Unsymmetric cisplatin-based Pt(IV) derivative containing 2-(2-propynyl)octanoate: a very efficient multi-action antitumor prodrug candidate

Elisabetta Gabano,[a]* Mauro Ravera,[a] Ilaria Zanellato,[a] Stefano Tinello,[a] Andrea Gallina,[a] Beatrice Rangone,[a] Valentina Gandin,[b] Cristina Marzano,[b] Maria Grazia Bottone,[c] Domenico Osella,[a]

[a.] *Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Viale Michel 11, 15121 Alessandria, Italy.*

[b.] *Dipartimento di Scienze del Farmaco, Università di Padova, Via Marzolo 5, 35131 Padova, Italy.*

[c.] *Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Università di Pavia, Via Ferrata 9, 27100 Pavia, Italy.*

ELECTRONIC SUPPLEMENTARY INFORMATION

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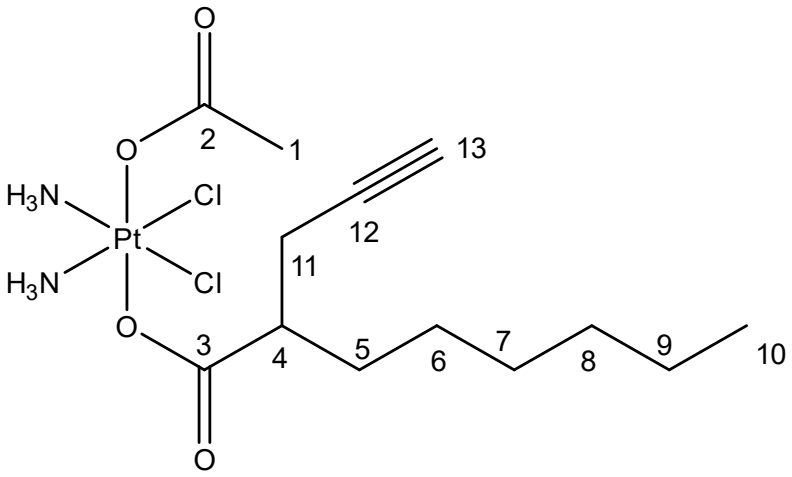

