



Fast and Reversible “Turn on” Fluorescent Sensors Based on Bisphenol-a for Zn²⁺ in Aqueous Solution

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Abstract

Two novel bisphenol-A derivatives (**R1** and **R2**) linked pyrene and naphthylthiazole moieties were synthesized via condensation reaction, and positively applied for the selective recognition of Zn²⁺ ion in EtOH/H₂O. Their optical properties were observed by using UV-vis and fluorescence measurements. **R1** and **R2** exhibited high selectivity and sensitivity towards Zn²⁺ over other metal ions. This fluorescence selectivity may be owing to inhibited excited-state intramolecular proton transfer (ESIPT) and photoinduced electron transfer (PET). The fluorescence titration analysis indicated detection limits of **R1** and **R2** for Zn²⁺ at 17.5 nM and 0.94 μM, respectively. Moreover, **R1** and **R2** were successfully applied to the detection of Zn²⁺ with different concentrations in water samples.

Keywords Fluorescence · Turn on · Bisphenol a · Zinc

Introduction

Zinc is a known trace element and cofactor of more than 300 human metalloenzymes involved in many physiological processes [1]. Moreover, its potential therapeutic activity for the treatment of various diseases is extensively investigated. However, many physiological and therapeutic mechanisms of action of zinc are not yet well understood, as the d10 configuration of Zn²⁺ hinders detection by usual spectroscopic techniques [2–5]. Modern industrial development has caused elevated concentrations of heavy metals, including Zn²⁺, which leads to toxicity of soil and inhibits plant and animal growth. Although Zn²⁺ plays vital roles in physiological and pathological processes [6], excessive amounts of zinc in human cause many severe diseases such as Alzheimer’s disease [7], ischemic stroke [8] and epilepsy [9]. Thus, there is a need for rapid response, effective detection and reliable identification of trace levels of Zn²⁺ in the environment and biological

samples. In this sense, the development of fluorescent sensors for the detection of zinc constitutes a very active field of research. In addition, fluorescence as a signaling technique outstrips other techniques in terms of simplicity, sensitivity and selectivity [10–16].

Fluorescent sensors are commonly composed of two structural subunits: a fluorophore and an ionophore, and both are connected through a spacer or also known as a linker [17, 18]. So far, fluorescence sensors bearing different fluorophores for Zn²⁺ have been reported, such as rhodamine [19], coumarin [20], BODIPY [21], fluorescein [22]. Bisphenol A (BPA), chemically designated as 2,2-bis(4-hydroxyphenyl) propane is an endocrine disrupting chemical, and although it is commonly used as intermediate in variety of consumer products like polycarbonate plastics and epoxy resins [23], the use of bisphenol-A as fluorophore is very scarce [24, 25]. Therefore, we think that the Schiff base (C=N) derivatives of bisphenol-A as fluorophore and pyrene or naphthylthiazole units as ionophores would be interesting to detect metal ion. In the excited states, an unfixed C=N structure is non-fluorescent owing to the predominant decay process of C=N isomerization [26]. After complexation with metal ions, its fluorescence increases strongly since it restricts the rotation of the C=N bond, which induce to the suppression of C=N isomerization.

We herein reported the synthesis and Zn²⁺ recognition properties of two new bisphenol-A based on fluorescent receptors (**R1** and **R2**). Receptors **R1** and **R2** exhibited selective

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emission responses to Zn^{2+} in a mixture of EtOH/H₂O solution with low detection limits.

Experimental

Materials and Method

The chemicals used in experiments were purchased from commercial suppliers. ¹H NMR (Proton Nuclear Magnetic Resonance), ¹³C NMR (Carbon Nuclear Magnetic Resonance), COSY (Correlation Spectroscopy) and APT (Attached Proton Test) spectra were recorded on Varian 400 MR in DMSO-*d*₆ (Dimethyl sulfoxide-*d*₆) and CDCl₃ (Chloroform-*d*) as solvents. Bruker FTIR (Fourier-Transform Infrared Spectroscopy) instrument was used to FTIR spectra analysis. Absorption spectra were measured by using a Shimadzu 1280 ultraviolet visible spectrophotometer at room temperature. A Perkin Elmer LS 55 instrument was used for fluorescence studies. The fluorescence spectra were recorded with the excitation wavelength of 365 nm by 5 nm slit. All pH measurements were made with a Crison Basic 20 pH meter.

