Supporting information

Benzorhodol derived far-red/near-infrared fluorescent probes for selective detection of butyrylcholinesterase activity in living cells and in non-alcoholic fatty liver of zebrafish

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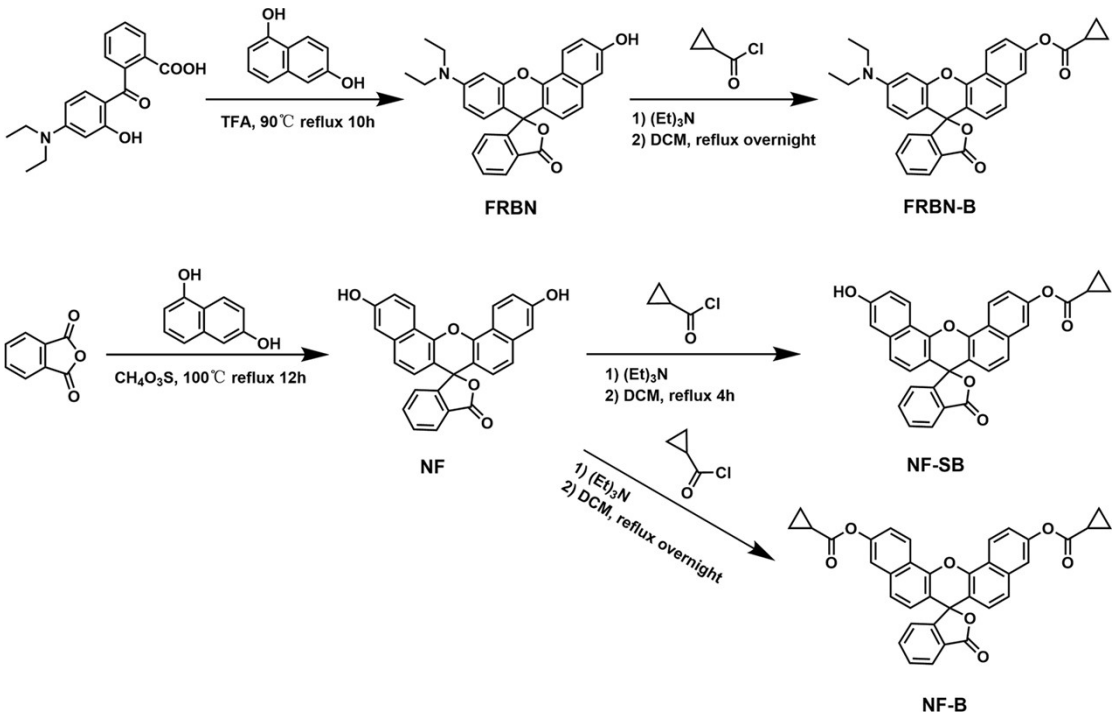
1. Synthesis of FRBN-B, NF-SB and NF-B



Scheme 1. Synthetic route of **FRBN-B, NF-SB and NF-B**

1.1. Synthesis of compound FRBN

2-(4-Diethylamino-2-hydroxybenzoyl)benzoic acid (1.25 g, 4.00 mmol) and 1, 6-dihydroxynaphthalene (0.640 g, 4.00 mmol) were placed in a flask, and trifluoroacetic acid (TFA, 20 mL) was added slowly under ice bath. The mixture was stirred at 90 ℃ for 10 h. The reaction was monitored using thin layer chromatography (TLC). The mixture was cooled to room temperature. During the stirring process, 40 mL ice water was mixed for 1 h. Then the saturated Na_2CO_3 solution is added to the reaction mixture slowly for neutralization until bubbles were no longer generated. A large amount of purple precipitate produced. The reaction liquid is filtered off, and the collected filter residue is dried in vacuo for 12 h to obtain the crude product. Then the crude product was dissolved in ethyl acetate for hot filtration, and the collected filter residue was dried for 2 h to obtain the purplish red solid product **FRBN** (1.24 g, 69%).

^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm) 10.12 (s, 1H), 8.45 (d, J = 9.1 Hz, 1H), 8.03 (d, J = 7.2 Hz, 1H), 7.81 – 7.70 (m, 2H), 7.36 (d, J = 8.8 Hz, 1H), 7.27 (t, J = 8.6 Hz, 2H), 7.15 (d, J = 2.4 Hz, 1H), 6.73 (d, J = 2.3 Hz, 1H), 6.61 – 6.49 (m, 3H), 3.41 (t, J = 7.0 Hz, 4H), 1.13 (t, J = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, DMSO-d_6), δ (ppm) 169.40, 157.67, 153.37, 152.36, 149.69, 147.31, 136.31, 136.04, 130.52, 129.11, 126.75, 125.04, 124.56, 124.22, 122.25, 119.36, 117.65, 110.12, 109.74, 109.44, 104.96, 97.64, 84.49, 44.25, 12.86.

LC-MS (ESI): m/z = 438.1455 $[M + H]^+$, calculated for $C_{28}H_{23}NO_4$=437.1627.

1.2. Synthesis of compound FRBN-B

Compound **FRBN** (0.500 g, 1.14 mmol) was dissolved in 10 mL dichloromethane (DCM), and triethylamine (TEA) (0.127 g, 1.25 mmol) was added under ice bath for 10 min of stirring. During the stirring process, cyclopropanecarbonyl chloride (0.132 g, 1.25 mmol) was mixed slowly to the reaction solution. After stirring for 30 min under the ice bath, the mixture was stirred constantly at room temperature overnight and the reaction was monitored by TLC. Finally, the rose-red solid **FRBN-B** (0.220 g, 35%) was obtained by silica gel column chromatography using a DCM as the elution solvent.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.62 (d, J = 9.1 Hz, 1H), 8.05 (d, J = 6.9 Hz, 1H), 7.66 – 7.59 (m, 2H), 7.54 (d, J = 2.3 Hz, 1H), 7.38 (t, J = 8.7 Hz, 2H), 7.16 (d, J = 7.0 Hz, 1H), 6.77 (d, J = 8.7 Hz, 1H), 6.68 – 6.62 (m, 2H), 6.43 (d, J = 8.9 Hz, 1H), 3.41 (q, J = 7.1 Hz, 4H), 1.94 – 1.88 (m, 1H), 1.21 (t, J = 6.9 Hz, 8H), 1.07 (dd, J = 7.8, 3.3 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 173.52, 169.85, 153.71, 152.44, 150.04, 149.59, 147.49, 135.01, 134.94, 129.55, 128.95, 127.00, 125.08, 124.92, 124.09, 122.59, 121.90, 121.51, 118.55, 112.64, 108.92, 104.96, 97.68, 84.42, 44.52, 13.13, 12.58, 9.46.

LC-MS (ESI): m/z = 506.1947 $[M + H]^+$, calculated for $C_{32}H_{27}NO_5$=505.1889.

1.3 Synthesis of probe NF

Phthalic anhydride (1.86 g, 12.56 mmol) and 1, 6-dihydroxynaphthalene (5.03 g, 31.43 mmol) were placed in a 500 mL round-bottomed flask and methanesulfonic acid (65 mL) was added to the flask slowly. The mixture was stirred at 100 ℃ for 12 h and the reaction was monitored by TLC. The reaction liquid was cooled to room temperature, and 250 mL of ice water was poured into the flask slowly for 5 h of stirring. A large amount of purple precipitate was generated during this process. The reaction liquid was filtered off and the filter residue was dried for 24 h to obtain the crude product. Finally, the crude product was further purified by silica gel column chromatography with the eluent being DCM/MeOH (200:1, v/v) to acquire the red solid product **NF** (1.65 g, 30%).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.20 (s, 2H), 8.69 (d, J = 9.1 Hz, 2H), 8.10 (d, J = 7.2 Hz, 1H), 7.82 – 7.71 (m, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.36 (dd, J = 9.0, 2.4 Hz, 2H), 7.31 (d, J = 8.1 Hz, 1H), 7.20 (d, J = 2.4 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 169.37, 157.84, 153.73, 146.53, 136.43, 136.30, 130.78, 126.26, 125.27, 124.71, 124.48, 124.29, 123.13, 119.79, 117.77, 109.87, 83.72.