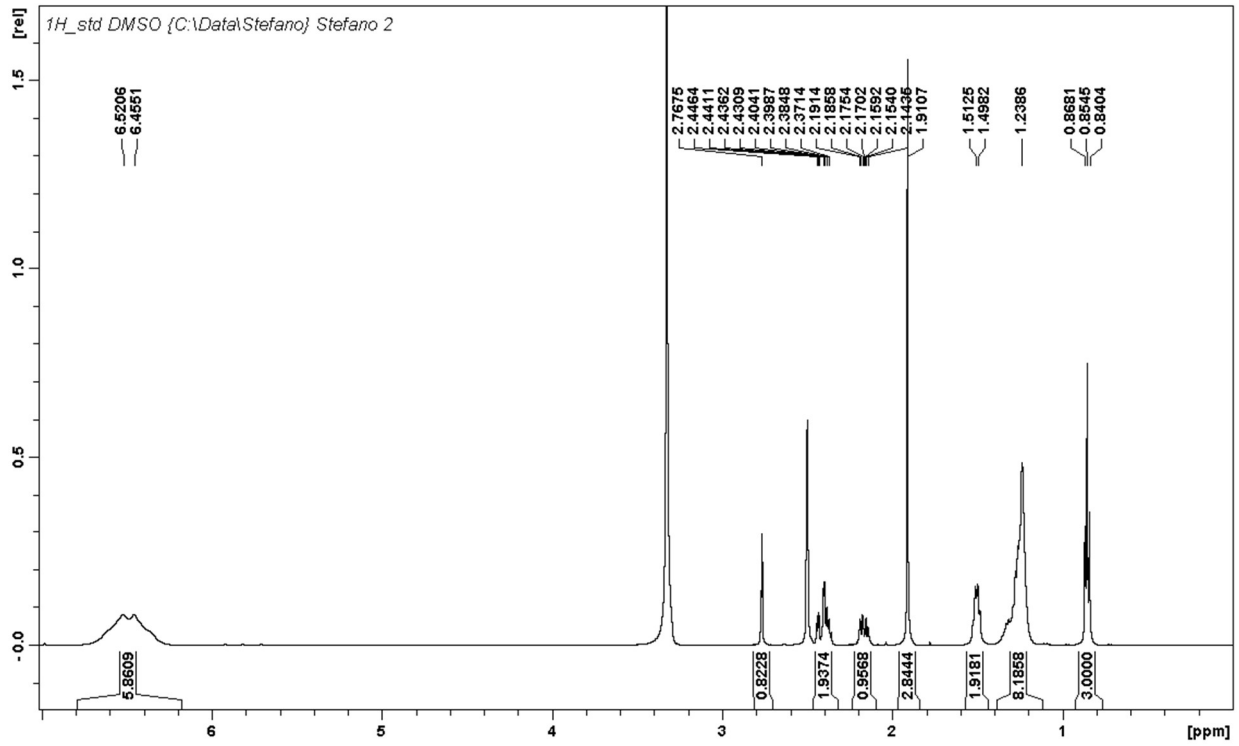

Figure S1. Numbering scheme for the assignment of NMR signals and ^{1}H-NMR spectrum of **1**

