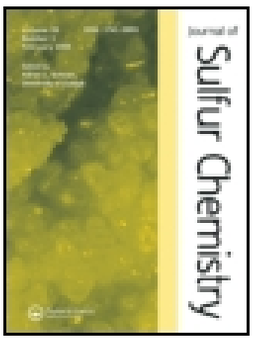

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Highly selective probes of copper(II) complexes for sulfide detection and cytotoxicity assay

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ABSTRACT

Two probes based on novel Copper(II) complexes were developed in order to obtain H_2S molecular probes with higher selectivity. The molecular structures of the complexes were characterized by ^1H NMR, HRMS, IR and elemental analysis. The interaction of the compounds with biologically important anions and amino acids was determined by UV-vis and Fluorescence titration experiments. Results indicated that the compounds showed the highest binding ability for HS^- among studied anions (AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and amino acids (GSH, HCys and Cys) accompanied by blue shift phenomenon in pure DMSO and aqueous solution. The possible mechanism of host–guest interaction may be that the Copper(II) ion of complex was captured by HS^- and free ligand released which showed a remarkable changes in UV-vis absorption. In addition, cytotoxicity of the synthesized compounds was studied on MCF-7 cells. Results indicated that the synthesized compounds had low cytotoxicity over a concentration range of 0–150 $\mu g \cdot mL^{-1}$, which exhibited that the synthesized probes could be used to detect H_2S *in vivo*.

The probes based on novel copper(II) complexes were synthesized and obtained by the reaction of substituent salicylaldehyde with amine derivatives, and then reacted with $Cu(CH_3COO)_2 \cdot H_2O$, respectively. Investigation on the interference from other species suggested that copper(II) complexes have high selectivity for HS^- over other anions (AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-), followed by the release of the copper ion to give a remarkable increase in UV–VIS absorption in pure DMSO solution and aqueous solution.

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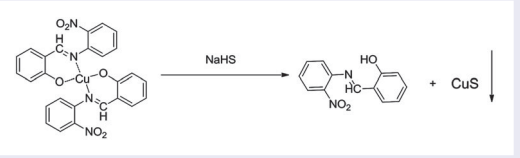


1. Introduction

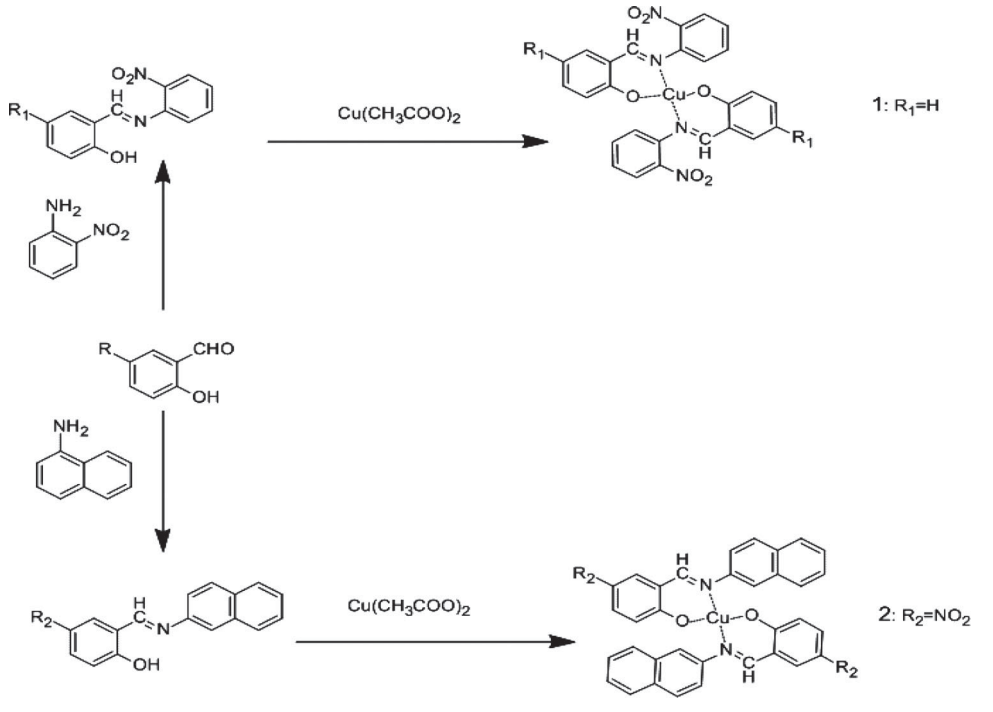
Hydrogen sulfide, a foul-smelling gas and commonly being perceived as a toxic industrial exhalation, is considered as the third signaling molecule *in vivo* as well as nitric oxide [1–10] and carbon monoxide [11]. H_2S can be produced by three typical pathways including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MPST) in different organs and tissues [3,12]. Studies have shown that the H_2S plays some important roles in various physiological processes *in vivo*, such as vasodilation apoptosis, neurotransmission ischemia/reperfusion-induced injury, insulin secretion and inflammation [4,13–18]. On the other hand, studies have established that the abnormal level of H_2S in cells may cause many types of disorders including Down's syndrome, Alzheimer's disease, liver cirrhosis and diabetes [1,8,19–23]. Therefore, it presents a significant research value to develop efficient methods that can detect H_2S in living biological systems. Traditional methods with complicated sample preparation and tissue or cell destruction including colorimetry, gas chromatography, electrochemical analysis and use of fluorescent have drawbacks for H_2S detection [24]. Herein, we synthesized copper complex for the detection of H_2S which could be applied in the sample of environment, pharmacy and so on with highly selective ability [25–28].

