Support Information

1. Materials and instruments

The chemicals needed for the experiment were purchased from Aladdin Reagent (Shanghai) Co., Ltd. and Shanghai Anaiji Chemical Co., Ltd., and the reagents needed were purchased from Shanghai Chemical Industry. All chemicals and solvents used were of analytical grade and no further purification was required. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05 mol/L Na_2HPO_4 water solution and 0.05 mol/L KH_2PO_4 water solution with the volume ratio 4:1.

Reaction processes were monitored on thin layer chromatography (TLC). TLC analysis was performed using precoated silica plates. Fluorescence spectra were carried out a HITACHI F-7000 spectrophotometer. UV-visible spectra were recorded with a HITACHI U-3900 spectrophotometer. Shanghai Huamei Experiment Instrument Plants (China) provided a PO-120 quartz cuvette (10 mm). NMR spectra were recorded on a JBruker AVANCE-600MHz spectrometer and chemical shifts were referenced relative to tetramethylsilane. Coupling constants (J values) are

reported in hertz. HR-MS was measured with an Thermo Scientific Q Exactive. Cell imaging experiments of living cells were carried out by Zeiss LSM880 Airborne confocal laser scanning microscopy.

2. Photochemical Properties of *RhB-NA* measurement

The stock solution of ***RhB-NA*** (2 mM) required for property testing was made by dissolving 0.0029 g probe in 2 mL DMSO. ATP stock solution, other ionic stock solutions (Cl^-; SO_4^{2-}; NO_3^-; CO_3^{2-}; Br^-; I^-; $H_2PO_4^-$; HPO_4^{2-}; Na^+; K^+)(200 mM) and other biomolecules stock solutions (AMP; ADP; CTP; GTP; UTP; GSH; Cys; Lys)(200 mM) were prepared by dissolving solid in PBS buffer solution. All the PBS solutions used in the experiment were prepared by dissolving in deionized water. Fluorescence measurements were performed on ***RhB-NA*** (λ_{em} = 562 nm, λ_{ex} = 550 nm, slit: 5 nm/ 5 nm). Both the fluorescence spectrum and UV-Vis were obtained in pure PBS buffer solution.

3. Calculation of the limit of detection (LOD)

The detection limit (DL) is calculated according to the following equitation: DL = 3σ / K. Where σ is the standard deviation of 10 blank sample, and K is the slope of the calibration curve.

4. Cell culture and imaging

Transplanted Hela cells into Dulbecco's Modified Eagle Medium which contained 10% Fetal Bovine Serum. The cells were incubated in the constant temperature incubator at 37℃ for about 24 h. Using Trypsin-EDTA solution (0.25%) digested the cells before transferring them to laser confocal petri dish.

CCK-8 test: HeLa cells growing at logarithmic phase were cultured in a 96-well plate. Cell density was adjusted to 5 × 104 cells/well. After adherence, the cells were incubated with 0、1、2.5、5、7.5、10、20 and 30 μM ***RhB-NA***. Incubation times were 6 hours and 12 hours. Cell viability was measured via CCK-8 assay. In brief, the cells were incubated with 10 μL CCK-8 for 1 h. The amount of CCK-8 formazan was determined at the reference wavelength of 450 nm by Microplate Reader.

First, the detection of ATP in HeLa cells by the probe was carried out. The probe (10 μM) was added to the petri dish of HeLa cells, incubated at 37°C for 10 min, and then the cells were imaged after washing 3 times with PBS. The effect of H_2S on intracellular ATP level was next performed. HeLa cells were pretreated with exogenous Na_2S (10 μM) for 10 min, washed 3 times with PBS, then the probe (10 μM) was added and incubated at 37℃ for 10 min, washed 3 times with PBS and imaged with a confocal microscope. As well as, the effect of H_2O_2 on intracellular ATP level was carried out. HeLa cells were pre-incubated with exogenous H_2O_2 (500 μM) for 10 min, washed 3 times with PBS, then added probe (10 μM) and incubated at 37℃ for 10 min, washed 3 times with PBS for imaging. On the basis of previous imaging, we carried out further research. HeLa cells were pretreated with exogenous H_2O_2 (500 μM) for 10 min, washed with PBS for 3 times, incubated with the probe for 8 min, washed with PBS for 3 times, and then added with exogenous ATP (1 mM). incubated for 10 min, washed 3 times with PBS, and imaged on a confocal microscope.