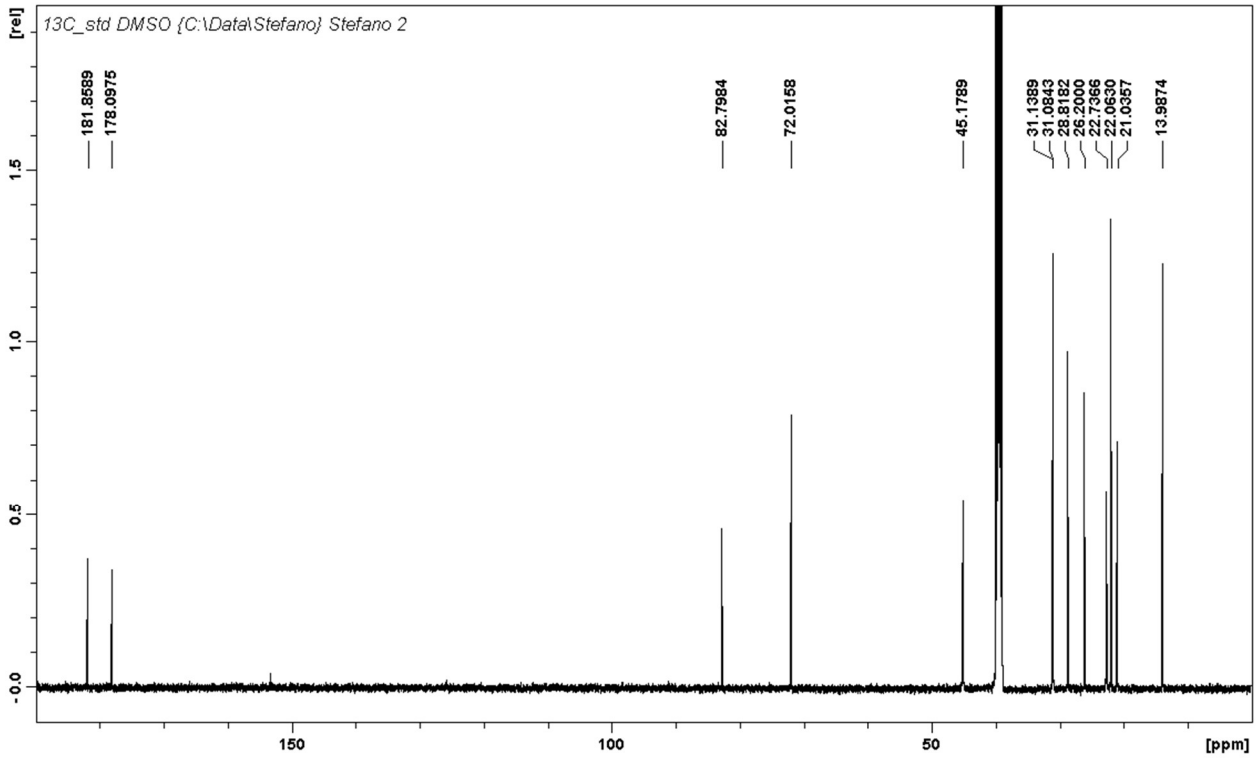

Figure S2. ^{13}C-NMR spectrum of **1**

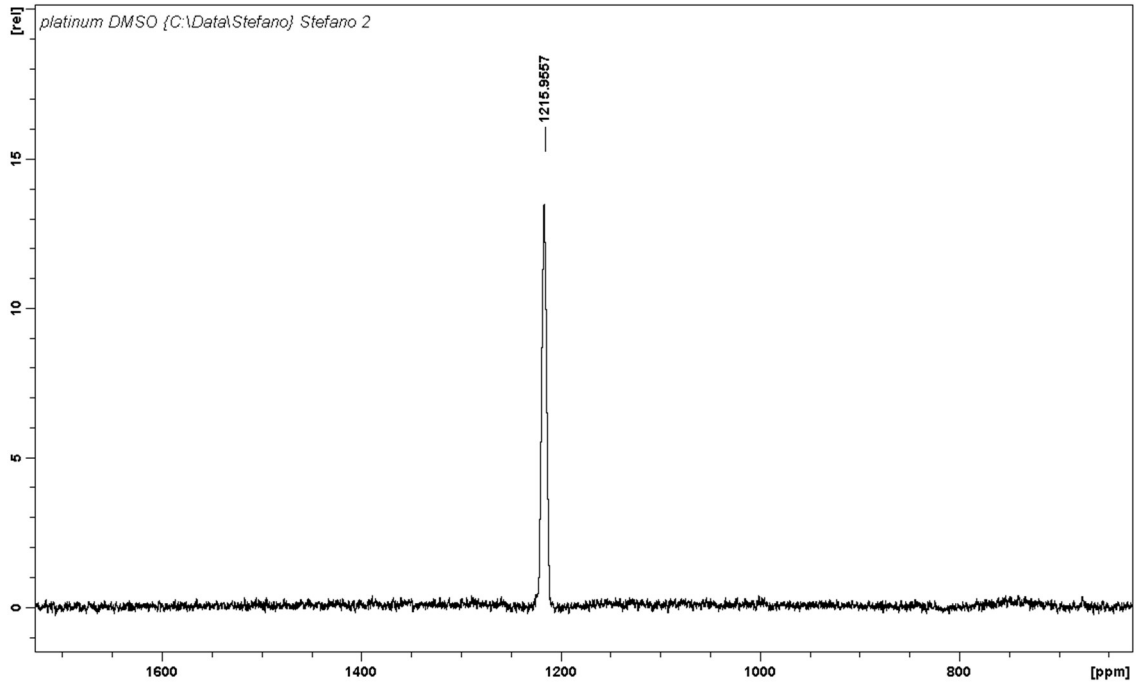

Figure S3. ^{195}Pt-NMR spectrum of **1**

