



Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

A colorimetric and turn-on fluorescent chemosensor for Al(III) based on a chromone Schiff-base



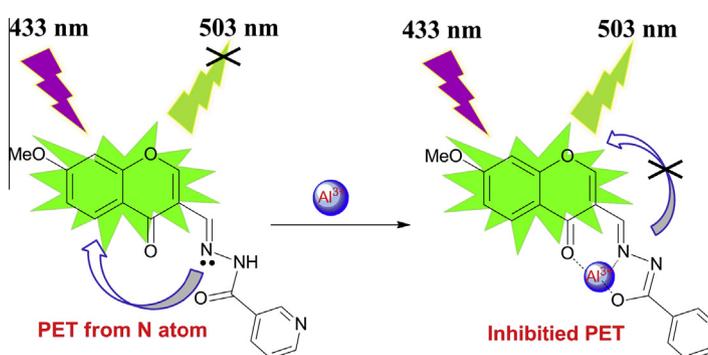
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HIGHLIGHTS

- A colorimetric and fluorescent Schiff base sensor for Al³⁺ was synthesized.
- The sensor exhibits higher sensitivity for Al³⁺ than other metal ions in ethanol.
- Al³⁺ resulted in an instant color change of **MCNH** from colorless to yellow–green.
- This probe features visible light excitation (433 nm) and emission (503 nm) profiles.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 26 June 2013

Received in revised form 12 September 2013

Accepted 16 September 2013

Available online 3 October 2013

Keywords:

Fluorescence sensor

Chromone

Colorimetric

Reversibility

Aluminum ions

Detection limit

ABSTRACT

A simple Schiff-base receptor 7-methoxychromone-3-carbaldehyde-(pyridylformyl) hydrazone (**MCNH**) was prepared. It exhibits an “off–on-type” mode with high sensitivity in the presence of Al³⁺. This compound could be used as Al³⁺ probe in ethanol and it features visible light excitation (433 nm) and emission (503 nm) profiles. Upon binding of Al³⁺, a significant fluorescence enhancement with a turn-on ratio over 800-fold was triggered. However, other metal ions had no such significant effect on the fluorescence. **MCNH** can also be used as a colorimetric chemosensor for Al³⁺, which is easily observed from colorless to yellow–green by the naked-eye. The detection limit of **MCNH** for Al³⁺ was as low as 1.9×10^{-7} M.

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Introduction

Natural abundance of aluminum in the biosphere is around 8% of the total mineral components. Al³⁺ exists widely in the environment due to acidic rain and human activities. It has been found that Al³⁺ ions exerted several neurotoxic effects in organisms a long time ago. For example, Al³⁺ has an important role in Alzheimer's disease, Parkinson's disease, bone softening, chronic renal

failure and smoking related diseases. Since there is a close association between Al³⁺ and human health, the investigation of Al³⁺ detection attracts more and more attention [1–8]. In recent decades, the fluorogenic sensing of different metal ions have been paid a great attention due to the advantages such as high sensitivity, selectivity [9], rapid response time and versatility [10–15]. The lack of spectroscopic characteristics and poor coordination ability of Al³⁺ are two main obstacles in the development of an Al³⁺ selective fluorescent probe [16–18].

Schiff-bases are an important class of compounds in medicinal and pharmaceutical fields. They have antibacterial, antifungal, antitumor, antioxidant, anti-inflammatory, antihypertensive,

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anti-HIV, antifilarial, anticonvulsant, herbicidal, insecticidal, schistosomicidal and anthelmintic activities [19–24]. Schiff-bases and their complexes have various applications in biological, clinical and analytical fields. Keeping this in mind, we have designed a Schiff-base ligand obtained by condensing 7-methoxychromone-3-carbaldehyde with nicotinic hydrazide. It is used as an efficient fluorescent probe for the determination of Al^{3+} in ethanol.

