Isatin appended rhodamine scaffold as an efficient chemical tool to detect selectively Al\(^{3+}\)

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5-methoxy isatin-appended rhodamine dye, (E)-3’-(diethylamino)-6’-(ethyl(methyl)amino)-2’-[(5-methoxy-2-oxindolin-3-ylidine)amino]spiro[isoindoline-1,9’-xanthen]-3-one (L) has been designed, synthesized, and characterized by different spectroscopic techniques. The chemosensor L shows high sensitivity towards Al\(^{3+}\) ions without interference from other biologically important cations in DMSO/H\(_2\)O (1/9, v/v) media. The Al\(^{3+}\)-ion promoted ring opening of the rhodamine spirolactam ring in chemosensor L evokes a fluorescence turn-on response via chelation-enhanced fluorescence (CHEF) process. Its lowest detection limit for Al\(^{3+}\) is 2.2 \times 10^{-8} M, and displays a significant color change from almost colorless to deep pink in the presence of Al\(^{3+}\). The titration results show a 1:1 complex formation between chemosensor L and Al\(^{3+}\).

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1. Introduction

Aluminum is a non-essential element for living systems and extensively used in modern life [1,2]. Intemperance of Al\(^{3+}\) deposition in brain is believed to cause neurodementia, such as Parkinson’s disease, Alzheimer’s disease, and dialysis encephalopathy [3–5]. Away from these, the toxicity of aluminium is a hazard towards aquatic life [6] and retards agricultural production in acidic soils [7]. Therefore, developing fast and selective methods for Al\(^{3+}\) detection is of vital significance in medical diagnostics, and has attracted much attention. Several methods for the detection of Al\(^{3+}\) at trace quantity levels in various samples such as graphite furnace atomic absorption spectrometry [8], electrochemical detection [9], mass spectrometry [10] and \(^{27}\)Al NMR technologies [11] are generally expensive and time-consuming in practice. Recently, the detection of heavy metal ions based on the fluorescent chemosensor method has been studied extensively because their real time monitoring capability, intrinsic selectivity, low cost and simple operation procedure. Although fluorescent chemosensors such as Schiff bases [12–14], triazoles [15,16], triazole-pyridyl [17–19], calixarene [20,21], and secondary/tertiary amines [22,23] and others have been reported for detecting Al\(^{3+}\), however, isatin appended rhodamine-based fluorescent chemosensor for Al\(^{3+}\) is scarce. During the last decade, many fluorescent probes for Al\(^{3+}\) have been reported for the purpose. Unfortunately many of these turn-on sensors are faced with the following problems: (i) sensing mechanisms operate only in an organic solvent or at non-physiological pH, (ii) potential off-target responses and poor selectivity, (iii) limited bio-activity studies (iv) behave as chemodosimeters i.e., irreversible binding to the receptor, and (v) interference caused by Fe\(^{3+}\) and Cu\(^{2+}\) with similar chemical properties. The poor coordination ability of Al\(^{3+}\) compared with common transition metal ions is the prime reason for the lesser development of Al\(^{3+}\) selective sensors [24]. For these reasons, we have taken care to design an isatin-appended rhodamine based scaffold as a chemosensor to overcome these problems. Rhodamine B and its derivatives (RhB) are extensively used as a fluorescent chemosensor because of their excellent photo-physical properties [25], where the signal transduction pathway mostly exploits its contrast structure–function correlation in lactonized–delactonized conformations [26]. This unique structure makes rhodamine dyes a good candidate in constructing a “turn-on” fluorescent probe for Al\(^{3+}\) [27–30].

Herein, we report a new turn-on fluorescent chemosensor based on isatin appended rhodamine dye (L), which can sensitively and selectively detect Al\(^{3+}\) in mixed solvent. It displays remarkably enhanced fluorescence intensity via CHEF [17,19,21,31] process and it shows prominent color changes upon recognition. The rhodamine B skeleton was used as the potential fluorophore and chromophore. The isatin part was chosen as the recognizing group to get better selectivity and solubility in water. To the best of
our knowledge, this is the first example of a fluorescent sensor based on a small molecule that allows the detection of Al$^{3+}$.

