A selective N,N-dithenoyl-rhodamine based fluorescent probe for Fe\(^{3+}\) detection in aqueous and living cells

Yi Liu\(^3,\)**, Cuixia Zhao \(^1,**\), Xiangyun Zhao \(^3\), Huili Liu \(^4\), Yibin Wang \(^2\), Yuguo Du \(^1,*\), Dongbin Wei \(^1,*\)

1 State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
2 Key Laboratory of Marine Ecology and Environmental Science and Engineering, First Institute of Oceanography, Ministry of Natural Resources (China), Qingdao 266061, China
3 School of Chemistry and Chemical Engineering, Yantai University, Yantai 264005, China
4 Key Laboratory of Molecular Pharmacology and Drug Evaluation, Ministry of Education, Yantai University, Yantai 264005, China

**Corresponding authors.**
E-mail addresses: liuyi@ytu.edu.cn (Y. Liu), duyuguo@rcees.ac.cn (Y. Du), weidb@rcees.ac.cn (D. Wei).
*The authors contributed equally.
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**Article info**

**ABSTRACT**

A novel N,N-dithenoyl-rhodamine based fluorescent and colorimetric Fe\(^{3+}\) probe 1 was designed and synthesized by only one step from Rhodamine B hydrazide and 2-thiophenecarbonyl chloride. The structure of probe 1 was characterized by \(^1\)H NMR/\(^{13}\)C NMR spectroscopy, IR spectroscopy, and HRMS spectrometry. Accompanying with significant changes in visual color and fluorescent spectrum, probe 1 displayed good sensitivity for Fe\(^{3+}\) with a broad pH span. The detection limit (3.76 \(\mu\)mol/L, 0.2 mg/L) for Fe\(^{3+}\) was lower than WHO recommended value (0.3 mg/L) for drinking water. Using two thiophene carbonyl groups as coordinating functional recognition group, probe 1 showed excellent selectivity towards Fe\(^{3+}\) over diverse coexistent metal ions and anions. The sensing mechanism between dithenoyl-substituted probe 1 and Fe\(^{3+}\) was further confirmed by \(^1\)H NMR and IR titration experiments, binding constants study, and Job’s plot analysis. Furthermore, probe 1 also exhibited good cell membrane permeability and could be used as an efficient Fe\(^{3+}\) probe in living human cells.

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**Introduction**

Besides being the 4th most plentiful element (about 150 mg/kg) in the earth crust, iron is widely abundant in almost all human tissues, and organs, and play essential roles as co-factors participated in various chemical and biological life processes (Ragsdale, 2006; Wu et al., 2019). In our body, most of iron were mainly as Fe\(^{3+}\) form storage in hemoglobin, ferritin, and hemosiderin (Moll and Davis, 2017). Fe\(^{3+}\) was...
always regarded as an essential component of many necessary enzymes which involves in various life activities, such as cell metabolism, DNA or protein synthesis, regulate osmotic pressure, maintain intracellular pH balance, etc. (Hentze et al., 2010; Murugan et al., 2018; Rouault, 2006). However, superfluous ingestion of iron will be harmful to our health, which may not only increase the disease risks of osteoporosis, hemochromatosis, diabetes, and other diseases, but also can cause various organ dysfunctions (Li et al., 2013). Recently, some reports had revealed that excess iron storage in liver could generate noxious hydroxyl radical (HO·) via Fenton and Haber-Weiss reaction (Pietragello, 2016). Subsequently, the hydroxyl radical can cause persistent injury to DNA, enzymes as well as cell membranes and finally result in serious liver fibrosis, long-term micronodular cirrhosis, and even hepatocellular carcinoma (Pietragello, 1996; Sumner and Kopelman, 2005; Pietragello, 2016). Conversely, iron deficiency can also cause many serious diseases, such as parkinson’s disease, metabolic process abnormality or organ growth retardation, etc. (Meng et al., 2018; Pippard, 2011).