LC-MS (ESI): m/z = 431.0863 $[M - H]^-$, calculated for $C_{28}H_{16}O_5$=432.0998.

1.4 Synthesis of probe NF-SB

Compound **NF** (0.500 g, 1.15 mmol) was dissolved in a containing 10 mL DCM, and TEA (0.113 g, 1.15 mmol) was added to the reaction solution under ice bath. The mixture is stirred for 10 min fully. During the procedure, cyclopropanecarbonyl chloride (0.120 g, 1.15mmol) was slowly added to within 30 min. After stirring in the ice bath for 30 min, the reaction liquid was stirred at room temperature for 4 h, and the reaction was monitored by TLC. Then, the crude product was purified

by silica gel column chromatography using DCM/n-hexane (1:1, v/v) as eluent for the yellow solid product **NF-SB** (0.170 g, 30%).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.23 (s, 1H), 8.89 (d, J = 9.0 Hz, 1H), 8.73 (d, J = 9.1 Hz, 1H), 8.16 – 8.10 (m, 1H), 7.83 – 7.76 (m, 3H), 7.68 (d, J = 8.5 Hz, 1H), 7.62 (dd, J = 9.1, 2.3 Hz, 1H), 7.49 (d, J = 8.6 Hz, 1H), 7.40 (dd, J = 9.0, 2.4 Hz, 1H), 7.32 (dd, J = 5.1, 2.0 Hz, 1H), 7.23 (d, J = 2.4 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 2.02 – 1.96 (m, 1H), 1.15 – 1.10 (m, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 173.41, 169.31, 157.92, 153.72, 150.44, 146.41, 146.28, 136.49, 136.34, 135.13, 130.85, 126.07, 125.34, 125.20, 124.68, 124.34, 124.25, 124.14, 123.37, 123.17, 121.67, 119.86, 119.51, 117.72, 112.81, 109.94, 109.78, 83.17, 70.25, 13.16, 9.69.

LC-MS (ESI): m/z = 501.1363 $[M + H]^+$, calculated for $C_{32}H_{20}O_6$=500.1260.

1.5 Synthesis of probe NF-B

Compound **NF** (0.500 g, 1.15 mmol) was dissolved in a 100 mL round-bottled flask containing 10 mL DCM, and TEA (0.260 g, 2.53 mmol) was added under ice bath. Cyclopropanecarbonyl chloride (0.270 g, 2.53 mmol) was mixed to the reaction solution slowly. After stirring in the ice bath for 10 min, the mixture reacted at room temperature overnight and the reaction was monitored by TLC. Finally, using DCM/n-hexane (1:2, v/v) as eluent, the crude product was further purified by silica gel column chromatography, and the yellow solid product **NF-B** (0.450 g, 70%) was obtained.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.91 (d, J = 9.1 Hz, 2H), 8.17 – 8.10 (m, 1H), 7.83 (d, J = 2.3 Hz, 2H), 7.78 (t, J = 3.7 Hz, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 2.3 Hz, 1H), 7.62 (d, J = 2.3 Hz, 1H), 7.37 – 7.31 (m, 1H), 6.93 (d, J = 8.8 Hz, 2H), 2.03 – 1.97 (m, 2H), 1.15 – 1.08 (m, 8H).

^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 173.44, 169.29, 153.75, 150.49, 146.10, 136.47, 135.18, 131.01, 125.83, 125.48, 125.15, 124.73, 124.43, 124.33, 123.30, 121.60, 119.57, 112.73, 82.61, 13.16, 9.73.

LC-MS (ESI): m/z = 569.1628 $[M + H]^+$, calculated for $C_{36}H_{24}O_7$=568.1522.

2. Spectral profiles

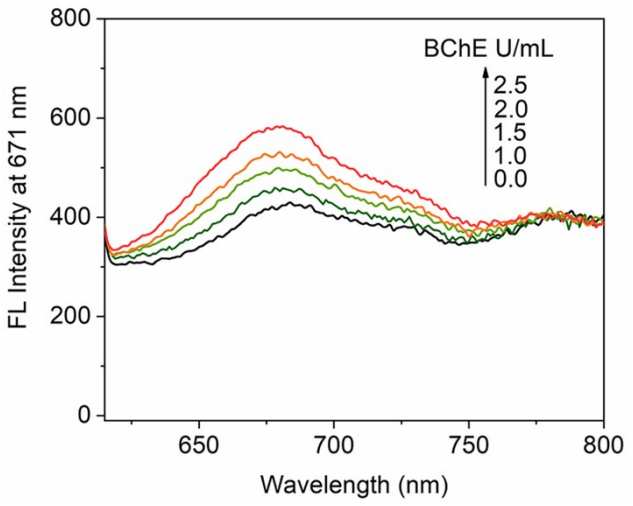


Fig. S1. Fluorescence spectrogram of the mixture containing **NF-B** (10 μM, λ_{ex} = 579 nm; Slit: 10 nm/10 nm) and the various concentrations of BChE (0 ~ 0.15 U/mL) in HEPES buffer (10 mM, pH = 7.4) at 37 ℃ for 30 min.

3. Mechanism of BChE towards FRBN-B and NF-SB

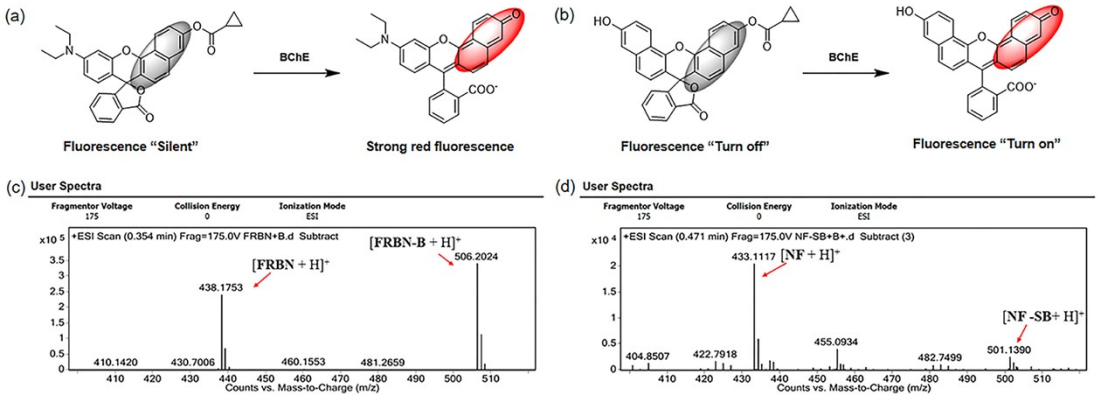


Fig. S2. Fluorescent probe activation mechanism of (a) FRBN-B and (b) NF-SB for BChE detection. (c) High-resolution mass spectrometry of FRBN-B and BChE. (d) High-resolution mass spectrometry of NF-SB and BChE.

