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Development of a simple pyrazine-derived “turn on” Al^{3+} fluorescent sensor with high selectivity and sensitivity

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ABSTRACT

In this article, a novel pyrazine-derived hydrazone Schiff-base ligand bearing the quinoline unit (**1**) has been designed, synthesized and evaluated as a “turn on” fluorescent chemosensor for Al^{3+} based on the photoinduced electron-transfer (PET) mechanism and the chelation-enhanced fluorescence (CHEF) phenomenon. The sensor **1** showed remarkably enhanced fluorescence intensity at 488 nm in the presence of Al^{3+} ion and it also showed high selectivity and sensitivity towards Al^{3+} ion over a wide range of metal ions in ethanol, for the detection limit of **1** for Al^{3+} could reach at 10^{-7} mol/L.

1. Introduction

The development of methods for the detection and recognition of biologically related metallic ions has attracted much interest due to their importance in the human body [1,2]. In recent years, many techniques, such as atomic absorption spectrometry (AAS) [3], atomic emission spectrometry (AES) [4], and electron paramagnetic resonance (EPR) [5], have been developed to detect metal ions. However, these techniques suffer from complicated operational sequence, sophisticated synthetic procedure and expensive operational cost in medicinal and environmental research [6]. Owing to the features of high sensitivity, facile operation, real time detection and instantaneous response, the fluorescence technique has received considerable attention in the field of detecting and recognizing metallic ions [7]. Consequently, fluorescent sensors have been recognized as powerful tools for monitoring biologically related metal ions [8–13].

The detection of Al^{3+} is of great importance because Al^{3+} is an essential metal ion in the biological and physical systems [14]. It is well known that aluminum is the most abundant metallic element in the earth’s crust and has widespread applications in modern life [15,16]. However, Al^{3+} can cause harm to human beings when it is exposed to high concentration levels [17]. Aluminum is regarded as a toxic element, because Al^{3+} can cause damage to central nervous system and immune system in human bodies [18], affecting the absorb and use of other trace elements [19], and induce many health problems, such as Alzheimer’s disease and Parkinson’s disease [20]. Nevertheless, there have been only a few reports about the development of fluorescent chemosensors for Al^{3+} because of its poor coordination ability [21]. Therefore, it is a great demand to design and synthesize Al^{3+} selective and sensitive fluorescent chemosensors [22–26].

For a sensor which is based on the photoinduced electron-transfer (PET) mechanism and the chelation-enhanced fluorescence (CHEF) phenomenon, it is evident that the PET from the amine group to the excited singlet state of fluorophore makes the fluorescence of the sensor quenched. However, when a suitable metal ion is added to the sensor solution, the inhibition of PET process will occur with the complexation of the sensor and this specific metal ion, which will cause a large chelation-enhanced fluorescence (CHEF) effect [27–30]. Thus, the enhancement of the fluorescence emission intensity of the sensor can be observed. However, some of the fluorescent sensors reported are lack of selectivity and sensitivity over other common biologically related metal ions, which restrains these sensors for practical application [31].

Herein, a novel pyrazine-derived hydrazone Schiff-base ligand called 2-Acetylpyrazine (8′-hydroxyquinolineyl-2′-acetyl) hydrazone (**1**) was designed and synthesized through a three-step reaction (Scheme 1). For this compound had so simple structure, it could be used as a fluorescent chemosensor to detect and recognize Al^{3+} ion. From the experimental process, we can observe that the ethanol solution of **1** was nearly nonfluorescent. However, when Al^{3+} was added to the solution of **1**, the fluorescence emission intensity at 488 nm would enhance remarkably. Furthermore, this fluorescent sensor **1** had high selectivity and

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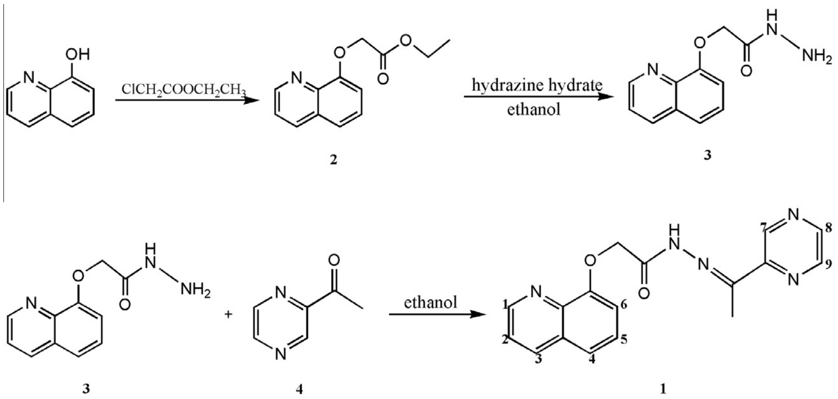


Scheme 1. The synthetic route of compound **1**.

sensitivity over a wide range of other common biologically related metallic ions tested, and could respond Al^{3+} in a reversible manner, which developed **1** for practical application.

