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A novel acidic pH fluorescent probe based on a benzothiazole derivative

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Abstract

A novel acidic pH fluorescent probe **1** based on a benzothiazole derivative has been designed, synthesized and developed. The linear response range covers the acidic pH range from 3.44 to 6.46, which is valuable for pH researches in acidic environment. The evaluated p*Ka* value of the probe **1** is 4.23. The fluorescence enhancement of the studied probe **1** with an increase in hydrogen ions concentration is based on the hindering of enhanced photo-induced electron transfer (PET) process. Moreover, the pH sensor possesses a highly selective response to H^+ in the presence of metal ions, anions and other bioactive small molecules which would be interfere with its fluorescent pH response. Furthermore, the probe **1** responds to acidic pH with short response time that was less than 1 min. The probe **1** has been successfully applied to confocal fluorescence imaging in live HeLa cells and can selectively stain lysosomes. All of such good properties prove it can be used to monitoring pH fluctuations in acidic environment with high sensitivity, pH dependence and short response time.

***Keywords*:** acidic pH fluorescent probe; benzothiazole derivative; enhanced PET process; cell imaging

1. Introduction

The determination of pH has a great interest in different areas due to comprehensive applications in numbers of processes, such as electrochemical sensing [1], biochemical and medical analyses [2,3], environmental monitoring [4], isomer analysis [5], clinical analysis [6], control and monitoring of biochemical reactions [7]. In cellular biology, pH plays pivotal role in many biological functions such as cellular proliferation and apoptosis [8], enzymatic activity [9,10], ion transport [11] and muscle contraction [12]. Abnormal intracellular pH values result in cellular dysfunction and cause some serious diseases such as cancer [13] and Alzheimer's disease [14]. Therefore, monitoring intracellular pH changes is very critical for the diagnosis of certain diseases and the measurement of therapeutic efficiencies.

So far, there are many approaches including electrochemistry [1], nuclear magnetic resonance (NMR) [15], absorbance spectroscopy [16] and fluorescence spectroscopy [17] for measuring the intracellular pH. Among these methods, fluorescence detection has attracted much attention due to its noninvasiveness, particularly high sensitivity, convenient operation, real-time detection and excellent specificity. As known to all, a cell can be divided into two parts: neutral cytosol (pH: 6.8-7.4) and acidic compartments (pH: 4.5-.6.0) [18]. Consequently, two kinds of pH fluorescent probes for the cell have been developed. One category is probes for cytosol which work at a pH of about 6.8-7.4 [19-23]. The other one is probes for weak acidic organelles such as lysomes and endosomes which function in the pH range of about 4.5-6.0 [24-27]. While a wide variety of fluorescent probes for the neutral pH range have been developed, there are only few acidic fluorescent probes, especially desirable ones for studying acidic organelles. Thus, the development of a new acidic pH fluorescent probe with high sensitivity and selectivity, short response time and membrane permeability is still in demand.

In the past decades, benzothiazole and its derivatives have gained increasing interest as fluorescent probes for pH [25,28-32], metal cations [33-36], anions [37-39] and small molecules [40-43] due to their excellent photophysical properties. So far, the design of fluorescent pH probe based on benzothiazole derivatives is mostly based on excited-state intramolecular proton transfer (ESIPT) [25] and intramolecular charge transfer (ICT) [28,30,32]. In order to further investigate the essential roles of acidity in cellular condition, there is a great need to develop other strategies for designing pH fluorescent probes based on

benzothiazole derivatives. Compared with the traditional single-atom electron donor like O, N, Se or Te for photoinduced electron transfer (PET) process, the more electron-rich groups can more effectively induce an "enhanced photoinduced electron transfer (PET)" process to a fluorophore and "switch off" its fluorescence [44]. The "enhanced PET" effect which gives lower background fluorescence and higher signal-to-noise ratio has been utilized to design the fluorescent probes for HClO [44-46] and reactive oxygen species [47]. Therefore, the anilino group as an electron-rich group was introduced to our target probe **1** (Scheme 1) to efficiently quench the fluorescence of the benzothiazole fluorophore through an enhanced PET process. The protonation of nitrogen atom of the anilino group in acidic medium results in disruption of the enhanced PET process, and thus the restoration of the emission from the excited fluorophore takes place.

In this article, we reported a new acidic pH fluorescent probe **1** based on enhanced PET process. This probe **1** with pKa 4.23 can sense acid pH ranging from 3.44 to 6.46 with excellent linear relationship, which was especially suited for quantitative determination of pH in this acidic pH window. This probe **1** responded to acidic pH with quick response time (less than 1 min), high selectivity and high sensitivity. Furthermore, this probe **1** has been successfully applied to fluorescence microscopic imaging in HeLa cells and displayed low cytotoxicity and excellent membrane permeability.

2. Experimental

2.1. Reagents and chemicals

4-(Diethylamino)salicylaldehyde and 2-aminothiophenol were purchased from Heowns Biochemical Technology Company. 4-Chloronitrobenzene was obtained from Aladdin Reagent Company. Anhydrous potassium carbonate, stannous chloride dihydrate and all other reagents were of analytical reagent grade, purchased from Sinopharm Chemical Reagent Company and used without further purification. Thin layer chromatography (TLC) was performed on silica gel 60 F254 visualized by UV, column chromatography was conducted over silica gel (200- 300 mesh), both of which were obtained from the Qingdao Ocean Chemicals (Qingdao, China). Water purified by a Milli-Q system (EMD Millipore, Darmstdt, Germany) was used for the preparation of all aqueous solutions. LysoTracker Red DND-99 was purchased from the Shanghai Qianchen Biotechnology Company.

2.2. Apparatus

The ^{1}H NMR and ^{13}C NMR spectra were recorded by employing a Bruker DRX-500 spectrometer with chemical shifts expressed in parts per million and using TMS as an internal standard. Electrospray ionization (ESI) mass spectrometry was obtained with Bruker maXis HD mass spectrometer (Bruker, Germany). All fluorescence measurements were determined on a Hitachi fluorescence spectrophotometer F-7000 (Tokyo, Japan). UV-vis absorption spectra were acquired on a UV-2600 spectrophotometer using a 1 cm quartz cell (Tokyo, Japan). Fluorescence images of living cells were obtained using an Olympus FV1200 laser scanning confocal microscope (Japan). The pH measurements were performed on a Mettler-Toledo Delta 320 pH meter which was calibrated with pH 4.00, pH 6.86 and pH 9.18 buffers before use. Data processing was performed on a Pentium Ⅳ computer with software of SigmaPlot. The fluorescence intensity was measured at excitation wavelength of 360 nm with both excitation and emission slit set at 2.5 nm.

2.3. Syntheses

The synthetic route for fluorescence probe **1** is shown in Scheme 1.

