Accepted Manuscript

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PII:	S1386-1425(17)30091-4
DOI:	doi: 10.1016/j.saa.2017.02.003
Reference:	SAA 14925
To appear in:	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy
Received date:	22 October 2016
Revised date:	31 January 2017
Accepted date:	2 February 2017

Please cite this article as: Hassan Zali-Boeini, Mohammad Zareh Jonaghani, Negar Fadaei, Hadi Amiri Rudbari , A new isoindoline–based highly selective "turn-on" fluorescent chemodosimeter for detection of mercury ion. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Saa(2017), doi: 10.1016/j.saa.2017.02.003

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A New Isoindoline–based Highly Selective "Turn-On" Fluorescent Chemodosimeter for Detection of Mercury Ion

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Abstract

A new isoindoline–based highly efficient turn-on Fluorescent chemodosimeter **S** with a thioamide functionality as a binding site for selective detection of Hg^{2+} ion has been developed. The chemodosimeter **S** showed an extreme selectivity for detection of Hg^{2+} ion among various two and three-valent metal ions in acetonitrile/water (70/30, v/v). It was found that, in the presence of Hg^{2+} ion the non-fluorescent chemodosimeter **S** was efficiently and rapidly desulfurized to the corresponding highly fluorescent amide **1**. A good linear relationship was shown between the fluorescence intensity and the concentration of Hg^{2+} within the range of 0-1 μ M, with a detection limit of 2.03×10^{-8} M.

Keywords

Fluorescent Chemodosimeter Mercury Ion Thioamide

1. Introduction

Recently, Fluorescent chemosensors and chemodosimeters have been attracted substantial attentions for detection of toxic heavy metal ions due to their simplicity, instantaneous response and high detection limit [1-2], when compared with other detection methods such as atomic absorption spectrometry [3-4], potentiometric ion-selective electrode [5], and inductively

coupled plasma mass spectrometry [6-9] and so on. Mercury is one of the most toxic and widespread global pollutants [10-11] among heavy metal ions. Due to presence of sulfur containing amino acids such as cysteine and N-acetyl cysteine in enzymes and proteins and high affinity of Hg²⁺ ion for sulfur atom, even very low concentrations of mercury ion, can causes serious health problems such as destroying endocrine systems, central nervous system and vision loss [12-15]. Hence, there have been significant efforts for selective and efficient detection of Hg²⁺ ion which is an important issue in environmental and food chemistry. Although some fluorescent chemosensors based on rhodamine [16-17], benzothiazole derivatives [18-19], fluorescein [20-21] and other fluorophores [22-32] have been synthesized and used for detection of mercury ion, nonetheless most of these sensors have some shortcomings such as weak fluorescence intensity, cross-sensitivities with other metal ions, fluorescence quenching upon Hg²⁺ addition, low selectivity and sensitivity, and difficult syntheses [33-38]. Therefore developing a new sensor with high selectivity and sensitivity for Hg²⁺ is still a valuable challenge. Chemodosimeter sensors, especially 'turn-on' fluorescence probes owing to their high sensitivity and selectivity have become interesting field in fluorescence sensing methods when compared with classical fluorescent chemosensors [39-45]. To date several fluorescent chemodosimeters have been developed for detection of Hg²⁺ ions based on the robust thiophilic character of Hg²⁺ ion and their mechanisms including: desulfurization reactions by hydrolysis [46-49], cyclization [50], and elimination reactions [51]. However, several significant limitations such as moderate sensitivity and selectivity, long reaction times, and operating at elevated temperatures still exist [52-54]. Intrigued to find a solution to these problems, we were interested in exploring a new chemodosimeter sensor for detection of Hg^{2+} ions in low temperatures, with a short response time, with high selectivity and sensitivity, and strong fluorescence emissions. In continuation of our studies on developing novel efficient sensors [55], herein, we report a new turn-on fluorescent chemodosimeter for detection of mercury ion based on desulfurization reaction of (Z)-3-(quinolin-2-ylmethylene)isoindoline-1-thione S.

