Modifying Charge and Hydrophilicity of Simple Ru(II) Polypyridyl Complexes Radically Alters Biological Activities: Old Complexes, Surprising New Tricks

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1) Experimental Descriptions

Flow cytometry analysis of apoptosis.

Given the spectral overlap between the emission of **1** and **2** with PI, apoptosis was also analyzed by flow cytometry using Hoechst as a nuclear stain, which resulted in dot plots with different numbers of quadrants. With PI and Annexin V staining, the cell population is divided into 4 quadrants, where PI-/Annexin V- is considered as viable cells, PI-/Annexin V+ is early apoptotic and PI+/Annexin V+ is late apoptotic and necrotic cells. Hoechst and Annexin V staining, however, gives 6 rectangular areas of cells populations, and there are cells distributed in R2 and R3 with much lower Hoechst intensity than viable cells. This is due to very little nuclear material, and thus these populations are considered as cell debris and are not included in calculations. The cell distribution is as follows: R4, R5, R6 and R7 representing lower left, lower right, upper left and upper right quadrants in PI/Annexin V staining.

For Figure S11 calculations were made excluding values from R2 and R3, and early apoptotic cells were identified from R5 for Hoechst/Annexin V staining. Dead cells were quantified from the population in R7.

Compound	2 hrs		24 hrs	
	Light	Dark	Light	Dark
1	11.64 ± 0.34%	5.37 ± 1.15%	23.30 ± 0.52%	15.42 ± 0.89%
2	0.35 ± 0.04%	0.20 ± 0.01%	0.71 ± 0.08%	0.42 ± 0.01%
Cisplatin	0.25 ± 0.01%		0.83 ± 0.03%	

Table S1. The % intracellular metal measured by ICP-OES. HL60 cells were treated with 5 μM **1**, 20 μM **2**, or 20 μM cisplatin for 2 hrs and 24 hrs. The metal levels in cell and media were measured by ICP-OES, and % intracellular levels was normalized in 10^7 cells. The metal levels were quantified by detection at three different wavelengths (n = 3).

Compound	2 hrs		24 hrs	
	Light	Dark	Light	Dark
1	$4.58 \pm 0.13 \times 10^7$	$2.15 \pm 0.46 \times 10^7$	$8.64 \pm 0.18 \times 10^7$	$6.42 \pm 0.36 \times 10^7$
2	$1.69 \pm 0.18 \times 10^7$	$0.93 \pm 0.03 \times 10^7$	$3.38 \pm 0.38 \times 10^7$	$2.05 \pm 0.03 \times 10^7$
Cisplatin	$3.22 \pm 0.52 \times 10^7$		$9.34 \pm 0.28 \times 10^7$	

Table S2. Metal atoms per cell measured by ICP-OES. HL60 cells were treated with 5 μM **1**, 20 μM **2**, or 20 μM cisplatin for 2 hrs and 24 hrs. The metal levels in cells and media was measured by ICP-OES, and the number of metal atoms per cell was calculated using the cell number obtained using trypan blue and manual counting. The metal levels were quantified by detection at three different wavelengths (n = 3).

