Electronic Supplementary Information for

Photophysical Properties and Intracellular Imaging of Water-Soluble Porphyrin Dimers for Two-Photon Excited Photodynamic Therapy

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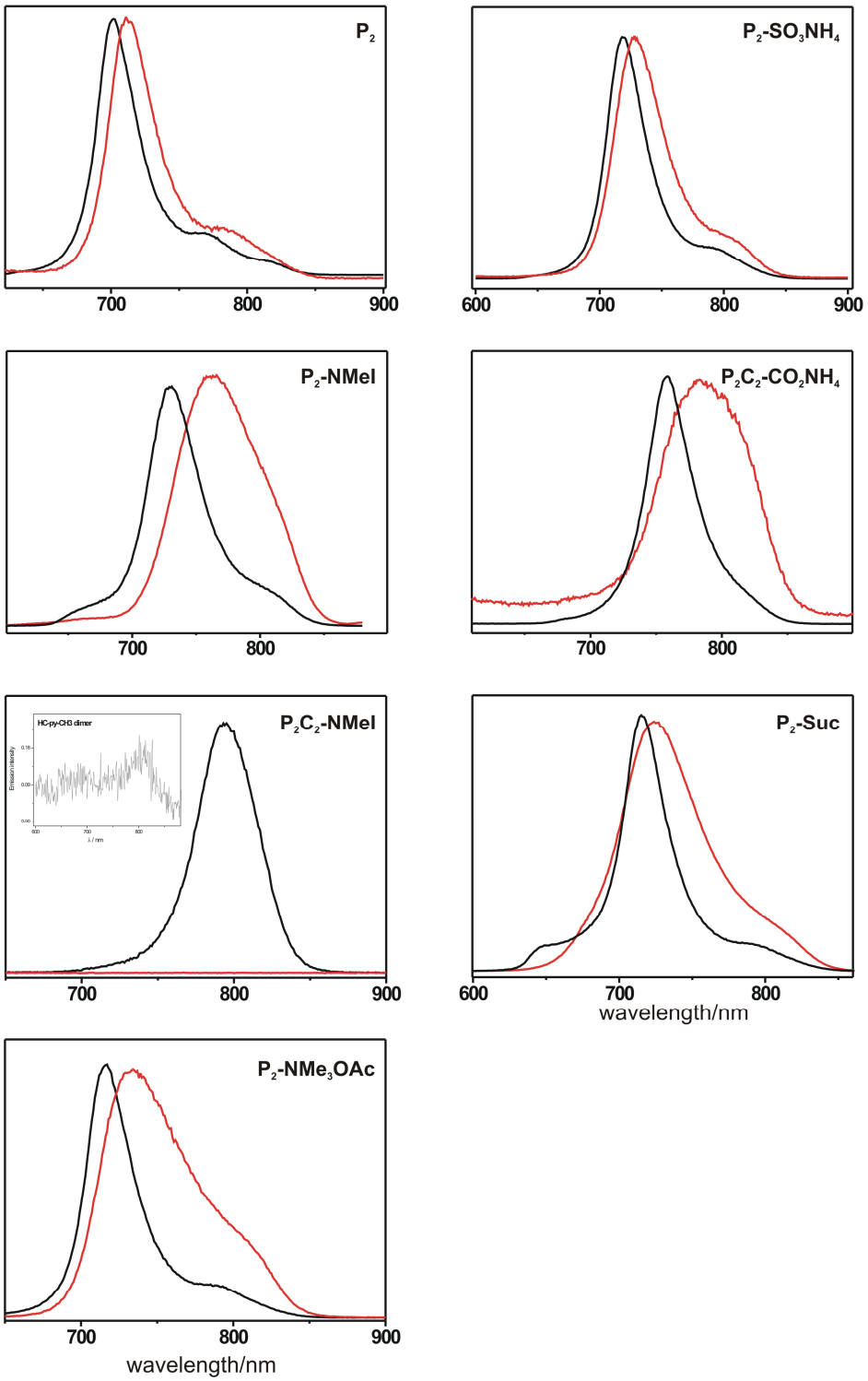

Fig S1. Normalised fluorescence spectra of the conjugated porphyrin dimers in DMSO (black) and water (red). The emission spectrum of **P_2C_2-NMeI** is water is not normalised due to its extremely low fluorescence intensity.

