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ORIGINAL ARTICLE

A New Schiff Base Chemodosimeter for Fluorescent Imaging of Ferric Ions in Living Cells

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Abstract A new and efficient chemodosimeter for ferric ions has been developed. The visual and fluorescent behaviors of the compound toward various metal ions were investigated: ferric ions are distinguished from other cations by selective color change and unusual fluorescence enhancement in mixed aqueous solution. Fluorescence microscopy experiments showed that this receptor is effective for detection of Fe^{3+} in vitro, developing a good image of the biological organelles. The sensing mechanism is shown to involve a hydrolysis process.

Keywords Cell imaging · Schiff base · Chemodosimeter · Fluorescence microscopy · Ferric ion detection

Introduction

Colorimetric and fluorescent sensing of metal ions by small organic molecules has received great attention in the last couple decades due to the vital roles played by these ions in biological and environmental contexts.[1–5] Iron is considered one of the most important trace elements in the body and an essential element in most biological systems.[6] It plays crucial roles in biological, environmental, and chemical contexts.[7, 8] There is increasing evidence that either its deficiency or overload can induce various disorders. Research shows that Fe^{3+} is implicated in the mechanisms of neurodegenerative diseases including Alzheimer's and Parkinson's disease.[9, 10] Therefore, the detection of trace amounts of Fe^{3+} ions is of critical interest. A quick glance at the literature reveals numerous works focusing on selective and sensitive detection of transition metal ions such as Cu^{2+}, Zn^{2+}, Pb^{2+}, Fe^{3+} and Hg^{2+} have been reported,[11–16] highlighting the importance and interest in this area of research. However, due to the paramagnetic nature of Fe^{3+}, its presence is most often signaled by fluorescence quenching,[17–19] which may limit the application of probes.[20] Thus, developing a turn-on fluorescent probe for Fe^{3+} has drawn increasing attention in recent years. For example, Ghosh et al. reported two Schiff base probes for Fe^{3+} based on traditional PET and ICT mechanisms.[21] Amplification of the fluorescence signal via chemodosimetric reaction is another potential route of interest to enable this counterintuitive fluorescence turn-on effect by Fe^{3+}.[22, 23] Earlier work in our laboratories established the design and synthesis of new receptors [24, 25] for selective sensing of various metal ions and anions. Accordingly, we recently reported a new coumarin based Schiff base chemosensor [26, 27] for Mg^{2+}, Fe^{3+} and a chemodosimeter [28] for HSO_4^- based on hydrolysis of Schiff base. Herein, we describe the preparation of a previously unreported compound, **Tz-1**, and its application as chemodosimeter, incorporating pyrene as a main fluorogenic element linked with 2-quinolinecarboxaldehyde via a hydrolysable imine functionality as shown in Scheme 1. Its affinity toward the imine framework and strong Lewis acid activity facilitate hydrolysis of the Schiff base **Tz-1** by Fe^{3+} ions over other metal cations.

Results and Discussion

The compound **Tz-1**, (*E*)-N-(pyren-1-yl)-1-(quinolin-2-yl) methanimine, was prepared by condensation of 1-

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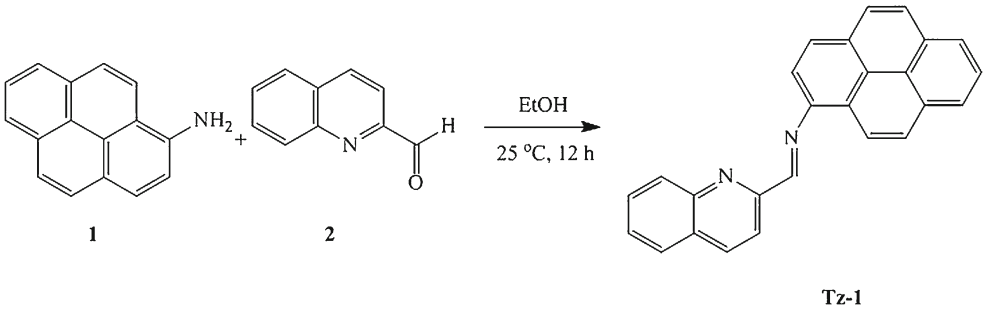
Scheme 1 Synthesis of chemodosimeter **Tz-1**