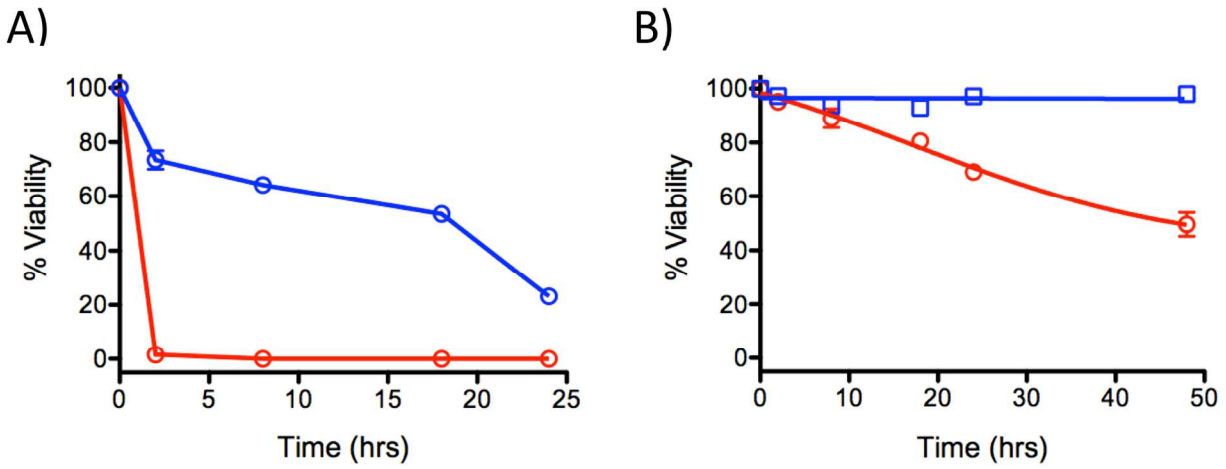

Figure S1. Time dependence of cell death in HL60 cells. A) Compound **1**; B) compound **2.** Blue, dark; red, irradiated. Viability was determined using Trypan Blue dye exclusion and manual counting.

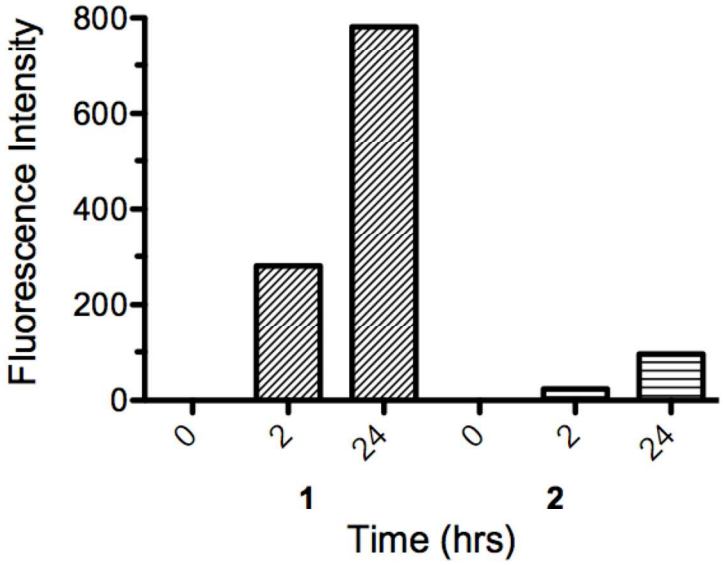

Figure S2. Time dependence of compound uptake and average emission intensity per cell in A549 cells determined by flow cytometry.

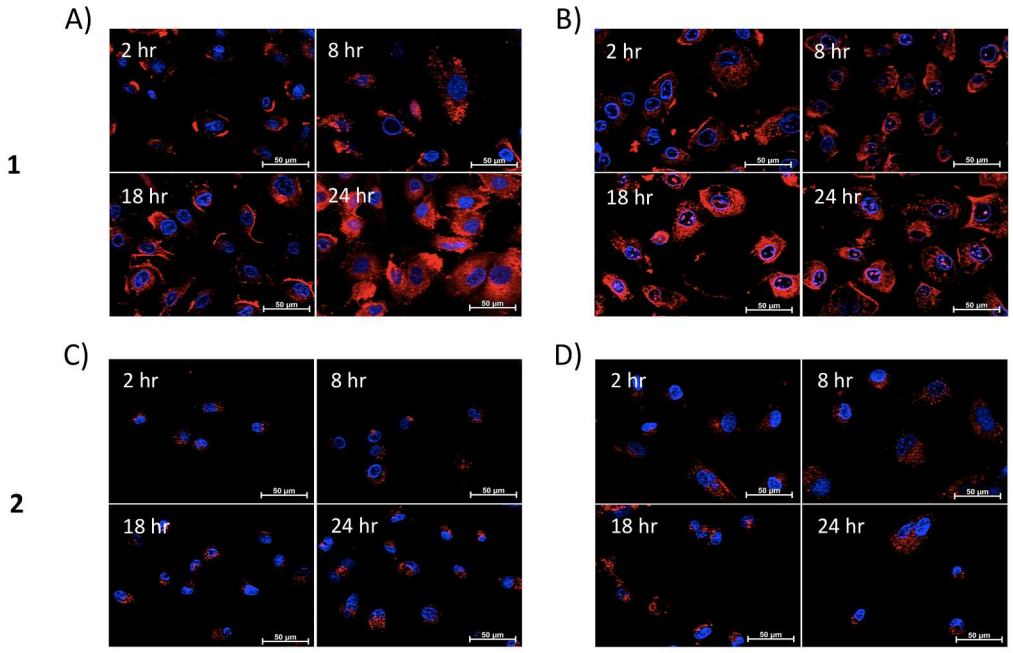

Figure S3. Time dependence of compound uptake in A549 cells measured by ApoTome miscroscope. The ruthenium compounds' emission are shown in red and Hoechst was used to stain the nucleus (blue). A) **1**, dark; B) **1**, light; C) **2**, dark; D) **2**, light.

