Title: A novel profluorescent probe for detecting oxidative stress induced by metal and H_2O_2 in living cells

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Supporting information

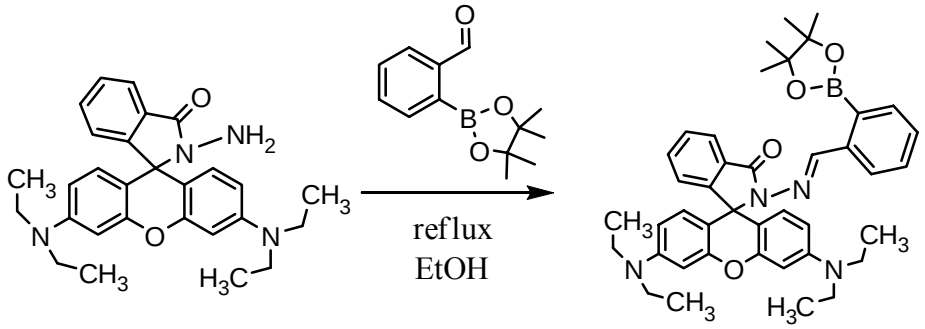
1. **Materials and instruments**
2. **Synthesis and characterization**
3. **Cell culture**
4. **Figs. S1-S11.**

1. Materials and instruments

2-formylphenylboronic acid, pinacol ester was purchased from Combi-Blocks, Inc. Rhodamine B and salicylaldehyde were purchased from Sigma-Aldrich. Other chemicals and solvents are of analytical pure grade. Solutions of Fe^{2+} and Fe^{3+} were prepared freshly by dissolving their chloride salts into 0.01 M HCl, while ferrous ammonium sulfate, $CuCl_2$ and solutions of other metal ions were prepared freshly in double-distilled water.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-300 spectrometer. ESI-MS analyses were performed using a Perkin-Elmer API 150EX mass spectrometer. UV/Vis spectra were recorded on a Perkin-Elmer Lambda 25 spectrometer at 298 K. Fluorescence spectra were recorded on a Perkin-Elmer LS55 luminescence spectrometer at 293 K. The excitation wavelengths and filters used were indicated in the figures. The pH measurements were carried out on a Corning pH meter equipped with a Sigma-Adrich micro combination electrode calibrated with standard buffer solution. The fluorescence responses of the probe in living cells were investigated under a Zeiss LSM 710 laser scanning confocal microscope. Excitation wavelength of laser was 510 nm and emission spectra were integrated over the range 520-580 nm. The REUSE function controlled by Zeiss software was applied to guarantee that all spectra were recorded under the same instrumental conditions.

2. Synthesis and characterization



RS-BE

Rhodamine B hydrozide and the active chelator **Rh-SBH** were synthesized according to the reported procedure and characterized by NMR and Mass spectra. [4]

Synthesis of **RS-BE**. 2-formylphenylboronic acid pinacol ester (1 mmol, 0.150 g) was added into 15 mL ethanolic solution of Rhodamine B hydrozide (1 mmol, 0.46 g), and then the solution was heated to flux under a nitrogen atmosphere for 3 h. The solution was concentrated through removing solvent by rotorevaporation, and then cooled down to room temperature. The obtained precipitate was further washed with ice-cold ethanol three times to afford the pure product (**RS-BE**) as pink solid (0.20 g, 30%). ^{1}H NMR spectrum is shown in Fig. S5. δ_H (300 MHz; $(CD_3)_2SO$; Me_4Si, δ, ppm) 9.4(1 H, s), 7.89(1 H, d, *J* 6.9), 7.81(1 H, d, *J* 7.8), 7.51-7.63(3 H, m), 7.45(1 H, t, *J* 6.5, 6.6), 7.32(1 H, m), 7.09(1 H, d, *J* 7.2), 6.39-6.46(4 H, m), 6.30-6.36(2 H, m), 3.31(8 H, q, *J* 7.8, 7.3), 1.23(12 H, s), 1.06(12 H, t, *J* 6.9); δ_C(CDCl3, Me_4Si) 165, 153, 148, 141, 135, 133, 130, 128, 127, 125.3, 123.4, 107.7, 107.1, 98.4, 83.7, 66.2, 44.3, 24.6, 12.6. ESI-MS: m/z, 671.3 $[M+H]^+$, 693.7 $[M+Na]^+$, $C_{41}H_{47}BN_4O_4$ requires 670.37. The purity of the compound was also confirmed by HPLC.