Herein, we report a chromone Schiff-base as a fluorescence “turn-on” sensor for distinct detections of Al^{3+} ions in ethanol. Selective fluorescence enhancement by Al^{3+} could be due to the effective coordination of Al^{3+} with **MCNH** over other metal ions. This restricts the photoinduced electron transfer (PET) process and enhances the fluorescence output of **MCNH** via chelation-enhanced fluorescence (CHEF) effect. The probe has been chosen because of its easy availability by classical organic syntheses and low detection limit. The free chemosensor **MCNH** shows no fluorescence emission. Upon binding of Al^{3+} ions, a significant fluorescence enhancement with turn-on ratios over 800-fold was achieved in ethanol.

Experimental

Materials and instrumentation

All chemicals were obtained from commercial suppliers and used without further purification. ^1H NMR, ^{13}C NMR spectra were measured on the Bruker 400 MHz instruments using TMS as an internal standard. ESI-MS were determined on a Bruker esquire 6000 spectrometer. UV-Vis absorption spectra were determined on a Shimadzu UV-240 spectrophotometer. Fluorescence spectra were recorded on a Hitachi RF-4500 spectrophotometer equipped with quartz cuvettes of 1 cm path length.

Synthesis

7-Methoxychromone-3-carbaldehyde was obtained according to the literature procedures [25]. Synthesis of **MCNH** was based on the following method (Scheme 1): an ethanol solution (10 mL) of nicotinic hydrazide (0.137 g, 1 mmol) was added dropwise to a solution (20 mL) of 7-methoxychromone-3-carbaldehyde (0.204 g, 1 mmol) in ethanol. Then the solution was reflux for 6 h under stirring and some white precipitant appeared. The mixture was filtered and dried under vacuum. Recrystallization from ethanol gave 7-methoxychromone-3-carbaldehyde-(pyridylformyl) hydrazone (**MCNH**), which was dried under vacuum. Yield, 58.3%, m.p: 171–173 °C. ^1H NMR (DMSO- d_6 , 400 MHz): δ 12.07 (s, 1H, —NH—), 9.08 (s, 1H, Py-H⁶), 8.78 (s, 1H, Ar-H⁴), 8.70 (d, $J = 6.8$ Hz, 1H, Py-H⁷), 8.61 (s, 1H, —N=CH⁵), 8.28 (d, $J = 8.0$ Hz, 1H, Py-H⁹), 8.04 (d, $J = 8.8$ Hz, 1H, Ar-H³), 7.59 (q, $J = 6.8$ Hz and

$J = 8.0$ Hz, 1H, Py-H⁸), 7.23 (d, $J = 2.0$ Hz, 1H, Ar-H²), 7.14 (dd, 1H, $J = 2.0$ Hz and $J = 8.8$ Hz, Ar-H¹), 3.92 (s, 3H, —CH₃) (Fig. S1). ^{13}C NMR (DMSO- d_6 , 400 MHz): 174.7 (8-C=O), 164.6 (C-2), 162.0 (12-C=O), 158.1 (6-C), 154.7 (9-C), 152.8 (11-C), 149.1 (13-C), 141.7 (17-C), 135.9 (15-C), 129.4 (14-C), 127.1 (5-C), 124.1 (16-C), 118.5 (7-C), 117.5 (3-C), 115.8 (10-C), 101.6 (4-C), 56.7 (1-C) (Fig. S2). MS (ESI) m/z 324.4 (M + H)⁺. IR (KBr) cm^{-1} : $\nu_{\text{C=O}}$ (Chromone): 1628. $\nu_{\text{C=N}}$ (Schiff-base): 1646. $\nu_{\text{C=N}}$ (Pyridine): 1591.

Analysis

Stock solutions (5 mM) of the nitrate salts of Ba^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , In^{3+} , Li^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , Pb^{2+} , Zn^{2+} , K^+ , Ga^{3+} , Ag^+ and Al^{3+} in ethanol were prepared. Stock solutions of **MCNH** (5 mM) were prepared in ethanol. Test solutions were prepared by placing 20 μL of the probe stock solution into cuvettes, adding an appropriate aliquot of each ions stock, and diluting the solution to 2 mL with ethanol solutions. Both the excitation and emission slit widths were 3.0 nm.

