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A coumarin-based fluorescent probe for specific and rapid detection of fluoride ions

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A simple and coumarin-based fluorescence probe has been designed and synthesized with silyl group as recognition group of fluoride ions (F−) in this study. The results showed that the fluorescence intensity of the probe displayed prominent enhancement with addition of F− at 445 nm with incubation of 1 min. There was an excellent linear relationship between fluorescence intensity and fluoride concentration from 0 to 30 μM (0∼0.57 ppm), which offered the important condition for the quantitative analysis. In addition, the highly selective response to fluorion, the low detection limit with 28 nM (0.532 ppb), low toxicity and bioimaging afforded an advantage for practical application and detecting fluoride in biological systerms.

Keywords: Fluorion, Fluorescence, Coumarin, Probe, Bioimaging.

INTRODUCTION

As one of the most abundant elements on the earth and the essential trace element for human being, fluoride plays a critical role in biological, medical, environmental and chemical fields, such as enamels formation, bone mineralization, osteoporosis treatment, and pharmaceutical ingredients[1–2]. However, everything has two sides and fluoride is not an exception. Fluoride is easily absorbed and accumulated by the body, and excess concentrations of fluoride may cause dental fluorosis, skeletal fluorosis, urolithiasis, immunotoxic effect, mitochondrial dysfunction and even cancer[2–3]. Moreover, fluorosis has been documented to result in significant health impairment because of continuing exposure to excessive fluoride levels in environment, especially groundwater[1, 4]. Therefore, it's quite valuable to explore a highly selective and sensitive method for the detection of biological and environmental fluoride. Recognition of fluoride has attracted growing interest based on its critical functionalities, and large pieces of research have been conducted in fluoride-detecting, such as electrode assay, nuclear magnetic resonance (NMR) analysis, electrochemical, colorimetric (ultraviolet-visible) and fluorescence sensing[5–7]. Compared with other methods, fluorescence sensor has more advantages of easy operation, high sensitivities and intracellular analysis. However, although the numerous probes with detection of fluorion have been reported[5, 8–16], these probes still have some issues for practical application, such as spectral overlap, poor selectivity and difficulty in bioimaging. Therefore, the development of new and effective fluorescent probe for detecting fluorion is still needed.

The identification group is the key to construction of fluorescence probes. *tert*-butyldimethylsily (TBDMS) was an excellent warhead of fluorescent probes for detecting fluorion based on the chemical affinity of fluoride and silyl group, which was firstly reported by Kim and Swager[17]. Moreover, coumarin is a satisfactory fluorophore because of favorable photophysical properties and low cytotoxicity[18–19]. Thus, in this work, we designed and synthesized fluorescent probe **1** (7-((*tert*-butyldimethylsilyl)oxy)-4- methyl-2*H*-chromen-2-one, P1) with the *tert*-butyldimethylsily as identify group and fluorophore. We hypothesize that the probe **1** is capable of detecting selectively fluoride ions (F^-).

MATERIAL AND METHODS

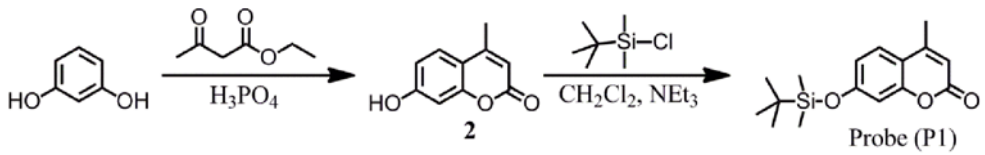
Material

The major material referred in the study, including dichloromethane, *tert*-butyldimethylsilyl chloride (TBSCl), triethylamine, ethyl acetate, acetonitrile, tetrabutylammonium fluoride (TBAF, $C_{16}H_{36}FN$), and N-ethylmaleimide,3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), were purchased from Sigma-Aldrich. The MCF7 cells were cultured in DMEM with 10% fetal calf serum.