3. Cell culture

Human SH-SY5Y neuroblastoma cells were obtained from ATCC (American Type Culture Collection). Cells were maintained in 1:1 mixture of Eagles Minimal Essential medium (ATCC) and Ham's F12 medium (ATCC) supplemented with 10% fetal bovine serum (ATCC) without any antibiotics and incubated at 37°C in a humidified atmosphere with 5% CO_2. The cells were routinely subcultured using 0.05% trypsin-EDTA solution (ATCC). The cells were seeded on 2-chamber slides for 48 hours at 5×10^4 cells/chamber and grown until each chamber was 20-30% confluent.

The sensor **RS-BE** was dissolved in acetonitrile at a concentration of 1 mM, and then was pre-diluted to a concentration of 10 μM in culture medium without fetal bovine serum. Cell culture medium was removed from the cells in the chamber and replaced with the fresh medium containing the sensor (10 μM).

Figures S1-S11

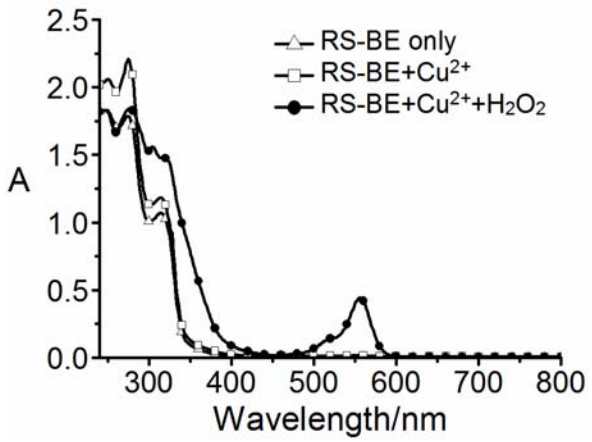


Fig S1. Absorption spectra of 50 μM **RS-BE**, with 50 μM Cu^{2+} before and after addition of 500 μM H_2O_2 (60 min) in acetonitrile/KPB buffer (10 mM, pH 7.5, v/v 1:1).

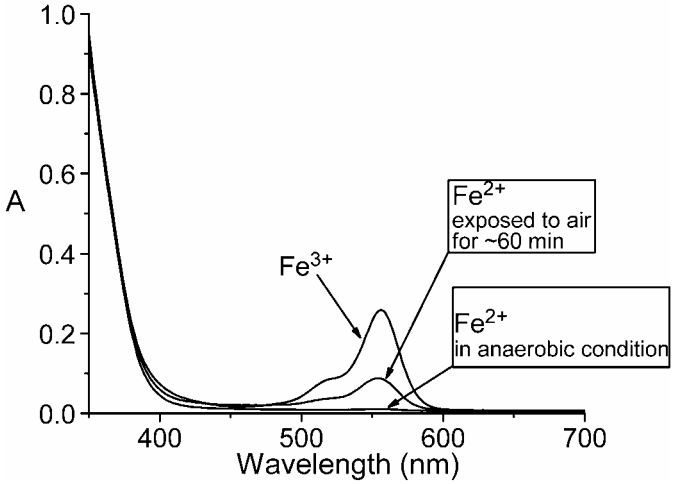


Fig. S2 Absorption spectra of 50 μM **Rh-SBH** incubated with 50 μM Fe^{3+} (top line) or Fe^{2+} (bottom line) under anaerobic conditions in acetonitrile/KPB buffer (10 mM, pH 7.5, v/v 1:1). The middle line is the spectrum of that with Fe^{2+} after exposed to air for ~ 60 min. As shown in this figure, Rh-SBH displayed full response to Fe^{3+} but little response to Fe^{2+} under anaerobic conditions in ACN/KPB buffer. However, after the solution with Fe^{2+} was exposed to air for ~60 min, the absorption peak at 554 nm appeared but with lower intensity compared to that with Fe^{3+}. This can be ascribed to the slow air-oxidation of Fe^{2+} to Fe^{3+}, that subsequently triggered the absorption response of Rh-SBH. These data suggest that Rh-SBH response to Fe^{3+}, not Fe^{2+}.

