Electronic Supplementary Material (ESI) for ChemComm.

A supramolecular photosensitizer system based on the host-guest complexation between water-soluble pillar[6]arene and methylene blue for durable photodynamic therapy

Kui Yang, Jia Wen, Shuang Chao, Jing Liu, Ke Yang, Yuxin Pei and Zhichao Pei*

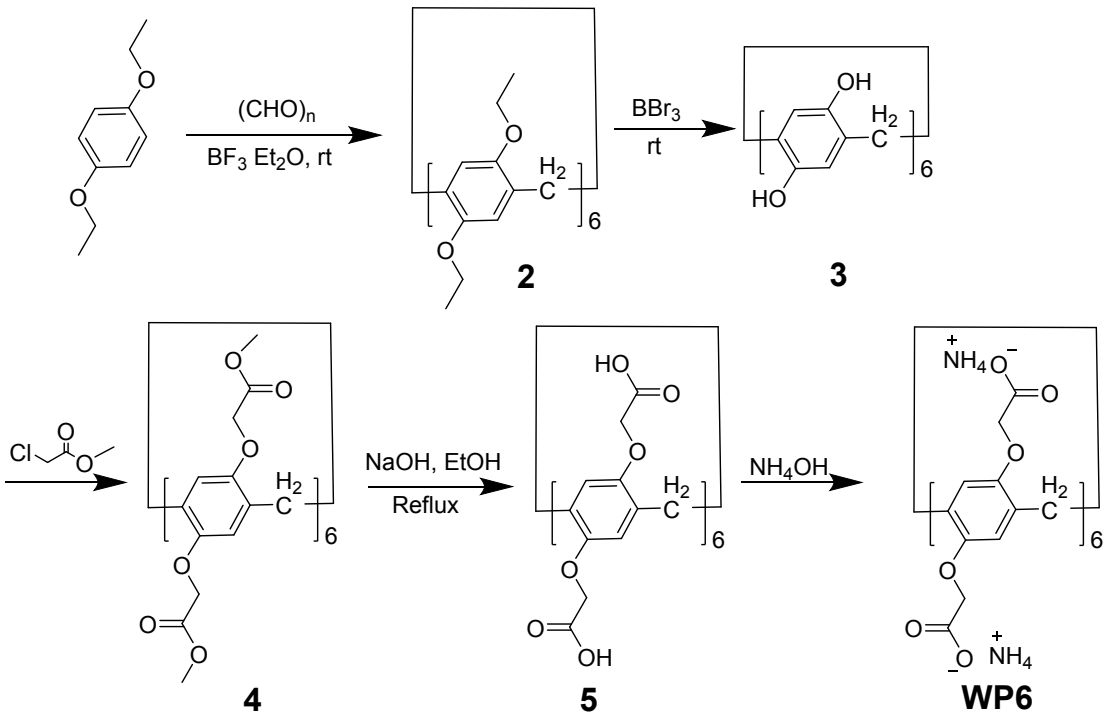
Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, Yangling 712100, P. R. China.

Supporting Information

1. Materials and methods

All reagents were purchased from commercial suppliers and used without further purification unless specified. Water used in this work was triple distilled. NMR spectra were recorded on a Bruker 500 MHz Spectrometer, with working frequencies of 500 MHz for ^{1}H and 125 MHz for ^{13}C respectively. Absorption spectra were collected by using a Shimadzu 1750 UV-visible spectrometer (Japan). Fluorescence spectra were measured with a Shimadzu RF-5301 fluorescence spectrometer (Japan). Cell culture was carried out in an incubator with a humidified atmosphere of 5% CO_2 at 37 ^{o}C. Cell toxicity was tested by microplate reader (KHB ST-360). The confocal laser microscope (CLSM) data were acquired using a CLSM (Andor REVOLUTION WD). The power of light is 25 mW/cm^2 at 630 nm.

2. Synthesis and characterization of the compounds



Scheme S1. Synthetic route of **WP6**.[S1-S3]

As shown in Scheme S1, **WP6** was synthesized according to the literatures.[S1-S3] The ^{1}H NMR spectrum of **2** was shown in Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.69 (s, 1H), 3.81 (m, 3H), 1.28 (t, J = 7.0 Hz, 3H). The ^{1}H NMR spectrum of

4 was shown in Figure S2. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.86 (s, 12H), 4.50 (s, 24H), 3.89 (s, 12H), 3.74 (s, 36H). The ^{1}H NMR spectrum of **5** was shown in Figure S3. ^{1}H NMR (500MHz, DMSO) δ 12.87 (s, 12H), 6.83 (s, 12H), 4.50 (s, 24H), 3.71 (s, 12H). The ^{1}H NMR spectrum of **WP6** was shown in Figure S4. ^{1}H NMR (500 MHz, D_2O) δ 6.61 (s, 12H), 4.05 (s, 24H), 3.84 (s, 12H).

