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RESEARCH ARTICLE

Synthesis, binding ability, and cell cytotoxicity of fluorescent probes for L-arginine detection based on naphthalene derivatives: Experiment and theory

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Abstract

Inspired by biological related parts, Schiff base derivatives and functional groups of chemical modification can provide efficient detection method of amino acids. Therefore, we have designed and prepared 4 compounds based on Schiff base derivatives involving —NO_2, —OH, and naphthyl group. Results indicated that compound **4** containing 2 nitro groups showed strong sensitivity and high selectivity for arginine (Arg) among normal 18 kinds of standard amino acids (alanine, valine, leucine, isoleucine, methionine, aspartic acid, glutamic acid, arginine, glycine, serine, asparagine, phenylalanine, histidine, tryptophan, proline, lysine, glutamine, and cysteine). Theoretical investigation also approved the strong binding ability of compound **4** for Arg. In addition, compound **4** displayed high combining ability of Arg and low cytotoxicity of MCF-7 cell in the 0 to 150 μg mL^{-1} of concentration range; it can be used for Arg in vivo detection of fluorescent probe.

KEYWORDS

arginine, cell cytotoxicity, fluorescent probe, naphthalene derivatives

1 | INTRODUCTION

In recent years, increasing attention in the field of host-guest chemistry has been devoted to the fast development of the molecular recognition system. The assay of amino acid is of particular importance in different food, biological, and chemical samples.[1-4] L-Arginine (Arg) ((2*S*)-2-amino-5-(diaminomethylideneamino)pentanoic acid) is classified as a semi-essential or conditionally essential amino acid that plays an important function in the metabolism of an organism.[5-8] L-Arginine, as the most basic amino acid, is found in particularly large amounts in protamines and histones. High concentration of free Arg is present in many plants including red algae, curcurbitaceae, and conifers where it functions as nitrogen storage and a transport form.[9-11] Because of this, Arg can be discovered in especially high concentrations in seedlings and reserve organs. L-Arginine is glucogenic and half-essential for humans and is an important metabolite of the urea cycle and also used as a drug in clinical therapy of endocrine diseases and hyperammonemia.[12-14] In addition, Arg plays important roles in cell division, the healing of wounds, the removal of ammonia from the body, the function of the immune system, the releasing of hormones, and in particular, gene regulation, glycoprotein targeting, and vesicle transport.[15-17] Consequently, the selective recognition of Arg is crucial in the fields of biochemistry and medical science.[18,19]

A variety of detection procedures for Arg analysis have been developed. Detection methods for Arg have been reported based on traditional determination, such as high-performance liquid chromatography, gas phase chromatography, ion exchange chromatography, and electrochemical method. The above methods have some deficiency, such as expensive instruments, poor repetitiveness, and selectivity.[20,21] The disadvantages of most used approaches are being time-consuming and expensive and requiring skillful labor techniques.[22-25] The design and synthesis of artificial receptors arise more attention due to high selectivity for the analytes. In this regard, fluorescence is a vital detection method because of its simplicity and high sensitivity. Recently, reports of fluorescent probe are numerous due to their outstanding features in biological environment.[26,27] However,

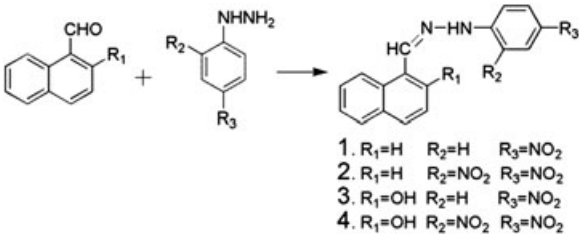
there are few reports about the application of fluorescent probe on the detection of amino acid.[28,29]

Based on the above consideration, we have rationally designed and synthesized a series of Schiff base derivative having an aldehyde group moiety as a signaling unit and conjugated imine functionality (C N) as a reaction unit and naphthalene as a fluorescent group (Scheme 1). Results indicated that the synthesized compounds showed the strongest response and high specificity for Arg among normal amino acids tested (alanine, valine, leucine, isoleucine, methionine, aspartic acid, glutamic acid, glycine, serine, threonine, asparagine, phenylalanine, histidine, tryptophan, proline, lysine, glutamine, tyrosine, and cysteine), which accompanied significant fluorescent strength. Therefore, the compounds can be used as a fluorescent probe for the detection of Arg.