In the present work, bis(salicylaldehyde-*o*-nitroaniline) copper(II) and bis(5-nitro alicylaldehyde-*α*-naphthylamine) copper(II) probes were synthesized to detect HS^- (Scheme 1). The results showed that the synthesized compounds especially bis(salicylaldehyde-*o*-nitroaniline) copper(II) have high selectivity and good sensitivity to HS^- among the tested anions (HS^-, AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and amino acids (such as GSH, HCys and Cys).

2. Results and discussion

2.1. UV–VIS titration

The binding properties of synthesized copper complexes (**1** and **2**) with various anions (HS^-, AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and amino acids (Cys, GSH and HCys) were investigated through UV–VIS spectroscopy in DMSO at 298 K. The UV–VIS spectral responses of copper complexes **1** during the titration with HS^- in DMSO are shown in Figure 1(a). In the absence of NaHS, compound **1** (4.0×10^{-5} mol L^{-1} in DMSO) revealed a strong absorption band at about 375 nm. With the increase in the amount of HS^-, the intensity of absorption band decreased gradually. At the same time, a new absorption peak appeared centered at about 340 nm and the intensity increased gradually. In



Scheme 1. Synthesis route for copper(II) complex.

general, the absorption peak shifted to the short wavelength direction with the addition of HS^-. As a result, the blue shift phenomenon occurred after compound **1** interacted with HS^-. One clear isosbestic point appeared at 375 nm which indicated that the interaction existed between compound **1** and HS^-. In addition, there was a certain stoichiometric ratio between the Cu(II) complex and HS^-. Analogous investigations were carried out on other normal anions (AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and amino acids (Cys, GSH and HCys). The addition of $H_2PO_4^-$, Cys, GSH and HCys induced similar spectral changes of Cu(II) complex compared with the addition of HS^- (supplementary material, http://dx.doi.org/10.1080/17415993.2018.1425410). The above results indicated compound **1** also interacted with $H_2PO_4^-$, Cys, GSH and HCys. However, the addition of AcO^-, F^-, Cl^-, Br^- and I^- did not induce any spectral responses which indicated that complex **1** showed almost no binding ability toward these anions or the binding abilities were very small and could be ignored.

The spectral changes of compound **2** with HS^- are also shown in Figure 1(b). We could see that the absorbance intensity of compound **2** at 375 nm decreased with the increase in HS^- concentration. At the same time, a new absorption peak developed at about 450 nm and the intensity increased gradually. In addition, one clear isosbestic point formed at 402 nm and the absorbance peak of compound **2** shifted to long wavelength direction gradually. The addition of $H_2PO_4^-$, AcO^-, Cys, GSH and HCys induced similar spectral changes compared with HS^- which showed $H_2PO_4^-$, AcO^-, Cys, GSH and HCys interacted with complex **2** (supplementary material, http://dx.doi.org/10.1080/17415993.2018.1425410). While, the additions of F^-, Cl^-, Br^- and I^- almost did not induce any spectral

