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RESEARCH ARTICLE

A ratiometric fluorescent chemosensor for the detection of cysteine in aqueous solution at neutral pH

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Abstract

A ratiometric fluorescent chemosensor 2-(2′-hydroxy-3′-formyl-5′-methoxyphenyl) benzothiazole (**1**) was developed for the detection of cysteine (Cys). In aqueous solution at neutral pH, **1** exhibited a ratiometric fluorescent response to Cys with a remarkable red-to-green shift in the emission wavelength. This fluorescence change was attributed to the cyclization reaction between the formyl group in **1** and the amino and sulfhydryl group in Cys in a stoichiometry of 1: 1 according to the proposed mechanism. At neutral pH, **1** displayed a significant fluorescence ratio signal enhancement with the addition of Cys. Furthermore, **1** showed good selectivity toward Cys. The detection limit and linear range were 5.6 and 0–100 μmol/L, respectively, which demonstrated that **1** could recognize relatively low concentrations of Cys and is a good candidate for applications in detecting Cys.

KEYWORDS

aqueous solution, chemosensor, cysteine, ratiometric fluorescence

1 | INTRODUCTION

Cysteine (Cys), an essential amino acid, has crucial roles in a variety of physiological processes.[1,2] An abnormal Cys content is clinically relevant to many diseases. For example, high levels of Cys may be associated with neurotoxicity, whereas a Cys deficiency can cause skin lesions, hair depigmentation and liver damage.[3–6] Therefore, the development of efficient methods for detecting and quantifying Cys is of great importance. To date, various strategies, including electrochemical voltammetry,[7] mass spectrometry,[8] high-performance liquid chromatography,[9] colorimetric[10] and phosphorescent,[11] have been developed for this purpose. As an alternative technique, the fluorescent chemosensor has received increasing attention in recent years due to its advantages of convenience, high sensitivity, selectivity and real-time response.[12–14]

The key to a fluorescence-sensing technique is to design a fluorescent chemosensor with specific moieties and characteristics. In the past few years, various fluorescent chemosensors for the detection of Cys have been developed.[15–21] Unfortunately, most exhibit fluorescence enhancement or fluorescence quenching upon the addition of analytes. These chemosensors, recognize analytes by increasing or decreasing only a single fluorescence intensity. However, the fluorescence intensity may be influenced by factors independent of the target species, for example, environmental or instrumental effects. By contrast, ratiometric fluorescent chemosensors effectively avoid this influence by measuring emission intensities at two different wavelengths and comparing the values.[22,23] This method could provide a built-in correction for the external environmental or instrumental effects, and supply more accurate data analysis compared with single-channel detection.[23] Thus, the development of ratiometric fluorescent chemosensors for the selective detection of Cys is urgently needed.

In this work, a ratiometric fluorescent chemosensor 2-(2′-hydroxy-3′-formyl-5′-methoxyphenyl)benzothiazole (**1**) was developed for the detection of Cys. In aqueous solution at neutral pH, a gradual decrease in the fluorescence intensity at 586 nm and a progressive increase in a new emission at 517 nm was observed with 1 h upon addition of Cys to **1**. A 17-fold enhancement in the emission ratio was achieved, which was desirable for ratiometric chemosensors. The proposed chemosensor exhibited good selectivity toward Cys. The detection limit and linear range were 5.6 and 0–100 μmol/L, respectively.

Abbreviations used: EtOH, Ethanol; HRMS, High-resolution mass spectrometry; NMR, Nuclear magnetic resonance; PBS, Phosphate-buffered saline; UV, Ultraviolet; Vis, Visible.