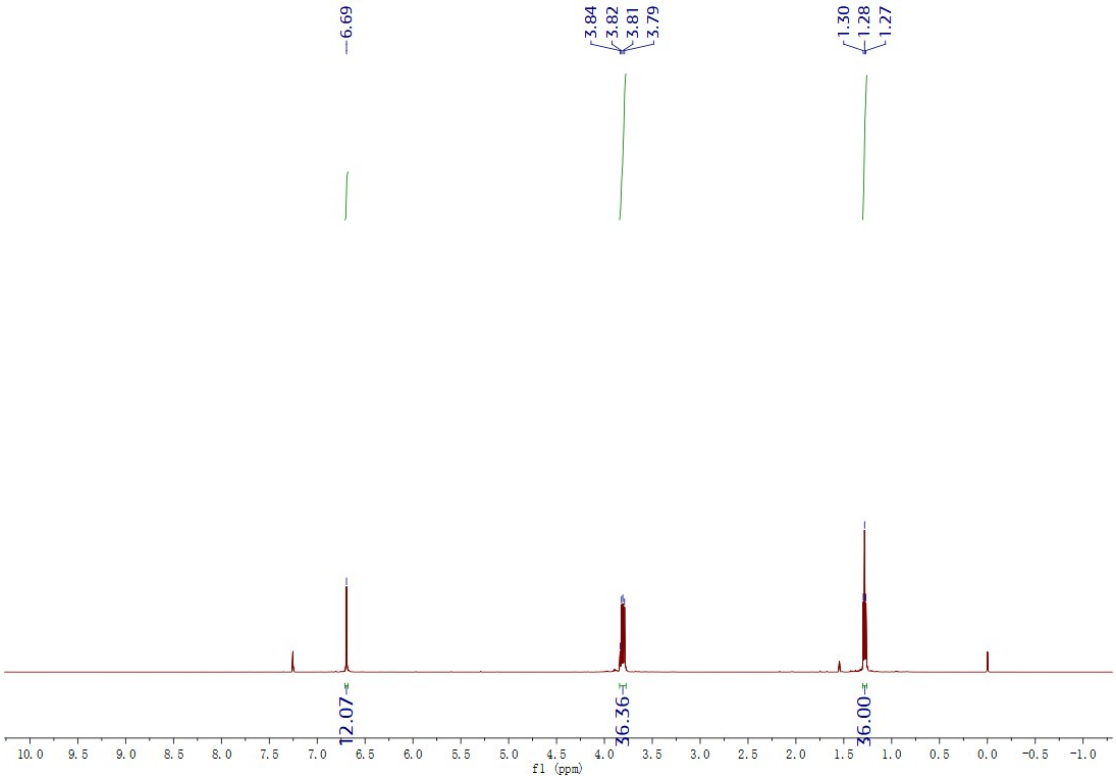


Figure S1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **2**.

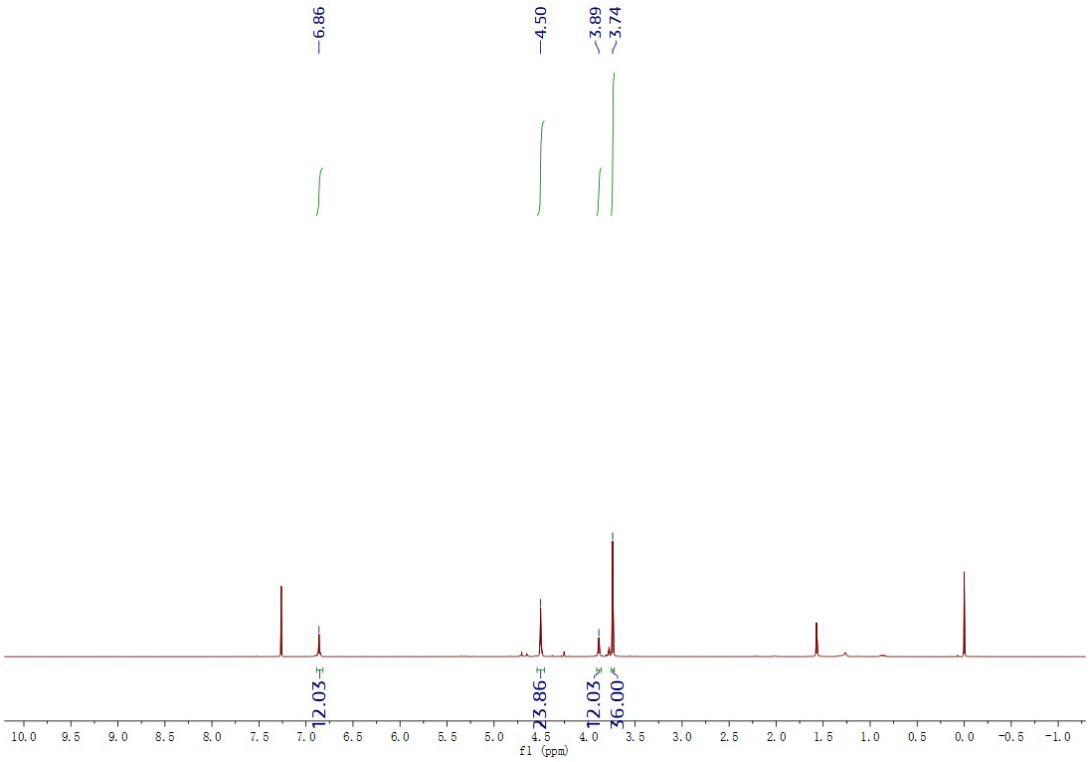


Figure S2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **4**.

