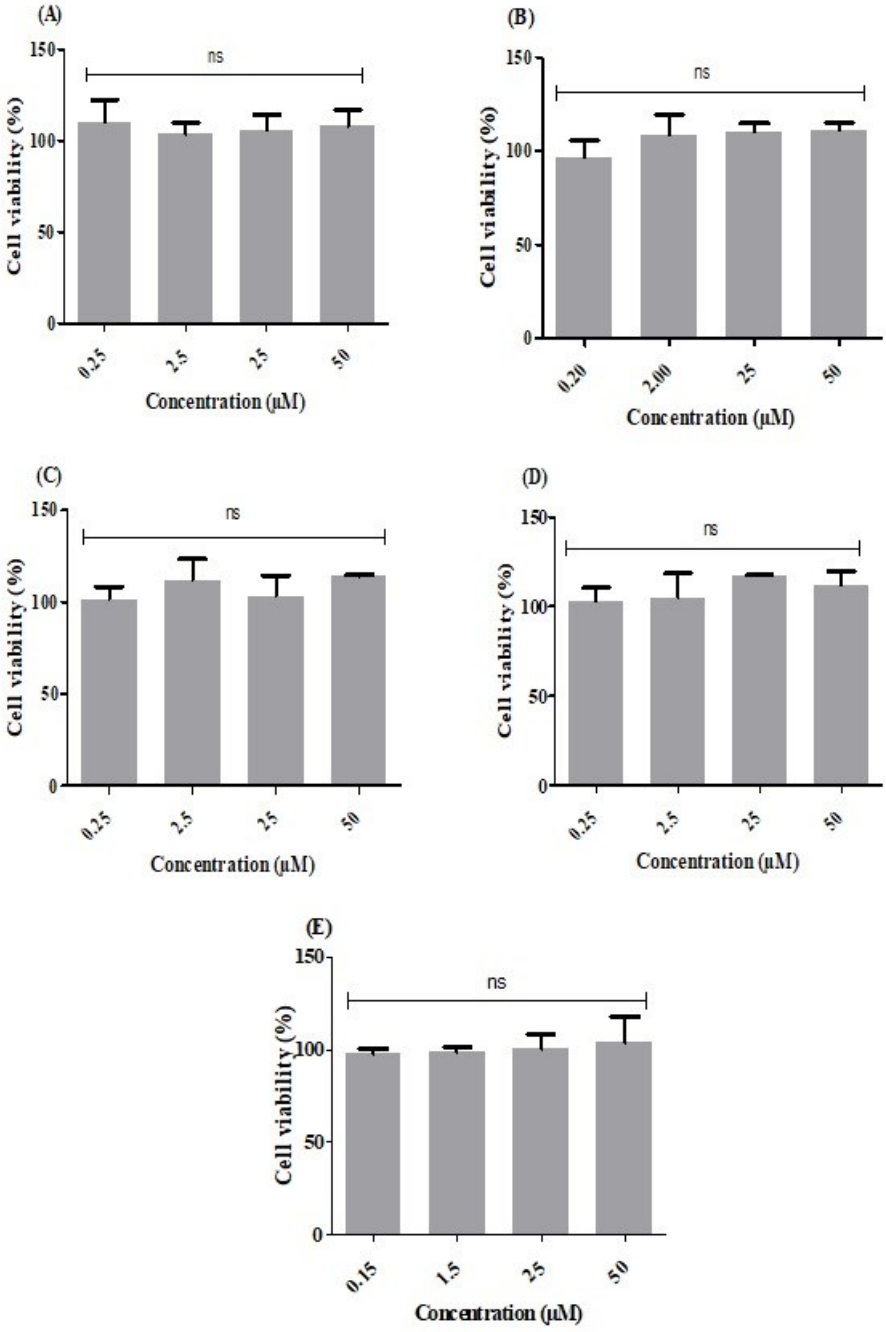

Figure S1. Cell viability in HCT116 cells after 48 hours exposure to Cu(I) complexes (**A – $[Cu(PPh_3)_{2(}NCCH_3)_2][BF_4]$; B – 1; C – 2; D – 3; E – 4**). Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of three independent biological assays. Statistical significance was calculated using t-student comparing each concentration relative to the control, 0.1% (v/v) DMSO. ns - statistically non-significant.

