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A diethylamino pyridine formyl Schiff base as selective recognition chemosensor for biological thiols

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ABSTRACT

A diethylamino pyridine formyl Schiff base compound was synthesized via typical condensation reaction between 4-*N*, *N*-diethyl amino salicylic aldehyde and 2-pyridine formyl hydrazine in good yields. Its photophysical properties and selective recognition properties for biological thiols in aqueous solution have been investigated. The results indicate that the compound exhibits quickly obvious UV–vis absorption and fluorescence turn-off response to glutathione (GSH) based on intermolecular hydrogen-bond interaction in PBS butter solution. The copper complex of the compound shows quickly obvious turn-on fluorescence response to cysteine (Cys) based on replacement reaction with copper in PBS butter solution. Then the compound can selectively recognize three biological thiols of GSH, Cys and homocysteine (Hcy) in aqueous solution. The compound also can be successfully applied for bioimaging in living cell.

1. Introduction

Biological thiols, such as cysteine (Cys) and glutathione (GSH), play key roles in various biological systems, and Cys is a metabolic product of homocysteine (Hcy) and a precursor of the antioxidant GSH [1–5]. Biological thiols have been considered as important biomarkers of liver damage, cancer and osteoporosis [6–12]. Since the fluorescence indicators for calcium ion were reported by Tsien in the early 1980s [13], fluorescence chemosensors have been recognized as the efficient molecular tools, which can help monitor and visualize trace amounts of samples because of their high sensitivity and high spatiotemporal resolution [14–24]. A large number of fluorescence chemosensors have been developed in recent years to recognize these biologically important species [25–31]. These chemosensors can highly selectively distinguish these biothiols from other amino acids, but most of them cannot distinguish Cys/Hcy/GSH from each other due to the similar structures and reactivity [32,33]. In fact, the discrimination between them has been a focal point and also a tough challenge for researchers, albeit some advances have been obtained.

Herein, we report a chemosensor for GSH/Cys by colorimetric and fluorescence detection mode in PBS butter solution. Chemosensor **1** can recognize GSH quickly while nearly has no response to Cys and Hcy based on intermolecular hydrogen-bond interaction. And copper complex of chemosensor **1** can recognize Cys based on replacement reaction with Cu^{2+}. In order to explore the recognition mechanism of the compound, a similar compound **2** without hydroxyl group was synthesized. The recognition properties of compound **1** and coordination mechanism between the compound and copper ion was also discussed.

2. Experimental

2.1. Synthesis and characterization

Compounds **1** and **2** were synthesized by the condensation reaction between diethylamine salicylaldehyde or diethylamine benzaldehyde and benzoyl hydrazine according to Ref. [34]. The starting materials diethylamine salicylaldehyde, benzoyl hydrazine, diethylamine benzaldehyde and Cupric chloride dihydrate ($CuCl_2{\cdot}2H_2O$) were purchased from Aladdin Industrial Corporation and used without further purification. NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer at room temperature. Mass spectra were recorded on an Agilent

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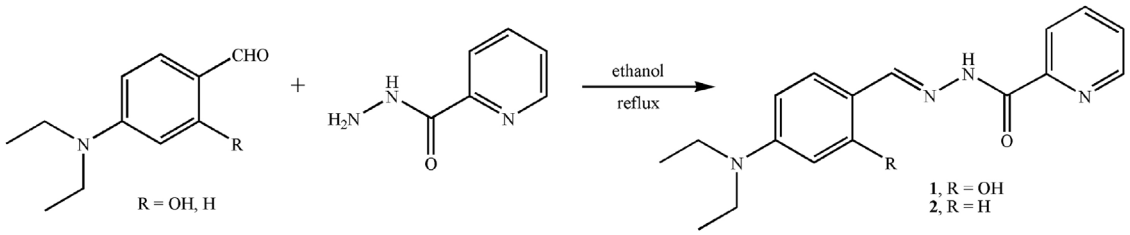

Scheme 1. Synthetic routes to compounds **1** and **2**.

Q-TOF6510 or LCMS-2010 spectrometer. The compounds were synthesized according to the procedures depicted in Scheme 1.

0.19 g (0.001 mol) of 4-*N*, *N*-diethylamino salicylic aldehyde and 0.14 g (0.001 mol) of 2-pyridine formyl hydrazine were dissolved in 20 mL of absolute ethanol. After the mixture was heated under reflux for 12 h, yellow precipitate was formed. The precipitate was filtered and washed with anhydrous ethanol several times. 0.24 g of compound with yield 78% was obtained. ^{1}H NMR (400 MHz, DMSO), δ: 1.11 (t, *J* = 7.2 Hz, 6H), 3.37 (q, *J* = 7.2 Hz, 4H), 6.13 (s, 1H), 6.28 (d, *J* = 8.8 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.67 (t, *J* = 6.8 Hz, 1H), 8.06 (t, *J* = 7.6 Hz, 1H), 8.11 (d, *J* = 6.8 Hz, 1H), 8.62 (s, 1H), 8.72 (d, *J* = 4.8 Hz, 1H), 11.59 (s, 1H), 12.21 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 12.82, 44.66, 98.48, 103.83, 106.47, 122.76, 126.67, 132.52, 137.78, 148.19, 149.42, 151.08, 152.00, 159.25, 160.85. $(M+H)^+$, Calcd exact mass: 313.1665, found 313.1668.

