Electronic Supplementary Information

A naphthalimide based solid state luminescent probe for ratiometric detection of aluminum ions: in vitro and in vivo applications

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EXPERIMENTAL SECTION

Materials and methods

4-Bromo-1,8-naphthalic anhydride, hydrazine hydrate, 4-formylphenylboronic acid and 2-hydroxy benzaldehyde were purchased from Sigma-Aldrich and used without any further purification. Absolute ethanol, tetrahydrofuran (THF), dichloromethane (DCM) were purchased from Merck, India and were of reagent grade. These solvents were dried before use. \( ^1 \)H NMR experiments was performed using Bruker AVANCE III HD 500 MHz spectrophotometer in CDCl\(_3\) and DMSO (d6) as solvent with tetramethylsilane as an internal standard. Bruker MicroTOF QII mass spectrometer was used for recording the mass spectra. Chemical shifts (d) were reported in ppm; coupling constants (\( J \)) are represented in Hz. Multiplicities are reported as follows: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. For the determination of fluorescence quantum yield of probe 4 and probe 4+ Al\(^{3+}\), fluorescein and diphenylanthracene were used as standard. Quantum yield calculations were performed according to Equation (1), in which \( \Phi_{fs} \) and \( \Phi_{fr} \) are the radiative quantum yields of the sample and the reference, \( A_s \) and \( A_r \) are the absorbance of the sample and the reference, \( D_s \) and \( D_r \) are the emission areas for the sample and the reference, \( L_s \) and \( L_r \) are the lengths of the absorption cells for the sample and the reference and \( N_s \) and \( N_r \) are the refractive indices of the sample and reference solutions, respectively.

\[
\Phi_{fs} = \Phi_{fr} \times \frac{1 - 10^{A_l r}}{1 - 10^{A_l r}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}
\]

Photo-physical Studies

For carrying out the photo-physical studies (UV/Vis and fluorescence spectroscopy), high purity HPLC-grade solvents were used. UV/Vis spectra was recorded on SHIMADZU UV-2450 spectrophotometer using quartz cuvettes (path length:1cm) and the cell holder was placed at 25\(^{\circ}\)C in a thermostat. The fluorescence spectral studies were done using SHIMADZU 5301PC spectrofluorimeter. The stock solution of probe 4 and compound 6, 7 and 8 was made in DMSO. Probe 4 (10 \( \mu \)M) in water containing 10% DMSO as co-solvent at 25\(^{\circ}\)C were used for the UV/Vis and fluorescence studies. For the titration experiments, a solution of the probe (3 mL), 10 \( \mu \)M in water: DMSO (9:1) was kept in a quartz cuvette (path length: 1cm) and the spectra
were recorded after the addition of the different analytes. For recording the fluorescence spectra, the emission of probe 4 was recorded using an excitation wavelength of 380 nm. The solutions of different metal ions used in the experiments were prepared in DMSO from their corresponding perchlorate salts which were of analytical grade and distilled water were used for carrying out all further experiments.

**Synthesis**

**Synthesis of compound 1**

Compound 1 has been synthesized according to the reported method.¹

**Synthesis of compound 2**

To a solution of compound 1 (500 mg, 1.285 mmol) and 4-formyl phenyl boronic acid (230 mg, 1.542 mmol) in dioxane, K₂CO₃ (710 mg, 5.14 mmol, dissolved in 1 ml water) and Pd(0) (326 mg, 0.2827 mmol) were added under a nitrogen atmosphere and the reaction mixture was heated at reflux for overnight. Dioxane was then removed under vacuum and the residue left was extracted with dichloromethane and then dried over anhydrous Na₂SO₄. Finally, the organic layer was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (CHCl₃/ CH₃OH, 9:1) to furnish compound 2 as light yellow solid (350 mg, 65% yields). ¹H NMR (500 MHz, CDCl₃ δ (ppm) = 2.61 (br, 4H, CH₂), 2.74 (t, J = 5 Hz, 2H, CH₂), 3.69 (br, 2H, CH₂), 4.38 (t, J = 5 Hz, 2H, CH₂), 7.70 (d, J = 5 Hz, 2H, Ar-H), 7.73 (t, J = 5 Hz, 2H, Ar-H), 8.11 (d, J = 5 Hz, 2H, Ar-H), 8.21 (d, J = 10 Hz, 1H, Ar-H), 8.67 (t, J = 5 Hz, 2H, Ar-H), 10.18 (s, 1H, Ar-H). ¹³C NMR (CDCl₃, 125 MHz): δ (ppm) = 37.32, 53.88, 56.16, 67.06, 122.57, 122.98, 127.33, 127.88, 128.70, 129.98, 130.61, 130.71, 131.42, 132.02, 136.16, 144.89, 145.22, 163.94, 164.14, 191.60. ESI-MS Calcd. for C₂₅H₂₂N₂O₄: 414.14; Found: 415.14 [M+H]⁺.