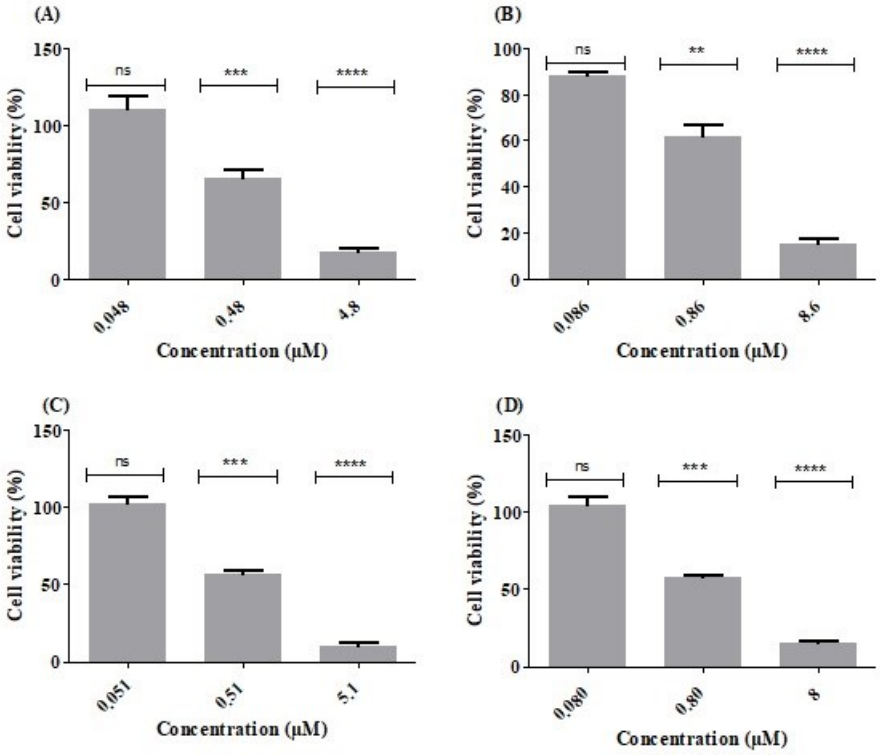

Figure S2. Cell viability in MCF-7 cells after 48 hours of exposure to the complexes (A –5; B – 6; C –7; D –8). Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of two independent biological assays. P-value was calculated using t-student comparing each concentration relative to the control, 0.1% (v/v) DMSO. ns - statistically non-significant, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

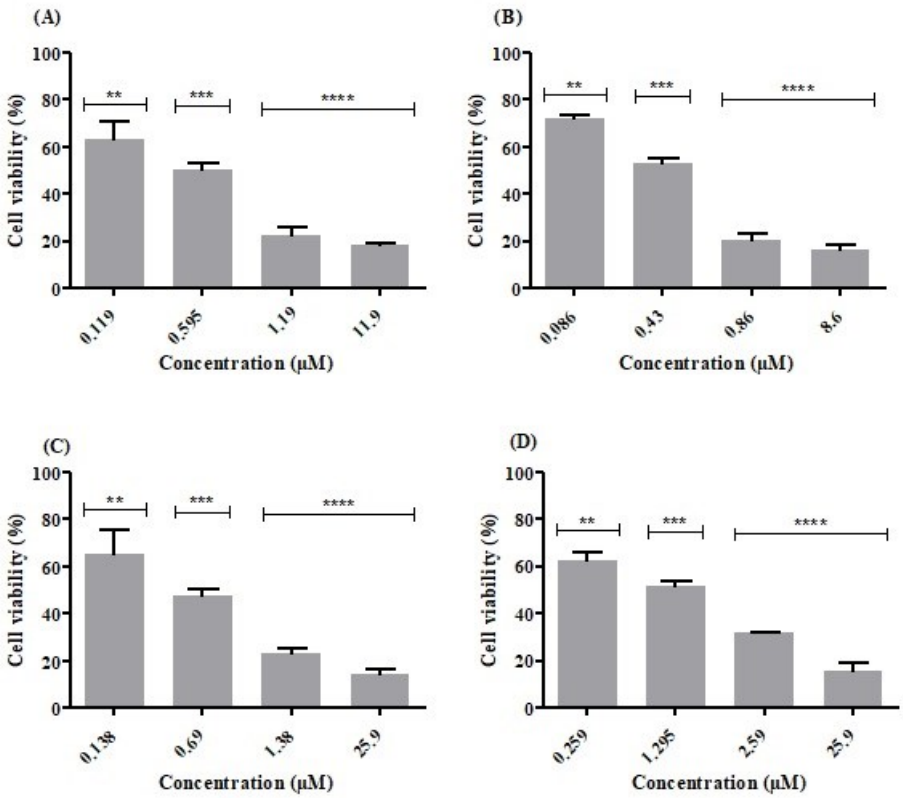

Figure S3. Cell viability in A2780 cell line after 48 hours exposure to study complexes (A – 5; B – 6; C – 7; D – 8). Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of two independent biological assays. P-value was calculated using t-student comparing each concentration relative to the control, 0.1% (v/v) DMSO. (** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