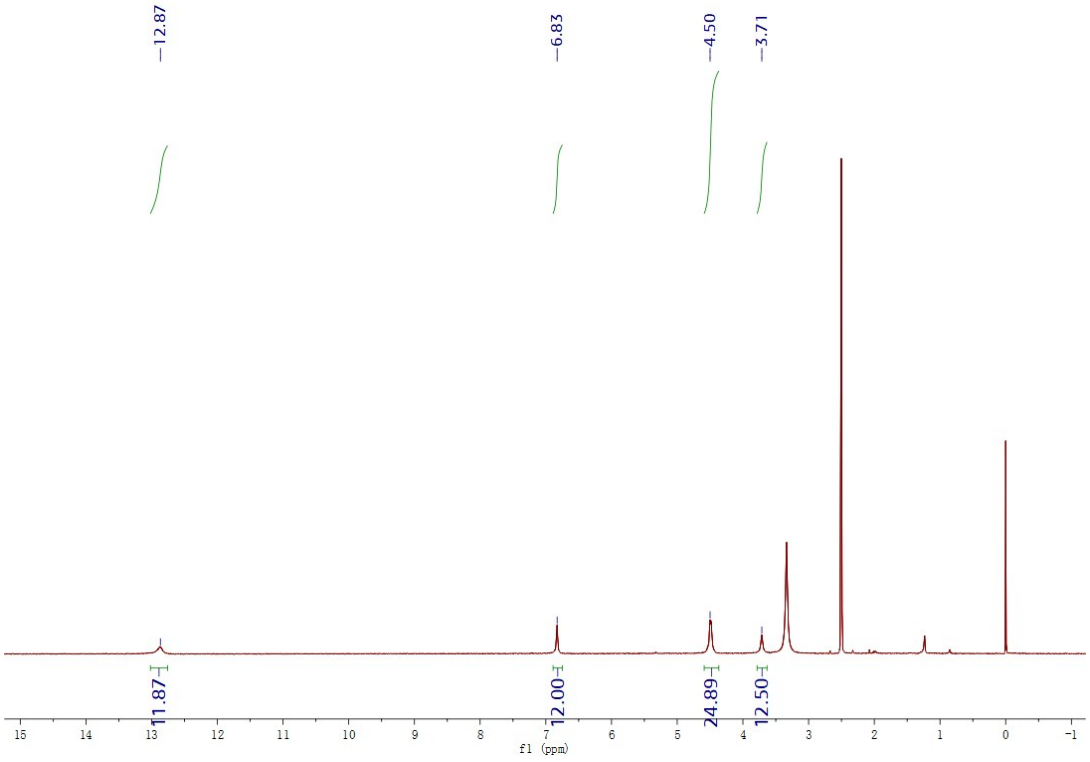


Figure S3. ^{1}H NMR spectrum (500 MHz, DMSO-*d*6) of **5**.

