Support information

Improving Fluorescence Brightness of Distyryl Bodipys by Inhibiting Twisted Intramolecular Charge Transfer Excited State

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1. Experimental methods

1.1 Regents and solvents

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated.

1.2 Equipment and measurements

Thin-layer chromatography (TLC) was used to monitor the reactions and silica gel (200–300 mesh) was used to column chromatography. Absorption spectra were measured on Varian Cary 4000 spectrophotometer. Fluorescence spectra were measured on Hitachi F-7000 fluorescence spectrometer. The $^1$H NMR and $^{13}$C NMR spectra were measured on a Bruker spectrometer, and recorded at 400 and 100 MHz, respectively. High resolution mass spectra were obtained on a Thermo Scientific Q Exactive Mass spectrometer. The fluorescence imaging assays in cells were performed in a Zeiss LSM 880+ Airyscan Laser Scanning Confocal Microscope with a 60×oil-immersion objective lens. The living animal imaging assays were performed in a Bruker In Vivo FX Pro small animal optical imaging system with an excitation filter of 650 nm and an emission filter of 750 nm.
1.3 Spectra test of **BDP1-4**

The stock solutions of **BDP1-4** (2 mM) were prepared by directly dissolving the solid compound into CH$_3$CN. The test solutions of **BDP1-4** (2 µM) were prepared by diluting 2 µL of their stock solution to 2 mL with the corresponding solvents, including CH$_2$Cl$_2$, CH$_3$CN, MeOH and buffered aqueous solution (10 mM PBS, pH 7.4, containing 50% CH$_3$CN). The acquired test solutions were subjected to spectra test on a Hitachi F-7000 fluorescence spectrometer or Varian Cary 4000 spectrophotometer.

1.4 Determination of Quantum Yields

Fluorescence quantum yields of all samples were determined via the relative determination method by using Cy5.5 (Φ = 0.23 in PBS) as the reference.

The quantum yields were calculated using the following Equation:

$$\Phi_u = \left(\frac{A_sFA_u\eta^2}{A_uFA_s\eta_0^2}\right)\Phi_s$$

Where $A_s$ and $A_u$ are the absorbance of the reference and sample solution at the reference excitation wavelength, $FA_s$ and $FA_u$ are the corresponding integrated fluorescence intensity, and $\eta$ and $\eta_0$ are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05. Reported values are averages ($n = 3$).

1.5 Photostability test

The photostability test were carried out by irradiating the CH$_3$CN solution of the dyes (2 µM) by a LED source (660 nm, 50 mW/cm$^2$) at room temperature for 10 min. The fluorescence intensities at the emission maxima of **BDP1-4**, **C-BDP** and **Cy5** were measured every one minute on a Hitachi F-7000 fluorescence spectrometer.

1.6 Preparation of the test solution for the selectivity test of **FP-BDP4**

The aqueous solutions of Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, Cu$^{2+}$, Zn$^{2+}$ were prepared from their chloride salts and the aqueous solutions of NO$_3^-$, Cl$^-$, Br$^-$, CO$_3^{2-}$ were prepared from their sodium salts. Hydrogen peroxide solution (H$_2$O$_2$) was prepared by dilution of commercial H$_2$O$_2$ solution in deionized water, and its concentration was determined by using an extinction coefficient of 43.6 M$^{-1}$cm$^{-1}$ at 240 nm. Hypochlorite solution (ClO$^-$) was prepared by the dilution of commercial NaClO
solution in deionized water, and its concentration was determined using an extinction coefficient of 350 M$^{-1}$cm$^{-1}$ at 293 nm. Superoxide solution ($O_2^-$) was prepared by dissolving KO$_2$ to dry dimethyl sulfoxide. Hydroxyl radical ($•OH$) was generated in situ through the Fenton reaction of Fe(ClO$_4$)$_2$ and H$_2$O$_2$. Singlet oxygen (‘$O_2$') was generated in situ by adding ClO$^-$ solution to 10 eq of H$_2$O$_2$ solution. Nitric oxide (NO) was generated from a commercial NO donor NOC-9 (dissolved in 0.1M NaOH solution). Peroxynitrite solution (ONOO$^-$) was prepared according to literature report$^1$, and the concentration of ONOO$^-$ was determined in 0.1 M NaOH by using an extinction coefficient of 1,670 M$^{-1}$ cm$^{-1}$ at 302 nm. The aqueous solutions of Cys, Hcy and GSH were freshly prepared by dissolving their solid in deionized water.

