Cellular Uptake and Phototoxicity Optimization of Arene Ruthenium Porphyrin Derivatives

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Supporting information

Table S1. UV-vis maximum absorption and molar extinction coefficient [λ (εx10^{-3}M^{-1}cm^{-1})] determined in ethanol. Tested compounds were dissolved in DMSO at concentration of 1000 µM and stock solutions were diluted in ethanol. Concentrations of 10 µM, 5 µM, 2.5 µM, and 1 µM were used for spectral analysis.

Compounds	Soret band	Q band I	Q band II	Q band III	ε (λ=420 nm)
Ru_4a	414.50 nm	510.00 nm	-	590.00 nm	25.942
Ru_4b	415.00 nm	510.50 nm	542.00 nm	589.00 nm	87.178
Ru_2a	414.00 nm	515.00 nm	545.00 nm	589.00 nm	232.35
Ru_2b	414.50 nm	512.00 nm	545.00 nm	589.00 nm	361.075
Zn-Ru_4a	424.50 nm	519.00 nm	558.50 nm	599.50 nm	231.800
Zn-Ru_4b	426.00 nm	519.00 nm	557.50 nm	598.00 nm	427.500
Zn-Ru_2a	425.50 nm	519.50 nm	557.00 nm	600.00 nm	475.300
Zn-Ru_2b	425.50 nm	519.50 nm	557.00 nm	600.50 nm	516.400

