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A NOVEL FLUORESCENT “TURN-ON” SENSOR FOR SELECTIVELY DETECTION OF Pb^{2+} [**]

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A distinctive fluorescent probe based on 2-(3-aminopropyl) isoindoline-1, 3-dione (probe ***1****) has been developed and synthesized. Probe* ***1*** *was applicable for sensing lead ion in C_2H_5OH with high binding constant (1.1×10^7 M^{-1}) and sensitivity down to 3.47 μM (3/slope) (limit of detection). The linear response was determined to be in the range of 0–9.0 μM. These results demonstrated highly selective and sensitive detection for lead ion of probe* ***1.***

Keywords: *detection technology, fluorescent probe, selectivity, sensitivity, limit of detection.*

ФЛУОРЕСЦЕНТНЫЙ ЗОНД ДЛЯ СЕЛЕКТИВНОГО ОБНАРУЖЕНИЯ Pb^{2+}

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*Разработан и синтезирован флуоресцентный зонд на основе 2-(3-аминопропил)изоиндолин-1,3-диона (****1****). Зонд* ***1*** *применен для зондирования ионов свинца в C_2H_5OH с высокой константой связывания (1.1×10^7 M^{-1}) и чувствительностью до 3.47 μM (3/наклон) (предел обнаружения). Отклик линеен в диапазоне 0–9.0 μM. Показано, что зонд* ***1*** *является высокоселективным и чувствительным датчиком для обнаружения иона свинца.*

Ключевые слова: *метод обнаружения, флуоресцентный зонд, селективность, чувствительность, предел обнаружения.*

Introduction. It is known to us that mercury, cadmium, and lead are highly poisonous heavy metal and play a useful role in the living body owing to the emissions of hazardous pollutants [1–4]. Lead accumulated in body could disturb a variety of body processes, and it is a persistent threat to human health due to the fact that lead has a deleterious effect on various issues and organs such as the cardiovascular, nervous, and immune systems [2, 5]. Light exposure to lead can bring about behavioral abnormalities, learning impairment, decreased hearing, high blood pressure, kidney disorders and neurological impairment ankles, fingers and reduces fertility in males [5–8]. An alarming is that children are the most threatened for lead exposure which can affect the brain development, neurochemical development and formation of the cerebral cortex [9–11]. Therefore, substantial attention has been paid in developing the realistic techniques for the detection of lead. Although traditional methods such as anodic stripping voltmeter (ASV) [12], atomic absorption spectrometry (AAS) [13], inductively coupled plasma mass spectrometry (ICP-MS) [14] and inductively coupled plasma atomic emission spectroscopy (ICP-AES) [15] have been proposed for the detection of Pb^{2+}, they are

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usually not suitable for deep level applications with various disadvantages like time-consuming, sophisticated and expensive. In contrast, fluorescence method has been enabled for lead ions with the development of new detection process, owing to the advantages of cost-effectiveness, high sensitivity, specificity and realistic operation [16–19].

It is pointed out that a large quantity of probes for lead ion [20–23]. However, there are also many disadvantages and challenges such as rigorous synthetic procedures, insufficient water solubility and low selectivity and sensitivity with metal ions. In addition, the turn-off probes which caused fluorescence quenching through electron transfer or energy [24] and the spin-orbit coupling effect [25] can always be disturbed by miscellaneous factors expressed by low selectivity and sensitivity [26–28]. Hence, searching for fluorescent probe of Pb^{2+} with fluorescence enhancement and specificity is still a promising as well as a challenging realm for the analytical chemists.

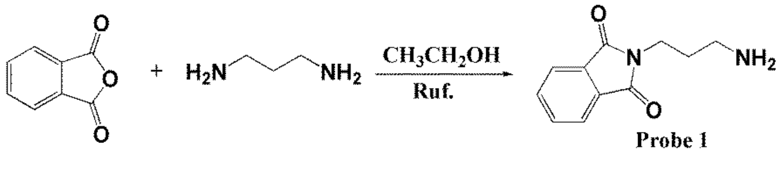
In this work, we reported the synthesis, spectroscopic characterization and mechanism research of efficient turn-on fluorescent probe **1** (Named: 2-(3-aminopropyl)isoindoline-1,3-dione). As expect, probe **1** could detect Pb^{2+} independently without any interference of metal ions as well as the other anions in C_2H_5OH solution.

