Supplementary Information

A dual-targeting, apoptosis-inducing organometallic half-sandwich iridium anticancer complex

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Cytotoxicyty testing:

MTT assay: Cell death was evaluated using a system based on the tetrazolium compound MTT. Briefly, after the treatment period, 10 µL of a freshly diluted MTT solution (2.5 mg mL^{-1}) was added to each well, and the plate was incubated at 37 °C in a humidified 5 % CO_2 atmosphere for 4 h. At the end of the incubation period, the medium was removed, and the formazan product was dissolved in 100 µL of dimethyl sulfoxide. Cell viability was evaluated by measurement of the absorbance at 570 nm, using a SUNRICE Tecan absorbance reader (Schoeller). Compound concentrations that produce 50 % cell growth inhibition (IC_{50}) were calculated from curves constructed by plotting cell survival (%) versus drug concentration (µM). The reading values were converted to the percentage of the control (percentage cell survival). Cytotoxic effects were expressed as IC_{50}. Concentrations of treated complexes in medium during treatment were verified by FAAS.

Sulphorhodamine B (SRB) assay. After the treatment period, the adherent cells were washed twice with PBS and fixed with 10% trichloroacetic acid (TCA). Suspension human leukemia HL-60 cells were fixed with 50% TCA added directly to the whole treated volume without any previous PBS washing. After 1 h, the fixed cells were washed with MiliQ water and incubated with 0.4% SRB for 30 min. Wells were washed three times with 1% acetic acid and SRB stained cellular proteins were dissolved with Tris base (pH 10.5). The absorbance at 570 nm was measured using a SUNRICE Tecan absorbance reader (Schoeller). IC_{50} values were calculated from curves constructed by plotting cell survival (%) versus drug concentration (µM). The reading values were converted to the percentage of control (% cell survival). Cytotoxic effects were expressed as IC_{50}. Concentrations of treated complexes in medium during treatment were verified by FAAS.

Neutral red (NR) assay: After the treatment period, 20 µL of a 0.33% solution of neutral red in phosphate buffered saline (PBS) was added to each well with adherent cells and the plate was incubated at 37 °C in a humidified 5 % CO_2 atmosphere for 2 h. Suspension human leukemia HL-60 cells were fixed prior the staining with 50% TCA. Afterwards, the dye containing medium was carefully removed and the cells were quickly rinsed with neutral red assay fixative (0.1% $CaCl_2$ in 0.5% formaldehyde). The incorporated dye was then solubilized in 200 µL of 1% acetic acid in 50% ethanol, allowed to stand for 10 min at room temperature and the absorbance was measured at λ=540 nm with a SUNRICE Tecan absorbance reader (Schoeller). The background absorbance of the plates at 690 nm was also measured and subtracted from 540 nm measurement. IC_{50} values were calculated from curves constructed by plotting cell survival (%) versus drug concentration (µM). The reading values were converted to the percentage of the control (percentage cell survival). Cytotoxic effects were expressed as IC_{50}. Concentrations of treated complexes in medium during treatment were verified by FAAS.

Table S1A: IC_{50} Values (µM) obtained by MTT assay for complex **1** and cisplatin after 72 h of treatment. Data are expressed as mean ± SD values of three independent experiments, each made in triplicate

	A2780	A2780cisR	RF[a]	HL-60	MCF-7	CHO-K1	TI[b]
1	0.78 ± 0.01	0.97 ± 0.03	1.3	0.41 ± 0.07	2.01 ± 0.11	32.5 ± 4.9	41.7
cisplatin	2.64 ± 0.47	15.0 ± 1.1	4.8	0.47 ± 0.07	22.9 ± 2.9	14.6 ± 1.6	5.5

[a]Resistance factor, defined as IC_{50} (resistant, A2780cisR)/IC_{50} (sensitive, A2780).
[b]Therapeutic Index calculated as a ratio of the IC_{50} of normal, noncancerous cells (CHO-K1 or HSF), to the IC_{50} obtained for cancer cells (A2780).