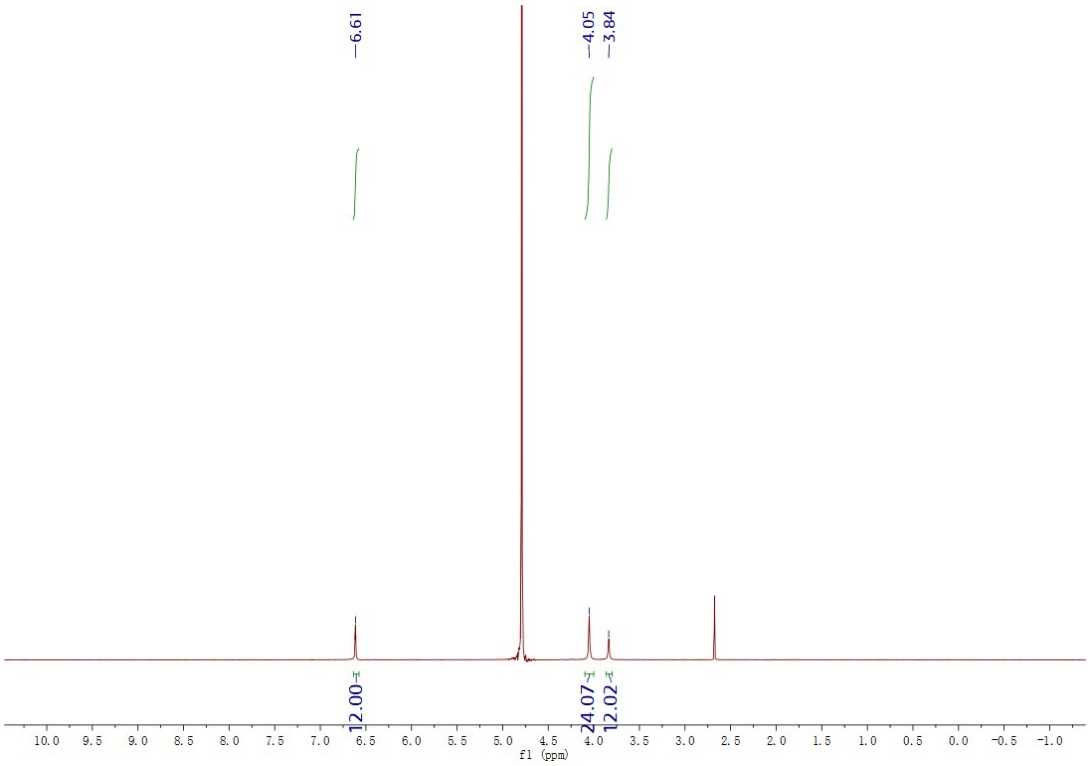


Figure S4. ^{1}H NMR spectrum (500 MHz, D_2O) of **WP6.**

3. UV-vis, fluorescence and NMR spectroscopy studies of host-guest complexation of WP6 and MB

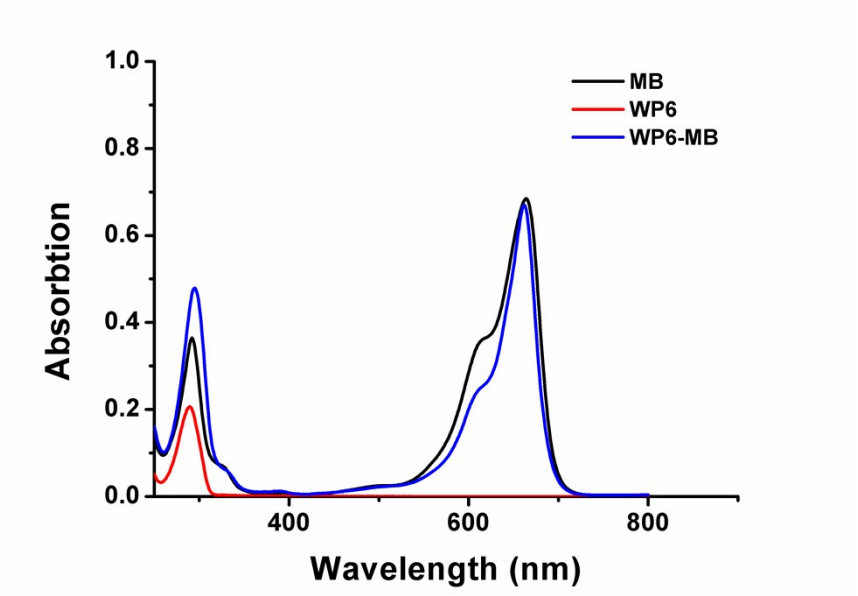


Figure S5. *UV-vis* spectra of **WP6**, **MB** and **WP6-MB** at the concentration of 10 μM.

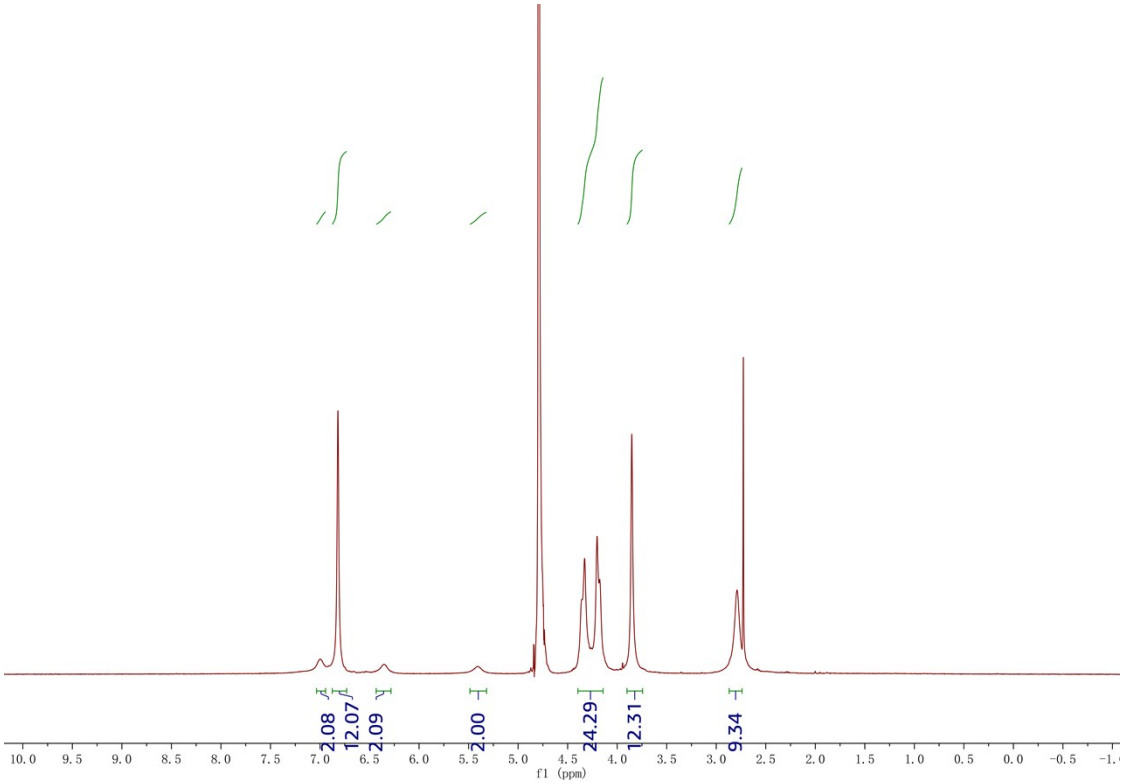


Figure S6. ^{1}H NMR spectrum (500 MHz, D_2O, 298 K) of **WP6**:**MB**=1:1.

