Electronic Supporting Information

for

An octabrominated Sn(IV) tetraisopropylporphyrin as a photosensitizer dye for singlet oxygen biomedical applications

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Materials

All reagents and solvents were procured form Sigma Aldrich. 4-isopropyl benzaldehyde, $SnCl_2$, 2′,7′-dichlorofluorescin diacetate (DCF-DA), N-acetyl-L-cysteine (NAC), 5,10,15,20-tetraphenylporphyrinato zinc(II) (ZnTPP), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), Triton-100 X were obtained from Sigma Aldrich. All solvents used were of analytical grade and were purified and dried by routine procedures immediately before use. Cultures of the MCF-7 cell were obtained from Cellonex®. 10% (v/v) heat-inactivated 10% fetal bovine serum (FBS) and 100 unit/mL penicillin-100 µg/mL streptomycin-amphotericin B were obtained from Biowest®. Dulbecco's phosphate-buffered saline (DPBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Lonza®. Nutrient agar and agar bacteriological BBL® Mueller Hinton broth were purchased from Merck and prepared according to the specifications provided. *S. aureus* (ATCC 25923) was obtained from Davies Diagnostics.

^{1}H NMR spectra were recorded with a Bruker 600 MHz instrument using trimethylsilane (TMS) as an internal standard. UV-visible absorption spectra were measured on a Shimadzu UV−2550 spectrophotometer. MALDI-TOF mass spectra were recorded on a Bruker® AutoFLEX III Smart-beam TOF/TOF mass spectrometer by using α-cyano-4-hydroxycinnamic acid as the matrix.

Steady state fluorescence spectra were obtained with a Varian Cary-Eclipse spectrofluorimeter. The fluorescence quantum yield (Φ_F) values were calculated by using a comparative method with ZnTPP (Φ_F = 0.039 in DMF[S1]) as the standard. Triplet state lifetimes were determined

in nitrogen saturated dimethylformamide (DMF) solutions at 500 nm by using an Edinburgh Instruments LP980 spectrometer and a pump beams of 425 and 470 nm for **SnH** and **SnBr**, respectively, provided by an Ekspla NT-342B laser fitted with an OPO (2.0 mJ/7 ns, 20 Hz).

Singlet oxygen quantum yields were deterimined by measuring singlet oxygen phosphorescecne (1270 nm) of the Sn(IV) porphyrins and reference compounds (ZnTPP, Φ_Δ = 0.53) in DMF as described in the literature.[S2] Steady-state phosphorescence measurements were carried out on a Picoquant Fluo Time 300 spectrometer equipped with a Near-IR PMT.

SnH and **SnBr** were found to be soluble in acetonitrile, chloroform, dichloromethane, dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and 1% DMSO-DMEM media, while they are only sparingly soluble in water.

Cell studies

The *in vitro* anticancer activities of the Sn(IV) porphyrins were evaluated against MCF-7 cell line by using the MTT assay.[S3] The MCF-7 cells cultured in Dulbecco's modified Eagle's medium (DMEM) containing l-glutamine and phenol red, and supplemented with 10% heat-inactivated fetal calf serum (FCS) and 100 unit/ml-penicillin–100 μg/ml-streptomycin-amphotericin B. When the cells were 80% confluent, they were collected and seeded in 96-well plates at a density of 1×10^4 cells per well 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 containing suitable aliquots of 1000 μM DMSO stock solutions of **SnH** and **SnBr** to prepare different concentrations (0.78−50 μM) with the DMSO diluted to < 1.0% in each case. 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 and 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 were prepared and no light treatment was performed. Cell viability was determined by MTT assay.[S3] 20 μL of MTT (5mg/mL) solution was added to each well and incubated for 3 h to form purple formazan crystals. The medium was discarded carefully and 200 μL of DMSO was added to dissolve formazan crystals. Data were quantified by measuring the absorbance at 540 nm with a Molecular Devices Spectra Max M5 plate reader. The

cytotoxicities of the complexes were measured as a percentage ratio of the absorbance of the treated cells relative to the untreated controls. The IC_{50} values were determined by nonlinear regression analysis (GraphPad Prism 5).

Cellular uptake

MCF-7 cells (1×10^5 cells) were seeded in 24-well cell culture plates and incubated for 24 h. The cells were exposed to **SnH** and **SnBr** in 10 μM in DMEM media (DMSO < 1.0%) 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 absorption at 430 nm for **SnH** and 480 nm for **SnBr** with an ELISA reader. Control experiments were carried out in the absence of Sn(IV)porphyrin treatment.

