Tetrahedron 73 (2017) 5189–5193

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Rhodamine based turn-on fluorescent sensor for Hg^{2+} and its application of microfluidic system and bioimaging

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ARTICLE INFO

Article history:
Received 28 April 2017
Received in revised form
4 July 2017
Accepted 8 July 2017
Available online 10 July 2017

Keywords:
Fluorescent sensor
Colorimetric sensor
Mercury ion
Rhodamine
Microfluidic system

ABSTRACT

In this paper, a new rhodamine derivative bearing thiazole and thiocarbonyl moieties **1** was synthesized and its sensing behaviors toward various metal ions were investigated by fluorescence spectroscopies and UV/Vis. The ring-opening process of spirolactam enables the selective fluorescence enhancement and colorimetric change for Hg^{2+} in CH_3CN-HEPES buffer (0.01 M, pH 7.4) (3:7, v/v). The selectivity and sensitivity of **1** towards Hg^{2+} lead to sensor **1** applied to microfluidic device and even used to functions Hg^{2+} sensing in Hela cells.

1. Introduction

Mercury, as a heavy-transition metal, has an extremely toxic impact on the human body or the environment.[1] Due to high bioaccumulation, easy absorptivity and persistence in the body, Hg^{2+} can cause human health problems such as central nervous system, serious cognitive and motion disorders, kidney disease, and brain damages.[2,3] Hence, in recent years, many efforts have been made to develop conventional detection methods for the quantitative analysis of Hg^{2+}.[4–6] Compared with many of the currents techniques for mercury detection such as atomic absorption spectroscopy; high performance liquid chromatography (HPLC) and electrochemical sensing, etc, the fluorescence detection method has been provided to be more convenient due to its simplicity, low cost, high sensitivity, high selectivity, suitable for on-site and real-time signaling.[7–15] Additionally, the fluorescence detection method can also be used to detect the concentration of the analytes which are in intact biosamples.[16–25] Recently, a number of the research groups have reported the application of fluorescence detection method on microfluidic system.[26–28] However, there is no much report for one fluorescent sensor applying on microfluidic system and in vivo imaging.

Rhodamine derivatives, as fluorescent or colorimetric chemosensors, have been actively utilized for detecting various metal ions such as Zn^{2+}, Fe^{3+}, Pb^{2+}, Cu^{2+}, Cr^{3+} and Hg^{2+}.[29,30] Most of rhodamine derivatives are colorless and non-fluorescent, whereas a pink color and a strong fluorescence emission is presented after ring-opening of the corresponding spirolactam produces. Strong dependence on these dramatic changes, coupled with relatively long emission wavelength which showed great advantages in fluorescence detection, contributes to the successful application of rhodamine derivatives as ion selective chemosensors.[31–41]

Herein, we design and synthesize a selective fluorescent and colorimetric chemosensor base on rhodamine derivative bearing thiolcarbonyl and thiazole groups. This novel fluorescent chemosensor can be used to detect mercury ion among many kinds other metal ions. In addition, the new chemosensor show a high sensitive of mercury ion so that can be successfully applied on microfluidic systems for detection of Hg^{2+}. The study of biological application using Hela cell was successfully demonstrated that this relatively low toxicity system can be employed for the using in cellular imaging to monitor the concentrations of Hg^{2+}.

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http://dx.doi.org/10.1016/j.tet.2017.07.012
0040-4020/

2. Experimental section

2.1. Synthesis

2.1.1. Materials and methods

Unless specially noted, all reagents and solvents were purchased from commercial suppliers and used without any purification. Deionized water was used throughout all experiments. Column chromatography was performed on silica gel. ^{1}HNMR and ^{13}C NMR spectra were collected on Bruker 2000. Mass spectra were obtained from Q-Tof mass spectrometer (Agilent 6530). Fluorescent spectra were measured on RF-5301/PC spectro-fluorophotometer. Absorption spectra were recorded on α-1860A UV–Vis spectrometer. MTT assay were carried out by Multiskan Go (51119200-VAN). The Microfluidic experiment using a fluorescent microscope (OlympusIX73). The width of the chip pipe is 500 μm. The cell imaging experiment were carried out using Confocal Scanning Microscopy (Leica, TCS sp5 II).

2.1.2. Synthesis of chemosensor **2**

A few drops of phosphorus oxychloride was added to a stirred mixture of rhodamine B (1.0 g, 2.3 mmol) and 2-aminobenzothiazole (0.27 g, 2.7 mmol) in 30 mL of anhydrous acetonitrile at room temperature, and heated to reflux overnight. After cooled to room temperature, the resulting solution was poured into 100 mL of cold water, and extracted with dichloromethane. The organic layer was then washed with aqueous NaOH solution and dried over anhydrous Na_2SO_4. Then the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography with CH_2Cl_2–methanol (50:1, v/v) as eluent to give **2** (0.5 g, 42%) as light yellow solid, Mp = 205 °C, ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 8.04 (d, J = 7.76 Hz, 1H), 7.60–7.52 (m, 3H), 7.10–7.19 (m, 2H), 6.76 (d, J = 3.16 Hz, 1H), 6.56–6.46 (m, 3H), 6.34 (d, J = 8.68 Hz, 1H), 6.27 (s, 1H), 3.28 (d, J = 7.2 Hz, 8H), 1.09 (t, J = 12.64 Hz, 12H); ^{13}CNMR (75 MHz,$CDCl_3$) δ165.5, 152.8, 147.7, 145.9, 137.5, 134.3, 133.0, 126.8, 123.6, 122.2, 111.4, 106.2, 105.4, 103.9, 96.5, 75.9, 43.2, 28.6, 11.6; ESI-MS m/z = 525.2325 (M+H) +, calcd for $C_{31}H_{32}N_4O_2S$ = 524.2225.