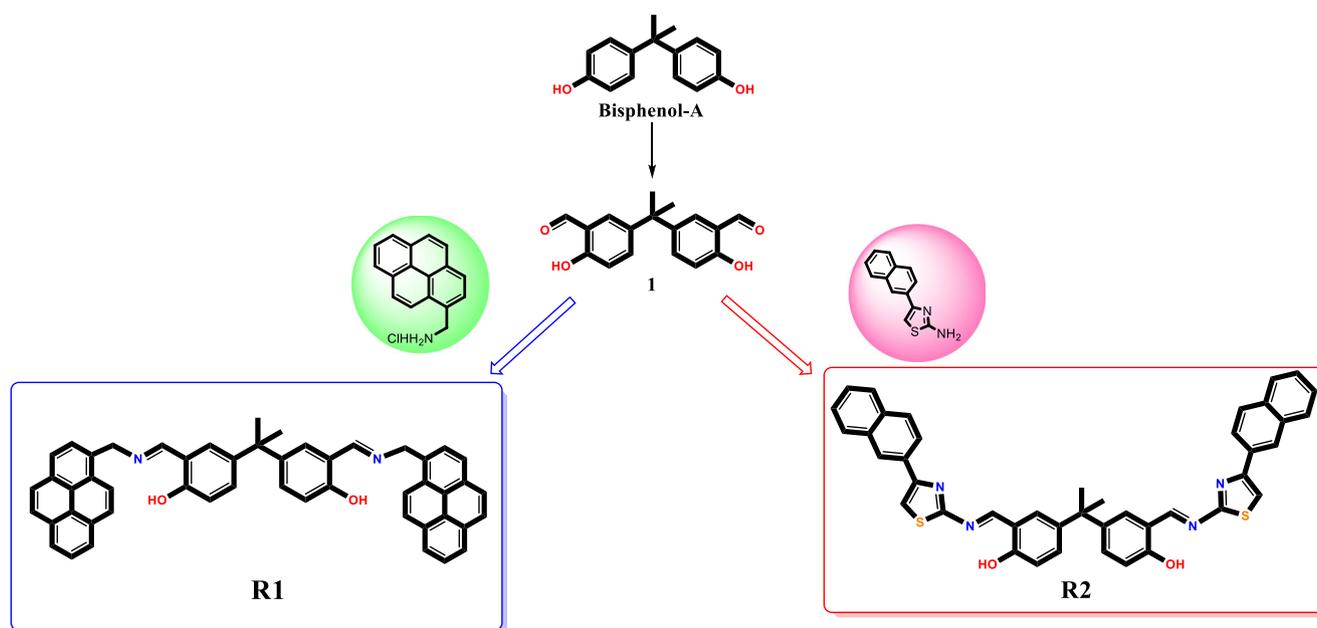
Synthesis Procedure of R1 and R2

The bisphenol A-dialdehyde (1) was synthesized according to the method reported in the previous work [25]. Then, to a solution of bisphenol-A dialdehyde (0.2 g, 0.70 mmol) in 30 mL ethanol, 1-pyrenemethylamine hydrochloride (0.38 g, 1.44 mmol) in the presence of triethylamine or 4-(naphthalen-

2-yl)thiazol-2-amine (0.32 g, 1.44 mmol) were added, and the mixture was stirred for 4 h at room temperature. At the end of this period, yellow residue was obtained and washed with anhydrous ethanol for three times, dried to give receptor **R1** and **R2** as yellow solids.

R1: Yield: % 81; Mp: 163–164 °C; FTIR (ATR): 1633 (C=N) cm^{-1} ; ¹H NMR (400 MHz CDCl₃): δ 13.35 (s, 2H), 8.31 (s, 2H), 8.26 (d, 2H, *J*=9.39 Hz), 8.17 (d, 4H, *J*=7.82 Hz), 8.10–8.14 (m, 4H), 8.03 (d, 4H, *J*=2.15 Hz), 7.97–8.01 (m, 2H), 7.90 (d, 2H, *J*=8.02 Hz), 7.09, 7.10 (dd, 2H, *J*=2.54, 2.34 Hz), 6.97 (d, 2H, *J*=2.34 Hz), 6.81 (d, 2H, *J*=8.80 Hz), 5.45 (s, 4H), 1.54 (s, 6H). ¹³C NMR (100 MHz CDCl₃): δ 165.86, 158.97, 140.87, 131.29, 131.21, 131.12, 131.06, 130.79, 129.28, 128.88, 128.16, 127.41, 126.88, 126.08, 125.39, 125.33, 124.99, 124.90, 124.76, 122.94, 118.18, 116.66, 60.55, 41.50, 30.91. Anal. Calcd for C₅₁H₃₈N₂O₂: % C, 86.17; H, 5.39; N, 3.94. Found: % C, 86.28; H, 5.41; N, 4.01.

R2: Yield: % 76; Mp: 158–160 °C; FTIR (ATR): 1652 (C=N), 1624 (C=N) cm^{-1} ; ¹H NMR (400 MHz *d*₆-DMSO): δ 11.46 (s, 2H), 9.39 (s, 2H), 8.53 (s, 2H), 8.16 (s, 2H), 8.09 (d, 2H, *J*=8.8 Hz), 7.88–7.99 (m, 8H), 7.47–7.52 (m, 4H), 7.31 (d, 2H, *J*=8.8 Hz), 6.94 (d, 2H, *J*=8.8 Hz), 1.68 (s, 6H). ¹³C NMR (100 MHz *d*₆-DMSO): δ 171.27, 164.50, 158.92, 152.93, 141.90, 134.48, 133.65, 133.19, 131.85, 128.76, 128.08, 127.05, 126.80, 125.25, 124.47, 119.28, 117.28, 114.26, 41.86, 30.89. Anal. Calcd for C₄₃H₃₂N₄O₂S₂: % C, 73.69; H, 4.60; N, 7.99; S, 9.15. Found: % C, 73.88; H, 4.67; N, 8.01; S, 9.18



Scheme 1 Synthesis route for receptors **R1** and **R2**

Analytical Procedure

The stock solutions of **R1** and **R2** were prepared at concentration of 0.2 μM and 5.0 μM for fluorescence and 10 μM and 20 μM for UV-vis measurements in EtOH/H₂O (95/5, v/v), respectively. Solutions of metal ions as perchlorate salts were prepared in H₂O with a concentration of 0.001 M for UV-vis absorption and fluorescence spectra analysis. Titration experiments were realized in 3 mL solution of **R1** and **R2** by addition of increasing metal ion concentration. The fluorescence spectra were performed with the excitation wavelength at 365 nm, slit 5.0/5.0 nm. To monitor the chemical shifts arising from the interaction of **R1** and **R2** with Zn²⁺, ¹H NMR experiments were also realized by addition of the known quantity of Zn²⁺ ion to solutions of **R1** (0.07 M) and **R2** (0.07 M).

