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Efficient rhodamine-thiosemicarbazide-based colorimetric/fluorescent 'turn-on' chemodosimeters for the detection of Hg^{2+} in aqueous samples

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ABSTRACT

This paper describes the optimized design, synthesis and application of three novel thiosemicarbazide-appended rhodamine spirolactam derivatives as 'turn-on' colorimetric and fluorescent chemodosimeters (**1**–**3**) for the detection of Hg^{2+} in aqueous samples with a broad pH range (5.0–9.4). The 'turn-on' change in the color and fluorescence signals of **1**–**3** is based on a Hg^{2+}-triggered reaction that converts the thiosemicarbazide form to the 1,3,4-oxadiazole form. The results of ^{13}C NMR studies indicated that the basicity of the 'C=S' can be varied from δ 183.05 (for nitro) to 181.3 ppm (for methyl) by changing the R group in the chemodosimeter. Electronic and spectrofluorimetric studies indicated that the order of the binding constants of these chemodosimeters with Hg^{2+} ions is **1** > **2** > **3**, which may be dependent on the substituent. Chemodosimeters **1**–**3** exhibit a stable response to Hg^{2+} from 2.5×10^{-7} to 2.0×10^{-5} M with a detection limit of 10^{-8} M. The response of these chemodosimeters to Hg^{2+} is highly selective and pH insensitive, and the response time is rapid. All of these unique features make these sensors particularly favorable for Hg^{2+} test strip applications. These sensors have been preliminarily used for the highly sensitive monitoring of Hg^{2+} levels in aqueous media with satisfactory resolution.

1. Introduction

Rhodamine derivatives are excellent chromophores/fluorophores and have attracted considerable interest due to their very good photophysical properties [1–3], such as long absorption and emission wavelengths, large extinction coefficients and high fluorescence quantum yields. Rhodamine derivatives with spirolactam structures are non-fluorescent; however, opening of the spirolactam ring results in a strong fluorescence emission [4]. The rhodamine framework is particularly suited for constructing OFF–ON fluorescent or colorimetric chemosensors [4,5] due to its special structural property. Several rhodamine-modified chemosensors have recently been developed for the detection of heavy and transition metal ions because of the widespread use of these metal ions and because of their harmful impacts on the environment and human health [6,7]. Among these cations, considerable attention has been devoted to the development of new Hg^{2+} chemosensors that are easy to handle and that possess high sensitivity and selectivity because of the high toxicity of Hg^{2+}. According to the list from the Agency for Toxic Substances and Disease Registry (ATSDR), Hg^{2+} is the third most commonly occurring and the second most toxic heavy metal [8,9]. Mercury contamination can occur through a variety of natural and anthropogenic sources, such as oceanic and volcanic emissions, gold mining and fossil fuel combustion. The extreme toxicity of mercury derivatives results from the high affinity of mercury for thiol groups in proteins and enzymes, which leads to the dysfunction of cells and consequently results in health problems, such as prenatal brain damage, serious cognitive issues, motion disorders and Minamata disease [10–12]. Therefore, the health concerns related to mercury exposure have motivated the search for selective and efficient methods for monitoring mercury in biological and environmental samples. Several analytical techniques, such as spectrophotometry [13], neutron activation analysis [14], anodic stripping voltammetry [15], X-ray fluorescence spectrometry [16], inductively coupled plasma mass spectrometry [17], electrothermal atomic absorption spectrometry [18] atomic fluorescence spectrometry [19] and cold vapor atomic absorption spectrometry [20], are available for the detection of mercury. However, these methods require expensive

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equipment and involve time-consuming and laborious procedures that can only be conducted by trained professionals. Because of its operational simplicity, low cost, real-time monitoring and high selectivity, colorimetric/fluorescent detection has become a promising strategy for the detection of Hg^{2+} [21–23]. Over the past few decades, considerable efforts have been devoted to the development of 'turn-on' colorimetric and fluorescent sensors, which are more suitable for test paper and bioimaging applications than those that exhibit Hg^{2+}-induced fluorescence quenching responses.

A survey of the literature revealed that xanthone-based derivatives exhibit a remarkable ring-opening reaction and that these derivatives are an ideal platform for constructing 'turn-on' chemosensor probes. Many of these probes have been developed for various ions over the past decade [4,24–27], but few probes have been constructed for Hg^{2+} [28,29]. These probes primarily follow two types of approaches for sensing Hg^{2+}: (i) simple reversible chemosensors, which involve achieving equilibrium between the spirolactam form (nonfluorescent) and the ring-opened amide form (fluorescent) due to Hg^{2+} coordination [30–32], and (ii) chemodosimeters, which are based on irreversible mercury-promoted desulfurization reactions, including hydrolysis, cyclization and elimination reactions, due to the strong thiophilic character of Hg^{2+} ions [33–37]. To date, various researchers have developed chemosensors (type i) that contain urea [38], naphthalimide [39], terphenyl [40], coumarin [41] and calix[4]arene [32] as binding functionalities due to their nucleophilic properties. However, many of these sensors are pH sensitive, which might result in poor affinity for Hg^{2+} under physiological conditions. In many cases, these reversible probes act as a dual sensor, which limits the selectivity to Hg^{2+}. For example, Das et al. reported a rhodamine-based chemosensor for the detection of Hg^{2+} and Cu^{2+} in a water–methanol (1:1, v/v) solution at pH 7.0 [42]. Tang and co-workers developed a novel rhodamine B derivative that enables the detection of both Cu^{2+} and Hg^{2+} through colorimetric and fluorescence methods, respectively [43]. However, irreversible chemodosimeters (type ii) have improved selectivity and sensitivity over other sensing strategies because of the use of a specific chemical reaction between the guest and host species [44], and these sensors might avoid the biological matrix effects for potential application in real-world samples using the irreversible desulfurization reaction. Very few fluorescent chemodosimeter molecules have been developed for Hg^{2+} using the irreversible Hg^{2+}-triggered reaction of thiosemicarbazides to form 1,3,4-oxadiazoles, which would serve as the foundation for a novel chemodosimeter for Hg^{2+}. Tae et al. [45,46] investigated the Hg^{2+} ion-sensing property of rhodamine 6G-based thiosemicarbazide systems. The results indicated that the sensing property of the chemosensor is primarily dependent on the substituent on the aniline ring. Shang et al. synthesized and reported a FRET-based chemosensor for Hg^{2+} ions using the chemodosimeter approach [47]. Similarly, Wei Guo and coworkers [48] developed a naphthalimide-appended rhodamine derivative as an irreversible chemosensor for Hg^{2+} based on the FRET process. Among them, few authors were developed sulfur functionalized silver nanoparticle to selectively sense Hg^{2+} ion [49,50]. All of the Hg^{2+}-triggered cyclization reactions occur via the formation of Hg–S complexes and desulfurization products as sequential intermediates. The formation of these complexes is the driving force for the cyclization reaction. The rate of the reaction depends on the ease of the coordination between 'S' and the incoming Hg^{2+} ion. Therefore, the basicity of the coordinating site 'S' has been tuned using various substituents to obtain better insight into the sensing mechanism of the Hg-triggered oxadiazole formation. We anticipate that a more efficient rhodamine-based chemodosimeter can be obtained if we optimize the basicity of the coordinating sites using an electronic effect.

