

Supporting information for

**Construction of a novel near infrared fluorescent probe  
with multiple fluorescence emission and its application  
for SO<sub>2</sub> derivative detection in cells and living zebrafish**

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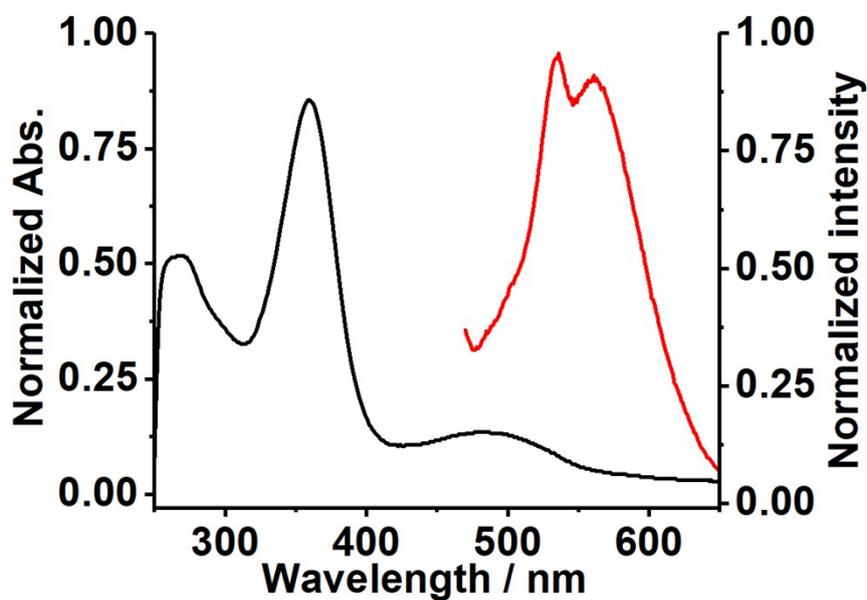
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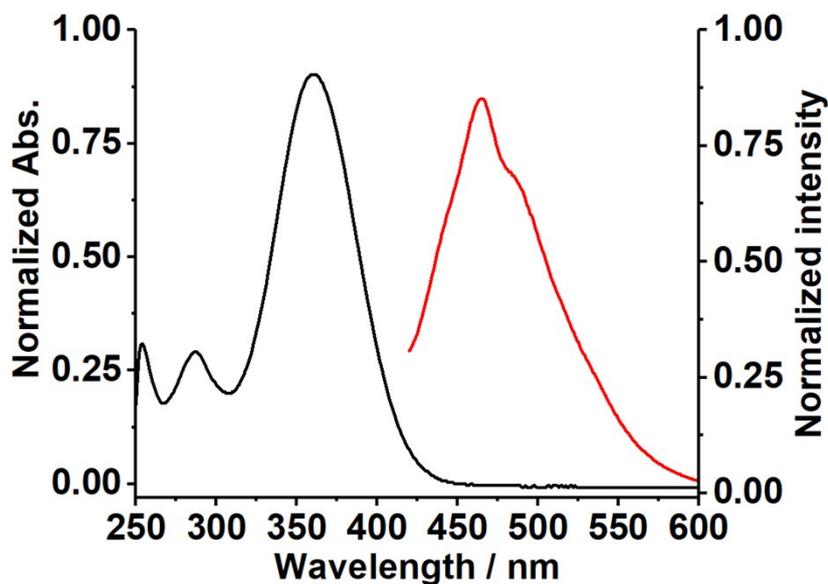
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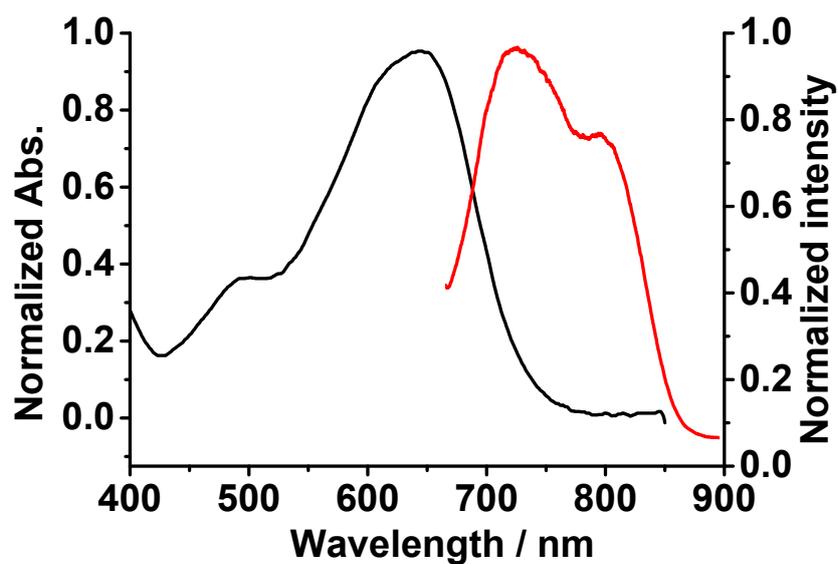
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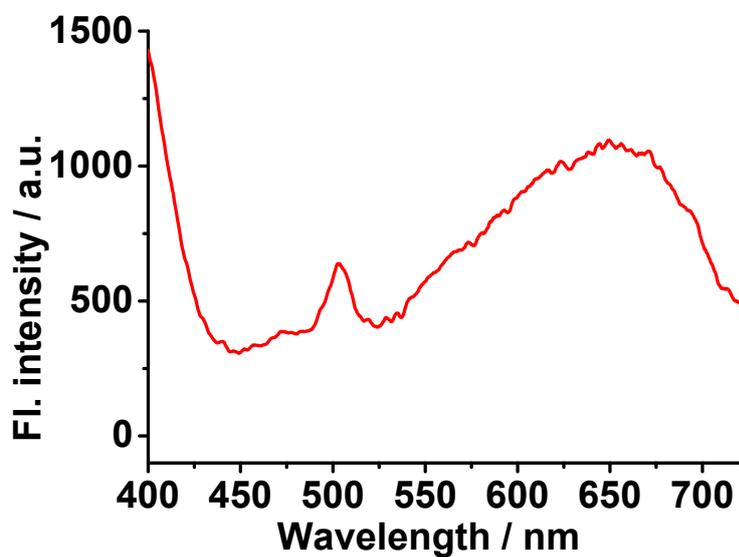
**Fig. S1** The normalized absorption and emission spectra of **compound 2**. 10  $\mu\text{M}$  of **compound 2** in 1 cm Cuvettes in PBS (pH 7.4, 10 mM). Excitation:  $\lambda = 450$  nm.