Results and Discussion

As shown in Scheme 1, **R1** and **R2** were synthesized via the condensation reaction of the aldehyde derivative of bisphenol-A (1) and pyrenemethylamine hydrochloride or 4-(naphthalen-2-yl)thiazol-2-amine in ethanol at room temperature, respectively. The formation of **R1** and **R2** was confirmed by appearance of imine protons at δ 8.31 and δ 9.39 ppm in ¹H NMR spectra (Fig. 1). The structures were also supported by ¹H NMR and ¹³C NMR, COSY, APT, FTIR and elemental analysis (Fig. S1–S10).

Metal Ion Selectivity and Competitiveness of **R1** and **R2**

The fluorescence properties of receptors **R1** and **R2** were observed in the presence of different metal ions in a mixture

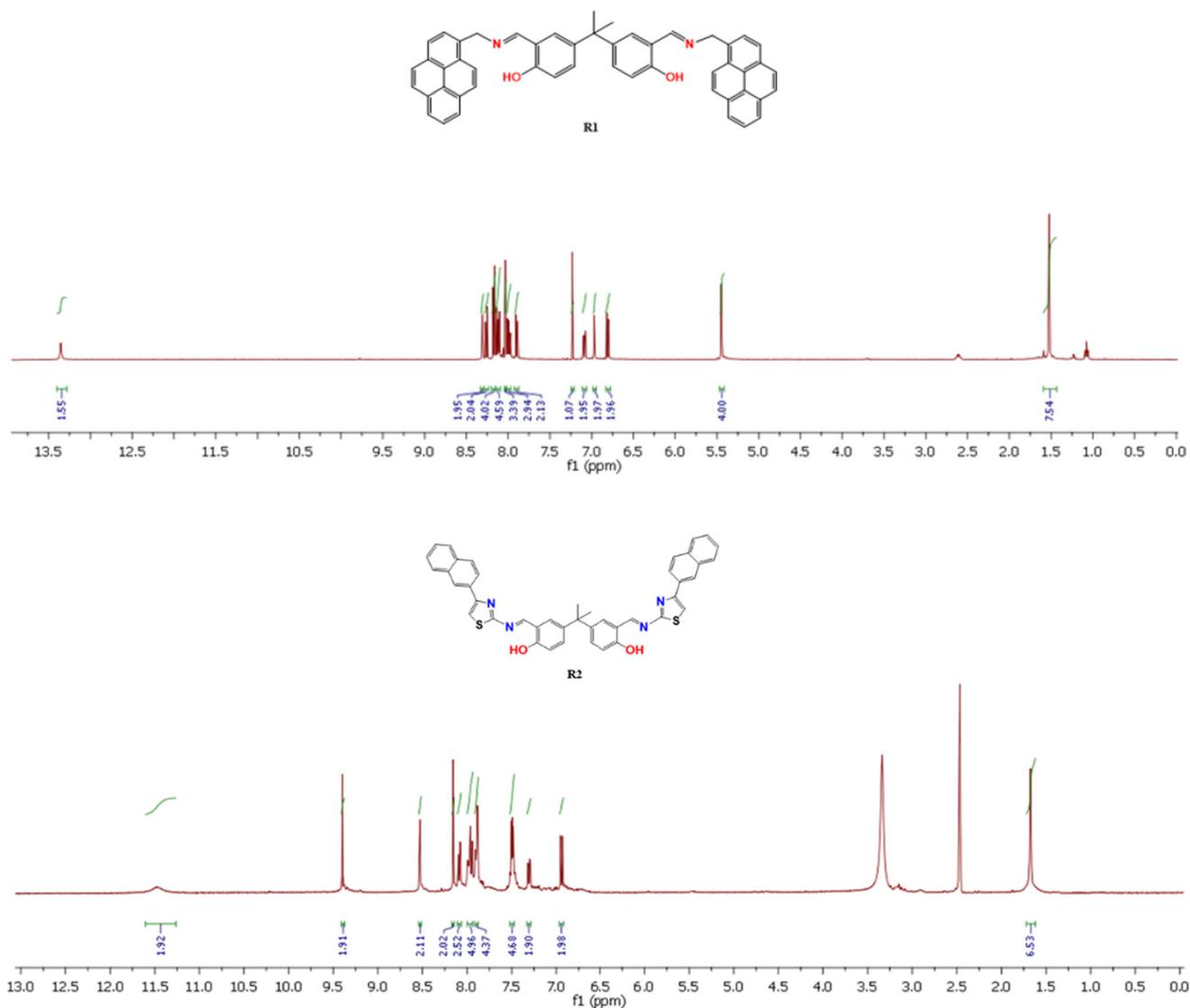


Fig. 1 ¹H NMR spectra of **R1** and **R2** in CDCl₃

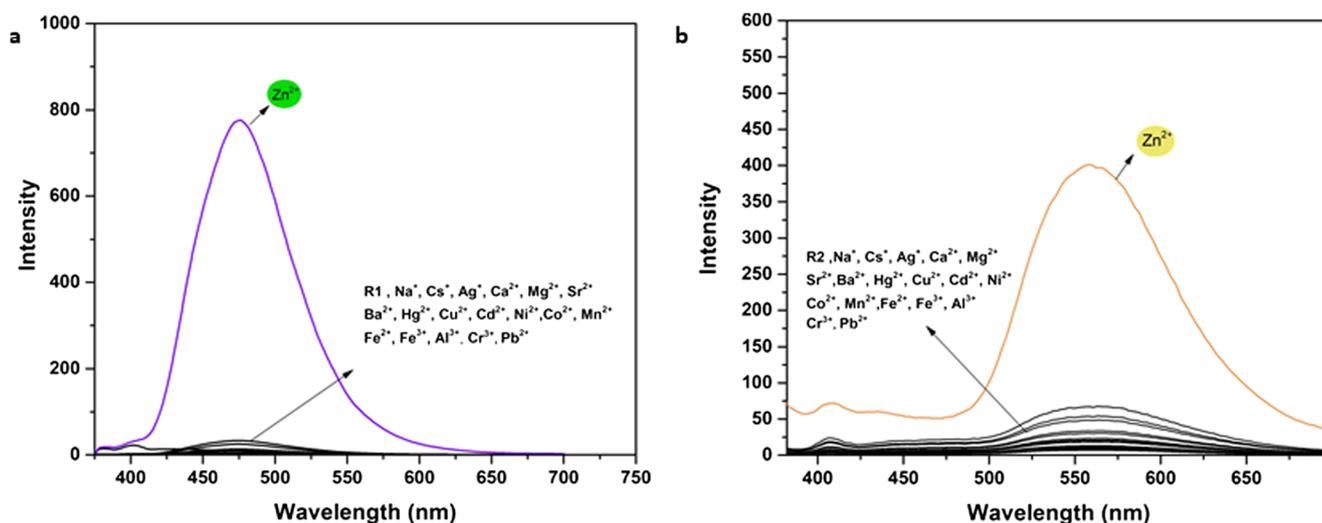


Fig. 2 Fluorescence spectra of (a) **R1** (0.2 μM) and (b) **R2** (5.0 μM) in EtOH/H₂O (95/5, v/v), in the presence of various metal ions

of EtOH/H₂O (95/5, v/v) solution. Receptors **R1** and **R2** exhibited any emission band at range of 400–700 nm. This phenomenon might be interested with the combined PET (Photo-induced electron transfer) effect and ESIPT (Excited state intramolecular proton transfer) process in the excited state.

