Electronic supplementary information

Heterobimetallic Ru(II)/Fe(II) complexes as potent anticancer agents against breast cancer cells, inducing apoptosis through multiple targets

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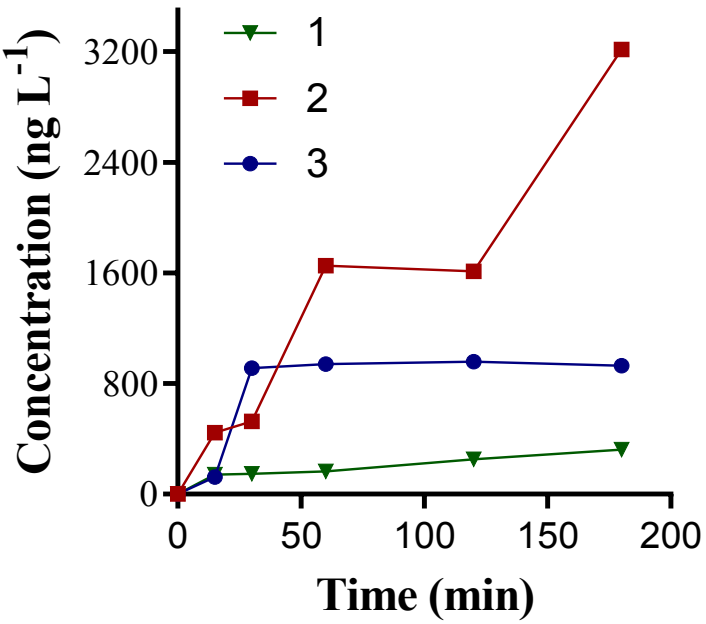
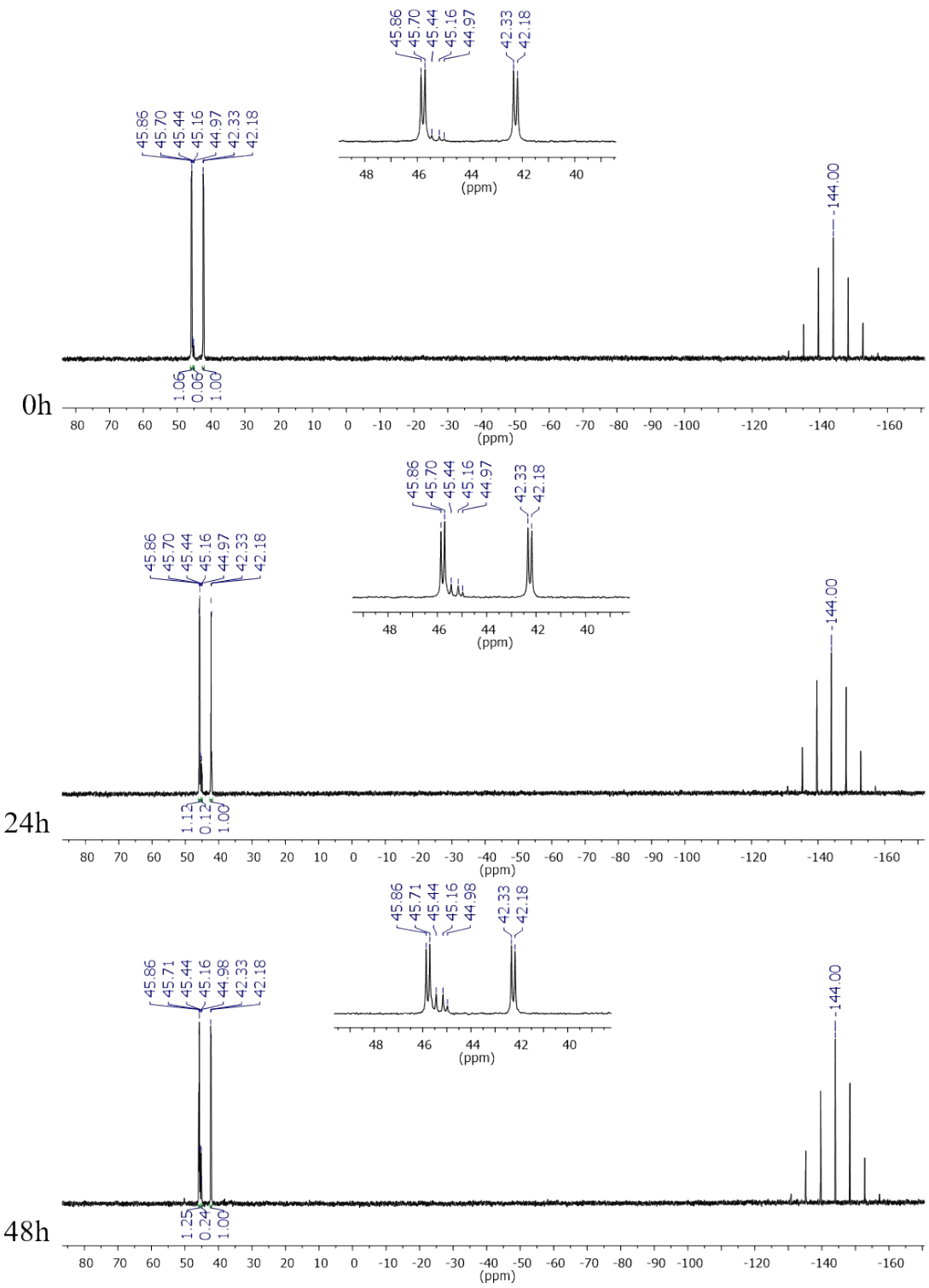
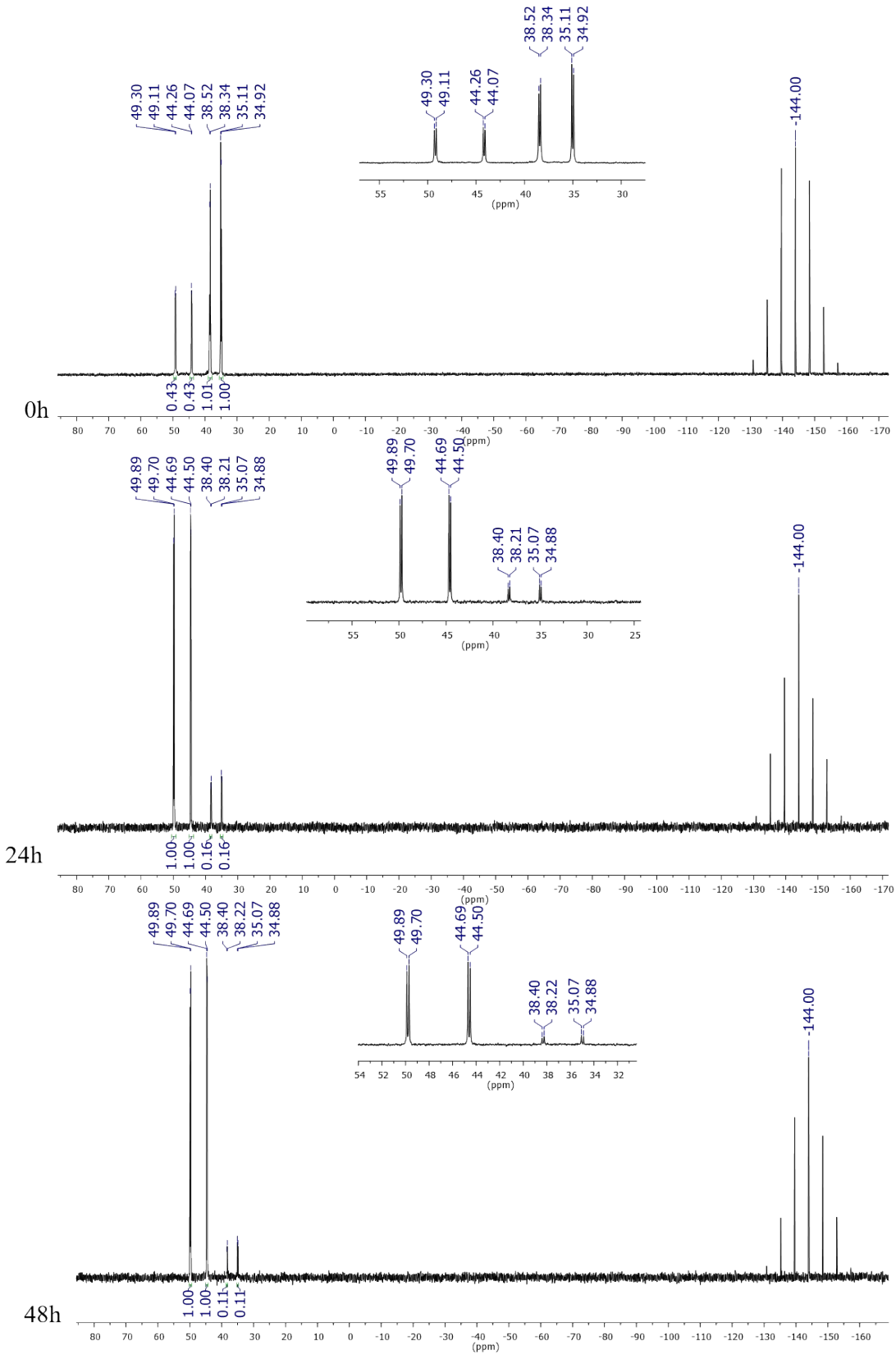