DCF-DA assay

The intracellular production of reactive oxygen species (ROS) was detected using the 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) assay.[S4] 1×10^5 MCF-7 cells were seeded in a 96 well plate and incubated for 24 h. Cells were incubated with 10 μM of **SnH** and **SnBr** in DMEM media (DMSO < 1.0%) for 4 h in dark. DCFDA (10 μM, final concentration) were added and incubated further for 30 min in the dark. Cells were washed three times with PBS to remove any extracellular compounds and DCF-DA and were irradiated at 660 nm with a Thorlabs M660L3 LED mounted into the housing of a Modulight 7710-680 medical laser system for 15 and 30 min. A similar 96 well plate is kept in dark. Cells were analysed using a multi-plate reader with excitation and emission wavelengths of 485 and 535 nm, respectively.[S4] The same experiment was also performed in the presence of 1 mM N-acetyl cysteine (NAC), as a ROS quencher. Hydrogen peroxide (H_2O_2) was used as a positive control and untreated cells were used as a negative control. For imaging purposes, cells were view under microscopy and images were captured.

Antimicrobial Studies

The preparation of the bacteria culture (*S. aureus*) and photodynamic antimicrobial studies were carried out by following the literature procedures with some modifications.[S5] For concentration optimisation studies, **SnBr** at different concentration (1, 2.5, 5, 10, 15 μM) dissolved in 1% DMSO/PBS is treated against *S. aureus* and irradiated for 60 min at 660 nm with a Thorlabs M660L3 LED and percentage cell survival was calculated. To study the time

dependent PACT activity, 5 mL aliquots of mixtures of the Sn(IV) porphyrin and *S. aureus* were prepared to provide the final study concentration of 5 μM. The mixtures were incubated in an oven equipped with a shaker for 30 min in the dark at 37°C. 2.5 mL of the incubated bacterial solutions were irradiated at 660 nm for 15, 30, 45, 60 min with a Thorlabs M660L3 LED mounted into the housing of a Modulight 7710-680 medical laser system (280 $mW.cm^{-2}$) in a 24 well plate, while the other 2.5 mL was kept on a plate in the dark to provide a control. After each irradiation, a 100 μL sample was pipetted out and spotted on agar plates with a micropipette. The plates were kept in the dark and were incubated at 37°C overnight for 24 h. A Scan 500 Automatic Colony Counter from Healthcare Technologies was used for the colony forming unit (CFU) determinations. The survival fractions were calculated through comparisons with a control solution containing no photosensitizer dye. The experiments were carried out in triplicate, so that standard deviations could be calculated.

Synthesis

5,10,15,20-Tetra(4-isopropylphenyl)porphyrin (**1H**)[S6a] and 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetra(4-isopropylphenyl)porphyrin (**1Br**)[S6b] were synthesized using methods described previously in the literature and **SnH** and **SnBr** were formed with a metal insertion reaction.

Dihydroxy[5,10,15,20-tetra(-4-isopropyl) phenyl porphyrinate] Sn(IV) (SnH, 1)

5,10,15,20-Tetra(4-isopropylphenyl) porphyrin (**1H**, 0.39 g, 0.5 mmol) and $SnCl_2$ (0.19 g, 1 mmol) were dissolved in 30 ml of pyridine and refluxed for 4 h. The reaction mixture was cooled and 15 ml of aqueous ammonia solution was added carefully and stirred for additional 1 h. 100 ml of water was added and the precipitate formed was filtered, dried and purified in neutral alumina (chloroform) to afford **SnH** as a purple solid (Yield: 0.38 g, 82 %). MS (MALDI-TOF): m/z for [M+H] = 935.44 (calc. 934.33), [M-2(OH)] = 900.46 (calc. 900.32). ^{1}H NMR ($CDCl_3$, 600 MHz): 9.19 (s, 8 H, β-pyrrolic), 8.22 (d, 8 H, 7.92 Hz, o-Ph-H), 7.66 (d, 8 H, 5.32 Hz, m-Ph-H), 3.29 (m, 4 H, isopropyl (CH)-H)), 1.57 (d, 24 H, 6.92 Hz, isopropyl (CH_3)-H) ppm.

Dihydroxy [2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetra (4-isopropyl) phenyl porphyrinate] Sn(IV) (SnBr, 2)

0.28 g (0.2 mmol) of $SnCl_2$ and 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetra(4-isopropylphenyl) porphyrin (**1Br**, 0.095 g, 0.5 mmol) were added to 5 ml of dry DMF and

refluxed for 15 min. The reaction mixture was cooled and added to 100 ml of NaCl solution to precipitate out the compound. The precipitate was collected by centrifugation, dried and subjected to column chromatography (neutral alumina, $CHCl_3$) to afford **SnBr** as a green solid (Yield: 0.22 g, 70%). MS (MALDI-TOF): m/z for [M+H] = 1566.75 (calc. 1565.60). ^{1}H NMR ($CDCl_3$, 600 MHz): 8.11 (d, 8 H, 8.00 Hz, o-Ph-H), 7.63 (d, 8 H, 7.96 Hz, m-Ph-H), 3.23 (m, 4 H, isopropyl (CH)-H)), 1.49 (d, 24 H, 6.88 Hz, isopropyl (CH_3)-H) ppm.