2 | MATERIAL AND METHODS

Most of the starting materials were obtained commercially, and all reagents and solvents used were of analytical grade. All amino acids were purchased from Aladdin Chemistry Co Ltd (Shanghai, China) and stored in a desiccator under vacuum and used without any further purification. Dimethyl sulfoxide (DMSO) was distilled in a vacuum after being dried with CaH_2. C, H, and N elemental analyses were made on a Vanio-EL instrument. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Unity Plus-400-MHz spectrometer. Electrospray ionization mass spectrometry was performed with a liquid chromatography mass spectrometry apparatus (Agilent, Palo Alto, CA, USA). Ultraviolet-visible (UV-Vis) titration experiments were made on a Shimadzu UV2550 spectrophotometer at 298 K. Fluorometric titration was performed on a Cary Eclipse Fluorescence Spectrophotometer at 298 K (Agilent, Palo Alto, CA, USA). The binding constant, K_s, was obtained by nonlinear least square calculation method for data fitting.

The cells at logarithmic growth phase were seeded in a 96-well plate at a density of 2.0×10^4 cells/well and cultured for 24 hours. After that, the culture media were replaced with 200 μL of Roswell Park Memorial Institute 1640 medium containing different concentrations of the compound, and the cells were further incubated for 24 hours. Next, the cells were washed with phosphate-buffered saline 3 times, and then 100 μL of culture medium and 20 μL of MTT solution were respectively added to each well. After the additional incubation (4 hours), the absorbance of each well was detected at 490 nm by 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.



SCHEME 1 Synthesis route for compounds

Compounds **1** to **4** were synthesized according to the route shown in Scheme 1.

Compound **1**: An organic ethanol solution of 4-nitrophenylhydrazine (10 mmol, 1.5314 g) was added to a 3-necked flask, and a hot ethanol solution of 1-naphthaldehyde (10 mmol, 1.7218 g) was added drop wise to the above solution, heating, magnetic stirring, so that completely dissolved, and soon, the color of the solution changed from light yellow to brick red. The mixture reacted at room temperature for 40 minutes and refluxed for 6 hours. Then, cooled and stood, brick-red solid appeared. Filtered, washed with anhydrous ethanol, and recrystallized by dichloromethane and anhydrous methanol in a volume ratio of 1:1 and brick-red solid obtained. Yield: 62%. Proton nuclear magnetic resonance (400 MHz, DMSO) δ 11.44 (s, 1H), 8.74 (d, J = 11.0 Hz, 2H), 8.19 (d, J = 9.4 Hz, 2H), 8.08 to 7.91 (m, 3H), 7.70 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H), 7.65 to 7.54 (m, 2H), 7.24 (d, J = 8.7 Hz, 2H). Elemental analysis: calc for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.42; found: C, 70.32; H, 4.19; N, 14.62. High-resolution mass spectrometry (*m*/*z*): 290.0921 $(M—H)^-$.

Compound **2**: The synthesis method was similar to the above procedure. Yield: 75%. Proton nuclear magnetic resonance (400 MHz, DMSO) δ 11.87 (s, 1H), 9.58 (s, 1H), 8.92 (d, J = 2.6 Hz, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 12.2 Hz, 1H), 8.21 to 8.13 (m, 2H), 8.08 (dd, J = 15.8, 8.2 Hz, 2H), 7.76 to 7.60 (m, 3H). Elemental analysis: calc for $C_{17}H_{12}N_4O_4$: C, 60.71; H, 3.60; N, 16.66; found: C, 60.47; H, 3.75; N, 16.89. High-resolution mass spectrometry (*m*/*z*): 335.0781 $(M—H)^-$.