**Synthesis of compound 3**

To a solution of hydrazine (204.72 mg, 4.0942 mmol) in absolute ethanol, add a solution of 2-hydroxybenzaldehyde (500 mg, 4.0942 mmol) in ethanol slowly in a drop-wise manner (within a period of 20 min) at room temperature. After complete addition of aldehyde, cool the reaction mixture to -20°C and allow it to stand for 20-30 min until, creamy white shiny crystals appear.² Filter and dry the crystals to furnish compound 3 in 66% yields (370 mg). ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 11.39 (s, 1H, -OH), 8.71 (s, 1H, -CH=N-), 7.42-7.34 (m, 2H, ArH), 7.05-6.94 (m, 2H, ArH). ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 164.39, 159.45, 133.07, 132.18, 119.34, 116.87, 116.75.
**Precautions:** 1) Do not reverse the order of addition i.e do not add hydrazine drop wise to a solution of aldehyde because it leads to the formation of dimer. 2) Do not leave the reaction mixture after complete addition of aldehyde at room temperature because dimer will be formed in maximum amount. 3) The formation of dimer can be monitored by observing the colour of crystals formed. If shiny yellow or light pink crystals are formed, this indicates the formation of dimer. Light pink crystals get later changed to yellow colour with time (within 1-2 hrs). Only the white/ pale yellow shiny crystals correspond to monomer formation. 4) The isolation of monomer out dimer is difficult, so it’s better to take these precautions seriously.

**Synthesis of compound 4**

A mixture of compound 2 (100 mg, 0.2412 mmol) and compound 3 (32.80 mg, 0.2412 mmol) in DCM: ethanol (4:6) was heated to reflux for 24 hours. The solid so obtained was filtered and dried to obtain crude product. The crude product was then recrystallized with ethanol to afford probe 4 as pure light yellow coloured solid in 53% yield (68 mg). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 2.66 (br, 4H, CH$_2$), 2.79 (t, $J = 5$ Hz, 2H, CH$_2$), 3.72 (br, 2H, CH$_2$), 4.41 (t, $J = 5$ Hz, 2H, CH$_2$), 7.00 (t, $J = 5$ Hz, 1H, Ar-H), 7.07 (d, $J = 10$ Hz, 1H, Ar-H), 7.41 (d, $J = 10$ Hz, 2H, Ar-H), 7.64 (d, $J = 5$ Hz, 2H, Ar-H), 7.77 (d, $J = 10$ Hz, 2H, Ar-H), 8.05-8.10 (m, 2H, Ar-H), 8.30 (t, $J = 10$ Hz, 1H, Ar-H), 8.67-8.70 (m, 2H, Ar-H), 8.76 (s, 1H, N=CH), 8.85 (s, 1H, N=CH), 11.74 (s, 1H, Ar-OH). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ = 37.19, 53.76, 56.04, 66.92, 116.62, 116.87, 117.53, 119.58, 122.05, 122.84, 127.13, 127.83, 128.64, 128.80, 128.86, 129.80, 130.42, 130.64, 131.18, 132.32, 132.55, 133.14, 133.74, 141.84, 145.78, 159.81, 161.54, 163.91, 164.11, 165.49. ESI-MS Calcd. for C$_{32}$H$_{28}$N$_{4}$O$_{4}$: 532.21; Found: 533.25 [M+H]$^+$.  