To date, many kinds of techniques such as inductively coupled plasma mass spectrometry, potentiometric membrane sensing and flame atomic absorption spectrometry had been developed for the determination of Fe$^{3+}$ (Liu et al., 2016). But these methods are usually not suitable for in situ detection of Fe$^{3+}$ in biological systems and require expensive instruments. Hence, due to its low cost, high sensitivity, quick response, more straightforward operation and, in situ measurement, fluorescent probe has become a promising strategy for Fe$^{3+}$ detection (Carter et al., 2014; Sahoo et al., 2012).

In the last decades, numerous studies on Fe$^{3+}$ ions determination based on fluorescent probes have been reported (Lan et al., 2017; Liu and Qian, 2017; Shen and Qian, 2018; Upadhyay et al., 2018; Wei et al., 2011; Yang et al., 2012; Zhou et al., 2017). However, due to the paramagnetic nature of Fe$^{3+}$, many reported probes underwent a fluorescence quenching mechanism which was difficult to be utilized as in-situ monitoring and in-vivo imaging (Cao et al., 2019; Yang et al., 2012). Thus, researchers paid increasing amount attentions to develop new ‘turn-on’ fluorescent probes for Fe$^{3+}$ detection. Up to now, multiple fluorescent materials such as naphthalimide, pyrene, fluorescein, quinazolinone, rhodamine or BODIPY have been used in the development of Fe$^{3+}$ ion probes (Lan et al., 2017; Liu and Qian, 2017; Shen and Qian, 2018; Upadhyay et al., 2018; Wei et al., 2011; Zhou et al., 2017). Among them, rhodamine dyes have been well-recognized for their remarkable fluorescence and physical properties and have been regarded as ideal probe precursors (Chen et al., 2012). However, one handicap for rhodamine-based Fe$^{3+}$ probes is that they were easily interfered by other transition metal ions (e.g., Hg$^{2+}$, Cu$^{2+}$, Al$^{3+}$, etc.), resulting in poor selectivity (Gupta et al., 2016; Jin et al., 2017; Rathinam et al., 2013; Wang et al., 2017). Therefore, it is still necessary to develop new architecture and highly selective ‘turn-on’ Fe$^{3+}$ ion fluorescent probe.

Thiophene heterocycles were usually represented as the key ion recognition group in fluorescence chemosensor designing (Mandal et al., 2013; Park et al., 2013; Wang et al., 2017; Xia et al., 2018; Yang et al., 2016, 2018). Some studies revealed that thiophene rings could be more strongly adsorbed onto the surface of the transition-metal ions due to its higher electron densities than other heterocycles (furan or pyrrole) (Camacho-Mendoza et al., 2014). Hence, multiple thiophene-rhodamine based probes had been reported and successfully applied to different cations/anions detection (Mandal et al., 2013; Wang et al., 2017; Xia et al., 2018; Yang et al., 2016, 2018). However, almost all of them contained only one thiophene group, except for one mercury ion probe (Park et al., 2013). We naturally envisaged that if we introduced more than one thiophene rings into the rhodamine skeleton might frame a novel fluorescent probe for Fe$^{3+}$ detection. From the structure view, two thiophene rings would provide the fit binding pocket and improve the affinity for Fe$^{3+}$ ions. Bearing these in mind, we designed and synthesized a novel N,N-dithienyl-rhodamine based probe 1 for Fe$^{3+}$ detection. This study will be an interesting exploration to investigate the effects of compact bisthiophene system on probe’s selectivity and sensitivity and to provide a new idea for designing novel probes.

1. Materials and methods

1.1. Chemicals and equipment

Rhodamine B (99% pure), 2-thiophenecarboxyl chloride (99% pure), benzoyl chloride (99% pure), cyclopropylcarbonyl chloride (97% pure) and other chemicals (AR grade) supplied by China Aladin Ltd. All NMR spectra, fluorescence spectra, IR spectra, and, high-resolution mass spectra (HRMS) were acquired by using Bruker spectrometers (400 and 100 MHz), Hitachi fluorescence spectrometer (F-7000), Infrared spectrophotometer (FTIR-850) and Q-TOF analyzer (Waters, USA), respectively. Cell image was obtained by a confocal microscope (Leica TCS SP5 II, Wetzlar, Germany). Ultrapure water was generated by a Milli-Q system (IQ 7000), and used as the solvent for all aqueous solution preparation.

