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Photodynamic activity of Sn(IV) meso-tetraacenaphthylporphyrin and its methyl- β -cyclodextrin inclusion complexes on MCF-7 breast cancer cells

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ABSTRACT: A novel Sn(IV) meso-tetraacenaphthylporphyrin (**SnTAcP**) has been synthesized and characterized. **SnTAcP** was complexed with methyl- β -CD (m β -CD), a nanocarrier that enhances water solubility, and the complexes were evaluated as PDT agents using MCF-7 breast cancer cells. A relatively low singlet oxygen quantum yield value of 0.36 was obtained in DMF, and the lowest energy Q band lies at 608 nm on the edge of the therapeutic window. **SnTAcP** was found to be non-toxic in the dark and phototoxic towards MCF-7 breast cancer cells with a half-maximal inhibitory concentration (IC₅₀) value of $11 \pm 1.1 \mu\text{g.mL}^{-1}$ after 30 min of irradiation with a 625 nm Thorlabs LED that provides a dose of 432 J.cm^{-2} . A higher IC₅₀ value of $21 \pm 1.1 \mu\text{g.mL}^{-1}$ was obtained for the m β -CD inclusion complex of **SnTAcP**.

KEYWORDS: porphyrins, singlet oxygen, methyl β -cyclodextrins, photodynamic therapy, inclusion complexes, photophysics.

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INTRODUCTION

Photodynamic therapy (PDT) is an alternative treatment modality that has been approved by the Food and Drug Administration for numerous tumors, including lung and breast cancers [1,2]. PDT utilizes visible or

near-infrared (NIR) absorbing photosensitizer (PS) dyes as photoactive chemicals which selectively accumulate in tumor cells due to the larger vasculature of these cells compared to normal cells [1-4]. The PS dyes interact with light of specific wavelength to produce reactive oxygen species (ROS) which eradicate tumor cells, rendering PDT a relatively non-invasive alternative for cancer treatment compared to conventional treatments like chemotherapy and radiotherapy with adverse side effects [3-9]. Amongst other PS dyes used for PDT, porphyrins have been used in this work due to their high photostability, ease of modification and unique optical properties. The insertion of central metals like zinc and tin to porphyrins enhances the intersystem crossing (ISC) from the singlet excited state to triplet state [10-13]. In the triplet state, the PS dye transfers energy to molecular oxygen in the tumor cells, so that ROS are produced [3,10,11], which can eradicate tumor cells *in situ*.

Ideally, a photosensitizer dye should be readily synthesized in a manner that can be readily scaled up. This means that symmetrical metal tetraarylporphyrin complexes with high singlet oxygen yields are obvious target compounds for applications such as PDT. A significant drawback with their use, however, is that the Q bands of conventional metal tetraphenylporphyrins lie outside the therapeutic window (620–1000 nm) where there is significant light penetration into human tissue. The *meso*-substituents can be modified to shift the Q-bands of porphyrins towards longer wavelengths to achieve maximum light-harvesting and deeper penetration of red region light in the tumor cells [11-14]. Recently we reported the synthesis and photodynamic activity of Sn(IV) *meso*-tetrathien-2-ylporphyrin complexes with a relatively high singlet oxygen quantum yield value ($\Phi_{\Delta} = 0.83$), which has a lower energy Q band that is red shifted to 613 nm in dimethylformamide (DMF) [14]. Favourable PDT properties were obtained during *in vivo* studies with MCF-7 breast cancer cells. In this study, a Sn(IV) *meso*-tetraacenaphthylporphyrin (**SnTAcP**) has been synthesized and characterized (Fig. 1), to determine whether a similar red shift of the Q band is observed and whether it is suitable for use as a photosensitizer dye for PDT. Puryltin® is a Sn(IV) chlorin that provides an example of a Sn(V) porphyrin analogue that has been developed as a photosensitizer dye for PDT [15-17].