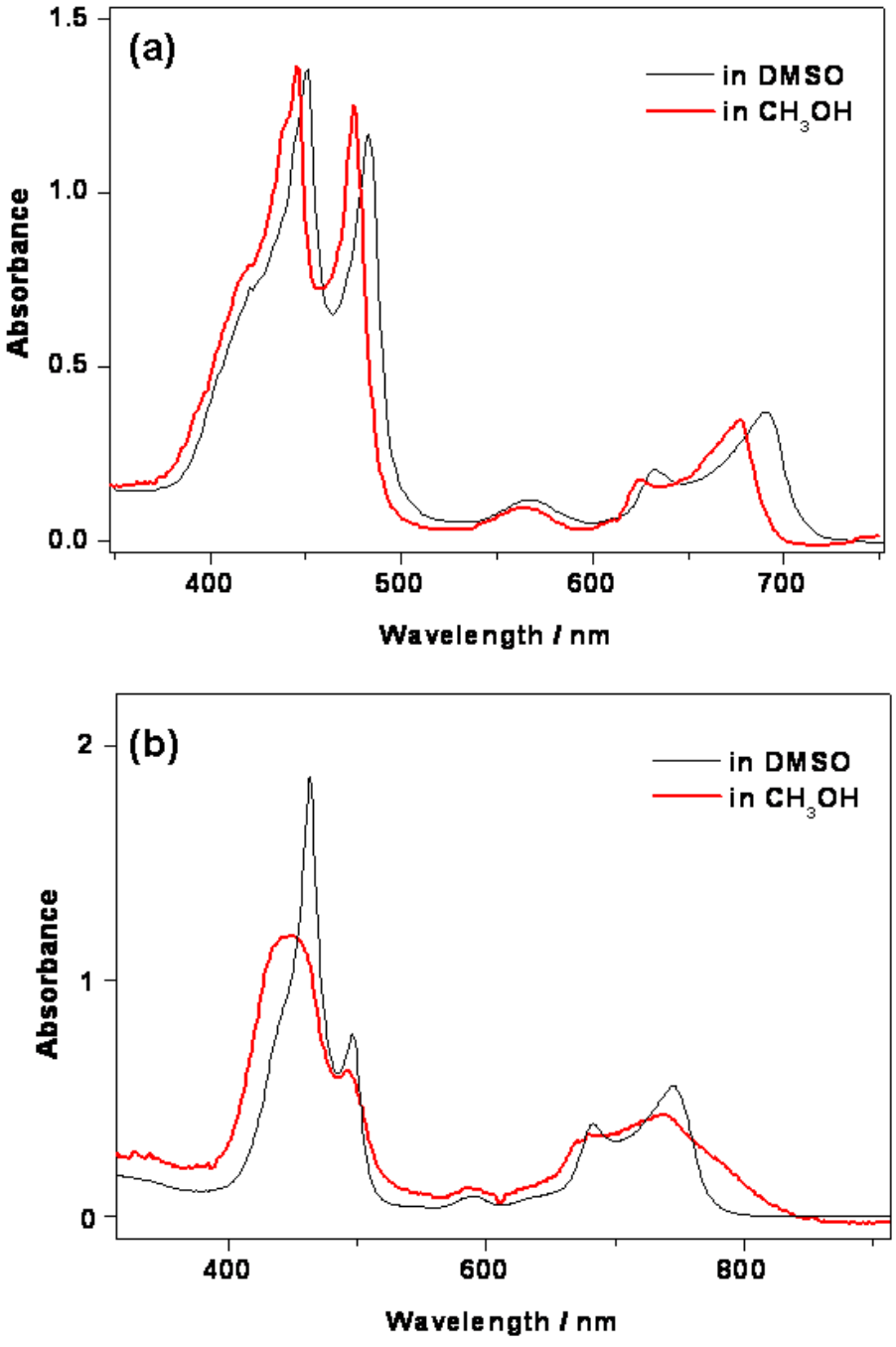

Fig S2. Absorption spectra of (a) $\mathbf{P_2}$ and (b) $\mathbf{P_2C_2\text{-}CO_2NH_4}$ in DMSO (black) and methanol (red). The broad spectrum of $\mathbf{P_2C_2\text{-}CO_2NH_4}$ in methanol is characteristic of the aggregated species.