5. Experimental Section

The probe was synthesized according to the route shown in Scheme 1 through 3 steps. Compound 2[1] and compound 5[2] was synthesized according to the procedure reported in the previous literature. Finally, compound 2 (213 mg, 1 mmol) and compound 5 (528 mg, 1 mmol) were dissolved in ethanol (15 mL), and the mixture was refluxed for 10h at 80℃. Later, distilled the solvent under reduced pressure, the crude product was purified with silica-gel column chromatography (CH_3OH/CH_2Cl_2, 1:20, v/v), affording the probe as a yellow solid (540 mg, yield 74%). ^{1}H-NMR and ^{13}C-NMR spectra and HR-MS of the probe are shown in Fig. S1-S3, respectively. ^{1}H-NMR (600 MHz, DMSO) δ 8.60 (d, J = 8.3 Hz, 1H), 8.37 (d, J = 7.3 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.76 (m, 1H), 7.63 (s, 1H), 7.53 (m, 2H), 7.42 (s, 2H), 7.00 (d, J = 8.3 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.32 (d, J = 19.9, 9.0 Hz, 6H), 3.92 (s, 2H), 3.27 (q, J = 7.1 Hz, 8H), 3.05 (d, J = 7.1 Hz, 2H), 2.70 (s, 2H), 2.23 (s, 2H), 1.03 (t, J = 7.0 Hz, 12H). ^{13}C-NMR (151 MHz, DMSO) δ 167.53, 164.27, 163.38, 153.78, 153.11, 153.10, 148.86, 134.31, 133.03, 131.37, 131.00, 130.17, 129.70, 128.71, 128.66, 124.37, 124.03, 122.71, 122.31, 119.84, 108.64, 108.59, 108.10, 105.55, 97.75, 64.47, 47.20, 47.04, 46.17, 44.10, 40.59, 40.47, 40.33, 40.19, 40.05, 39.91, 39.77, 39.63, 39.09, 33.74, 15.21, 12.80. HR-MS: m/z calcd. for the probe ($C_{44}H_{46}N_6O_4$, $[M+H]^+$), 723.3581; found, 723.3646.

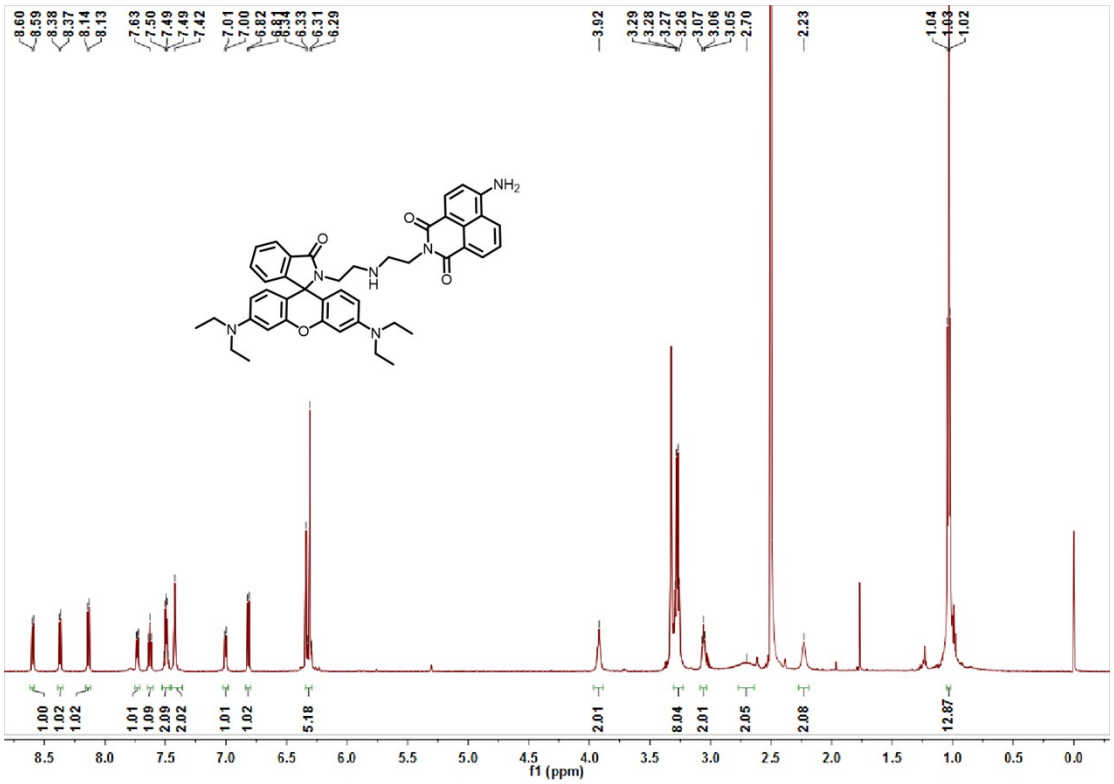

Fig. S1: ^{1}H NMR (600MHz) of ***RhB- NA*** in DMSO-d_6.