2. Experimental

2.1 Materials and methods

All reagents and solvents were obtained commercially and used without further purification. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker-Avance400MHz spectrophotometer

using DMSO- d_6 and residual solvent as an internal standard. Infrared spectra (IR) spectra were recorded on a Jasco, FT/IR-6300 spectrometer. Absorption spectra were determined on a Shimadzu UV-160. Fluorescence spectra were recorded with a Shimadzu RF-5000 Spectrofluorometer.

2.2 Preparation of (Z)-3-((quinolin-2-yl)methylene)isoindolin-1-one (1)

In a two-neck round bottom flask equipped with magnetic stirrer and a condenser, phthalimide (10 mmol, 1.47 g), quinaldine (5 mmol, 0.72 g), dry ZnCl₂ (21 mmol, 2.86 g) and *N*,*N*-dimethylaniline (15.0 ml) were charged and heated under nitrogen ventilation at 150 °C for 24 h. After completion (TLC), the reaction mixture was poured in HCl (10%, 600 mL) and stirred at 95 °C for 1 h. After cooling to 50 °C, the reaction mixture was filtered and washed with water (2×50 mL). The product was dried under vacuum to obtain (Z)-3-((quinolin-2yl)methylene)isoindolin-1-one as a fine yellow powder in good yield (1.1 g, 81%); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.07 (s , 1 H), 7.66-7.74 (m, 2H), 7.80-7.92 (m, 4H), 8.05 (d, , *J*= 7.4 Hz, 1H), 8.15 (d, , *J*= 7.4 Hz, 1H), 8.33 (d, , *J*= 8.4 Hz, 1H), 11.49 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 101.2, 121.1, 123.1, 123.2, 126.1, 126.7, 127.2, 127.9, 128.5, 130.5, 130.7, 132.8, 137.6, 138.1, 139.3, 145.8, 154.9, 168.1; IR (KBr); 3289(NH), 1702(C=O), 1651, 1591, 1296, 1200, 846, 757.

2.3 Preparation of (Z)-3-((quinolin-2-yl)methylene)isoindoline-1-thione(S)

In a round bottom flask, Compound **2** (2 mmol, 0.54 g) and 2,4-bis(4-methoxyphenyl)-1,3,2,4dithiadiphosphetane-2,4-dithione (Lawesson's Reagent) (2 mmol, 0.81 g) were dissolved in diethylene glycol diethyl ether and the reaction mixture was heated for 2h at 80°C. After cooling, the reaction mixture was poured over crushed ice. Then, the crude precipitated product was filtered and subjected to flash chromatography using alumina as the stationary phase and petroleum ether/ethyl acetate (2:1) as eluent to afford compound **S** as an orange solid (0.35 g, 61% yield); ¹H NMR (400 MHz, DMSO- d_6) δ : 7.21 (s, 1 H), 7.65-7.76 (m, 3H), 7.81-7.87 (m, 2H), 7.95-8.01 (m, 2H), 8.14 (t, *J*= 6.8 Hz, 1H), 8.47 (d, *J*= 8.4 Hz, 1H), 13.01 (s, 1H); ¹³C NMR (100 MHz, DMSO- d_6) δ : 104.4, 120.7, 123.8, 123.9, 126.4, 127.0, 128.0, 128.3, 128.6, 130.5, 130.6, 131.5, 132.5, 135.4, 137.4, 155.2, 170.3; IR (KBr); 3219(NH), 1173(C=S), 1642, 1589, 1476, 1403, 1340, 1294, 737.

3. Results and discussion

At the beginning of our study, (*Z*)-3-((quinolin-2-yl)methylene)isoindolin-1-one1 was synthesized from reaction of phthalimide and quinaldine in the presence of $ZnCl_2$ at rather elevated temperature. Then, the corresponding thioamide chemodosimeter **S** was prepared by the thionation reaction of compound **1** with Lawesson's reagent in diethylene glycol diethyl ether as solvent (yield 61%, Scheme 1). The structure of compounds **1** and **S** were established by ¹H NMR and ¹³C NMR spectroscopy. Moreover, the structure of compound **1** was further confirmed by X-ray analysis (Figure 1, CCDC1502691).