**Synthesis of compound 5**

To a solution of hydrazine (183.8 mg, 3.672 mmol) in ethanol, add 2-methoxybenzaldehyde (500 mg, 3.672 mmol) in a drop-wise manner. After complete addition of aldehyde, reaction mixture was stirred for 6 hrs at room temperature. The solid so obtained was filtered and finally recrystallized with ethanol to get compound 5 as yellow solid in 70% yields (385 mg). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ (ppm) = 9.08 (s, 1H, -CH=N-), 8.10 (d, 1H, $J = 10$ Hz, ArH), 7.42 (t, 2H, $J = 15$ Hz, ArH), 7.04-6.93 (m, 2H, ArH), 3.89 (s, 3H, -OCH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ (ppm) = 159.24, 157.58, 132.38, 127.36, 122.86, 120.79, 111.18, 55.52.
Synthesis of compound 6
A mixture of compound 2 (50 mg, 0.1206 mmol) and compound 5 (18.2 mg, 0.1206 mmol) in DCM: ethanol (1:9) was heated to reflux for 24 hours. The solid so obtained was filtered and dried to obtain crude product. The crude product was then recrystallized with ethanol to afford compound 6 as light yellow coloured solid in 54% yields (35 mg). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ (ppm) = 2.64 (br, 4H, CH$_2$), 2.77 (t, $J = 5$ Hz, 2H, CH$_2$), 3.72 (br, 2H, CH$_2$), 3.94 (s, 3H, CH$_3$), 4.41 (t, $J = 5$ Hz, 2H, CH$_2$), 7.00 (d, $J = 10$ Hz, 1H, Ar-H), 7.07 (t, $J = 5$ Hz, 1H, Ar-H), 7.48 (t, $J = 5$ Hz, 1H, Ar-H), 7.63-7.67 (m, 2H, Ar-H), 7.78 (d, $J = 10$ Hz, 2H, Ar-H), 8.06-8.17 (m, 3H, Ar-H), 8.31 (t, $J = 5$ Hz, 1H, Ar-H), 8.66-8.71 (m, 2H, Ar-H), 8.79 (s, 1H, N=CH), 9.17 (s, 1H, N=CH).

$^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) = 37.33, 53.84, 55.66, 56.17, 67.07, 111.27, 120.86, 122.05, 122.52, 122.92, 127.09, 127.40, 127.84, 128.71, 128.76, 128.89, 129.93, 130.34, 130.45, 130.82, 131.32, 132.48, 132.81, 134.58, 141.31, 141.76, 146.12, 158.64, 159.28, 160.54, 164.08, 164.30. ESI-MS Calcd. for C$_{33}$H$_{30}$N$_4$O$_4$: 546.23; Found: 547.26 [M+H]$^+$. 

Synthesis of Compound 7:
To the solution of compound 2 (100 mg, 0.2412 mmoles) in anhydrous ethanol, add aniline (24 mg, 0.2412) and allow it to reflux for overnight. The solid so obtained is filtered and dried. The solid is then recrystallized in methanol to yield the compound 7 as light brown solid in 67% yields (80 mg). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 2.63 (br, 4H, CH$_2$), 2.74 (t, $J = 6.9$ Hz, 2H, CH$_2$), 3.71 (t, $J = 8.9$ Hz, 4H, CH$_2$), 4.39 (t, $J = 6.9$ Hz, 2H, CH$_2$), 7.29-7.31 (m, 2H, Ar-H), 7.45 (t, $J = 8.9$ Hz, 2H, Ar-H), 7.63-7.77 (m, 5H, Ar-H), 8.11 (d, $J = 8.2$ Hz, 2H, Ar-H), 8.29 (d, $J = 8.4$ Hz, 1H, Ar-H), 8.59 (s, 1H, N=CH), 8.66 (t, $J = 8.2$ Hz, 2H, Ar-H).

$^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ = 37.42, 54.02, 56.35, 67.26, 121.12, 122.35, 123.19, 126.64, 127.43, 128.71, 128.76, 129.04, 129.36, 129.58, 130.29, 130.70, 131.14, 131.67, 132.76, 136.68, 142.00, 146.38, 152.10, 159.79, 164.44, 164.64. ESI-MS Calcd. for C$_{31}$H$_{27}$N$_3$O$_3$: 489.2052; Found: 490.2495 [M+H]$^+$. 

