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A simple and pH-independent and ultrasensitive fluorescent probe for the rapid detection of Hg^{2+}

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ABSTRACT

Development of fluorescent probes for Hg^{2+} has become a hot topic in modern chemical research due to its high toxicity. In this paper, we for the first time report the synthesis and application of a thioether spirocyclic rhodamine B derivative (**TR**) as an efficient fluorescent probe for Hg^{2+}. **TR** was synthesized using a simple procedure under mild condition. By employing a thioether spirocycle instead of classic spirolactam as recognition unit, our proposed probe **TR** is acidity-insensitive, and exhibits a pH-independent and ultrasensitive response to Hg^{2+}. The probe works well within a wide pH range from 3.5 to 11.5, and exhibits a 350-fold fluorescence enhancement upon 0.5 equiv of Hg^{2+} triggered, with a detection limit of 2.5 nM estimated for Hg^{2+}. In virtue of the strong thiophilic characteristic of Hg^{2+}, the response of the probe to Hg^{2+} is instantaneous and highly selective, which make it favorable for cellular Hg^{2+} imaging applications. It has been preliminarily used for highly sensitive monitoring of Hg^{2+} level in living cells with satisfying resolution, demonstrating its value of the practical applications in biological systems.

1. Introduction

It is well-known that mercury is one of the most toxic heavy metal elements, and a very small amount of mercury ions could cause serious damage to the central nervous and endocrine systems [1,2]. Therefore, efficient monitoring of trace amount of Hg^{2+} in biological samples with high spatial resolution is remarkably important for human health. Fluorescence method is highly sensitive, non sample-destructing or less cell-damaging, and can offer fast analysis with spatial resolution. These unique features make it favorable for both detection and imaging of Hg^{2+} in biological samples [3–11]. As a consequence, the design and synthesis of fluorescent probes, in particular turn-on type probes for Hg^{2+} [7–11], has become a hot topic in modern chemical research, since such probes are more suitable for bioimaging applications than those showing Hg^{2+}-induced fluorescence quenching responses.

Rhodamine dyes possess several excellent spectroscopic properties, such as large molar extinction coefficient and high fluorescence quantum yield, and have been widely applied to construct fluorescence turn-on probes for various analytes by employing different fluorescence signal transduction strategies [12–19]. Quite a few rhodamine-based probes have also been developed for fluorescence turn-on detection of Hg^{2+} in the past decade [20–31]. Some of them show high sensitivity towards Hg^{2+} with detection limit located at nM level [30,31], unfortunately, most of these probes are based on the Hg^{2+}-triggered ring-opening reaction of rhodamine spirolactam derivatives (see Fig. 1), which are acidity sensitive and result in pH-dependent response of the probes to Hg^{2+}, and are not convenient for detection of Hg^{2+} in practical diversified samples. It might also result in a poor affinity of the probes for Hg^{2+} under physiological neutral conditions. Few of probes which show pH-independent response to Hg^{2+} have also been reported [22,31], however, they suffered slight interference from other metal ions such as Ag^{+} and Zn^{2+}. Therefore, the design of pH-independent rhodamine probes with high sensitivity and selectivity for Hg^{2+} is desired if these probes are to be used in complex biological or environmental samples.

Since the ether bond is more stable in acidic condition than that of amide bond, and Hg^{2+} exhibits a strong thiophilic affinity, we envisioned that a more acidic stable and sensitive probe might be achieved if we optimized the molecular structure of the rhodamine probe by using a more simple thioether spirocycle instead of classic spirolactam as recognition unit. Herein we reported the design, synthesis and application of a novel thioether

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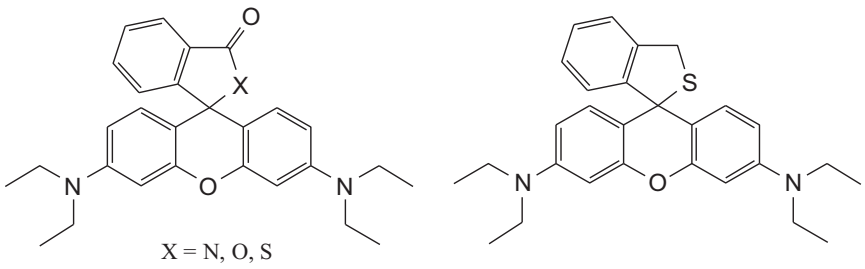

Spirolactam Rhodamine B Thioether spirocyclic Rhodamine B

Fig. 1. The structures of spirolactam rhodamine B and thioether spirocyclic rhodamine B.

spirocyclic rhodamine B derivative **TR** (see Fig. 1) as a fluorescent probe for Hg^{2+} with improved recognition performance. In our new designed probe molecule, a more simple and stable thioether spirocycle was chosen as the Hg^{2+} recognition module. It was synthesized using a simple procedure under mild condition. Such a structure-optimized molecular probe shows pH-insensitive, turn-on fluorescent response to Hg^{2+} in aqueous solution. The probe responses well to Hg^{2+} within a wide pH range from 3.5 to 11.5, exhibits a 350-fold fluorescence enhancement upon 0.5 equiv of Hg^{2+} triggered, and exhibits high sensitivity for Hg^{2+} with a response concentration range from 1.0×10^{-8} to 1.0×10^{-6} M, and a detection limit of 2.5 nM for Hg^{2+}. Owing to the strong thiophilic characteristic of Hg^{2+}, the probe also shows a high selectivity toward Hg^{2+} with a very fast response time. It has been applied for highly sensitive imaging of Hg^{2+} in living cells with satisfying results.