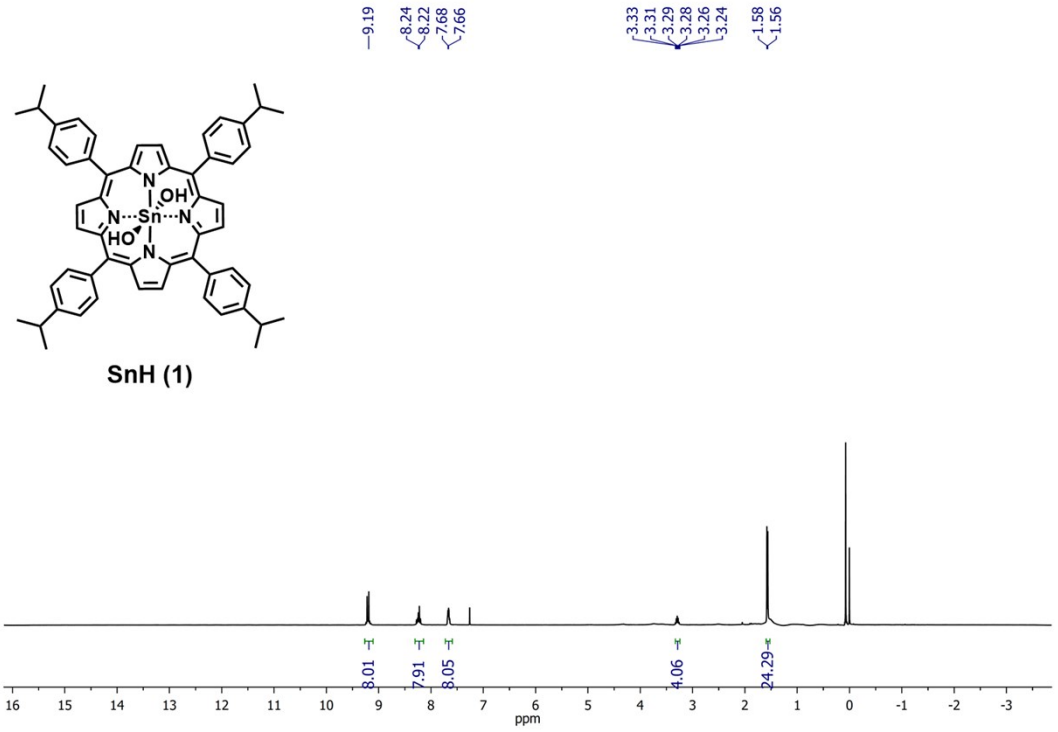

Figure S1. ^{1}H NMR (600 MHz) spectrum of **SnH** in $CDCl_3$.

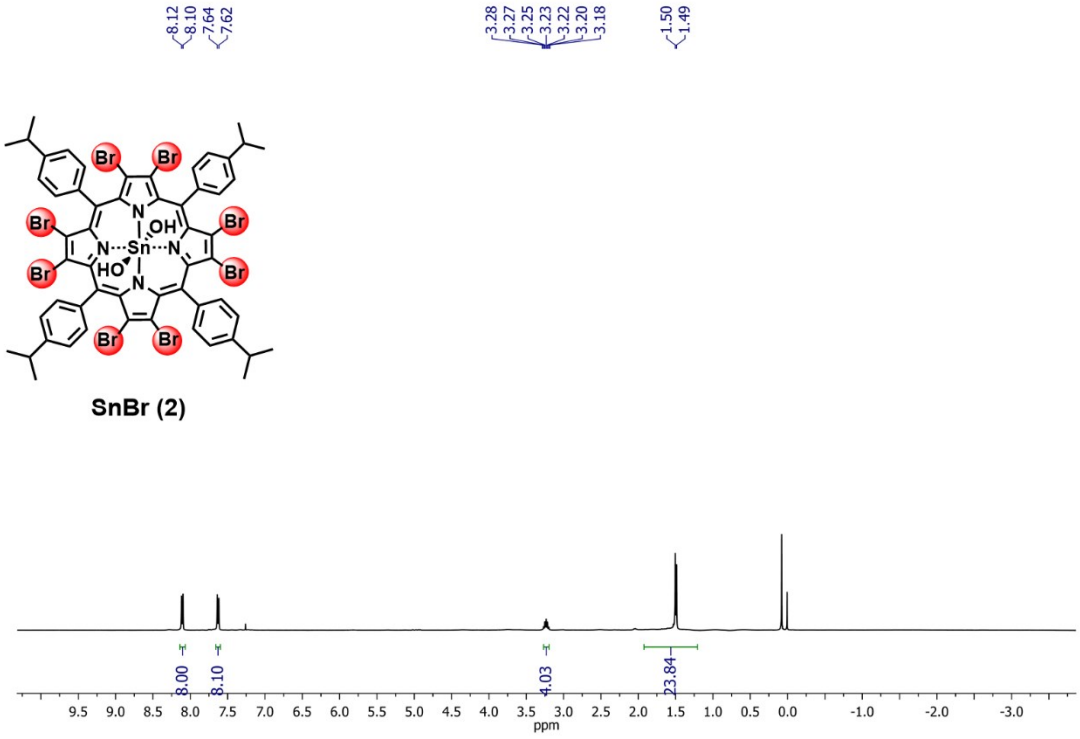

Figure S2. ^{1}H NMR (600 MHz) spectrum of **SnBr** in $CDCl_3$.

