ABSTRACTS

Two new acetylenic compounds were designed and synthesized as the probes to realize the detection and recognition of Au^{3+} . The latent fluorophores was rearranged into the fluorescent coumarin derivatives with strong fluorescence by a gold ion-mediated hydroarylation reaction. In the meanwhile, the generated fluorescent substances were also isolated and characterized. The probes identified Au^{3+} ions highly selectively over other biologically relevant metal ions, and detection concentration of Au^{3+} was as low as 0.1 μM or less. Fluorescent imaging of Au^{3+} in living cells was also successfully demonstrated.

Keywords: Au^{3+} ; Acetylenic compounds; Fluorescent detection; Bioimaging

Introduction

Gold, a valuable precious metal, as a kind of special metal is of the special physical and chemical properties and has been applied in a variety of fields like catalysis, pharmaceuticals, foods and electronic industry (Zhang et al., 2011; Park et al., 2012; Kargari et al., 2006). Gold ion shows an excellent appropriation and biocompatibility for its application in medication. Gold-based drugs are valuable for the treatment of a wide variety of diseases including rheumatic arthritis, asthma, tuberculosis, malaria, cancer, HIV, and brain lesions (Adhikari et al., 2015; Patil et al., 2012; Yuan et al., 2011; Jeong, et al., 2012).

At the same time, on account of its extensive use has also produced a number of problems of pollution can not be neglected. Gold ion has potential toxicity to the human body due to its damage to the liver, kidneys, and peripheral nervous system (Ott et al., 2009; Li et al., 2008; Chen et al., 2012). Some residues of gold which is in the gold-catalyzed reaction and other areas may enter human body with food or drug and cause serious damage to the human health. Due to its extensive use in the field of food, gold is easy to access to biological body and concentrate through food chains, which often results in denaturation of protein and will produce toxicity to human and animals. The gold in environment can also be washed into rivers, lakes and seas, which is likely to be absorbed by human body, Gold ion can also combine with enzyme in the human body to form complex, causing the enzyme to lose its activity. We usually call it the "gold pollution".

It becomes a very serious issue how to monitor the presence of gold species both in the living and environmental settings (Choi et al., 2013; Hutchings et al., 2008; Yang et al., 2009; Yang et al., 2013; Connor et al., 2005). We try to develop several methods for efficient and convenient detection of Au^{3+} in various samples. Fluorescent probes are powerful tools for monitoring biologically relevant species in vitro and/or in vivo due to their simplicity and high sensitivity. It is a challenge to develop rapidly responsive fluorescent probes which can function in water for bio-applications (Jandl

et al., 1957; Luckey et al., 1975; Yang et al., 2013; Seo et al., 2012; Wang, et al., 2012). A few well-designed fluorescent probes for gold ions in organic solvents or aqueous-organic media have been reported, and some probes have been employed successfully to detect gold ions in cells (Dong et al., 2010; Egorova et al., 2010; Jou et al., 2009; Park et al., 2012; Patil et al., 2012; Wang et al., 2011).

Gold ions are known to activate carbon-carbon multiple bonds, especially alkynes, toward nucleophilic addition. Owing to the characteristic alkynophilicity of the gold ion, a variety of organic transformations have been investigated by employing gold ions (Hashmi et al., 2008; Jun et al., 2011; Kambama et al., 2015; Liu et al., 2010; Pina et al., 2008; Wang et al., 2009; Wang et al., 2012) and based on gold-ion-promoted cyclizations from the non-fluorescent apocoumarin to the strongly fluorescent coumarin (Do et al., 2010).

With these considerations in mind, herein, we design latent fluorescence probes based on apocoumarins for detection of the Au^{3+} , which is based on the well-studied julolidine fluorophore. Latent fluorophore **1**, **2** are activated by a reactive and alkynophilic Au(III) ion, followed by the intramolecular electrophilic aromatic substitution with a Michael acceptor, and will complete the hydroarylation reaction to afford a fluorescent compounds **1-1**, **2-1**, where the gold(III) ion is regenerated. In particular, the probes exhibit several meritorious features as follows: (i) high sensitivity and selectivity to Au (III) ion; (ii) significant fluorescence enhancement based on their structures; (iii) rapid response; and (iv) good cell membrane permeability and excellent live cell imaging. These desirable attributes render the sensor suitable for detection of Au^{3+} .