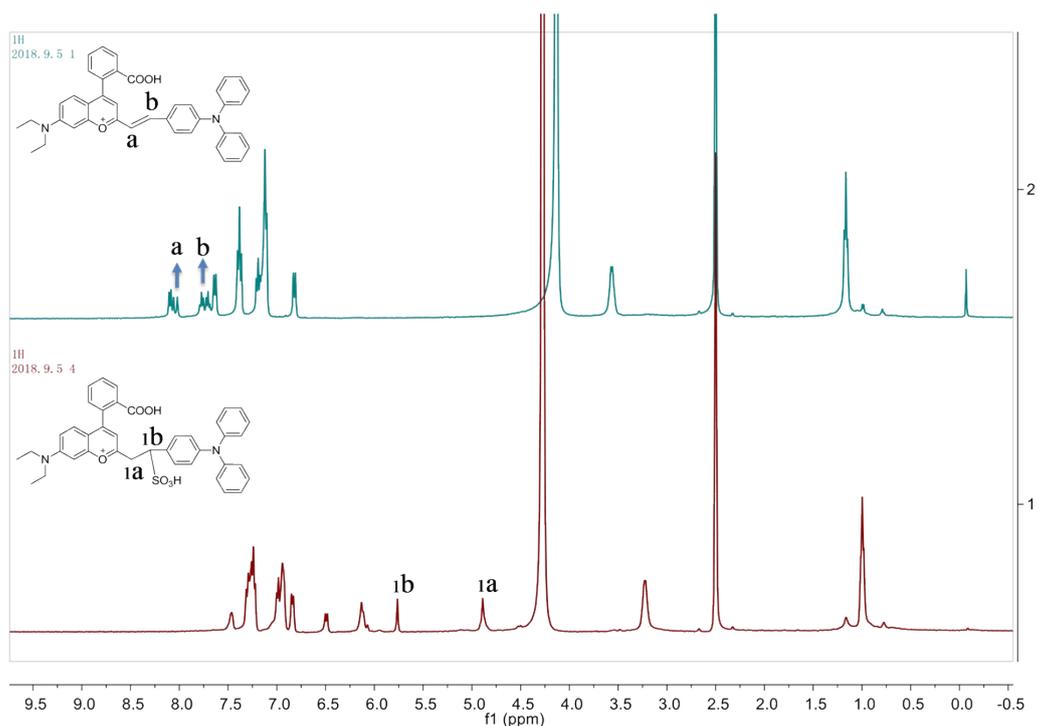
**Fig. S2** The normalized absorption and emission spectra of **compound 3**. 10  $\mu\text{M}$  of **compound 3** in 1 cm Cuvettes in PBS (pH 7.4, 10 mM). Excitation:  $\lambda = 400$  nm.