2 | EXPERIMENTAL

2.1 | Materials and methods

All the reagents in this work were of analytical grade and used without further purification. 2-Hydroxy-4-methoxybenzaldehyde, 2-hydroxy-4-methylbenzaldehyde, urotropine, 2-aminothiophenol and trifluoroacetic acid were from J&K Chemical Co. (Beijing, China). All the other materials were purchased from Sinopharm Chemical Reagent Co. (Beijing, China). Deionized water was used throughout. Phosphate-buffered saline (PBS) (50 mmol/L) at known pH was prepared by adding Na_2HPO_4, NaH_2PO_4 and HCl/NaOH to deionized water and adjusting with a pH meter. Unless mentioned, all the absorption and fluorescence spectra were recorded at 25°C.

Absorption spectra were recorded on a JASCO-750 UV/Vis spectrophotometer, 5 cm quartz cell. Fluorescence spectra were measured on a JASCO-FP-8300 fluorescence spectrophotometer, 1 cm quartz cell. The pH was adjusted by a METTLER TOLEDO FE20/EL20 pH meter. ^{1}H and ^{13}C NMR spectra were recorded by a Bruker 400 Avance NMR spectrometer operated at 400 MHz. High-resolution mass spectrometry (HRMS) was undertaken using a Shimadzu LCMS-IT/TOF liquid chromatograph mass spectrometer without using the LC part. Photographs were taken using a Nikon D5500 camera.

2.2 | Synthesis and characterization the compounds

The synthetic route for compounds **1a**, **2a**, **1** and **2** are shown in Scheme 1.

2.2.1 | 2-(2′-Hydroxy-5′-methoxyphenyl)benzothiazole (1a)

2-Hydroxy-4-methoxybenzaldehyde (4.56 g, 30 mmol) was dissolved in 30 ml EtOH, followed by the addition of 2-aminothiophenol (3.75 g, 30 mmol) and 5 ml 30% H_2O_2. The mixture was stirred at 80°C for 12 h to form a yellow precipitate. The product was then filtered and collected. After being dried under reduced pressure, 5.01 g of **1a** was obtained (yield 65%). HRMS spectrometry: *m/z* calcd for $[M + H]^+$: 258.0583; found: 280.0585. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.06 (s, 1H), 8.14 (d, J = 7.56 Hz, 1H), 8.08 (d, J = 8.25 Hz, 1H), 7.73 (s, 1H), 7.55 (t, J = 7.89 Hz, 1H), 7.47 (t, J = 7.92 Hz, 1H), 7.05 (s, 1H), 7.04 (s, 1H), 3.81 (s, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm): 164.88, 152.80, 151.96, 150.93, 135.29, 126.92, 125.51, 122.72, 122.49, 120.22, 119.21, 118.56, 111.61, 56.11.

2.2.2 | 2-(2′-Hydroxy-3′-formyl-5′-methoxyphenyl) benzothiazole (1)

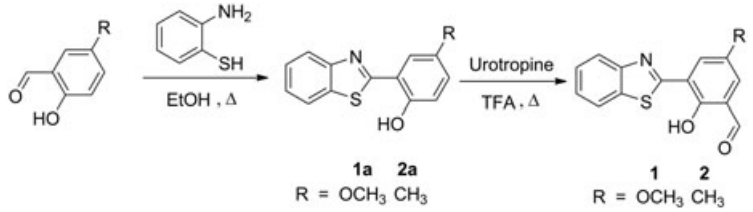
1a (2.57 g, 10 mmol) was mixed with 30 ml trifluoroacetic acid, then stirred and heated to 75°C until it dissolved. Urotropine (4.2 g, 30 mmol) was then added and the mixture was stirred at 80°C for 12 h. The solvent was removed by vacuum distillation to obtain a crude product. The crude product was purified on a silica-gel column using petroleum ether/ethyl acetate (5: 1 *v*/v) as the eluent. 0.77 g of **1** was obtained with a yield of 27%. HRMS spectrometry: *m/z* calcd for $[M + H]^+$: 286.0532; found: 286.0533. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.34 (s, 1H), 10.33 (s, 1H), 8.20 (d, J = 7.20 Hz, 1H), 8.13 (d, J = 7.89 Hz, 1H), 7.94 (d, J = 3.45 Hz, 1H), 7.60 (t, J = 6.87 Hz, 1H), 7.52 (d, J = 6.87 Hz, 1H), 7.47 (m, 1H), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm): 190.25, 166.95, 155.30, 152.25, 151.46, 133.13, 127.09, 126.15, 124.32, 122.53, 121.71, 121.62, 119.48, 115.02, 56.22.