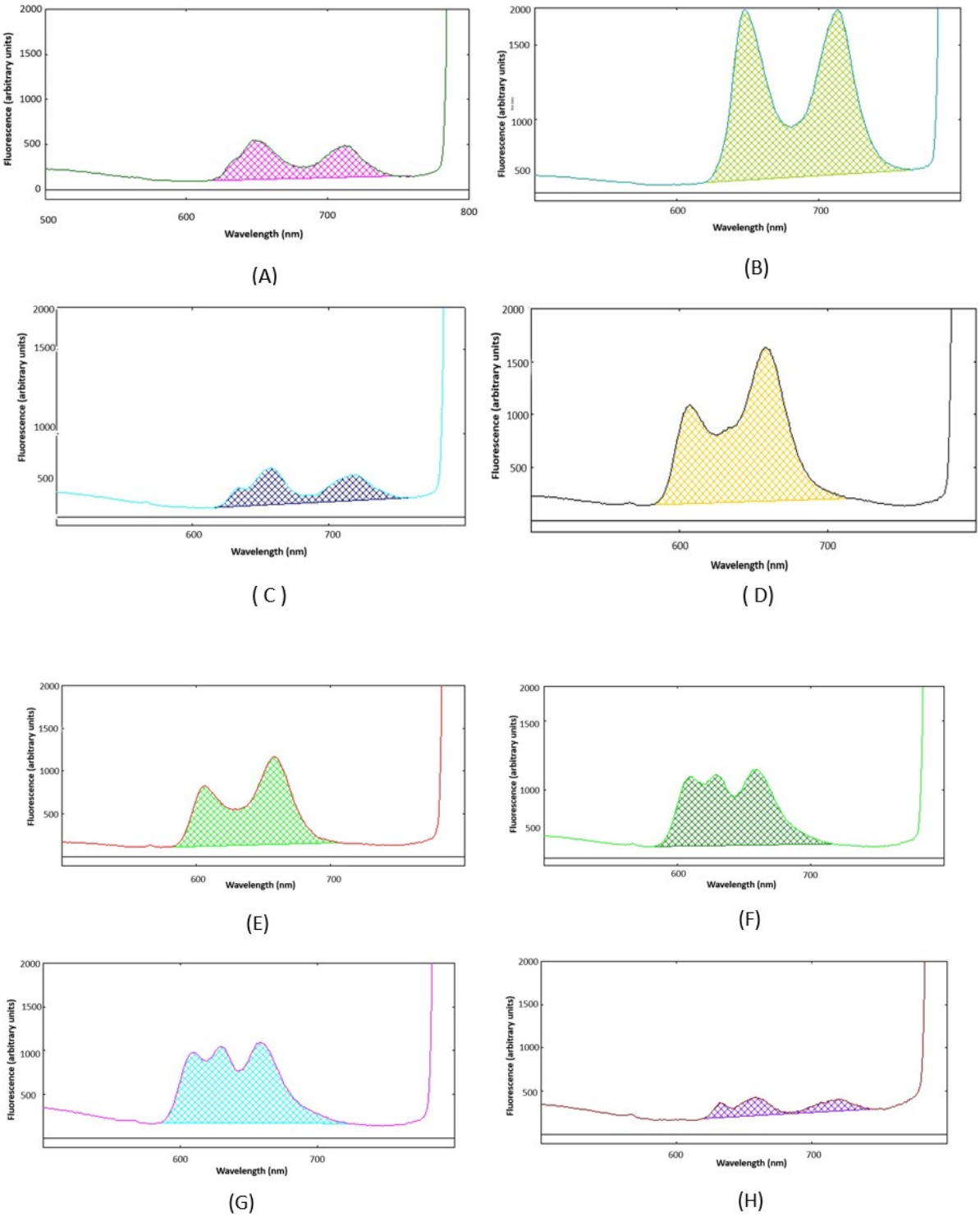


Fig S1. Emission spectra of solubilized cells incubated with arene ruthenium porphyrins at 5 µM determined by spectrofluorometry to determine the cellular uptake of PSs. (A) **Ru**$_{4a}$, (B) **Ru**$_{4b}$, (C) **Ru**$_{2a}$, (D) **Ru**$_{2b}$, (E) **Zn-Ru**$_{4a}$, (F) **Zn-Ru**$_{4a}$, (G**) Zn-Ru**$_{4b}$, (H) **Zn-Ru**$_{2b}$.

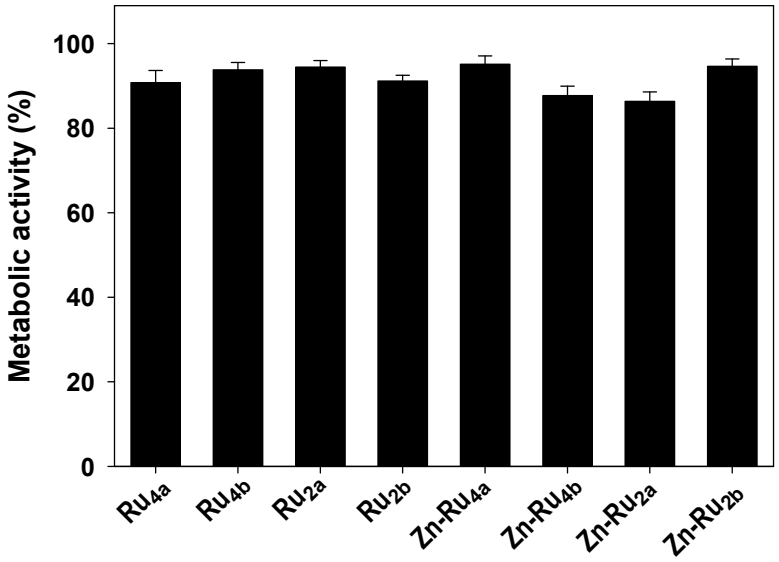
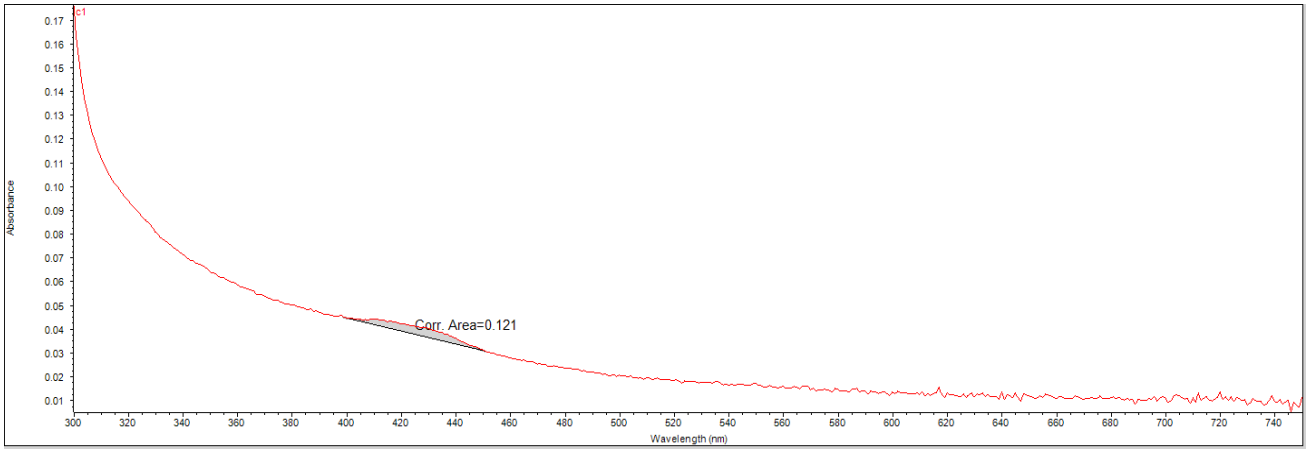
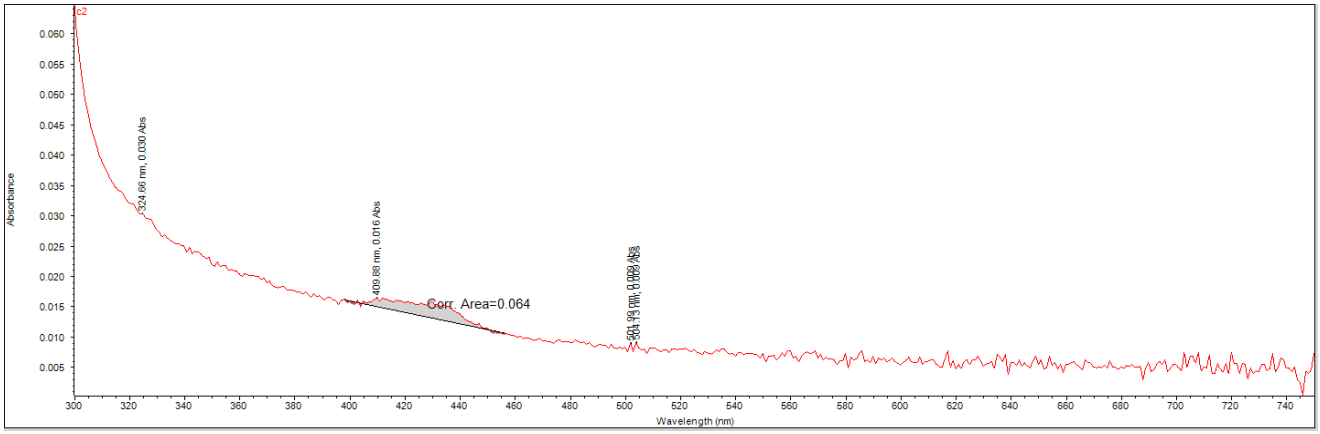