However, the addition of Zn²⁺ (5.0 equiv.) caused a dramatic fluorescence enhancement at 472 nm for **R1** and 560 nm for **R2** (Fig. 2a, b), showing formation of R1-Zn²⁺ and R2-Zn²⁺ complexes. After the complexation, the PET process and ESIPT might be inhibited, leading to fluorescence

Fig. 3 Visual fluorescence changes upon addition of various metal ions to **R1** and **R2** solutions



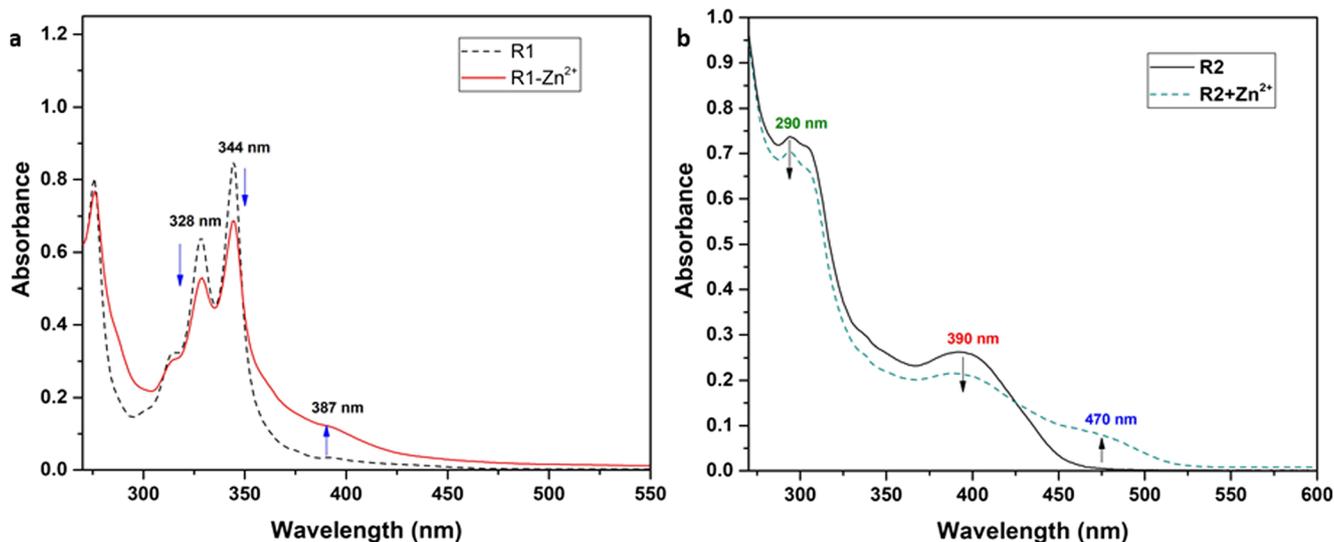


Fig. 4 UV-vis changes of (a) **R1** (10 μM) and (b) **R2** (20 μM) with 2.0 equiv. of Zn²⁺ in EtOH/H₂O (95/5, v/v)

enhancement. When other common metal ions were added to the solution of **R1** and **R2**, no obvious fluorescent changes were observed under the same conditions. In addition, initiatory test for the detection of Zn²⁺ was performed as fluorometric test in UV light. As seen in Fig. 3, **R1** and **R2** was suddenly emitted blue and yellow fluorescent with Zn²⁺ among other metal ions, respectively. Otherwise, in the UV-vis studies (Fig. 4), whereas **R1** indicated three main absorbance bands at 275, 328 and 344 nm, and **R2** indicated two bands at 290 and 390 nm. When Zn²⁺ ion was added to solutions of **R1** and **R2**, these bands decreased and new absorbance bands were revealed at 387 nm for **R1** and 470 nm for **R2**, indicating formation of R1-Zn²⁺ and R2-Zn²⁺ complexations (Fig.4a, b).