2.2.3 | 2-(2′-Hydroxy-5′-methylphenyl)benzothiazole (2a)

2-Hydroxy-4-methylbenzaldehyde (4.1 g, 30 mmol) was dissolved in 30 ml EtOH, followed by the addition of 2-aminothiophenol (3.75 g, 30 mmol) and 5 ml 30% H_2O_2. The mixture was stirred at 80°C for 12 h to form a yellow precipitate. The product was then filtered and collected. After being dried under reduced pressure, 4.77 g of **2a** was obtained (yield 59%). HRMS spectrometry: *m/z* calcd for $[M + H]^+$: 242.0634; found: 280.0630. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.39 (s, 1H), 8.14 (d, J = 7.89 Hz, 1H), 8.06 (d, J = 7.56 Hz, 1H), 7.98 (s, 1H), 7.55 (t, J = 7.23 Hz, 1H), 7.45 (t, J = 7.23 Hz, 1H), 7.23 (d, J = 8.22 Hz, 1H), 7.00 (d, J = 8.25 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm): 165.87, 154.74, 152.00, 134.77, 133.77, 128.94, 128.82, 126.97, 125.56, 122.58, 122.52, 118.44, 117.41, 20.57.

2.2.4 | 2-(2′-Hydroxy-3′-formyl-5′-methylphenyl) benzothiazole (2)

2a (2.41 g, 10 mmol) was mixed with 30 ml trifluoroacetic acid, stirred and heated to 75°C until it dissolved. Urotropine (4.2 g, 30 mmol) was added and the mixture was stirred at 80°C for 12 h. The solvent was removed by vacuum distillation to obtain a crude product. The crude product was purified on a silica-gel column using petroleum ether/ethyl acetate (5: 1 *v*/v) as the eluent. 0.65 g of **2** was obtained with a yield of 24%. HRMS spectrometry: *m/z* calcd for $[M + H]^+$: 270.0583; found: 270.0584. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.75 (s, 1H), 10.33 (s, 1H), 8.21 (m, 2H), 8.12 (d, J = 8.25 Hz, 1H), 7.72 (s, 1H), 7.60 (t, J = 7.80 Hz, 1H), 7.51 (t, J = 6.87 Hz, 1H), 2.39 (s, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm): 190.79, 166.98, 158.54, 151.52,



SCHEME 1 Synthesis of the compounds

135.09, 133.10, 132.48, 128.90, 126.88, 125.86, 123.78, 122.39, 121.62, 118.64, 77.59, 20.37.

3 | RESULTS AND DISCUSSION

3.1 | Ratiometric fluorescence response of 1 toward Cys

The reaction between **1** and Cys was first investigated using the time-dependent fluorescence and absorption spectra. As shown in Figure 1(a), the absorption peak at 467 nm decreased gradually and a new band at 359 nm appeared within 1 h after the addition of Cys (500 μmol/L) to **1** (10 μmol/L). Two isobestic points at 395 and 332 nm could be observed, which suggested that new compound was formed. Analogous changes could also be found in the fluorescence spectra of **1** upon the addition of Cys (Figure 1b). **1** exhibited a fluorescence peak at 586 nm in the absence Cys. When Cys was added, this peak (586 nm) decreased slowly, and a new emission peak at 517 nm emerged and gradually grew intense, indicating a ratiometric fluorescence response of **1** to Cys. A 17-fold enhancement in the emission ratio (I_{517}/I_{586}) was achieved after the addition of Cys for 1 h. The fluorescence response of red to green can be observed by naked eye upon UV light irradiation (Figure 1b inset), which demonstrated that **1** could serve as a 'naked-eye' chemosensor for Cys.

