Supporting Information

"Turn-on trivalent cation selective chemodosimetric probe to image native cellular iron pools"

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1) Instruments and experimental information:

General information:

All chemicals were purchased from merck, S.D. Fine and Sigma Aldrich and were used without further purification. THF (AR grade) was used for the spectral studies. A freshly prepared metal nitrates(Fe$^{3+}$, Cr$^{3+}$, Al$^{3+}$, Fe$^{2+}$, Pb$^{2+}$, Ag$^{+}$, Ni$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Na$^{+}$, Hg$^{2+}$, Mg$^{2+}$ and Co$^{2+}$) were used as (Hg$^{2+}$ was used as chloride salt due to solubility issue) standard solutions (10$^{-3}$ M) in THF/H$_2$O(8:2 v/v). FT-IR spectra in KBr were recorded on a perkinelmer spectrum 2. $^1$H and $^{13}$C NMR spectra in DMSO-d$_6$ and DMSO-d$_6$+D$_2$O(8:2) were recorded on Bruuker-Avance 400 MHz using tetra methyl silane as an internal standard. Absorption spectra were recorded with SHIMADZU UV-2450 spectrophotometer, with a quartz cuvette. The fluorescence spectra were recorded with Cary Eclipse spectro photometer (slit widths at excitation and emission of the spectro photometer are 5-10 ) respectively. LSMS-ESI and HRMS-ESI spectra were recorded on a Q-TOF micromass and WATERS-QTof Premier spectrometer, respectively.

The details and conditions for live cell imaging:

The exponentially growing Candida cells were centrifuged at 5000rpm for 5 mins, the cell pellet was suspended in HEPES buffer and 1ml of cell suspension was distributed in three tubes. The first set cells were directly treated with 50 μM ligand solution for 30 mins without any salt treatment and then washed with buffer solution three times, centrifuged and washed pellets were suspended in HEPES buffer for photography. The second set cells were treated with 5 mM EDTA for one hour, washed by centrifugation using the same buffer as above and then treated with the probe 1(50 μM) for 30 mins. The third set washed cells were treated with 50 μM Fe$^{3+}$
salt for 30 mins, washed and incubated with 50 μM ligand solution for 30 mins, washed to remove excess unabsorbed ligand. Cells of all three sets were separately mounted on clean, grease free slides and photographed under high power objective of fluorescence microscope.

**Imaging of banana pith:** The freshly collected banana pith was sectioned and washed carefully with HEPES buffer (pH 7.2). Then the sections were treated with ligand solution (50 μM), washed and then photographed. Another set of sections were treated first with EDTA to chelate the iron present in the pith and then with ligand, and finally photographed.

**Synthetic routes and characteristic data:**

**Synthesis of (1):**

To a stirred solution of 3-amino-3-ethyl cabazole (0.100 g, 0.473 mmol) in methanol, imidazole 2-carboxaldehyde (0.045 g, 0.463 mmol) and few drops of acetic acid were added. The resulting mixture was then refluxed at 80°C for 5 h to yield a bright yellow precipitate. The precipitate was filtered-off and simultaneously washed thoroughly with methanol for several times. The washed residue was dried under reduced pressure inside a desiccator. The product was obtained as yellow solid in 62% (0.085 g) yield.

Yield: 62%. Melting point: 232-236°C. FT-IR (KBr, ν in cm⁻¹): 3112, 3047, 2976, 2895, 1634, 1612, 1477, 1444, 1233, 746. ¹H NMR (DMSO-d₆, 400 MHz): 8.6 (m, 2H), 8.19 (m, 2H), 7.65-7.59 (m, 2H), 7.53-7.51 (m, 2H), 7.48-7.45 (m, 1H), 7.20-7.28 (m, 1H), 4.46-4.44 (m, 2H), 3.41 (s, 1H), 1.34-1.30 (m, 3H). ¹³C NMR (DMSO-d₆): δ = δ = 147.8, 145.4, 142.5, 140.1, 138.4, 125.9, 122.8, 122.3, 120.7, 120.3, 119.7, 118.8, 112.3, 109.5, 109.3, 37.1, 13.7. MS (HRMS): m/z calculated for C₁₈H₁₆N₄[MH]⁺ 289.1453, found 289.1451.
Scheme S1. Synthesis of compound 1.

Figure S1. $^1$H-NMR Spectrum of 1 in DMSO-d$_6$. 
Figure S2. $^{13}$C-NMR Spectrum of 1 in DMSO-d$_6$.

Figure S3. UV-vis spectra of 1(10µM) in THF-H$_2$O (8:2).
**Figure S4a.** UV-vis spectra of 1(10µM) in presence of Fe³⁺/Al³⁺/Cr³⁺ (360 µM) in THF-H₂O (8:2).

![UV-vis spectra of 1(10µM) in presence of Fe³⁺/Al³⁺/Cr³⁺ (360 µM) in THF-H₂O (8:2).](image)

**Figure S4b.** UV-vis spectra of 1(10µM) in presence of Fe³⁺/Al³⁺/Cr³⁺ (360 µM) in MeOH-H₂O (8:2).

![UV-vis spectra of 1(10µM) in presence of Fe³⁺/Al³⁺/Cr³⁺ (360 µM) in MeOH-H₂O (8:2).](image)
**Figure S4c.** UV-vis spectra of 1 (10 µM) in presence of Fe$^{3+}$/Al$^{3+}$/Cr$^{3+}$ (360 µM) in DMSO-H$_2$O (8:2).