Compound **3**: The synthesis method was similar to the above procedure. Yield: 64%. Proton nuclear magnetic resonance (400 MHz, DMSO-d_6, 298 K) δ 11.42 (s, 1H), 8.99 (s, 1H), 8.77 (d, J = 8.6 Hz), 8.20 (d, J = 9.3 Hz), 7.88 (d, J = 8.7 Hz), 7.69 to 7.52 (m), 7.39 (dd, J = 21.6, 14.5 Hz), 7.24 (d, J = 8.9 Hz), 7.12 (d, J = 9.0 Hz). Elemental analysis: calc for $C_{17}H_{13}N_3O_3$: C, 66.44; H, 4.26; N, 13.67; found: C, 66.50; H, 4.19; N, 13.93. High-resolution mass spectrometry (*m*/*z*): 306.0887 $(M—H)^-$.

Compound **4**: The synthesis method was similar to the above procedure. Yield: 80%. Proton nuclear magnetic resonance (400 MHz, DMSO-d_6, 298 K) δ 12.23 (s, 1H), 9.33 (s, 1H), 9.23 (d, J = 8.5 Hz), 8.89 (dd, J = 9.6, 2.7 Hz), 8.35 (dd, J = 25.1, 9.2 Hz), 8.15 to 8.00 (m), 7.85 (t, J = 7.5 Hz), 7.68 (d, J = 8.9 Hz). Elemental analysis: calc for $C_{17}H_{12}N_4O_5$: C, 57.96; H, 3.43; N, 15.90; found: C, 58.04; H, 3.51; N, 15.77. High-resolution mass spectrometry (*m*/*z*): 351.0769 $(M—H)^-$.

3 | RESULTS AND DISCUSSION

3.1 | Ultraviolet-visible titration

The binding abilities of synthesized compounds with amino acids were investigated by using UV-Vis absorption method in DMSO-H_2O (1:1, *v*/*v*) at 298 K. The UV-Vis spectral changes of the compounds were shown in Figure 1 during the titration with Arg. In the absence of Arg, compound **4** (4.0×10^{-5} mol L^{-1} in DMSO) exhibited an obvious peak at 420 nm. With the increase of Arg, the intensity of absorption peak at 420 nm decreased remarkably and a new absorption peak appeared centered at about 510 nm. As a result, the absorption peak shifted to the long wavelength direction gradually and red-shift

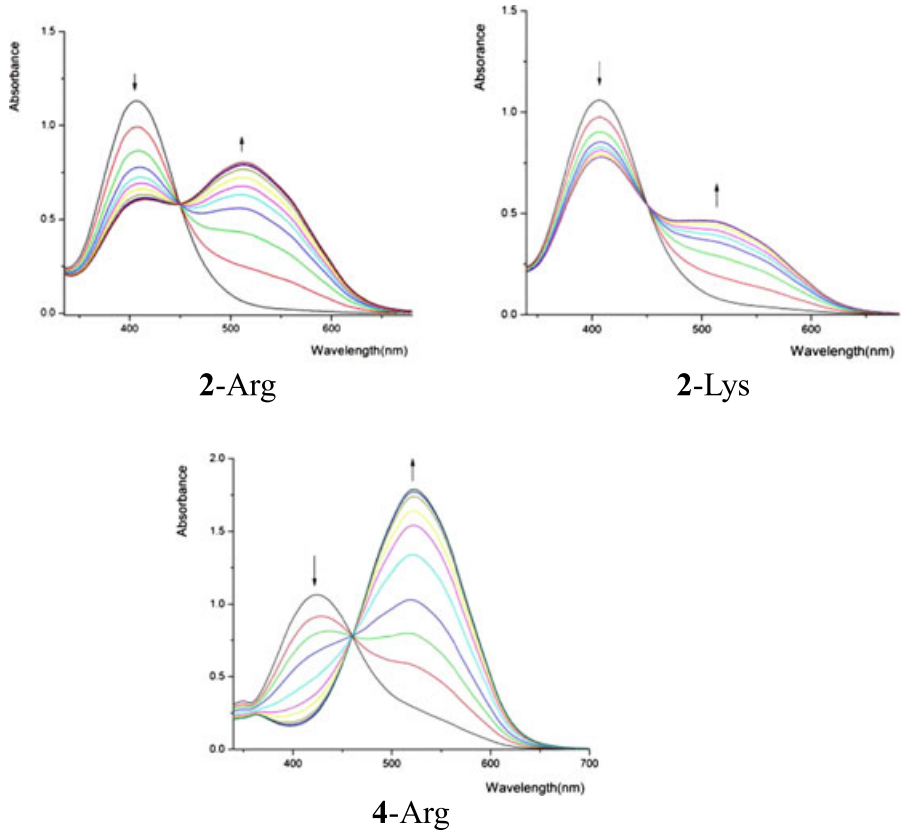


FIGURE 1 Ultraviolet-visible (UV-Vis) spectral changes of the compounds upon the addition of arginine (Arg). [compound] = 4.0×10^{-5} mol L^{-1}; [Arg] = 0 to 1.6×10^{-3} mol L^{-1}. The arrows indicate the direction of increasing anion concentration