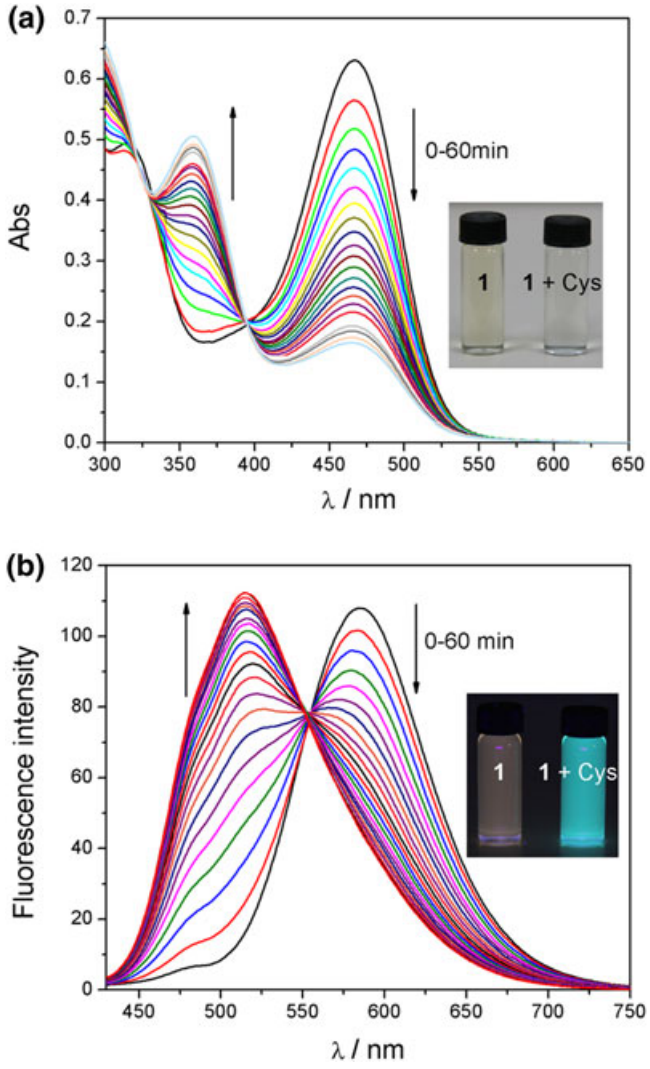


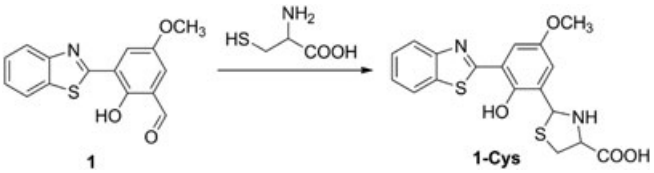
FIGURE 1 (a) absorption and (b) fluorescence spectra of **1** (10 μmol/L) upon addition of Cys (500 μmol/L). (inset) **1** in the absence and presence of Cys in a glass cuvette excited by (a) sunlight and (b) UV light. Conditions: 90% water/DMF (v/v) at pH 7.0 buffered by 50 mmol/L PBS. Excitation was at 415 nm

3.2 | Proposed mechanism for the detection of Cys

According to reports, the formyl group is an active site in the reaction of chemosensors with Cys.[5,24] Thus, a proposed mechanism is shown in Scheme 2. After a cyclization reaction between the formyl group in **1** and the amino and sulfhydryl group in Cys, **1** became **1-Cys** along with a blue-shift in the fluorescence. To verify this proposed mechanism, a series of experiments were performed. First, the product of **1** with Cys was characterized by HRMS. As shown in Figure 2, a clear peak at *m/z* 389.0625 ([**1-Cys** + H]$^+$, cald. 389.0624) was observed after the reaction between **1** and Cys. More importantly, the isotopic patterns corresponded very well with those calculated for **1-Cys**. This result clearly revealed that the product of the reaction between **1** and Cys was **1-Cys**.

