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A selective '*Off–On*' fluorescent sensor for Zn^{2+} based on hydrazone–pyrene derivative and its application for imaging of intracellular Zn^{2+}

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ABSTRACT

A simple and effective fluorescent sensor based on hydrazone–pyrene has been synthesized. This probe displays a highly selective fluorescent enhancement with Zn^{2+}, and application of this probe to detect the intrinsic Zn^{2+} ions present in pancreatic β-cells was successfully demonstrated.

Fluorescent sensors are powerful tools to monitor ions because of the simplicity and high sensitivity of fluorescence.[1] In particular, the development of a fluorescent probe for zinc ion in the presence of a variety of other metal ions has received great attention.[2,3] Zn^{2+} is involved in a variety of physiological and pathological processes, such as Alzheimer's disease, epilepsy, ischemic stroke, and infantile diarrhea.[4] It is also reported that zinc ion is a potent killer of neurons via oxidative stress.[5] In particular, compared to other tissues, pancreatic islets contains relatively high concentrations of Zn^{2+}, which play a critical role in insulin biosynthesis, storage and secretion.[6] A decrease in the concentration of Zn^{2+} can cause a reduction of the ability of the islet cells to produce and secrete insulin.[7] Accordingly, development of Zn^{2+} selective fluorescent sensors and convenient methods to detect intracellular Zn^{2+} ion are certainly important issues in recent years. The total concentration of Zn^{2+} in different cells varies from the nanomolar range up to about 0.3 mM,[8] which means that optimized chemical probes are required to monitor zinc concentration ranging from nanomolar to micromolar. For example, most of the fluorescence-based probes for Zn^{2+} suffered limitations due to tight binding affinity or lack of sufficient selectivity to detect intrinsic levels of Zn^{2+} in pancreatic islets.

Herein, we report a relatively simple fluorescent sensor **1**, which displayed a selective fluorescence enhancement with Zn^{2+} among various metal ions examined at pH 7.4. Furthermore, we successfully demonstrated that probe **1** can detect the intrinsic Zn^{2+} ions present in pancreatic β-cells and can be used as a potential real time sensor to monitor the intrinsic Zn^{2+} level in pancreatic β-cells.

As shown in Scheme 1, intermediate **4** was synthesized from 1-bromopyrene in three steps by modifying the reported procedure[9] with an improved yield of 45%. Compound **4** was then reacted with hydrazine to give the pyrene–hydrazone derivative **1**[10] in 46 % yield. The detailed experimental procedures and ^1H NMR, ^{13}C NMR spectra are explained in Supplementary data.

To obtain an insight into the sensing properties of **1** towards metal ions, the emission changes were examined with different ions such as Ag^+, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Pb^{2+} and Zn^{2+} in CH_3CN–HEPES buffer (0.02 M, pH 7.4) (4:6, v/v). Fluorescence spectra were obtained by exciting probe **1** at 450 nm. As shown in Figure 1, probe **1** exhibited a selective fluorescence enhancement only with Zn^{2+} with maximum emission at 527 nm (Fig. 1). In contrast, other metal ions did not induce significant fluorescence enhancement of **1**. Probe **1** displayed a remarkable fluorescence enhancement of 12-fold upon the addition of Zn^{2+} (Fig. 2). A Job plot analysis confirmed 1:1 stoichiometry between **1** and Zn^{2+} (Fig. 2). From the fluorescence titration experiments as shown in Figure 2, the association constant was calculated as 2.43×10^4 M^{-1}.[11] The partial ^1H NMR spectra of **1** upon the addition of various amounts of Zn^{2+} are illustrated in the supporting information (Fig. S1). The OH proton and NH_2 protons became broad at room temperature and imine C–H peak as well as pyrene peaks move to upfield region as shown in Figure S1, which support two nitrogens on hydrazone moiety as well as phenolic oxygen may participate in binding with Zn^{2+}.

