Supporting Information

A I and II type compatible vinyl-pyridine modified BODIPY dimer photosensitizer for photodynamic therapy in A-549 cells

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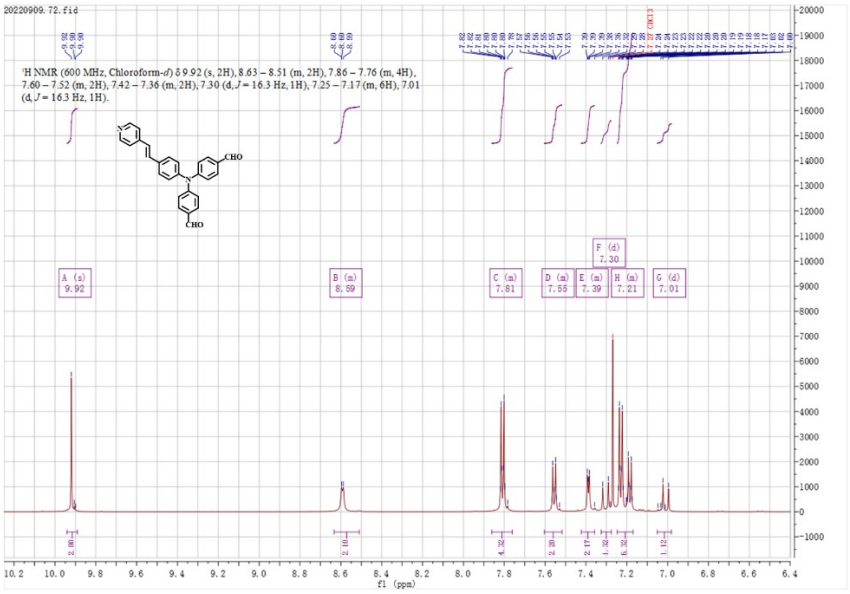
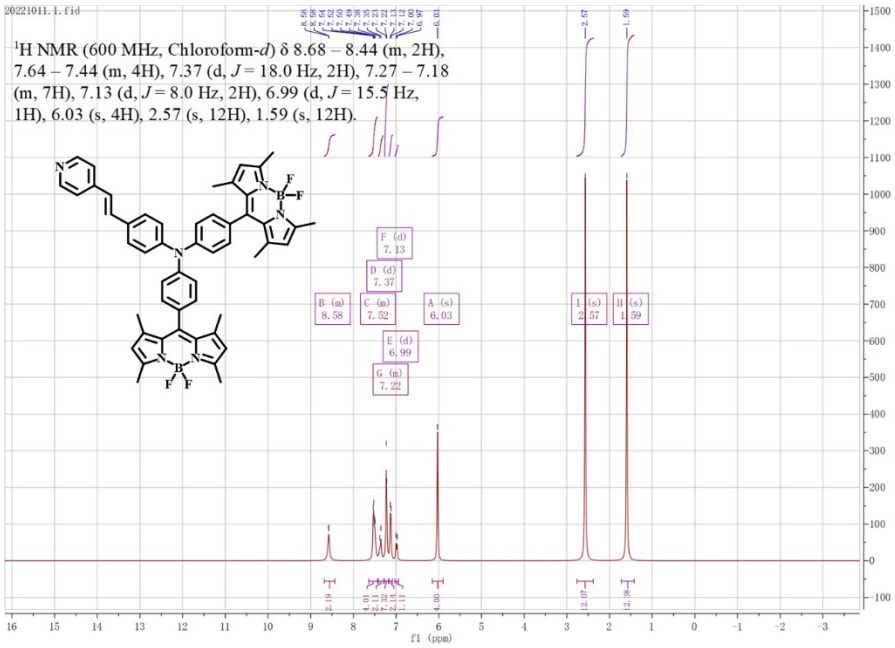
1. The ^{1}H NMR spectra of (E)-4,4'-((4-(2-(pyridin-4-yl)-vinyl)-phenyl)-azanediyl) dibenzaldehyde.



^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.92 (s, 2H), 8.63 – 8.51 (m, 2H), 7.86 – 7.76 (m, 4H), 7.60 – 7.52 (m, 2H), 7.42 – 7.36 (m, 2H), 7.30 (d, *J* = 16.3 Hz, 1H), 7.25 – 7.17 (m, 6H), 7.01 (d, *J* = 16.3 Hz, 1H).

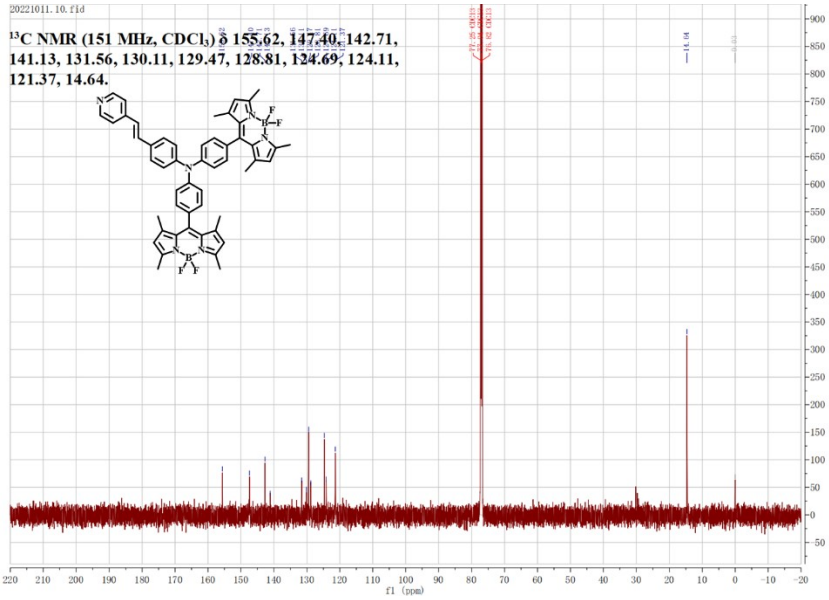
Fig. S1 The ^{1}H NMR spectra of the 4-(4-formyl-N-[3-[(E)-2-(4-pyridyl)vinyl]phenyl]anilino)benzaldehyde.