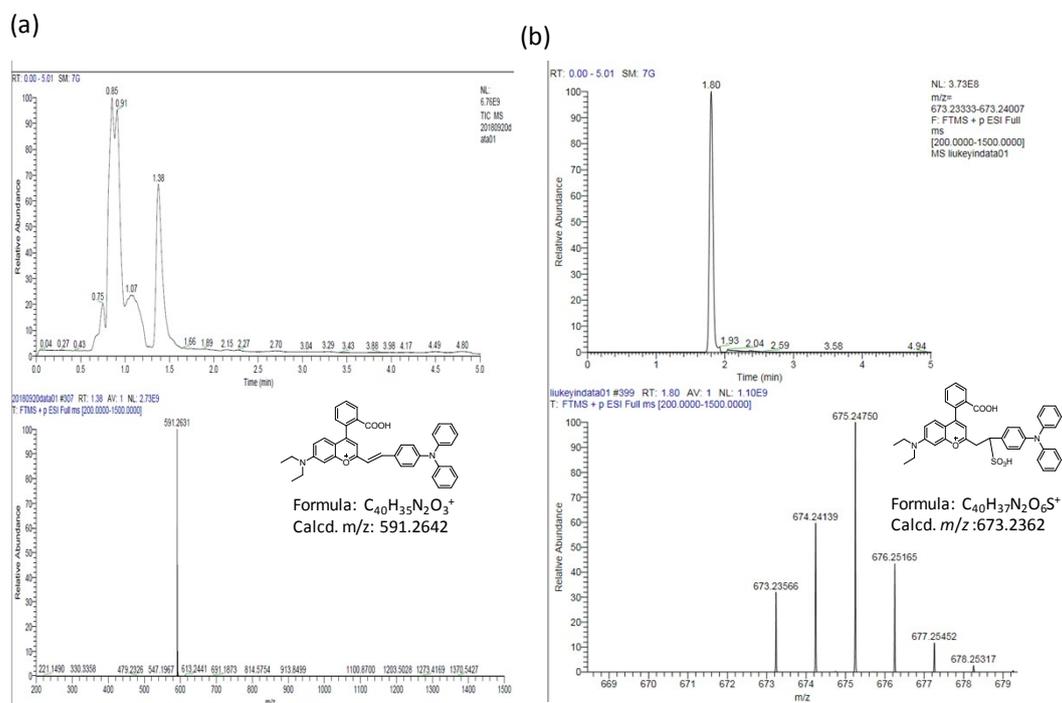
**Fig. S3** The normalized absorption and emission spectra of **Rh-TPA**. 10  $\mu\text{M}$  of **Rh-TPA** in 1 cm Cuvettes in PBS (pH 7.4, 10 mM). Excitation:  $\lambda = 640$  nm.



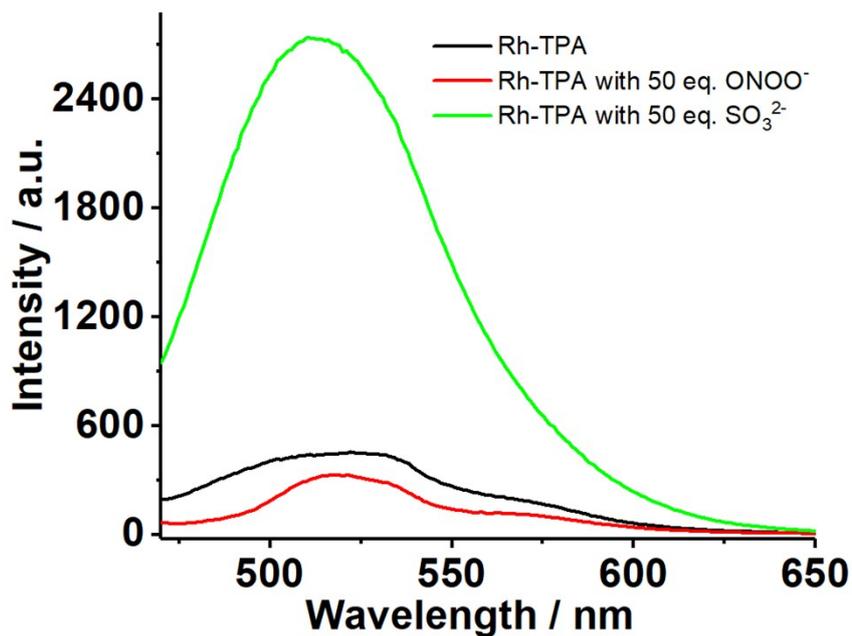
**Fig. S4** The excitation spectrum of **Rh-TPA**. 10  $\mu\text{M}$  of **Rh-TPA** in 1 cm Cuvettes in PBS (pH 7.4, 10 mM). Emission:  $\lambda = 740$  nm.