Insertion of Scheme 1

Synthesis of compound 3

The synthesis of compound **3** was accordance with the previous literature [43]. ^{1}H NMR (500 MHz, $CDCl_3$), δ(ppm): 12.53 (1H, s), 7.84 (1H, d, J = 8.1 Hz), 7.79 (1H, d, J = 7.9 Hz), 7.45 (1H, d, J = 8.5 Hz), 7.41 (1H, t, J = 7.4 Hz), 7.28 (1H, t, J = 7.5 Hz), 6.27 (2H, s), 3.39 (4H, q, J = 7.1 Hz), 1.20 (6H, t, J = 7.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$), δ(ppm): 169.55, 159.78, 152.21, 151.30, 131.89, 129.82, 126.21, 124.14, 121.18, 120.99, 105.77, 104.13, 97.88, 44.54, 12.66. MS (ESI) m/z: 299.1212 $(M+H)^{+}$.

Synthesis of compound 2

Compound **3** 1.49 g (5 mmol) and anhydrous potassium carbonate 3.46 g (25 mmol) were dissolved in 100 mL N, N-dimethylformamide (DMF). The above mixture was stirred at room temperature for 30 min followed by the addition of 4-chloronitrobenzene 0.79 g (5 mmol), and the reaction mixture was stirred at 80 ℃ for 9 h. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (petroleum ether/ ethyl acetate = 8:1, v/v) to give compound **2** as an orange solid (0.95 g, yield 45 %). ^{1}H NMR

(500 MHz, $CDCl_3$), δ(ppm): 8.42 (1H, d, J = 7.9 Hz), 8.20 (2H, m), 7.98 (1H, d, J = 7.9 Hz), 7.75 (1H, d, J = 8.0 Hz), 7.40 (1H, m), 7.27 (1H, d, J = 7.4 Hz), 7.12 (2H, dd, J = 2.0 Hz, 7.3 Hz), 6.69 (1H, dd , J = 2.5 Hz, 9.1 Hz), 6.25 (1H, d, J =2.4 Hz), 3.38 (4H, q, J = 7.1 Hz), 1.18 (6H, t, J = 7.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$), δ(ppm): 162.67, 162.47, 153.72, 152.33, 150.87, 142.83, 135.05, 131.40, 125.99, 125.95, 124.22, 122.15, 121.10, 116.81, 113.10, 109.59, 103.04, 44.66, 12.52. MS (ESI) m/z: 420.1355 $(M+H)^+$.

Synthesis of compound 1

Under nitrogen atmosphere, compound **2** 0.46 g（1 mmol）and stannous chloride dihydrate 2.26 g（10 mmol）were dissolved in the mixture of 30 mL anhydrous ethanol and 30mL concentrated hydrochloric acid, and the above mixture was left stirring violently at 60 ºC for 5 h. Then the anhydrous ethanol was removed, and the pH adjusted to 9.0 with 6 mol/L NaOH. This mixture was then filtered and the filtrate was extracted by ethyl acetate (50 mL × 3). Organic layer was collected, washed with saturated brine (50 mL × 3) and then dried by anhydrous Na_2SO_4. Then the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel chromatography (petroleum ether/ethyl acetate = 6:1, v/v) to compound **1** as a white solid (0.30 g, yield 77 %). ^{1}H NMR (500 MHz, DMSO-d_6), δ(ppm): 8.24 (1H, d, J = 9.1 Hz), 7.97 (1H, d, J = 7.7 Hz), 7.91 (1H, d, J = 8.0 Hz), 7.44 (1H, m), 7.30 (1H, m), 6.86 (2H, dd, J = 2.1 Hz, 6.7 Hz), 6.61 (2H, dd, J = 2.1 Hz, 6.6 Hz), 6.56(1H, dd, J =2.5 Hz, 9.1 Hz), 5.98 (1H, d, J = 2.5 Hz), 5.01 (2H, s), 3.27 (4H, q, J = 7.1 Hz), 1.02 (6H, t, J = 7.1 Hz). ^{13}C NMR (125 MHz, DMSO-d_6), δ(ppm): 162.73, 158.20, 151.99, 150.33, 145.61, 144.85, 134.65, 129.98, 125.84, 123.75, 121.47, 121.36, 120.50, 114.77, 109.99, 106.48, 98.38, 43.95,12.31. MS (ESI) m/z: 390.1631 $(M+H)^+$.

2.4. Spectrophotometric experiments

The fluorescence measurement experiments were measured in Britton–Robinson (B-R) buffer solution. The pH of Britton–Robinson buffer solution used was between 3.00 and 8.00, which was achieved by adding 0.04 mol/L boric acid, 0.04 mol/L phosphoricacid, 0.04 mol/L acetic acid and 0.20 mol/L sodium hydroxide. The solutions of metal ions for fluorescence discrimination were prepared respectively by dissolving $HgCl_2$, $CuSO_4·5H_2O$, $MnCl_2·4H_2O$, $Pb(CH_3COO)_2·3H_2O$, $ZnSO_4·7H_2O$, $CaCl_2·2H_2O$, $AgNO_3$, NaCl, $BaSO_4$, $AlCl_3$, $NiCl_2·6H_2O$, K_2CO_3, $MgSO_4$, $FeCl_3·6H_2O$, Li_2CO_3, $CoSO_4·7H_2O$ and $CrCl_3·6H_2O$ in deionized water with

final concentrations of 5×10^{-6} mol/L for them. As well as, glutathione (GSH), cysteine (Cys), homocysteine (Hcy), arginine (Arg), histidine (His) and alanine (Ala) solutions were prepared respectively using twice-distilled water with final concentrations of 5×10^{-6} mol/L. All tested samples were prepared at room temperature and rested for 5 min before UV-vis and fluorescence determination.

2.5. Cytotoxicity assay

The cytotoxic effects of compound **1** were assessed using a tetrazolium-based colorimetric assay (MTT assay) [48]. HeLa cells were cultured in Dulbecco□ s Modified Eagle□ s medium (DMEM) containing high glucose supplemented with 10% fetal bovine serum, 100 units· mL^{-1} penicillin and 100 μg · mL^{-1} streptomycin. Approximately 1×10^5 cells were seeded in a 96-well microplate to a total volume of 100 μl/ well. After 24 h of cell attachment the culture media was removed and washed by Dulbecco□ s phosphate buffered saline (DPBS). The cells were then cultured in a medium with different concentrations of compound **1** for 24 h, subsequently the culture media was removed and the cells were washed with the culture medium. Cells in a culture medium without the fluorescent probe **1** were used as the control. Fresh medium (100 μL) containing MTT (10 μL, 5 mg · mL^{-1}) was added to each well and incubated for another 4 h to allow the formation of formazan dye. After removed the supernatant carefully, 150 μL dimethyl sulphoxide (DMSO) was added to each well to dissolve the purple products. The plate was shaken for 10 min and the absorbance values (A) were read at a wavelength of 570 nm by microplate reader (Synergy 2, Bio Tek Instruments Inc.). Cell viability was calculated by $A/A_0\times$ 100% (A and A_0 are the absorbance of experimental group and control group, respectively).