The test solution of FP-BDP4 (4 µM) was prepared by diluting 4 µL of the FP-BDP4 stock solution (2 mM in CH$_3$CN) into 2 mL buffer solution (PBS / CH$_3$CN = 1:1, 10 mM, pH = 7.4) containing various biological species. The resulting solutions were kept at 37°C for 60 min and then the fluorescence intensities were measured.

1.7 Cell Culture and Fluorescence Imaging.

All cell lines were purchased from GeneFull Biotech co., Ltd (China). Raw 264.7 cells were grown in RPMI 1640 medium and HeLa cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % FBS (Fetal Bovine Serum), 100 U/mL sodium penicillin G and 100 µg/mL streptomycin at 37 ºC in humidified environment of 5% CO$_2$. Cells were plated on glass bottom cell culture dish (30 mm) and allowed to adhere for 12 hours.

For imaging experiments with BDP1-4 and C-BDP, HeLa cells were treated with the corresponding dye (1 µM) in DMEM medium for 60 min. After washing the cells with PBS for three times, the fluorescence imaging assays were performed. Emissions were collected at 650−750 nm ($\lambda_{ex} = 633$ nm).

To evaluate the subcellular localization of BDP2 and BDP3, HeLa cells were first incubated with BDP2 or BDP3 (0.5 µM) for 1 h in DMEM and then washed with PBS for three times, followed by treating with cytoplasm membrane dye DiO (5 µM) for 15 min. After washing the cells with PBS for three times, fluorescence imaging was performed. For BDP2 and BDP3, emissions were collected at 650−750 nm ($\lambda_{ex} = 633$ nm); for DiO, at 500−600 ($\lambda_{ex} = 488$ nm).

For the selectivity test of probe FP-BDP4 in cells, the Raw 264.7 macrophage cells were first
incubated with **FP-BDP4** (1 μM) for 1 h at 37 °C and then washed with PBS for three times, followed by treating with various ROS/RNS (100 μM). After 30 min, the cells were washed again and subjected to fluorescence imaging.

To image endogenous H$_2$O$_2$, Raw 264.7 macrophage cells were pretreated with PMA (2 μg/mL) in culture medium for 12 h. The cells were washed with PBS and then incubated with **FP-BDP4** (1 μM) for 60 min. After washing three times with PBS, the fluorescence imaging was performed. For the inhibition assay, the cells were pretreated with PMA (2 μg/mL) for 12 h, washing with PBS and then incubated with NAC (2 mM) for 1 h. Then, the cells were washed three times with PBS, followed by incubating with **FP-BDP4** (1 μM) for 60 min. After washing with PBS for three times, the imaging assays were performed.

1.8 Imaging H$_2$O$_2$ in inflamed mouse models

Female BALB/c nude mice (6-8 weeks old) were obtained from Beijing Virtal River Laboratory Animal Technology Co., Ltd. All the animal experiments were carried out in accordance with the relevant laws and guidelines issued by the Ethical Committee of Shanxi University. For imaging exogenous H$_2$O$_2$, the mice were first intraperitoneally (i.p.) injected with **FP-BDP4** (50 μM, 100 μL, 50% DMSO in saline) for 10 min, and then i.p. injected with H$_2$O$_2$ (2 mM, 100 μL) for 60 min. For imaging endogenous H$_2$O$_2$, the mice were first i.p. injected with LPS (2 mg/mL, 100 μL) for 24 h, and then i.p. injected with **FP-BDP4** (50 μM, 100 μL, 50% DMSO in saline) for 60 min.