Results and discussion

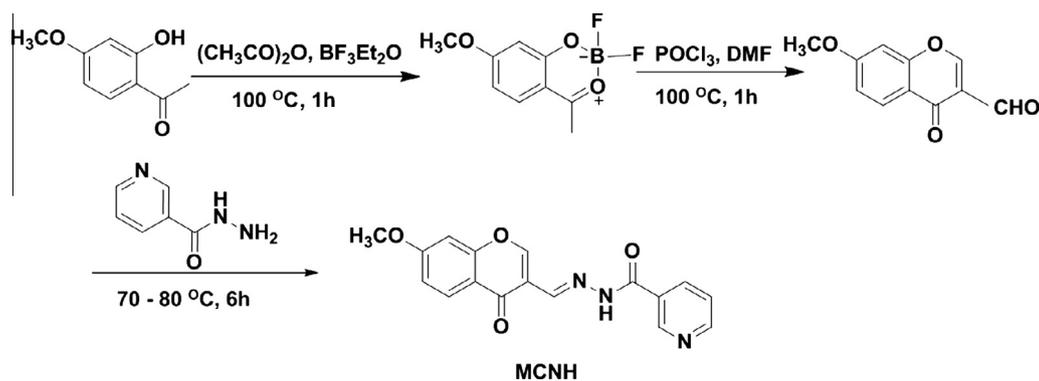
Absorption studies

Aluminum ions induced a significant change in the absorption spectrum of the reagent (Fig. 1). The colorless solution of **MCNH** exhibited a broad band around 280 nm. Upon addition of Al^{3+} ions, the absorbance bands at 408 nm, 433 nm and 460 nm enhanced. The significant change to wavelength resulted in a color change from colorless to yellow, which can be easily detected by the naked-eye. Addition of other representative metal ions such as Ba^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , In^{3+} , Li^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , Pb^{2+} , Zn^{2+} , K^+ , Ga^{3+} , Ag^+ did not give rise to significant color changes. These results suggest that **MCNH** could be served as a potential naked-eye chemosensor selective for Al^{3+} [26–29].

Emission studies

MCNH forms colorless and nonfluorescent solutions either in aqueous media or organic solvents, including (dimethyl formamide) DMF, (tetrahydrofuran) THF, (dimethylsulfoxide) DMSO, ethanol, methanol and acetonitrile. Addition of aluminum ions to **MCNH** in ethanol and methanol leads to the development of a yellow-green color and yellow-green fluorescence (Fig. S3).

A fluorescence study was further used to determine the selectivity and sensitivity of **MCNH** in the presence of various metal ions, such as Ba^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , In^{3+} , Li^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , Pb^{2+} , Zn^{2+} , K^+ , Ga^{3+} and Ag^+ . **MCNH**



Scheme 1. Synthesis of 7-methoxychromone-3-carbaldehyde-(pyridylformyl) hydrazone (**MCNH**).