Besides the HRMS results, more evidence for the reaction products of **1** and Cys was obtained from ^{1}H NMR experiments. Figure 3 shows the aromatic proton peaks in the ^{1}H NMR spectra of **1** before and after the reaction with Cys. As shown in Figure 3(a), the formyl in **1** resonated at 10.33 ppm (proton a). After addition of Cys, new peaks were seen at 5.72 and 5.91 ppm (proton b), which were assigned to the methine proton of the thiazolidine diastereomer in **1-Cys** (Figure 3b and c, proton b).[25] Moreover, the integrations revealed the transition from proton a to proton b visually: when 0.5 equiv. Cys was added to **1** and reacted for 1 h, the integration of proton a was 0.49 and the integration of proton b was 0.51, which indicated that half of compound **1** has become **1-Cys**. When 1 equiv. Cys was added and reacted for 1 h, the peak at 10.33 ppm disappeared and the integration of proton b increased to 1.00, demonstrating that all of compound **1** had become **1-Cys**. These facts also proved that the cyclization reaction between the chemosensor and Cys was in a stoichiometric ratio of 1: 1.

According to the proposed mechanism, the methoxy unit was not the response group for Cys. However, it has an important influence on the fluorescence wavelength of **1**. To understand this phenomenon, control compound **2** was designed and synthesized, in which the methoxy group was replaced by methyl group. As can be seen in Figure 4, **2** exhibited similar ratiometric fluorescence response to Cys. Nevertheless, the emission wavelengths of **2** before and after the addition of Cys were shorter than that of **1**. These results indicated



SCHEME 2 Proposed mechanism for the reaction of **1** with Cys

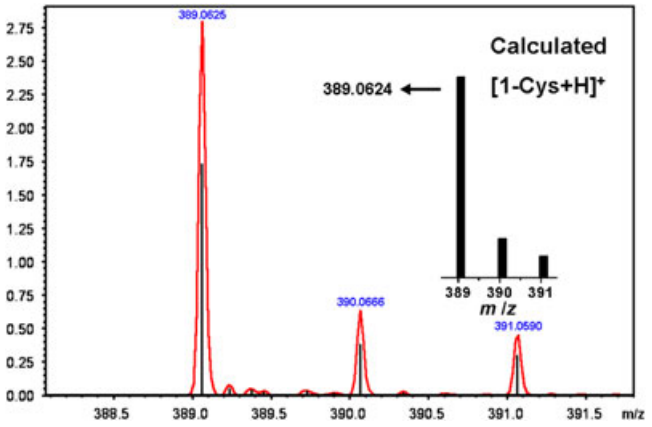


FIGURE 2 HRMS spectrum of the product of **1-Cys**. The inset shows calculated isotopic patterns