2. Experimental

2.1. Synthetic procedure

Rhodamine B hydrazide was prepared according to the literature procedure [32,33] as described previously and was characterized by $^1$H NMR, mass data and FT-IR. A mixture of rhodamine B hydrazide (0.5 g, 1.095 mmol) and 5-methoxy-1H-indole-2,3-dione (0.194 g, 1.095 mmol) was taken in 10 ml of dry methanol. The resulting solution was stirred for 12 h under reflux using a fused CaCl$_2$ guard tube. A dark maroon precipitate was formed within the reaction flask (Scheme 1). Then, it was cooled and the resulting precipitate was filtered off, washed with methanol/ether (1:1) by three times and dried under vacuum. Yield: 0.375 g, 56%. M.P./C$_{240}$ oC (decomp.). IR (KBr, cm$^{-1}$): 400–4000: $\nu$ (cm$^{-1}$) = 3373, 3217, 2969, 2359, 1724, 1680, 1617, 1513, 1482, 1300, 1268, 1222, 1184, 1117, 1020; $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ (ppm), 1.09 (t, 12H, NCH$_2$CH$_3$, $J$ = 5 Hz), 3.32 (q, 8H, NCH$_2$CH$_3$, $J$ = 5 Hz), 3.66 (s, 3H, isatin-OMe), 6.37–6.35 (m, 2H, xanthene-H), 6.41 (d, 2H, xanthene-H, $J$ = 2 Hz ), 6.58 (s, 2H, xanthene-H), 6.72 (d, 1H, isatin-H, $J$ = 5 Hz), 6.80 (d, 1H, isatin-H, $J$ = 10 Hz), 6.98 (t, 1H, isatin-H, $J$ = 5 Hz), 7.08 (d, 1H, Ar-H, $J$ = 10 Hz), 7.55 (d, 2H, Ar-H, $J$ = 5 Hz), 7.88 (d, 1H, Ar-H, $J$ = 7 Hz), 10.55 (s, 1H, NH); $^{13}$C-NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm), 12.89, 44.09, 56.09, 68.09, 97.89, 105.78, 108.54, 111.57, 114.55, 118.21, 124.56, 128.22, 129.62, 134.73, 138.80, 148.91, 152.21, 154.57, 160.50, 164.31; ESI-MS: m/z calculated for C$_{37}$H$_{38}$N$_5$O$_4$ [M + H$^+$] = 616.28, found 616.13; Anal. Calc. for C$_{37}$H$_{38}$N$_5$O$_4$: C, 72.29; H, 6.13; N, 11.41; Found: C, 72.17; H, 6.06; N, 11.37.

Fig. 1. Absorption changes of L (10 $\mu$m) in DMSO/H$_2$O (1/9, v/v) using HEPES buffer at pH 7.2 at 25 °C, by addition of 100 $\mu$m different metal ions (Na$^{+}$, Mg$^{2+}$, Cd$^{2+}$, Al$^{3+}$, Ga$^{3+}$, In$^{3+}$, Hg$^{2+}$, Cr$^{3+}$, Co$^{2+}$, Mn$^{2+}$, Fe$^{3+}$, Cu$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, and Ni$^{2+}$).

Fig. 2. UV–vis spectra of chemosensor L (10 $\mu$m) in DMSO/H$_2$O (1/9, v/v) using HEPES buffer at pH 7.2 at 25 °C, in the presence of different amount of Al$^{3+}$ (0–100 $\mu$m). Insert: absorbance at 559 nm as a function of Al$^{3+}$ concentration, indicating a 1:1 metal–ligand ratio.

