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A Highly Sensitive and Selective Turn-on Fluorescent Probe for Pb(II) ion based on Coumarin-quinoline Platform

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A new fluorescent probe T1, which contained coumarin fluorophore with a triazole substituted 8-hydroxyquinoline (8-HQ) receptor and a Schiff base spacer, was rationally designed and synthesized. It displayed a high affinity towards Pb^{2+} ions with a dissociation constant (K_d) of 0.1 μM and possessed a high selectivity for Pb^{2+} ions. A visually detectable colour change from colourless to yellow and 30-fold fluorescence enhancement were observed upon addition of Pb^{2+}. Experiments also indicated that a 1:1 stoichiometry of T1 with Pb^{2+} inhibited photo-induced electron transfer (PET) process, resulting in the increase of fluorescence intensity.

Lead ions, extremely toxic heavy metal ions, could cause great threat to human health because they can enter into the human body and accumulate through the food chain, and they are difficult to be excreted. [1-2] Exposure to even small amounts of lead ions can cause health problems, such as digestive, neurological and cardiovascular disorders. For children, it can bring some particular problems including slowed motor responses, decreased IQs, and hypertension. [3] The safety concentration of lead ions in aquatic organisms is 0.16 mg/L and the normal level in human body should be below 0.1 mg/L. Fluorescent probes are becoming popular because of their ease of application in solution as well as their high sensitivity and selectivity for trace metal ions, [4] anions [5] and small molecules. [6] However, Pb^{2+}, as well as Cu^{2+} ions and Hg^{2+} ions, is known as a kind of inherent fluorescence quenching metal ion, [7-8] so few studies were available for "OFF-ON" fluorescent probes which can recognize Pb^{2+} ions [9] with high selectivity and sensitivity.

We herein presented a novel "OFF-ON" fluorescent probe **T1** for Pb^{2+} ions detection. **T1** displayed high selectivity for Pb^{2+} ions over other metal ions and displayed visually detectable colour change from colourless to yellow with 30-fold fluorescence enhancement. Receptor is an important factor when designing new fluorescent probes. As a fluorophore, 8-hydroxyquinoline (8-HQ) was widely used in fluorescent probes presenting great advantages in bio-imaging. [10] But it had some intrinsic disadvantages such as short emission wavelength, low fluorescence quantum yield, poor stability and solvent polarity-dependent emission. Recently 8-HQ was introduced into fluorescent probes acting as a metal ion chelating agent for metal ions detection [11], but most of these probes were using 2-/7- modified 8-HQ as the receptors. 8-substituted 8-HQ was rarely involved in fluorescent probes construction because the alkylation of 8-OH in 8-HQ might sacrifice efficient ligating hydroxyl group. Previously, our group reported two fluorescent probes for cadmium ion [12] recognition in which 8-OH moiety was substituted by 2-methylpyridine. Although both of these two probes could specifically recognize cadmium ions, the low affinity might limit their practical applications.

Motivated by these studies, we used triazole to substitute 8-OH in 8-HQ as the receptor. Schiff base structure was built between 2-formyl substituted 8-HQ and 3-amino coumarin. Trizole substituted 8-HQ could furnish an extra binding site, and might inhibit the possible excited-state intramolecular proton transfer (ESIPT) event. 8-HQ derivative was connected with coumarin by C=N structure. Coumarin dye was employed as fluorophore due to its outstanding photophysical properties and easy modification [13]. The C=N structure might provide another binding state, which could improve the selectivity. As expected, the probe **T1** presented excellent selectivity and sensitivity towards Pb^{2+} that caused an "OFF-ON" response with a 30-fold fluorescence enhancement.

The elaborately designed probe **T1** was synthesized based on the route listed in **Scheme 1**. 8-hydroxyquinoline-2-carboxaldehyde (8-HQC) was obtained from 2-methyl-8-hydroxyquinoline oxidation product by using SeO_2 as oxidizer. The hydroxyl group of 8-HQC substituted by bromoacetylene gave 8-pAl-HQC. Triazole was successfully introduced after alkynyl reacted with NaN_3 based on 'click reaction' [14] principle. 8-BnT-HQC was obtained after this step. At last, the reaction between formyl group of 8-BnT-HQC and amino group of coumarin devirative afforded the desired product **T1**,