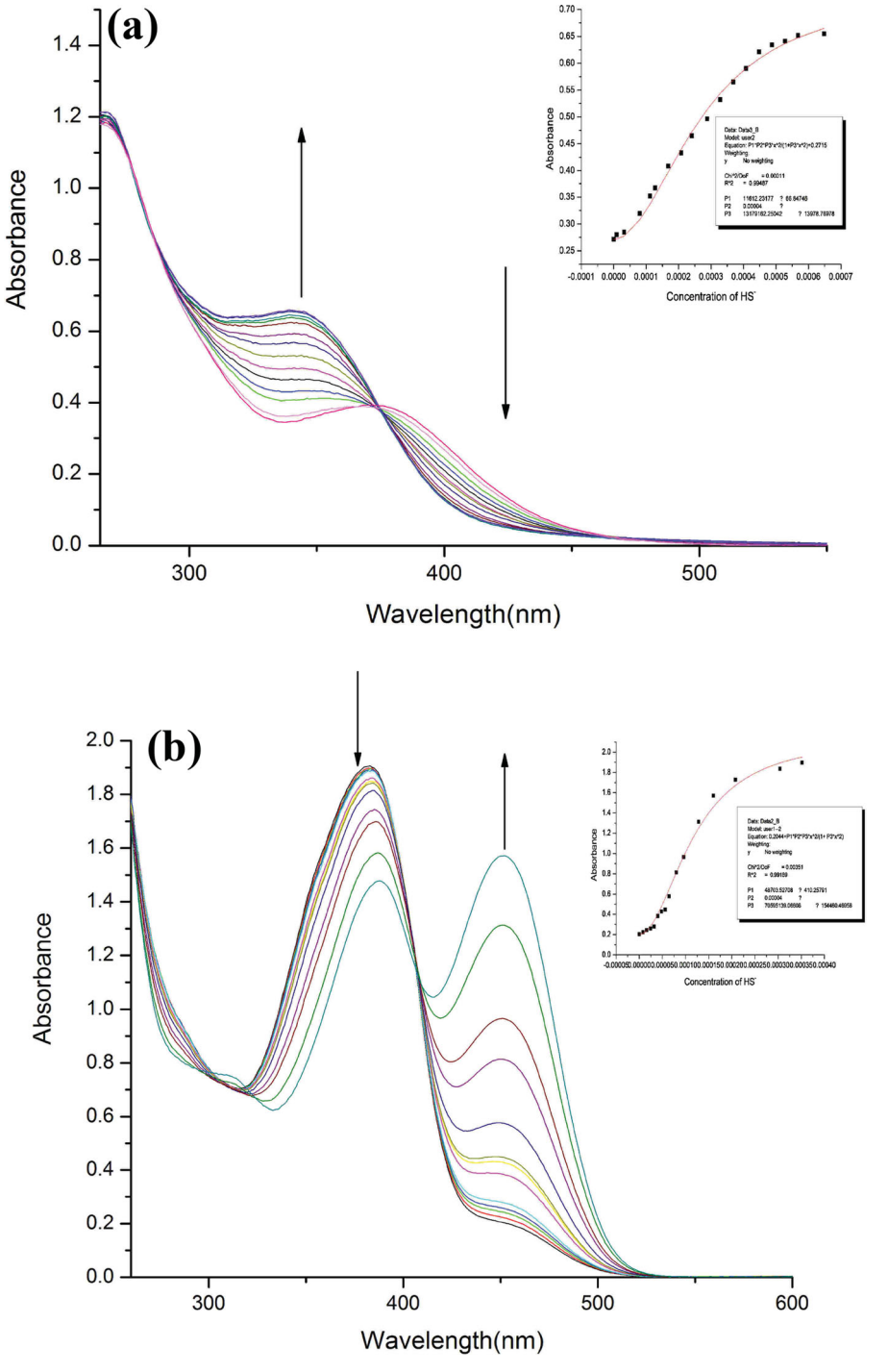


Figure 1. UV–VIS spectra of compounds (**1** and **2**) (4.0×10^{-5} mol L^{-1}) with the addition of HS^- in DMSO solution. (a) Compound **1** (HS^- (0–64.8) $\times 10^{-5}$ mol L^{-1}) and (b) compound **2** (HS^- (0–25.6) $\times 10^{-5}$ mol L^{-1}). Arrows indicate the direction of increasing HS^- concentration.

changes of compound **2** which indicated the binding abilities were very weak and could be ignored.

Owing to complexes (**1** and **2**) having the strongest binding ability for HS^- among anions tested (HS^-, AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and in pure DMSO solvent, complex **1** was tested in the DMSO–H_2O (1:1, v/v) solution shown in supplementary material (http://dx.doi.org/10.1080/17415993.2018.1425410). The addition of HS^- induced similar spectral changes that were found in the DMSO–H_2O (1:1, v/v) solution compared with pure DMSO solvent. However, the response of UV–VIS spectra was weaker than that of pure DMSO solvent which showed that the binding ability was weak to a certain degree. The addition of $H_2PO_4^-$ induced weak spectral changes at 381 nm compared with pure DMSO solvent. However, the other anions (AcO^-, F^-, Cl^-, Br^- and I^-) induced a murky phenomenon. The above results exhibited that complex **1** could be used as a sensor for the detection of HS^- in aqueous solution.

Besides, the binding abilities of compound **1** and **2** with HS^- (Figure 2(a,b)) were also determined in HEPES buffer solution. Results indicated that the interaction existed in host–guest. Similar spectral changes of complexes (**1** and **2**) with anions tested (AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and amino acids (Cys, GSH and HCys) in pure DMSO solvent compared with HS^- were reported; and data are given in the supplementary material (http://dx.doi.org/10.1080/17415993.2018.1425410). Results indicated that two compounds showed different binding abilities for Cys, GSH and HCys which were weaker than that of HS^-. The reason may be that the copper(II) ion of the complex was captured by HS^- and the free ligand was released after the copper complex interacted with HS^- [29,30]. UV–VIS spectral responses of compounds (**1** and **2**) (4.0×10^{-5} mol L^{-1}) upon the addition of anions (AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and GSH are shown in Figure 3 and **(S-12)**, respectively. Results indicated that the high selectivity and sensitivity of the copper(II) complex for HS^- were not affected by the existence of other anions and GSH.

Furthermore, the detection limit of compound **1** (4.0×10^{-5} mol L^{-1}) was carried out in pure DMSO solution through UV–VIS titration as shown in Figure 4. In the absence of NaHS, the absorbance value of compound **1** (4.0×10^{-5} mol L^{-1}) was 0.515 at 375 nm. The absorption coefficient was calculated and the value was 10.4 mol cm. Compound **1** (4.0×10^{-5} mol L^{-1}) and the concentration of HS^- (0–5.6×10^{-5} mol L^{-1}) have a good linear relationship by UV–VIS intensity which indicated the quantitative detection of HS^- ranging from 0 to 5.6×10^{-5} mol L^{-1}. The addition of HS^- (3.2×10^{-5} mol L^{-1}) to compound **1** leads to 1.3 times increase in the corresponding UV–VIS intensity. The above results exhibited the detection limit of compound **1** for HS^- was 3.2×10^{-5} mol L^{-1}. Analogously, the detection limit of compound **1** (4.0×10^{-5} mol L^{-1}) was also determined (supplementary material, http://dx.doi.org/10.1080/17415993.2018.1425410). The value of the absorption coefficient for compound **2** (4.0×10^{-5} mol L^{-1}) was 44.5 mol cm and the detection limit of compound **2** (4.0×10^{-5} mol L^{-1}) for HS^- was 4×10^{-6} mol L^{-1}.