2. The ^{1}H NMR spectra, ^{13}C NMR spectra and mass spectrometry of VP-BDP$_2$



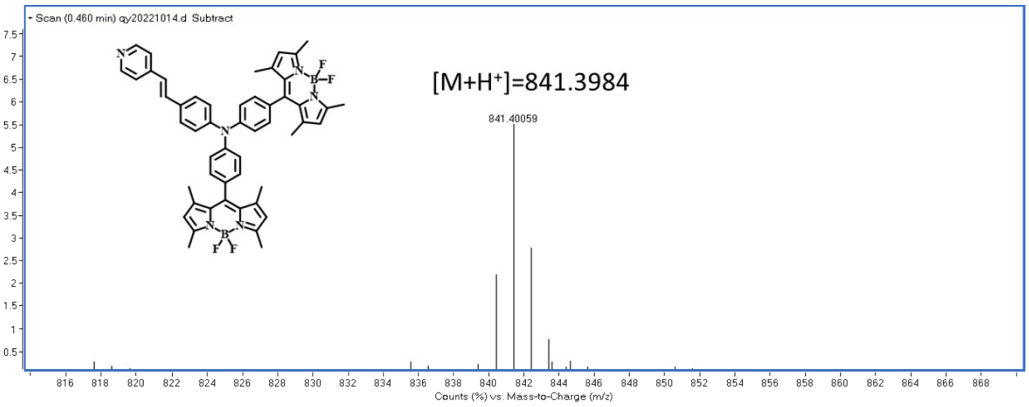
^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.68 – 8.44 (m, 2H), 7.64 – 7.44 (m, 4H), 7.37 (d, *J* = 18.0 Hz, 2H), 7.27 – 7.18 (m, 7H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.99 (d, *J* = 15.5 Hz, 1H), 6.03 (s, 4H), 2.57 (s, 12H), 1.59 (s, 12H).

Fig. S2 The ^{1}H NMR spectra of VP-BDP$_2$



^{13}C NMR (151 MHz, $CDCl_3$) δ 155.62, 147.40, 142.71, 141.13, 131.56, 130.11, 129.47, 128.81, 124.69, 124.11, 121.37, 14.64.

Fig. S3 The ^{13}C NMR spectra of VP-BDP$_2$



calcd for VP-BDP_2 $[M+H]^+$=841.3984, found $[M+H]^+$=841.4006

Fig. S4 The Mass spectra of VP-BDP_2

3. The synthesis methods of (E)-4,4'-((4-(2-(pyridin-4-yl) vinyl) phenyl) azanediyl) dibenzaldehyde

Synthesis of Compound 3:

The synthesis methods used in the below reaction could be referred to our previous work[1, 2] and the reaction dosage is as followed.

Compound 2 (200 mg, 0.468 mmol), 4-vinylpyridine (59 mg, 0.562 mmol), palladium acetate (10.5 mg, 0.045 mmol), tri(o-methylphenyl) phosphine (28.5 mg, 0.094 mmol), potassium phosphate (139 mg, 0.655mmol), DMF 6 mL, reaction time 6h, the product PY-TPA 99 mg, yield 52 %.

Synthesis of VP-BDP_2:

Compound 3 (100 mg,0.25 mmol) and 2, 4-dimethylpyrrole (94 mg,1 mmol) were added to 150 mL anhydrous dichloromethane and 1 drop of trifluoroacetic acid was added as catalyst and the solution turned brown red gradually. Then, the reaction was tracked by thin-layer chromatogram. After the Compound 3 was almost consumed, the tetrachloro-p-benzoquinone (490 123 mg,0.5 mmol) was added and react for 2 h. Then, Et_3N, 0.5mL was added into the system, after 15 min, the $BF_3{\cdot}OEt_2$ 0.75mL was added. After 12 h reaction, the mixture was washed with saturated sodium chloride brine, and was extracted with dichloromethane/water system. The organic phase (dichloromethane) was combined and purified by silica gel column chromatography.

VP-BDP_2 3.8 mg, yield 1.8 %.