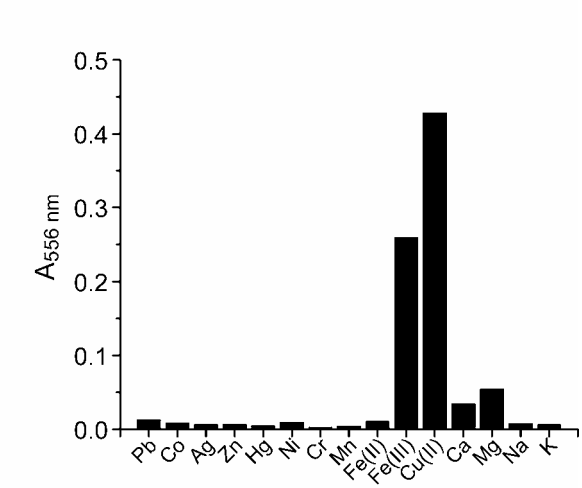


Fig. S3. Absorption responses (556 nm) of 50 μM of Rh-SBH to various metal ions including 50 μM of Pb^{2+}, Co^{2+}, Ag^{+}, Zn^{2+}, Hg^{2+}, Ni^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, as well as 1 mM of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} in ACN/KPB buffer (10 mM, pH 7.5, v/v 1:1).

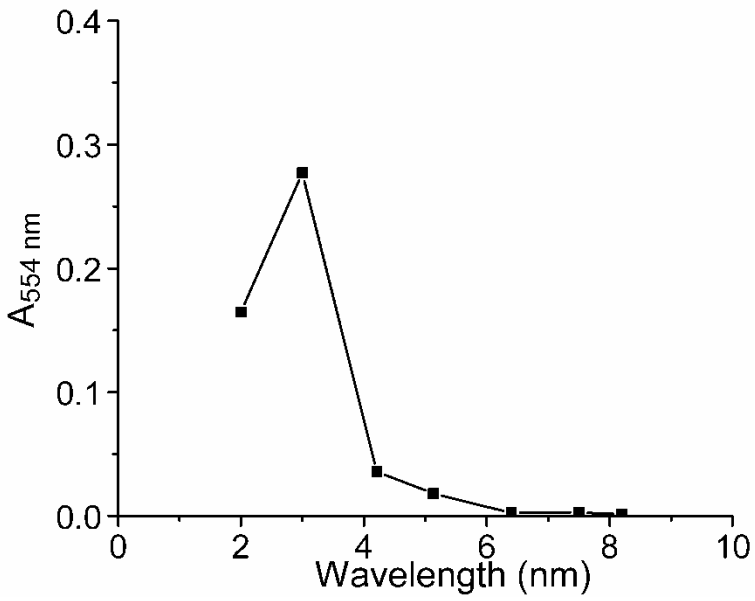


Fig. S4. Absorption profile of 50 μM of Rh-SBH under varied pH values of 2, 3, 4.21, 5.13, 6.4, 7.50 and 8.20. Little characteristic color of rhodamine could be observed for Rh-SBH between pH 5.0 and 8.2, suggesting that the spirolactam form is stable in physiological relevant pH range.