2.2. Fluorescence response

The fluorescence properties of two compounds (**1**–**2**) after the addition of anions and amino acids tested were also investigated in pure DMSO solution. Compound **1** exhibited

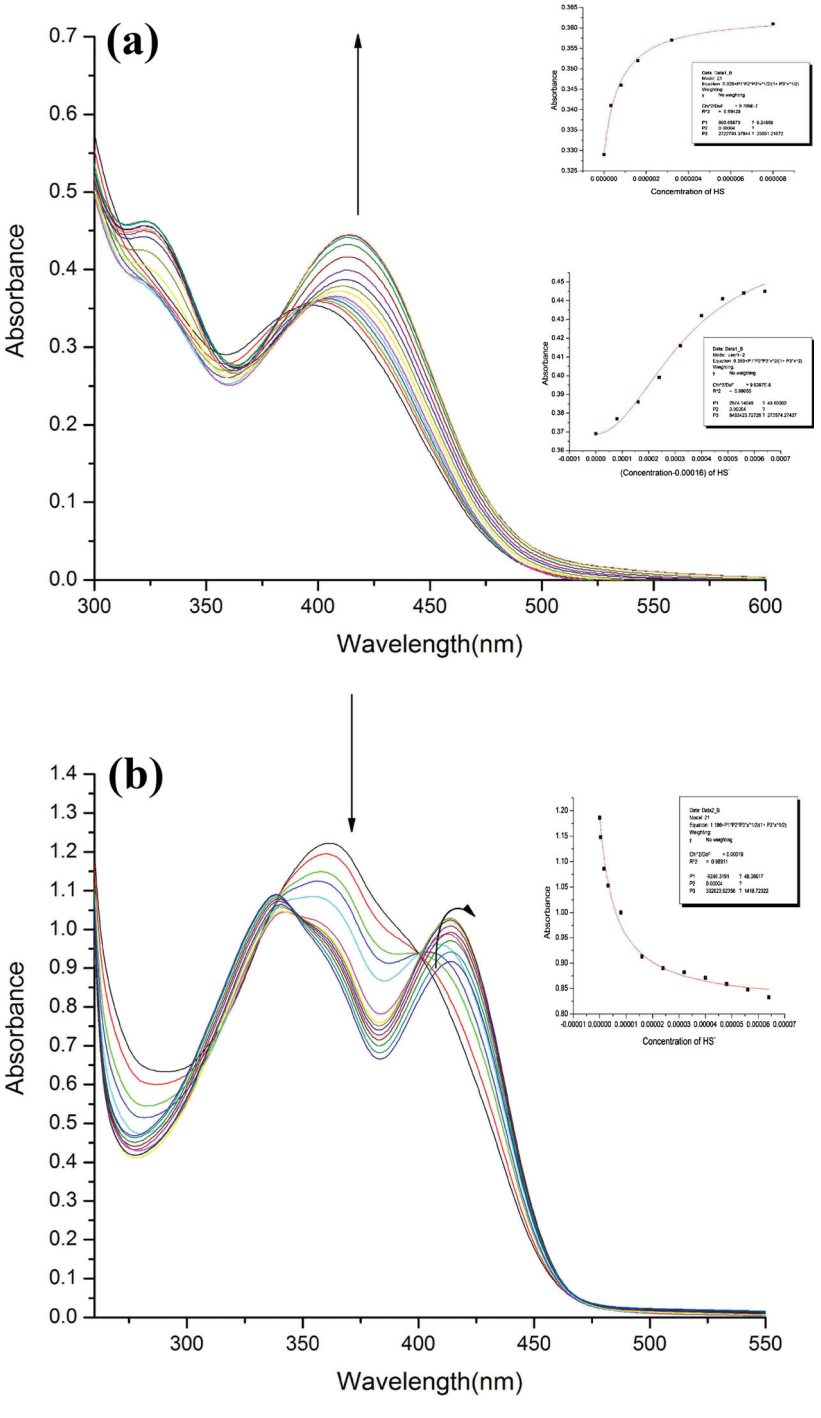


Figure 2. UV–VIS spectra of compound (**1** and **2)** (4.0×10^{-5} mol L^{-1}) toward HS^- ((0–80) $\times 10^{-5}$ mol L^{-1}) was recorded in DMSO–H_2O (1:1, v/v 0.04 mol L^{-1} HEPES buffer at pH 7.38). (a) compound **1** (HS^- (0–80) $\times 10^{-5}$ mol L^{-1}) and (b) compound **2** (HS^- (0–80) $\times 10^{-5}$ mol L^{-1}). Arrows indicate the direction of increasing HS^- concentration.

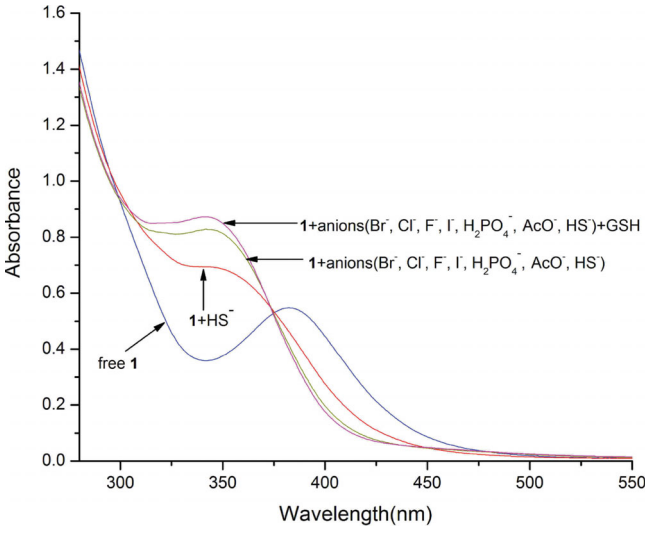


Figure 3. UV–vis spectral responses of compound **1** (4.0×10^{-5} mol L^{-1}) upon the addition of 8 equiv anions (AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) and 8 equiv GSH.