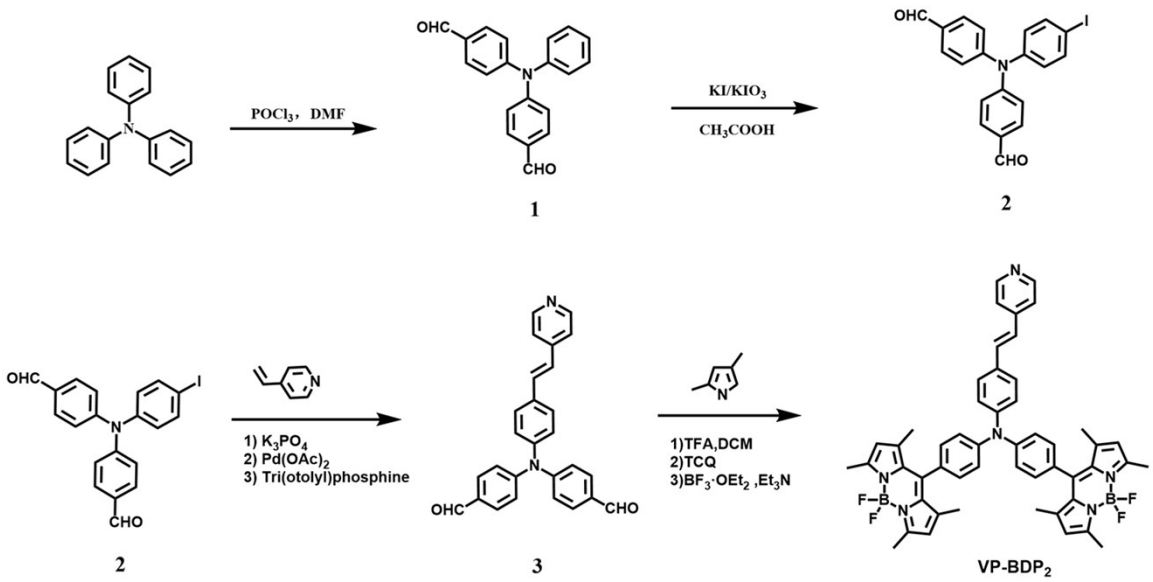


Fig. S5 The synthetic procedure of the VP-BDP_2 photosensitizer.

4. The fluorescence spectra of VP-BDP_2 in tetrachloromethane

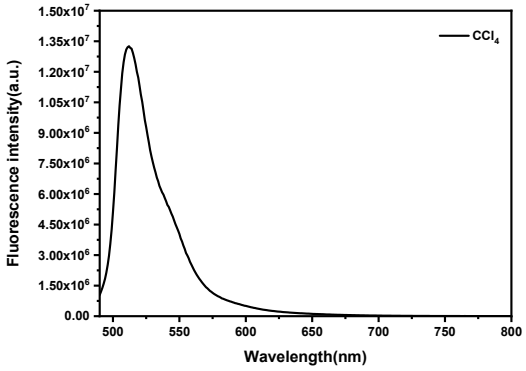


Fig. S6 The fluorescence spectra of VP-BDP_2 in tetrachloromethane

5. The photo stability test of ABDA and DPBF

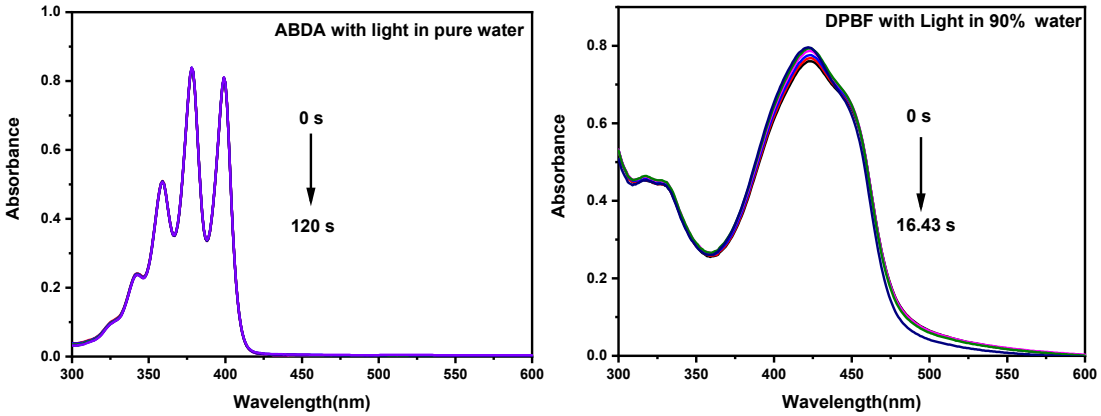


Fig. S7 The photostability test of ABDA and DPBF (EtOH:H_2O=1:9) in water system

6. Singlet oxygen test of VP-BDP_2 and T-BDP_2 in 90% water content solvent system

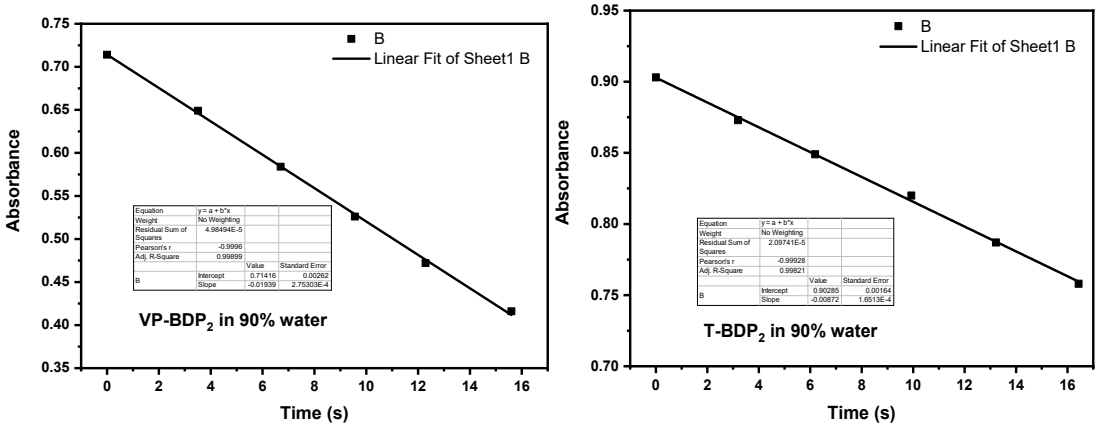


Fig. S8 The Singlet oxygen test of VP-BDP_2 and T-BDP_2 in 90% water content solvent system