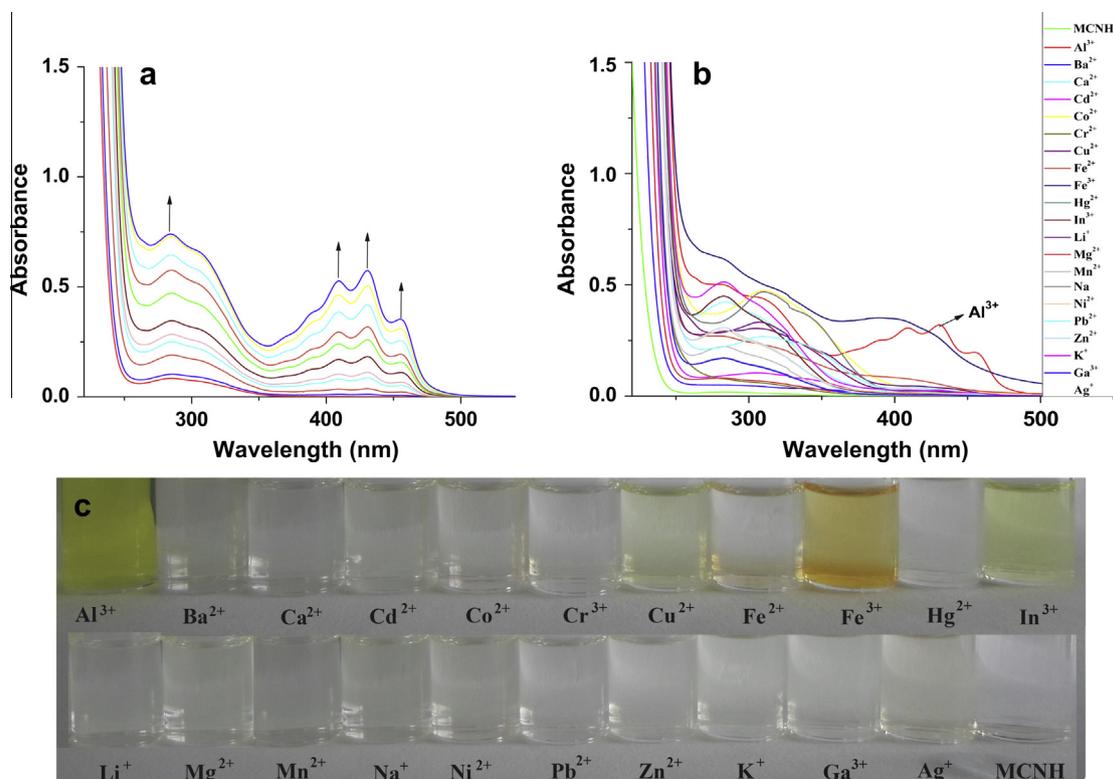


Fig. 1. (a) The absorbance of **MCNH** in ethanol (50.0 μM) after addition of Al³⁺ (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 equiv.); (b) Absorption changes of **MCNH** upon addition of different metal ions (100.0 μM); (c) The photograph of **MCNH** after addition of different metal ions.

itself was non-fluorescent in ethanol. Fascinatingly, treatment of **MCNH** (50.0 μM) with Al³⁺ (50.0 μM) resulted in a strong increase in the fluorescence intensity in the emission spectrum at 503 nm upon excitation at 433 nm (Fig. 2). Interestingly, no such effect was observed when **MCNH** was treated with a large number of other cations (500.0 μM). The probe exhibited high selectivity for Al³⁺ over other metal ions.

PET mechanism involves the deactivation of the excited state of a fluorophore by addition of an electron to one of its excited-state frontier orbitals. This leaves the fluorophore in a nonemissive state. For example, the free electron pair of a functional group attached to the fluorophore may quench its fluorescence intramolecularly due to a **PET** process. Metal coordination to this group renders it

a less efficient electron donor, thereby interrupting the **PET** process and enhances the fluorescence output via chelation-enhanced fluorescence (**CHEF**) effect [30,31]. Here, the emission intensity of **MCNH** is very low, and with a low quantum yield value of 0.051 in ethanol. It is due to quenching by the Schiff-base nitrogen long pair electrons through a **PET** mechanism. With increasing of Al³⁺ (0–1.0 equiv.), the emission intensity of **MCNH** (50.0 μM) at 503 nm increased more than 800-fold (Fig. 3), and the fluorescence quantum yield get to 0.389. The chelation of Al³⁺ with the carbonyl of chromone moiety, the nitrogen atom of the —C=N group and the carbonyl of pyridylformyl hydrazone moiety lead to the **PET** process was suppressed, the fluorescence of the system was restored. [32,33].

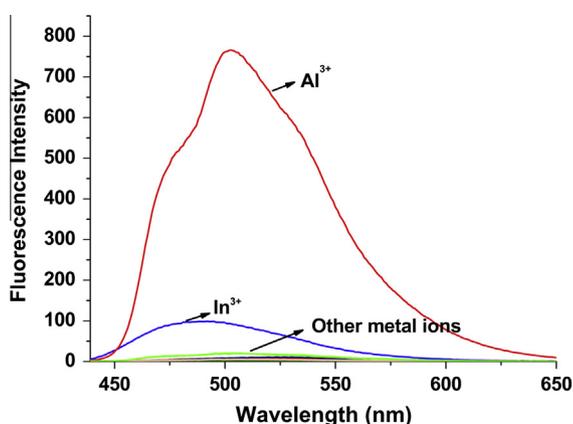


Fig. 2. Fluorescence responses of **MCNH** (50.0 μM) in ethanol with 500.0 μM of Ba²⁺, Ca²⁺, Cd²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe²⁺, Fe³⁺, Hg²⁺, In³⁺, Li⁺, Mg²⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, Zn²⁺, K⁺, Ga³⁺, Ag⁺ and 50.0 μM of Al³⁺. Excitation wavelength was 433 nm.