2.6. Confocal imaging in living cells

HeLa cells were plated on 35-mm glass-bottomed dishes and cultured in DMEM containing high glucose supplemented with 10% fetal bovine serum for 24 h. Before the experiments, the cells were washed with DPBS, followed by treated with the medium containing 5.0 μM probe **1** and 1.0 μM LysoTracker Red DND-99 for 30 min at 37 ℃, then by washing with DPBS three times and imaged. LysoTracker Red DND-99 (1.0 μM) was used to co-stain the cells to lysosome. The confocal fluorescence images were observed by an Olympus FV1200 laser scanning confocal microscope with 40× objective lens. Confocal fluorescence images were

observed at different detection channels (probe **1** channel, λ_{ex} = 405 nm; LysoTracker Red DND-99 channel, λ_{ex} = 555 nm)

3. Results and discussion

3.1. Spectroscopic analytical performance of probe 1

We studied the fluorescence spectra of probe **1** (5.0 μM) in 0.04 mol/L B-R buffer (DMF/water=1: 99, v/v) with various pH (Figure 1). From Figure 1, one can see that probe **1** showed almost no fluorescence at pH 6.46. However, fluorescence intensity increased dramatically when the pH value gone down from 6.46 to 3.44. It displays an approximately 19-fold increase in the emission intensities at around 432 nm within the pH range 6.46-3.44, which is valuable for studying acidic pH.

Insertion of Figure 1

The time-dependent fluorescence responses of probe **1** (5.0 μM) were also determined at pH 3.44, 4.96 and 6.46 (Figure S1). As shown in Figure S1, when probe **1** was added into the solutions with different pH, the fluorescence intensity reached equilibrium in a short time (less than 1 min). Therefore, the probe **1** can be used to detect pH of samples immediately.

The UV-vis absorption spectra of probe **1** (5.0 μM) at pH 3.44, 4.96 and 6.46 were recorded in Figure 2. The probe **1** (5.0 μM) in 0.04 mol/L B-R buffer (DMF/water=1: 99, v/v) with pH 6.46 exhibited weak absorption near 370 nm. At pH 3.44, the absorption band centered 370 nm increased obviously and did not accompany the shift of the band at 370 nm.

Insertion of Figure 2

3.2. Principle of operation and the basis of quantitative assay

The ability of the probe **1** to recognize proton was investigated at an excitation wavelength of 360 nm, with results illustrated in Figure 3. As shown in Figure 3, the linear response range covers the acidic pH range from 3.44 to 6.46. The relationship between the pH value and the fluorescence intensity at λ_{em} = 432 nm can be expressed quantitatively using a Henderson-Hasselbalch type equation (Equation (1)) [49]:

$$\log[\frac{I_{\max} - I}{I - I_{\min}}] = pH - pK_a \quad (1)$$

where I is the observed fluorescence intensity, I_{max} and I_{min} represent the maximum and minimum of the fluorescence intensity of the probe **1** in its acid (pH 3.00) and conjugate base

(pH 8.00), respectively.

Therefore, the linear response in Figure 3 can be expressed by the following equation (2) of the calibration line:

$$\log[\frac{I_{max}-I}{I-I_{min}}] = 0.9912\,pH - 4.2311 \quad (R^2=0.9853) \quad (2)$$

where R is the linear correlation coefficient.

According to the equation (2), the calculated pK_a value of probe **1** was 4.23.

Insertion of Figure 3

In order to determine the bonding site of proton, we got ^{1}H NMR and ^{13}C NMR spectra of probe **1** + CF_3COOD. The downfield shift of H(3") and H(5") 0.32 ppm at pH 3.44 indicated the protonation of nitrogen atom of the primary anilino group (Figure 4). The downfield shift of C(3") and C(5") 4.86 ppm at pH 3.44 also indicated the protonation of nitrogen atom of the primary anilino group (Figure 5). The HSQC spectra of probe **1** and probe **1**+CF_3COOD at pH 3.44 were shown in electronic supplementary information (ESI). To further confirm the proton sensing mechanism, we also got the electrospray mass spectrum of probe **1** + HCOOH in acetonitrile-water (1: 99) at pH 3.44 (Figure 6). A peak at *m/z* 391.2823 corresponding to the $[\mathbf{1} + H + H]^+$ was clearly observed, which indicated the formation of a **1**/ H^+ adduct of 1:1 stoichiometry at pH 3.44 (Figure 6). So we drew a conclusion that nitrogen atom of the primary anilino group was protonated and the nitrogen atoms of benzothiazole ring and diethylamino group were not protonated in acidic solution. The protonation process of probe **1** in acidic condition was shown in Scheme 2. Therefore, the primary anilino group as an electron-rich group efficiently quenched the fluorescence of the benzothiazole fluorophore through an enhanced PET process, giving a non-fluorescent compound. After protonation of nitrogen atom of the primary anilino group in acidic solution, the fluorescence enhancement of probe **1** occurred for the hindering of enhanced PET process.

Insertion of Figure 4

Insertion of Figure 5

Insertion of Figure 6

Insertion of Scheme 2

3.3. Effect of ionic strength

For a fluorescence pH probe, ionic strength is also regarded as a limiting factor for its application in real samples. The effect of ionic strength was examined by adjusting the buffer solution of pH 3.44 and 6.46 to ionic strength from 0.01 mol/L to 2.0 mol/L with sodium chloride (Figure S2). From Figure S2, it was found that with increasing the ionic strength the fluorescence intensity remained almost constant. So it was not necessary to maintain a certain ionic strength in later experiments.

3.4. Selectivity

In complex biological samples, a highly selective response to the target specie over other potentially competing species is necessary. To explore if probe **1** could still work well in complex environments, an additional examination was taken to test the selective response of common ions, anions and amino acids which were regard as potential interference substances. The relative fluorescence intensity of the probe **1** in the absence or presence of competitive species was shown in Figure 7. As displayed in Figure 7 there were no metal ions, anions or bioactive molecules cause significant changes in fluorescence intensity. These results reveal that probe **1** exhibited high selectivity toward H^+ over competitive species, which makes it feasible for practical purposes.

Insertion of Figure 7

3.5. Cytotoxicity assays and confocal imaging in living cells

In order to explore potential applications in real biological samples. First, the cytotoxicity of compound **1** was evaluated by the MTT assay (Figure S3). As shown in Figure S3, about 90% cell viability could be observed after the HeLa cells were treated with compound **1** for 24 h at concentrations between 0 μM and 16 μM, which indicated that probe **1** had almost no cytotoxicity to HeLa cells. Next, colocalization experiments were performed in HeLa cells using probe **1** and a commercially available lysosome-specific staining probe (LysoTracker Red DND-99) to confirm if probe **1** could be a lysosome-target probe. The HeLa cells were incubated with probe **1** (5.0 μM) and LysoTracker Red DND-99 (1.0 μM) for 30 min at 37 ℃, then the cells were washed with DPBS three times to remove the extracellular remaining probe **1** and imaged (Figure 8). As shown in Figure 8, the distribution of blue fluorescence from probe **1** co-localized with red fluorescence from LysoTracker Red DND-99, which suggest that probe **1** selectively stains lysosomes in living cells.

Insertion of Figure 8

4. Conclusions

In conclusion, a novel acidic pH fluorescent probe **1** based on a benzothiazole derivative has been reported. The synthesis of this probe **1** is facile and also it displays high sensitivity and selectivity, short response time, good membrane permeability and low cytotoxicity. The linear response range covers the acidic pH range from 3.44 to 6.46, which is applicable to investigate acidic environment. The evaluated p*Ka* value of the probe **1** is 4.23. The fluorescence enhancement of the studied probe **1** with an increase in hydrogen ions concentration is based on the hindering of enhanced PET process. Furthermore, this probe **1** was successfully applied to confocal fluorescence imaging in live HeLa cells and can selectively stain lysosomes. All in all, this probe **1** has good ability in detecting acidic pH conditions in solutions and it will be great beneficial to study chemical and biological systems.