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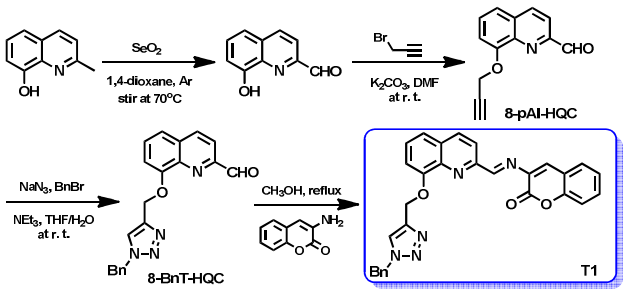
and it was well characterized by ^{1}H-NMR, MS and IR. Detailed experimental steps and related characterizations were shown in ESI† (Fig. S1-S3).



Scheme 1 Synthesis route of T1.

As shown in **Fig. 1**, **T1** in aqueous HEPES (CH_3CN: H_2O = 95:5, v/v, 10 mM HEPES, pH 7.2) exhibited two main absorption bands located at 240 nm ($\varepsilon = 5.3 \times 10^4$ M^{-1} cm^{-1}) and 323 nm ($\varepsilon = 3.1 \times 10^4$ M^{-1} cm^{-1}), respectively. Both of these two bands were attributed to π-π* transition resulted from PET (photo-induced electron transfer) process. [12] Upon addition of Pb^{2+}, the absorbance at 240 nm and 323 nm decreased gradually, along with the emergence of two bands at 260 nm and 385 nm. The presence of three well-defined isobestic points at 250 nm, 307 nm and 340 nm indicated the formation of **T1**-Pb^{2+} complex. These changes were associated with a visually detectable colour change from colourless to bright yellow (inset of Fig. 1).

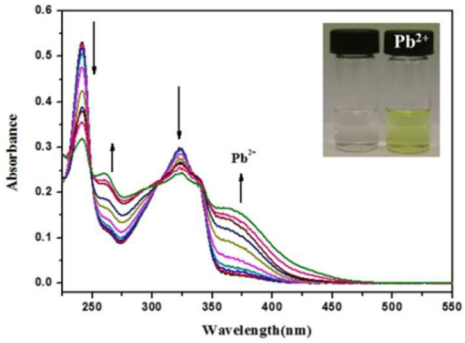


Fig. 1 UV-Vis absorption spectral changes of **T1** (20 μM) in buffer solution (CH_3CN:H_2O = 95:5, v/v, 10 mM HEPES, pH 7.2) upon addition of Pb^{2+} (0 to 40 μM). Inset: visible colour changes of **T1** in the absence (left) and presence (right) of Pb^{2+} (2 equiv.).

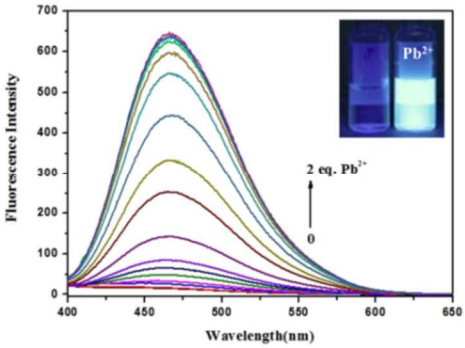


Fig. 2 Fluorescence spectral changes of **T1** (20 μM) in buffer solution (CH_3CN:H_2O = 95:5, v/v, pH 7.2) upon addition of Pb^{2+} (0 to 40 μM), λ_{ex}=385 nm. Inset: Fluorescence color changes of **T1** in the absence (left) and presence (right) of Pb^{2+} (2 equiv.) using a handheld UV lamp.