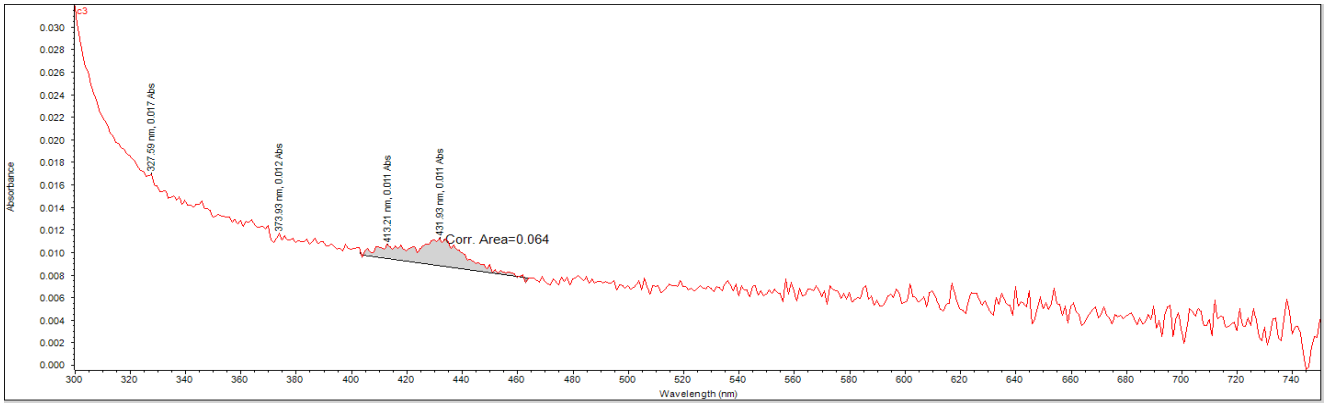
Fig S2. Toxicity in the dark. Cells were preincubated with 5 μM of PSs for 24h in the dark and were kept 30 min wrapped in aluminum foil on the illumination platform. Metabolic activity was determined by the MTT assay and presented as a percentage of MTT reduction to formazan, compared to controls. Mean of three independent experiments ± S.D. is shown.


