Supporting information

ESIPT-based fluorescent probe for sensitive detection of hydrazine in aqueous solution

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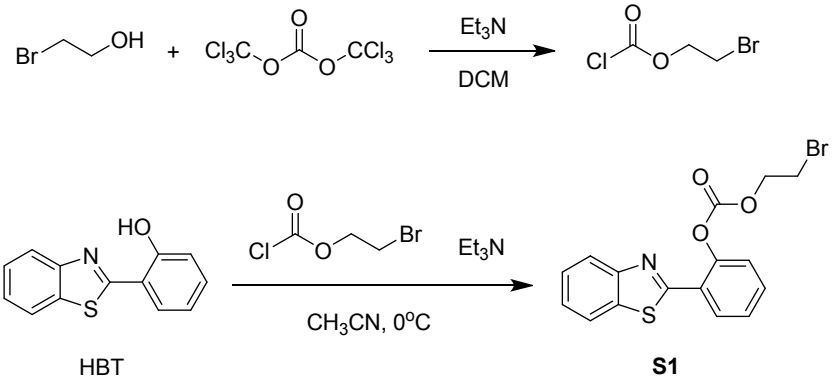
1. Instruments

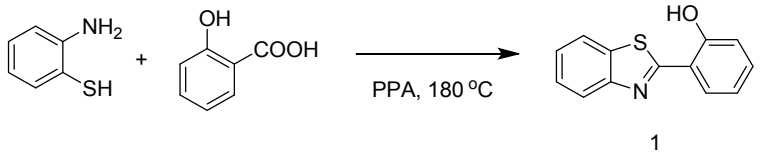
Instruments: ^{1}H-NMR and ^{13}C-NMR spectra were taken in $CDCl_3$ and DMSO-d_6 at 25 ^{o}C on a Bruker AV-400 spectrometer in NMR Facility of East China University of Science and Technology (ECUST). The chemical shifts were reported in ppm (TMS as internal standard). Mass spectra were performed in the Analysis Center of East China University of Science and Technology (ECUST).

2. Synthesis



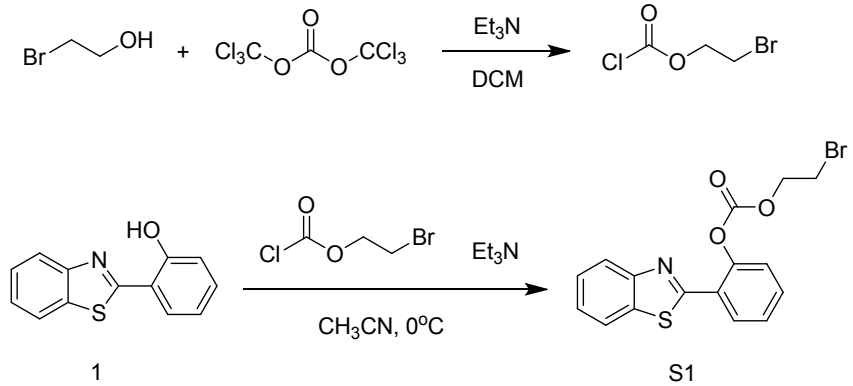
Scheme 1 The synthesis of the probe **S1**

Synthesis of compound 1



To a solution of salicylic acid (1.1 g，8 mmol) in 20 mL of polyphosphoric acid was added 2-aminobenzenethiol (1 g，8 mmol), and the mixture was heated to 180 ^{o}C for 3 hours. Then the mixture was poured into ice water. The precipitate was filtered, washed with water for 3 times, and the filter cake was dried off to give the title compound (1.55 g, 85%).

Synthesis of compound **S1**



To a solution of 2-bromoethanol (124 mg, 1 mmol) and triethylamine (101 mg, 1 mmol) in CH_2Cl_2 (10 mL) was added a solution of triphosgene (110 mg, 0.37 mmol) in CH_2Cl_2 (8 mL) at 0 °C under Ar. The reaction was kept at 0 °C for half an hour and then warmed to room temperature for an hour.