phenomenon occurred after compound **4** interacted with Arg. One clear isosbestic point appeared at 450 nm, indicating the formation of stable complexation (**4**-Arg). Analogous investigations were carried out on other normal amino acids. However, the additions of histidine, asparagine, lysine, threonine, and tryptophan proline, leucine, phenylalanine, alanine, glycine, valine, methionine, aspartic acid, glutamic acid, isoleucine, serine, cysteine, tyrosine, and glutamine induced very weak spectral response. The above results indicated that compound **4** showed no binding ability toward these amino acids or the binding abilities were very small and could be ignored (Figure 2).

The type of the compounds can be adjusted by changing the electronic properties of the ortho-, meta-, or para-substituents according to the resonance structure, and the binding ability of the amino acid can also be changed accordingly.[30] Therefore, compounds **1**, **2** (*o*-NO_2), and **3** (*m*-OH) were synthesized, and the influence of the electronic properties of the substituents on the interaction between the host and guest was studied. The results showed that the absorption spectra of compound **2** showed remarkable changes with Arg and lysine additions, whereas compounds **1** and **3** did not change (Figure 2). In addition, red-shift phenomenon occurred at different levels, and a distinct allele appeared, indicating that both compounds **2** and **4** interact with Arg. Compared with compound **2**, the red-shift effect of compound **4** and Arg was obvious and may be related to the space geometries of host-guest. And the following theoretical studies have also confirmed that.

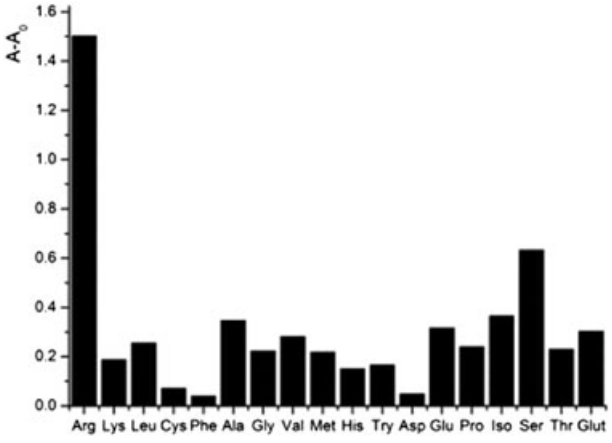


FIGURE 2 Ultraviolet-visible (UV-Vis) spectral changes of compound **4** upon the additions of various amino acids

3.2 | Fluorescent response

The photophysical responses of the 4 compounds to the added amino acids were also investigated in DMSO-H_2O (1:1, *v*/*v*) solution. By the addition of Arg gradually, the fluorescence intensity of compound **4** centered at 570 nm increased (Figure 3). According to the literature,[31-33] fluorescence enhancement may be related to the change of free energy. In the synthetic compound, the receptor is isolated from the fluorophore by a —OH and —NH—N spacer as an interaction site. In addition, the intramolecular hydrogen bonds were present between —C N— and near —OH. When compound **4** interacted with Arg, electron transfer occurred in —OH to —C N—. Typically, the emission peak was enhanced after the addition of Arg. Thus, the fluorescence intensity of compound **4** enhanced after it interacted with Arg

