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LETTER

A novel "turn-on" fluorescent probe for Fe^{3+} in aqueous media based on C═N isomerization†

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A new type of fluorescent probe (L) for Fe^{3+} based on C═N isomerization was synthesized. It shows fluorescence enhancement (turn-on) response for Fe^{3+} in aqueous media (v(CH_3CN): v(H_2O) = 1:9). The complexation mode and the corresponding fluorescent enhancement mechanism were elucidated by IR spectra, transient spectra, Job plot, and DFT calculations.

Fe^{3+} is the most abundant transition metal in cellular systems and is of outstanding importance due to its biological functions, including essential roles in oxygen uptake, oxygen metabolism, electron transfer, and transcriptional regulation.[1] Fe^{3+} deficiency and overload can induce various diseases, such as Parkinson's disease, Alzheimer's disease and cancer.[2] Therefore, development of a Fe^{3+}-amplified fluorescent probe, as an efficient and economic method for the analysis of Fe^{3+}, is very important in biological systems and environmental science. However, the paramagnetic nature of Fe^{3+} always leads to the fluorescent quenching,[3] so the probes of Fe^{3+}-amplified emission in aqueous media are very rare examples,[4] most of which are based on opening rhodamine derivatives spiro ring *via* ion binding. Therefore, design of a new type of Fe^{3+}-amplified fluorescent probe is still a challenge.

Recently, C═N isomerization as a signaling mechanism has been actively developed to design fluorescent probes.[5] Since C═N isomerization is the predominant decay process of excited states, compounds containing unbridged C═N bonds are usually non-fluorescent. However, when metal cations were added to the group, the C═N isomerization may be inhibited, thereby leading to strong fluorescence emission. Following this strategy, a number of excellent C═N isomerization-based probes have been exploited for sensing various metal cations (*e.g.* Zn^{2+}, Cd^{2+}, Cu^{2+}, Mg^{2+}) as well as ClO^-, HSO_3^-, Cys and Hcy.[6] Herein we reported a Fe^{3+}-amplified fluorescent probe based on C═N isomerization. The structure of probe **L** was confirmed by X-ray crystallography, ^1H NMR, ^{13}C NMR and MS data. The details of the synthesis are described in the ESI.† This probe exhibits a fluorescence turn-on signal toward Fe^{3+} in CH_3CN–H_2O buffer solution with high sensitivity and selectivity compared with other metal ions examined.

The single-crystal of compound **L** was obtained from acetonitrile solution by slow evaporation at ambient temperature. The crystal structure with atom-numbering is shown in Fig. 1. The chromone-ring and the N2, N3/C11–C13 ring are nearly coplanar with a dihedral angle of 4.3 (2)°, the semirigid structure with the potential binding sites (O2, N1, O3) may create suitable space to better match the corresponding ions.

The UV-vis titration of the Fe^{3+} was conducted by using 20 μM of **L** in CH_3CN–water (1/9, v/v). As shown in Fig. S1 (ESI†), free **L** reveals three absorption bands at 238, 284 and 334 nm (molar extinction coefficient $\varepsilon_{238} = 3.7 \times 10^4$ M^{-1} cm^{-1}, $\varepsilon_{284} = 3.1 \times 10^4$ M^{-1} cm^{-1} and $\varepsilon_{334} = 3.3 \times 10^4$ M^{-1} cm^{-1}), upon addition of Fe^{3+}, the band at 334 nm gradually weakens and a new band appears with an isosbestic point at 372 nm, indicating formation of a new complex between **L** and Fe^{3+}.

The acid titration control experiments revealed that no significant fluorescence response of **L** was observed in the pH range from 6.0 to 10.0, suggesting that **L** was insensitive to the pH under neutral or alkali conditions (Fig. S2, ESI†). Thus, the probe was expected to work well under physiological conditions. Simultaneously, considering the hydrolysis property of Fe^{3+}, pH 7.0 was chosen as the optimal test condition.[7]

The fluorescence titration of the probe (20 μM) was examined in acetonitrile and acetate buffer solution (v/v = 1/9, 10 mM, pH = 7.0). As shown in Fig. 2, obviously, the free **L** is non-fluorescent because the C═N isomerization is the predominant decay process in the excited states[5] but the fluorescence intensity

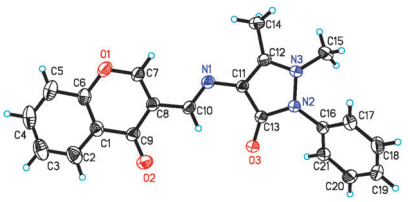

Fig. 1 View of the structure of **L** with displacement atomic ellipsoids drawn at the 30% probability level.

