

Research Article

Yucun Liu*, Miao Wu, Jihan Zhao, Yuan Wang, and Yongling Zhang

A simple salicylaldehyde-bearing pyrazine as a turn-on fluorescent chemosensor for Al^{3+} and Zn^{2+} recognition and its applications

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Abstract: A simple fluorescent chemosensor, 5-(diethylamino)-2-((2-(pyrazin-2-yl) hydrazono)methyl)phenol, has been synthesized by Schiff-base condensation reaction. The chemosensor exhibited highly selective and sensitive “off-on” fluorescent responses toward Al^{3+} and Zn^{2+} but the signal of fluorescence emission varies. The detection limits were found to be 2.33×10^{-7} M for Al^{3+} and 1.68×10^{-7} M for Zn^{2+} , respectively. The binding mechanisms between chemosensor and Al^{3+} or Zn^{2+} ions were supported by Job's, ^1H NMR, Fourier transform infrared spectra, and MS experiments. The sensing behavior was also studied with molecular logic functions of OR, AND, and NOT gates. In addition, the chemosensor was able to detect Al^{3+} and Zn^{2+} by producing distinct color changes observed by the naked eye on sensor-coated swabs. Moreover, the chemosensor was successfully applied to effectively detect Al^{3+} and Zn^{2+} in actual water and drug samples.

Keywords: salicylaldehyde, pyrazine, chemosensor, $\text{Al}^{3+}/\text{Zn}^{2+}$, spectroscopic analysis

1 Introduction

Fluorescent chemosensors for selective detection of interesting analytes, such as ions and toxicants, have recently

attracted great attention and are widely used in biological, industrial, and environmental analyses [1,2], because fluorescent chemosensors have some advantages of convenient operation, high selectivity, instantaneous response, and nondestructive detection [3–6]. As we all know, metal ions play very significant roles in biological and environmental systems. Among metals, as the third most widespread element, aluminum has been widely used in our daily life including food additives, aluminum-based medicines and storage/cooking utensils, and so on [7]. Therefore, these factors provide chances for increasing the concentration of Al^{3+} in the environment and biosome. As a non-essential element of the human body, nevertheless, excessive aluminum is harmful to the human nervous system and causes severe human health issues, such as Alzheimer's disease, Parkinson's disease, colic, rickets, and anemia [8,9]. In addition, the high content of Al^{3+} can impact the growth of plant roots and aquatic wildlife [10]. Consequently, the development of effective fluorescent chemosensors to detect Al^{3+} in biological and environmental systems is of important significance [11,12]. Likewise, Zn^{2+} , being the second most abundant micronutrient transition metal ion plays multifunctional roles in fundamental biological processes including brain function and pathology, gene transcription and expression, cellular apoptosis, and neural signal transmission [13,14]. Moreover, Zn^{2+} is recognized to be an important structural cofactor and catalytic center of Zn^{2+} -containing enzymes and DNA-binding proteins [15]. However, disruption of Zn^{2+} homeostasis may be associated with various pathological processes such as Alzheimer's disease, diabetes, Parkinson's disease, and hypoxia-ischemia [16]. And, Zn^{2+} is also a waste product derived from agriculture and food in the environment, and the excessive Zn^{2+} content may reduce the activity of microbes present in soil [17]. Therefore, designing selective and sensitive chemosensors for monitoring the presence of Zn^{2+} concentration level is becoming highly needed and is still full of challenges.

Owing to the significance of Al^{3+} and Zn^{2+} , numerous fluorescent chemosensors for Al^{3+} or Zn^{2+} have been developed based on single-analyte responsive systems [18–23]. However, the development of a single fluorescent chemosensor for

* Corresponding author: Yucun Liu, Department of Chemistry, Jilin Normal University, Siping, 136000, China, e-mail: liuyc7889@jlnu.edu.cn

Miao Wu, Jihan Zhao, Yuan Wang: Department of Chemistry, Jilin Normal University, Siping, 136000, China

Yongling Zhang: Department of Information Technology, Jilin Normal University, Siping, 136000, China

multi-analytes with distinct fluorescence responses is gradually becoming new research hot spot and is extremely challenging. In addition, single chemosensors for multi-analytes have some advantages, such as potential cost reduction and saving analytical time [24,25]. At present, although a few fluorescent chemosensors have been designed for simultaneous detection of Al^{3+} and Zn^{2+} based on different experimental conditions [26–30], the development of a single fluorescent chemosensor that can selectively detect and identify both Al^{3+} and Zn^{2+} is still a challenging work. In these considerations, as an easily composable group, Schiff base derivatives are well known to be good ligands and thereby can be developed a potential single chemosensor for the detection multi-analytes simultaneously.

With this in mind, we synthesized a simple pyrazinyl-salicylimine Schiff base fluorescent chemosensor. This chemosensor displayed favorable selectivity in the recognition and detection of Zn^{2+} and Al^{3+} under different emission wavelengths. The presence of other competitive metal ions and the effects of the system pH on detection were also studied. The chemosensor displayed a low detection limit for Zn^{2+} or Al^{3+} and showed good reversibility by the addition of Zn^{2+} or Al^{3+} and ethylenediamine tetraacetic acid (EDTA) that could be applied to build molecular logic circuits. Moreover, the chemosensor toward these two metal ions was also utilized for real water and drug samples quantitative analysis; also, cotton swabs containing the chemosensor were prepared for the rapid detection of Zn^{2+} and Al^{3+} .

2 Experimental

2.1 Materials and instrumentations

All starting materials, metal ions, nitroaromatics, and reagents were obtained from the best-known commercial sources (Aladdin and Macklin) and were used without further purification. ^1H NMR and ^{13}C NMR spectra were recorded in d_6 -DMSO with a Bruker 400 MHz spectrometer operating at 400 and 100 MHz for ^1H and ^{13}C NMR spectroscopy, respectively. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane. The ultraviolet (UV)-Vis absorption spectra were recorded on a Shimadzu UV-2550 spectrometer in 1 cm path length quartz cell. Fluorescence emission spectra were carried out on a Perkin Elmer fluorescence spectrophotometer equipped with quartz cuvettes of 1 cm path length. Fourier transform

infrared spectra (FT-IR) were recorded on a Perkin Elmer FT-IR spectrophotometer using a KBr pellet and were reported in wavenumber (cm^{-1}). Single crystal X-ray data were collected on Bruker Smart Apex II single-crystal X-ray diffractometer using graphite-monochromated Mo-K_α radiation ($0.71073 \text{ \AA}$) at 25°C . Images were generated using Mercury software.

