IN VITRO EVALUATION OF SCHIFF BASE DECORATED PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY IN PC-3 PROSTATE CANCER CELLS

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SUPPORTING INFORMATION

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1. Materials

4-methoxybenzaldehyde, 4-aminophenol, anhydrous zinc(II) acetate, anhydrous indium(III) chloride, anhydrous potassium carbonate, glacial acetic acid, methanol, *N,N'*-dimethylformamide (DMF), n-pentanol, n-hexane, tetrahydrofuran (THF) and dimethylsulfoxide (DMSO) were purchased from Merck. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and phosphorous pentoxide were purchased from Sigma Aldrich. Absolute ethanol and 4-nitrophthalonitrile were purchased from Honeywell and Acros Organics, respectively.

2. Instrumentation

Absorption spectra in the UV-visible region were recorded with a Shimadzu 2001 UV spectrophotometer. FT-IR spectra were measured with a Perkin Elmer Spectrum One Spectrometer. The mass spectra were acquired on a Bruker Daltonics (Bremen, Germany) Microflex mass spectrometer equipped with an electron spray ionization (ESI) source. ^{1}H-NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ solutions on a Varian 500 MHz spectrometer. Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluorometer using 1 cm path length cuvettes at room temperature.

Photo-irradiations were measured using a General Electric quart line lamp (70W). A 600 nm glass cut off filter (Schott) and a water filter were used to filter off ultraviolet and infrared radiations respectively. An interference filter (Intor, 700 nm with a band width of 40 nm) was additionally placed in the light path before the sample. Light intensities were measured with a POWER MAX5100 (Mol electron detector incorporated) power meter.

3. Procedures applied for photophysical, photochemical and sono-photochemical Experiments

3.1. Fluorescence quantum yields

Fluorescence quantum yields (Φ_F) were determined by the comparative method (Eq. 1) [S1, S2],

$$\Phi_F = \Phi_{F_{(std)}} \frac{F.A_{std}.\eta^2}{F_{std}.A.\eta_{std}^2} \qquad (1)$$

where F and F_{Std} are the areas under the fluorescence emission curves of the samples and the standard, respectively. A and A_{Std} are the respective absorbances of the samples and standard at the excitation wavelengths, respectively. n^2 and n_{Std}^2 are the refractive indices of solvents used for the sample and standard, respectively. Unsubstituted ZnPc in DMSO (Φ_F = 0.20) [S3] was employed as the standard. All samples and standard were excited at the same wavelength. The absorbances of the sample and standard at the excitation wavelength was about 0.05.

3.2. Singlet oxygen quantum yields

Singlet oxygen quantum yield (Φ_Δ) was analyzed by using the interested method with ZnPc in DMSO as standard. DPBF was used as chemical quenchers for singlet oxygen determination in DMSO. Eq. (2) was used for the calculations:

$$\Phi_\Delta = \Phi_\Delta^{std} \frac{R.I_{abs}^{std}}{R^{std}.I_{abs}} \qquad (2)$$

where Φ_Δ^{Std} is the singlet oxygen quantum yield for the standard unsubstituted ZnPc (Φ_Δ^{Std} = 0.67 in DMSO) [S4]. R and R_{Std} are the DPBF photobleaching rates in the presence of the

respective samples (**3bH$_2$Pc**, **Zn3b** and **In3b**) and standard, respectively. I_{abs} and I_{abs}^{Std} are the rates of light absorption by the samples (**3bH$_2$Pc**, **Zn3b** and **In3b**) and standard, respectively. The sensitizer solutions that contain DPBF were prepared in the dark and irradiated in the Q band region using the set-up described above. DPBF degradation at 417 nm was observed. The light intensity 8.83 x 10^{15} photons s^{-1} cm^{-2} was used for Φ_{Δ} determinations.

3.3. Photodegradation quantum yields

Photodegradation quantum yields were determined using Eq. (3) [S5, S6],

$$\Phi_d = \frac{(C_0 - C_t).V.N_A}{I_{abs}.S.t} \qquad (3)$$

where ''C_0'' and ''C_t'' are the samples concentrations before and after irradiation respectively, ''*V*'' is the reaction volume, "N_A" is the Avogadro's constant, ''*S*'' is the irradiated cell area, ''*t*'' is the irradiation time and "I_{abs}" is the overlap integral of the radiation source light intensity and the absorption of the samples. A light intensity of 2.52x10^{16} photons s^{-1} cm^{-2} was employed for Φ_d determinations.

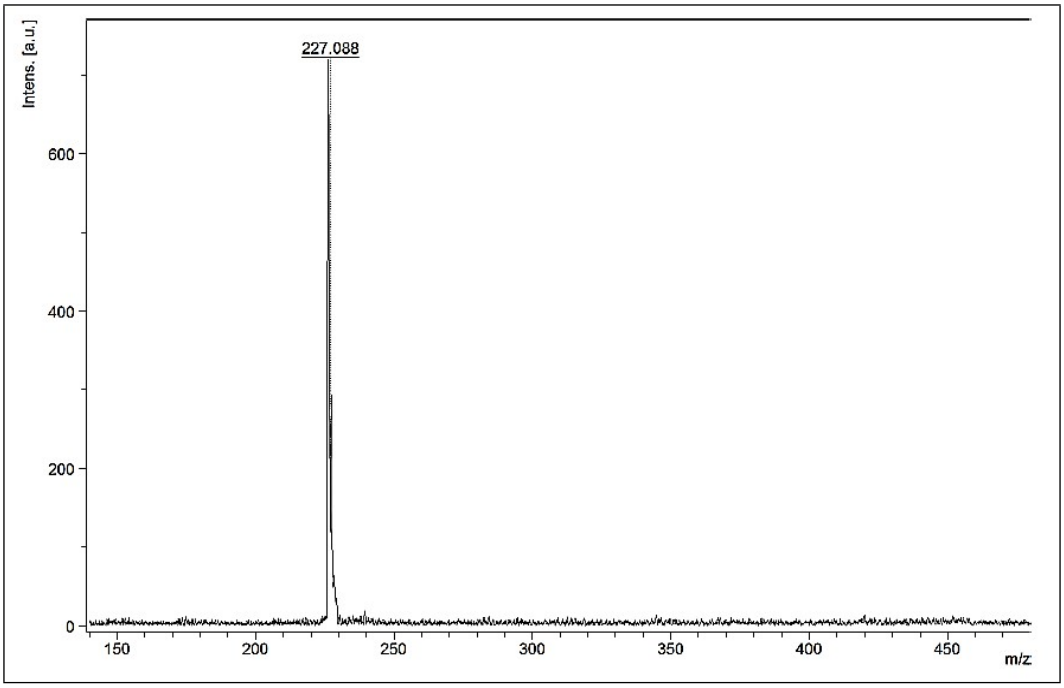

Figure S1. Mass spectra of compound **3a**.