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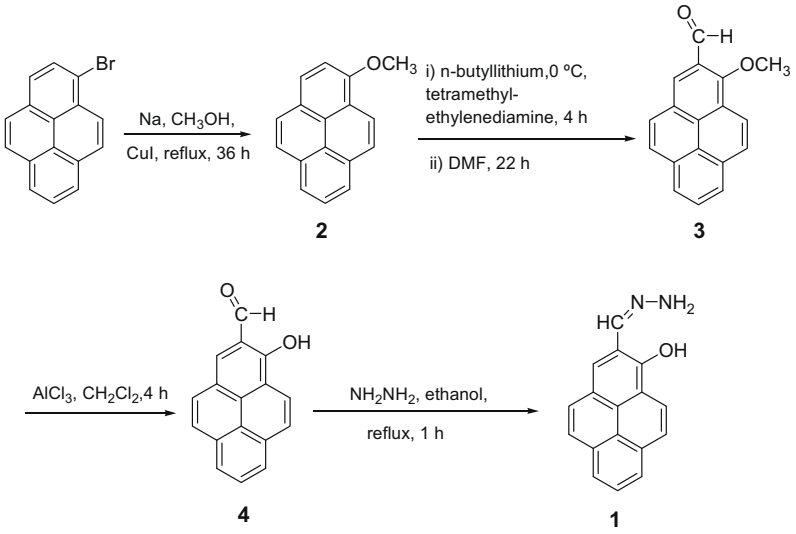

Scheme 1. Synthesis of fluorescent probe **1**.

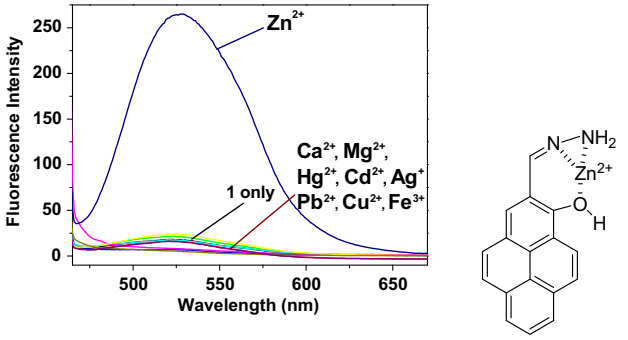

Figure 1. Fluorescent spectra of **1** (20 μM) in CH_3CN–HEPES buffer (0.02 M, pH 7.4) (4:6, v/v) with 20 equiv of various metal ions, such as Ag^+, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Pb^{2+} and Zn^{2+}. Excitation wavelength was 450 nm.

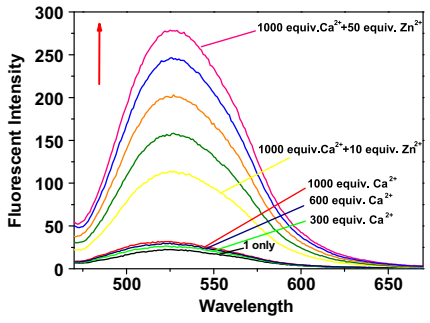

Figure 3. Effect of calcium ion on the binding of zinc ion to probe **1**. Zinc ions (0–50 equiv) were added to solution of **1** (20 μM) containing a large excess of calcium ions (1000 equiv) in CH_3CN–HEPES buffer (0.02 M, pH 7.4) (4:6, v/v).

As shown in Figure S5, there was not any significant change in the absorption spectra upon the addition of different amounts of Zn^{2+}. The weak fluorescence of probe **1** in the absence of Zn^{2+} and the absence of any significant change in absorption spectra upon the addition of Zn^{2+} indicate that large fluorescence enhancement with Zn^{2+} can be attributed to the blocking of photo-induced electron transfer (PET) process from nitrogen in hydrazone moiety to pyrene.[12] As shown in Figure 3, this probe did not respond to Ca^{2+}, a problem associated with other Zn^{2+} sensors. Similar fluorescent intensity of **1** with Zn^{2+} was observed in the presence of large excess Ca^{2+}.

