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RESEARCH ARTICLE

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The arginine detection and cytotoxicity of fluorescent probes based on naphthalene derivatives

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Abstract

From a biological point of view, Schiff base probes containing fluorescent groups are chemically modified to provide an effective method for the detection of amino acids. According to this method, three molecular probes containing Schiff base and hydroxyl group have been designed and synthesized. UV-Vis and fluorescent data indicated that compound **2** showed strong sensitivity and high selectivity for arginine (Arg) among normal twenty kinds of amino acids. In addition, compound **2** displayed high combining ability with Arg and low cytotoxicity in MCF-7 cell from 0 to 150 μg/mL. The above results showed the synthesized probes also can be used a biosensor for the Arg detection in vivo.

1 | INTRODUCTION

In recent years, more and more attentions have been paid to the research and application field of host–guest chemistry, which boosted the rapid development of molecular recognition.[1–3] Amino acids are important components of organisms. In particular, biosensors, as tools for detecting amino acids, have gained more and more attentions.[4,5] So, the detection of amino acids is very vital in organisms.[6,7]

Arginine (Arg) has an important physiological role in cell division, gene regulation, wound healing, hormone release, and immune system function.[8–10] In addition, Arg is also clinically

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used as a treatment for diseases caused by endocrine and high ammonia reactions.[11,12] Lacking of Arg will also lead to an autosomal recessive inherited disease that is the final step in enzymatic urea cycling.[13–15] Therefore, the selective recognition of Arg is crucial in biochemical and clinical applications.

A variety of detection procedures for Arg have been developed. More traditional detection methods, such as high-performance liquid, ion exchange, gas chromatography, have been reported on the detection of Arg.[16–18] However, the traditional detection methods have some shortcomings, such as complex and expensive instruments, inconvenient operation, poor selectivity, precision, sensitivity, and high repeatability.[19] The design of artificial receptors becomes attractive due to its high selectivity and sensitivity. And the fluorescence probe has been reflected in this regard as a convenient detection method.[20,21] In recent years, more reports on fluorescent molecular probes are based on their advantages in the biological environment and their clinical potential applications.[22–24] However, there are few reports based on the detection and application of fluorescent probes in amino acids, especially in Arg.

In addition, Arg has a certain basicity due to its guanidine moiety, which can form hydrogen bonds. Based on the above consideration, we have rationally designed and synthesized a series of Schiff base derivatives obtained by the reaction of aldehyde groups and amino groups. By detecting the reaction of compounds with different amino acids, it was found that the synthesized compounds showed the highest selectivity and the highest specific binding ability to Arg among amino acids tested. The changes of fluorescence intensity were observed after the Arg was added to the synthesized compounds.

2 | MATERIAL AND METHODS

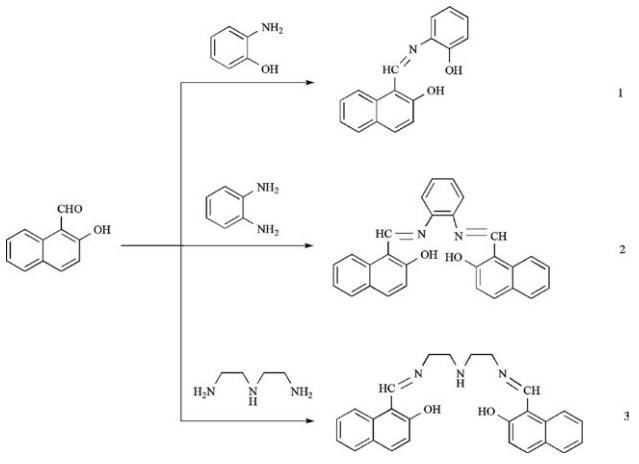
Some of the materials were commercially available and the reagents and solvents used are analytical. All amino acids were from Aladdin Chemical Co. Ltd. (Shanghai, China) and stored in a desiccator for use without purification. Dimethyl sulfoxide (DMSO) was dried over CaH_2, vacuum distilled and stored for use. C, H, and N elemental analyzes were performed using a Vanio-EL instrument. ^{1}H NMR and ^{13}C NMR spectra were determined using a Unity Plus-400-MHz spectrometer. ESI-HRMS was measured using a Mariner instrument. UV-Vis titrations were measured using a Shimadzu UV2600. Fluorescence spectra were measured using an Eclipse fluorescence spectrophotometer (Agilent, USA). The binding constant K_s was fitted by a nonlinear least square method. All experiments were conducted on the basis of pH = 7.4.