Therefore, the main objective of the current study is to tune the electronic cloud (basicity) of the binding unit 'S' by varying the substituents on the aromatic ring to selectively sense Hg^{2+} ions in an aqueous-organic medium. In addition, the sensitivities of the synthesized chemodosimeters **1–3** were investigated by varying the substituent. To the best of our knowledge, this is the first report on the effect of substituents on the Hg^{2+} ion-sensing property of these rhodamine derivatives, which sense via the Hg^{2+}-triggered conversion of the thiosemicarbazide form to the oxadiazole form. In this study, three new thiosemicarbazide-based rhodamine derivatives were prepared, characterized and employed as chemodosimeters to detect Hg^{2+} ions. Various spectral techniques, including UV–vis, fluorescence spectroscopy, FT-IR, FAB-Mass, ^{1}H NMR, and ^{13}C NMR, were used to investigate the interaction between the chemodosimeter and Hg^{2+} ions.

2. Experimental

2.1. Materials and methods

Rhodamine 6G hydrazide was synthesized according to established literature procedures [51]. All of the other reagents, cations (as perchlorate salts) and solvents were purchased from commercial sources and were used without further purification. Double-distilled water was used in all of the experiments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 spectrometer operating at 300 and 75 MHz, respectively, using tetramethylsilane as the internal standard. The chemical shifts in the ^{1}H NMR spectra are expressed in units of ppm (normalized integration, multiplicity and the value of J in Hz). FAB-MS spectra were obtained using a JEOL JMS 700 mass spectrometer. FT-IR spectra were recorded on an ALPHA-P spectrometer (Bruker, Germany). Melting points were determined using a Barnstead electrothermal apparatus (UK) and are reported uncorrected. UV–vis spectra were recorded using an Agilent 8453 spectrophotometer (1 cm quartz cell) at 25 °C. Fluorescence spectra were recorded using a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell) at 25 °C. The excitation and emission slit widths were 3 nm. Thin layer chromatography (TLC) analyses were performed on silica gel plates, and flash column chromatography was conducted using silica gel (230–400 mesh).

2.2. Synthesis

2.2.1. *N-((3′,6′-bis(ethylamino)-2′,7′-dimethyl-3-oxospiro[isoindoline-1,9′-xanthen]-2-yl) carbamothioyl)-4-methylbenzamide (**1**) [52]*

A solution of *p*-toluoylchloride (1.0 g, 6.4 mmol) in acetonitrile (25 mL) was added drop wise to a suspension of potassium thiocyanate (0.628 g, 6.4 mmol) in anhydrous acetonitrile (25 mL). The reaction mixture was refluxed for 1 h and then cooled to room temperature. A solution of rhodamine 6G hydrazide (2.4 g, 6.4 mmol) in acetonitrile was added, and the resulting mixture was refluxed for 1 h and then cooled to room temperature. After being filtered under reduced pressure, the solid was washed with cold acetonitrile and dried to afford crude **1** as a light pink solid. The crude material was purified using a 230–400 mesh flash column with 10% methanol in chloroform to afford the pure product as a pale white solid (yield 69%). Mp 202–204 °C; FT-IR (KBr), ν cm^{-1}: 3431, 3249, 2981, 2931, 2863, 1717, 1618, 1514, 1338, 1195, 1079, 728. ^{1}H NMR (DMSO-d_6, 300 MHz), δ(ppm): 1.20 (t, 6H, J = 6.9 Hz), 1.88 (s, 6H), 2.3 (s, 3H), 3.11 (m, 4H), 5.08 (t, 2H, J = 5.1 Hz), 6.22 (s, 2H), 6.40 (s, 2H), 7.02–7.05 (m, 1H), 7.27 (d, 2H, J = 8.1 Hz), 7.56–7.61 (m, 2H), 7.72 (d, 2H, J = 8.4 Hz), 7.87–7.90 (m, 1H,), 11.48 (s, 1H), 11.79 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz), δ (ppm): 182.3, 168.3, 163.4, 152.17, 151.9, 148.4, 144.2, 134.1, 129.4, 129.3, 129.1, 129.0, 128.4, 124.3,