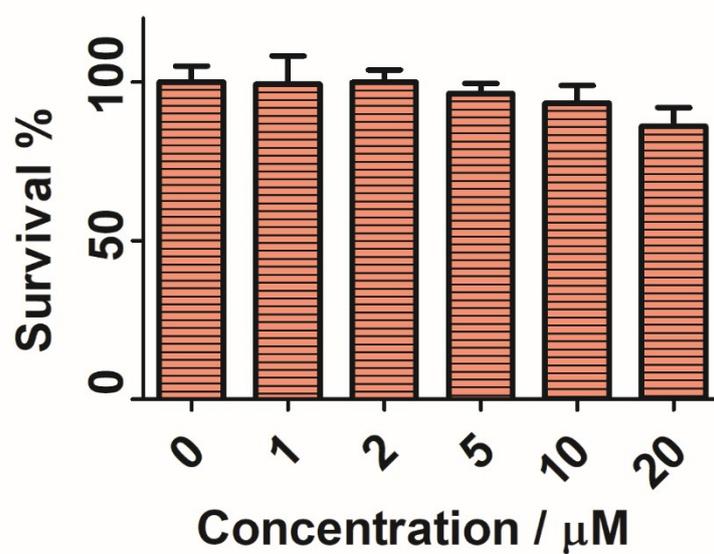
**Fig. S5** The  $^1\text{H}$  NMR spectra of Rh-TPA (upper spectrum) and with the addition of 30 eq. of  $\text{Na}_2\text{SO}_3$  in DMSO- $d_6$ /D $_2$ O (3:1) solution (lower spectrum).



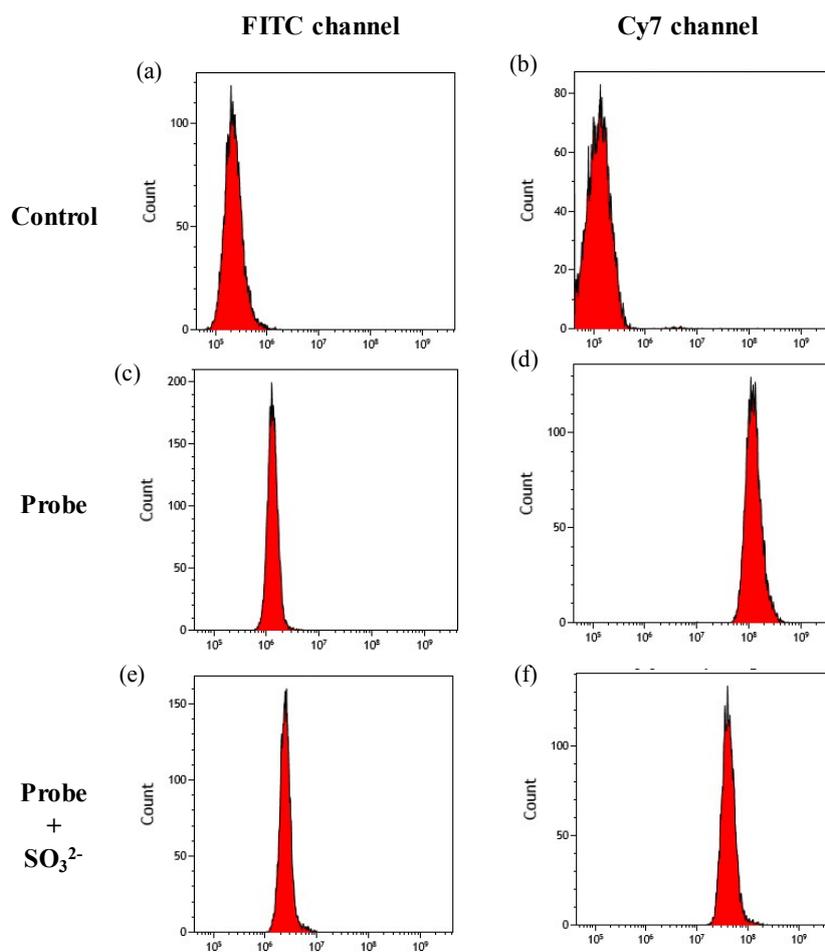
**Fig. S6** The LC-MS spectra of Rh-TPA (a) and Rh-TPA interacted with 30 equivalents of  $\text{SO}_3^{2-}$  (b).