To a solution of compound 1 (227 mg, 1 mmol) in anhydrous CH_3CN (15 mL) was added the above acyl chloride solution at 0 °C. After the addition, triethylamine (101 mg, 1 mmol) was added and the reaction was kept at 25 °C for about 9 hours. When the reaction was over, the mixture was concentrated in vacuo. Then water and CH_2Cl_2 was added. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel flash chromatography to give the title compound **S1** (208 mg, 55%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (dd, J_1 = 1.2 Hz, J_2 = 6.4 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.54-7.49 (m, 2H), 7.44-7.39 (m, 2H), 7.34 (d, J = 8.0 Hz, 1H), 4.59 (t, J = 6.0 Hz, 2H), 3.61 (t, J = 6.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.40, 153.12, 152.74, 148.34, 135.41, 131.60, 130.42, 126.99, 126.39, 126.15, 125.49, 123.47, 123.18, 121.47, 68.03, 27.79. HRMS (ESI): Calcd for $C_{16}H_{13}BrNO_3S$ ($M+H^+$) 377.9800; Found, 377.9802.

3. Methods and data

1) HPLC data

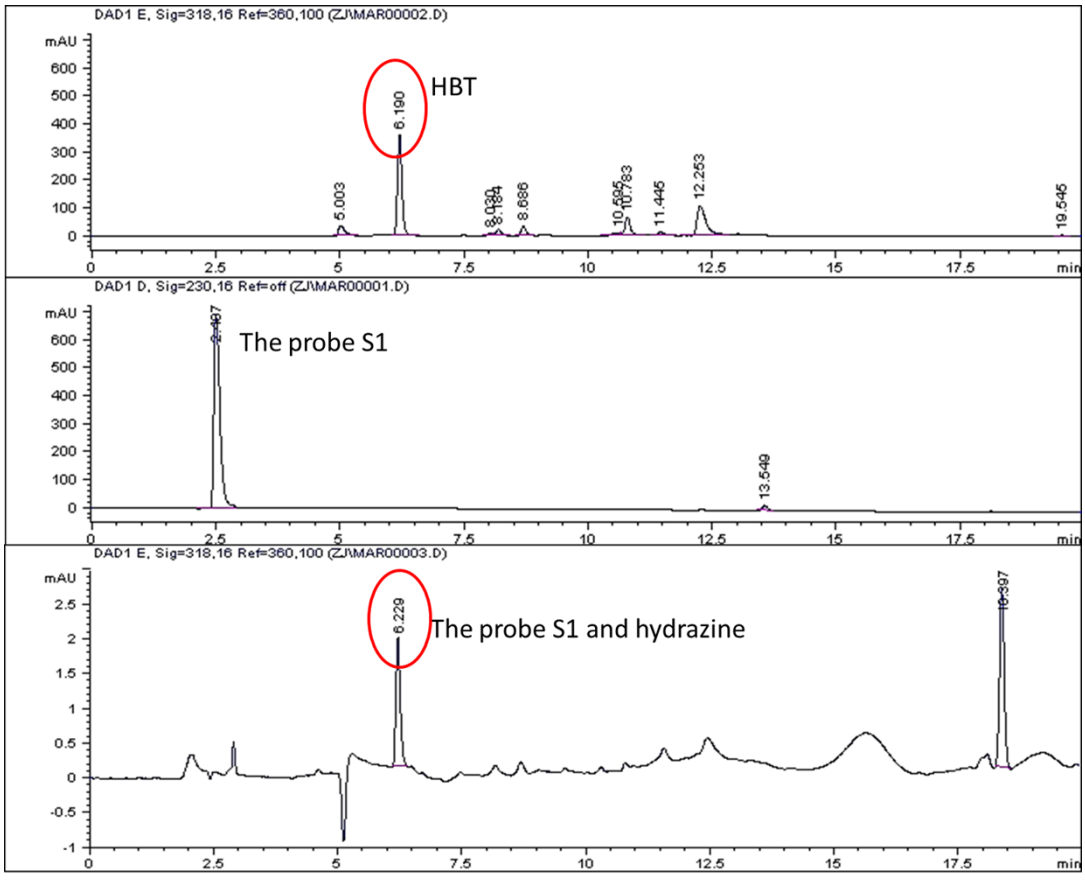


Fig 1 The HPLC data of the detection. In the third picture, the concentration of the probe was 20 μM and 20 μM hydrazine was added.

HPLC was performed on a ZoRBAX RX-C18 column (Analytical 4.6×250mm 5-Micron, Agilent) with a HP 1100 system. The HPLC solvents employed were acetonitrile and buffer (acetic acid and ammonium acetate pH 6.0). HPLC conditions were as follows: solvent A: solvent B = 10:90 (0 min)-100:0 (20 min), flow rate 1 mL/min, detection by UV (230 nm and 318 nm).