The cultured cells were seeded in 96-well plates at a density of 2.0×10^4 cells/well and cultured. Different concentrations of probes solution were added to the cells and further incubated for 24 hours. 96-well plates were tested for absorbance at 490 nm using a microplate reader (Thermo Multiscan MK3, Thermo Fisher Scientific, MA, USA). Cell viability was calculated from the following formula: Cell viability (%) = (average absorbance of drug group − average absorbance of blank) − (average absorbance of control group − average absorbance of blank).[25]

Compounds **1**-**3** were synthesized according to the route shown in Scheme 1.

Compound **1**: *o*-aminophenol (1.0910 g, 10 mmol) was added to absolute ethanol (20 mL) and the mixture was stirred. Then, 2-hydroxy-1-naphthaldehyde (1.7220 g, 1 mmol) in the ethanol solution (20 mL) was added to the above solution. After the mixture was heated for 6 hours, yellow precipitate was obtained by hot filter, then washed three times by hot ethanol, dried in vacuum. Yield: 80%. M.p.: 261~263°C. ^{1}HNMR (400 MHz, DMSO-d_6, 298 K) δ 15.70 (t, J = 8.8 Hz, 1H), 10.34 (s, 1H), 9.46 (dd, J = 28.9, 23.2 Hz, 1H), 8.35 (t, J = 25.3 Hz, 1H), 8.02-7.86 (m, 1H), 7.78 (t, J = 19.3 Hz, 1H), 7.72 (s, 1H), 7.46 (m, 1H), 7.25 (dt, J = 21.0, 11.4 Hz, 1H), 7.20 (s, 1H), 7.07-6.84 (m, 2H), 6.84 (s, 1H). ESI-HRMS (m/z): 264.1017 $(M + H)^+$.

Compound **2**: *o*-phenylenediamine (0.3460 g, 3.2 mmol) was dissolved in anhydrous methanol at room temperature. The hot methanol solution (10 mL) containing 2-hydroxy-1-naphthaldehyde (1.0331 g, 6.0 mmol) was slowly added to the above solution followed by heating and stirring, the color of the mixture rapidly changed from colorless to orange. The mixed solution was refluxed for 3 hours at 65°C, then stood and cooled, orange powder was precipitated. The powder was washed twice by CH_3Cl and CH_3OH (1:1, v:v) and recrystallized to give an orange needle-like solid (0.9964 g). Yield: 79.8%. M.p. 235~236°C, ^{1}HNMR (400 MHz, DMSO-d_6, 298 K) δ 15.13 (d, J = 4.0 Hz, 1H), 9.71 (d, J = 3.9 Hz, 1H), 8.56 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.84 (m, 2H), 7.56 (s, 1H), 7.46 (dd, J = 6.0,



