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# A simple hydrazone as a multianalyte ( $\text{Cu}^{2+}$ , $\text{Al}^{3+}$ , $\text{Zn}^{2+}$ ) sensor at different pH values and the resultant $\text{Al}^{3+}$ complex as a sensor for $\text{F}^{-}$ †

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A new colorimetric and fluorescence molecular chemosensor based on triazole hydrazone can be used as a multi-probe for selective detection of  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cu}^{2+}$  by monitoring changes in the absorption and fluorescence spectral patterns. Results show that  $\text{Al}^{3+}$  and  $\text{Zn}^{2+}$  ions can induce remarkable fluorescence enhancement at pH 6.0 and pH 10.0, respectively, while the addition of  $\text{Cu}^{2+}$  ions leads to a significant UV-visible absorption enhancement in the visible range at pH 6.0. In addition, the resultant  $\text{Al}^{3+}$  complex could act as an 'on-off' fluorescence sensor for  $\text{F}^{-}$ . The fluorescence sensor was also used to monitor intracellular  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$ , and  $\text{F}^{-}$  in Hela cells.

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## Introduction

Fluorescence methods based on molecular chemosensors are important tools for the recognition of metal ions owing to the operational simplicity and low detection limits.<sup>1,2</sup> A new chemosensor design concept of a 'single sensor for multiple analytes' has been introduced recently.<sup>3</sup> Compared to one-to-one analysis methods, a chemosensor with different responses toward multiple metal ions and good bioimaging ability is highly desirable for practical applications.<sup>4</sup>

Aluminum is the most abundant metal element on earth, accounting for about 8% of total mineral components, and it plays an important role in many fields.<sup>5</sup> However, excess aluminum can damage the human nervous system and induce several health hazards.<sup>5,6</sup> The World Health Organization limits  $\text{Al}^{3+}$  concentration in drinking water to  $200 \mu\text{g L}^{-1}$  ( $7.41 \mu\text{M}$ ).<sup>7</sup> In general,  $\text{Al}^{3+}$  prefers hard donor sites (*e.g.*, O and N) in its coordination sphere because of its strong acidity, and the strong hydration of  $\text{Al}^{3+}$  in aqueous media leads to its weak coordination ability.<sup>8,9</sup> Besides, the detection of  $\text{Al}^{3+}$  can be easily interfered with by the matrix, leading to limited selectivity and sensitivity.<sup>10–12</sup> Thus, designing a selective and sensitive fluorescence probe for  $\text{Al}^{3+}$  in aqueous media remains a challenge.

Unlike  $\text{Al}^{3+}$  ion,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  ions are essential elements in biological systems.<sup>13–16</sup> However, at high concentrations,  $\text{Zn}^{2+}$ / $\text{Cu}^{2+}$  ion could also cause disorders associated with neurodegenerative diseases.<sup>17,18</sup> For fluorescent detection of  $\text{Zn}^{2+}$ , most of the reported sensors are affected by cross interference of  $\text{Cd}^{2+}$  owing to their closely related electronic and binding properties.<sup>19,20</sup> On the other hand, excessive  $\text{Cu}^{2+}$  is harmful to the environment,<sup>21</sup> and colorimetric probes for  $\text{Cu}^{2+}$  determination are widely used because they can monitor  $\text{Cu}^{2+}$  both in solution and on test strips, which can be evaluated by the naked eye.<sup>22,23</sup>

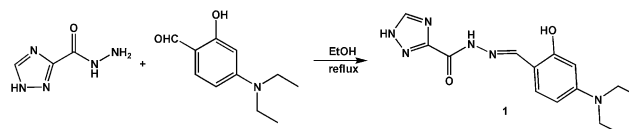
It has been noted that Schiff base sensors deserve special attention due to their simplicity, sensitivity, easy operation, and low cost.<sup>15</sup> Although a number of sensors towards two of  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Zn}^{2+}$  ions have been developed,<sup>5,6,17,23–27</sup> simultaneous selective detection of all three ions by a molecular chemosensor based on a Schiff base scaffold is rarely reported.

Considering the above circumstance, we report here an acylhydrazone derivative **1** (Scheme 1) bearing a triazole ring as a fluorescent turn-on probe toward  $\text{Al}^{3+}$  and  $\text{Zn}^{2+}$  ions at different pH values, and also as a colorimetric probe for  $\text{Cu}^{2+}$  detection. Noticeably, the **1** +  $\text{Al}^{3+}$  system is able to detect  $\text{F}^{-}$  ions by fluorescence quenching, as the resultant metal-probe complex used as secondary sensor is one of the most promising design strategies for the detection of fluoride.<sup>28–30</sup> In addition, the application of the fluorescent probe in living cell image is demonstrated.

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Scheme 1 Synthesis route of the probe 1.