The fluorescence titration spectra of **T1** with Pb^{2+} were shown in **Fig. 2**. **T1** exhibited very weak fluorescence with a low fluorescence quantum yield (0.0038) using quinine sulfate in 0.5 M H_2SO_4 as the reference [15] when excited at 385 nm. It was ascribed to the PET process from lone electron pair in nitrogen atom of triazole and quinolone to coumarin, which quenched fluorescence of **T1**. Upon addition of Pb^{2+}, **T1** showed a turn-on response and the emission band located at 466 nm increased gradually. Titration of **T1** with Pb^{2+} (0-2 equiv.) in buffer solution resulted in a 30-fold enhancement in fluorescence intensity. The clear "OFF-ON" response was attributed to that the complexation of **T1** and Pb^{2+} suppressed the PET process from triazole-quinolone to coumarin, the electron-donating ability of nitrogen atom was effectively suppressed, restraining PET process and resulting in a significant increase in fluorescence emission. Additionally, the addition of Pb^{2+} made the fluorescence colour clearly change from colourless to blue. The apparent disassociation constant (K_d) of **T1** with Pb^{2+} was 0.1 μM implied that Pb^{2+} had a high affinity toward Pb^{2+}. This value is better than that of most reported fluorescent probes (Table S1). The reaction of **T1** with Pb^{2+} followed a 1 : 1 stoichiometry, as confirmed by a Job's plot of the emission spectra as a function of molar fraction of **T1** (Fig. S4) and ESI-MS (Fig. S2). The fluorescence intensity of T1 shows a good linear response to Pb^{2+} in the concentration range of 6 to 20 μM, with a regression equation of I_{466} = 32.098 × [Pb^{2+}] (μM) - 44.1 (Fig. S6). The detection limit (3S/m [16], where S is the standard deviation of 15 blank measurements, and m is the slope of the linear equation) was determined to be as low as 33.6 nM, which is comparable with other groups' work. [17] Besides, the stability of the imine of T1 in buffer solution was also investigated. The fluorescent intensity at 466 nm of T1 and T1-Pb^{2+} barely changed within 3 h, suggesting that the monitoring system is stable (Figure S7) [18].

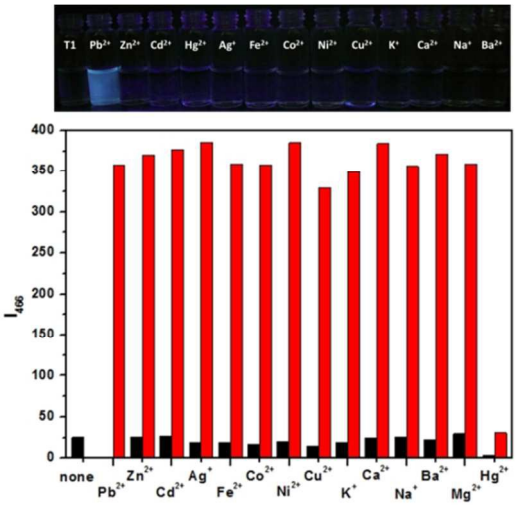


Fig. 3 Selectivity and competition studies of **T1** (20 μM) with 1 eq. Pb^{2+} in the presence of 2 equiv. other metal ions (Zn^{2+}, Cd^{2+}, Ag^+, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, K^+, Ca^{2+}, Na^+, Ba^{2+}, Mg^{2+}, Hg^{2+}) in buffer solution (CH_3CN:H_2O = 95:5, v/v, 10 mM HEPES, pH 7.2). Top: Fluorescence changes of **T1** in the presence of various metal ions using a handheld UV lamp.

Selectivity is an important indicator to evaluate a fluorescent probe. To verify specific recognition to Pb^{2+} ions, the emission spectra of **T1** response to other heavy, transition, and main group metal ions were recorded as well. As shown in **Fig. 3**, addition of

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Pb^{2+} ions caused remarkable fluorescence changes, whereas other metal ions Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^{+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, K^{+}, Ca^{2+}, Na^{+} and Ba^{2+} exerted no or little change on emission of **T1** which testified that **T1** had a good selectivity towards Pb^{2+}. Moreover, a competition experiment was also carried out to further explore the utility of **T1** as an ion-selective probe for Pb^{2+} ions. As illustrated by the red bars in **Fig. 3** below, a significant level of fluorescence was observed for Pb^{2+} ions as compared with other metal ions. The coexistent metal ions had a negligible influence on the fluorescence intensity, whereas the existent Hg^{2+} ions didn't cause fluorescence enhancement. Possibly, it is because Hg^{2+} possessed a higher affinity than Pb^{2+} toward **T1**, and Hg^{2+} could quench fluorescence via CHEQ (chelation-enhanced fluorescence quenching) effect which was observed in other probes. [19] Both C=O in coumarin and triazole provided binding sites, causing a stronger binding of T1 with Hg^{2+} [20]. The distinct responses of selectivity and competition studies indicated that **T1** could be used to distinguish Pb^{2+} ions over many other metal ions.