Figure 1. ORTEP representation of compound **1**. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii. Hydrogen bonds are shown as dashed lines.

3.1 Absorption and Fluorescence spectral responses

Absorption and Fluorescence spectral responses of S toward various metal cations were investigated in aqueous solution of acetonitrile (CH₃CN-H₂O 70/30, v/v). Hence, UV absorption spectra of S was taken alone and in the presence of various metal cations (Figure 2A). As shown in figure 2, absorption spectrum of free chemodosimeter S displayed a maximum placed at 426 nm with shoulders at 407 nm and 449 nm. When a solution containing 2.5 equiv. of Zn²⁺, Sn²⁺, Cr^{3+} , Al^{3+} , Cd^{2+} , Mn^{2+} , Cu^{2+} , Ba^{2+} , Co^{2+} , Ca^{2+} , Ni^{2+} , Pb^{2+} or Ag^{+} metal ions were added, the absorption spectra of S did not change, whereas in the presence of Hg^{2+} ion intensity of the absorption maxima exhibited a little increase with a notable blue shift ($\Delta\lambda = 25$ nm). When chemodosimeter S (10 μ M) was excited at 426 nm, a very weak emission at 529 nm (quantum yield, Φ = 0.007) in aqueous CH3CN solution at room temperature was observed. Upon the addition of Hg^{2+} (10 μ M), a remarkable enhancement in the fluorescence intensity (quantum yield, $\Phi = 0.56$, Fig. 2B and) was detected. To examine the selectivity of S toward mercury ion, fluorescence emission spectra of S in the presence of 50 equiv. of various metal ions in aqueous solution of acetonitrile (CH₃CN-H₂O 70/30, v/v) were recorded. As shown in figure 2B the bare chemodosimeter S (0.1 μ M) displays no fluorescence emission (fluorescence off-state) whereas, in the presence of Hg^{2+} ion intensively fluoresces at 529 nm. The signaling mechanism is more likely based on desulfurative oxidation of thioamide functionality of chemodosimeter S with mercury ion to corresponding amide 1 which shows strong green fluorescence emission (fluorescence on-state). None of all other metal ions including monovalent, divalent, and trivalent cations show measurable effect on S.



Figure 2. (A) UV-vis absorption spectra of the chemodosimeter **S** (20 μ M) with various metal ions in CH₃CN/H₂O (70:30). [Mⁿ⁺] = 50 μ M; (B) Fluorescence spectra of **S** (0.1 μ M) in the presence of various metal ions in CH₃CN/H₂O (70:30, v/v) at room temperature [Mⁿ⁺] = 5 μ M.



Scheme 2. The proposed signaling mechanism of chemodosimeter S with Hg^{2+} .

The proposed mechanism for the operation of chemodosimeter **S** is shown in scheme 2. In order to approve the operation mechanism of chemodosimeter **S**, Infrared and ¹H NMR spectrum of **S** was recorded before and after addition of mercury ion. As shown in the figure 3A upon the addition of Hg^{2+} ion appearance of a new peak in IR spectrum corresponding to the carbonyl group at 1702 cm⁻¹ was evident for the formation of amide **1**. This explicitly confirms the proposed desulfurative mechanism for conversion of thioamide functionality of chemodosimeter **S** to the analogous amide **1**. In the ¹HNMR spectrum of compound **S**, there is a singlet signal at 13.1 ppm (1H) which belongs to NH group of thioamide functionality. During addition of

mercury ion, this characteristic signal was shifted drastically to upfield region (11.8 ppm, $\Delta\delta$ = 1.3 ppm) which further approves the proposed operation mechanism of chemodosimeter **S** (Figure 3).



Figure 3. (A) FT IR spectra of the S and $S + Hg^{2+}$; (B) ¹H NMR spectra of 1, S, and S + 1.0 equiv. of HgCl₂ in DMSO-*d*₆ at room temperature.

