Supporting Information

New application of phthalocyanine molecules: from photodynamic therapy to photothermal therapy by means of structural regulation rather than formation of aggregates

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Experimental Procedures

Materials. N,N-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), n-pentanol, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), 2,7-dichlorofluorescin diacetate (DCF), indocyanine green (ICG), methylene blue (MB), and Cremophor EL (CEL) were purchased from Sigma-Aldrich. Fetal bovine serum (FBS), RPMI 1640 medium were obtained from HyClone (USA). 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Genview (USA). PcA1, PcA2, PcA3, PcB1, PcB2, PcC2, PcD1, and PcD2 were synthesized according to previous procedures. In the whole text, only the major C4h isomers were showed for tetrasubstituted phthalocyanines, which likely present the other isomers. If there was no specific annotation, all the Pc compounds were dissolved in DMF (10 mM) first and then diluted into water or culture media by using 0.1% CEL as co-solvent.

Photophysical and photochemical properties. Electronic absorption and fluorescence spectra were measured on a Shimadzu UV-2450 UV-vis spectrophotometer and an Edingburgh FL900/FS920 spectrofluorometer, respectively.

The fluorescence quantum yields ($\Phi_F$) were determined by the equation: $\Phi_F^{(\text{sample})} = \frac{F_{\text{sample}}}{F_{\text{ref}}} \cdot \frac{A_{\text{ref}}}{A_{\text{sample}}} \cdot \frac{n_{\text{sample}}^2}{n_{\text{ref}}^2} \cdot \Phi_F^{(\text{ref})}$, where $F$, $A$, and $n$ are the measured fluorescence (area under the fluorescence spectra), the absorbance at the excitation position (610 nm), and the refractive index of the solvent, respectively. Use the unsubstituted ZnPc in DMF as the reference $\Phi_F^{(\text{ref})} = \ldots$
The singlet oxygen quantum yields ($\Phi_{\Delta}$) were measured by a steady-state method with DPBF as the scavenger. The concentration of DPBF was monitored spectroscopically at 414 nm with time of irradiation. The data is obtained by the equation: 

$$\Phi_{\Delta(\text{sample})} = \left( \frac{k_{\text{sample}}}{k_{\text{ref}}} \right) \left( \frac{A_{\text{ref}}}{A_{\text{sample}}} \right) \Phi_{\Delta(\text{ref})},$$

where $k_{\text{sample}}$ and $k_{\text{ref}}$ are the photobleaching rates of DPBF in the presence of the samples and reference, respectively; $A_{\text{ref}}$ and $A_{\text{sample}}$ are the absorbance at Q band (area under the absorption spectra in 610-800 nm) of the samples and reference, respectively. All measurements were performed in DMF and referenced to ZnPc ($\Phi_{\Delta(\text{ref})} = 0.56$).

**Synthesis and characterization of PcC1**

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme_S1.png}
\end{center}

Scheme S1. Synthetic route of PcC1.

A mixture of C1\textsuperscript{3} (0.58 g, 2 mmol) and $K_2CO_3$ (1.0 g, 7 mmol) in n-pentanol (30 mL) was stirred at 90 °C for 30 min, and then anhydrous CuCl\textsubscript{2} (0.13 g, 1 mmol) and DBU (0.6 mL) were added. The resulting mixture was heated to 135 °C under reflux for 12 h. After removing the volatiles in vacuo, the residue was dissolved in DMF (4 mL), and then poured into 150 mL ice-water to give blue precipitate, which was collected by filtration. The crude product was further purified by silica gel column chromatography using DMF/ethylacetate (1:5, v/v) as eluent to remove yellow impurity substance, and the dark blue product was collected with DMF/acetic acid (5:1, v/v) as eluent. The solvent was evaporated, and washed with water, ethanol and acetone successively, followed by freeze-drying to give a final solid PcC1 (203 mg, 36%). IR (KBr, cm\textsuperscript{-1}): 1400, 1481, 1501, 1582, 1604 (C=C, C=N-); 1685 (-C=O); 2925 (O-H); 1241 (Ar-O-Ar). HRMS (ESI): m/z calcd for C\textsubscript{60}H\textsubscript{32}CuN\textsubscript{8}O\textsubscript{12} [M-H\textsuperscript{-}]: 1118.1363. found: 1118.1395. Anal. Calcd for C\textsubscript{60}H\textsubscript{32}CuN\textsubscript{8}O\textsubscript{12}·3H\textsubscript{2}O: C, 61.54; H, 3.51; N, 9.56. Found: C, 61.36; H, 3.26; N, 9.54.