2. Experimental

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma-Aldrich (St. Louis, MO). All reagents employed for synthesis were obtained commercially and used without further purification. All of the solvents used were of analytical reagent grade. Deionized water was used to prepare all aqueous

solutions. The solutions of metal ions were prepared from their chloride or nitrate salts. Chromatography was carried out on silica gel (200–300 mesh). Thin layer chromatography (TLC) was carried out using silica gel GF₂₅₄ plates with a thickness of 0.20–0.25 mm.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. The ¹H-NMR (600 MHz) and ¹³C-NMR (150 MHz) spectra were recorded in CDCl₃ at 20 °C on Bruker spectrometer, and tetramethylsilane (TMS) as an internal standard. UV–visible spectra were performed on a Thermo Scientific NanoDrop 2000 spectrophotometer. Fluorescence spectra were measured on a Hitachi F-7000 fluorescence spectrophotometer. Mass spectra were carried out using Ultra-Apex Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics Inc). The fluorescence images were obtained using Olympus confocal laser scanning microscopy (Olympus Fluoview FV1000).

2.3 General procedure for analysis

Stock solutions of probe **1**, **2** (2 mM) was prepared in DMSO. Stock solutions of analytes (20 mM) were prepared in distilled water. The stock solutions of analytes were diluted to desired concentrations with distilled water when needed. UV–vis and fluorescence spectra were obtained in ethanol -HEPES buffer (10 mM, pH 7.4, 1:1, v/v) solution at 25°C. All fluorescence measurements were carried out on a Hitachi Fluorescence Spectrophotometer F-7000. The samples were excited at 400 nm with the excitation and emission slit widths set at 5.0 nm. The emission spectrum was scanned from 425 nm to 650 nm at 1200nm/min. The photo multiplier voltage was set at 700V.

2.4 Cell culture and imagings

The MCF-7 (human breast cancer cell line) cell was maintained in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose and 10% fetal bovine serum in a water-jacketed CO₂ incubator (Forma Series II 3110, Thermo Fisher Scientific Inc., USA) providing a humidified atmosphere containing 5% CO₂ at 37 °C, then

washed with PBS buffer three times. Fluorescence imaging of MCF-7 cells was obtained with a lypus Fluoview FV1000 microscope.

3. Results and Discussion

3.1. Synthesis and characterization

The synthesis of compounds **1**, **2** are summarized in Scheme 1. Compounds of **1**, **2** and **3** were synthesized by a one-step reaction between 8-Hydroxyjulolidine and either of phenylpropionic acid (for **1**), 2-Butynoic acid and (for **2**) in dichloromethane containing 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and 4-dimethylaminopyridine(DMAP)[33]. 8-Hydroxyjulolidine (1 mmol), phenylpropionic acid (1.1 mmol), EDC (1 mmol) and DMAP (0.5 mmol) were dissolved in 5 mL CH₂Cl₂ in a flask. The reaction mixture was stirred at 0 °C for an hour, then kept at room temperature for another 4 hours. After evaporating all the volatiles under reduced pressure, purification by flash column chromatography on silicagel, to give compounds **1**, and **2** as a light yellow powder in 86% and 52% yields, respectively. The detail characterization data and experimental procedure were shown in the Figure S2 in the Supporting Information.

The hydroarylation of **1-1**, **2-1** were monitored in ethanol –water (v:v =1:1) by applying a catalytic amount of Au³⁺ under a handheld UV-vis lamp. The reactions were detected by thin-layer chromatography (TLC). After the reaction was complete, the fluorescent compounds were separated by column chromatography and proven to be hydroarylation products **1-1**, **2-1** (**Scheme 1**), whose structure was confirmed by ¹H and ¹³C-NMR and mass spectral analyses (Figure S2 in the Supporting Information).

