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References
1. General Information

1.1 Materials
All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05mol/L Na₂HPO₄ water solution and 0.05mol/L KH₂PO₄ water solution with the volume ratio 4:1. Hydrazine and various analytes were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All chemicals and solvents used were of analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

1.2 Instruments
TLC analysis was performed using precoated silica plates. Ultraviolet–visible (UV–vis) spectra were recorded on U-3900 UV-Visible spectrophotometer. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ¹H NMR and ¹³C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI-MS was measured with an Thermo Scientific Q Exactive. The cell imaging experiments were measured by a Zeiss LSM880 Airyscan confocal laser scanning microscope. Mice images were carried out by the Institute of Pharmacology and Toxicology Academy of Military Medical Sciences PLA, Peop. Rep.

1.3 In vivo imaging

Cell Culture and Imaging. The HepG 2 cells were grown in Dulbecco’s Modified Eagle’s medium supplemented with 12% Fetal Bovine Serum and 1% antibiotics at 37 °C in humidified environment of 5% CO₂. Cells were plated on 6-well plate and allowed to adhere for 24 h. Before the experiments, cells were washed with PBS 3 times.

Living mice imaging. Balb/c type mouse (12-14 weeks, male) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Experiments were conducted according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Institute of Pharmacology and Toxicology Academy of Military Medical Sciences PLA, Peop. Rep. China Ethical Committee on Animal Care and Use, and all efforts were made to minimize animal suffering and reduce the number of animals used for the experiments. Imaging procedures were conducted with adult nude mice under general anesthesia by injection of sodium pentobarbital (0.5 mL/0.03%). Firstly, Balb/c mouse was treated with depilatory paste, and washed with deionized water for further experiment. Then, probe NIR-SP and Na₂SO₃ was carefully injected into Balb/c type mouse according to designed experiment. Images were taken using an excitation laser of 475 nm (530 nm) and emission 490±20 nm (650±20 nm), respectively. It should be pointed out that, the autofluorescence of mouse mainly attribute to the hair induced background interferes.
2. Experimental Section

Scheme S1. Synthesis route of probe NIR-SP and a series of D-\(\pi\)-A-\(\pi\) structured NIR dyes.

Scheme S2. Synthesis route of SD (SO\(_2\) donor) and release of SO\(_2\).
Synthesis of Compound 1

Cyclohexanone (6.1 mL, 60 mmol) was added dropwise to the concentrated H₂SO₄ (80 mL), and the solution was stirred at 0 °C until the end, then 4-(diethylamino)-2-hydroxybenzaldehyde (5.89 g, 31 mmol) was added. The mixture was further heated at 90 °C for 2 h, after cool down to room temperature the mixture was added slowly to ice-water (500 mL), then 7.5 ml HClO₄ was added and the result suspension was precipitated and filtered off to washed with water, and dried in vacuum, thus a desired compound 1 was obtained for the next step without further purification.

Synthesis and Characterization of probe NIR-SP

Compound 1 (0.711 g, 2 mmol) and terephthalaldehyde (0.536 g, 4 mmol) was dissolved in 25 mL CH₃COOH. The mixture was heated at 110 °C for 2 h. After the reaction was completed, the solvent was removed to give the crude product. Then, dried and subjected to purification by flash chromatography (CH₂Cl₂: CH₃OH; 20:1) to give probe NIR-SP as a black solid (0.601 g, 64%). ¹H NMR (600 MHz, DMSO) δ 10.08 (s, 1H), 8.60 (s, 1H), 8.11 (s, 1H), 8.04 (d, J = 8.0 Hz, 2H), 7.96 (d, J = 9.5 Hz, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 9.4 Hz, 1H), 7.35 (s, 1H), 5.76 (s, 2H), 3.75 (s, 4H), 2.97 (d, J = 4.7 Hz, 2H), 2.91 (t, J = 5.8 Hz, 2H), 1.88 (dt, J = 11.8, 6.0 Hz, 2H), 1.28 (s, 6H). ¹³C NMR (151 MHz, DMSO) δ 193.15 (s), 160.91 (s), 159.24 (s), 149.35 (s), 141.35 (s), 136.25 (s), 133.68 (s), 132.79 (s), 131.43 (s), 130.14 (s), 124.43 (s), 120.58 (s), 119.76 (s), 95.93 (s), 55.39 (s), 27.14 (d, J = 9.7 Hz), 21.61 (s).