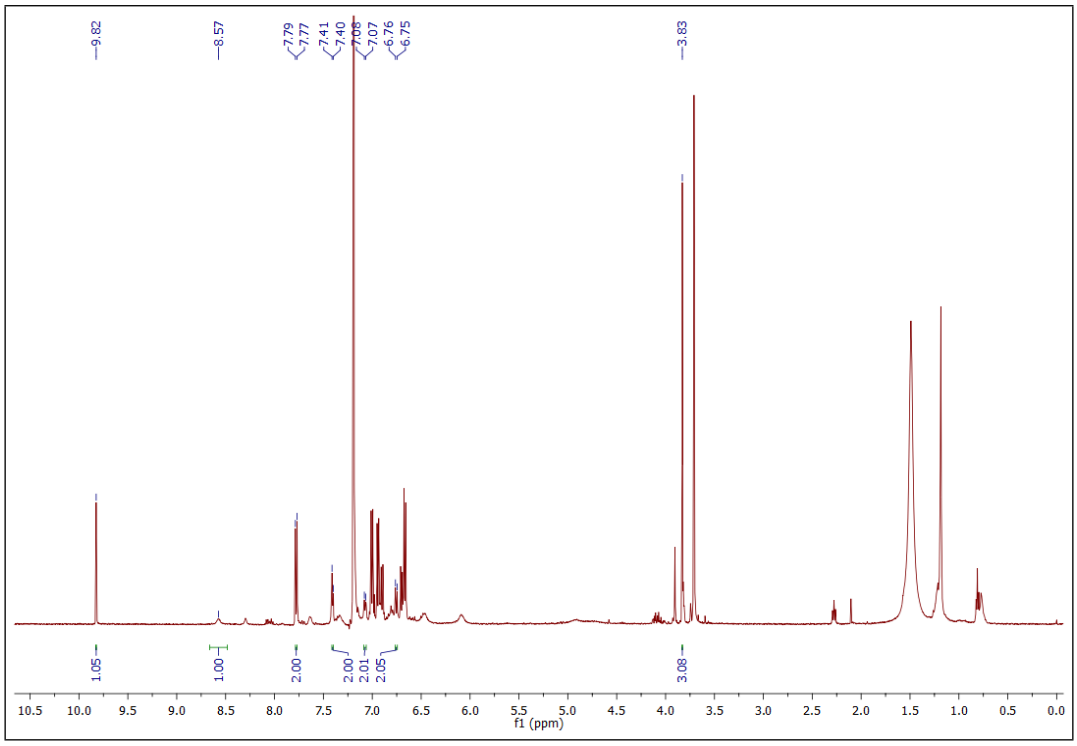

Figure S2. ^{1}H-NMR spectra of compound **3a**.

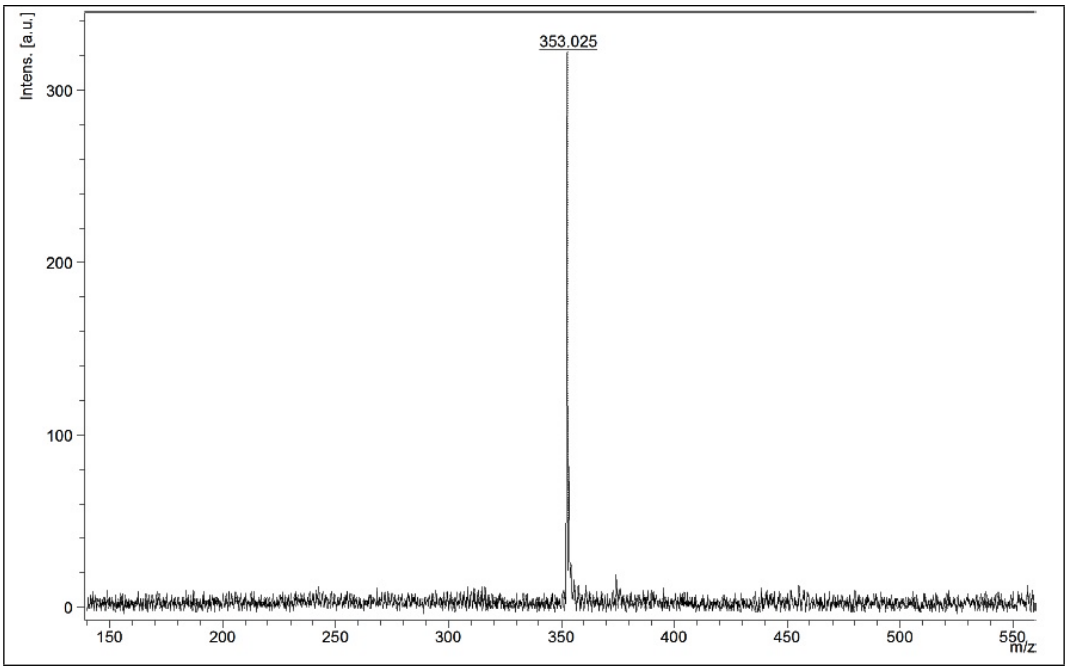

Figure S3. Mass spectra of compound **3b**.