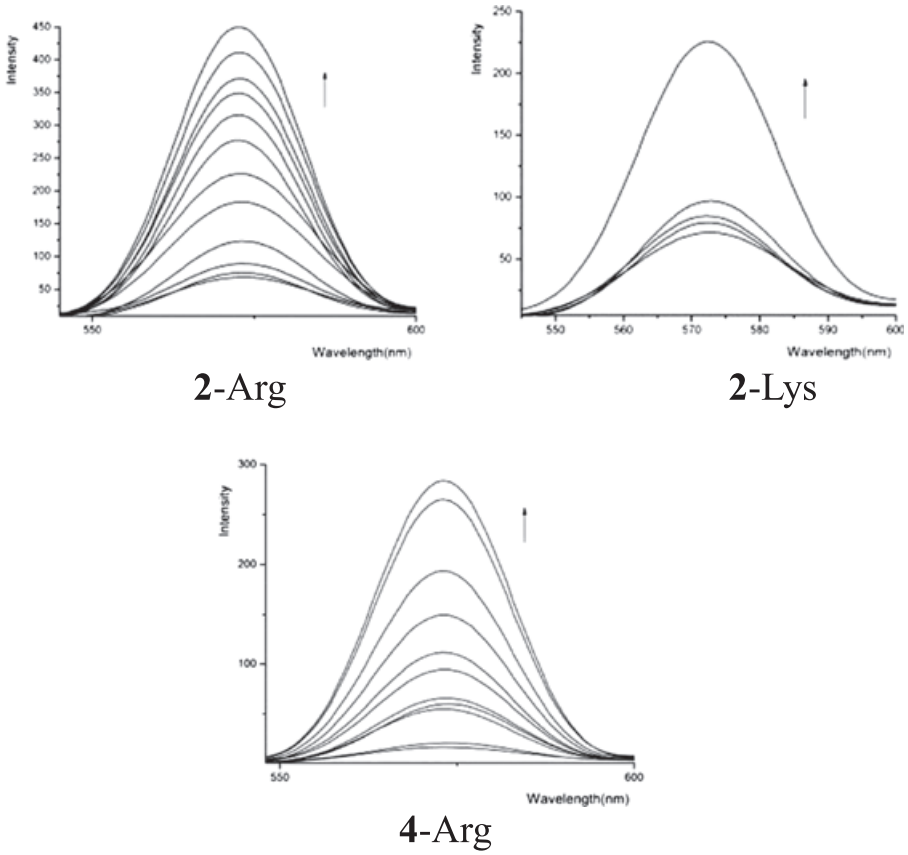


FIGURE 3 Fluorescence response of the compounds (4.0×10^{-5} mol L^{-1}) upon the addition of arginine (Arg; 0-2.0×10^{-3} mol L^{-1}). The arrows indicate the increase direction of Arg concentration

and compound **4** can be used as fluorescent probes for the detection of Arg. In the addition of leucine, phenylalanine, alanine, glycine, valine, methionine, histidine, tryptophan, aspartic acid, glutamic acid, cysteine, proline, isoleucine, serine, threonine, glutamine, tyrosine, asparagine, and lysine, the fluorescence response was very small that indicated that compound **4** showed no binding ability toward these amino acids (Figure 4). According to UV-Vis and fluorescence data, the synthesized compound **4** showed high selectivity and specificity for Arg. Therefore, compound **4** can be used as fluorescence probe for the detection of Arg.

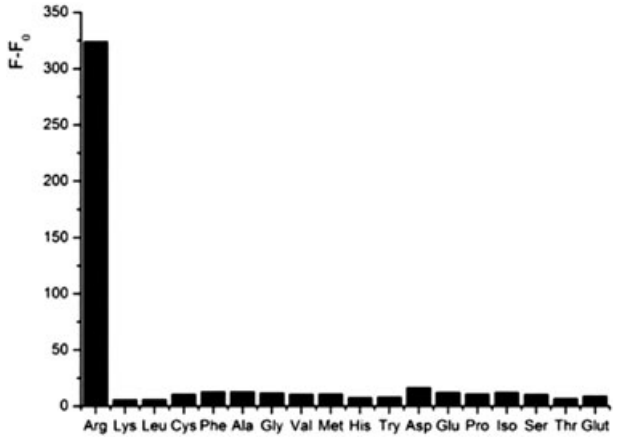


FIGURE 4 Changes in fluorescence intensity of compound **4** in presence of 10 equiv of amino acids tested

Similarly, compound **2** showed remarkable fluorescence responses for Arg and Lys, while compound **2** showed very weak fluorescence responses for other amino acids tested. The above results indicated that compound **2** showed strong binding abilities for Arg and Lys, and almost no binding ability for other amino acids or the binding ability was very weak and could be ignored. At the same time, compounds **1** and **3** showed almost no fluorescence response for all amino acids tested, which suggested that the binding abilities were very weak and could be ignored.