The fluorescence titration spectra of **R1** and **R2** in presence of different added concentrations of Zn²⁺ were depicted in

Fig. 5. As the concentration of Zn²⁺ was increased (0–5 equiv.), there was distinct emission enhancing of the bands centred at 472 nm for **R1** and 560 nm for **R2**, which showed the isomerization of C=N bond, increased the rigidity of the molecule, and produced the chelated enhanced fluorescence (CHEF) effects [27]. With the concentration of Zn²⁺ ions up to 2.0 equiv., the fluorescence intensities of **R1** and **R2** were gradually increased, and then the fluorescence intensity did not increase any further and a plateau was reached. To reveal the stoichiometry ratios of **R1** and **R2** with Zn²⁺, Job's plot experiments were realized. In Fig. 6, the emission intensities at 472 and 560 nm are plotted against the molar fractions of **R1** and **R2**, respectively. The emission intensities at 472 and 560 nm were reached maximum at 0.33 of molar fractions of **R1** and **R2**, showing 1:2 stoichiometries of **R1** and **R2** with Zn²⁺ (Fig. 6). The values of the linearly dependent coefficient

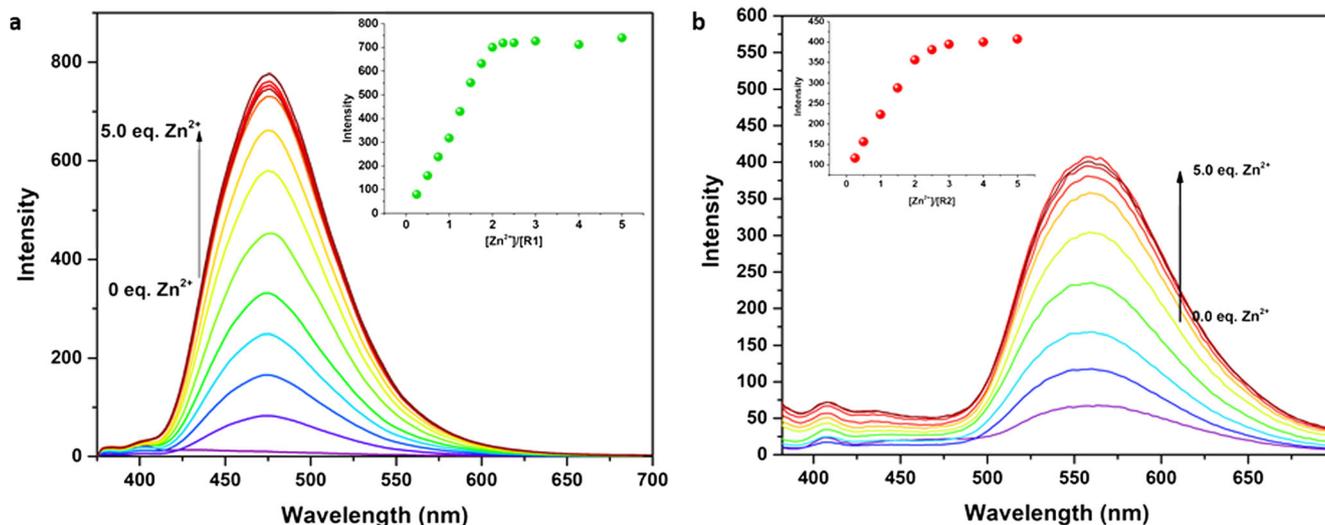


Fig. 5 Fluorescence changes of (a) **R1** and (b) **R2** with various amounts of Zn²⁺ (0.0–5.0 equiv.) in EtOH/H₂O (95/5, v/v) (insets in a and (b) titration profile indicate the formation of a 1:2 complex