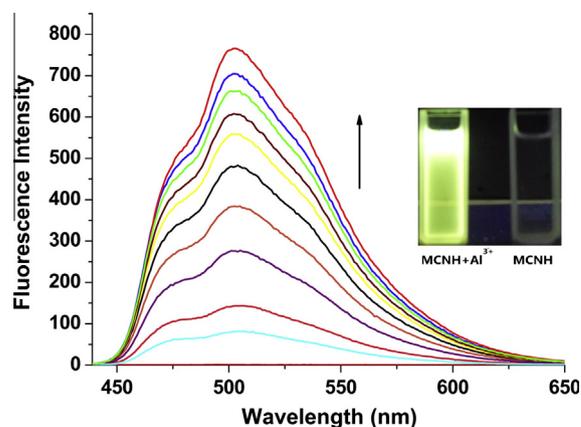


Fig. 3. Fluorescence emission spectra of **MCNH** (50.0 μM) in ethanol upon the addition of Al(NO₃)₃ (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 equiv.) with an excitation of 433 nm. Inset: color of **MCNH** and **MCNH** + Al³⁺ system under UV lamp (λ_{ex} = 433 nm, λ_{em} = 503 nm).

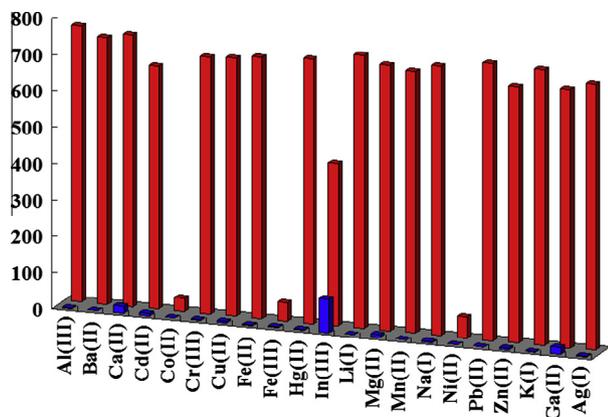


Fig. 4. Relative fluorescence of **MCNH** and its complexation with Al^{3+} in the presence of various metal ions. Blue bar: **MCNH** (50.0 μM) and **MCNH** with 10.0 equiv. of Ba^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , In^{3+} , Li^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , Pb^{2+} , Zn^{2+} , K^+ , Ga^{3+} , Ag^+ stated. Red bar: 50.0 μM of **MCNH** and 1.0 equiv. of Al^{3+} with 10.0 equiv. of metal ions stated ($\lambda_{\text{ex}} = 433 \text{ nm}$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

To explore the possibility of using **MCNH** as a practical ion selective fluorescent chemosensor for Al^{3+} , competition experiments were carried out. For this purpose, **MCNH** was treated with 1.0 equiv. of Al^{3+} in the presence of 10.0 equiv. of other metal ions. Relatively low interference was observed for the detection of Al^{3+} in the presence of other metal ions (Fig. 4). **MCNH** responding for

Al^{3+} in the presence of Co^{2+} , Fe^{3+} and Ni^{2+} are relatively low but clearly detectable. Therefore, **MCNH** was shown to be a promising selective fluorescent sensor for Al^{3+} in the presence of most competing metal ions.