1.9 Cytotoxicity Assays.

HeLa cells were seeded into a 96-well plate in DMEM medium supplemented with 10% FBS and 1% antibiotics at 37°C in a humidified environment of 5% CO$_2$. After 24 h of cell attachment, the cells were washed with PBS, followed by addition of increasing concentrations of the dye in DMEM medium. The final concentrations of the dyes were kept from 0 to 10 μM. The cells were then incubated at 37 °C in an atmosphere of 5% CO$_2$ and 95% air for 24 h, followed by MTT assays. Untreated assays with DMEM medium (n = 6) were also conducted under the same conditions.
2. Synthesis and characterization of the compounds

Scheme S1. The synthetic routes of BDP1-3 and C-BDP.

General procedures of Knoevenagel-type reactions for the synthesis of compound C-BDP, BDP1 and intermediate 2 and 4

BODIPY 1\(^{[2]}\) (1 equiv) and aromatic aldehyde (1-4 equiv) were dissolved in dry N, N-dimethylformamide (20 mL/mmol BODIPY). Glacial acetic acid (1 mL/mmol BODIPY) and piperidine (1 mL/mmol BODIPY) were added to the reaction mixture. Then, the reaction mixture was heated for 1-3 h at 130 °C. After cooling to room temperature, the mixture was poured into water and extracted with ethyl acetate for three times. The combined organic layer was dried over Na\(_2\)SO\(_4\) and then filtered. The solvent was evaporated, and the residue was purified by silica gel chromatography.

**C-BDP:** Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY 1 (162 mg, 0.5 mmol) and 4-(diethylamino) benzaldehyde (354 mg, 2.0 mmol). The crude
product was purified by column chromatography (PE: EA=5:1) affording a deep green solid (208 mg, 65%).

$^1$H-NMR (CDCl$_3$, 400 MHz) δ (ppm): 7.56 (s, 1H), 7.52-7.50 (m, 5H), 7.47-7.45 (m, 3H), 7.33-7.30 (m, 2H), 7.19 (s, 1H), 7.15 (s, 1H), 6.68-6.66 (d, $J = 8.8$ Hz, 4H), 6.58 (s, 2H), 3.43-3.38 (q, $J = 7.2$ Hz, 8H), 1.41 (s, 6H), 1.21-1.84 (t, $J = 7.2$ Hz, 12H); 13C NMR (100 MHz, CDCl$_3$) δ 152.8, 148.2, 140.7, 136.2, 135.9, 135.7, 132.9, 128.9, 128.8, 128.5, 124.2, 117.1, 114.4, 111.4, 44.5, 14.6, 12.7; ESI-MS [M+H]$^+$: calcd for 643.3778, Found 643.3787.

**BDP1**: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY 1 (162 mg, 0.5 mmol) and 4-(1,1-dioxidothiomorpholino) benzaldehyde (480 mg, 2.0 mmol). The crude product was purified by column chromatography (PE: EA=2:1) affording a dark blue solid (231 mg, 59%).

$^1$H-NMR (CDCl$_3$, 400 MHz) δ (ppm): 7.64-7.60 (d, $J = 16$ Hz, 2H), 7.57-7.55 (d, $J = 8.8$ Hz, 4H), 7.51-7.48 (m, 3H), 7.32-7.30 (m, 2H), 7.22-7.18 (d, $J = 16$ Hz, 2H), 6.90-6.88 (d, $J = 8.8$ Hz, 4H), 6.64 (s, 2H), 3.93-3.90 (t, $J = 4.4$ Hz, 8H), 3.10-3.07 (t, $J = 4.4$ Hz, 8H), 1.43 (s, 6H); 13C NMR (100 MHz, CDCl$_3$) δ 152.5, 147.5, 141.9, 138.1, 135.3, 135.1, 133.2, 129.2, 129.1, 129.0, 128.9, 128.5, 125.5, 117.6, 117.2, 116.8, 115.6, 50.4, 46.9, 14.6; ESI-MS [M+Na]$^+$: calcd for 789.2523, Found 789.2528.

