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Supporting Information

A Resorufin-based Fluorescent Probe for Hydrazine Detection and Its Application in Environmental Analysis and Bioimaging

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1. General Information

All chemicals and reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by dried and distilled from drying agents under an inert atmosphere. Double distilled water was utilized in all experiments. The NMR spectra were recorded on a Bruker AVANCE III HD 400 and 500 MHz spectrometer, using $SiMe_4$ as an internal standard. ^1H NMR data of chemical shifts (δ) were given in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) using $CDCl_3$ (δ = 7.26 ppm) as reference. ^{13}C NMR data of chemical shifts (δ) were reported in ppm with $CDCl_3$ at δ 77.0 as internal standard. The HRMS were measured on the AB Sciex TripleTOF® 4600 high-resolution mass spectrometer and the MS were measured on the AmaZon SL mass spectrometer. The fluorescence spectra were measured using F97 Pro fluorescence spectrophotometer. The absorption spectra were measured using UV-1920 UV-vis spectrophotometer.

Preparation for N_2H_4 test. A stock solution of **RFT** (1 mM) was prepared in 100% DMSO and was subsequently diluted to prepare appropriate concentration solutions of the probe in PBS buffer (10 mM, pH 7.4, containing 20% DMSO), respectively. Hydrazine for testing is prepared from purchased 80% hydrazine hydrate.

The stock solutions (10 mM) of analytes were prepared in H_2O. The analytes used in the stock aqueous solutions of analytes were (1) Cl^-; (2) Br^-; (3) I^-; (4) SO_4^{2-}; (5) Mg^{2+}; (6) Ca^{2+}; (7) NO^{2-}; (8) $S_2O_3^{2-}$; (9) NH_4^+; (10) CO_3^{2-}; (11) triethylamine; (12) thiourea; (13) diethylamine; (14) phenylamine; (15) HPO_4^{2-}; (16) $H_2PO_4^-$; (17) AcO^-; (18) normal hexyl amine; (19) N_2H_4. All absorption and fluorescence measurements were performed in DMSO/PBS buffer (1 : 4, v/v, 10 mM, pH 7.4) at room temperature. In the selectivity studies, the test samples were prepared by adding appropriate amount of individual stock solution of analytes to 4 mL solution of **RFT** (10 μM). In the titration experiment, solutions of **RFT** (10 μM) were incubated with different concentrations of N_2H_4.

The fluorescence spectra were recorded with the excitation at 525 nm and the emission was collected at 550-750 nm.

The detection limit. The detection limit was calculated from fluorescence titration. In

the absence of N_2H_4, the fluorescence emission spectra of **RFT** were measured by five times and the standard deviation of blank measurement was achieved. To gain the slop, the fluorescence intensity at 590 nm was plotted to the concentration of N_2H_4. The detection limit was calculated according to equation showing below:

$$\text{Detection limit} = 3\ \sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of N_2H_4.

Cell culture and cell imaging. The HepG2 cells were cultured in high-glucose Dulbeccos Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1% penicillin-streptomycin and incubated under an atmosphere containing 5% CO_2 at 37 ℃ humidified air for 24 h. In exogenous experiment, the HepG2 cells were incubated with probe **RFT** for 30 min in the absence and presence of N_2H_4 (1 mM) in a cell culture plate, then shifting the culture medium and washing with PBS twice, the fluorescence images of cells were set out. Cell imaging was performed by confocal fluorescence microscope after washing the cells with PBS for three times. To avoid the artifacts that occur during fixation procedures, all experiments were conducted in live cells.

MTT assay experiment. MTT Cytotoxicity assays. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) experiment was performed in 96-well plate to assess the cytotoxicity of **RFT**. HepG2 cells were cultured in 96-well plate at a density of 1 × 105 cells/well and incubated for 24 h prior to experimental treatments. Then, **RFT** in various concentrations (0, 2, 5, 10, 20 and 30 μM) was added to the well and incubated for 24 h. MTT solution (1 mg/mL) was added to each well and continuously incubated for 4 h at 37 °C. Subsequently, MTT solution was removed and DMSO (100 μL/well) was added to each well to dissolve the dark blue formazan crystals. The absorbance was measured at 570 nm by a microplate reader. Cell viability was expressed as a percentage of MTT reduction with the untreated cells as 100%.