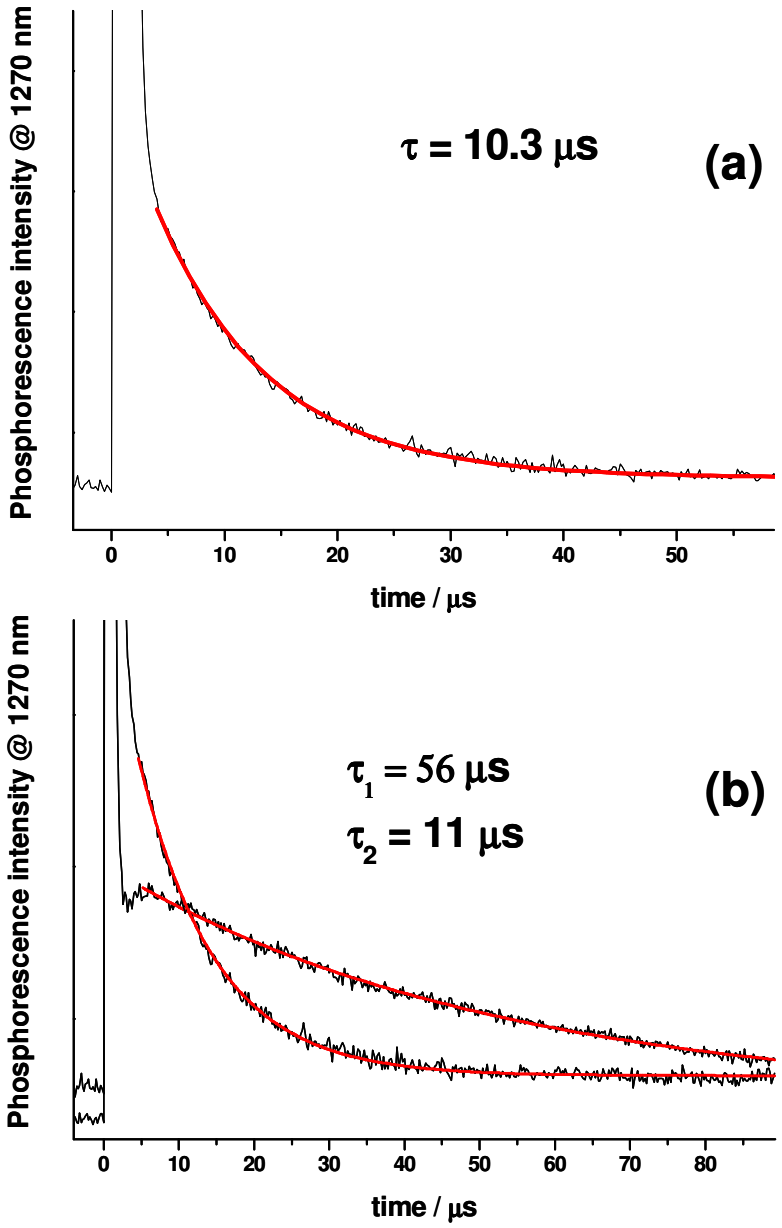

Fig S3. Decay traces showing the phosphorescence of singlet oxygen (recorded at 1270 nm) (a) following excitation of **P_2** in methanol (similar traces were obtained for all the dimers) and (b) for **P_2C_2-NMeI** (τ = 11 μs) and **P_2-SO_3NH_4** (τ = 56 μs) in D_2O containing 1% DMSO. The fast decay component of all the traces (the dominant signal between 0–5 μs) arises from the fluorescence of the dimers, whilst the longer decay component corresponds to the decrease in the intensity of the singlet oxygen phosphorescence.