c1
Absorbance
Wavelength (nm)
Corr. Area=0.121

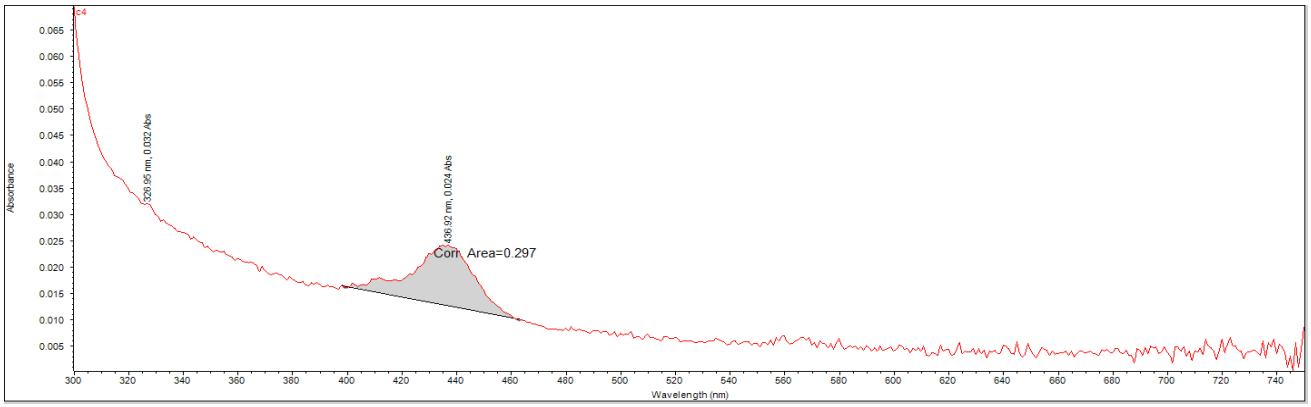
(A)


c2
Absorbance
Wavelength (nm)
324.66 nm, 0.030 Abs
409.88 nm, 0.016 Abs
Corr. Area=0.064

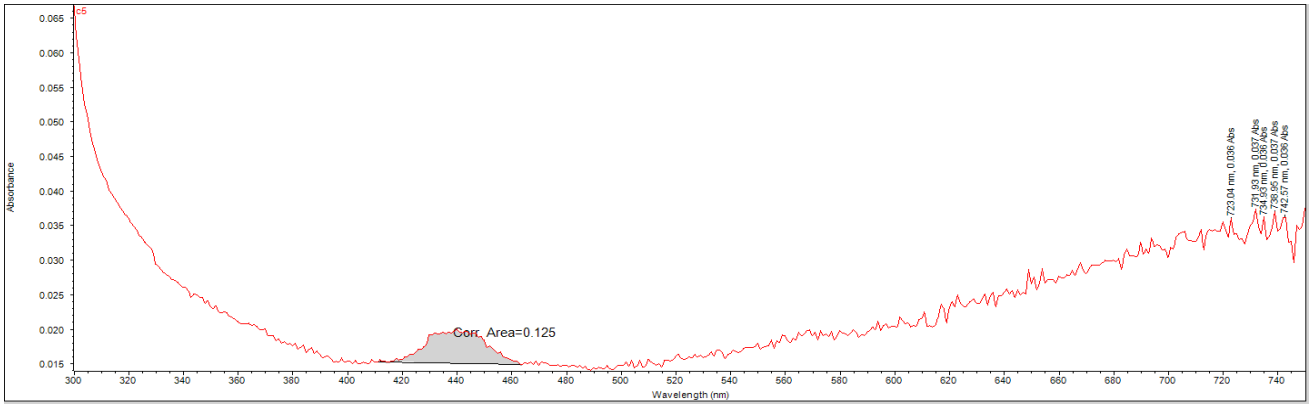
(B)


c3
Absorbance
Wavelength (nm)
327.59 nm, 0.017 Abs
373.93 nm, 0.012 Abs
413.21 nm, 0.011 Abs
431.93 nm, 0.011 Abs
Corr. Area=0.064