2. Experimental

2.1. Materials

8-Hydroxyquinoline, ethyl chloroacetate, hydrazine hydrate, acetyl pyrazine, absolute ethanol and salts of Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+} were obtained from commercial suppliers and used without further purification.

2.2. Methods

Distilled water was used throughout all experiments. ^{1}H NMR spectra were measured on the JNM-ECS 400 MHz instruments using TMS as an internal standard in $CDCl_3$. The ESI-MS data were obtained in ethanol from a Bruke Esquire 6000 spectrometer. FT-IR spectra were recorded with a VERTEX-70 spectrometer. Elemental analyses were performed using a VarioEL Cube V1.2.1 analyzer. UV–Vis absorption spectra were recorded on a Perkin Elmer Lamda 35 UV–Vis spectrophotometer in ethanol medium at 298 K. Fluorescence spectra were generated on a Hitachi RF-4500 spectrophotometer equipped with quartz cuvettes of 1 cm path length. Melting points were determined on a Beijing X-4 microscopic melting point apparatus.

2.3. Synthesize

2.3.1. Synthesize of compound **1** (2-acetylpyrazine (8′-hydroxyquinolineyl-2′-acetyl) hydrazone)

Ethyl 2-(quinolin-8-yloxy) acetate (**2**) and 2-(quinolin-8-yloxy) acetohydrazide (**3**) were prepared by the reported method [32]. A solution of the 2-(quinolin-8-yloxy) acetohydrazide (0.199 g, 0.968 mmol) in ethanol was added to a solution containing acetyl pyrazine (0.118 g, 0.968 mmol) in ethanol. The mixture was refluxed for 26 h, and the obtained light yellow solution was then cooled to room temperature. Then the light yellow product was filtered, washing three times with distilled water and absolute ethanol, respectively. Finally, the solid was dried under drying oven overnight to give the desired product **1** as a white powder (0.24 g, 77.24%) (Scheme 1). m.p. 213–215 °C, ^{1}H NMR (400 MHz, $CDCl_3$) (Fig. S1): 11.26 (s, 1H, –NH–), 9.49 (d, 1H, $J = 1.6$ Hz, H_1), 8.94 (dd, 1H, $J = 4.0$ Hz, 1.6 Hz, H_3), 8.55–8.50 (m, 2H, $H_{2,\,4}$), 8.28 (d, 1H, $J = 8.0$ Hz, H_5), 7.59–7.51 (m, 3H, $H_{7,\,8,\,9}$), 7.29 (dd, 1H, $J = 7.2$ Hz, 1.6 Hz, H_6), 5.03 (s, 2H, $–CH_2–$), 2.63 (s, 3H, $–CH_3$). MS (ESI) (Fig. S2): *m/z* 322.1219 $[M+H^+]^+$, 344.1037 $[M+Na^+]^+$, 665.2140 $[2M+H^+]^+$. FT-IR (KBr pellet, cm^{-1}) (Fig. S3): 3441 (N–H), 1695 (C=O), 1622 (C=N), 1275 (C–O), 1119 (C–N). UV–Vis (nm) (Fig. 1): 295, 350. *Anal.* Calcd. for $C_{17}H_{15}N_5O_2$ (%): C, 63.54; H, 4.71; N, 21.79; O, 9.96. Found: C, 63.04; H, 4.57; N, 21.40; O, 10.99.

2.4. Analysis

Stock solutions (10 mM) of the salts of Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+} in ethanol were prepared. Stock solution of the compound **1** (10 mM) in dimethyl sulfoxide (DMSO) was also prepared. Test solutions were prepared by placing 10 μL of the probe stock solution into cuvettes, adding an appropriate aliquot of each metal ion stock, and diluting the solution to 2 mL with ethanol. For all measurements, excitation wavelength was at 350 nm; the excitation slit width was 5.0 nm, and the emission slit width was 3.0 nm.