We have already performed the HPLC test. And we found that after adding hydrazine, the retention time of the product was as same as the time of the HBT. Therefore it indicated that the detection was based on ESIPT mechanism and substitution-cyclization-elimination cascade.

2) The detection limit

The fluorescence intensity of **S1** was measured by 10 times and the standard deviation was calculated. The fluorescence intensity at 465 nm was plotted as a concentration of hydrazine. By using detection limit 3σ/k, the detection limit was calculated as 0.147 μM. σ is the standard deviation of the fluorescence intensity of **S1**, k is the slope between the fluorescence intensity at 465 nm versus the hydrazine concentration.

3) The detection of hydrazine in different systems

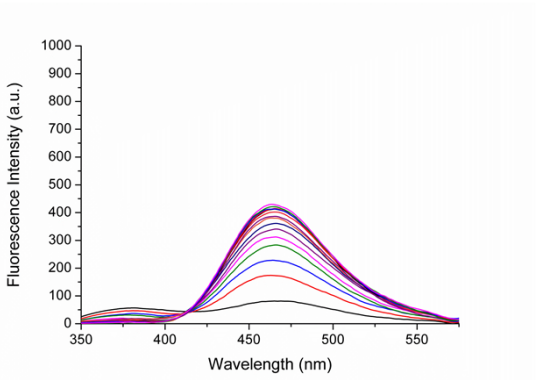


Fig 2 The fluorescence responses of the probe **S1** (5 μM) to hydrazine (20 μM) in 20 min. The detection system was PBS buffer (pH 7.4) with 1% DMSO as a cosolvent. Slit: 5 nm, 5 nm.

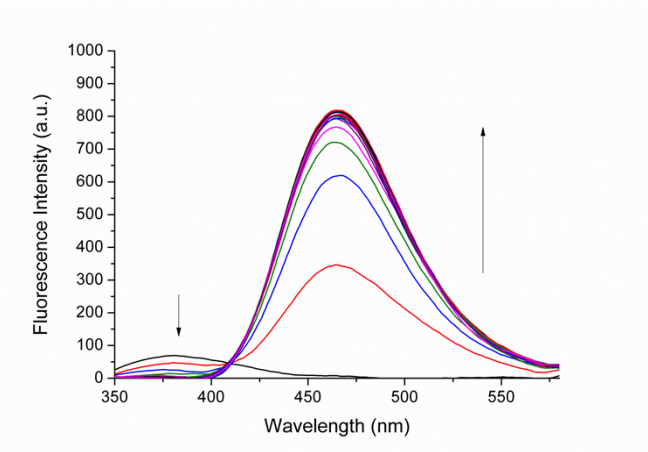


Fig 3 The fluorescence responses of the probe **S1** (5 μM) to hydrazine (20 μM) in 15 min. The detection system was PBS buffer (pH 7.4) with 1% ethanol as a cosolvent. Slit: 5 nm, 5 nm.

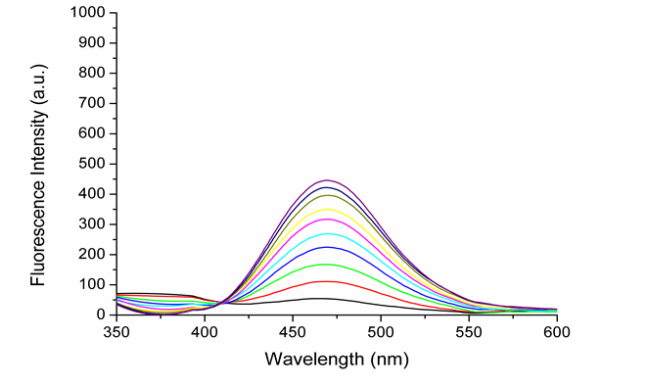


Fig 4 The fluorescence responses of the probe **S1** (5 μM) to hydrazine (20 μM) in 20 min. The detection system was PBS buffer (pH 7.4) with 1% CH_3CN as a cosolvent. Slit: 5 nm, 5 nm.

4) The selectivity of the probe (some common species in the human body)

We investigated the selectivity of the probe, choosing some common species in the human body. The results showed that the probe didn't respond to GSH, NaSH, Cys or Hcy. This indicated that the probe had the potential of detecting hydrazine in the biological system.