Methods

1. Synthesis of the probe (Scheme 1)

7-((tert-butyldimethylsilyl)oxy)-4-methyl-2H-chromen-2-one (Probe, P1) To a solution of compound 2 (0.2643 g, 10 mmol) in dichloromethane (50 mL) were added *tert*-butyldimethylsilyl chloride (TBSCl) (1.81 g, 12 mmol) and triethylamine (1.7 mL, 12 mmol) . The mixture was stirred at room temperature for 12h. Subsequently, the reaction was quenched with water (30 mL), and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography. 1H-NMR (400 MHz, DMSO-d_6): δ = 0.24 (s, 6 H), 0.95 (s, 9H), 2.43 (s, 3 H), 6.23 (s, 1 H), 6.83 (d, J =2.4 Hz, 1 H), 6.88 (dd, J = 8.8, 2.4 Hz, 1 H), 7.66 (d, J = 8.8, Hz, 1 H) ppm; 13C-NMR (100 MHz, DMSO-d6): δ = 18.0, 25.4, 107.1, 111.6, 114.0, 116.9, 126.6, 153.2, 154.4, 158.6, 160.0 ppm; HRMS (ESI) m/z calcd for $C_{16}H_{22}O_3Si$ (M+H): 291.9411. Found: 291.1403, error: 2.7 ppm.



Scheme 1. Synthesis of probe (P1)

2. Absorption and fluorescence spectroscopy

Absorption spectra and fluorescence spectra were measured by a Thermo (BioMate 3S) UV/Vis spectrophotometer and the F97pro fluorospectrophotometer, respectively.

The stock solution of probe **1** (20 μM) was prepared in acetonitrile solution. The tetrabutylammonium fluoride (TBAF, $C_{16}H_{36}FN$) (100 μM) were dissolved in acetonitrile as stock solution. The above stock solutions need to be mixed with the volume ratio of 1/1, when the absorption and fluorescence spectroscopy were detected. For the detection of fluorescence spectra, the excitation wavelength was fixed at 387 nm with scan speed 1000 nm min^{-1} and excitation and emission width 10 nm.

3. Assay for cytotoxic activity of the probe

The cytotoxic activity was evaluated by the MTT assay, which is based on the intensity of a purple formazan dye by the mitochondrial succinate dehydrogenase of active cells. MCF7 cells (10×10^4 /mL) were subcultured in 96-well flat microtiter plates for 24 h, and then the cells were incubated with gradient concentrations (1μM, 5 μM, 10 μM, 20 μM, 60 μM) of probe 1 for another 24 h followed. Subsequently, 110 μL culture medium mixed with MTT (10/1, v/v) were added to each well. After the plate was incubated for another 4 h at 37°C in the dark, the culture medium was removed followed by the addition of 100 μL DMSO. The absorbance was measured at 490 nm using a Thermo (Multiskan MK3) microplate reader. The percentage of cell viability was calculated relative to control wells designated as 100% viable cells.

4. Fluorescence image of probe 1 in MCF7 cells

MCF7 cells (10×10^4/mL) were seeded in a 6-well flat microtiter plate for adherence for 24 h. Cells were incubated with P1 (10 μM) for 30min, and then washed with D-hanks solution for 3 times. Treated with NaF (0.2, 1 and 5 mM, respectively) for another 30 min, and washed the cells with D-hanks solution 3 times as followed. Subsequently, fluorescence images of the cells were obtained by a fluorescence microscope.

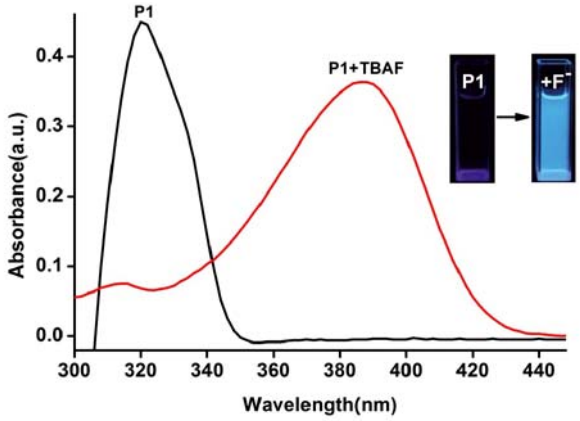


Figure 1. UV-vis absorption spectra of P1 (10 μM) in the absence/presence of Tetrabutylammonium fluoride (TBAF) (50 μM) in acetonitrile. Insets: the image of P1 without and with F− under UV light