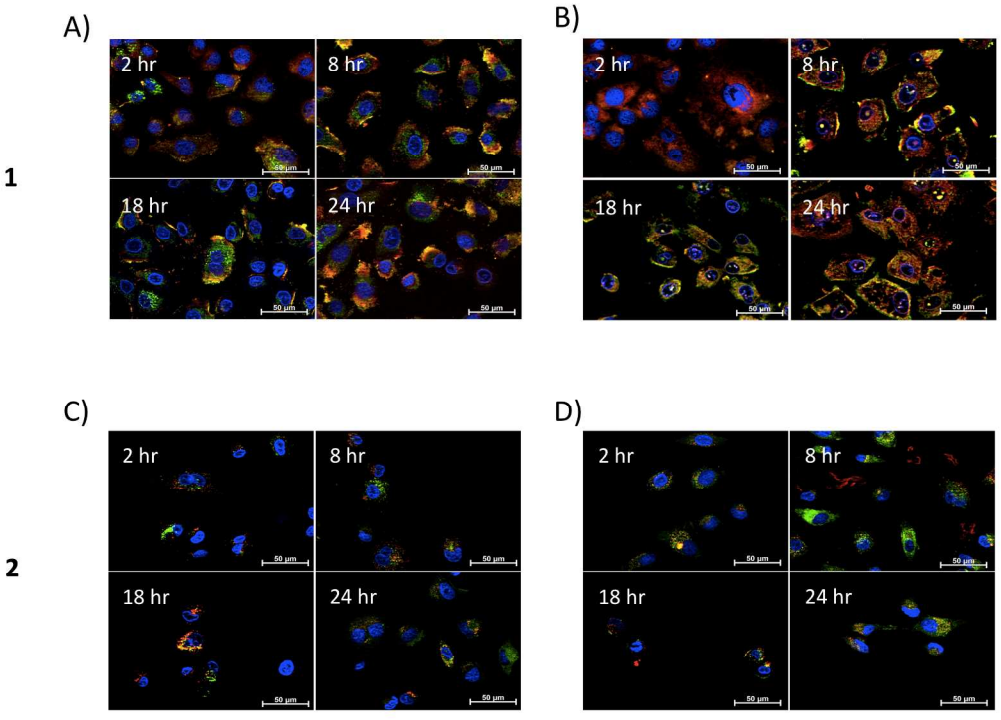

Figure S4. Time dependence of compound localization in lysosomes in A549 cells measured by ApoTome miscroscope. Lysotracker green was used to visualize lysosomes. A) **1**, dark; B) **1**, light; C) **2**, dark; D) **2**, light.

