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Electronic Supplementary Information

A two-photon-activated prodrug for therapy and drug release monitoring

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Figure. S1. ¹H NMR spectrum (in DMSO- d_6) of **1**.



Figure. S2. ¹H NMR spectrum (in CDCl₃) of **2**.



Figure. S3. ¹H NMR spectrum (in CDCl₃) of 3.



Figure S4. ¹H NMR spectrum (in CDCl₃) of 4.



Figure S5.¹³C NMR spectrum (in DMSO- d_6) of 4.

Spectrum from sample P2.wiff (sample 1) - Sample002, Experiment 1, +TOF MS (100 - 1000) from 0.068 to 0.101 min
C35H54N2O6Si2 +H



Figure S6. HRMS spectrum of 4. HRMS (ESI): calcd for $C_{35}H_{55}N_2O_6Si_2$ ([M+H]⁺) 655.3593, found 655.3598.



Figure S7. ¹H NMR spectrum (in DMSO- d_6) of 5.



Figure S8.¹³C NMR spectrum (in DMSO- d_6) of 5.



Figure S9. HRMS spectrum of 5. HRMS (ESI): calcd for $C_{23}H_{27}N_2O_6$ ([M+H]⁺) 427.1864, found 427.1872.



Figure S10. ¹H NMR spectrum (in CDCl₃) of 6.





Figure S12. HRMS spectrum of 6. HRMS (ESI): calcd for $C_{29}H_{41}N_2O_6Si$ ([M+H]⁺) 541.2728, found 541.2724.



Figure S13. ¹H NMR spectrum (in DMSO-*d*⁶) of DCM.



Figure S14. ¹H NMR spectrum (in CDCl₃) of 7.





Figure S16. HRMS spectrum of 7. HRMS (ESI): calcd for $C_{50}H_{52}N_5O_8Si$ ([M+H]⁺) 878.3580, found 878.3584.



Figure S17. ¹H NMR spectrum (in DMSO- d_6) of 8.



Figure S18.¹³C NMR spectrum (in DMSO- d_6) of 8.



Figure S19. HRMS spectrum of 8. HRMS (ESI): calcd for $C_{44}H_{38}N_5O_8$ ([M+H]⁺) 764.2715, found 764.2717.



Figure S20. ¹H NMR spectrum (in CDCl₃) of the prodrug (compound 9).



Spectrum from sample P7.wiff (sample 1) - Sample001, Experiment 1, +TOF MS (100 - 1500) from 0.082 to 0.115 min © C65H51N7O13 +H



Figure S22. HRMS spectrum of the **prodrug (compound 9)**. HRMS (ESI): calcd for $C_{65}H_{52}N_7O_{13}$ ([M+H]⁺) 1138.3618, found 1138.3630.



Figure S23. Absorption spectra of CPT, coumarin (compound **3**, a control), prodrug and prodrug (10 μ M) exposed to blue light for 30 min in pH 7.4 PBS buffered water solution (containing 20% DMSO).



Figure S24. Photostability test of DCM-NH2 (10 μ M, Excitation wavelength: 500 nm.) in pH 7.4 PBS buffered water containing 20% DMSO. The change of the fluorescence intensity ratio for DCM-NH2 is less than 5 % under continuous light irradiation using a blue LED lamp (with the wavelength range of 400–450 nm at the power of 10 mW cm⁻²)



Figure S25. (A) Fluorescence spectra of the prodrug (10 μ M) irradiated with blue light irradiation for different time periods; (B) Fluorescence response of prodrug at 436 nm as a function of irradiation time. (Excitation wavelength: 365 nm.)



Figure S26. (A) HPLC chromatogram of prodrug, compound **3** and CPT. (B) HPLC chromatogram for the prodrug (10 μ M) upon one-photon irradiation (blue light) for different time periods. Peaks in the chromatograms were detected by monitoring the absorption at 365 nm. The mobile phase was 80/20 acetonitrile/water at a flow rate of 1.0 mL/min.

The prodrug, coumarin (compound **3**) and CPT give rise to a peak at 7.08, 1.87 and 1.64 min respectively in HPLC chromatogram (A). Upon light irradiation (B, one-photo irradiation), the peak intensity at 7.08 min (corresponding to the prodrug) decreases and two new peaks emerge at 1.87 and 1.64 min which well match that for compound **3** and CPT respectively.



Figure S27. Percentage of CPT release (as determined by HPLC) as a function of two-photon irradiation time.



Figure S28. HRMS spectra of the photolysis products. The photolysis products were purified and separated by column chromatography on silica gel, and then subjected to HRMS analysis, respectively.



Figure S29. Relationship between CPT-release percentage (as determined by HPLC) and irradiation/dark time (one-photon).



Figure S30. DLS analysis for the prodrug in aqueous solution (10 μ M in PBS solution containing 20% (upper panel) or 1% DMSO (lower panel), pH = 7.4, 10 mM).



Figure S31. Flow cytometry profiles for HeLa cells in the absence (the control) and presence of the prodrug for 4 h, irradiated with blue light for 10 min or 30 min.



Figure S32. Viability of HeLa and A549 cell lines (without being pretreated with prodrug) with/without blue light irradiation.



Figure S33. Cell viability profiles for HeLa and A549 cell lines treated with CPT and the prodrug of varied concentrations under dark or blue light irradiation.

Determination of quantum yield for prodrug:

Quantum yields of the prodrug was determined according to literature procedures, using an Rhodamine B as the reference (rhodamine B in methanol, 0.70, 25 °C) $^{[1, 2]}$.

The quantum yield (φ) of prodrug was calculated by comparing their integrated fluorescence intensities (excitation at 490 nm) and absorbance values at 490 nm with those of rhodamine B. Rhodamine B ($\varphi_r = 0.70$) was dissolved in methanol (refractive index: 1.3284) and prodrug was dissolved in DMSO (refractive index: 1.4795).



Figure S34. Plot of integrated fluorescence intensity vs. absorbance of prodrug.

The integrated fluorescence intensity is the area under the fluorescence curve in the wavelength range from 500 to 700 nm. Quantum yield can be calculated according to the following equation:

$$\varphi_s = \varphi_r \times \left[\frac{Grad_s}{Grad_r}\right] \times \frac{n_s^2}{n_r^2}$$

where φ is the fluorescence quantum yield of fluorophore, Grad is the slope of the plot of integrated fluorescence intensity vs absorbance (as shown in Figure S25); n is the refractive index at 25°C of the solvent. Subscript 'r' stands for reference, and 's' stands for samples. In order to minimize the re-absorption effects, absorbance values in the 10 mm fluorescence cuvettes was maintained under 0.1 at the excitation wavelength. Excitation and emission slit widths were set at 5.0 nm when recording their fluorescence spectra.

The φ_s of prodrug was therefore calculated to be 0.024, and fluorescence quantum yields of DCM were calculated as 0.12 using a reported method with Rhodamine B in previous work ^[1, 2].

Reference:

[1] T. Karstens, K. Kobs, J. Phys. Chem., 1980, 84, 1871.

[2] P. S. Zhang, X. F. Jiang, X. Z. Nie, Y. Huang, F. Zeng, X. T. Xia, S. Z. Wu. *Biomaterials*, 2016, **80**, 46.