The efficacy of PS dyes in PDT depends in part on their solubility in aqueous media, a property which many porphyrins lack, since this determines whether they can be effectively delivered to tumor cells intravenously. Various nanocarriers have been utilized in drug delivery mainly to improve the solubility of porphyrin-related drug molecules and to avoid adverse side effects [18]. For example, cyclodextrins (CDs) have been used to solubilize hydrophobic drug molecules [19-21] and have been studied in the context of porphyrins [22]. CDs are a class of naturally occurring cyclic oligosaccharides derived from the degradation of starch. The structures of the three main types of CDs, α -, β - and γ -CD comprise six, seven and eight glucopyranose units, respectively [23-25]. These glucopyranose units are linked by α -(1–4) bonds and form a truncated conical structure with a hydrophobic central cavity and hydrophilic rims of the cone. β -CDs and their derivatives have an appropriate cavity size to host a variety of hydrophobic molecules to form inclusion complexes [23-25]. The CD of interest in this work was a partially methylated β -CD (m β -CD) which is a derivative of β -CD with methyl substituents substituting hydroxyl groups on the hydrophilic rim, which can interact through hydrogen bonding to form a crystalline lattice. Chemically modified β -CDs eliminate this and have been found to form inclusion complexes with guest molecules that have enhanced solubility [26]. In this study, the *meso*-acenaphthyl groups of **SnTAcP**

were complexed with methyl- β -CD (m β -CD), and the inclusion complex formed was also evaluated for use as a photosensitizer dye for PDT by carrying out *in vitro* studies using MCF-7 breast cancer cells. The meso-tetraacenaphthylporphyrin complex was selected since the large meso-aryl rings were expected to enhance the interaction with the m β -CD rings.

EXPERIMENTAL

Materials

Ultra-pure water was obtained from a Milli-Q Water System (Millipore). 5-Acenaphthenecarboxaldehyde, pyrrole, tin(II) chloride dihydrate, 1,3-diphenylisobenzofuran (DPBF), anthracene-9,10-bis-methylmalonate (ADMA), dimethylformamide (DMF), bioreagent grade m β -CD (1.5–2.1 methyl groups per glucose unit), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Trypan blue were purchased from Sigma-Aldrich and used without further purification. Ultrapure water from a Milli-Q Water System (Millipore Corp, Bedford, MA, USA) was used throughout. Dry solvents were used for all reactions and air, and moisture-sensitive reactions were carried out under argon gas. MCF-7 cells cultures were obtained from Cellonex®. 100 unit/mL penicillin-100 μ g/mL streptomycin-amphotericin B and heat-inactivated fetal calf serum (FCS) were obtained from Biowest®. Dulbecco's modified Eagle's medium (DMEM) and Dulbecco's phosphate-buffered saline (DPBS) were obtained from Lonza®.

Instrumentation

A Shimadzu UV–2550 spectrophotometer was used to record UV-visible absorption spectra, while fluorescence emission spectra were recorded on a Varian Eclipse spectrofluorimeter. ^1H NMR measurements were recorded on a Bruker AMX 600 MHz. Mass spectral data were acquired on a Bruker Auto-FLEX III Smartbeam MALDI-TOF mass spectrometer operated in positive ion mode using α -cyano-4-hydroxycinnamic acid as a matrix. Fourier transform-infrared (FT-IR) spectra were obtained from a Bruker Alpha FT-IR spectrometer with a universal attenuated total reflectance (ATR) sampling accessory. Monochromatic laser light from an Ekspla NT 342B-20-AW pulsed laser (2.0 mJ / 5 ns, 20 Hz) was used for quantifying the singlet oxygen quantum yields of compounds with DPBF and ADMA used as $^1\text{O}_2$ scavengers. Powder X-ray diffraction (XRD) data were obtained from a Bruker D8 Diffractometer fitted with a Lynx Eye detector under Cu K_α ($\lambda = 1.5405 \text{ \AA}$) with a 192 s per step scanning rate. Zero background silicon wafer slides were used as sample holders. A Perkin Elmer TGA 8000 thermogravimetric analyzer was used to carry out thermogravimetric analysis (TGA). PDT studies were conducted using a Modulight® Medical Laser system (ML) 7710–680 fitted with a Thorlab M625L3 light-emitting diode (LED).

Synthesis

Free base tetraacenaphthylporphyrin (H₂TAcP)

H₂TAcP has been reported previously [27] and was synthesized (Scheme 1) by following a modified literature procedure [28,29]. 5-Acenaphthenecarboxaldehyde (1.024 g, 5.620 mmol) and distilled pyrrole (0.390 mL,

5.620 mmol) in 200 mL of propionic acid were heated to reflux for 4 h. The reaction mixture was cooled, filtered and then washed with cold methanol. The solid obtained was purified by neutral alumina column chromatography using chloroform as the eluent to give a solid crystalline product.