4. Optimization of experimental conditions

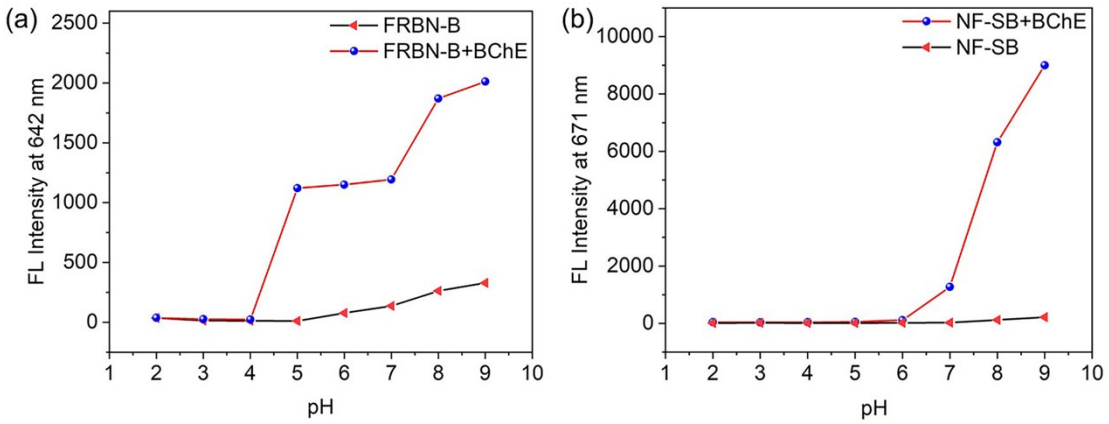


Fig. S3. Fluorescence intensity curve of (a) **FRBN-B** (10 μM, λex = 579 nm; Slit: 5 nm/5 nm) and (b) **NF-SB** (10 μM, λex = 579 nm; Slit: 10 nm/10 nm) in different pH values of HEPES buffer solution in the presence and absence of BChE (0.5 U/mL).

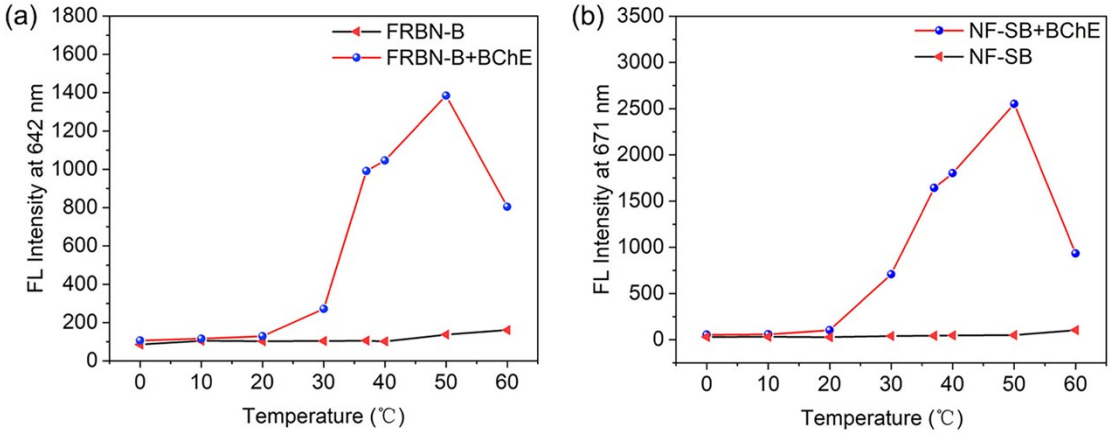


Fig. S4. Fluorescence intensity curve of (a) **FRBN-B** (10 μM, λex = 579 nm; Slit: 5 nm/5 nm) and (b) **NF-SB** (10 μM, λex = 579 nm; Slit: 10 nm/10 nm) at different temperatures in the presence and absence of BChE (0.5 U/mL)

5. Cytotoxicity of the probe

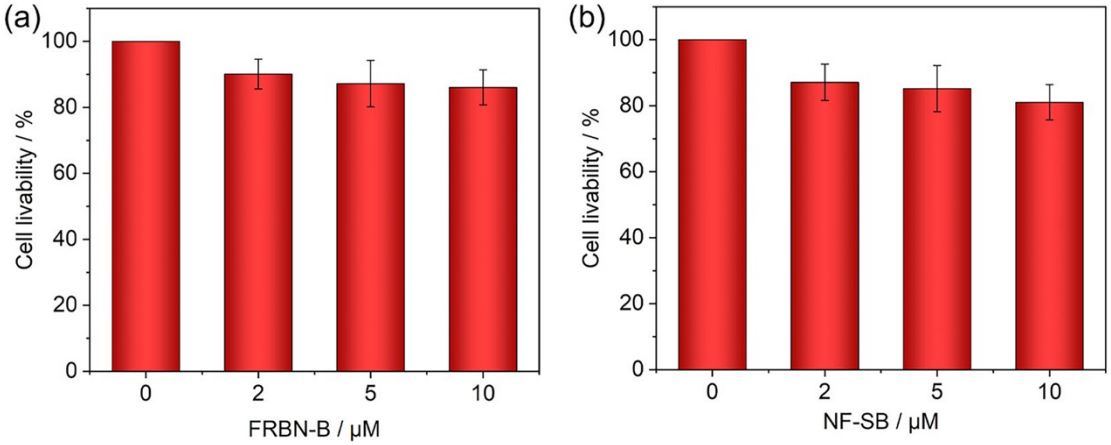


Fig. S5. Cell viabilities of HeLa cells treated with varying concentrations (0, 2, 5, 10 μM) of (a) **FRBN-B** and (b) **NF-SB** for 6 h. Values are mean ± SD (n=3).