**Fig. S7** The fluorescence spectra of 10  $\mu\text{M}$  of **Rh-TPA** (black line), and 10  $\mu\text{M}$  of **Rh-TPA** in the presence of 50 eq. of  $\text{ONOO}^-$  (red line) or  $\text{SO}_3^{2-}$  (green line) respectively. In 1 cm Cuvettes in PBS (pH 7.4, 10 mM). Excitation:  $\lambda = 450$  nm.

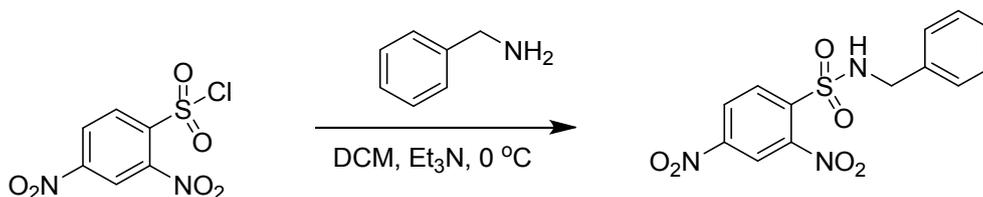


**Fig. S8** The MTT assay of **Rh-TPA** incubated with HeLa cells for 24 h.



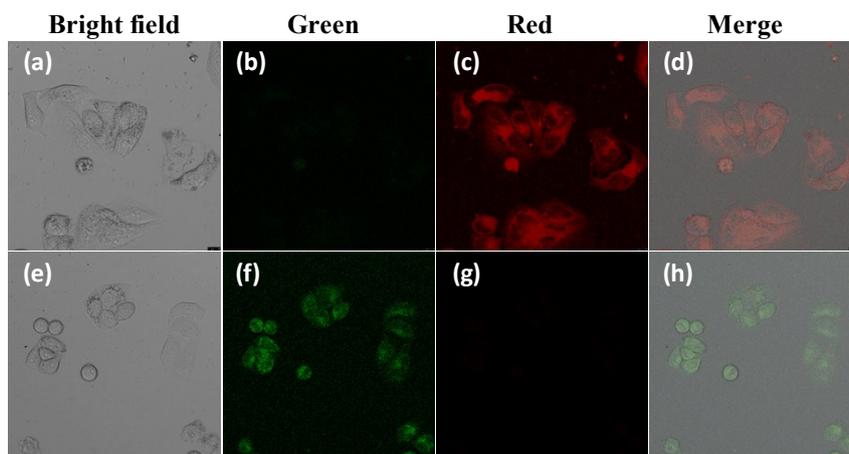
**Fig. S9** The histogram of flow cytometry assay of **Rh-TPA** in HeLa cells. Control group (a, b), HeLa cells only. Probe group (c, d), HeLa cells were incubated with 10  $\mu$ M probe for 20 min. Probe with  $\text{SO}_3^{2-}$  group (e, f), HeLa cells were incubated with 10  $\mu$ M probe and 50  $\mu$ M  $\text{SO}_3^{2-}$  for 20 min. FITC channel was excited by 488 nm, Cy7 channel was excited by 640 nm.