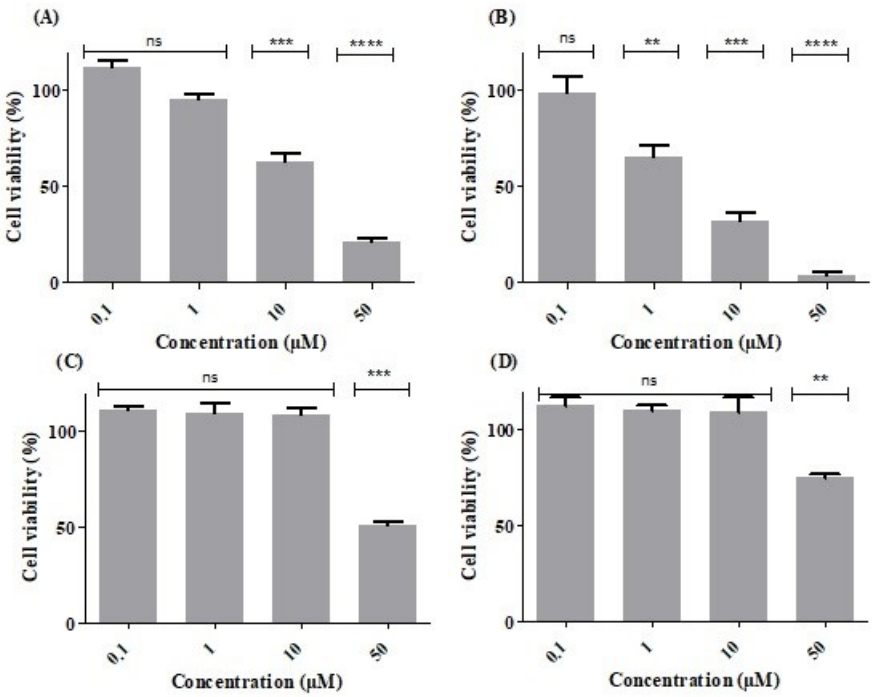

Figure S4. Cell viability in fibroblasts after 48 hours exposure to study complexes (A –5; B – 6; C – 7; D – 8). Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of two independent biological assays. P-value was calculated using t-student comparing each concentration relative to the control, 0.1% (v/v) DMSO. (ns - statistically non-significant, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