2.2 Fluorescence experiments

First, the stock solutions of the chemosensor **1** ($1 \times 10^{-2} \text{ M}$) and the different metal ions (K^+ , Al^{3+} , Na^+ , Ca^{2+} , Co^{2+} , Cu^{2+} , Mn^{2+} , Fe^{3+} , Tb^{3+} , Pb^{2+} , Mg^{2+} , Ni^{2+} , Ag^+ , Zn^{2+} , Cd^{2+} as perchlorates, $1 \times 10^{-2} \text{ M}$) were prepared in ethanol and water (95:5, v/v). Then, the solution of **1** was diluted with ethanol-water (95:5, v/v) to $1 \times 10^{-4} \text{ M}$. For the analytes selectivity experiments, 2.0 equiv. of the respective metal ions were added in 1.5 mL of **1** ($1 \times 10^{-4} \text{ M}$), and these mixed solutions were all diluted to 3 mL with ethanol-water (95:5, v/v) as the experimental subjects. For the competitive experiment of metal ions, the appropriate amounts of Al^{3+} or Zn^{2+} and a competitive metal ion were mixed with 1.5 mL of **1** solution, and then, these solutions were diluted with ethanol-water (95:5, v/v) to maintain the final concentration of **1** to be $5 \times 10^{-5} \text{ M}$. In titration experiments, 15 mL solution of **1** ($1 \times 10^{-2} \text{ M}$) was taken in a quartz optical cell; then, the Al^{3+} or Zn^{2+} stock solutions were added gradually using a pipette, and the final concentration of **1** was $5 \times 10^{-5} \text{ M}$. As for the reversible experiments, the chemosensor **1** and Al^{3+} or Zn^{2+} were first mixed, in which the EDTA was added after 5 min.

2.3 Calculations for detection limit

The detection limit (LOD) of the chemosensor **1** for Al^{3+} or Zn^{2+} was calculated based on $3\sigma/k$, where σ is the standard deviation of the blank solutions, and k is the slope of the calibration curve.

2.4 Job's plot experiments

Job's continuation method was carried out to determine the sensing stoichiometry of **1** with Al^{3+} or Zn^{2+} using fluorescence emission spectroscopy. The concentrations

of **1** and Al^{3+} or Zn^{2+} were varied but the sum of their concentrations was maintained at 5×10^{-5} M. Fluorescence intensity changes were plotted as a function of the mole fraction of Al^{3+} or Zn^{2+} . The inflection points in the resulting Job's plots corresponded to the mole fraction of Al^{3+} or Zn^{2+} in the complexes.

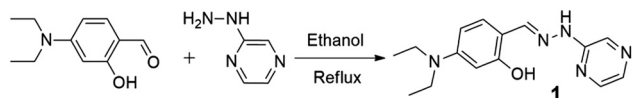
2.5 Synthesis of (*E*)-5-(diethylamino)-2-((2-(pyrazin-2-yl)hydrazono)methyl)phenol (**1**)

Into a 100 mL single-necked flask, 2-hydrazinopyrazine (0.20 g, 1.82 mM) and 4-(diethylamino)salicylaldehyde (0.35 g, 1.82 mM) were added and dissolved in 30 mL anhydrous ethanol. The mixture solution was stirred at 80°C for 6 h. The solid residue was collected by filtration and washed with cold ethanol, and then, the crude product was purified by recrystallization. After drying, chemosensor **1** was obtained as a yellow solid (0.45 g, 86%). FT-IR (KBr, cm^{-1}) 3,439, 3,336, 3,179, 3,088, 3,049, 2,973, 1,632, 1,580, 1,523, 1,433, 1,352, 1,245, 1,126, 1,002, 919, 823, 782, 668. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz), δ (ppm): 10.91 (s, 1H), 10.47 (s, 1H), 8.30 (s, 1H), 8.18 (s, 1H), 8.09 (d, $J = 4$ Hz, 1H), 7.92 (d, $J = 4$ Hz, 1H), 7.31 (d, $J = 8$ Hz, 1H), 6.25 (d, $J = 8$ Hz, 1H), 6.13 (s, 1H), 3.35 (d, $J = 8$ Hz, 4H), 1.11 (d, $J = 8$ Hz, 6H). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz), δ (ppm): 157.90, 152.22, 149.27, 143.09, 142.03, 133.95, 129.95, 129.41, 107.40, 103.89, 97.42, 43.77, 12.57. Electrospray ionization-mass spectrometry (ESI-MS) (m/z): 286.26 $[\text{M} + \text{H}]^+$.

3 Results and discussion

3.1 Synthesis and single crystal of **1**

The chemosensor **1** was facilely synthesized by an one-pot aldehyde-hydrazine condensation reaction of 4-(diethylamino)salicylaldehyde and 2-hydrazinopyrazine in the presence of ethanol with excellent yield and high purity (Scheme 1). The molecular structure of **1** was confirmed



Scheme 1: Synthesis of the chemosensor **1**.

using ^1H NMR, ^{13}C NMR, MS, and also single-crystal X-ray diffraction analyses.

A reasonable single crystal of chemosensor **1** for X-ray diffraction analysis was obtained from the slow evaporation of methanol- CH_2Cl_2 mixture (CCDC Number: 2121363). An oak ridge thermal ellipsoid plot (ORTEP) view (by Mercury software) and single-cell arrangement of **1** are given in Figure 1. The presence of the pyrazine and salicylaldehyde moieties could be easily observed and were linked by a hydrazone bond in the compound. It was also found that compound **1** was almost planar. An intramolecular hydrogen bond is present between N(3) and H(1) with a bond distance of 2.7 Å. The crystallographic data, refinement parameters (Table S1 in Supplementary material), and bond lengths and angles (Table S2) are presented in ESI.