Fig. S1 Permeation across Caco-2 cells, represented by concentration after permeation of complexes 1-3 through the Caco-2 cell membrane per time, quantified by ICP-MS. Each value represents the mean derived from at least three individual experiments in triplicate (mean ± SD).

(A)


0h
24h
48h
45.86
45.70
45.44
45.16
44.97
42.33
42.18
45.71
44.98
-144.00
1.06
0.06
1.00
1.13
0.12
1.25
0.24
(ppm)

(B)


0h
49.30
49.11
44.26
44.07
38.52
38.34
35.11
34.92
-144.00
0.43
0.43
1.01
1.00
24h
49.89
49.70
44.69
44.50
38.40
38.21
35.07
34.88
-144.00
1.00
1.00
0.16
0.16
48h
49.89
49.70
44.69
44.50
38.40
38.22
35.07
34.88
-144.00
1.00
1.00
0.11
0.11
(ppm)

(C)

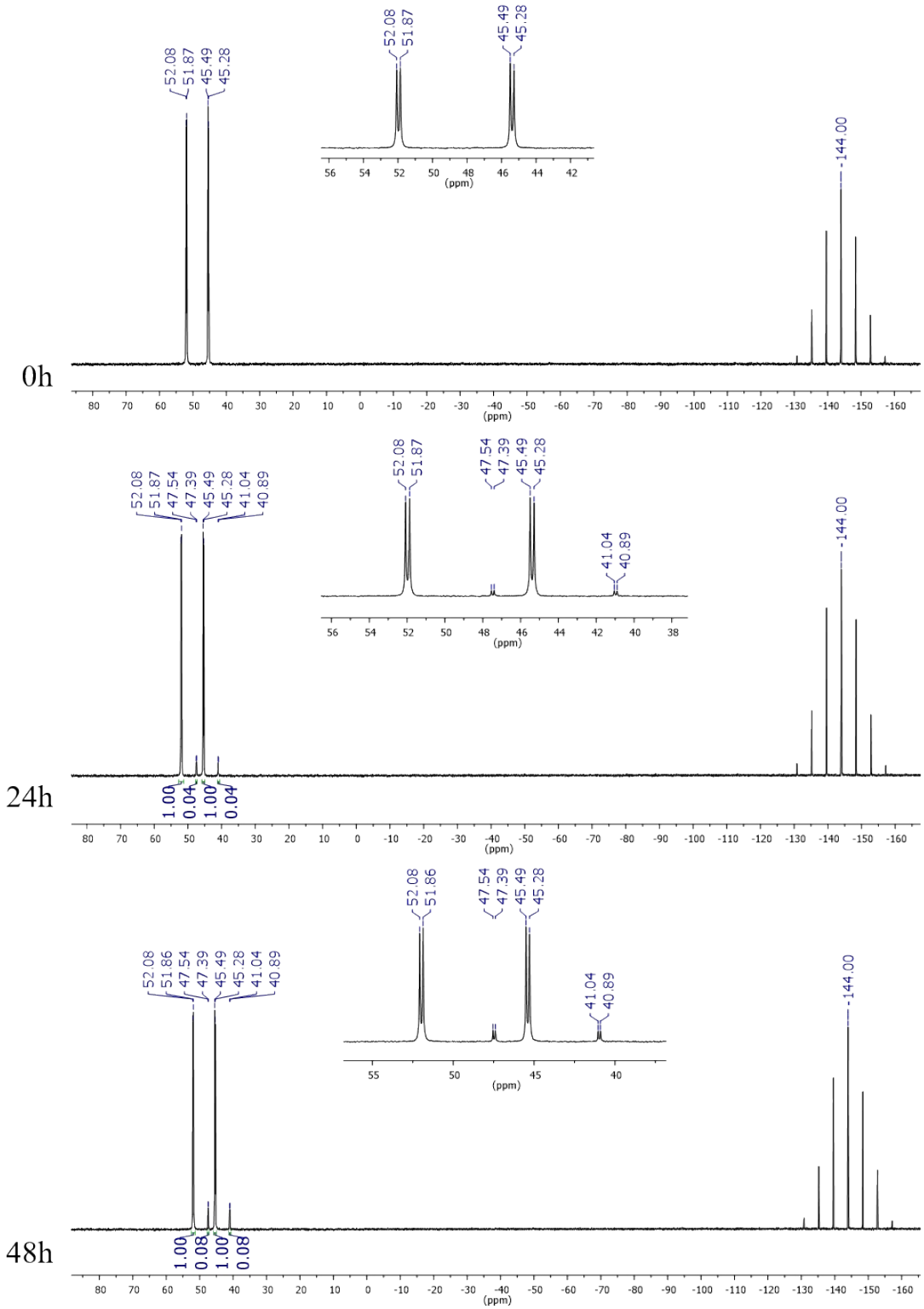


Fig. S2 Stability Study. ^{31}P NMR (^{1}H) spectra of complexes **1** (A), **2** (B) and **3** (C) in culture medium / DMSO solution (20/80%(v/v)).