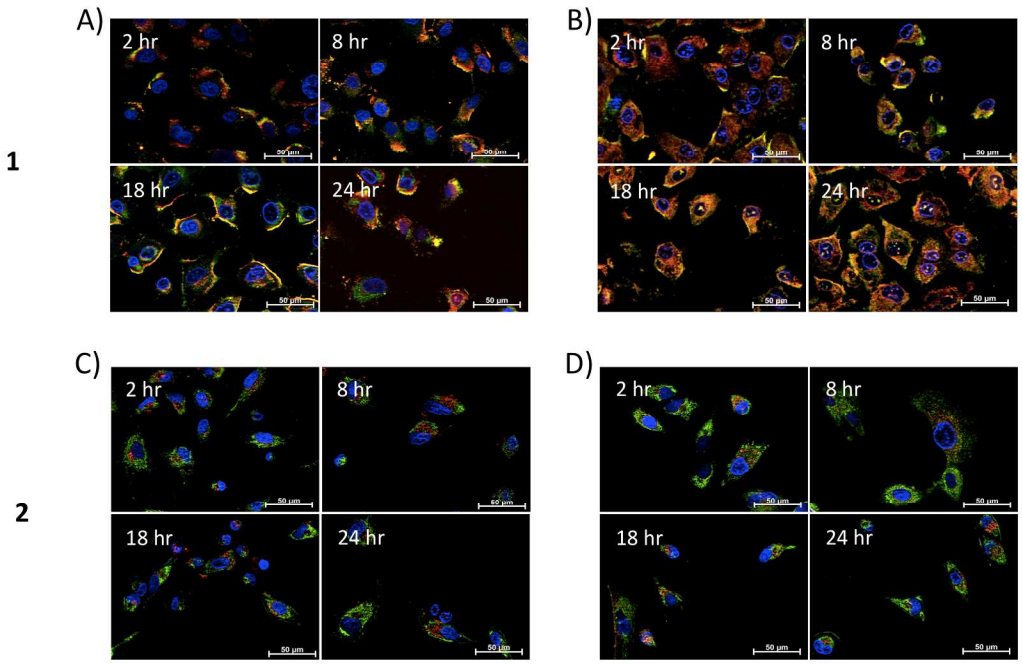

Figure S5. Time dependence of compound localization in mitochondria in A549 cells measured by ApoTome miscroscope. Mitotracker green was used to visualize mitochondria. A) **1**, dark; B) **1**, light; C) **2**, dark; D) **2**, light.

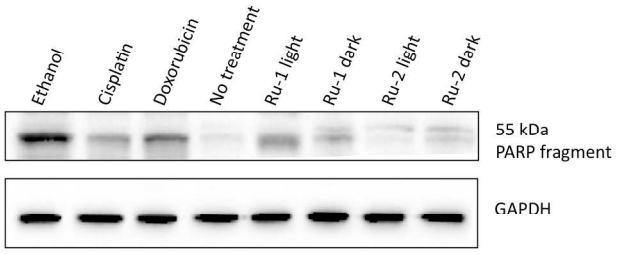

Figure S6. Western blotting of 55 kDa PARP fragment shows contribution from necrotic cell death for 10% ethanol, cisplatin, doxorubicin, **1**, and **2**.

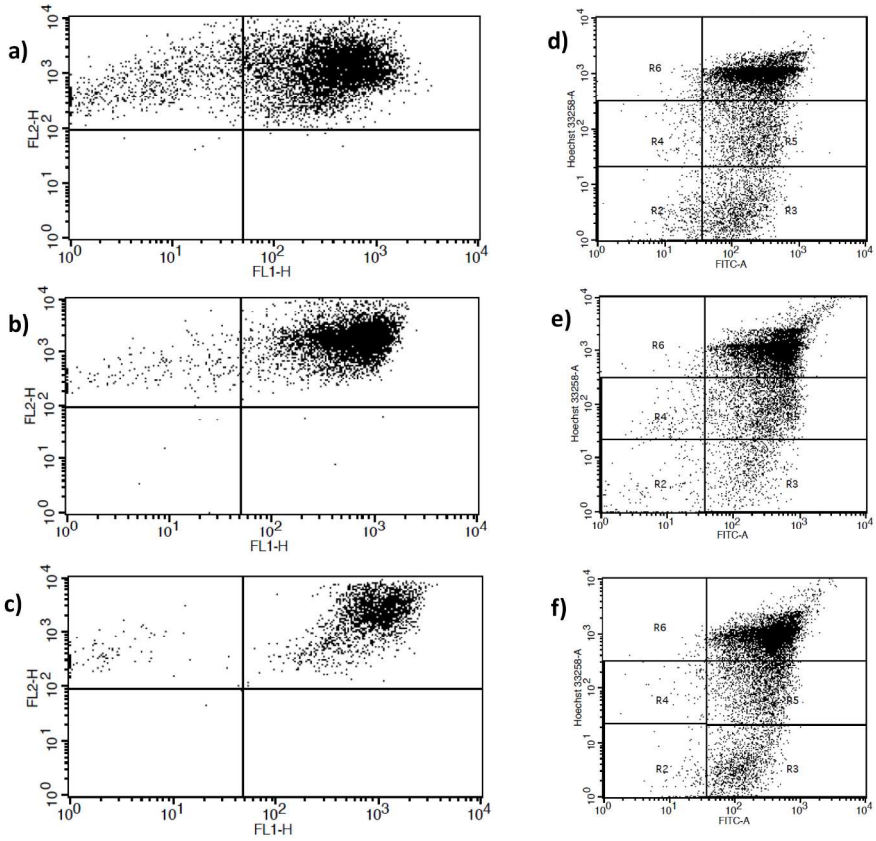

Figure S7. Flow cytometry analysis of apoptosis induced by **1** after light exposure. Annexin V was used in conjunction with Propidium Iodide (left) or Hoechst (right) staining. A) and D) 2 hours; B) and E) 8 hours; C) and F) 24 hours. Induction of cell death is rapid, and while there are many cells that are classified as late apoptotic (or necrotic), a very small percentage can be classified as early apoptotic (Annexin V positive cells, both PI or Hoechst negative).