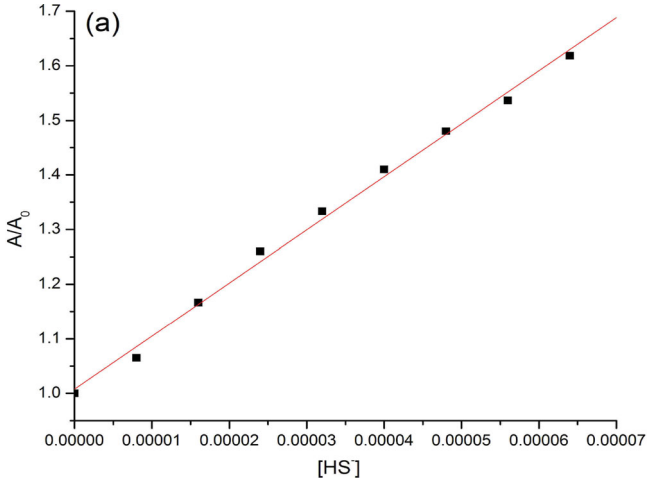


Figure 4. UV–VIS intensity of compound **1** in pour DMSO solution as a function of concentrations of HS^-.

an emission peak at about 340 nm. With the increasing concentration of HS^-, the fluorescence emission was decreased obviously at about 343 nm (Figure 5(a)). The addition of Cys, GSH and HCys induced similar fluorescence spectral changes of compound **1** compared with the addition of HS^- (supplementary material, http://dx.doi.org/10.1080/17415993.2018.1425410). However, the additions of $H_2PO_4^-$, AcO^-, F^-, Cl^-, Br^- and I^- produced a nominal change in the fluorescence spectral of the probe.

For compound **2**, the fluorescence emission centered at 433 nm was gradually strengthened upon the addition of HS^- (Figure 5(b)), which was because the addition of HS^- could coordinate with Cu^{2+} and release the free ligand. Upon the addition of other anions ($H_2PO_4^-$, AcO^-) and amino acids (Cys, GSH and HCys) into the solution of compound

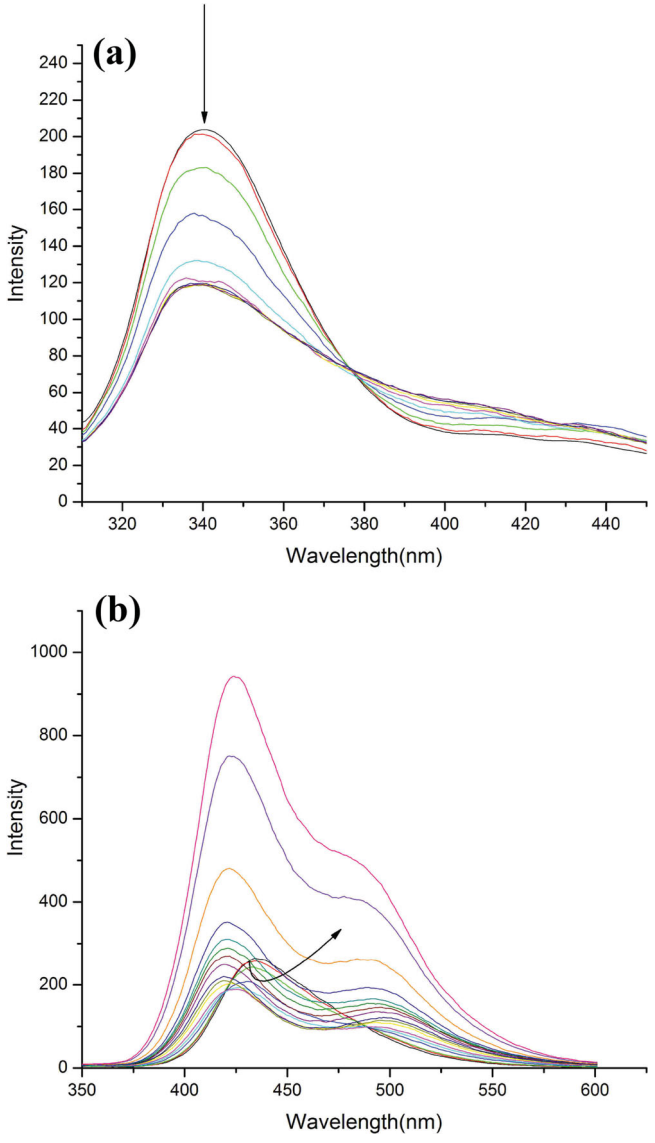


Figure 5. Fluorescence response of compound (**1** and **2**) (4.0×10^{-5} mol L^{-1}) up on the addition of HS^- in pure DMSO solution. (a) Fluorescence response (λ_{ex} 289 nm, slit widths: 10 nm/10 nm) of compound **1** up on the addition of HS^- ((0–80) $\times 10^{-5}$ mol L^{-1}) and (b) Fluorescence response (λ_{ex} 327 nm, slit widths: 2.5 nm/5 nm) of compound **2** up on the addition of HS^- ((0–80) $\times 10^{-5}$ mol L^{-1}). Arrows indicate the direction of increasing HS^- concentration.