1.2. Synthesis of probes 1 and its analogies 2, 3

1.2.1. Synthesis of dithienyl-substituted probe 1

At 0 ºC, a mixture of rhodamine hydrazide (Dujols et al., 1997) (137 mg, 0.3 mmol) and Et$_3$N (0.41 mL, 3.0 mmol) in 8 mL dry acetoni trile was carefully added excess 2-thiophenecarbonyl chloride (0.26 mL, 2.4 mmol) drop by drop. Then, the mixture was gradually heated to 76 ºC (oil bath) and reflux lasted for further 5 hr. Upon cooling to room temperature and removing the solvent, dithienyl-substituted compound 1 as brown solid was obtained in 56% yield (378 mg) via flash chromatography purification. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.04 (dd, H$_7$, 1H, J = 6.4, 1.2 Hz), 7.60 (dd, H$_{2,7}$, 2H, J = 7.6, 1.2 Hz), 7.42 (dd, H$_{4,9}$, 2H, J = 3.6, 1.2 Hz), 7.36 (dd, H$_{5,6}$, 2H, J = 4.8, 1.2 Hz), 7.23 (dd, H$_{6,7}$, 1H, J = 6.4 Hz, 1.2 Hz), 6.87 (d, H$_3$, 1H, J = 3.6 Hz), 6.86 (d, H$_5$, 1H, J = 3.6 Hz), 6.34 (d, H$_{9,9'}$, 2H, J = 8.0 Hz), 6.26 (d, H$_{10,10'}$, 2H, J = 2.4 Hz), 6.05 (dd, H$_{11,11'}$, 2H, J = 8.0, 2.4 Hz), 3.29 (q, CH$_2$CH$_3$, 8H, J = 7.2 Hz), 1.13 (t, CH$_3$CH$_2$, 12H, J = 7.2 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 164.8, 162.7, 154.7, 150.2, 149.2, 136.5, 133.7, 132.7, 132.3, 130.8, 130.1, 128.8, 127.2, 124.9, 123.9, 107.7, 104.4, 97.4, 68.5, 44.5, 12.8; IR (film) 760, 1615, 1712, 2972, 3084 cm$^{-1}$.
ESI-HRMS calcd: C_{38}H_{37}N_{4}O_{4}S_{2} ([M + H]^+) 677.2251, found 677.2249.

1.2.2. Synthesis of dibenzo-substituted analog 2
Using rhodamine hydrazide (137 mg, 0.3 mmol), Et₃N (0.41 mL, 3.0 mmol) and benzoyl chloride (0.27 mL, 2.4 mmol) as substrates, dibenzo-substituted analog 2 was obtained (51%, 339 mg) as brown solid. Synthesis procedure and physical data were described in Appendix A Text S1.

1.2.3. Synthesis of dicyclopentyl-substituted analog 3
Using rhodamine hydrazide (137 mg, 0.3 mmol), Et₃N (0.42 mL, 3.0 mmol) and cyclopropylcarbonyl chloride (0.26 mL, 2.4 mmol) as substrates, dicyclopentyl-substituted analog 3 was obtained (45%, 266 mg) as brown solid. Synthesis procedure and physical data were shown in Appendix A Text S2.

1.3. Fluorescence measurements
The stock solutions (10 mmol/L) of three analogs (1, 2, and 3) were prepared in methanol with requisite amount. During testing, the stock solutions of 1–3 were diluted with MeOH/H₂O (1/1, V/V, pH 7.2) to a concentration of 50 μmol/L. HCl (100 mmol/L and NaOH (100 mmol/L) aqueous solutions were used to modulate different pH value. All the cations (including Fe³⁺, Fe²⁺, Ca²⁺, Na⁺, Mg²⁺, Ag⁺, Zn²⁺, Mn²⁺, Ba²⁺, Al³⁺, Cu²⁺, Cr³⁺, Cd²⁺, Hg²⁺) were prepared from their sulfate or chloride salts. All the anions (including Cl⁻, ClO₄⁻, HPO₄²⁻, OH⁻, SO₄²⁻, NO₃⁻, SO₃⁻) were prepared from their sodium salts. All salts were dissolved in ultrapure water to corresponding concentrations.