3.2 Fluorescence response to metal ion

As an excellent chemosensor, the selective sensing behavior of **1** for various metal ions is a very important parameter, and the fluorescence experiment was investigated upon the addition of several metal ions such as Na^+ , K^+ , Ag^+ , Al^{3+} , Ca^{2+} , Pb^{2+} , Co^{2+} , Cd^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} , Ni^{2+} , Fe^{3+} , Cu^{2+} , and Tb^{3+} in ethanol- H_2O (v:v = 95:5) at ambient conditions. As shown in Figure 2, upon excitation at 366 nm, **1** exhibited negligible fluorescence emission at 525 nm due to the photoinduced electron transfer (PET) process [31]. When **1** was treated with 2.0 equiv. of metal ions mentioned previously, Al^{3+} and Zn^{2+} induced apparent fluorescence enhancements. However, Al^{3+} and Zn^{2+} ions could be easily distinguished from the respective fluorescence emission wavelengths centered at 582

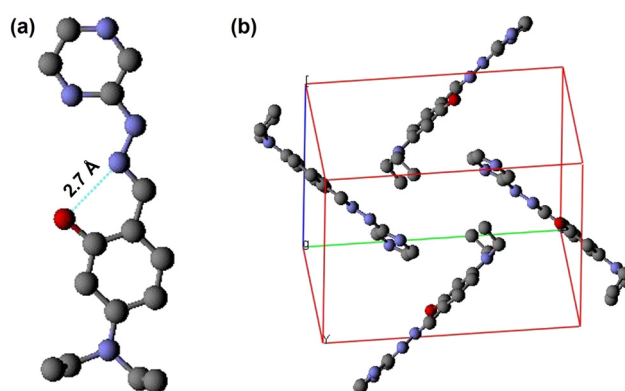


Figure 1: (a) ORTEP view and the intramolecular hydrogen bond of the chemosensor **1**; (b) unit cell of **1** in a single crystal. Hydrogen atoms were omitted for clarity.

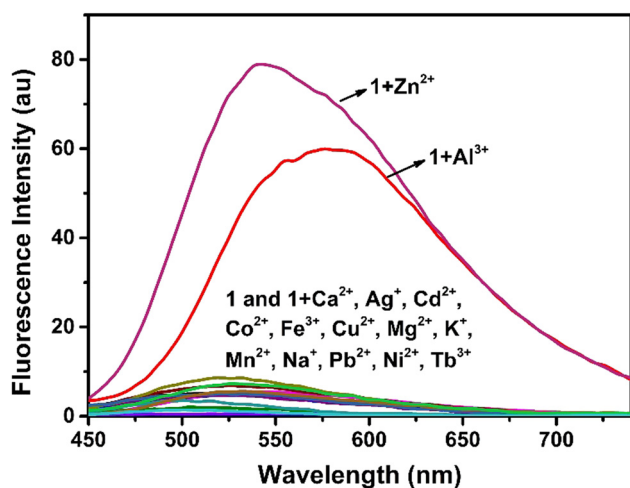


Figure 2: Fluorescence spectrum changes of **1** (50 μM) on addition of various metal ions (2.0 equiv.) in ethanol–water (95:5, v/v) ($\lambda_{\text{ex}} = 366 \text{ nm}$).

and 541 nm. The observed fluorescence enhancement should be attributed to the chelation-enhanced fluorescence (CHEF) effect by complexation of **1** with Al³⁺ and Zn²⁺ via the imine bond N, the phenolic hydroxyl O, and the pyrazine N [32,33], which inhibited of free rotation of **1** as well as eliminated the PET process. Such selective fluorescence changes of chemosensor **1** were not observed in the presence of other tested metal ions, which revealed a good selectivity of **1** toward Al³⁺ and Zn²⁺.

The specificity fluorescence responses of **1** toward Al³⁺ and Zn²⁺ were examined by interference ion experiments, which were performed by taking the fluorescence of **1** with 2.0 equiv. of Al³⁺ or Zn²⁺ in the presence of 2.0 equiv. of other metal ions. As the fluorescence intensity of **1**

increased at 582 nm and 541 nm in the presence of Al³⁺ and Zn²⁺, respectively, we monitored emission wavelength at 582 nm and 541 nm, respectively, to check the influence of other metal ions on the emission intensity. As shown in Figure 3a, except for the slight interference of Cu²⁺ and Fe³⁺, no significant fluorescence quenching at 582 nm was observed in **1**–Al³⁺ solution upon the addition of other metal ions. This experiment showed that **1** could be used to detect Al³⁺ even in the presence of other relevant metal ions. Simultaneously, **1** was treated with Zn²⁺ in the presence of other metal ions, except for Cu²⁺, Fe³⁺, which quenched the fluorescence intensity at 541 nm; other metal ions did not significantly affect the intensity of the **1**–Zn²⁺ solution (Figure 3b). It suggested that chemosensor **1** had a stronger binding affinity toward Cu²⁺ and Fe³⁺ than Zn²⁺. Although Cu²⁺ and Fe³⁺ caused certain interference, the chemosensor had potential application in the recognition of Zn²⁺ owing to its excellent selectivity [34]. From the competitive experiment, even coincidentally, the original fluorescence intensities were both enhanced to a certain extent by either Zn²⁺ added to **1**–Al³⁺ solution or Al³⁺ added to **1**–Zn²⁺ solution, indicating that there might be a certain synergistic effect between Al³⁺ and Zn²⁺ in fluorescence emission.

In order to verify the sensitivity of the chemosensor **1** toward Al³⁺ and Zn²⁺, quantitative fluorescence titration experiments of Al³⁺ and Zn²⁺ to **1** had been carried out in ethanol–H₂O (v:v = 95:5), respectively. The results are shown in Figure 4. For the titration of Al³⁺, the maximum emission wavelength red-shifted from 525 to 582 nm and a significant fluorescence emission enhancement at 582 nm was detected upon the progressive addition of Al³⁺. With an

