Supporting information

for

Photodynamic activity of Sn(IV) tetrathien-2-ylchlorin against MCF-7 breast cancer cells

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Equipment

UV-visible absorption spectra were measured on a Shimadzu UV−2550 spectrophotometer, while a Varian Eclipse spectrofluorimeter was used to record the fluorescence emission spectra. Triplet state lifetimes were determined at 500 nm in N_2 saturated DMF solutions using an Edinburgh Instruments LP980 spectrometer with a pump beam of 430 nm provided by an Ekspla NT-342B laser fitted with an OPO. MS data were obtained on a Bruker® AutoFLEX III Smart-beam TOF/TOF mass spectrometer in positive ion mode by using α-cyano-4-hydroxycinnamic acid as the matrix. ^{1}H and ^{13}C NMR spectra were measured with a Bruker Avance 400 (400 MHz) at room temperature with $CDCl_3$ as the solvent. Steady-state phosphorescence of $^{1}O_2$ at *ca.* 1270 nm was measured on a Picoquant FluoTime 300 spectrometer equipped with a Near-IR PMT. For photobleaching studies, a solution of **SnC** in 1% DMF/H2O was irradiated for 30 min using a 660 nm Thorlabs M660L3 LED in the arrangement used for the *in vitro* cell studies, and absorption spectra were recorded at regular intervals.

Cell studies

The *in vitro* anticancer activities of the Sn(IV) porphyrins were evaluated against MCF-7 cancer cells by MTT assay in the dark and under illumination.[S1] MCF-7 cell lines were grown in Dulbecco's modified Eagle's medium (DMEM), and were supplemented with 10% fetal bovine serum (FBS) and 0.01% antibiotics (penicillin and streptomycin-amphotericin B) in 75 cm^2 cell culture flasks incubated at 37 °C and 5% CO_2. The cells were seeded (1×10^4/well) in 96-well plates in DMEM medium. The cells were then incubated for 24 h at 37°C in the incubator under 5% CO_2. The medium was replaced with DMEM media containing compounds at different concentrations (0.3−25 μM), and the cells were incubated for 24 h in the dark. Control cells were given fresh DMEM medium. After 24 h, the original medium was removed, and fresh DMEM with no phenol red was added.

Cells were irradiated at 660 nm with a Thorlabs M660L3 LED mounted into the housing of a Modulight 7710-680 medical laser system (280 $mW.cm^{-2}$) for 30 min. Fresh DMEM medium was added, and cells were incubated for a further 24 h in the dark. A separate set of cells treated with the compounds was prepared, and no light treatment was performed. After 24 h, the medium was removed, and the cells were incubated for 3 h with a 5 mg/mL MTT solution added to each well of the plate. The medium was discarded carefully after the incubation period, and the formazan crystals were solubilized in 200 μL of DMSO. Absorbance was read

at 540 nm with a Molecular Devices Spectra Max M5 plate reader. An analysis of control cells with no treatment and cells treated only with irradiation with no photosensitizer was also carried out. The percentage of cell viability was measured as a percentage ratio of the absorbance of the treated cells relative to that of the untreated controls. The IC_{50} values were determined by nonlinear regression analysis. Experiments were performed in triplicate.

Cellular uptake

For time-dependent cellular uptake experiments with the MCF-7 cell line, approximately 1×10^5 cells were seeded in 24-well cell culture plates and incubated for 24 h. Sn(IV) complexes (10 μM) were added to the cells at regular time intervals (6, 12, 24, 48 h). After the incubation time, the cells were washed three times with PBS, lysed with 30 μL of Triton-100X, and solubilized in 70 μL of DMSO. The relative cellular uptake was measured by determining the absorbance at 435 nm with an ELISA reader. Control experiments were carried out in the absence of the Sn(IV) complexes.

DCF-DA assay

The intracellular production of reactive oxygen species (ROS) was detected by following a previously reported protocol using 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA).[S2]

Theoretical Calculations

Geometry optimizations for **SnC** and **SnP** were carried out at the B3LYP/SDD level of theory by using the Gaussian 09 software package.[S3]