Synthesis of Compound 8:
To the solution of compound 3 (100 mg, 0.349) in anhydrous ethanol, add benzaldehyde (78.05 mg, 0.7349) and allow it to reflux for 1 hr. The solid so obtained is filtered and dried. The solid is then recrystallized in methanol to yield the compound 8 as yellow solid in 51% yields (85 mg). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 6.93-7.05 (m, 2H, Ar-H), 7.36 (d, $J = 6$ Hz, 2H, Ar-H), 7.457-7.51 (m, 3H, Ar-H), 7.84-7.87 (m, 2H, Ar-H), 8.64 (s, 1H, -N=CH-), 8.79 (s, 1H, -N=CH-),
\begin{eqnarray*}
11.78 \text{ (s, 1H, -OH).} \quad \delta = 117.30, 119.81, 129.01, 129.20, 131.95, \\
132.73, 133.29, 133.86, 160.21, 162.84, 165.51.
\end{eqnarray*}

**Preparation of detection kit**

For the preparation of detection kit, a pre-coated silica plate is cut into 7x5 cm dimension. Take a black paper and puncture it in order to make holes. Cover the silica plate with this black paper. To the holes, load 10 µM solution of probe 4 and let it dry. Observe the fluorescence of probe coated kit under UV lamp. For the detection purpose, add sample containing aluminium and observe fluorescence change under UV lamp. In our case, we have added different concentration of aluminium (1.5, 10 and 50 µM). Other metal ions are also tested (50 µM solution used for other metal ions).

**Solid State Detection**

For the solid state fluorescence detection, Fluoromax-4 Spectrofluorometer was used with vertical sample holder. For the detection purpose, place a 20 mg of probe 4 on the black paper and observe it under UV lamp. To it, add solid aluminium (30 mg) and gently mix it and observe under UV to observe the fluorescence changes in solid state. Similar procedure is followed for observing the solid state emission of model compound 5.

**Procedure of NMR (Stability of probe 4 in aqueous medium)**

For examining the stability of probe 4 in aqueous medium in presence of aluminium ions, \(^1\)H-NMR study was carried out. For this, 6 mg of probe 4 was taken in a 25 ml round bottom flask and add 1 ml of DMSO (\(d_6\)) and 8 ml of D\(_2\)O and stir the solution for 2 min. Add to it solid aluminium 7 mg into the reaction mixture and allow the reaction mixture to stir for 2 hrs. After 2 hrs, extract the reaction mixture with CDCl\(_3\) (5 ml) and pass it through sodium sulphate and allow the CDCl\(_3\) to evaporate at room temperature until it gets reduced to one-forth. The solution left was used for carrying out \(^1\)H NMR.

**MTT Assay**

To determine the cell viability after addition of probe 4, MTT assay was done. MTT assay is based on the reduction of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, yellow in color) to formazan (blue color) by mitochondrial succinate dehydrogenase. C6 glioblastoma cells (10000 cells/mL) were incubated in 96 well plate for 24 hours. Cells were exposed to different concentration of test probe 4 for 24 hours in CO2 incubator. After 24 hours of incubation, a freshly prepared MTT solution (100 µL) was added in each well. After the
incubation period of 2 hours, medium containing MTT was removed, followed by the addition of 100 µL of DMSO to dissolve the formazan crystals. Absorbance was recorded at 595 nm by an ELISA Plate Reader (Biotek Synergy HT). Untreated cells were considered as control. All experiments were performed in triplicate and the cell viability after addition of probe 4 was determined by using given formula:

\[
\text{% Cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100
\]

**Cell culture**

For the biological studies, the C6 glioblastoma cells were obtained from the National Centre for Cell Sciences, Pune, India. C6 Cells were maintained in DMEM supplemented with 1X PSN (GIBCO), 10% FBS (Biological Industries) at 37°C and humid environment containing 5% CO₂. The seeding of cells were done in 12 well plates and 90 mm petri dishes with a cell density of 10,000 cells/ml for the purpose of fluorescence imaging.

**Details of treatments and fluorescence Detection in living cells**

For the purpose of bio-imaging of aluminium ions in cells, three groups were chosen: (1) Control; (2) C6 glioblastoma cells treated with 2.5 µM of probe 4 for 30 min; (3) Exogenous detection: C6 glioblastoma cells treated with 10 µM of Al³⁺ for 30 min followed by 2.5 µM of probe 4 for 30 min, (Aluminum perchlorate was used as source for aluminium). After treatment, these cell groups were washed thrice for 5 minutes with 1X PBS and images were taken using A1R Nikon Laser Scanning Confocal microscope at blue and green channels with excitation, \(\lambda_{ex}= 405\) nm laser source.