The binding mode of **T1** and Pb^{2+} was further investigated by ^{1}H-NMR in CD_3CN. As shown in **Fig. 4**, the chemical shifts of **T1**-Pb^{2+} had significant down field shift from 8.39 ppm to 8.82 ppm for H_a and from 5.08 ppm to 5.20 ppm for $H_{b,\ c}$, whereas other protons showed no or little changes. Similar phenomena were also found in other reported probes. [16b] Therefore, we deduced that one nitrogen atom of triazole close to methylene, oxygen atom, nitrogen atom of quinolone and nitrogen atom of HC=N structure together participated in binding with a Pb^{2+} ion. However, oxygen atom of carbonyl group in coumarin might be another binding site, and this hypothesis couldn't be confirmed from ^{1}H-NMR titration results. Then IR was employed to examine the binding mode (Fig. S2). The distinct vibration located at ~3260-3330 cm^{-1} and ~1713 cm^{-1} in IR spectrum of **T1** were respectively assigned to C-H stretching vibration of HC=N structure and C=O stretching vibration of coumarin. When binding with Pb^{2+} ion, a new significant vibration located at ~1088 cm^{-1} appeared which was attributed to C=N stretching vibration of HC=N structure. It is because C=N was fixed and showed strong IR signal upon binding with Pb^{2+} ions. The vibration wavelength corresponding to C=O stretching vibration kept stable before and after binding with Pb^{2+} ions, which indicated that C=O didn't provide a Pb^{2+} binding site. Additionally, the vibration located at ~3260-3330 cm^{-1} exhibited an obvious change. The MS results further verified the binding ratio of 1:1 between **T1** and Pb^{2+} as mentioned above (Fig. S2).

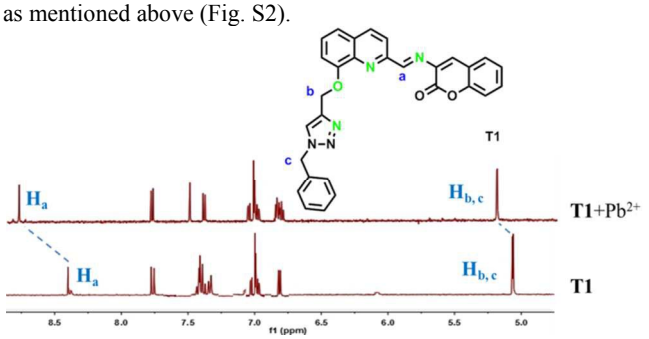

Fig. 4 ^{1}H NMR spectra of T1 in the absence and presence of Pb^{2+} in CD_3CN.

Based on the results of ^{1}H-NMR, IR and MS, we speculated that Pb^{2+} bond with **T1** as N-O-N-N-Pb^{2+} model, as shown in Fig. 5. The existence of a PET process from triazole-substituted 8-HQ to coumarin led to weak fluorescence of **T1**. Upon binding with Pb^{2+}, PET process was effectively suppressed, causing a considerable increase in fluorescence intensity and realizing an "OFF-ON" response toward Pb^{2+}. The colour changes from colourless to bright yellow in absorption, colourless to blue in emission were visually detectable.

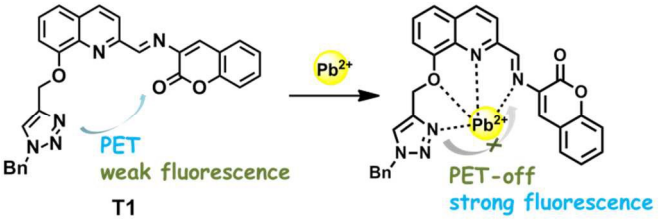

Fig. 5 Proposed binding mode of T1 toward Pb^{2+}.

In conclusion, we have designed and synthesized a new fluorescence probe for Pb^{2+} based on a PET mechanism. Probe **T1** exhibited high selectivity and sensitivity towards Pb^{2+} with 30-fold fluorescence intensity enhancement (OFF-ON response) because of the formation of Pb^{2+}-**T1** complex with a binding ratio of 1:1. It could availably distinguish Pb^{2+} over other metal ions. The binding event between **T1** and Pb^{2+} resulted in detectable colour change from colourless to bright yellow in absorption, colourless to blue in emission. Hence, probe **T1** possessed great potential in preliminary rapid detection of Pb^{2+}.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Detailed experimental procedures, synthesis and characterization data, additional spectroscopic data available. See DOI: 10.1039/b000000x/

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