Scheme 1 The mechanism of the reaction.

3.2 Selectivity of probes for Au^{3+}

To evaluate the specific nature of probe for Au^{3+} , the spectral changes in probe toward other metal ions were explored. The effect of a wide range of environmentally and physiologically active metal ions was investigated for compounds **1** and **2**. Fluorescence spectra of **1** and **2** before and after addition of various metal ions, including Co^{2+} , Ba^{2+} , Pb^{2+} , Au^{3+} , Ni^{2+} , Ag^{+} , Yb^{3+} , Al^{3+} , Er^{3+} , Cu^{2+} , Zn^{2+} , Mn^{2+} , Cr^{3+} , Fe^{3+} , Sm^{3+} , Ho^{3+} , Zr^{4+} , Eu^{3+} , Mg^{2+} , Nd^{3+} (10equiv.) et al were recorded. Under identical conditions, only Au^{3+} causes a notable fluorescent response of **1** and **2**. **Figure 1** shows that compounds **1** and **2** shows no fluorescence emission before the addition of many metal ions, when Au^{3+} is added, a strong fluorescence emission at 511 nm for compound **1** and at 467 nm for compound **2**. We also note that the color of the solution changed from colorless to green for compounds **1** and from colorless to cyan under illumination with a 365-nm UV lamp.

(b)

Figure 1 (a) Fluorescence spectra of probe **1** (1 μM) in the presence of various metal ions in ethanol/HEPES solution (1:1, v/v, pH 7.4) ($\lambda_{\text{ex}} = 415 \text{ nm}$, $\lambda_{\text{em}} = 511 \text{ nm}$, slit: 5 nm/5 nm), Insert: Optical density of the probe **1** (1 μM) at 511 nm in the presence of 10 μM various metal ions and a visual fluorescence change photograph for Au^{3+} and other cations under illumination with a 365 nm UV lamp. (b) Fluorescence spectra of probe **2** (1 μM) in the presence of various metal ions in ethanol/HEPES solution (1:1, v/v, pH 7.4) ($\lambda_{\text{ex}} = 400 \text{ nm}$, $\lambda_{\text{em}} = 467 \text{ nm}$, slit: 5 nm/5 nm), Insert: Optical density of the probe **2** (10 μM) at 467 nm in the presence of 10 μM various metal ions and a visual fluorescence change photograph for Au^{3+} and other cations under illumination with a 365 nm UV lamp.

3.3 Spectroscopic response of **1** and **2** to Au^{3+}

The fluorescent properties of **1** and **2** towards Au^{3+} were studied. First, the probe **1** and **2** show a good response to Au^{3+} in the pH range pH 5–8, and physiological pH region (pH 7.4) was selected for further investigation (Fig. S2, EI). Moreover, The kinetic study showed that the reaction was complete within 5, and 10 min for Au^{3+} with **1**, and **2**, respectively. The changes in the fluorescence spectra of probe **1** (1 μM) in the absence or presence of Au^{3+} (0–30 μM) and probe **2** (1 μM) in the absence or presence of Au^{3+} (0–30 μM) in ethanol/HEPES solution (1:1, v/v, pH 7.4) are displayed in Figure 2. Also, the detection limit of **1** and **2** for thiol was investigated. A concentration of Au^{3+} also were 0.10 μM and 0.12 μM can be detected by using **1** and **2**. As it can be seen the addition of Au^{3+} caused

changes in the fluorescence spectra; i.e. an enhancement of the emission intensity at 511 nm and 467 nm were observed.

We also investigated the absorbance changes in the UV-Vis spectra when Au^{3+} was added to ethanol/HEPES solution (1:1, v/v, pH 7.4) containing **1** (20 μM) or **2** (30 μM). There is a new absorption peak appeared at 400 nm, and the color of solution from colorless to yellow(Fig. S3, EI).