Titration experiments were carried out by adding 2 mL of corresponding concentration probe solution into a quartz cuvette. Then, corresponding cations or anions aqueous solutions were incrementally added into the corresponding quartz cuvette by micropipettes. The fluorescence spectra were recorded at room temperature after complete addition of different cations or anions solutions under shaking for 200 sec. The emission spectra were recorded range from 550 to 700 nm (excited at 566 nm). All spectroscopic data were obtained in triplicate and were averaged.

1.4. Cell culture and fluorescence microscopy imaging

Human HepG2 cell was provided by ATCC. Cell culture conditions: Dulbecco’s Modified Eagle’s medium containing 4.5 g/L glucose, 10,000 U/L streptomycin, 10,000 U/L penicillin, 10% fetal bovine serum at 37°C with 5% CO₂. The cytotoxicity test of probe 1 to human HepG2 cells was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The HepG2 cells in 96-well plates were cultured in a 96-well plate (150 μmol/L/well). Firstly, the dithenoyl-substituted probe 1 with different concentrations (0, 20, 30, 40, and 50 μmol/L) were carefully added into the corresponding well (1 × 10⁵ cells per well).

After incubating at 37°C under an atmosphere of 5% CO₂ for 6 hr, CCK-8 (100 μL/well) was added to each well and incubated for further 0.5 hr at 37°C under an atmosphere of 5% CO₂. Then we measured the absorbance value at 450 nm and each value was achieved from the average of three parallel experiments. Cell viability was finally calculated by the ratio of the corresponding absorbance value of treatment group to control group.

In another 8-well plate, HepG2 cells were first co-cultured with Fe³⁺ (50 μmol/L) for 2 hr, washed three times with PBS buffer, and then treated with probe 1 (50 μmol/L) and incubated for another 2 hr in the culture medium. Finally, after washing three times with PBS buffer, the HepG2 cells in the culture medium were imaged with a Leica TCS-SP5 Spectral Laser Scanning Confocal Microscope.

2. Results and discussion

2.1. Synthesis of disubstituted-rhodamine analogs 1, 2, and 3

As shown in Scheme 1, using rhodamine hydrazide and corresponding acyl chlorides as substrates, dithenoyl probe 1 and its analogs 2 and 3 were easily synthesized in one step with 56%, 51%, and 45% yield, respectively. The synthesized rhodamine hydrazide and disubstituted-rhodamine analogs 1–3 were characterized by HRMS, ¹H NMR, and ¹³C NMR spectra (Appendix A Fig. S1-S10) which were well consistent with expected chemical structures.

2.2. Stability of dithenoyl-substituted probe 1

The stability of probe 1 was evaluated under different pH conditions (Appendix A Fig. S11). Under the pH range of 6–10, no visible change in absorption was observed. Conversely, a strong and obvious fluorescence absorption appeared at 588 nm when the pH value was lower than 6. Along with the decreasing of the pH values, the absorption was increasing. These results confirmed the opening of the spirolactam ring of dithenoyl-substituted probe 1. In this context, the probe 1 could work within a wide pH range from 6 to 10. Considering the typical pH values of natural water and intracellular are near neutral, therefore, our further fluorescent studies chose pH 7.2 as the suitable experiment pH value. In addition, in order to evaluate the stability during the storage of probe. The solution of probe 1 still exhibited excellent sensitivity even after 6 months of storage at room temperature, indicating that it has good stability and practicability (Appendix A Fig. S12).