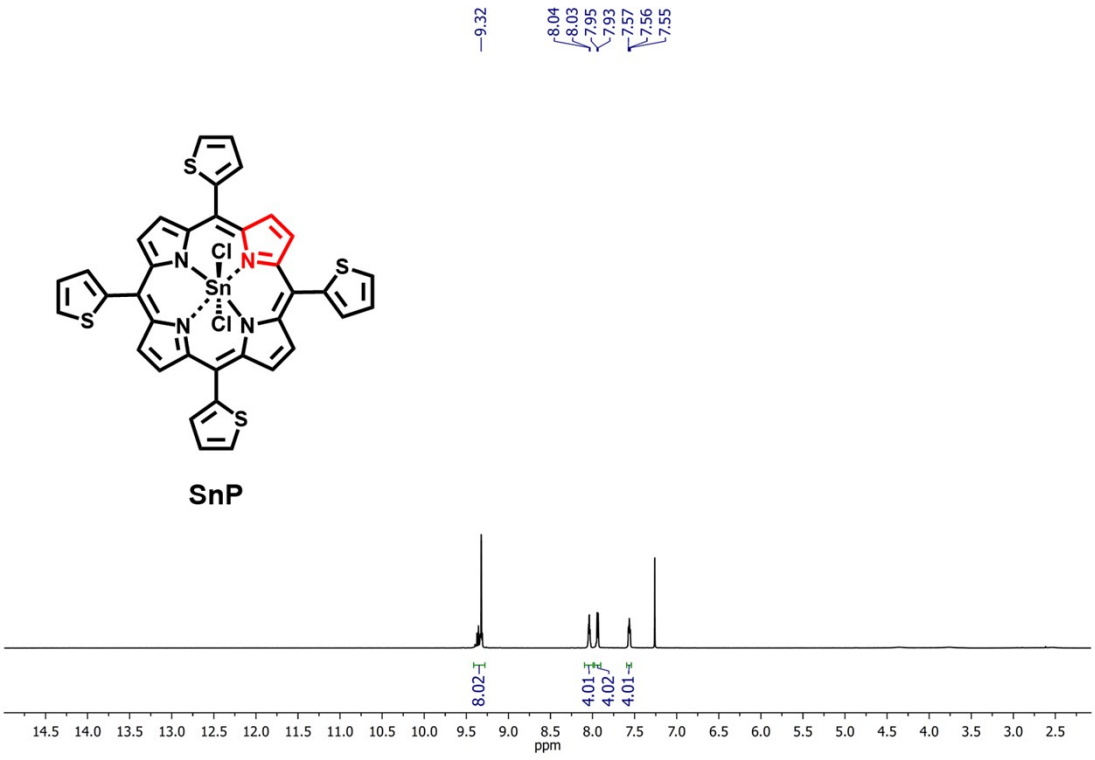

Figure S1. ^{1}H NMR (400 MHz) spectrum of **SnP** in $CDCl_3$.

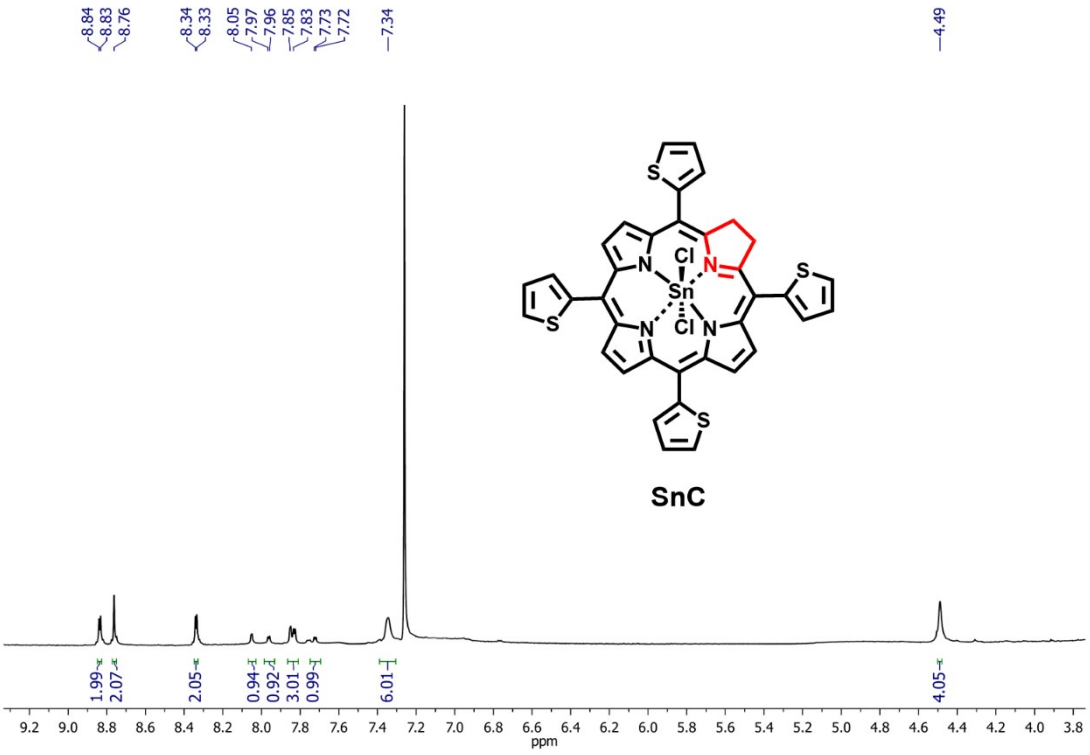

Figure S2. ^{1}H NMR (400 MHz) spectrum of **SnC** in $CDCl_3$.