Table S1B: IC_{50} Values (µM) obtained by Neutral red assay for complex **1** and cisplatin after 72 h of the treatment. Data are expressed as mean ± SD values of three independent experiment, each made in triplicate

	A2780	A2780cisR	RF[a]	HL-60	MCF-7	CHO-K1	TI[b]
1	0.45 ± 0.07	0.46 ± 0.06	1.0	0.26 ± 0.08	1.20 ± 0.04	53.0 ± 6.5	117.8
cisplatin	0.66 ± 0.15	2.65 ± 0.21	4.0	0.61 ± 0.08	7.9 ± 1.2	34.6 ± 4.8	52.4

[a]Resistance factor, defined as IC_{50} (resistant, A2780cisR)/IC_{50} (sensitive, A2780).
[b]Therapeutic Index calculated as a ratio of the IC_{50} of normal, noncancerous cells (CHO-K1 or HSF), to the IC_{50} obtained for cancer cells (A2780).

Table S2: IC_{50} Values (µM) in A2780 cells obtained by MTT assay for complex **1** and cisplatin as dependent on treatment interval. The data are expressed as mean ± SD values of three independent experiments, each made in triplicate

	24 h	48 h	72 h
1	1.84 ± 0.45	0.83 ± 0.06	0.78± 0.01
cisplatin	20.8 ± 6.4	2.79 ± 0.65	2.64 ± 0.47

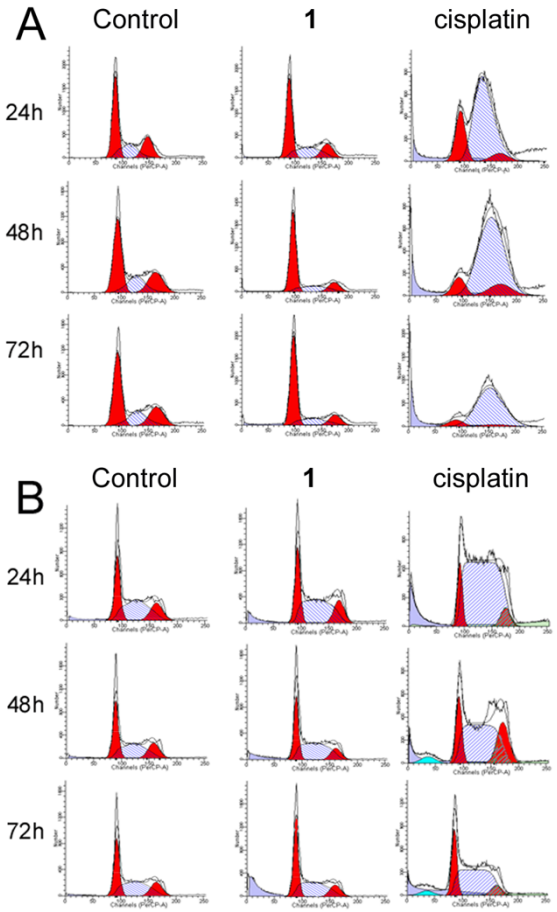


Fig. S1. *Flow cytometric detection of the cell-cycle profiles in A2780 and HL60 cells. Effects of equitoxic doses (IC_{50}) of* ***1*** *and cisplatin on cell cycle distribution of A2780 (A) or HL-60 (B) cells. Left panels, untreated (control) A2780 or HL-60 cells; middle panels, A2780 or HL 60 cells treated with 0.8 µM or 0.5 µM* ***1,*** *respectively, for 24, 48 or 72 h; right panels, A2780 or HL-60 cells treated with 3.1 µM or 0.5 µM cisplatin for 24, 48 or 72 h.*