Zebrafish Culture and in vivo Imaging. Zebrafish embryos were pro-vided by Institute for Brain Research and Rehabilitation, South China Normal University, (Guangzhou, China). Zebrafish embryos were housed at 28℃ on a 14 : 10 light–dark

photoperiod under semi-static conditions with charcoal filtered water. All fluorescence images were obtained on a stereomicroscope under the confocal laser scanning microscope at an excitation wavelength of 525 nm and collection window of 550–750 nm.

Fluorescence imaging in mice. All operations were performed according to the guidelines by the Institutional Animal Care and Use Committee (Reference number: HUSOM2022-097) The live imaging was monitored using the fluorescence imaging system (VISQUE in Vivo Smart).

In the exogenous experiment, mice were subcutaneously injected with hydrazine (1 mM) and 10 minutes later subcutaneously injected with **RFT** (1 mM, 40 μL) at the same site. Then fluorescence imaging was performed after incubation for 0, 10, 20, 30, 40, 50, 60 minutes. All groups within study contained n = 3 mice.

2. Supplementary Date

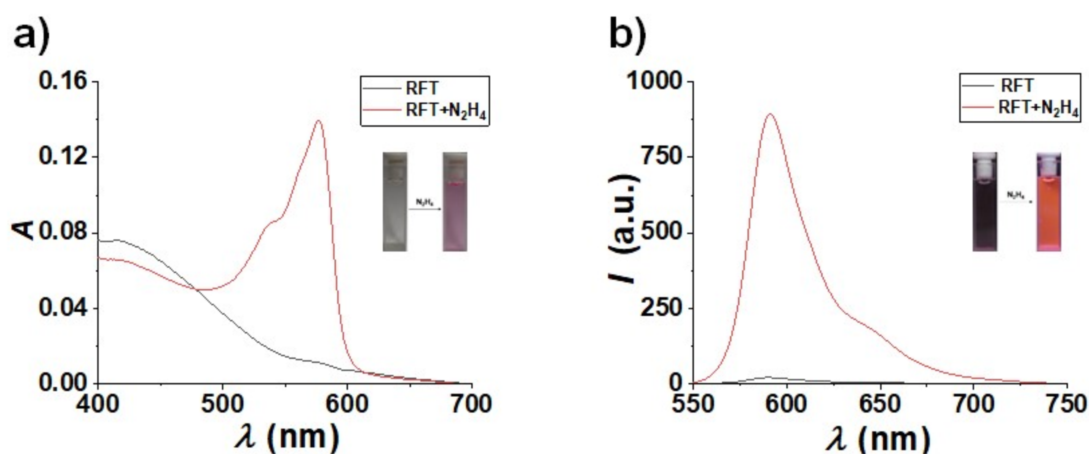

Fig. S1 (a) Absorption spectra and (b) fluorescence spectra of **RFT** (10 μM), in the absence and presence of 10 equiv. N_2H_4 in DMSO/PBS buffer (1 : 4, v/v, 10 mM, pH = 7.4) at room temperature. The inset shown upon addition of N_2H_4 a) the color change of probe solution at daylight. b) fluorescent color change at 365 nm lamp.