## Experimental section

### Materials and instrumentation

Solvents and starting materials for syntheses were purchased commercially and used as received. Elemental analyses were carried out on an Elemental Vario EL analyzer.  $^1\text{H}$  NMR spectra are recorded on a Bruker AV400 NMR spectrometer in  $\text{DMSO-}d_6$  solution. The UV spectra were recorded on a Purkinje General TU-1800 spectrophotometer. Fluorescence spectra were determined on a Varian CARY Eclipse spectrophotometer. ESI-MS spectra were obtained on a Bruker Daltonics Esquire 6000 mass spectrometer. Fluorescent images were taken on a Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope. The X-ray diffraction measurement for  $1 \cdot 0.5\text{H}_2\text{O}$  was performed on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromatized  $\text{MoK}\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ) by using  $\phi$ - $\omega$  scan mode. Semi-empirical absorption correction was applied to the intensity data using the SADABS program.<sup>31</sup> The structure was solved by direct methods and refined by full matrix least-square on  $F^2$  using the SHELXTL-97 program.<sup>32</sup> All non-hydrogen atoms were refined anisotropically. All H atoms were positioned geometrically and refined using a riding model.

### Synthesis of 1

A quantity of 1,2,4-triazole-3-carbohydrazide (1.27 g, 10 mmol) was added to an EtOH solution (50 mL) containing 4-(diethylamino)-2-hydroxybenzaldehyde (1.93 g, 10 mmol). The mixture was refluxed for 3 h with two drops of acetic acid. After cooling to room temperature, the separated solid was filtered, washed with EtOH, and then dried in air. Yield 79%. Anal. calc. for  $\text{C}_{15}\text{H}_{19}\text{N}_5\text{O}_2$ : C, 59.79; H, 6.36; N, 23.24. Found: C, 59.89; H, 6.48; N, 23.11%.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ ),  $\delta$  (ppm): 14.79 (s, 1H, OH), 12.13 (s, 1H, NH), 11.41 (s, 1H, NH-triazole), 8.62 (s, 1H, CH-triazole), 8.54 (s, 1H, CH=N), 7.14–7.16 (d, 1H, aryl-H), 6.26–6.29 (dd, 1H, aryl-H), 6.12–6.13 (d, 1H, aryl-H), 3.33–3.39 (q, 4H, 2 $\text{CH}_2$ ), 1.09–1.12 (t, 6H, 2 $\text{CH}_3$ ). ESI-MS:  $m/z = 303.1804$  for  $[\text{M} + \text{H}]^+$ . Crystals of  $1 \cdot 0.5\text{H}_2\text{O}$  (Fig. 1) suitable for X-ray diffraction analysis were obtained by recrystallization of compound 1 from EtOH solution. Crystal data for  $\text{C}_{14}\text{H}_{19}\text{N}_6\text{O}_{2.5}$ : crystal size:  $0.45 \times 0.17 \times 0.15 \text{ mm}$ , orthorhombic, space group  $Pbca$ .  $a = 18.204(19) \text{ \AA}$ ,  $b = 15.339(15) \text{ \AA}$ ,  $c = 23.87(2) \text{ \AA}$ ,  $V = 6666(11) \text{ \AA}^3$ ,  $Z = 16$ ,  $T = 296(2) \text{ K}$ ,  $\theta = 1.71$ – $25.00^\circ$ , 31 591

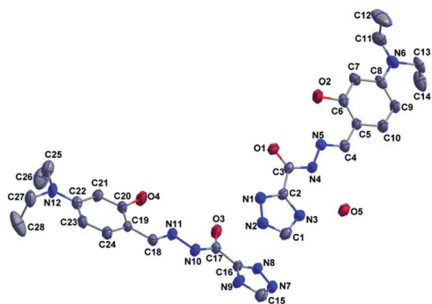


Fig. 1 Crystal structure of  $1 \cdot 0.5\text{H}_2\text{O}$ .

reflections measured, 5856 unique ( $R_{\text{int}} = 0.1615$ ). Final residual for 415 parameters and 5856 reflections with  $I > 2\sigma(I)$ :  $R_1 = 0.0711$ ,  $wR_2 = 0.1843$  and  $\text{GOF} = 1.040$ .

### General UV-vis and fluorescence spectra measurements

The spectral analyses were accomplished in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  (10 mM, 1/1, v/v, pH = 6.0 and or 10.0 for the detection of  $\text{Al}^{3+}/\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , respectively) solution at room temperature. The concentration of the probe 1 for UV-vis and fluorescence measurement was  $5 \mu\text{M}$ . Nitrate salts of different cations ( $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Zn}^{2+}$ ) and sodium or ammonium salts of different anions ( $\text{AcO}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CN}^-$ ,  $\text{SCN}^-$ ,  $\text{N}_3^-$ ,  $\text{ClO}_4^-$ ,  $\text{HSO}_4^-$ ,  $\text{HSO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{S}^{2-}$  and  $\text{PO}_4^{3-}$ ) were used for different titration experiments. UV-vis and fluorescence spectrophotometric titration were conducted directly in 2 mL cuvette by successive addition of corresponding chemical reagent using a microliter syringe. Upon addition of every aliquot, the solution was well mixed then the spectrum was measured.