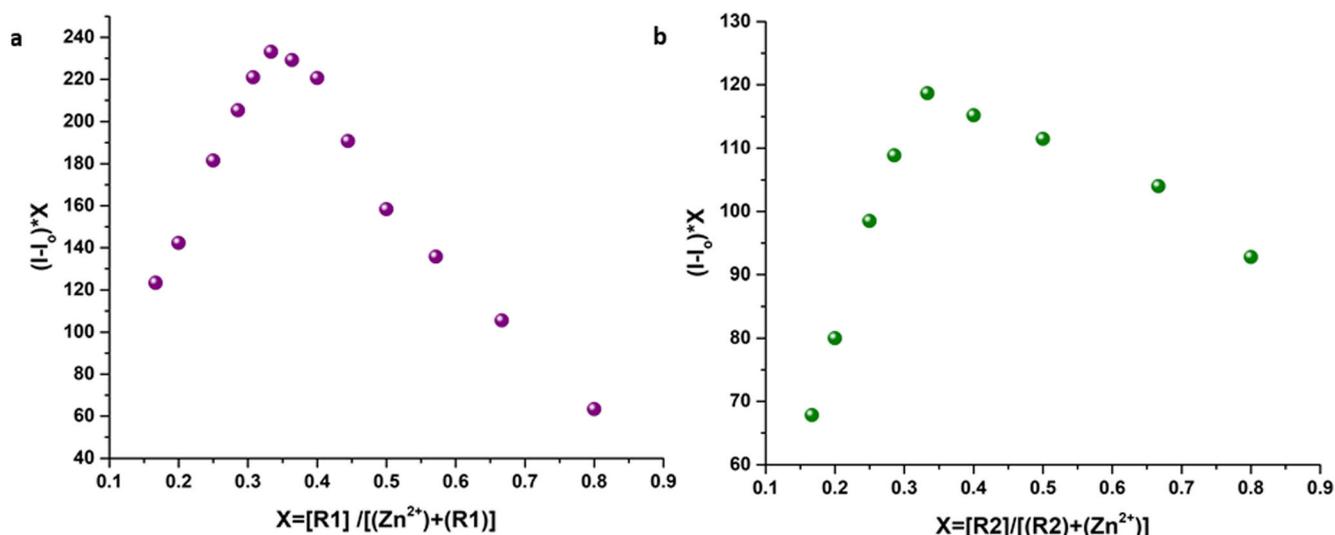


Fig. 6 Job plots of R1-Zn²⁺ (a) and R2-Zn²⁺ (b) complexations

(R²) were found to be 0.99, and the detection limits by fluorescent titration data were also calculated as 17.5 nM for **R1** and 0.94 μM for **R2** with 3σ/slope, (Fig. S11). Based on 1:2 binding stoichiometry, the binding constants (logK) for **R1** and **R2** towards Zn²⁺ ion was calculated using the Benesi-Hildebrand eq. [28] and found to be 13.46 and 10.68, respectively (Fig. S12).

Considering multiple metal ions in samples, fluorescence interference experiments for **R1** and **R2** were subsequently carried out in presence of various competing metal ions to explore the possibility of using **R1** and **R2** as a practical ion selective fluorescent sensor for Zn²⁺. The effect of foreign metal ions on the fluorescence intensities of R1-Zn²⁺ and R2-Zn²⁺ systems was presented in Fig. S13. As seen in Fig. S13, the foreign metal ions did not interfere on the detection of Zn²⁺ by **R1** and **R2**. These results suggest that receptors **R1** and **R2** can be an excellent fluorescent sensor for sensing of

Zn²⁺ ion with highly selectivity and anti-interference performance among the other relevant metal ions.

In addition, the energy-optimized structures of **R1** and **R2** were obtained on density functional theory (DFT) calculations at B3LYP/6-31G (d, p) level by using Gaussian 16 program [29–31]. The optimized structures, the energy of MOs and contours of selected HOMO and LUMO orbitals of **R1** and **R2** have been shown in Fig. 7. The HOMO was localized on pyrene ring in **R1**, while the LUMO was located on the pyrene and benzene in bisphenol-A. Meanwhile, the π electrons of HOMO of **R2** are mainly distributed on the bisphenol-A fragment. The LUMO is distributed on the naphthylthiazole unit. The HOMO to LUMO energy gaps for **R1** and **R2** were determined as 3.327 eV and 1.631 eV, respectively. This difference in the energy gaps of **R1** and **R2** may ascribed to the lack of π-conjugation in **R1** unlike **R2**.

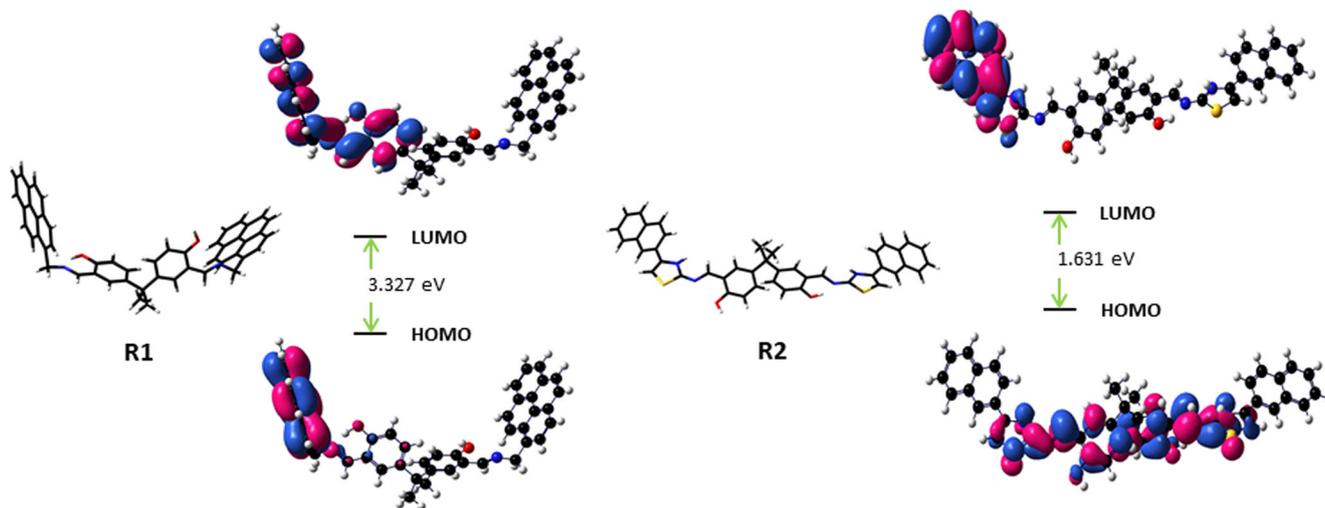


Fig. 7 Optimized structures and the HOMO and LUMO energy gaps levels of **R1** and **R2**: Calculations are based on optimized ground state geometry by DFT at the B3LYP/6-31G (p,d) /level using Gaussian 16

Table 1 Determination of Zn^{2+} in the spiked tap water samples by the present method

Sample ^a	Added (μM)	Measured (μM)	Recovery (%)	RSD ($n = 3$) (%)
T1	5	4.97 (R1)	99.4	0.76
		4.79 (R2)	95.8	1.09
T2	10	10.07 (R1)	100.7	0.92
		9.89 (R2)	98.9	1.03
T3	15	14.95 (R1)	99.6	1.22
		14.81 (R2)	98.7	1.45