6. Bio-imaging of intracellular BChE in living cells

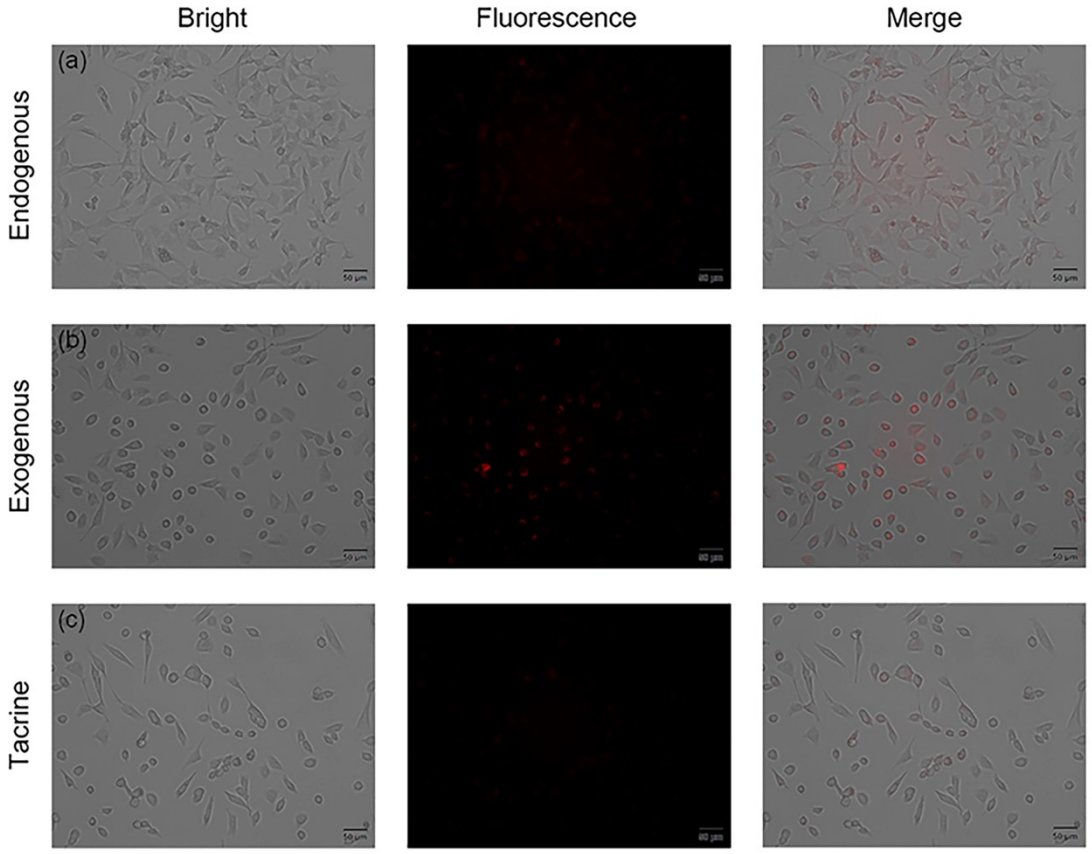


Fig. S6. Hela cells imaging of **NF-SB** (5 μM at 37 °C) with endogenous (a) and exogenous (b) with BChE (1 U/mL) for 30 min. (c) Hela cells incubated with tacrine (20 μM) for 30 min and then incubated with **NF-SB** (10 μM) for 30 min.

7. ^{1}H, ^{13}C NMR and ESI-MS spectra

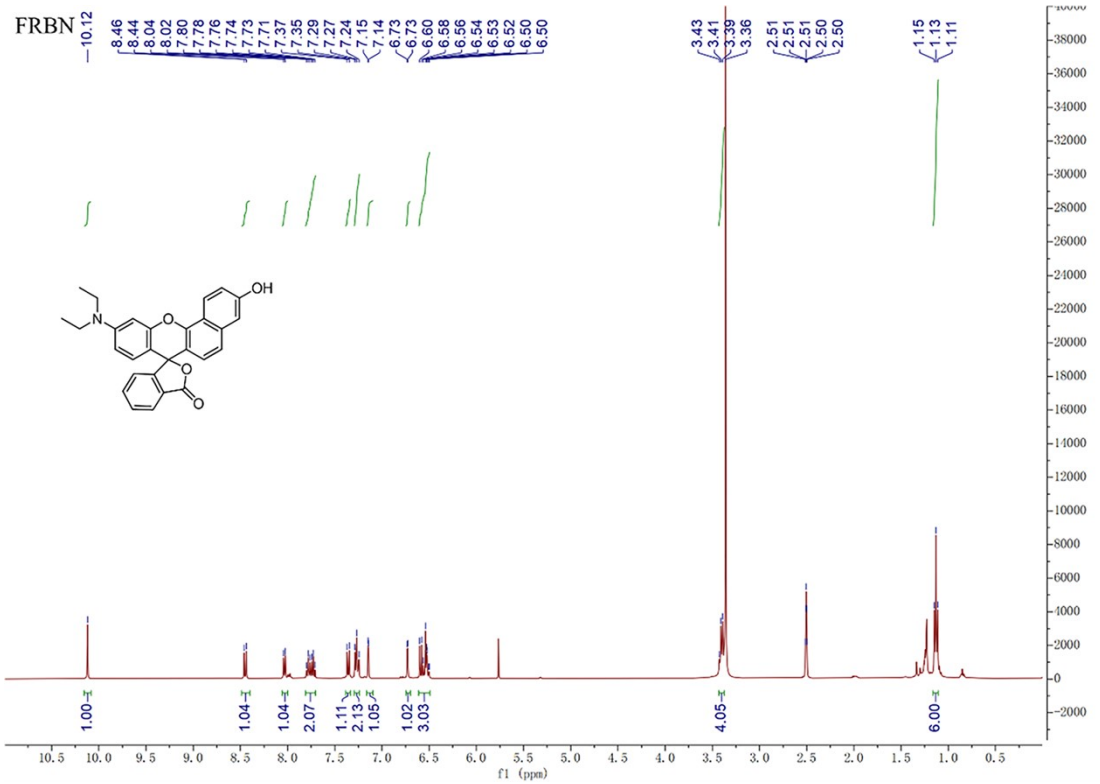


Fig. S7. ^{1}H NMR spectrum of **FRBN** in DMSO-d_6.

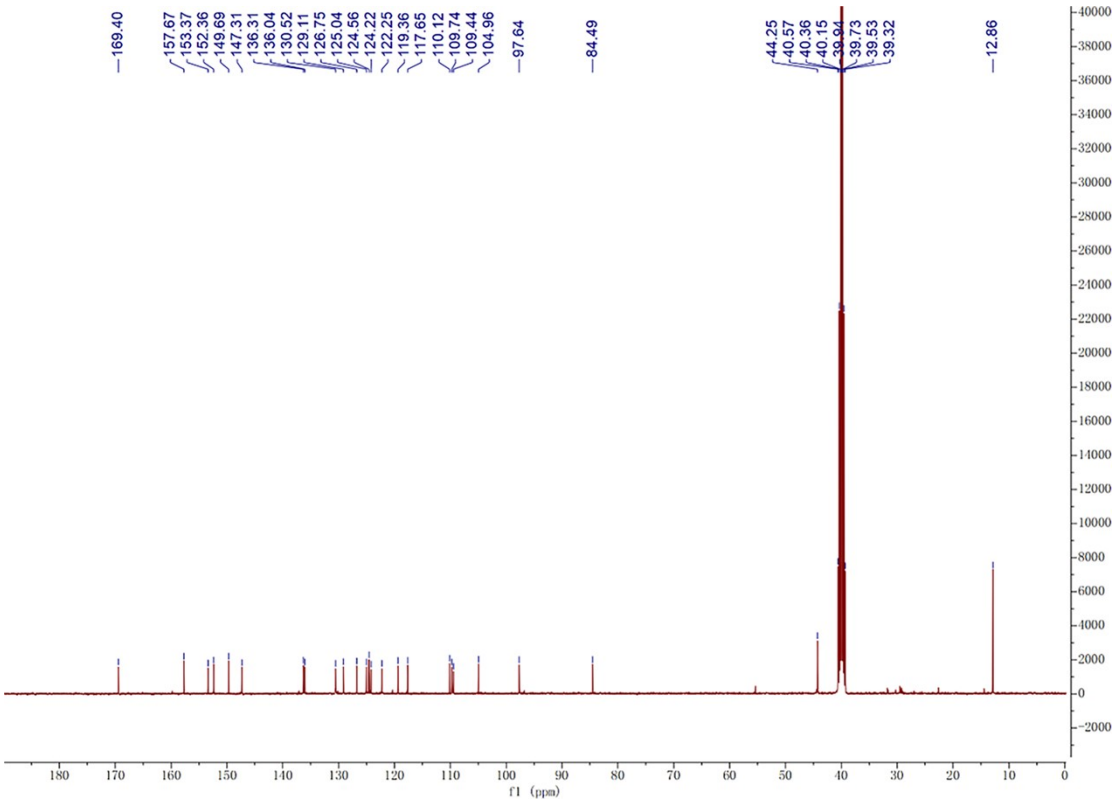


Fig. S8. ^{1}C NMR spectrum of **FRBN** in DMSO-d_6.