2.3. Selectivity of dithenoyl-substituted probe 1

The selectivity of dithenoyl-substituted probe 1 was investigated by addition 10.0 equiv. of different cations (K⁺, Ca²⁺, Na⁺, Mg²⁺, Ag⁺, Zn²⁺, Mn²⁺, Ba²⁺, Al³⁺, Cu²⁺, Cr³⁺, Cd²⁺, Hg²⁺ and Fe³⁺). As shown in Fig. 1a, all of the added cations except Fe³⁺ ions did not show obvious change in fluorescence spectra, which demonstrated the rhodamine scaffold was kept in the ring closed isomeric form. Remarkably, numerous possible competitive metal ions, such as Fe²⁺, Hg²⁺, Cu²⁺, Al³⁺ also did not induce any shift in fluorescence intensity. In contrast, when Fe³⁺ ions were added into the solution, a strong fluorescence band was observed, the colorless probe solution exclusively changed to pink. These results confirmed
the Fe$^{3+}$ ions could promote the ring-opening reaction of rhodamine spirolactam.

Considering the mono-thiophene-rhodamine derivative (Xia et al., 2018) had been reported to be an ideal sensor for hypochlorite ion, 10.0 equiv. of H$_2$O$_2$ and several anions such as ClO$^-$, Cl$^-$, CO$_3^{2-}$, H$_2$PO$_4^-$, HPO$_4^{2-}$, OH$^-$, S$_2$O$_3^{2-}$, NO$_3^-$, SO$_4^{2-}$ were further tested for the selectivity with our dithenoyl-substituted probe 1 in MeOH/H$_2$O solution (1/1, V/V). Also, dithenoyl-substituted probe 1 did not give any observable response for ClO$^-$ and other anions (Fig. 1b). After replacing the hydrogen atom on hydrazide with thiophene group, ClO$^-$ could not oxidize the N,N-diacylhydrazine group and induce the ring-opening of rhodamine spirolactam. All these results indicated that this new architecture N,N-dithenoyl-rhodamine based probe 1 had excellent recognition and selectivity for Fe$^{3+}$ ion.

### 2.4. Fluorescence response for Fe$^{3+}$ ion

A fluorescence titration experiment was performed to examine the fluorescence response of probe 1 to different concentrations of Fe$^{3+}$ (0–50 μmol/L) in MeOH/H$_2$O (1/1, V/V, pH 7.2) solution. It is easy to find that, without Fe$^{3+}$, nearly no obvious fluorescence signal appearance (Fig. 2). However,
upon increasing the Fe$^{3+}$ amount gradually, an apparent peak at 588 nm emerged and continuously increased, indicating ring-opening of the rhodamine spirolactam and the increasing coordination interactions between Fe$^{3+}$ and dithenoyl-substituted probe 1. Also, along with the addition of Fe$^{3+}$, the color of the solution in quartz cuvette changed from colorless to pink, which makes this probe possible to detect Fe$^{3+}$ in naked-eye (Appendix A Fig. S13-S14). The maximal fluorescence enhancement appeared when the addition of 1.0 equiv. of Fe$^{3+}$ (50 μmol/L) into the system.

Based on the fluorescence intensity at 588 nm, the detection limit of the synthesized probe 1 for Fe$^{3+}$ in aqueous system was determined using both S/N test method and serial dilution method. It was found that the detection limit was 3.74 μmol/L (0.21 mg/L, based on S/N = 3), the relative standard deviation of ten runs was 4.8%. A good linear regression curve ($R = 0.9903$) was achieved between Fe$^{3+}$ concentration (range from 5.0 to 30.0 μmol/L) and the fluorescence intensity at 588 nm. Considering the World Health Organization (WHO) had suggested 0.3 mg/L as the maximum value of Fe$^{3+}$ in drinking water (Yoder and Kisaalita, 2006), our dithenoyl-substituted probe 1 was just capable of detecting both qualitatively and quantitatively of Fe$^{3+}$.

Real-time determination is also an important factor for this dithenoyl-substituted probe. As shown in Appendix A Fig. S15, the response of 1 towards Fe$^{3+}$ was fast (less than 200 sec) and could be used as a real-time probe for Fe$^{3+}$ detection.