In vitro studies demonstrated the ability of **1** to detect Zn^{2+} with excellent selectivity. To examine whether this ability is preserved for biological application, HaCaT cells were first used to monitor intracellular Zn^{2+} ions. Human keratinocyte cell line, HaCaT were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin. Cells were incubated with Zn^{2+} (10 μM) for

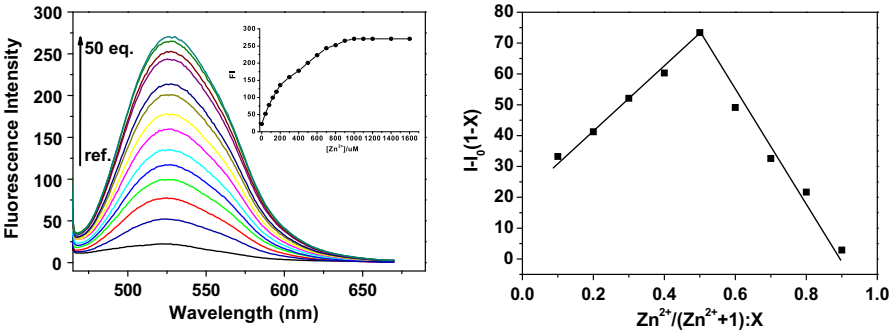

Figure 2. Left: Fluorescent emission spectra of **1** (20 μM) in the presence of different concentrations of Zn^{2+} in CH_3CN–HEPES buffer (0.02 M, pH 7.4) (4:6, v/v). Excitation wavelength was 450 nm. Inset: the fluorescent intensity as a function of Zn^{2+} concentration. Right: the Job Plot using fluorescent intensity at 527 nm of **1** and Zn^{2+}in CH_3CN–HEPES buffer (0.02 M, pH 7.4) (4:6, v/v). Fluorescent intensity was measured at 527 nm and total concentration of [**1**] and [Zn^{2+}] was 100 μM.

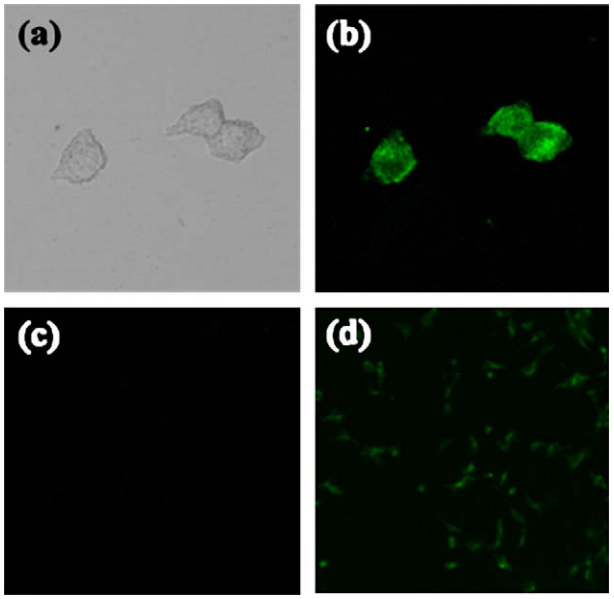

Figure 4. (a) Bright field images of HaCaT cells treated with Zn^{2+} (10 μM) and probe **1** (20 μM). (b) Fluorescence images of (a). (c) Fluorescence images of pancreatic β-cells without probe **1**. (d) Fluorescence images of pancreatic β-cells with probe **1** (20 μM).