Experimental details. *Reagents and equipment.* All chemical reagents used in the research were analytical grade without further purification. Cation solutions were prepared from $CrCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, KCl, NaCl, $CaCl_2{\cdot}6H_2O$, $MgCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, and so on. Similarly, the anions solutions were prepared from NaCl, NaBr, NaF, $NaNO_3$, $NaNO_2$, $Na_2C_2O{\cdot}4H_2O$, Na_3PO_4, and so on. All the salt above being purchased from Shanghai, China.

The ^1H NMR spectra was conducted in DMSO-d_6 on a Bruker DRX-400 spectrometer with TMS as the internal standard. Electrospray ionization mass spectra (ESI-MS) were recorded on a Triple TOF^{TM}5600+ system. Fluorescence spectra were measured on Varian Cary 20 Eclipse spectrophotometer.

Synthesis of probe 1. The probe **1** was synthesized following the reported procedure with some modification. Isobenzofuran-1, 3-dione (296.02 mg, 2.0 mmol) was dissolved in CH_3CH_2OH (20 mL) and propane-1,3-diamine (334 μL, 4.0 mmol) diluted with CH_3CH_2OH (10 mL) was added in. Then the resulting mixture was stirred for about 12 h. The crude product of Probe **1** was obtained and washed by distilled H_2O and CH_3CH_2OH for three times respectively. Elemental analysis for $C_{11}H_{12}N_2O_2$: calculated (%): C 64.69, H 5.92, N 13.72; found (%): C 64.65, H 6.08, N 13.71. IR (cm^{-1}): 3511 m, *ν* (N-H), 2878 s, *ν* (C-H), 1715 m, *ν* (C=O). ESI-MS *m/z*: calculated $C_{11}H_{12}N_2O_2$ (204.0899); Experimental: 237.1239, [probe **1** + H + $CH_3OH]^+$. ^1H NMR (400 MHz, DMSO-d_6); δ 8.19 (d, *J* = 8.9 Hz, 2H), 7.66 (dt, *J* = 10.2 Hz, 2H), 3.23 (d, *J* = 8.5 Hz, 2H), 2.77 (dd, *J* = 6.4, 3H), 3.07 (dd, *J* = 2.1 Hz, 2H), 2.45–2.40 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 139.24, 128.55, 123.62, 56.50, 29.93, 29.11.

Results and discussion. *Synthesis and structural characterization of probe 1.* As shown in Scheme 1, probe **1** was obtained by the reaction of isobenzofuran-1,3-dione and propane-1,3-diamine in CH_3CH_2OH:



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

the structure of probe **1** was confirmed by FT-IR spectra (IR) analysis, ^1H NMR, ^{13}C NMR, elemental analyses (EAs) and electrospray ionization mass spectra (ESI-MS).

Fluorescence study of probe 1 with various metal ions. The selective ability of probe **1** toward Pb^{2+} was investigated in C_2H_5OH at room-temperature with the concentration of Pb^{2+} was 1.0 μM. A wide range of metal ions were investigated using fluorescence spectra with the solutions containing probe **1** (1.0 μM) and the metal ions (20 equiv.) in C_2H_5OH. As shown in Fig. 1a, the tested metal ions such as Ba^{2+}, Ca^{2+}, Cr^{3+}, Mg^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, Na^+, K^+, Ni^{2+} and so on did not induce any apparent changes in fluorescence emission when exited at 483 nm. While the Pb^{2+} was added, a notable fluorescence enhancement was found at 528 nm. Therefore, probe **1** was a good probe for Pb^{2+} in C_2H_5OH. Following the same method, the anions (PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, SO_4^{2-}, $C_2O_4^{2-}$, CO_3^{2-}, ClO^-, et al.) could not cause any fluorescence signal when they are added to probe **1** in C_2H_5OH. Besides, we developed the selectivity performance of probe **1** in the presence of various metal ions. From Fig.1b, nearly 50-folds fluorescence enhancement was observed when Pb^{2+} was added in.

