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Selective and sensitive fluorescent and colorimetric chemosensor for detection of CO_3^{2-} anions in aqueous solution and living cells

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ABSTRACT

A new colorimetric and fluorescent chemosensor for visual determination of carbonate ions was developed by the microwave assisted solvent free synthesis of 7,8-dihydroxy-3-(4-methylphenyl) coumarin (**DHMC**). The structural characterization of **DHMC** was confirmed by microanalysis and spectroscopy methods (MALDI-TOF, FT-IR, ^{1}H NMR, ^{13}C NMR, and 2D HETCOR). The binding behaviors of **DHMC** were investigated towards various anions by UV–vis and fluorescence spectroscopy. **DHMC** showed a selective and sensitive fluorometric and colorimetric responses towards carbonate ion over other anions. The detection limit of CO_3^{2-} was found to be 1.03 μM. Moreover, the fluorescence imaging in living cells suggests that **DHMC** has a great potential in the biological imaging application. It has been demonstrated that **DHMC** can be used as a rapid and reliable sensor for the determination of carbonate anion in a variety of practical applications.

1. Introduction

The development of selective and sensitive sensor systems to determine various anion species have been great attention. In recent years, noticeable efforts have been made to prepare artificial optical chemosensor for anion recognition because of their significant place in biological, medical, industrial, and environmental applications [1–3]. Among various anions, carbonate ions are substantial minerals due to their beneficial roles in the different types of areas, especially in geological and industrial materials [4–7]. Carbonates are commonly formed when atmospheric carbon dioxide reacts with water forming carbonic acid and consequently transform to bicarbonate and carbonate. Calcium carbonate takes part in among the most common minerals in soil, chalk, limestone and marble. It has commonly been used in various industrial fields such as plastics, adhesives and papers. Some analytical methods for determination of carbonate ions have been developed including Fourier transform infrared spectroscopy (FT-IR) [8], ion-selective electrodes [9] and acoustic method [10]. Among these reported methods, fluorescence molecular sensing methods remain attractive due to their high sensitivity, simplicity, low cost, quick response and real-time monitoring. These methods can be classified into two different types: Fluorescence enhancement (turn on) and fluorescence quenching (turn off). The “turn on” fluorescence process has several advantages than “turn off” process in terms of the lower detection limit value. Although many anion sensors based on colorimetric and fluorescence detection have been developed including fluoride [11], acetate [12] and cyanide [13], only a few sensors for fluorimetric detection of carbonate anion exist. The lack of efficient receptors for the carbonate anion in literature can be seen. There is an urgent need to develop new carbonate fluorescence chemosensors.

Coumarin derivates are extensively used as a fluorescent sensors due to their excellent fluorescent properties such as high quantum yield, large scale stokes shift, and great photo-stability [14]. Many fluorescence sensors based on coumarin derivates have been synthesized including Zn^{2+} [15], Cu^{2+} [16], Fe^{3+} [17], CN^{-} [18], F^{-} [14], and glutathione [19]. However, there is no coumarin derivative fluorescent carbonate sensor in the literature.

Herein, we report the design and synthesis of a novel coumarin based **DHMC** (7,8-dihydroxy-3-(3-chlorophenyl) coumarin) (Scheme 1), which showed naked eye and emission “turn on” respond to the carbonate ions in mixture of water/acetonitrile (H_2O/ACN; 99/1). The photophysical behaviors in the absence and presence of different anions

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have been evaluated by fluorescence and NMR experiments.

2. Experimental

2.1. Materials and instruments

FT-IR NMR, MS spectra and microanalyses were recorded using a Perkin Elmer spectrum one FT-IR, a Bruker DPX-400 spectrometer, a Bruker Daltonics microflex mass spectrometer and a CHNS-932 (LECO) instruments, respectively. In the NMR measurements, $CDCl_3$ for TMAN and DMSO-d6 for **DHMC** were used as deuterated solvents. UV–vis experiments were performed on a Perkin Elmer Lambda 25 UV spectrophotometer using quartz cuvette. Fluorescence studies were carried out with Hitachi 7000 spectrophotometer. All materials and reagents used in the experiments were of standard analytical grade from Merck and Sigma-Aldrich without further purification.