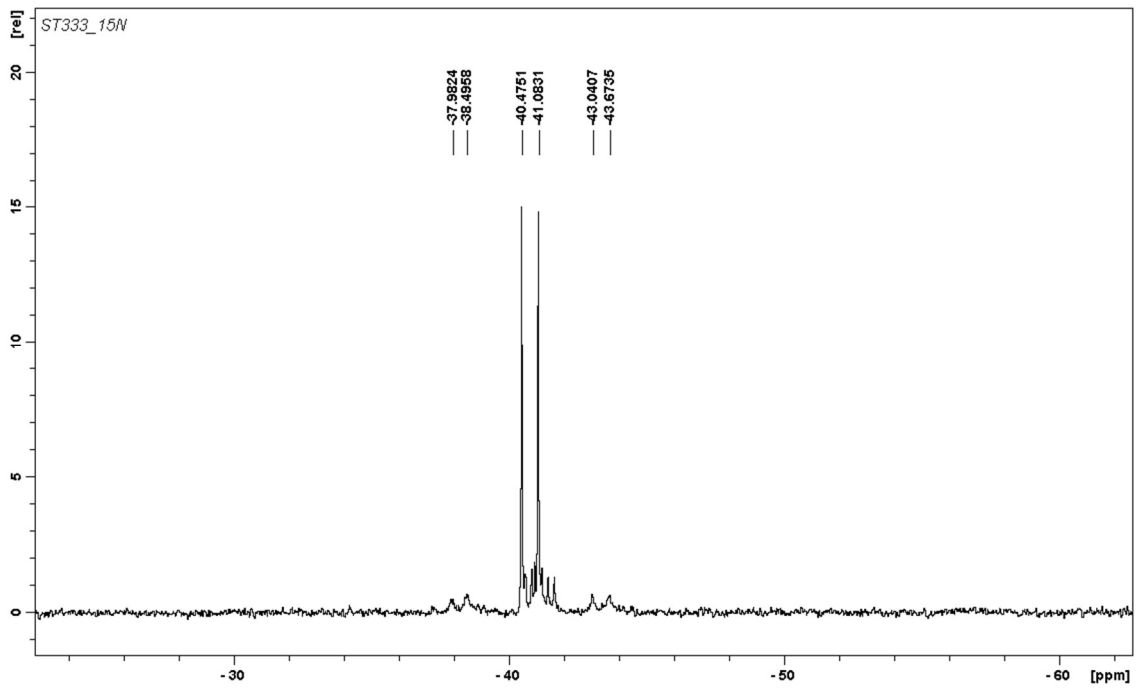

Figure S4. ^{15}N-DEPT-NMR spectrum of **1-^{15}NH$_3$**

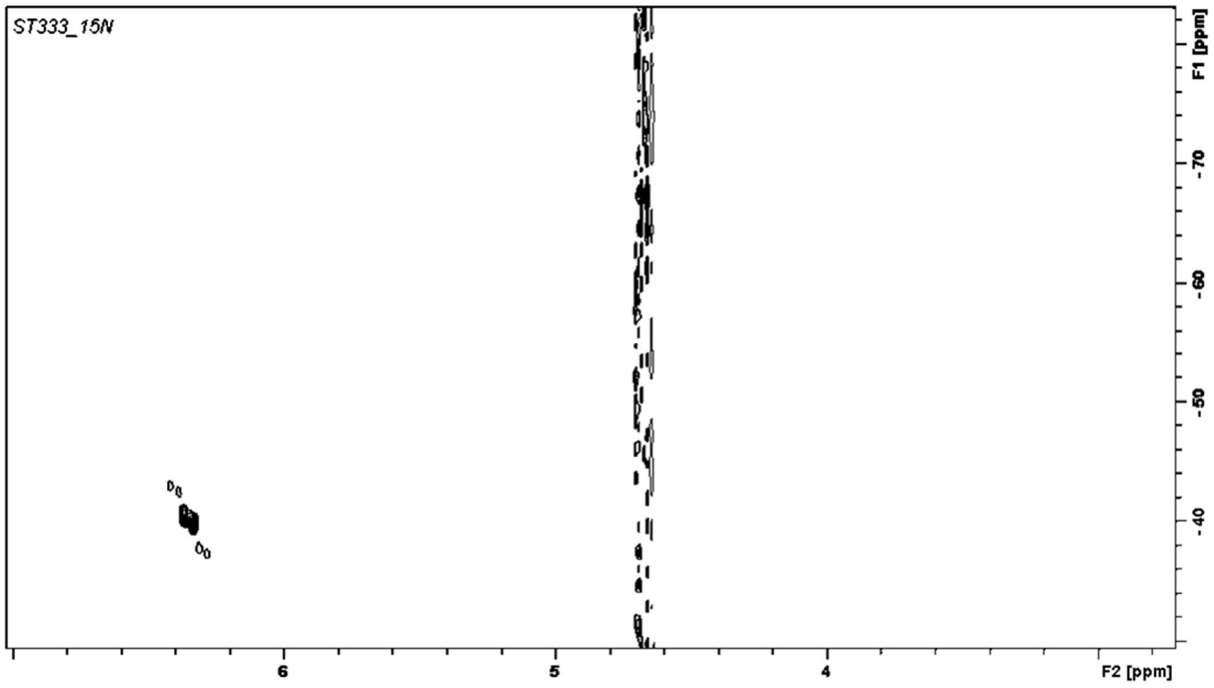

Figure S5: ^{1}H-^{15}N-HSQC NMR