7. Singlet oxygen test of VP-BDP_2 and T-BDP_2 in different solvent system

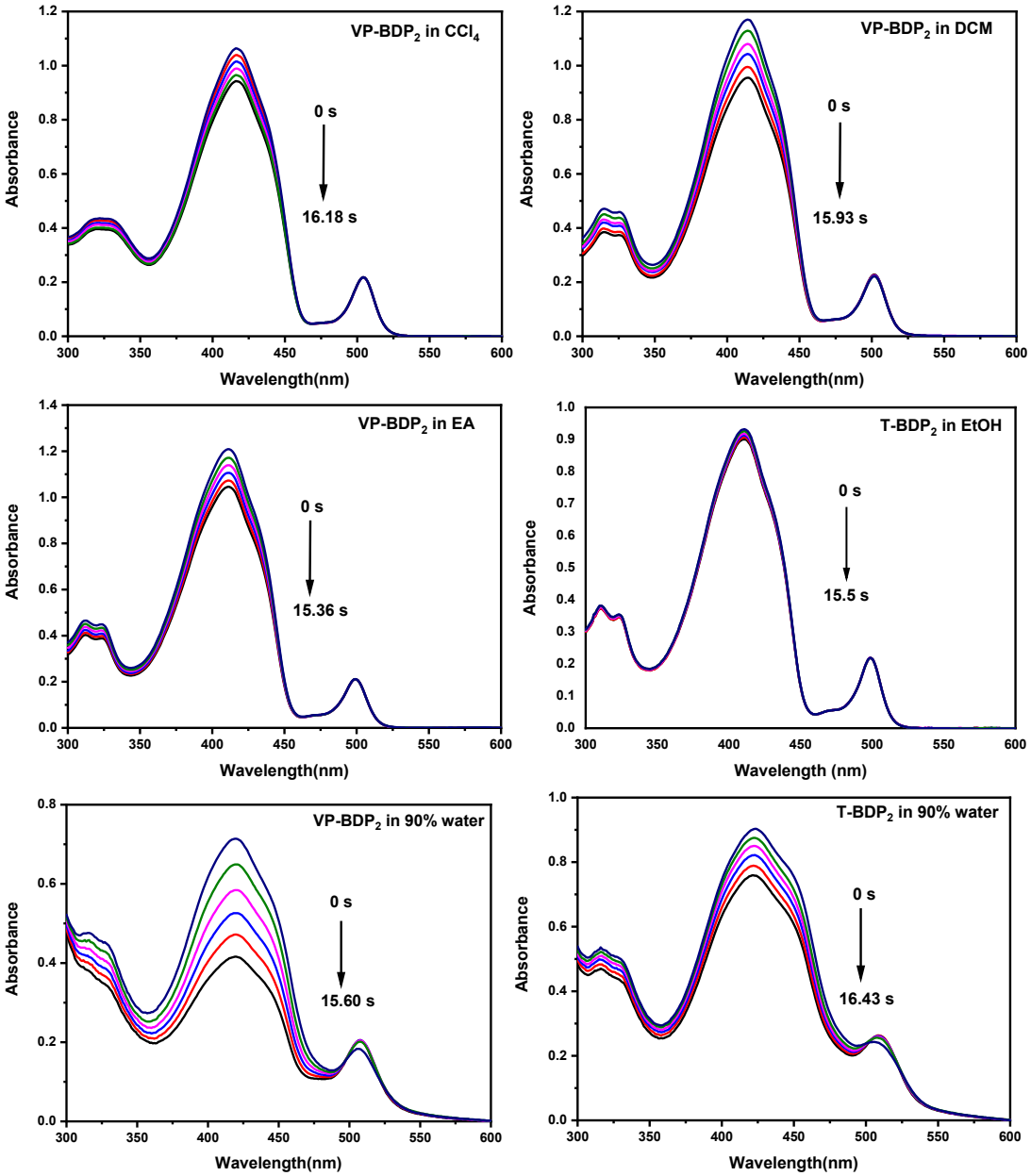


Fig. S9 The Singlet oxygen test of VP-BDP_2 and T- BDP_2 in different solvent system.