**Compound 2**: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY 1 (325 mg, 1.0 mmol) and 4-(1,1-dioxidothiomorpholino) benzaldehyde (239 mg, 1.0 mmol). The crude product was purified by column chromatography (DCM: EA=1:5) affording a dark blue solid (257 mg, 47%).

$^1$H-NMR (CDCl$_3$, 400 MHz) δ (ppm): 7.57-7.32 (d, $J = 3.2$ Hz, 2H), 7.20-7.17 ($J = 10.8$ Hz, 1H), 6.91-6.90 (d, $J = 4.8$ Hz, 2H), 6.61 (s, 1H), 6.02 (s, 1H), 3.95 (s, 4H), 3.13 (s, 4H), 2.61 (s, 3H), 1.44 (s, 3H), 1.40 (s, 3H); 13C NMR (100 MHz, CDCl$_3$) δ 154.7, 153.1, 147.5, 142.5, 142.4, 140.0, 135.4, 135.1, 132.2, 129.1, 129.0, 128.9, 128.5, 125.5, 117.6, 117.2, 116.8, 115.6, 50.4, 47.0, 14.6, 14.3; ESI-MS [M+Na]$^+$: calcd for 568.2012, Found 568.2014.

**Compound 4**: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY 1 (162 mg, 0.5 mmol) and 4-(4-methylpiperazin-1-yl) benzaldehyde (408 mg, 2.0 mmol). The crude product was purified by column chromatography (CH$_2$Cl$_2$: Et$_3$N=30:1) affording a dark green solid, which was further washed with PE to give a pure product (130 mg, 37%).

$^1$H-NMR (CDCl$_3$, 400 MHz) δ (ppm): 7.61 (s, 1H), 7.57-7.53 (m, 5H), 7.48-7.47 (m, 3H), 7.33-7.31 (m, 2H), 7.20-7.16 (d, $J = 16$ Hz, 2H), 6.92-6.90 (d, $J = 8.8$ Hz, 4H), 6.60 (s, 2H), 3.33-3.30 (t, $J = 4.8$ Hz,
8H), 2.61-2.58 (t, $J = 4.8$ Hz, 8H), 2.37 (s, 6H), 1.42 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 152.7, 151.4, 141.3, 135.9, 135.4, 128.9, 128.8, 128.7, 128.6, 127.8, 117.4, 116.3, 115.2, 54.9, 48.1, 46.1, 14.6; ESI-MS [M+H]$^+$: calcd for 697.3996, Found 697.3994.

**Compound 3:** Compound 2 (100 mg, 0.18 mmol) and 4-(4-methylpiperazin-1-yl) benzaldehyde (75 mg, 0.36 mmol) were dissolved in dry N, N-dimethylformamide (10 mL). Glacial acetic acid (0.18 mL) and piperidine (0.18 mL) were added to the reaction mixture. Then, the reaction mixture was heated for 2 h at 130 °C. After cooling to room temperature, the mixture was poured into water and extracted with ethyl acetate for three times. The combined organic layer was dried over Na$_2$SO$_4$ and then filtered. The solvent was evaporated, and the residue was purified by silica gel chromatography (CH$_2$Cl$_2$: MeOH=50:1) affording 3 as a dark green solid (35 mg, 27%). $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 7.64-7.55 (m, 6H), 7.52-7.47 (m, 3H), 7.33-7.31 (m, 2H), 7.23-7.14 (d, $J = 16$ Hz, 2H), 6.92-6.89 (d, $J = 8.8$ Hz, 4H), 6.62-6.60 (d, $J = 8.0$ Hz, 2H), 3.94-3.92 (t, $J = 4.8$ Hz, 4H), 3.34-3.31 (t, $J = 4.8$ Hz, 4H), 3.12-3.10 (t, $J = 4.8$ Hz, 4H), 2.60 (s, 4H), 2.38 (s, 3H), 1.43 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 153.5, 151.5, 147.3, 137.6, 136.5, 135.3, 134.5, 129.3, 129.2, 128.9, 128.8, 128.5, 127.6, 117.7, 117.5, 117.2, 116.1, 115.7, 115.2, 54.8, 50.4, 48.0, 47.1, 46.0, 14.6, 14.5; ESI-MS [M+H]$^+$: calcd for 732.3350, Found 732.3357.