SCHEME 1 Synthesis route for compounds

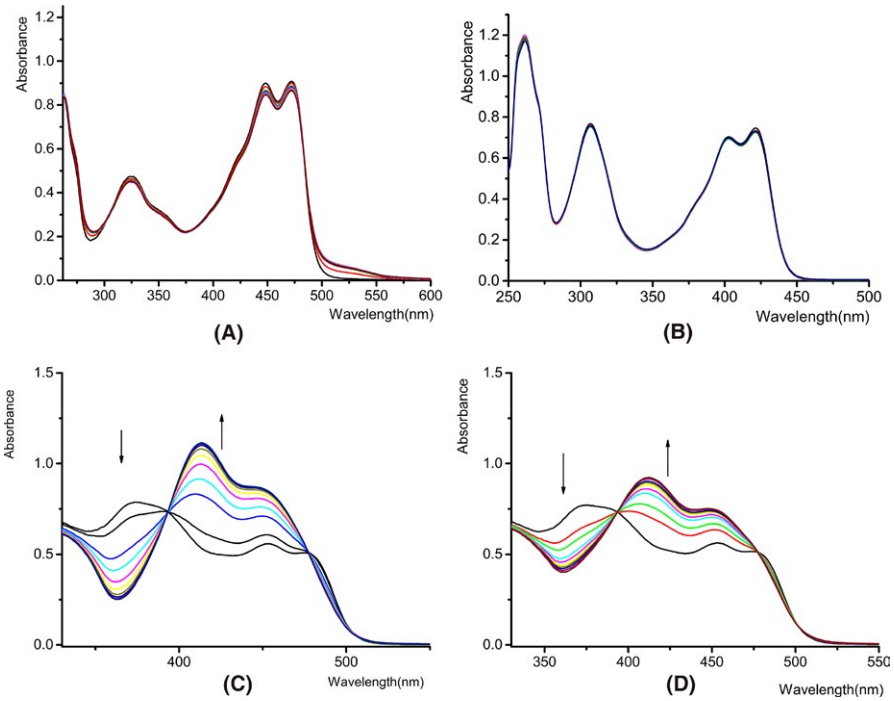


FIGURE 1 UV-Vis spectral changes of compound **1**(A), **2**(C, D), **3**(B) upon the addition of Arg (A, B, C) and Lys (D). [compounds] = 4.0×10^{-5} mol/L; [Arg] = 0-1.6×10^{-3} mol/L; [Lys] = 0-1.4×10^{-3} mol/L

3.4 Hz, 1H), 7.38 (s, 1H), 7.08 (d, $J = 9.2$ Hz, 1H). ESI-HRMS (m/z): 439.1415 $(M + Na)^+$.

Compound **3**: Diethylenetriamine (0.3301 g, 3.2 mmol) was dissolved in anhydrous methanol (20 mL) and then added slowly to the methanol solution (10 mL) containing 2-hydroxy-1-naphthaldehyde (1.0503 g, 6.1 mmol). The mixture was heated and stirred magnetically until completely resolving. After the reaction was carried out for 45 minutes, the color of the mixture solution changed from colorless to orange. Then, the mixture solution was refluxing for 3 hours. The mixture was stood and cooled at room temperature. The yellow solid was filtered, washed twice with anhydrous ethanol, and recrystallized from trichloromethane. The yellowish solid (0.6769 g) was obtained. Yield: 51.9%. M.p.: 273~275°C. ^{1}H NMR (400 MHz, DMSO-d_6, 298K) δ 13.98 (s, 1H), 9.08 (d, $J = 10.6$ Hz, 1H), 8.04 (d, $J = 8.4$ Hz, 1H), 7.70 (d, $J = 9.4$ Hz, 1H), 7.61 (d, $J = 7.7$ Hz, 1H), 7.38 (t, $J = 7.6$ Hz, 1H), 7.16 (t, $J = 7.4$ Hz, 1H), 6.70 (d, $J = 9.4$ Hz, 1H), 3.71 (dd, $J = 10.9$, 5.4 Hz, 2H), 3.37 (s, 1H), 2.90 (t, $J = 5.9$ Hz, 2H). ESI-HRMS (m/z): 434.1843 $(M + Na)^+$.

