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Highly selective and sensitive fluorescent probe for mercury ions based on a novel rhodol-coumarin hybrid dye

Kun Huang [a], Xiaojie Jiao [b], Chang Liu [a, b], Qing Wang [a], Xiaoying Qiu [b], Dasheng Zheng [b], Song He [b], Liancheng Zhao [a, b], Xianshun Zeng [a, b, *]

[a] School of Materials Science and Engineering, Harbin Institute of Technology, Harbin, 150001, China
[b] Tianjin Key Laboratory for Photoelectric Materials and Devices, School of Materials Science & Engineering, Tianjin University of Technology, Tianjin, 300384, China

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ABSTRACT

A fluorescent probe **RCHg** based on a novel rectilinear π-extended rhodol-coumarin hybrid dye has been designed and synthesized for the detection of Hg^{2+} ions. Via a specific Hg^{2+}-promoted ring-opening and desulfurization cascade reaction, **RCHg** shows high selectivity and sensitivity for Hg^{2+} detection and with a detection limit down to the nanomolar range. At the same time, the probe **RCHg** is living cell membrane permeable and should be suitable for practical application in vitro.

1. Introduction

Since a number of metal ions can have extremely harmful effects on humans and the environment, the development of highly selective and sensitive probes for environmentally and biologically relevant metal ions is a continued attractive research theme with prime importance [1]. Mercury species are a particular concern in many aspects due to their high toxicity, which are considered as ubiquitous toxic pollutants because they can be converted to highly toxic organomercury compounds, such as methylmercury, by bacteria in the environment, which are subsequently bioaccumulated through the food chain [2]. Aberrant accumulation of mercury in the human body can elicit various diseases including serious cognitive and motion disorders, prenatal brain damage, etc. [2a,3], via damnification of central nervous system, mitosis, and the endocrine system [4]. As a result, simple, inexpensive and reliable detection methods for mercury detection with high selectivity and sensitivity are urgently required.

By taking full use of their advantages in sensitivity, simplicity and real-time analysis, small-molecule fluorescent probes afford a promising approach to tracing metal ions, organic molecules, and biological analytes [5]. In the past decade, a number of fluorescent probes based on various fluorophores such as coumarin, pyrene, anthracene, benzoxadiazole, phenothiazine, etc. [6], have been developed for mercury detection. However, many of them suffered from some limitations such as low quantum yield, short emission wavelength, fluorescence quenching, lack selectivity toward Hg^{2+} over other metal cations, poor water solubility, being unfavorable for the applications of biological systems. As a result, it is highly desired and challenging to create novel probes that meet the criteria of appropriate selectivity and optical sensitivity for detection of Hg^{2+} ions in biological samples.

In 2005, Tae and coworkers [7] demonstrated that rhodamine-spirolactam-based thiosemicarbazide derivative exhibited high selectivity and sensitivity for Hg^{2+} ions detection and imaging via a specific Hg^{2+}-promoted spiroring-opening process and desulfurization cascade reaction. Since then, a number of thiosemicarbazide derivatives of rhodamines have been designed and synthesized for sensing of Hg^{2+} ions due to their excellent photophysical properties [8]. These probes possess a special nonfluorescent circular spirolactam structure, which block the fluorescent signal being realized. Upon the specific spiroring-opening process and

* Corresponding author. School of Materials Science and Engineering, Harbin Institute of Technology, Harbin, 150001, China.
E-mail address: xshzeng@tjut.edu.cn (X. Zeng).

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desulfurization cascade reaction with Hg^{2+} ions, the nonfluorescent spirocyclic forms of the probes were converted irreversibly to the ring-opened fluorescent xanthylium species, resulting in the highly sensitive "turn-on" detections of Hg^{2+} ions. Meanwhile, compared with coumarin, pyrene, anthracene, etc., rhodamine exhibits a red-shifted emission wavelength value (over 550 nm), which establishes the foundation to serve as fluorophores for analyte to avoid the influence of the background fluorescence. However, many of these Hg^{2+} probes suffer from the high detection limit and poor membrane permeability for application in biological systems.

Herein, we design and synthesize a new fluorescent probe **RCHg** for highly sensitive "turn-on" fluorescent detection of Hg^{2+} ions in aqueous solution (Scheme 1). To synthesize the probe **RCHg**, we firstly prepared a novel rectilinear π-extended hybrid rhodol-coumarin dye **RC**. Because **RC** inherits a facile derivatizable spirolactone structure from rhodamine dyes, it provides a versatile platform for the design of various probes by introducing an analyte-responsive elements on the spirolactone structure. Then, the probe **RCHg** was synthesized from **RC** by introducing a hydrazide moiety, followed by the reaction with methyl 2-isothiocyanatobenzoate. The probe **RCHg** functioned as a highly Hg^{2+}-selective fluorescent probe with a highly sensitive response (5.5 nM). Meanwhile, the probe **RCHg** could be used as a cell membrane permeable probe for Hg^{2+} imaging in biological systems.

