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A novel imidazo[1,5-a]pyridine-rhodamine FRET system as an efficient ratiometric fluorescent probe for Hg^{2+} in living cells

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ABSTRACT

An imidazo[1,5-a]pyridine-rhodamine-based ratiometric fluorescent probe was designed and successfully synthesized. The probe shows a large Stokes shift (204 nm), high sensitivity and high selectivity. The detection limit was calculated to be as low as 0.93 nM. The probe could quickly (5 min) detect Hg^{2+} over a wide pH range from 4 to 10. Furthermore, it could be used for imaging Hg^{2+} in living cells.

1. Introduction

Mercury ions, one of the most commonly found and most dangerous toxic elements in the environment, can cause serious damage to brain, heart, kidney and immune system [1–4]. Therefore, accurate, rapid and cheap detection of mercury ion in biological and environmental systems with high selectivity and sensitivity is highly demanded.

Rhodamine dyes are widely used as fluorescent probes because of their high fluorescence quantum yield and high absorption coefficient [5]. Spirolactam-type rhodamine derivatives are nonfluorescent and colorless, whereas spirolactam ring-opening gives rise to strong fluorescence emission and a pink color. Since Tae reported the first Hg^{2+} probe based on rhodamine derivative [6], a number of intensity-based Hg^{2+} probes were synthesized [3,5,7–14]. However, a primary limitation of these simple “turn off” or “turn on” probes is that variations in probe concentration, probe environment, or excitation intensity may influence the fluorescence intensity measurements. To reduce the influence of such factors, ratiometric fluorescent probes are designed [15–17].

Besides internal charge transfer (ICT) mechanism [18–21], Förster resonance energy transfer (FRET) has become one of the most extensively used [22–33]. Prominent among these is the rhodamine-based FRET system. Previously, we successfully synthesized imidazo[1,5-a]pyridines via a novel tandem reaction [34,35]. The compounds were easy to prepare with good yield and the max emission wavelength is about 460 nm which has good and efficient spectral overlap with the rhodamine acceptor absorption (Fig. S1). So, it is suitable to construct a new imidazo[1,5-a]pyridine-rhodamine based FRET platform to dedect ions or other species.

In order to further improve the biocompatibility, accuracy and detection efficiency of a probe for Hg^{2+}, we successfully synthesized a new FRET-based ratiometric probe **TMUHg-1** for identifying Hg^{2+}. The probe employed an imidazo[1,5-a]pyridine as a donor, rhodamine as an acceptor, and piperazine as a linker. As expected, **TMUHg-1** could quickly (5 min) detect Hg^{2+} with high sensitivity and selectivity in a wide pH range from 4 to 10. The detection limit of 0.93 nM was achieved. More importantly, **TMUHg-1** could be used for imaging Hg^{2+} in living cells. The photophysical properties of typical FRET-based Hg^{2+} probes are summarized in Table S1.

2. Experimental section

2.1. Materials and equipments

UV–vis spectra and fluorescence spectra were recorded on a U-3900 UV–vis spectrometer (Hitachi) and RF-5301PC luminescence

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spectrophotometer (Shimadazu) at room temperature, respectively. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance 400 (400 MHz) spectrometer ($CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard). Chromatographic separations were done by column chromatography using 200–300 mesh silicagel. All reagents and solvents were purchased from commercial sources and used without further purification. The solutions of metal ions were prepared from chlorizated salts which were dissolved in deionized water. Deionized water was used throughout the process of absorption and fluorescence determination. All samples were prepared at room temperature, and rested for 30 min before UV–vis and fluorescence determination.

2.2. Cell culture and imaging

Glioma cells were cultured in RPMI-1640 containing 10% fetal bovine serum at 37 °C in a 5% CO_2/95% air incubator. For living cells imaging experiments, the growth medium was removed and replaced with RPMI-1640 without FBS. The cells were treated and incubated with 2 μM of **TMUHg-1** at 37 °C under 5% CO_2 for 1 h. The cells were washed three times with PBS and then cell images were obtained via a confocal microscope from FV1000 (Olympus) at an excitation of 405 nm.

2.3. Synthesis

2.3.1. Synthesis of compound *1* and *2*

Compound **1** and **2** were synthesized according to the literature [36–39].

2.3.2. Synthesis of compound *3*

Compound **1** (253 mg, 1 mmol) was dissolved in CH_2Cl_2 (20 mL), and then EDC (288 mg, 1.5 mmol) and DMAP (30 mg, 0.2 mmol) were added. Subsequently, compound **2** (470 mg, 1 mmol) was added, and the reaction mixture was stirred at room temperature for 6 h. Then the solvent was removed under reduced pressure to afford crude compound **3**, which was purified on a silica gel column (C_2H_5OH: CH_2Cl_2 = 1: 200) to afford pure compound **3** (549 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (m, 1H), 7.72 (dd, J = 4.0 Hz, 8.0 Hz, 1H), 7.51–7.46 (m, 3H), 7.10–7.08 (m, 1H), 6.70–6.67 (m, 2H), 6.56 (m, 2H), 6.46 (d, J = 12.0 Hz, 1H), 6.42 (d, J = 4.0 Hz, 1H), 6.31 (dd, J = 2.8 Hz, 8.0 Hz, 1H), 3.82 (s, 4H), 3.34 (q, J = 8.0 Hz, 4H), 3.28 (s, 4H), 2.96 (d, J = 4.0 Hz, 4H), 1.82 (m, 2H), 1.62 (s, 2H), 1.44 (m, 2H), 1.17 (t, J = 8.0 Hz, 6H), 0.97 (t, J = 8.0 Hz, 3H). ^{13}C NMR ($CDCl_3$,100 MHz) δ 168.29, 166.22, 153.55, 153.52, 151.60, 151.21, 149.00, 138.51, 132.66, 129.88, 128.38, 128.21, 124.44, 123.77, 123.11, 120.79, 117.43, 112.41, 112.21, 110.18, 108.34, 104.20, 103.18, 97.92, 65.56, 48.81, 44.39, 29.03, 26.29, 22.46, 13.78, 12.59.