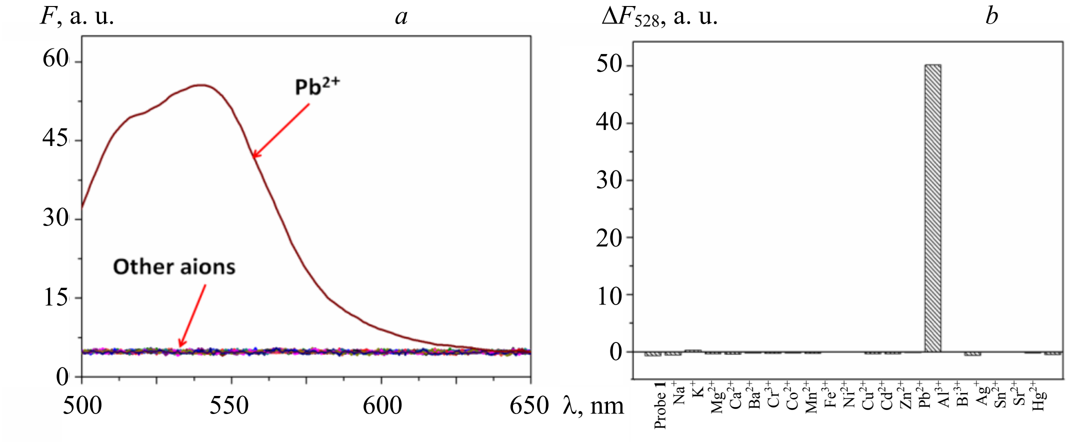


Fig. 1. a) Fluorescence responses of probe **1** upon the addition of several metal ions in C_2H_5OH; b) Fluorescence intensity of the complexation probe **1**-Pb^{2+} in the presence of various metal ions in C_2H_5OH; $\lambda_{ex} = 483$ nm, $\lambda_{em} = 528$ nm, slit: 3 nm/5 nm.

*Fluorescence spectra of probe **1** and Pb^{2+}*. The sensing ability of probe **1** towards Pb^{2+} was conducted in C_2H_5OH at room-temperature. The fluorescence spectra titration for Pb^{2+} was obtained by probe **1** (2 μM) was put in a quartz cell (10.0 mm width) and 4 μL Pb^{2+} (1×10^{-3} M) was added gradually. As shown in Fig. 2, probe **1** exhibited no obvious fluorescent signal when excited at 483 nm in C_2H_5OH. However, the fluorescence of probe **1** in C_2H_5OH was dramatically increased with the addition of Pb^{2+}. The fluorescence absorption peak shifted from 540 to 528 nm with the concentration of Pb^{2+} increasing. We could see an obvious color change from colorless to pink under illumination with a 365 nm UV lamp.

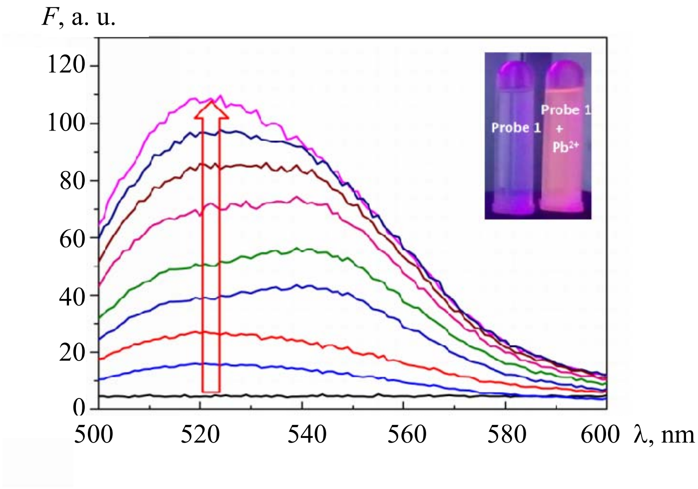


Fig. 2. Fluorescent spectral changes of probe **1** (2 μM) upon addition of Pb^{2+} in C_2H_5OH at room-temperature. Inset: The visible fluorescence changes upon UV irradiation.

*UV-vis titration spectra of probe **1** and Pb^{2+}*. The UV-vis titration spectra of probe **1** and Pb^{2+} was assayed in C_2H_5OH at room-temperature for describing the interaction effect in details. A solution of probe **1** (2 μM) was placed in a quartz cell (10.0 mm width) with 2000 μL C_2H_5OH and 2 μL Pb^{2+} (1×10^{-3} M) was added gradually, then the UV-vis spectra titration for Pb^{2+} was obtained. As shown in Fig. 3, a peak at 279 nm was gradually reinforced with the incremental addition of Pb^{2+}. The phenomena indicated that probe **1** can combine with Pb^{2+} to form a stable complex for detecting technology.

