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Deep-red to near-infrared fluorescent dyes: Synthesis, photophysical properties, and application in cell imaging

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ABSTRACT

More and more attention has been paid to the design of new fluorescent imaging agents with good photostability and water solubility, especially those with emissions in the deep-red and near-infrared regions. In this work, we designed and synthesized four novel fluorescent dyes with deep-red or NIR fluorescence by hybridizing coumarin and pyronin moieties based on our previous work. Introduction of carboxylic acid in the dyes not only imparted the dyes with water solubility but also provided a versatile sensing platform for designing the fluorescent probes and sensors of biomolecules. The photophysical properties of these new dyes were investigated through absorption and fluorescence spectroscopy. Cell imaging experiments showed that esterification products could selectively stain lysosomes with good photostability, thereby indicating that they could be useful in the development of fluorescent probes for bioimaging.

1. Introduction

Visualization of cellular activities, which is becoming increasingly convenient and detailed, allows the description of various biological phenomena. Fluorescent imaging technology is an effective technique of cell imaging with special fluorescent probes; in this technique, small-molecule probes can permeate the cells and image living cells [1–14]. This technology is essential to designing and synthesizing fluorescent dyes containing active substitutes for fluorescent label and imaging. Deep-red and near-infrared (NIR) dyes present a number of advantages over short-wavelength fluorophores in biodetection and imaging because of their minimal photodamage to biological samples, negligible interference from autofluorescence in living systems, and deep-tissue penetration [15–18]. Much effort has been exerted to exploit new dyes for imaging [19–32]. However, NIR fluorescent dyes with excellent properties, such as good water solubility and high photostability, are very rare.

To date, most available fluorophores are non-hydrophilic or slightly soluble in related aqueous solution. Fluorescent dyes may effectively suppress or prevent aggression of hydrophobic and rigid core structures in polar media after gaining water solubility [33]. Water solubility can be enhanced by introducing water-solubilizing groups to the dye structure [34–38]. However, chemically converting hydrophobic fluorescent dyes into water-soluble compounds is not easy because most structural modifications in fluorescent dyes also compromise some of their photophysical properties [39].

In our previous work, we developed a series of deep-red emissive **CP** dyes by hybridizing coumarin and pyronin moieties [40,41]. These **CP** dyes showed large Stokes shifts, excellent photostability, and good cell-membrane permeability. In the present work, we designed and synthesized another series of deep-red or NIR and water-soluble fluorescent **CPC** dyes (**CPC1**and **CPC2**, Scheme 1). Carboxylic groups were introduced to the dyes to improve their water solubility and facilitate labeling. **CPC1** maintained good photophysical properties compared with the corresponding **CP** dye. **CPC2** emitted NIR fluorescence because of the introduction of stronger electron-donating groups (julolidine). **CPC1** and **CPC2** were further esterified to **CPC1E** and **CPC2E**, which may locate in lysosomes and do not enter the nucleus because of steric hindrance at the end of their molecular structures [3,15,23,40,41]. The properties of the **CPC** dyes were investigated through absorption and fluorescence spectroscopy and confocal laser scanning microscopy.

2. Experimental section

2.1. Materials and apparatus

All commercial chemicals were used without further purification, but water was utilized after being purified by a Millipore filtration system. The stoke solutions of **CPC** dyes were stored in dimethyl sulfoxide (DMSO; 1.0×10^{-3} M). Cresyl violet in methanol ($\Phi = 0.54$) was chosen as the reference standard to calculate the fluorescence quantum yield. All measurements were taken at room temperature. Absorption