Binding studies

In order to determine the stoichiometry between **MCNH** and Al^{3+} , the method of continuous variation (Job's plot) was used [34]. The total concentration of the **MCNH** and Al^{3+} was constant (10.0 μM), with a continuous variable molar fraction of guest ($[\text{MCNH}]/([\text{MCNH}] + [\text{Al}^{3+}])$). Fig. S4 shows the Job's plot of **MCNH** with Al^{3+} (at 503 nm), the **MCNH**- Al^{3+} complex concentration approaches a maximum when the molar fraction of Al^{3+} is 0.5, which means **MCNH** and Al^{3+} forms a 1:1 complex. In addition, the formation of a 1:1 complex between **MCNH** and Al^{3+} was further conformed by the appearance of a peak at m/z 457.4, assignable to $[\text{MCNH} + \text{Al}^{3+} + \text{C}_2\text{H}_5\text{OH} + \text{NO}_3^- - \text{H}]^+$ in the ESI-MS spectra (Fig. S5).

In IR spectra, the stretching frequency of the carbonyl of chromone moiety and the $-\text{C}=\text{N}$ decreased by $\sim 22 \text{ cm}^{-1}$ and $\sim 49 \text{ cm}^{-1}$, the $\nu(\text{C}=\text{O})$ vibration at 1646 cm^{-1} and the sharp $\nu(\text{NH})$ vibration of 3191 cm^{-1} for **MCNH** are absent in the complex, indicating the possibility of enolization of the amide functionality and subsequent proton replacement by the aluminum, which suggests coordination to the two carbonyl oxygen atoms and the nitrogen atom of $-\text{C}=\text{N}$ group. The band at 635 cm^{-1} or so was assigned to $\nu(\text{Al}-\text{O})$, which demonstrated formation of a coordina-

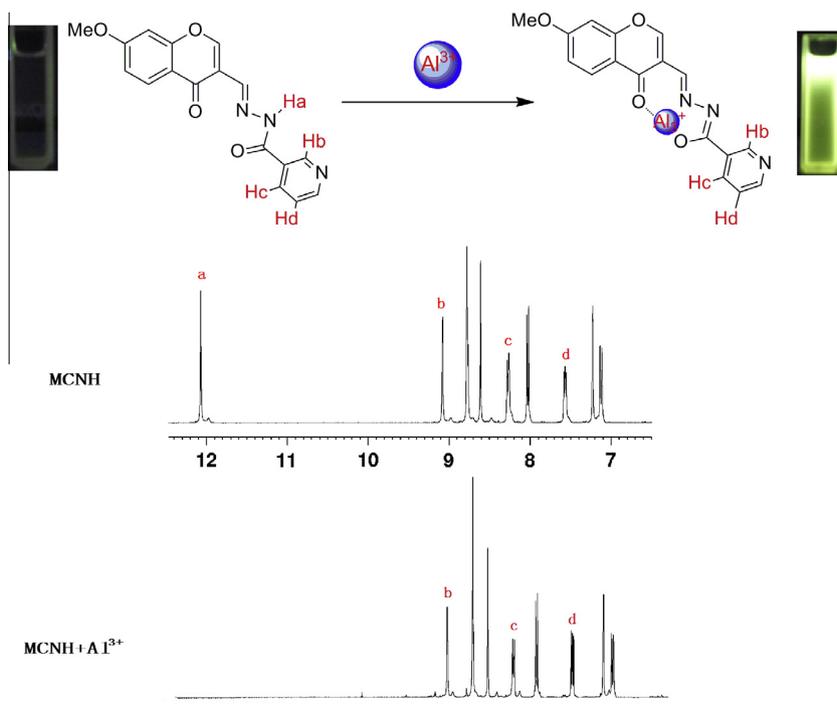


Fig. 5. Proposed binding mode for interaction of Al^{3+} with **MCNH** and ^1H NMR spectra of **MCNH** with Al^{3+} in $\text{DMSO}-d_6$: (I) **MCNH** (top); (II) **MCNH** with Al^{3+} (bottom).

Table 1
Changes in chemical shift of related protons in NMR titration.