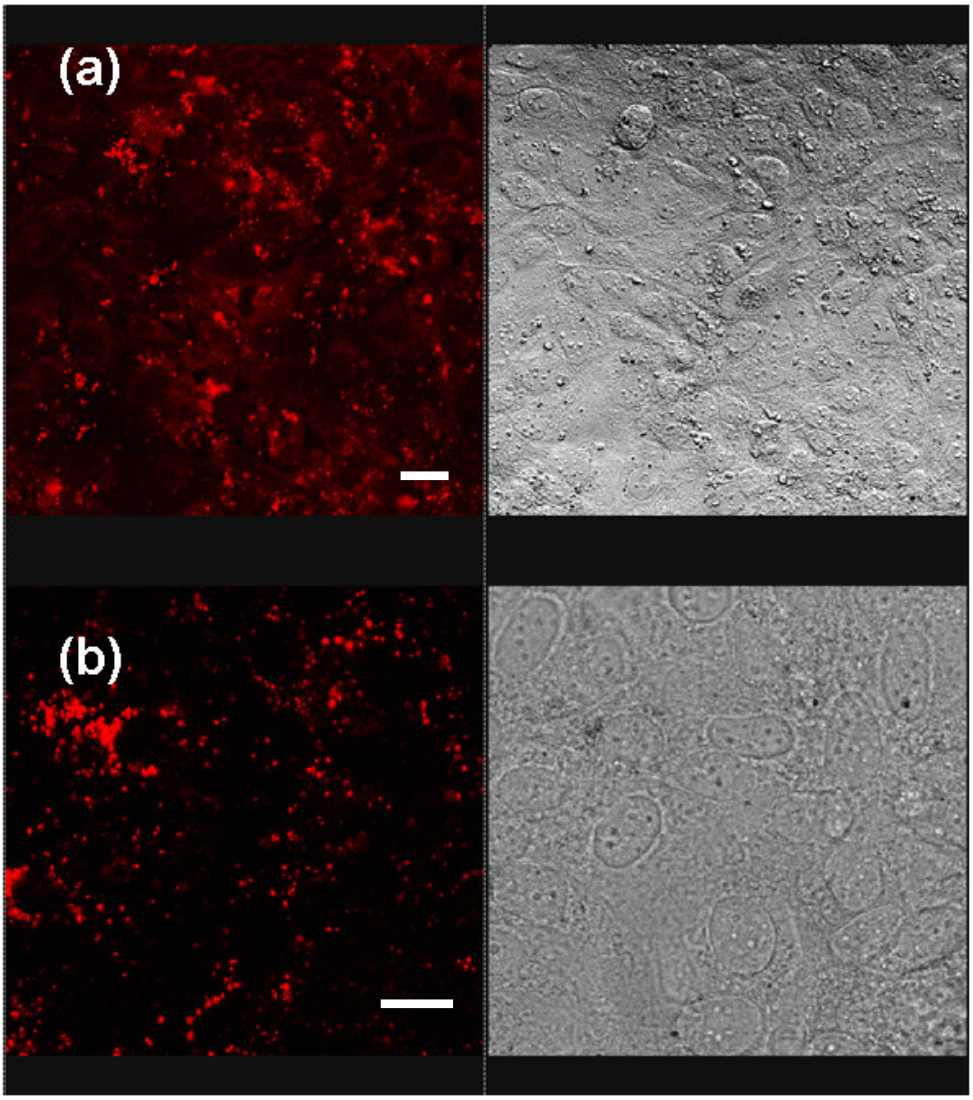

Fig S4. Confocal images of SK-OV-3 cells incubated for 6 h with 10 μM of **P_2** (a) diluted from a 1 mM stock solution in DMSO, scale bar = 20 μm or (b) formulated in dioleoylphosphatidylcholine liposomes prior to delivery to the cells (a 100:1 ratio of lipid to porphyrin dimer was used), scale bar = 20 μm. Left: 650–750 nm fluorescence images and right: transmission images. There are indications of the dark toxicity of **P_2** towards SK-OV-3 cells under prolonged incubation (>6 h) or higher concentration (>10 μM) with both delivery formulations, tested by the CellTiter 96® AQueous one solution cell proliferation assay (Promega).