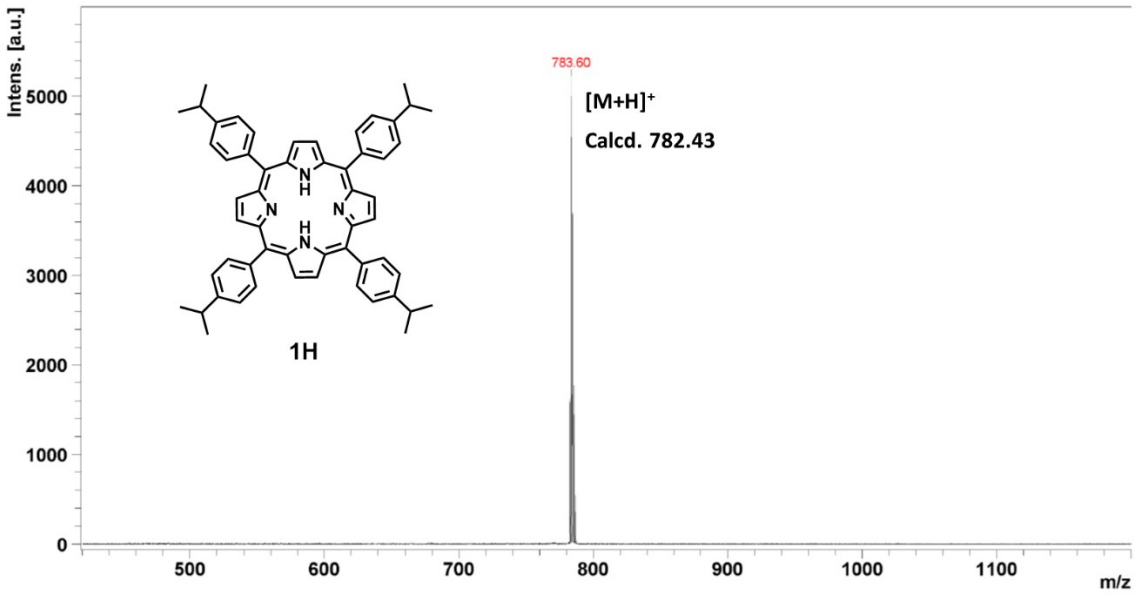

Figure S3. MALDI-TOF MS data for **1H**.

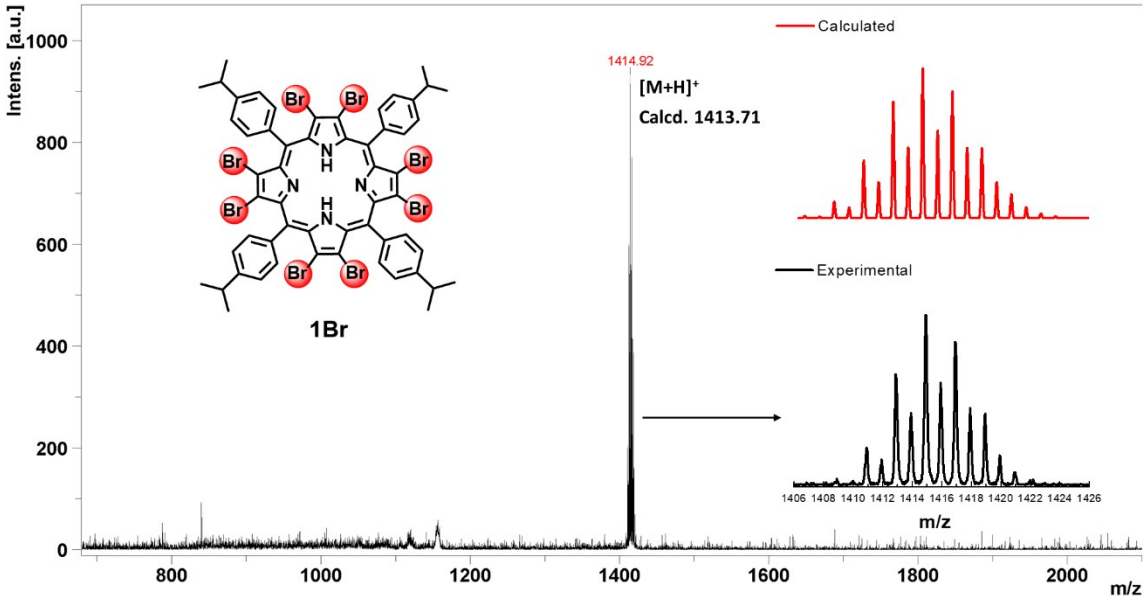

Figure S4. MALDI-TOF MS data for **1Br**.