The binding constant values were determined from the emission intensity data following the modified Benesi–Hildebrand Eq. (1) [33]:

$$\frac{1}{F - F_{\min}} = \frac{1}{K(F_{\max} - F_{\min})[AL^{3+}]} + \frac{1}{F_{\max} - F_{\min}}. \tag{1}$$

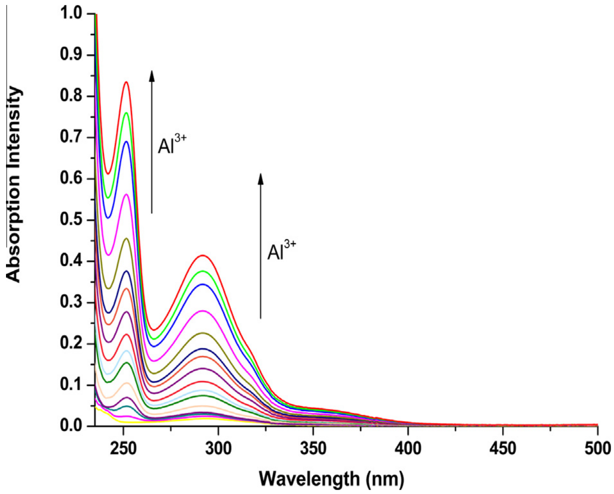


Fig. 1. UV–Vis absorption of compound **1** (100 μM) measured in ethanol upon addition of various concentration of Al^{3+} (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0 *equiv.*, respectively).

where $F_{\min}$, F, and $F_{\max}$ are the emission intensities of the organic moiety considered in the absence of aluminum ion, at an intermediate aluminum concentration, and at a concentration of complete interaction, respectively, and where K_a is the binding constant concentration.

3. Results and discussion

3.1. UV–Vis analysis of compound **1**

Fig. 1 depicts the UV–Vis absorption spectra of compound **1** (100 μM) in ethanol upon the gradual addition of Al^{3+}. The compound **1** in the absence of any metal ion displayed a weak absorption band at 295 nm with a shoulder at 350 nm, which was possibly assigned to the absorption band of pyrazine. However, when Al^{3+} was added to the solution of **1**, the absorption band at 295 nm enhanced with increasing Al^{3+} amount. Simultaneously, a new absorption band which was centered at 251 nm appeared with increasing intensity (Fig. 1), which suggested that Al^{3+} coordinated with pyrazine, and this compound **1** could respond for Al^{3+} in the UV–Vis absorption spectra. According to the UV–Vis Absorption spectra of compound **1**, we chose the shoulder (350 nm) as excitation wavelength in the following fluorescence emission measurements.

3.2. Selectivity of compound **1** for Al^{3+} over other metal ions

The fluorescence emission spectra of compound **1** upon addition of various metal ions (Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) in ethanol was illustrated in Fig. 2. The addition of Al^{3+} into the solution of **1** caused the remarkably enhanced fluorescence intensity at 488 nm. However, upon addition of Fe^{3+} and Cr^{3+}, it resulted in relatively weak enhancement in fluorescence intensity and was significantly lower than that of Al^{3+}. Nevertheless, no significant change of the fluorescence emission was observed in the presence of other metal ions investigated (Fig. 2). From the results above, it was concluded that compound **1** had high selectivity for Al^{3+} over other biologically related metal ions.

3.3. Fluorescence titration of compound **1** with Al^{3+}

As shown in Fig. 3, the compound **1** without any metal ion showed very weak fluorescence at 391 nm in ethanol upon excitation at 350 nm, and there was no emission at longer wavelength.

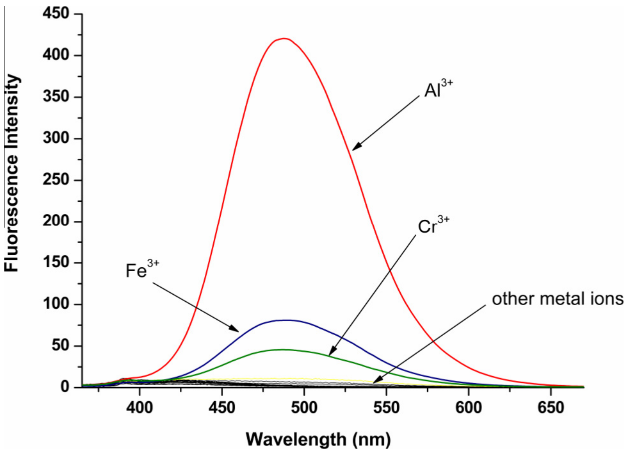


Fig. 2. Fluorescence spectra of **1** (50 μM) upon addition of various metal ions (1 *equiv.*) in ethanol with an excitation at 350 nm.

