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Pyrene based fluorescent probes for detecting endogenous zinc ions in live cells

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ABSTRACT

Two unique pyrene derivatives are reported as Zn^{2+} selective fluorescent probes, in which two pyrenes bearing imine and OH groups are connected via an ether linker or thioether linker. Upon the addition of Zn^{2+}, the resulting phenolate group can induce internal charge transfer (ICT) peaks, which can cause selective turn-on fluorescence of each probe at 550 nm. Bathochromic shifts of their emissions are observed upon the binding with Zn^{2+}. Each probe can successfully image exogenous Zn^{2+} ions as well as free zinc ions released during apoptosis. These results using live cells suggest that these pyrene derived probes can be very effective probes in zinc biology studies.

1. Introduction

Zn^{2+} ions are one of the essential metal ions in many biological processes. More specifically, they are known to be involved in Alzheimer's disease, Parkinson's disease and in ischemic stroke [1]. It is also reported that Zn^{2+} ions are released from intracellular metalloproteins during apoptosis (programmed cell death) [2]. Accordingly, fluorescent probes, which can visualize the mobile zinc ions in the cell and those from apoptosis, are currently of great importance. On the other hand, relative high concentrations of Zn^{2+} are naturally present in pancreatic islets, in which a lowered concentration of Zn^{2+} can reduce the ability of the islet cells to produce and secrete insulin [3]. For this purpose, fluorescent probes [4] have been extensively studied for Zn^{2+} due to their high selectivity and simplicity [5,6].

In the current study, we utilized two unique pyrene derivatives (**1** and **2**) bearing imine and OH groups, in which two pyrene moieties are connected via either an ether linker (**1**) or a thioether linker (**2**). These two hosts contain nice binding pockets for Zn^{2+}, which are composed of two imine moieties, two phenolate groups and additional oxygen/sulfur in the linker. The resulting phenolate groups can induce nice internal charge transfer (ICT) peaks, which can cause selective turn-on emissions upon the binding with Zn^{2+}. Probes **1** and **2** can successfully image exogenous Zn^{2+} ions, which was confirmed by the fluorescence quenching upon the treatment of membrane-permeable zinc ion chelator tetrakis(2-pyridylmethyl) ethylenediamine (TPEN). Then, these two probes were applied to sense free zinc ions released during apoptosis.

2. Experimental

2.1. Materials and equipment

General methods unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions with tetramethylsilane (TMS) as an internal standard using a Bruker AM-300 spectrometer. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). Fourier transform infrared (FT-IR) spectra were recorded on a Scimitar Series model Varian 800 FT-IR spectrometer by the standard KBr disk method. For the X-ray crystallography, the diffraction data for probe **1** were collected on a Bruker SMART AXS diffractometer using Mo Kα (λ = 0.71073 Å). The crystal was

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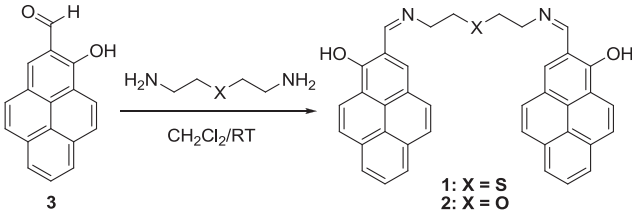

Scheme 1. Synthesis of probe **1** and **2**.

mounted on a glass fiber under epoxy. The CCD data were integrated and scaled using a Bruker SAINT, and the structures were solved and refined using SHELXTL. Hydrogen atoms were located in the calculated positions. The crystallographic data are listed in Table S1. The bond lengths and angles are listed in Table S2. The CIF deposition number: CCDC 763173 for **1**. UV–vis spectra were obtained using a Scinco 3000 spectrophotometer (1 cm quartz cell) at 25 °C. Fluorescence spectra were recorded on RF-5301/PC (Shimada) fluorescence spectrophotometer (1 cm quartz cell) at 25 °C. Deionized water was used to prepare all aqueous solutions. Compound **3** was prepared modifying the reported literature procedures [7].

2.2. Synthesis

2.2.1. Compound 1

1-Hydroxypyrene-2-carbaldehyde **3** (100 mg, 0.41 mmol) was dissolved in CH_2Cl_2 and 2,2′-thiobis (ethylamine) (0.24 mL, 0.20 mmol) was added to the reaction mixture. The reaction mixture was stirred at RT for 12 h. The precipitate formed was filtered and washed with cold CH_2Cl_2 to give **1** as a red solid (168 mg, 71.0%). m.p. = 203 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 14.42 (s, OH), 8.64 (s, 2H), 8.47 (d, J = 9.00 Hz, 2H), 8.06–7.90 (m, 8H), 7.69 (s, 2H), 7.60 (d, J = 8.98 Hz, 2H), 7.50 (d, J = 8.95 Hz, 2H), 3.96 (t, J = 6.28 Hz, 4H), 3.06 (t, J = 6.40 Hz, 4H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 167.04, 157.42, 132.86, 132.56, 129.96, 127.47, 127.41, 127.32, 127.12, 126.51, 125.14, 124.86, 124.48, 123.52, 121.95, 115.35, 80.03, 59.34, 33.54, 32.15, 31.16, 29.16, 14.35. HRMS (FAB) calcd for $C_{38}H_{29}N_2O_2S$ $[M + H]^+$ 577.1871; found 577.1950.

