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A new fluorescent chemodosimeter for ultra-sensitive determination of toxic thiophenols in environmental water samples and cancer cells

Feng Wu [a,1], Hongying Wang [a,1], Junchao Xu [a], Hou-Qun Yuan [b], Lintao Zeng [a,*], Guang-Ming Bao [b,*]

[a] Tianjin Key Laboratory of Organic Solar Cells and Photochemical Conversion, School of Chemistry & Chemical Engineering, Tianjin University of Technology, Tianjin 300384, PR China
[b] School of Animal Science and Technology, Jiangxi Agricultural University, Nanchang 330045, PR China

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ABSTRACT

A novel fluorescent chemodosimeter for thiophenols has been readily developed from a red emitting **Indole-BODIPY**. The chemodosimeter displays a significant fluorescence turn-on response towards thiophenols based on nucleophilic substitution reaction. A linear relationship was obtained between the fluorescence intensity and the concentrations of thiophenols ranging from 0 to 1.8 μM. The chemodosimeter can determine thiophenols (4-fluorothiophenol, 4-methoxybenzenethiol, *p*-toluenethiol) with high selectivity, fast response (within 8 min) and extremely low limit of detection (9.8 nM). The chemodosimeter was successfully used to determine the level of thiophenol (PhSH) in industrial wastewater and Bohai Sea water with good recoveries. Furthermore, the chemodosimeter was employed to image thiophenols in cancer cells with good performance and low cytotoxicity. Therefore, this chemodosimeter has great potential application in environment.

1. Introduction

Thiophenols are a class of highly toxic chemicals, which are widely used for preparation of agrochemicals, pharmaceuticals and industrial products [1–3]. Some researchers have revealed that thiophenols are poisonous to aquatic organisms and animals. The median lethal concentration (LC_{50}) of thiophenol is ranging from 0.01 to 0.4 mM in fish and the median lethal concentration (LD_{50}) of thiophenol is 46.2 $mg \cdot kg^{-1}$ in mouse [4,5]. Moreover, it is well established that the presence of thiophenol in water and soil has a bad impact on natural habitats [6]. Hence, thiophenol has been listed as a category of the most important pollutants by the United States Environment Protection Agency (EPA waste code P014) [5]. Thiophenol can easily invade the human body by inhalation or skin absorption. These thiophenols target some organs such as eyes, skin, respiratory system, central nervous system, kidney, liver and spleen, which induces serious damage to the central nervous system and other related symptoms [5,7,8]. Therefore, it is very necessary to monitor trace thiophenol in environmental and biological systems.

High performance liquid chromatography (HPLC) [9,10] and ultraviolet-visible spectrometry [11] are the most widely used methods for the detection of thiophenol in environmental samples. However, these methods have some disadvantages such as time-consuming, low sensitivity as well as complicated pretreatments. Fluorescent chemodosimeters have emerged as a promising technique due to high sensitivity, visual detection, low cost, fast response and no need of complicated pre-treatment [12]. Wang reported the first fluorescent chemodosimeter for thiophenol through thiolysis reaction of dinitrobenzenesulfonylamide, which utilized 4-amino-7-nitro-2,1,3-benzoxadiazole (NBD) as the fluorophore [13]. Although this chemodosimeter was creative, it showed weak fluorescence ($\Phi = 0.02$) and low sensitivity. Recently, several other fluorescent chemodosimeters for thiophenols have been developed based on thiolysis reaction of dinitrobenzenesulfonylamides [14–28] or dinitrophenyl ethers [29–36]. Among these reported fluorescent chemodosimeters, some displayed poor water solubility [30,35,37] and high pH-dependence [21,33], some exhibited relatively weak fluorescence intensity [13,30] and low sensitivity [13], and most of them needed excitation/emission in the ultraviolet or visible region [14], which might be suffered from the interference from background and some fluorescent species.

* Corresponding authors.
E-mail addresses: 407650531@qq.com, zlt1981@126.com (L. Zeng), bycb2005@gmail.com (G.-M. Bao).
[1] These authors contributed equally to this work.

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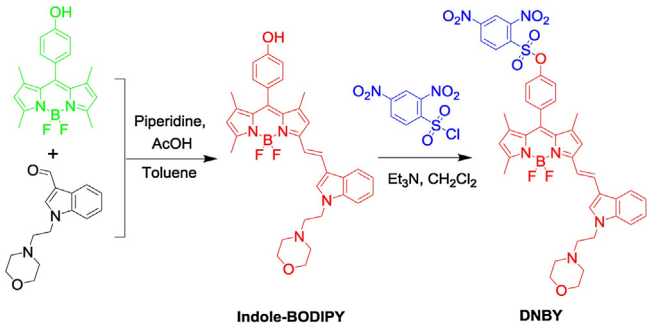

Scheme 1. Synthetic route of the chemodosimeter **DNBY**.

Thus, it is highly desired to develop applicable fluorescent chemodosimeters with good water solubility, high brightness, red or near-infrared emission, high sensitivity as well as rapid response.

In this work, we report a red emitting fluorescent chemodosimeter for the detection of thiophenols, which utilize a water soluble red emitting **Indole-BODIPY** as a reporter and the 2,4-dinitrobenzenesulfonyl (DNBS) group as recognition unit (Scheme 1). It was envisioned that the DNBS group would be removed by thiophenols *via* thiolysis reaction [38]. As a result, the **Indole-BODIPY** was released and produced fluorescence turn-on signal to indicate PhSH. To prove the feasibility of our design concept, we evaluated the ability of **DNBY** for quantitative detection of thiophenols in environmental water samples and living cells.

2. Experimental section

2.1. Materials

All chemicals were purchased from commercial suppliers (Aladdin-Reagent, Sigma-Aldrich, TCI), and used directly without further purifications. Double-distilled water was used in all experiments.