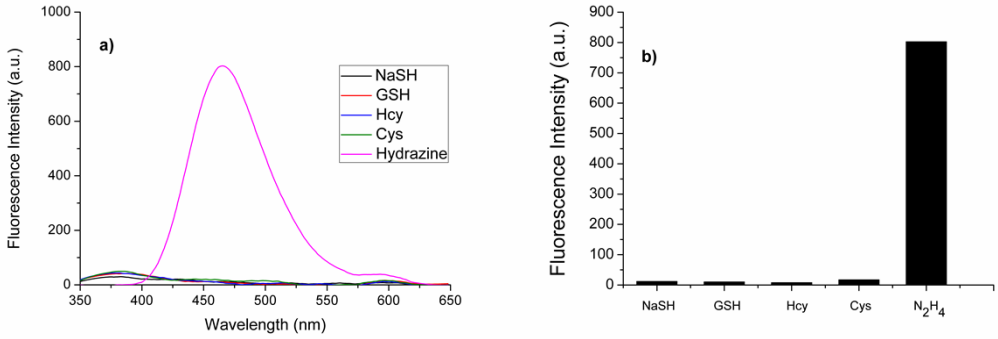
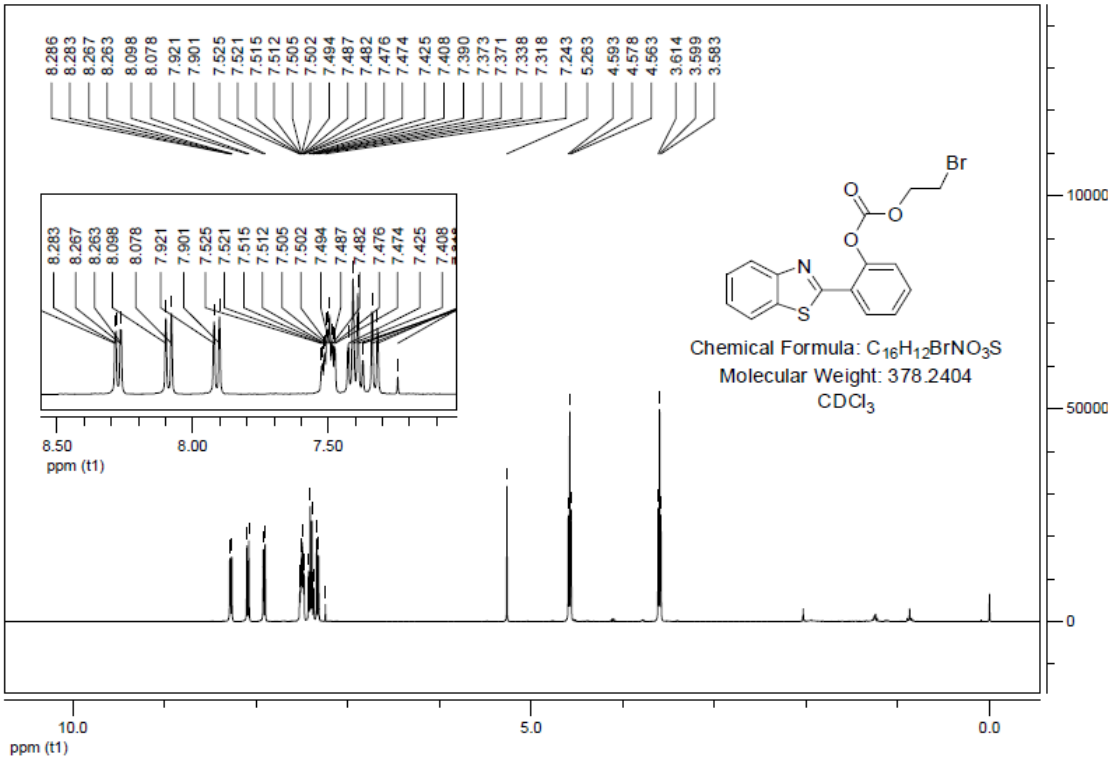
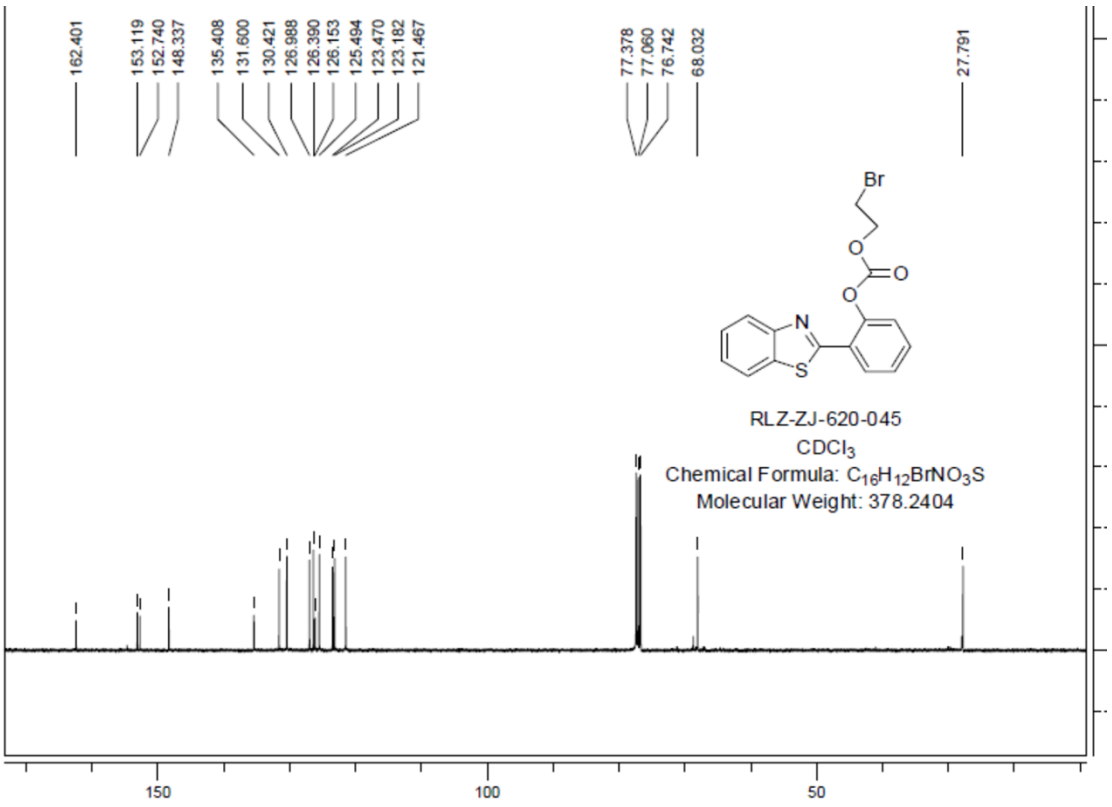