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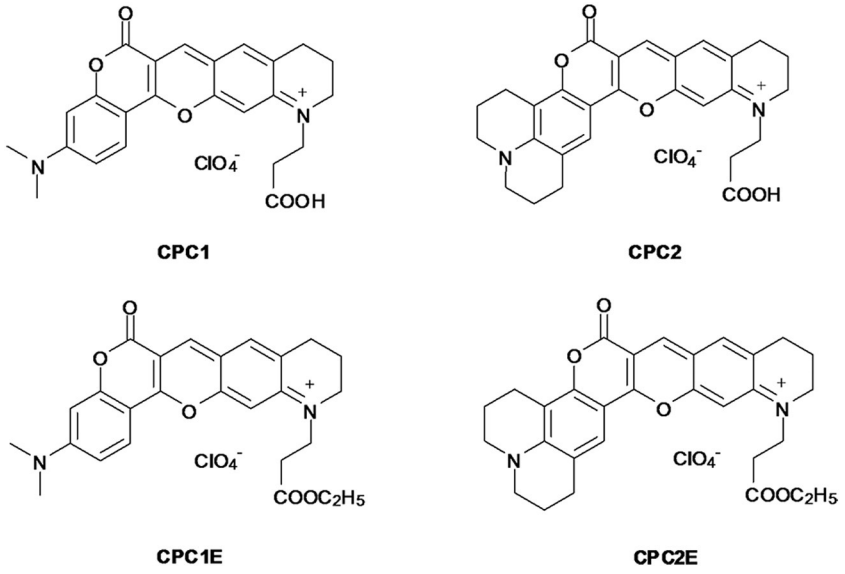

Scheme 1. Structure of **CPC** dyes.

and fluorescence spectra of all compounds were recorded in a Hitachi U-3010 absorption spectrometer and a Hitachi F-4500 fluorescence spectrometer, respectively. All fluorescence spectra were excited under 570 nm. ^{1}H NMR and ^{13}C NMR spectra were gained at 400 and 100 MHz, respectively. HRMS spectra were obtained on a Bruker Apex IV Fourier transform mass spectrometer.

2.2. Synthesis and characterization of the fluorescent dyes

2.2.1. Synthesis of compound ***2***

A modified method was followed to synthesize compound **2** [33]. 7-hydroxy-1, 2, 3, 4-tetrahydroquinoline (1, 0.5 g, 3.35 mol) were weighed in a round-bottomed flask, then acrylic acid (230 μL, 3.35 mol) and water (3 mL) was added. The reaction components were heated for 6 h at 70 °C. After cooling to room temperature, the reaction mixture was extracted with ethyl acetate, and then the solvent was evaporated under reduced pressure. The residual material was purified by column chromatography using 30:1 (v/v) dichloromethane/methanol as the eluent to afford **2** as a pale yellow solid (585.26 mg, 79%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (s, 1H), 8.82 (s, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.05 (s, 1H), 5.97 (d, J = 7.9 Hz, 1H), 3.46 (t, J = 7.1 Hz, 2H), 3.21 (t, J = 6.1 Hz, 2H), 2.56 (t, J = 6.2 Hz, 2H), 2.48 (t, J = 7.1 Hz, 2H), 1.81 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.07, 156.36, 145.09, 129.19, 112.80, 102.73, 97.75, 48.42, 46.64, 30.73, 26.62, 22.03. ESI-HRMS. Calcd for $[C_{12}H_{15}NO_3 + H]^+$: m/z 222.1125. Found: m/z 222.1121.

2.2.2. Synthesis of compound ***6***

Compound **6** was synthesized from compound **4** according to our previous report [15]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.74 (s, 1H), 7.15 (s, 1H), 5.21 (s, 1H), 3.22 (m, 4H), 2.70 (m, 4H), 1.87 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.55, 162.79, 150.98, 145.97, 119.85, 117.29, 105.34, 103.07, 85.79, 49.20, 48.67, 26.94, 20.99, 20.08. ESI-HRMS. Calcd for $[C_{15}H_{15}NO_3 + H]^+$: m/z 258.1125. Found: m/z 258.1117.

2.2.3. Synthesis of compound ***7***

Compound **7** was synthesized from compound **3** according to our previous report [41]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.30 (s, 1H), 7.86 (d, J = 9.3 Hz, 1H), 6.71 (dd, J = 9.3, 2.5 Hz, 1H), 6.44 (d, J = 2.5 Hz, 1H), 3.16 (s, 6H)·^{13}C NMR (100 MHz, $CDCl_3$): δ 187.12, 159.95, 156.18, 155.57, 154.28, 129.12, 111.58, 110.77, 108.06, 97.07, 40.45. ESI-HRMS. Calcd for $[C_{12}H_{10}ClNO_3 + H]^+$: m/z 252.0422. Found: m/z 252.0418.

