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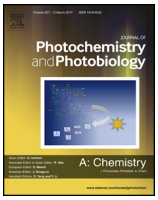

Invited feature article

Synthesis of 13-substituted derivatives of berberine: Aggregation-induced emission enhancement and pH sensitive property

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ABSTRACT

Five kinds of berberine derivatives with hydroxy, methyl, ethyl, benzyl and (4-methyl)benzyl group at 13-position have been synthesized and characterized. 13-hydroxyberberine chloride exhibits aggregation-induced emission enhancement (AIEE) property because of hydroxy group, which is beneficial to increase the rigidity of molecule by enlarging conjugated system. Optical properties in pure solution, CH_3OH/H_2O mixed solution, amorphous and crystalline state were comparatively investigated. Polymeric morphology and particle size of 13-hydroxyberberine chloride with different water fractions (0–90 vol%) were obtained by scanning electron microscope (SEM) and dynamic light scattering (DLS) method respectively, which provided reasonable explanation that the formation of small globular nanoparticles in mixed solution is conducive to the fluorescence emission. The single crystal structure of 13-hydroxyberberine chloride was determined by single-crystal X-ray diffraction. Crystallographic data indicated that the main mechanism of the AIEE phenomenon is the existences of J-aggregation (head to tail dipole stacking) combined with molecular planarization. The calculation done by DFT showed that the HOMO–LUMO bandgap is in accordance with experimental data. To further explore the biomedical application of 13-hydroxyberberine, its cell viability and cell imaging performance were examined, which demonstrate that 13-hydroxyberberine shows definite fluorescent intensity. In all, 13-hydroxyberberine should be a promising candidate for different biomedical application such as pH fluorescence probe because of its response to the stimuli of pH value.

1. Introduction

As we all know, an increasing number of scientists pay more attention to the development of nano- and microsized fluorescent materials [1]. Fluorescent materials possess highly fluorescence efficiency in the state of nanoaggregation, solid and crystalline, namely aggregation-induced emission enhancement (AIEE) property, which is opposite to the "aggregation-caused quenching" (ACQ). Nevertheless, a majority of fluorescent materials are subjected to aggregation-caused quenching (ACQ) effect because of intensive π-π stacking and dipole–dipole interactions, which bring about low fluorescence emission, limiting the application of fluorescent materials. As a kind of AIE(E) fluorescent material, small organic molecules attract tremendous attention due to their strong emission in the poor solvents [2–4]. For example, siloles compound is considered as the most representative of an aggregation-induced emission (AIE) compound in earlier study of Tang's group [5]. Following closely behind are tetraphenylethylene (TPE) compound [6], intramolecular charge transfer (ICT) compound [7], and hydrogen bonding compound [8], which are also exploited as the new AIE molecules. Tang's group has also reported the luminescence mechanisms of AIE(E) which involve inter- and intramolecular phenomena, such as molecular conformation distortion, J-aggregation, $C-H\cdots\pi$ interaction and so forth [9–12].

Recently, this kind of organic molecule is considered to have great potential application in the field of photocatalysts [13], organic light-emitting diodes (OLEDs) [14,15] and optical devices [16], especially as chemical or biological probe [17]. Many important physiological processes of the body are related to pH

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value, and the fluorescence property of some compounds can indicate the change of acid-base property of medium. Therefore, the study of fabricating novel chemical or biological fluorescence probe with AIE(E) molecules is valuable. In comparison to traditional pH fluorescence probe with the problem of aggregation-caused quenching (ACQ), new pH fluorescence probe has higher sensitivity and faster response speed [18,19].

Based on our previous researches [20–22], our group synthesized a series of 13-substituted derivatives of berberine (Scheme 1) and their spectroscopic properties in solution, nanosuspensions, amorphous and crystalline were investigated [23,24]. With further exploration of 13-hydroxyberberine chloride assembly feature, we researched its polymeric morphology and particle size by scanning electron microscope (SEM) and dynamic light scattering (DLS) method respectively. Crystal structure elucidates the mechanism of enhanced emission in the aggregation state. The geometrical and electronic structures were obtained to evidence the AIEE study of compound **1**. Moreover, the relationships between photoluminescence (PL) intensity and pH values were explored [25]. To further examine the biological potential of compound **1** as pH fluorescence probe, the cell uptake behavior as well as cell viability of it was further evaluated.