The quantum yield of probe (NIR-SP) in EtOH was calculated to be 0.15. The fluorescence quantum yield (Φ) was calculated using: Φₓ = Φₛ × (Aₓ/Aₛ) × (Dₓ/Dₛ) × (nₛ²/nₓ²) where the subscripts s and x refer to standard (Indocyanine Green: Φₛ = 0.13 in DMSO) and the test samples, respectively. Thus, Φₛ and Φₓ are the respective quantum yields; nₛ and nₓ are the refractive indices of the solvents used; Aₛ and Aₓ are the absorption intensities at the respective excitation wavelengths of the standard and test samples; and Dₛ and Dₓ are integrals of the fluorescence intensities.

Synthesis and Characterization of NIR-OH and NIR-B (OH)₂

The synthesis route of NIR-OH and NIR-B (OH)₂ were similar as described above. The crude product was recrystallized with ethanol to get a black solid.

**NIR-OH:** ¹H NMR (600 MHz, DMSO) δ 10.30 (s, 1H), 8.52 (s, 1H), 8.06 (s, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.59 (d, J = 7.8 Hz, 2H), 7.45 (d, J = 8.8 Hz, 1H), 7.29 (s, 1H), 6.93 (d, J = 7.4 Hz, 2H), 3.70 (s, 4H), 2.94 (s, 2H), 2.87 (s, 2H), 1.86 (s, 2H), 1.25 (s, 6H). ¹³C NMR (151 MHz, DMSO) δ 161.98 (s), 159.17 (s), 156.65 (s), 149.35 (s), 135.70 (d, J = 16.5 Hz), 132.64 (s), 131.10 (s), 130.01 (s), 129.27 (s), 129.12 (s), 123.89 (s), 119.95 (s), 119.33 (s), 95.92 (s), 46.19 (s), 27.21 (s), 27.04 (s), 21.60 (s), 12.96 (s).

**NIR-B (OH)₂:** ¹H NMR (600 MHz, DMSO) δ 8.59 (s, 1H), 8.09 (s, 1H), 7.94 (d, J = 9.4 Hz, 1H), 7.65 (d, J = 7.1 Hz, 2H), 7.53 (d, J = 4.4 Hz, 2H), 7.47 (d, J = 6.9 Hz, 1H), 7.34 (s, 1H), 3.73 (s, 4H), 2.96 (s,
2H), 2.90 (s, 2H), 1.89 (d, J = 28.0 Hz, 2H), 1.27 (s, 6H). $^{13}$C NMR (151 MHz, DMSO) δ 163.45 (s), 160.16 (s), 158.80 (s), 156.03 (s), 148.60 (s), 137.58 (s), 133.99 (s), 132.26 (s), 126.90 (s), 125.41 (s), 123.39 (s), 118.77 (s), 118.43 (s), 116.41 (s), 95.84 (s), 45.96 (s), 27.22 (d, J = 4.3 Hz), 21.45 (s), 13.04 (s).