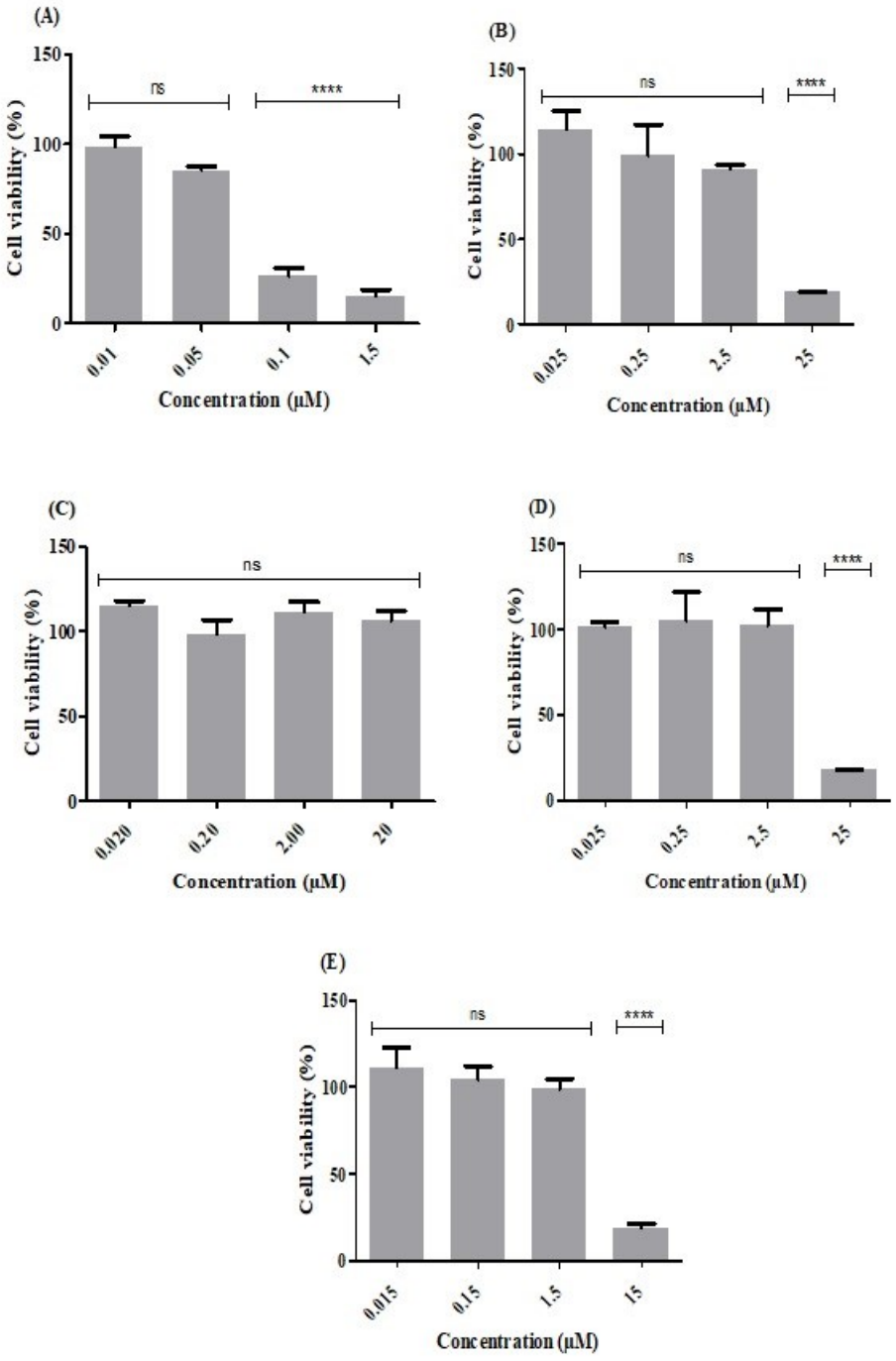

Figure S5. Cell viability in HCT116 cell line after 48 hours exposure to the starting complex (SC) (A) and the ligands B –dptyz; C – dpp; D – bipy; E – Me_2bpy. Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of two independent biological assays. P-value was calculated using t-student comparing each concentration relative to the control, 0.1% (v/v) DMSO. (ns - statistically non-significant, **** $p \leq 0.0001$).

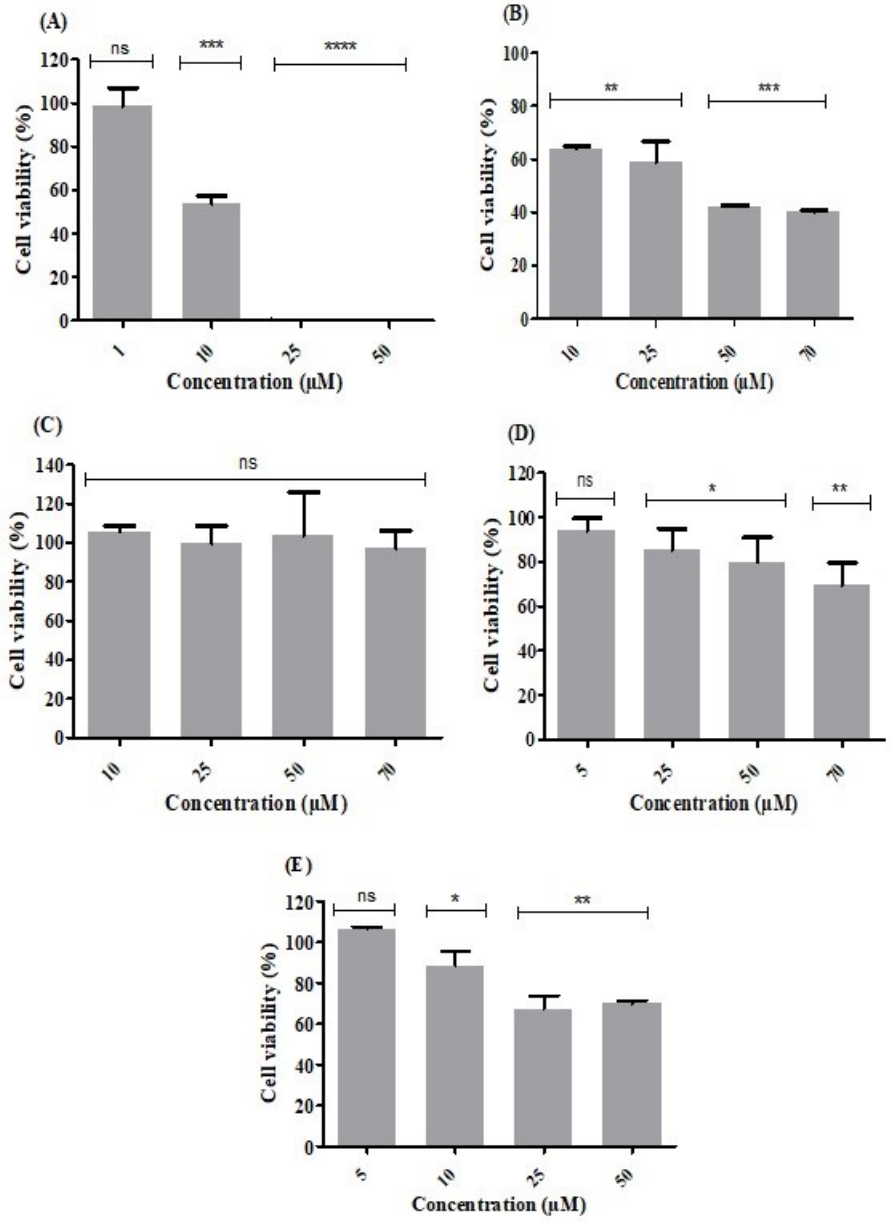

Figure S6. Cell viability fibroblasts after 48 hours exposure to the starting complex (SC) (A) and the ligands B –dptyz; C – dpp; D – bipy; E – Me_2bpy. Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of two independent biological assays. P-value was calculated using t-student comparing each concentration relative to the control, 0.1% (v/v) DMSO. (ns - statistically non-significant, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