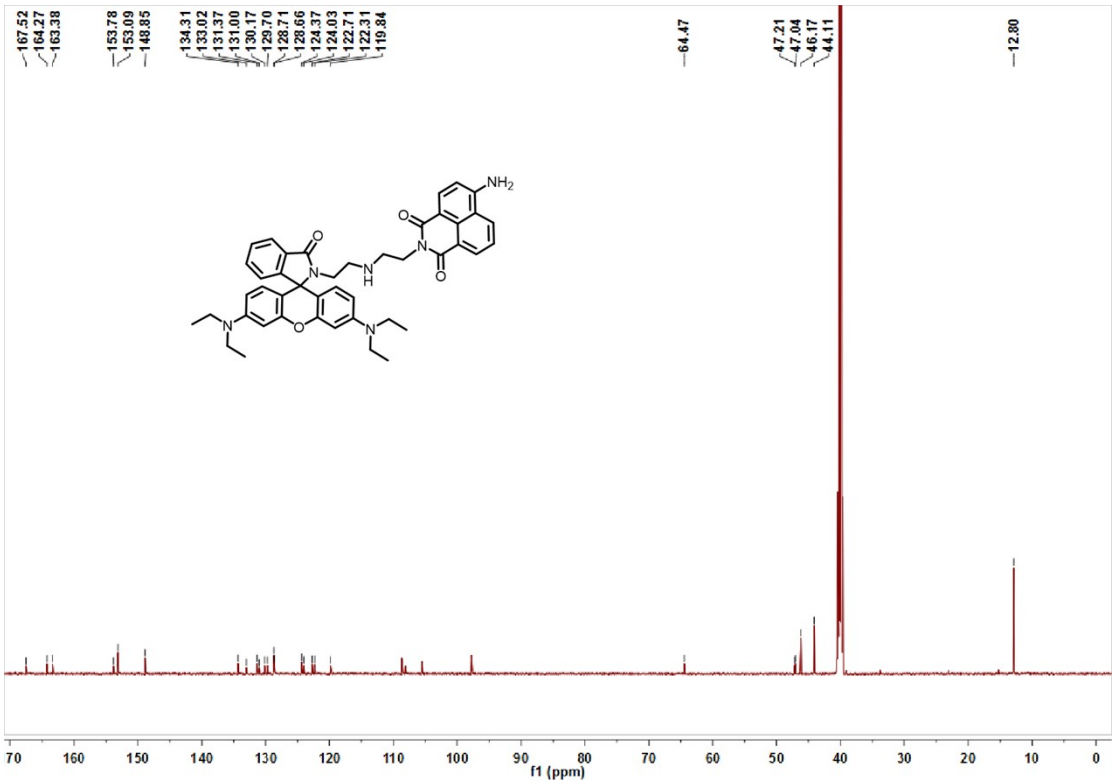

Fig. S2: ^{13}C NMR of ***RhB-NA*** in DMSO-d_6.