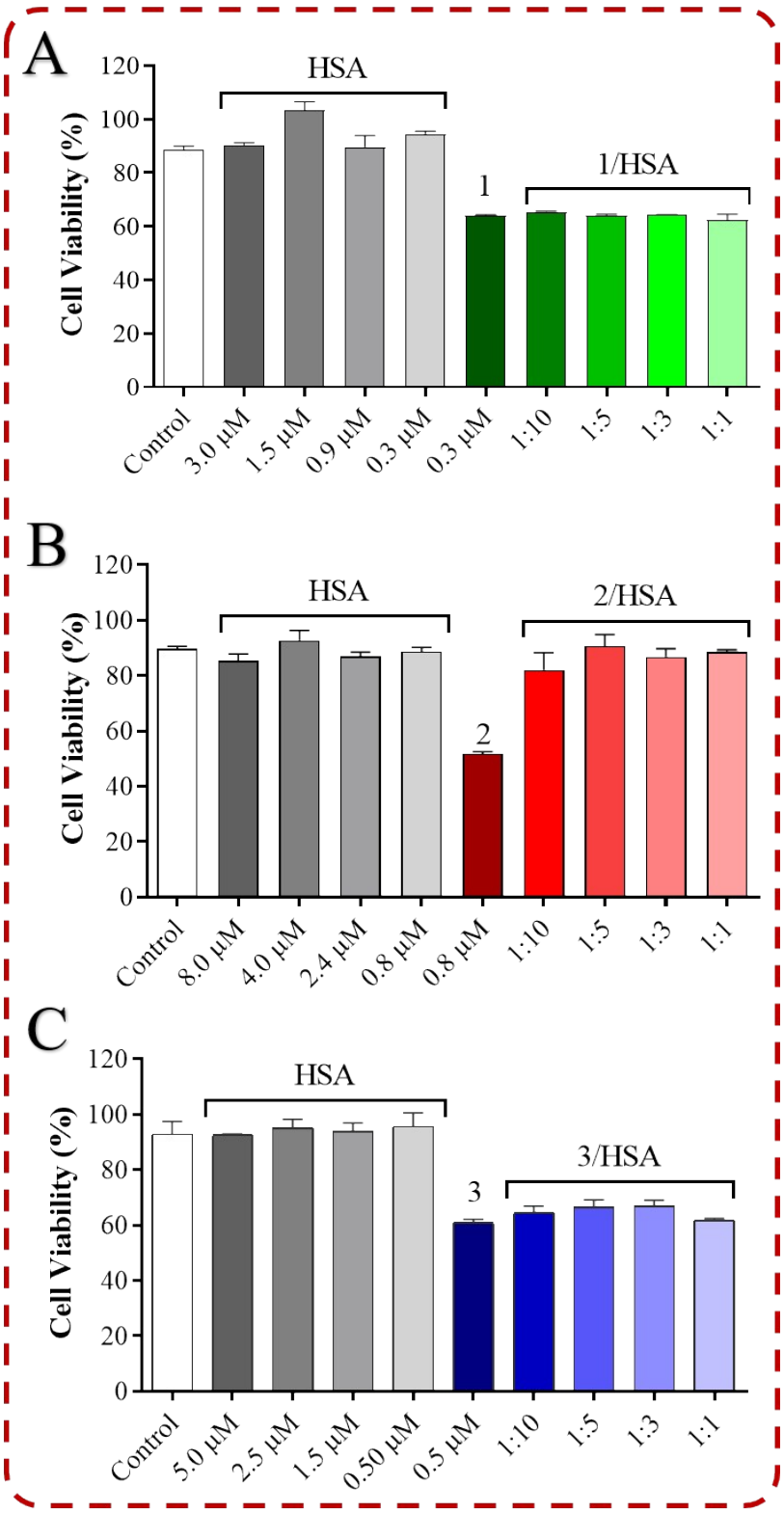


Fig. S3 Effect of HSA on the cytotoxicity of complex 1 (A), 2 (B) and 3 (C) against breast tumor cell (MDA-MB-231) after 48h. MDA-MB-231 cells were treated with complex **1** at 0.3 μM (A), complex **2** at 0.8 μM (B) and complex **3** at 0.5 μM (C) pre-incubated with HSA