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A highly selective HBT-based "turn-on" fluorescent probe for hydrazine detection and its application

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ABSTRACT

As one of the important industrial chemicals, hydrazine (N_2H_4) can be inhaled through the skin, leading to many serious health issues. In this paper, we constructed a novel turn-on fluorescent probe HBTM for N_2H_4 detection based on ESIPT and ICT mechanism by incorporating the methyl dicyanvinyl group to 2-(2′-hydroxylphenyl) benzothiazole (HBT) fluorophore. The probe showed the following advantages: high sensitivity with detection limit of 2.9×10^{-7} M, high selectivity over other related interfering species, wide linear range of 0–140 μM and pH value adaptation. Moreover, the probe could detect N_2H_4 on paper strips and image N_2H_4 in living cells.

Introduction

Hydrazine (N_2H_4), as a typical reducing agent and foaming agent, is widely used in pharmaceutical, agricultural industries and preparing polymer foams.[1] In view of its high enthalpy of combustion, N_2H_4 is also used as a propellant in various rocket fuels.[2] However, it should be noted that N_2H_4 is highly poisonous to humans and animals.[3] Toxicological studies have shown that prolonged expose to high levels of N_2H_4 can irreversibly damage the liver, lungs, kidneys and central nervous system.[4] Additionally, N_2H_4 has been turned out to show potential carcinogenic and mutagenic effects by the United States Environmental Protection Agency (EPA). The threshold limit value of N_2H_4 is 0.312 μM.[5] Therefore, it is of profound significance to design highly sensitive and selective N_2H_4-specific sensors.

Several traditional methods have been employed for detection of N_2H_4 levels, but they cannot be applied to monitor N_2H_4 in living cells.[6] In consideration of high sensitivity, operability and real-time monitoring, fluorescent probes have been recognized as promising tools for detection of N_2H_4 in biological and practical samples. To date, a large number of fluorescent probes capable of monitoring N_2H_4 have been designed. The reactive sites of the reported N_2H_4-specific fluorescent probes mainly include 4-bromo butyrate,[7] trifluoroacetyl acetone,[8] acetyl,[9] 2-fluoro-5-nitro-benzoic ester,[10] aldehyde,[11] aliphatic[12] and malononitrile.[13] Most of the probes suffer from poor selectivity, high detection limit and narrow linear range. In this work, we first describe a new type of turn-on fluorescent probe HBTM for N_2H_4 detection by integrating excited state intramolecular proton transfer (ESIPT) and intramolecular charge transfer (ICT) characteristics (Scheme 1).[14] Upon treatment with N_2H_4, malononitrile group of probe HBTM is reduced to the hydrazone product, resulting in a fluorescence turn-on response at 510 nm by blocking ICT characteristics. Surprisingly, the probe displays a wide linear range of 0–140 μM, which is better than most of the reported N_2H_4 probes. Also, the probe can be applied to detect N_2H_4 over a wide pH range of 4.0–11.0 with high sensitivity. Moreover, the probe has been successfully used to test N_2H_4 levels on paper strips and image N_2H_4 in J774 cells.

Experimental

Chemicals and apparatus

All reagents and solvents were obtained from commercial sources and used without further purification. Double-distilled water was prepared through Milli-Q water purification system. The fluorescence spectra were performed using a Shimadzu RF-5301 spectrofluorimeter. UV–vis absorption spectra were obtained by a Shimadzu UV-2700 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were acquired on a BRUKER 400 spectrometer. All pH measurements were carried out on a PHS-3C acidometer. ESI-MS were measured with an Agilent 1260-6224 Liquid Chromatography Mass Spectrometer. Cells images were recorded on CLSM TCS SP5 (Leica, Germany).

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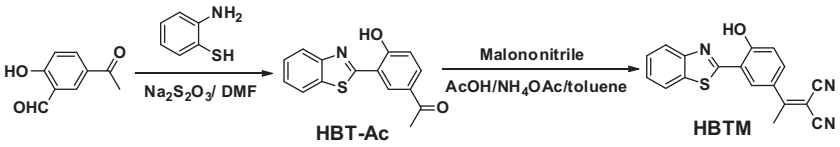

Scheme 1. Synthesis of the target fluorescent probe HBTM.

Synthesis of HBTM

In a 50 ml round-bottom flask, 5-acetyl-2-hydroxybenzaldehyde (97.0 mg, 0.59 mmol) and 2-aminothiophenol (73.6 mg, 0.59 mmol) was dissolved in DMF (5 ml), and then anhydrous sodium thiosulfate (111.6 mg, 7.08 mmol) was added. The mixture was stirred at 110 °C under nitrogen atmosphere. The progress of the reaction was monitored by TLC. After the reaction was completed, the organic mixture was poured into deionized water and extracted with ethyl acetate (30 ml × 3). Then the collected organic phase was dried with anhydrous sodium sulfate, filtered and concentrated. Purification by column chromatography (petroleum ether/ethyl acetate 8:1) gave desired product HBT-Ac as a white solid (128 mg, yield 81%). ^{1}H NMR(400 MHz, d_6-DMSO): δ 12.32 (s, 1H), 8.86 (s, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 8.01 (dd, J = 8.6, 2.2 Hz, 1H), 7.55 (t, J = 7.3 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 8.6 Hz, 1H), 2.60 (s, 3H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 196.1, 163.2, 159.9, 151.4, 134.9, 132.4, 129.3, 128.9, 126.5, 125.2, 122.4, 122.0, 118.8, 116.9, 26.5; LC-MS TOF: Calcd for $C_{15}H_{11}NO_2S$ $[M+H]^+$: 270.0510; Found: 270.0665.