8. Singlet oxygen test of T-BDP_2 with ABDA as detector

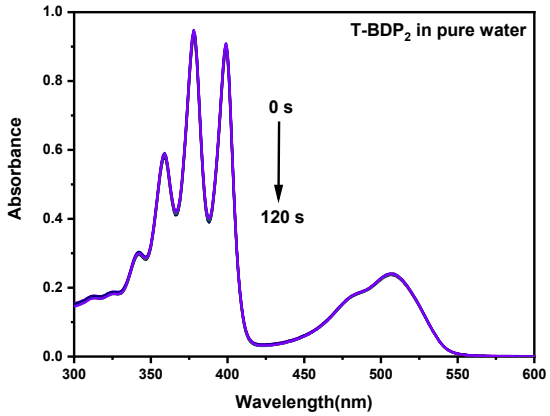


Fig. S10 The singlet oxygen test of T-BDP_2 with ABDA as detector

9. The fluorescence imaging of VP-BDP_2 in zebrafish

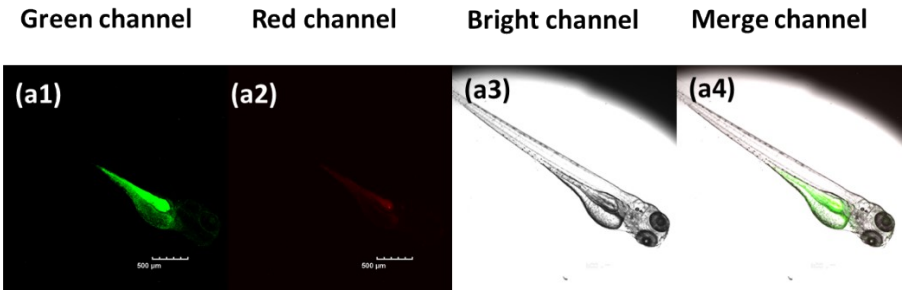


Fig. S11 The fluorescence imaging of VP-BDP_2 in zebrafish

10. Singlet oxygen test of Compound 2I-Ph-BDP

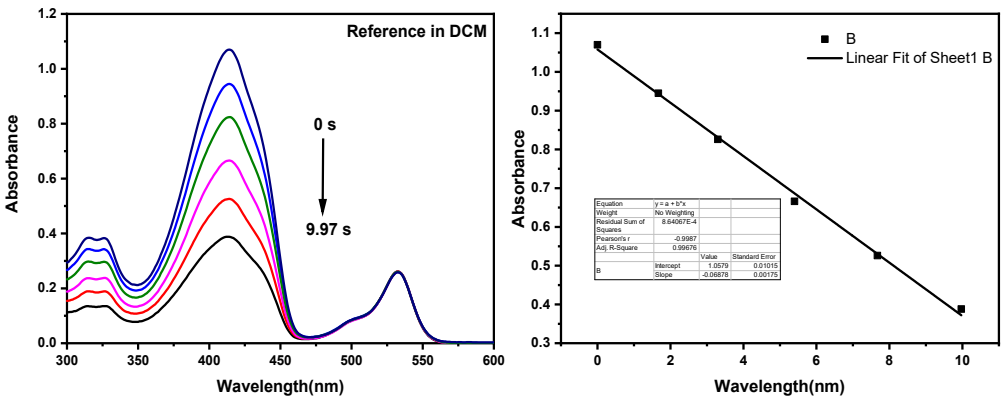


Fig. S12 Singlet oxygen test of Compound 2I-Ph-BDP(reference)

11. The experiment procedure of singlet oxygen yield test[3], superoxide radical detection[4] and fluorescence quantum yield[5]

Firstly, the absorbance of the photosensitizer was adjusted to about 0.3. Then, the suitable DPBF solution was added to the above solution and make the absorbance of DPBF near 1.0. Afterward, the mixed solution was exposed to corresponding green monochromatic light for a different interval seconds and the ultraviolet spectra was recorded by photospetrometer immediately. Taking the decrease in max absorbance of DPBF as the horizontal coordinate and the time interval as vertical coordinate to obtained the slope for the calculation of the singlet oxygen yield. The value of pearson coefficient of fitted line was used to verify whether the concentration of oxygen or DPBF is saturated during the period of experiments. The reference also was tested in the same method. The singlet oxygen quantum yield was calculated according to the following equation:

$$\phi_{\triangle} = \phi_r \times \frac{k_s}{k_r} \times \frac{1 - 10^{-OD_r}}{1 - 10^{-OD_s}}$$

Where $\boldsymbol{\phi}_{\triangle}$ represent the singlet oxygen yield, the "r" represent the reference sample, "s" represent the test sample, "k" was the slope of absorbance decrease of DPBF with the time interval and "OD" was stand for absorbance correction factor.

In the superoxide radical tests, the DHR123 was used as the fluorescent indicator of superoxide radical($O_2^{\cdot-}$)[4, 6]. When DHR123 reacts with superoxide radical($O_2^{\cdot-}$), its green fluorescence will be significantly enhanced. The detection method is to add moderate DHR123 into the solution of VP-BDP_2 at about the concentration of 15 μM. After different time illumination, the fluorescence intensity of DHR123 was detected through fluorescence emission spectra to determinate whether the superoxide radical ($O_2^{\cdot-}$) was produced. The detection of superoxide radical($O_2^{\cdot-}$)

DHR123 was performed in the same period of our last work[1], thus, the data of the optical stability test of DHR123 also was used in this work.

In the fluorescence quantum yield test, the Rh B in ethanol solution ($\boldsymbol{\phi}$r=0.50) was selected as the reference. The absorbance of both sample and reference was adjusted to about 0.05, then the wavelength at the intersection was used to excite the sample and reference, and the fluorescence quantum yield was calculated by the following formula.

$$\phi_f = \phi_r \times \frac{A_r}{A_s} \times \frac{F_s}{F_r} \times \frac{n_s^2}{n_r^2}$$

Where $\boldsymbol{\phi}_f$ is stand for the fluorescence quantum yield, the "r" represent the reference sample, "s" represent the test sample A is stand for the absorbance, F is stand for integration of fluorescence spectra, n is stand for the refractive index.