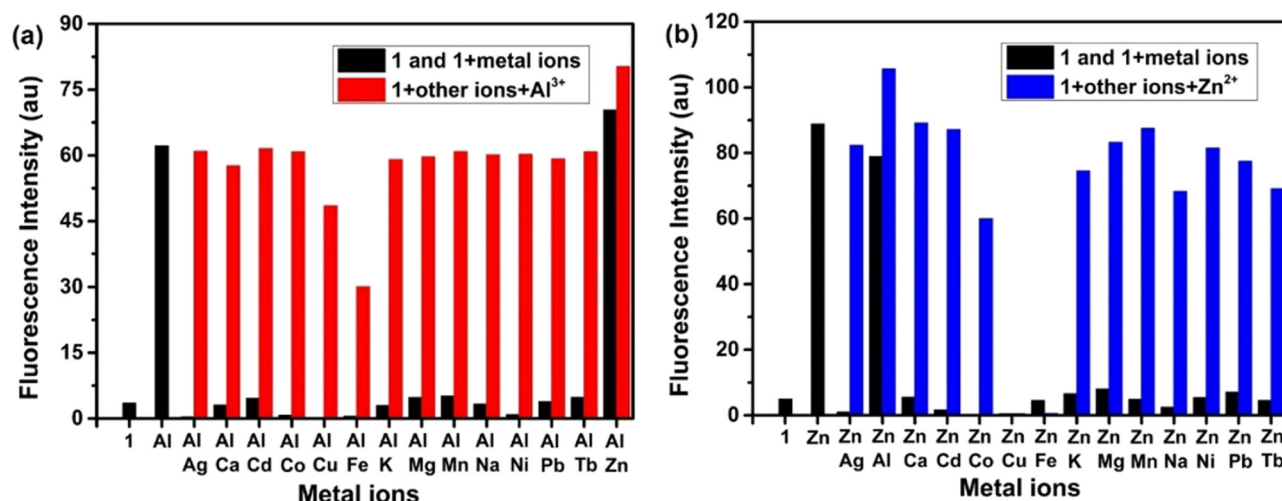


Figure 3: Competitive selectivity of **1** toward (a) Al³⁺ and (b) Zn²⁺ in the presence of other metals (2 equiv.).

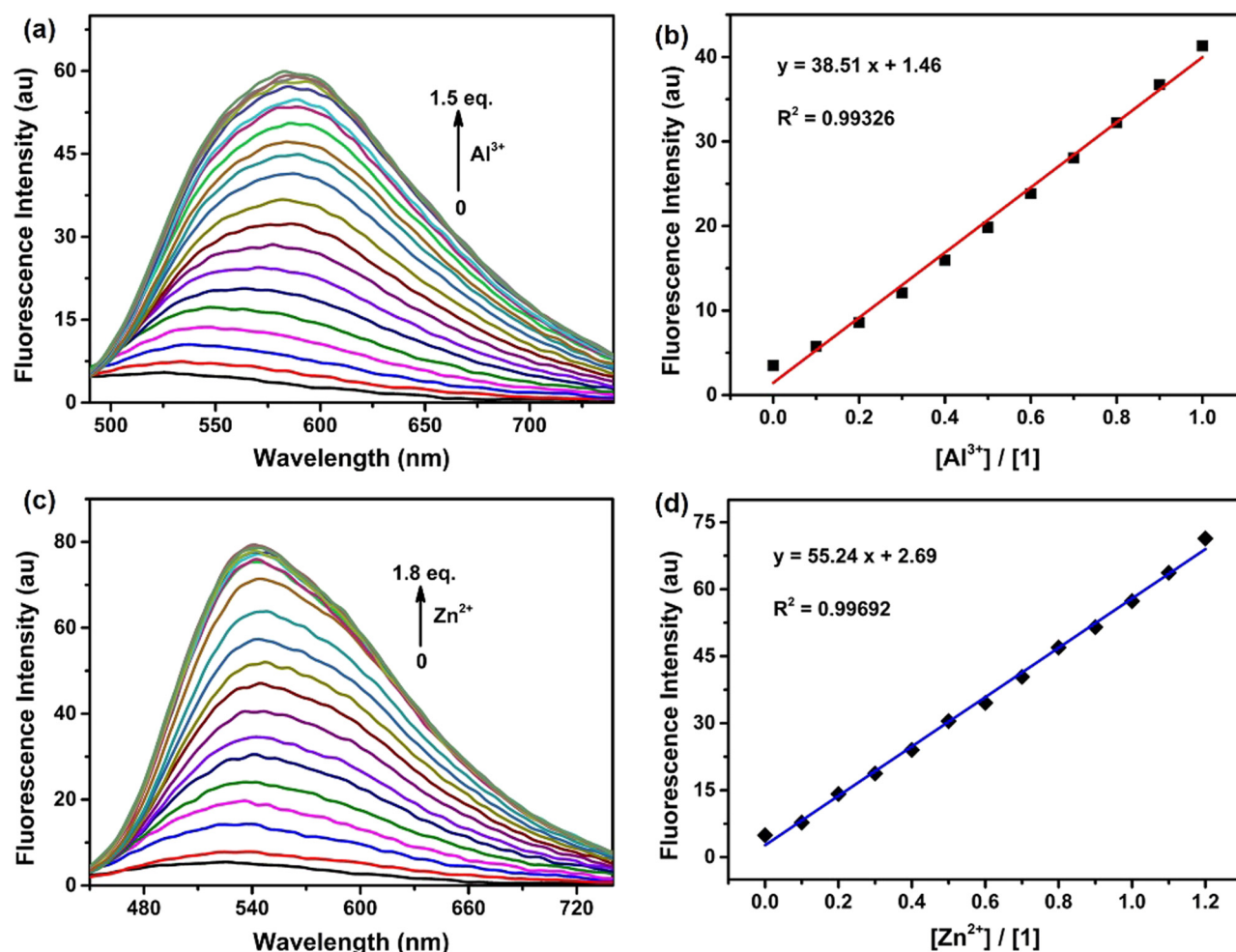


Figure 4: Fluorescence spectra changes of 1 (50 μM) upon incremental addition of (a) Al³⁺ (0–1.5 equiv.) and (c) Zn²⁺ (0–1.8 equiv.), the linear relationship the fluorescence intensity at (b) 582 nm versus the equivalents of Al³⁺ and (d) 543 nm versus the equivalents of Zn²⁺.

increase in Al³⁺ concentration up to 75 mM (1.5 equiv.), the fluorescence intensity showed almost no change. Plotting of the fluorescence emission intensity of 1–Al³⁺ at 582 nm versus the concentration of Al³⁺ (0–1.0 equiv.) showed a good linear relationship ($R^2 = 0.99326$), demonstrating that 1 could potentially be performed as a quantitative fluorescent chemosensor for detecting Al³⁺. The binding constant for the formation of the 1–Al³⁺ complex was calculated to be $2.03 \times 10^4 \text{ M}^{-1}$ using the Benesi–Hildebrand equation [35]. The detection limit was determined as low as $2.33 \times 10^{-7} \text{ M}$ for Al³⁺ on the basis of the IUPAC recommendation of the $3\sigma/k$ method [36]. This detection limit was significantly below the enforceable drinking water standard for Al³⁺ (7.4 μM) proposed by the World Health Organization (WHO) [37]. Similarly, the binding ability of chemosensor 1 toward Zn²⁺ was also investigated by a fluorescence titration experiment in ethanol–H₂O (v:v = 95:5), as shown in Figure 3b. Upon the addition of Zn²⁺ (0–2.0 equiv.) to the solution of 1, the fluorescence emission