H₂TAcP: obtained as a black solid in 20% yield. ¹H NMR (600 MHz, CDCl₃) δ, ppm 8.61–8.50 (m, 8H), 8.23–16 (m, 4H), 7.63 (d, J = 6.6 Hz, 4H), 7.17–7.14 (m, 4H), 7.01–6.87 (m, 8H), 3.72–3.64 (m, 16H), –2.30 (s, 2H). MALDI-TOF MS: Calculated: 918.37; Found: 919.79 [M+H⁺].

Sn(IV) tetraacenaphthylporphyrin (SnTAcP)

The metallation of the **H₂TAcP** with a Sn(IV) ion (Scheme 1) was carried out by following a previously reported procedure [14,28]. **H₂TAcP** (0.7192 g, 0.782 mmol) and SnCl₂·2H₂O (0.1764 g, 1.564 mmol) were dissolved in 50 mL pyridine and refluxed for 12 h. The reaction mixture was cooled, and ammonia solution (20 mL) carefully added then refluxed for an additional 1 h. The reaction mixture was cooled, and 100 mL of water was added. The green precipitate formed was filtered and washed with water. The filter cake was dissolved in CHCl₃, filtered and dried to give **SnTAcP** as a green solid. **SnTAcP**: obtained as a black solid in 83% yield (695 mg). ¹H NMR (600 MHz, CDCl₃) δ, ppm 8.89–8.85 (m, 8H), 8.45–8.26 (m, 4H), 7.71–7.68 (m, 4H), 7.21–7.14 (m, 12H), 3.74–3.66 (m, 16H). MALDI-TOF MS: Calculated: 1070.25; Found: 1071.25 [M+H⁺].

Preparation of solid inclusion complexes of SnTAcP/mβ-CD

TGA was first used to determine the water content of mβ-CD and **SnTAcP/mβ-CD**. **SnTAcP** was found to have 3% water content, while mβ-CD had 0% water content. The amount of water in each sample was accounted for in calculating the molar ratios of the dye and cyclodextrin. A molar ratio of 1:4 for **SnTAcP** to mβ-CD was used for complexation. The modified complexation method was adapted from Miclea *et al.* [30]. mβ-CD was dissolved in a minimum amount of water and heated up to 100°C. When the CDs were completely dissolved, **SnTAcP** was dissolved in DMSO instead of ethanol and added gradually. The solutions were left to stir for 24–72 h then cooled to ambient temperature. The solutions were filtered, and the product recrystallized in water. The DMSO was removed with a VirTis bench-top K freeze dryer, and the products were stored at room temperature for further use.

Cell culture and in vitro studies on MCF-7 breast cancer cells

The cell culturing and in vitro studies were carried out as reported in the literature [14,31]. Human breast adenocarcinoma cells (MCF-7 cells) were used for in vitro cytotoxicity studies where dark studies were performed prior to in vitro photodynamic therapy studies. DMEM containing 4.5 g·L⁻¹ glucose with L-glutamine and Phenol red, supplemented with 10% (v/v) heat-inactivated FCS and 100 unit/mL penicillin-100 µg·mL⁻¹ streptomycin-amphotericin B were used for culturing MCF-7 cells. The MCF-7 cells were cultured in 25 cm² vented flasks (Corning®) then subcultured in 75 cm² vented flasks (Corning) in a humidified atmosphere incubator with 5% CO₂ at a physiological temperature of 37°C (Heal Force). Standard trypsinization was used for sub-culturing the cells. Viable trypsinized cells were counted using trypan blue dye exclusion assay (0.4%) with a hemocytometer. Approximately 10,000 cells were seeded in supplemented DMEM containing Phenol red

in 96-well tissue culture plates (Porvair®). The cells were incubated at 37°C under 5% CO₂ for 24 h to facilitate cell adherence to the wells. The adhered cells were washed with 100 µl DPBS twice, and then 100 µl of supplemented DMEM containing appropriate aliquots of 1000 µg.mL⁻¹ DMSO stock solutions of **H₂TAcP**, **SnTAcP** and **SnTAcP/mβ-CD** to obtain a range of different concentrations of photosensitizer dye (0, 3.13, 6.25, 12.5, 25, 50 µg.mL⁻¹). Three separate wells were filled at each concentration for all three photosensitizers. The same solvent was used to so a direct comparison of the data can be made. As control experiments, placebo cells were incubated with supplemented DMEM only and with supplemented DMEM containing 5% DMSO (v/v), which was the maximum percentage of DMSO used. No significant cytotoxic effects were observed in the MCF-7 cells in the absence of photosensitizer dyes in the dark. After 24 h treatment, the wells were rinsed with 100 µL DPBS twice, supplemented DMEM with Phenol red was added and the plates re-incubated for 24 h.