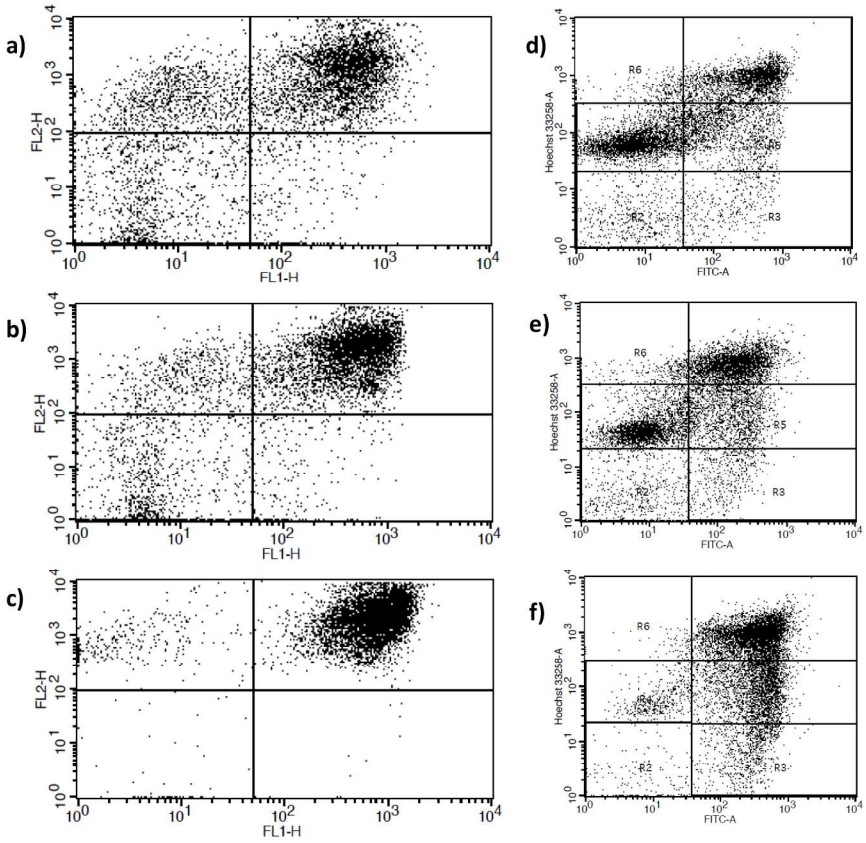

Figure S8. Flow cytometry analysis of apoptosis induced by **1** protected from light. Annexin V was used in conjunction with Propidium Iodide (left) or Hoechst (right) staining. A) and D) 2 hours; B) and E) 8 hours; C) and F) 24 hours. At all time points there are many cells that are classified as late apoptotic (or necrotic), but at 2 and 8 hours there are Annexin V negative cells that are both PI or Hoechst positive, indicating necrosis, along with some fraction of early apoptotic cells (Annexin V positive cells, both PI or Hoechst negative).

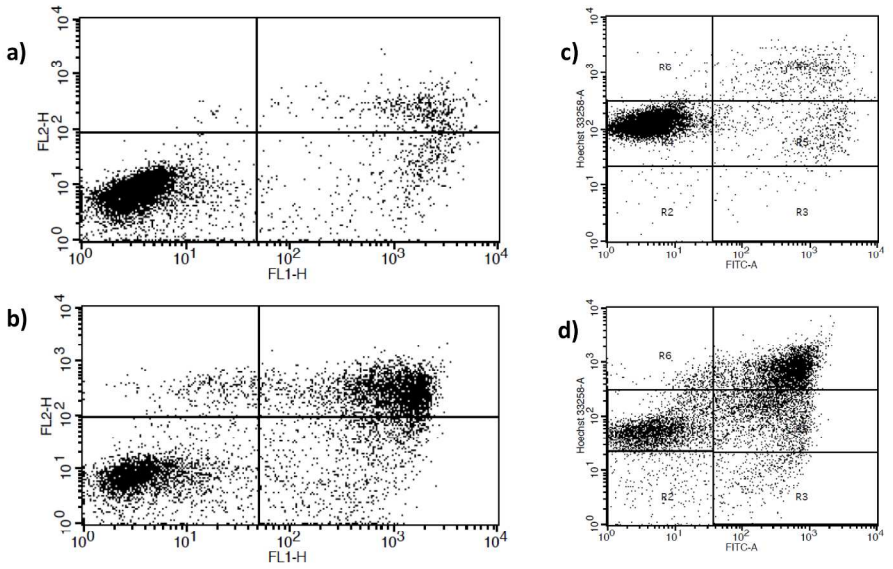

Figure S9. Flow cytometry analysis of apoptosis induced by **2** after light exposure. Annexin V was used in conjunction with Propidium Iodide (left) or Hoechst (right) staining. A) and C) 2 hours; B) and D) 24 hours. There are a significant fraction of early apoptotic cells (Annexin V positive cells, both PI or Hoechst negative).