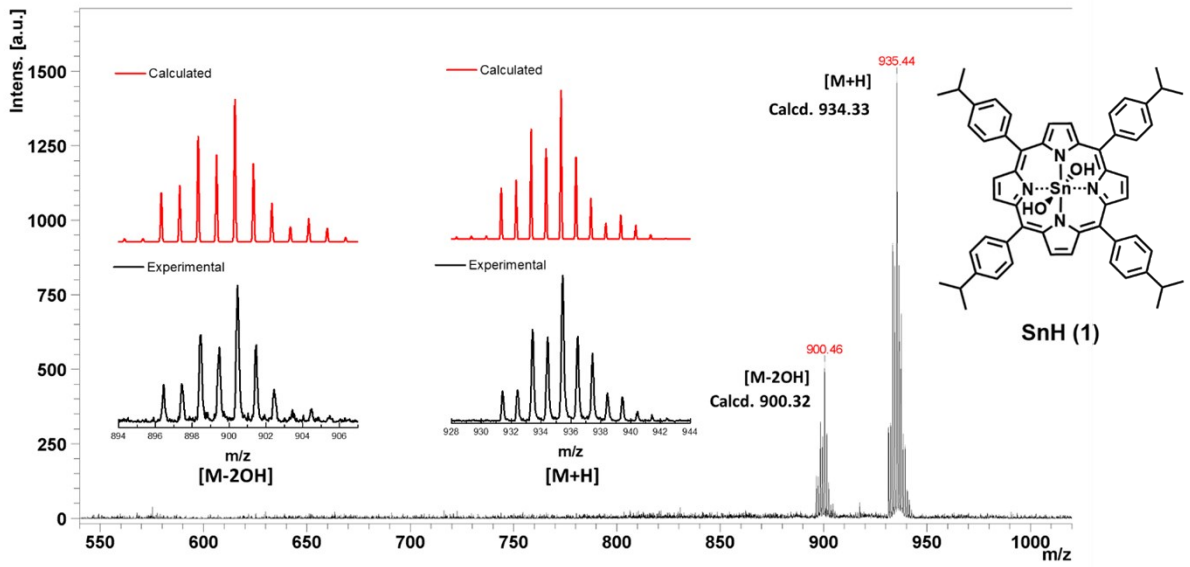

Figure S5. MALDI-TOF MS data for **SnH** (**1**).

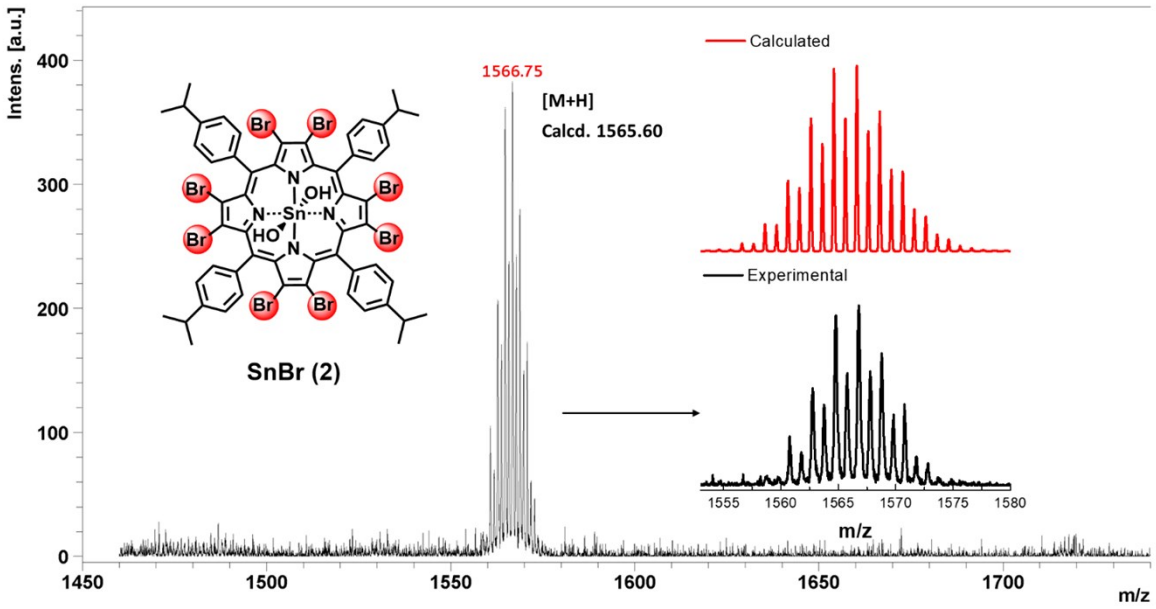

Figure S6. MALDI-TOF MS data for **SnBr** (**2**).