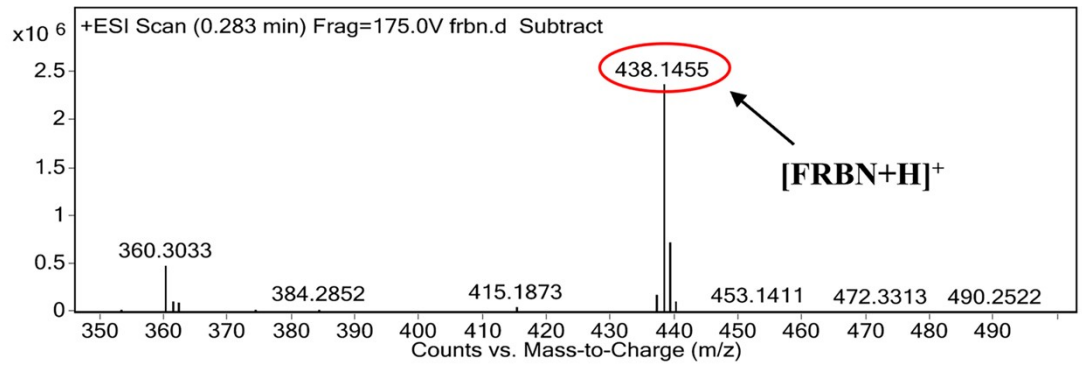


Fig. S9. ESI-MS of compound **FRBN**.

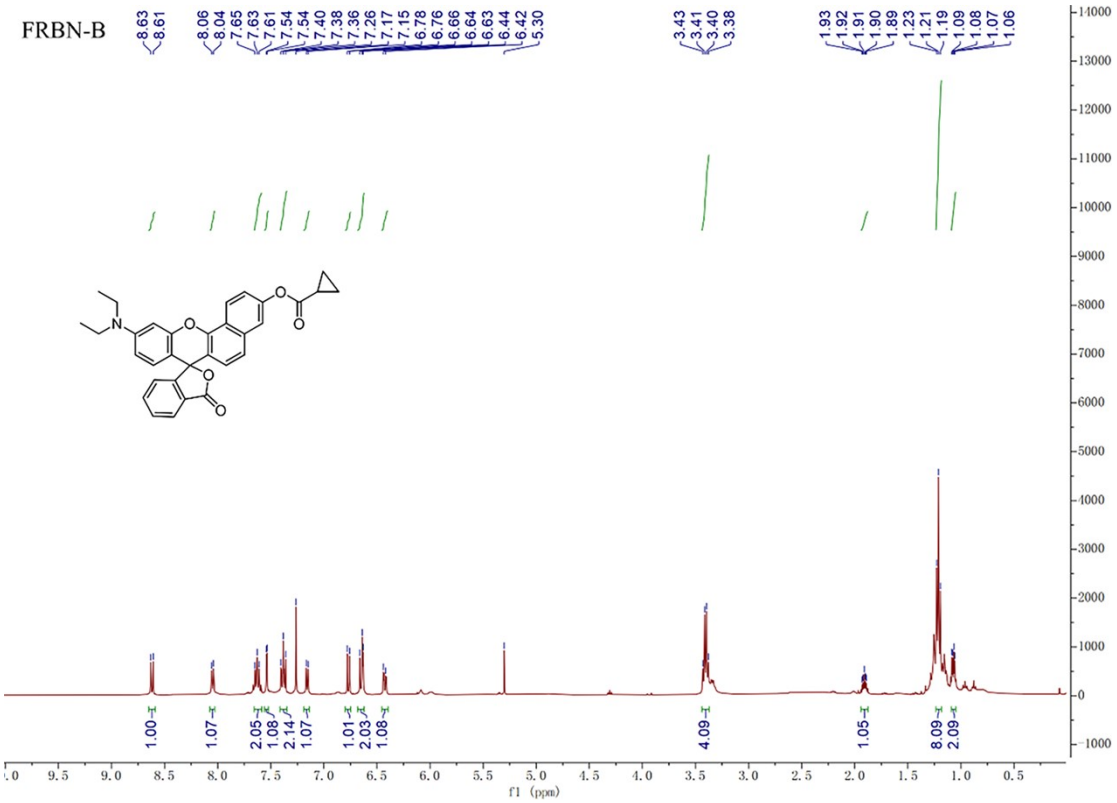


Fig. S10. ^{1}H NMR spectrum of **FRBN-B** in $CDCl_3$.

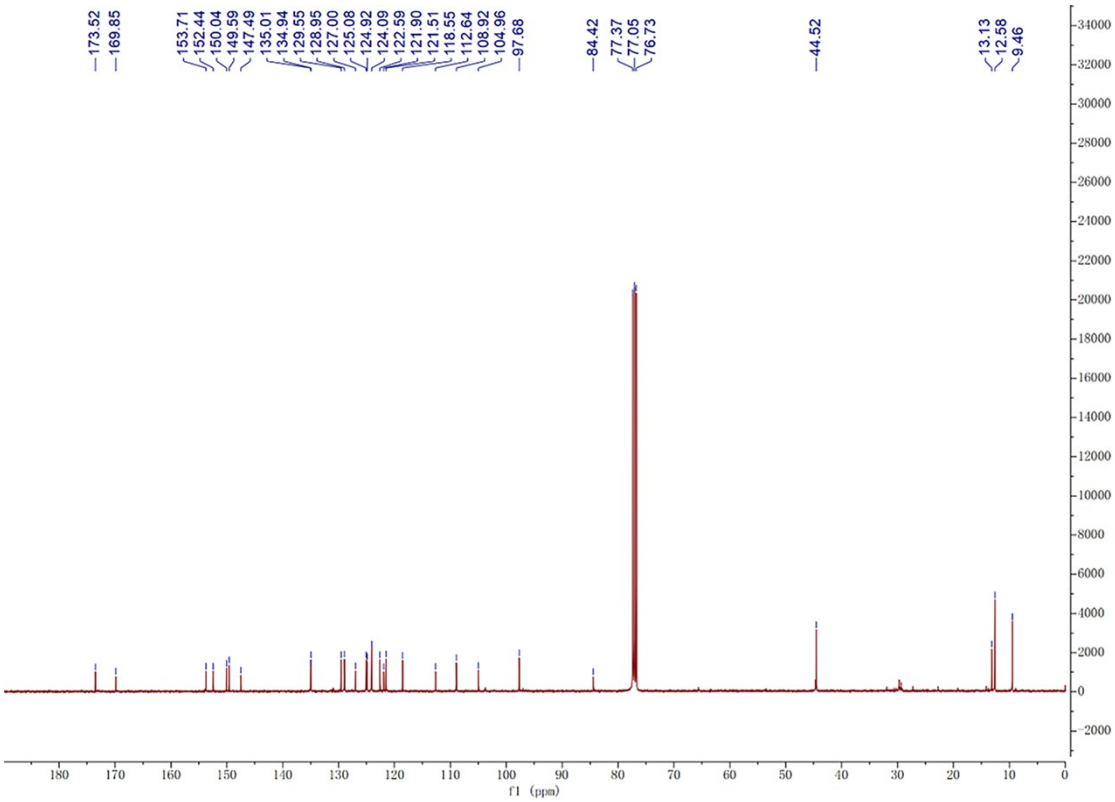


Fig. S11. ^{1}C NMR spectrum of **FRBN-B** in $CDCl_3$.

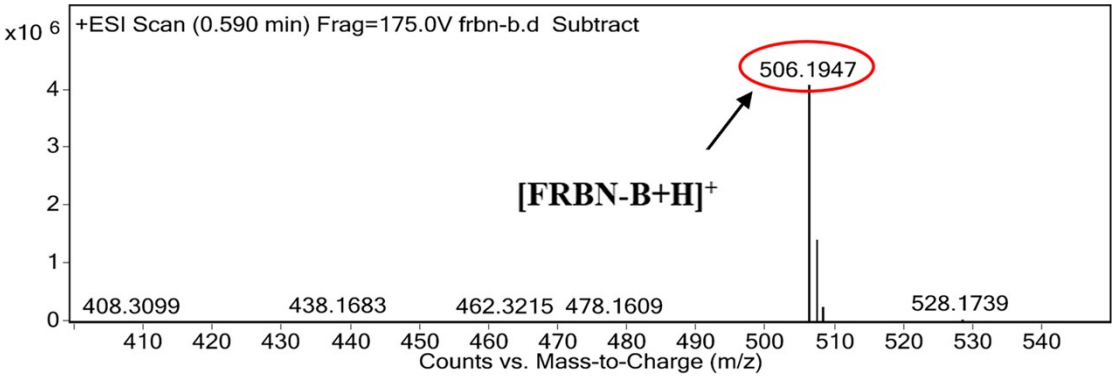


Fig. S12. ESI-MS of compound **FRBN-B**.

