**Supplementary Information**

**A Chemosensor for Al\(^{3+}\) Ion in Aqueous Ethanol Medium: Photophysical and Live Cell Imaging Studies**

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Experimental section

UV-vis and fluorescence spectroscopic studies.

Stock solution of the sensor \( L \) was prepared at the concentration of \( 10^{-3} \) M in 10 mL of EtOH and then diluted to a desired concentration. Stock solutions of various ions were prepared at the concentration of \( 10^{-3} \) M in 10 mL distilled water. In titration experiments, a quartz optical cell of 1 cm optical pathlength was filled with a 2 mL solution of the sensor \( L \) (10^{-5} \) M) to which the metal ion stock solutions were gradually added using a micropipette. Spectral data were recorded within 5 min after addition of the respective metal ions. In selectivity experiments, the test samples were prepared by placing appropriate amounts of the cations stock into 3 mL of \( L \) (10^{-5} \) M). For fluorescence measurements, excitation was provided at 520 nm and emission was acquired from 540 nm to 700 nm. The association constant was determined from the change in fluorescence intensity resulting from the titration of dilute solution (~10^{-5} \) M) of the dye against \( Al^{3+} \) ion concentration. The reported values gave good correlation coefficients (>0.97).

Determination of Quantum yield:

Fluorescence quantum yields were determined by comparing the corrected spectra with that of pure rhodamine B\(^1\) in ethanol taking the total area under the fluorescence band using the equation (i)^2

\[
\Phi_S = \Phi_R \left( \frac{F_SA_R}{F_RA_S} \right) \times \left( \eta_S/\eta_R \right)^2 \quad \text{(i)}
\]

Where, \( \Phi \) stands for quantum yield, \( F \) stands for area under the fluorescence spectra, \( A \) stands for absorbance value and \( \eta \) stands for the refractive index value. The subscript ‘R’ indicates the value of the parameter for reference (i.e. Rhodamine-B) and ‘S’ subscript indicates the value of the parameter for the sample.
Determination of Detection Limit:

For the evaluation of the detection limit of the probe L with Al\(^{3+}\) ion, the probe was treated with different concentration of trivalent Al\(^{3+}\) ion and the emission intensity at 580 nm was plotted against the Al\(^{3+}\) ion concentration in the lower region. Detection limit was calculated using the following equation:

\[
\text{Detection limit} = \frac{3\sigma}{S}
\]

Where \(\sigma\) is the standard deviation of the eight blank measurements, \(S\) is the slope of the curve of fluorescence emission versus metal ion concentration.

X-ray crystallography:

Single-crystal X-ray data of compound L was collected at 100 K on a Bruker SMART APEX CCD diffractometer using graphite monochromated MoK\(_\alpha\) radiation (\(\lambda = 0.71073\) Å). The linear absorption coefficients, scattering factors for the atoms, and the anomalous dispersion corrections were taken from the International Tables for X-ray Crystallography.\(^3\) The data integration and reduction were carried out with SAINT software\(^4\). Empirical absorption correction was applied to the collected reflections with SADABS\(^5\) and the space group was determined using XPREP\(^6\). The structure was solved by the direct methods using SHELXL-97 and refined on \(F^2\) by full-matrix least-squares using the SHELXL-97 program. In this compound all the non-hydrogen atoms were refined anisotropically.
Fig. S1 500 MHz $^1$H NMR spectrum of sensor L.

Fig. S2 125 MHz $^{13}$C NMR spectrum of sensor L.
Fig. S3 ESI MS spectrum of sensor L.

Fig. S4 125 MHz $^{13}$C NMR spectrum of Al$^{3+}$ complex of L.
Fig. S5 IR spectra of (a) sensor L and (b) Al$^{3+}$ complex of L.

Fig. S6 ESI MS spectrum of Al$^{3+}$ complex of L.
Fig. S7 Fluorescence intensity profile statistics: Untreated control cells (B), L6 cells + sensor (H) and L6 cells + Al^{3+} sensor (N).
**MTT assay for cell viability assessment.** MTT assay was conducted to evaluate whether the sensor itself induced any cytotoxicity in normal cells. Different concentrations of the sensor, ranging from 10µl through 60µl of stock solution (7×10^{-5}M), and/or solvent of same order, were added to the cultured cells (10^6 cells/mL) in 96-well micro plates and incubated for 24h. A set of untreated control cellswere also kept which were devoid of any exposure to any concentration of sensors. MTT was added into each well at the end of incubation period. After 3h of incubation at 37ºC, dimethyl sulfoxide (DMSO) was added into each well. The absorbance of each well was then measured at 595nm using ELISA reader (Thermo scientific, Multiiskan ELISA, USA). The percentage cell survivability was calculated as: (mean experimental absorbance/mean control absorbance) × 100%. No significant difference was observed between the percentages of viable cells in the sensor-treated and the solvent-treated lots, indicating thereby that the sensor was non-cytotoxic and safe for biological use.

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![Cellular Cytotoxicity Assessment](image1)

**Fig. S8** Cellular cytotoxicity assessment of different concentrations of sensor L and solvent system in L6 cell lines.

![Optimized Structures](image2)

**Fig. S9** Optimized structures of ligand L and L− Al^{3+} complexes

[S8]
Table 1 Crystal Data and details of refinements for the sensor $L_3$

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tr>
<td>Empirical Formula</td>
<td>C$<em>{35}$H$</em>{36}$N$_4$O$_5$</td>
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<tr>
<td>Formula Weight</td>
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<td>c (Å)</td>
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<td>$\gamma$ (°)</td>
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<td>Independent Reflections</td>
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<td>Goodness-of-fit</td>
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<tr>
<td>wR1 [I &gt; 2.0 $\sigma$(I)]</td>
<td>0.1732</td>
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References:


4 SAINT+, version 6.02; Bruker AXS: Madison, WI, 1999.