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References

[1] J. Zhou, L. Zhang, Y. Tian, Anal. Chem. 88 (2016) 2113-2118.

[2] W. Pan, H. Wang, L. Yang, Z. Yu, N. Li, B. Tang, Anal. Chem. 88 (2016) 6743-6748.

[3] X. Gao, Y. Zhang, B. Wang, Org. Lett. 24 (2003) 4615-4618.

[4] Z.Z. Li, C.G. Niu, G.M. Zeng, Y.G. Liu, P.F. Gao, G.H. Huang, Y.A. Mao, Sens. Actuators, B-Chem. 114 (2006) 308-315.

[5] J.H. Cheng, Y.H. Zhang, X.F. Ma, X.G. Zhou, H.F. Xiang, Chem. Commun. 49 (2013) 11791-11793.

[6] S.A. Grant, K. Bettencourt, P.K. Rulevitch, J. Hamilton, R. Glass, Sens. Actuators, B-Chem. 72 (2001) 174-179.

[7] A.S. Jeevarajan, S.Vani, T.D. Taylor, M.M. Anderson, Biotechnol. Bioeng. 78 (2002) 467-472.

[8] A. Roos, W.F. Boron, Physiol. Rev. 61 (1981) 296-434.

[9] E.J. Blott, G.M. Griffiths, Nat. Rev. Mol. Cell Biol. 3 (2002) 122-131.

[10] J. Stinchcombe, G. Bossi, G.M. Griffiths, Science 305 (2004) 55-59.

[11] K.R. Hoyt, I.J. Reynolds, J. Neurochem. 71 (1998) 1051-1058.

[12] E.R. Chin, D.G. Alllen, J. Physiol. 512 (1998) 831-840.

[13] H. Izumi, T. Torigoe, H. Ishiguchi, H. Uramoto, Y. Yoshida, M. Tanabe, T. Ise, T. Murakami, T. Yoshida, M. Nomoto, K. Kohno, Cancer Treat. Rev. 29 (2003) 541-549.

[14] T.A. Davies, R.E. Fine, R.J. Johnson, C.A. Levesque, W.H. Rathbun, K.F. Seetoo, S.J. Smith, G. Strohmeier, L. Volicer, L. Delva, Biochem. Biophys. Res. Commun. 194 (1993) 537-543.

[15] R.J. Gillies, K. Ugurbil, J.A. Denhollander, R.G. Shulman, Proc. Natl. Acad. Sci. USA 78 (1981) 2125-2129.

[16] J.R. Chaillet, K. Amsler, W.F. Boron, Proc. Natl. Acad. Sci. USA 83 (1986) 522-526.

[17] J. Yin, Y. Hu, J. Yoon, Chem. Soc. Rev. 44 (2015) 4619-4644.

[18] J. Han, K. Burgess, Chem. Rev. 110 (2010) 2709-2728.

[19] B.K. McMahon, R. Pal, D. Parker, Chem. Commun. 49 (2013) 5363-5365.

[20] R. Gui, X. An, W. Huang, Anal. Chim. Acta 767 (2013) 134-140.

[21] Q. Lu, X.H. Su, L.Y. Wang, W. Sun, Y.B. Lei, Z.Y. Wen, J. Lumin. 154 (2014) 124-130.

[22] W. Pan, H. Wang, L. Yang, Z. Yu, N. Li, B. Tang, Anal. Chem. 88 (2016) 6743-4748.

[23] H. Yu, G. Li, B. Zhang, X. Zhang, Y. Xiao, J. Wang, Y. Song, Dyes Pigm. 133 (2016) 93-99.

[24] H.S. Lv, S.Y. Huang, B.X. Zhao, J.Y. Miao, Anal. Chim. Acta 788 (2013) 177-182.

[25] Q. Wang, L. Zhou, L. Qiu, D. Lu, Y. Wu, X.B. Zhang, Analyst 140 (2015) 5563-5569.

[26] X.J. Cao, L.N. Chen, X. Zhang, J.T. Liu, M.Y. Chen, Q.R. Wu, J.Y. Miao, B.X. Zhao, Anal. Chim. Acta 920 (2016) 86-93.

[27] K. Tanaka, T. Kumagai, H. Aoki, M. Deguchi, S. Iwata, J. Org. Chem. 66 (2001) 7328-7333.

[28] Z. Liu, C. Zhang, W. He, F. Qian, X. Yang, X. Gao, Z. Guo, New J. Chem. 34 (2010)

656-660.

[29] Y. Li, Y. Wang, S. Yang, Y. Zhao, L. Yuan, J. Zheng, R. Yang, Anal. Chem. 87 (2015) 2495-2503.

[30] J. Chao, Y. Liu, J. Sun, L. Fan, Y. Zhang, H. Tong, Z. Li, Sens. Actuators B 221 (2015) 427-433.

[31] E. Faggi, J. Serra-Vinardell, M.D. Pandey, J. Casas, G. Fabriâs, S.V. Luis, L. Alfonso, Sens. Actuators B 234 (2016) 633-640.

[32] M. Sun, L. Du, H. Yu, K. Zhang, Y. Liu, S. Wang, Talanta 162 (2017) 180-186.

[33] S. Liu, L. Zhang, X. Liu, New J. Chem. 37 (2013) 821-826.

[34] H. Wen, Q. Huang, X.F. Yang, H. Li, Chem. Commun. 49 (2013) 4956-4958.

[35] N. Khairnar, K. Tayade, S.K. Sahoo, B. Bondhopadhyay, A. Basu, J. Singh, N. Singh, V. Gite, A. Kuwar, Dalton Trans. 44 (2015) 2097-2012.

[36] A. Singh, A. Singh, N. Singh, D.O. Jang, Tetrahedron 72 (2016) 3535-3541.

[37] L. Geng, X.F. Yang, Y. Zhong, Z. Li, H. Li, Dyes Pigm. 120 (2015) 213-219.

[38] S. Erdemir, B. Tabakci, M. Tabakci, Sens. Actuators B 228 (2016) 109-116.

[39] L. Tang, Y. Zou, K. Zhong, Y. Bian, RSC Adv. 6 (2016) 48351-48356.

[40] Q. Huang, X.F. Yang, H. Li, Dyes Pigm. 99 (2013) 871-877.

[41] C. Liu, Z. Cao, Z. Wang, P. Jia, J. Liu, Z. Wang, B. Han, X. Huang, X. Li, B. Zhu, X. Zhang, Sens. Actuators B 220 (2015) 727-733.

[42] X. Ren, F. Wang, J. Lv, T. Wei, W. Zhang, Y. Wang, X. Chen, Dyes Pigm. 129 (2016) 156-162.

[43] S. Feng, X. Li, Q. Ma, B. Liang, Z. Ma, Anal. Methods 8 (2016) 6832-6839.

