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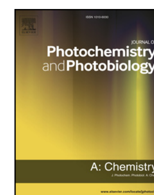
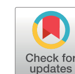


A Schiff-based fluorescence sensor for the detection of Cu^{2+} and its application in living cells



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ABSTRACT

A novel fluorescent and colorimetric probe **FL** was designed and synthesized for selective Cu^{2+} detection in aqueous solutions. The detection limit of probe **FL** for Cu^{2+} was determined to be 1.09 μM. Furthermore, probe **FL** was successfully used to recognize Cu^{2+} in living cells, indicating its high potential in biological imaging applications.

1. Introduction

Among various crucial analytes, heavy metal ions tend to be highly toxic and harmful [1]. Cu^{2+} is an essential trace element and plays a crucial role in various physiological processes in the human body [2]. However, the high accumulation of copper can damage the central nervous system and increase the risk of Alzheimer's and Parkinson's diseases [3,4]. In addition, Cu^{2+} is an environmental pollutant that can hinder animal and plant growth when present in excessive amounts [5]. Therefore, developing probes for Cu^{2+} recognition in environmental applications is highly crucial. Numerous fluorescent probes for Cu^{2+} have been developed in the past decades [6–15]. For rapid analysis and environment-friendly reasons, a probe that detects Cu^{2+} by simple methods and color changes is highly desirable.

In this study, a probe **FL** (Scheme 1) was synthesized and its ion recognition ability was examined for a series of metal ions. Probe **FL** exhibited a high sensitivity and selectivity toward Cu^{2+}. In addition, fluorescence microscopy experiments indicated that probe **FL** can be used to detect Cu^{2+} present in living cells.

2. Experimental section

2.1. Materials and general methods

All reagents and solvents were obtained commercially and used without further purification. Thin-layer chromatography (TLC) was performed using TLC plates precoated with silica gel 60 F254. The ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AM300 spectrometer and referenced to the solvent signals. High-resolution mass spectroscopy (HRMS) and electrospray ionization (ESI) spectra were obtained using a mass spectrometer and Orbitrap mass spectrometer (Thermo Scientific™), respectively. UV–vis spectra were measured using a Jasco V360 spectrophotometer with a diode array detector, and the resolution was set at 1 nm. Fluorescence spectra were recorded using a Jasco FP-8300 fluorescence spectrometer. Melting points were measured using a melting point apparatus without calibration. pH was measured using a digital pH meter. Fluorescence microscopy was performed using an IX-71 fluorescence microscope (Olympus, Center Valley, PA, USA).

2.1.1. Synthesis of (9H-fluoren-9-ylidene)hydrazine (FL-1)

A solution of 9-fluorenone (0.50 g, 2.78 mmol) was dissolved in 10 mL of ethanol, and 98 % aqueous N_2H_4 (0.8 mL) was added into the resulting solution. The reaction mixture was heated under reflux for 10 h. The reaction mixture was then cooled to room temperature (RT), and the precipitate was filtered and dried to give a yellow solid (0.49 g, 90 %). ^{1}H NMR (Supporting information, Fig. S1) (300 MHz, DMSO-d_6): δ/ppm 8.18 (d, J = 6.9 Hz, 1 H), 7.98 (s, 2 H), 7.88 (d, J = 1.2 Hz, 1 H), 7.86–7.76 (m, 1 H), 7.68–7.65 (m, 1 H), 7.45–7.37 (m, 2 H), 7.35–7.28 (m, 2 H); ^{13}C NMR (Supporting information, Fig. S2) (75 MHz, DMSO-d_6): δ/ppm 139.7, 139.6, 138.4, 137.3, 129.8, 129.1, 128.1, 128.0, 127.8, 125.5, 120.7, 120.2, 120.1.

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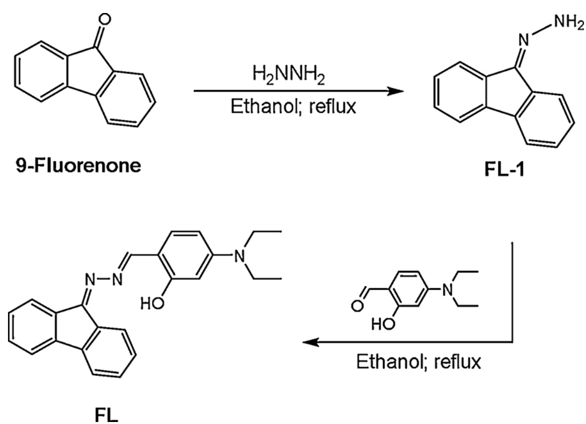

Scheme 1. Synthesis of probe **FL**.