	$-\text{NH}(\text{a})$ δ (ppm)	$-\text{N}=\text{CH}(\text{b})$ δ (ppm)	Change in δ	$-\text{Hc}$ δ (ppm)	Change in δ	$-\text{Hd}$ δ (ppm)	Change in δ
MCNH	12.071	9.084		8.284		7.588	
MCNH + Al^{3+}	–	9.098	0.014 (up-field)	8.324	0.040 (up-field)	7.623	0.035 (up-field)

tive bond between aluminum and oxygen. Correspondingly, the $\nu(\text{Al}-\text{N})$ was at 549 cm^{-1} or so (Fig. S6) [35].

NMR studies provide additional evidence of the interaction between **MCNH** and Al^{3+} . ^1H NMR spectra of **MCNH** were recorded in $\text{DMSO}-d_6$ upon the addition of Al^{3+} . Significant spectral changes were observed as shown in Fig. 5. The δ 12.071 signal for $-\text{NH}(\text{a})$ disappeared upon the addition of Al^{3+} , the signals of H_b , H_c and H_d was up-field shifted 0.014, 0.040 and 0.035 respectively, while the signals of the other protons remained nearly unchanged (Table 1). This indicated that the direct involvement of the carbonyl of chromone moiety, the nitrogen atom of the $-\text{C}=\text{N}$ group, the carbonyl of pyridylformyl hydrazone moiety coordinate with Al^{3+} .

As shown in Fig. S7, the association constant K_a of the complex was calculated to be $1.33 \times 10^4\text{ M}^{-1}$ with a linear relationship by the Benesi–Hildebrand equation (Eq. (1)) [36], where F_{max} , F_{min} , and F are fluorescence intensities of **MCNH** in the presence of Al^{3+} at saturation, free **MCNH**, and any intermediate Al^{3+} concentration. The association constant K_a of the complex was also determined using modified Benesi–Hildebrand equation: $(F_{\text{max}} - F_{\text{min}})/(F - F_{\text{min}}) = (1/K) 1/([\text{Al}^{3+}]) + 1$, A plot of $(F_{\text{max}} - F_{\text{min}})/(F - F_{\text{min}})$ vs $1/[\text{Al}^{3+}]$ yields the association constant value as $1.43 \times 10^4\text{ M}^{-1}$ (Fig. S8) [37,38].

$$\frac{1}{F - F_{\text{min}}} = \frac{1}{K(F_{\text{max}} - F_{\text{min}})[\text{Al}^{3+}]} + \frac{1}{F_{\text{max}} - F_{\text{min}}} \quad (1)$$

The detection limit of **MCNH** as a fluorescent sensor for the analysis of Al^{3+} was determined from a plot of normalized fluorescence intensity as a function of the concentration of the added metal ions (Fig. S9) and it was found that **MCNH** has a detection limit of $1.9 \times 10^{-7}\text{ M}$ for Al^{3+} , which is low for the detection of Al^{3+} ions found in many chemical systems [39,40].

Reversibility is a prerequisite in developing novel chemosensors for practical application. The reversibility of the recognition process of **MCNH** was performed by adding an Al^{3+} bonding agent, Na_2EDTA . The addition of Na_2EDTA to a mixture of **MCNH** and Al^{3+} resulted in diminution of the fluorescence intensity at 503 nm, which indicated the regeneration of the free chemosensor **MCNH** (Fig. S10). Such reversibility and regeneration are important for the fabrication of devices to sense the Al^{3+} ions.

Conclusion

In summary, we have developed a novel chemosensor **MCNH** based on **PET** and **CHEF** mechanism by a facile one-step reaction. The probe exhibited high selectivity for Al^{3+} over other metal ions with 800-fold fluorescence enhancement and high sensitivity with the detection limit reaching at 10^{-7} M level in ethanol. The chemosensor shows an excellent turn-on fluorescence signal with high sensitivity in the presence of Al^{3+} , allowing its reversible detection in the presence of a wide range of environmentally relevant competing metal ions. More importantly, the detection limit was sufficiently low to detect the sub-micromolar concentration of the Al^{3+} . Thus, we believe **MCNH** has the ability to serve as a practical sensor for Al^{3+} detection in biological systems and environment. This strategy may provide a general way for designing new **PET** sensors to detect other environmentally and biologically relevant species.

Acknowledgment

This work is supported by the National Natural Science Foundation of China (81171337).

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.saa.2013.09.062>.

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