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Synthesis and evaluation of a new furfuran-based rhodamine B fluorescent chemosensor for selective detection of Fe^{3+} and its application in living-cell imaging

Tong Zhou[a,b], Xiangxue Chen[a], Qinghan Hua[a], Wu Lei[b,*], Qingli Hao[b], Baojing Zhou[b], Chang Su[b], Xiaofeng Bao[a,*]

[a] School of Enviromental and Biological Engineering, Nanjing University of Science & Technology, 200 Xiaolingwei, Nanjing 210094, PR China
[b] School of Chemical Engineering, Nanjing University of Science & Technology, Nanjing 210094, PR China

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ABSTRACT

A new furfuran-based rhodamine B fluorescent probe (**RBFF**) has been designed and synthesized, and its sensing behavior towards various metal ions was evaluated via UV–vis and fluorescence spectroscopic techniques. **RBFF** exhibits a highly sensitive and selective "turn-on" fluorescent response toward Fe^{3+} ion and a fluorescence "turn-off" response when added $B_4O_7^{2-}$ to the **RBFF**-Fe^{3+} in the EtOH/H_2O solution (1:1, v/v, HEPES, 1 mM, pH 7.20). The detection limit of **RBFF** for Fe^{3+} was calculated to be 0.025 μM. The fluorescence microscopy experiment suggested that **RBFF** could also be served as a biological fluorescence probe for the detection of Fe^{3+} in human cervical carcinoma cells (HeLa).

1. Introduction

The design and synthesis of selective and sensitive sensors towards transition metals has tremendously gained in importance, because transition metals are essential in biological and environmental processes for their diverse functions, such as electron transit, metabolism, and catalysis [1–3]. Fe^{3+}, transition metal ions play an indispensable role in the growth and development of living systems [4,5]. Numerous enzymes use iron as a catalyst [6,7] for oxygen metabolism, electron transfer, and DNA and RNA synthesis. Deficient Fe^{3+} ions [8] can cause anemia, affecting the process of cell metabolism in vivo, and excessive Fe^{3+} ions [9,10] with in the body have been associated with the development of severe diseases including various cancers, osteoporosis, hemochromatosis, and dysfunction of organs. Clearly, it is essential to detect the iron pool in biological systems.

According to reports in the literature, a lot of technologies have been used to detect Fe^{3+}, such as inductively coupled plasma atomic emission spectrometry (TCP-AES) [11], potentiometric stripping voltammetry [12] and ion chromatography(IC) [13]. However, above methods are relatively large modern instrumental analysis techniques. Not only the operation of instruments are quite complex, but also purchasing the machine and late machine maintenance are always too expensive. Fluorescence detection technology [14–17] is gradually attracting people's attention for high sensitivity, good selectivity, wide linear range and uncomplicated equipments. Thus, there is an urgent need to develop some highly selective fluorescent chemosensors for Fe^{3+} to satisfy the biological and environmental needs.

A series of probes used to detect Fe^{3+} have been reported in the previous literature [18–25]. Early fluorescent probes exhibited a fluorescence quenching response to the Fe^{3+}, which limits their biological application [26–31]. Therefore, for biological applications, enhanced fluorescent probes are more advantageous than quenched fluorescent probes. Rhodamine B derivatives are the widely used dyes in the field of organic small molecule probes [32–36] due to their excellent spectroscopic properties of large Molar absorption coefficient, high fluorescence quantum yield, good photostability and long absorption and emission wavelength elongated to be visible. The spirolactam form of rhodamine derivatives is colorless and hardly fluorescent in common solvents. When they bind to some mental cations under the particular solvent system, the spirolactam turned into the open-ring form with big strong fluorescence and obvious pink color. In view of these facts, many new rhodamine derivatives as the fluorescent probes towards

* Corresponding authors.
E-mail addresses: leiwuhao@njust.edu.cn (W. Lei), baoxiaofeng@mail.njust.edu.cn (X. Bao).