^a T1-T3 are known concentrations of Zn^{2+} solution sample

Effect of pH and Reversibility Tests

Figure S14 shows the fluorescence intensities of **R1**, **R2** and their Zn^{2+} complexes at different pH in EtOH/H₂O (95/5, v/v). The pH of the solutions was adjusted using either NaOH or HCl solutions. The fluorescence intensities of **R1** and **R2** were not remarkably changed over the pH range tested. By the addition of Zn^{2+} , **R1** and **R2** solutions showed strong fluorescence

emission in the range of pH 6 and 8. Nevertheless, the fluorescence intensities towards Zn^{2+} of **R1** and **R2** were apparently decreased at high pH 9.0, since -OH ions interacted with Zn^{2+} ions ($K_{sp} = 3 \times 10^{-17}$ for $Zn(OH)_2$) [32]. Consequently, these receptors would be an ideal sensor for monitoring Zn^{2+} in the pH range of 6–8.

To increase the applicability of sensors, the reversibility is a significant point in the design of novel optical sensors. Thus, we

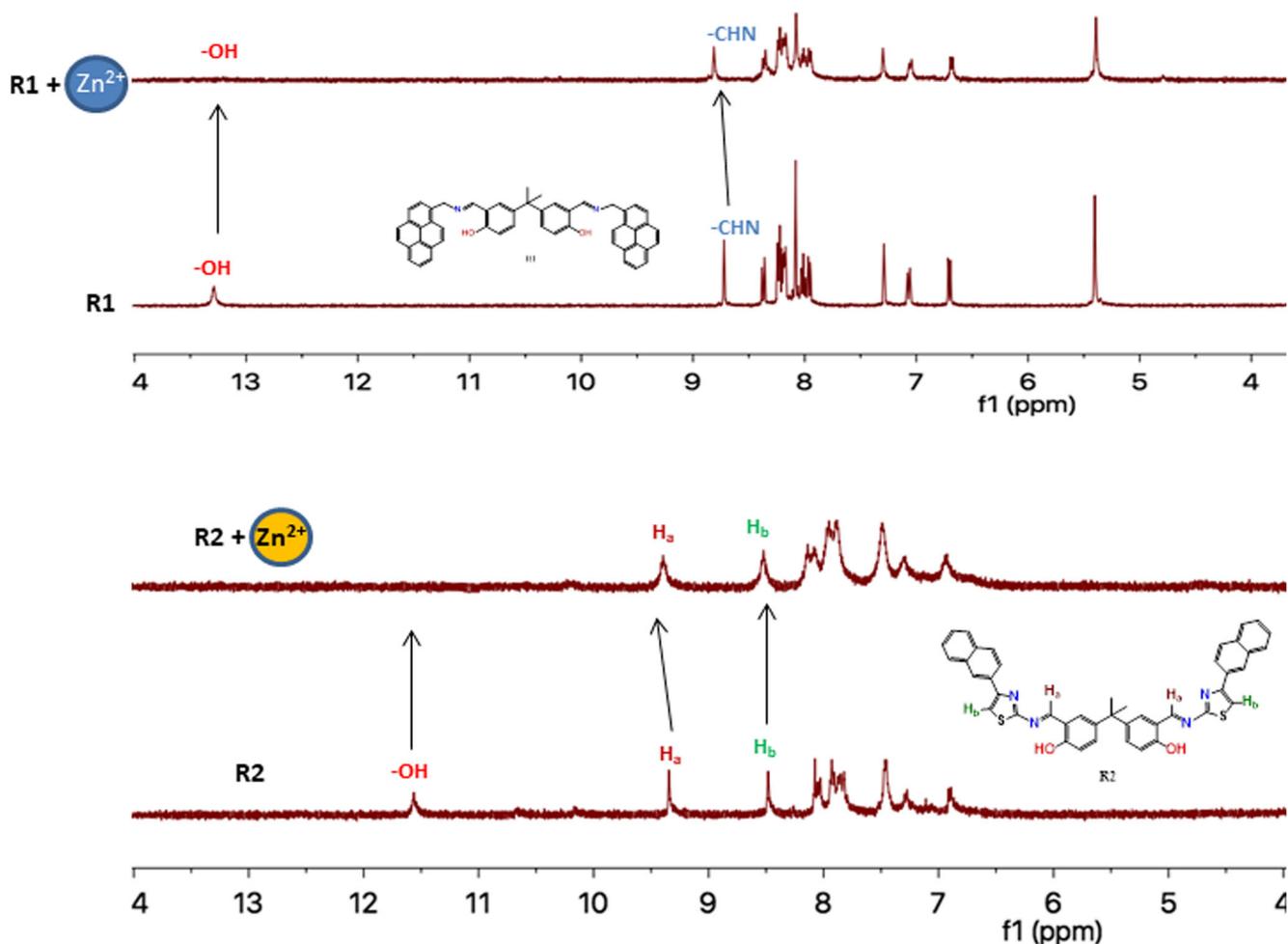
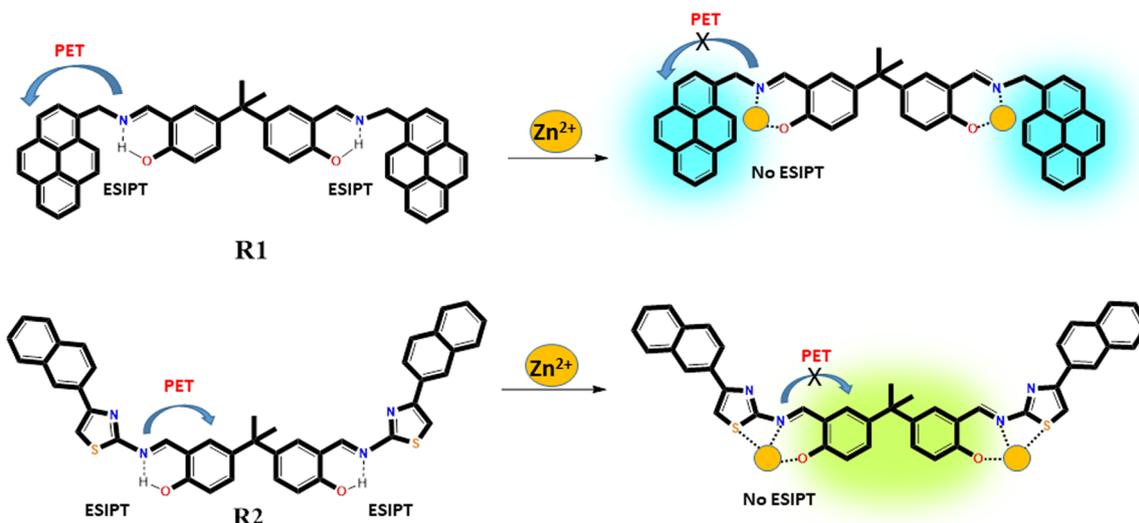