## Synthesis of SO<sub>2</sub> donor



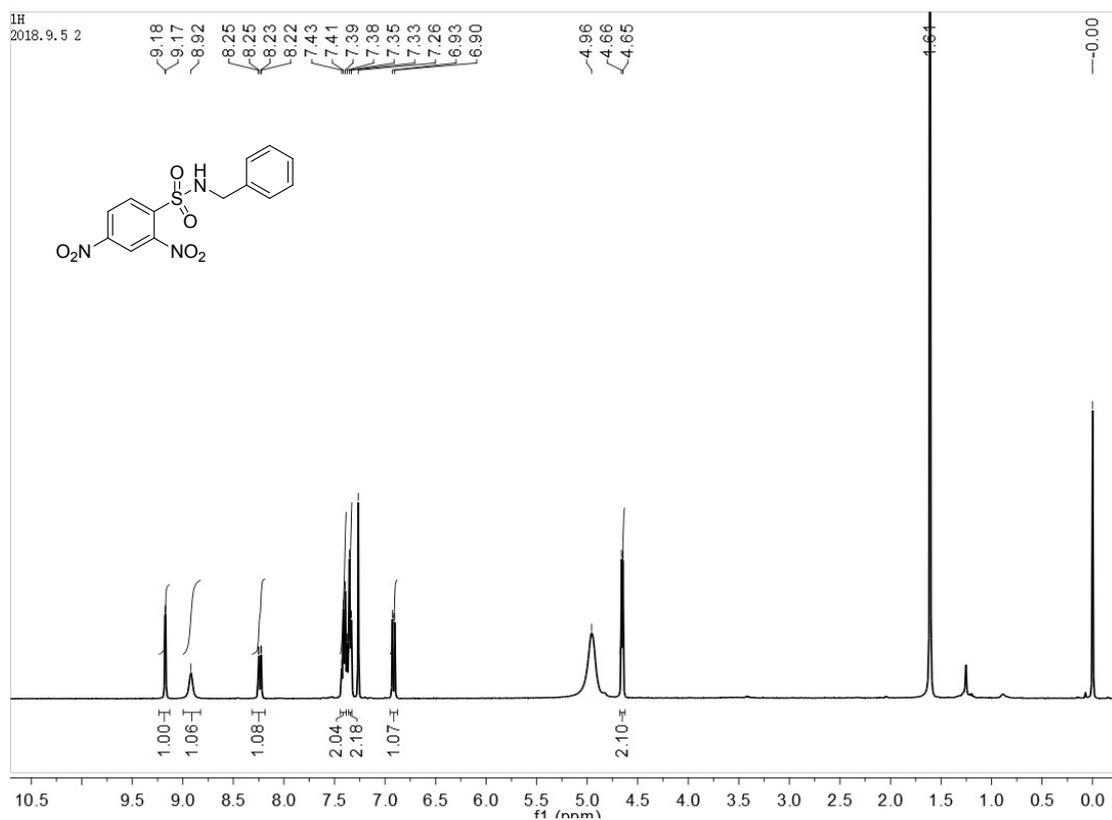
**Scheme S1** The synthesis route of SO<sub>2</sub> donor.

Benzylamine (0.5 g, 4.67 mmol) was dissolved in 5 mL dichloromethane, and 0.5 mL triethylamine was added. The solution was cooled to 0 °C in an ice-bath, then 2,4-dinitrobenzene-1-sulfonyl chloride (1.18 g, 4.44 mmol) in 5 mL dichloromethane was added dropwise. Warmed the solution to room temperature and stirred overnight. The organic solvent was removed by rotary evaporator and the crude product was purified by silica gel column, eluted by petroleum ether and ethyl acetate (v/v=10/1). The final compound was obtained in yellow color (1.12 g, 3.32 mmol), yield: 74.8%. <sup>1</sup>H NMR (400 MHz, CCl<sub>3</sub>D) δ 9.17 (d, *J* = 2.5 Hz, 1H), 8.92 (s, 1H), 8.23 (dd, *J*<sub>1</sub> = 9.3 Hz, *J*<sub>2</sub> = 2.3 Hz, 1H), 7.42 (m, 2H), 7.35 (m, 2H), 6.91 (d, *J* = 9.3 Hz, 1H), 4.95 (s, 1H), 4.65 (d, *J* = 5.6 Hz, 1H).