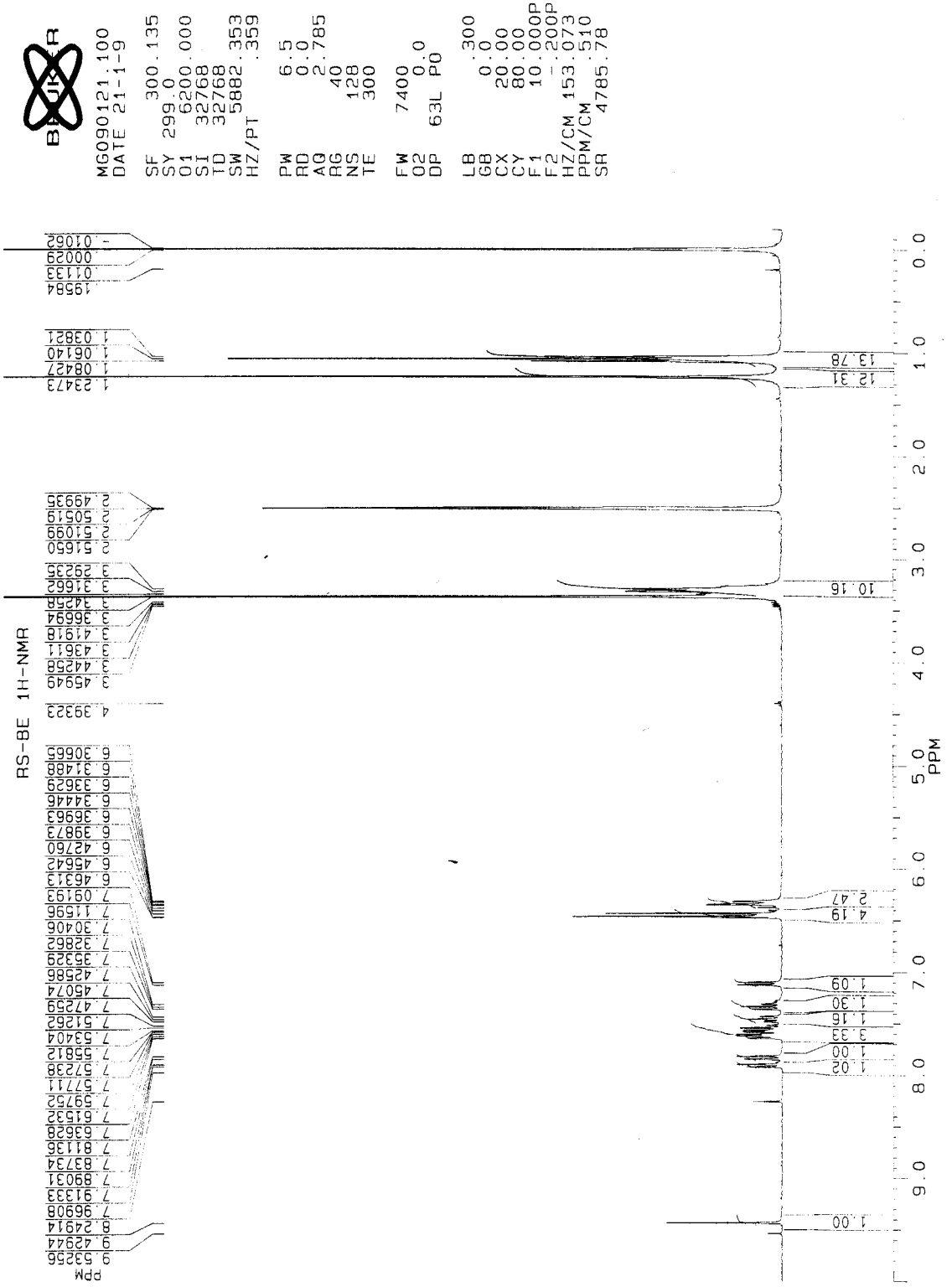


Fig. S5 ^{1}H NMR spectrum of 5 mM RS-BE in $(CD_3)_2SO$.