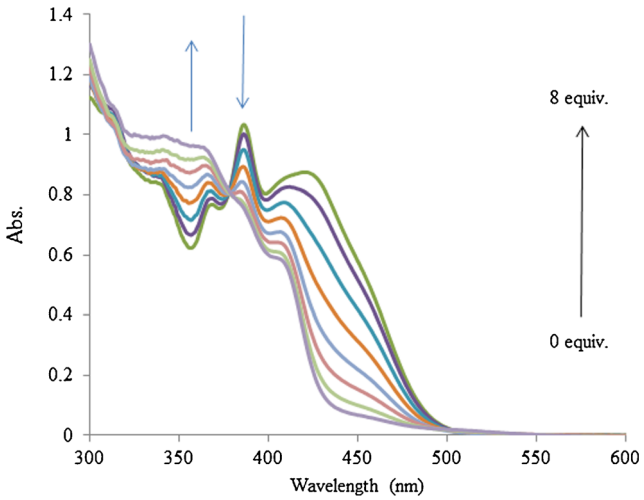


Fig. 1 Family of spectra taken in the course of the titration of **Tz-1** (5.0×10^{-5} M) in DMSO/H_2O (v/v=70:30) with a standard solution of Fe^{+3} at 25 °C

aminopyrene (1) with 2-quinolinecarboxaldehyde (2) in moderate yield (Scheme 1). This compound was characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

UV–vis Spectroscopic Studies

To examine the feasibility of **Tz-1** as a chemodosimeter for Fe^{3+}, we first investigated its chromogenic character in the absence and presence of Fe^{3+} ions. The compound **Tz-1** was stable in a solvent mixture of DMSO/H_2O (v/v=70:30). In this solvent system, **Tz-1** showed characteristic strong absorption peaks at 386 and 425 nm. Upon treatment with Fe^{3+} ions, the absorption at these two peaks decreased markedly, with some increase in absorption at lower wavelengths and a clear isosbestic point observed at 379 nm (Fig. 1). The color of the solution changed from yellow to colorless (Fig. 2b).

An important feature of sensing molecules such as chemodosimeters are their high selectivity toward the analyte

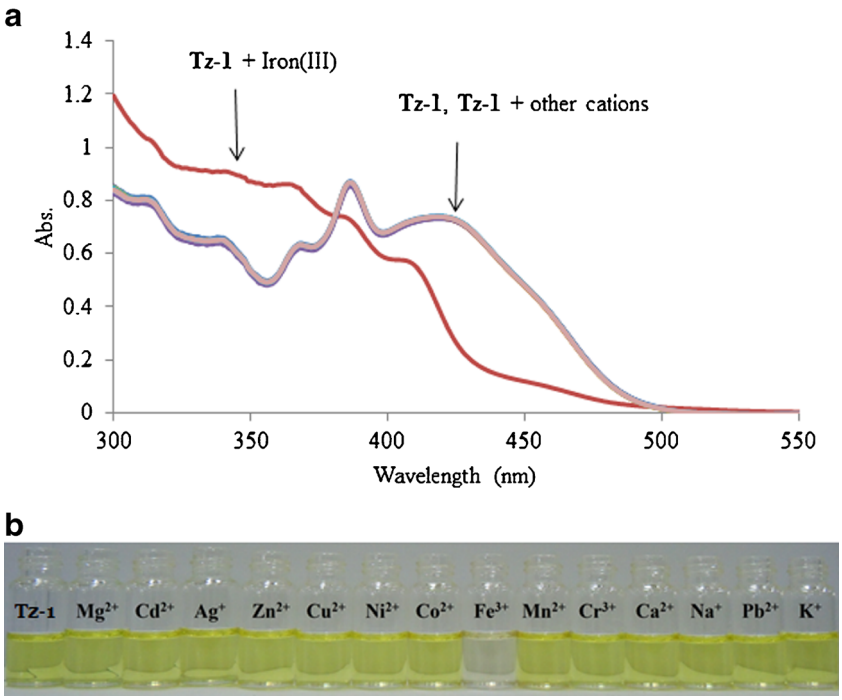


Fig. 2 **a** UV–Vis spectra of **Tz-1** (5.0×10^{-5} M) in DMSO/H_2O (v/v=70:30) in the presence of 8 equiv. of Fe^{3+} ion and other cations including Mg^{2+}, Cd^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Ca^{2+}, Na^{+}, Pb^{2+}, and K^{+}, respectively. **b** Color changes of **Tz-1** (5.0×10^{-5} M) upon addition of 8.0 eq. of various cations in DMSO/H_2O (v/v=70:30)