2.1.3. Synthesis of **1**

Compound **2** (0.11 g, 2 mmol) and Lawesson's Reagent (0.08 g, 2 mmol) were dissolved in dry toluene, and the reaction mixture was refluxed for 6 h under N_2 atmosphere, after removal of toluene, the residue was purified by flash chromatography with CH_2Cl_2–hexane = (20:1, v/v) as eluent to obtain compound 1 (0.07 g, 63%) as a yellow solid, Mp = 225 °C, ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.14 (ppm) (d, J = 5.8 Hz, 1H), 7.45 (s, 2H), 7.27–7.06 (m, 3H), 6.75 (s, 1H), 6.26–6.35 (m, 3H), 6.07 (s, 2H), 3.23 (d, J = 5.64 Hz, 8H), 1.07 (t, J = 13.6 Hz, 12H); ^{13}CNMR (75 MHz, $CDCl_3$) δ188.8, 152.7, 137.0, 136.1, 132.3, 128.7, 127.6, 126.9, 124.4, 124.1, 122.9, 111.6, 106.3, 96.6, 75.9, 43.3, 28.6, 11.5; ESI-MS m/z = 541.2074 $(M+H)^+$, calcd for $C_{31}H_{32}N_4OS_2$ = 540.2018.

2.2. Preparation of metal ion solutions for fluorescent study

The probe **1** was dissolved in CH_3CN (1 mM) and maintained in room temperature. Stock solutions (0.01 M) of the perchlorate salts of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Fe^{2+}, Sr^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} ions in distilled water were prepared. Test solutions were prepared by placing 30 mL of the chemosensor stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 3 mL with 0.01 M HEPES (pH 7.4) and CH_3CN.

2.3. Fluorescence detection of Hg^{2+} ions in microfluidic device

The Microfluidic experiment using a fluorescent laser scanning microscope (OlympusIX73), The dimension of the microfluidic channel was 500 μM wide and 2000 μm long. For detection of Hg^{2+}, 0.25 mM chemosensor **1** was prepared in distilled water from stock solution (50 μM) in CH_3CN and different concentrations of $Hg(ClO_4)_2$ solutions were prepared in distilled water as well. Then, 5 mL of 50 μM each chemodosimeter were mixed with 5 mL of various concentrations (10 nM-8μM) of $Hg(ClO_4)_2$ in a 1.5 mL polystyrene test tube and the mixture was manually introduced into the microchannel. Microscopic observation of fluorescence in the microchannel was done using a confocal laser scanning microscope with a 560 nm excitation and 570–640 nm emission filters at 200 magnifications. The fluorescence images were captured at least three times and their mean fluorescence intensities were measured by Image-J program (NIH, Bethesda, MD) and the data was evaluated by Origin Pro7.5 (Origin Lab, Northampton, MA).

2.4. Imaging of Hela cell previously inducted by $Hg(ClO_4)_2$

HeLa cells, purchased from Nanjing Cobioer Biosciences Company, were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (V/V) Fetal Bovine Serum (FBS, Gibco), 100U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO_2 at humidified incubator. Cells were transferred to culture dishes and incubated for 20 h. Two groups were studied as follows: (I) HeLa cells were incubated with probe **1** (5 μM) for 30min (II) HeLa cells were pretreated with probe **1** (5 μM) for 30 min and then exposed to Hg^{2+} (8 μM) for another 30min. Cell imaging was carried out after washing the cells in PBS (pH7.4). Cells were imaged by confocal scanning microscopy. The titration experiment of fluorescence were carried out by mixing hydrogel beads with various concentrations of Hg^{2+}, and the fluorescence intensity values were obtained by Imaging Software. The detection limit was calculated with the equation:

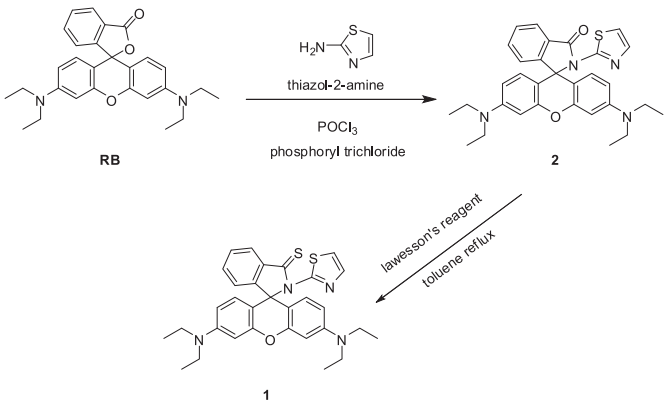
$$\text{Detection limit} = 3\sigma_{bi}/m$$

where σ_{bi} is the standard deviation of blank measurements, and m is the slope between fluorescent intensity and sample concentration.

3. Results and discussion

3.1. Synthesis

Probe **1** was synthesized in two steps (Scheme 1). For the synthesis of probe **1**, intermediate product **2** was synthesized in 42% yield from rhodamine B and thiazol-2-amine in anhydrous



Scheme 1. Synthesis of probe **1**.