**Synthesis and characterization of PcC3**
Scheme S2. Synthetic route of PcC3.

A mixture of C3 (552 mg, 2 mmol), K$_2$CO$_3$ (840 mg, 6 mmol) and DMF (2 mL) in n-pentanol (30 mL) was heated to 90 °C under reflux for 30 min, and then CuCl$_2$ (134 mg, 2 mmol) and DBU (0.6 mL) were added to it for further stirring at 135 °C overnight. The solvent was then removed in vacuo, and the residue was purified by silica gel column chromatography using EA/DMF (5:1, v/v) as eluent, followed by size-exclusion chromatography using DMF as the eluent to give a blue solid (113 mg, 19.3%). IR (KBr, cm$^{-1}$): 1599, 1490, 1451 (C=N, C=C); 2872.28, 1384.10 (C-H); 1091.55 (C-O-C); 1280.10 (Ar-O-R); 3415.26 (-OH). HRMS (ESI): m/z Calcd for C$_{56}$H$_{65}$CuN$_8$O$_{16}$ [M+H]$^+$ 1168.3809, found: 1168.3813. Anal. Calcd for C$_{56}$H$_{65}$CuN$_8$O$_{16}$·2H$_2$O: C, 54.21; H, 5.85; N, 9.03. Found: C, 54.48; H, 5.61; N, 9.15.

Cell culture. Human hepatocarcinoma (HepG2) cells were purchased from ATCC. The cells were cultured in RPMI 1640 medium containing 10% FBS, streptomycin (50 μg·mL$^{-1}$), and penicillin (50 units·mL$^{-1}$). They were maintained in an incubator at 37 °C under a 5% CO$_2$ atmosphere.

In vitro therapeutic efficacy test. HepG2 cells were seeded in 96-well plates with RPMI 1640 culture media. After 24 h, cells were incubated with Pc compounds (0 μM, 5 μM, or 10 μM) for 2 h. After irradiation with a laser (630, 685, or 730 nm) for 5 or 10 min, cells were incubated for another 24 h. The cell viability was valuated by MTT method via a Spectramax Microwell plate reader.

In vitro therapeutic efficacy test (using an ice-bath to inhibit the temperature elevation of cells during laser treatment). HepG2 cells were seeded in 96-well plates with RPMI 1640 culture media. After 24 h, cells were incubated with Pc compounds (0 μM, 5 μM, or 10 μM) for 2 h. And then 96-well plates were standed on an ice-bath and treated with a laser irradiation (630, 685, or 730 nm) for 5 or 10 min, during which the temperature of cells were controlled at below 30 °C. After that, cells were incubated for another 24 h. The cell viability was valuated by MTT method via a Spectramax Microwell plate reader.

In vitro ROS generation test. HepG2 cells were incubated with Pc compounds (0 μM, 5 μM, or 10 μM) for 2 h and stained with DCF (5 μM) for 30 min. After irradiation with a laser (630, 685, or 730 nm) for 5 or 10 min, cell images were acquired using a fluorescent confocal microscope (Nikon). ROS probe was excited at 488 nm and monitored at 516-536 nm.
In vivo studies. Female mice (~20 g) were purchased from Vital River Co., Ltd, China. All animal experiments were approved by the local Ethics Committee.

In vivo PTT activities. S180 cancer cells (about 1×10⁷ cells per mouse) were subcutaneously injected into male mice. When the tumor volumes reached about 100 mm³, two groups of mice (5 mice per group) were treated with PcC1 (200 μM, 50 μL) by intratumoral injection as test groups. Two groups of mice treated with an equal volume of saline (containing with 0.1% CEL) were used as control groups. After 10 min post-injection, two groups of mice were irradiated with a 685 nm laser (0.2 W·cm⁻², 10 min). Temperature of tumor sites during laser irradiation were detected using an IR thermal camera. Tumor size was determined by using a caliper for a duration of 14 day and calculated using the following formula: volume = (tumor length) × (tumor width) × (tumor height). Body weight of mice before and after treatment were also observed.

Results and Discussion

Figure S1. Simplified Jablonski diagram illustrating the possible activated processes of Pc. Green and red stars show ground and excited state Pcs, respectively.

Table S1. The photophysical and photochemical properties of phthalocyanines in DMF.

<table>
<thead>
<tr>
<th>Compound</th>
<th>λₘₐₓ /nm</th>
<th>λₑᵣ /nm</th>
<th>Φᵣ</th>
<th>Φₐ</th>
<th>PET effect</th>
<th>Paramagnetic metal</th>
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<tr>
<td>PcA1¹</td>
<td>695</td>
<td>708</td>
<td>0.03</td>
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<tr>
<td>PcA2¹</td>
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<td>×</td>
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<tr>
<td>PcA3¹</td>
<td>688</td>
<td>700</td>
<td>0.14</td>
<td>0.56</td>
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<td>×</td>
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<tr>
<td>PcB1²</td>
<td>680</td>
<td>681</td>
<td>0.02</td>
<td>0.15</td>
<td>√</td>
<td>×</td>
</tr>
<tr>
<td>PcB2²</td>
<td>685</td>
<td>690</td>
<td>0.22</td>
<td>0.49</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>PcC1</td>
<td>688</td>
<td>-</td>
<td>0</td>
<td>0.16</td>
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<td>√</td>
</tr>
<tr>
<td>PcC2³</td>
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<td>698</td>
<td>0.14</td>
<td>0.68</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>PcC3</td>
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<td>710</td>
<td>0</td>
<td>0.12</td>
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<tr>
<td></td>
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<td>705</td>
<td>0.14</td>
<td>0.82</td>
<td>×</td>
<td>×</td>
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</table>