Figure 4A shows the fluorescence behavior of the chemodosimeter **S** upon addition of diverse metal ions. It was distinctly observed that, after addition of Hg^{2+} ion, the nonfluorescent solution of **S** was immediately turned to a bright green fluorescent solution under UV light. Figure 4B shows the fluorescence emission of compound **1** and a mixture of compound **1** and Hg^{2+} ion. This clearly indicates that the intense green emission is only related to compound **1** which is not affected by probable complexation between **1** and Hg^{2+} ion.



Figure 4. (A) Fluorescence changes upon addition of various metal cations to **S** in CH₃CN/water under UV light (365nm); (B) Fluorescence emission of compound **1** alone and in the presence of Hg^{2+} .

The fluorescence and absorbance titration experiments of the chemodosimeter **S** with Hg²⁺ ion were performed in CH₃CN/H₂O mixture (70/30, v/v) (Figure 5A, and 5B). In the absorption spectra, intensity of the band centered at 426 nm decreased and shifted to 401 nm during the addition of Hg²⁺ ion. A good linear detection range was observed in the emission spectra at 529 nm upon addition of 0.2–1 μ M of Hg²⁺ (Figure 5C). The detection limit of the chemodosimeter **S** for Hg²⁺ ion was estimated to be 2.03×10⁻⁸ M. Figure 5D obviously demonstrate extreme selectivity of **S** toward Hg²⁺ ion over other metal ions.



Figure 5. (A) Fluorescence spectra of **S** (0.1 μ M) in the presence of 0.2-5 μ M of Hg²⁺; (B) UV-vis absorption spectra of the chemodosimeter **S** (20 μ M) with various concentrations of Hg²⁺ ion ([Hg²⁺]= 0-50 μ M); (C) Plot of fluorescence intensities of **S** at 529 nm against 0.2-1.2 μ M concentrations of Hg²⁺; (D) Emission intensity of **S** (0.1 μ M) in the presence of Hg²⁺ and other metal ions (5 μ M) in CH₃CN/H₂O (70/30, v/v) at room temperature

Since response time is an important factor in chemodosimeter sensors, the time dependence for the interaction of **S** with Hg²⁺ ion was studied and the results are shown in Figure 6A. The fluorescence intensity at 529 nm was increased rapidly upon addition of 5 μ M Hg²⁺ ion to 0.1 μ M **S** and then remained constant after 1 min. The stoichiometric ratio between the **S** and Hg²⁺ was determined by Job's plots. As shown in figure 6B, a 1:1 stoichiometry for the interaction of **S** with Hg²⁺ ion was observed.



Figure 6. (A) Time dependence of fluorescence emission of **S** (0.1 μ M) to addition of Hg²⁺ ion 5 μ M); (B) Job's plot diagram of chemodosimeter sensor **S** for Hg²⁺ (the total concentration of **S** and Hg²⁺ was 2 μ M)

Conclusion

In summary, (*Z*)-3-((quinolin-2-yl)methylene)isoindoline-1-thione **S** as a new compound was synthesized in two simple steps from readily available starting materials. The compound **S** was shown to be as an efficient, fast, and highly selective chemodosimetersensor for detection of Hg^{2+} ion among various mono, di, and tri-valent metal ions. It was found that, the presented chemodosimeter worked based on irreversible desulfurization reaction of thioamide functionality of **S** to amide functional group in the presence of mercury ion. The selectivity of **S** for detection of Hg^{2+} ion was attributed to high affinity of Hg^{2+} toward sulfur atom of thioamide moiety in the chemodosimeter **S**. Fortunately, the vivid green fluorescent response of **S** to Hg^{2+} can be even sensed and persuaded by the naked eye. This property make this simple molecule as a promising chemodosimeter sensor for the facile visualization of trace amounts of Hg^{2+} ion which is an important issue in environmental chemistry.

Acknowledgements

We are grateful to the University of Isfahan research council for financial support of this work.

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Highlights

- A new isoindoline–based highly efficient turn-on Fluorescent chemodosimeter for selective detection of Hg^{2+} ion has been developed.

- A desulfurative reaction is proposed for the operational mechanism of chemodosimeter.

- Sensing protocol and the mechanism of the chemodosimeter is supported by ¹H NMR, IR, and Fluorescence studies.