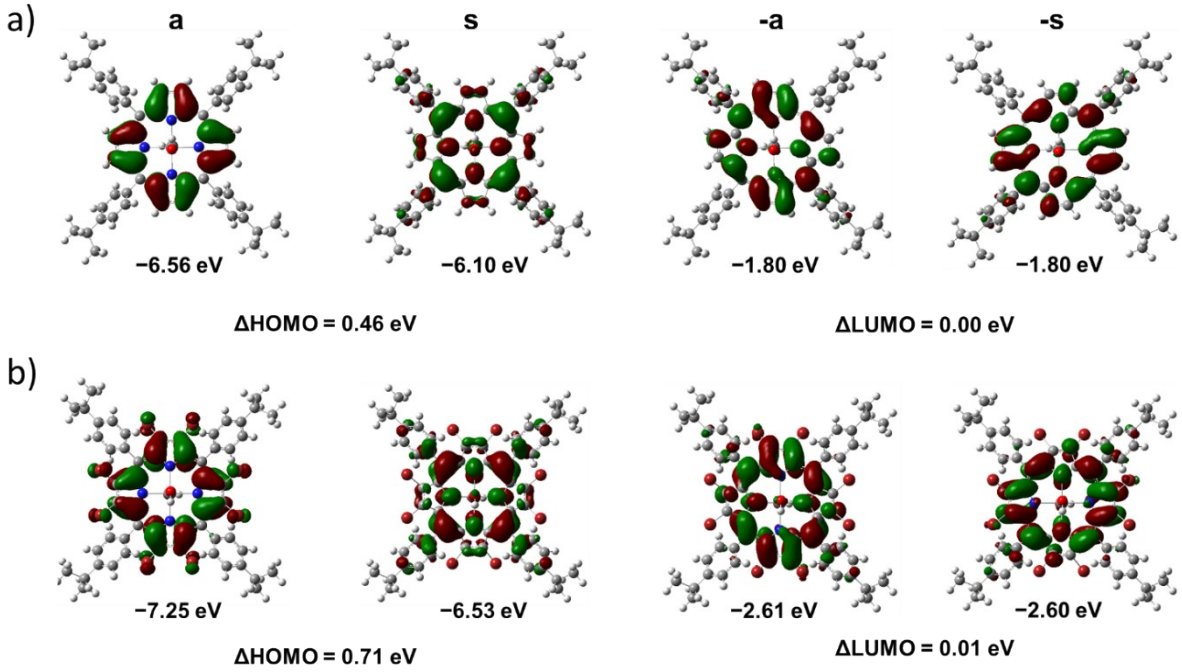

Figure S7. The angular nodal patterns and MO energy values of the **a**, **s**, **-a** and **-s** MOs of (a) **SnH** and (b) **SnBr** at an isosurface value of 0.02 a.u.