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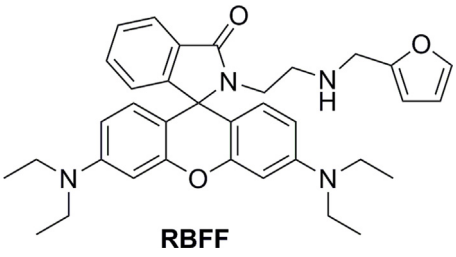

Fig. 1. Chemical structure of **RBFF**.

mental ions are getting more and more popular with researchers [37–44]. Meanwhile, rhodamine-based enhanced probes for Fe^{3+} have been designed and discussed during recent years [45–49]. Most of these probes have difficult problems to solve, such as expensive raw materials, complex synthesis steps, poor water solubility or poor biocompatibility etc. Furfuran and its derivatives [50,51] are important containing oxygen heterocyclic compounds. Its skeleton structure exists widely in nature, which possesses characteristics of anti-bacteria, antiviral and antitumor. Therefore, the derivatives of furan can exhibit excellently bio-compatibility in the cell. Unfortunately, it was rarely reported that the design of based furan as a fluorescence sensor detected content of metal cations from the cell.

Herein, we introduced that a rhodamine B derivative based on furfural had simple synthesis steps, which was named **RBFF** (Fig. 1). **RBFF** was successfully used as a selective and sensitive fluorescent and colorimetric sensor for Fe^{3+} in an EtOH/H_2O solution (1:1, v/v, HEPES, 1 mM, pH 7.20). Most importantly, this sensor could be applied for vivo imaging in HeLa cells to confirm that our **RBFF** probe can availably detect Fe^{3+} in living cells.

2. Experimental

2.1. Materials and general methods

All reagents and organic solvents were of ACS grade or higher and were used without further purification. Unless otherwise noted, all of the chemicals were purchased from J&K Scientific (Shanghai, China) and were used as received. All of the solvents were of analytical grade, and double distilled water was used in all of the experiments. The salts used in the stock solutions of metal ions were $FeCl_3{\cdot}6H_2O$, $AlCl_3$, $SnCl_2{\cdot}2H_2O$, $CrCl_3{\cdot}6H_2O$, $PbCl_2$, $CoCl_2{\cdot}6H_2O$, $CaCl_2$, $ZnCl_2$, $MgCl_2{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, $HgCl_2$, $Ba(NO_3)_2$, $AgNO_3$, KCl, $MnCl_2{\cdot}4H_2O$, $CuCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $LiCl{\cdot}H_2O$ and NaCl. The reactions were performed under an argon atmosphere using standard Schlenk techniques. Thin-layer chromatography was performed on a HAIYANG silica gel F254 plate, and the compounds were visualized under UV light (λ = 254 nm). Column chromatography was performed using HAIYANG silica gel (type: 200–300 mesh ZCX-2). ^1H (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded on an Avance 500 spectrometer (Bruker; Billerica, MA, USA). The chemical shifts are reported in δ units (ppm) downfield relative to the chemical shift of tetramethyl silane. The abbreviations br, s, d, t, and m denote broad, singlet, doublet, triplet, and multiplet, respectively. Mass spectra were obtained with a Finnigan TSQ Quantum LC/MS Spectrometer. High-resolution mass spectra (HRMS) were acquired under electron ionization conditions with a double-focusing high-resolution instrument (Autospec; Micromass Inc.). The pH levels of the stock solutions were measured using a PHS-25C Precision pH/mV Meter (Aolilong, Hangzhou, China). UV–vis and fluorescence spectra were obtained on a UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Japan) and an Edinburgh FLS920 fluorescence spectrophotometer (Livingston, UK), respectively, at room temperature.

2.2. Synthesis of 2-(2-aminoethyl)-3′,6′-bis(diethylamino)spiro[isoindoline-1,9′-xanthen]-3-one (1)

To a solution of rhodamine B (1.82 g, 3.81 mmol) in 5 mL of ethanol, ethane-1, 2-diamine (2.29 mL, 38.1 mmol) was added. The mixture was refluxed for 12 h and then dried under vacuum. The residue was dissolved in CH_2Cl_2 and then washed with H_2O and brine. The organic layer was dried with $MgSO_4$. After removal of the solvent, flash chromatography (silica gel; MeOH/CH_2Cl_2 = 3/97,v/v) of the residue yielded 1 as a pink solid (1.49 g, 81%). ^1H NMR ($CDCl_3$), δ 7.90 (dd, J = 5.6, 3.0 Hz, 1H), 7.61 –7.38(m, 2H), 7.09 (dd, J = 5.6, 2.9 Hz, 1H), 6.43 (d, J = 8.8 Hz, 2H), 6.37(d, J = 2.5 Hz, 2H), 6.27 (dd, J = 8.9, 2.6 Hz, 2H), 3.38–3.27 (m, 8H),3.19 (t, J_1 = 6.65 Hz, J_2 = 6.6 Hz, 2H), 2.41 (t, J_1 = 6.65 Hz, J_2 = 6.6 Hz, 2H), 1.16 (t, J_1 = 7.0 Hz, J_2 = 7.1 Hz, 12H) ppm. ^{13}C NMR ($CDCl_3$), δ168.59, 153.35, 148.79, 132.38, 131.20, 128.64, 128.02, 123.80, 122.71, 108.13, 105.65, 97, 64.91, 44.36, 44.06, 40.73, 12.56 ppm. ESI–MS $[M+1]^+$ found, 485.13; calculated for $C_{30}H_{37}N_4O_2^+$, 485.29.