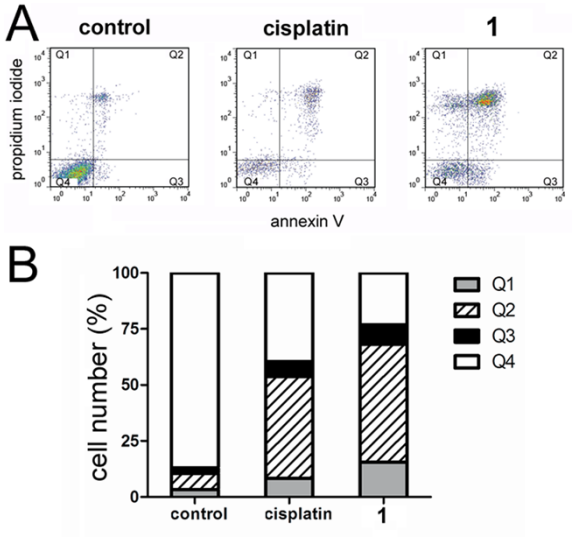


***Fig. S2**. Quantification of apoptosis after 48-h exposure of A27820 cells to 1 or cisplatin. A. Induction of apoptosis in A2780 cells after treatment with **1 or** cisplatin. Cells were untreated (control) or treated with 20 µM ($IC_{90,48h}$) cisplatin or 2.6 µM ($IC_{90,48h}$) **1** for 48 h. B. Quantitative evaluation. Quadrant 1, PI-positive (cells undergoing necrosis); Quadrant 2, Annexin V-positive /PI-positive (cells in the late period of apoptosis and undergoing necrosis); Quadrant 3, Annexin V positive/ PI-negative (cells in the early period of apoptosis); Quadrant 4, Annexin V –negative/ PI-negative (living cells). The experiment was made in triplicate.*

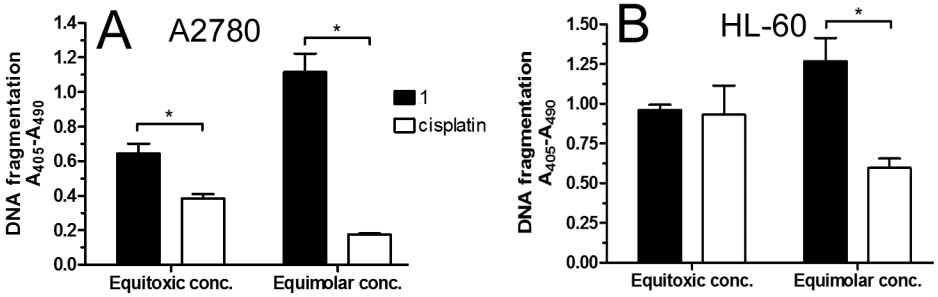
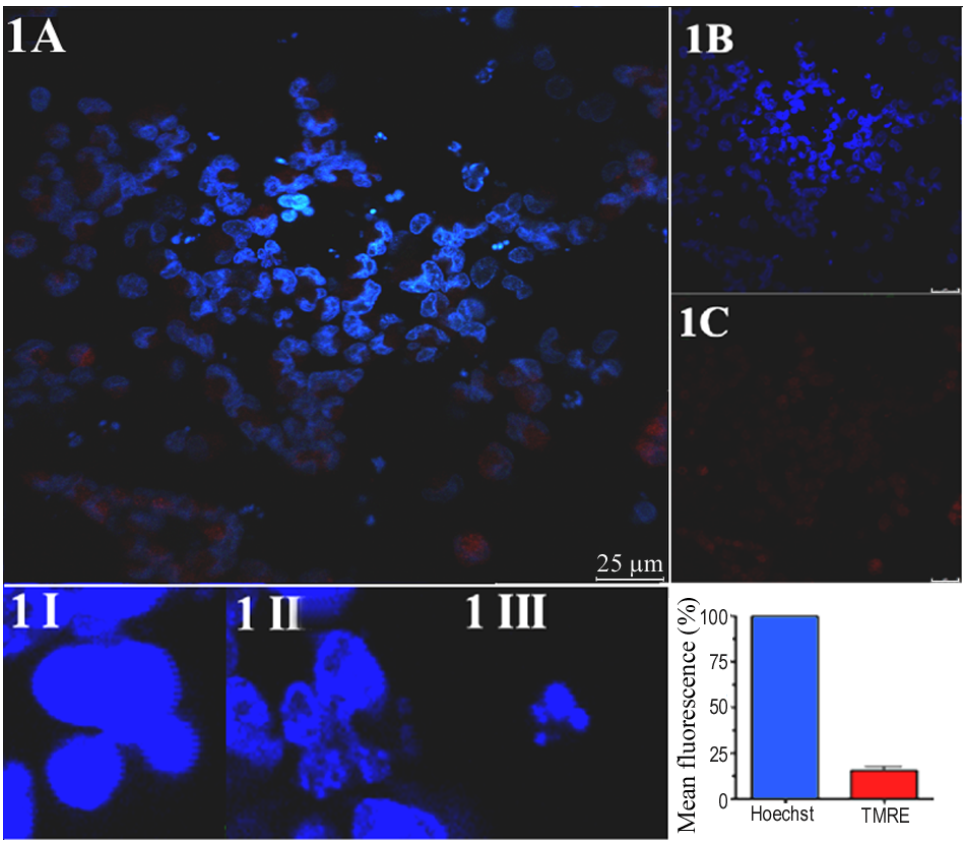