12. The experiment procedure of photo/dark cytotoxicity test[7]

After the cultivation of A-549 cells in two 96-well plate (about 1×10^4 cells/well) for 24 h, the photosensitizer with different concentrations (0-16 μM) were added into one of 96- well plates. After another 24 h incubation, the medium tested sample was replaced to remove dead cells and excess photosensitizer. The phototoxic cell experiment group was conducted by irradiating the 96-well plates with green monochromatic light for 15 minutes. Dark toxicity tested sample still was placed in the incubator. Afterward, after 12 h incubation, the MTT solution (thiazolyl blue ,10 μL; 5 mg/mL) was added into both 96-well plates and were incubated at proper environment for 4 h. At last, the MTT solution was replaced with 150 μL DMSO in each well. The absorbance at 570 nm of each well was measured with the enzyme-labeled instrument.

Cell viability = (Mean absorbance of test wells – Mean absorbance of medium control wells) / (Mean absorbance of untreated wells - Mean absorbance of medium control well) ×100%

13. The experiment procedure of fluorescence imaging and AO/EB staining test in cells[8-10]

In the cell fluorescence imaging, the A-549 cells were seeded into petri dish with 2 mL 1640 culture medium. After 24 h of cell cultivation, 10 μL photosensitizer (10^{-3} M) solution was added into the petri dish and incubate with cells for 4 h. Then, the fluorescence imaging was perform on Olympus FV3000 laser scanning confocal microscope.

In the superoxide radical($O_2^{\cdot-}$) detection experiment, the A-549 cells were seeded into petri dish with 2 mL 1640 culture medium. After 24 h of cell cultivation, 10 μL VP-BDP_2 photosensitizer (10^{-3} M) solution was added into the petri dish and incubate with cells for 4 h. Once the confocal microscope confirms that the photosensitizer has entered the cell, then, the DHE 10 uL (10^{-3}M) was added into the petri dish and incubate with cells for 10 min in dark condition. After the corresponding irradiated treatment of the cells, the Olympus FV-3000 laser scanning confocal microscope was used to detect the superoxide radical($O_2^{\cdot-}$) generation.

In the intracellular photodynamic experiments, the AO/EB stained experiment was perform on A-549 cells and the AO/EB was used to indicate the apoptosis condition of the cells. Firstly, three petri dishes filled with A-549 cells were named A,B,C and the following three independent experiment was performed on them respectively. The A petri dish was placed under illumination for 15 min. The 10 μL VP-BDP_2 photosensitizer (10^{-3} M) was added to B petri dish and incubate for 4 hours. The 10 μL VP-BDP_2 photosensitizer (10^{-3} M) was added to C petri dish and incubated for 4 h, then, treat with 15 min irradiation. Finally, the 10 time-diluted 5 μL AO and 5 μL EB were added to these three dishes severally. Then, according to the excitation wavelength on the instruction manual of AO/EB, the Olympus FV-3000 laser scanning confocal microscope was used to analyzing the apoptosis condition of A-549 cells.

14. The fluorescence imaging experiment of VP-BDP_2 in zebrafish[11]

In fluorescence imaging of zebrafish, the purchased zebrafish seedling was incubated in melanin inhibitor containing medium at 28.5℃ environment and zebrafish egg will become fish-shaped within 24-48 hours. Then, the 20 μL VP-BDP_2 photosensitizer (10^{-3} M) was added to the zebrafish containing medium and incubated for 5 hours. After that, 30 μL MS222 anesthetic was added to that petri dish and incubated for another 15 minutes. Until no obvious swimming was observed, the zebrafish was used to fluorescence imaging. The detection of $O_2^{\cdot-}$ (Superoxide radical) was similar to the method used in cells and the dosage also is 20 μL photosensitizer (10^{-3} M).