2.5. Proposed sensing mechanism

The binding stoichiometry of dithenoyl-substituted probe 1 with Fe$^{3+}$ ion was determined using the Job’s plot method (En et al., 2014). In the experiment, the total concentration of Fe$^{3+}$ ions and probe 1 was kept at 50 μmol/L, whereas the mole ratios between probe 1 and Fe$^{3+}$ were varied for 10 different fractions. As seen in Appendix A Fig. S16, the fluorescence intensity of probe 1-Fe$^{3+}$ complex achieved the maximum mole fraction of 50% at 588 nm, suggesting the stoichiometry of dithenoyl-substituted probe 1 with Fe$^{3+}$ ions should be 1:1. This result also consistent with the finding that probe 1 gave the maximal fluorescence enhancement after treatment with 1 equiv. Fe$^{3+}$ ions. Next, the binding association constant (K$_a$) for probe 1-Fe$^{3+}$ in MeOH/H$_2$O (V/V, 1/1, pH 7.2) solution was calculated (3.4 × 10$^4$ (mol/L)$^{-1}$) by using the linear curve fitting procedure and the Benesi-Hildebrand plot (Appendix A Fig. S17).

To further understand the sensing mechanism, $^1$H NMR and FT-IR spectroscopic titration experiments were investigated between probe 1 and different concentrations of Fe$^{3+}$.

Fig. 2 — Gradient changes in emission spectra. The dithenoyl-substituted probe 1 (50 μmol/L) was treated with various concentrations of Fe$^{3+}$ (0–50.0 μmol/L) in H$_2$O/MeOH solution (V/V, 1/1, pH 7.2).

![Gradient changes in emission spectra](image.png)

Fig. 3 — $^1$H NMR titration experiment of probe 1 (5.0 mmol/L) by added increasing amounts of Fe$^{3+}$ in d-DMSO.
As shown in Fig. 3, after respective addition of 0.2, 0.5, 0.8, and 1.0 equiv. of FeCl$_3$ into dithenoyl-substituted probe 1 in DMSO, the proton signals of thiophene (H$_{2,2'}$, H$_{3,3'}$, H$_{4,4'}$) were obvious shifted up-field. Especially, when 1.0 equiv of Fe$^{3+}$ was added into probe 1 in DMSO, the protons on thiophene rings (H$_{2,2'}$, H$_{3,3'}$, H$_{4,4'}$) were shifted up-field by 0.074 ppm, 0.043 ppm, and 0.038 ppm, respectively. These up-field shifts of proton signal were attributed to the change in electron density of thiophene group, indicating that the sulfur atom on the thiophene ring participated into the coordination with Fe$^{3+}$ ions. As illustrated in Appendix A Fig. S18, the absorption of carbonyl (C=O) group shifted from 1691 to 1686 cm$^{-1}$ after treatment with 1.0 equiv. of Fe$^{3+}$ ions, indicating the electropositivity of carbonyl decreased and the oxygen atom on carbonyl group involved in coordination with Fe$^{3+}$ ions. Notably, a strong NH absorption appearance at 3360 cm$^{-1}$ showed the ring-open of the rhodamine spirrolactam. In addition, the characteristic absorption of thiophene shifted from 3084 to 3040 cm$^{-1}$ and the ring stretching vibration peak dramatical decreased near 1545 cm$^{-1}$ and 1516 cm$^{-1}$ also suggested that the thiophene ring participated in the Fe$^{3+}$ detection.

To further evaluate the important roles of thiophene ring in probe 1, two control compounds (using phenyl group and cyclopropyl group respectively replace the thiophene ring of dithenoyl-substituted probe 1) were synthesized and used to detect Fe$^{3+}$ ions under the same conditions (Scheme 1). As expected, upon addition of 1.0 equiv. of Fe$^{3+}$ ions, both analogs 2 and 3 displayed no emission signal at 588 nm (Fig. 4). These control experiments further indicated that the thiophene groups played crucial roles in Fe$^{3+}$ ions recognition and chelation processes.