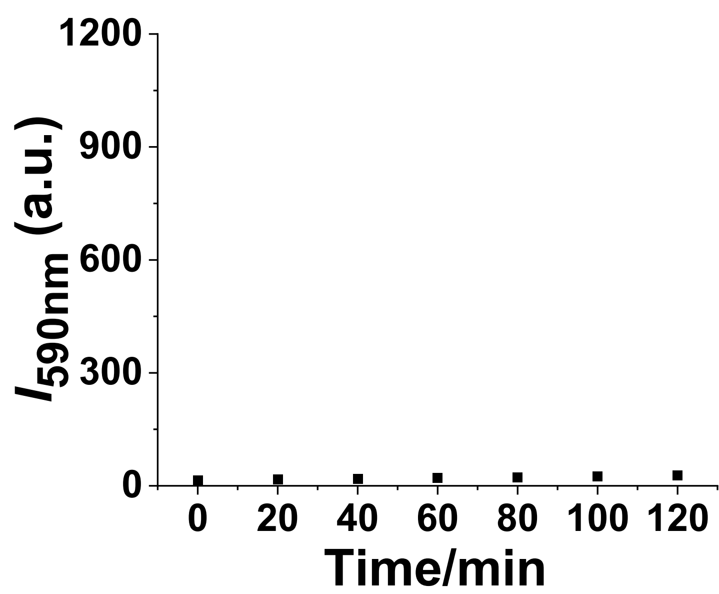

Fig. S2 Fluorescence spectra of probe **RFT** (10 μ() at 590 nm in DMSO/PBS (1:4,v/v, 10 mM, pH = 7.4) buffer at room temperature.