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† Electronic supplementary information (ESI) available: Synthesis, characterization and spectroscopic and binding studies of compounds **L** and **L**–Fe^{3+}. CCDC 894138 for **L**. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2nj40737a

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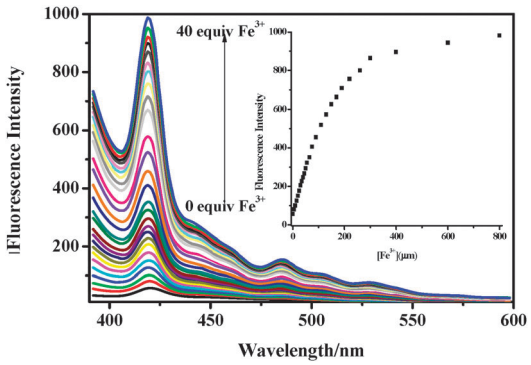

Fig. 2 Fluorescence titration spectra of **L** (20 μM) in CH_3CN–water (1/9, v/v, pH = 7.0) solution upon the addition of Fe^{3+}, from bottom to top: 0–40 equiv. Fe^{3+} (λ_{ex} = 372 nm). Inset: changes in fluorescence intensity at 420 nm against concentration of Fe^{3+}.

(λ_{em} = 420 nm) increases significantly upon addition of Fe^{3+}. Upon increasing the concentration of Fe^{3+} up to 0.8 mM (40 equivalents), the solution showed an impressive fluorescence enhancement with a quantum yield of 0.32 (quinine sulfate in 0.5 M H_2SO_4 as a standard, Φ_f = 0.55) and an approximately 16-fold enhancement in the fluorescence intensity at 420 nm. These spectral properties indicate that its complexation with Fe^{3+} ions restricts the rotation of C=N bonds and results in the suppression of C=N isomerization so that its fluorescence is drastically increased. In order to obtain insight into the C=N isomerization-based decay process, the fluorescence lifetime (τ) was measured by single photon counting and it shows a good single-exponential decay. The lifetime of free **L** is 0.69 ns and the radiative decay rate constant (k_r) and nonradiative decay rate constant (k_{nr}) are calculated to be about 2.9×10^7 s^{-1} and 1.4×10^9 s^{-1}, indicating that the nonradiative decay is the predominant process in the excited states of **L**. When Fe^{3+} was added, the lifetime of **L** increases to 0.88 ns, which was longer than that of free **L**, and the radiative and nonradiative decay rate constants changed to 3.6×10^8 s^{-1} and 7.7×10^8 s^{-1}, respectively; both radiative and nonradiative decay processes became comparative so that the fluorescence was increased.[6h]

The stoichiometry of the **L**–Fe^{3+} complex was determined using Job's plot, which indicated the formation of a 1 : 1 complex (Fig. S3, ESI†). By nonlinear least-squares fitting of fluorometric titration data based on the 1 : 1 binding model, the association constant was calculated to be 1.07×10^4 M^{-1}.[8] To further explore the complexation model, IR spectrometry was used to investigate the free ligand **L** and its Fe^{3+}-complex (Fig. S4, ESI†). One can observe a shift of the stretch vibration absorption of the C=N band from 1631 cm^{-1} (free **L**) to 1616 cm^{-1} (**L**–Fe^{3+}), and the stretch vibration absorption of the carbonyl group of **L** (1661 cm^{-1}) almost disappeared in the Fe^{3+}-complex. So these data indicate that all the carbonyl groups and the C=N bond took part in coordination with Fe^{3+}.

We further investigated the ability of the probe to quantify Fe^{3+} in water solution. Fig. 3 demonstrates that there is a good linear dependence of fluorescence intensity on Fe^{3+} concentration (0–90 μM), and the regression equation is F_{420nm} = 62.0302 + 4.5389 [Fe^{3+}] (μM), with R^2 = 0.9965.

Ion selectivity study was performed in acetonitrile and acetate buffer solution (v/v = 1/9, 10 mM, pH = 7.0). From

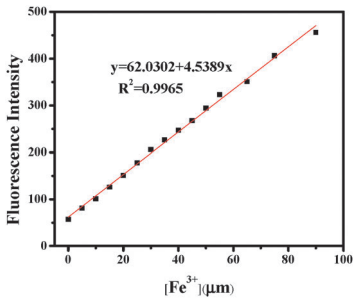

Fig. 3 Plot of emission intensities *vs.* [Fe^{3+}] ([**L**] = 20 μM, [Fe^{3+}] = 0–90 μM).

