Materials Science and Engineering C 51 (2015) 279–286

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Synthesis and cytotoxicity of azo nano-materials as new biosensors for L-Arginine determination

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ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form 3 March 2015
Accepted 8 March 2015
Available online 10 March 2015

Keywords:
Arginine
Biosensor
Nano-material
KYSE450

ABSTRACT

Inspired from biological counterparts, chemical modification of azo derivatives with function groups may provide a highly efficient method to detect amino acid. Herein, we have designed and prepared a series of azo nano-materials involving $-NO_2$, –COOH, $-SO_3H$ and naphthyl group, which showed high response for Arginine (Arg) among normal twenty kinds of (Alanine, Valine, Leucine, Isoleucine, Methionine, Aspartic acid, Glutamic acid, Arginine, Glycine, Serine, Threonine, Asparagine, Phenylalanine, Histidine, Tryptophan, Proline, Lysine, Glutamine, Tyrosine and Cysteine). Furthermore, theoretical investigation further illustrated the possible binding mode in the host-guest interaction and the roles of molecular frontier orbitals in molecular interplay. In addition, nano-material **3** exhibited high binding ability for Arg and low cytotoxicity to KYSE450 cells over a concentration range of 5–50 μmol · L^{-1} which may be used a biosensor for the Arg detection in vivo.

1. Introduction

With the increasing attention of host-guest chemistry, recognition and sensing of various molecular and ionic analytes have recently emerged as a key research field [1–22]. In particular, the detection of amino acid using biosensor has received an increased interest because amino acids, as the building blocks for proteins, play vital roles in the metabolic processes with living bodies [23–25]. As an important amino acid, Arginine (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 [26]. Consequently, the selective recognition of Arg is crucial in the fields of biochemistry and medical science.

Detection methods for Arg have been reported based on traditional determination, such as HPLC, gas phase chromatography, ion exchange chromatography, electrochemical method, etc. [27,28]. The above methods have some deficiency, such as the expensive instruments, the poor repetitiveness and selectivity. 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 [29,30]. Even though considerable efforts have been devoted to develop fluorescent chemosensors for various guests, there have been relatively few reports on Arg selective receptors which show the fluorescent changes or color changes.

Azobenzol derivative is an important organic material and shows unique optical function [31]. As a potential host, azobenzene has been broadly exploited in many areas because of its efficient inclusion ability and easy derivatization by functional groups [32]. In addition, receptors containing aldehyde group show sensitive response for Arg according to literature [33]. Recently, reports of nano-materials are numerous due to their outstanding features in biological environment [34–36]. However, there are few reports about the application of small organic nano-material on the detection of amino acid.

In addition, Arg has an outstanding basicity attributed to its guanidine moiety, which can form a hydrogen-bonding interaction [37]. Based on the above consideration, we synthesized a series of azo derivatives containing aldehyde groups (Scheme 1) and prepared their nano-materials. The detection of amino acid using these biosensors was also studied in neutral aqueous solution and cell cytotoxicity of five nano-materials was evaluated on KYSE450. Results indicated that these nano-materials showed high response for Arg among normal amino acids tested (Alanine, Valine, Leucine, Isoleucine, Methionine, Aspartic acid, Glutamic acid, Arginine, Glycine, Serine, Threonine, Asparagine, Phenylalanine, Histidine, Tryptophan, Proline, Lysine, Glutamine, Tyrosine and Cysteine).

2. Material and methods

All reagents and solvents used were of analytical grade and most of the starting materials, especially for all amino acids, were purchased

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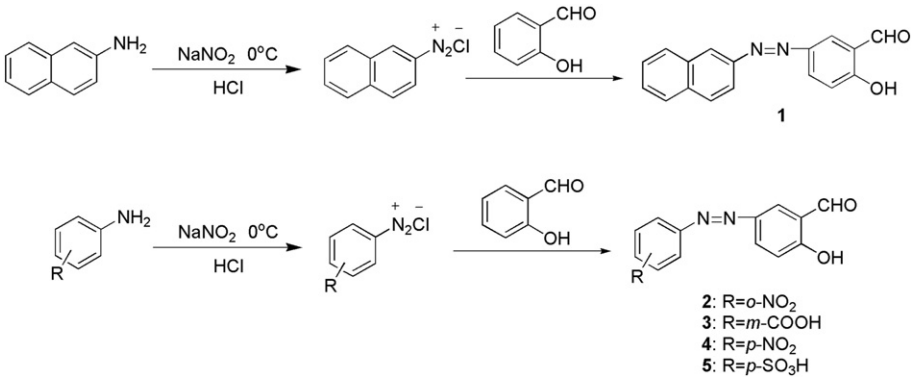
http://dx.doi.org/10.1016/j.msec.2015.03.005
0928-4931/



Scheme 1. Synthesis route for compounds.

from Aladdin Chemistry Co. Ltd (Shanghai, China). All 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. C, H, and N elemental analyses were made on a Vanio-EL instrument. ^{1}H NMR spectra were recorded on a Unity Plus-400-MHz spectrometer. ESI-MS was performed with a Mariner apparatus. 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. The SEM images were obtained by Quanta TM450 FEI coating it with Au. The optimized geometries of compounds were used density functional theory at the B3LYP/3-21G level with Gaussian03 program. The binding constant, K_s, was obtained by non-linear least squares calculation method for data fitting.

Compounds **1**–**5** were synthesized according to the route shown in Scheme 1.