**Statistical analysis for fluorescence intensity of cell and tissue images**

For the fluorescence quantification analysis in cell and tissue images, NIS-Elements Viewer software was used. Further, for carrying out intensity analysis, several regions of interest (ROI) in each image were chosen and corresponding intensity was determined for every ROI in each image using software. Further, Sigma Stat for Windows (version 3.5) was used to analyze the results by One way ANOVA (Holm-Sidak post hoc method), in order to determine the significance of the means. The values are expressed as Mean ± Standard Error of Mean (SEM). Values with \(P\) value \(\leq 0.05\) were considered as statistically significant.
Procedure of tissue imaging

Wistar strain rat was first decapitated and its brain was cautiously removed. The freshly dissected brain was then embedded in the cryomatrix contained in the mold and snap freezing was done using chilled isopentane for 5 min. Next, the cryomatrix-embedded brain was taken out from the mold carefully, mounted and finally coronal sections of 60 μm thickness were made directly on the microscopic glass slide using freezing cryomicrotome. For the staining of tissue, the sections were washed with 1X PBS thrice for 5 min each. Then, two groups were made for: I, brain sections were incubated with 4 μM of probe 4 for 1 hour at 37°C. II, the probe 4 pre-treated sections were exposed to 15 μM of exogenous aluminium source for another 1hour. After treatment, the tissue images were taken at 10X magnification using the A1R Nikon Laser Scanning Confocal microscope under an excitation of 405 nm in blue and green channel. To observe the changes in the fluorescent intensity of tissue sections at variable depth along the Z axis, optical Sectioning was done at the interval of 5.0 μm thickness.

Note: The official permission to conduct animal experiments was acquired from the Institutional Animal Ethical committee, Reg. No. of Animal house: 226/CPCSEA. All experimental protocols involving animals were performed in agreement with the guidelines of ‘Animal Care and Use’ laid down by Institutional Animal Ethical Committee, Guru Nanak Dev University.

In vivo toxicity of aluminium ions

In vivo toxicity of Al^{3+} ions in nematodes was determined by choosing seven different concentrations of Al^{3+} ions. Nematodes were exposed with different concentration of Al^{3+} ions and after 24 hrs, the numbers of alive and dead nematodes were determined via visual inspection under a microscope. The nematodes were considered dead if no movement was observed in them. This experiment was independently repeated at least three times (Figure S50).

Imaging of aluminium in Nematodes

For this purpose of detection of aluminium in nematodes, two groups were chosen as follows: (I) Nematodes treated with probe 4 (5.0 μM) for 3 hrs. (II) Nematodes treated with probe 4 (5.0 μM) for 3 hrs followed by washing with 1X and exposed to aluminium (10 uM) for 2 hrs. Nematode Images were taken using A1R Nikon Laser Scanning Confocal microscope at 405 and 488 nm channels using 405 nm excitation laser.
Figure S1: Representation of synthetic route of probe 4 and model compounds
Figure S2: $^1$H NMR spectra of compound 2 in CDCl$_3$ as solvent (500 MHz)

Figure S3: $^{13}$C NMR spectra of compound 2 in CDCl$_3$ as solvent (125 MHz)
Figure S4: $^1$H-NMR spectrum of compound 3 in CDCl$_3$ as solvent in 500 MHz

Figure S5: $^{13}$C-NMR spectrum of compound 3 in CDCl$_3$ as solvent
Figure S6: $^1$H NMR spectra of probe 4 using CDCl$_3$ as solvent (500 MHz)

Figure S7: $^{13}$C NMR spectra of probe 4 in CDCl$_3$ as solvent (125 MHz).
Figure S8: $^1$H-NMR spectrum of compound 5 in CDCl$_3$ as solvent (500 MHz)

Figure S9: $^1$H-NMR spectrum of compound 5 in CDCl$_3$ as solvent (125 MHz)
Figure S10: Mass spectrum of compound 2

Figure S11: Mass spectrum of probe 4
Figure S12: $^1$H-NMR spectrum of compound 6 in CDCl$_3$ as solvent

Figure S13: $^{13}$C-NMR spectrum of compound 6 in CDCl$_3$ as solvent
Figure S14: $^{13}$C-NMR spectrum of compound 6 in CDCl$_3$ as solvent (magnified region)

Figure S15: Mass spectra of compound 6
Figure S16a: $^1$H-NMR spectrum of compound 7 in CDCl$_3$ as solvent in 300 MHz.