2.2. Reagents and apparatus

All reagents were purchased from Aldrich and were used without further purification. Elemental analyses (carbon, hydrogen and nitrogen) were performed with a Perkin-Elmer CHN analyzer 2400. Melting points were determined using a Buchi 530 melting apparatus. NMR spectra were recorded on a Bruker spectrometer at 500 (1H NMR) MHz in DMSO-$d_6$. Chemical shifts ($\delta$ values) were reported in ppm down field from internal Me$_4$Si. Mass spectra were recorded in methanol solvent in Qtof Micro YA263. The electronic spectra were recorded in DMSO-water solution on a Hitachi model U-3501 spectrophotometer. IR spectra (KBr pellet, 400–4000 cm$^{-1}$) were recorded on a Perkin-Elmer model 883 infrared spectrophotometer. Emission spectra were measured by Perkin-Elmer (Model LS-50B) fluorimeter. Fluorescence lifetimes...
were measured by the method of Time Correlated Single-Photon Counting (TCSPC) using a HORIBA JobinYvon Fluorocube-01-NL fluorescence lifetime spectrometer. The sample was excited using a nanosecond laser diode at 340 nm and the signals were collected at the magic angle of 54.7° to eliminate any considerable contribution from fluorescence anisotropy decay [34]. The typical time resolution of the experimental set-up is ~800 ps. The decays were deconvoluted using DAS-6 decay analysis software. The acceptability of the fits was judged by \( x^2 \) criteria and visual inspection of the residuals of the fitted function to the data. Mean (average) fluorescence lifetimes were calculated using the following equation:

\[
\tau_{av} = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i}
\]

in which \( \alpha_i \) is the pre-exponential factor corresponding to the \( i \)th decay time constant, \( \tau_i \).

2.3. Quantum yield measurements

The fluorescence quantum yields are determined using anthracene as a reference with a known \( \Phi_S \) value of 0.27 in EtOH [35]. The area of the emission spectrum is integrated using the software available in the instrument and the quantum yield is calculated according to the following equation [36]:

\[
\Phi_S/\Phi_R = (A_\text{S}/A_\text{R})[(\text{Abs})_\text{S}/(\text{Abs})_\text{R}]^{\eta_\text{S}}/\eta_\text{R}^2,
\]

where \( \Phi_S \) and \( \Phi_R \) are the fluorescence quantum yield of the sample and reference, respectively; \( A_\text{S} \) and \( A_\text{R} \) are the area under the fluorescence spectra of the sample and the reference, respectively; \( (\text{Abs})_\text{S} \) and \( (\text{Abs})_\text{R} \) are the corresponding optical densities of the sample and the reference solution at the wavelength of excitation; \( \eta_S \) and \( \eta_R \) are the refractive index of the sample and the reference, respectively [37].

2.4. Association constant

Calculation for the binding constant [38] using spectrophotometric titration data.

The binding constant of the \( \text{Al}^{3+} \text{-L} \) complex was evaluated using the Benesi–Hildebrand (B–H) plot.

\[
1/(A - A_0) = 1/(K(A_{\text{max}} - A_0)C) + 1/(A_{\text{max}} - A_0)
\]

where \( A_0 \) is the absorbance of \( \text{L} \) at absorbance maxima (\( \lambda = 559 \text{ nm} \)), \( A \) is the observed absorbance at that particular wavelength in the presence of a certain concentration of the metal ion (C), \( A_{\text{max}} \) is the maximum absorbance value that was obtained at \( \lambda = 559 \text{ nm} \) during titration with varying [C], K is the association constant (M\(^{-1}\)) and was determined from the slope of the linear plot, and [C] is the concentration of the \( \text{Al}^{3+} \) ion added during titration studies. The goodness of the linear fit of the B–H plot of \( 1/(A - A_0) \) vs. (\( [\text{L}]_0 - [\text{L}]_0[-\text{Al}] \)) is the absorbance at that particular wavelength in the presence of varying [C].

2.5. Absorption measurements

The absorbance of the sample and the reference solution at the wavelength of excitation was determined using a scanning UV–vis spectrophotometer.

Fig. 3. Photographs of chemosensor L (10 \( \mu \text{m} \)) in DMSO/H\(_2\text{O} \) (1/9, v/v) using HEPES buffer at pH 7.2 at 25 °C. In the presence of different metal ions (100 \( \mu \text{m} \)) under (a) visible light and (b) UV light.