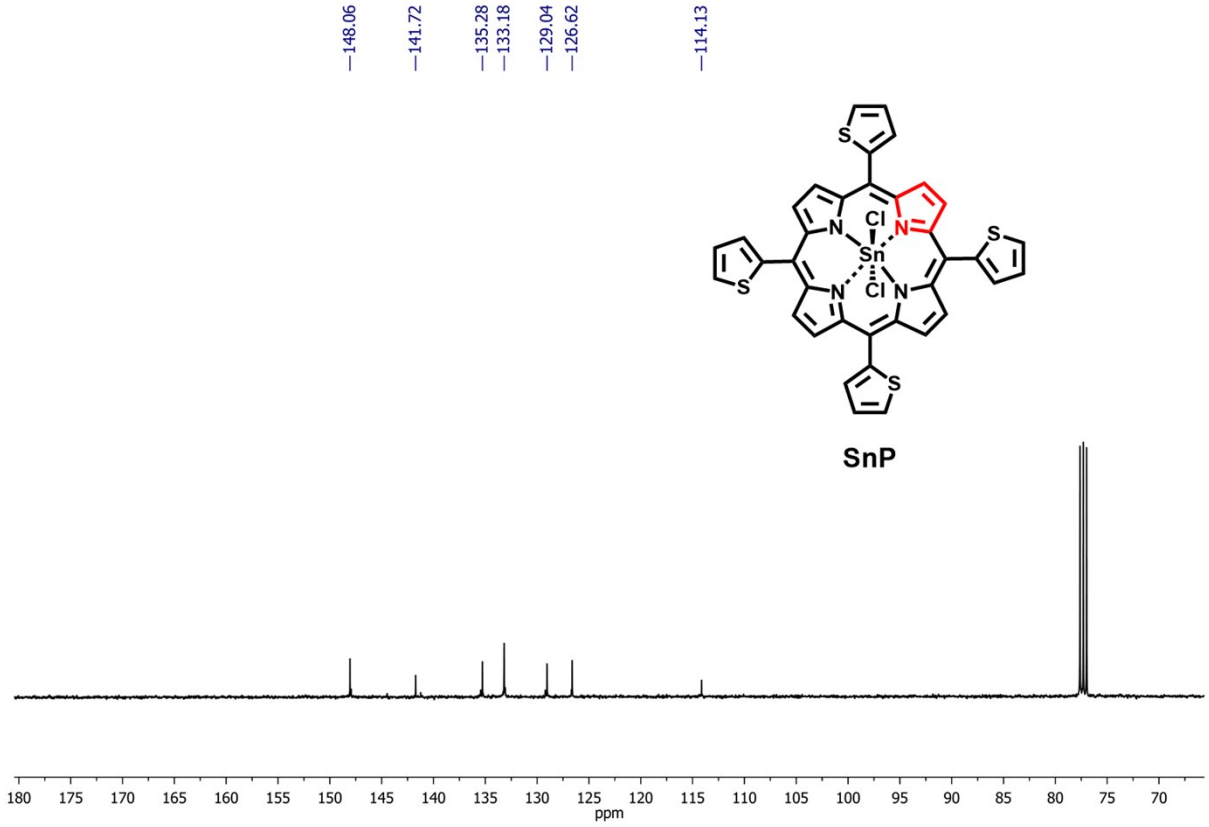

Figure S3. ^{13}C NMR (400 MHz) spectrum of **SnP** in $CDCl_3$.

