# Supporting information
for
# Cationic benzylidene cyclopentanone photosensitizers for selective photodynamic inactivation of bacteria over mammalian cells

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**Materials**

N-ethyl-N-hydroxyethylaniline, N-phenyl-diethanolamine, Cyclopentanone were purchased from Energy Chemical Corporation. 4-(diethylamino)benzaldehyde and 9,10-anthracene-diyl-bis (methylene)dimalonic acid (AMDA) were purchased from Sigma Aldrich Chemical Company. Phosphate buffered saline (PBS) solution (pH=7.4) and Dubach's modified Eagle's medium (DMEM, containing 4.5 g/L glucose, 100 unit/mL penicillin, 100 μg/mL streptomycin) were obtained from Beijing Solarbio Science & Technology Co. Ltd. Cell Counting Kit-8 (CCK-8), lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100) and Mito-tracker green were purchased from Beyotime Institute of Biotechnology. Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Co. Ltd. Luria Bertani (LB) broth and nutrient agar were purchased from Qingdao Hope Biol-Technology Co. Ltd. Pyridine and other materials were purchased from Beijing Chemical Co. Ltd. Solvents used for spectroscopy experiments were spectrophotometric grade.

**Characterization methods**

UV-Vis spectra were measured by a Hitachi U-3900 spectrophotometer. Steady-state fluorescence was carried out by using Hitachi F-4500 spectrometer. The $^{1}H$ NMR spectra were gained from a Bruker DPX 400 spectrometer. The High Resolution Mass Spectrometer (HR-MS) analyses were conducted by a Bruker APEX 7.0 E mass spectrometer. Elemental analyses were studied on a Flash EA 1112 elemental analyzer. The detailed methods for the water solubility, octanol-water partition coefficient are the same with our previous report unless otherwise noted.[1,2,3]

**Synthesis of P1-P3**

**4-((2-chloroethyl)(ethyl)amino)benzaldehyde (C1).** $PCl_5$ (52.06 g, 0.25 mol) was added slowly to DMF (120 mL) and the solution was cooled in an ice bath. After stirring at 0°C for 30min, the solution was continuously stirred at room temperature for another 15 min, and then N-ethyl-N-hydroxyethylaniline (20.00 g, 0.12 mol) was slowly added. After stirring at 45°C for 12h, the solution was added to ice water and neutralized with saturated sodium bicarbonate solution. The aqueous layer was extracted three times with $CHCl_3$ (3×100 mL) and the extraction liquid was washed with a large amount of water. The organic phase was dried over anhydrous $MgSO_4$ and rotary evaporated to give **C1** (24.90 g, yield 98.00%). $^1$HNMR (400MHz $CDCl_3$): δ (ppm) 1.23 (t, J = 7.1 Hz, 3H), 3.52 (q, J = 7.1 Hz, 2H), 3.62–3.66 (m, 2H), 3.71–3.74 (m, 2H), 6.72 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 9.74 (s, 1H).

**4-(bis(2-chloroethyl)amino)benzaldehyde (C2).** N-phenyl-diethanolamine (20.00 g, 0.11 mol), DMF (150 mL) and $PCl_5$ (95.50 g, 0.46 mol) was used in the synthesis of **C2**, and the synthetic route was similar to that of **C1**. **C2** (26.30 g, yield 98.22%), $^1$HNMR (400MHz $CDCl_3$): δ (ppm) 3.68 (t, J = 6.9 Hz, 4H), 3.85 (t, J = 6.9 Hz, 4H), 6.75 (d, J = 8.9 Hz, 2H), 7.77 (d, J = 8.9 Hz, 2H), 9.79 (s, 1H).

**2-(4-((2-chloroethyl)(ethyl)amino)benzylidene)-5-(4-(di-ethylamino)benzylidene)cyclopentanone (D1). DEA** (2.43 g, 0.01mol), **C1** (2.11 g, 0.01 mol) was dissolved in 15 mL methanol, and the catalyst LiOH• $H_2O$ (0.13 g, 3 mmol) was added to the solution. After stirring at 35°C for 18h, the crude product was purified by silica gel column chromatography to give **D1** (3.93 g, 90.03%). $^1$HNMR (400MHz, $CDCl_3$): δ (ppm) 1.19–1.23 (m, 9H), 3.06 (s, 4H), 3.39–3.51 (m, 6H), 3.61–3.70 (m, 4H), 6.71 (t, J = 9.0

Hz, 4H), 7.52 (t, J = 7.4 Hz, 6H).