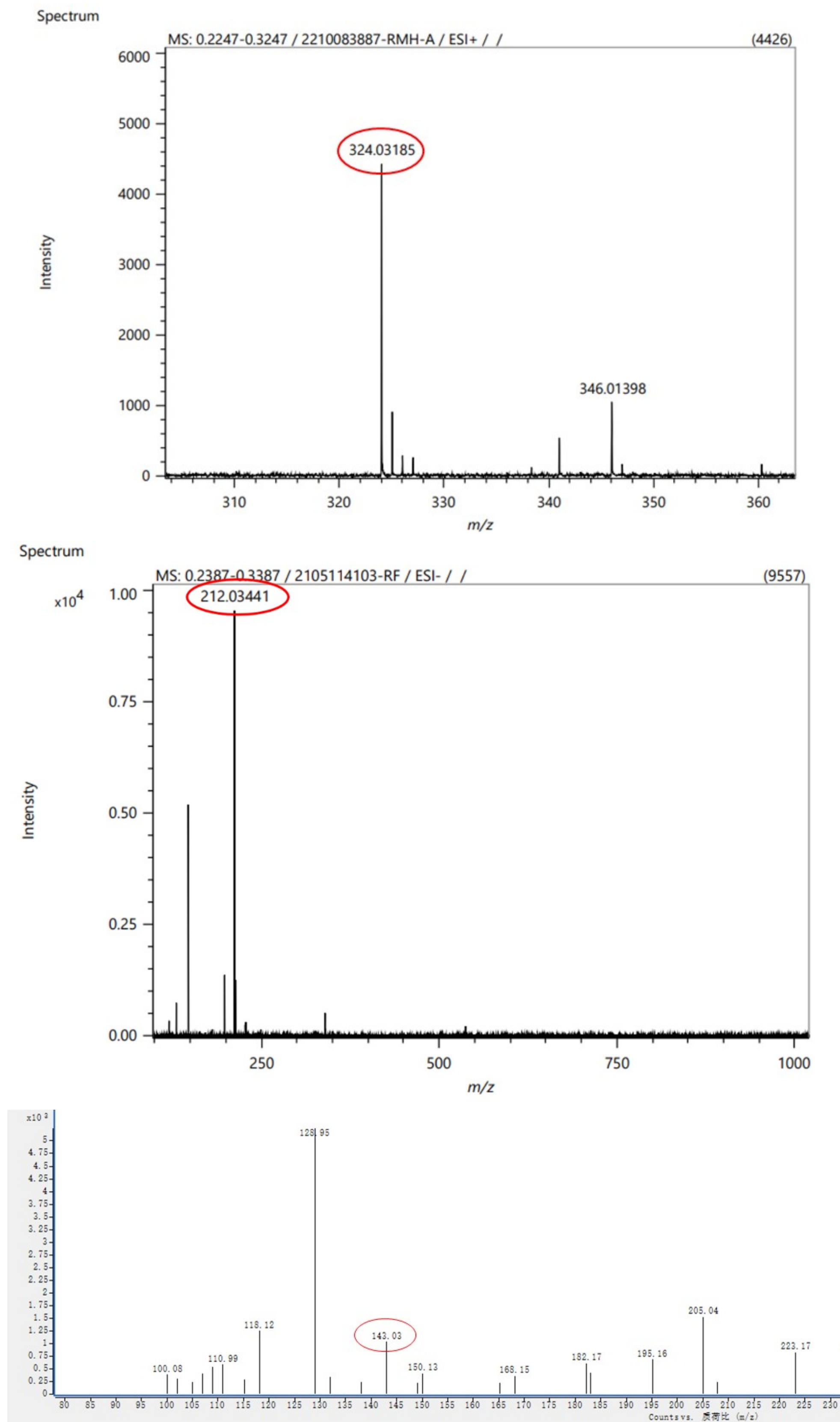

Fig. S3 HR-MS spectra before and after the **RFT** reacts with N_2H_4.

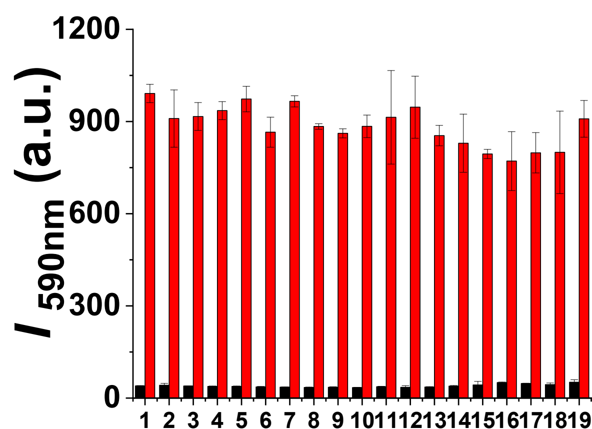

Fig. S4 Spectroscopic data measured for **RFT** (10 μM) in DMSO/PBS buffer (1 : 4, v/v, 10 mM, pH =7.4 , λ_{ex} = 525 nm) at RT. Using 100 μ After processing a single analyte, add 100 more μM hydrazine, monitor fluorescence intensity at 590nm. (1) Blank; (2) Cl^-; (3) Br^-; (4) I^-; (5) SO_4^{2-}; (6) Mg^{2+}; (7) Ca^{2+}; (8) NO^{2-}; (9) $S_2O_3^{2-}$; (10) NH_4^+; (11) CO_3^{2-}; (12) triethylamine; (13) thiourea; (14) diethylamine; (15) phenylamine; (16) HPO_4^{2-}; (17) $H_2PO_4^-$; (18) AcO^-; (19) normal hexyl amine; (20) N_2H_4.

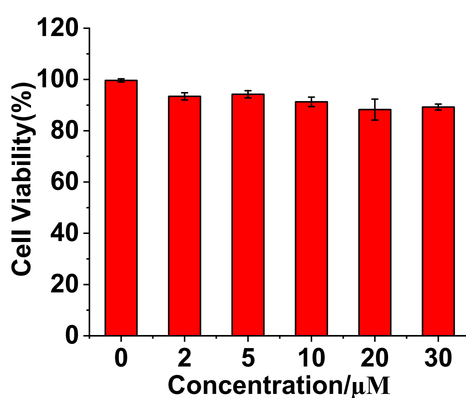

Fig. S5 Cytotoxicity assays of **RFT** in living HepG2 for 12 h.