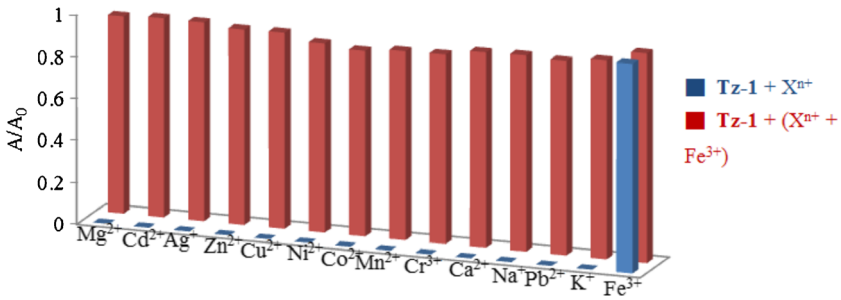


Fig. 3 UV–vis absorption response of **Tz-1** (5.0×10^{-5} M) to various cations (8 equiv.) in DMSO/H_2O (v/v=70:30). Bars represent the intensity ratios of the absorption at 356 nm and were calculated according to: $A=A_{Host+Xn+} - A_{Host}$ and $A_0=A_{Host+Fe(III)} - A_{Host}$

over other competitive species. Variations of UV–vis spectral and visual color changes of **Tz-1** in DMSO/H_2O (v/v=70:30) solutions caused by miscellaneous cations (in the form of perchlorate salts) including Mg^{2+}, Cd^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, Cr^{3+}, Ca^{2+}, Na^{+}, Pb^{2+}, and K^{+} were recorded in Fig. 2. Among the miscellaneous competitive cations, Fe^{3+} produced the most obvious change in absorption. Other cations showed no significant change in absorption, and did not interfere with the signal induced by Fe^{3+} (Fig. 3).

The colorimetric detection limit of **Tz-1** was calculated [29–31] from UV–vis titration data (Fig. 4a) on the basis of 3σ/K to be 3.22×10^{-5} M. (Detection limit is taken to be 3σ/K, where σ refers to the standard deviation of the blank solutions and K refers to the slope of the linear regression curve.)

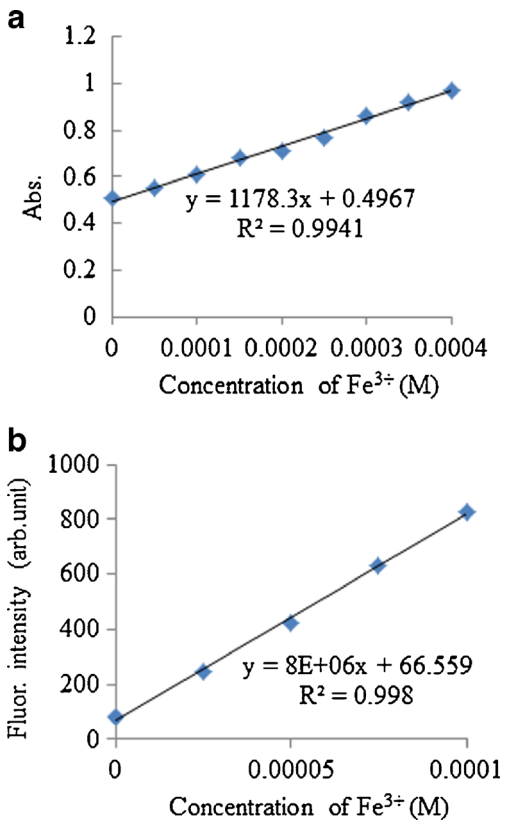


Fig 4 **a** Absorbance of **Tz-1** versus Fe^{3+} concentrations. [**Tz-1**]=5.0×10^{-5} M. **b** Fluorescence intensity of **Tz-1** versus Fe^{3+} concentrations. [**Tz-1**]=5.0×10^{-6} M