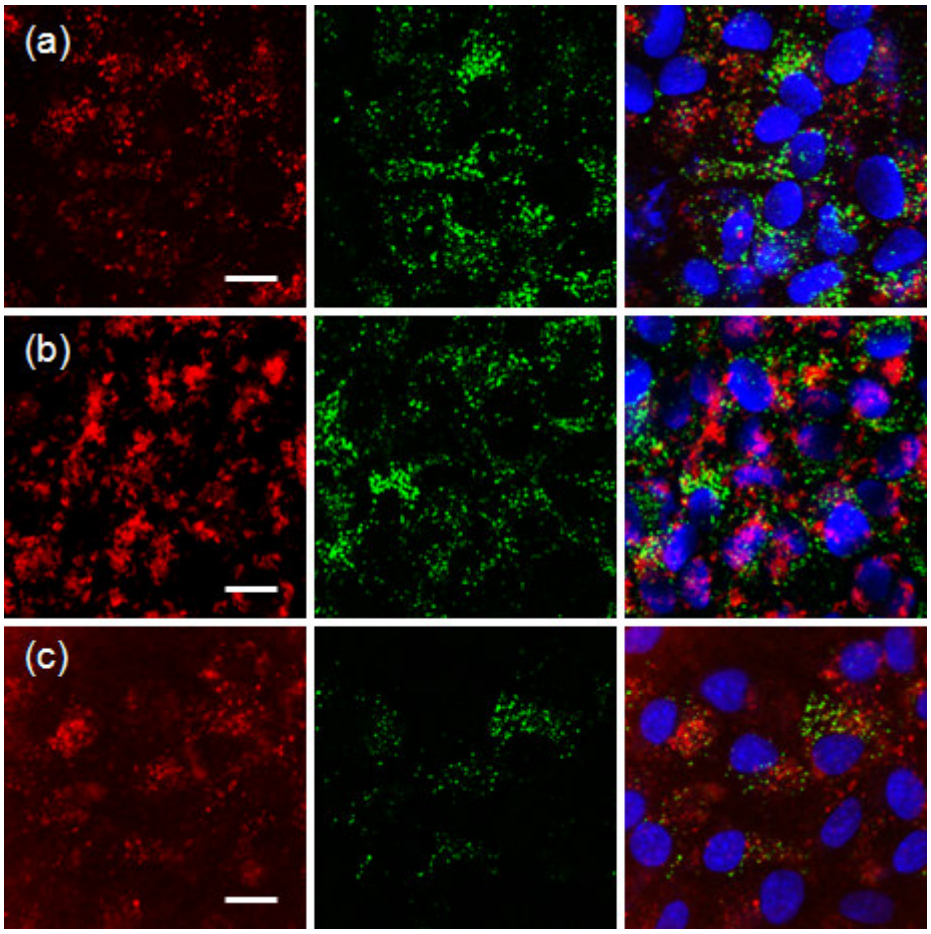

Fig S5. Punctate fluorescence of porphyrin dimers (a) **P_2-NMeI**, (b) **P_2-NMe_3OAc** and (c) **P_2C_2-NMeI** in SK-OV-3 human ovarian cancer cells with nuclear stain Hoechst 33258 and the lysosome marker LysoTracker yellow. Left: emission of the porphyrins (red, 10 μM, 4 h incubation, λ_{ex} = 458 nm, λ_{em} = 650–710 nm), middle: emission of LysoTracker (green, 50 nM, 1 h incubation, λ_{ex} = 488 nm, λ_{em} = 535–590 nm) and right: overlaid porphyrin, marker and Hoechst (blue, λ_{ex} = 354 nm, λ_{em} = 435–485 nm) images. Scale bar = 20 μm.

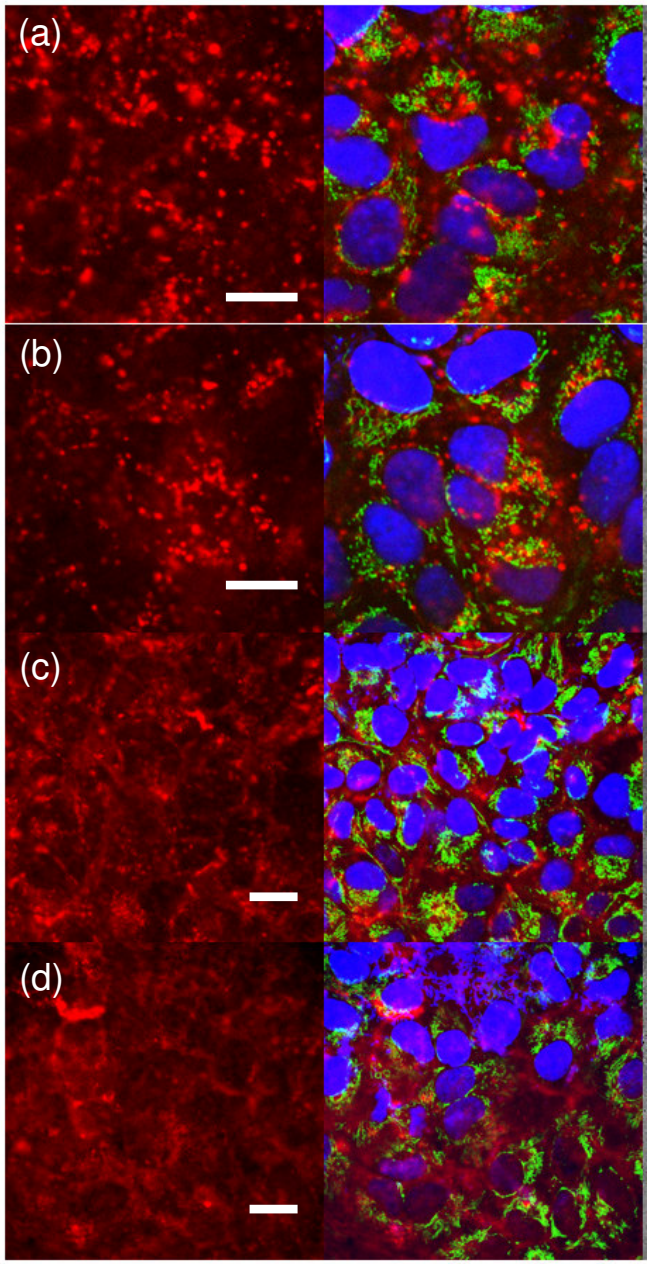

Fig S6. Punctate fluorescence of porphyrin dimers (a), **P_2-NMeI** (b) **P_2C_2-NMeI**, (c) **P_2-SO_3NH_4** and (d) **P_2C_2-CO_2NH_4** in SK-OV-3 human ovarian cancer cells with nuclear stain Hoechst 33258 and the mitochondria marker rhodamine 123. Left: emission of the porphyrins (red, 10 μM, 4 h incubation, λ_{ex} = 458 nm, λ_{em} = 650–710 nm), middle: emission of rhodamine 123 (green, 10 μg mL^{-1}, 0.5 hr incubation, λ_{ex} = 488 nm, λ_{em} = 505–530 nm) and right: overlaid porphyrin, marker and Hoechst (blue, λ_{ex} = 354 nm, λ_{em} = 435–485 nm) images. Scale bars = 20 μm.