**Figure S2.** Temperature variation profile of control group (only water with 0.1% CEL) after being exposed to different laser irradiations (630 nm, 685 nm, and 730 nm, power densities are all controlled at 1.0 W cm\(^{-2}\)).

**Figure S3.** Electronic absorption of PcA1 and PcA2 (both at 4 μM) in water with 0.1% CEL.

**Figure S4.** (a) Electronic absorption of PcA1 (4 μM) in water with 0%, 0.1%, and 10% CEL. (b) Temperature variation profile of PcA1 (10 μM) in water with 0%, 0.1%, and 10% CEL after being exposed to 685nm laser irradiation (1.0 W cm\(^{-2}\)) for 10 min.
**Figure S5.** (a) Electronic absorption of PcA2 (4 \( \mu \text{M} \)) in water with 0%, 0.1%, and 10% CEL. (b) Temperature variation profile of PcA2 (10 \( \mu \text{M} \)) in water with 0%, 0.1%, and 10% CEL after being exposed to 685nm laser irradiation (1.0 W cm\(^{-2}\)) for 10 min.

**Figure S6.** (a) Chemical structure of PcA3. (b) Electronic absorption of PcA3 (4 \( \mu \text{M} \)) in water with 0%, 0.1%, and 10% CEL. (c) Temperature variation profile of PcA3 (10 \( \mu \text{M} \)) in water with 0%, 0.1%, and 10% CEL after being exposed to 685nm laser irradiation (1.0 W cm\(^{-2}\)) for 10 min.
**Figure S7.** Electronic absorption (left) and fluorescence spectra (right, excited at 610 nm) of PcB1 and PcB2 in DMF (both at 4 μM).

**Figure S8.** Temperature variation profile of (a) PcB1 and (b) PcB2 in water with 0.1% CEL (at 10 μM) after being exposed to different laser irradiations (630 nm, 685 nm, and 730 nm, power densities are all controlled at 1.0 W·cm⁻²).

**Figure S9.** (a) Electronic absorption of PcB1 (4 μM) in water with 0%, 0.1%, and 10% CEL. (b) Temperature variation profile of PcB1 (10 μM) in water with 0%, 0.1%, and 10% CEL after being exposed to 685nm laser irradiation (1.0 W·cm⁻²).

**Figure S10.** Electronic absorption spectra (a) and fluorescence spectra (b, excited at 610 nm) of PcC1, PcC2, and PcC3 in DMF (all at 4 μM).
Figure S11. Temperature variation profile of (a) PcC1 and (b) PcC2 (both at 10 μM) in water with 0.1% CEL after being exposed to the laser irradiations (630 nm, 685 nm, and 730 nm, power densities are all controlled at 1.0 W·cm⁻²).

Figure S12. (a) Electronic absorption of PcC1 (4 μM) in water with 0%, 0.1%, and 10% CEL. (b) Temperature variation profile of PcC2 (10 μM) in water with 0%, 0.1%, and 10% CEL after being exposed to 685nm laser irradiation (1.0 W·cm⁻²).

Figure S13. Cytotoxic effect of PcC1 and PcC2 (5 μM, containing 0.1% CEL) on HepG2 cells in the presence and absence of laser irradiation (630 nm, 1.0 W·cm⁻², 5 min). The temperature of cells was controlled at below 30 °C via an ice-bath during laser treatment.
Figure S14. Temperature variation profile of PcD1 and PcD2 (both at 10 μM) in water with 0.1% CEL after being exposed to the laser irradiation (685 nm, 1.0 W·cm⁻²).

Figure S15. Electronic absorption spectra of PcC1, ICG, and MB in aqueous solution (all at 10 μM). Left: in water with 0.1% CEL. Right: in water with 10% CEL.

Figure S16. Temperature variation profile of ICG and MB in water with 10% CEL (both at 10 μM) after being exposed to different laser irradiations (630 nm, 685 nm, and 730 nm, power densities are all controlled at 1.0 W·cm⁻²). Ctrl is only water with 10% CEL.
Figure S17. Temperature variation profile of ICG in aqueous solution (10 µM) after being exposed to 808 nm laser irradiations (1.0 W·cm⁻²). Left: in water with 0.1% CEL. Right: in water with 10% CEL.

Figure S18. Body weights of mice after different treatments.

References