The supplemented DMEM with Phenol red was replaced with 50 mM phosphate buffer (pH 7.4) containing 150 mM NaCl (PBS). The MCF-7 cancer cells plated in 96 wells and treated with the compounds were irradiated for 30 min using the illumination kit of a Modulight® 7710–680 Medical Laser fitted with a Thorlabs 625 nm LED with an irradiance of 240 mW.cm⁻² (determined with a Coherent FieldmaxII TOP energy/power meter and a Coherent Powermax PM10 sensor). After irradiation, PBS was replaced with 10% DMEM and cells were left in the dark for 24 h followed by the addition of 25 µL of 4 mg mL⁻¹ of MTT to each well then incubation for 3 h. The culture medium was removed, and DMSO (200 µL) was added to dissolve the purple formazan crystals. A Synergy 2 multi-mode microplate reader (BioTek®) was used to determine the number of viable cells after treatment at an excitation wavelength of 540 nm. Triplicate measurements were made for all three wells to provide a total of nine measurements at each concentration for each photosensitizer dye both in the dark toxicity measurements and the measurements made after 30 min irradiation with the 625 nm LED. No significant effects were observed in light control experiments with placebo cells in supplemented DMEM only and with supplemented DMEM containing 5% DMSO (v/v). The cytotoxicity and IC₅₀ values of the compounds were calculated by nonlinear regression analysis (GraphPad Prism 5).

Fluorescence and singlet oxygen quantum yields

The fluorescence quantum yields (Φ_F) and singlet oxygen quantum yields (Φ_Δ) were determined using the comparative method [32,33] following literature procedures, using zinc phthalocyanine ($\Phi_\Delta = 0.20$ [34], $\Phi_F = 0.67$ in DMSO [35]) and zinc tetrasulfophthalocyanine ($\Phi_\Delta = 0.56$ in water) [36] as the standards, with DPBF and ADMA as the singlet oxygen scavengers in DMSO and water, respectively.

RESULTS AND DISCUSSION

Synthesis and characterization

Free base porphyrin **H₂TAcP** was synthesized (Scheme 1) from 5-acenaphthenecarboxaldehyde and pyrrole following literature procedures [28]. Sn(IV) meso-tetraacenaphthylporphyrin (**SnTAcP**) was prepared (Scheme 1) through the use of SnCl₂.2H₂O in pyridine after which aqueous ammonia was used for the hydrolysis step [37]. The reagents were heated at reflux in chloroform for 4 h. Neutral alumina column chromatography was used to isolate the targeted compounds. The compounds were characterized by MALDI-TOF MS, and ¹H NMR,

UV-visible absorption and emission spectroscopy. The calculated mass of 918.37 amu for **H₂TAcP** matches the observed parent peak at 919.79 m/z in the MALDI-TOF mass spectrum (Fig. 1). The eight β -proton peaks lie between 8.61–8.50 ppm region in the ¹H NMR spectrum, while the four proton signal between 8.19–8.22 ppm arises from the aromatic part of the *meso*-acenaphthyl rings, along with those at 7.62–7.64 and 7.17–7.14 ppm that integrate to four and eight protons, respectively. The sixteen proton signals resonating between 3.72–3.64 ppm were assigned to the -CH₂ protons of the *meso*-acenaphthyl rings. The two protons resonating upfield at -2.30 ppm correspond to the two pyrrole hydrogens in the porphyrin cavity. MALDI-TOF MS confirmed the structure of **SnTAcP**, since the calculated mass of 1070.25 amu agrees exactly with the observed parent peak at 1071.25 m/z when an [M+H]⁺ species is taken into consideration (Fig. 1). Upon inserting the Sn(IV) ion, the peak for the two inner N-H protons at -2.30 ppm was no longer observed, confirming the successful metallation of the porphyrin. It proved impossible to identify the hydroxyl proton peak in a similar manner to what has been reported previously in other recent studies with Sn(IV) tetraarylporphyrin complexes [38-42].