3.3 | Binding constant

According to the job-plot analysis, compounds **2** and **4** interacted with amino acids as the ratio of 1:1. By the method of nonlinear least squares calculation, the binding constants could be obtained and listed in Table 1[33-35] based on the UV-Vis data. From Table 1, the fluorescent probe **4** showed the strongest binding ability for Arg among amino acids tested. The reason may be that (i) the —C N and —OH or $—NO_2$ group of the fluorescent probe could interact with the guanidine group of the Arg through hydrogen-bond and (ii) conjugate effect and conjugate system because of its outstanding basicity. However, the fluorescent probe of compound **4** showed very weak emission changes for leucine, phenylalanine, alanine, glycine, valine, methionine, histidine, tryptophan, aspartic acid, glutamic acid, proline, isoleucine, serine, threonine, glutamine, and cysteine and the binding ability could be ignored. The reason may be that π-π staking possibly existed between the 4 compounds and amino acids. For Arg, the binding ability

TABLE 1 Binding constants of compounds with various amino acids

Amino Acid	K_s (1)	K_s (2)	K_s (3)	K_s (4)
Arginine	<10	$(9.78 \pm 0.08) \times 10^6$	<10	$(1.82 \pm 0.09) \times 10^7$
Lysine	ND	$(1.44 \pm 0.02) \times 10^6$	<10	<10
Leucine	ND	<10	<10	$(1.63 \pm 0.02) \times 10^3$
Phenylalanine	ND	<10	ND	<10
Alanine	ND	<10	ND	$(2.96 \pm 0.05) \times 10^3$
Glycine	ND	ND	<10	$(1.31 \pm 0.10) \times 10^3$
Valine	ND	ND	<10	$(3.89 \pm 0.04) \times 10^3$
Methionine	ND	ND	ND	$(1.84 \pm 0.09) \times 10^3$
Histidine	ND	<10	ND	$(4.54 \pm 0.01) \times 10^2$
Tryptophan	ND	ND	ND	<10
Aspartic acid	ND	ND	<10	<10
Glutamic acid	ND	<10	ND	<10
Proline	ND	<10	ND	$(7.22 \pm 0.30) \times 10^2$
Isoleucine	ND	<10	ND	$(2.83 \pm 0.01) \times 10^3$
Serine	<10	<10	<10	$(1.03 \pm 0.05) \times 10^3$
Threonine	ND	ND	ND	$(2.06 \pm 0.01) \times 10^3$
Glutamine	ND	<10	<10	<10
Cysteine	ND	ND	ND	ND

ND: the binding constant could not be determined.

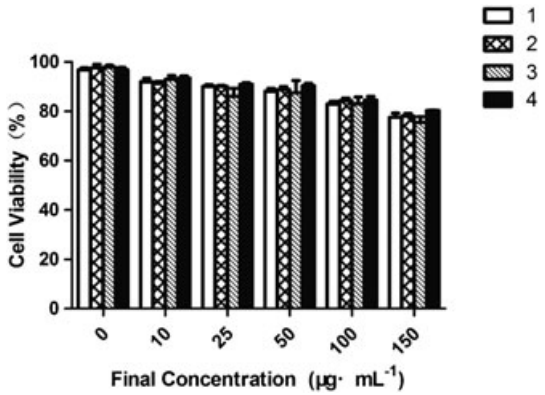


FIGURE 5 Cell viability values (%) estimated by an MTT proliferation test versus incubation concentration of fluorescence probe. MCF-7 cells were cultured in the presence of 0 to 200 μg mL^{-1} of fluorescence probe at 37°C for 24 hours. Cell viability (expressed in %) was calculated considering 100% growth in the absence of fluorescence probe

followed the order of **4** > **2**. Compound **4** showed the strongest binding ability high selectivity for Arg among 4 compounds. The reason may be the well match of space geometry. Therefore, compound **4** could be used as a good biosensor for the detection of Arg.