2.1.2. Synthesis of probe FL

(*E*)-2-(((9*H*-Fluoren-9-ylidene)hydrazono)methyl)-5-(diethylamino) phenol (**FL**) Compound **FL-1** (0.50 g, 2.576 mmol) was dissolved in 20 mL ethanol followed by addition of 4-(diethylamino)salicylaldehyde (0.497 g, 2.576 mmol). The resulting solution was then heated under reflux for 12 h. After the reaction mixture was cooled to RT, the solvent was removed using rotary evaporation and the residue was purified by column chromatography on silica. The appropriate fractions, which were eluted with ethyl acetate–hexane (1:4, v/v), were combined to evaporated under reduced pressure to give probe **FL** as a red solid (0.76 g, 80 %). ^{1}H NMR (Supporting information, Fig. S3) (300 MHz, DMSO-d_6): δ/ppm 11.18 (s, 1 H), 8.87 (s, 1 H), 8.41(d, J =7.5 Hz, 1 H), 7.92–7.81 (m, 3 H), 7.63–7.34 (m, 5 H), 6.44–6.39 (m, 1 H), 6.21 (d, J =2.4 Hz, 1 H), 3.45–3.38 (m, 4 H), 1.14 (t, J =2.4 Hz, 6 H); ^{13}C NMR (Supporting information, Fig. S4) (75 MHz, DMSO-d_6):δ/ppm 163.8, 161.6, 157.4, 152.6, 142.0, 140.7, 137.1, 132.9, 131.9, 131.3, 131.0, 128.8,128.7, 128.5, 122.5, 121.2, 120.9, 107.6, 105.3, 97.3, 44.5, 13.0; HRMS (EI) (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{23}ON_3$ 369.1841, found: 369.1843.

2.1.3. Cell culture and imaging

The MCF-7 cell line was routinely cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with fetal bovine serum (10 %) and antibiotic penicillin/streptomycin (1 %). All cells were cultivated under a humidified atmosphere with 5 % CO_2 and 95 % air at 37 °C. For fluorescence imaging of the probe in the cell environment, MCF-7 cells were incubated with **FL** (20 μM) and phosphate buffer solution (PBS) for 1 h at 37 °C. The cells were then washed twice with PBS to remove the excess probe **FL**, and PFA was added to fix the cells for 30 min. The fluorescence images were captured using an Olympus IX-71 fluorescence microscope.

2.1.4. Detection of Cu^{2+} in real water samples

To evaluate the practicality of the present method, lake water samples were examined.

The lake water samples were obtained from the White Sand Lake of Changhua University of Education, Changhua, Taiwan. The lake water samples were filtered through a 0.20 μm filtered membrane and then centrifugedat 12,000 rpm for 10 min. $Cu(ClO_4)_2$ (10 equiv) were first dissolved in water of the above source, followed by the addition of probe **FL** into each water sample.

3. Results and discussion

3.1. UV/vis and fluorescence spectra of the probe FL for various metal ions

We used by UV/vis and fluorescence measurements to investigate the chemosensor behavior of probe **FL** with metal ions in DMSO/water

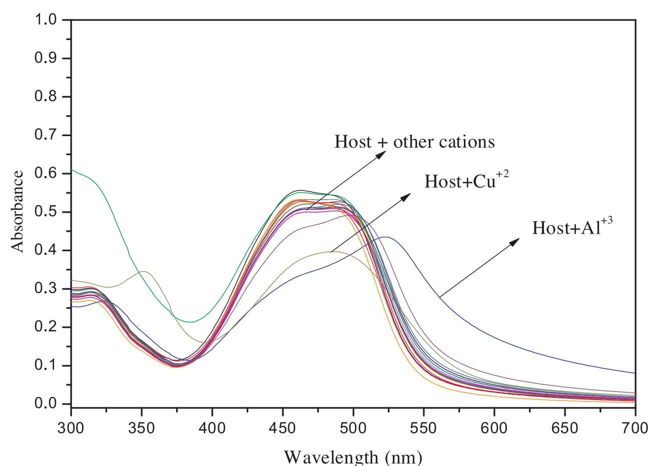

Fig. 1. Absorbance responses of **FL** (20 μM) with various metal ions (10 eq.) in DMSO/water (2:8, v/v).