The characteristic Q and B (or Soret) bands of porphyrins [43,44] are observed in the UV-visible absorption spectra of **H₂TAcP** and **SnTAcP** (Fig. 2). The B band of **H₂TAcP** lies at 427 nm. There is a slight red shift of the B band to 432 nm upon metallation with Sn(IV). As is characteristic of freebase porphyrins, four Q-bands are observed at 517, 546, 593 and 653 nm in the spectrum of **H₂TAcP** in DMF. Upon metallation to form **SnTAcP**, only two Q-bands are observed at 566 and 608 nm as would normally be anticipated for metal porphyrin complexes with a four-fold symmetry axis. It is significant that the lower energy Q-band of **SnTAcP**, which is the most important in the context of PDT, lies at 608 nm and is hence significantly red-shifted compared to that of the analogous *meso*-tetraphenyl complex that has been reported previously [14], which lies at 600 nm in DMF. The red shift of the spectral bands of **SnTAcP** is hence similar to what is observed for the Sn(IV) *meso*-tetrathien-2-ylporphyrin complex that we reported recently [14], which has a Q band at 613 nm in DMF. Significant broadening of the Q and B bands is observed in the spectra of **H₂TAcP** and **SnTAcP** relative to what is normally observed for analogous tetraphenylporphyrins, especially at higher concentrations. This can be attributed to the tendency of the bulky *meso*-acenaphthyl substituents to aggregate through π - π stacking.

Characterization of SnTAcP/m β -CD inclusion complexes

The inclusion complexes of the porphyrin (**SnTAcP**/m β -CD) were prepared using a procedure derived from the ethanol-water method described previously [30]. DMSO was used to dissolve the dyes instead of ethanol, since **SnTAcP** was not sufficiently soluble in the latter solvent. Thermogravimetric analysis was used to confirm that the inclusion complex was formed in an approximate 1:4 ratio. Further evidence for the formation of the inclusion complex was obtained by XRD and IR and UV-visible absorption spectroscopy. In contrast with **SnTAcP**, the **SnTAcP**/m β -CD inclusion complex is readily soluble in aqueous solvents (Fig. 3). The B band of **SnTAcP** shifts from 431 nm in DMF to 465 nm upon inclusion in m β -CD, while the Q bands lie at 575 and 614 nm. The XRD data for **SnTAcP**, m β -CD, a physical mixture of **SnTAcP** and m β -CD and **SnTAcP**/m β -CD inclusion complex are shown in Figure 4. **SnTAcP** exhibits crystalline behavior, while m β -CD is amorphous. The diffractogram of the physical mixture of **SnTAcP** and m β -CD is a superimposition of those of **SnTAcP** and m β -CD, while in contrast, the diffractogram for **SnTAcP**/m β -CD exhibits a different crystalline behavior from the parent dye thus confirming the formation of an inclusion complex. The new crystalline material has

relatively intense peaks below 30°. This clearly demonstrates that a new material has been prepared. The IR spectra of **SnTAcP**, m β -CD, physical mixture of **SnTAcP** and m β -CD, and **SnTAcP**/m β -CD inclusion complex are also shown in Figure 4. The IR spectrum of **SnTAcP**/m β -CD inclusion complex is broadly similar to that of m β -CD and differs markedly from that of **SnTAcP**.

Photophysical and photochemical properties

The Φ_F value of **H₂TAcP** was found to be 0.10 in DMF, while **SnTAcP** has a Φ_F value of less than 0.01 due to the heavy atom effect (Table 1). These values are comparable to the values we reported recently for Sn(IV) *meso*-tetrathien-2-ylporphyrin complexes [14]. The Φ_A value of **H₂TAcP** is 0.15 in DMF, while **SnTAcP** has a higher value of 0.36 (Table 1). These values are significantly lower than the value of 0.83 in DMF that was reported recently for or Sn(IV) *meso*-tetrathien-2-ylporphyrin complexes [14]. The most likely explanation is that nonradiative decay occurs readily due to aggregation effects. A significantly lower Φ_A value of 0.09 was obtained for the **SnTAP**/m β CD inclusion complex in water when ADMA was used as a singlet oxygen scavenger [45], since oxygen is less soluble in water than it is in organic solvents, and water more rapidly quenches singlet oxygen formed by water-soluble photosensitizer dyes [46].