2. Experimental

2.1. Apparatus

Hitachi F-4500 fluorescence spectrometer was used for the determination of the fluorescence with both excitation and emission slits set at 5.0 nm. Shimadzu MultiSpec-1501 UV–visible spectrophotometer was used for the determination of UV–vis absorption spectra. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA-400 spectrometer operating at 400 MHz, 100 MHz respectively, with tetramethylsilane as an internal reference. The pH value of the solution was measured by the Mettler-Toledo Delta 320 pH meter.

2.2. Chemicals

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), PBr_3, 95% $LiAlH_4$, phenyl isothiocyanate and rhodamine B were all purchased from Shanghai Sinopharm Reagent Company. Stock solutions of Fe^{3+}, Al^{3+}, Hg^{2+}, Cu^{2+}, Mn^{2+}, K^{+}, and Ca^{2+} were prepared from their chloride salts; solutions of Zn^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Ag^{+}, and Mg^{2+} were prepared from their nitrate salts with distilled water. These solutions were then diluted with HEPES buffer solution (pH 7.4) for detection. Column chromatography was conducted over silica gel (100–200 mesh) and thin layer chromatography (TLC) was carried out using silica gel 60 F254, both of which were obtained from the Qingdao Ocean Chemicals (Qingdao, China). Water used in all experiments was double distilled and purified by a Milli-Q system (Millipore, USA).

2.3. Spectrophotometric Experiments

A 20 μM stock solution of Probe **TR** was prepared by dissolving an appropriate amount of **TR** in absolute ethanol, which was protected from light and kept at 4 °C for further use. $HgCl_2$ solution was diluted stepwise with HEPES buffer solution (pH 7.4) to give the standard solution of the Hg^{2+} (8×10^{-4} M). 100 μL of **TR** and 900 μL of Hg^{2+} standard solution were combined to afford 1 mL complex solution of Hg^{2+} and **TR**, which contained 2×10^{-6} M of probe **TR** and 5.0×10^{-6}–1.0×10^{-8} M of Hg^{2+}. Blank solution of **TR** was prepared under the same conditions without Hg^{2+}. For all measurements of fluorescence spectra, excitation was fixed at 520 nm, and emission spectra were recorded within the wavelength range of 530–650 nm. Complex systems were allowed to stand for 10 min to ensure complete formation of metal–ligand complex.

2.4. Cell culture and imaging experiments

HeLa living cells for experiment were offered by Biomedical Engineering Center of Hunan University. Initially, the cells were first washed with phosphate buffered saline (PBS), incubated for 30 min with 2×10^{-6} M probe **TR** (1% DMSO, HEPES, pH 7.4) at 37 °C, washed with PBS three times to wash away the free probe, incubated with 1×10^{-6} M of Hg^{2+} (HEPES, pH 7.4) at 37 °C for 30 min, and last washed with PBS three times. The HeLa living cells were then used for fluorescence imaging experiments with an Olympus FV500-IX70 confocal laser microscope.

2.5. Synthetic details

Compound **3**, **2**, **1** were synthesized according to the literature methods [32].

2.5.1. Synthesis of compound *4*

Phenyl isothiocyanate (270 mg, 2 mmol) in DMF was added dropwise into excessive ethylenediamine at room temperature. After stirring for 6 h, the solution was diluted with CH_2Cl_2 and washed with water (50 mL × 3), then dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a yellow oily crude product. At last, the crude product was recrystallized from acetonitrile to give a yellow solid (145 mg, yield: 50%). ESI-MS: $[M]^{+}=195.9$.

2.5.2. Synthesis of compound *3*

To a stirred solution of rhodamine B (5.0 g, 10.3 mmol) in absolute tetrahydrofuran (150 mL), 95% $LiAlH_4$ (0.8 g, 20.1 mmol) was added carefully under nitrogen, and the resulting mixture was then stirred for one day at room temperature. Water (50 mL) was carefully added to quench the reaction. The solution was extracted with CH_2Cl_2 and washed with water (100 mL × 3), brine, dried over anhydrous $MgSO_4$, filtered, concentrated to give crude product which was subjected to flash chromatography (silica gel, CH_2Cl_2/EtOH, 100:1, v/v) to yield pure product as a light pink foamy solid (2.2 g, yield: 44%). ESI-MS: $[M+H]^{+}=431.2$.