2 induced similar fluorescence spectral changes of compound **2** compared with the addition of HS^- (supplementary material, http://dx.doi.org/10.1080/17415993.2018.1425410). There were no significant spectral changes observed upon the titration of compound **2** with (Cl^-, Br^- and I^-).

Then fluorescence spectral changes data of compounds (**1**–**2**) upon the addition of various anions (HS^-, $H_2PO_4^-$, AcO^-, F^-, Cl^-, Br^- and I^-) and amino acids (Cys, GSH and

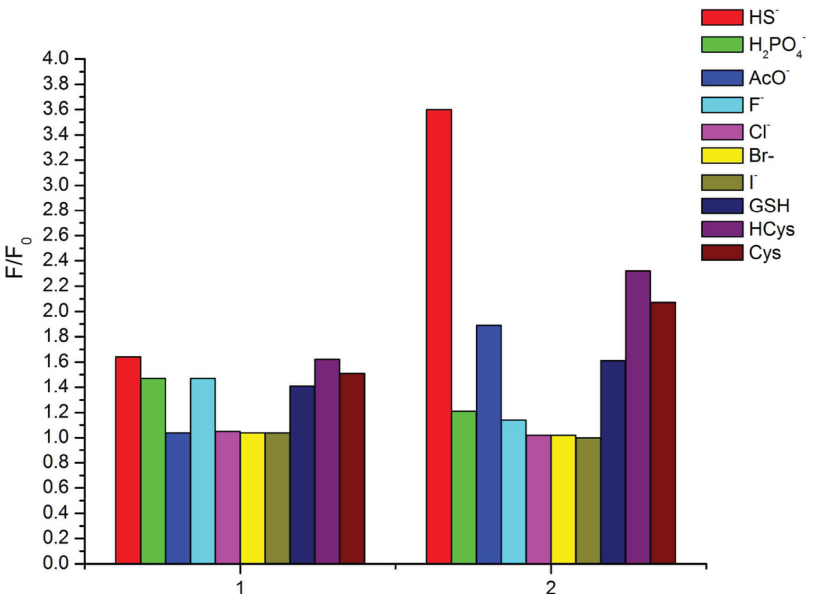


Figure 6. Fluorescence spectral changes of compounds (**1**–**2**) upon the addition of various anions and amino acids in pure DMSO solution. Fluorescence response (λ_{ex} 289 nm, slit widths:10 nm/10 nm) of compound **1** (4×10^{-5} mol L^{-1}) upon the addition of various anions (HS^-, AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) ((0–80) $\times 10^{-5}$ mol L^{-1}) and amino acids (GSH, HCys and Cys) ((0–80) $\times 10^{-5}$ mol L^{-1}). Fluorescence response ($\lambda_{ex,}$ 327 nm, slit widths: 2.5 nm/5 nm) of compound **2** (4×10^{-5} mol L^{-1}) up on the addition of various various anions (HS^-, AcO^-, $H_2PO_4^-$, F^-, Cl^-, Br^- and I^-) ((0–80) $\times 10^{-5}$ mol L^{-1}) and amino acids (GSH, HCys and Cys) ((0–80) $\times 10^{-5}$ mol L^{-1}).

HCys) were analyzed by using the bar graph in pure DMSO solution as shown in Figure 6. The results also indicated that compounds (**1**–**2**) exhibited a different binding ability with various anions (HS^-, $H_2PO_4^-$, AcO^- and F^-) and amino acids (Cys, GSH and HCys), and complexes (**1**–**2**) showed a stronger binding ability for HS^- than other anions ($H_2PO_4^-$, F^-, AcO^-, Cl^-, Br^- and I^-) amino acids (Cys, GSH and HCys).

Besides, a comparison of spectra of compounds (**1**–**2**) measured in the presence of HS^- with the spectra of plain ligands is shown in Figure 7. And the spectra of compounds (**1**–**2**) in the presence of HS^- and plain ligands had the same emission. The result also indicated the reason may be that the copper(II) ion of the complex was captured by HS^- and free ligand was released after the copper complex interacted with HS^-.

2.3. Binding constants

Complexes **1** and **2** interacted with various anions as the ratio of 1:1 or 1:2 according to the Job-plot curve (supplementary material, http://dx.doi.org/10.1080/17415993.2018.1425410).

The binding constants of compounds with various anions and amino acids in DMSO and DMSO/H_2O solution were calculated using the method of non-linear least square calculation [31–33]. The obtained binding constants are listed in the Table 1 and in the supplementary material (http://dx.doi.org/10.1080/17415993.2018.1425410) according to

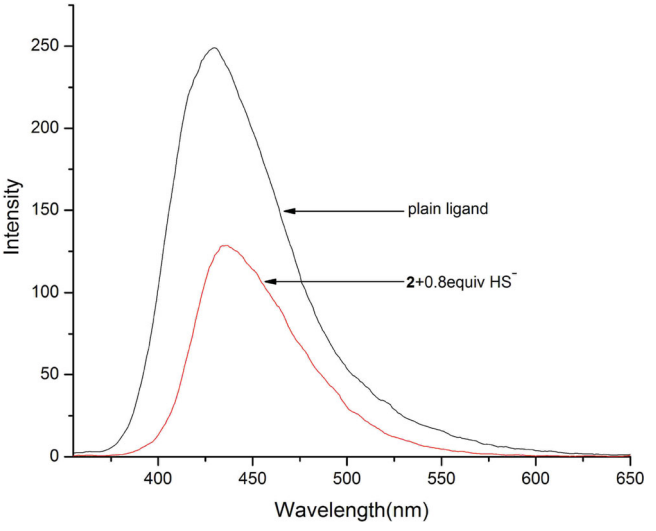


Figure 7. A comparison of fluorescence spectra of compounds **2** (4.0×10^{-5} mol L^{-1}) in the presence of 0.8 equiv HS^- with the spectra of plain ligand (4.0×10^{-5} mol L^{-1}).