RESULTS AND DISCUSSION

Probe (P1) was synthesized (Scheme 1) (The synthesis method of compoud **2** has been reported in our previous study[20]) and confirmed by NMR and HRMS (The details of the ^{1}H NMR, ^{13}C NMR and HRMS of P1 are available upon request). Subsequently, we investigated the UV-vis absorption spectra of the P1 (10 μM) in the absence /presence of tetrabutylammonium fluoride (TBAF) (50 μM) in acetonitrile (Figure 1). The results indicated that the absorption peak of the free P1 showed one prominent absorption peak at about 320 nm, but there was a red shift to around 387 nm when TBAF was added to the solution of P1 and the color of the solution turned from colorlessness to blueness under UV

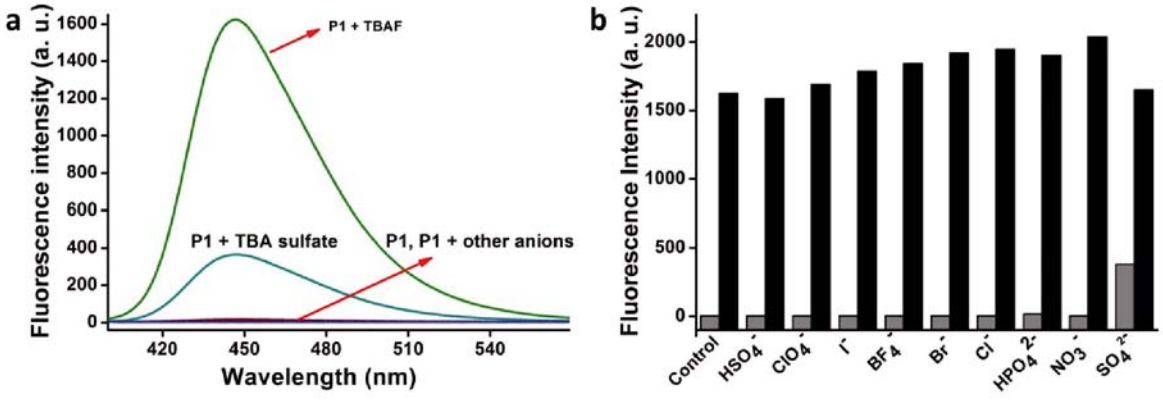


Figure 2. a) Fluorescence spectra of P1 (10 μM) toward various anions (50 μM) in acetonitrile with incubation of 1 min, λex = 387 nm. b) Fluorescence intensity response of P1 (10 μM) with various analytes in acetonitrile, λex = 387 nm. grey bar: probe + analytes (50 μM); Black bar: probe + F− (50 μM) + analytes (50 μM)