15. Transient absorption spectra of VP-BDP_2 and attenuation curves of major absorption peaks in the pure water solvent.

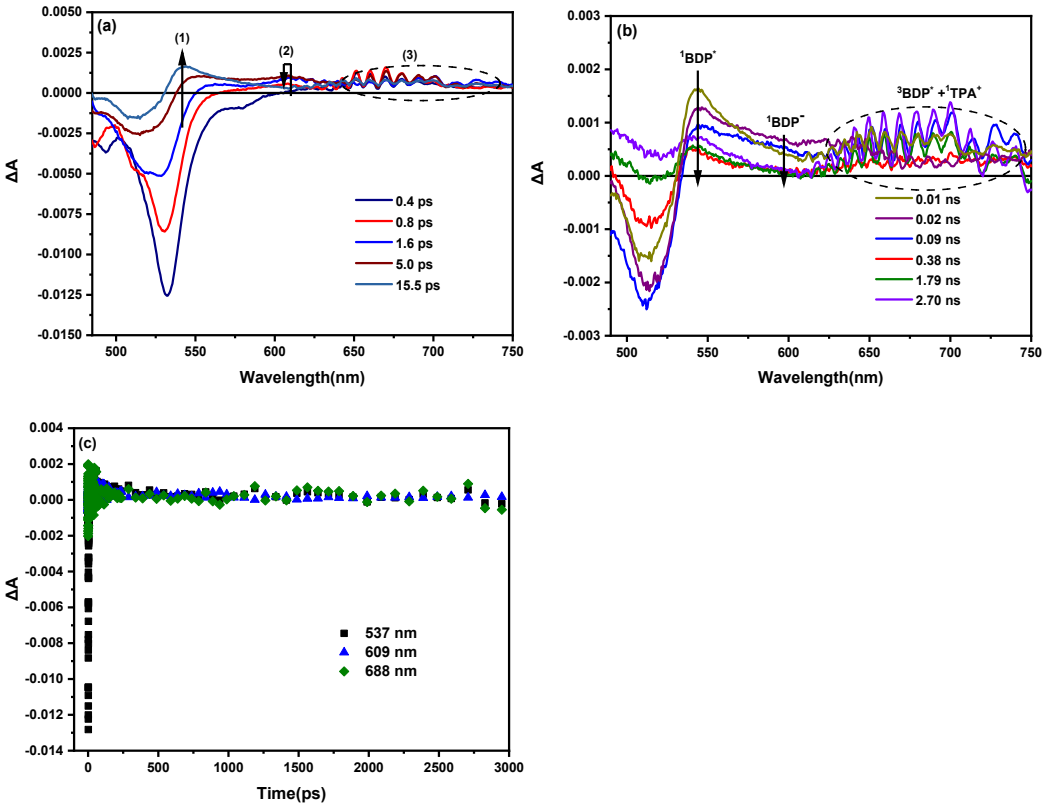


Fig. S13 Transient absorption spectra of VP-BDP_2 and attenuation curves of major absorption peaks

in the pure water solvent.

16. Transient absorption spectra of T-BDP_2 and attenuation curves of major absorption peaks in the dichloromethane solvent.

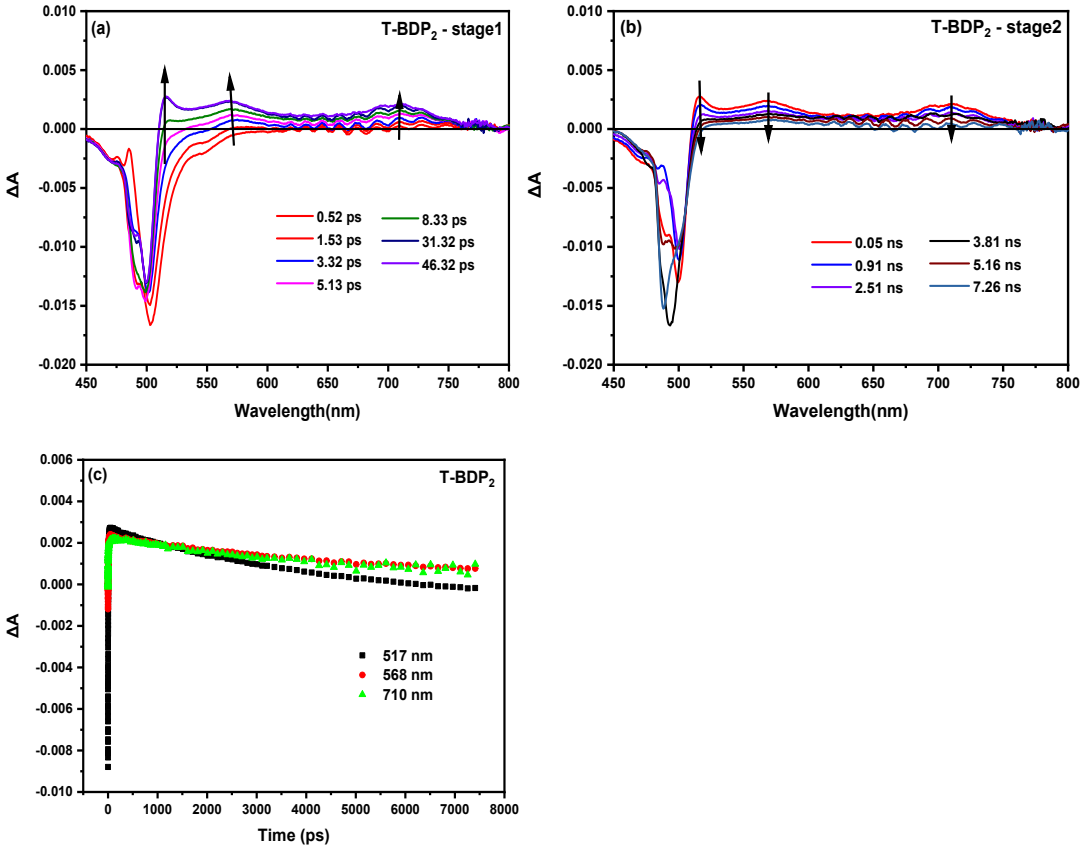


Fig. S14 Transient absorption spectra of T-BDP_2(a,b,c) and attenuation curves of major absorption peaks in the anhydrous dichloromethane solvent at same concentration.

17. The vertical excitation energy of T-BDP_2 in the pure water condition.

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Summary of excited states:
State:    1    Exc. Energy:   1.538 eV   Multi.: 3    MO pairs:      4
State:    2    Exc. Energy:   1.538 eV   Multi.: 3    MO pairs:      4
State:    3    Exc. Energy:   2.260 eV   Multi.: 3    MO pairs:      2
State:    4    Exc. Energy:   2.260 eV   Multi.: 3    MO pairs:      2
State:    5    Exc. Energy:   2.266 eV   Multi.: 1    MO pairs:      2
State:    6    Exc. Energy:   2.266 eV   Multi.: 1    MO pairs:      2
State:    7    Exc. Energy:   2.738 eV   Multi.: 3    MO pairs:      4
State:    8    Exc. Energy:   2.738 eV   Multi.: 3    MO pairs:      4
State:    9    Exc. Energy:   2.786 eV   Multi.: 1    MO pairs:      4
State:   10    Exc. Energy:   2.786 eV   Multi.: 3    MO pairs:      4
State:   11    Exc. Energy:   2.786 eV   Multi.: 1    MO pairs:      4
State:   12    Exc. Energy:   2.786 eV   Multi.: 3    MO pairs:      4
State:   13    Exc. Energy:   2.913 eV   Multi.: 3    MO pairs:      2
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Fig. S15 The vertical excitation energy of T-BDP_2 in the pure water condition.