spectrum of **1-^{15}NH$_3$**

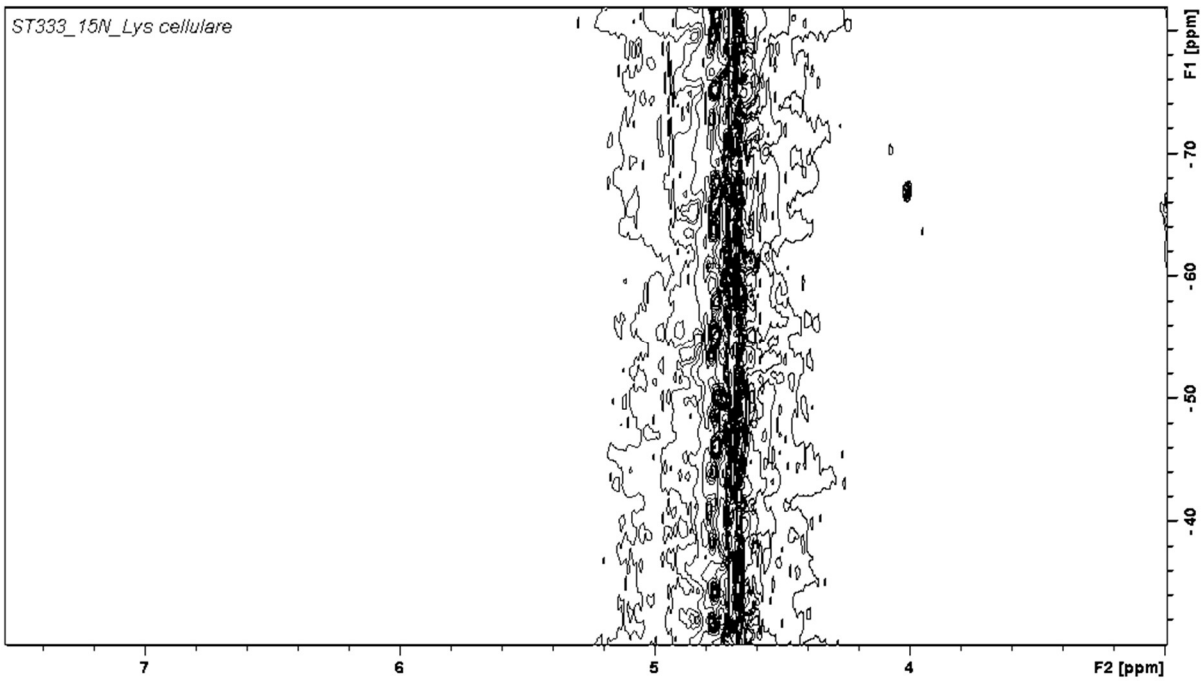

Figure S6: ^{1}H-^{15}N-HSQC NMR spectrum of **1-^{15}NH$_3$** upon reduction with cellular lysate