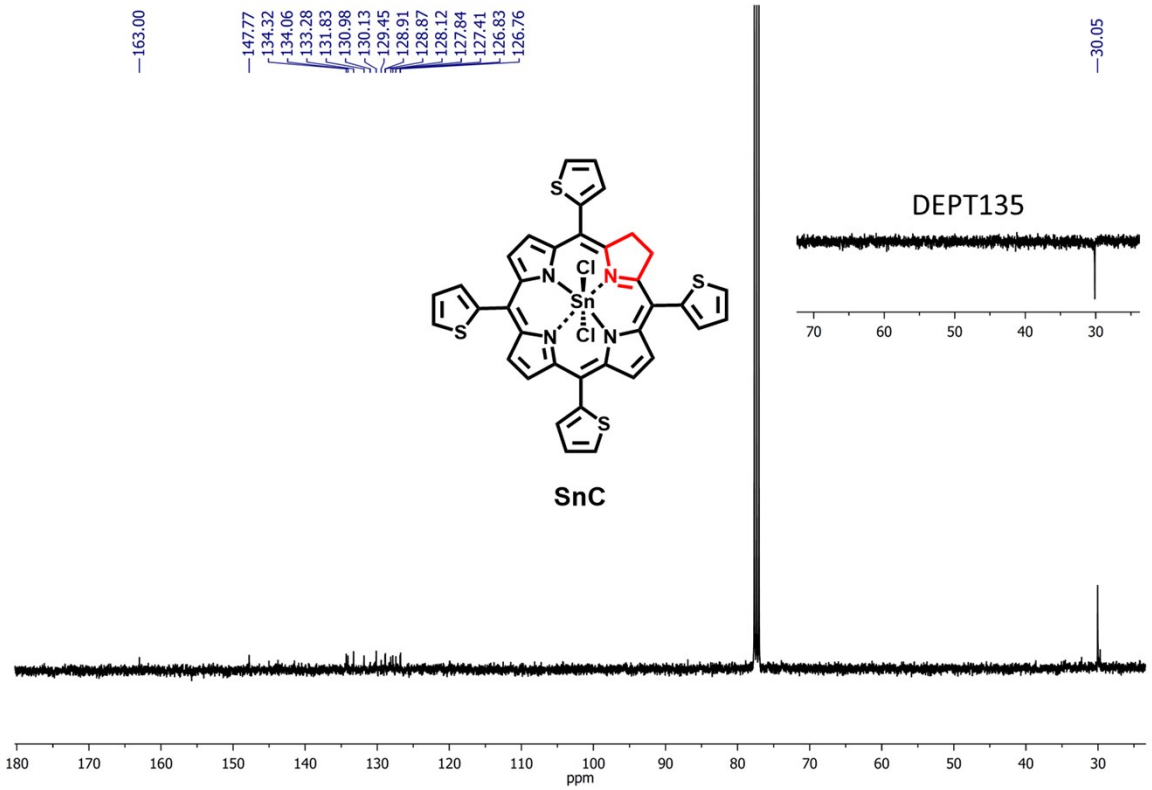

Figure S4. ^{13}C NMR (400 MHz) spectrum of **SnC** in $CDCl_3$. [Inset: partial DEPT135 NMR showing pyrrolinic carbon ($-CH_2-CH_2-$) inverse peaks].

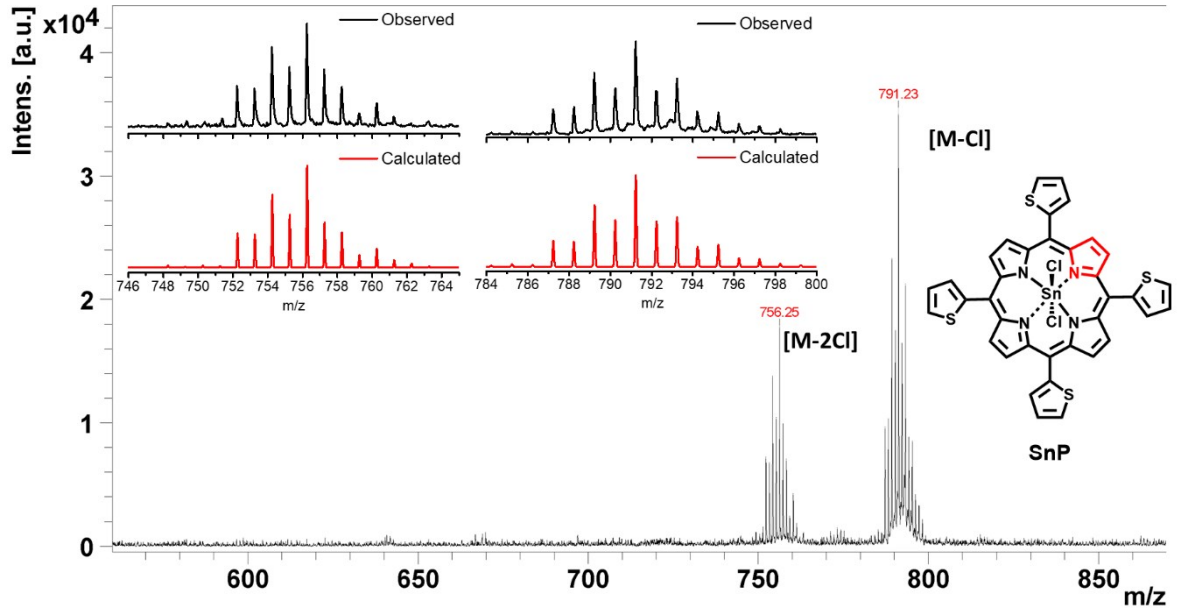

Figure S5. MALDI-TOF MS data for **SnP**.

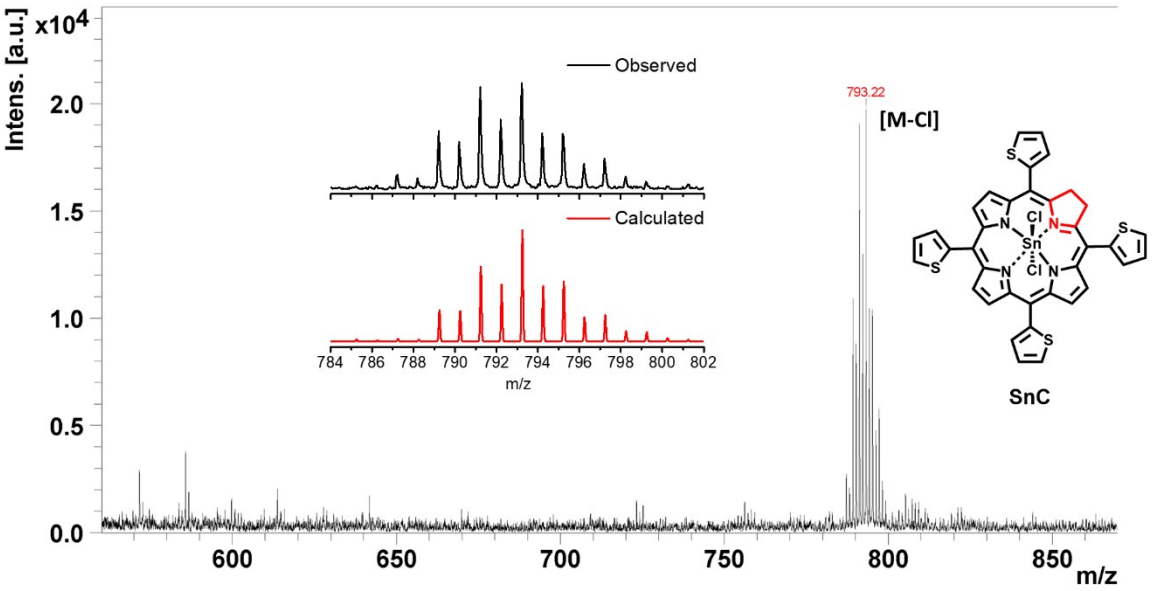

Figure S6. MALDI-TOF MS data for **SnC**.