[44] H. Zhu, J. Fan, J. Wang, H. Mu, X. Peng, J. Am. Chem. Soc. 136 (2014) 12820-12823.

[45] Y.X. Liao, M.D. Wang, K. Li, Z.X. Yang, J.T. Hou, M.Y. Wu, Y.H. Liu, X.Q. Yu, RSC Adv. 5 (2015) 18275-18278.

[46] X.H. Zhou, Y.R. Jiang, X.J. Zhao, D. Guo, Talanta 160 (2016) 470-474.

[47] Y. Koide, Y. Urano, S. Kenmoku, H. Kojima, T. Nagano, J. Am. Chem. Soc. 120 (2007) 10324-10325.

[48] R. I. Freshney, Culture of animal cells: a manual of basic technique, 5th ed., John Wiley & Sons Inc, Hoboken, 2005.

[49] Q.J. Ma, H.P. Li, F. Yang, J. Zhang, X.F. Wu, Y. Bai, X.F. Li, Sens. Actuators B 166-167 (2012) 68-74.

Captions of figures

Scheme 1. Synthesis of fluorescent probe **1**: (a) $Na_2S_2O_5$ aqueous solution, DMF, 90 ℃, 12 h, 82%; (b) 4-chloronitrobenzene, K_2CO_3, DMF, 80 ℃, 9 h, 45%; (c) $SnCl_2{\cdot}2H_2O$, EtOH-HCl (1:1, v/v), 60 ℃, 5 h, 77%.

Scheme 2. Proposed protonation process of probe **1** in acidic condition.

Figure 1. Fluorescence spectra of probe **1** (5.0 μM) in 0.04 mol/L Britton-Robinson buffer (DMF/water=1: 99, v/v) with various pH: (1) 8.00, (2) 7.47; (3) 6.67, (4) 6.46, (5) 6.14, (6) 5.90, (7) 5.70; (8) 5.33, (9) 4.96, (10) 4.80, (11) 4.52, (12) 4.31, (13) 4.10, (14) 3.83, (15) 3.66, (16) 3.44, (17) 3.20, (18) 3.00. The excitation wavelength is 360 nm. Inset: pH dependence of the fluorescence emission intensity of probe **1**.

Figure 2. UV-vis absorption spectra of probe **1** (5.0 μM) in 0.04 mol/L Britton-Robinson buffer (DMF/water=1: 99, v/v) with pH 3.44, 4.96 and 6.46.

Figure 3. Plot of $\log[(I_{max}-I)/(I-I_{min})]$ as a function of the pH.

Figure 4. ^{1}H NMR spectra of probe **1** (a) and probe **1**+CF_3COOD at pH 3.44 (b).

Figure 5. ^{13}C NMR spectra of probe **1** (a) and probe **1**+CF_3COOD at pH 3.44 (b).

Figure 6. Electrospray mass spectrum of probe **1** + HCOOH in acetonitrile-water (1: 99) at pH 3.44.

Figure 7. Fluorescence intensity of probe **1** (5.0 μM) in the presence of various analytes in 0.04 mol/L Britton-Robinson buffer (DMF/water=1: 99, v/v) with pH 3.44 (a) and 6.46 (b). (1) blank, (2) Li^+, (3) Na^+, (4) K^+, (5) Mg^{2+}, (6) Ca^{2+}, (7) Al^{3+}, (8) Hg^{2+}, (9) Cu^{2+}, (10) Pb^{2+}, (11) Zn^{2+}, (12) Ag^+, (13) Mn^{2+}, (14) Ba^{2+}, (15) Co^{2+}, (16) Ni^{2+}, (17) Cr^{3+}, (18) Fe^{3+}, (19) $H_2PO_4^-$, (20) CH_3COO^-, (21) PO_4^{3-}, (22) HCO_3^-, (23) Cl^-, (24) SO_4^{2-}, (25) NO_3^-, (26) CO_3^{2-}, (27) GSH, (28) Cys, (29) Hcy, (30) Ala, (31) His, (32) Arg. The excitation wavelength is 360 nm and the emission wavelength is 432 nm. The concentration is 5.0 μM for all the analytes.

Figure 8. Fluorescence microscope images of living HeLa cells co-stained with 5.0 μM probe **1** and 1.0 μM LysoTracker Red DND-99. (a) Blue emission from probe **1**; (b) red emission from LysoTracker Red DND-99; (c) overlay of (a) and (b), areas of co-localization are visualized in burgundy.