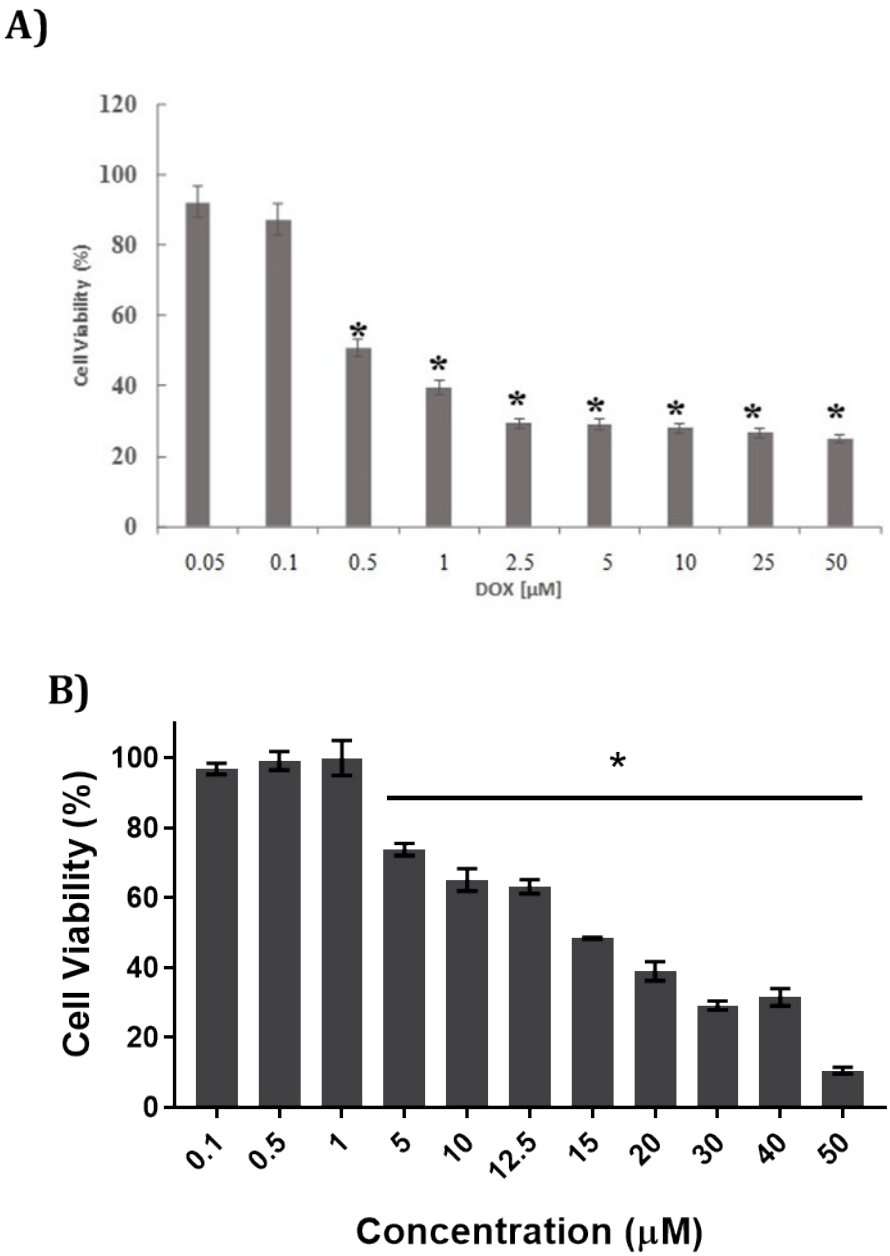

Figure S7. Cell viability of HCT116 cells after 48 hours of exposure to doxorubicin (A) or cisplatin (B). Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of three independent biological assays. P-value was calculated using t-student comparing each concentration relative to the control, 0.1% (v/v) DMSO. * $p \leq 0.01$.

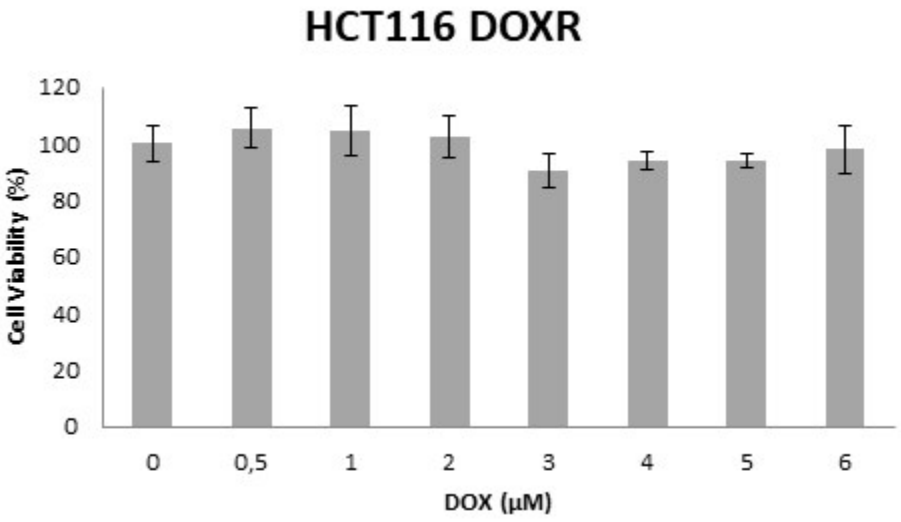

Figure S8. Cell viability of HCT116 DoxR cells after 48 hours of exposure to doxorubicin. Values of cell viability were normalized relative to the control vehicle in (0.1% (v/v) DMSO. Data are represented as the mean ± SD of three independent biological assays.