## Results and discussions

### UV-vis spectroscopic studies of 1 with metal ions in buffered $\text{CH}_3\text{CN}/\text{HEPES}$ solution (10 mM, 1/1, v/v) at pH 6.0

The UV-vis spectrum of probe 1 ( $5 \mu\text{M}$ ) in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1 v/v, pH 6.0) features only one band centered at 376 nm, which should be assigned to the  $\pi$ - $\pi^*$  transition of the imine units.<sup>33</sup> Upon addition of metal ions (1 eq.), including  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Zn}^{2+}$  to the solution of 1, dramatic change in the UV spectra could be obtained only in the case of  $\text{Cu}^{2+}$ . As shown in Fig. 2, a  $\sim 20 \text{ nm}$  redshift selectively for  $\text{Cu}^{2+}$  was observed, corresponding for the green color. In addition, the presence of the other competitive metal ions did not lead to any significant changes in the absorbance of  $1 + \text{Cu}^{2+}$  at 398 nm (Fig. S1, ESI $^\dagger$ ), thereby establishing that the other metal ions do not interfere with  $\text{Cu}^{2+}$  detection. As expected, 1 is

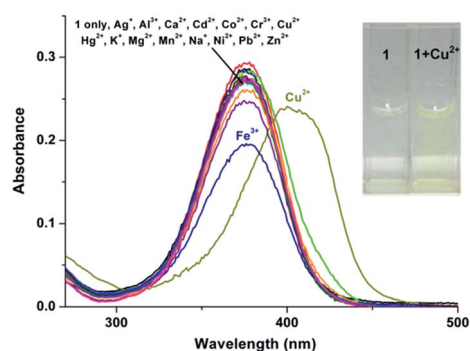


Fig. 2 UV-vis spectra of  $5 \mu\text{M}$  probe 1 in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 6.0) with 1 eq. of metal ions:  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  ions and blank. The insert shows the color of probe 1 only and with the addition of  $\text{Cu}^{2+}$  ion.

a “naked-eye” colorimetric probe with high selectivity towards  $\text{Cu}^{2+}$ .

To evaluate the sensing properties of **1** ( $5 \mu\text{M}$ ) as a receptor, a titration experiment was carried out with gradual addition of  $\text{Cu}^{2+}$  ions (0–2 eq.). As shown in Fig. 3, the absorbance at 376 nm decreases gradually upon addition of  $\text{Cu}^{2+}$ , along with a concomitant generation of a new peak at 398 nm. The appearance of the isosbestic point at 390 nm indicates that the coordination reaction is clean and straightforward.<sup>20</sup> In addition, the probe **1** showed a good linear relationship between the absorbance ratio of  $A_{398}/A_{376}$  and the concentration of  $\text{Cu}^{2+}$  ions from 0 to  $5 \mu\text{M}$  (Fig. 3, inset). Thus, sensor **1** is potentially applicable for quantitative analysis of  $\text{Cu}^{2+}$ . The detection limit of **1** for  $\text{Cu}^{2+}$  is 106.4 nM, which was calculated using the equation  $\text{DL} = 3S_{\text{bi}}/S$  (where  $S_{\text{bi}}$  is the standard deviation of blank measurements and  $S$  is the slope of the intensity *versus* sample concentration).<sup>27</sup> Job's plot experiment result suggests that the binding of **1** to  $\text{Cu}^{2+}$  is of 1 : 1 stoichiometry (Fig. S2, ESI†). The association constant ( $K_{\text{a}}$ ) was calculated to be  $5.50 \times 10^4 \text{ M}^{-1}$  by fitting the data to the Benesi–Hildebrand expression (Fig. S3, ESI†). The probe **1** can function in the pH range of 5.0–12.0 (Fig. S4, ESI†), which is relatively wide when compared to some rhodamine based  $\text{Cu}^{2+}$  ion chemosensors.<sup>34,35</sup>

#### Fluorescence spectroscopic studies of **1** with metal ions in buffered $\text{CH}_3\text{CN}/\text{HEPES}$ solution (10 mM, 1/1, v/v) at pH 6.0

As shown in Fig. 4, excitation of **1** ( $5 \mu\text{M}$ ) at 390 nm in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v) at pH 6.0 shows weak emission at 460 nm (the pass width of emission and excitation being 2.5 nm). Addition of  $\text{Al}^{3+}$  to **1** solution induces a significant fluorescence enhancement (light-blue emission under 365 nm UV lamp, Fig. 4, inset). By contrast, the addition of the other metal ions does not lead to any noticeable spectral change (except for  $\text{Zn}^{2+}$ , which showed a slight fluorescence enhancement). In addition, the fluorescence of the **1** +  $\text{Al}^{3+}$  complex could be maintained in the presence of most of the other metal ions (Fig. S5, ESI† except for  $\text{Cu}^{2+}$ , because of its inherent magnetic property; and  $\text{Cr}^{3+}$ , probably due to its strong Lewis acidity, resulting in the hydrolytic cleavage of the imine bond<sup>36</sup>).