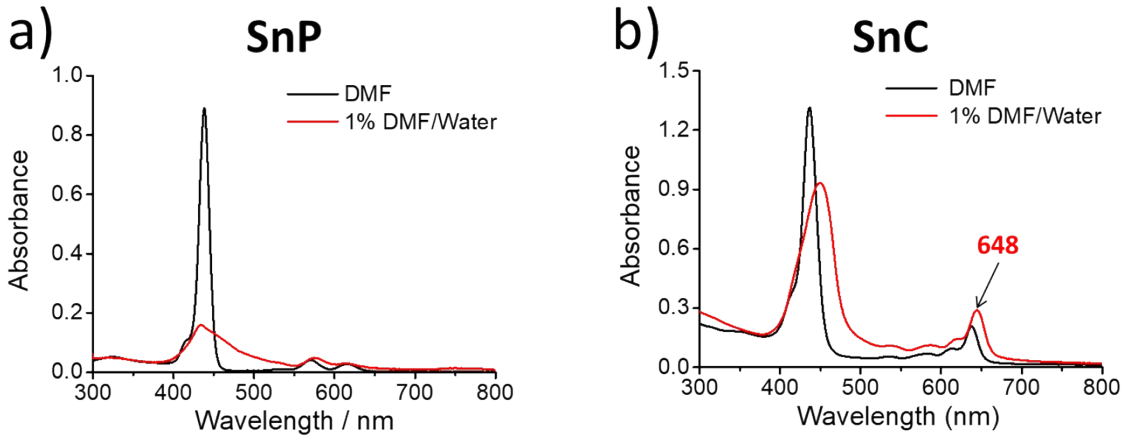

Figure S7. Absorbance spectra of (a) **SnP** and (b) **SnC** in DMF and 1% DMF:H_2O.

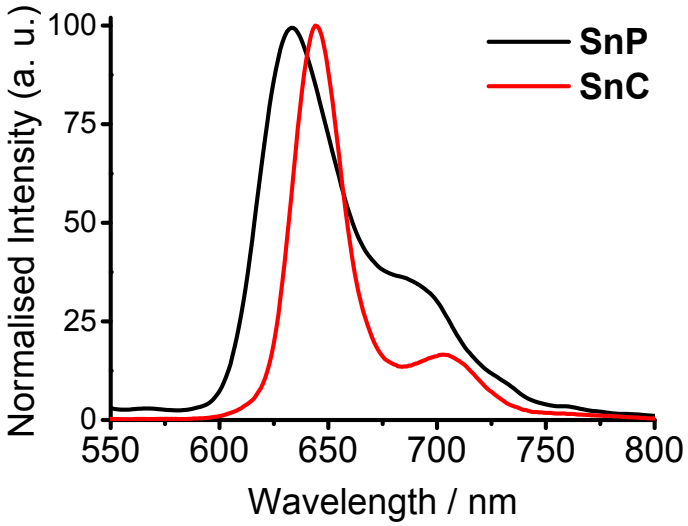

Figure S8. Emission spectra of **SnP** (black) and **SnC** (red) in DMF (λ_{ex} at the B band maximum).

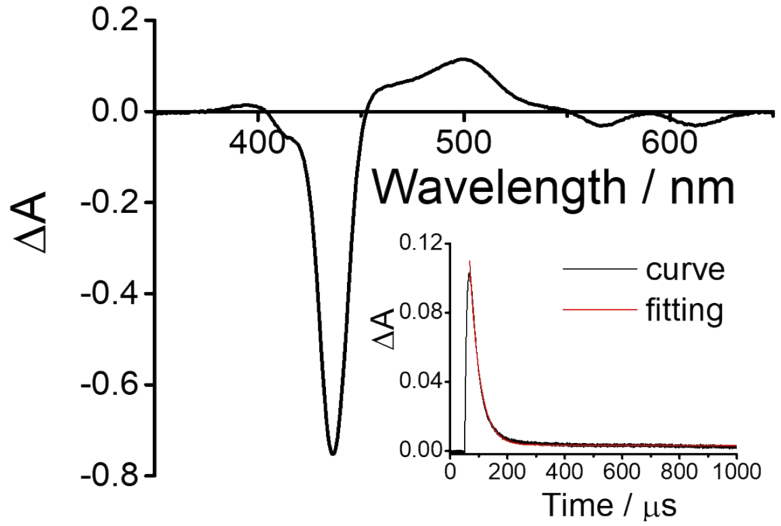

Figure S9. Transient absorption spectra for **SnP** excited at the B band maximum in N_2 purged DMF (Inset: triplet absorption decay curve).