**Figure S4d.** UV-vis spectra of 1 (10 µM) in presence of Fe$^{3+}$/Al$^{3+}$/Cr$^{3+}$ (360 µM) in DMF-H$_2$O (8:2).
**Figure S5.** UV-vis spectra of I (10µM) in presence of Fe$^{3+}$/Al$^{3+}$/Cr$^{3+}$ (360µM) in THF.

![UV-vis spectra](image1)

**Figure S6.** UV-vis spectra of I (10µM) in presence of Fe$^{3+}$/Al$^{3+}$/Cr$^{3+}$ (360 µM) and 3-amino n-ethyl carbazole (10µM).

![UV-vis spectra](image2)
Scheme S2. Metal mediated hydrolysis of probe 1.

Figure S7. Fluorescence spectra of 1(1µM) in the presence of 51µM, 75 µM and 63 µM for Fe$^{3+}$/Al$^{3+}$/Cr$^{3+}$ respectively, and carbazole amine (1µM) in THF/H$_2$O(8:2 v/v).
**Figure S8.** Fluorescence spectra of 1(1µM) in presence of various metals in THF

![Fluorescence spectra](image)

**Figure S9.** (a) $^1$H-NMR titration spectrum of 1, (b) after immediate addition of Al$^{3+}$ and (c) after 15 minutes addition of Al$^{3+}$ in DMSO-d$_6$ + D$_2$O(8:2v/v).

![1H-NMR spectra](image)
**Figure S10.** MS of 1 and ESI-Ms of 1 in presence of Al$^{3+}$/Fe$^{3+}$/Cr$^{3+}$.

All mass spectra were recorded after 15 minutes of adding Al$^{3+}$/Fe$^{3+}$/Cr$^{3+}$ in 1.

**Figure S10a.** MS of 1.
Figure S10b. ESI-Ms of 1 in presence of Fe$^{3+}$.

Figure S10c. ESI-Ms of 1 in presence of Al$^{3+}$. 
The details information for the determination of the limit of detection:

Detection limit was calculated on the basis of fluorescence titration, using the equation LOD = (3σ/m) at S/N = 3, where σ is the standard deviation of the blank solution, calculated from 10 blank measurements and m is the slope of the fluorescence intensity vs M$^{3+}$ concentration plot.
**Figure S11a.** Plot of fluorescence intensity of 1 (1 μM) in THF:H$_2$O (8:2, pH = 7.2) at 330 nm as a function of the Cr$^{+3}$.

**Figure S11b.** Plot of fluorescence intensity of 1 (1 μM) in THF:H$_2$O (8:2, pH = 7.2) at 330 nm as a function of the Fe$^{+3}$. 
**Figure S11c.** Plot of fluorescence intensity of 1 (1 μM) in THF:H₂O (8:2, pH = 7.2) at 330 nm as a function of the Al³⁺.

**Figure S12a.** The change in fluorescence intensity (λex = 330 nm) of 1 with pH in a THF/water mixture (8:2 v/v) was measured. pH of the solution was adjusted with NaOH and HCl.
**Figure S12b.** The change in fluorescence intensity ($\lambda_{\text{ex}} = 330$ nm) of 1 and 1 in presence of Fe$^{3+}$, Al$^{3+}$, Cr$^{3+}$ (5 equiv) with pH in a THF/water mixture (8:2 v/v) was measured. pH of the solution was adjusted with NaOH and HCl.

**Figure S13a.** Time-dependent change in fluorescence intensity ($\lambda_{\text{ex}} = 330$ nm) of 1 in THF/water mixture (8:2 v/v; pH 7.2) in the presence of 9, 6 and 3 eq of Fe$^{3+}$. 
Figure S13b. Time-dependent change in fluorescence intensity ($\lambda_{ex} = 330$ nm) of 1 in THF/water mixture (8:2 v/v; pH 7.2) in the presence of 9, 6 and 3 eq of Al$^{3+}$.

![Figure S13b](image)

Figure S13c. Time-dependent change in fluorescence intensity ($\lambda_{ex} = 330$ nm) of 1 in THF/water mixture (8:2 v/v; pH 7.2) in the presence of 9, 6 and 3 eq of Cr$^{3+}$.

![Figure S13c](image)
Figure S13d. Time-dependent changes in fluorescence intensity ($\lambda_{\text{ex}} = 330$ nm) of 1 in THF/water mixture (8:2 v/v; pH 7.2) in the presence of 19 eq, 25 eq, 21 eq for Fe$^{3+}$/Al$^{3+}$/Cr$^{3+}$ respectively.

![Graph showing time-dependent changes in fluorescence intensity](image)

Figure S13e: The change in fluorescence intensity ($\lambda_{\text{ex}} = 330$ nm) of 1 in THF/water mixture (8:2 v/v; pH 7.2) in the presence of 1 equiv of Fe$^{3+}$.

![Graph showing comparison of fluorescence intensity](image)