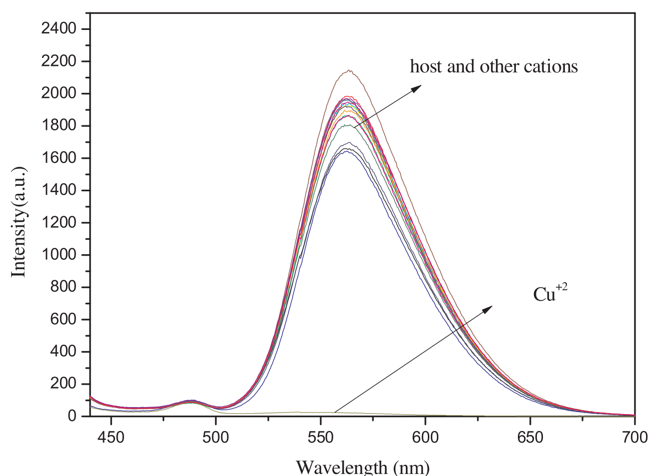

Fig. 2. Fluorescence emission spectra of probe **FL** (20 μM) recorded in DMSO/water (2/8, v/v) (λ_{em} =565 nm) after addition of 10 equiv. of various metal ions.

(2/8, v/v). The solution of probe **FL** showed a major absorption band at 475 nm (Fig. 1). In the presence of Cu^{2+} and Al^{3+}, the absorption spectra of probe **FL** showed a major band at 480 and 525 nm with a red-shift, respectively. However, in the fluorescence spectra of probe **FL** (Fig. 2) indicated that the probe **FL** alone and other cations except for Cu^{2+} exhibited a considerably strong single fluorescence emission band at 565 nm when excited at 420 nm. On adding Cu^{2+}, probe **FL** exhibited prominent fluorescence quenching. A color change from yellow to orange was observed only in the probe **FL** solution with Cu^{2+}, which can be easily detected with the naked eye, as depicted in Fig. 3. The observed color change and fluorescent quenching may be attributed to the binding of probe **FL** with Cu^{2+} through the hydroxyl oxygen and imine nitrogen that chelate Cu^{2+}. The fluorescence quenching and color change were not observed when other ions were added to probe **FL**. The results indicated that probe **FL** is a sensitive probe that can be used to detect Cu^{2+} with the naked eye in environmental analyses.

3.2. Fluorescence titration and binding studies

The fluorescence titration profiles (Fig. 4) indicate that the addition of 0.5 equivalents of Cu^{2+} instantly led to a decrease of the fluorescence intensity at 565 nm by more than 80 %. The association constant for the probe **FL**-Cu^{2+} complex in DMSO/water (2/8, v/v) was determined to be 4.61 × 10^5 M^{-2} by using the Stern–Volmer plot (Fig.

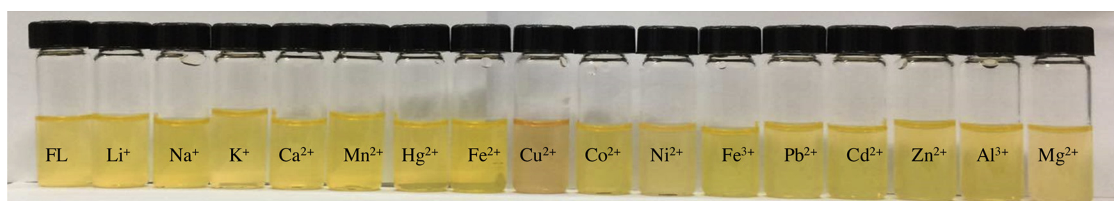

Fig. 3. The color changes observed by naked eye of probe **FL** (20 μM) upon addition of 10 equiv. of various metal ions in DMSO/water (2/8, v/v).