3 | RESULTS AND DISCUSSION

3.1 | UV-Vis titration

The binding abilities of three Schiff base compounds with amino acids were determined by UV-Vis absorption spectroscopy at 298 K. The UV-Vis spectral of compound **2** with Arg was shown in Figure 1. In the absence of Arg, the probe **2** (4.0×10^{-5} mol/L in DMSO-H_2O (1:1, v/v)) exhibited an obvious peak at 360 nm. With the addition of Arg, the intensity of absorption peak at 360 nm decreased gradually and formed a trough at 345 nm. At the same time, a new absorption peak at about 420 nm appeared and the intensity increased. As a result, the red-shift phenomenon of compound **2** appeared interaction with Arg. A clear isosbestic point appeared at 398 nm, indicating that a stable complex (**2**-Arg) had formed between host and guest. After the addition of lysine (Lys), the similar spectral change was observed compared with Arg (Figure 1C) which showed compound **2** also interacted with Lys (Figure 1D). In addition, red-shift phenomenon occurred at different degree, and one clear isosbestic point appeared which might be related to the space

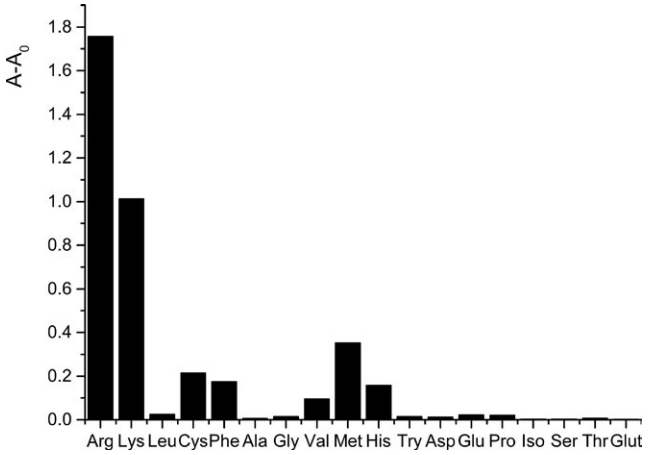


FIGURE 2 UV-Vis spectral changes of compound **2** (4×10^{-5} mol/L) upon the additions of 10 equiv of various amino acids (2×10^{-2} mol/L) at the wavelength of 420 nm

geometries of host–guest. Other amino acids were similarly studied. However, the additions of histidine, asparagine, threonine, tryptophan, proline, leucine, phenylalanine, alanine, glycine, valine, methionine, aspartic acid, glutamic acid, isoleucine, serine, cysteine, tyrosine, and glutamine induced very weak spectral responses which indicating compound **2** did not show any effect on these amino acids or the binding abilities were very weak and could be ignored (Figure 2).

In order to research the difference of binding abilities induced by the different substituents, the structures of this kind of compounds can be tuned by changing the electron property of the substituents on the –C=N– and the near phenolic hydroxyl group position. Therefore, compounds **1** and **3** were also synthesized. UV-Vis spectra showed that compounds **1** and **3** changed little or almost did not change (Figure 1A,B).

3.2 | Fluorescent response

The photophysical responses of three compounds to the added amino acids were also surveyed in DMSO-H_2O (1:1, v/v) solution. With the increasing of Arg, the fluorescence intensity of compound **1** with 575 nm increased (Figure 3A). Similarly, the fluorescence intensity of compound **3** centered at 460 nm also strengthened upon the addition of Arg (Figure 3C). After other amino acids (leucine, phenylalanine, alanine, glycine, valine, methionine, histidine, tryptophan, aspartic acid, glutamic acid, cysteine, proline, isoleucine, serine, threonine, glutamine, tyrosine, asparagine, and lysine) were added, the fluorescence responses were very small which indicated that compounds (**1** and **3**) showed almost no binding abilities toward these amino acids (Figure 4). Nevertheless, the fluorescence intensity of compound **2** was unchanged after Arg was added (Figure 3B). That is to say, upon the addition of Arg,