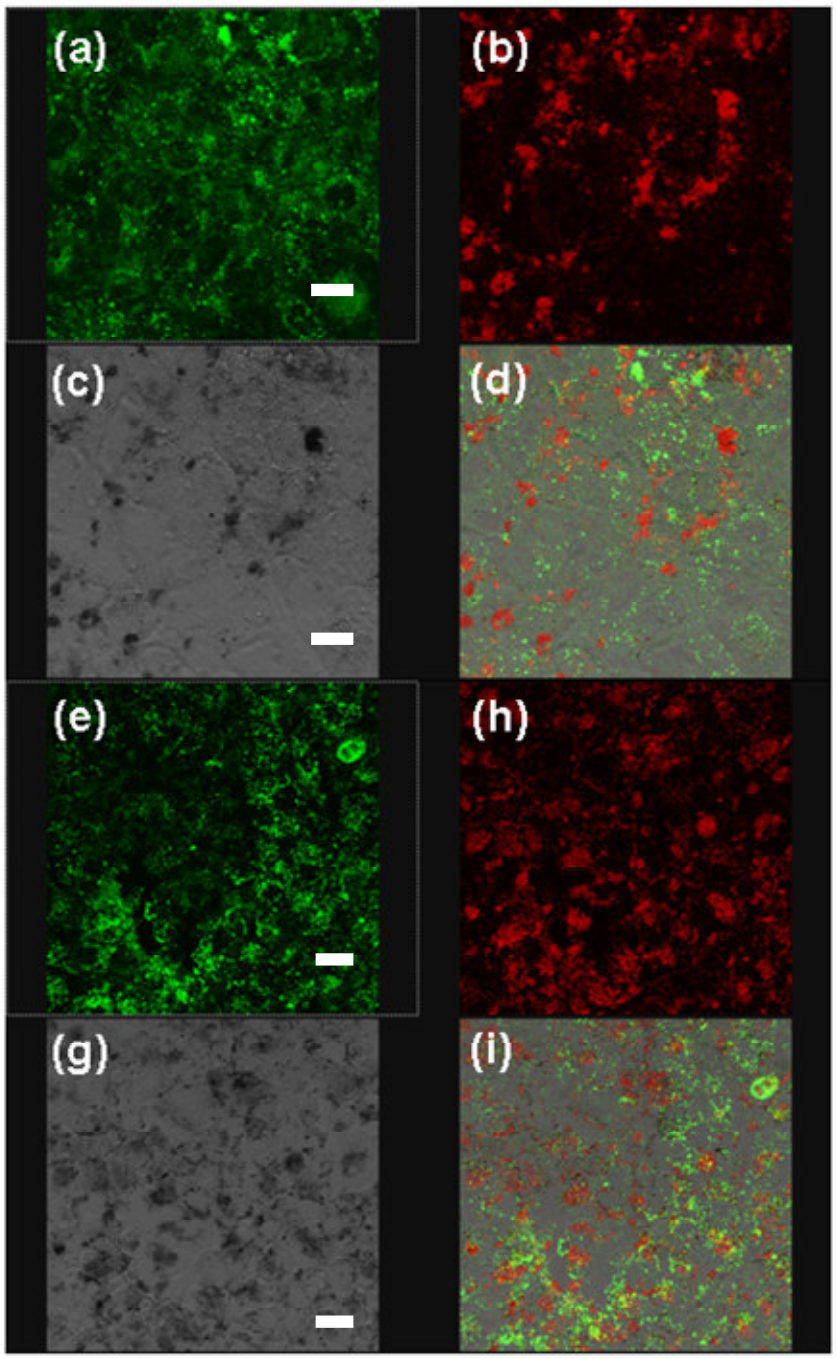

Fig S7. Confocal fluorescence images of dimer **P_2-NMe_3OAc** with lysotracker yellow after (a-d) 4 h incubation and (e-i) 18 h incubation. The dimer localisation is shown in red (λ_{ex} = 488 nm, λ_{em} = 650–750 nm), Lysotracker in green (50 nM, 1 h incubation, λ_{ex} = 488 nm, λ_{em} = 535–590 nm), overlapping regions appear yellow**.** Scale bars = 20 μm.