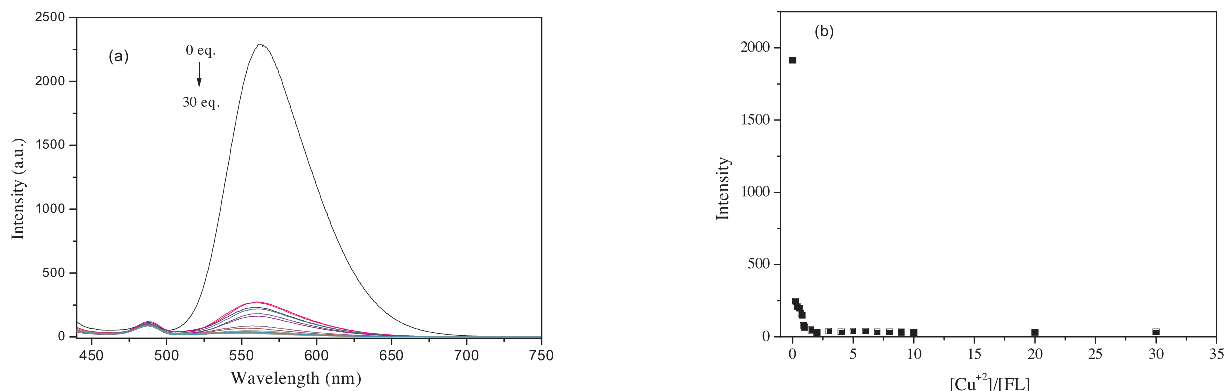

Fig. 4. (a) Fluorescence spectra of probe **FL** (20 μM) in DMSO/water (2/8, v/v) upon addition of increasing concentrations of Cu^{2+}; (b) Intensity (λ_{em} = 565 nm) of probe **FL** as a function of Cu^{2+} equiv.

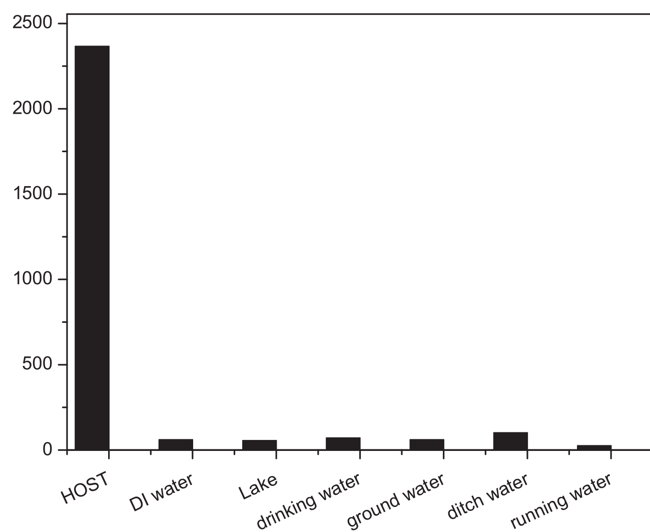

Fig. 5. Fluorescence responses of **FL** (20 μM; λ_{em} = 565 nm) with Cu^{2+} (10 eq.) in different water source.

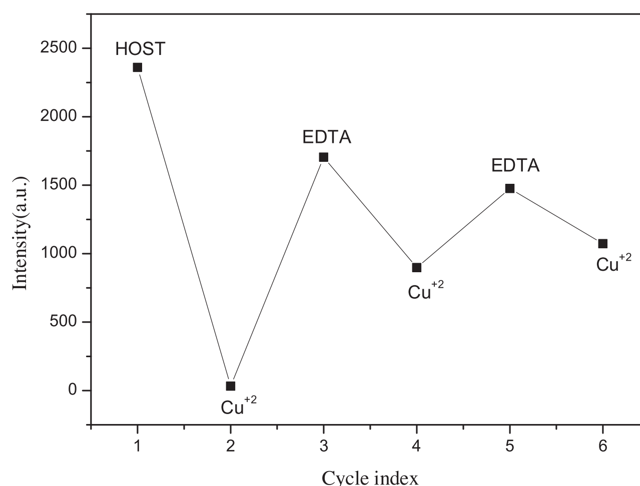

Fig. 6. Fluorescence responses of **FL** (20 μM; λ_{em} = 565 nm) with Cu^{2+} (10 eq.) and EDTA in DMSO/water (2/8, v/v).

S5). The detection limit of probe **FL** for the analysis of Cu^{2+} ions was estimated to be 1.09 μM by using the expression 3σ/*S*, where σ is the standard deviation of the control sample and S is the slope of the calibration curve (Fig. S6). A Job plot indicated a 2:1 stoichiometric complexation of probe **FL** with Cu^{2+} (Fig. S7). In addition, the formation of 2:1 complex between probe **FL** and Cu^{2+} was further confirmed by the appearance of a peak at *m/z* 860, assignable to [2probe **FL** + Cu^{2+} + $2H_2O$ + Na^+] (Fig. S8).