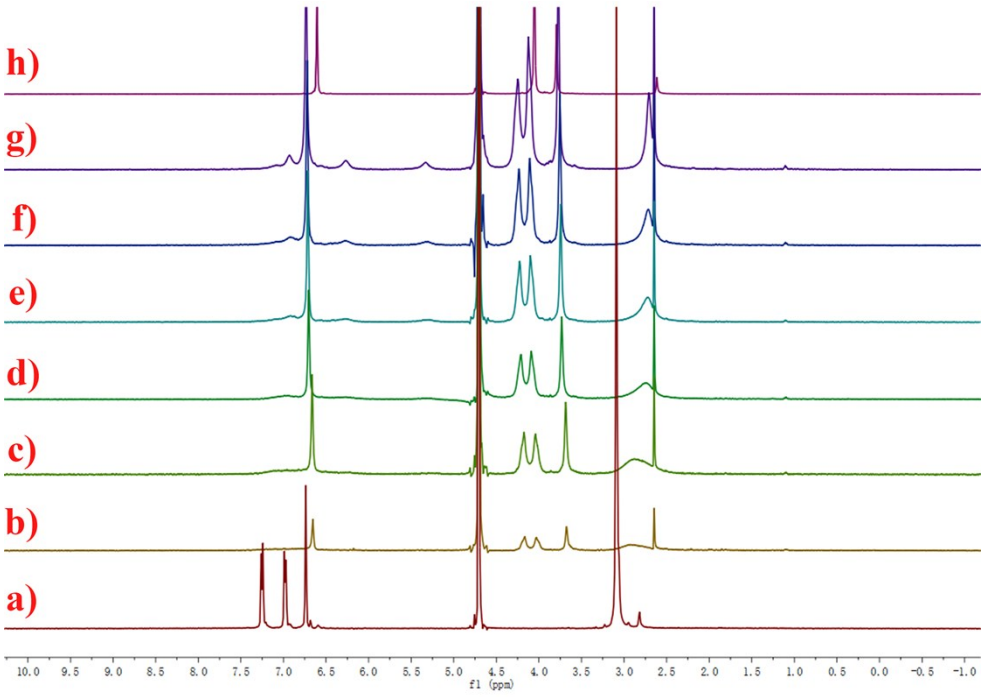


Figure S7. ^{1}H NMR spectra (500 MHz, D2O, 298 K) of **MB** at a constant concentration of 10 mM with different concentrations of **WP6** (mM): (a) 2.0, (b) 4.0, (c) 6.0, (d) 8.0, (e) 10, (f) 15. (g) ^{1}H NMR spectra of **WP6** at the concentration of 10 mM.

To determine the association constant for the complexation between **WP6** and **MB**, fluorescence titration experiments were carried out in solutions which had a constant concentration of **WP6** (1×10^{-5} M) and varying concentrations of **MB**. The association constant (K_a) of **WP6⊃MB** was estimated through a non-linear curve-fitting method,.

The non-linear curve-fittings were based on the equation:

$$\Delta F = (\Delta F\infty/[G]_0)\ (0.5[H]_0 + 0.5([G]_0+1/K_a)-(0.5\ ([H]_0^2+(2[H]_0(1/K_a - [G]_0)) + (1/K_a + [G]_0)^2)^{0.5})) \quad \text{(eq. 1)}$$

Where ΔF is the fluorescence intensity changes at 682 nm at $[G]_0$, $\Delta F\infty$ is the fluorescence intensity changes at 682 nm when **WP6** is completely complexed, $[H]_0$ is the initial concentration of **WP6**, and $[G]_0$ is the fixed initial concentration of **MB**.[S4]

4. Determination of quantum yield (QY)

The QY of **WP6-MB** was determined according to the literature.[S5]

$$\emptyset_x = \emptyset_r(\frac{A_r(\lambda_r)}{A_x(\lambda_x)}\frac{I(\lambda_r)}{I(\lambda_x)})(\frac{n_x^2}{n_r^2})(\frac{D_x}{D_r})$$

Where Φ is quantum yield; the subscripts x and r refer to the unknown and the standard respectively; λ is the excitation wavelength; A is absorbance at the excitation wavelength λ; I is the intensity of the exciting light at wavelength λ; n is the refractive index of the solution; D is the integrated area under the corrected emission spectrum. According to the literatures,[S6,S7] the QY of **MB** is 0.52. And the QY of **WP6-MB** is determined using **MB** as the standard, which is 0.21.

5. Evaluation of reactive oxygen species (ROS)

The level of ROS was monitored by using probe 2', 7'-dichloro-fluorescein diacetate (DCFH-DA). A solution of DCFH-DA in DMSO was added into the solution of **WP6-MB** and **MB** respectively. The resulting solutions containing 10 μM **WP6-MB** or **MB** and 10 μM DCFH-DA were photoirradiated with red light (630 nm) for different periods of time. And the changes in the fluorescence spectra of DCFH-DA were recorded with excitation and emission wavelength at 488 nm and 520 nm, respectively.