5 − (2′ − Azo − naphthalene) − salicylaldehyde (1)

5-(2′-Azo-naphthalene)-salicylaldehyde was synthesized according to the literature [38]. HCl (37%, 6 mL) was added slowly to a solution of 2-amine-naphthalene (5 mmol, 715 mg) in a small quantity of water at 0–5 °C. Then, $NaNO_2$ (20%, 20 mL) was added to the above-mentioned mixture and the solution was stirred for 1 h to give a bright yellow solution. Salicylaldehyde (5 mmol) dissolved in the solution of Na_2CO_3 (18 g Na_2CO_3 and 150 mL H_2O) was added dropwise to the bright yellow solution for 1 h. After stirring for 4 h, the reaction mixture was neutralized with HCl. The deep-red crude solid was filtered and recrystallized from ethanol to afford a pure product. Yield: 87%. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ 11.39 (s, 1H, –OH), 10.08 (s, 1H, –CHO), 8.92 (d, 1H, ph-H), 8.32 (dd, 2H, ph-H), 8.01–7.94 (dd, 2H, ph-H), 7.84 (d, 1H, ph-H), 7.69–7.56 (m, 3H, ph-H), 7.19 (d, 1H, ph-H). Elemental analysis: Calc. for $C_{17}H_{12}N_2O_2$: C, 73.90; H, 4.38; N, 10.14; Found: C, 73.88; H, 4.61; N, 9.84. ESI-MS (*m*/*z*): 275.3 (*M*-H)$^-$.

5 − (o − Nitro − phenylazo) − salicylaldehyde (2)

The synthesis method was similar to the above procedure. Yield: 77%. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ 11.47 (s, 1H, –OH), 10.02 (s, 1H, –CHO), 8.23–8.16 (m, 2H, ph-H), 7.94 (d, 1H, ph-H), 7.70 (d, 2H, ph-H), 7.61–7.58 (m, 1H, ph-H), 7.15 (d, 1H, ph-H). Elemental analysis: Calc. for $C_{13}H_9N_3O_4$: C, 57.57; H, 3.34; N, 15.49; Found: C, 57.73; H, 3.21; N, 15.67. ESI-MS (*m*/*z*): 270.2 (*M*-H)$^-$.

5 − (*m* − Carboxyl − phenylazo) − salicylaldehyde (3)

The synthesis method was similar to the above procedure. Yield: 91%. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ 13.32 (s, 1H, –OH), 11.64 (s, 1H, –CHO), 10.36 (s, 1H, ph-H), 8.33 (s, 1H, ph-H), 8.21 (s, 1H, ph-H), 8.14–8.07 (m, 3H, ph-H), 7.73–7.69 (t, sH, ph-H), 7.21 (d, 1H, ph-H). Elemental analysis: Calc. for $C_{14}H_{10}N_2O_4$: C, 62.22; H, 3.73; N, 10.37; Found: C, 61.94; H, 4.05; N, 10.56. ESI-MS (*m*/*z*): 269.4 (*M*-H)$^-$.

5 − (*p* − Nitro − phenylazo) − salicylaldehyde (4)

The synthesis method was similar to the above procedure. Yield: 73%. δ 11.48 (s, 1H, –OH), 10.06 (s, 1H, –CHO), 8.41 (d, 2H, ph-H), 8.30–8.21 (m, 2H, ph-H), 8.04 (d, 2H, ph-H), 7.18 (d, 1H, ph-H). Elemental analysis: Calc. for $C_{13}H_9N_3O_4$: C, 57.57; H, 3.34; N, 15.49; Found: C, 57.81; H, 3.59; N, 15.14. ESI-MS (*m*/*z*): 270.1 (*M*-H)$^-$.

5 − (*p* − Sulfonic − phenylazo) − salicylaldehyde (5)

The synthesis method was similar to the above procedure. Yield: 81%. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ 11.55 (s, 1H, –OH), 10.35 (s, 1H, –CHO), 8.18 (d, 1H, ph-H), 8.11–8.08 (dd, 1H, ph-H), 7.82–7.74 (m, 4H, ph-H), 7.20 (d, 1H, ph-H). Elemental analysis: Calc. for $C_{13}H_{10}N_2O_5S$: C, 50.98; H, 3.29; N, 9.15; Found: C, 50.61; H, 3.58; N, 8.99. ESI-MS (*m*/*z*): 304.2 (*M*-H)$^-$.

Nano-materials of five compounds were prepared by reprecipitation method [39,40]. The DMSO and the water solution of CTAB (hexadecyl trimethyl ammonium bromide) were good solvent and poor solvent, respectively. In the experiment, the good solvent containing compound (0.35 mL, 4 mmol·L^{-1}) was poured into the poor solvent containing CTAB (100 mL, 3 mmol·L^{-1}). The mixture was placed for 24 h and centrifuged. The expected solid was washed with water and dried in vacuum.

3. Results and discussion

3.1. SEM images of compounds

The SEM images were obtained by Quanta TM450 FEI coating it with Au (Fig. 1).

As shown in Fig. 1, compound **1** could be assembled into long-thin flakiness on the entire compound. The width of flakiness was nanometer according to the scale. Compound **2** was formed into an oval platelet. Two compounds (**3** and **4**) were both formed into ribbon although the substituent located in different positions. For compound **5** containing *o*-NO_2, it was assembled into flaky and like a flower entirely. Although compounds **2** and **5** have the same substituent (–NO_2), SEM images were remarkably different due to the different locations of nitro group. Therefore, SEM image was related with space configuration.