**BDP2:** Compound 3 (30 mg, 0.04 mmol) was dissolved in CH$_3$CN (10 mL) followed by addition of CH$_3$I (0.5 mL). The reaction mixture was stirred at 80 °C for 12 h and then evaporated to dryness. The residue solid was washed with ethyl acetate to remove material and then collected as a dark green solid (31 mg, 88%). $^1$H-NMR (CD$_3$CN, 400 MHz) $\delta$ (ppm): 7.62-7.57 (m, 6H), 7.54-7.50 (m, 1H), 7.43-7.41 (m, 3H), 7.38-7.37 (d, $J = 4.0$ Hz, 1H), 7.08-7.06 (d, $J = 8.8$ Hz, 4H), 6.78 (s, 2H), 3.96-3.93 (t, $J = 5.2$ Hz, 4H), 3.64-3.62 (t, $J = 4.4$ Hz, 4H), 3.55-3.52 (t, $J = 4.4$ Hz, 4H), 3.19 (s, 6H), 3.11-3.08 (t, $J = 6.0$ Hz, 4H), 1.47 (s, 6H); $^{13}$C NMR (100 MHz, CD$_3$CN) $\delta$ 158.0, 157.4, 155.1, 153.7, 147.7, 143.7, 141.8, 141.3, 141.2, 140.1, 138.3, 138.2, 134.4, 134.3, 134.2, 134.1, 134.0, 133.9, 133.8, 132.9, 121.2, 121.0, 120.9, 120.7, 66.3, 56.5, 55.4, 51.6, 47.3, 19.1.; ESI-MS [M]$^+$: calcd for 746.3506, Found 746.3510.

**BDP3:** Compound 4 (28 mg, 0.04 mmol) was dissolved in CH$_3$CN (10 mL) followed by addition of CH$_3$I (0.5 mL). The reaction mixture was stirred at 80 °C for 12 h and then evaporated to dryness. The residue solid was washed with CH$_2$Cl$_2$ to remove material and then collected as a dark green
solid (38 mg, 97%). $^1$H-NMR (CD$_3$CN, 400 MHz) $\delta$ (ppm): 7.63-7.60 (d, $J = 8.8$ Hz, 4H), 7.59-7.56 (m, 4H), 7.52 (s, 1H), 7.43-7.39 (m, 4H), 7.09-7.07 (d, $J = 8.8$ Hz, 4H), 6.80 (s, 2H), 3.64-3.63 (t, $J = 4.8$ Hz, 8H), 3.56-3.54 (t, $J = 4.8$ Hz, 8H), 3.20 (s, 12H), 1.47 (s, 6H); $^{13}$C NMR (100 MHz, CD$_3$CN) $\delta$ 152.4, 149.8, 142.3, 138.6, 136.2, 134.7, 133.0, 129.1, 128.6, 128.5, 115.8, 61.0, 51.2, 41.9, 13.8; ESI-MS [M]$^+$: calcd for 853.3432, Found 853.3424.

**Synthesis of compound BDP4 and FP-BDP4:**

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{B} \\
\text{F} & \quad \text{F} \\
\text{OHC} & \quad \text{DMF}, \text{Piperidine}, \text{AcOH} \\
130 & \quad ^\circ \text{C}, 2h \\
\end{align*}
\]

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{B} \\
\text{Br} & \quad \text{OH} \\
\text{OH} & \quad \text{CH}_3 \\
\end{align*}
\]

Scheme S2. The synthetic routes of FP-BDP4.