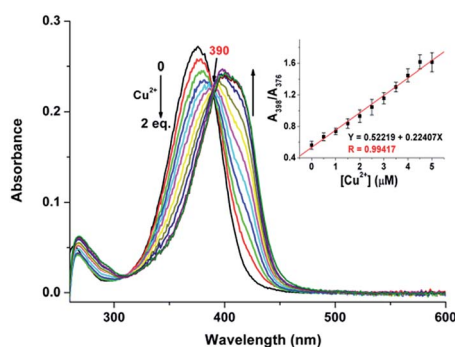


Fig. 3 Absorption spectra of  $5 \mu\text{M}$  probe **1** upon the addition of  $\text{Cu}^{2+}$  (0–2 eq.) in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 6.0). The inset shows the absorbance ratio of  $A_{398}/A_{376}$  as a function of  $\text{Cu}^{2+}$  concentration (0– $5 \mu\text{M}$ ).

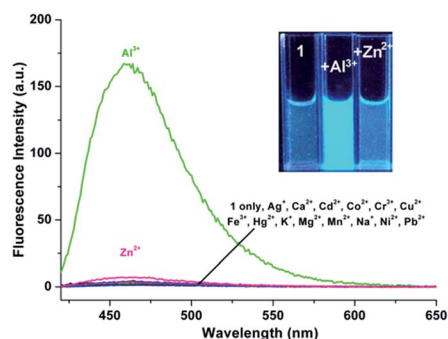


Fig. 4 Fluorescence emission spectra of  $5 \mu\text{M}$  probe **1** in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 6.0) with 1 eq. of metal ions:  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  ions and blank, excitation wavelength was 390 nm (the pass width of emission and excitation being 2.5 nm). The inset shows the change of the color of probe **1** only and with the addition of  $\text{Al}^{3+}$  and  $\text{Zn}^{2+}$  under 365 nm UV lamp, respectively.

The titration experiment of the probe with increasing concentration of  $\text{Al}^{3+}$  (Fig. 5) showed that the fluorescence intensity at 460 nm of a  $5 \mu\text{M}$  solution of **1** increased linearly with incremental addition of  $\text{Al}^{3+}$  ions (1– $6.5 \mu\text{M}$ ). The detection limit of **1** for  $\text{Al}^{3+}$  is calculated to be 22.5 nM. The quantum yields of the solutions of **1** ( $5 \mu\text{M}$ ) with and without 1 eq.  $\text{Al}^{3+}$  ions are 0.28 and 0.02, respectively, using quinine sulfate as standard ( $\Phi_{\text{F}} = 0.546$  in  $0.5 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ ).<sup>20</sup> Furthermore, Job's plot experiment shows that the binding of **1** to  $\text{Al}^{3+}$  is of 1 : 1 stoichiometry (Fig. S6, ESI†), whose association constant ( $K_{\text{a}}$ ) was calculated to be  $1.01 \times 10^5 \text{ M}^{-1}$  (Fig. S7, ESI†).

#### Fluorescence spectroscopic studies of **1** with metal ions in buffered $\text{CH}_3\text{CN}/\text{HEPES}$ solution (10 mM, 1/1, v/v) at pH 10.0

During the experimental process, we found that the fluorescence intensity of the solution of **1** may be enhanced by adding  $\text{Zn}^{2+}$  to certain amount. Therefore,  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v) at pH 10.0 was employed as a tested media to

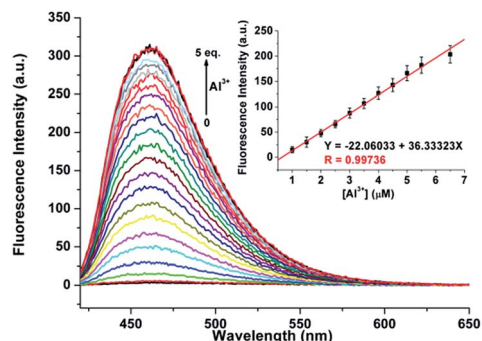


Fig. 5 Fluorescence emission spectra of  $5 \mu\text{M}$  probe **1** upon the addition of  $\text{Al}^{3+}$  (0–5 eq.) in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 6.0). The inset shows the fluorescence intensities at 460 nm as a function of  $\text{Al}^{3+}$  concentration (1– $6.5 \mu\text{M}$ ). Excitation wavelength was 390 nm (the pass width of emission and excitation being 2.5 nm).

detect  $\text{Zn}^{2+}$  ion (the pass width of emission and excitation being 5 nm). It is observed that only the presence of  $\text{Zn}^{2+}$  ions induces clear fluorescent enhancement of **1** (Fig. 6), which indicates the high selectivity of probe **1** for  $\text{Zn}^{2+}$  ion. Unfortunately, several transition metal ions, such as  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Ni}^{2+}$  ions could interfere with the  $\text{Zn}^{2+}$  detection (Fig. S8, ESI†). The titration experiment shows that the fluorescence intensity at 470 nm increased linearly over the  $\text{Zn}^{2+}$  concentration range of 0.5–4  $\mu\text{M}$  (Fig. 7). The detection limit of **1** for  $\text{Zn}^{2+}$  is 102.5 nM, which is sufficiently low for  $\text{Zn}^{2+}$  detection in many chemical and biological systems.<sup>20</sup>