6. Determination of ROS quantum yield

For ROS quantum yield (Φ_{Δ}) measurement, 1,3-diphenylbenzofuran (DPBF) was used as a probe, and **MB** was used as a reference compound ($\Phi_{\Delta MB}$ = 0.5) (*Phys. Chem. Chem. Phys.*, 2002, **4**, 2320-2328; *Adv. Powder Technol.*, 2018, **29**, 341-348). The solutions of **MB** (10 μM) and **WP6-MB** (10 μM) containing DPBF (30 μM) were irradiated at 630 nm (25 mW/cm^2) for 120 min. The absorbance of the solutions was measured for different period of time (Fig. S8). $\Phi_{\Delta WP6\text{-}MB}$ was calculated using the equation $\Phi_{\Delta WP6\text{-}MB} = (\Phi_{\Delta MB} \times W_{WP6\text{-}MB} \times I_{MB})/(W_{MB} \times I_{WP6\text{-}MB})$, where $W_{WP6\text{-}MB}$ and W_{MB} are the DPBF photobleaching rates (415 nm) in the presence of **WP6-MB** and **MB**, respectively. $I_{WP6\text{-}MB}$ and I_{MB} are the rates of light absorption by **WP6-MB** and **MB**, respectively.

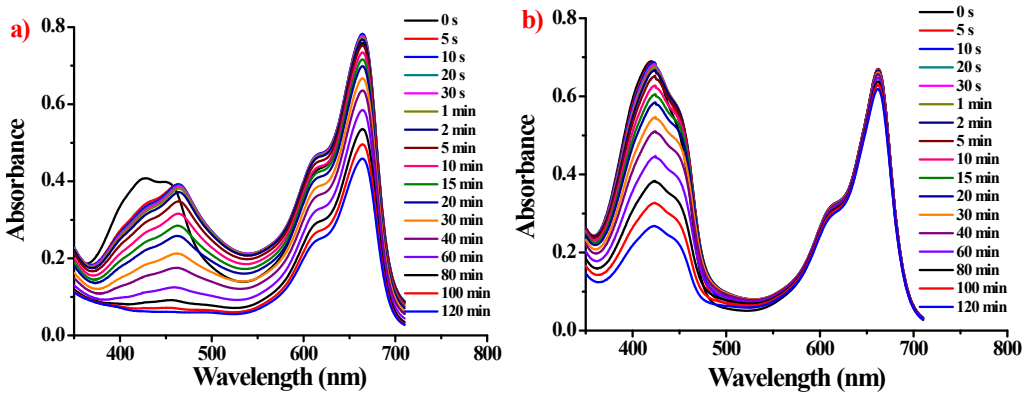


Figure S8. Determination of ROS quantum yield of **MB** (a) and **WP6-MB** (b) through the absorption of DPBF upon irradiation by laser at 630 nm for different time.

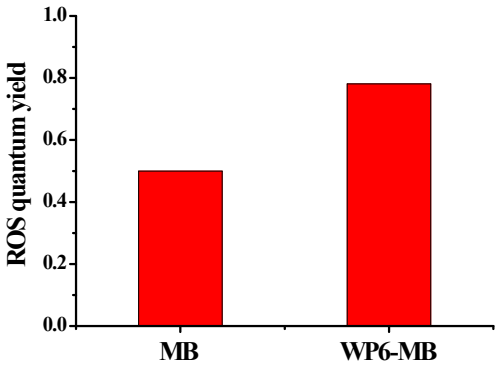


Figure S9. Quantum yields of **MB** and **WP6-MB** to generate ROS under 630 nm irradiation at 25 mW cm^{-2}.

7. Confocal laser scanning microscopy (CLSM)

MCF-7 cells were seeded in 35 mm plastic bottomed μ-dishes for 24 h. The medium was replaced with a fresh one. Then the cells were incubated with **MB, WP6-MB** for 4 h at the concentration of 10 μM, respectively. Next, the dishes were washed with PBS for three times. After washing with PBS, the cells were stained with Hoechst 33342 for 10 min. Finally, the cells were washed with PBS and then observed under a CLSM.

The intracellular level of ROS was monitored by using DCFH-DA, with excitation wavelength at 488 nm. The whole procedure was performed as below: MCF-7 cells were seeded in 35 mm plastic bottomed μ-dishes for 24 h. The medium was replaced with a fresh one. Then the cells were incubated with **WP6-MB** and **MB** for 4 h at the concentration of 10 μM, respectively. Next, the dishes were photoirradiated with red light (630 nm) for 40 min. Then the medium containing 10 μM DCFH-DA was refreshed. After 20 min incubation, the cells were washed with PBS and then observed under a CLSM.

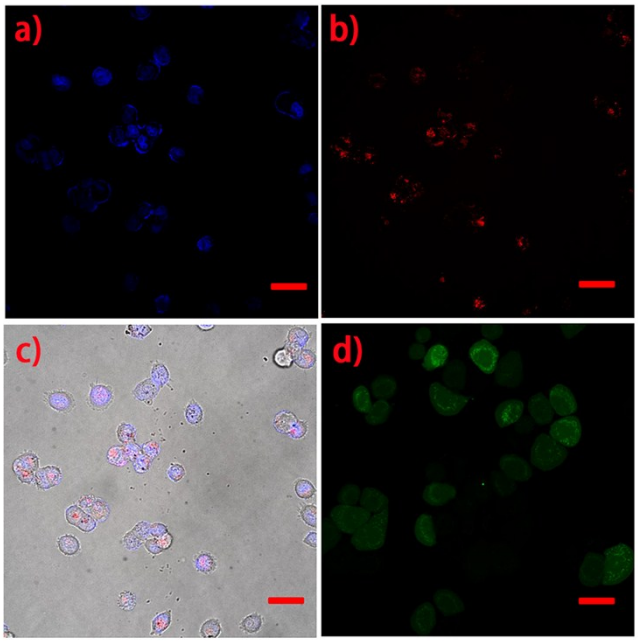


Figure S10. CLSM of MCF-7 cells incubated with **MB**. The scale bar is 50 μm. (a) Hoechst 33342 channel. (b) **MB** channel. (c) Merged. (d) ROS detection by DCFH-DA probe.