at 1:1, 1:3, 1:5 and 1:10 complex-to-protein molar ratio. Control indicated cells without treatment.

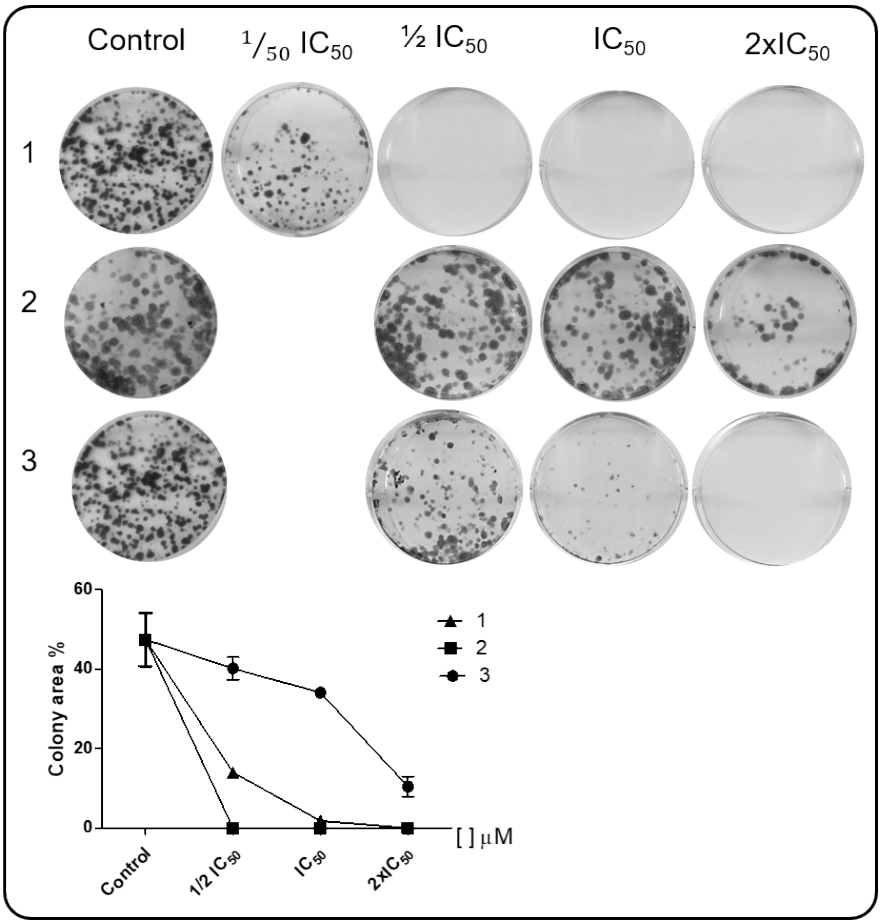


Fig. S4 Effects of complexes **1**, **2** and **3** at different concentrations on MDA-MB-231 colony formation after 48 h treatment and quantification of colony area %.