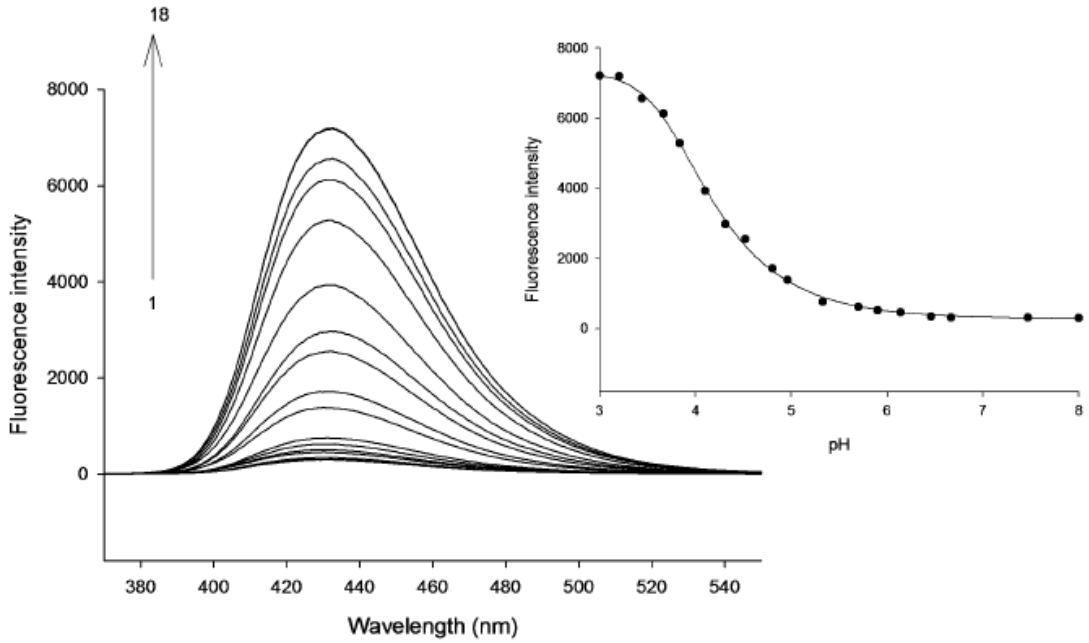


Figure 1

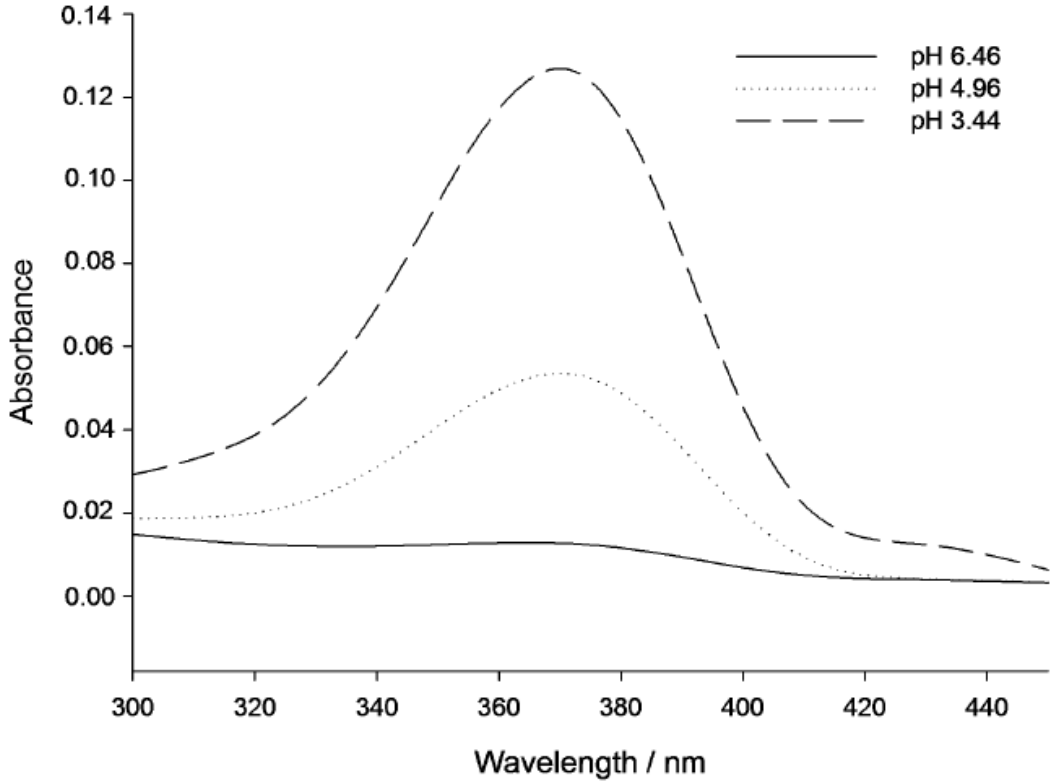


Figure 2

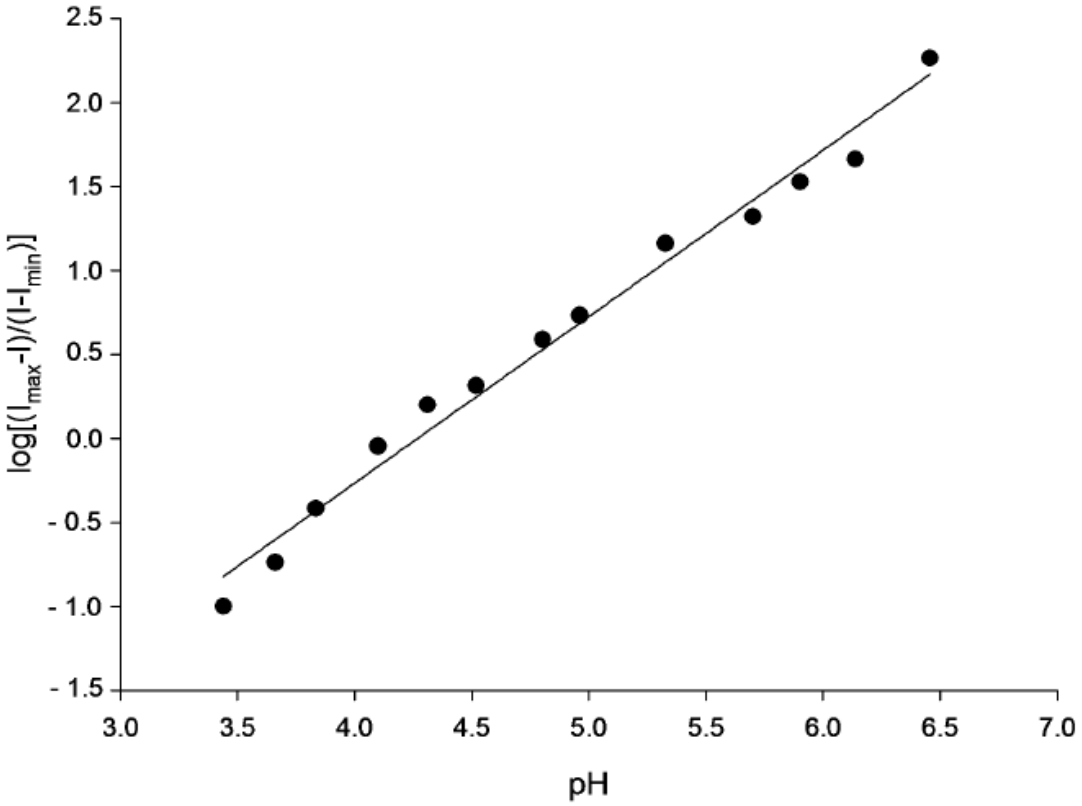


Figure 3

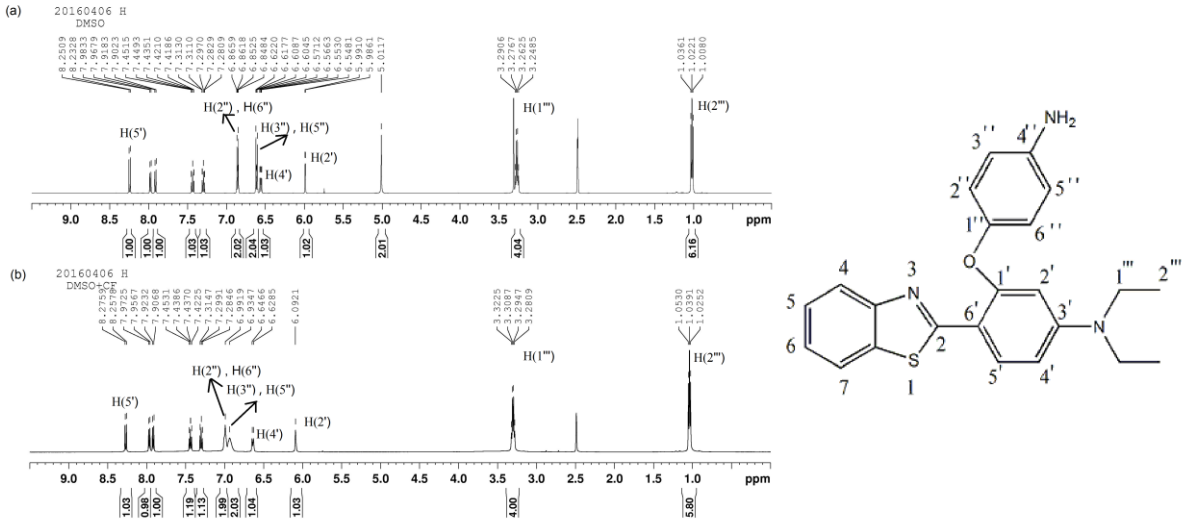


Figure 4

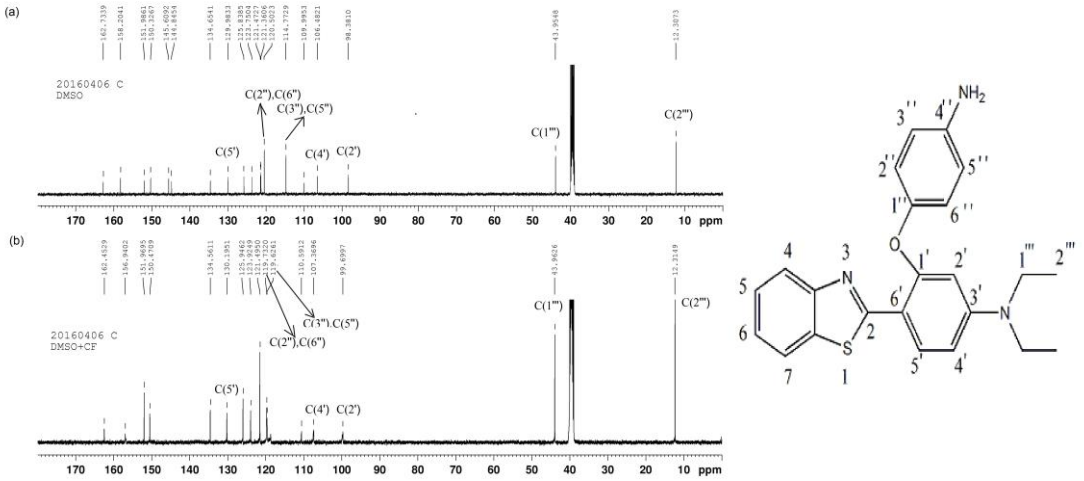


Figure 5

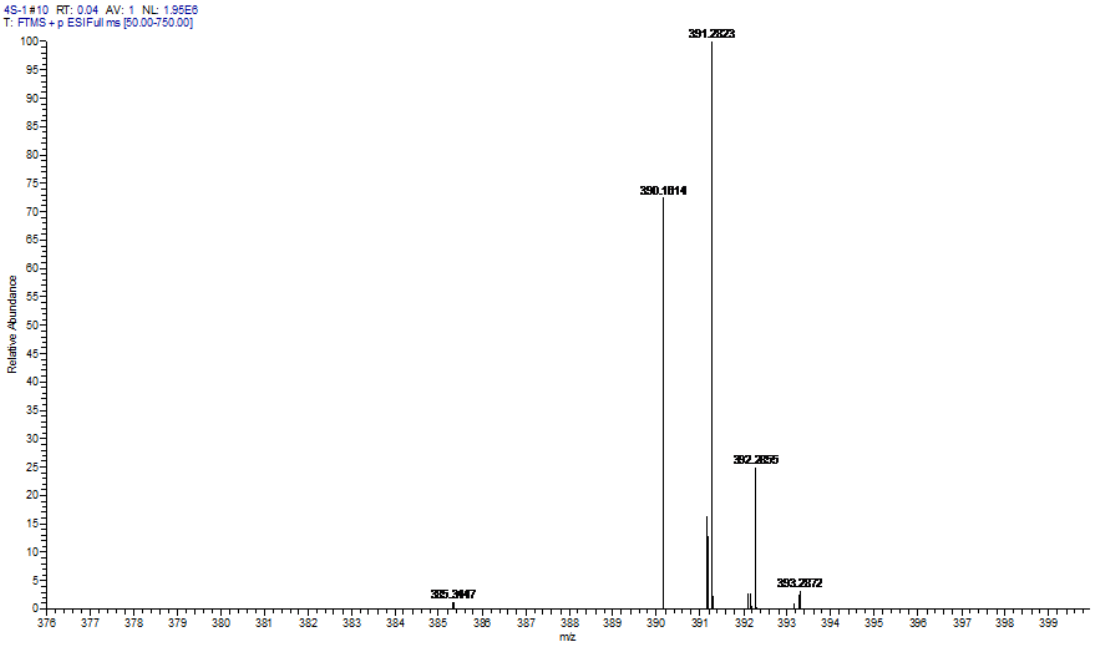


Figure 6

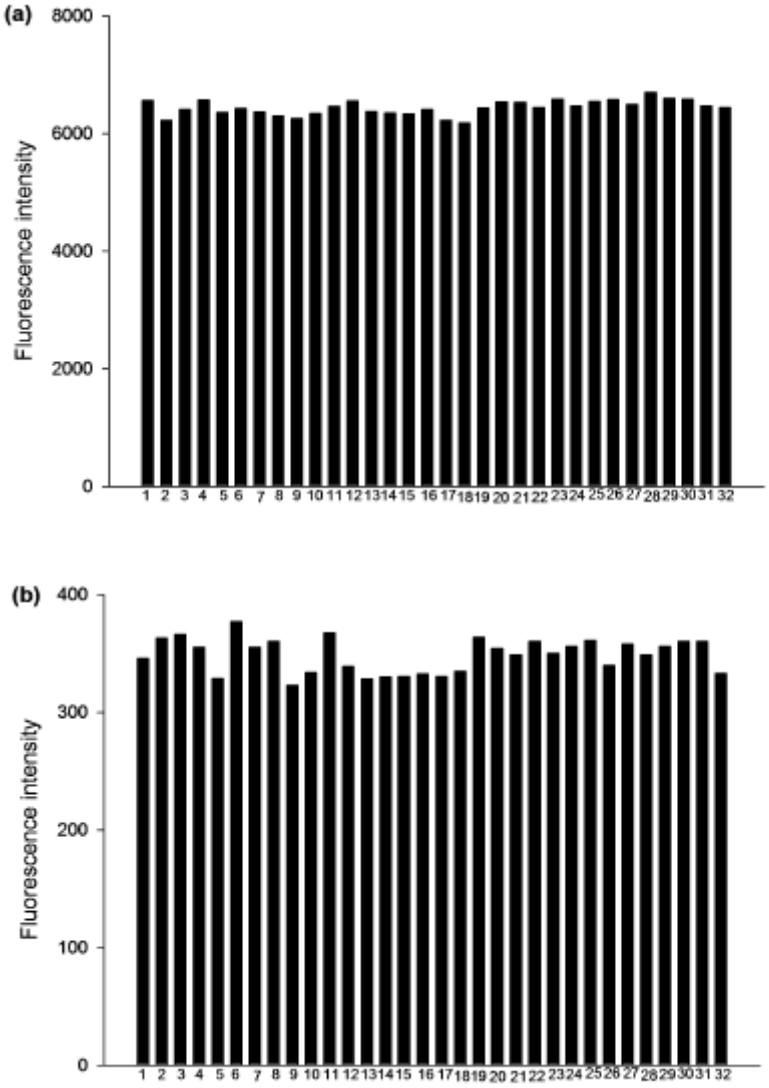