3.4 | Cytotoxicity assay

Scientific research suggests that glutathione peroxidase is an important selenoprotein that is expressed in most tissues. However, glutathione peroxidase is not found in human breast cancer cells (MCF-7). Therefore, we chose MCF-7 cell, the special cell lines.[36] Cytotoxicity of the compounds toward a cervical cancer cell line (MCF-7 cell) was evaluated by using a conventional MTT assay. No remarkable differences in the proliferation of the cells were observed in the absence and presence of fluorescence probe (0-150 μg mL^{-1}; Figure 5). The cellular viability was estimated to be 80% after 48 hours of incubation with the fluorescence probe <150 μg mL^{-1}. The anticipated cytotoxicity of fluorescence probe (<150 μg mL^{-1}) was expected below. Combination with binding the constants, compound **4** showed high binding ability and low cell cytotoxicity and may be used to detect Arg in vivo.

3.5 | Theoretical investigation

The geometries of compound **4** and the combination product **4**-Arg were optimized (Figure 6) by using Hartree-Fock method with basis sets 3-21G. The calculation was performed with Gaussian03 program.[37] From Figure 6, the intramolecular hydrogen bond indeed existed in compound **4**. The distance between the hydrogen atom of imine (—HN—N) and the oxygen atom of nitro group was 1.761 Å. According to literatures,[38,39] the existence of intramolecular hydrogen bond and electron-withdrawing group could strengthen the host-guest binding ability. The stronger the electron-withdrawing effect was, the higher the anion binding ability was. The combination product between compound **4** and Arg was also optimized, which was shown in Figure 6. After compound **4** interacted with Arg, the structure of combination product **4**-Arg changed compared with compound **4**. The intramolecular hydrogen-bond between imine and nitro groups disappeared, and a new intramolecular hydrogen-bond formed between the nitrogen atom of —CH N— and oxygen atom of hydroxyl group, where the distance of the hydrogen bond was 1.604 Å. Meantime, the angle of 2 nitrogen atoms in the nitro group changed slightly from 122.8° to 122.0°, which approved that the space structure of compound **4** changed after it interacted with Arg. In addition, the intermolecular hydrogen bond also formed between nitrogen atoms in the nitro group of compound **4** and hydrogen atom in the carboxyl group

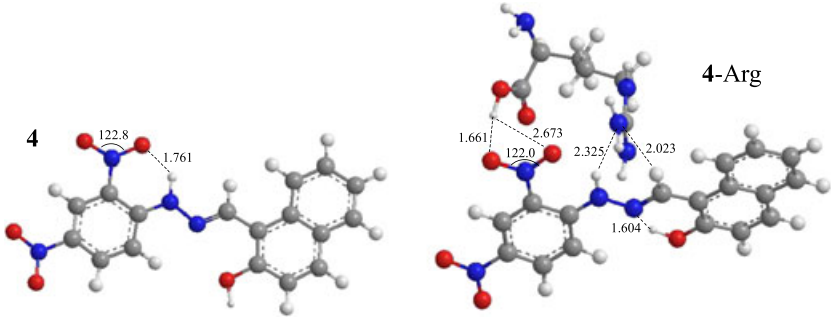


FIGURE 6 The optimized structures of compound **4** and the combination product **4**-Arg

of Arg. Especially, guanidine group interacted with —NH—N CH— of compound **4**, which approved the experimental results. The above theoretical investigation approved the experimental results, where the stable host-guest complex formed also explained the reason why compound **4** showed strong binding ability for Arg.

4 | CONCLUSION

In conclusion, the 4 compounds were developed and demonstrated a highly sensitive and selective absorption assay for Arg among 18 standard amino acids. Theoretical and experimental data both indicated that compound **4** containing hydroxyl and 2 nitro groups exhibited the strongest binding ability for Arg and showed low cytotoxicity to MCF-7 cells over a concentration range of 0 to 150 μg mL^{-1}, which may be used as a biosensor for the Arg detection in vivo. This work is of great importance in gaining a better understanding of the special properties of Schiff base in the presence of amino acids and also greatly expands the scope of reagents that may find future application in the development of analytical methods for the selective determination of those amino acids such as Arg.

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