***Fig S3**. DNA apoptotic fragmentation induced by 1 and cisplatin quantified by a specific ELISA colorimetric kit. Comparison of the efficiency of **1** and cisplatin in inducing apoptosis in A2780 (A) or HL60 (B). Cells were treated with equimolar (3 µM) or equitoxic ($IC_{50,24h}$) concentrations for 24 h. The symbol (*) denotes significant difference ($p < 0.01$). Experiments were made in triplicate.*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1B
1C
25 µm
1 I
1 II
1 III
Mean fluorescence (%)
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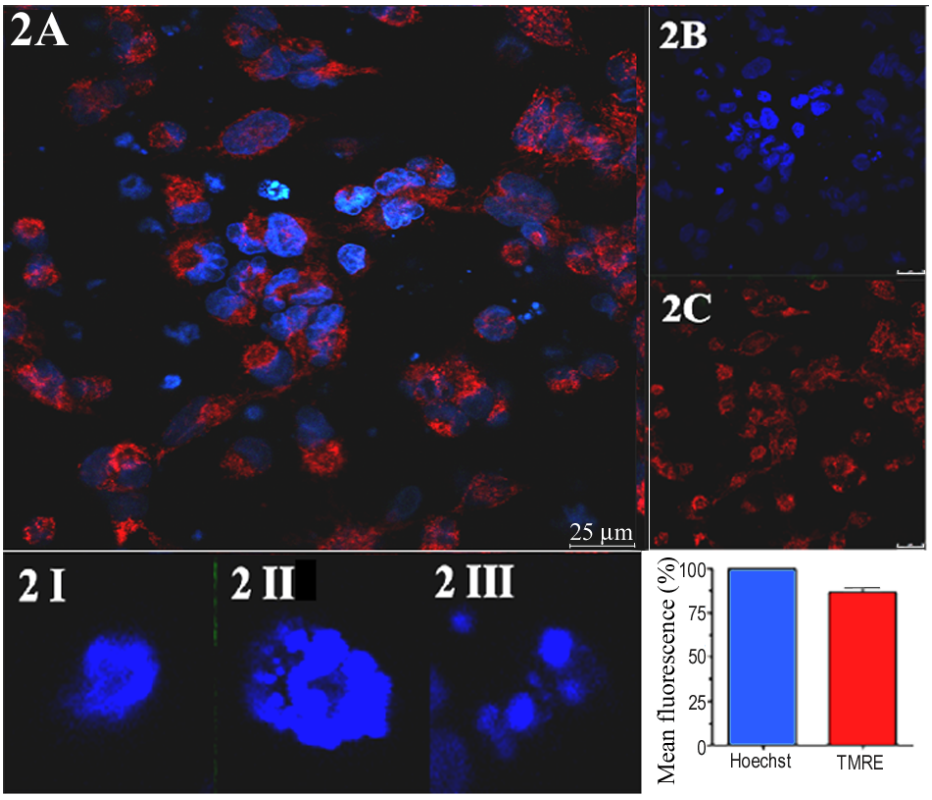
75
50
25
0
Hoechst
TMRE