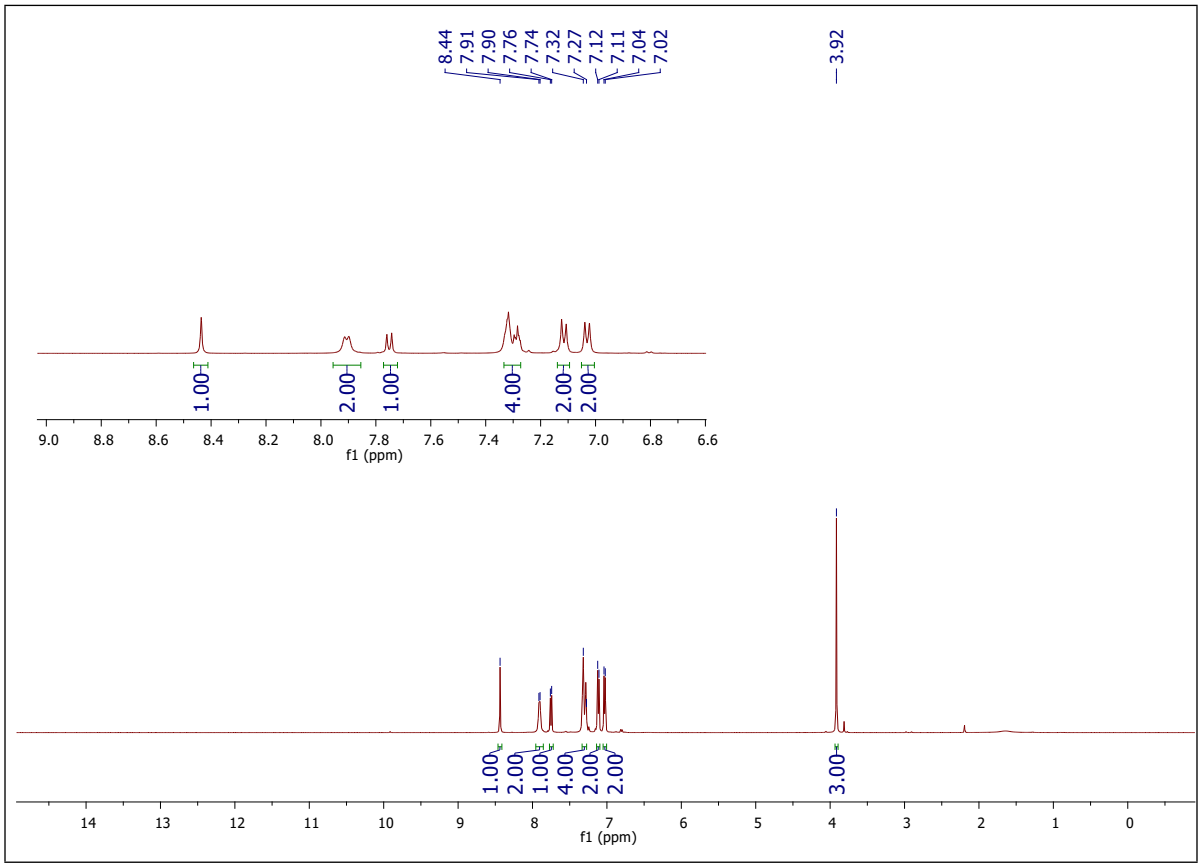

Figure S4. ^{1}H-NMR spectrum of compound **3b**.

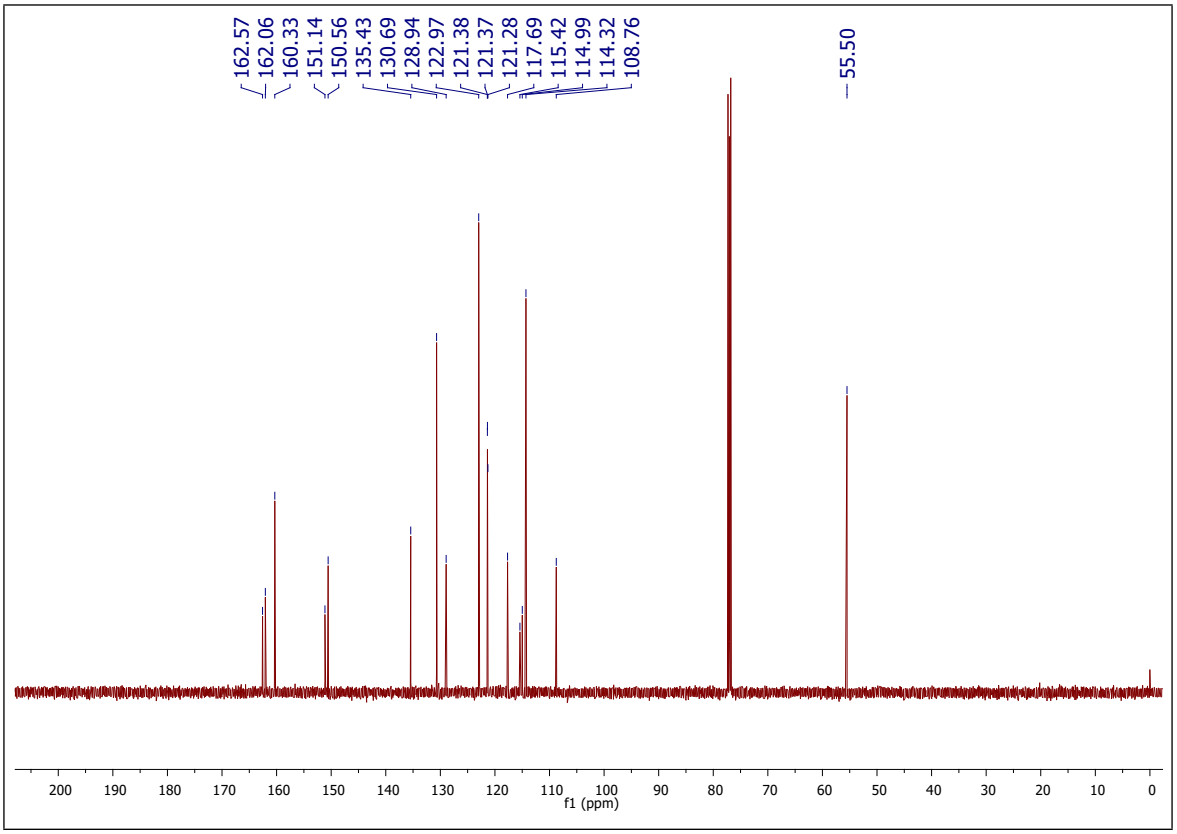

Figure S5. ^{13}C-NMR spectrum of compound **3b**.

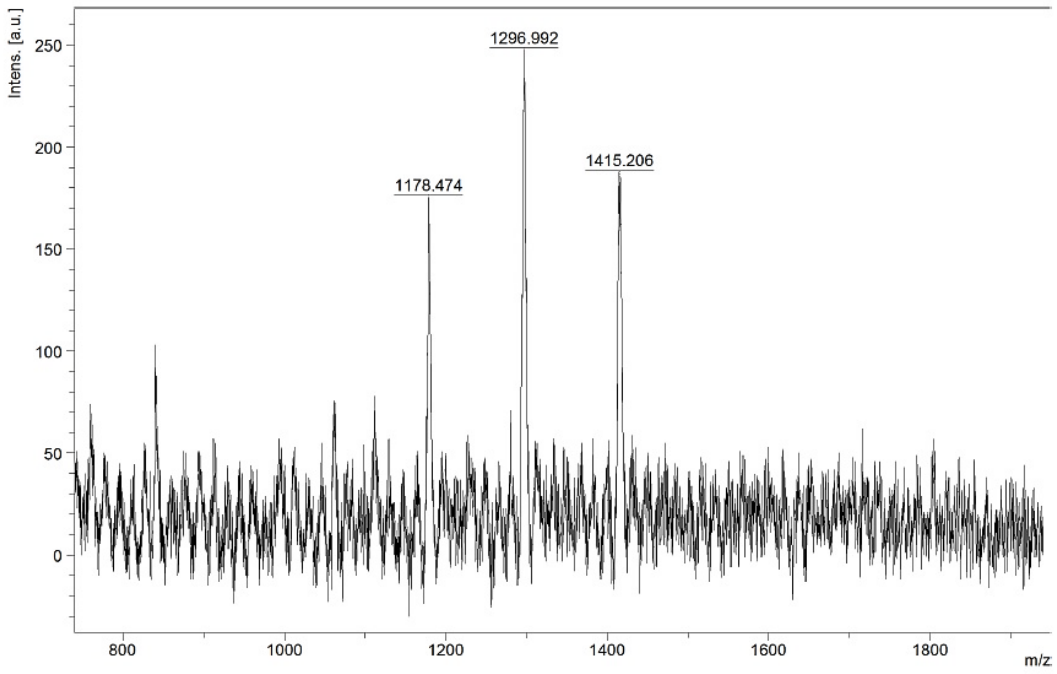

Figure S6. Mass spectra of compound **3bH$_2$Pc**.