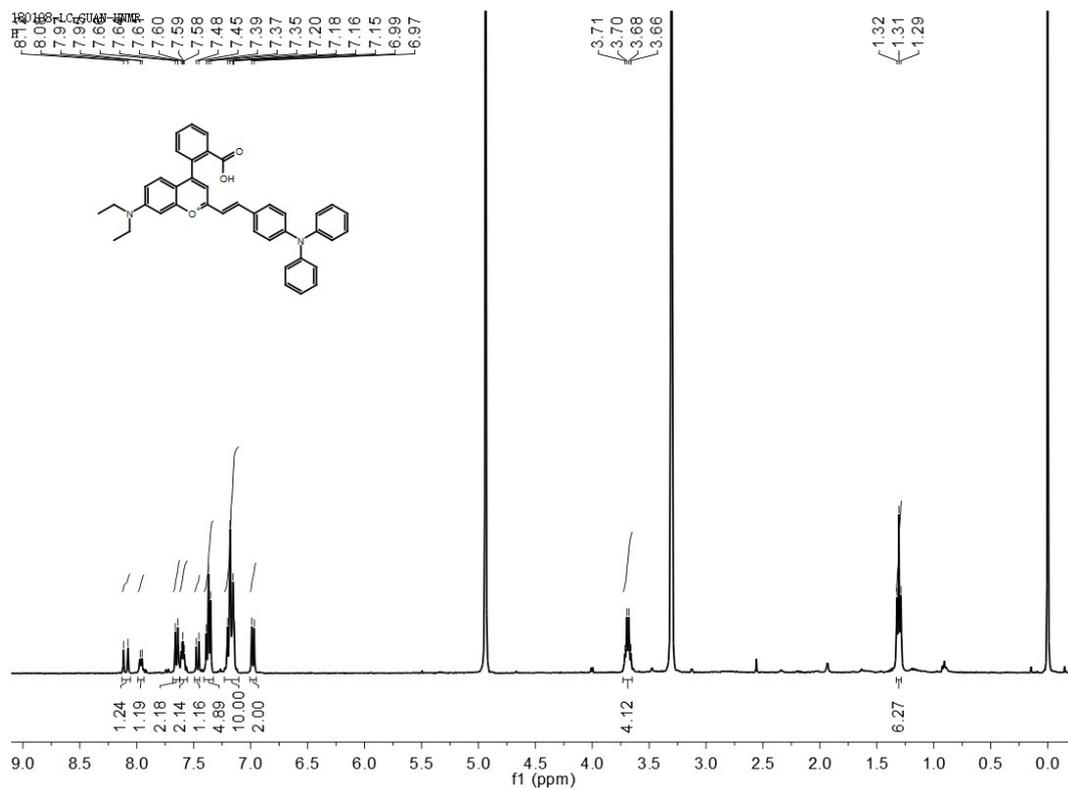


**Fig. S10** The confocal fluorescence images of endogenous SO<sub>2</sub> in HeLa cells. (a-d) HeLa cells were incubated with 10 μM of **Rh-TPA** for 20 min; (a) Bright

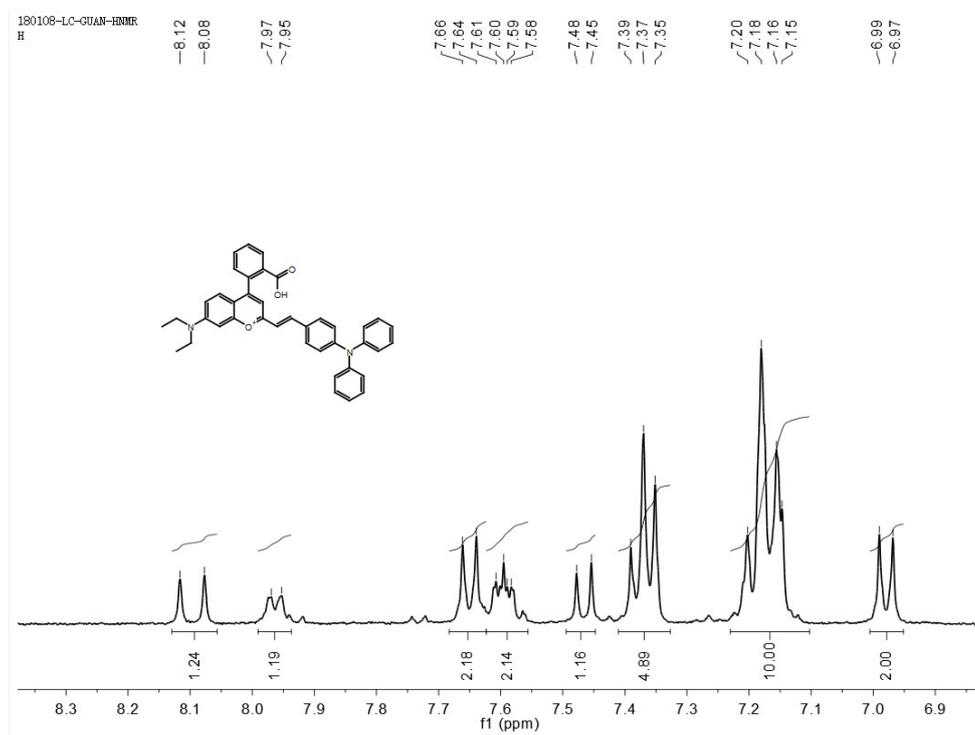
field, (b) Green channel, (c) Red channel, (d) Merge. (e-h) HeLa cells were preincubated with SO<sub>2</sub> donor (N-benzyl-2,4-dinitrobenzenesulfonamide, 40 μM) and Cys (200 μM) for 40 min then incubated with **Rh-TPA** for 20 min before fluorescence imaging; (e) Bright field, (f) Green channel, (g) Red channel, (h) Merge. Scale bar: 20 μm.