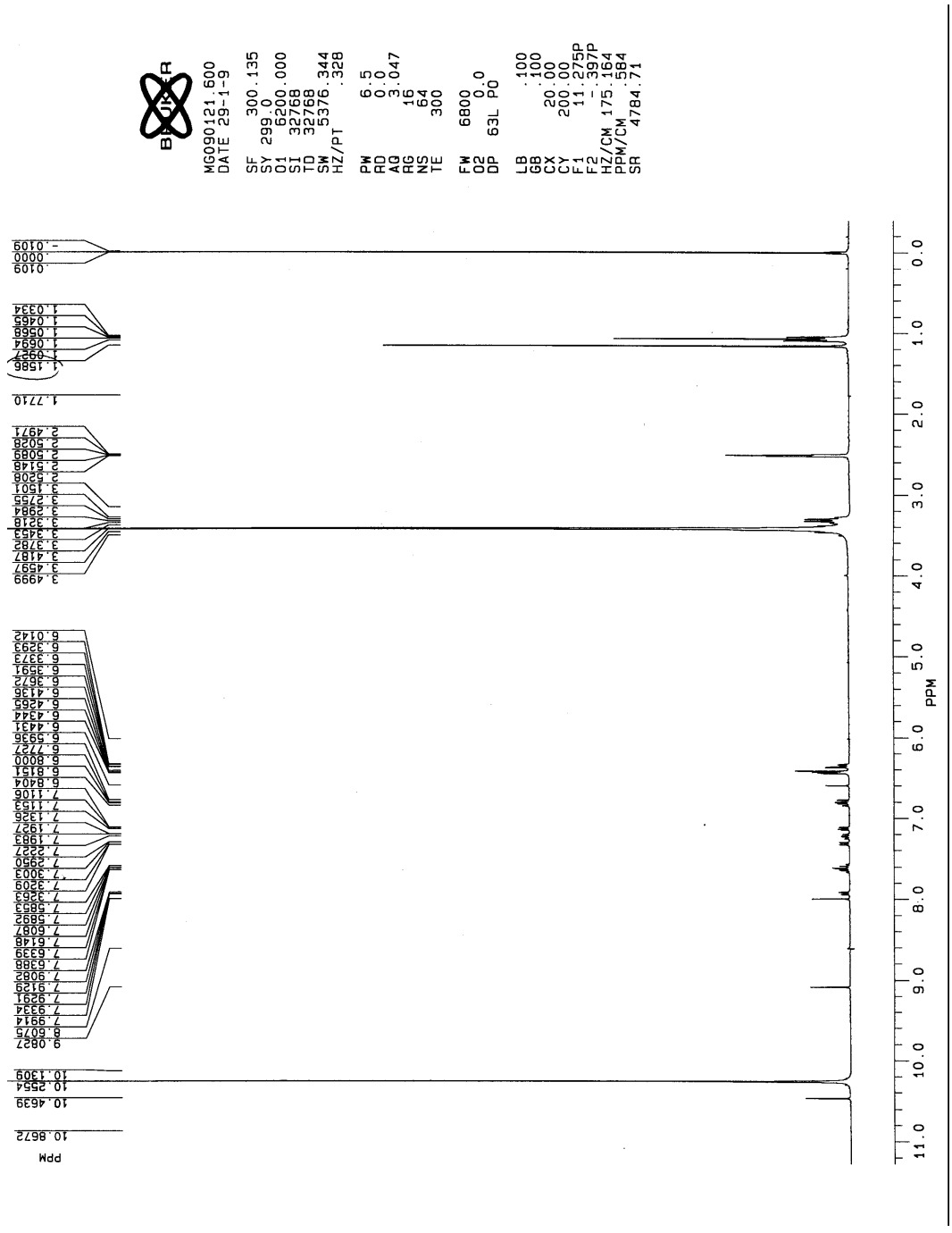


Fig. S6 ^{1}H NMR spectrum of reaction 5 mM RS-BE with 500 mM H_2O_2 in $(CD_3)_2SO$ after 2 h.