Fluorescence Studies

To further examine the usefulness of **Tz-1** as a chemodosimeter for Fe^{3+} detection, we investigated its fluorescence properties. In DMSO/H_2O (v/v=70:30), **Tz-1** showed a moderate fluorescence emission at 440 nm. Titration by Fe^{3+} (Fig. 5) led to a marked increase in this emission peak, yielding a signal quite obvious instrumentally or by naked eye observation under UV lamp (Fig. 6) The color of fluorescence emission changed from blue to Olympic blue (Fig. 6b). No obvious spectral changes could be observed when other cations like Mg^{2+}, Cd^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Ca^{2+}, Na^{+}, Pb^{2+}, and K^{+} were added, respectively (Fig. 6a). Moreover, in the presence of miscellaneous competitive cations, similar fluorescence changes were recorded, indicating that interference problems for this chemodosimeter are minimal (Fig. 7). Altogether, these results indicate that the selectivity of **Tz-1** for Fe^{3+} ions over other competitive cations in the aqueous medium is remarkably high.

The fluorescent detection limit of **Tz-1** was calculated [29–31] from fluorescence titration data (Fig. 4b) on the basis of 3σ/K to be 1.37×10^{-6} M.

Live Cell Imaging

The application of receptor **Tz-1** in live cell imaging of RAW264.7 cells was investigated. The images of cells were obtained using a Nikon fluorescence microscope. When RAW 264.7 cells were incubated with **Tz-1** (20 μM), no fluorescence was observed (Fig. 8). After treatment with Fe^{3+}, bright blue fluorescence was observed in the RAW264.7 cells (Fig. 8). An overlay of fluorescence and bright-field images shows that the fluorescence signals are localized in the intracellular area, indicating a subcellular distribution of Fe^{3+} and good cell-membrane permeability of **Tz-1**.

Mechanistic Studies

The distinct color change and large fluorescence enhancement of receptor **Tz-1** is proposed to involve coordination of Fe^{3+} to the Schiff base functionality, followed by hydrolytic cleavage

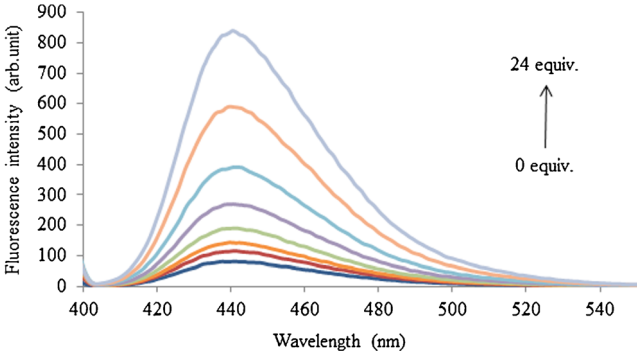


Fig 5 Fluorescence emission spectra of **Tz-1** (5.0×10^{-6} M) in the presence of different equivalents of Fe^{3+} in DMSO/H_2O (v/v=70:30), λ_{ex}=396 nm and λ_{em}=440 nm

of the imine linkage to generate 1-aminopyrene (1) and 2-quinolinecarboxaldehyde (2) as depicted in Scheme 2. Catalytic ion-induced hydrolysis reactions are known to be the operating principle of other reported Schiff base chemodosimeters. To prove the above process chemodosimetric reaction was carried out with **Tz-1** and Fe^{3+}. The hydrolysis products, 1-aminopyrene (1) and 2-quinolinecarboxaldehyde (2) were separated, isolated and confirmed by ^{1}H NMR spectroscopy and comparison with authentic compounds. In addition, gradual ^{1}H NMR titration of the host was carried out, showing steady formation of the aldehyde peak of **2** (at δ=10.12 ppm) on addition of increasing amounts of Fe^{3+} (Fig. 9).

Experimental

General Method

In titration experiments, all the cations in the form of perchlorate salts were purchased from Sigma-Aldrich, USA and stored in a vacuum desiccator. All chemicals were of analytical grade and used as received with the exception of DMSO, which was distilled over calcium hydride.