2. Experimental

2.1. Materials and equipments

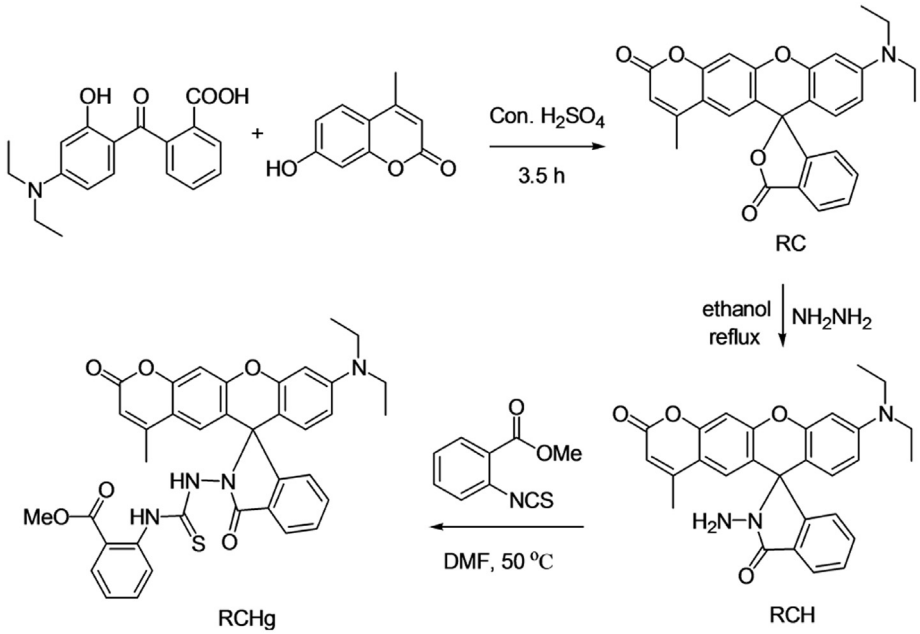
All chemicals were purchased from Sigma-Aldrich Chemical Company or TCI. Solvents were commercial products and were used without further purification unless otherwise mentioned. NMR spectra were recorded on a Bruker spectrometer at 400 (^{1}H NMR) MHz and 100 (^{13}C NMR) MHz. Chemical shifts (d values) were reported in ppm down field from internal Me_4Si (^{1}H and ^{13}C NMR). High resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. UV absorption spectra were recorded on a UV-2550 UV/Vis spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed using an F-4600 fluorescence spectrophotometer (Hitachi, Japan) and a quartz cell 1 cm. Melting points were recorded on a Boethius Block apparatus and pH measurements were carried out on a Mettler Toledo MP 220 pH meter. Cells were imaged on a confocal microscope (Olympus FV1000-IX81).

2.2. Preparation of stock solutions

Stock solutions (10 mM) of the nitrate salts of Ag^+, Al^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, NH_4^+, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} ions were prepared in distilled water. Stock solutions of **RC** (5 mM), **RCH** (5 mM), and **RCHg** (5 mM) were prepared in DMF. Test solutions were prepared by placing appropriate volume of the stock solution into a 5 mL vial, adding an appropriate aliquot of each metal stock, and diluting the solution to 3 mL with CH_3CN–H_2O (1/1, v/v). Unless otherwise stated, all data were collected 10 min after the addition of guest ions.

2.3. Synthesis of *RC*

7-Hydroxyl-4-methylcoumarin (1.85 g, 10.5 mmol) and 2-(4-diethylamino-2-hydroxy)benzoylbenzoic acid (3.13 g, 10 mmol) were heated to 90 °C in 5 mL concentrated H_2SO_4 for 3.5 h. After cooling to room temperature, the mixture was slowly poured into 100 g ice and neutralized by dicarbonate. The aqueous layer was extracted into dichloromethane (3 × 50 mL). The combined organic layer was washed with saturated salt solutions, dried over anhydrous magnesium sulfate and then filtered. The solvent was removed under reduced pressure and the final product **RC** was obtained as a light pink powder in 65% yield by column chromatography on silica gel (CH_2Cl_2/PE = 1/1); Mp: 193–195 °C. HRMS (ESI): *m/z* $[M+H]^+$ = 454.1658; Calcd for $[M+H]^+$: 454.1654; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.05 (d, *J* = 7.0 Hz, 1H), 7.71–7.60 (m, 2H), 7.21–7.18 (m, 2H), 6.96 (s, 1H), 6.58 (d, *J* = 8.8 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.39 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.14 (d,



Scheme 1. Synthesis of **RC**, **RCH** and **RCHg**.