2.2. Synthesis

2-(2,3,4-trimethoxyphenyl)-1-(4-methylphenyl) acrylonitrile (TMAN) and 7,8-dihydroxy-3-(3-chlorophenyl) coumarin (**DHMC**) were obtained as following our previously reported method [20–22]. The structural characterization results of compounds TMAN and **DHMC** are given in detail below.

2.2.1. Synthesis of 2-(2,3,4-trimethoxyphenyl)-1-(4-methylphenyl) acrylonitrile (TMAN)

2,3,4-Trimethoxybenzaldehyde (1) (1.06 g, 5.4 mmol) and 4-methylphenylacetonitrile (0.67 g, 5.4 mmol) were dissolved in ethyl alcohol (50 mL). The mixture was refluxed for 30 min under argon atmosphere and then cooled to room temperature. After that, 30% sodium hydroxide solution was added to the mixture until the sight of blur. The reaction mixture was poured into cold water, and the precipitated solid was filtered and washed with hot water. Finally, the product was crystallized from ethyl alcohol and a yellow-colored solid (TMAN) was obtained (1.47 g, 88%), Anal. Calc. for $C_{19}H_{19}NO_3$ (MW: 309.36): C, 73.77; H, 6.19; N, 4.53; Found C, 73.80; H, 6.22; N, 4.59%. MALDI-MS: *m/z* calc. 309.36; found: 306.66. FT-IR (KBr, cm-1): 3038 νC-H (Ar), 2936 νO-CH3, 2212 νC≡N, 1587, 1510, 1461 νC=C. ^{1}H NMR (400 MHz, DMSO-d6): δ 2.42 (3H, s, H17), 3.92 (3H, s, H9), 3.95 (3H, s, H8), 3.96 (3H, s, H7), 6.82 (1H, d, J = 9.2 Hz, H4), 7.28 (2H, d, J = 8 Hz, H15), 7.61 (2H, d, J = 8.4 Hz, H14), 7.83 (1H, s, H10), 8.05 (1H, d, J = 9.2 Hz, H5). ^{13}C NMR (400 MHz, DMSO-d6): δ 21.23 C17, 56.11 C9, 60.97 C8, 61.83 C7, 107.47 C4, 109.95 C11, 118.67 C12, 121.07 C6, 123.34 C5, 125.80 C14, 129.69 C15, 132.14 C13, 135.71 C10, 138.97 C16, 141.98 C2, 153.11 C1 and 155.74 C3. MALDI-MS: *m/z* calc. 309.36; Found: 306.66.

2.2.2. Synthesis of 7,8-dihydroxy-3-(3-methylphenyl)coumarin (DHMC)

TMAN (0.4 g, 1.3 mmol), silicagel (15 g), and pyridinium hydrochloride (5 g) were mixed at solvent-free conditions. Afterwards, the reaction mixture was applied microwave irradiation (320 W) for 25 min. Reaction mixture was quenched with 2 M hydrochloric acid and then was subjected to filtration. The filtrate was dissolved in acetone (50 mL) and silica gel filtration was applied. After solvent evaporation, residue was washed with distilled water and then dried under vacuum. In order to precipitate the solid in n-hexane (200 mL), it was dissolved in ethyl acetate (10 mL). The column chromatography (chloroform:hexane) was performed to purify crude solid. A pale yellow-colored solid (**DHMC**) was obtained (0.26 g, 74%), Anal. Calc. for $C_{16}H_{12}O_4$ (MW: 268.26): C, 71.64; H, 4.51; Found C, 71.67, H; 4.53%. FT-IR (KBr, cm-1): 3357, 3227 νO-H, 3027 νC-H(Ar), 1723 νC=O, 1619, 1581, 1503 νC=C. ^{1}H NMR (400 MHz, DMSO-d6): δ 2.35 (1H, s, H16), 6.85 (1H, d, J = 8 Hz, H4), 7.11 (1H, d, J = 8.4 Hz, H5), 7.26 (2H, d, J = 8.4 Hz, H14), 7.62 (2H, d, J = 8.4 Hz, H13), 8.09 (1H, s, H9), 9.47 (1H, s, H7) and 10.15 (1H, s, H8). ^{13}C NMR (400 MHz, DMSO-d6): δ 21.19 C16, 113.22 C6, 113.30 C4, 119.55 C5, 122.32 C10, 128.61 C13, 129.20 C14, 132.26 C12, 132.75 C15, 137.84 C2, 141.51 C9, 143.56 C1, 149.91 C3, 160.55 C11. MALDI-MS: *m/z* calc. 268.26; Found: 268.706 $[M]^+$, 291.0 $[M + Na]^+$ and 307.28 $[M + K]^+$.