Synthesis and Characterization of **NIR-Acrylate**

**NIR-OH** (0.459 g, 1 mmol) and acryloyl chloride (0.090 g, 0.096 mL, 1.2 mmol) was dissolved in 10 mL CH$_2$Cl$_2$ with Et$_3$N (0.121 g, 0.166 mL, 1.2 mmol). The mixture was stirred overnight at 0-5 °C in ice-water. After the reaction was completed, removing the solvent under reduced pressure gave a solid which was purified by flash chromatography (CH$_2$Cl$_2$ : CH$_3$OH; 20:1) to give **NIR-Acrylate** as a black solid (0.43 g, 85%). $^1$H NMR (600 MHz, CDCl$_3$) δ 8.39 (s, 1H), 8.12 (s, 1H), 7.93 (d, J = 9.3 Hz, 1H), 7.67 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 7.10 (s, 1H), 6.66 (d, J = 17.3 Hz, 1H), 6.36 (dd, J = 17.5, 10.4 Hz, 1H), 6.09 (d, J = 10.5 Hz, 1H), 3.73 (dd, J = 13.9, 6.9 Hz, 4H), 2.98 (s, 4H), 1.95 (t, J = 8.4 Hz, 2H), 1.40 (s, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 164.22 (s), 162.67 (s), 159.16 (s), 156.51 (s), 151.42 (s), 148.92 (s), 136.57 (s), 133.24 (s), 133.04 (s), 132.65 (s), 132.32 (s), 127.91 (s), 127.64 (s), 123.51 (s), 121.88 (s), 119.73 (s), 118.40 (s), 95.88 (s), 46.53 (s), 27.45 (s), 27.13 (s), 21.50 (s).
Synthesis route of SD
SD was synthesized according to the literature methods with modifications.\textsuperscript{1} Synthesis of the probes is described as Scheme S2.

2.2 Characterization data for synthesis.
**Figure S1:** Structure characterization of probe NIR-SP.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{s1}
\caption{$^1$H-NMR spectrum of probe NIR-SP in DMSO-$d_6$}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{s2}
\caption{$^{13}$C-NMR spectrum of probe NIR-SP in DMSO-$d_6$}
\end{figure}
MS (ESI) spectrum of probe **NIR-SP**
Figure S2: Structure characterization of NIR-OH.

$^1$H-NMR spectrum of NIR-OH in DMSO-$d_6$

$^{13}$C-NMR spectrum of NIR-OH in DMSO-$d_6$
Figure S3: Structure characterization of NIR-B (OH)₂.

^1^H-NMR spectrum of NIR-B (OH)₂ in DMSO-d₆

^1^3^C-NMR spectrum of NIR-B (OH)₂ in DMSO-d₆
Figure S4: Structure characterization of NIR-Acrylate.

\(^1\)H-NMR spectrum of NIR-Acrylate in CDCl\(_3\)

\(^{13}\)C-NMR spectrum of NIR-Acrylate in CDCl\(_3\)
**Figure S5**: Fluorescence spectra of the probe NIR-OH with Na₂SO₃ (a: 0-50 µM) and (b: 0-500 µM).

**Figure S6**: Fluorescence spectra of the probe NIR-B (OH)₂ with Na₂SO₃ (a: 0-50 µM) and (b: 0-500 µM).

**Figure S7**: Fluorescence spectra of the probe NIR-Acrylate with Na₂SO₃ (a: 0-50 µM) and (b: 0-500 µM).
**Figure S8:** Time-dependent of the probe **NIR-SP** with Na$_2$SO$_3$ at 660 nm (a) and 465 nm (b).

**Figure S9:** pH interference of the probe **NIR-SP** with Na$_2$SO$_3$ at 660 nm (a) and 465 nm (b).
Figure S10: The $^1$H NMR titration of NIR-SP with Na$_2$SO$_3$. 

(a) 
+ Na$_2$SO$_3$ 5 equal
   -→ CHO

+ Na$_2$SO$_3$ 2 equal
   -→ CHO

+ Na$_2$SO$_3$ 1 equal
   -→ CHO

+ Na$_2$SO$_3$ 0.5 equal
   -→ CHO

Probe NIR-SP
   -→ CHO

(b) 
+ Na$_2$SO$_3$ 20 equal (with trace CF$_3$COOH)

Probe NIR-SP
Figure S11: ESI-MS of NIR-SP with Na$_2$SO$_3$.

Mass spectrum of probe NIR-SP with Na$_2$SO$_3$. 

**Product 1**

**Product 2**
**Figure S12:** The cytotoxicity test.

![Cytotoxicity Test Graph]

**Figure S13:** Cell-imaging of SD with Cys.

![Cell-imaging Figures]
Figure S14: ESI-MS of SD with Cys.

Byproduct 1 of SD with Cys

Mass spectrum of SD with Na₂SO₃.

Byproduct 2 of SD with Cys
References