intensity at 542 nm increased gradually and then reached the maximum when the addition of Zn²⁺ was 90 μM (1.6 equiv.). And a good linear correlation between the emission intensity at 542 nm of 1–Zn²⁺ and the concentration ratio of $[\text{Zn}^{2+}]/[\text{1}]$ was observed in the range of 0–1.2 equiv. of Zn²⁺, which could be used for the determination of unknown Zn²⁺ in an aqueous solution containing ethanol. According to the titration profile, the corresponding detection limit was also calculated to be $1.68 \times 10^{-7} \text{ M}$ for Zn²⁺, which value was much lower than the WHO suggested tolerance level (76 μM) in drinking water [30]. And the binding constant between the chemosensor 1 and Zn²⁺ was calculated to be $1.15 \times 10^4 \text{ M}^{-1}$. From these results, therefore, chemosensor 1 had high sensitivity for Al³⁺ and Zn²⁺ and could be potentially used for the detection of Al³⁺ and Zn²⁺ in practical samples.

Generally, the solution pH value is also a primary factor affecting the response of the chemosensor. Thus, the effect of pH in the pH range 2.0–13.0 on the

fluorescence emission of chemosensor **1** was evaluated in the absence and presence of Al^{3+} and Zn^{2+} separately (Figure S1 in Supplementary material). The fluorescence emission intensity of free chemosensor **1** showed little dependence on pH from 2.0 to 13.0. For Al^{3+} and Zn^{2+} , emission intensity was monitored at 582 and 542 nm, respectively. In the presence of Al^{3+} , significant fluorescence enhancements of **1** were observed upon the addition of Al^{3+} in the pH range from 4.0 to 10.0. By contrast, the fluorescence intensity decreased gradually in acidic (pH, < 4.0) and alkaline (pH, > 10.0) solutions. A similar fluorescence phenomenon was also found in the **1**– Zn^{2+} system, and a drastic enhancement in emission intensity of **1** was caused in a range of pH 5.0–9.0 in the presence of Zn^{2+} . The pH experiment suggested that the chemosensor **1** could form stable complexes with Al^{3+} or Zn^{2+} in weakly acidic, neutral, and weakly alkaline environments. We speculated that the strongly acidic conditions could induce dissociation of **1**– Al^{3+} or **1**– Zn^{2+} complexes because of the protonation of **1**, and the strongly alkaline solutions could provide adequate hydroxyl ions to extract Al^{3+} or Zn^{2+} from complexes to form the corresponding hydroxide [38]. Taking the aforementioned results into consideration, these appropriate pH ranges of **1** toward Al^{3+} and Zn^{2+} made it suitable as a selective fluorescent chemosensor to recognize both Al^{3+} and Zn^{2+} in environmental systems.

3.3 Naked eye identification

Naked eye detection, as a simple method, was more practical for the rapid detection of target ions because it

needed an uncomplicated program. Therefore, photographs of the single **1** and **1** with the above metal ions had been recorded under 365 nm UV light (Figure 5a). Except for Al^{3+} and Zn^{2+} , single **1** and **1** with other metal ions did not produce any change in color under 365 nm irradiation. The chemosensor **1** exhibited yellow and greenish-yellow coloration in the presence of Al^{3+} and Zn^{2+} , respectively, indicating that the chemosensor **1** could be used for a naked eye detection of Al^{3+} and Zn^{2+} over other metal ions by solution process. In addition, various concentrations of Al^{3+} or Zn^{2+} were added separately to verify the performance of **1** as an efficient chemosensor (Figure S2). The color of the solution was changed significantly in the presence of different Al^{3+} or Zn^{2+} . With increasing amounts of Al^{3+} and Zn^{2+} to 0.1 and 0.02 equiv., respectively, an obvious color change from pale yellow to yellow of **1** solution was observed under visible light and an enhancement of emission intensity was shown under UV 365 nm irradiation, showing that Al^{3+} and Zn^{2+} complexed with **1** to prevent the PET process and enhance the fluorescence intensity. These results indicated that chemosensor **1** could be conveniently used for the practical estimation of Al^{3+} and Zn^{2+} concentrations. Furthermore, for convenient use in an onsite analysis, swab-based chemosensor **1** for Al^{3+} and Zn^{2+} detection was also developed. As shown in Figure 5b, when the swab was dipped into the Al^{3+} and Zn^{2+} solution, respectively, a yellow and greenish-yellow emission was observed separately by the naked eye under a 365 nm UV lamp, indicating that Al^{3+} and Zn^{2+} complexed effectively with the chemosensor **1**. Furthermore, when the swab was dipped in different ion solutions, the fluorescence of the swab dipped in Al^{3+} and Zn^{2+} strengthened and the fluorescence of the other swabs remained the same (Figure S3). The

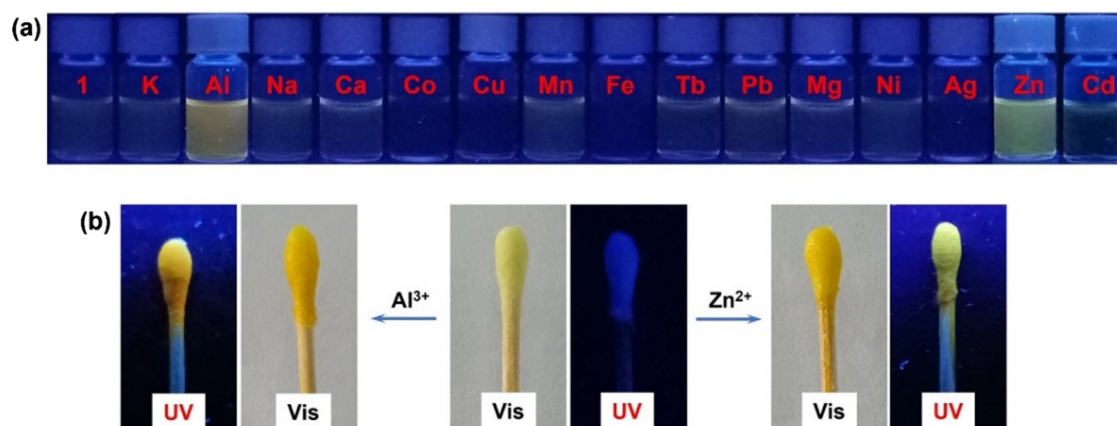


Figure 5: (a) Photos of fluorescence changes of **1** on the addition of various metal ions (2.0 equiv.) in ethanol–water (95:5, v/v) under UV (365 nm) lamp. (b) The color changes of the **1**-coated cotton swab in the absence and presence of Al^{3+} and Zn^{2+} .

experiment of the swabs demonstrated that **1** was a portable chemosensor for the rapid detection of Al^{3+} and Zn^{2+} . Undoubtedly, these data suggested that chemosensor **1** showed promising potential for the rapid and onsite identification of Al^{3+} and Zn^{2+} .