With above experimental results in hand and referring previously reported studies (Jin et al., 2017; Wang et al., 2017; Wu et al., 2019), a conceivable mechanism of Fe$^{3+}$ complex with dithenoyl-substituted probe 1 was proposed in Fig. 5. We speculated the oxygen atom of all three amides around the hydrazide group and one sulfur atom on the thiophene group participated into the recognition and coordination with Fe$^{3+}$ ions. The compact rigid dithenoyl-substituted structure was considered to be more favorable for ‘S’ atom and ‘O’ atoms oriented to the Fe$^{3+}$ ion.

2.6. Confocal fluorescent images in living HepG2 cells

Considering the favorable spectroscopic properties to Fe$^{3+}$, we next explored the applications of this novel dithenoyl-substituted probe 1 for Fe$^{3+}$ detection in living HepG2 cells. The cytotoxicity study of probe 1 was first carried out on HepG2 cells to investigate its practicality. As shown in Appendix A Fig. S19, after treatment with probe 1 for 6 hr under different concentration conditions (20–50 µmol/L), the HepG2 cell viability remained close to 100% (>95%), even at the highest concentration (50 µmol/L). This result proved that the dithenoyl-substituted probe 1 was safety for further laser confocal microscopy imaging study in living HepG2 cells.

Subsequently, the potential biological application of probe 1 was explored. As shown in Fig. 6, the HepG2 cells displayed negligible intracellular background fluorescence under fluorescence microscopy (emission at 550–700 nm, excitation at 543 nm) and exhibited normal morphology with good health (Fig. 6 A1-A3). In contrast, an observable red fluorescence appeared after the HepG2 cells were supplemented 2 hr with 50 µmol/L probe 1 at 37°C (Fig. 6 B1). In another well, HepG2 cells were first incubated with 50 µmol/L Fe$^{3+}$ ions for 2 hr in the growth medium, followed by treated with 50 µmol/L probe 1 for another 2 hr at 37°C, a stronger red fluorescence was observed (Fig. 6C1). Also, this color change could be obverted in bright field. Overlay the fluorescence and bright field images.

![Fig. 4](image.png)  **Fig. 4** – The fluorescence spectra of dithenoyl-substituted probe 1 (50 µmol/L), analogs 2 (50 µmol/L) and 3 (50 µmol/L) by treated with 50 µmol/L Fe$^{3+}$ in MeOH/H$_2$O (1/1, V/V, pH 7.2) solution.

![Fig. 5](image.png)  **Fig. 5** – Proposed sensing mechanism of probe 1 with Fe$^{3+}$ ion.
could see that the HepG2 cells emitted red fluorescence after being treated with Fe$^{3+}$ ions and probe 1. These results indicated that this novel N,N-dithenoyl-rhodamine based probe 1 has well cell membrane-permeable and could efficiently localize and image for Fe$^{3+}$ ions in the living HepG2 cells (Fig. 6C3).

3. Conclusions

In this study, a novel architecture N,N-dithenoyl-rhodamine based probe 1 was synthesized and characterized, which showed good sensitivity and excellent selectivity towards Fe$^{3+}$ over other cations and anions. Probe 1 showed fast fluorescence response within 200 sec and a low detection limit of 0.21 mg/L for Fe$^{3+}$. The stoichiometry ratio of probe 1 binding with Fe$^{3+}$ was verified as 1:1. The spirolactam-ring opening mechanism of the probe 1-Fe$^{3+}$ complex was confirmed by the $^1$H NMR and FTIR titration experiments. Interestingly, two control analogs 2 and 3 were synthesized and tested showed no responsible to Fe$^{3+}$, indicating the two thiophene rings in probe 1 scaffold played important roles in recognition and coordination with Fe$^{3+}$ ion. What’s more, HepG2 cell confocal fluorescence imaging experiment successfully indicated that dithenoyl probe 1 had not only ideal physical and spectroscopic properties, but also possessed good, fast cell-membrane permeability, low toxicity and could be used as dual-mode monitoring intracellular Fe$^{3+}$ bioavailability in living cells.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.12.005.

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