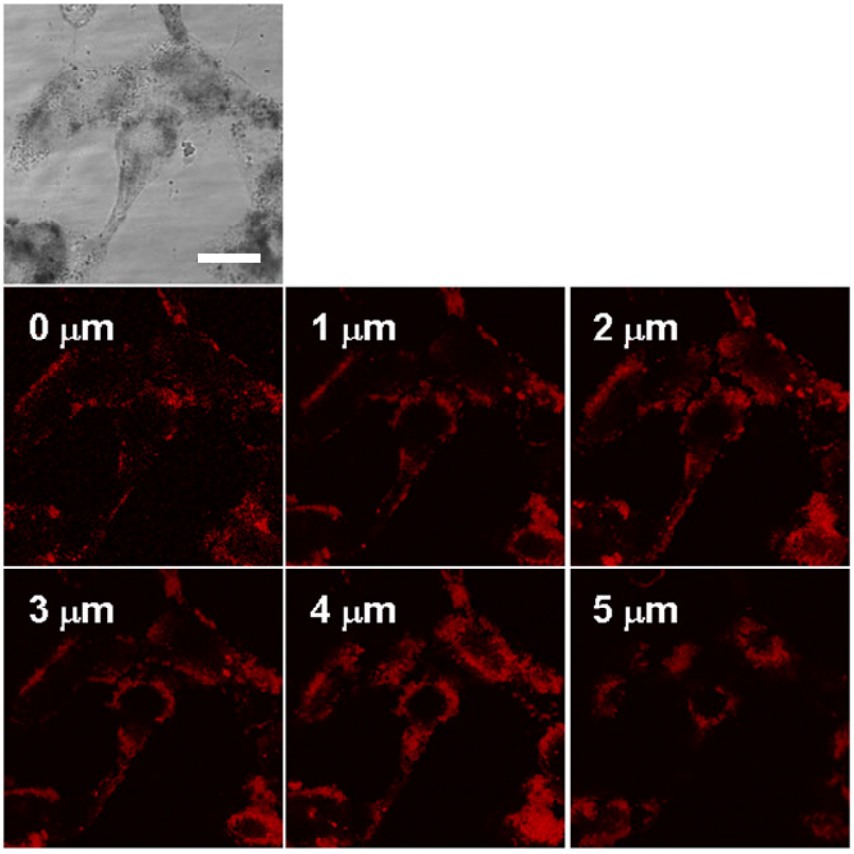

Fig S8. Confocal fluorescence images of dimer **P_2-NMe_3OAc** (10 μM, 24 h incubation, λ_{ex} = 488 nm, λ_{em} = 650–750 nm) recorded as a series of 1 μm slices through the cell layer. Scale bar = 20 μm.