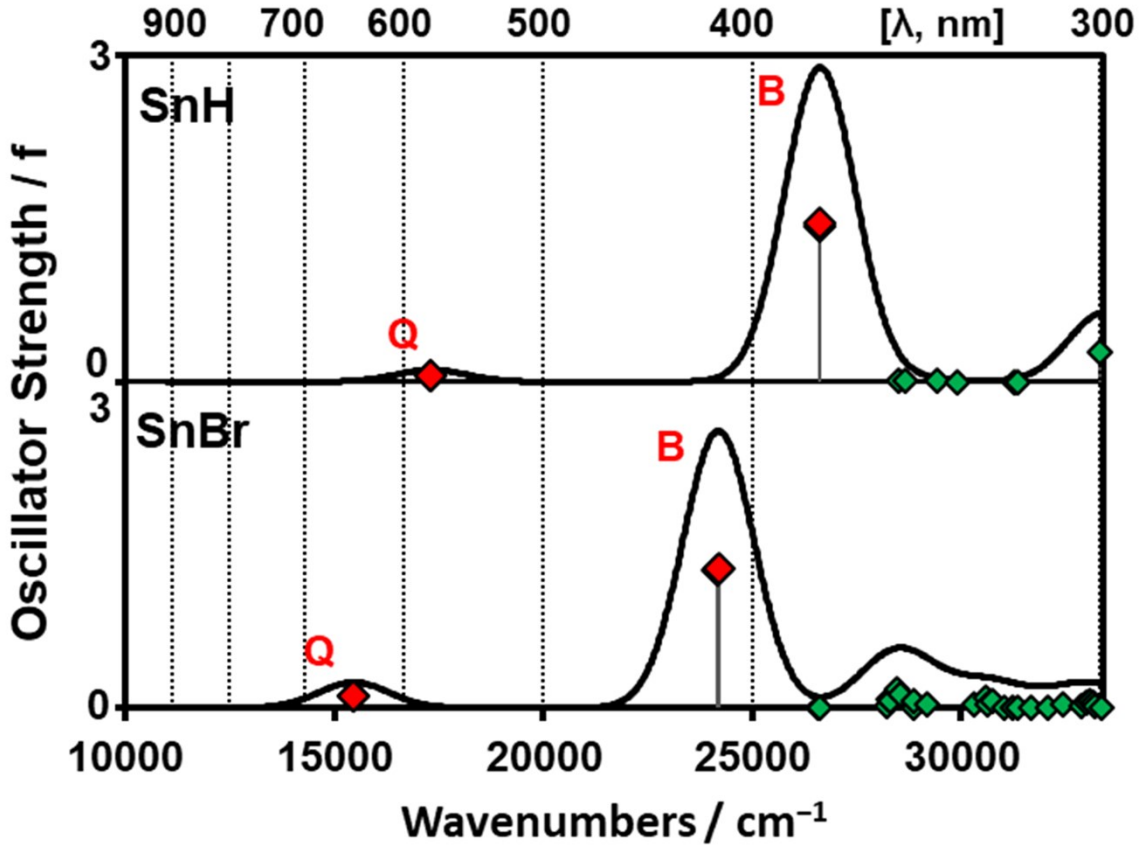

Figure S8. Calculated TD-DFT spectra for B3LYP-optimized geometries of **SnH**, **SnBr** at the CAM-B3LYP/SDD level of theory. Red diamonds are used to highlight the Q and B bands of Gouterman's 4-orbital model. Simulated spectra were generated using the Chemcraft program with a fixed bandwidth of 2000 cm^{-1}.

Table S1. The calculated UV-visible absorption spectra of the B3LYP optimized geometry of **SnH** and **SnBr** obtained by using the CAM-B3LYP functional of the Gaussian 09 software package [S7] with 6-31G(d) basis sets.

			SnH		
	#[a]	**λ_{exp}[b]**	**λ_{calc}[c]**	***f*[d]**	**Wavefunction =[e]**
Q	1	605	578	0.05	**57% s → -s; 30% a → -a; 8% s → -a;** …
Q	2		577	0.06	**57% s → -a; 30% a → -s; 8% s → -s;** …
B	3	429	376	1.44	**57% a → -a; 29% s → -s; 7% a → -s;** …
B	4		376	1.46	**58% a → -s; 29% s → -a; 7% a → -a;** …
			SnBr		
	#[a]	**λ_{exp}[b]**	**λ_{calc}[c]**	***f*[d]**	**Wavefunction =[e]**
Q	1	675	647	0.12	**46% s → -a; 28% s → -s; 15% a → -s; 10% a → -a**; …
Q	2		647	0.12	**46% s → -s; 28% s → -a; 16% a → -a; 10% a → -s**; …
B	3	478	414	1.27	**67% a → -a; 22% s → -s**; …
B	4		413	1.28	**67% a → -s; 22% s → -a**; …

[a]Excited state number assigned in increasing energy in the TD-DFT calculations. [b]Experimental wavelengths in nm, recorded in Table 1. [c]Calculated wavelengths in nm. [d]Calculated oscillator strengths. [e]Wavefunctions describing the MOs involved in the transition based on eigenvectors predicted by TD-DFT. Only one-electron transition contributions of more than 5% are included. **a**, **s**, **-a** and **-s** refers to the MO nomenclature of Michl's perimeter model. One-electron transitions between these four MOs are highlighted in bold.

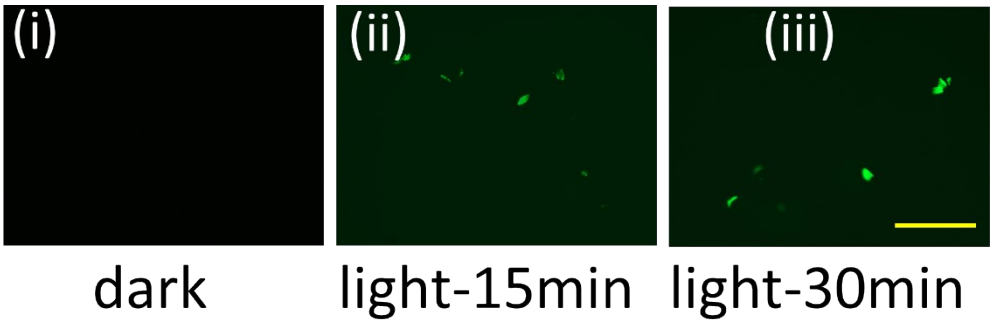

Figure S9. Fluorescent images of MCF-7 cells for the detection of ROS generation by **SnBr** with the DCF-DA assay, (i) in the dark, (ii) after 15 min light irradiation, and (iii) 30 min light irradiation. [Scale:200 μm]