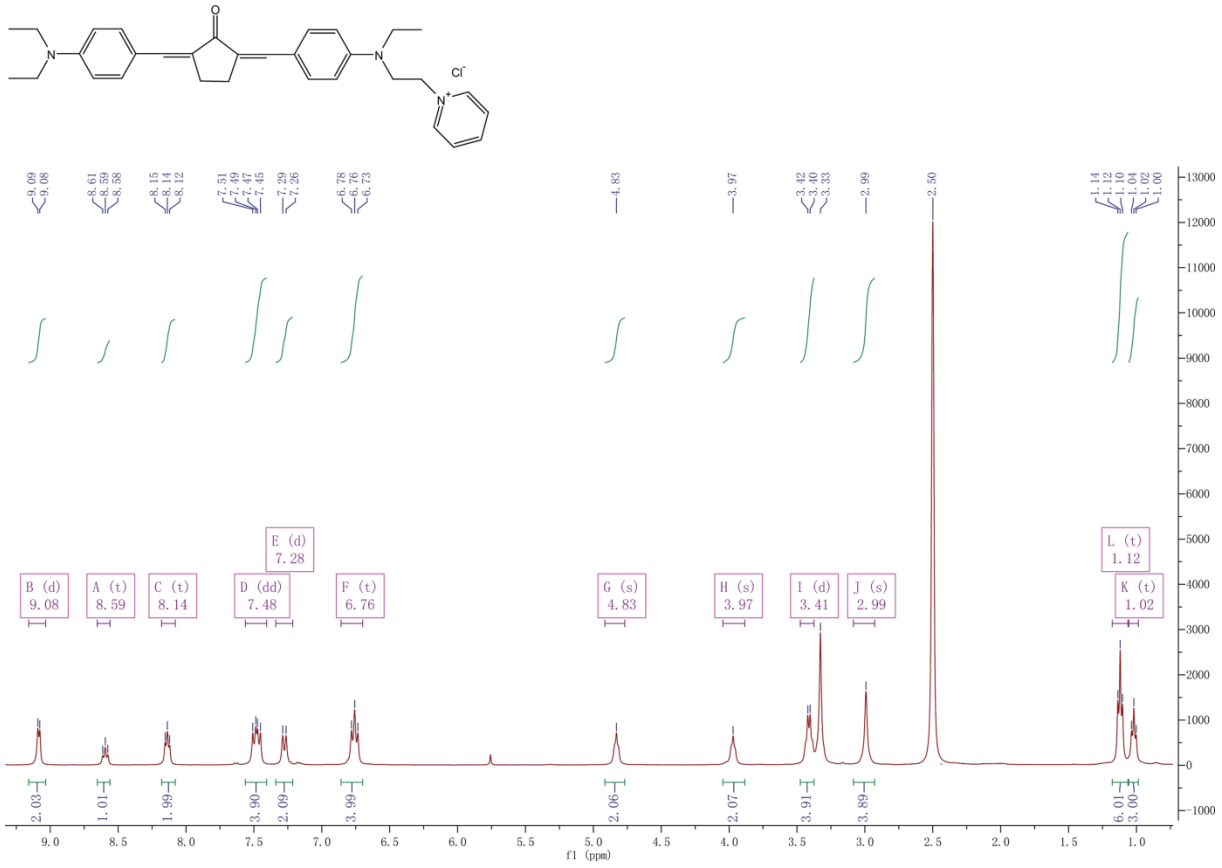
**2,5-bis(4-((2-chloroethyl)(ethyl)amino)benzylidene)cyclo-pentanone (D2)** and **2-(4-(bis(2-chloroethyl)amino)benzyli-dene)-5-(4-(diethylamino)-benzylidene)cyclopentanone (D3)**. The synthetic routes for **D2** and **D3** are similar to that for **D1**. **D2** (yield 95.35%): $^1$HNMR (400MHz, $CDCl_3$): δ (ppm) 1.22 (t, J = 7.0 Hz, 6H), 3.07 (s, 4H), 3.49 (q, J = 7.0 Hz, 4H), 3.62–3.71 (m, 8H), 6.75 (d, J = 8.0 Hz, 4H), 7.52–7.55 (m, 6H). **D3** (yield 91.42%): $^1$HNMR (400MHz, $CDCl_3$): δ (ppm) 1.21 (t, J = 7.04 Hz, 6H), 3.06 (s, 4H), 3.42 (q, J = 6.99 Hz, 4H), 3.66 (t, J = 6.9 Hz, 4H), 3.79 (t, J = 7.0 Hz, 4H), 6.69–6.76 (m, 4H), 7.49–7.56 (m, 6H).

**1-(2-((4-((3-(4-(diethylamino)benzylidene)2-oxocyclopen-tylidene)methyl)phenyl)(ethyl)amino)ethyl)-pyridin-1-ium-chloride (P1). D1** (0.22 g, 0.50 mmol) was added to 10mL dry pyridine, and the solution was stirred at 100°C for 24 h. After cooling the system cooling down to room temperature, the crude product was obtained by rotary evaporation. The deep red compound **P1** was recrystallized from toluene. **P1** (0.18 g, yield 70.18%): $^1$HNMR (400MHz, DMSO-$d_6$): δ (ppm) 1.02 (t, J = 6.7 Hz, 3H), 1.12 (t, J = 6.8 Hz, 6H), 2.99 (s, 4H), 3.41 (d, J = 6.9 Hz, 4H), 3.97 (s, 2H), 4.83 (s, 2H), 6.76 (t, J = 9.4 Hz, 4H), 7.28 (d, J = 9.5 Hz, 2H), 7.48 (dd, $J_1$ = 8.8 Hz, $J_2$ = 13.8 Hz, 4H), 8.14 (t, J = 6.7 Hz, 2H), 8.59 (t, J = 7.6 Hz, 1H), 9.08 (d, J = 5.5 Hz, 2H). HR-MS (ESI): m/z $[M]^+$: Calcd for $[C_{32}H_{38}N_3O]^+$ 480.30094; found 480.30075. Elem. Anal.: Calcd for $C_{32}H_{38}ClN_3O$: C, 74.41; H, 7.42; N, 8.14. Found: C, 74.37; H, 7.49; N, 8.09.