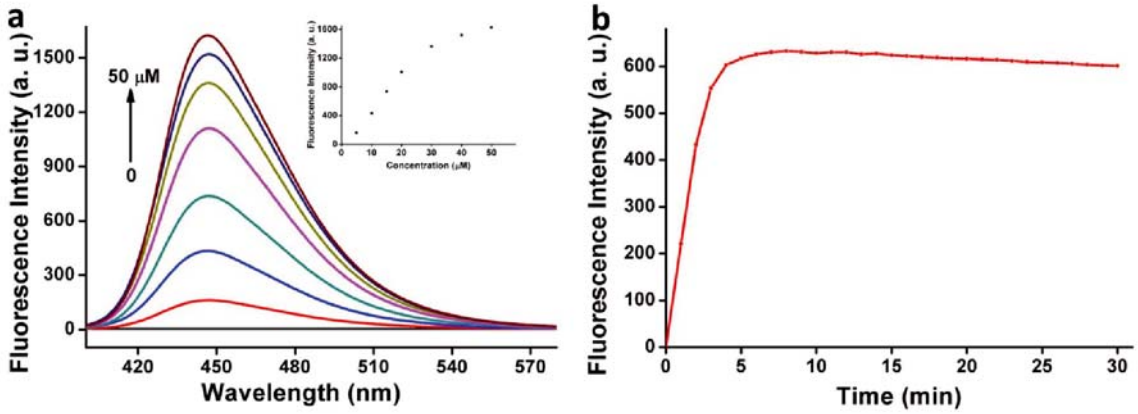


Figure 3. a) Fluoride concentration-dependent fluorescence intensity change of P1 in acetonitrile. b) Time-dependent fluorescence intensity change of P1 (10 μM) with 10 μM TBAF, λex = 387 nm

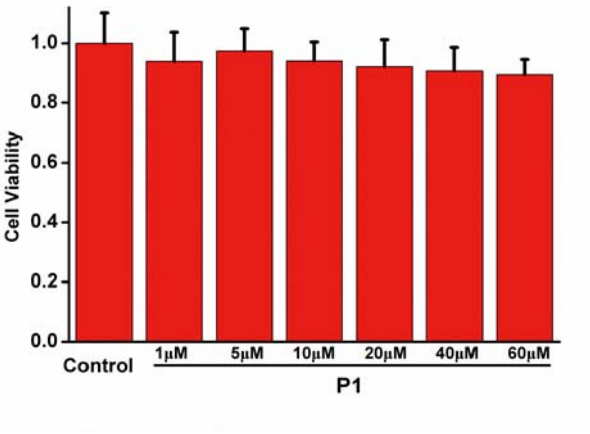


Figure 4. Cytotoxicity of probe for MCF7 cells

light. Therefore, the 387 nm was conformed as excitation wavelength of P1 toward the fluorion.

Next, we further explored the optical properties of fluorescent probe. The fluorescence spectra of P1 toward various anions (TBA salts of F^-, NO_3^-, HPO_4^{2-}, HSO_4^-, ClO_4^-, I^-, BF_4^-, Br^-, Cl^-, SO_4^{2-}, 50 μM) were investigated (Figure 2a). The results showed that the fluorescence intensity of P1 displayed prominent enhancement with addition of F^- at 445 nm with incubation of 1 min. However, the other anions displayed negligible change. Although sulphate showed the slight enhancement of fluorescence intensity, this was insignificant for detecting F^-. Meanwhile, the probe itself did not show significant change in fluorescence intensity. Thus, these