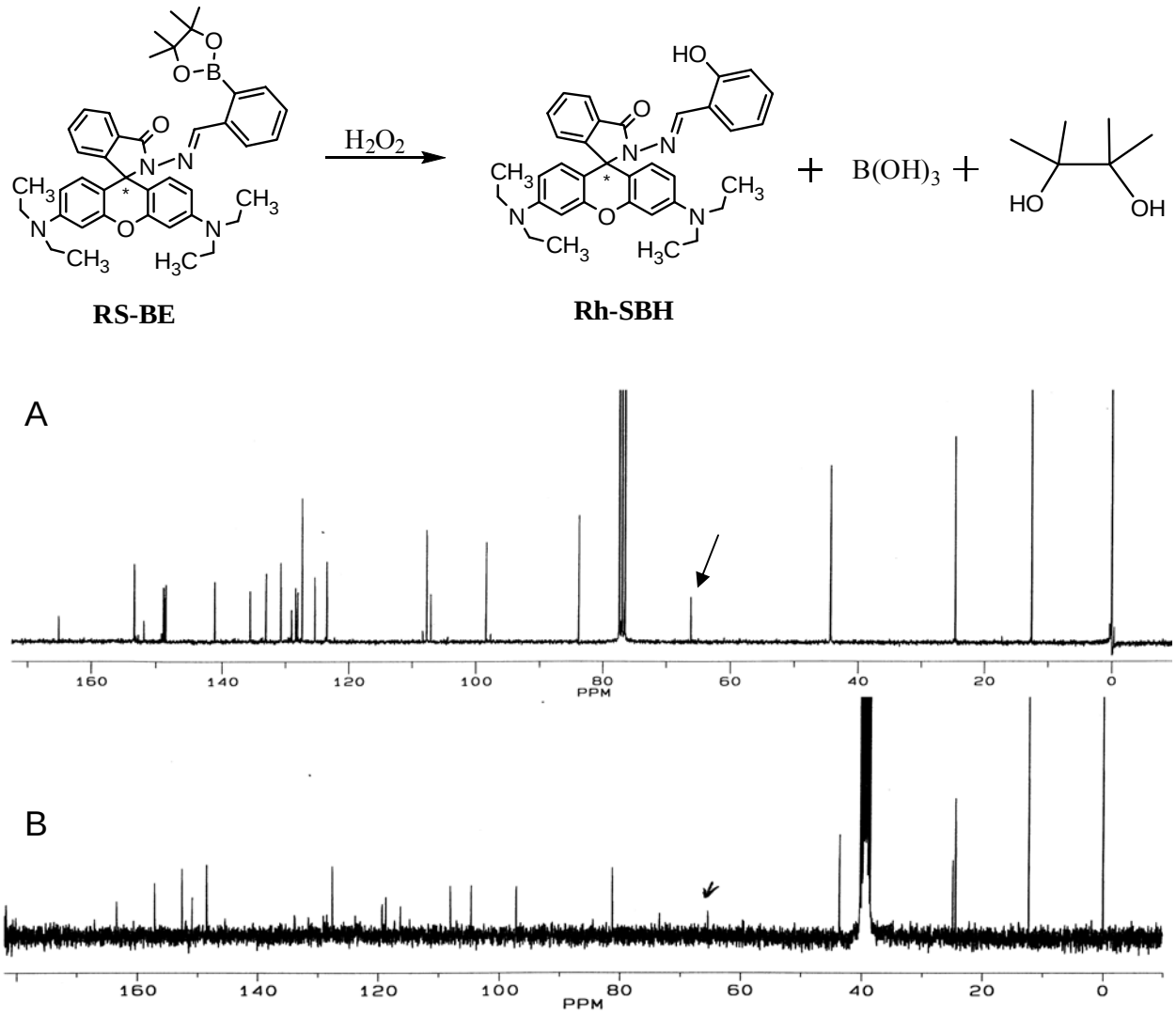


Fig. S7 ^{13}C NMR spectra of (A) 5 mM **RS-BE** only in $CDCl_3$ and (B) 5 mM **RS-BE** with 50 mM H_2O_2 incubated in $(CD_3)_2SO$ for 24 h. The characteristic peak of the 9-carbon (marked with *) at ~ δ 66.2 ppm was still discernible after the reaction with H_2O_2, suggesting that H_2O_2 did not induce the conversion of RS-BE/Rh-SBH from the spirolactam (ring-closed) form to the ring-opened amide form.

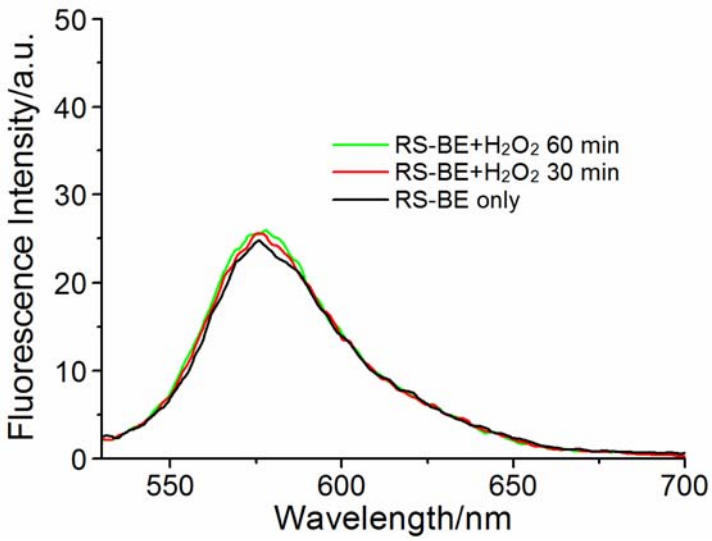


Fig. S8 Fluorescent spectra (E_x, 510 nm; E_m, 580 nm) of 50 μM RS-BE (black line) and after the addition of 500 μM H_2O_2 for 30 min (red line) and 60 min (green line) in acetonitrile/KPB buffer (10 mM, pH 7.5, v/v 1:1).