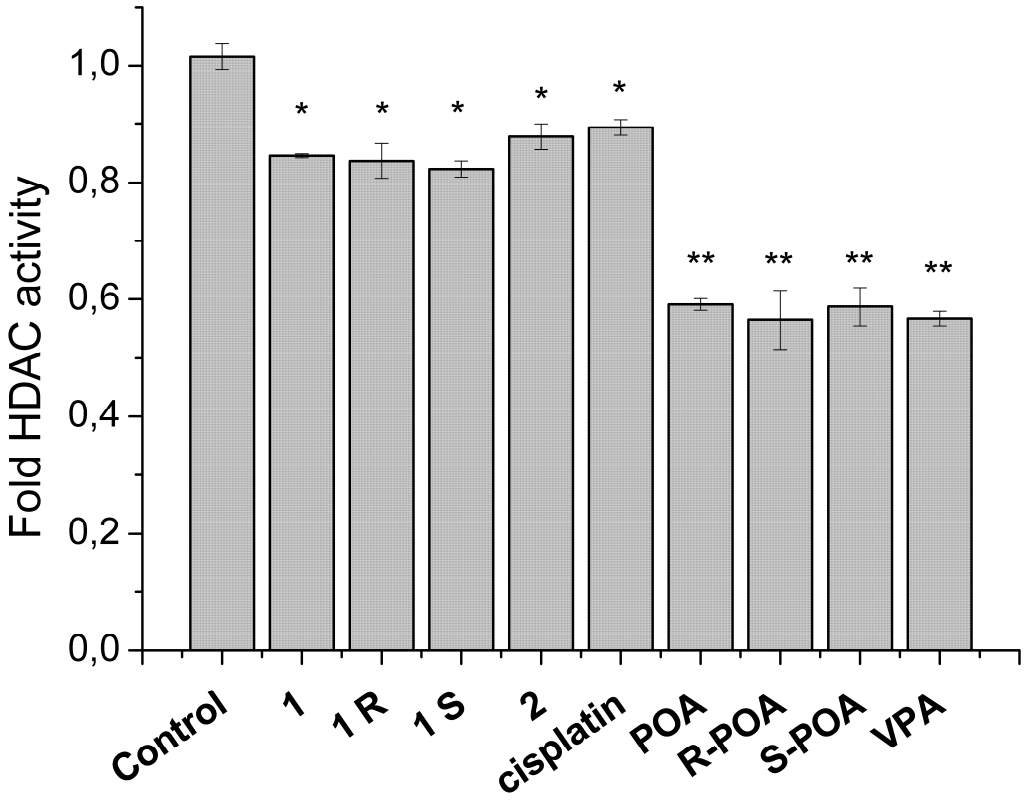

Figure S7. HDAC activity (fold decrease). Data are means ± standard deviations of three experiments performed in triplicate and were compared by means of a two-tailed t-test (* $p<0.05$; ** $p<0.01$; *** $p<0.001$). All the Pt complexes were 1 μM, whereas all MCFAs were 5 mM. The treatments were prolonged up to 24 h.

Chromatin staining procedure

2×10^5 A2780 cells were seeded on Nunc™ Lab-Tek™ 4-chamber slides and allowed to attach for 24 h. The treatment was performed with 1 μM complex **1** or cisplatin, or 5 mM POA. After 4 h, the medium was replaced with the staining solution, consisting of 5 ng mL^{-1} Hoechst 33342 in Earle's Balanced Salt solution (EBSS). Cells were incubated in the dark for 5 minutes, washed threefold with EBSS and immediately observed using a standard DAPI filter set (at 350/461 nm Exc/Em) of a fluorescence microscope (Zeiss Axiolab), equipped with a digital camera (Nikon digital Sights, DS-U3). Pictures were taken at 10X magnification. Figure S8 shows the results.