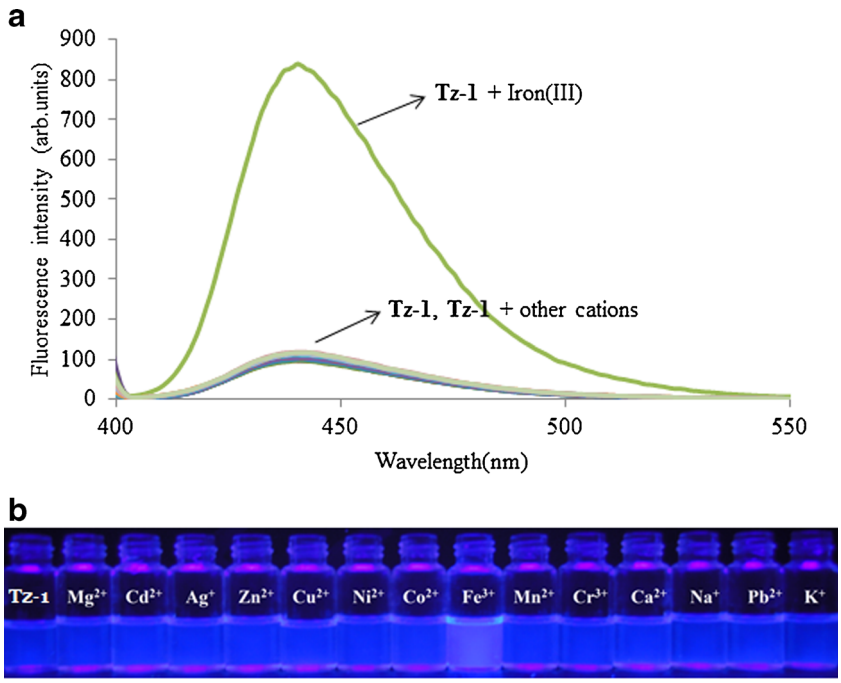


Fig. 6 **a** Fluorescence emission spectra of **Tz-1** (5.0×10^{-6} M) in presence of various cations (24 equiv.) in DMSO/H_2O (v/v=70:30). **b** Fluorescence changes of **Tz-1** (5.0×10^{-6} M) in presence of various cations (24 equiv.) in DMSO/H_2O (v/v=70:30) as observed under UV lamp

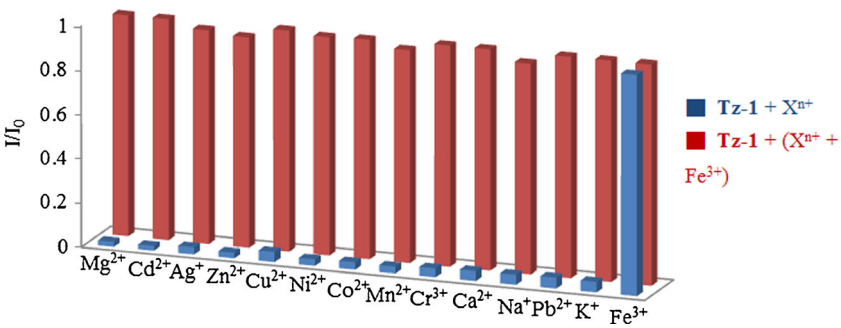


Fig. 7 Fluorescence response of **Tz-1** (5.0×10^{-6} M) to various cations (24 equiv.) in DMSO/H_2O (v/v=70:30). Bars represent the intensity ratio of the emission at 440 nm and were calculated according to: $I=I_{Host+Xn+} - I_{Host}$ and $I_0=I_{Host+Fe(III)} - I_{Host}$