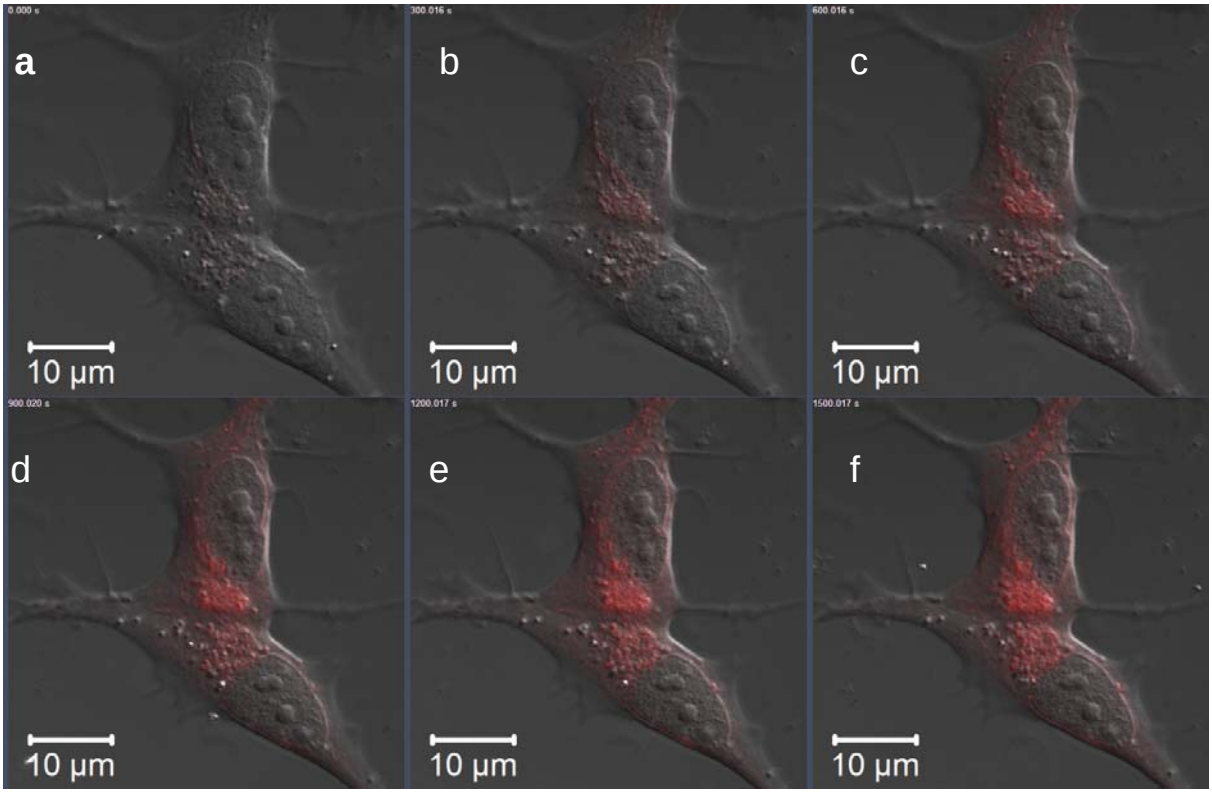


Fig. S9 Time course of the confocal fluorescence images of live human SH-SY5Y neuroblastoma cells pretreated with RS-BE and Fe(8-HQ) (10 μM each) after the addition of 100 μM H_2O_2 for 0, 5, 10, 15, 20, 25 min (a-f).