18.The fluorescence spectra of T-BDP2 in different water content solvent system.

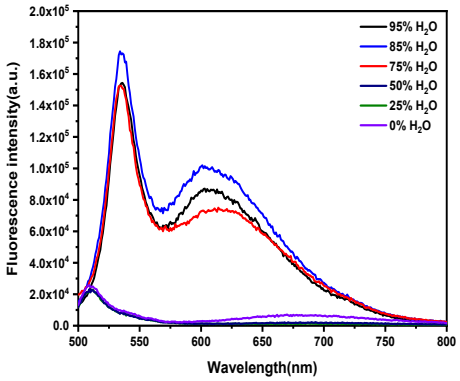

Fig. S16 The vertical excitation energy of T-BDP_2 in the pure water condition.

19.The fluorescence imaging in cell of T-BDP_2 photosensitizer

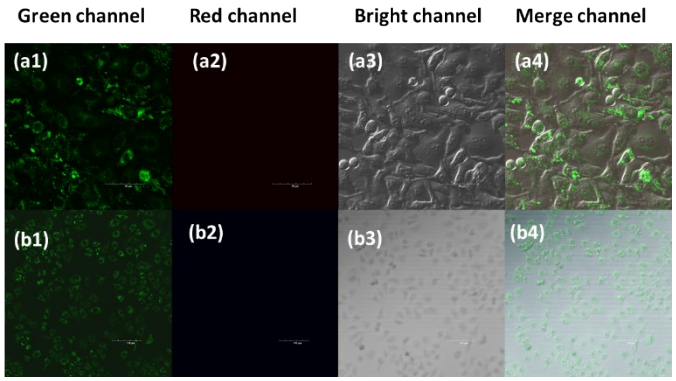

Fig. S17 The fluorescence imaging in cell of T-BDP_2 photosensitizer

20.The experiment result of open Z-scanning experiment

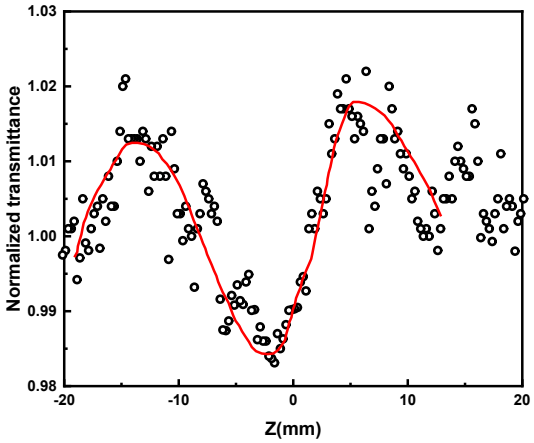

Fig S18 The experiment result of open Z-scanning experiment at 800 nm.(red line : hand fitting)

21. The calculation of the power density of the monochrome LED light at green waveband

The illuminance at 15 cm away from the light was about 1.6w lux, the power density of the monochrome green light was 2.3 mW/cm^2 according the transformational relation at green waveband.

1. L. Wang and Y. Qian, *Biomater Sci*, 2023, 11, 1459-1469.
2. L. Wang and Y. Qian, *Dyes Pigm.*, 2022, 198.
3. X. Zhao, Q. Yao, S. Long, W. Chi, Y. Yang, D. Tan, X. Liu, H. Huang, W. Sun, J. Du, J. Fan and X. Peng, *J Am Chem Soc*, 2021, 143, 12345-12354.
4. M. Li, J. Xia, R. Tian, J. Wang, J. Fan, J. Du, S. Long, X. Song, J. W. Foley and X. Peng, *J Am Chem Soc*, 2018, 140, 14851-14859.
5. K. Nawara and J. Waluk, *Anal Chem*, 2017, 89, 8650-8655.
6. L. Feng, C. Li, L. Liu, Z. Wang, Z. Chen, J. Yu, W. Ji, G. Jiang, P. Zhang, J. Wang and B. Z. Tang,

ACS Nano, 2022, 16, 4162-4174.

7. D. H. Wang, L. J. Chen, X. Zhao and X. P. Yan, *J Mater Chem B*, 2021, 9, 9900-9907.
8. S. Li, K. Chang, K. Sun, Y. Tang, N. Cui, Y. Wang, W. Qin, H. Xu and C. Wu, *ACS Appl Mater Interfaces*, 2016, 8, 3624-3634.
9. P. He, W. Han, C. Bi, W. Song, S. Niu, H. Zhou and X. Zhang, *ACS Nano*, 2021, 15, 6961-6976.
10. I. Matai, A. Sachdev and P. Gopinath, *Biomater. Sci.*, 2015, 3, 457-468.
11. K. D. Addisu, W. H. Hsu, B. Z. Hailemeskel, A. T. Andrgie, H. Y. Chou, C. H. Yuh, J. Y. Lai and H. C. Tsai, *ACS Biomater Sci Eng*, 2019, 5, 5453-5469.