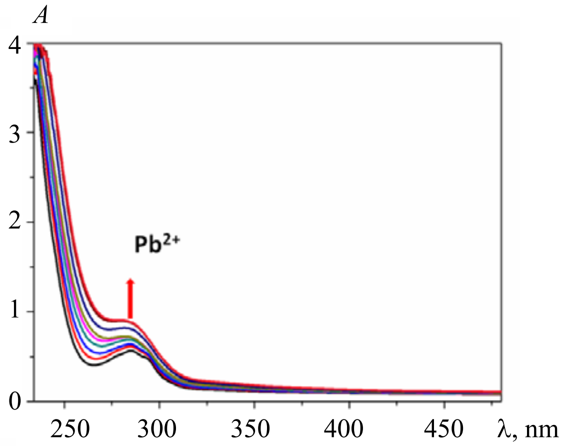
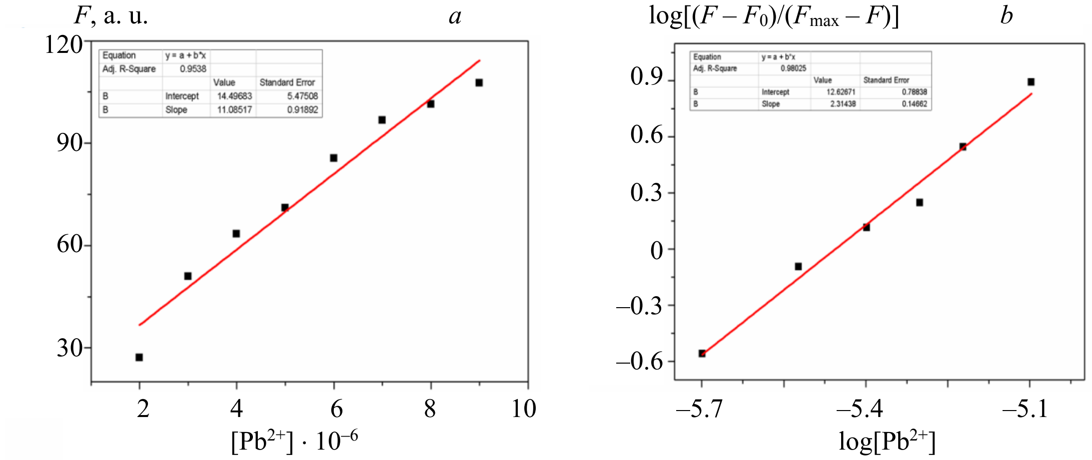


Fig. 3. UV–vis absorbance spectra of probe **1** (2 μM) in the presence of different amounts of Pb^{2+} (0–6 μM) in C_2H_5OH.

The determination of LOD and binding constant. As shown in Fig. 4, the different concentration of Pb^{2+} linear response range from 0 to 200 nM for quantitative determination was observed. A nonlinear least-squares curve fitting of the plot of Log$\{(F - F_0)/(F_{\max} - F)\}$ to Log$[Pb^{2+}]$ indicated the binding ratio between probe **1** and Pb^{2+} was 1: 1, and the binding constant (K) was calculated as 1.1×10^7 M^{-1}. Accordingly, the detection limit was 3.47 μM for probe **1**-Pb^{2+} complex which was calculated by linear fitting of fluorescence titration results in Benesi-Hildebrand equation.



Equation	y = a + b*x		
Adj. R-Square	0.9538		
		Value	Standard Error
B	Intercept	14.49683	5.47508
B	Slope	11.08517	0.91892

Equation	y = a + b*x		
Adj. R-Square	0.98025		
		Value	Standard Error
B	Intercept	12.62671	0.78838
B	Slope	2.31438	0.14662

Fig. 4. a) Fluorescent spectral changes of probe **1** (1.0 μM) upon addition of Pb^{2+} in C_2H_5OH at room-temperature; b) plot of $\log[(F - F_0)/(F_{\max} - F)]$ vs $\log[Pb^{2+}]$ for probe **1**; $\lambda_{ex} = 483$ nm.

Proposed mechanism. The ESI-MS is validly used to analysis the product between probe **1** and Pb^{2+} in CH_3OH. Figure 5 exhibited the complex P**1** between Probe **1** and Pb^{2+}, the peak at $m/z = 285.9432$, agreeing to $[P1 + CH_3OH + H_2O + H]^+$ (calc. 285.9372) was undoubtedly detected, which suggested that the -NH_2 of probe **1** was oxidized to -NO_2 due to the oxidizability of Pb^{2+}. Then the proposed mechanism of probe **1** and Pb^{2+} was deduced as Fig. 6.

Comparison with other probes. Above all, probe **1** possesses specificity and high associativity in detecting trace Pb^{2+} under C_2H_5OH solution. These appearances are similar to or better than most of the recently reported methods. As showed in Table 1, while there are several probes possess lower LOD, they may have the progress of fluorescent quenching compared with those reported. However, the application of probe **1** in living cells would remain to be further investigated.