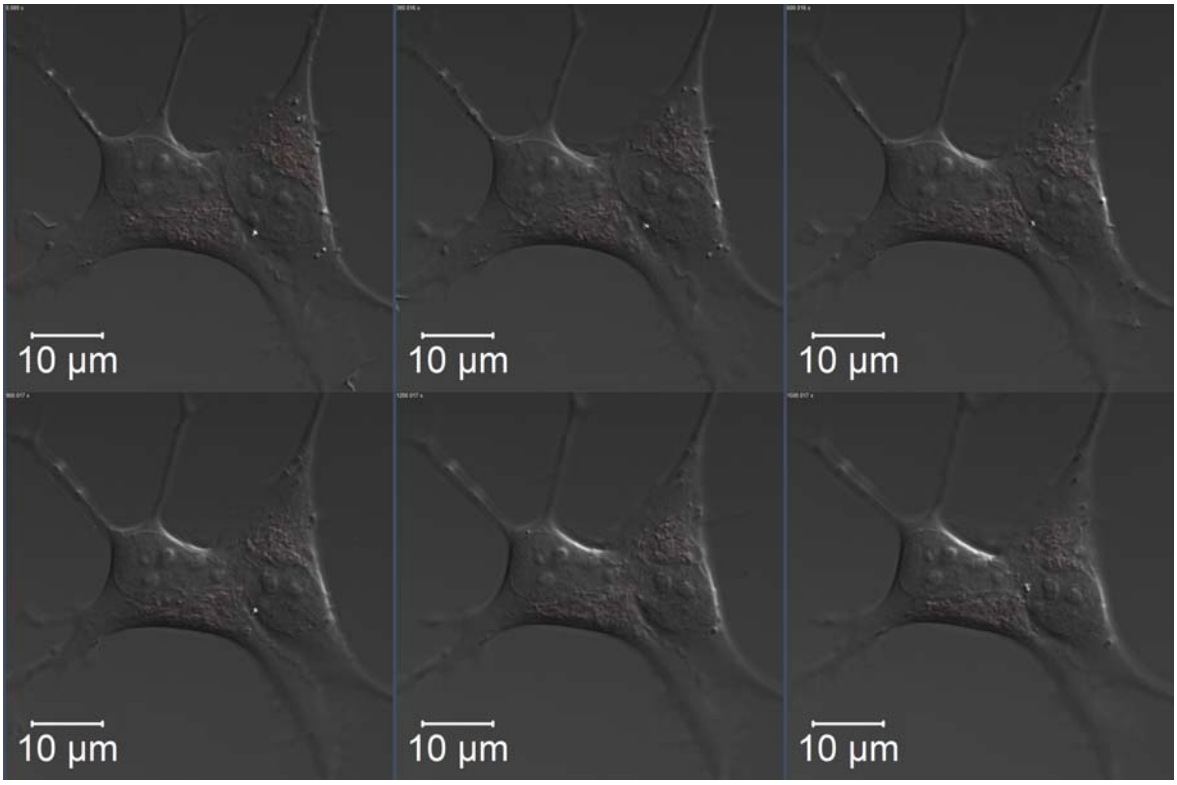


Fig. S10 Time course of the confocal fluorescence images of live human SH-SY5Y neuroblastoma cells pretreated with RS-BE and $CuCl_2$ (10 µM each) after the addition of 100 µM H_2O_2 for 0, 5, 10, 15, 20, 25 min (a-f). Little intracellular fluorescence enhancement was observed after the addition of H_2O_2 to the cells.

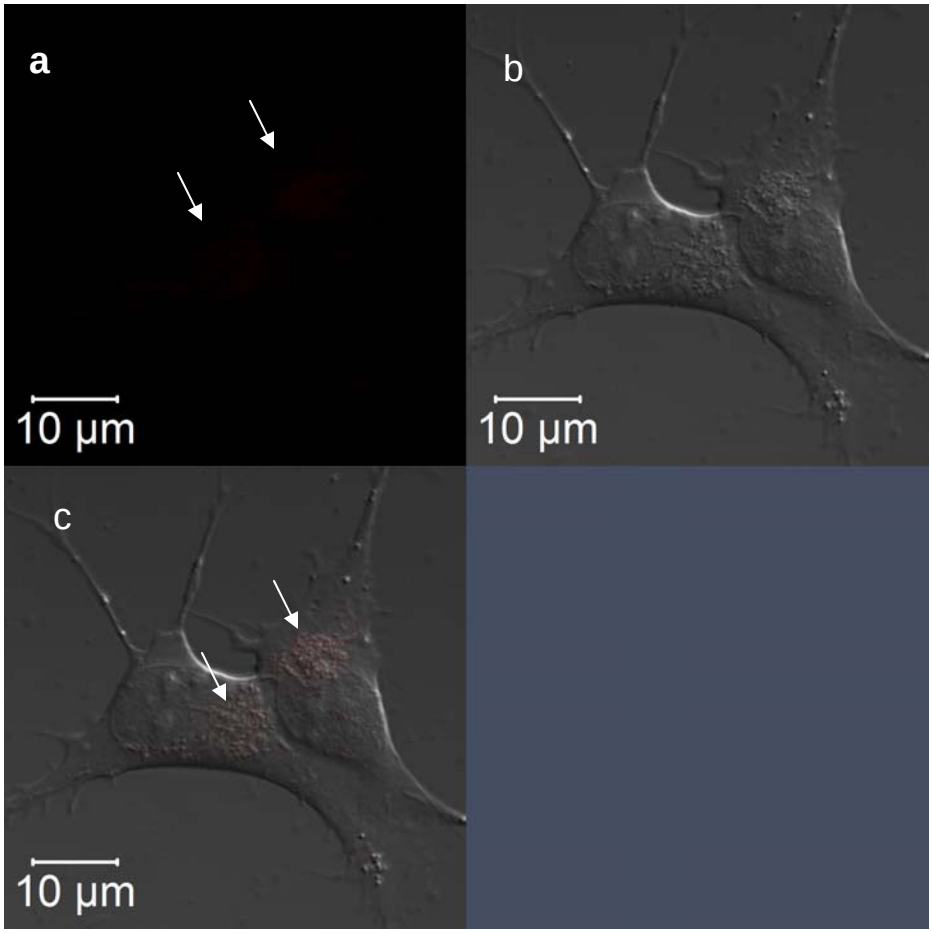


Fig. S11 Confocal fluorescence images of live human SH-SY5Y neuroblastoma cells pretreated with RS-BE (10 μM) and $CuCl_2$ (50 μM) after the addition of 500 μM H_2O_2 for 30 min. (a) confocal image, (b) DIC, (c) overlay of (a) and (b). Weak intracellular fluorescence enhancement (indicated by arrows) was observed after the addition of H_2O_2 to the cells.