Compound **2** was synthesized similar to **1** with yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.19 (t, *J* = 7.2 Hz, 6H), 3.42 (q, *J* = 7.2 Hz, 4H), 6.66 (d, *J* = 9.2 Hz, 2H), 7.44-7.48 (m, 1H), 7.67 (d, *J* = 9.2 Hz, 2H), 7.88 (td, *J* = 7.6 Hz, 1.6 Hz, 1H), 8.13 (s, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 8.57 (d, *J* = 4.8 Hz, 1H), 10.78 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$), δ: 12.71, 44.54, 111.10, 120.37, 122.85, 126.50, 129.85, 137.65, 148.07, 149.66, 149.71, 149.81, 159.64.

2.2. Photophysical properties and recognition for biological thiols

The titrations were carried out in 10 mm quartz cuvettes at 25 °C on a Shimadzu UV2550 spectrophotometer and a Horiba Fluoromax-4 fluorescence spectrometer, respectively. **1**-Cu^{2+} was obtained by mixing compound **1** and Cupric chloride dihydrate with mole ratio of 1:1 in acetonitrile at 25 °C. The spectral changes were monitored with the addition of GSH/Cys/Hcy solution in PBS buffer solution. The cell imaging experiment was carried out on a LSM 710 Laser confocal microscopy.

3. Results and discussion

3.1. Photophysical properties of compound 1 and response to GSH

UV-vis absorption and fluorescence spectra of compound **1** in PBS buffer solution (10 μM) upon the addition of different concentrations of GSH are shown in Fig. 1. Compound **1** exhibits strong absorption in ultra-violet region with the main absorption peak at 383 nm ($\varepsilon = 4.28 \times 10^4$ M^{-1} cm^{-1}). It exhibits fast response to GSH aqueous solution (<5 s). As shown in Fig. 1(a), with the increase of GSH, the absorption peak of compound **1** at 383 nm decreases gradually and is red-shifted to 420 nm.

Compound **1** (10 μM) in buffer solution displays fluorescence emission at 488 nm upon excitation at 383 nm. The incremental addition of GSH to the solution of compound **1** results in a decrease in fluorescence intensity. At last, fluorescence decreases to about 13% of the original. The change also can be observed by naked eyes in natural light and 365 nm UV-light (insert of Fig. 1). The compound changes from yellow to colorless and blue fluorescence is almost quenched. The detection limits [35] of chemosensor **1** with absorption and fluorescence as detected signal for GSH in PBS butter solution are 1.2 μM (absorbance) and 7.4×10^{-3} μM (fluorescence), respectively.

The selectivity and competitive experiments of compound **1** toward various amino acids in PBS butter solution were conducted in the presence of GSH mixing with different excess amino acids. As displayed in Fig. 2(a), the reaction of all interfering species with compound **1** results in negligible change of absorbance. Notably, the addition of GSH induces much greater decrease in absorbance than the other two homogeneous biothiols (Cys/Hcy) even at very high concentration. These results indicate the excellent selectivity of compound **1** toward GSH.

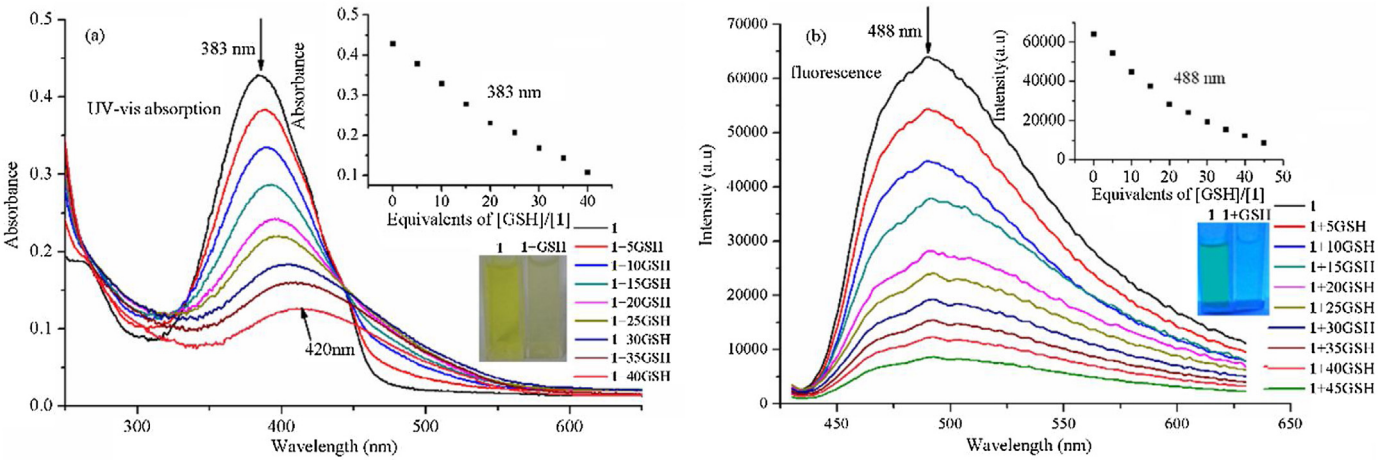

Fig. 1. UV–vis absorption (a) and fluorescence (b, λ_{ex} = 383 nm) spectra of compound **1** in PBS buffer solution (pH = 7.4, *C* = 10 μM) upon the addition of different concentrations of GSH.