Fig.5 (a) Fluorescence responses of the probe **S1** (5 μM) to some common species in the human

body in the PBS buffer (with 1% ethanol) at room temperature. (b) The column chart of the selectivity of the probe. Excitation wavelength was 300 nm. Slit: 5 nm, 5 nm. The concentrations of NaSH, GSH, Hcy and Cys were 500 μM. The concentration of hydrazine was 20 μM.

4. NMR data





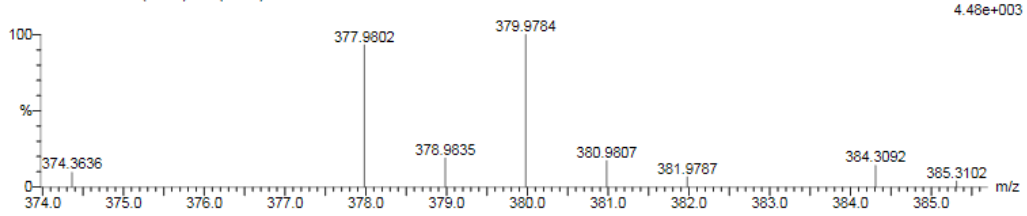
5. HRMS data

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
100 formula(e) evaluated with 6 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-21 H: 0-40 N: 0-1 O: 0-5 S: 0-1 Br: 0-1

WP-ZHU ECUST institute of Fine Chem 08-May-2014 19:27:15
ZWP-ZJ-620-045 78 (0.573) Cm (70:79) 1: TOF MS ES+ 4.48e+003



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
377.9802	377.9800	0.2	0.5	10.5	6.9	0.0	C16 H13 N O3 S Br