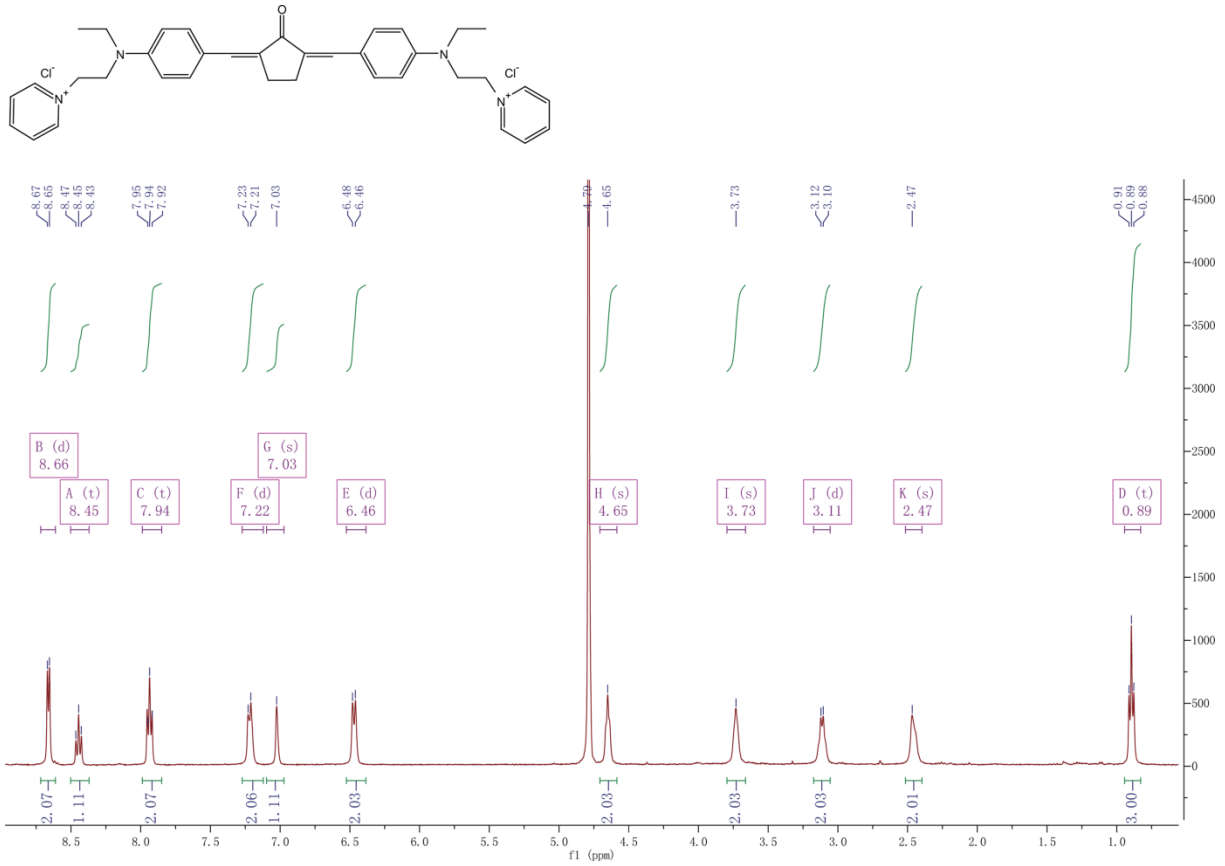
**1,1'-(((((2-oxocyclopentane-1,3-diylidene)bis(methanylyli-dene))bis(4,1-phenylene))bis(ethylazanediyl))-bis(ethane-2, 1-diyl))bis(pyridin-1-ium)chloride (P2)** and **1,1'-(((4-**