The spectral response of **1** (5  $\mu\text{M}$ ) with or without  $\text{Al}^{3+}/\text{Zn}^{2+}$  (1 eq.) in  $\text{CH}_3\text{CN}/\text{HEPES}$  (v/v = 1 : 1) solution at different pH values was evaluated at room temperature. As shown in Fig. S9 and S10, ESI†, the sensor **1** can detect  $\text{Al}^{3+}$  ions in the pH range of 4.0–8.0, while 7.0–12.0 in the case of  $\text{Zn}^{2+}$  detection. All results show that sensor **1** may be used as a candidate for detecting  $\text{Al}^{3+}$  and  $\text{Zn}^{2+}$  in semi-aqueous media.

### Spectroscopic studies of the **1** + $\text{Al}^{3+}$ system in the presence of $\text{F}^-$ ions

The advantage of the reversibility of the probe based on coordination reaction (*vide infra*) was taken into consideration.<sup>30</sup> The rupture of the **1** +  $\text{Al}^{3+}$  species to regenerate **1**, and the subsequent change in the optical properties of **1** +  $\text{Al}^{3+}$  system in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v) at pH 6.0, were studied by introducing a variety of anions ( $\text{AcO}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CN}^-$ ,  $\text{SCN}^-$ ,  $\text{N}_3^-$ ,  $\text{ClO}_4^-$ ,  $\text{HSO}_4^-$ ,  $\text{HSO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{S}^{2-}$  and  $\text{PO}_4^{3-}$ ). The significant quenching of fluorescence in the **1** +  $\text{Al}^{3+}$  system due to regeneration of **1** was observed only in the presence of  $\text{F}^-$  ions (Fig. 8, the pass width of emission and excitation being 2.5 and 5 nm, respectively). In addition, the presence of competitive anions do not interfere with  $\text{F}^-$  detection (Fig. S11, ESI†). It is suggested that the strong interaction of  $\text{Al}^{3+}$  and  $\text{F}^-$  ions possibly facilitates the rupture of the **1** +  $\text{Al}^{3+}$  complex in support of a quenching phenomenon, by the reduction in fluorescence intensity at 460 nm, which is also

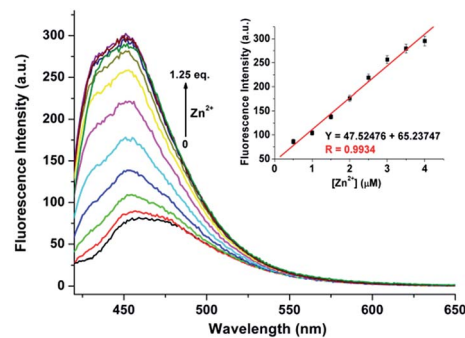


Fig. 7 Fluorescence emission spectra of 5  $\mu\text{M}$  probe **1** upon the addition of  $\text{Zn}^{2+}$  (0–1.25 eq.) in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 10.0). The inset shows the fluorescence intensities at 460 nm as a function of  $\text{Zn}^{2+}$  concentration (0.5–4  $\mu\text{M}$ ). Excitation wavelength was 390 nm (the pass width of emission and excitation being 5 nm).

supported by UV spectrum (Fig. S12, ESI†). The titration experiment (Fig. 9) showed that the fluorescence intensity at 460 nm of **1** +  $\text{Al}^{3+}$  system decreased linearly with gradual addition of  $\text{F}^-$  ions (1–10  $\mu\text{M}$ ). Based on the above fluorescence titration analysis, the detection limit is found to be 30.6 nM, which is quite lower than some of the reported resultant metal-probe complex sensors for  $\text{F}^-$  ions.<sup>25</sup> Reaction mechanism of **1** with  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$  and  $\text{Zn}^{2+}$ . The reaction mechanism of **1** with  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Zn}^{2+}$  was explored using ESI-MS analysis. The ESI-MS spectra of **1** +  $\text{Cu}^{2+}$ , **1** +  $\text{Al}^{3+}$ , and **1** +  $\text{Zn}^{2+}$  exhibit peaks at  $m/z$  363.0863, 390.1046, and 406.0924 (Fig S13–S15, ESI†), which should be assigned to  $[\text{Cu}(\text{1-2H})]$ ,  $[\text{Al}(\text{1-2H})(\text{NO}_3)]$ , and  $[\text{Zn}(\text{1-2H})(\text{CH}_3\text{CN})]$ , respectively. Moreover, the  $^1\text{H}$  NMR spectral differences between **1** and **1** +  $\text{Al}^{3+}/\text{Zn}^{2+}$  is shown in Fig. 10. The proton peaks at 14.79 and 12.13 should be assigned to OH and  $\text{NH}-\text{C}=\text{O}$  of **1**, respectively. Both peaks disappeared in the spectra of **1** +  $\text{Al}^{3+}$  and **1** +  $\text{Zn}^{2+}$ , confirming the metal binding of the phenolic hydroxyl and the enolized hydrazone carbonyl