3.3. Application in real samples

For biological applications, probe **FL** can operate in a wide range of pH (Fig. S9). The time course of fluorescence changes of probe **FL** in the presence of 10.0 equivalents of Cu^{2+} in DMSO/water (2/8, v/v) was examined, and the result indicated that the recognition interaction was nearly completed within 1 min of Cu^{2+} addition (Fig. S10). The practical application of probe **FL** for the selective detection of Cu^{2+} in different water sources, such as lake, ground and tap water, was also demonstrated. Cu^{2+} was introduced to the water of different sources (200 μM). When probe **FL** was added to each water sample solution, the fluorescence intensity was quenched immediately (Fig. 5). This result indicated that probe **FL** is a sensitive sensor that can be used in environmental analyses.

3.4. Reversibility and competition experiments

The reversibility of probe **FL** toward Cu^{2+} was analyzed in the presence of EDTA (200 μM). As expected, the fluorescence intensity of probe **FL**, which was quenched in the presence of Cu^{2+}, recovered to approximately 75 % of its original value upon the addition of EDTA (Fig. 6). This reversibility is essential for the fabrication of devices for detecting Cu^{2+}.

To confirm the binding site, NMR titration experiments were

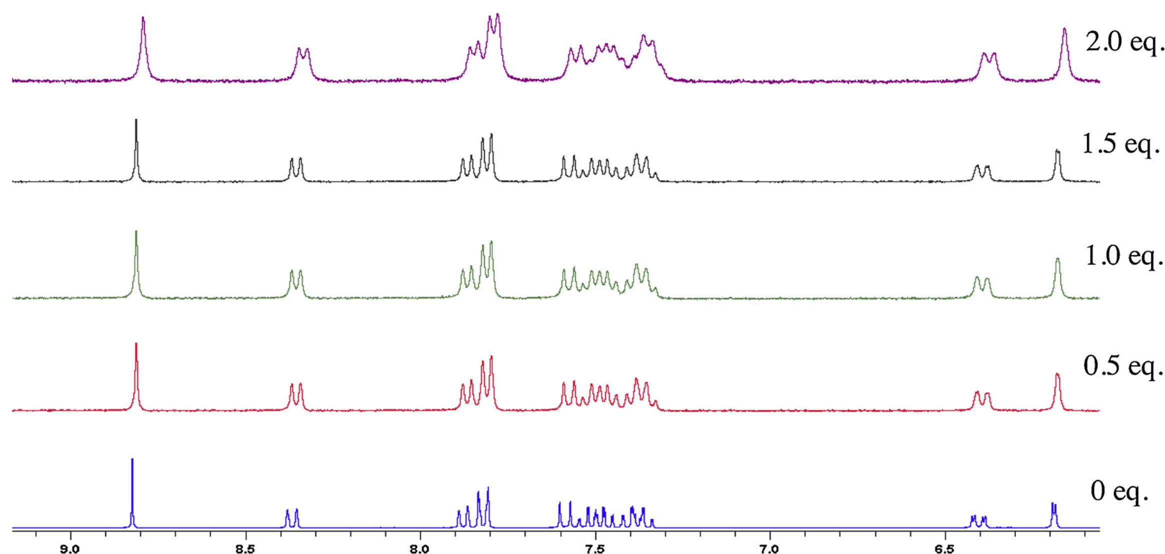

Fig. 7. ^{1}H NMR titration plots of probe **FL** with Cu^{2+} in DMSO-d_6.