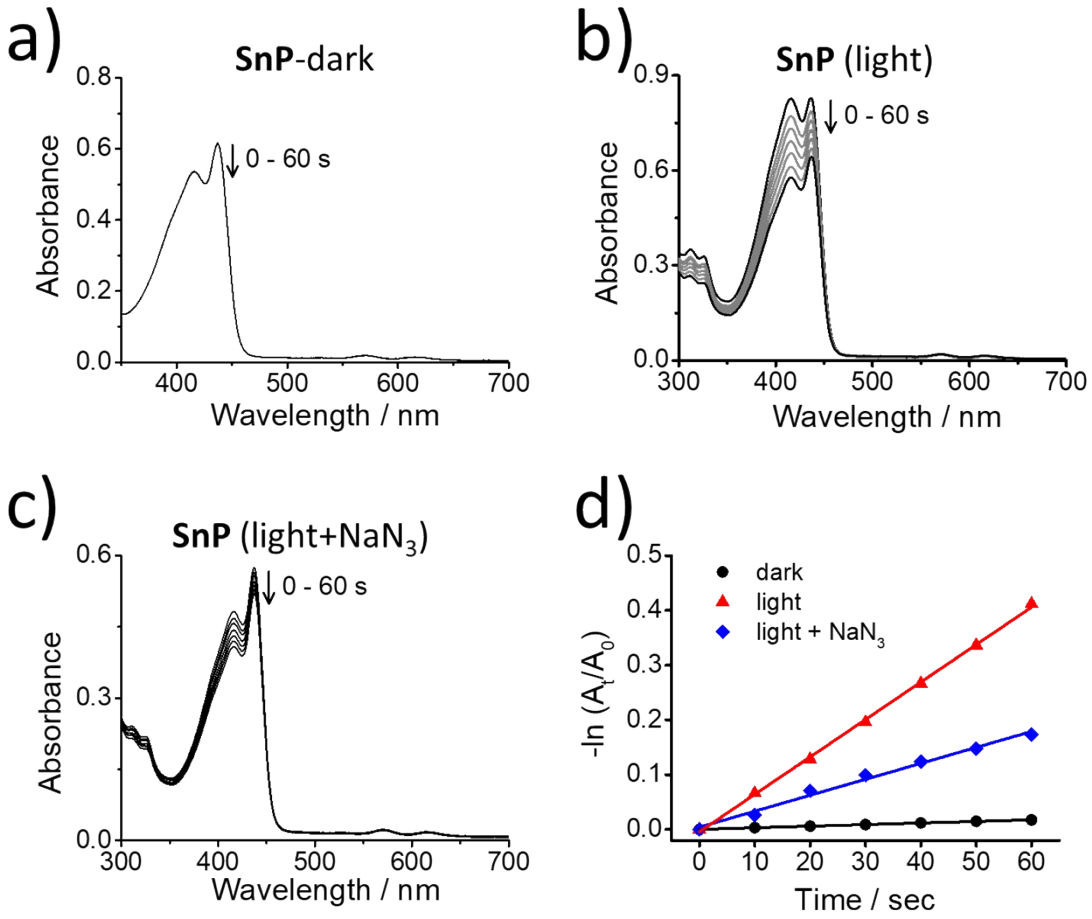

Figure S10. Spectral changes of DPBF in the presence of **SnP**, (a) when kept in the dark, (b) when under light irradiation, and (c) when under light irradiation in the presence of NaN_3; (d) A plot of absorbance change of DPBF at 418 nm *vs* photoirradiation time in the dark, and under illumination in the presence and absence of NaN_3 in DMF.