a physiological application point of view, the interference from other metal ions should be taken into account. Hence, the fluorescence change in the **L**–Fe^{3+} ion reaction in the presence of other competitive metal ions was investigated as illustrated in Fig. 4. No change in fluorescence emission was observed after adding Na^+ and K^+ (both in 100 mM), and the data also show that Co^{2+}, Cd^{2+}, Mn^{2+}, Hg^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Ba^{2+}, Sr^{2+}, Pb^{2+}, Li^+ and Ag^+(all in 0.8 mM) caused

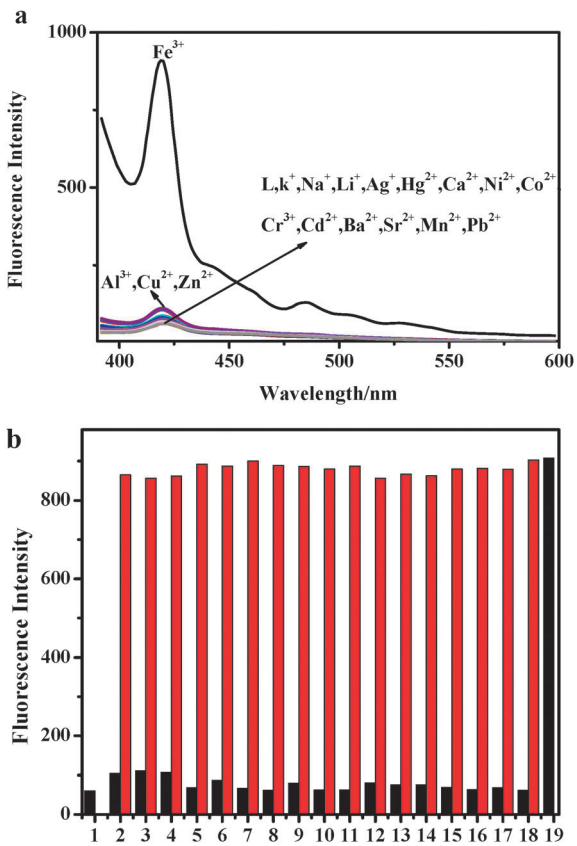

Fig. 4 (a) Fluorescence spectra of **L** (20 μM) upon the addition of various metal ions in CH_3CN–water (1/9, v/v, pH = 7.0) solution with an excitation at 372 nm. (b) Metal ions selectivity of **L** (20 μM) (λ_{ex} = 372 nm, λ_{em} = 420 nm). The black bars represent the fluorescence emission of **L** and various metal ions. The red bars represent the fluorescence changes that occur upon addition of 40 equiv. Fe^{3+} to the solution containing **L** and other metal ions. 1, blank; 2, Al^{3+}; 3, Cu^{2+}; 4, Zn^{2+}; 5, Mg^{2+}; 6, Cr^{3+}; 7, Ca^{2+}; 8, Ba^{2+}; 9, Pb^{2+}; 10, Hg^{2+}; 11, Co^{2+}; 12, Ni^{2+}; 13, Mn^{2+}; 14, Cd^{2+}; 15, Ag^+; 16, Li^+ (above ions were all 0.8 mM); 17, K^+ (100 mM); 18, Na^+ (100 mM); 19, Fe^{3+}.

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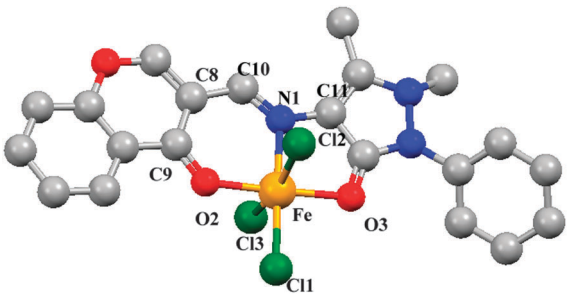

Fig. 5 B3LYP/6-31G* optimized structure for the **L**–Fe^{3+} complexes.

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negligible response to the fluorescence of **L**. Of all the tested metal ions, only the addition of Cu^{2+}, Zn^{2+} and Al^{3+} (all in 0.8 mM) gave a limited increase in fluorescence intensity. The results indicate that **L** can potentially be used to detect Fe^{3+} in environmental and biological systems, even in the presence of other competitive pollutant cations.