3. NMR Data

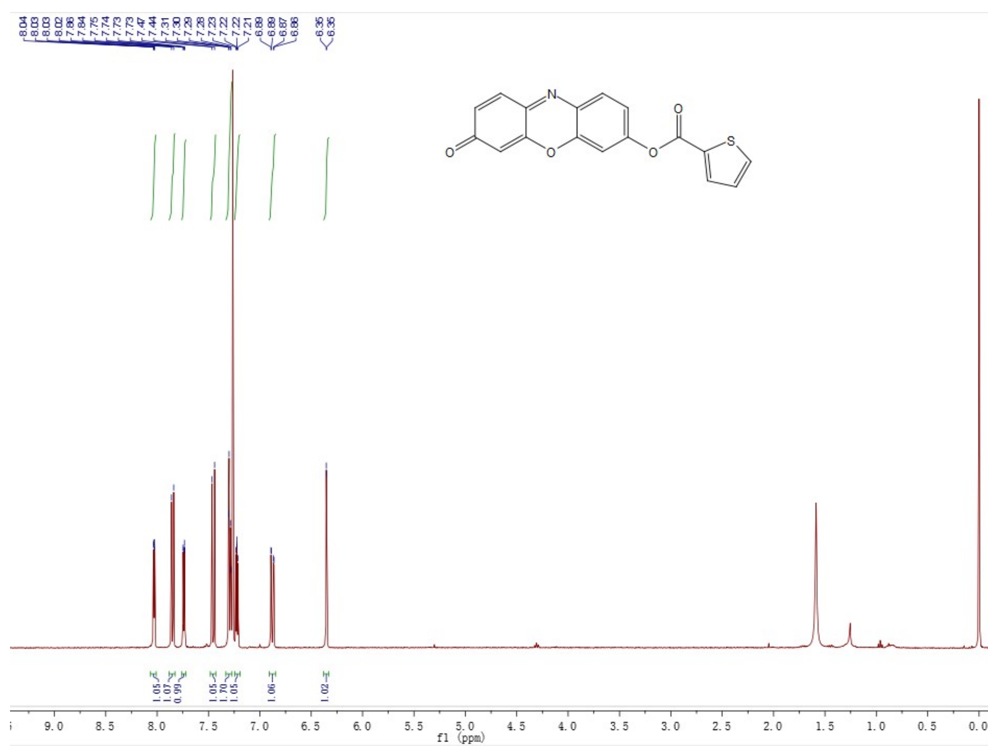

Fig. S6 ^{1}H NMR of **RFT** in $CDCl_3$.

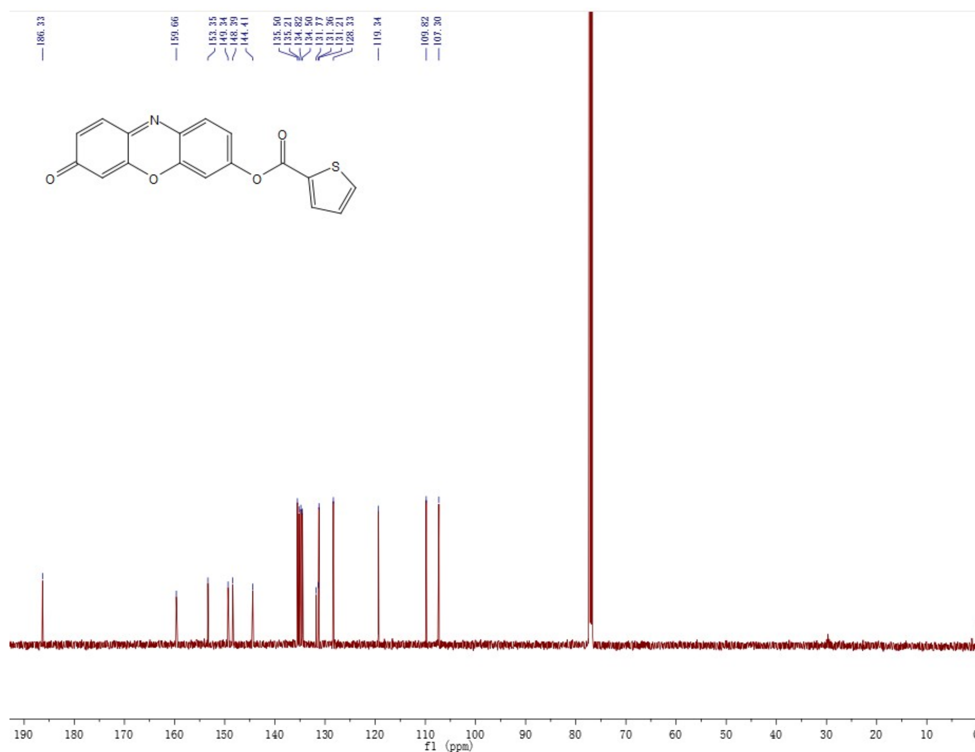

Fig. S7 ^{13}C NMR of **RFT** in $CDCl_3$.