3.4 Reversibility for Al^{3+} and Zn^{2+}

Reversibility, as an important factor to determine whether the chemosensor could be recycled, plays a significant role in practical applications. Therefore, the metal complexing agent EDTA was used as a chelating ligand and was added to the solutions of **1**- Al^{3+} and **1**- Zn^{2+} , respectively (Figure 6a and b). In the case of **1**- Al^{3+} solution, after the addition of 2.0 equiv. of EDTA, the yellow emission of the solution vanished accompanied by an obvious decrease in the fluorescence intensity at 582 nm, suggesting that EDTA extracted Al^{3+} from the **1**- Al^{3+} complex to release the **1** free (Figure S4). Subsequently, when Al^{3+} was added again to the earlier mixture, the yellow emission as well as fluorescence intensity was both regenerated, and this reversible detection could be repeated at least four times by alternate addition of EDTA and Al^{3+} . Coincidentally, in UV-Vis spectra, the free chemosensor **1** displayed a maximal absorption peak at 365 nm which was attributed to π - π^* transition. Upon the addition of Al^{3+} to the solution of **1**, the initial absorption band at 365 nm was decreased accompanied by a 15 nm blue-shift, and a simultaneous increase at 437 nm was observed. The new band at 437 nm could be attributed to metal to ligand charge transfer (MLCT). After then, the absorption spectrum of **1**- Al^{3+} was similar to that of the free **1** upon the addition of EDTA (Figure S5). The same reversible cycle experiment was also implemented for the

1- Zn^{2+} system, and the greenish-yellow emission and fluorescence intensity at 542 nm could achieve “off-on-off” mode. In the absorption spectrum, the π - π^* transition absorption wavelength of free **1** was decreased along with the shift of the 365 nm peak to 346 nm with the addition of Zn^{2+} ; in addition, a new MLCT absorption band at 429 nm emerged. Subsequently, the MLCT band disappeared, and the π - π^* transition band was recovered upon the addition of EDTA. These results indicated that **1** was an effectively reversible chemosensor for Al^{3+} and Zn^{2+} detections.

According to the aforementioned results, a molecular logic gate was further built, setting Al^{3+} , Zn^{2+} , and EDTA as multiple inputs. The group of Al^{3+} and Zn^{2+} was set as an OR logic gate, and EDTA merged the earlier logic gate to form an AND logic gate [39]. For input, the presence and absence of Al^{3+} , Zn^{2+} , and EDTA were assigned as 1 and 0, respectively. For output, turn-on fluorescence at 582 or 542 nm was recorded as 1, and quenched fluorescence was recorded as 0. As shown in Figure 7, in the absence of EDTA input, the fluorescence intensity of the chemosensor was enhanced (output = 1) either by Al^{3+} and Zn^{2+} input separately or by Al^{3+} and Zn^{2+} input together, and other input signals could cause the significant fluorescence quenching (output = 0).

3.5 Sensing mechanism for Al^{3+} and Zn^{2+}

To better understand the recognition mechanism of the chemosensor **1** to Al^{3+} and Zn^{2+} , the binding stoichiometry of the complexation of **1** with Al^{3+} and Zn^{2+} , respectively, was first verified using Job's method. As shown in Figure 8, when the mole fraction of Al^{3+} or Zn^{2+} was

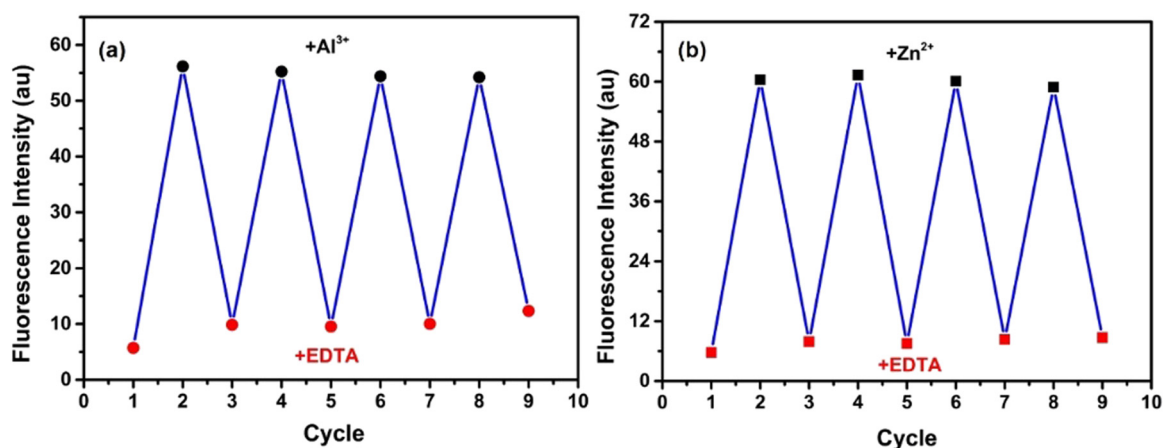


Figure 6: Fluorescence intensity of **1** at (a) 589 nm by alternate addition of Al^{3+} and EDTA and (b) 543 nm by alternate addition of Zn^{2+} and EDTA.