**((3-(4-(diethylamino)- benzylidene)-2-oxocyclopentylidene)methyl)-phenyl)azanediyl)bis(ethane-2,1-diyl))bis(pyridin-1-ium) chloride (P3)**. Compounds **P2** and **P3** were obtained by a similar procedure as described above for **P1**. **P2** (yield 55.03%): $^{1}$HNMR (400MHz, $D_2O$): δ (ppm) 0.89 (t, J = 6.9 Hz, 6H), 2.47 (s, 4H), 3.11 (d, J = 6.5 Hz, 4H), 3.73 (s, 4H), 4.65 (s, 4H), 6.46 (d, J = 8.2 Hz, 4H), 7.03 (s, 2H), 7.22 (d, J = 8.0 Hz, 4H), 7.94 (t, J = 7.2 Hz, 4H), 8.45 (t, J = 7.8 Hz, 2H), 8.66 (d, J = 5.9 Hz, 4H). HR-MS (ESI): m/z $[M]^+$: Calcd for $[C_{37}H_{42}N_4O]^+$ 593.30417; found 593.30499. Elem. Anal.: Calcd for $C_{37}H_{42}Cl_2N_4O$: C, 70.58; H, 6.72; N, 8.90. Found: C, 70.53; H, 6.74; N, 8.91. **P3** (yield 90.32%): $^{1}$HNMR (400MHz, $D_2O$): δ (ppm) 1.02 (t, J = 7.0 Hz, 6H), 2.62 (s, 4H), 3.20 (d, J = 6.9 Hz, 4H), 3.85 (s, 4H), 4.60 (s, 4H), 6.59 (dd, $J_1$ = 8.6 Hz, $J_2$ = 20.58 Hz, 4H), 7.07 (s, 1H), 7.14 (s, 1H), 7.31 (t, J = 9.96 Hz, 4H), 7.94 (t, J = 7.0 Hz, 4H), 8.44 (t, J = 8.0 Hz, 2H), 8.65 (d, J = 5.9 Hz, 4H). HR-MS (ESI): $[M]^+$: Calcd for $[C_{37}H_{42}N_4O]^+$ 593.30417; found 593.30354. Elem. Anal.: Calcd for $C_{37}H_{42}Cl_2N_4O$: C, 70.58; H, 6.72; N, 8.90. Found: C, 70.55; H, 6.75; N, 8.88.

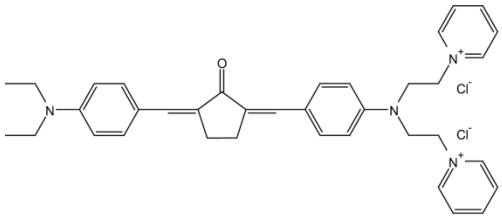
**$^1$H NMR spectra of P1**

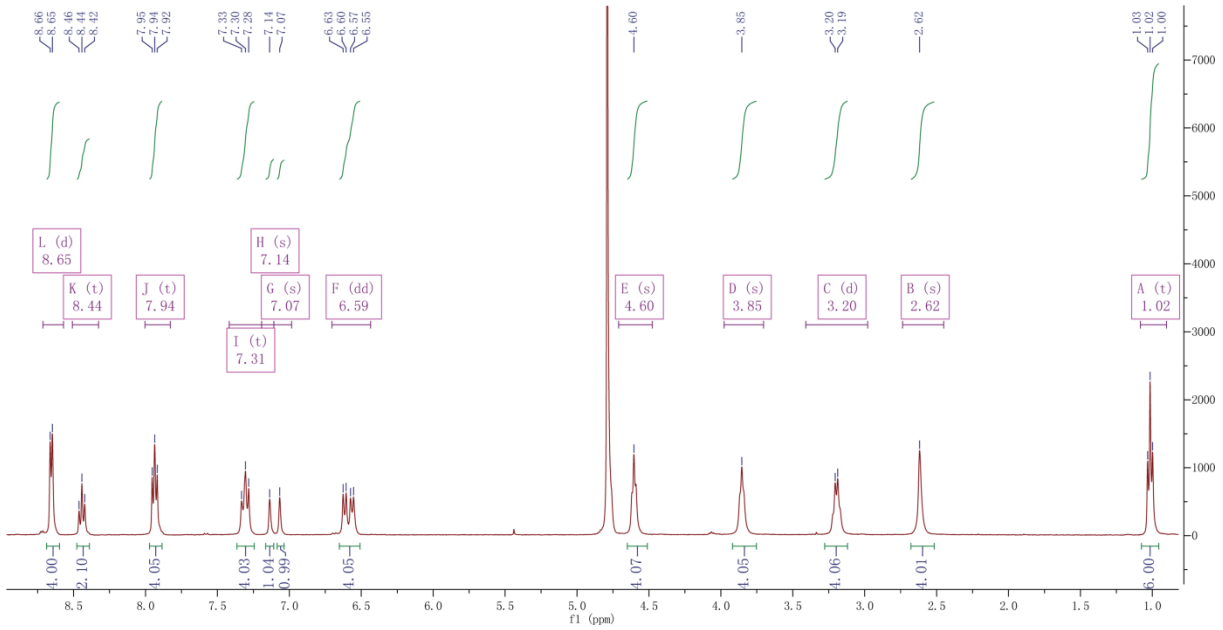


## $^1$H NMR spectra of P2



**$^{1}$H NMR spectra of P3**



**HPLC analysis of P1–P3**

Liquid Chromatography (HPLC) was performed with a SHIMADZU LC-6AD series liquid chromatography equipped with a diode array detector. PSs were analyzed on SHIMADAZU (Shim-pack: PRC-ODS, Serial No. EC0693) C18 column. Detection was achieved at 254 nm. All chromatography runs were performed at 40°C with a mobile phase flow rate of 10 mL $min^{-1}$. Isocratic elution was performed with 95:5 (**P1**) and 60:40 (**P2** and **P3**) MeOH/aqueous solution.