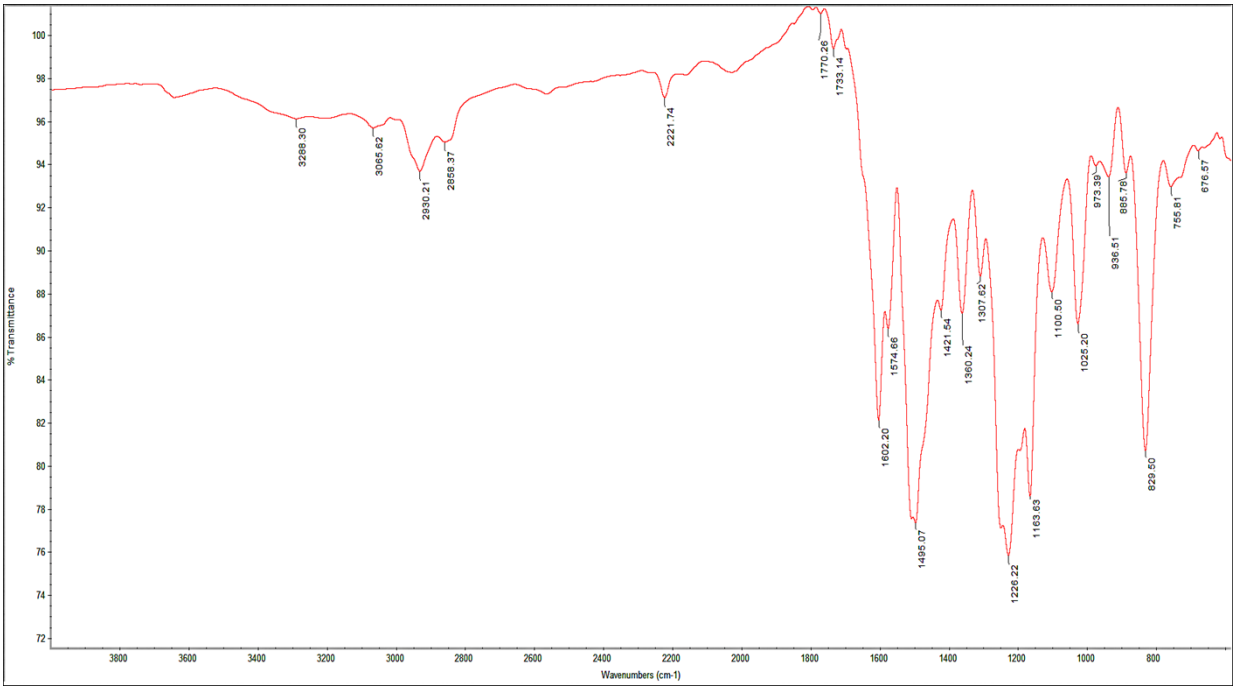

Figure S7. FT-IR spectra of compound **3bH$_2$Pc**.

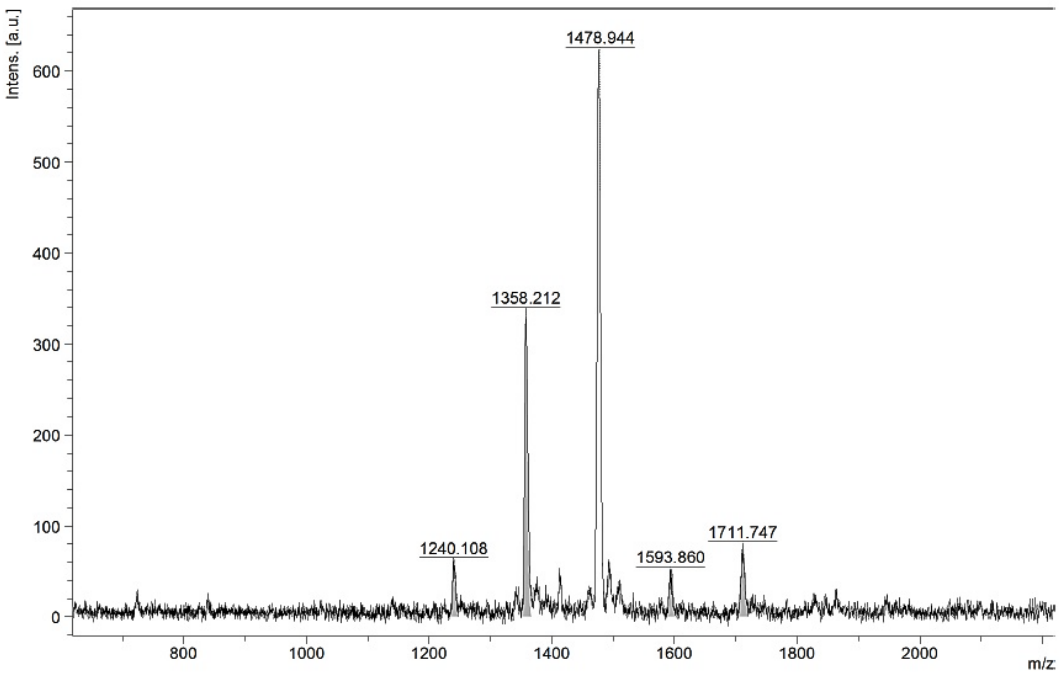

Figure S8. Mass spectra of compound **Zn3b**.