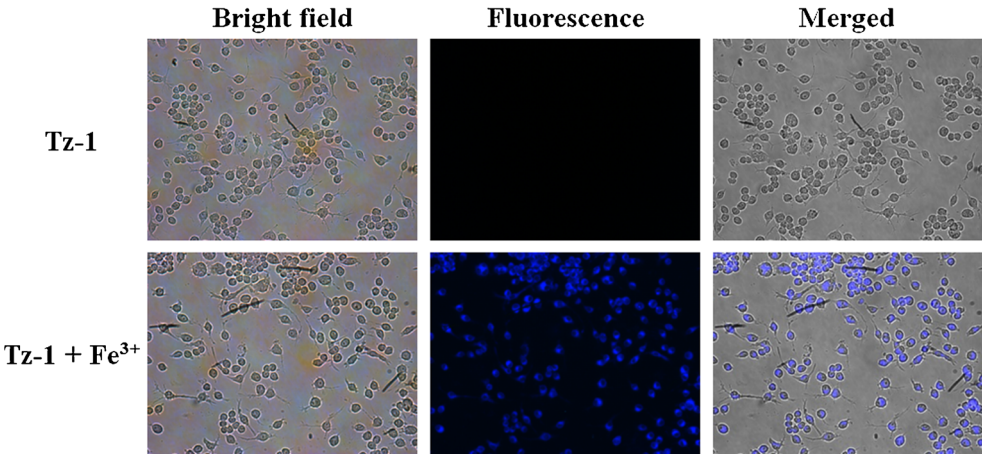
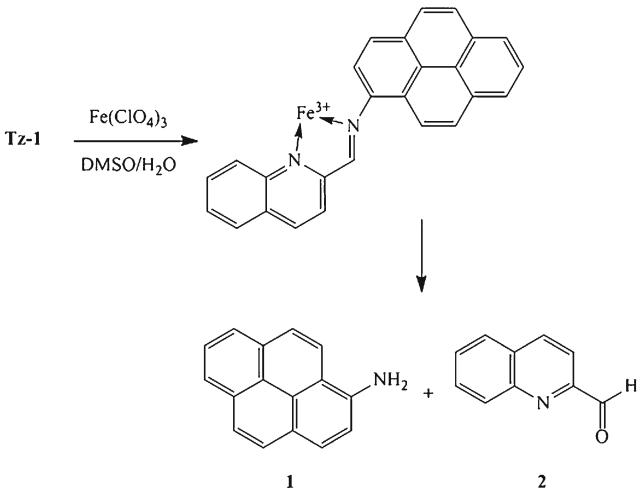


Fig. 8 Fluorescence images of living RAW264.7 cells treated with **Tz-1** and Fe^{3+}. (*Left*) bright field image; (*center*) fluorescence image; and (*right*) merged image



Scheme 2 The proposed mechanism for sensing of Fe^{3+} by **Tz-1**

Melting points were determined on a Fargo MP-2D melting point apparatus in open capillaries and are uncorrected. ^{1}H and ^{13}C NMR were recorded at 400 and 100 MHz on a Bruker spectrometer using trimethylsilane (TMS) as an internal standard. UV–Vis spectra were performed in a 1 cm path length quartz cell using a Cary 300 UV–vis spectrophotometer. Fluorescence spectra were measured with a Perkin Elmer LS-50B.

The mouse macrophage-like cell line RAW264.7 cells were purchased from the Bioresource Collection and Research Centre (FIRDI, Hsinchu, Taiwan). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10 % heat-inactivated fetal bovine serum, 100 units/mL of penicillin and 100 μg/mL of streptomycin and incubated at 37 °C in a humidified atmosphere with 5 %