![Fig. 3. Photographs of chemosensor L (10 \( \mu \text{m} \)) in DMSO/H\(_2\text{O} \) (1/9, v/v) using HEPES buffer at pH 7.2 at 25 °C. In the presence of different metal ions (100 \( \mu \text{m} \)) under (a) visible light and (b) UV light.](image)

Table 1

<table>
<thead>
<tr>
<th>Chemosensor, ( K_a )</th>
<th>Absorption, ( K_a )</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( L + \text{Ca}^{2+} )</td>
<td>0.81 ( \times ) 10(^3)</td>
<td>0.69 ( \times ) 10(^3)</td>
</tr>
<tr>
<td>( L + \text{Co}^{2+} )</td>
<td>0.99 ( \times ) 10(^3)</td>
<td>0.89 ( \times ) 10(^3)</td>
</tr>
<tr>
<td>( L + \text{Cr}^{3+} )</td>
<td>1.11 ( \times ) 10(^3)</td>
<td>1.02 ( \times ) 10(^3)</td>
</tr>
<tr>
<td>( L + \text{Ni}^{2+} )</td>
<td>1.23 ( \times ) 10(^3)</td>
<td>1.16 ( \times ) 10(^3)</td>
</tr>
<tr>
<td>( L + \text{Fe}^{3+} )</td>
<td>1.39 ( \times ) 10(^3)</td>
<td>1.25 ( \times ) 10(^3)</td>
</tr>
<tr>
<td>( L + \text{Cu}^{2+} )</td>
<td>1.49 ( \times ) 10(^3)</td>
<td>1.41 ( \times ) 10(^3)</td>
</tr>
<tr>
<td>( L + \text{Al}^{3+} )</td>
<td>2.91 ( \times ) 10(^3)</td>
<td>2.86 ( \times ) 10(^3)</td>
</tr>
</tbody>
</table>

Fig. 4. Emission spectra of chemosensor L (10 \( \mu \text{m} \)) in the presence of increasing concentrations of \( \text{Al}^{3+} \) (0, 15, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 100 \( \mu \text{m} \)) in DMSO/H\(_2\text{O} \) (1/9, v/v) using HEPES buffer at pH 7.2 at 25 °C. Excitation was performed at 350 nm. Inset: fluorescence intensity at 590 nm as a function of \( \text{Al}^{3+} \) concentration.
vs \(1/\text{[Al}^{3+}\text{]}\) for 1:1 complex formation confirms the binding stoichiometry between \(L\) and \(\text{Al}^{3+}\).

Calculation for the binding constant \([38]\) using emission titration data

\[
1/(l-l_0) = 1/K(l_{\text{max}}-l_0)/C + 1/(l_{\text{max}}-l_0)
\]

(Meq) was determined from the Job’s Plot. In the case of evaluation of the binding constant from the results of fluorescence titration, a modified B–H equation (Eq. 4) was used, where \(l_0, l_{\text{max}}\) and \(l\) represent the emission intensity of free \(L\), the maximum emission intensity observed in the presence of added metal ion at 590 nm (\(\lambda_{\text{ex}}=350\) nm) and the emission intensity at a certain concentration of the metal ion added.

2.5. Calculation for detection limit

The detection limit (DL) of \(L\) for \(\text{Al}^{3+}\) was determined from the following equation:

\[
\text{DL} = 3\text{Sb}/S
\]

Where Sb is the standard deviation of the blank solution; S is the slope of the calibration curve.

2.6. General procedure for fluorescence and UV–vis titrations

Stock solution of the chemosensor (\(L\)) was prepared in DMSO/H\(_2\)O (1/9, v/v) in the concentration range of \(10^{-3}\) mol/L. 2 ml of the ligand solution was taken in the cuvette. Stock solutions of guests in the concentration range of \(10^{-4}\) were prepared in the same solvents and were individually added in different amounts to the receptor solution. Upon addition of metal ions, the change in emission of the receptor was noted. The same stock solution of receptor and guests were used to perform the UV–vis and fluorescence titrations at 25°C. Fluorescence measurements were carried out with excitation and emission slit of 2.5 nm and 2.5 nm (\(\lambda_{\text{ex}}=350\) nm).