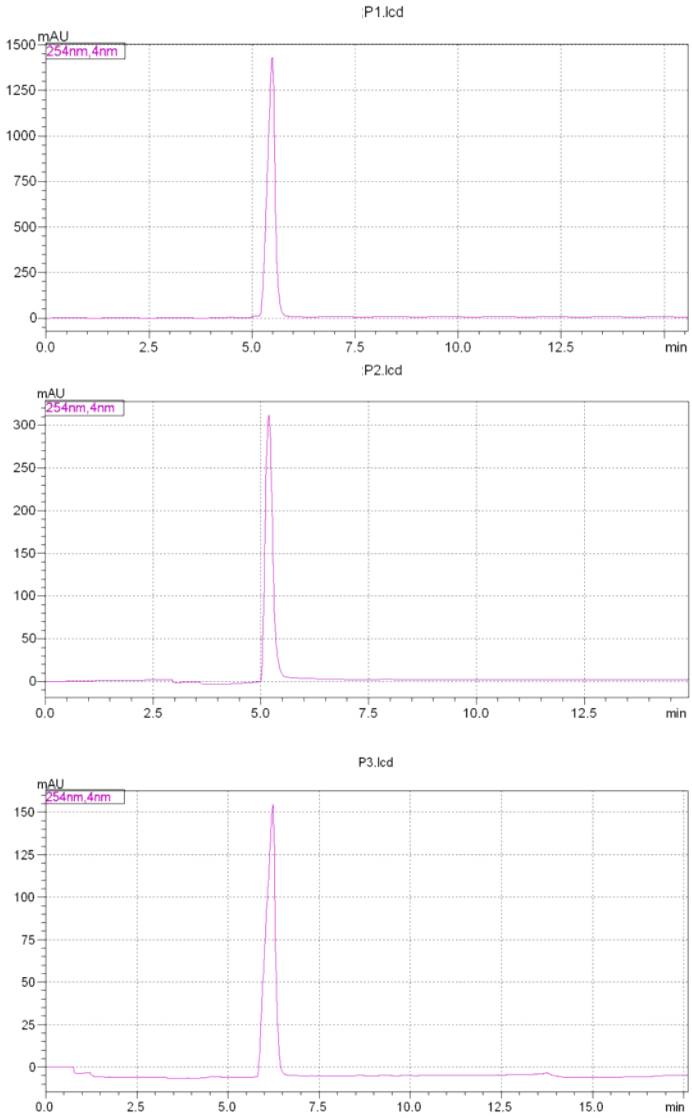



**Figure S1.** High performance liquid chromatography of **P1** – **P3** detected at 254 nm.

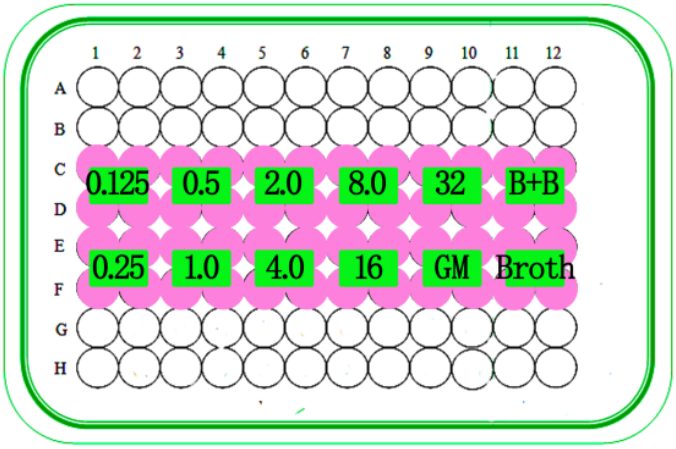



**Figure S2.** Schematic diagram denoting the concentrations of PSs in each well.

**Singlet oxygen quantum yield:**

The singlet oxygen quantum yield ($\Phi_\Delta$) was determined using Rose bengal (RB) as the reference with a yield of 0.75 in PBS. A mixed solution of a photosensitizer and AMDA was prepared. In the solution, the concentration of the photosensitizer was adjusted to possess the same absorbance (1.0) at 532 nm and the absorbance of AMDA at 402 nm was also adjusted to 1.0. In the experiment, the solution was stirred vigorously to ensure the saturation of air. When the solution was irradiated by a 532 nm diode laser, the bleaching of the absorption band of AMDA at 402 nm was monitored. The solution of AMDA alone was also irradiated to diminish the errors origin from the photo-activation. The $\Phi_\Delta$ of each compound was calculated by the following equation:

$$\Phi_\Delta^S = \frac{k_S}{k_R} \times \Phi_\Delta^R$$

where k is the slope of the photodegradation rate of AMDA, S means the sample, R means the reference, and $\Phi_\Delta^R$ is the singlet oxygen quantum yield of the reference.