Figure 7

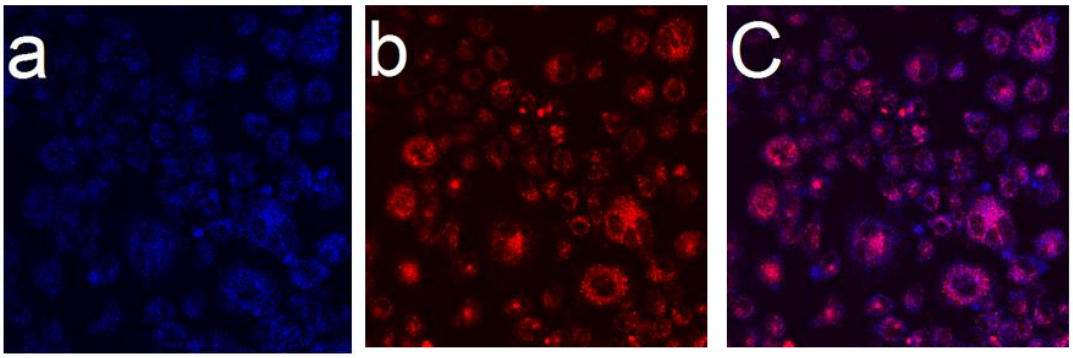
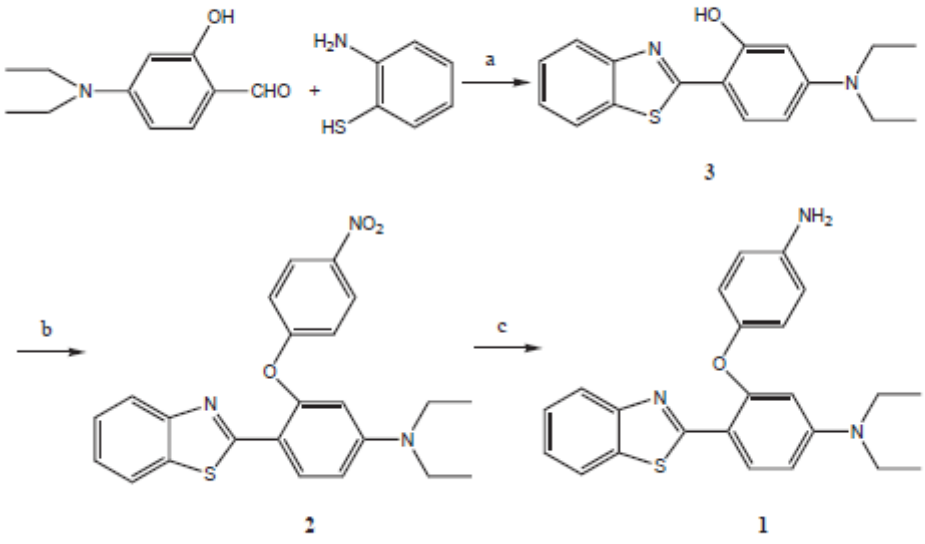
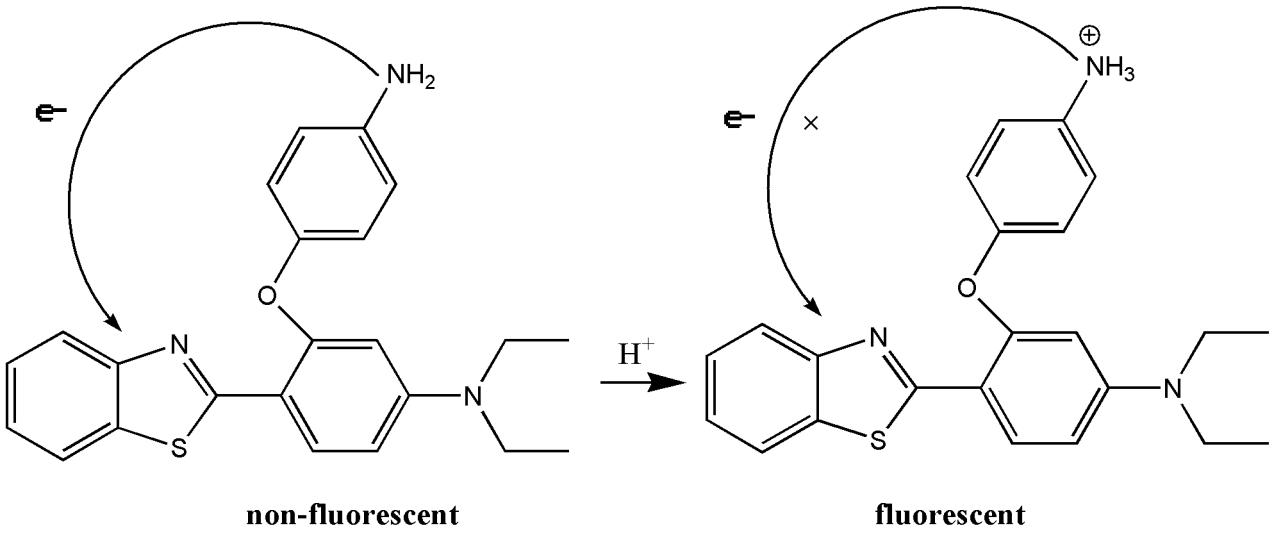


Figure 8

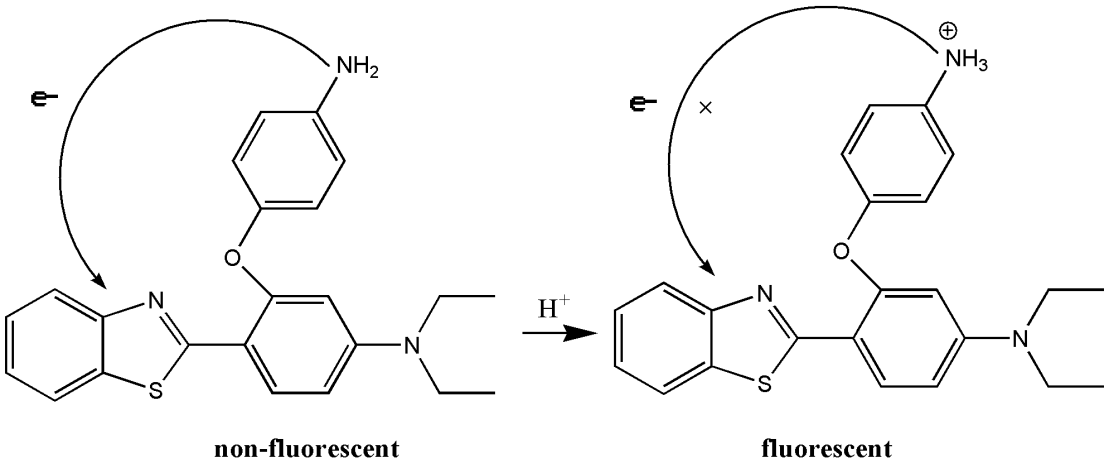


Scheme 1



non-fluorescent **fluorescent**

Scheme 2



non-fluorescent **fluorescent**

Graphical abstract

Highlights

1) A novel acidic pH fluorescent probe has been reported.

2) The linear response range covers the acidic pH range from 3.44 to 6.46.

3) The design of fluorescent pH probe is based on enhanced PET process.

4) The probe displays high sensitivity, high selectivity and short response time.

5) The probe has been successfully applied to fluorescence imaging in living cells.