Table 1. Binding constants of Cu(II) complex with various anions.

Anion	K_s(**1**)(DMSO)	K_s(**1**)(DMSO:H_2O (1:1v/v))	K_s(**2**)(DMSO)
HS^-	$(1.32 \pm 0.01) \times 10^{7a}$	$(1.99 \pm 0.03) \times 106^{a}$	$(7.06 \pm 0.02) \times 10^{7a}$
$H_2PO_4^-$	$(7.85 \pm 0.05) \times 10^{3b}$	$(1.95 \pm 0.07) \times 104^{ab}$	$(4.86 \pm 0.12) \times 103^{b}$
AcO^-	ND	ND	$(4.27 \pm 0.04) \times 103^{b}$
F^-	ND	ND	ND
Cl^- (Br^- or I^-)	ND	ND	ND

[a]The binding ratio of host–guest is 1:2.
[b]The binding ratio of host–guest is 1:1.

UV–VIS data. The binding ability trend of two complexes to anions and amino acids followed the order of HS^- > Cys, GSH and HCys > $H_2PO_4^-$ > AcO^- ≫ F^-. In addition, the binding constants of compounds with various anions and amino acids in HEPES solution were also calculated (supplementary material, http://dx.doi.org/10.1080/17415993.2018.1425410). Results indicated that the binding ability trend was the same as the DMSO solvent. In general, both the two complexes showed the strongest selectivity for HS^- among anions and amino acids tested. The reason may be that the copper(II) ion of the complex was captured by HS^- and the free ligand was released after the copper complex interacted with HS^-. These binding constants could provide a theoretical basis for the optimization of sensor and so the calculation of binding constant was necessary.

2.4. Cytotoxicity assay

Scientific research suggests that glutathione peroxidase (GPx1) is an important selenoprotein which is expressed in most tissues. However, GPx1 is not found in human breast cancer cells (MCF-7). Therefore, we chose the MCF-7 cell, the special cell lines [34,35]. Cytotoxicity of the compounds toward a cervical cancer cell line (MCF-7 cell) was evaluated using a conventional MTT assay. No remarkable differences in the proliferation of the cells were observed in the absence and presence of the fluorescence probe

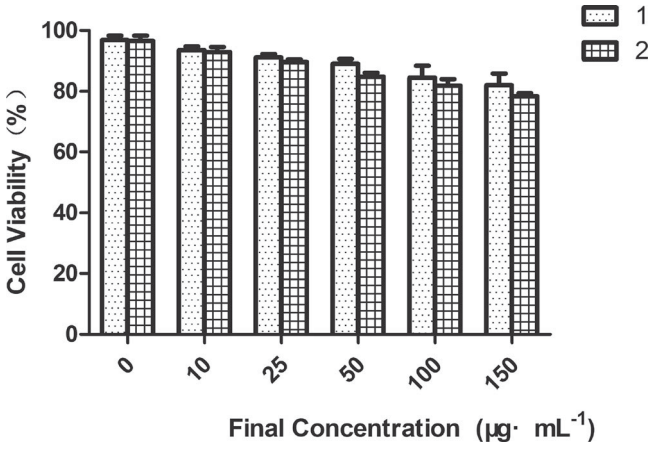


Figure 8. Cell viability values (%) estimated by an MTT proliferation test versus incubation concentration of fluorescence probe. MCF-7 cells were cultured in the presence of 0–150 μg mL^{-1} of fluorescence probe at 37°C for 24 h. Cell viability (expressed in%) was calculated considering 100% growth in the absence of fluorescence probe.

(0–150 μg mL^{-1}) (Figure 8). The cellular viability was estimated to be 80% after 24 h of incubation with the fluorescence probe < 150 μg mL^{-1}. The anticipated cytotoxicity of the fluorescence probe (< 150 μg mL^{-1}) was expected to be low. In combination with binding constants, compound **1** showed high binding ability and low cell cytotoxicity and may be used to detect H_2S *in vivo*.

3. Conclusion

In summary, we reported the development probes of copper complex for H_2S detection which were designed and synthesized based on an inorganic reaction. The probes, especially complex **1**, exhibited highly sensitivity and selectivity to H_2S among various relevant anions and amino acids species in HEPES buffer solution. In addition, both two compounds showed low cytotoxicity to MCF-7 cells. Therefore, these probes for hydrogen sulfide could explain the function of this signaling molecular in the broader biological environment and may assist to build up new diagnostic and therapeutic agents in neurodegenerative and cardiovascular disease. Furthermore, the synthesized small molecular compound can be used as an efficient probe to investigate functions that are related to H_2S.