2. Experimental

2.1. Materials and instruments

All the reagents were obtained commercially, analytical grade and used without further purification. Berberine chloride, Sodium borohydride ($NaBH_4$), formaldehyde solution, benzyl bromide and acetaldehyde solution were obtained from Sigma-Aldrich, and 4-Methylbenzyl bromide was got from Macklin. NMR spectra were record on a Bruker Avance III 400 MHz spectrometer in DMSO-*d*6 as the internal standard. Mass spectra were measured on Shimazu LCMS-IT-TOF mass spectrometers. Ultraviolet (UV) absorption was recorded on a Shimadzu UV-2600 spectrometer. Photoluminescence (PL) spectra were taken using an (Edinburgh Instruments) FLS920 spectrophotometer. Absolute PL quantum yields and fluorescence lifetime were collected on an (Edinburge Instruments, England) FLS 980 spectrometer. Scanning electron microscope (SEM) images were obtained with a Zeiss Merlin (Zeiss Co., Germany) emission scanning electron microscope. Particle size was got by dynamic light scattering (DLS) method on a Brookhaven BI-90 plus particle size analyzer. Single crystal X-ray diffraction (XRD) patterns were taken on a Xcalibur Nova (Agilent Technologies (China) Co., Ltd) diffractometer. The geometrical and electronic structures were obtained at the B3LYP/6-31G** level. Cell viability was analyzed with a Flex Station 3 microplate reader (Moleculardevices, America). Cell imaging performance was examined on a laser scanning confocal microscopy 710 (Zeiss, Germany).

2.2. Synthetic procedures

The molecular structures and synthesis routes of 13-substituted berberine are exhibited in Scheme 1. All the derivatives had a high-quality synthesis and marked as compounds **1-5** (Table 1). Additionally, an oxidation reaction using N-bromosuccinimide (NBS) gave the reaction products **2-5** in good yield [26,27]. 13-hydroxyberberine chloride was synthesized by a sequence of nucleophilic substitution reactions with acetone and $KMnO_4$.

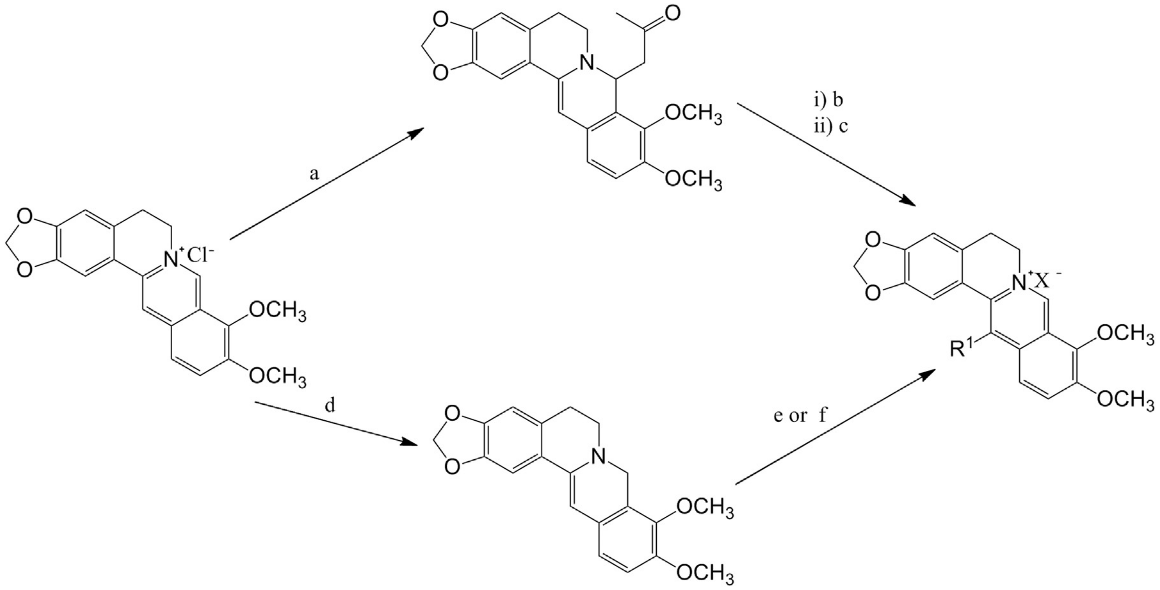
2.2.1. Compound *1*

Berberine chloride (3.71 g, 10 mmol) was dissolved in deionized water (4 mL) and acetone (4 mL). 50 wt% NaOH (5.6 mL) was added in the mixture with vigorous stirring at the room temperature for 30 min. The mixture was filtered and the residue was washed with deionized water (15 mL) and methanol (15 mL) to obtain brown intermediate **6a** (3.85 g, yield: 53%).

The suspension of compound **6a** (2.32 g, 5.9 mmol) was dissolved in acetone (100 mL) and cooled to minus ten degrees

Table 1
Structures of compounds **1-5**.

Compound	R^1	X
1	OH	Cl
2	CH_3	Cl
3	C_2H_5	Cl
4	CH_2Ph	Br
5	CH_2PhCH_3	Br



Scheme 1. Synthetic routes of 13-substituted berberine derivatives. (a) acetone, 50 wt% NaOH, room temperature; (b) $KMnO_{4(aq)}$, acetone, −10 °C; (c) HCl $_{(concd)}$, methanol, reflux; (d) $NaBH_4$, K_2CO_3, methanol, room temperature; (e) RCHO, HOAc, 80% ethanol, 85–95 °C; (f) $RPhCH_2Br$, NaI, CH_3CN, room temperature, reflux, NBS.