Mitochondrial potential

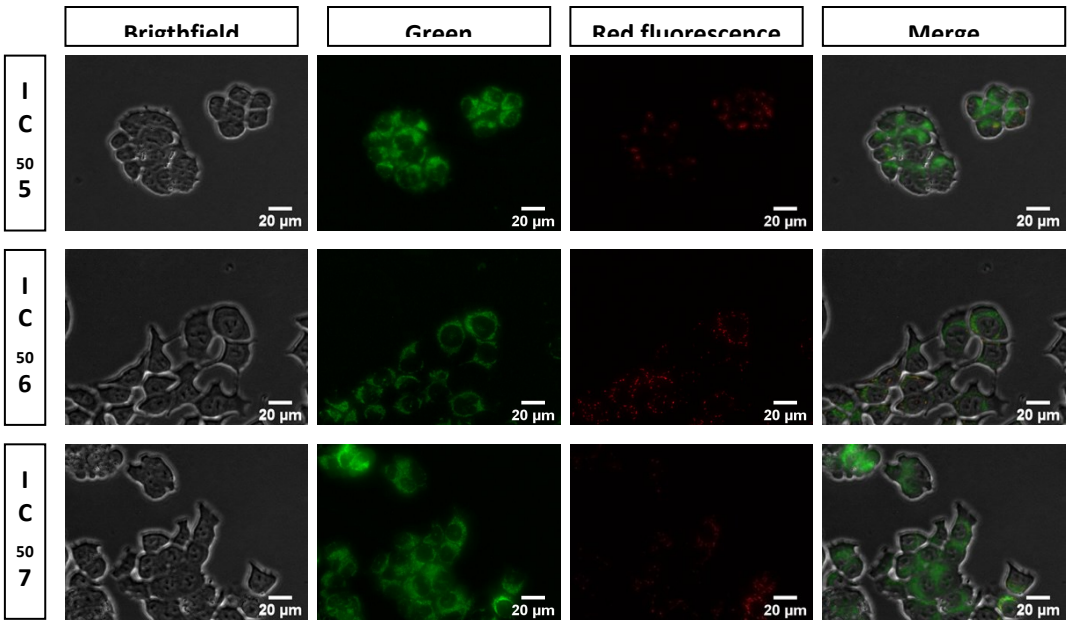

Figure S9. HCT116 cells labeled with JC-1 after 48 h exposure to the IC_{50} of complexes (**5, 6** e **7**). At least 5 images were analyzed for each condition under study.