Compound **BDP4**: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY 5$^{[3]}$ (162 mg, 0.5 mmol) and 4-(1,1-dioxidothiomorpholino) benzaldehyde (360 mg, 1.5 mmol). The crude product was purified by column chromatography (PE: EA=2:1) affording a dark blue solid (231 mg, 60%). $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 8.80-8.79 (m, 2H), 7.62-7.57 (m, 6H), 7.37-7.36 (m, 2H), 7.19 (s, 1H), 7.19 (s, 1H), 6.92-6.90 (d, $J = 8.8$ Hz, 4H), 6.65 (s, 2H), 3.96-3.94 (t, $J = 4.8$ Hz, 8H), 3.13-3.10 (t, $J = 4.8$ Hz, 8H), 1.47 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 153.1, 150.5, 147.6, 143.8, 141.1, 136.0, 133.7, 132.2, 129.4, 128.8, 123.9, 118.1, 117.0, 115.5, 50.4, 46.9, 14.9. ESI-MS [M+H]$^+$: calcd for 768.2656, Found 768.2652.

Compound **FP-BDP4**: BDP4 (192 mg, 0.25 mmol) and (4-(bromomethyl) phenyl) boronic acid (160 mg, 0.75 mmol) were dissolved in CH$_3$CN (30 mL) and then refluxed under N$_2$ for 24 h. After cooling to about 40 $^\circ$C, the reaction mixture was filtered, and the filter cake was washed with a small amount of CH$_3$CN for several times. The residue solid was collected and dried in vacuum affording a dark green solid (200 mg, 82%). $^1$H-NMR (CD$_3$CN, 400 MHz) $\delta$ (ppm): 8.91 (d, $J = 4.0$ Hz, 2H), 8.20-8.19 (d, $J = 4.0$ Hz, 2H), 7.89-7.88 (d, $J = 5.2$ Hz, 2H), 7.58-7.57(d, $J = 5.2$ Hz, 4H), 7.42-7.41(d, $J = 1.6$ Hz, 4H), 7.04-7.02 (d, $J = 4.8$ Hz, 4H), 6.80 (s, 2H), 6.26 (s, 2H), 5.85 (s, 2H), 5.42 (s, 2H), 3.94 (s, 8H), 3.07 (s, 8H), 1.46 (s, 6H); $^{13}$C NMR (100 MHz, DMSO) $\delta$ 153.6, 148.8, 146.4, 140.6, 138.4, 136.5, 135.3, 134.4, 131.2, 129.6, 127.7, 127.0, 125.8, 119.4, 115.7, 115.0, 64.0, 63.3, 50.3, 46.3, 15.4. ESI-MS [M]$^+$: calcd for 902.3195, Found 902.3206.
3. Supplementary Figures

**Figure S1.** Normalized absorption (A) and emission (B) spectra of BDP1-4 and C-BDP in CH₃CN at 25 °C. \( \lambda_{ex} = 615 \text{ nm} \); slits, 5/5 nm.

**Figure S2.** Normalized absorption (A) and emission (B) spectra of BDP1-4 and C-BDP in CH₃OH at 25 °C. \( \lambda_{ex} = 615 \text{ nm} \); slits, 5/5 nm.
Figure S3. Normalized absorption (A) and emission (B) spectra of BDP1-4 and C-BDP in buffered aqueous solution (10 mM PBS, pH 7.4, containing 50% CH$_3$CN) at 25 °C. $\lambda_{ex} = 615$ nm; slits, 5/5 nm.

Figure S4. Fluorescence intensity changes of C-BDP, BDP1-4, and Cy5 in CH$_3$CN when continuously irradiated by a LED source (660 nm, 50 mW/cm$^2$) for 10 min.
Figure S5. The solubility comparison of BDP1-4 (10 μM) with C-BDP (10 μM). Absorbance spectra were obtained in CH$_3$CN (solid line) or H$_2$O/CH$_3$CN (v/v 1:1, dashed line).

Figure S6. Percentage of viable HeLa cells after treatment with BDP1-4, respectively, at varied concentrations for 12 hr, which is measured by MTT assays.
Figure S7. Time-dependent fluorescence (A) and absorption (B) changes of **FP-BDP4** (4 µM) upon treated with 100 µM H$_2$O$_2$ in PBS (10 mM, pH = 7.4, containing 50% CH$_3$CN). $\lambda_{ex}$ = 615 nm; slits: 5/5 nm.