3. Results and discussion

3.1. Synthesis and structural characterization of \(L\)

Chemosensor \(L\) was synthesized by condensing rhodamine B hydrazide with 5-methoxyindoline-2,3-dione, as depicted in Scheme 1, and were characterized by \(^1\text{H NMR,}^{13}\text{C NMR, IR and mass spectrometry techniques (Figs. S1–S4, in Supporting information).}

3.2. Absorption spectroscopic studies

The recognition behavior of chemosensor \(L\) (10 μm) toward different cations (\(\text{Na}^+, \text{Mg}^{2+}, \text{Cd}^{2+}, \text{Al}^{3+}, \text{Ga}^{3+}, \text{In}^{3+}, \text{Hg}^{2+}, \text{Cr}^{3+}, \text{Co}^{2+}, \text{Mn}^{2+}, \text{Fe}^{3+}, \text{Cu}^{2+}, \text{Pb}^{2+}, \text{Zn}^{2+}, \text{and Ni}^{2+}\) ) was investigated by UV–vis and fluorescence spectroscopy in DMSO/H\(_2\)O (1/9, v/v) using HEPES buffer at pH 7.2 at 25°C. In the absence of metal ions, chemosensor \(L\) (10 μm) displayed no absorption in the visible region, indicating that the rhodamine core is in the ring closed isomeric form. Significant changes in intensities of the absorption bands were observed in the UV–vis spectra of \(L\) upon addition of the aforementioned metal ions as depicted in Fig. 1. In the cases of \(\text{Na}^+, \text{Mg}^{2+}, \text{Mn}^{2+}, \text{Ga}^{3+}, \text{In}^{3+}, \text{Zn}^{2+}, \text{Pb}^{2+}, \text{or Hg}^{2+}\) no detectable changes were observed in their absorption spectrum. However, any of the \(\text{Al}^{3+}, \text{Fe}^{3+}, \text{Cd}^{2+}, \text{Co}^{2+}, \text{Cu}^{2+}, \text{Ni}^{2+}, \text{and Cr}^{3+}\) ion induced a red shift of the absorption band at 557–559 nm, compared to that of the bare ligand at 365 nm. Upon gradual addition of \(\text{Al}^{3+}\) ion (0–100 μm) to chemosensor \(L\) (10 μm) in DMSO/H\(_2\)O (1/9, v/v), a concomitant red shift in the spectral position at 559 nm was observed along with an increase in the absorption intensity which may be due to the opening of spir-oactum ring of the rhodamine moiety (Fig. 2). As depicted in inset

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**Fig. 5.** Fluorescence spectra (excitation at 350 nm) of \(L\) (10 μm) in DMSO/H\(_2\)O (1/9, v/v) using HEPES buffer at pH 7.2 at 25°C, by addition of 100 μm different metal ions (\(\text{Na}^+, \text{Mg}^{2+}, \text{Cd}^{2+}, \text{Al}^{3+}, \text{Ga}^{3+}, \text{In}^{3+}, \text{Hg}^{2+}, \text{Cr}^{3+}, \text{Co}^{2+}, \text{Mn}^{2+}, \text{Fe}^{3+}, \text{Cu}^{2+}, \text{Pb}^{2+}, \text{Zn}^{2+}, \text{and Ni}^{2+}\)).

**Fig. 6.** Job plot of \(L\) and \(\text{Al}^{3+}\) ([\(L\)]+[\(\text{Al}^{3+}\)]=100 μm) in DMSO/H\(_2\)O (1/9, v/v) using HEPES buffer at pH 7.2 at 25°C.
of Fig. 2, the absorbance at 559 nm band as a function of Al$^{3+}$ concentration predicted 1:1 stoichiometric complex between L with Al$^{3+}$ ion. Surprisingly a huge enhancement of absorbance (24 fold) at $\lambda_{\text{max}}$=559 nm was observed upon addition of 100 $\mu$m of Al$^{3+}$. Comparatively less increase of absorbance at 557 nm was also detected when the same amount (100 $\mu$m) of Fe$^{3+}$, Cd$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, or Cr$^{3+}$ were added one at a times. It may be due to their low binding affinity to L compared to that of Al$^{3+}$.