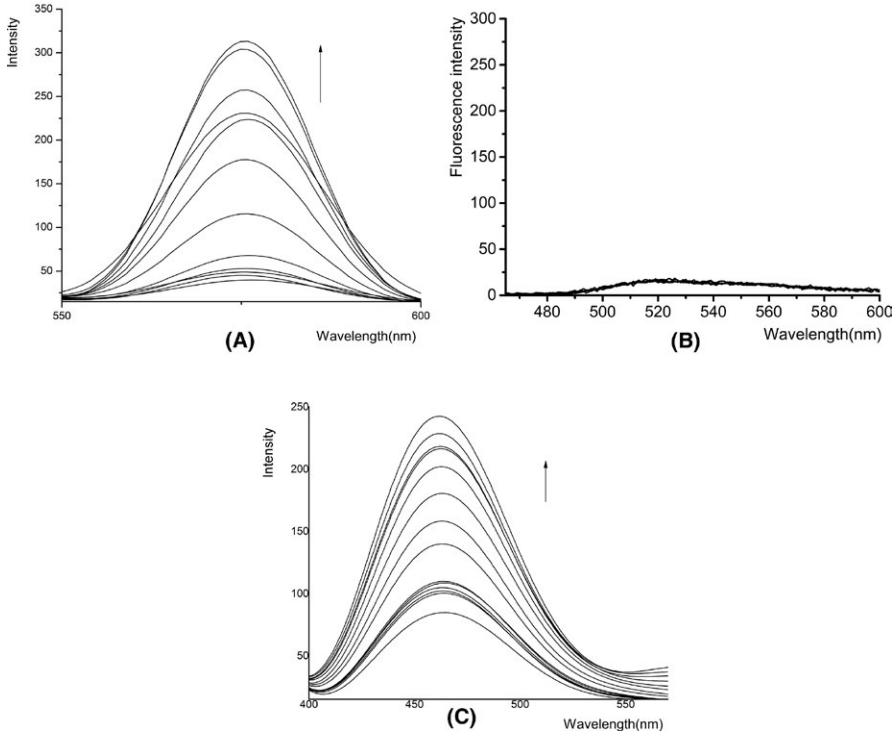


FIGURE 3 Fluorescence response of compound **1**(A), **2**(B), **3**(C) (4.0×10^{-5} mol/L) with the addition of Arg (0-2.0×10^{-3} mol/L)

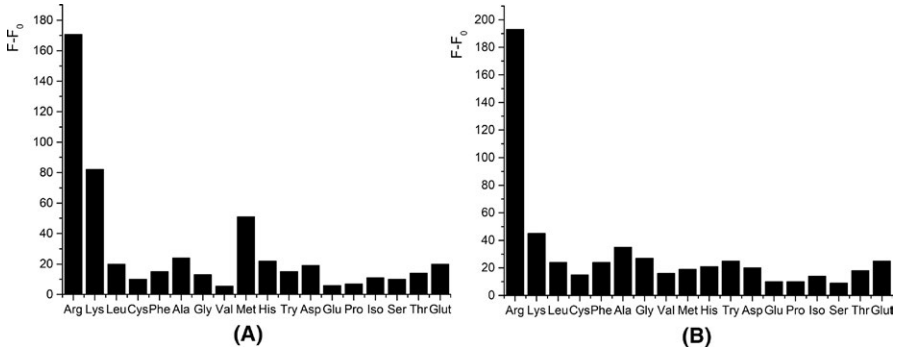


FIGURE 4 Changes in fluorescence intensity of compounds **1** (A) and **3** (B) (4.0×10^{-5} mol/L) in presence of 10 equiv of amino acids tested

TABLE 1 The binding constant of the interaction between the host and the guest

Amino acid	*Ks* (1) M^{-1}	*Ks* (2) M^{-1}	*Ks* (3) M^{-1}
Arg	<10	$(3.90 \pm 0.38) \times 10^6$	ND
Lys	ND	$(5.73 \pm 0.32) \times 10^3$	<10
Leu	ND	<10	<10
Phe	ND	<10	ND
Ala	ND	ND	ND
Gly	ND	<10	<10
Val	ND	<10	<10
Met	ND	<10	ND
His	ND	<10	ND
Try	ND	ND	ND
Asp	ND	ND	<10
Glu	<10	ND	ND
Pro	ND	ND	ND
Iso	ND	ND	ND
Ser	ND	ND	<10
Thr	ND	ND	ND
Gln	ND	ND	<10
Cys	ND	<10	ND

ND: the spectral changes were very small and could not be determined.

the UV-Vis of compound **2** changed; however, there was no fluorescence response of it. While the fluorescence spectra of **1** and **3** changed, their UV-Vis spectra did not change.