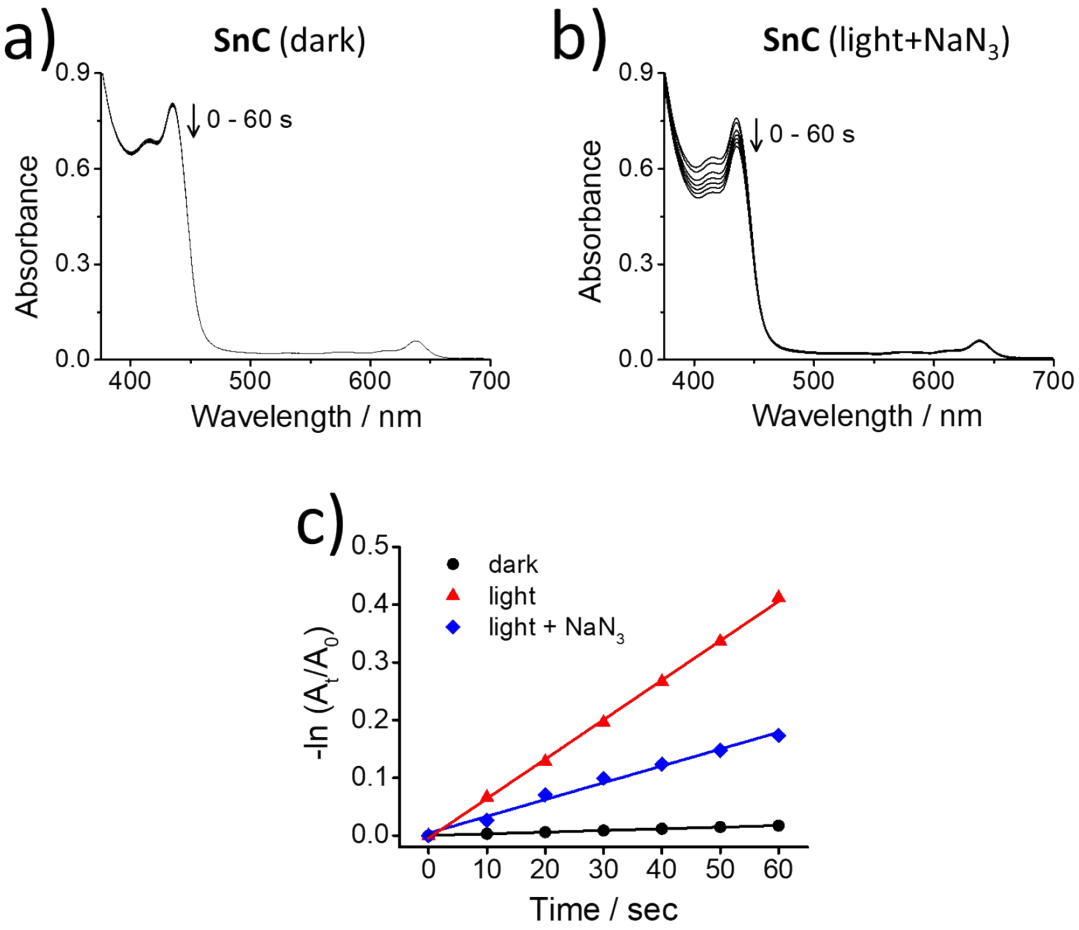

Figure S11. Spectral changes of DPBF in the presence of **SnC**, (a) when kept in the dark (dark), and (b) when under light irradiation in the presence of NaN_3; (c) A plot of the absorbance change of DPBF at 418 nm *vs* photoirradiation time for **SnC** in the dark, and under illumination in the presence and absence of NaN_3 in DMF.

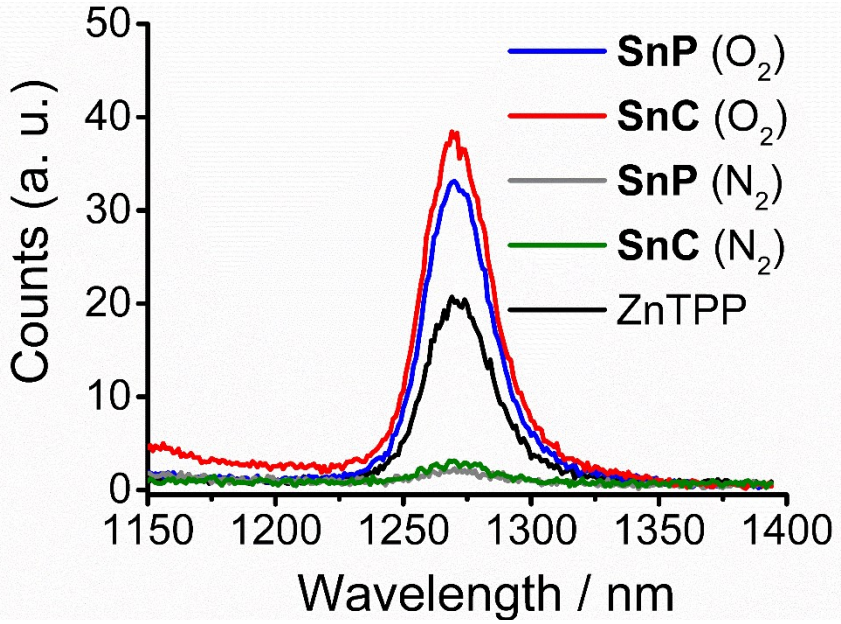

Figure S12. Singlet oxygen phosphorescence signals generated by **SnP** and **SnC** in air-saturated and N_2 purged DMF along with that of the ZnTPP standard.