2.3. UV–visible/fluorescence and ^{1}H NMR titration studies

The stock solutions of **DHMC** (10 mM) in H_2O/ACN (99/1, v/v) solvent mixture and guest anions (10 mM) in H_2O were prepared. A quartz cuvette with a 30 mm path length was used for the UV–vis and fluorescence measurements. Absorption and fluorescence spectra were acquired by transferring various amounts of ion solutions to the **DHMC** solutions. For the **DHMC**, the excitation wavelength at room temperature was determined to be suitable at 390 nm. ^{1}H NMR titrations were also performed on a Varian 400 MHz spectrometer at room temperature. The **DHMC** solution (0.112 M in d_6-DMSO-D_2O (9/1)) was titrated with concentrated sodium carbonate solution and changes in the chemical shifts of **DHMC** were followed. All titrations were carried out at least three times to obtain consistent values.

2.4. Computational details

To support experimental results, computational studies were carried out utilizing GAUSSIAN 16 program. The geometry optimization of the ligand was performed using the DFT/B3LYP function of the Gaussian program and the LANL2DZ basis set 6-31G. Electronic transition calculations for the ligand were performed using the TDDFT/B3LYP function and LANL2DZ basis set [23–27]. Water was preferred as the solvent medium in all calculations. To calculate solvent effect, CPCM model was used [28].

2.5. Cell culture and imaging

Intracellular imaging potential of **DHMC** was investigated with yeast cells (Saccharomyces cerevisiae) as a model organism. Accordingly, cells were grown in 2% Saborand dextroz agar for 12 h at 37 °C with continuous shaking. After reaching expected confluency, they were incubated with 100 μM solution of **DHMC** prepared with DMSO-PBS buffer (5% DMSO, 95% PBS) for one hour at room temperature and then were washed with PBS two times. After that, they were further incubated with 100 μM of sodium carbonate solution which was also prepared with DMSO-PBS. Before and after **DHMC** and carbonate incubation, bright field phase contrast images of the cells were obtained using fluorescent cell imaging system (ZOE, Bio-Bad, Germany). Bright field and fluorescent images were merged with the software of the imaging device.

3. Results and discussion

3.1. Synthesis

Solvent free synthesis of 7,8-dihydroxy-3-(4-methylphenyl) coumarin (**DHMC**) was prepared in high yield by the reaction of TMAN with pyridinium hydrochloride using silica gel as support material following our previously reported method [21]. The structure of crude compound was purified with column chromatography and then crystallized. General synthetic pathway of TMAN and **DHMC** is shown in Scheme 1. The structure characterizations of synthesized molecules have been carried out using a combination of MS, FT-IR, elemental analysis, ^{1}H, ^{13}C, and 2D HETCOR NMR techniques (Supplementary material, Figs. S1–S8). The methoxy functional groups in the structure of TMAN were converted to hydroxyl groups. The FT-IR spectra of **DHMC** shows an obvious OH stretching vibrations at 3368 and 3170 cm^{-1} which are clear indications the OH formation from the methoxy of TMAN. The -C=O stretching vibrations were observed at 1723 cm^{-1} which is the characteristic peak for lactone ring of coumarin