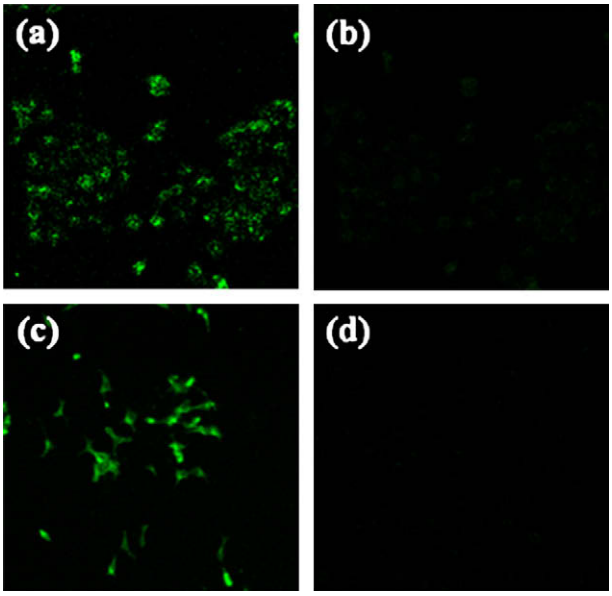

Figure 5. (a) Fluorescence images of HaCaT cell treated with Zn^{2+} (10 μM) and probe **1** (20 μM). (b) Fluorescence images of HaCaT cell treated with Zn^{2+} (10 μM) and probe **1** (20 μM) and subsequent treatment of the cells with 25 μM TPEN. (c) Fluorescence images of pancreatic β-cells with Zn^{2+} (5 μM) and probe **1** (20 μM). (d) Fluorescence image of (c) incubated with TPEN (50 μM).

90 min at 37 °C and washed three times with PBS to remove the remained Zn^{2+} ion. As shown in Figure 4b, cells incubated with 5 μM Zn^{2+} and **1** (20 μM) displayed clear green fluorescence.

These results encouraged us to use our probe **1** to detect biologically relevant intracellular Zn^{2+} ions. For the application of detecting intrinsic Zn^{2+} ion in live cells, pancreatic β-cells (Rin-m cell line), which contains intrinsic Zn^{2+} ions for storage of insulin,[6] were used. Pancreatic β-cell line (Rin-m) were cultured in RPMI1640 medium (containing 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/l glucose, and 1500 mg/l sodium bicarbonate) supplemented with 10% fetal bovine serum at 37 °C in a humidified incubator. The detailed procedures are explained in the supporting information. The results of fluorescence microscopy experiments demonstrate that intracellular Zn^{2+} ions in pancreatic β-cells can be fluorescently detected by using probe **1** (Fig. 4d). In addition, even stronger green fluorescence was detected in presence of exogenous Zn^{2+} with **1** as shown in Figure 4c, which means this probe can be used to detect increase or decrease of intracellular Zn^{2+} ion levels in the live cells.

Then, the cells exposed to **1** and Zn^{2+} were further treated with a membrane-permeable zinc chelator (*N,N,N′,N′*-tetrakis(2-pyridylmethyl) ethylenediamine, TPEN), which is known to decrease the intracellular level of zinc.[13] As shown in Figure 5b, the TPEN treated cells displayed very weak fluorescence, indicating that green fluorescence is caused by response of **1** to intracellular zinc ions. Fluorescence images of pancreatic β-cells with additional exogenous Zn^{2+} (5 μM) and probe **1** (20 μM) are explained in Figure 5c. The results reveal that cells treated with both **1** (20 μM) and external Zn^{2+} ions (5 μM) have a brighter fluorescence response as compared to the case without adding external Zn^{2+} ions. The TPEN treatment also induced the substantial decrease of green fluorescence (Fig. 5d).

In conclusion, we successfully demonstrated that our relatively simple probe **1** a highly selective fluorescence enhancement with Zn^{2+} at pH 7.4. In addition, this probe was successfully applied for imaging intracellular Zn^{2+} ions. Most importantly, we demonstrated this probe can monitor the level of intrinsic Zn^{2+} in pancreatic β-cells.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.028.

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10. *(Z)-2-(Hydrazonomethyl)-4,6-dihydropyren-1-ol* (**1**): A stirred solution of 4 (0.1 g, 0.4 mmol) and 50 μL of hydrazine hydrate in 25 ml ethanol was heated to reflux for 1 h under a nitrogen atmosphere. After the ethanol was evaporated under reduced pressure, the residue was purified by silica gel column chromatography using dichloromethane as eluent to obtain orange powder of **1** in a yield of 80% (0.83 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.62 (s, 2H), 7.87–8.19 (m, 7H), 8.27 (s, 1H), 8.48–8.52 (d, 1H), 12.05 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 121.6, 124.2, 124.9, 125.3, 126.4, 126.5, 127.0, 133.3, 138.2, 147.2, 152.4. HRMS (FAB): m/z = 260.0945 $[M+H]^+$, calcd for $C_{17}H_{12}N_2O$ = 260.0950.
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