8. Cytotoxicity evaluation

MCF-7, HeLa and 293T cells were cultured in DMEM medium containing 10% FBS, 1% penicillin/streptomycin (complete DMEM) in 5% CO_2 at 37 °C. HepG2 and HL7702 were cultured in RPMI 1640 medium containing 10% FBS, 1% penicillin/streptomycin (complete RPMI 1640 medium) in 5% CO_2 at 37 °C. The relative cytotoxicities of **WP6**, **MB** and **WP6-MB** were evaluated *in vitro* by MTT assay, respectively. The cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100 μL complete medium. After 4 h grew at 37 °C, **WP6-MB** and **MB** groups were irradiated with 630 nm light for 20 min, 40 min and 80 min respectively. Subsequently, cells were incubated for another 24 h. The cells were washed and the fresh medium containing MTT (0.5 mg/mL) was added into each plate. The cells were incubated for another 4 h. After that, the medium containing MTT was removed and dimethyl sulfoxide (100 μL) was added to each well to dissolve the formazan crystals. Finally, the plate was gently shaken for 10 min and the absorbance at 490 nm was recorded with a microplate reader.

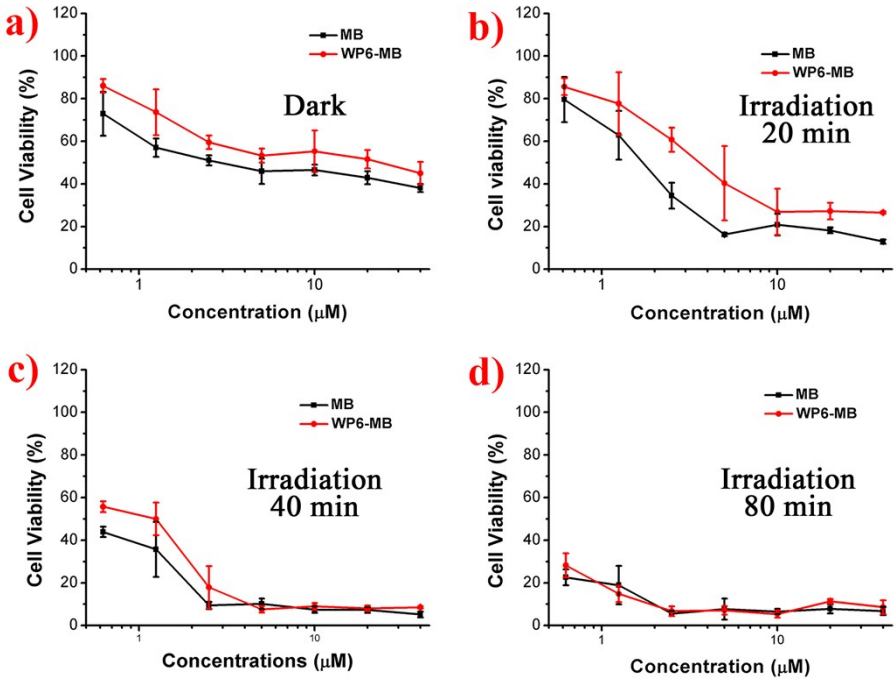


Figure S11. Relative cell viability of HeLa cells (cancer cells) after treatment with **WP6-MB** and **MB** at different concentrations without (a) and with 20 min (b), 40 min (c) and 80 min (d) light irradiation.

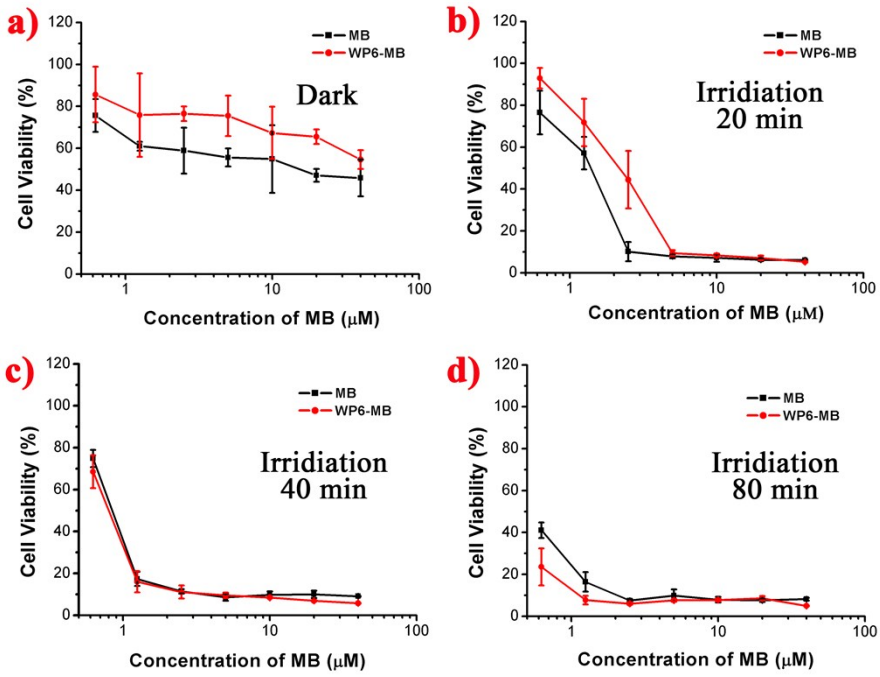


Figure S12. Relative cell viability of HepG2 cells (cancer cells) after treatment with **WP6-MB** and **MB** at different concentrations without (a) and with 20 min (b), 40 min (c) and 80 min (d) light irradiation.