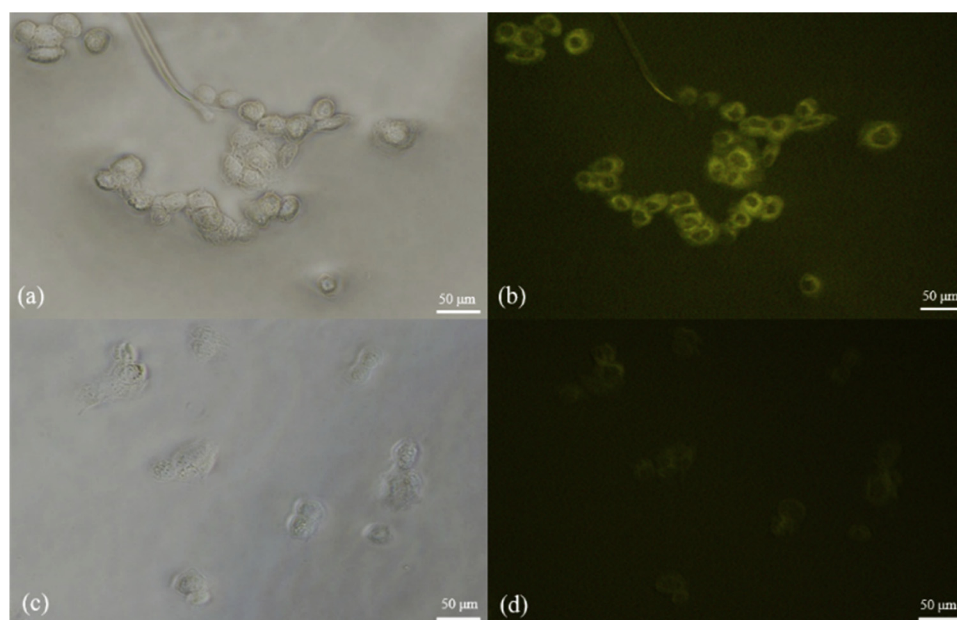

Fig. 8. (a) Bright field images of MCF-7 cells incubated with probe **FL**, (b) confocal fluorescence microscopy images of MCF-7 cells incubated with probe **FL**, (c) bright field images of MCF-7 cells incubated with probe **FL**-Cu^{2+} and (d) confocal fluorescence microscopy images of MCF-7 cells incubated with probe **FL**-Cu^{2+}.

performed. The ^{1}H NMR spectra of probe **FL** indicated that an enamine proton signal at 8.81 ppm broadened and shifted to 8.79 ppm. Other proton signals also exhibited similar marginal shifts (Fig. 7). These observations suggested the formation of the probe **FL**-Cu^{2+} complex.

Competition experiments were performed to study the influence of other ions on the binding ability of the probe **FL**-Cu^{2+} complex. Some interference was observed for the detection of Cu^{2+} in the presence of Hg^{2+} and Fe^{+3} (10.0 equiv). The fluorescence quenching of probe **FL**-Cu^{2+} may be due to the displacement of Cu^{2+} by Hg^{2+} and Fe^{+3} from probe **FL**-Cu^{2+} complex. (Fig. S11).

3.5. *Cell viability and fluorescence imaging of FL in live cells*

To demonstrate the practical applications of probe **FL**, experiments were conducted to examine its ability in detecting Cu^{2+} in MCF-7 cells. As displayed in Fig. 8, incubation of probe **FL** with MCF-7 cells without Cu^{2+} gave yellow fluorescence (Fig.8b). However, the intensity of intracellular yellow fluorescence was quenched in the presence of Cu^{2+} (Fig. 8d). Furthermore, the cytotoxicity of probe **FL** on MCF-7 cells was evaluated using the MTT assay. As displayed in Fig. 9, the results indicated a cell viability of approximately 90 % after treatment with probe **FL** and 48-h incubation. Thus, probe **FL** can be used for detecting Cu^{2+} in living cells with minimal cytotoxicity. Taken together, probe **FL** demonstrated advantages in practical applications in biological systems.

4. Conclusion

In summary, a simple probe **FL** was designed and synthesized as a selective fluorescent sensor for Cu^{2+}. Probe **FL** exhibited selectivity toward Cu^{2+} over other ions in DMSO/water (2/8, v/v) and demonstrated an obvious color change with Cu^{2+}. The living cell image experiments indicated that probe **FL** can be used to detect intracellular Cu^{2+} ions in living cells.

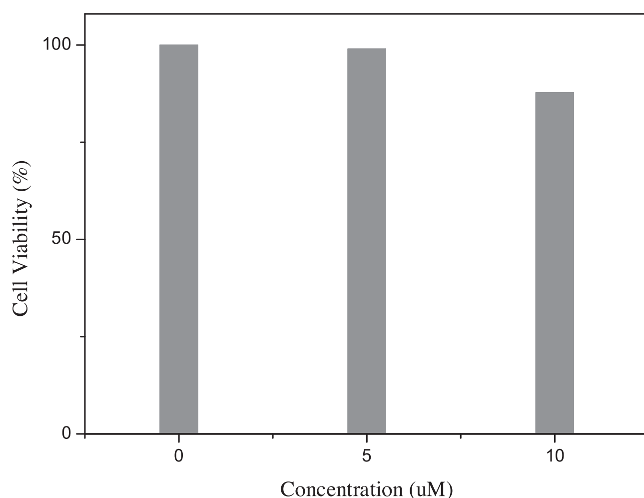

Fig. 9. Cytotoxicity assay of **FL** toward MCF-7 cells. MCF-7 cells were incubated with 5 and 10 μM of probe **FL** for 48 h.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jphotochem.2019.112326.

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