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Trimethine cyanine dyes with an indole nucleus: Synthesis and spectral properties studies

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ABSTRACT

A series of trimethine quinoline cyanine dyes with an indole nucleus were designed and synthesized based on the chemical structures of Cy_3 and TO. The structures of all dyes were confirmed by ^{1}HNMR and MS. Studies on their spectral properties showed that the trimethine cyanine dyes had properties of both the embedded dye TO and the bonded dye Cy_3. The maximum fluorescence wavelength of the final cyanine dyes shifted red compared with those of Cy_3 and TO alone.

When we changed the substituent groups on the indole ring of the dye, both the fluorescence intensity and wavelength exhibited corresponding changes. The fluorescence wavelength of indole quinoline with an electron donor group shifted red and the fluorescence intensity was enhanced with good photostability. When labeled with BSA, a significant fluorescence enhancement could be observed.

1. Introduction

Fluorescent cyanine dyes, based on thiazole or indole, have been widely used in the testing of biomacromolecules, determination of trace amounts of metal ion, infrared laser dyes and manufacture of optical data storage discs among other applications. [1–4]

The dyes with quaternary ammonium structure are of particular interest due to their relative stability, high molar extinction coefficients, narrow spectrum width, high sensitivity, and low aggregation [5]. They can be used to label biological molecules in aqueous solution without losing any activity of the biological molecules. Cyanine dyes, as probes applied in biological labeling, are usually divided into two major classes in terms of the relationship between fluorescent dye and labeled biomolecules. One class includes bound fluorescent probes that are covalently bonded to biomolecules by an active group. These dyes include polymethylene chain cyanine dye and its derivatives. They have many advantages for bioanalytical assays including having strong spectral signals in the longer wavelength region with a minimal background compared with biomolecules and high sensitivity. [6,7] For example, the Cy_3, $Cy_{5.5}$, and Cy_7 dyes have been used for the labeling of a variety of biological/biomedical macromolecules and nanoparticles [8–10].

The other class includes embedded fluorescent probes, which can be inserted into DNA molecules tightly due to high affinity, such as thiazole orange (TO), oxazole yellow (YO), and their dimers (TOTO and YOYO). Compared with bound fluorescent probes, the embedded ones are more beneficial because there is no background interference in the detection process. They have a lower quantum yield and weaker fluorescence in solution, while a larger fluorescence enhancement is observed when they are bound to a DNA or RNA partner. This kind of dye has a higher affinity for tumors than normal cells [11]. They have been studied widely [12–13] and can be further used in the early stage labeling of cancer cells.

An increasing scientific and commercial interest in the synthesis and application of near-infrared cyanine dyes have received much attention in recent years. Near-infrared (NIR) fluorescence-based imaging is becoming a key tool for early disease diagnosis, therapeutic applications, and biochemical analysis. [14,15]

Both TO and Cy dye have their own shortcomings. Although TO has a high affinity for DNA, its fluorescence intensity is weak and the maximum emission wavelength is too far from the near infrared region and the signs disturbed by ultraviolet in an organism. The fluorescence wavelength of Cy is in the near infrared region, but the fluorescence intensity decreases when bound to DNA. Due to its bright fluorescence and NIR emission, its fluorophore is receiving considerable interest as possible components for new NIR optical imaging agents. [16–18]

In the present work, we designed and synthesized a series of indole quinoline trimethine cyanines simultaneously containing both a Cy_3 and TO group (Fig. 1). The presence of the Cy group can ensure that the maximum emission wavelength of these dyes is in

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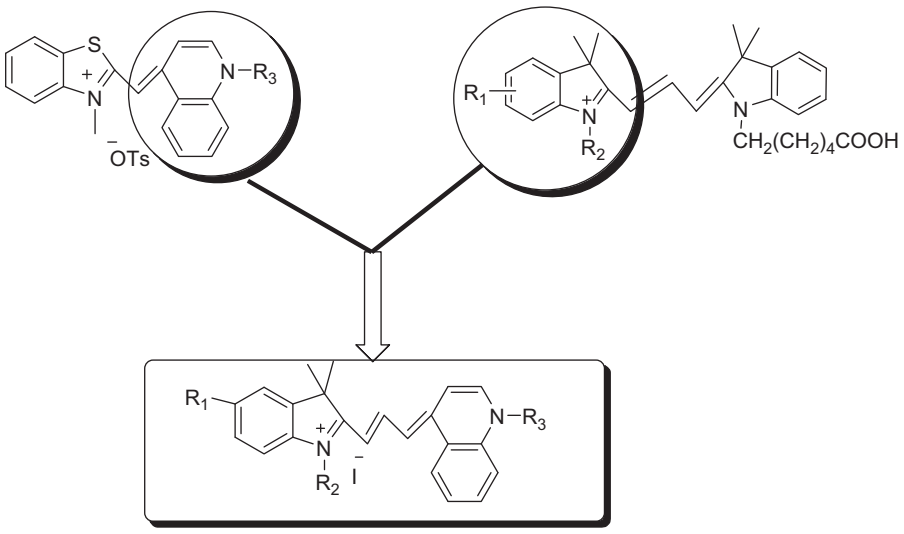

Fig. 1. The design of indole quinoline dyes.

the near infrared region, while the TO group is capable of intercalating tightly into DNA grooves.