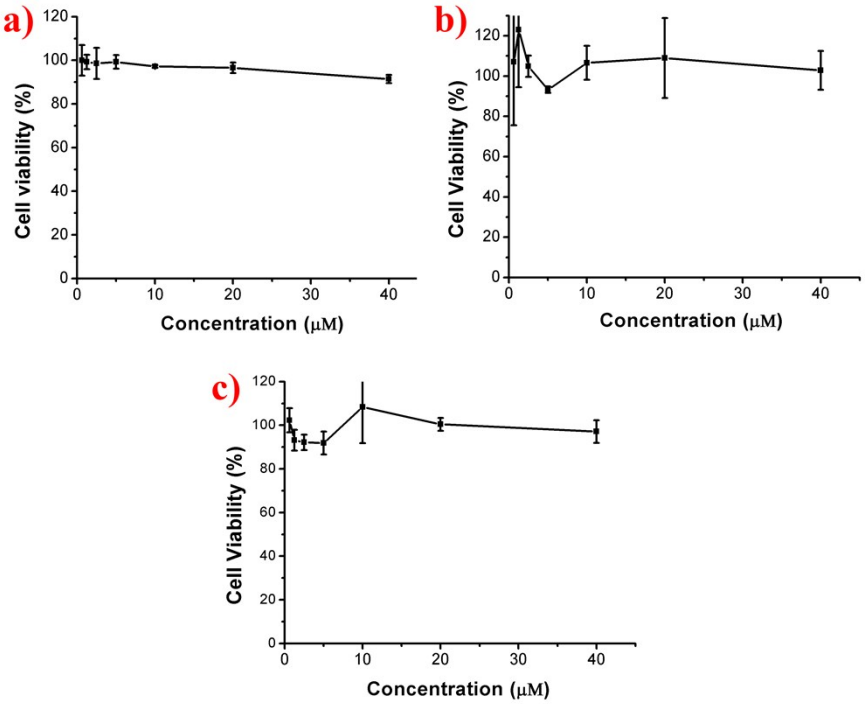


Figure S13. Relative cell viability of MCF-7 cells (a), HeLa cells (b) and 293T cells (c) after treatment with **WP6** at different concentrations.

9. Flow cytometry

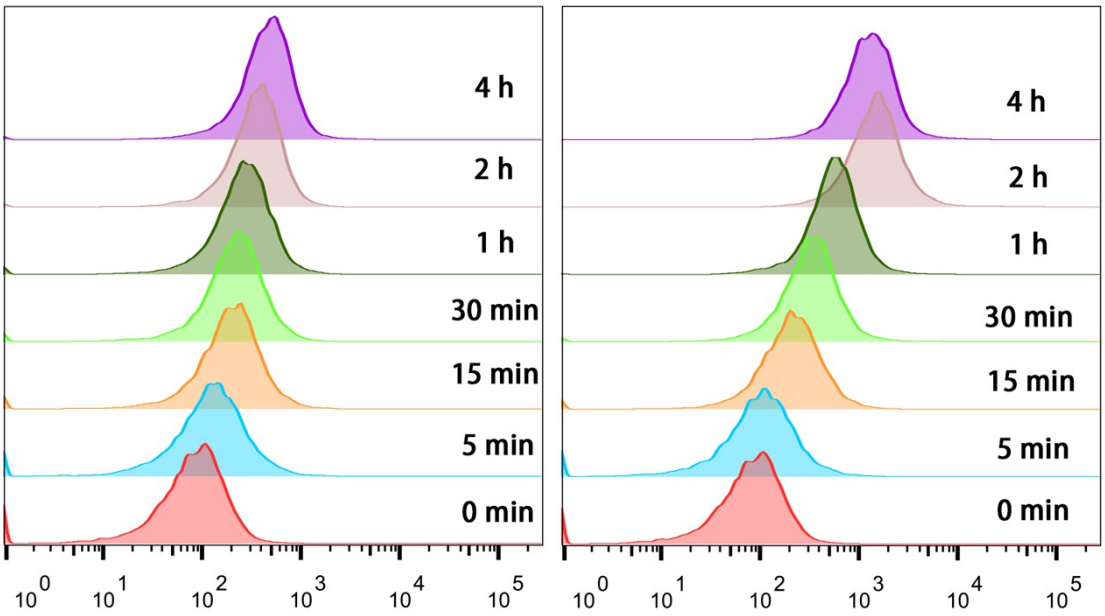


Figure S14. Flow cytometry diagrams of the uptake of **WP6-MB** (left) and **MB** (right) by MCF-7 cells.

10. References

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S7. W. Wang, L. Yang, H. Sun, Z. Yang, Q. Du and C. Li, *Adv. Powder Technol.*, 2018, **29**, 341-348.