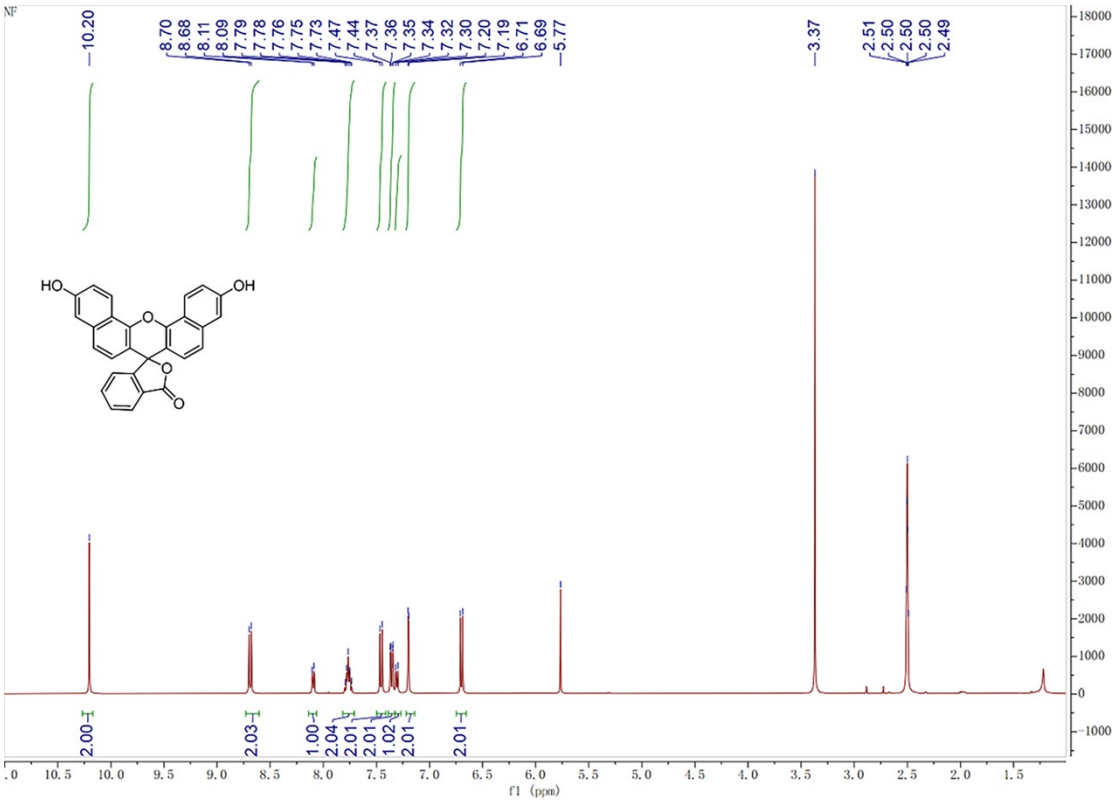


Fig. S13. ^{1}H NMR spectrum of **NF** in DMSO-d_6.

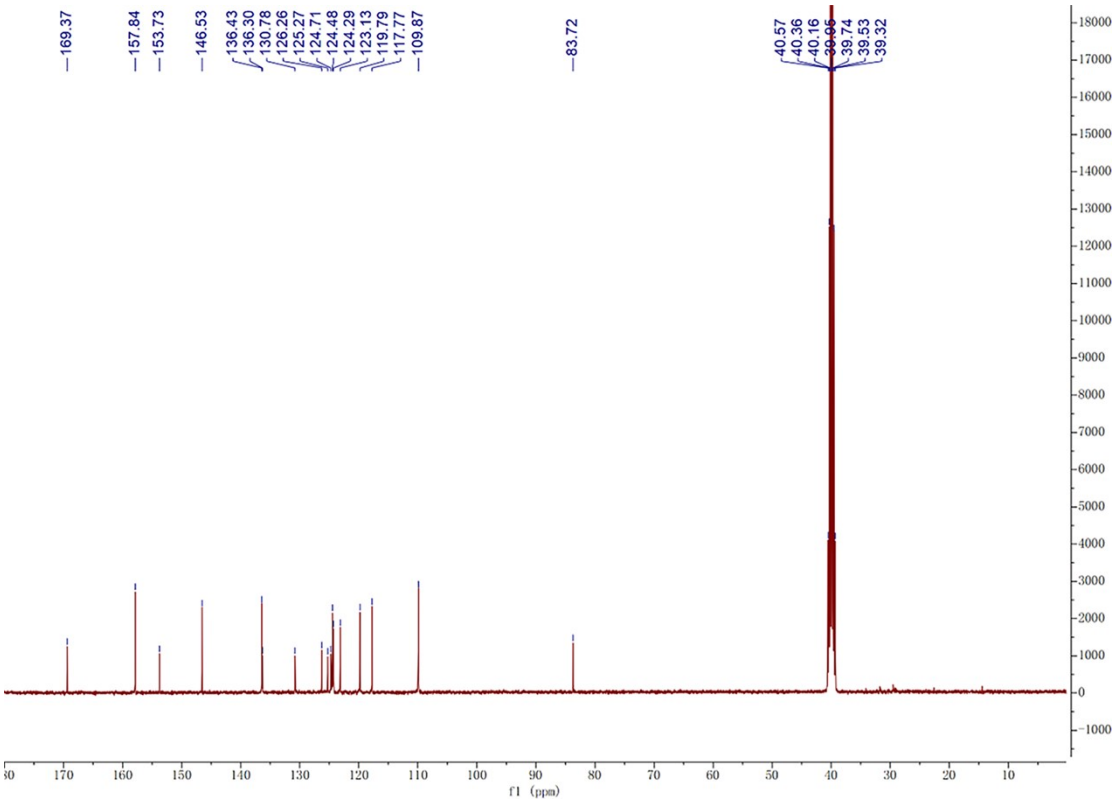


Fig. S14. ^{1}C NMR spectrum of **NF** in DMSO-d_6.

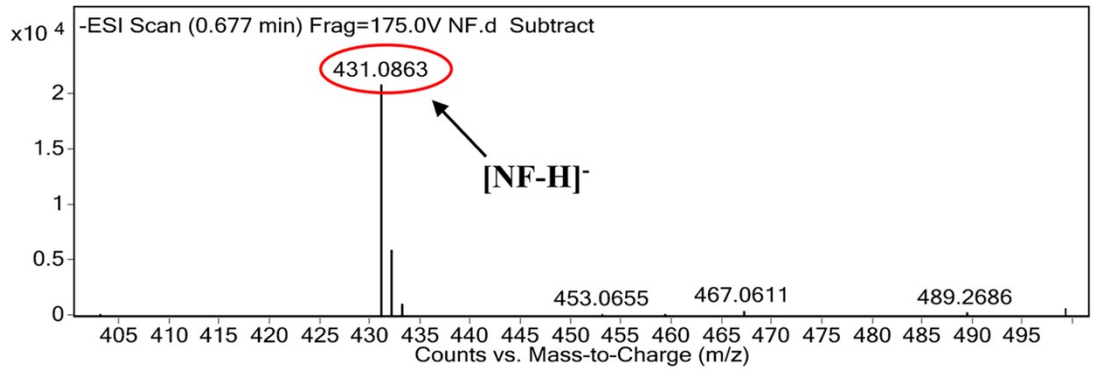


Fig. S15. ESI-MS of probe **NF**.