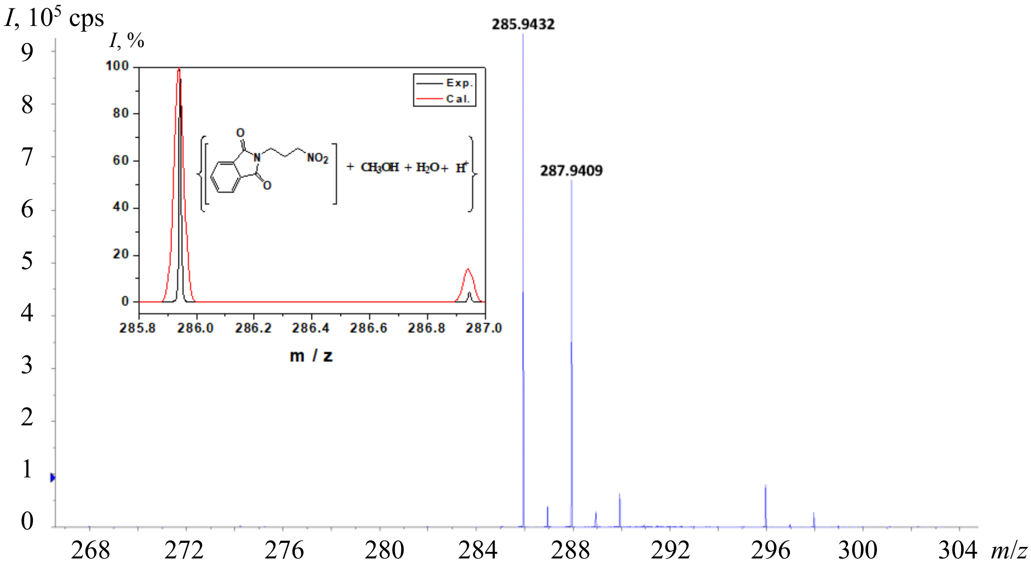


Fig. 5. The ESI-MS spectra of probe **1** and Pb^{2+} positive ion mode in methanol.

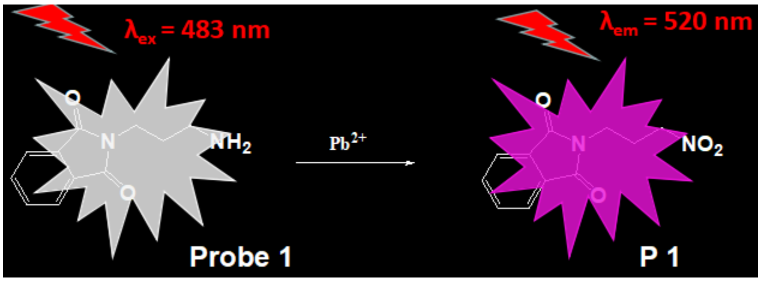


Fig. 6. The proposed determination mechanism of probe **1** and Pb^{2+}.

TABLE 1. The Comparison of Probe **1** with Other Reported Probes in the Literature

LOD, μM	Linear range, μM	Reaction media	Binding constant, M^{-1}	Change of signal	Methods
–	0–2.3	Acetonitrile:water (99:1)	–	Turn on	[29]
200	–	CH_3CN	–	Turn on	[30]
–	–	H_2O:DMSO (3:2)	7.7×10^3	Turn off	[31]
0.5	–	Acetonitrile: H_2O (9:1)	–	Turn on	[32]
0.83	1.6–10.0	Ethanol:HEPES (1:5, pH 7.4)	–	Turn off and red shift	[33]
2.7×10^{-3}	0.01–10	HEPES solution	7.86×10^8	Turn on	[34]
0.38	–	CH_3CN:H_2O (95:5)	2.1×10^4	Turn on	[35]
0.0173	–	CH_3CN	6.68×10^4	Turn off	[36]
3.47	0.2	C_2H_5OH	1.1×10^7	Turn on	This work

Conclusion. A novel sensor for fluorescent “turn-on” detection of Pb^{2+} in C_2H_5OH with high sensitivity and selective specificity was obtained. The detection limit was reckoned at 3.47 μM displaying a higher sensitivity ability towards Pb^{2+} than some reported probes. The binding constant (K) of probe **1** and Pb^{2+} was determined to be 1.1×10^7 M^{-1} which specified that probe **1** displayed high affinity toward Pb^{2+}.

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