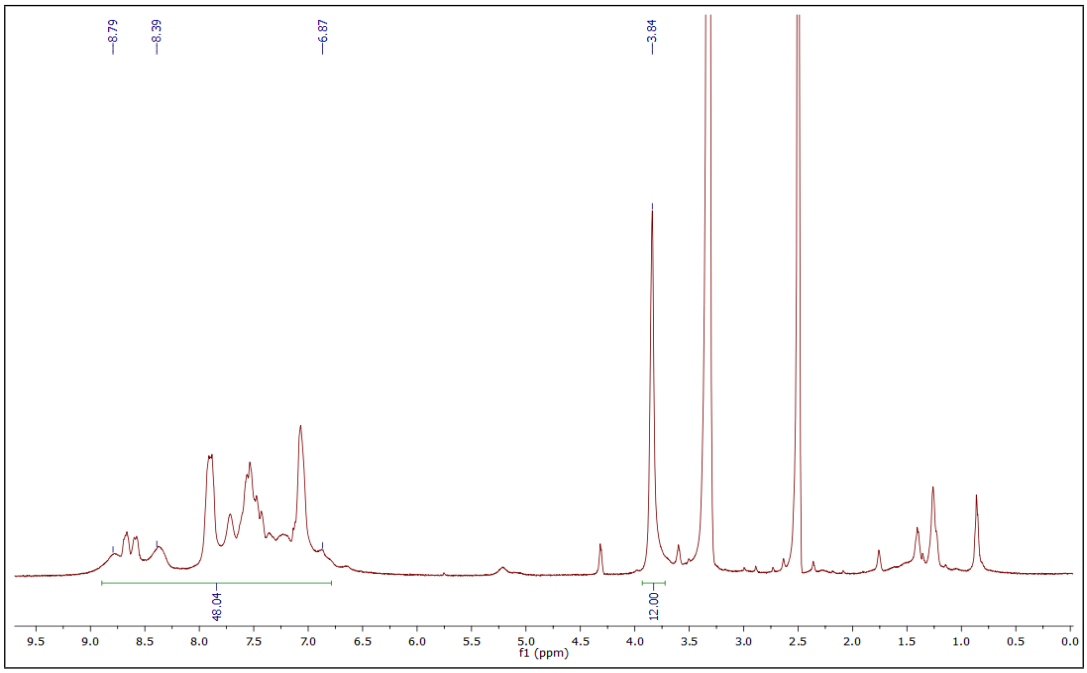

Figure S9. ^{1}H-NMR spectra of compound **Zn3b**.

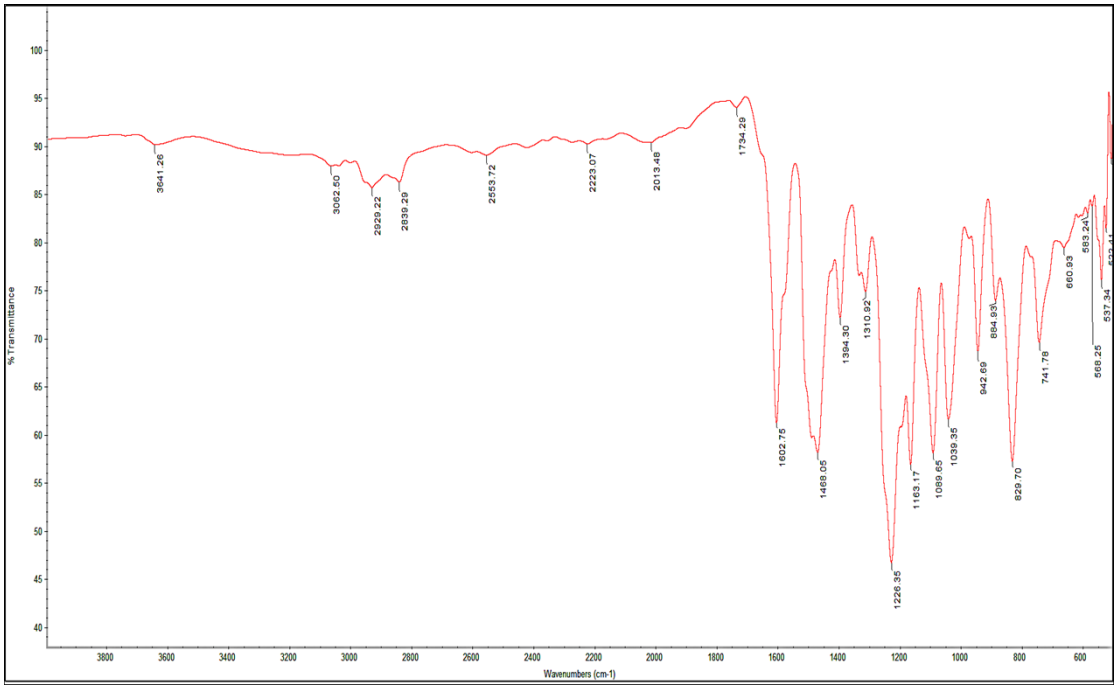

Figure S10. FT-IR spectra of compound **Zn3b**.

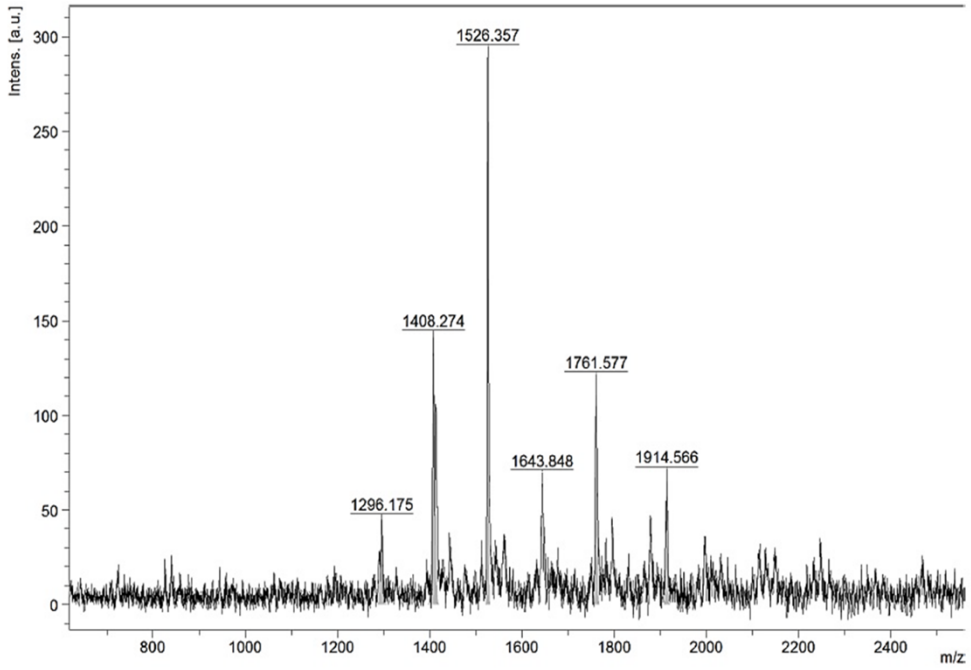

Figure S11. Mass spectra of compound **In3b**.