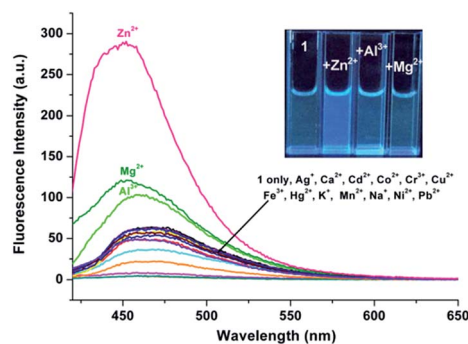


Fig. 6 Fluorescence emission spectra of 5  $\mu\text{M}$  probe **1** in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 10.0) with 1 eq. of metal ions:  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  ions and blank, excitation wavelength was 390 nm (the pass width of emission and excitation being 5 nm). The insert shows the change of the color of probe **1** only and with the addition of  $\text{Al}^{3+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Zn}^{2+}$  under 365 nm UV lamp, respectively.

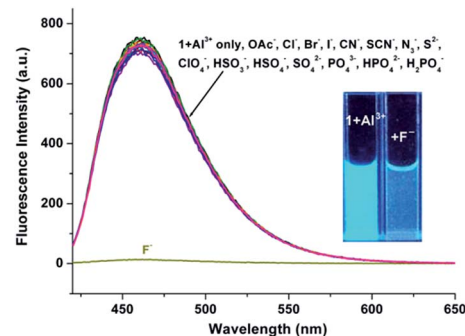


Fig. 8 Fluorescence emission spectra of 5  $\mu\text{M}$  probe **1** +  $\text{Al}^{3+}$  (1 eq.) in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 6.0) with 10 eq. of common anions:  $\text{AcO}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CN}^-$ ,  $\text{SCN}^-$ ,  $\text{N}_3^-$ ,  $\text{S}^{2-}$ ,  $\text{ClO}_4^-$ ,  $\text{HSO}_4^-$ ,  $\text{HSO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{HPO}_4^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ , and blank, excitation wavelength was 390 nm (the pass width of emission and excitation being 2.5 and 5 nm, respectively). The insert shows the change of the color of **1** +  $\text{Al}^{3+}$  only and with the addition of  $\text{F}^-$  under 365 nm UV lamp.

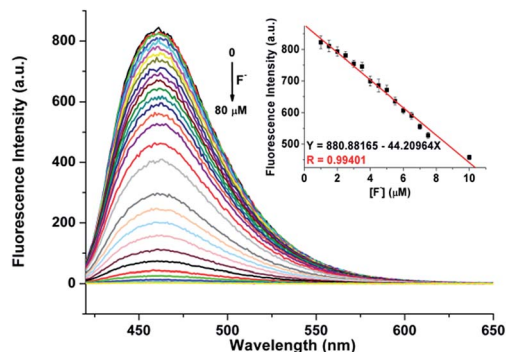


Fig. 9 Fluorescence emission spectra of 5  $\mu\text{M}$  probe **1** +  $\text{Al}^{3+}$  (1 eq.) upon the addition of  $\text{F}^-$  (0–80  $\mu\text{M}$ ) in buffered  $\text{CH}_3\text{CN}/\text{HEPES}$  solution (10 mM, 1/1, v/v, pH = 6.0). The inset shows the fluorescence intensities at 460 nm as a function of  $\text{F}^-$  concentration (0–4  $\mu\text{M}$ ). Excitation wavelength was 390 nm (the pass width of emission and excitation being 2.5 and 5 nm, respectively).

groups. Although the  $\text{CH}=\text{N}$  signal gives almost no shift, the triazole NH of **1** shifts to lower field in the presence of  $\text{Al}^{3+}/\text{Zn}^{2+}$ , probably due to the coordination of imine N atom according to the previous results.<sup>37</sup> Therefore, it is suggested that **1** could chelate metal ions (such as  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Zn}^{2+}$ ) to form 1 : 1 complex with  $\text{O}_2\text{N}$  donor set (Scheme 2).