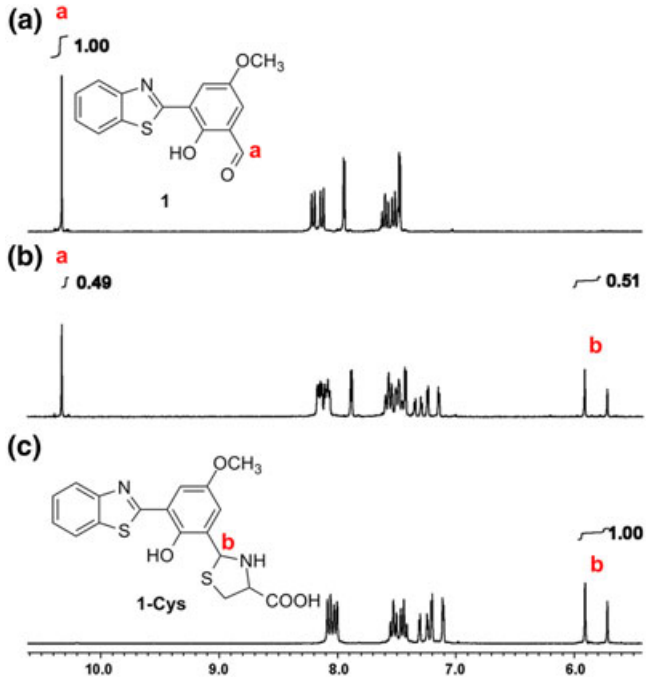


FIGURE 3 ^{1}H NMR spectra of **1** in DMSO-d_6 after the addition of (a) 0, (b) 0.5 and (c) 1 equiv. Cys

that an electron donating group in **1** is beneficial to its long wavelength emission.

3.3 | Effect of reaction time and pH values

The dynamics of the reaction between **1** and Cys was investigated by studying the time-dependent fluorescence spectra at 517 nm. As shown in Figure 5, the fluorescence intensity at 517 nm increased almost linearly within 1 h in the presence of 0–100 μmol/L Cys. It can also be seen that increases in the concentration of Cys accelerate the reaction rate. In the presence of 500 μmol/L Cys, the fluorescence intensity at 517 nm for **1** increased quickly at first, and barely changed after reacting for 1 h. Because a long reaction time does is not favorable for practical detection, an appropriate reaction time of 1 h was preferred in these experiments to obtain a good signal magnification.

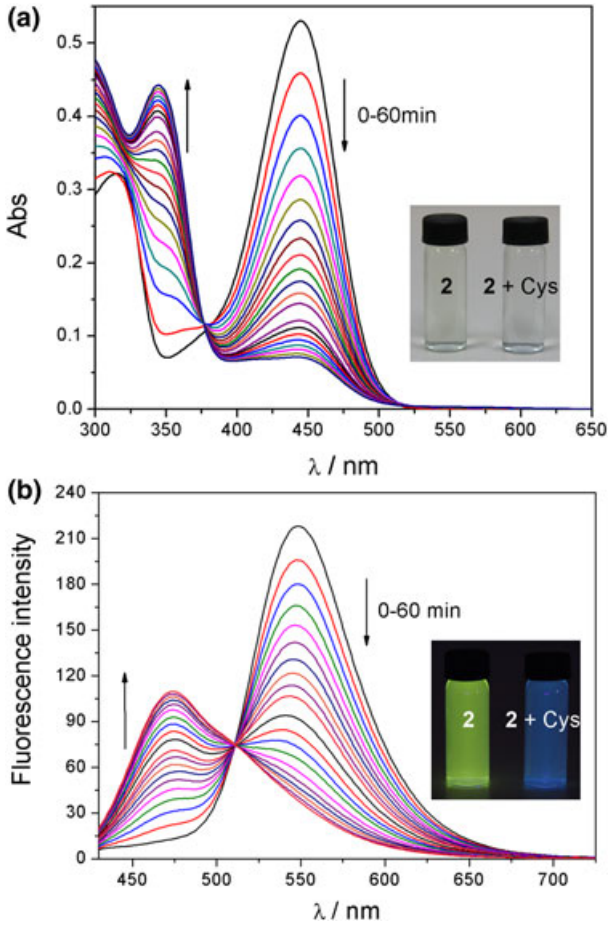


FIGURE 4 (a) absorption and (b) fluorescence spectra of **2** (10 μmol/L) upon addition of Cys (500 μmol/L). (inset) **2** in the absence and presence of Cys in a glass cuvette excited by (a) sunlight and (b) UV light. Conditions: 90% water/DMF (*v*/v) at pH 7.0 buffered by 50 mmol/L PBS. Excitation was at 415 nm