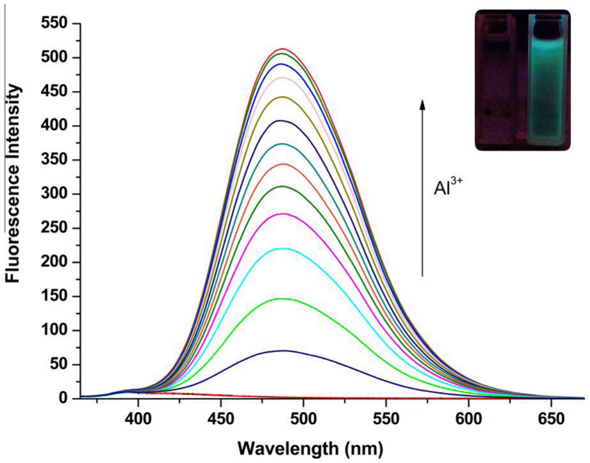
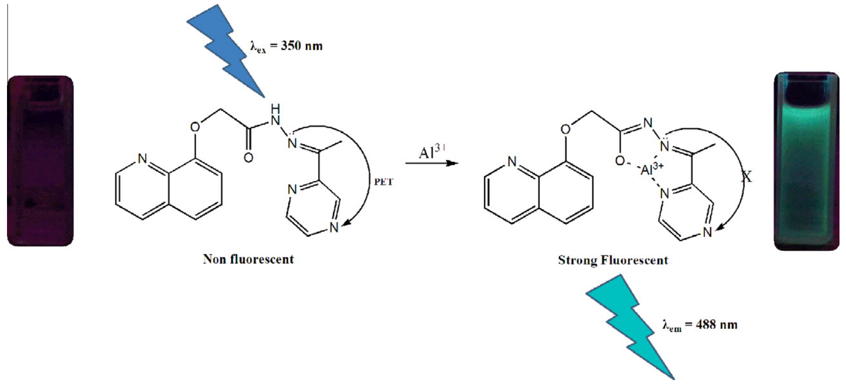


Fig. 3. Change in fluorescence spectra of **1** (50 μM) measured in ethanol upon addition of various concentration of Al^{3+} (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 *equiv.*, respectively) with an excitation at 350 nm. Inset: fluorescence color of **1** in the absence (left) and presence (right) of Al^{3+} in ethanol under a UV lamp.

We hypothesized that the lone pair electrons from the Schiff-base nitrogen atom to the pyrazine contributed to the photoinduced electron-transfer (PET) phenomenon, which made the fluorescence emission of compound **1** quenched. Upon addition of less than 0.2 *equiv.* of Al^{3+}, there was almost no change in the fluorescence emission spectrum. Nevertheless, when the amount of Al^{3+} was more than 0.2 *equiv.*, the fluorescence emission intensity at 488 nm increased with increasing amount of Al^{3+} (Fig. S4). The reason was attributed to the inhibition of the PET phenomenon upon complexation of **1** with Al^{3+}, which made the quenched fluorescence recur by occurring highly efficient chelation-enhanced fluorescence (CHEF) effect [34–36] (Scheme 2). As a result, the fluorescence emission intensity at 488 nm enhanced remarkably by about 230-fold with a large red-shift in the presence of 1 *equiv.* of Al^{3+}. Simultaneously, the fluorescence color of ethanol solution of **1** turned light green upon addition of Al^{3+} under a UV lamp, which could be easily detected by the naked eye (Fig. 3). Furthermore, the binding constant of **1** with Al^{3+} was determined to be 1.35×10^4 from the fluorescence titration profile using the Benesi–Hildebrand Eq. (1) (Fig. S5), and the detection limit of this sensor **1** for Al^{3+} was estimated as 10^{-7} mol/L level conducted by using fluorescence spectra (Fig. S6), which was lower than some reported Al^{3+} selective and sensitive fluorescent sensors [37–40]. It demonstrated that the detection limit was low enough for this sensor to detect and control Al^{3+} in the environmental and biological systems.