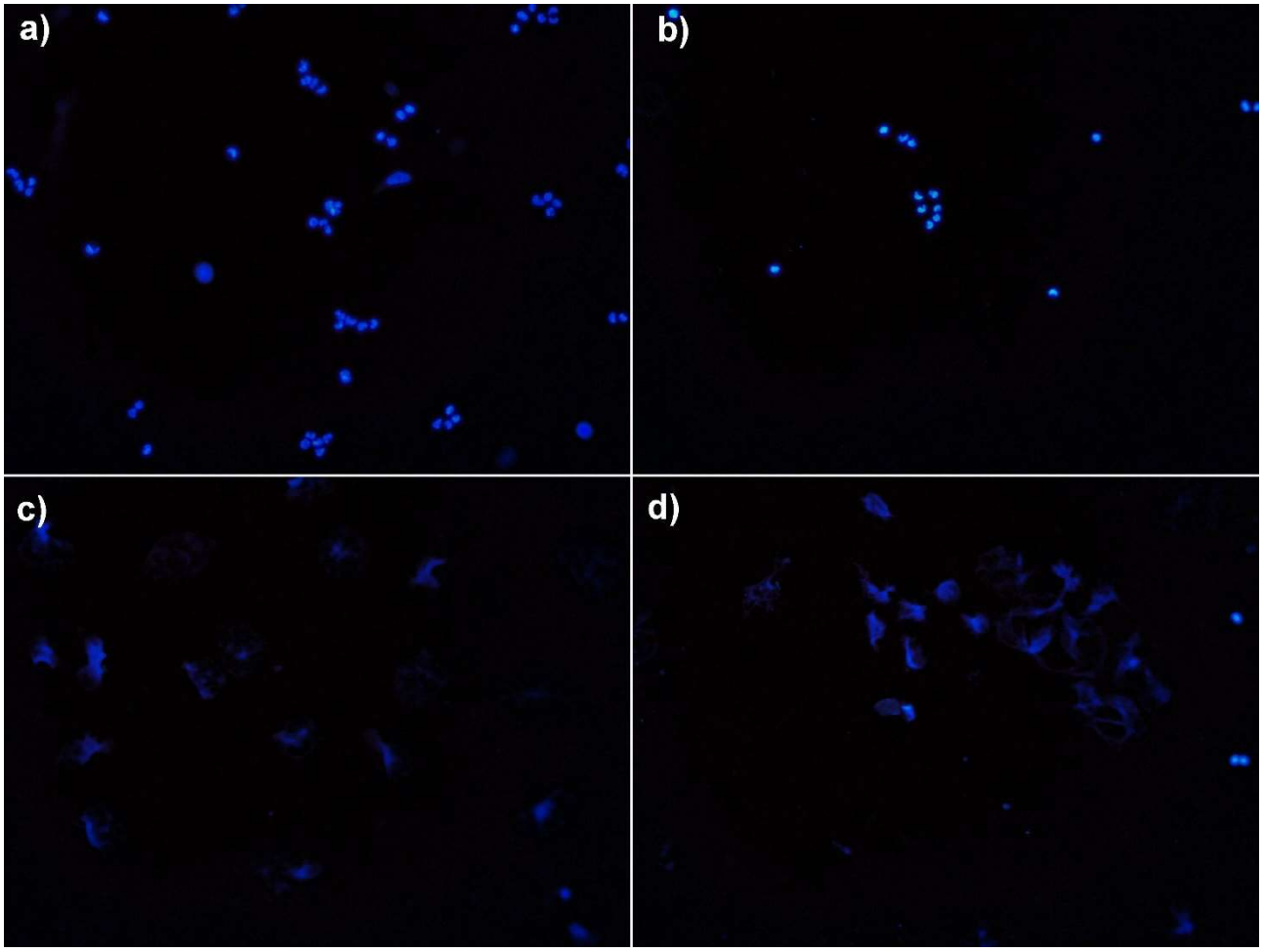

Figure S8. Representative pictures of Hoechst 33342 stained A2780 cells after a 4 h treatment: a) control; b) 1 μM cisplatin; c) 1 μM complex **1**; d) 5 mM POA. Cells treated with **1** or POA clearly showed chromatin decondensation. On the contrary, treatment with cisplatin induces typical apoptosis-related chromatin condensation (i.e. pycnosis).

Table S1. Analysis of relative gene expression data using real-time quantitative PCR. The NCBI accession number is reported along with the 5'-3' sequence of the forward and reverse primer and the expected product length.

Gene	Accession n.	Forward	Reverse	Product lenght (bp)
Cyclin D1 (CCND1)	NM_053056.2	TGAGGGACGCTTTGTCTGTC	GCCTTTGGCCTCTCGATACA	75
p21 (CDKN1A)	NG_009364	GCGACTGTGATGCGCTAATG	GAAGGTAGAGCTTGGGCAGG	141
COX-2	M90100.1	CCCTGAGCATCTACGGTTTG	CATCGCATACTCTGTTGTGTTC	107
Cyclin A2 (CCNA2)	NM_001237.4	TGGTGGTCTGTGTTCTGTGA	TGCCAGTCTTACTCATAGCTGA	136
Cyclin E (CCNE)	NM_001238	GCAGGATCCAGATGAAGAAATG	TAATCCGAGGCTTGCACGTT	173
GAPDH	NG_007073.2	ATCCCTGAGCTGAACGGGAA	GGCAGGTTTTTCTAGACGGC	99
HPRT1	NM_000194.2	TTGCTTTCCTTGGTCAGGCA	ATCCAACACTTCGTGGGGTC	85
TP53	NG_017013.2	GCCCCTCCTCAGCATCTTATC	CTCATAGGGCACCACCACAC	99
RNA18SN1	NR_145820.1	CGTCTGCCCTATCAACTTTCG	TGCCTTCCTTGGATGTGGTAG	124