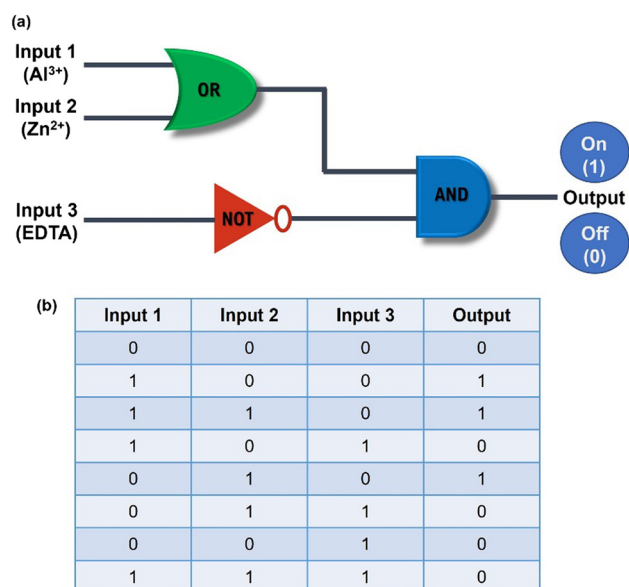


Figure 7: (a) The possible logic gate circuit and (b) the truth table.

reached around 0.33 and 0.50, respectively, the fluorescence intensity of the complex of **1**– Al^{3+} at 582 nm and **1**– Zn^{2+} at 542 nm reached the maximum, indicating the 2:1 stoichiometry for **1**– Al^{3+} and 1:1 stoichiometry for **1**– Zn^{2+} complex. Also, the linearity of the Bensí–Hildebrand (BH) graph showed the 1:1 bonding mechanism between **1** and Zn^{2+} , whereas a poor linearity of BH graph was shown between **1** and Al^{3+} (Figure S6). In addition, the stoichiometric ratio has been confirmed using the free bindfit software, where a 2:1 and 1:1 stoichiometry of chemosensor **1** with Al^{3+} and Zn^{2+} , respectively, gave highly satisfactory results (<http://supramolecular.org>). However, the fitting of the assumed conjugation of 1:2 and 1:1 resulted in lower values for binding constants of

1 with Al^{3+} as well as 1:2 and 2:1 resulted in negative values for binding constants of **1** with Zn^{2+} (Figure S7). In UV-Vis titration experiment, a strong absorbance maximum at 366 nm assigned to the π – π^* transition of **1** was gradually weakened along with a simultaneous increase at 434 and 428 nm was observed upon progressive addition of Al^{3+} and Zn^{2+} , respectively. And a clear isosbestic point at 398 nm for **1**– Al^{3+} and 396 nm for **1**– Zn^{2+} suggested the formation of a stable product (Figure S8). The results of UV-Vis absorption spectra indicated that the phenolic oxygen and imine nitrogen atoms in **1** might be involved in coordination with Al^{3+} and Zn^{2+} [40]. Furthermore, the signal peak of MALDI-TOF-MS at $m/z = 595.3238$ corresponding to $[\mathbf{21} + \text{Al} \cdot 2\text{H}]$ (calcd. m/z 595.2838) and $m/z = 627.7, 871$ corresponding to $[\mathbf{1} + \text{Zn} + \text{CH}_3\text{OH} + 2\text{ClO}_4 + 2\text{Na}]$ (calcd. m/z 627.6, 469) confirmed the 2:1 and 1:1 binding mode of **1** with Al^{3+} and Zn^{2+} , respectively (Figure S9).

In addition, ^1H -NMR titration experiment had been implemented for an in-depth understanding of the binding mode. In the case of Zn^{2+} (Figure 9), upon the addition of Zn^{2+} , the phenolic-OH proton (H_a) peak at 10.47 ppm was weakened gradually and downfield shifted by 0.09 ppm. The signal at 10.91 ppm assigned to NH (H_b) exhibited a 0.10 ppm downfield shift. Meanwhile, the $\text{CH}=\text{N}$ proton (H_c) at 8.30 ppm had been shifted downfield to 8.33 ppm. Also, the pyrazinyl proton signal H_g shifted to a lower field of about 0.04 ppm, and H_h and H_i had a little shift of about 0.02 ppm. Of all the benzene ring proton, H_d , H_e , and H_f shifted downfield about 0.08 ppm, 0.07 ppm, and 0.06 ppm, respectively, as well as these signals became blunt peaks because of the coordination reaction. Similarly, except for the proton signals of the benzene ring shifted upfield, other proton signals of **1** showed a trend of shifting

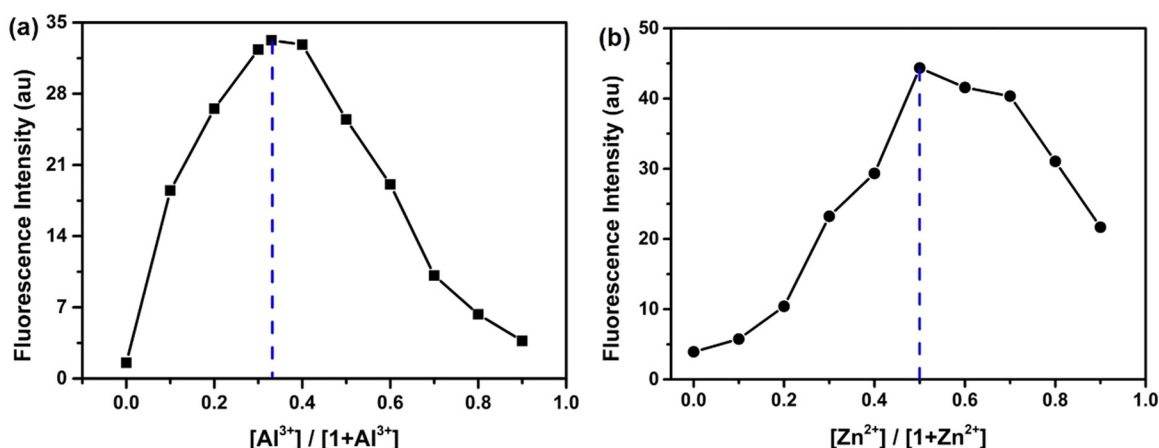


Figure 8: Job's plot for determining the stoichiometry of **1** with (a) Al^{3+} and (b) Zn^{2+} with 366 nm excitation wavelength.