PDT studies

In vitro studies were conducted using human breast adenocarcinoma MCF-7 cells and a 625 nm Thorlabs LED that provides a dose of 432 J.cm⁻² over 30 min (Fig. 5). The evaluation of the PDT activity was conducted through the calculation of the half-maximal inhibitory concentration (IC₅₀) value, which is a measure of the effectiveness of a compound in inhibiting a particular biological behavior. The IC₅₀ values are used to quantify the concentration of the dyes or their complexes that is needed to inhibit the MCF-7 cell growth by 50%. A summary of the IC₅₀ values for all the compounds studied is provided in Table 2. The *in vitro* photodynamic activity of **H₂TAcP**, **SnTAcP** and the **SnTAcP**/m β -CD inclusion complex was determined by using the MTT assay on MCF-7 cells following a protocol reported previously in the literature [47,48]. No significant dark toxicity was observed across the studied concentration range (Fig. 5). An IC₅₀ value of 19 ± 1.1 µg.mL⁻¹ was obtained for **H₂TAcP**, while the metallated **SnTAcP** derivative had an IC₅₀ value of 11 ± 1.1 µg.mL⁻¹. The higher PDT activity of **SnTAcP** can be attributed to its higher Φ_A value. The encapsulated analogue, **SnTAcP**/m β -CD exhibited decreased PDT activity (IC₅₀ = 21 ± 1.1 µg.mL⁻¹). The IC₅₀ values obtained for **SnTAcP** are significantly higher than the 5.6 ± 1.1 µg.mL⁻¹ value that was obtained for a Sn(IV) *meso*-tetrathien-2-ylporphyrin complex with a Φ_A value of 0.83 using the same experimental setup with a shorter irradiation time of 20 min and a dose of 288 J.cm⁻² [14]. This can be attributed to lower cellular uptake due to aggregation effects related to the bulkier nature of the *meso*-aryl substituents [49,50], the smaller red shift of the lower energy Q band and/or the significantly lower Φ_A values obtained for **SnTAcP**.

CONCLUSIONS

In the context of the *meso*-tetraacenaphthylporphyrin studied in this works, the incorporation of *meso*-acenaphthyl rings resulted in significant red shifts of the Q and B bands of **SnTAcP** compared to the corresponding compound with *meso*-phenyl rings, and this means that the lower energy Q band lies on the outer

edge of the therapeutic window. However, the spectral bands of **SnTAcP** are significantly broadened since the bulky acenaphthyl rings provide scope for π - π stacking. When the photodynamic activity of the porphyrins was evaluated on MCF-7 breast cancer cells, an IC_{50} value of $11 \pm 1.1 \mu\text{g.mL}^{-1}$ was obtained. This value is significantly higher than that obtained previously for a Sn(IV) meso-tetrathien-2-ylporphyrin complex [14], probably due to the relatively low Φ_{Δ} values that are obtained for **SnTAcP**. The cyclodextrin inclusion complexes formed with **SnTAcP** enhance the solubility of the photosensitizer dye in aqueous solvents. Although favorable photodynamic activity results were not obtained in the context of the **SnTAcP**/m β -CD inclusion complex, further PDT related research on the m β -CD complexes of other readily synthesized Sn(IV) tetraarylporphyrins with red-shifted Q bands is merited.

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Schemes

Scheme 1. The synthesis of H_2TAcP and SnTAcP .

Figures

Figure 1. MALDI-TOF MS data for **H₂TAcP** and **SnTAcP**.

Figure 2. UV-visible absorption spectra of H₂TAcP and SnTAcP. Solvent = DMF.

Figure 3. UV-visible absorption spectrum of the SnTAcP/mβ-CD inclusion complex in deionized water.

Figure 4. XRD diffractograms of SnTAcP, mβ-CD, a physical mixture of SnTAcP and mβ-CD, and the SnTAcP/mβ-CD inclusion complex (A), and their IR spectra (B).

Figure 5. Cell viability plots showing the cytotoxic effect of porphyrin **H₂TAcP** (A), **SnTAcP** (B) and **SnTAcP/mβ-CD** (C) after irradiation with a 625 nm Thorlabs LED for 30 min (red symbols) and in its absence when the cells are kept in the dark (black symbols). Error bars set at $\pm 2\sigma$ are provided based on nine measurements that were made for each photosensitizer.

Tables**Table 1.** Summary of the spectroscopic and physicochemical properties of **H₂TAcP** and **SnTAcP** in DMF.

	Q bands / nm	B band / nm	λ_{em} / nm	Φ_F	Φ_A
H₂TAcP	517, 546, 593, 653	427	655, 720	0.10	0.15
SnTAcP	566, 608	431	---	< 0.01	0.36

Table 2. IC₅₀ values for **H₂TAcP** and **SnTAcP**.

	Dark IC₅₀ (µg.mL⁻¹)^a	Light IC₅₀ (µg.mL⁻¹)^b
H₂TAcP	> 50	19 ± 1.1
SnTAcP	> 50	11 ± 1.1
SnTAcP/mβ-CD	> 50	21 ± 1.1

^a Cells kept in the dark. ^b After irradiation with a 625 nm Thorlabs LED for 30 min.