2. Experimental section

2.1. General

Fluorescence spectra were scanned using a fluorescence spectrophotometer, Cary Eclipse, USA. Mass spectral analysis was carried out using an electrospray ionization (ESI) mass spectrometer. Melting points were taken on a Yanaco apparatus and were uncorrected. ^{1}HNMR spectra were recorded on a Varian 400 M spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from TMS (tetramethylsilane), using $CDCl_3$ as a solvent. All the reagents were analytically pure. The UV–visible absorption spectra were collected within the range of 250–1000 nm using a Shimadzu UV2550 (Japan) spectrophotometer.

2.2. Synthesis

2.2.1. Synthesis of 2,3,3-trimethyl-3H-indole (1a–1c)

1 mol of phenylhydrazinium chloride and 150 mL of glacial acetic acid were added to a three-necked flask under a flow of nitrogen. The solution was stirred and refluxed in the dark before 44 ml of 3-methyl-2-butanone was added to the flask and kept refluxing for 8 h. After the solvent was removed using a rotary evaporator, the residue was washed with saturated sodium bicarbonate solution for some time to adjust the pH value to 7. The crude product was extracted with chloroform and the organic layer was dried with anhydrous magnesium sulfate overnight. The solvent was evaporated on a rotary evaporator and the residue was purified by column chromatography with eluent of dichloromethane/petroleum ether 1:3 (v/v) to obtain compounds 1a–1c.

2.2.2. Preparation of the quaternary salts (2a, 2b, 2d)

The intermediates 2a, 2b, and 2d were synthesized according to the following procedure.

The 2,3,3-trimethylindole derivative 1a–1c (0.02 mol) and bromide (0.03 mol) were refluxed in the proper solvent (2a and 2d in CH_3CN, 2b in o-dichlorobenzene) for 12 h. The mixture was cooled to room temperature and the solvent was removed using a rotary evaporator. Crude products were obtained by washing the residue with ether or ethyl acetate.

2.2.3. Synthesis of compound 3(3a, 3b, 3d)

A mixture of N, N′-bis-(4-methoxyphenyl)formamidine (1.280 g, 5 mmol), 1,2,3,3-tetramethyl-3H-indolium iodide derivative 2 (5 mmol) and glacial acetic acid (3 mL) was heated under reflux for 12 h. Upon cooling, the solvent was removed under a vacuum and the mixture was precipitated from diethyl ether. After filtration the solid was washed with ice-cold acetone and diethyl ether and then dried under vacuum to yield crude compound 3 as yellow/orange crystals.

2.2.4. Synthesis of dyes (4a–4d)

A mixture of 4-methyl quinolinium bromide (1.5 mol) and triethylamine (0.1 mol) was added to compound 3 (1 mol) in CH_3OH at room temperature under vigorous stirring and reacted for 12 h under nitrogen in the dark. The color of the solution turned to dark blue from deep yellow. After the solvent was removed by rotary evaporator, the crude product was purified by TLC. The data of dyes 4a–4d were shown below.

Dye 4a: ^{1}HNMR(400 MHz, $CDCl_3$): δ1.23(s, 6H), 1.15–1.56(t, J=4.40 Hz, 3H), 1.86–1.92 (m, 2H), 3.94–4.00(m, 2H), 4.65–5.00(m, 2H), 7.27–7.31(m, 2H), 7.52–7.55 (m, 2H),7.61–7.71(m, 2H),7.75–7.80(m, 1H), 8.03(t, J=7.00 Hz, 1H), 7.97(d, J=7.20 Hz, 2H), 8.06(d, J=11.20 Hz, 1H), 8.42(d, J=12.00 Hz, 1H), 8.80(d, J=6.40 Hz, 1H). ESI-MS: m/e 413.37 (M^+), 414.41 (M^++1).

Dye 4b: ^{1}HNMR(400 MHz, CD_3Cl_3): δ 1.67(s, 3H). 1.69(s, 6H), 2.12–2.14 (m, 2H), 3.26(t, J=6.60 Hz, 2H), 3.82(t, J=6.60 Hz, 2H), 4.93(t, J=6.60 Hz, 1H), 5.92(t, J= 13.80 Hz, 1H), 6.75(t, J=6.80 Hz, 1H), 6.93 (t, J=11.40 Hz, 1H), 7.23 (s, 1H), 7.66(d, J=10.80 Hz, 1H), 7.85–7.89(t, J=7.20 Hz, 1H), 7.93(d, J=8.00 Hz, 1H), 7.99(d, J=9.20 Hz, 1H), 8.20(d, J=12.40 Hz, 1H), 8.36(d, J=8.40 Hz, 1H), 9.48(d, J=8.00 Hz, 1H).ESI-MS: m/e 495.2 (M^+), 497.3 (M^++2).

Dye 4c: ^{1}HNMR(400 MHz, DMSO-d_6): δ1.36–1.40(m, 2H), 1.56–1.59(m, 2H), 1.69 (s, 3H), 1.71(s, 6H), 1.86–1.89 (m, 2H), 2.27–2.33(m, 2H), 3.96–4.00(m, 2H), 4.73–4.76(m, 2 H), 6.30(d, J=12.80 Hz, 1H), 7.19(d, J=8.40 Hz, 1H), 7.341–7.41 (m, 2H), 7.66(s, 1H), 7.81–7.84(d, J=7.60 Hz, 1H), 8.05–8.08(t, J=7.80 Hz, 1H), 8.17(d, J=6.80 Hz, 1H), 8.25–8.28(t, J=7.40 Hz, 1H), 8.33(d, J=12.80 Hz, 1H), 8.66 (d, J=8.40 Hz,1H), 8.73(d, J=6.80 Hz, 1H). ESI-MS: m/e 489.8 (M^+), 491.8 (M^++2).