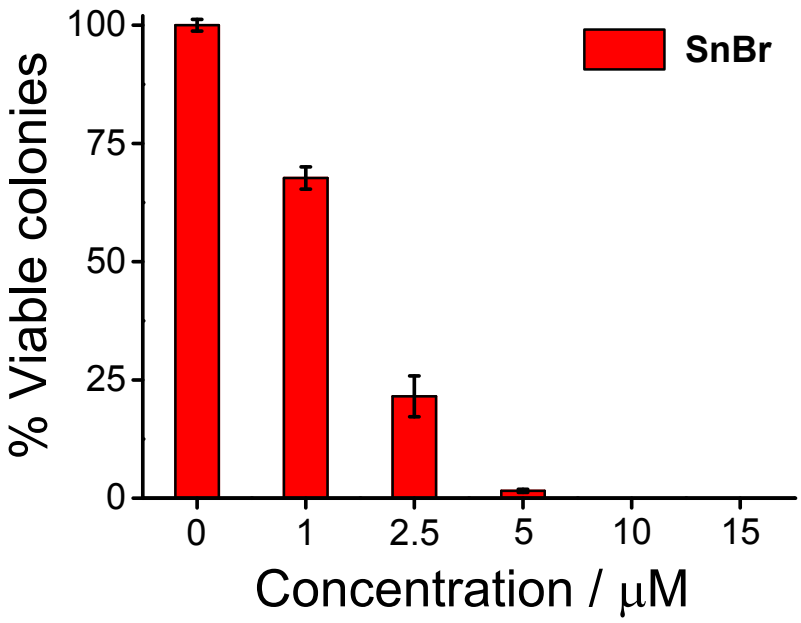

Figure S10. Antimicrobial activities of **SnH** (0−15 μM) towards *S. aureus* when illuminated at 660 nm with a Thorlabs M660L3 LED (280 mW.cm^{-2}) for 60 min.

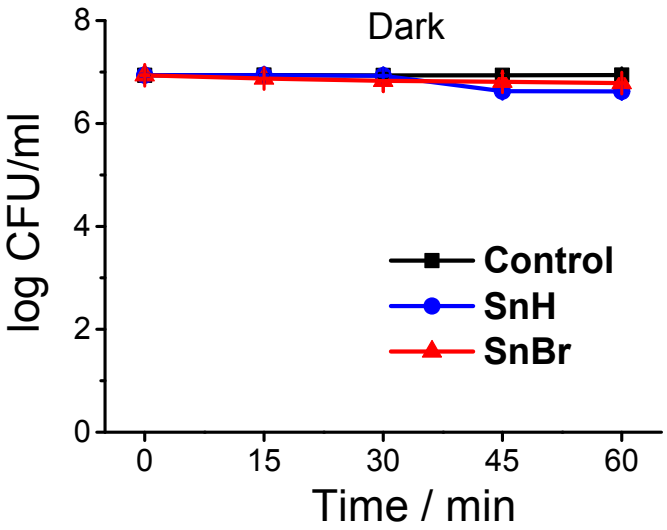

Figure S11. Logarithmic reduction of *S. aureus* treated with Sn(IV) porphyrins (**SnH** and **SnBr**) at 5 μM in the dark for 60 min.

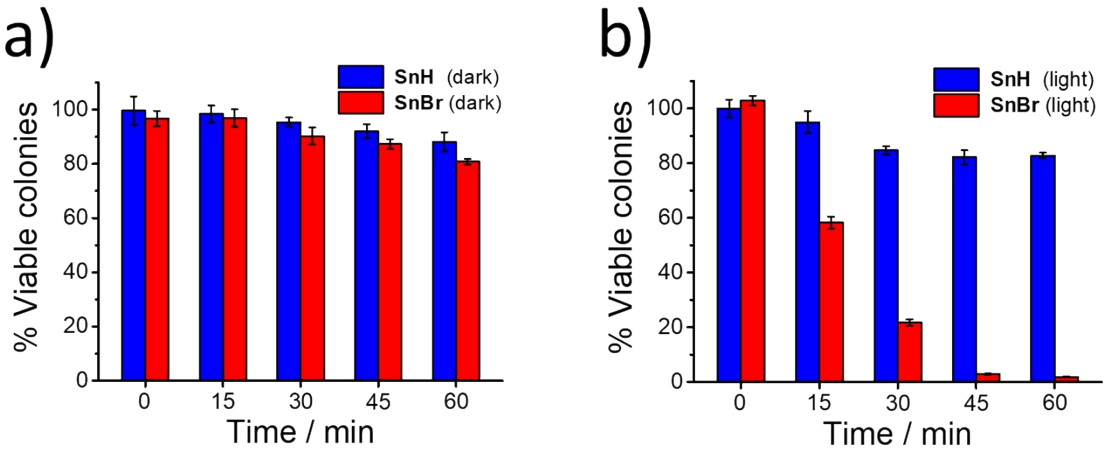

Figure S12. Antimicrobial activities of 5 μM of **SnH** and **SnBr** towards *S. aureus* in the (a) dark and (b) upon light irradiation at 660 nm with a Thorlabs M660L3 LED (280 $mW.cm^{-2}$).

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