To a solution of HBT-Ac (100 mg, 0.37 mmol), malonitrile (48.9 mg, 0.74 mmol) and toluene (5 ml), NH_4OA_C (71.4 mg, 0.92 mmol) dissolved in AcOH (0.14 ml, 1.5 mmol) was added. After the mixture was refluxed for 8 h, the organic mixture was poured into deionized water and extracted with ethyl acetate (30 ml × 3). Then the collected organic phase was dried with anhydrous sodium sulfate, filtered and concentrated. Purification by column chromatography (petroleum ether/ethyl acetate 8:1) gave desired product HBTM as a yellow solid (47 mg, yield 40%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.41 (s, 1H), 8.65 (d, J = 2.5 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 8.09 (d, J = 7.6 Hz, 1H), 7.79 (dd, J = 8.7, 2.5 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.47 (t, J = 7.6 Hz, 1H), 7.24 (d, J = 8.7 Hz, 1H), 2.67 (s, 3H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 175.5, 162.9, 159.3, 151.3, 134.9, 131.9, 128.8, 127.1, 126.5, 125.2, 122.4, 122.1, 119.1, 117.3, 113.9, 113.7, 81.4, 23.9; HRMS (ESI$^+$): Calcd for $C_{18}H_{11}N_3OS$ $[M+H]^+$: 318.0623; Found: 318.0691.

General procedure for the spectra measurement

The stock solution of probe (1 mM) was prepared in DMSO. Solutions of various analytes (GSH, Hcy, Cys, $H_2NCH_2CH_2NH_2$, Et_3N, Thiosemicarbazide, Benzylamine, $C_6H_5NH_2$, ClO^-, SO_3^{2-}, I^-, Br^-, SH^-, HPO_4^{2-}, Mg^{2+}, Zn^{2+}, K^+, Cr^{3+}, Al^{3+}, Fe^{3+}, Na^+) were prepared in double-distilled water. The resulting solution was kept at 25 °C and then the absorption and fluorescence spectra were performed.

Cell culture and imaging

J774 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (10%), penicillin (100 μg/ml) and streptomycin (100 μg/ml) at 37 °C in a CO_2 incubator. In the fluorescence imaging experiment, the cells were treated with HBTM (10 μM) for 30 min followed by treatment with N_2H_4 (100 μM) for 60 min at 37 °C. In the control experiment, J774 cells were incubated with HBTM (10 μM) for 30 min at 37 °C. Before cell fluorescence imaging experiments, the cells should be washed three times with PBS buffer.

Results and discussion

Spectral properties of probe HBTM

With the probe in hand, we first evaluated the time-dependent fluorescence changes of the probe (5 μM) in the presence of 150 μM N_2H_4 in DMSO/H_2O solution (8:2, v/v) at 25 °C. As shown in Fig. 1, after treatment with 150 μM N_2H_4, a gradual increase in the fluorescence intensity was observed, which reached steady state at approximately 55 min. The pseudo first-order reaction rate constants between probe HBTM and N_2H_4 was determined to be 0.0355 min^{-1} (Fig. S1).

Encouraged by the initial test results, we then investigated the absorption and emission spectra of HBTM in DMSO/H_2O solution (8:2, v/v) at 25 °C. As described in Fig. 2A, the probe showed two major absorption peaks at 458 nm and 382 nm, which was assigned to ketone and enol structure of HBTM. Upon the addition of N_2H_4 (150 μM) to the HBTM solution, the absorption at 458 nm and 382 nm gradually decreased, whereas the absorption band (300–340 nm) increased. Additionally, the isosbestic point at 340 nm indicated the reaction of HBTM with N_2H_4 was one-to-one conversion to form a new compound. Subsequently, fluorescence analysis experiment showed that the probe displayed weak fluorescence in the absence of N_2H_4. The fluorescence quantum yield ($Ø_{fl}$) of HBTM was 0.014 using fluorescein as a standard. As shown in Fig. 2B, upon addition of increasing concentrations of N_2H_4, the fluorescence intensity at 510 nm enhanced gradually and reached a plateau in the presence of 150 μM N_2H_4 ($Ø_{fl}$ = 0.071). Meanwhile, the fluorescence intensity at 510 nm exhibited an excellent linear relationship (R^2 = 0.991) with the

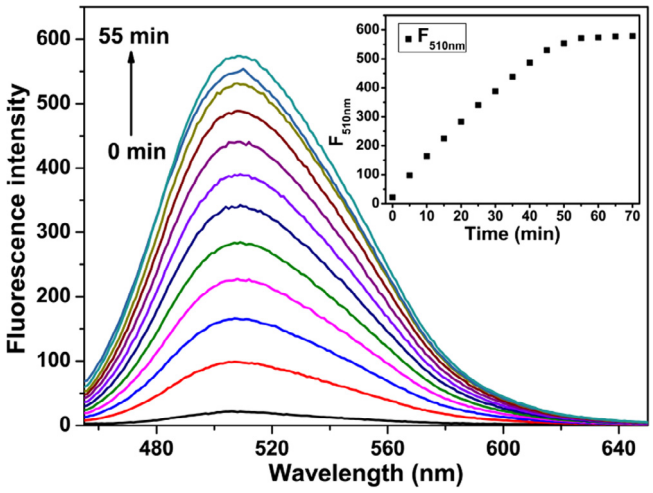

Fig. 1. Time-dependent fluorescence changes of probe HBTM (5 μM) in DMSO/H_2O (8:2, v/v) in the presence of 150 μM N_2H_4 at 25 °C. Inset: plot of fluorescence intensity at 510 nm versus time for the reaction of probe HBTM with N_2H_4. λ_{ex} = 440 nm. Slit width: d_{ex} = 3 nm, d_{em} = 5 nm.