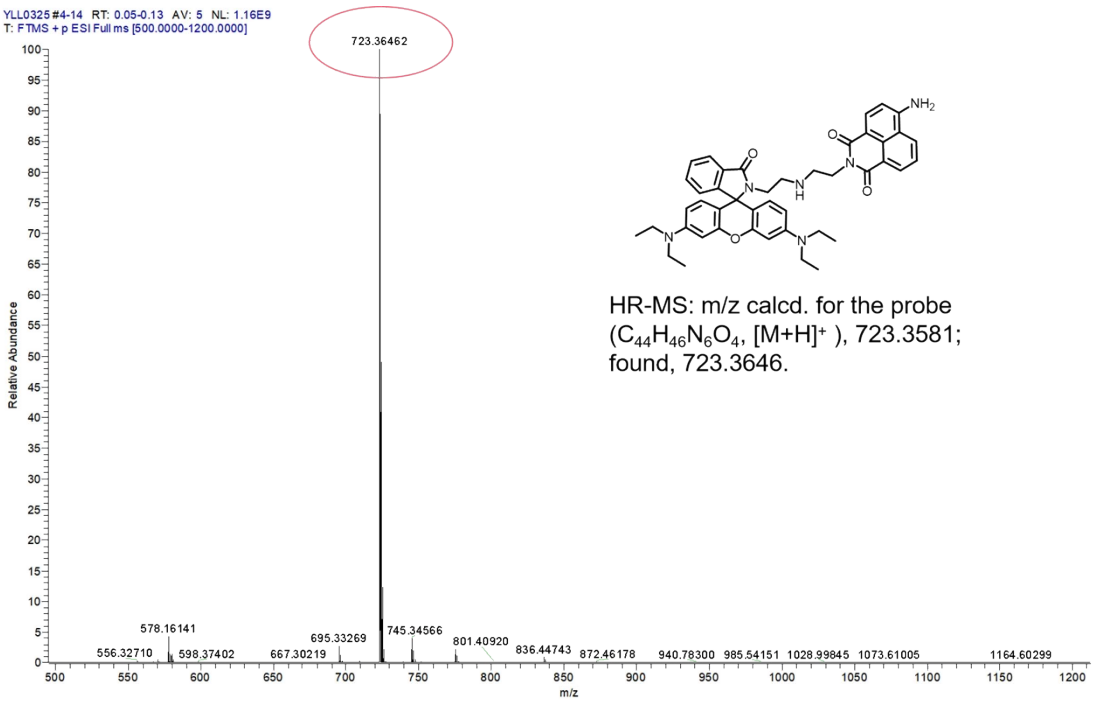

Fig. S3: The HR-MS of ***RhB-NA*****:** *m/z*: $[M+H]^+$ Calcd. for $C_{44}H_{46}N_6O_4$, 723.3581; found, 723.3646.

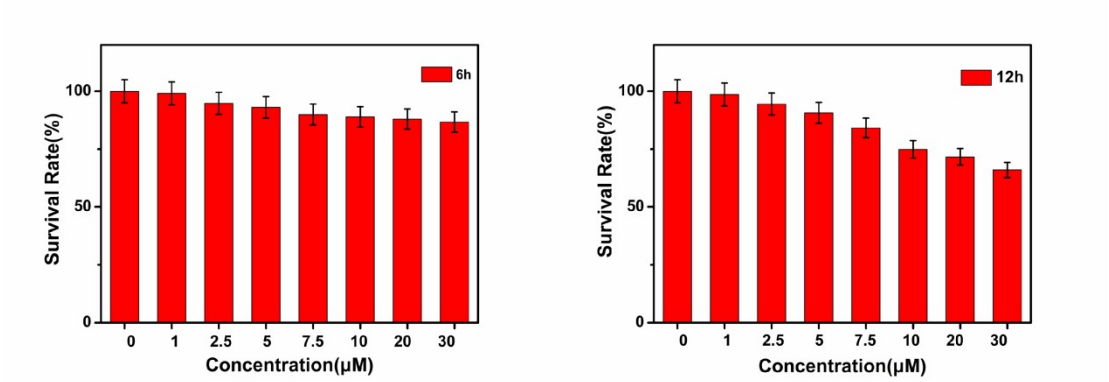

Fig. S4: Cytotoxicity data of ***RhB-NA*** (0、1、2.5、5、7.5、10、20、30 μM) in HeLa cells (a) 6 hours (b) 12 hours.

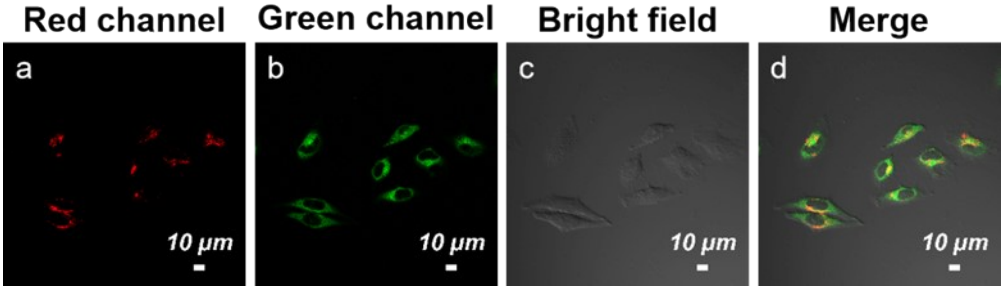

Fig. S5: Confocal microscopy fluorescence images in living HeLa cells incubated with ***RhB-NA*** (10 μM). (Red channel: λ_{ex}= 561 nm, λ_{em}= 580-620 nm; Green channel:

λ_{ex}= 458 nm, λ_{em}= 510-550 nm, scale bar: 10 μm).

References

1. B. Bag and B. Biswal, *Org Biomol Chem*, 2012, **10**, 2733-2738.
2. Y. Fang, W. Shi, Y. Hu, X. Li and H. Ma, *Chem Commun (Camb)*, 2018, **54**, 5454-5457.