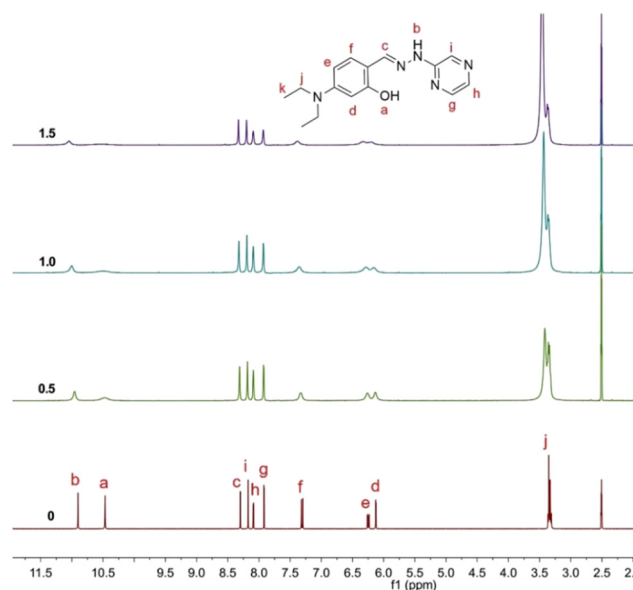
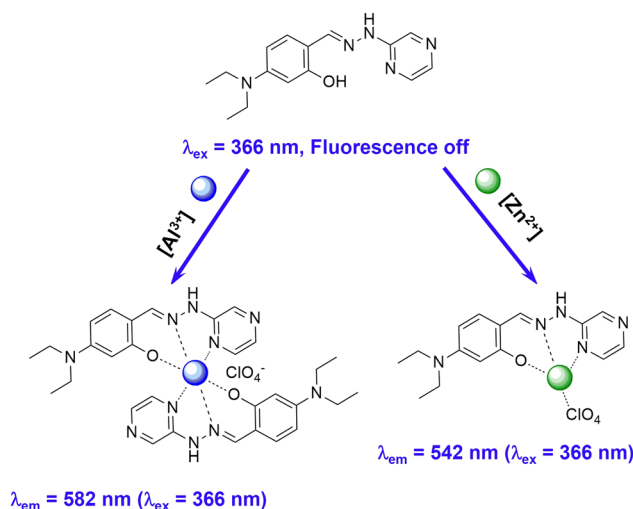


Figure 9: ^1H NMR spectra (400 MHz) measured during the titration of **1** with Zn^{2+} in d_6 -DMSO.

downfield in the Al^{3+} titration experiment (Figure S10). In the FT-IR spectrum, the $-\text{OH}$ stretching of **1** at $3,439\text{ cm}^{-1}$ disappeared upon the addition of Al^{3+} and Zn^{2+} with the appearance of a band at $3,370\text{ cm}^{-1}$. The peak at $1,634\text{ cm}^{-1}$ ascribed to $\text{C}=\text{N}$ stretching moved toward a lower wave-number around $1,628$ and $1,624\text{ cm}^{-1}$ after complexation with Al^{3+} and Zn^{2+} , respectively (Figure S11). The earlier results further demonstrated that phenol oxygen and imine nitrogen atoms of **1** are involved in the binding with Al^{3+} and Zn^{2+} . Therefore, according to Job's plot, MS, UV-Vis, ^1H -NMR, and FT-IR results, the chemosensor **1** could provide three metal binding sites (phenol O, imine N, and



Scheme 2: Proposed reaction mechanism of **1** with Al^{3+} and Zn^{2+} .

pyrazine N) for Al^{3+} and Zn^{2+} , and the reasonable coordination mode of **1** for Al^{3+} , and Zn^{2+} is proposed in Scheme 2.

3.6 Practical applications

To explore the practical application as well as the sensitivity of chemosensor **1**, experimental verification was implemented to detect Al^{3+} and Zn^{2+} in potable water and drug samples (Tables 1 and 2). All the results were measured three times in parallel. For the water samples, the accuracy was investigated via adding a known concentration of standard Al^{3+} or Zn^{2+} to the samples, and the results were analyzed using the linear relationship of the previously calculated fluorescence titrations.

Table 1: Determination of Al^{3+} recovery in real samples

Samples	Al^{3+} added (mM)	Al^{3+} found ($n = 3$, mM)	Recovery ($n = 3$, %)	RSD (%)	Relative error (%)
Water	20	19.14	95.70	0.79	-0.86
	30	28.66	95.53	1.52	-1.34
	40	39.41	98.53	1.07	-0.59
Drug	—	10.15	—	0.98	—

Table 2: Determination of Zn^{2+} recovery in real samples

Samples	Zn^{2+} added (mM)	Zn^{2+} found ($n = 3$, mM)	Recovery ($n = 3$, %)	RSD (%)	Relative error (%)
Water	30	30.26	100.89	1.32	0.26
	45	45.58	101.28	0.87	0.58
	55	53.78	97.78	1.65	-1.22
Drug	—	10.07	—	0.77	—

The results suggested that chemosensor **1** had excellent recoverability for the detection of Al^{3+} and Zn^{2+} in the tested water. Simultaneously, antacid and zinc granule supplementation drugs were selected as the test object for Al^{3+} and Zn^{2+} , respectively. The content of Al^{3+} and Zn^{2+} in the drugs was calculated to be 0.954 and 42.81 $\text{mg}\cdot\text{g}^{-1}$, respectively, which was in good agreement with the actual content (42.81 $\text{mg}\cdot\text{g}^{-1}$ for Al^{3+} and 0.954 $\text{mg}\cdot\text{g}^{-1}$ for Zn^{2+}). The results exhibited the effective content detection for target ions of the chemosensor **1**. These results showed beyond doubt that chemosensor **1** could be used for the analysis and detection of Al^{3+} and Zn^{2+} in real water and drug samples.

4 Conclusions

In summary, we have designed and developed a simple pyrazinyl-salicylimine Schiff base fluorescent chemosensor **1**, which could achieve the identification and detection of Al^{3+} and Zn^{2+} . Chemosensor **1** exhibited excellent sensitivity and selectivity toward Al^{3+} and Zn^{2+} in mixed-aqueous media (ethanol: H_2O = 95:5) with distinct fluorescence “turn-on” signals. The fluorescence enhancement mechanisms of sensing Al^{3+} and Zn^{2+} were attributed to the CHEF effect and the stoichiometric ratios of **1** with Al^{3+} and Zn^{2+} appeared to be 2:1 and 1:1, respectively. Moreover, chemosensor **1** could reversibly identify Al^{3+} and Zn^{2+} upon the addition of EDTA, and thus, a reasonable logic circuit was constructed with fluorescence emission as an output signal. In addition, the easy-to-prepare **1**-coated swabs could offer direct and rapid Al^{3+} and Zn^{2+} detection in real time by the naked eye. Importantly, chemosensor **1** was successfully applied to quantitative analysis of Al^{3+} and Zn^{2+} in actual samples. These findings provide a useful strategy for the design and construction of simple fluorescent chemosensors with multiple detection modes and potential utility in fluorescence analysis.

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