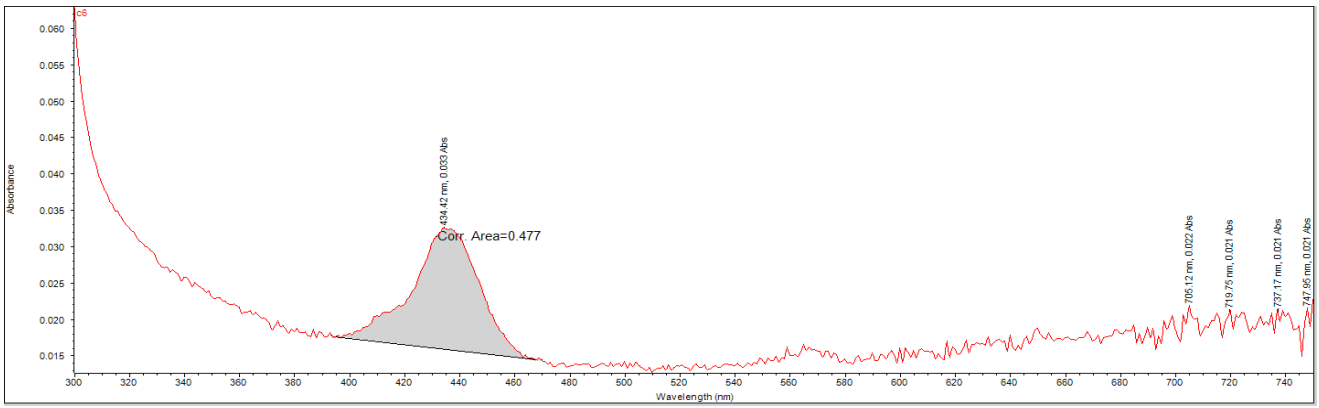
(C)


c4
Absorbance
Wavelength (nm)
320.95 nm, 0.032 Abs
436.92 nm, 0.024 Abs
Corr. Area=0.297

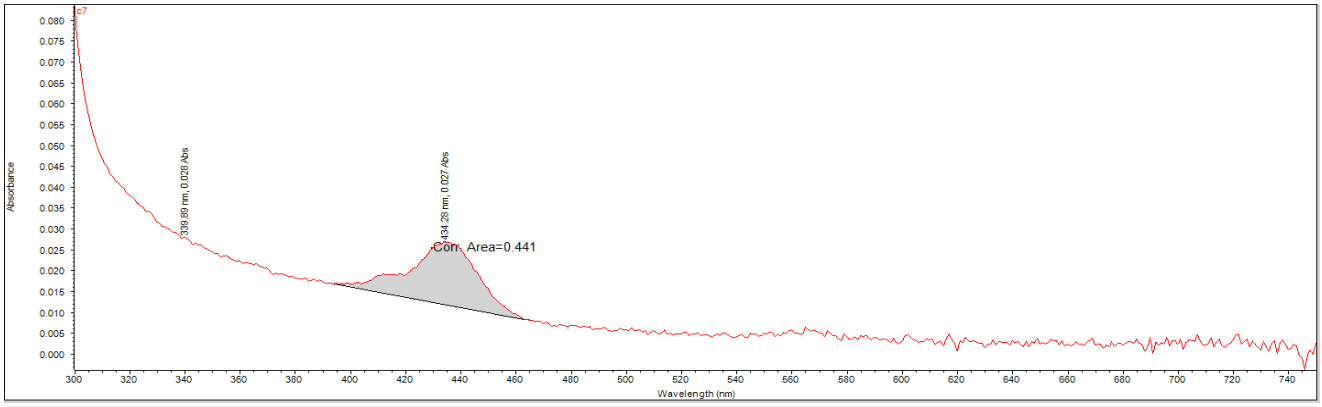
(D)


c5
Absorbance
Wavelength (nm)
723.04 nm, 0.036 Abs
731.93 nm, 0.037 Abs
734.93 nm, 0.036 Abs
739.57 nm, 0.037 Abs
742.57 nm, 0.036 Abs
Corr. Area=0.125