To further clarify the configuration of **1** and corresponding complexes, we performed density functional theory (DFT) calculations with the Becke-3-Lee-Yang-Parr (B3LYP) exchange function using the Gaussian 09 package.<sup>38</sup> Results show that probe **1** adopts a 1 : 1 binding stoichiometry with  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Al}^{3+}$  ions (Fig. 11). In all three complexes, **1** uses the N atom of imine and two deprotonated O atoms of both hydroxyl and enolized carboxyl groups to coordinate with metal ions (an additional coordinated  $\text{CH}_3\text{CN}$  molecule for  $\text{Zn}^{2+}$ , whereas a bidentate nitrate anion in the case of  $\text{Al}^{3+}$ ), thus acting as a tridentate dianionic ligand. The coordination bond lengths of **1**- $\text{Cu}^{2+}$ , **1**- $\text{Zn}^{2+}$  and **1**- $\text{Al}^{3+}$  range from 1.833–1.947, 1.944–2.179, and 1.775–1.973 Å, respectively. As shown in Scheme 3, the free Schiff base sensor **1** displayed weak fluorescence emission primarily due to the  $\text{C}=\text{N}$  isomerization. Binding with  $\text{Zn}^{2+}/\text{Al}^{3+}$  ions inhibits the isomerization of  $\text{C}=\text{N}$ , thus enhancing the

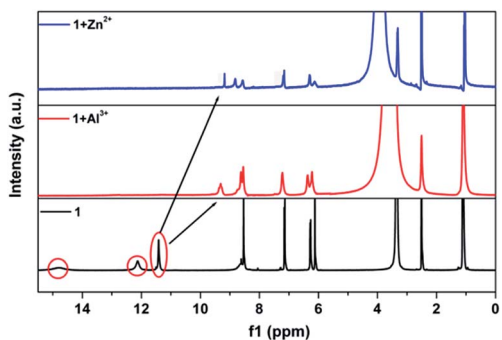
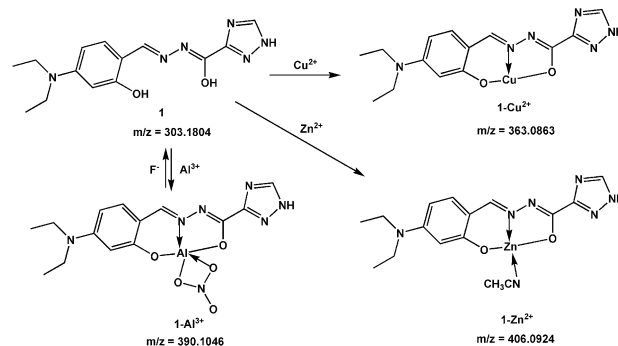


Fig. 10  $^1\text{H}$  NMR spectral changes of **1** with the addition of  $\text{Al}^{3+}/\text{Zn}^{2+}$  in  $\text{DMSO}-d_6$  solution.



Scheme 2 The proposed reaction mechanism of **1** with  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Zn}^{2+}$  ions.

fluorescence intensity through the chelation-enhanced fluorescence (CHEF) mechanism.<sup>39</sup>

The spatial distributions and orbital energies of the HOMO and LUMO of **1**, **1**- $\text{Cu}^{2+}$ , **1**- $\text{Al}^{3+}$ , and **1**- $\text{Zn}^{2+}$  complexes were also generated (Fig. 11). The 3D isosurface HOMO and LUMO diagram showed that the electron densities of HOMOs for four compounds are more or less similar, and mostly located on the probe moiety, whereas those of LUMOs are separated to the coordination solvent (for **1**- $\text{Zn}^{2+}$ ) or anion (for **1** +  $\text{Al}^{3+}$ ). Both HOMO and LUMO of **1**- $\text{Cu}^{2+}$  are concerned with the metal ion, clearly establishing the charge transfer process from probe **1** to  $\text{Cu}^{2+}$  ion. In addition, LUMO and HOMO energies are calculated to be  $-1.38$  eV and  $-5.07$  eV, respectively, for probe **1**. In **1**- $\text{Cu}^{2+}$ , **1**- $\text{Zn}^{2+}$ , and **1**- $\text{Al}^{3+}$  complexes, the respective LUMO and HOMO energies are  $-1.61$  and  $-5.23$  eV,  $-0.95$  and  $-4.51$  eV,  $-2.14$  and  $-5.55$  eV, respectively. The corresponding energy difference  $\Delta E$  of **1**, **1**- $\text{Cu}^{2+}$ , **1**- $\text{Zn}^{2+}$ , and **1**- $\text{Al}^{3+}$  are 3.69, 3.62, 3.56 and 3.41 eV, respectively, suggesting that the complexes are more stable than the free probe **1**.

### Fluorescence imaging of intercellular $\text{Al}^{3+}$ , $\text{Zn}^{2+}$ and $\text{F}^-$

The ability of the fluorescence chemosensor **1** to detect  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$  and  $\text{F}^-$  in HeLa cells was examined. The cells were added with **1** (5  $\mu\text{M}$ ) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum for 30 min at 37 °C,