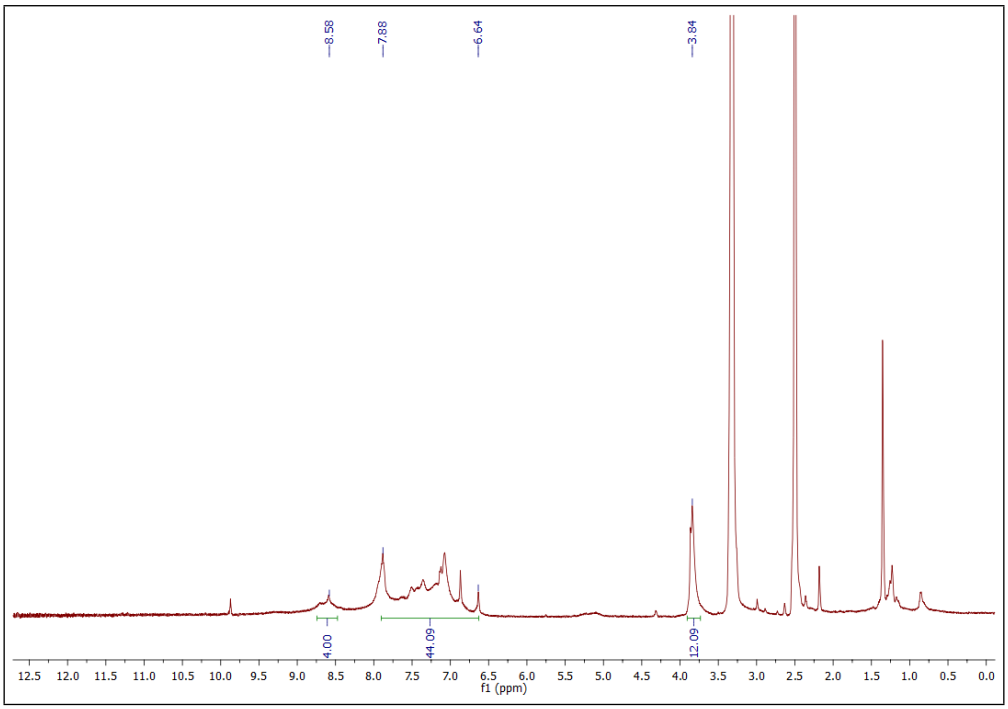

Figure S12. [1]H-NMR spectra of compound **In3b**.

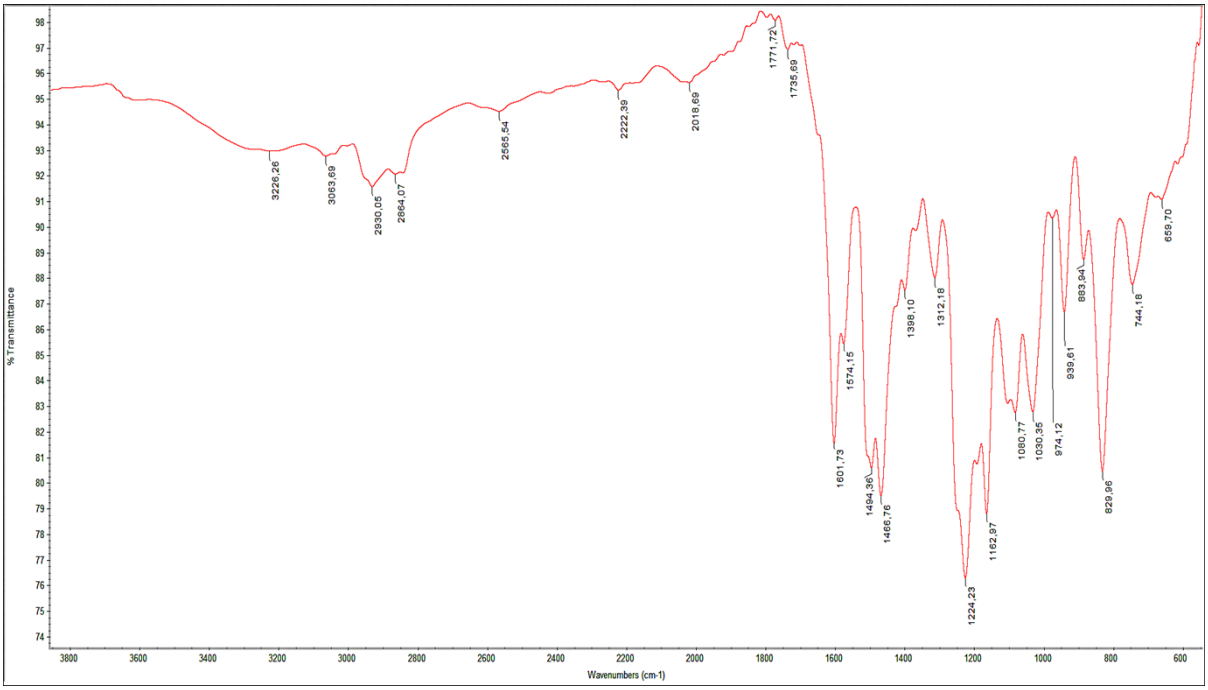

Figure S13. FT-IR spectra of compound **In3b**.