Figure S8. HRMS spectra of the mixture of **FP-BDP4** and H$_2$O$_2$. 
Figure S9. Prediction of the d-PeT effect in FP-BDP4 and BDP4 by DFT/TDDFT calculations. (A) Frontier orbital energy representation of the d-PeT process in FP-BDP4. (B) Frontier orbital energy representation of the d-PeT inhibition in BDP4. As showed in (A), the calculated LUMO level of the pyridinium unit lies between the HOMO and LUMO levels of BDP unit, thus allowing the electron transfer from the excited BDP unit to the pyridinium unit via d-PeT (Donor-excited PeT with fluorophore acts as the electron donor)\(^4\) that results in the fluorescence quenching. In contrast, as shown in (B), the calculated LUMO level of the pyridyl unit is higher than that of BDP unit, and thus the d-PeT is inhibited and the fluorescence is restored.

Figure S10. (A) Fluorescence spectra changes of FP-BDP4 (4 μM) in PBS / CH\(_3\)CN (v/v= 1:1, 10 mM, pH = 7.4) upon addition of H\(_2\)O\(_2\) (0-200 μM). The spectra were measured at 37 °C with a reaction time of 60 min. (B) The linear relationship between fluorescence intensity (F\(_{720}\) nm) and H\(_2\)O\(_2\) concentration.
Figure S11. Fluorescence responses of FP-BDP4 (4 μM) toward various biological species including various ions (1 mM), biothiols (1 mM), and ROS/RNS (100 μM). 0–21: blank, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺, NO₃⁻, Cl⁻, Br⁻, CO₃²⁻, Cys, GSH, Hcy, NO, HClO, O₂⁻, ONOO⁻, •OH, O₂⁻ and H₂O₂ in PBS (10 mM, pH 7.4, containing 50% CH₃CN).

Figure S12. Fluorescence responses of FP-BDP4 (4 μM) toward H₂O₂ (100 μM) at different pH values.
Figure S13. Percentage of viable HeLa cells after treatment with the indicated concentrations of FP-BDP4 for 12 hours, which is measured by MTT assays.
4. $^1$H NMR, $^{13}$C NMR and HRMS spectra

$^1$H NMR spectra of C-BDP in CDCl$_3$.

$^{13}$C NMR spectra of C-BDP in CDCl$_3$. 
HRMS spectra of compound C-BDP.

$^1$H NMR spectra of BDP1 in CDCl$_3$. 
$^{13}$C NMR spectra of BDP1 in CDCl₃.

HRMS spectra of compound BDP1.
$^1$H NMR spectra of compound 2 in CDCl$_3$.

$^{13}$C NMR spectra of compound 2 in CDCl$_3$. 
HRMS spectra of compound 2.

1H NMR spectra of compound 3 in CDCl₃.
$^{13}$C NMR spectra of compound 3 in CDCl$_3$.

HRMS spectra of compound 3.
$^1$H NMR spectra of BDP2 in CD$_3$CN.

$^{13}$C NMR spectra of BDP2 in CD$_3$CN.
HRMS spectra of BDP2.

$^1$H NMR spectra of compound 4 in CDCl$_3$. 
$^{13}$C NMR spectra of compound 4 in CDCl$_3$.

HRMS spectra of compound 4.
$^1$H NMR spectra of BDP3 in CD$_3$CN.

$^{13}$C NMR spectra of BDP3 in CD$_3$CN.
HRMS spectra of BDP3.

$^1$H NMR spectra of BDP4 in CDCl$_3$. 
$^{13}$C NMR spectra of BDP4 in CDCl$_3$.

HRMS spectra of BDP4.
$^1$H NMR spectra of FP-BDP4 in CD$_3$CN.

$^{13}$C NMR spectra of FP-BDP4 in DMSO-d$_6$. 
5. Reference