Thus, L behaves as an efficient colorimetric probe for the detection of these ions owing to the fact that the color changes can be distinguished easily by naked eye. The solution of the chemosensor L showed a dramatic color change from colorless to a fluorescent orange one in the presence of Al$^{3+}$ ion under UV lamp which could easily be detected by the naked-eye, while the addition of other metal ions did not show any detectable color change (Fig. 3). The binding affinity for each of these metal ions towards L was evaluated from the linear fit ($R^2=0.99$) of Benesi–Hildebrand (B–H) plot for 1:1 complexation (Fig. S5 in Supporting information). The binding interactions follow the order Al$^{3+}$ > Cu$^{2+}$ > Fe$^{3+}$ > Ni$^{2+}$ > Cr$^{3+}$ > Co$^{2+}$ > Cd$^{2+}$. Both the association constants [38] have comparable values with a good agreement (Table 1). The order of higher $K_a$ for Al$^{3+}$ signifies its superior binding interaction and selectivity compared to the coordinatively competing and biologically co-existing other metal ions.

### 3.3. Fluorescence spectroscopic studies

The metal-free L initially displayed no appreciable emission band above 500 nm ($\Phi=0.01$), indicating the existence of only the lactam form in mixed media upon excitation at 350 nm. Addition of Al$^{3+}$ ions triggered formation of an emission band with a maximum at 590 nm. The color of the solution changed from colorless to pink. Appearance of a new emission spectral band at
~590 nm on binding to Al$^{3+}$ ion suggested the opening of the spirolactam ring and generation of the delocalized xanthene moiety followed by the formation of complex (L–Al$^{3+}$) by metal ion coordination. Incremental addition of Al$^{3+}$ (0–100 μM) provided a steady fluorescence turn-on (Fig. 4) with red shift in the emission maximum and the emission quantum yield $\Phi$ with Al$^{3+}$ may be attributed to the formation of a rigid system after binding. The enhancement of luminescent intensity of the new peak, upon binding to analyte, is usually preferred to promote the selectivity of the sensor. The observed fluorescent enhancement may be attributed to the formation of a rigid system after binding with Al$^{3+}$ ion, causing the chelation-enhanced fluorescence (Scheme 2). Addition of even large excess of other metal ions did not induce an appreciable fluorescence turn-on, although Fe$^{3+}$, Cd$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, and Cr$^{3+}$ showed an obvious absorption response (Fig. 5). The paramagnetic nature of these ions most likely quenched the fluorescence of the resulting L complexes. These findings indicated that L behaved as a highly sensitive and selective fluorescent Al$^{3+}$ sensor. Relative fluorescence enhancement of L in the absence and presence of various other metal ions and thereby its selectivity for Al$^{3+}$ was shown in Fig. S6 in Supporting information. The Job’s plot [41] in Fig. 6 gives a 1:1 stoichiometric ratio between L and Al$^{3+}$ for the newly formed species, corroborated by QTOF mass spectroscopy in which the peak at m/z = 661.44 in the mass spectrum is assignable to the mass of [(L+Al$^{3+}$+H$_2$O+1)$^+$] (Fig. 9).

The interference of other competing metal ions was investigated by the competition experiment which was conducted with 2.0 equiv. of Al$^{3+}$ ion in the presence of other metal ions in the same concentration (Fig. S7 in Supporting information). It was observed that the detection of Al$^{3+}$ in the presence of other metal ions was not hampered. So L could be used as a selective and sensitive colorimetric as well as a fluorimetric sensor for Al$^{3+}$ ions. The results indicate the selectivity of L towards Al$^{3+}$ remained unaffected even in the presence of massive biologically abundant metal ions. For practical application, the detection limit [42] of L was also estimated. The fluorescence titration profile of L (10 μM) with Al$^{3+}$ demonstrated that the detection limit of Al$^{3+}$ is 2.2 × 10$^{-8}$ M which is far below the WHO acceptable limit (0.05 mg L$^{-1}$ or 1.85 μM of Al$^{3+}$) in the drinking water (Fig. S8 in Supporting information).