Autophagic potential

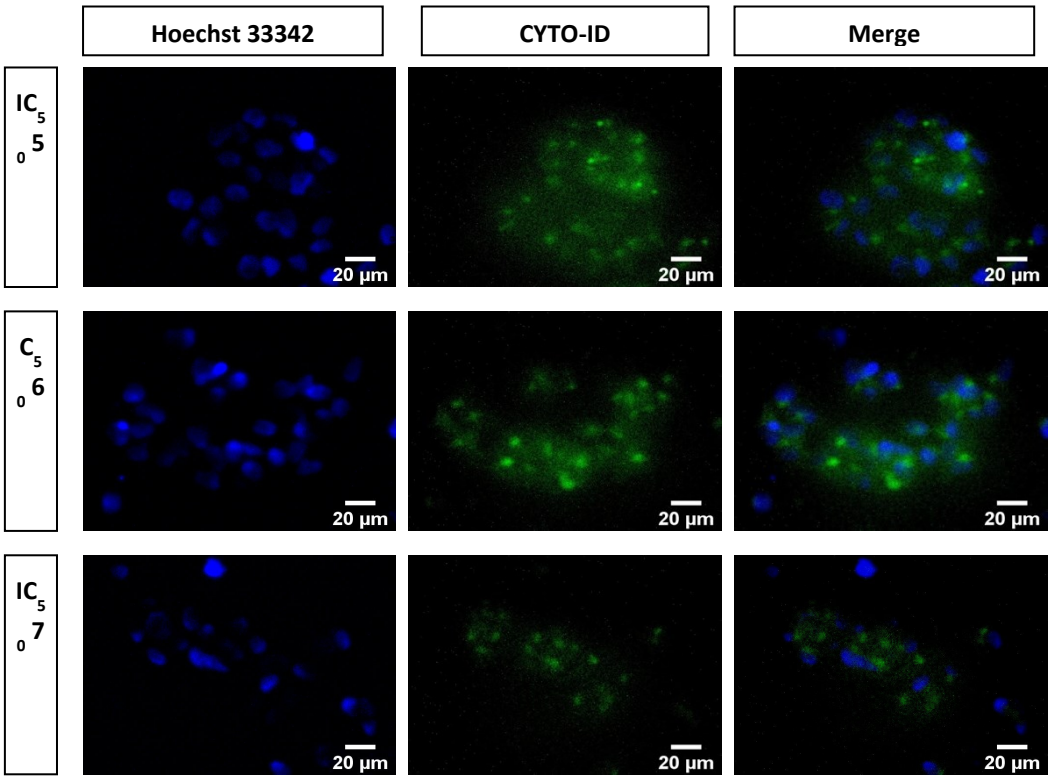

Figure S10. HCT116 cells labelled with Cyto-ID probe and Hoechst 33342 for analysis of autophagic components after 48 h exposure to the IC_{50} of complexes **5**, **6** and **7**. At least 5 images were analyzed for each condition under study.

Reactive Oxygen Species (ROS)

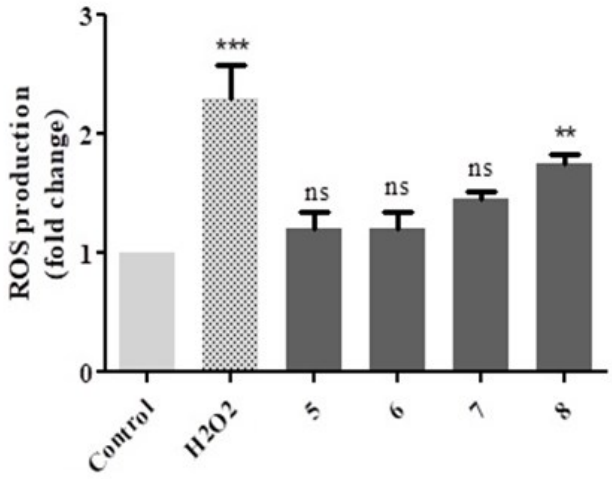

Figure S11. ROS levels quantified by flow cytometry in HCT116 tumor cell line after exposure to IC_{50} of complexes, 0.1% (v/v) DMSO or 25 µM H_2O_2 for 48 hours. Values were normalized to vehicle control (DMSO). Data are represented as the mean ± SD of three independent biological

assays. Statistical significance was assessed relative to control (DMSO) by the one-way ANOVA method (*** $p \leq 0.001$, ** $p \leq 0.01$, ns - statistically not significant).

pDNA cleavage

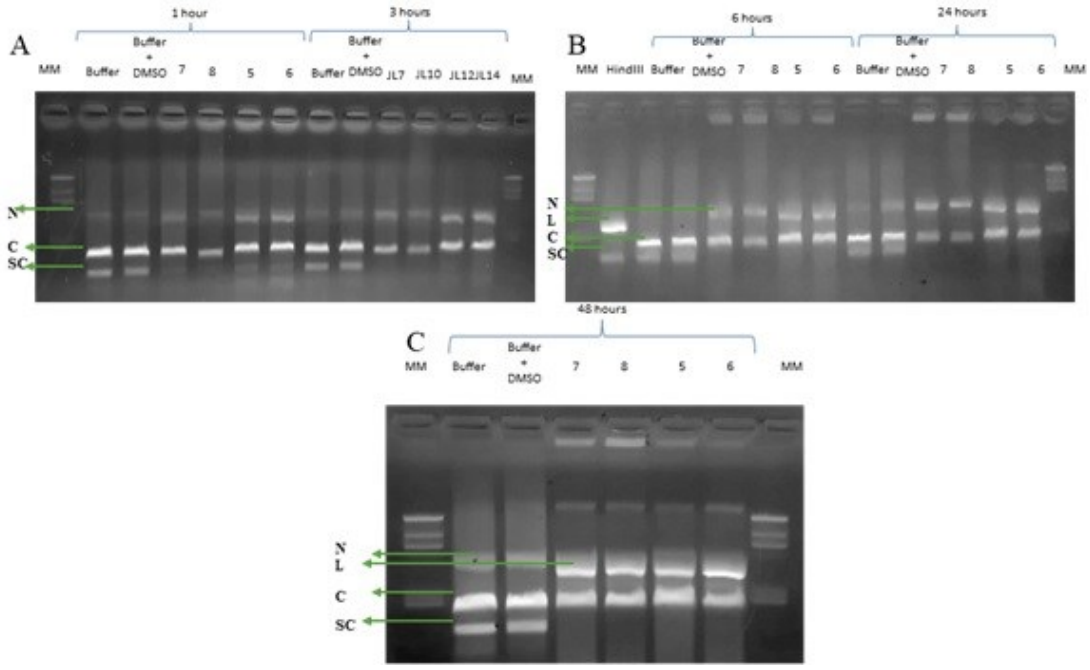

Figure S12. Plasmid DNA (100 ng pUC18) was exposed to a fixed concentration of each complex (25 μM) with varying exposure times (1, 3, 6, 24 and 48 hours). The electrophoresis conditions were: 1.0% agarose gel in 1x TAE with 0.0015% GelRed, 40 V for 3 hours. The molecular weight marker used was the lambda *HindIII*; and the legend for images A, B and C is above the wells. The buffer solution was composed by 5 mM Tris-HCl, 50 mM NaCl, pH 7.2. The green arrows correspond to the isoforms of the plasmid pUC18: N – nicked form; L - linear form; C - coiled form; SC - supercoiled form.