Figure S16b: $^1$H-NMR spectrum of compound 7 (expanded region).
Figure S17: $^{13}$C-NMR spectrum of compound 7 in CDCl$_3$ as solvent in 75 MHz.

Figure S18: Mass spectra of compound 7
Figure S19: $^1$H-NMR spectrum of compound 8 in CDCl$_3$ as solvent in 300 MHz.

Figure S20: $^{13}$C-NMR spectrum of compound 8 in CDCl$_3$ as solvent in 75 MHz.
Figure S21: Fluorescence spectra of probe 4 (10 µM) in different Water: THF fractions from 0% to 100%, ($\lambda_{ex} = 380$ nm), slit width 5:5.

Figure S22: (a) Fluorescence spectra of compound 6 (10µM, in H$_2$O: DMSO, 9:1) in presence of 60 eq. of Al$^{3+}$ ions; $\lambda_{ex}$= 360 nm, slit width: 3:5. (b) Image of compound 6 (solid) under UV lamp.
**Figure S23**: UV-vis spectra of probe 4 (10.0 µM) in different water: THF mixture, showing the leveling off tail.

**Figure S24**: DLS data of probe 4 (10 µM) in water (containing 10% DMSO as co-solvent) with average particle size of 101.6 nm.
**Figure S25:** SEM, TEM and DLS data of probe 4. (A and E) SEM and TEM image of probe 4 in H$_2$O (10% DMSO as a co-solvent). (B) DLS data of probe 4. (C and F) SEM and TEM image of probe 4 + Al$^{3+}$ ions (60 equiv) in H$_2$O (10% DMSO as a co-solvent). (D) DLS data of probe 4 + Al$^{3+}$ ions under similar set of conditions.

**Figure S26:** DLS data of probe 4 (10 µM) in water (containing 10% DMSO as co-solvent) in the presence of 60 equivalents of aluminum ions.
Figure S27: Plausible representation of the change in self-assembly of probe 4 in water and on addition of Al$^{3+}$ ions
Detection limit

Figure S28: Detection limit plot of probe 4, representing the equation of $y = mx + C$.

$R^2 = 0.9964$

Equations: $y = mx + C$, where $m$= slope

Thus, $y = 28005x + 0.9311$

Here, slope $(m) = 28005$

Standard deviation (SD) of the free ligand (measured by making repetition of 10 times) $= 0.024$

Calculation of detection limit:

\[ DL = 3 \times \frac{SD}{Slope} \]

\[ = 3 \times \frac{0.024}{28005} \]

\[ = 2.82 \, \mu M \]
Figure S29: Fluorescence spectra of probe 4 (10µM) at different pH buffers. Spectra recorded using excitation wavelength of 380 nm. Slit 5:5.

Figure S30: Fluorescence spectra of probe 4 (10µM) in buffer of a) pH = 7.5 to 5.5 in presence of 30 eq. of Al^{3+} ions. Spectra recorded using excitation wavelength of 380 nm, Slit 5:5.
Figure S31: Fluorescence spectra of probe 4 (10μM) in buffer of pH= 4.5 in presence of 30 eq. of Al$^{3+}$ ions. Spectra recorded using excitation wavelength of 380 nm, Slit 5:5.

Figure S32: Fluorescence spectra of probe 4 (10μM) in buffer of pH= 8.5 in presence of 30 eq. of Al$^{3+}$ ions. Spectra recorded using excitation wavelength of 380 nm, Slit 5:5.

Figure S33: Fluorescence response of probe 4 (10.0 μM) on addition of 60 eq. of (a) Cd$^{2+}$ and b) Co$^{2+}$ in water: DMSO (9:1), λ$_{ex}$ = 380 nm, Slit 5:5.
**Figure S34**: Fluorescence response of probe 4 (10.0 μM) with addition of 60 eq. of (a) Cu$^{2+}$ and b) Fe$^{2+}$ in water: DMSO (9:1), $\lambda_{ex} = 380$ nm, Slit 5:5.