The chemical reversibility behavior of L was studied to examine the reusability of the receptor. Now to demonstrate this fact the emission titration experiment was conducted using the Al$^{3+}$ complex of L (L–Al$^{3+}$) with Na$_2$EDTA (ethylenediaminetetraacetic acid disodium salt). From this experiment it was revealed that the pink colour of L–Al$^{3+}$ disappeared with increasing concentration of Na$_2$EDTA and the emission intensity of the L–Al$^{3+}$ complex also gradually decreased (Fig. S9 in Supporting information). It indicates the decomposition of L–Al$^{3+}$ as Na$_2$EDTA strips away Al$^{3+}$ from the binding zone.

### 3.4. TCSPC studies

Fluorescence decay measurements often served as a sensitive indicator of the local environment of a fluorophore, and it was responsive to excited state affairs. Fig. 7 represented the time resolved fluorescence decay profile of L and its metal complex in DMSO-aqueous solvent using a 340 nm nano-LED as the excitation source and the relevant data were compiled in Table 2. Emission decay traces ($\lambda_{emn}=590$ nm) for L (10 μM) could be best
fitted with a triexponential functions with time constants $\tau_1 = 1.85$ (52%), $\tau_2 = 3.73$ (16%) and $\tau_3 = 0.48$ (32%) with $\chi^2 = 1.08$ ns, whereas the same probe showed a triexponential decay with the time constants $\tau_1 = 1.05$ (29%), $\tau_2 = 3.98$ (63%) and $\tau_3 = 2.98$ (8%) having $\chi^2 = 1.10$ ns in the presence of the Al$^{3+}$ ion. The minor and short-lived component was assigned to the decay time for the excited state related to the spirolactam moiety, while the long-lived major component was attributed to the xanthene form of the chemosensor L [43]. The relative increase in the percentage of the opening xanthene form upon the addition of Al$^{3+}$ confirms the interaction of Al$^{3+}$ with the receptor.

3.5. Effects of pH

For practical applicability, the appropriate pH conditions for successful operation of the L were explored. At acidic conditions (pH < 5), the ring opening of rhodamine took place for free L because of the strong protonation. When pH > 5, no significant ring opening was observed. However, in the presence of the Al$^{3+}$ ion, there was an obvious fluorescence OFF-ON change between pH 5 and 10 (Fig. S10 in Supporting information). Thus, L can detect the Al$^{3+}$ ion with a wide pH span (5 to 10) because in this region L with the Al$^{3+}$ ion induces a remarkable fluorescence OFF-ON, whereas L without the Al$^{3+}$ ion does not lead to such a change.

The complexation mode of L toward the Al$^{3+}$ ion, with NMR spectral differences upon binding of the metal to the dye, is depicted in Fig. 8. The apparent downshift of $H_2$ and $H_3$ in the presence of 1.0 equiv. of Al$^{3+}$ suggests the formation of the ring opened form of rhodamine. The downshift of $H_3$ indicates that the oxygen atom of the amide group participates in the binding process. In addition, the $H_3$ downshift from 7.87 to 7.95 is due to the “O” on the hydrazide group participating in the binding. Other protons of the rhodamine moiety also downfield shifted because of the strong coordination between L and Al$^{3+}$ ion.

4. Conclusions

In conclusion, we report easily synthesized highly sensitive and selective “turn-on” fluorescent sensors for Al$^{3+}$ based on the CHEF mechanism. Interaction of Al$^{3+}$ with L enhances the fluorescence emission at 590 nm in mixed solvent and induces a turn-on response in the electronic and fluorescence spectra in the visible region. It should be noted that this sensing ensemble has the following advantages: (1) the detection for Al$^{3+}$ can be use in mixed solvent; (2) it is a fluorescence “turn ON” response probe and the sensing process can also be observed by naked eyes; (3) the components of the ensemble are easily synthesized and obtained; (4) the sensing mode to Al$^{3+}$ is highly efficient and novel and there is no interference with other metal ions; and (5) it is chemically reversible and used in a wide pH range (pH 5-10). Moreover, compared with the reported probes for Al$^{3+}$, this is the first chemosensor based on a small molecule that can detect Al$^{3+}$ at $2.2 \times 10^{-8}$ M level. We believe that the L can be used for many practical applications in chemical, environmental and biological systems.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jlumin.2014.05.003.