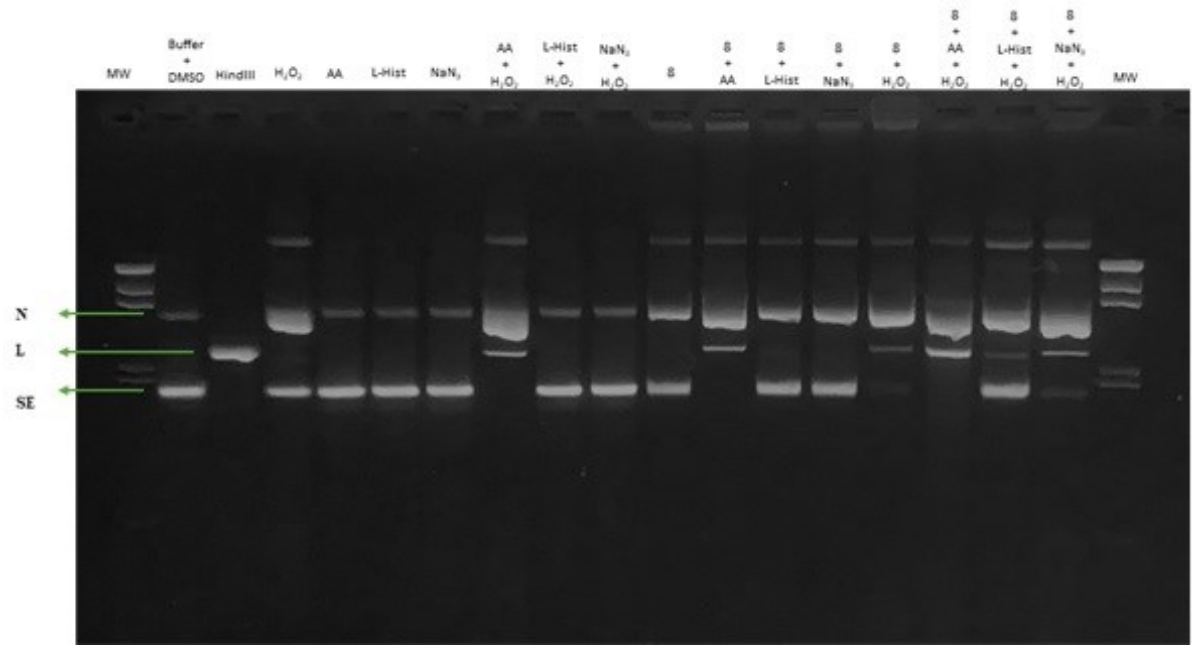

Figure S13. Agarose gel electrophoresis to determine the DNA cleavage mechanisms induced by complex 8. The electrophoresis conditions were: 1.0% agarose gel in 1x TAE with 0.0015% GelRed, 90 V for 90 minutes. Legend: MM- lambda molecular weight marker *HindIII*; 1- pUC18 exposed to buffer solution (5 mM Tris-HCl, 50 mM NaCl) and 25% (v/v) DMSO; 2- pUC18 exposed to *HindIII*; 3- pUC18 exposed to 100 µM H_2O_2; 4- pUC18 exposed to 50 µM ascorbic acid; 5- pUC18 exposed to 50 µM NaN3; 6- pUC18 exposed to 50 µM of L-Histidine; 7- pUC18 exposed to 50 µM ascorbic acid and 100 µM H_2O_2; 8- pUC18 exposed to 50 µM NaN3 and 100 µM H_2O_2; 9- pUC18 exposed to 50 µM of L-histidine and 100 µM of H_2O_2; 10- pUC18 exposed to 25 µM of 8; 11- pUC18 exposed to 25 µM of 8 and 50 µM of ascorbic acid; 12-pUC18 exposed to 25 µM 8 and 50 µM NaN3; 13- pUC18 exposed to 25 µM of 8 and 50 µM of L-Histidine; 14- pUC18 exposed to 25 µM of 8 and 100 µM of H_2O_2; 15- pUC18 exposed to 25 µM of 8, 50 µM of ascorbic acid and 100 µM of H_2O_2; 16- pUC18 exposed to 25 µM 8, 50 µM NaN3 and 100 µM H_2O_2; 17- pUC18 exposed to 25 µM of 8, 50 µM of L-histidine and 100 µM of H_2O_2. The green arrows represent the pUC18 isoforms identified in the gel: N – nicked form; L - linear form; C - coiled form; SC - supercoiled form.