3.4. Selectivity of compound **1** for Al^{3+} in the presence of other metal ions

Competition experiments were conducted to further explore the selectivity of this fluorescent sensor **1** for Al^{3+} in the presence of other biologically related metal ions. The fluorescence responses of compound **1** in the presence of Al^{3+} mixed with a variety of other metal ions (Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) in ethanol was shown in Fig. 4. It was evident that two magnetic metal ions Cu^{2+} and Ni^{2+} made the fluorescence intensity at 488 nm completely quenched. However, Cd^{2+}, Co^{2+}, Fe^{2+} and Fe^{3+} induced relatively slight quenching at 488 nm. Nevertheless, in the presence of other metal ions investigated, the fluorescence emission spectra displayed a similar



Scheme 2. Proposed mechanism for detection of Al^{3+} by **1**.

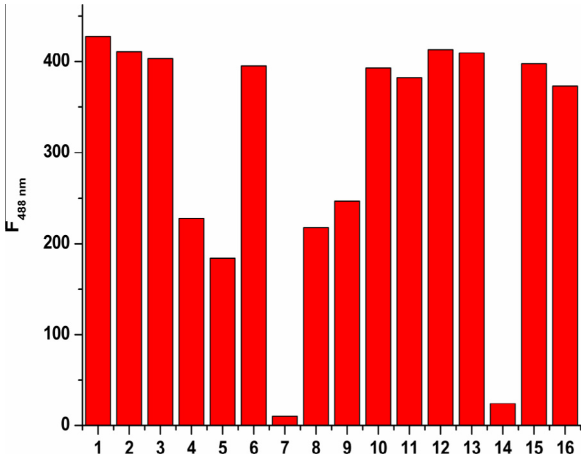


Fig. 4. Fluorescence responses of **1** (50 μM) to Al^{3+} (1 *equiv.*) in the presence of various metal ions (5 *equiv.*) under the same conditions at a wavelength of 488 nm. Bars represent the fluorescence intensity at 488 nm. ((1) Al^{3+}; (2) $Al^{3+} + Ba^{2+}$; (3) $Al^{3+} + Ca^{2+}$; (4) $Al^{3+} + Cd^{2+}$; (5) $Al^{3+} + Co^{2+}$; (6) $Al^{3+} + Cr^{3+}$; (7) $Al^{3+} + Cu^{2+}$; (8) $Al^{3+} + Fe^{2+}$; (9) $Al^{3+} + Fe^{3+}$; (10) $Al^{3+} + K^{+}$; (11) $Al^{3+} + Mg^{2+}$; (12) $Al^{3+} + Mn^{2+}$; (13) $Al^{3+} + Na^{+}$; (14) $Al^{3+} + Ni^{2+}$; (15) $Al^{3+} + Pb^{2+}$; (16) $Al^{3+} + Zn^{2+}$).

pattern to that with Al^{3+} only. (Fig. 4). Thus, most competing metal ions did not interfere with the detection of Al^{3+}. Therefore, this compound **1** was shown to be a promising selective fluorescent sensor for Al^{3+} in the presence of most biologically related metal ions.

*3.5. The reversibility and regeneration of the binding of Al^{3+} by **1***

In order to develop this fluorescent sensor **1** for practical application, the reversibility and regeneration of compound **1** were investigated. For this purpose, the Al^{3+} complex was treated with $EDTANa_2$ that was a good chelating agent with Al^{3+}. As illustrated in Fig. 5, the addition of $EDTANa_2$ to a mixture of compound **1** and Al^{3+} made the fluorescence emission intensity quenched completely at 488 nm. It seemed that the decomplexation of **1**-Al^{3+} and the formation of Al^{3+}–$EDTANa_2$ complex made compound **1** free from **1**-Al^{3+} solution. As a result, the fluorescence emission spectrum was nearly equal to free **1**. However, when the excessive Al^{3+} was added to the solution above, the fluorescence intensity at 488 nm enhanced again (Fig. 5). Therefore, we concluded that this fluorescent sensor **1** could be developed for practical application, for the reversibility and regeneration of **1** were perfect.

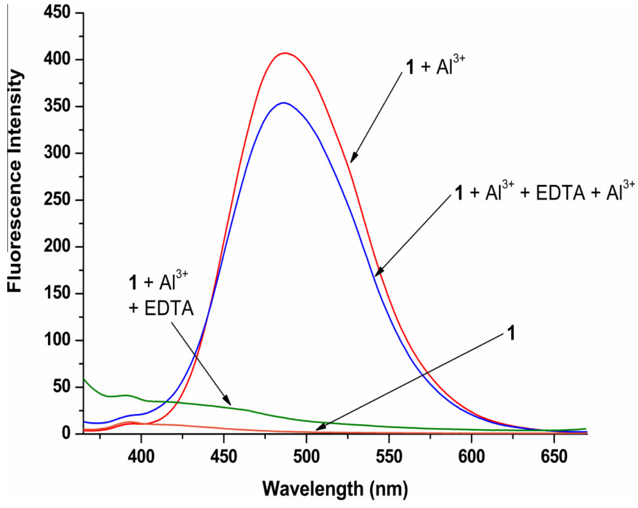


Fig. 5. Fluorescence response of ethanol solution of **1** (50 μM) and Al^{3+} (1 *equiv.*) upon addition of $EDTANa_2$ (1 *equiv.*) with an excitation at 350 nm.