2.3. Synthesis of 3′, 6′-bis (diethylamino)-2-(2-((furan-2-ylmethyl) amino) ethyl) spiro [isoindoline-1, 9′-xanthen]-3-one (RBFF)

To a solution of 2-(2-aminoethyl)-3′,6′-bis(diethylamino)spiro[isoindoline-1,9′-xanthen]-3-one (96.8 mg, 0.2 mmol) in 5 mL of absolute alcohol was added furan-2-carbaldehyde (24 μL, 0.24 mmol). The reacting mixture was refluxed at 78 °C for 12 h, and then under control of 0 °C, sodium borohydride (38 mg, 1 mmol) was put in the flask to stir for 20 min. Subsequently the reaction mixture was stirred at room temperature for 5 h, and taken to dryness under vacuum. The residue was dissolved in CH_2Cl_2 and then washed with H_2O. The organic phase was dried with Magnesium sulfate and then concentrated. Flash chromatography (silica gel; MeOH/CH_2Cl_2 = 0/100 to 1/99, v/v) of the crude mixture afforded **RBFF** (78 mg) in 69% yield as a creamy-white solid. ^1H NMR($CDCl_3$), δ7.89 (dd, J = 5.7, 2.9 Hz, 1H), 7.45–7.41(m, 2H),7.27 (d, J = 1.1 Hz, 1H), 7.07 (dd, J = 5.6, 2.9 Hz, 1H), 6.41 (d, J = 8.9 Hz, 2H), 6.37 (d, J = 2.6 Hz, 2H), 6.26 (d, J = 2.6 Hz, 1H), 6.24–6.22(m, 2H), 6.06(d, J = 2.9 Hz, 1H), 3.60(s, 2H), 3.37–3.28 (m, 10H), 2.44 (t, J_1 = 3.3 Hz, J_2 = 6.6 Hz, 2H), 1.16 (t, J_1 = 3.7 Hz, J_2 = 7.1 Hz, 12H) ppm. ^{13}C NMR ($CDCl_3$), δ168.70, 153.76, 153.41, 148.92, 141.72, 132.52, 131.24, 128.86, 128.13, 123.93, 122.91, 110.11, 108.27, 106.92, 105.66, 65.12, 47.29, 45.61, 44.50, 40.34, 29.83, 12.74 ppm. HRMS $[M+1]^+$ found, 565.3190; calculated for $C_{35}H_{41}N_4O_3^+$, 565.3179.

2.4. Stock solution preparation for spectral detection

Stock solutions (1 mM) of the chloride or nitrate salts of Fe^{3+}, Al^{3+}, Sn^{2+}, Cr^{3+}, Pb^{2+}, Co^{2+}, Ca^{2+}, Zn^{2+}, Mg^{2+}, Fe^{2+}, Hg^{2+}, Ba^{2+}, Ag^+, K^+, Mn^{2+}, Cu^{2+}, Ni^{2+}, Li^+ and Na^+ in EtOH/H_2O (1:1, v/v, pH = 7.20) were prepared. A stock solution of **RBFF** (1 mM) was prepared in EtOH/H_2O (1:1, v/v, pH 7.20). The working solutions of **RBFF** were freshly prepared by diluting the highly concentrated stock solution to the desired concentration prior to spectroscopic measurements.

2.5. UV–vis and fluorescence spectral studies

All experiments were carried out in an EtOH/H_2O solution (1:1, v/v, HEPES, 1 mM, pH 7.20). In all of the spectroscopy experiments, the spectral data were recorded 25 min after the addition of the ions. To investigate the metal ion selectivity, the test samples were prepared by placing 5 equiv. of the cation stock solution in 3 mL of the **RBFF** solution (20 μM). For the fluorescence measurements,