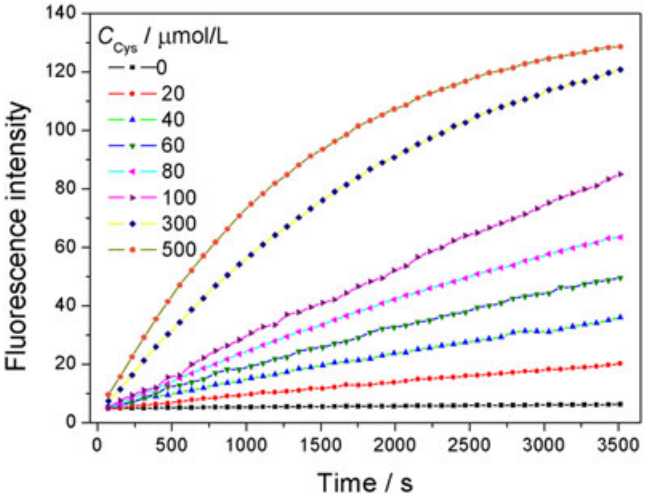


FIGURE 5 The fluorescence intensity at 517 nm of **1** (10 μmol/L) in the presence of different concentrations of Cys as a function of reaction time. Conditions: 90% water/DMF (*v*/v) at pH 7.0 buffered by 50 mmol/L PBS. Excitation was at 415 nm

The pH of the solution was essential to the reaction between **1** and Cys. To investigate the pH effect, the fluorescence intensity ratio I_{517}/I_{586} in the absence and presence of 100 μmol/L Cys was examined

over a pH range of 4.0–11.0. As shown in Figure 6, the fluorescence intensity ratio signals before (**1**) and after (**1-Cys**) the addition of Cys increased with pH. Signal magnification reached a maximum at pH 7.0. Thus, pH 7.0 was suggested as suitable for Cys quantification using **1** as a chemosensor.

3.4 | Selectivity of 1 toward Cys over other amino acids

To further evaluate the selectivity of **1** toward Cys over other amino acids, the fluorescence intensity ratio (I_{517}/I_{586}) of **1** was measured in the presence of amino acids essential for the human body (Figure 7). Upon the addition of amino acids to solutions of **1**, a remarkable increase in the fluorescence intensity ratio was observed for Cys. By contrast, no significant changes in fluorescence were induced by other amino acids expect for Lys, which was due to a cyclization reaction between its amino groups and the formyl group in **1**. This result suggested that **1** is a good Cys-selective chemosensor, which could distinguish Cys from most other amino acids.

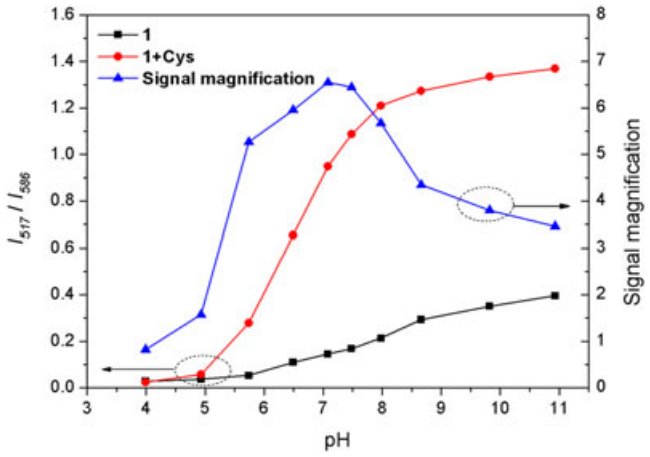


FIGURE 6 The fluorescence intensity ratio (I_{517}/I_{586}) of **1** (10 μmol/L) in the absence and presence of Cys (100 μmol/L) as a function of pH. The blue line shows the corresponding signal magnifications. Conditions: 90% water/DMF (v/v) at different pH values buffered by 50 mmol/L PBS. Excitation was at 415 nm, and the reaction time was 1 h