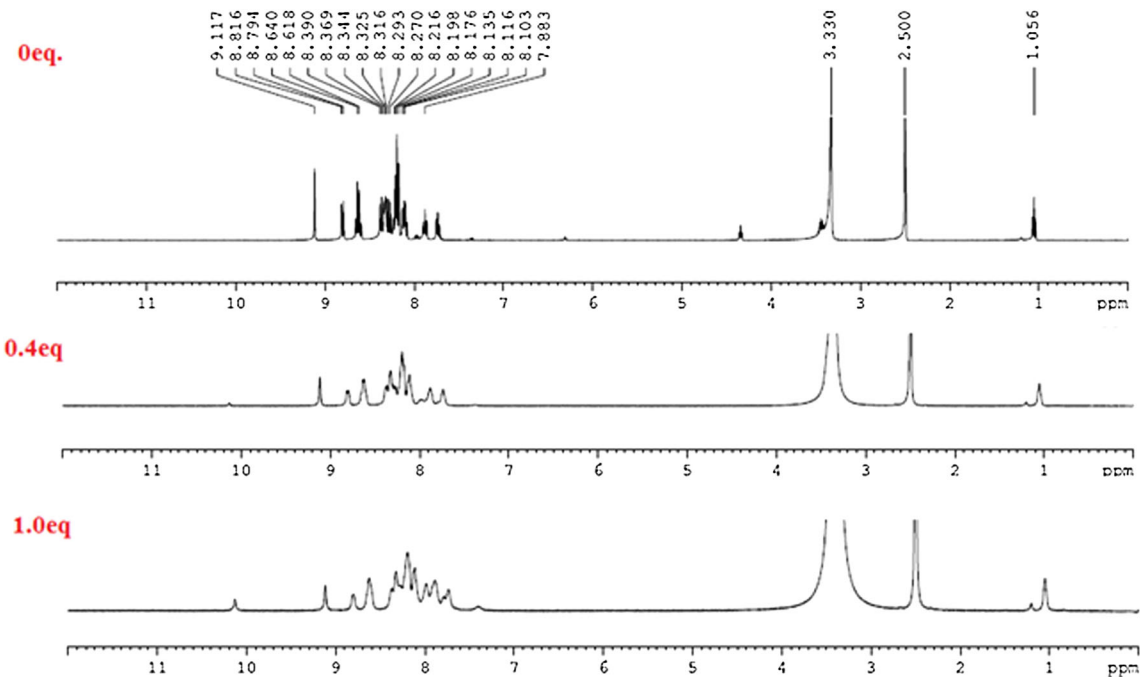


Fig. 9 ^{1}H NMR spectra showing the gradual titration of **Tz-1** with different equivalents of Fe^{3+}

CO_2. The RAW264.7 cells were seeded on glass slides and allowed to adhere for 24 h.

Compound **Tz-1** or iron (III) perchlorate were dissolved into a 1.0×10^{-2} M stock in sterile DMSO and water mixed at a ratio of 70:30. To examine the fluorescence activity of **Tz-1** in live cells, the RAW264.7 cells were seeded on glass slides and allowed to adhere for 48 h. The cells were treated with DMEM containing 2.0×10^{-5} M iron (III) perchlorate for 30 min and then washed with PBS (phosphate buffered saline) three times. Following washing the cells were loaded with 2.0×10^{-5} M **Tz-1** in DMEM for another 30 min and washed again with PBS three times to remove the remaining sensor. Fluorescence or bright-field microscopy was done with a Nikon (Düsseldorf, Germany) Eclipse 50i microscope. For epifluorescence microscopy, a UV2A filter block (EX 330 to 380, DM 400, and BA 420; Nikon, Düsseldorf, Germany) was used.

Synthesis

Synthesis of (*E*)-N-(pyren-1-yl)-1-(quinolin-2-yl) methanimine (**Tz-1**). To a stirred solution of 1-aminopyrene (1) (217 mg, 1.0 mmol) in ethanol (20 mL), 2-quinolinecarboxaldehyde (2) (173 mg, 1.1 mmol) was added at room temperature. The reaction mixture was stirred for 12 h and the solid residue was filtered off. The resulting precipitate was collected and the crude product was further washed with ethanol several times to afford the pure product **Tz-1**. Yield: 225 mg, (63 %). mp: 146–148 °C.

^{1}H NMR (DMSO-d_6, 400 MHz) δ : 7.72 (t, 1H, *J*=6.8 Hz), 7.86 (t, 1H, *J*=6.8 Hz), 8.08 (m, 2H), 8.17–8.24 (m, 4H), 8.27 (d, 1H, *J*=9.2 Hz), 8.31 (q, 2H), 8.37 (d, 1H, *J*=8.4 Hz), 8.60 (m, 2H), 8.79 (d, 1H, *J*=9.2 Hz), 9.12 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ : 116.3, 119.1, 123.3, 124.4, 124.9, 125.8, 125.9, 126.0, 126.5, 127.1, 127.7, 127.8, 128.0, 128.5, 128.7, 129.1, 129.7, 130.5, 130.8, 131.3, 131.5, 137.6, 143.7, 148.0, 155.2, 161.7. HRMS (EI) m/z=356.1321 [M^+], calcd for $C_{26}H_{16}N_2$=356.1313.

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