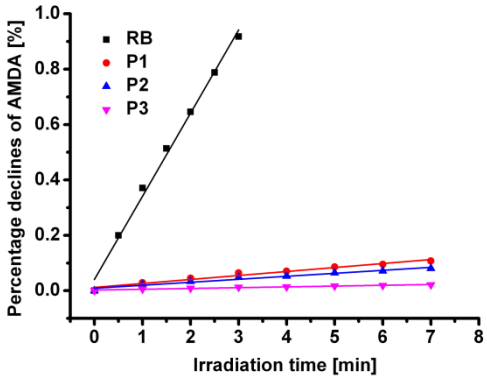



**Figure S3.** Percentage declines of AMDA in the presence of photosensitizers or rose bengal (RB).

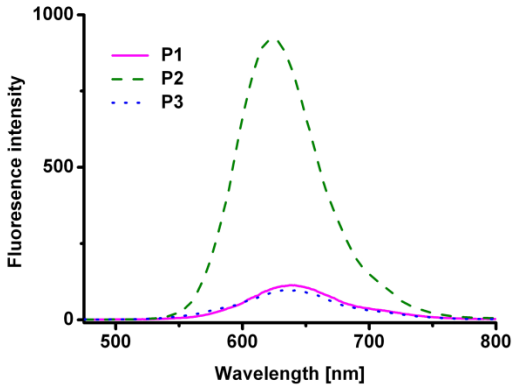



**Figure S4.** The fluorescence emission spectra of **P1**-**P3** in PBS at the concentration of 25 μM. Excited wavelength is 460 nm.

**Table S1.** Diameters of the inhibition zone of PSs against *S. aureus*, *E. coli* and *E. coli* (CA-31) after incubation for 24 h.

| Strains | C | 0 | 4 | 8 | 16 | 32 | 50 | 64 |
|---|---|---|---|---|---|---|---|---|
| *S. aureus* (Dark) | **P1** | 4.50 ± 0.05 | 5.25 ± 0.45 | 5.76 ± 0.35 | 6.5 ± 0.38 | 7.45 ± 0.39 | 7.99 ± 0.42 | 8.45 ± 0.29 |
| | **P2** | 4.50 ± 0.05 | 6.09 ± 0.40 | 6.58 ± 0.42 | 7.36 ± 0.42 | 8.33 ± 0.42 | 8.80 ± 0.45 | 9.26 ± 0.50 |
| | **P3** | 4.50 ± 0.05 | 5.37 ± 0.05 | 5.89 ± 0.45 | 6.65± 0.45 | 7.56 ± 0.42 | 8.12 ± 0.48 | 8.60 ± 0.46 |
| *S. aureus* (Light) | **P1** | 4.50 ± 0.05 | 8.34 ± 0.39 | 9.08 ± 0.38 | 10.09 ± 0.38 | 11.41 ± 0.44 | 12.53 ± 0.46 | 13.14 ± 0.55 |
| | **P2** | 4.50 ± 0.05 | 8.13 ± 0.38 | 8.87 ± 0.38 | 9.88 ± 0.39 | 11.15 ± 0.48 | 12.27 ± 0.48 | 12.84 ± 0.38 |
| | **P3** | 4.50 ± 0.05 | 8.61 ± 0.47 | 9.59 ± 0.45 | 10.53 ± 0.40 | 12.12 ± 0.42 | 13.22 ± 0.44 | 14.03 ± 0.48 |
| *E. coli* (Dark) | **P1** | 4.50 ± 0.05 | 4.55 ± 0.05 | 5.05 ± 0.39 | 5.78 ± 0.38 | 6.72± 0.42 | 7.25 ± 0.38 | 7.70 ± 0.40 |
| | **P2** | 4.50 ± 0.05 | 4.97 ± 0.10 | 5.46 ± 0.38 | 6.17 ± 0.39 | 7.17 ± 0.45 | 7.69 ± 0.48 | 8.09 ± 0.50 |
| | **P3** | 4.50 ± 0.05 | 5.18 ± 0.03 | 5.71 ± 0.39 | 6.44 ± 0.45 | 7.44 ± 0.39 | 7.92 ± 0.40 | 8.36 ± 0.44 |
| *E. coli* (Light) | **P1** | 4.50 ± 0.05 | 8.15 ± 0.44 | 8.88 ± 0.40 | 9.89 ± 0.55 | 11.20 ± 0.42 | 12.30 ± 0.45 | 12.90 ± 0.46 |
| | **P2** | 4.50 ± 0.05 | 8.00 ± 0.52 | 8.72 ± 0.42 | 9.72 ± 0.38 | 11.02 ± 0.50 | 12.11 ± 0.48 | 12.70 ± 0.47 |
| | **P3** | 4.50 ± 0.05 | 8.28 ± 0.40 | 9.24 ± 0.38 | 10.15 ± 0.39 | 11.71 ± 0.44 | 12.83 ± 0.42 | 13.56 ± 0.38 |
| *E. coli* (CA-31) (Dark) | **P1** | 4.50 ± 0.05 | 4.50 ± 0.05 | 4.50 ± 0.40 | 4.65 ± 0.38 | 5.64 ± 0.42 | 6.20 ± 0.46 | 6.54 ± 0.50 |
| | **P2** | 4.50 ± 0.05 | 4.52 ± 0.05 | 4.88 ± 0.39 | 5.22 ± 0.44 | 5.90 ± 0.19 | 6.59 ± 0.38 | 7.03 ± 0.42 |
| | **P3** | 4.50 ± 0.05 | 4.58 ± 0.05 | 4.92 ± 0.38 | 5.38 ± 0.42 | 6.00 ± 0.44 | 6.70 ± 0.45 | 7.34 ± 0.46 |
| *E. coli* (CA-31) (Light) | **P1** | 4.50 ± 0.05 | 7.17 ± 0.41 | 8.21 ± 0.40 | 8.76 ± 0.42 | 10.09 ± 0.50 | 11.23 ± 0.48 | 11.72 ± 0.50 |
| | **P2** | 4.50 ± 0.05 | 7.01 ± 0.54 | 8.06 ± 0.41 | 8.50 ± 0.40 | 9.96 ± 0.48 | 10.92 ± 0.48 | 11.40 ± 0.46 |
| | **P3** | 4.50 ± 0.05 | 8.10 ± 0.40 | 8.86 ± 0.39 | 9.86 ± 0.38 | 10.97 ± 0.44 | 11.76 ± 0.46 | 12.63 ± 0.38 |