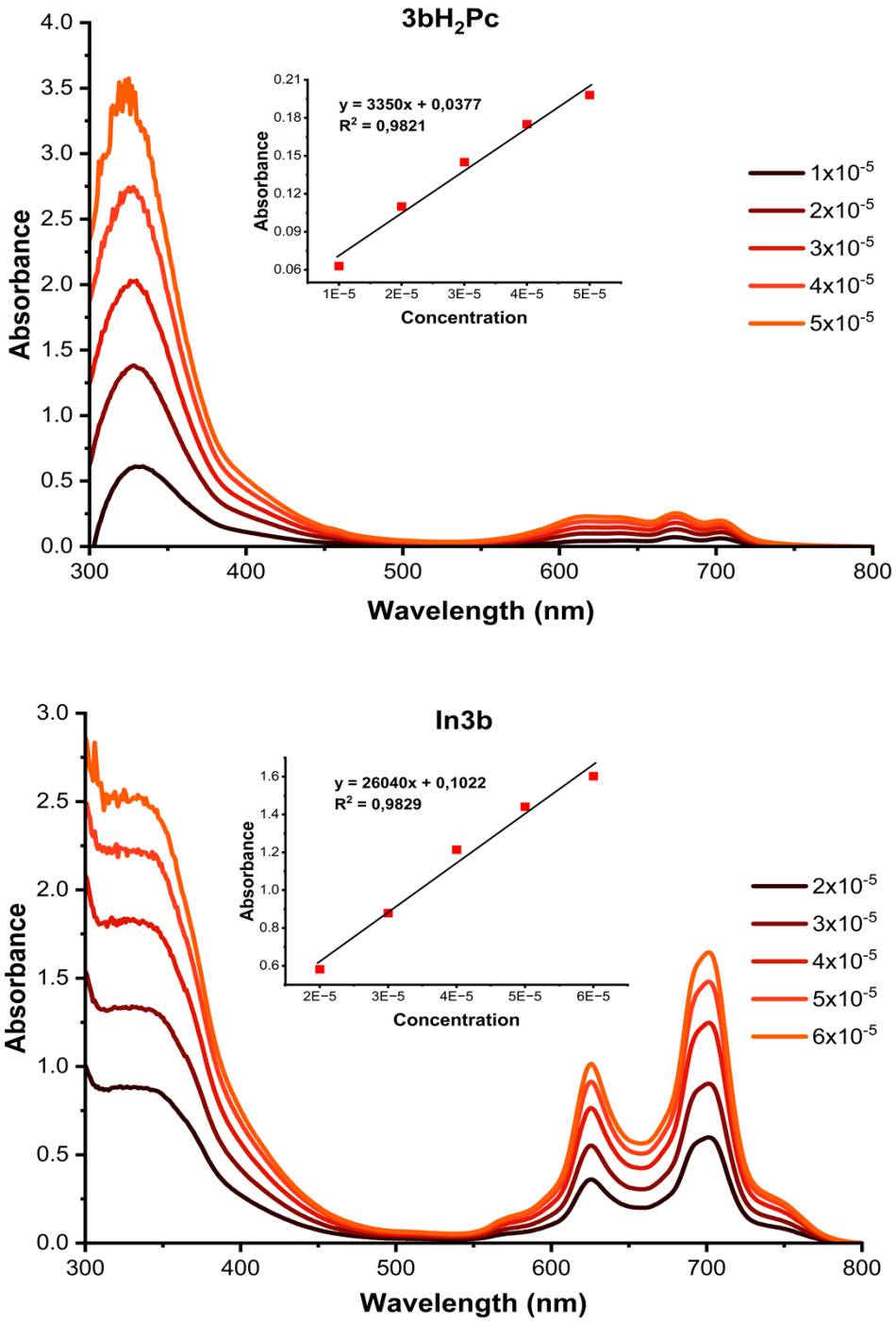

Figure S14. Absorbance changes of **3bH$_2$Pc** and **In3b** in DMSO at different concentrations

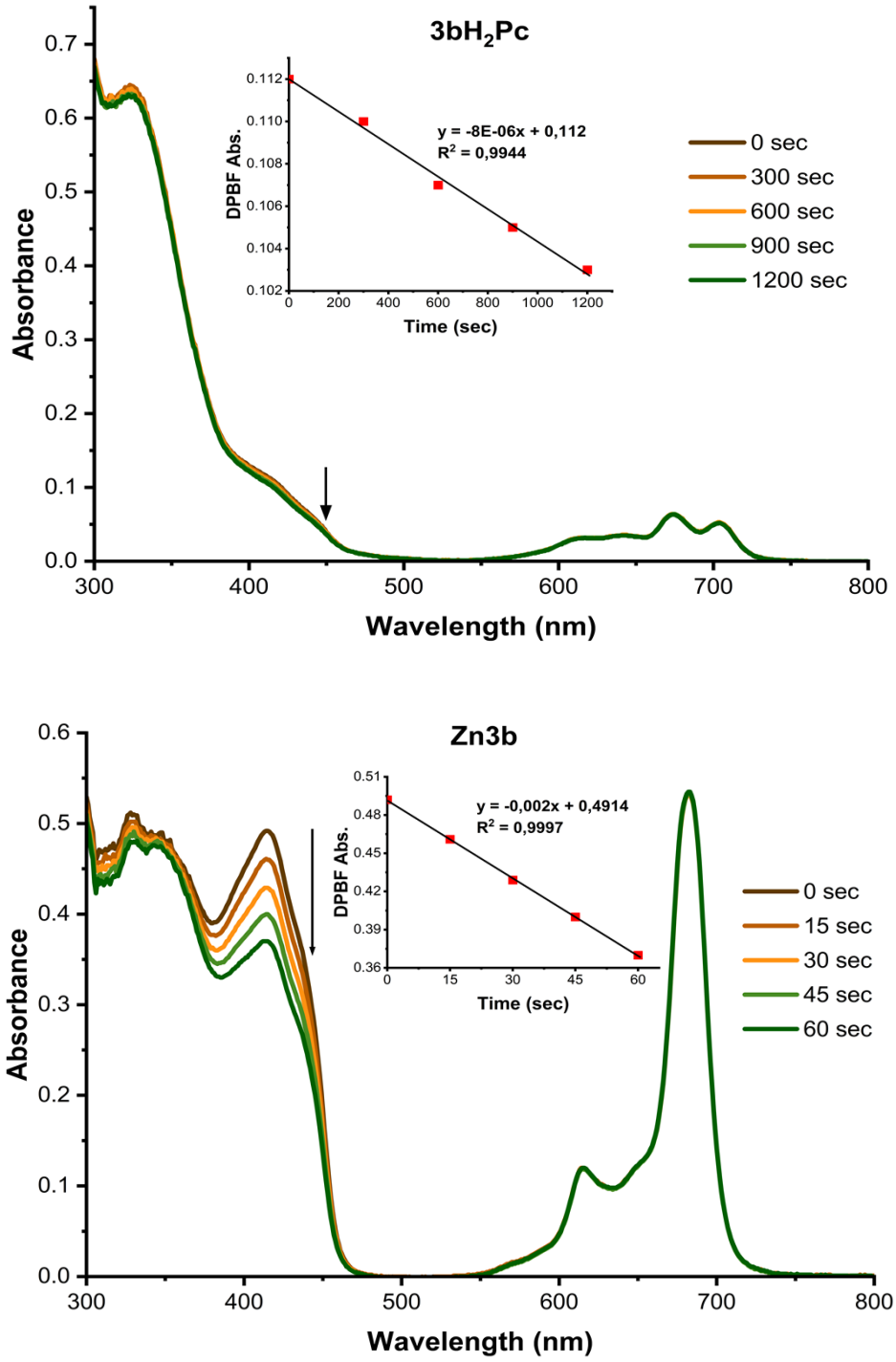

Figure S15. A typical spectrum for the determination of singlet oxygen quantum yield for: **3bH$_2$Pc** and **Zn3b** in DMSO using DPBF as a singlet oxygen quencher.