2.2. Equipments and methods

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as the internal standard. The chemical shift was recorded in ppm and the following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet. HR-MS spectrum was measured by a HP-1100 LC–MS spectrometer. UV–vis spectra were recorded on a Hitachi UV 3310 spectrometer. Fluorescence spectra were recorded on a Hitachi FL-4500 fluorometer. Fluorescent images were acquired on a Nikon A1 confocal laser-scanning microscope with a 100 objective lens. Numerical Aperture of the objective: 100x/1.4 Oil (DIC N2), OFN 25, Plan Apo VC, Nikon Company. Immersion oil, type: NF, nd = 1.515 (23 °C). Microscope: Ti Microscope, Light path: L100, Condenser: 3 (DIC N 2), Zoom: 1.00x, Nikon Company. Column chromatography was performed on silica gel (mesh 200–300), which was purchased from Qingdao Ocean Chemicals Corporation. The chromatography system consisted of a LC-10ATVP pump and SPD-10AVP UV–vis detector (Shimadzu, Kyoto, Japan) with an injector (10 μL sample loop). The analysis was performed on an Optima Pak C_{18} column (5 μm, 150 × 4.6 mm, RS tech Corporation, Daejeon, Korea) and Chromatography Data System N2000 (Surwit Technology, Hangzhou, China).

2.3. Synthesis of chemodosimeter *DNBY* [39]

Indole-BODIPY was synthesized according to our previously reported literature [40]. Indole-BODIPY (100.0 mg, 0.17 mmol) and triethylamine (70.0 μL, 0.50 mmol) were dissolved in 20 mL of anhydrous CH_2Cl_2. 2,4-dinitrobenzenesulfonyl chloride (55.1 mg, 0.21 mmol) in 2 mL of anhydrous CH_2Cl_2 was dropwise added into this solution for 0.5 h at 0 °C. The temperature was raised to 40 °C, and the reaction mixture was stirred for 2 h. After the reaction completed, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/EtOH = 100/1) to afford **DNBY** as a dark blue solid (122.6 mg, 0.15 mmol, 87.8%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ/ppm* 8.68 (d, *J* = 2. 2Hz, 1H), 8.48 (dd, *J* = 8.6, 2.2 Hz, 1H), 8.18 (d, *J* = 8.6 Hz, 1H), 8.07–8.00 (m, 1H), 7.67 (d, *J* = 16. 2Hz, 1H), 7.58 (s, 1H), 7.53 (d, *J* = 16.2 Hz, 1H), 7.44–7.30 (m, 7H), 6.67 (s, 1H), 5.99 (s, 1H), 4.29 (s, 2H), 3.75 (s, 4H), 2.82 (s, 2H), 2.62 (s, 3H), 2.55 (s, 4H), 1.40 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ/ppm* 155.85, 153.07, 150.97, 149.15, 142.27, 139.71, 137.27, 135.82, 135.65, 133.93, 130.72, 126.13, 123.00, 122.84, 121.45, 120.71, 120.56, 120.32, 117.88, 114.43, 109.88, 66.89, 57.93, 53.83, 44.16, 14.90, 14.61, 14.39. HR-MS (ESI): calculated for $C_{40}H_{38}BF_2N_6O_8S^+(M^+)$ 811.2533; Found 811.2523.

2.4. Fluorescence analysis

The solutions of various testing species including NaF, NaCl, NaBr, Na_2SO_4, $NaHSO_3$, $Na_2S_2O_3$, CH_3COONa, $NaHCO_3$, Na_2HPO_4, NaH_2PO_4, Na_3PO_4, $NaNO_2$, $NaNO_3$, NaClO, glutathione (GSH), L-cysteine (Cys), homocysteine (Hcy), Na_2S, H_2O_2 were prepared in double-distilled water. Stock solutions of thiophenol, phenol, aniline, 4-fluorothiophenol, 4-methoxybenzenethiol, *p*-toluenethiol, 1-propanethiol were prepared in DMSO/PBS solution (v/v = 3/7, pH = 7.4), respectively. The fluorescence measurements were carried out in 10 mM PBS buffer solution (pH = 7.4) with 30% fraction of DMSO. For all measurements, the excitation wavelength was 595 nm, the excitation and emission slit widths were 5 nm.

2.5. Study of the sensing mechanism by HPLC

The sensing mechanism of **DNBY** for PhSH was studied by HPLC. The standard solutions of PhSH (10.0 μg·mL^{-1}), **Indole-BODIPY** (10.0 μg·mL^{-1}) and **DNBY** (10.0 μg·mL^{-1}) were prepared in HPLC-grade methanol, respectively. **DNBY** solution (10.0 μg·mL^{-1}) was treated with 1.0 μg·mL^{-1} of PhSH in HPLC-grade methanol. Then, these solutions were separately injected into HPLC for analysis. The component of **DNBY**+ PhSH and its reaction products can be identified by comparison of their retention time with PhSH, **Indole-BODIPY** and **DNBY** under the same chromatographic conditions.

2.6. Determination of the detection limit

The fluorescence spectrum of **DNBY** (2.0 μM) was measured for five times to obtain the standard deviation of a blank measurement. Upon addition of increasing amount of PhSH, the fluorescence spectra of **DNBY** were measured. By plotting the fluorescence intensity at 634 nm versus the concentration of PhSH, a linear relationship was obtained. The limit of detection for PhSH was calculated based on signal to noise ratio (S/N = 3).

2.7. Measurements of thiophenol in water samples

The practical water samples (Bohai Sea water and industrial wastewater) were collected from Bohai Sea and river surrounding the Bohai Chemical Cop. Int. The pH values of the water samples (50 mL) were adjusted to 7.4, and then DMSO was added to form