Fig. 8 ¹H NMR (400 MHz DMSO-d₆) spectra changes of **R1** and **R2** upon addition of Zn^{2+} (2.0 equiv.)



Scheme 2 The proposed interaction model between Zn^{2+} with **R1** and **R2**

observed the reversibility of **R1** and **R2** for Zn^{2+} ion using EDTA solution. As seen in Fig. S15 and S16, an immediate fluorescence quenching was observed upon the addition of EDTA to **R1**- Zn^{2+} and **R2**- Zn^{2+} systems. Also, the fluorescence enhancing capability of **R1** and **R2** for Zn^{2+} kept relatively stable values within 4 cycles with little emission intensity loss (Fig. S15b and S16b). It could be found that EDTA could bleach the “signal-on” fluorescence emission bands for Zn^{2+} . Moreover, the response time of **R1** and **R2** for Zn^{2+} was performed, and the results were showed in Fig. S17. In the presence of Zn^{2+} , the fluorescence emission intensities of **R1** and **R2** reached the maximum values within 10 s, and remaining constant from 10 s to 5 min, which shows quickly responsive “turn-on” fluorescent sensor for Zn^{2+} . Moreover, to assess the practical efficacy of **R1** and **R2**, we used them to determine the concentration of Zn^{2+} in tap water samples. For this purpose, the water samples were centrifuged and filtrated, and then added to Zn^{2+} solutions with the concentrations of 5, 10 and 15 μM . Briefly, the Zn^{2+} spiked water sample (0.15 mL) was mixed with 2.85 mL of EtOH, then 0.6 μL for **R1** and 15 μL for **R2** of the EtOH solution of receptors (**R1** or **R2**) was added and the fluorescence spectra was recorded. As shown in Table 1, the recovery was between 95.8% and 100.7% with lower relative standard deviation ($\text{RSD} < 2\%$), which clearly shows the reliability and accuracy of the proposed method.

^1H NMR Experiments

To insight the binding interaction of receptors **R1** and **R2** with Zn^{2+} , ^1H NMR experiments were performed by addition of 2.0 equiv. Zn^{2+} ion to solutions of **R1** and **R2** in DMSO-d_6 (Fig. 8) In the ^1H NMR of the free **R1** and **R2**, the peaks at δ 13.28 or δ 11.56 ppm and δ 8.72 or δ 9.37 ppm correspond to the protons of phenolic-OH and imine groups, respectively.

Upon the addition of Zn^{2+} with 2.0 equiv. concentration, the phenolic-OH signals at δ 13.28 and δ 11.56 ppm disappeared, indicating that OH was effective a group in the complexation between Zn^{2+} and **R1** or **R2**. Also, the signals of imine protons (CHN) at δ 8.72 ppm for **R1** and δ 9.37 ppm for **R2** shifted to 8.81 ppm and 9.40 ppm while Zn^{2+} existed. On the other hand, the CH signal (H_b) at δ 8.51 ppm belongs to thiazole moiety of **R2** was slightly downfield shifted to δ 8.54 ppm in presence of Zn^{2+} , suggesting thiazole ring-metal coordination. Very little change can be observed for the other aromatic protons. These data show that the phenolic-OH and imine for **R1**, and thiazole ring as well as the phenolic-OH and imine groups for **R2** are efficient on formation of their Zn^{2+} -complexes. According to these data, the proposed interaction models between Zn^{2+} with **R1** and **R2** were depicted in Scheme 2.

Conclusion

In summary, fluorescence sensing abilities of two novel synthesized receptors, i.e. **R1** and **R2** were explored for efficient, rapid and selective sensing of Zn^{2+} . Bisphenol-A based sensors (**R1** and **R2**), containing pyrene and naphthylthiazole units, displayed high selectivity and sensitive towards Zn^{2+} ion with little interference observed from other coexistent metal ions. The 1:2 interactions between **R1** and **R2** of Zn^{2+} ion with detection limits 17.5 nM and 0.94 μM were proved from fluorescence spectral data, respectively. The achieved reversibility tests indicated that **R1** and **R2** could be reused with proper treatment. Moreover, the practical applicability of **R1** and **R2** in water samples for Zn^{2+} detection also makes them lucrative and worthwhile. These data exemplified a new possibility for the exploration of novel fluorescence sensors with high selectivity and specificity.

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