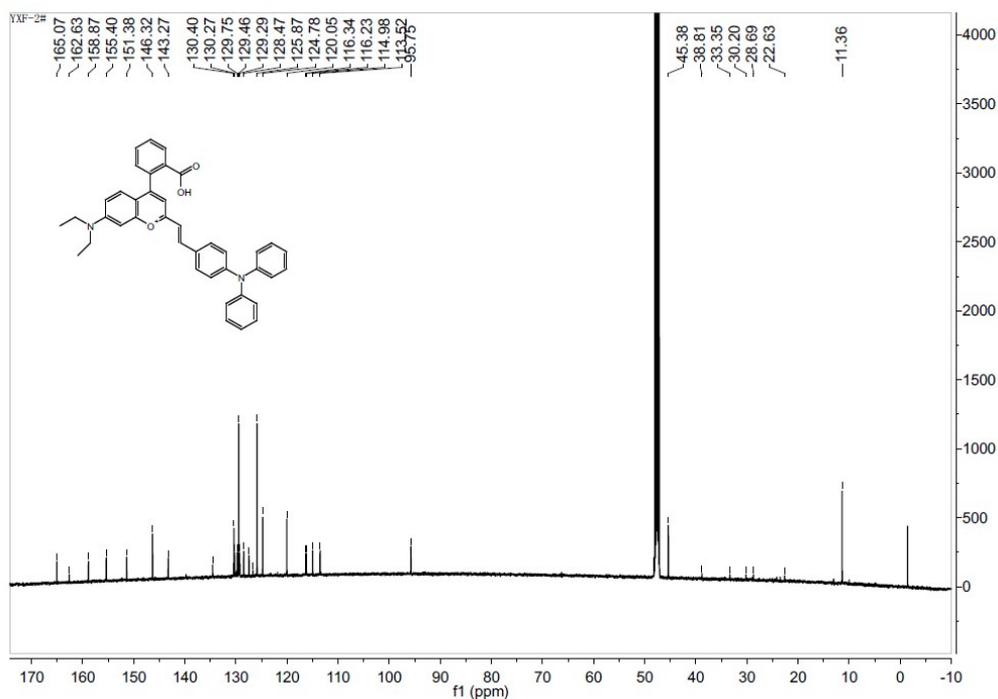
**Fig. S11** <sup>1</sup>H NMR spectrum (400 MHz) of SO<sub>2</sub> donor in CDCl<sub>3</sub>.



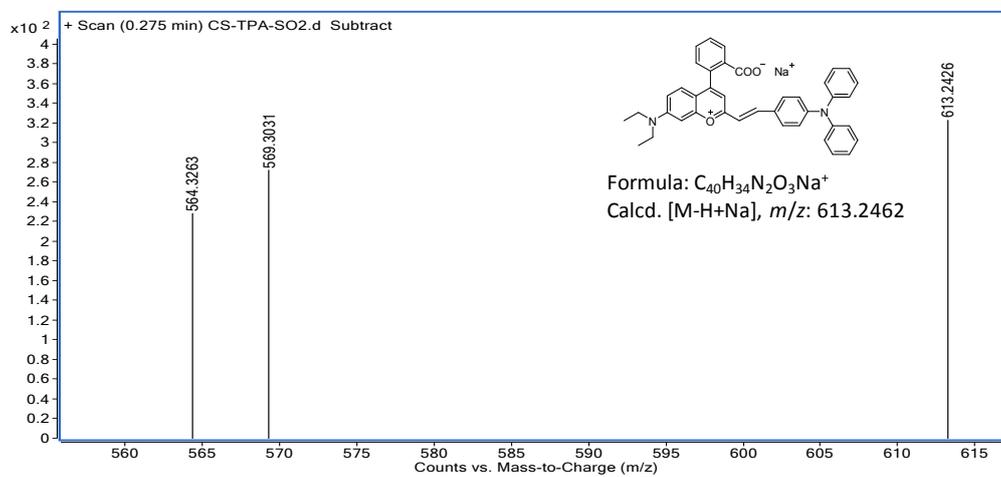
**Fig. S12**  $^1\text{H}$  NMR spectrum (400 MHz) of Rh-TPA in methanol- $\text{d}_4$ .



**Fig. S13**  $^1\text{H}$  NMR spectrum (400 MHz) of Rh-TPA in methanol- $\text{d}_4$ .



**Fig. S14**  $^{13}\text{C}$  NMR spectrum (100 MHz) of **Rh-TPA** in methanol- $\text{d}_4$ .



**Fig. S15** The HR-MS spectrum of **Rh-TPA**.