4. Material and methods

All reagents and solvents used were of analytical grade. Sodium hydrosulfide hydrate, all anions in the form of tetrabutylammonium salts (such as $(n\text{-}C_4H_9)_4NCl$, $(n\text{-}C_4H_9)_4NBr$, $(n\text{-}C_4H_9)_4NI$, $(n\text{-}C_4H_9)_4NAcO$ and $(n\text{-}C_4H_9)_4NH_2PO_4$), all amino acids (such as GSH, HCys and Cys) and HEPES were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). All anions and amino acids were stored in a desiccator under vacuum and used without any further purification. Dimethyl sulfoxide (DMSO) was distilled in vacuum after being dried with CaH_2. ^1H NMR spectra were recorded on a Unity Plus-400-MHz

spectrometer. HRMS was performed with a Mariner apparatus. UV–VIS titration experiments were made on a Shimadzu UV2550 Spectrophotometer (Shimadzu, Japan) at 298 K. Fluorometric titration was performed on a Cary Eclipse Fluorescence Spectrophotometer (Agilent, USA) at 298 K. IR was performed with a Shimadzu IRT racer-100 (Shimadzu, Japan). The binding constant, K_s, was obtained by the non-linear least squares calculation method for data fitting.

The cells at the logarithmic growth phase were seeded into a 96-well plate at a density of 2.0×10^4 cells/well and cultured for 24 h. After that, the culture media were replaced with 200 μL of RPMI 1640 medium containing different concentrations of the compound and the cells were further incubated for 24 h. Next, the cells were washed with PBS three times, and then 100 μL of the culture medium and 20 μL of MTT solution were, respectively, added to each well. After additional incubation (4 h), the absorbance of each well was detected at 490 nm using the microplate reader (Thermo Multiscan MK3, Thermo Fisher Scientific, MA, USA) with the plain cell culture media as the control. The survival curves were plotted and the IC50, defined as the compound concentrations required for 80% inhibition of cell growth, was calculated based on the survival curves.

4.1. Synthesis of Cu(II) complex

- Cu(II) complexes were synthesized according to the route shown in Scheme 1.
- Bis(salicylaldehyde-*o*-nitroaniline) copper(II) (compound **1**).

Salicylaldehyde-*o*-nitroaniline was synthesized according to the literatures [36–38]. Salicylaldehyde (1.22 g, 10 mmol) was dissolved in ethanol solution (25 mL) and the ethanol solution (15 mL) containing nitroaniline (1.38 g, 10 mmol) was added slowly. Then, the mixture was refluxed for 5 h and the red crude was obtained. The precipitate was recrystalled by ethanol, washed with water and dried under vacuum. Yield: 85%. m.p. 111–113°C. ^{1}H NMR (400 MHz, DMSO) δ 14.49 (s, 1H, minus;OH), 8.75 (s, 1H, –CH−N–), 8.43 (s, 1H, ph-H), 8.30 (d, J = 7.5 Hz, 1H, ph-H), 7.50 (t, J = 7.3 Hz, 2H, ph-H), 7.38 (dd, J = 15.0, 7.4 Hz, 3H, ph-H), 7.13 (d, J = 9.1 Hz, 1H, ph-H) **(S-1).** Elemental analysis: Calc. for $C_{13}H_{10}N_2O_3$: C, 64.46; H, 4.16; N, 11.56; O, 19.82 Found: C, 64.35; H, 4.15; N, 11.54; O, 19.80.

Then, bis(salicylaldehyde-*o*-nitroaniline) copper(II) was also synthesized according to the above similar procedure. To a stirred solution of salicylaldehyde-*o*-nitroaniline (242 mg, 1 mmol) in ethanol (15 mL), copper acetate hydrate (99 mg, 0.5 mmol) was added slowly. Then, plenty of the red precipitate was produced at room temperature, separated by filtration, washed with water and dried under vacuum. Yield: 82%. IR: C−C (Ph) 1445, 1591, 1601 and 1513 cm^{-1}; −CH: 749 cm^{-1}; NO_2:1514 and 1342 cm^{-1} **(S-2)**. Elemental analysis: Calc. for $C_{26}H_{18}CuN_4O_6$: C, 57.19; H, 3.32; Cu, 11.64; N, 10.26; O, 17.58 Found: C, 57.35; H, 3.32; Cu, 11.65; N, 10.24; O, 17.60. MS-HRMS (*m/z*): 568.0400 $(M + Na)^+$ **(S-3)**.

Bis(5-nitroalicylaldehyde-α-Naphthylamine) was synthesized according to the above similar procedure. Yield: 75%. m.p. 174–176°C. ^{1}H NMR (400 MHz, DMSO) δ 14.57 (s, 1H, –OH), 8.82 (s, 1H, –CH−N–), 8.48 (d, J = 2.7 Hz, 1H, ph-H), 8.34 (dd, J = 9.2, 2.7 Hz, 1H, ph-H), 8.26–8.18 (m, 1H, ph-H), 7.97–7.85 (m, 2H, ph-H), 7.65–7.52 (m, 3H, ph-H), 7.26 (s, 1H, ph-H), 7.20 (d, J = 9.2 Hz, 1H, ph-H) **(S-4)**. Elemental analysis: Calc. for

$C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58; O, 16.42; Found: C, 70.01; H, 4.13; N, 9.60; O, 16.40.

Bis(5-nitroalicylaldehyde-α-Naphthylamine) copper(II) was also synthesized according to the above similar procedure. Yield: 78%. IR: C−C (ph): 1468, 1492, 1573 and 1605 cm^{-1}; −CH: 773 and 795 cm^{-1}; NO_2: 1312 and 1492 cm^{-1} **(S-5)**. Elemental analysis: Calc. for $C_{34}H_{22}CuN_4O_6$: C, 63.20; H, 3.43; Cu, 9.84; N, 8.67; O, 14.86. Found: C, 63.25; H, 3.42; Cu, 9.83; N, 8.66; O, 14.88. MS-HRMS (*m/z*): 668.0694 $(M + Na)^+$ **(S-6)**.

Disclosure statement

No potential conflict of interest was reported by the authors.

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