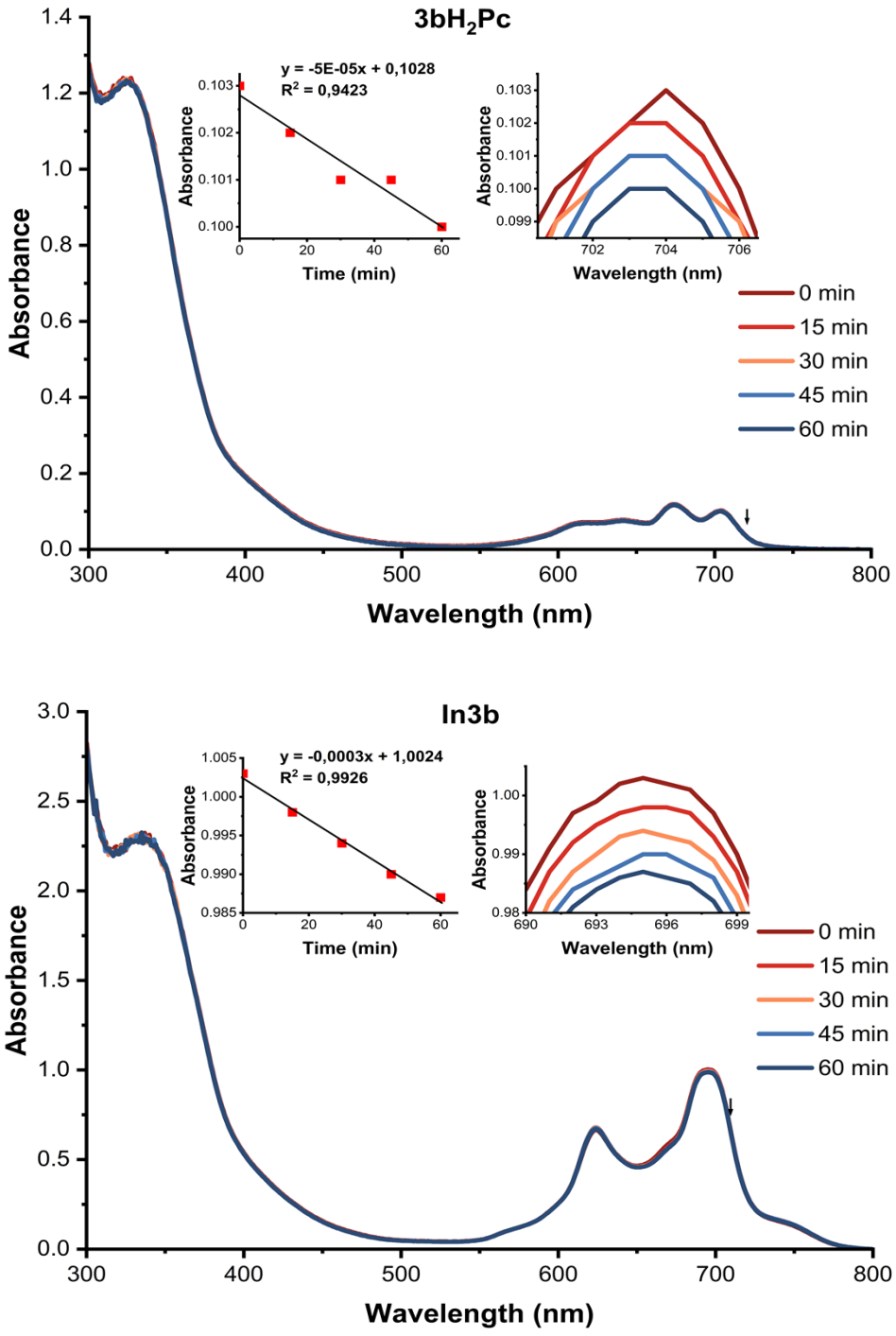

Figure S16. A typical spectrum for the determination of photodegradation for: **3bH$_2$Pc** and **In3b** in DMSO.

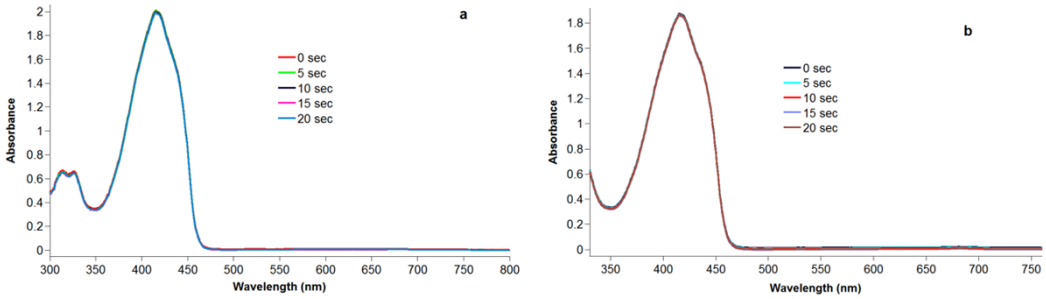

Figure S17. A spectrum for the determination of singlet oxygen quantum yield of **DPBF** after a) sonochemical irradiation, b) sono-photochemical irradiation.

4. In vitro Studies

4.1. Cell Culture

The human prostate cancer cell line PC3 (CRL-1435, ATCC) was used in this study. PC3 cells were cultured in RPMI-1640 medium (Capricorn Scientific, Germany) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine (Capricorn Scientific, Germany). Cells were maintained at 37 °C in a humidified incubator under 5% CO_2 atmosphere and routinely subcultured for experimental use.

4.2. Cytotoxicity assay

The MTT (M6494, Invitrogen, USA) assay was performed according to the protocol described in our previous study [S7]. Cells were treated with phthalocyanine derivatives at varying concentrations, and cell viability was evaluated 24 h after treatment. The highest concentration that did not induce a statistically significant toxic effect compared to the control group was selected as the treatment dose for subsequent experiments.

4.3. Light irradiation

PC3 cells were incubated with phthalocyanine derivatives at a concentration of 5 μM in the culture medium for 4 h under dark conditions. Following incubation, the cells were washed

with phosphate-buffered saline (PBS; Cat. No. 10010023, Gibco), and fresh culture medium was added. The cells were then irradiated at an irradiance of 0.5 mW/cm² for 15 min, corresponding to a total light dose of 0.45 J/cm², using an Abet solar simulator equipped with optical filters (long-pass >600 nm and short-pass <800 nm).

4.4. Apoptosis detection

Apoptosis was evaluated using Annexin V–FITC/PI staining 24 h after PDT treatment. The analysis was performed by flow cytometry using NovoCyte flow cytometer (Agilent, USA). Cells were stained according to the manufacturer's instructions using the Annexin V–FITC/PI Apoptosis Detection Kit (Cat No: E-CK-A211 Elabscience, USA). Following staining, samples were incubated for 20 min at 37°C in the dark, and apoptotic cell populations were subsequently analyzed by flow cytometry.

4.5. Cellular ROS Analysis

Cellular ROS levels were determined using dihydroethidium (DHE; D11347, Invitrogen). Cells were incubated with 10 μM DHE in serum-free medium for 30 min at 37 °C in the dark. Immediately after PDT treatment, cells were subjected to ROS analysis to capture early oxidative stress responses. As a positive control, cells were treated with tert-butyl hydroperoxide (tBHP) to induce intracellular ROS generation. DHE fluorescence was analyzed using a NovoCyte flow cytometer (Agilent) and detected in the PE fluorescence channel. Data were analyzed using FlowJo software, and ROS levels were expressed as the percentage of DHE-positive cells.

4.6. Statistical analysis:

Statistical analyses were performed using GraphPad Prism 9 software (GraphPad Software, USA). Normality of the data distribution was assessed using the Shapiro–Wilk test. For

normally distributed data sets, one-way analysis of variance (One-way ANOVA) followed by Tukey's post hoc test was applied. For data that did not show a normal distribution, the Kruskal–Wallis test was used. A value of $p < 0.05$ was considered statistically significant.

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