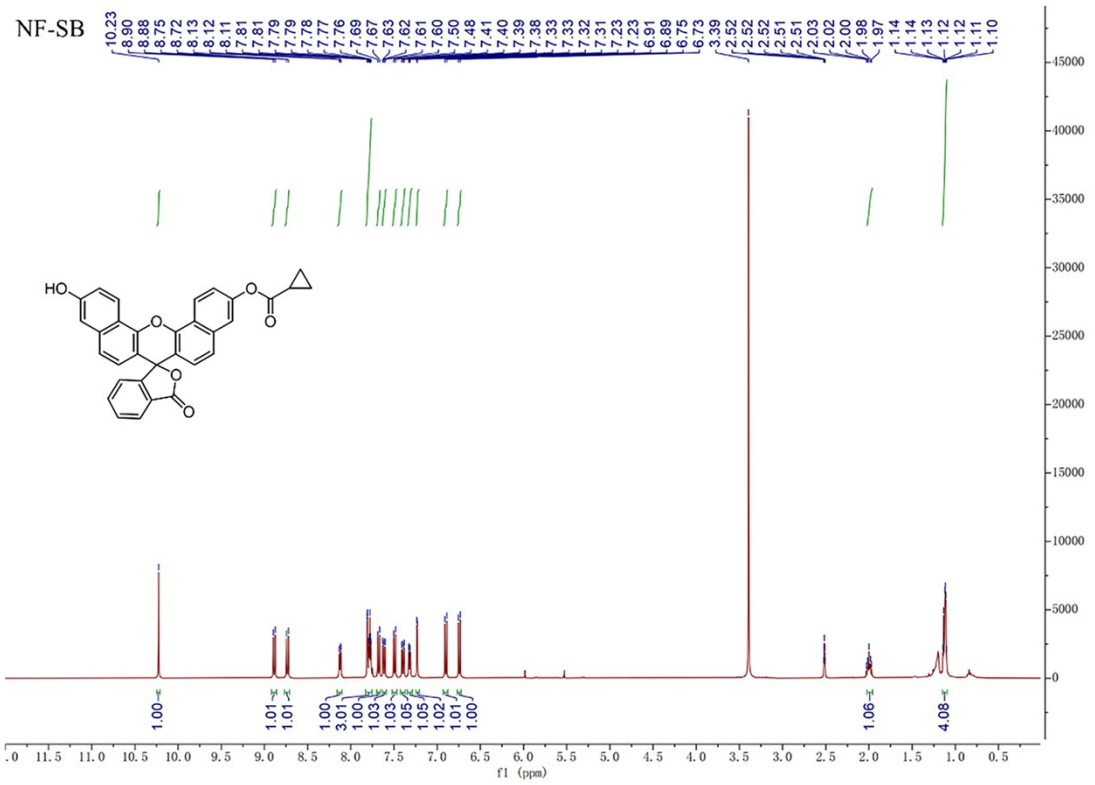


Fig. S16. ^{1}H NMR spectrum of **NF-SB** in DMSO-d_6.

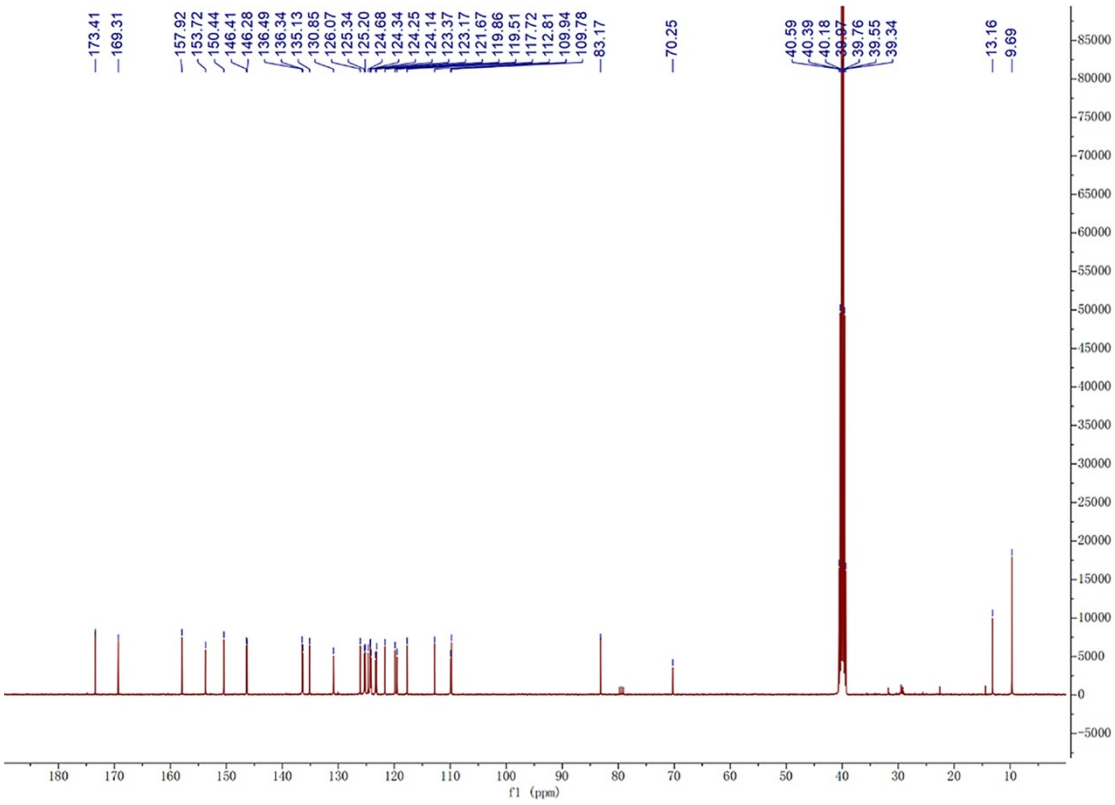


Fig. S17. ^{1}C NMR spectrum of **NF-SB** in DMSO-d_6.

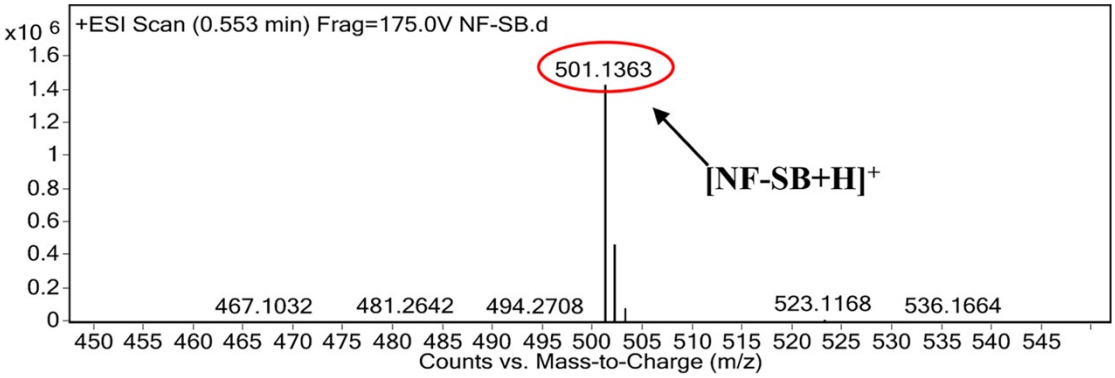


Fig. S18. ESI-MS of probe **NF-SB**.