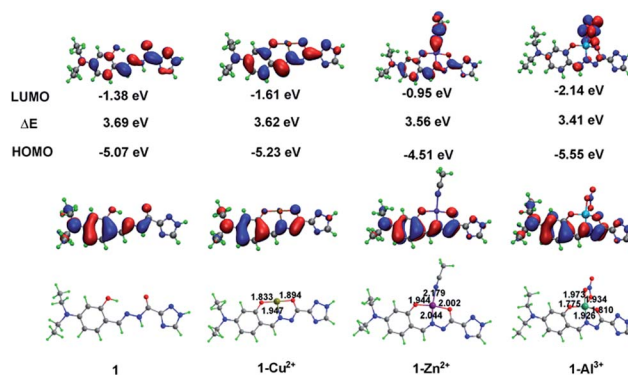
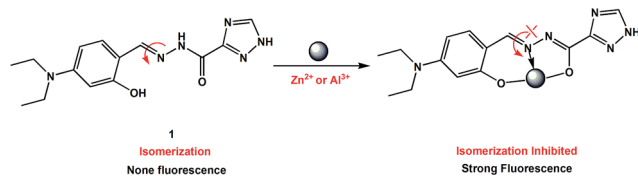


Fig. 11 Optimized structures and HOMO/LUMO of **1**, complexes **1**- $\text{Cu}^{2+}$ , **1**- $\text{Al}^{3+}$  and **1**- $\text{Zn}^{2+}$  by DFT calculation.



Scheme 3 Possible luminescence mechanism of **1** with  $\text{Al}^{3+}/\text{Zn}^{2+}$ .

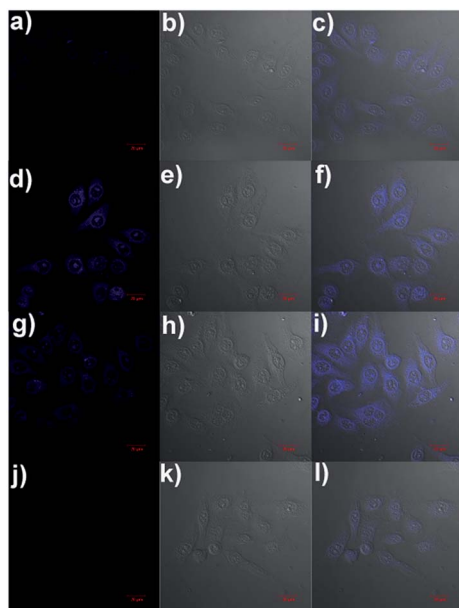


Fig. 12 Confocal fluorescence images of HeLa cells: confocal fluorescence (a), brightfield (b), and overlay (c) images of HeLa cells incubated with  $5 \mu\text{M}$  of **1** for 30 min at  $37^\circ\text{C}$ ; confocal fluorescence (d), brightfield (e), and overlay (f) images of HeLa cells incubated with  $5 \mu\text{M}$  of **1** for 30 min at  $37^\circ\text{C}$  and then incubated with  $5 \mu\text{M}$   $\text{Al}^{3+}$  for another 30 min at  $37^\circ\text{C}$ ; confocal fluorescence (g), brightfield (h), and overlay (i) images of HeLa cells incubated with  $5 \mu\text{M}$  of **1** for 30 min at  $37^\circ\text{C}$  and then incubated with  $5 \mu\text{M}$   $\text{Zn}^{2+}$  for another 30 min at  $37^\circ\text{C}$ ; confocal fluorescence (j), brightfield (k), and overlay (l) images of HeLa cells incubated with  $5 \mu\text{M}$  of **1** and  $\text{Al}^{3+}$  ( $5 \mu\text{M}$ ) for 30 min at  $37^\circ\text{C}$  and then incubated with  $20 \mu\text{M}$   $\text{F}^-$  for another 30 min at  $37^\circ\text{C}$ .

leading to weak intracellular fluorescence as determined by laser scanning confocal microscopy (Fig. 12a). Free **1** was removed by rinsing the cells with DMEM. Then the cells were added with  $5 \mu\text{M}$   $\text{Al}^{3+}/\text{Zn}^{2+}$  in DMEM supplemented with 10% fetal bovine serum and incubated for another 30 min at  $37^\circ\text{C}$  under same imaging condition. A significant increase in the fluorescence from the intracellular area was observed (Fig. 12d and g). In addition, the cells supplemented with **1** and  $\text{Al}^{3+}$  were further treated with  $\text{F}^-$  ( $20 \mu\text{M}$ ), the strong intracellular fluorescence became weak (Fig. 12j). All facts suggest that sensor **1** can be used to image intracellular  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$  and  $\text{F}^-$  in living cells.

## Conclusion

In summary, a simple Schiff base has been presented as a fluorescent off-on sensor for  $\text{Al}^{3+}$  and  $\text{Zn}^{2+}$  under different pH conditions, as well as a colorimetric probe for  $\text{Cu}^{2+}$  in semi-

aqueous media. The sensor functions by forming stable complexes with  $\text{Cu}^{2+}/\text{Zn}^{2+}/\text{Al}^{3+}$ , and the detection limit of the sensor is at the parts per million level for each of the metal ions. In addition, the resultant **1** +  $\text{Al}^{3+}$  complex could act as an 'on-off' fluorescent sensor for  $\text{F}^-$ . The probe **1** was also used to determine  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$  and  $\text{F}^-$  in living cells.

## Conflicts of interest

There are no conflicts to declare.

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