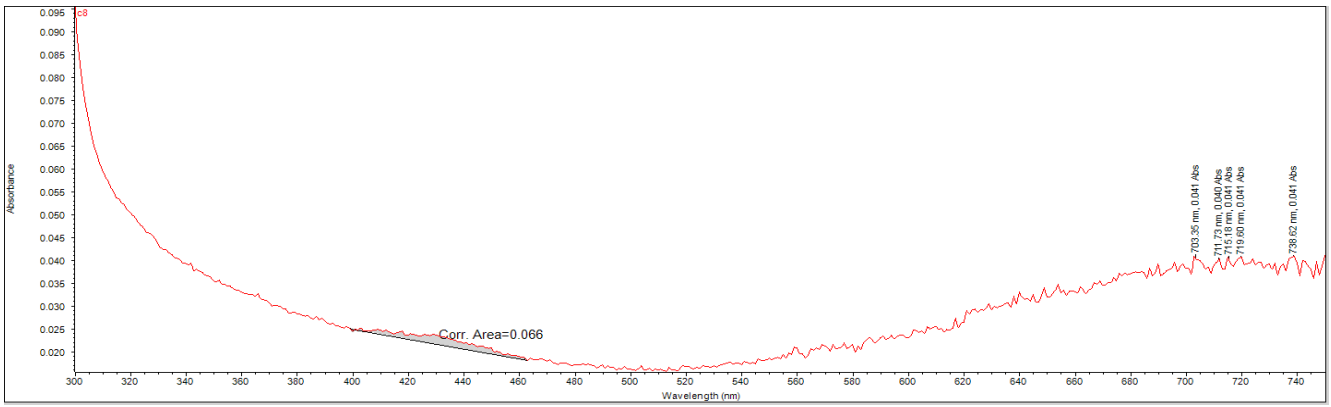
(E)


c6
Absorbance
Wavelength (nm)
434.42 nm, 0.033 Abs
Corr. Area=0.477
705.12 nm, 0.022 Abs
719.75 nm, 0.021 Abs
737.17 nm, 0.021 Abs
747.95 nm, 0.021 Abs

(F)



(G)



(H)

Fig S3. Absorbance spectra of solubilized cells incubated with arene ruthenium porphyrins for 24 h at 5 µM. The area under the peak at the Soret band corresponds to the amount of compound taken up by the cells. (A) **Ru$_{4a}$**, (B) **Ru$_{4b}$**, (C) **Ru$_{2a}$**, (D) **Ru$_{2b}$**, (E) **Zn-Ru$_{4a}$**, (F) **Zn-Ru$_{4a}$**, (G) **Zn-Ru$_{4b}$**, (H) **Zn-Ru$_{2b}$**.

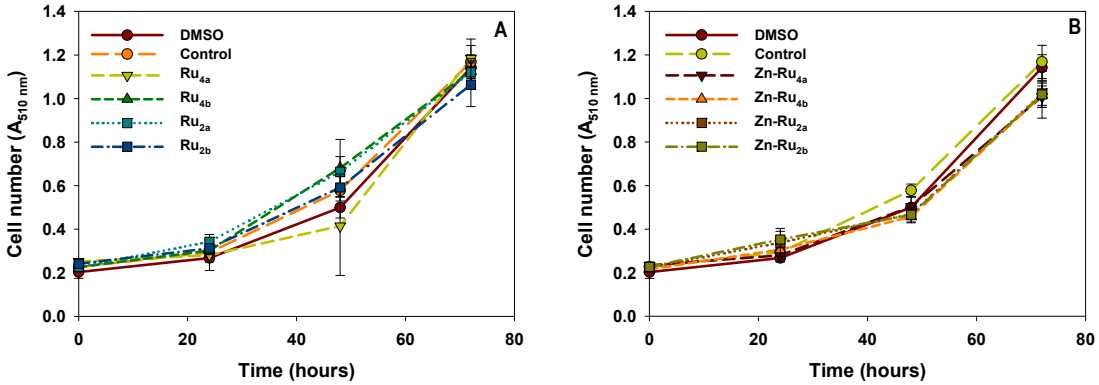


Fig S4. Effect arene ruthenium porphyrins on cell proliferation. Cell cultures were preincubated with 5 µM PSs for 24 h, kept in the dark, and SRB assay was then performed. **Panel A,** Zn-free Ru-porphyrins; **Panel B,** Zn-Ru derivatives. Results are presented as $OD_{510\ nm}$ corresponding to the number of adhered viable cells. Data are expressed as mean ± S.D. of three independent experiments.