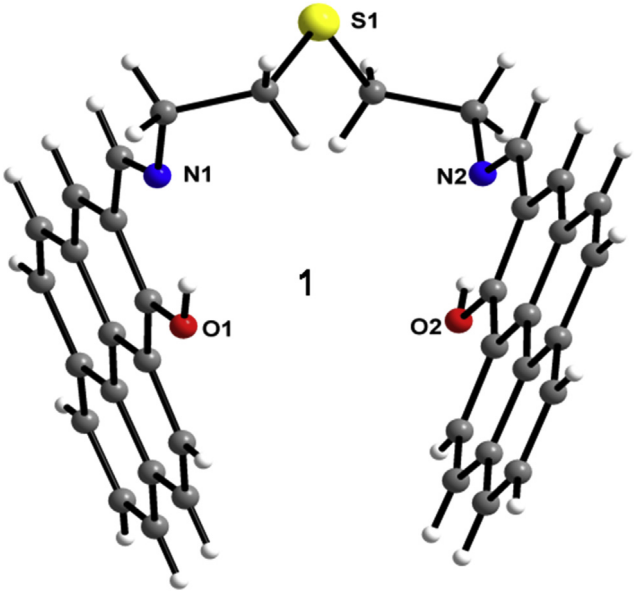

Fig. 1. X-ray crystal structure of probe **1**.

2.2.2. Compound 2

1-Hydroxypyrene-2-carbaldehyde **3** (100 mg, 0.41 mmol) was dissolved in CH_2Cl_2 and 2,2′-oxybis (ethylamine) (0.02 mL, 0.20 mmol) was added to the reaction mixture. The reaction mixture was stirred at RT for 12 h. The precipitate formed was filtered and washed with cold CH_2Cl_2 to give **2** as a yellow solid (176 mg, 76.6%). m.p. = 200 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 14.53 (s, OH), 8.61 (s, 2H), 8.32 (d, J = 9.10 Hz, 2H), 7.99–7.80 (m, 8H), 7.54 (s, 2H), 7.35 (d, J = 8.98 Hz, 2H), 3.91 (s, 8H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 167.16, 156.66, 132.52, 132.18, 127.00, 127.12, 126.88, 126.81, 126.66, 125.97, 124.77, 124.20, 123.90, 122.96, 121.49, 119.52, 115.34, 69.91, 58.44, 30.92. HRMS (FAB) calcd for $C_{38}H_{29}N_2O_3$ $[M + H]^+$ 561.2100; found 561.2178.

2.3. Preparation of stock solution for fluorescent study

Stock solutions of probe **1** and probe **2** (0.4 mM) was prepared in DMSO. Metal ions stock solutions (10 mM) of the perchlorate salts of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Sr^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} ions in distilled water were prepared.

The test solutions were prepared by placing 75 μL of probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting these stock solutions to 3 mL with distilled water and DMSO.

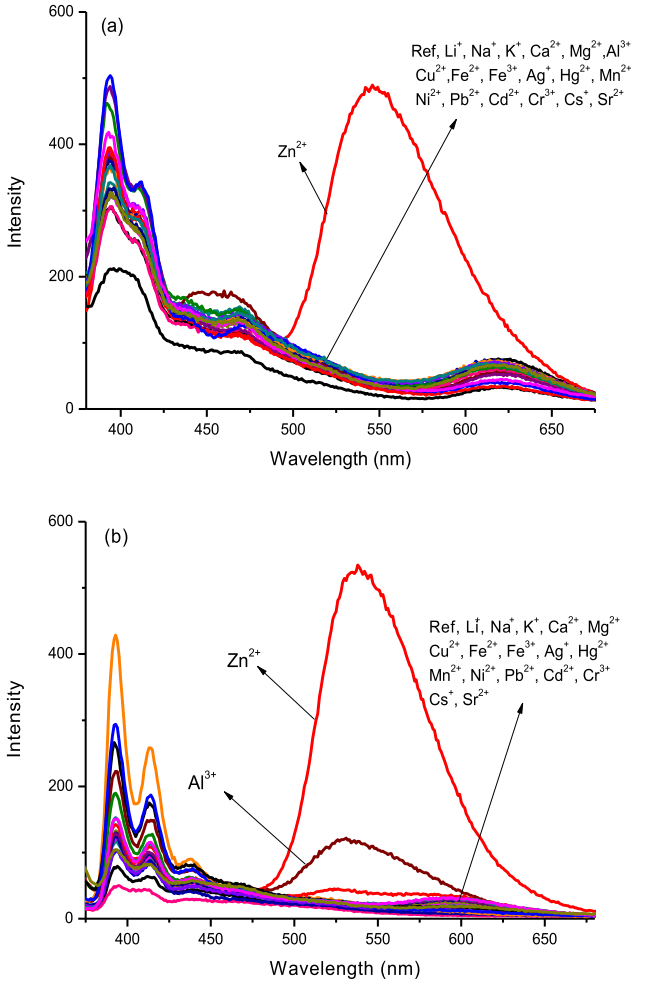

Fig. 2. Fluorescence emission changes of probe **1** (10 μM) (a) and **2** (10 μM) (b) with various metal ions (100 μM) in DMSO-H_2O (1:1, v/v). (λ_{ex} = 355 nm, slit: 5 nm/5 nm).