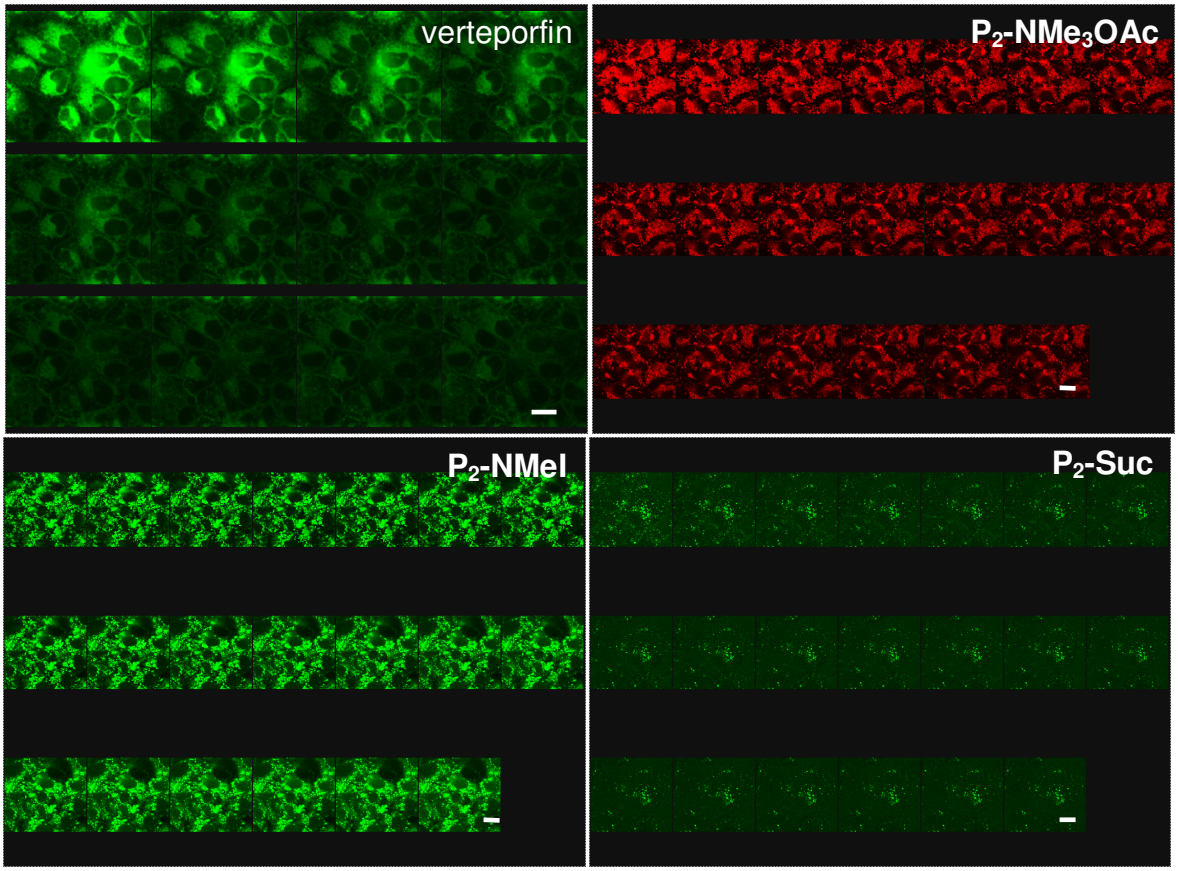

Fig S9. Example series of confocal fluorescence images recorded following photobleaching of **P_2-NMeI, P_2-NMe$_3$OAc**, **P_2-Suc** and verteporfin using 488 nm irradiation, 650–750 nm emission detection. The SK-OV-3 cells were incubated with 10 μM solutions of the photosensitisers for 24 h before irradiation. Scale bars = 20 μm.

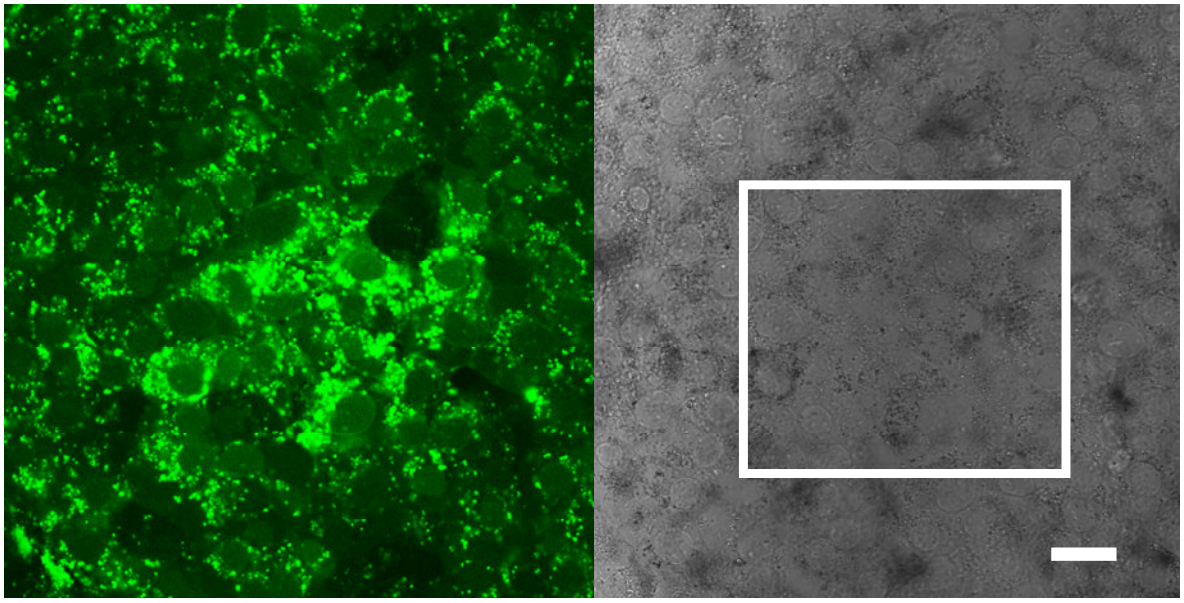

Fig S10. Confocal fluorescence and transmission images of SK-OV-3 cells incubated for 24 h with 10 μM solution of **P_2-NMeI**, and then irradiated for 3 min with 488 nm laser light (1 mW), emission detection 650–750 nm. The irradiated area is bounded by the white box in the transmission image. Scale bar = 20 μm.