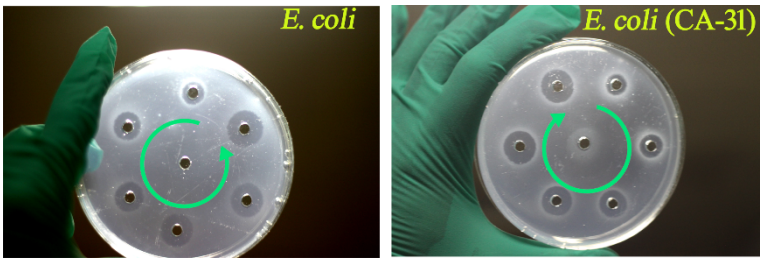



**Figure S5.** Diameters of the inhibition zone of PS **P3** against *E. coli* and *E. coli* (CA-31) after incubation for 24 h, the concentrations are 4.0, 8.0, 16.0, 32.0, 50.0, and 64.0 μM, respectively.

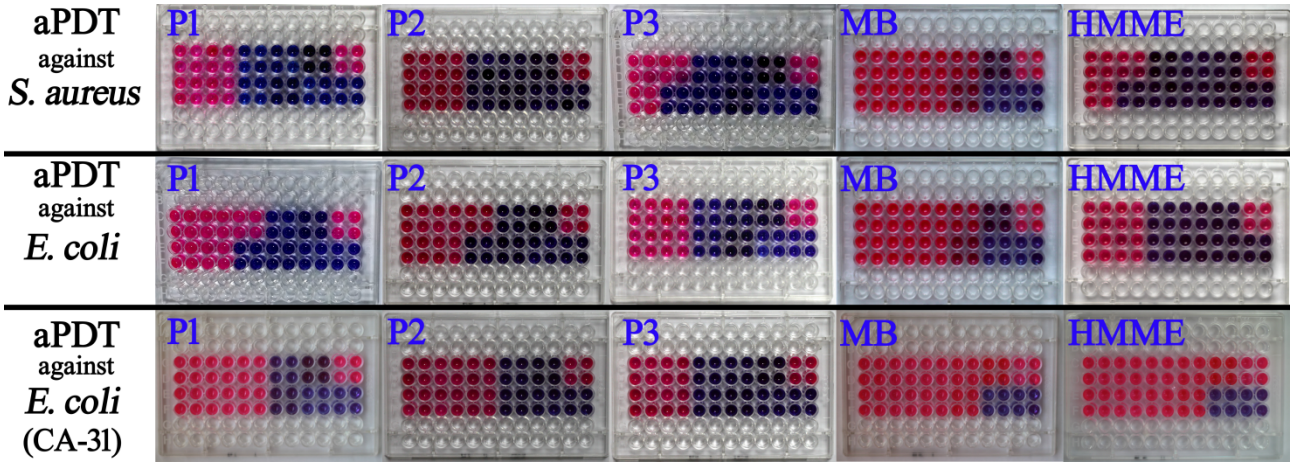



**Figure S6.** The color variation observed for the resazurin test of PSs against *S. aureus*, *E. coli*, and *E. coli* (CA-31) after being irradiated with laser (30 J $cm^{-2}$) and incubated for another 24 h.

**MIC test by spread plate method**

As shown in the schematic diagram (Figure S2), the concentrations of four neighboring wells in a sterile 96-well plate, for example C1, C2, D1, and D2, are same. 100 μL of the photosensitizers at different concentrations were added into the corresponding wells on 96-well plates. The **GM** group (100 μL 1% gentamicin in each well) and the **B+B** group (100 μL broth with bacteria) were used as the positive-control and negative-control groups, respectively. All the wells described above contained 10 μL of bacterial suspension (~ $5\times10^6$ CFU per mL) to achieve a final concentration of $5\times10^5$ CFU per mL except of the **Broth** group, which contained 10 μL of nutrient broth as the blank-control group. These 96-well plates were irradiated with a 532 nm laser for 10 min (the total light dose was approximately 30 J $cm^{-2}$) and incubated at 37°C for another 18 h. 200 μL bacterial suspensions with **P3** at the concentrations of 2.0, 4.0, 8.0, and 16.0 μM were used to spread plates, respectively. Meanwhile, the bacterial suspension without **P3** was diluted 10,000 fold before spreading plates. After incubating all plates overnight, the colony numbers were counted. Three replicates were done at the same concentration.