2.2.4. Synthesis of compound ***8***

3 mL of dry dimethylformamide was taken out in a glass vial and 2 mL of phosphoroxychloride was slowly added. The resulting mixture was vortexed and reacted for 30 min at 50 °C. Compound **6** (1.23 g, 4.8 mmol) was dissolved in 15 mL of dimethylformamide, and the stoke solution was added dropwise into the mixture. The mixture was stirred for 6 h at 60 °C, and poured into 20 mL of ice water to obtain an brick red solid (1.45 g, 91.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.28 (s, 1H), 7.45 (s, 1H), 3.38 (m, 4H), 2.79 (m, 4H), 1.99 (m, 4H)·^{13}C NMR (100 MHz, $CDCl_3$): δ 187.32, 160.36, 153.70, 151.56, 149.77, 125.05, 120.52, 109.75, 107.45, 105.72, 50.56, 50.12, 27.67, 21.08, 20.22, 20.11. ESI-HRMS. Calcd for $[C_{16}H_{14}ClNO_3 + H]^+$: m/z 304.0735. Found: m/z 304.0730.

2.2.5. Synthesis of ***CPC1*** and ***CPC2*** dyes

Compound **7** (1 mmol) or **8** (1 mmol) and compound **2** (1.2 mmol) were weighed in a two-necked flask, and 3 mL of acetic acid was added. The reaction mixture was heated to 85 °C for 3 h. After cooling to room temperature, 2.0 mL of perchloric acid (70%) was added to the mixture. A blue-violet power solid was precipitated by the dropwise addition of water, and then the precipitate was filtered and dried in vacuo to obtain a blue-violet powder. The product was further purified by column chromatography eluting with 40:1 (v/v) CH_2Cl_2/CH_3OH and recrystallized from absolute methanol to afford **CPC1** and **CPC2** dyes as deep-green and soil gold powders, respectively.

CPC1. Yield: 42.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (s, 1H), 8.73 (s, 1H), 7.97 (d, J = 9.2 Hz, 1H), 7.70 (s, 1H), 7.33 (s, 1H), 6.98 (d, J = 9.2 Hz, 1H), 6.71 (s, 1H), 3.87 (m, 2H), 3.70 (m, 2H), 3.19 (s, 6H), 2.83 (m, 2H), 2.74 (m, 2H), 1.93 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.82, 158.57, 157.31, 157.02, 156.87, 156.36, 143.60, 131.09, 128.34, 126.13, 117.21, 111.92, 105.44, 100.42, 98.05, 97.03, 56.50,55.38, 48.84, 31.18, 27.11, 20.34, 19.03. ESI-HRMS. Calcd for $[C_{24}H_{23}N_2O_5]^+$: m/z 419.1601. Found: m/z 419.1602.

CPC2. Yield: 40.7%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.56 (s, 1H), 8.69 (s, 1H), 7.66 (s, 1H), 7.61 (s, 1H), 7.25 (s, 1H), 3.84 (m, 2H), 3.67 (m, 2H), 3.46 (m, 4H), 2.80 (m, 4H), 2.73 (m, 4H), 1.91 (m, 6H)·^{13}C NMR (100 MHz, DMSO-d_6): δ 172.38, 160.87, 158.17, 156.84, 155.40, 151.54, 150.78, 143.39, 130.59, 127.03, 121.15, 115.83, 106.09, 104.89, 99.19, 96.43, 56.01, 54.90, 50.20, 49.62, 48.17, 30.69, 26.67, 20.25, 19.98, 19.55, 19.18, 18.55. ESI-HRMS. Calcd for $[C_{28}H_{27}N_2O_5]^+$: m/z 471.1914. Found: m/z 471.1913.