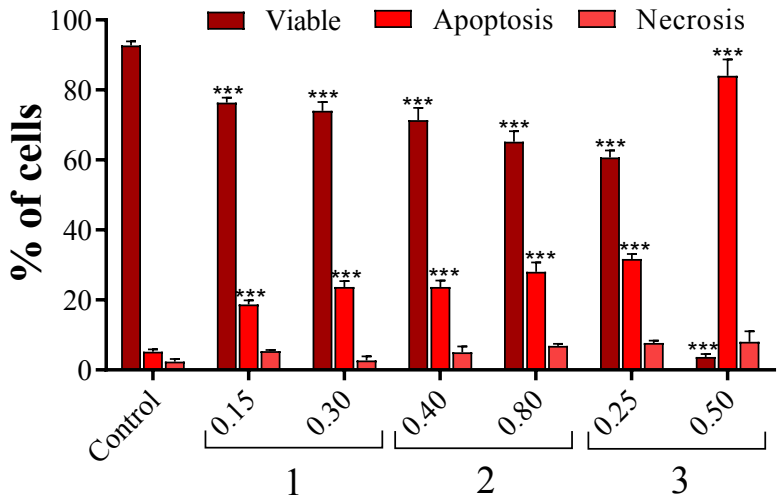


Fig. S5 Percentage of apoptosis and necrosis after treatment with ½ IC_{50} and IC_{50} of the complexes 1-3 by double staining with HO/PI. Data show the means ± SD of experiments performed in triplicate. Significant differences from the control are indicated by *$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$.

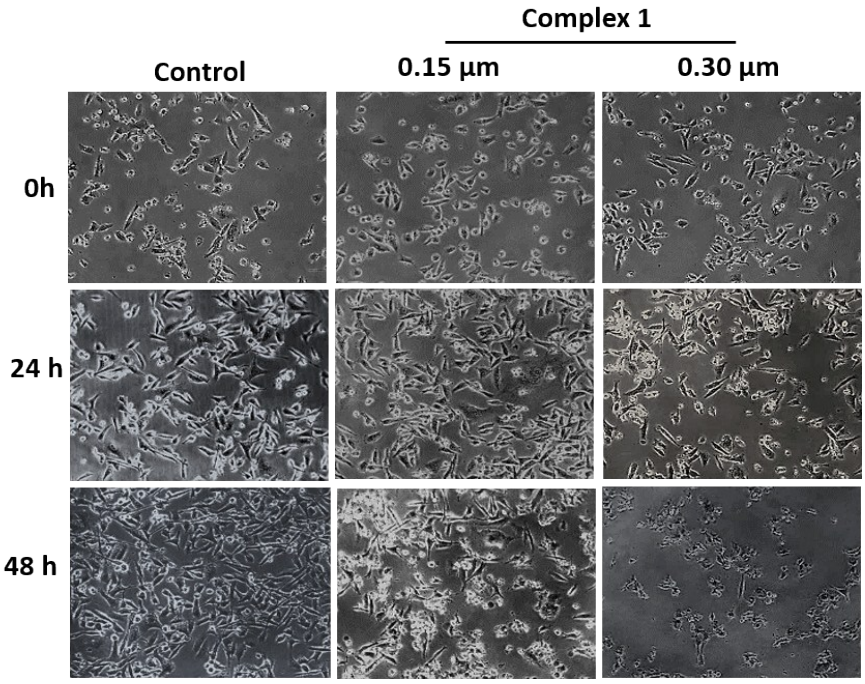


Fig. S6 Morphology of MDA-MB-231 cells treated with complex 1 (0.15 and 0.30 µM) by 0, 24 and 48 h.