**Figure S35**: Fluorescence response of probe 4 (10.0 μM) with addition of 60 eq. of (a) Fe$^{3+}$ and b) Zn$^{2+}$ in water: DMSO (9:1), $\lambda_{ex} = 380$ nm, Slit 5:5.

**Figure S36**: Fluorescence response of probe 4 (10.0 μM) with addition of 60 eq. of (a) Hg$^{2+}$ and b) Na$^+$ in water: DMSO (9:1), $\lambda_{ex} = 380$ nm, Slit 5:5.
**Figure S37**: Fluorescence response of probe 4 (10.0 μM) with addition of 60 eq. of (a) Ca²⁺ and b) Pd²⁺ in water: DMSO (9:1), λₑₓ=380 nm, Slit 5:5.

**Figure S38**: a) Fluorescence response of probe 4 (10.0 μM) with addition of 60 eq. of (a) K⁺ and b) La³⁺ in water: DMSO (9:1), λₑₓ=380 nm, Slit 5:5.

**Figure S39**: Fluorescence spectra of probe 4 (10μM) in water 9:1 Water: DMSO in presence of 60 eq. of Cr³⁺ ions. Spectra recorded using excitation wavelength of 380 nm and Slit of 5:5.
Figure S40: $^1$H-NMR titration of probe 4 in presence of aluminum ions (0, 5 and 10eq.) in DMSO (d$_6$) in 500 MHz.

Figure S41: $^1$H-NMR of probe 4 in presence in CDCl$_3$ (to check the stability of probe 4 in aqueous medium in the presence of Al$^{3+}$ ions in 300 MHz. Procedure is provided in experimental section.
No significant change in emission intensity in presence of Al$^{3+}$ ions

**Figure S42:** Fluorescence spectra of compound 7 (10 µM) in presence of Al$^{3+}$ ions (60 eq.) in water: DMSO (9:1). Spectra recorded using excitation of 360 nm with slit width of 3:3.

**Figure S43:** Fluorescence spectra of compound 8 (10µM) in 9:1, water: DMSO in presence of 60 eq. of Al$^{3+}$ ions. Spectra recorded using excitation wavelength of 340 nm. Slit = 5:5
**Figure S44:** (a) Solid state thin layer fluorescence of probe 4 using excitation wavelength of 380 nm using (1:1, slit width). (b, c) Solid state fluorescence of probe 4 when excited using 405 nm laser. (d) Probe 4 under day light.

**Figure S45:** XRD pattern of probe 4 and probe 4+ Al$^{3+}$
Figure S46: Images representing bulk phase detection of aluminium in solid state. a) Probe alone  b) Gentle mixing of sample for 30 seconds leads to the appearance of blue fluorescence. c) On further mixing for 2 min, enhanced blue fluorescence was observed. Images captured under UV lamp illumination.

Figure S47: (a) Percentage viability data of different concentration of aluminum ions in C6 glial cells. (b) Percentage viability data of different concentration of probe 4 in C6 glial cells
Figure S48: Histograms depict the relative expression of the probe 4 in different groups in ratio of blue channel/green channel. * $p \leq 0.05$ Control v/s Probe alone and Probe + Al$^{3+}$, # $p \leq 0.05$ Probe alone v/s Probe + Al$^{3+}$, Holm-Sidak method after one-way ANOVA.

Figure S49: Plot of the change in fluorescent intensity v/s depth at intervals of 5 µm along z-axis, * $p \leq 0.05$ Probe alone v/s Probe + Al$^{3+}$, Holm-Sidak method after one-way ANOVA. (a) Intensity of 3D images of probe 4; (b) probe 4 +Al$^{3+}$ (in green and blue channel).
Figure S50: a) Percentage survival of nematodes on exposure to different concentration of aluminium ions; b) Plot of number of individual survival versus different concentration of aluminium ions; c) Light microscopic images of the different groups under 10X zoom.
Figure S51: Imaging of nematodes using compound 6 (5 μM, as negative control). First row, images on treated with compound 6 for 3 hrs. Second row represent the images having compound 6 treatment first and then exposure to 10μM of Al^{3+} ions for 2 hrs. Images captured keeping 405 and 488 nm laser ‘ON’ with Ex= 405 nm.

References