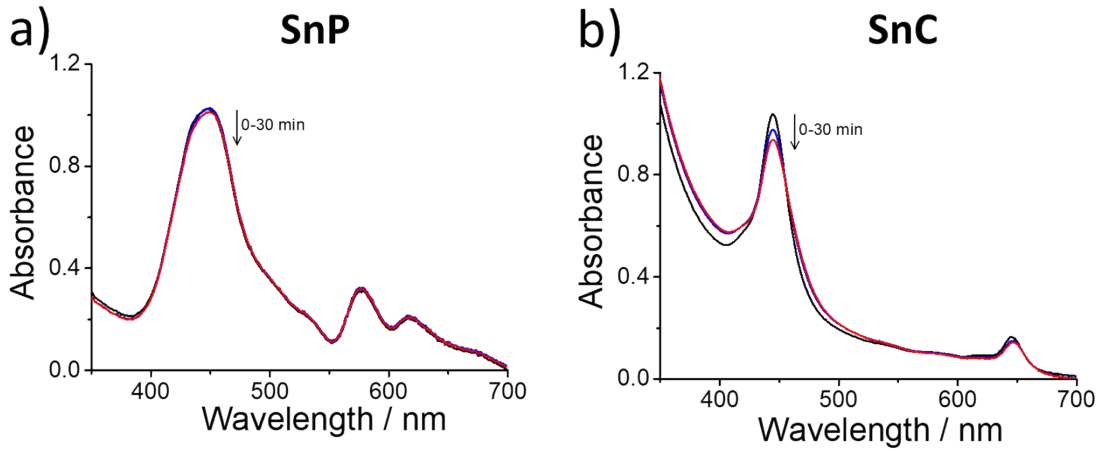

Figure S13. Photostability of **SnP** and **SnC** in 1% DMF:H_2O under irradiation at 660 nm for 30 min with a Thorlabs M660L3 LED under the same conditions used for the *in vitro* cell studies against MCF-7 breast cancer cells.

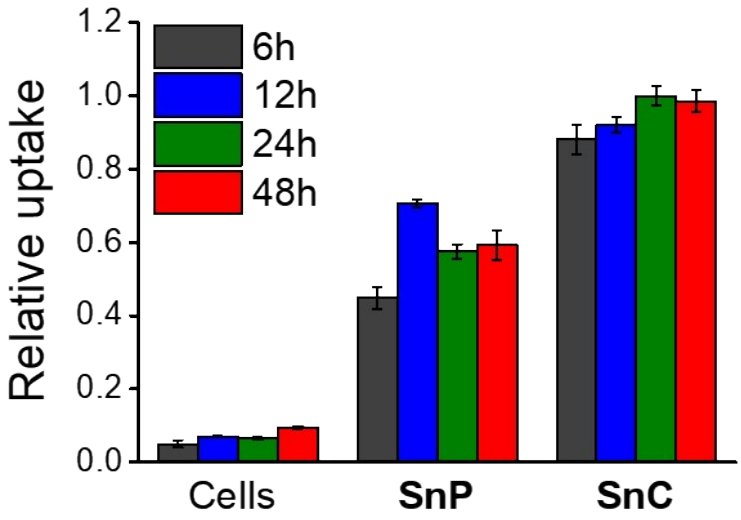

Figure S14. Time-dependent relative cellular uptake of **SnP** and **SnC** at 10 μM by MCF-7 cells determined by the UV-visible absorption spectroscopy of the lysed cells. Error bars represent the mean standard deviation.

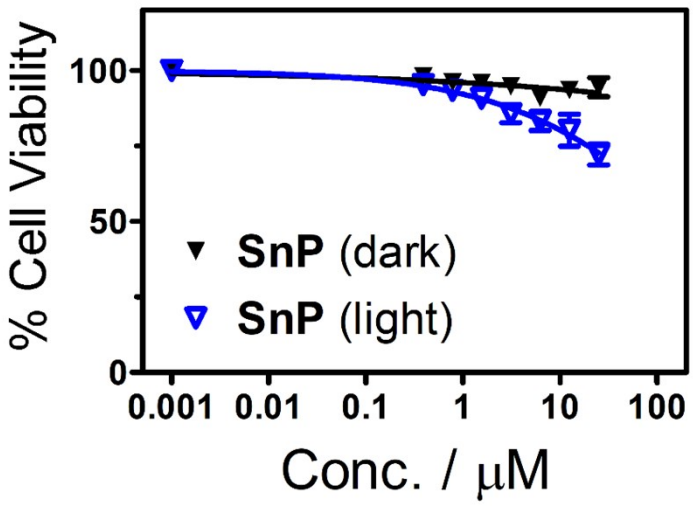

Figure S15. MTT-assay cytotoxicity profiles of **SnP** in MCF-7 cells after 24 h incubation in the dark and under photoirradiation for 30 min (red circle) with a 660 nm (280 mW.cm^{-2}) LED. Error bars represent the mean standard deviation.

References

S1. M. V Berridge, P. M. Herst and A. S. Tan, *Biotechnol. Annu. Rev.*, 2005, **11**, 127–152.

S2. M. Oparka, J. Walczak, D. Malinska, L.M.P.E. van Oppen, J. Szczepanowska, W.J.H. Koopman, M.R. Wieckowski, *Methods,* 2016, **109**, 3–11.

S3. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, G. a. Petersson, H. Nakatsuji, X. Li, M. Caricato, A.V. Marenich, J. Bloino, B.G. Janesko, R. Gomperts, B. Mennucci, H.P. Hratchian, J.V. Ortiz, A.F. Izmaylov, J.L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V.G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M.J. Bearpark, J.J. Heyd, E.N. Brothers, K.N. Kudin, V.N. Staroverov, T.A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A.P. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, J.M. Millam, M. Klene, C. Adamo, R. Cammi, J.W. Ochterski, R.L. Martin, K. Morokuma, O. Farkas, J.B. Foresman, D.J. Fox, (2009) Gaussian 09, Revision E.01, Gaussian, Inc., Wallingford.