2A
2B
2C
25 µm
2 I
2 II
2 III
Mean fluorescence (%)
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75
50
25
0
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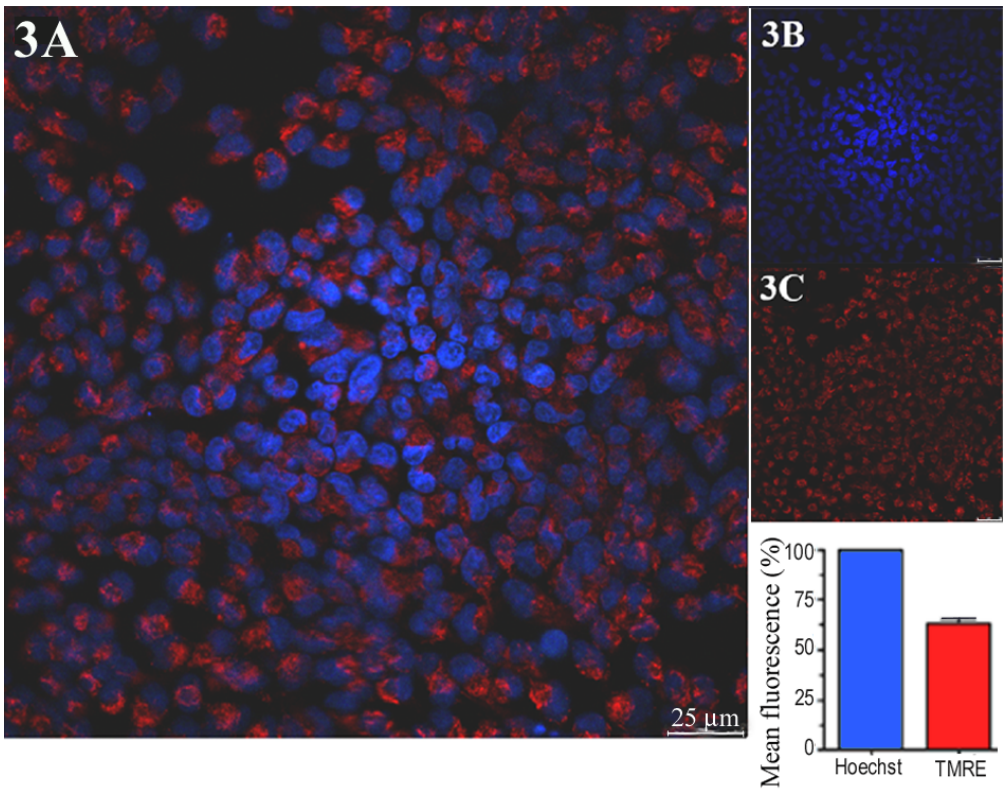

Fig. S4. *Multi-parameter apoptosis assay. Confocal microphotographs obtained after the treatment of A2780 cells with complex* ***1*** *(1), cisplatin (2) at the concentration corresponding to* $IC_{90,48hr}$ *or for untreated cells (3). A – a merge output of both fluorescence channels, B - cell nuclei stained with Hoechst dye, C - fluorescence signal of TMRE for detection of mitochondrial membrane potential. Typical features of apoptosis are shown at panels 1 and 2: I - pyknosis (nucleus condensation), II - karyorrhexis (nucleus fragmentation) and III - presence of apoptotic bodies. Fluorescence analysis is on the right bottom part of the picture, signals from the individual channels were normalized to fluorescence of Hoechst dye.*