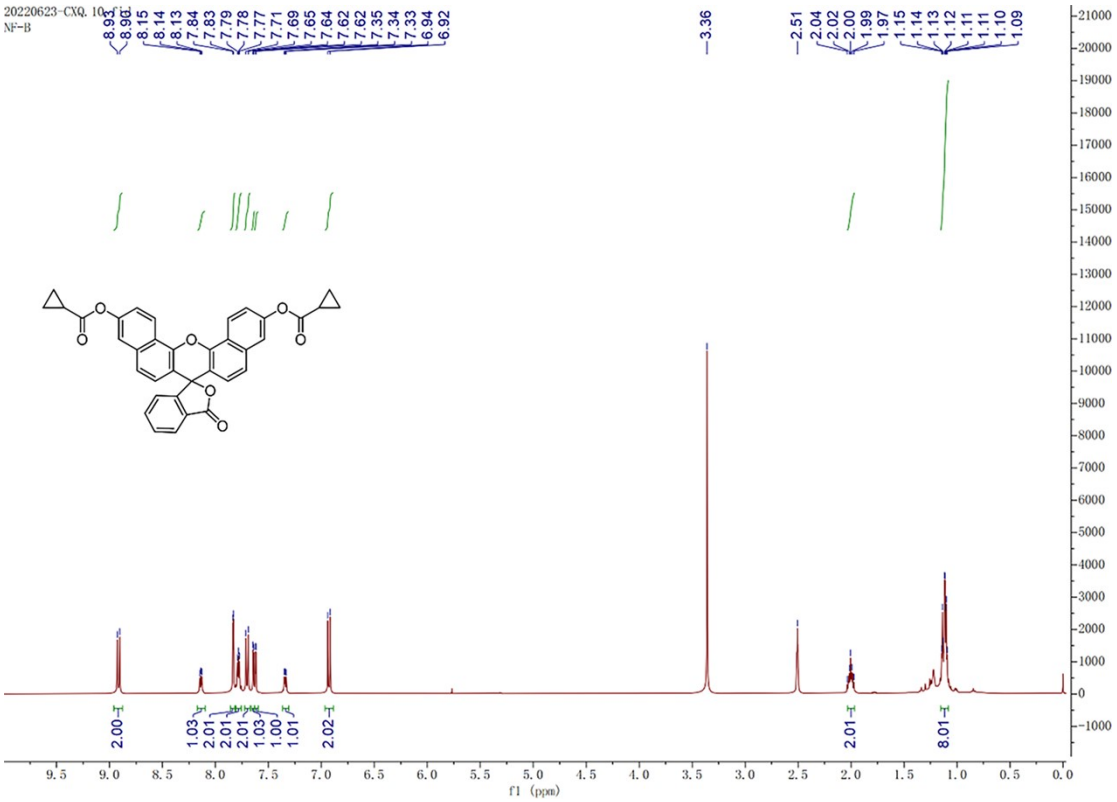


Fig. S19. [1]H NMR spectrum of **NF-B** in DMSO-d_6.

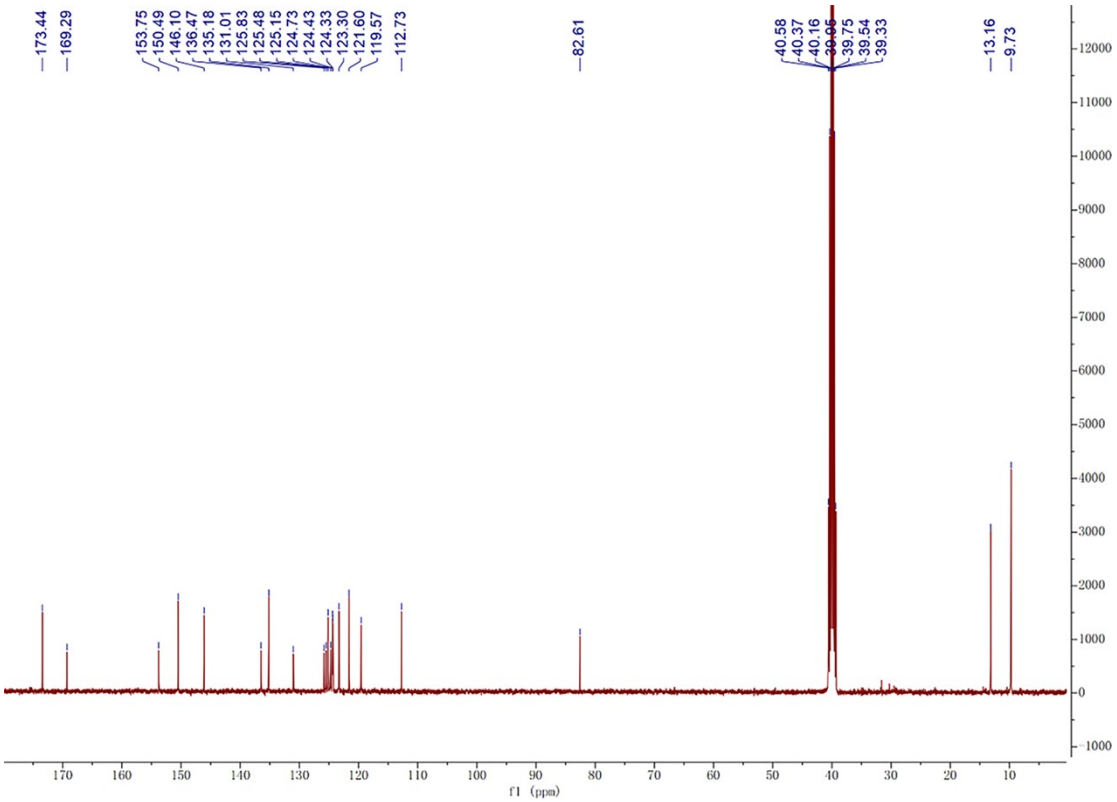


Fig. S20. [1]C NMR spectrum of **NF-B** in DMSO-d_6.

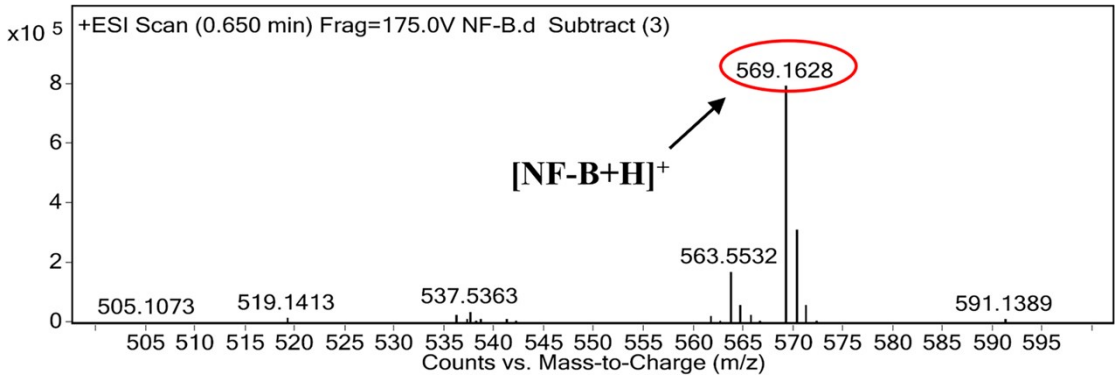


Fig. S21. ESI-MS of probe **NF-B**.