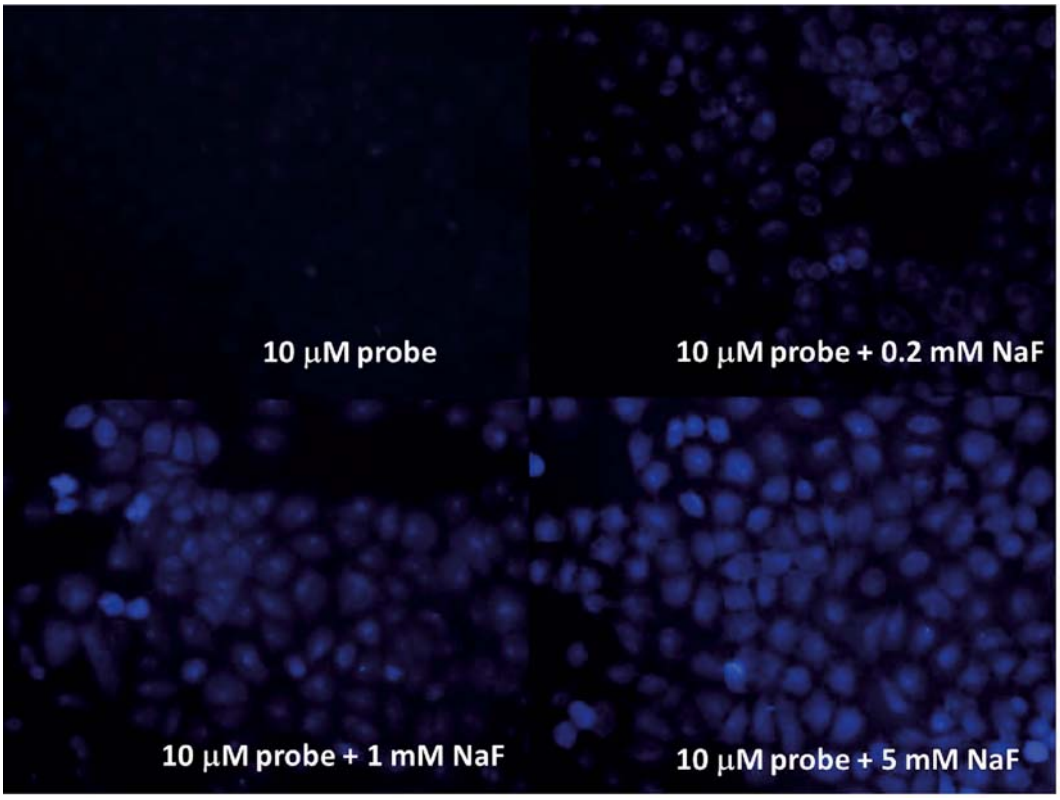


Figure 5. Fluorescence images of probe in MCF-7 cells. (The MCF7 cells were incubated with P1 (10 μM) for 30 min, and then washed with D-hanks solution three times. Sebuseqently, the cells were treated with NaF (0.2, 1 and 5 mM, respectively) for another 30min, and washed with D-hanks solution three times as followed.)

results verified the probe possessed the excellent selectivity for F^- detection. Moreover, to explore the influence of other ions for the determination of fluoride, we carried out the competition experiment (Figure 2b). Thrillingly, the results indicated the existence of other anions does not affect the determination of fluoride. Encouraged by these results, we further completed the quantitative analysis of the fluoride concentration (Figure 3a). The fluorescence intensity was gradually enhanced with the increasement of the fluoride concentration from 0 to 50 μM. In addition, there was a good linear relationship between fluorescence intensity and fluoride concentration from 0 to 30 μM (0∼0.57ppm) ($y = 45.56\,x + 7.40$, R^2=0.9967) with a relatively wider line range in comparison with previous reports[9], which provided a possibility for quantitative analysis of fluoride content. The reaction time is related to whether the probe can monitor the fluoride content in real time. Therefore, we detected the time to compete reaction. The results showed the reaction reached saturation period after 5 min, which is faster than that of previous research[10, 21] and provided the condition for rapid detection of F^-. Meanwhile, the detection limit with 28 nM (0.532 ppb) (3 σ/y) also showed advantage for detecting fluoride content.

We studied the application of probes in biological imaging. To evaluate the potentials of P1 in biological applications in living systems, a fluorescence microscopy imaging experiment was conducted after investigating the cytotoxic activity of P1 in MCF7 cells by MTT assay. Obviously, after the MCF7 cells were incubated with higher concentration of P1 (60 μM) for 24h, the survival rates of cells are above 90% (Figure 4). As shown in Figure 5, the presence of F^- could trigger obvious blue fluorescence in MCF7 cells compared to the MCF7 cells in the absence of F^-. More interestingly, the fluorescence intensity of P1 was enhanced clearly along with increasement of NaF concentration. Thus, this probe can detect the F^- in biological systems.

CONCLUSIONS

In summary, we designed and synthesized a simple probe, which can selectively and rapidly detect F^-. Meanwhile, under optimal detection conditions, there was the excellent linear relationship between fluorescence intensity and F^- concentration over 0∼30 μM (0∼0.57ppm). Furthermore, bioimaging and a low detection limit also afforded a possibility for detecting F^- in biological systerms.

ACKNOWLEDGEMENTS

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