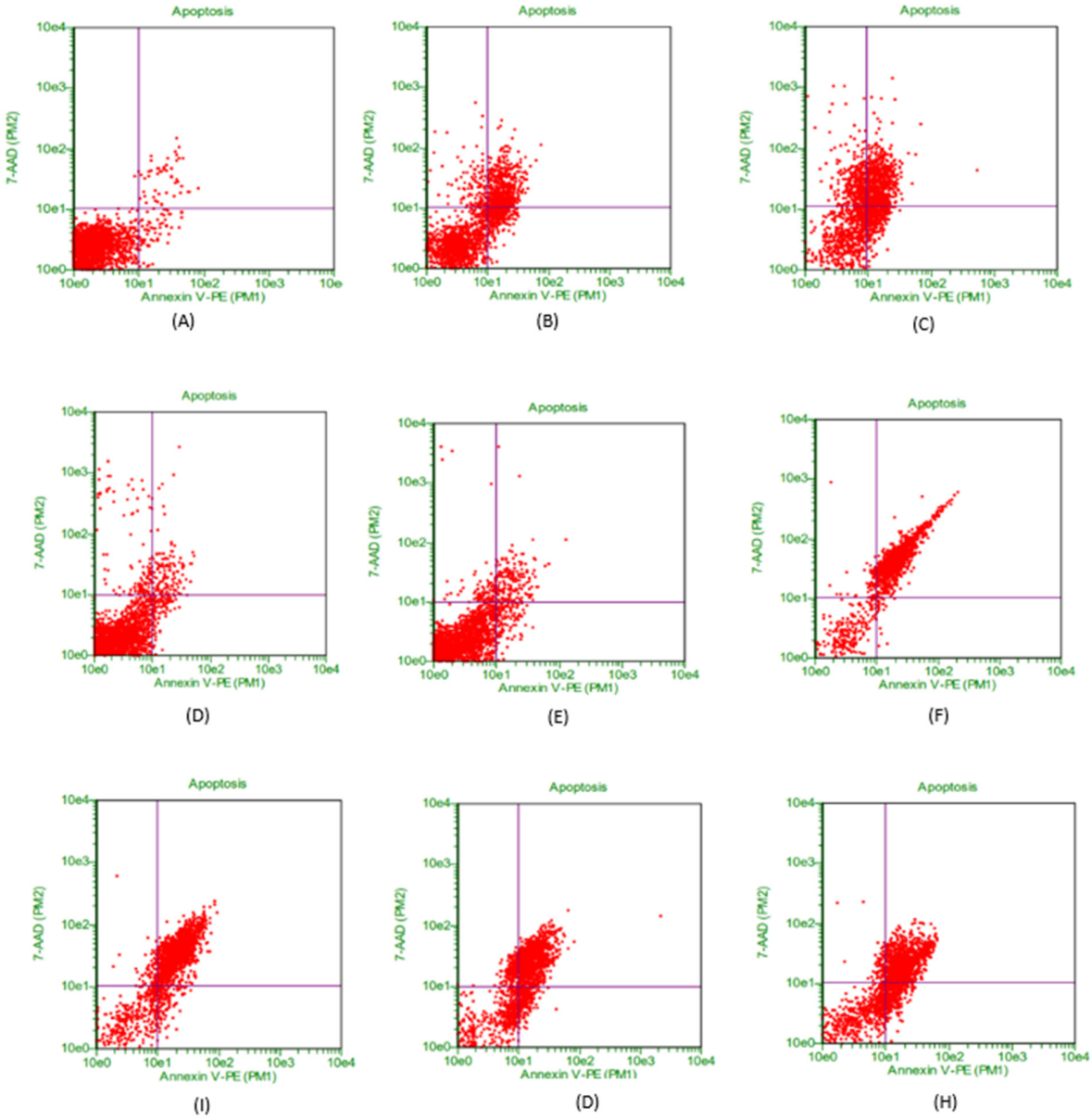


Fig S5. **Flow cytometry analysis of illuminated cells.** Colo205 cells were preincubated with 5 μM of the PSs for 24 h and then illuminated for 30 min. The readings were taken after 24 h. **Control** (A), **Ru_{4a}** (B), **Ru_{4b}** (C), **Ru_{2a}** (D), **Ru_{2b}** (E), **Zn-Ru_{4a}** (F), **Zn-Ru_{4b}** (G), **Zn-Ru_{2a}** (H), **Zn-Ru_{2b}** (I). The lower left quadrant represents viable cells, the lower right quadrant represents early apoptotic cells, the upper right quadrant represents late apoptotic cells, and the upper left quadrant represents necrotic cells. Results of one representative experiment are shown.