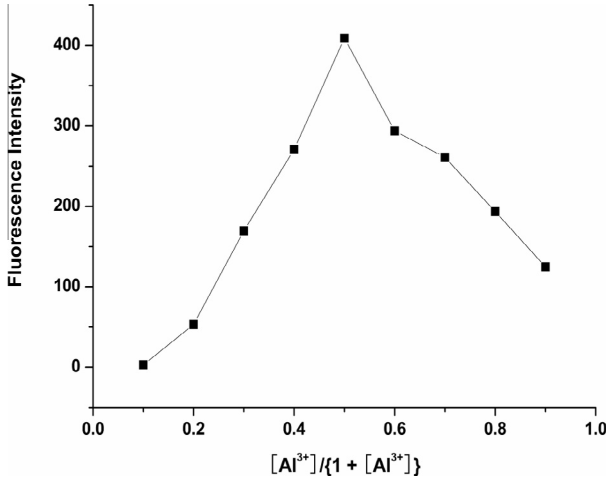


Fig. 6. Job's plot for determining the stoichiometry between **1** and Al^{3+} in ethanol ($X_{Al} = [Al^{3+}]/([Al^{3+}] + [\mathbf{1}])$, the total concentration of **1** and $Al^{3+}$was 100 μM).

*3.6. Binding stoichiometry between compound **1** and Al^{3+}*

The binding stoichiometry between compound **1** and Al^{3+} was evaluated from a Job's plot which was obtained from the fluorescence emission intensity at 488 nm. As can be seen from Fig. 6, the maximum fluorescence emission intensity was observed when the amount of Al^{3+} and **1** was equimolar, which indicated that compound **1** formed a 1:1 complex with Al^{3+} (Fig. 6). Furthermore, from the electrospray ionization mass spectra (ESI-MS), we could observe a peak at 411.3369 ($[\mathbf{1} + Al^{3+} + H_2O + CH_3CH_2OH - 2H^+]^+$), which further supported 1:1 binding stoichiometry between **1** and Al^{3+} (Fig. S7).

Finally, in order to demonstrate the proposed binding mode of compound **1** towards Al^{3+}, we carried out ^1H NMR analysis (Fig. S8). Upon addition of Al^{3+} into $CDCl_3$ solution of compound **1**, the proton signal of the imino group (at δ 11.26 ppm) was slightly down-field shifted to δ 11.30 ppm, and the fifth proton signal H_5 of the quinoline group (at δ 8.28 ppm) was also sightly down-field shifted to δ 8.34 ppm. At the same time, the proton signals of the pyrazine group were sightly down-field shifted, while the signals of other protons remained nearly unchanged. These results suggested that the oxygen atom of the carbonyl group, the nitrogen atom of the Schiff-base and one nitrogen atom of the pyrazine group were simultaneously coordinated with Al^{3+} (Scheme 2), and they were corresponded well with the UV–Vis analysis.

4. Conclusion

In summary, a simple structure fluorescent sensor **1** based on PET and CHEF mechanism has been successfully designed and synthesized through a facile three-step reaction. This fluorescent sensor **1** had high selectivity for Al^{3+} over other interfering metal ions with large fluorescence enhancement, and it also had high sensitivity towards Al^{3+} with the detection limit reaching at 10^{-7} mol/L level in ethanol. Furthermore, this sensor **1** could respond Al^{3+} in a reversible manner, which developed **1** for practical application, and the 1:1 stoichiometry between **1** and Al^{3+} was confirmed by job's plot and electrospray ionization mass spectrometry (ESI-MS). These results indicated that compound **1** could be used as a fluorescent chemosensor for detection and recognition of Al^{3+} and might accelerate the development of novel simple structure sensors. Moreover, the applications of novel sensors in real samples for detecting biologically and environmentally important metal ions are ongoing in our laboratory.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2015.02.004.

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