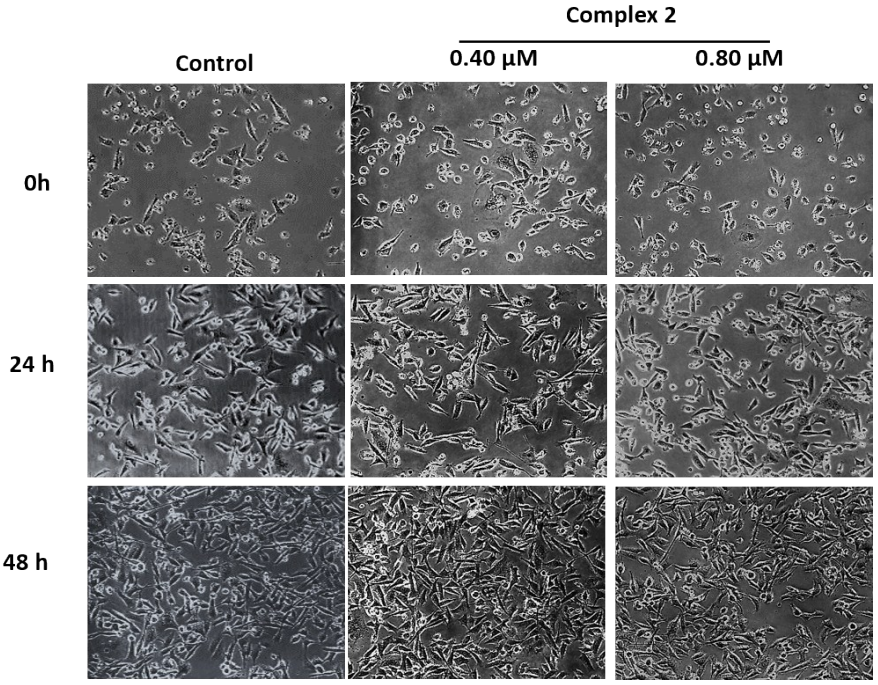


Fig. S7 Morphology of MDA-MB-231 cells treated with complex 2 (0.40 and 0.80 µM) by 0, 24 and 48 h.

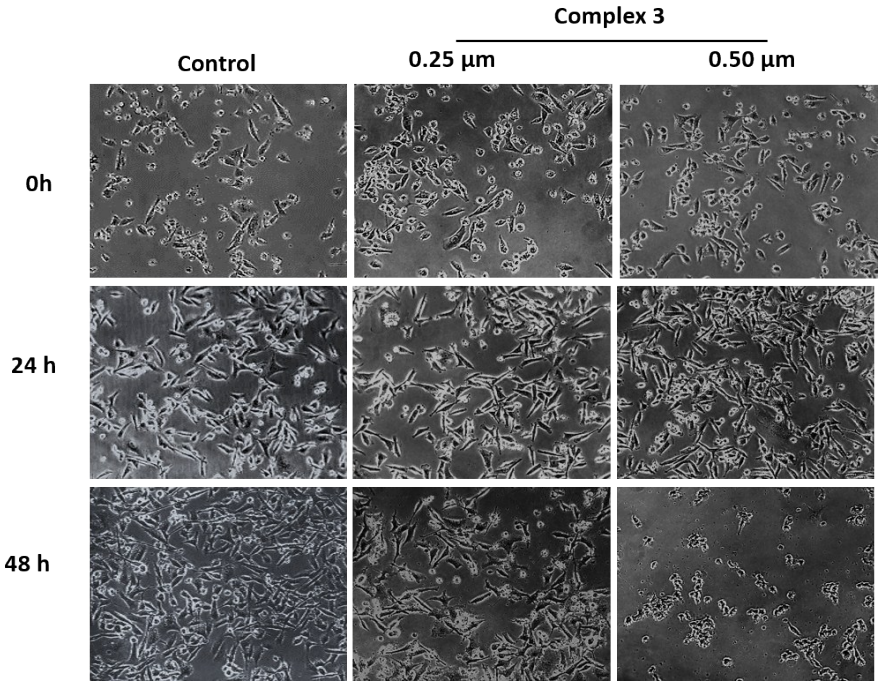


Fig. S8 Morphology of MDA-MB-231 cells treated with complex 3 (0.25 and 0.50 µM) by 0, 24 and 48 h.