The photophysical properties and IR spectrometry revealed that a 1 : 1 complex was formed between **L** (binding sites O2, N1, O3) and Fe^{3+}. More direct evidence was obtained from the MALDI-TOF mass spectrum of the system **L**–Fe^{3+} (Fig. S5, ESI†), and its isotopic distribution pattern suggests the existence of a [**L**–Fe^{3+}] species in the gaseous state. To further verify the configuration of $FeLCl_3$, we carried out the density functional theory (DFT) calculations with B3LYP by using the Gaussian03 package.[9] The 6-31G* basis sets were used for the H, C, N, O and Cl atoms, the exception was for the Fe atom, where the LANL2DZ effective core potential (ECP) was employed. The optimized configuration is shown in Fig. 5, which shows that the Fe is six-coordinated with an octahedral geometry. The tridentate ONO planar ligand **L** coordinates the iron(III) center in a meridional fashion. The three monodentate chloride ligands occupy the remaining meridional sites. The Fe–O2, Fe–O3 bond lengths are 1.942 Å and 1.996 Å, and the Fe–N1 bond length is 2.049 Å. The Fe–Cl2 (axial) and Fe–Cl3 (axial) bond lengths are 2.357(1) and 2.362(1) Å longer than the Fe–Cl1 (equatorial) distance (2.254 Å) which is similar in reported $FeCl_3$ complexes.[10] These data indicate that **L** could provide suitable space to better accommodate iron(III).

In conclusion, we have developed a new type of "turn-on" fluorescent sensor **L** based on the chromone fluorophore. It shows high sensitivity and selectivity toward Fe^{3+} in aqueous solution. When **L** bound with Fe^{3+}, the C=N isomerization was eliminated, thus, it induced an enhancement in the fluorescence intensity of **L**. The present work also provides a novel concept for design of Fe^{3+} fluorescent probes. The complexation mode and the corresponding enhancement mechanism were elucidated by IR spectrometry, transient spectra, Job plot and DFT calculations.

Experimental section

Materials and instruments

All the materials and solvents were purchased from commercial suppliers and used without further purification. The solution of metal ions was prepared from chloride salts of Ni^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, Hg^{2+}, Na^{+}, Ca^{2+}, Zn^{2+}, Mg^{2+}, Pb^{2+}, K^{+}, Co^{2+}, Li^{+}, Cd^{2+}, Cr^{3+}, Al^{3+}, Sr^{2+}, Ba^{2+}, and nitrate salts of Ag^{+}. Stock solutions of metal ions (20 mM) were prepared in deionized water.

A stock solution of **L** (200 μM) was prepared in CH_3CN. In selectivity experiments, the test samples were prepared by adding appropriate amount of metal ion stock into 3 ml solution of **L** (20 μM). Absorbance spectra were measured using a Purkinje general UV-1901 spectrophotometer. Fluorescence spectra measurements were performed on a Cary Eclipse fluorescence spectrophotometer. ^{1}H NMR, ^{13}C NMR spectra were obtained on a Bruker Avance 400 MHz NMR spectrometer with tetramethylsilane (TMS) as internal standard. X-ray data were collected on Bruker Smart APEX II CCD diffractometer with graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation.

Synthesis of L

A solution of chromone-3-carboxaldehyde (0.34 g, 2 mmol) and 4-aminoantipyrine (0.40 g, 2 mmol) in 30 ml ethanol was stirred at 50 °C for 2 h. After completion of the reaction, the obtained yellow precipitate was filtered and washed several times with cold ethanol. After drying under reduced pressure, the reaction afforded 0.58 g (79%) a yellow solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.67 (s, 1H), 8.92 (s, 1H), 8.14 (d, J = 8 Hz, 1H), 7.85 (t, J = 7.2 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.52–7.56 (m, 4H), 7.39 (t, J = 6 Hz, 4H), 3.19 (s, 3H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, d-$CDCl_3$): δ (ppm) 175.83, 160.52, 156.18, 154.68, 151.71, 150.47, 134.79, 133.65, 129.19, 126.29, 125.49, 124.65, 124.36, 122.18, 118.91, 118.22, 35.87, 10.18. MALDI-TOF MS: ([M + H]$^+$), 360.204.

Crystal structure determination of L

A crystal (0.2 × 0.2 × 0.1 mm) was mounted on a Bruker Smart APEX II CCD equipped with graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation. T_{max} = 0.991 and T_{min} = 0.982. The relevant crystal data and structural parameters are: f_w = 359.38; space group $R\bar{3}$ is trigonal; a = b = 34.465(13) Å, c = 7.825(3) Å, α = β = 90°, γ = 120°; V = 8050(5) (Å^3); Z = 18; ρ = 1.334 g cm^{-3}; μ(Mo Kα) = 0.091 cm^{-1}, the intensities were collected at 296(2) K. 13 438 reflections were measured and after merging (R_{int} = 0.0215), some 3152 reflections (of which 2409 were labelled as observed) were used in the refinement. The final R_1 ($R_1 = \sum||F_o| - |F_c||/\sum|F_o|$) value was 0.0415, wR_2 ($[\sum(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$) value was 0.1007.

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