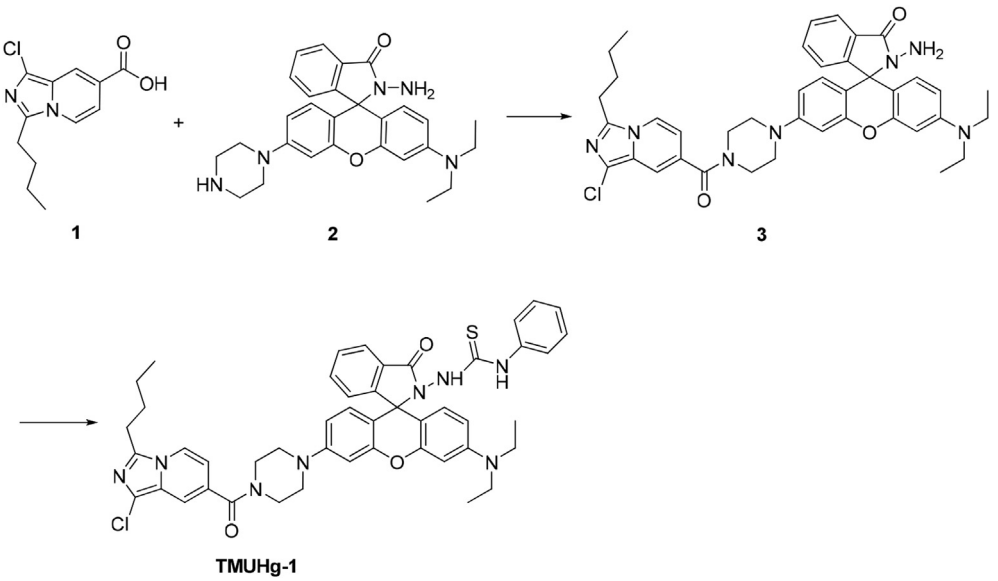
2.3.3. Synthesis of the probe *TMUHg-1*

Compound **3** (150 mg, 0.2 mmol) in DMF (1.5 mL) was added to a solution of phenyl isothiocyanate (0.1 mL, 0.65 mmol) in DMF (1.5 mL). The reaction mixture was stirred for 12 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was column chromatographed on silica gel (C_2H_5OH: CH_2Cl_2 = 1: 200) to give the 101 mg (60%) of **TMUHg-1**. mp: 100.5–101 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (d, J = 8.0 Hz,1H), 7.74–7.60 (m, 3H), 7.50 (d, J = 4.0 Hz, 2H), 7.28 (s, 1H), 7.20 (m, 2H), 7.11 (m, 1H), 7.06 (d, J = 8.0 Hz, 2H), 6.92 (s, 1H), 6.69 (m, 2H), 6.53 (s, 3H), 6.45 (s, 1H), 6.32 (d, J = 8.0 Hz, 1H), 3.81 (s, 4H), 3.35 (q, J = 8.0 Hz, 4H), 3.27 (s, 4H), 2.96 (t, J = 8.0 Hz, 2H), 1.82 (m, 2H), 1.65 (s, 2H), 1.43 (m, 2H), 2.57 (s, 3H), 1.16 (t, J = 8.0 Hz, 6H), 0.96 (t, J = 8.0 Hz, 3H). ^{13}C NMR ($CDCl_3$,100 MHz) δ 182.4, 168.3, 167.0, 154.0, 153.9, 152.1, 149.6, 138.5, 134.5, 129.4, 128.3, 127.8, 126.1, 124.8, 124.7, 124.4, 124.1, 123.7, 120.8, 117.5, 112.4, 103.4, 66.8, 48.4, 29.0, 26.3, 22.5, 13.8, 12.4. HRMS: 839.2864 ($[M+H]^+$); Calcd for $C_{47}H_{48}ClN_8O_3S$: 839.3259. *Anal.* Calcd for $C_{47}H_{47}ClN_8O_3S$: C, 67.17; H, 5.76; N, 13.33. Found: C, 67.29; H, 5.82; N, 13.35.

3. Results and discussion

3.1. Synthesis of the probe *TMUHg-1*

The synthetic route to access probe **TMUHg-1** is shown in Scheme 1. The reaction of Rhodamine B hydrazide **3** and phenyl isothiocyanate in DMF afforded probe **TMUHg-1** as pink powder in 60% yield. The probe **TMUHg-1** was characterized by ^{1}H NMR and ^{13}C NMR (see Supporting Information).



Scheme 1. Synthesis of chemosensor **TMUHg-1**.