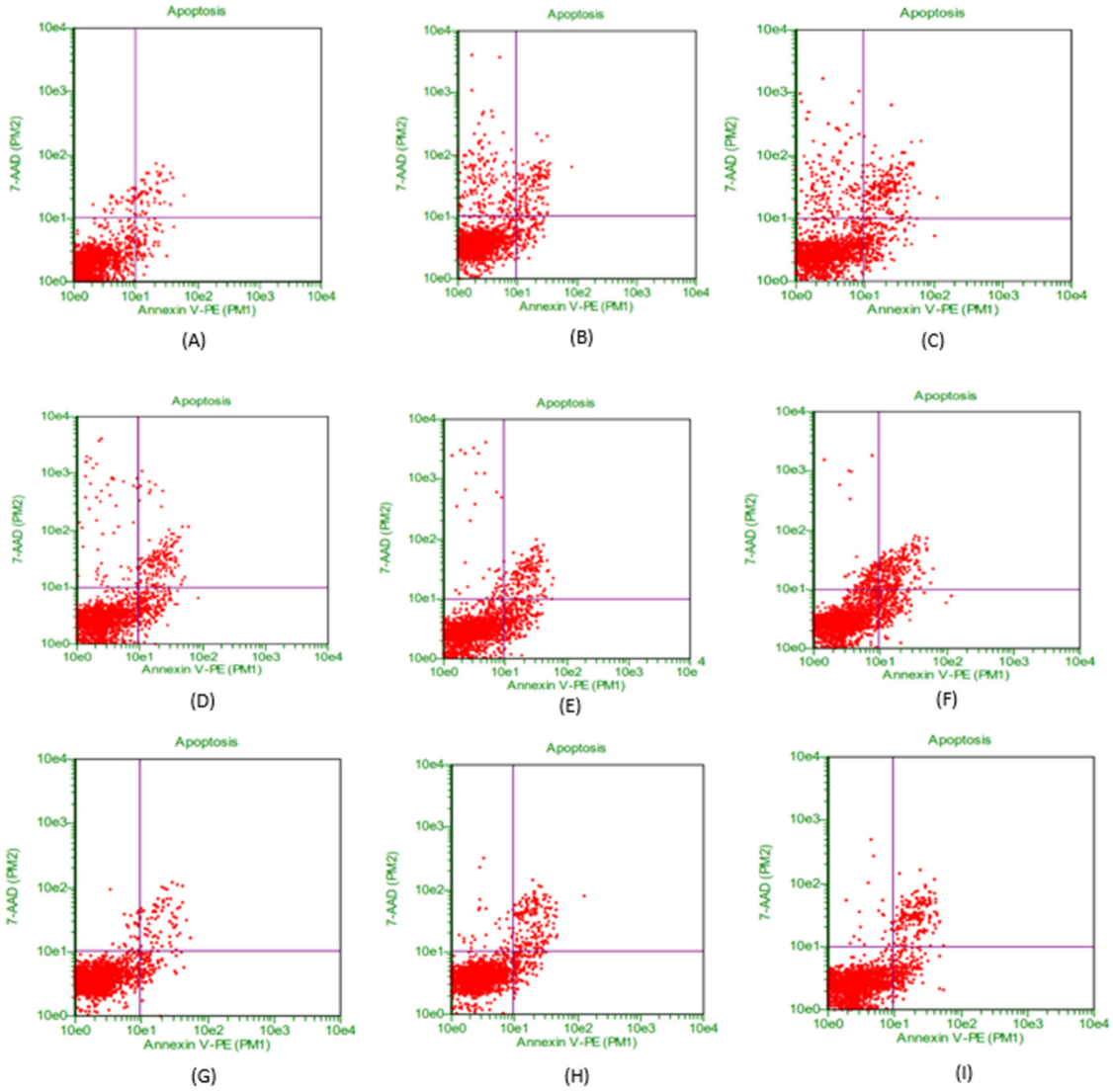


Fig S6. **Flow cytometry profiles of non-illuminated cells.** Colo205 cells preincubated with 5 μM of the PSs for 24 h and kept in the dark. The readings were taken after 24 h. **Control** (A), **Ru$_{4a}$** (B), **Ru$_{4b}$** (C), **Ru$_{2a}$** (D), **Ru$_{2b}$** (E), **Zn-Ru$_{4a}$** (F), **Zn-Ru$_{4b}$** (G), **Zn-Ru$_{2a}$** (H), **Zn-Ru$_{2b}$** (I). The lower left quadrant represents viable cells, the lower right quadrant represents early apoptotic cells, the upper right quadrant represents late apoptotic cells, and the upper left quadrant represents dead necrotic cells. Results of one representative experiment are shown.