**Table S2.** The inactivation efficiency of **P3** against *E.coli* (25922).

| The concentration of **P3** (μM) | The colony number | Inactivation efficiency (%) |
|---|---|---|
| 0 | $(211 \pm 28) \times 10^4$ | 0 |
| 2.0 | 115 ± 26 | 99.9945 |
| 4.0 | 2 ± 5 | 99.9999 |
| 8.0 | No detected | 100 |
| 16.0 | No detected | 100 |

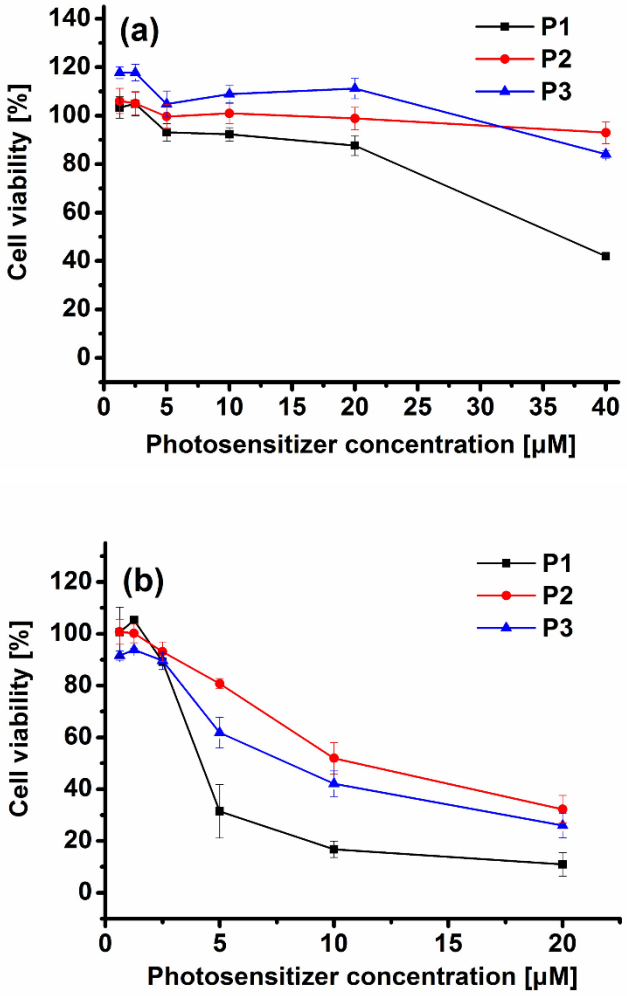



**Figure S7.** Viability of B16 cells incubated with different concentration of PSs. (a) In dark. (b) Irradiated with a 532 nm laser (30 J $cm^{-2}$) for 10 min.

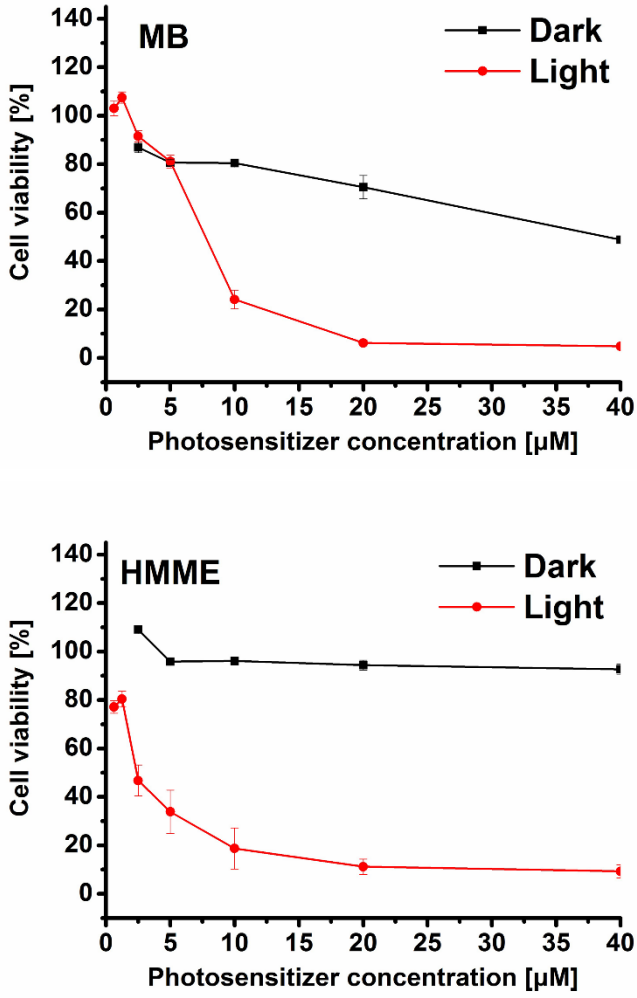



**Figure S8.** Viability of HepG2 cells incubated with different concentration of PSs, **MB** and **HMME**. In dark (black points) and irradiated with a 532 nm laser (30 J $cm^{-2}$) for 10 min (red points).

References

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