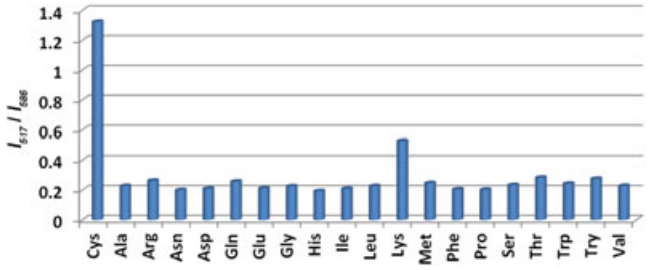


FIGURE 7 The fluorescence intensity ratio (I_{517}/I_{586}) of **1** (10 μmol/L) in the presence of different amino acids (100 μmol/L). Conditions: 90% water/DMF (v/v) at pH 7.0 buffered by 50 mmol/L PBS. Excitation was at 415 nm, and the reaction time was 1 h

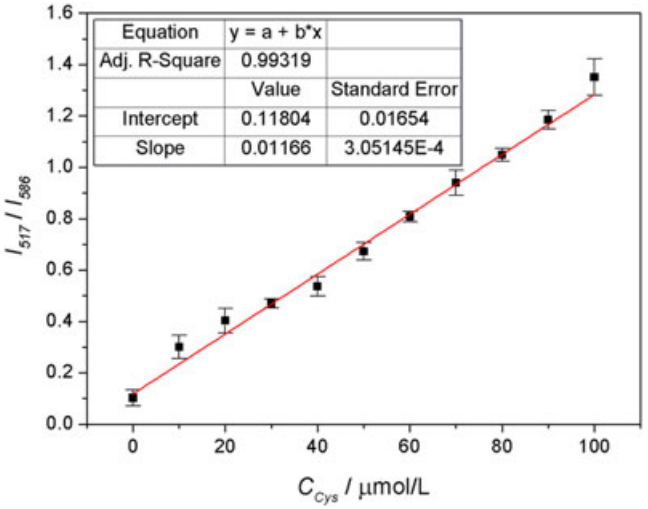


FIGURE 8 Effects of different concentrations of Cys (0–100 μmol/L) on the fluorescence intensity ratio (I_{517}/I_{586}) of **1** (10 μmol/L). Conditions: 90% water/DMF (v/v) at pH 7.0 buffered by 50 mmol/L PBS. Excitation was at 415 nm, the reaction time was 1 h

3.5 | Analytical applications

The linear response and detection limit of **1** upon addition of Cys were measured under the optimal conditions at pH 7.0. As shown in Figure 8, a linear relationship (0–100 μmol/L) was observed between the fluorescence intensity ratio (I_{517}/I_{586}) and the Cys concentration. The correlation coefficient (R^2) was calculated to be 0.99 (n = 3). Furthermore, according to the equation of $C_{DL} = 3S_b m^{-1}$ defined by IUPAC (where S_b is the standard deviation from 10 blank solutions and m is the slope of the calibration curve), the detection limit was 5.6 μmol/L. Thus, it can be concluded that **1** exhibited high sensitivity toward Cys and could be used to recognize Cys at a fairly low concentration.

4 | CONCLUSION

In conclusion, we have developed **1** as a new fluorescent chemosensor for Cys detection. Benefitting from the cyclization reaction with Cys in a stoichiometric ratio of 1: 1, chemosensor **1** displayed high sensitivity and selectivity ratiometric fluorescence in response to Cys in aqueous solution at neutral pH. A remarkable red-to-green shift in emission wavelength was observed with 17-fold enhancement in the emission ratio. A broad linear range of 0–100 μmol/L and a low detection limit of 5.6 μmol/L were obtained, which demonstrated that **1** could be used as a good ratiometric fluorescent chemosensor for detecting Cys at relatively low concentrations.

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