Figure 2 (a) Fluorescence spectra of probe **1** (1 μM) in the presence of increasing amounts of Au^{3+} (0–30 equiv.) in ethanol/HEPES solution (1:1, v/v, pH 7.4). Inset: A

linear calibration curve of the fluorescent intensity at 511 nm versus the concentration of Au^{3+} over the range 0 to $30\mu\text{M}$. ($E_x = 415\text{ nm}$); (b) Fluorescence spectra of probe 2 ($1\mu\text{M}$) in the presence of increasing amounts of Au^{3+} (0–30 equiv.) in ethanol/HEPES solution (1:1, v/v, pH 7.4). Inset: A linear calibration curve of the fluorescent intensity at 467 nm versus the concentration of Au^{3+} over the range 0 to $30\mu\text{M}$. ($E_x = 400\text{ nm}$).

3.4. Proposed mechanism

As has been well demonstrated in previous studies (Do et al., 2010), utilized the Au^{3+} -mediated hydroarylation reaction of a latent fluorophore of the alkyne derivative to yield a strongly fluorescent coumarin derivative. Based on the observation summarized above and the results arising from previous investigations, it is plausible that compound 1 and 2 are play high sensitivity and selectivity towards Au^{3+} .

The detection mechanism is shown in Scheme 2. Firstly, the transition state i and ii were formed by coordination between the gold (III) ion and alkynyl group, then the transition state was involved in an intramolecular Michael reaction, which is occurred in the para position of dialkylamino group from the probe as an electron donor and functionalized alkynyl group through phenyl ester as electron acceptor, resulted in transition state iii. Through the reaction, the fluorescent coumarin derivatives were formed, and the gold (III) ion is regenerated as catalyst.

The reaction rate is strongly affected by Michael receptor which is associated with the stability of carbocation of the transition state ii. Due to the conjugative effect of phenyl, methyl group and hydrogen, the detected rate of the reaction was following the order $1 > 2$.

Scheme 2 The synthesis of the compounds.

3.5. Cellular Imaging

Since probe 1 and 2 shows increased fluorescence induced by Au^{3+} and bioimaging detection of Au^{3+} may become possible. Cytotoxicity is an important indicator of the applicability of probe in biological systems. Therefore, the effect of 1 and 2 on cell proliferation was determined by MTT assay in MCF-7 cells (Fig. S5, ESI). These results implied that the two probes were suitable for Au^{3+} detection in live cells. The optical windows at 500–540nm, 425–475 nm and 555–655 are chosen for confocal imaging of 1 and 2. We tested the capability of **1**, **2** to image Au^{3+} in living cells. As shown in Figure 3, MCF-7 cells were incubated with different concentrations Au^{3+} (0 μM , 5 μM , 1 μM , and 30 μM) for 30 min at 37 $^{\circ}\text{C}$ and then incubated with the same concentrations probe 1 and 2 separately, These cell experiments indicated that **1** and **2** can successfully permeate through cell membranes.

Figure 3 Confocal fluorescence images of MCF-7 cells incubated with different concentrations Au^{3+} (0 μM , 5 μM , 15 μM , and 30 μM) for 30 min at 37 $^{\circ}\text{C}$ and then incubated with the same concentrations probe 1(1 μM) and 2(1 μM) at 37 $^{\circ}\text{C}$ for 30 min (A, B, C, D, G, H, I, J), and their overlay images(A1, B1, C1, D1, G1, H1, I1, J1),

Co-stained with Lyso Tracker Red (LTR) (E and K), Overlay of probe 1, 2 image and LTR image (F and L) respectively (scale bar: 40 μm).

4. Conclusions

In summary, two new colorimetric and turn-on fluorescent probes for rapid and highly selective Au^{3+} detection in HEPES buffer solution (pH7.4) were designed and synthesized. It also provided a method for the synthesis of coumarin compounds. Moreover, fluorescence imaging of MCF-7 cells was carried out successfully, which indicates the cell permeability and bio-compatibility of probes. It is anticipated that the new fluorescent probes will serve as a tool in Au^{3+} -related chemical and biological studies.

Acknowledgments

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Abstract Graphic

The Title:

Fluorescent imaging of Au^{3+} in living cells with two new high selective Au^{3+} probes

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