The fluorescence changes of compounds may be related to free energy.[26] In the structure of the compounds, the receptor was separated from the fluorophore by two interacting site –OH spacers. Therefore, the electron may be transferred from –OH to –C=N– after interaction between compounds and Arg. The evidence came from ^{1}H NMR titrations of compounds with Arg. When Arg was added to compounds, the loss of proton (H^+) in compound **1** was derived from the phenolic hydroxyl group on the benzene, and the loss of proton (H^+) in the compound **2** was came from the phenolic hydroxyl group on the naphthalene. But, the ^{1}H NMR spectrum of compound **3** did not change after Arg was added.

3.3 | Binding constant

The job-plot analysis showed that the ratios between compounds **1**, **2**, **3**, and Arg were 1:1, 1:2, and 2:3, respectively (Figure S10). The obtained binding constants were listed in Table 1. The nonlinear least square method was fitted according to UV-Vis data and fluorescence data.[27–29] From Table 1, the probes showed the strongest binding ability with Arg among all tested amino acids. The reason may be that based on the C=N and –OH, the probes can interact with the guanidine group of Arg.[30] However, among twenty amino acids, only Arg, Lys, and His belong to basic amino acids, and their basic chemical groups are guanidyl, amino, and imidazolyl. Guanidyl has a relatively strong basicity and the imidazolyl group has the smallest basicity. And other amino acids are acidic amino acids and neutral amino acids; they are not easy to react with compounds (**1**, **2**, **3**). So, the binding ability of the synthesized compounds to other amino acids was very weak.

Data analysis of UV-Vis, fluorescence, ^{1}H NMR titrations and binding constants showed that compounds **1** and **3** gave negligible response to Arg. Despite significant response was observed in the corresponding fluorescent titrations, compound **2** had the strongest binding capacity for Arg.

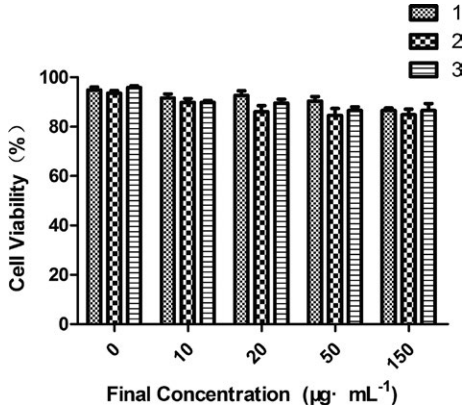


FIGURE 5 Cell viability value (%) is compared with the concentration. MCF-7 was incubated at 37°C for 24 h with a probe at 0-150 μg/mL. In the absence of probes, cell viability (in%) was calculated in view of 100% growth

3.4 | Cytotoxicity assay

Studies have shown that glutathione peroxidase (GPx1) and selenium binding protein (SBP1) are two important selenoprotein that are expressed in most tissues. However, GPx1 is not expressed in human breast cancer cells (MCF-7), so a special cell line MCF-7 was chosen.[31] The MTT assay was used to evaluate the cytotoxicity of drugs on human breast cancer cell lines (MCF-7 cells).[32] When the concentration of probes was in the range (0-150 μg/mL), the proliferation of cells did not appear much difference (Figure 5). Probes (<150 μg/mL) were found to be 80% viable after the incubation was 24 hours. Based on the above analysis, compound **2** showed high binding ability and low cytotoxicity, and could be used for in vivo detection of Arg.

4 | CONCLUSION

In conclusion, the three naphthalene-based derivatives herein showed high selectivity and sensitivity to Arg among natural amino acids. Compound **2** demonstrated the strongest binding

capacity for Arg and low cytotoxicity to MCF-7 cells, so it can be used to detect Arg in vivo. This work broadens the application fields of probes and provides the basis for clinical application.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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