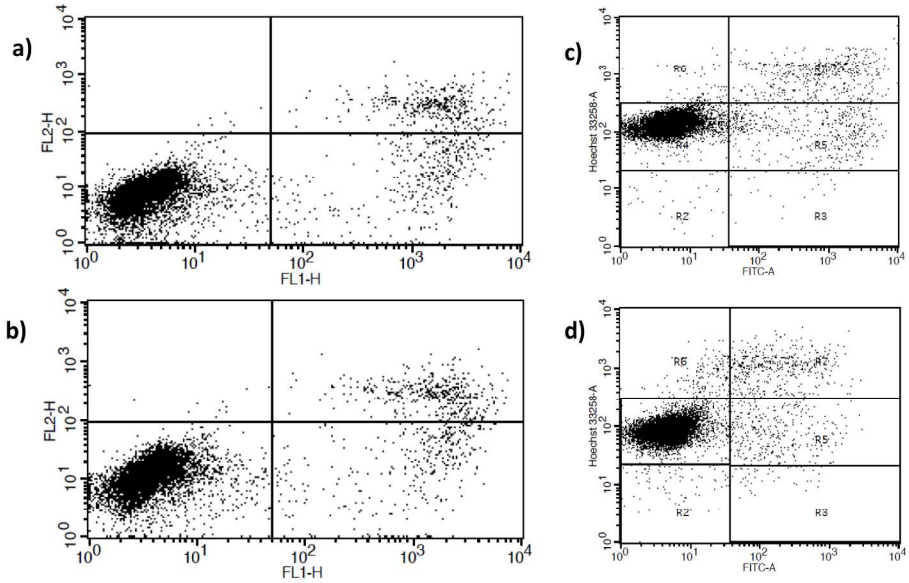

Figure S10. Flow cytometry analysis of apoptosis induced by **2** protected from light. Annexin V was used in conjunction with Propidium Iodide (left) or Hoechst (right) staining. A) and C) 2 hours; B) and D) 24 hours. The compound does not induce significant cell death.

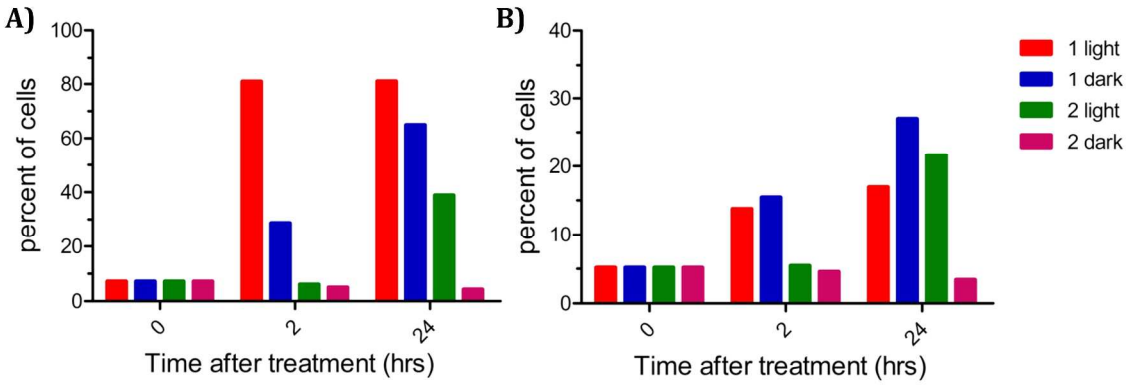

Figure S11. Cell death study by flow cytometry comparing ratio of early apoptotic vs. dead cells. HL60 cells were treated with 5 μM **1** or 20 μM **2** for 2 hrs and 24 hrs and stained by Hoechst 33342 and FITC conjugated Annexin V. The percent of A) dead cells and B) early apoptotic cells were measured by flow cytometry.