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ORIGINAL PAPER

Synthesis and Spectra of a Kind of Novel Longer-Wavelength Benzoxazole Indole Styryl Cyanine Dye with a Carbazole-Bridged Chain

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Abstract Based on cyanine dye probe oxazole yellow (YO) and Cy_3, a series of novel styryl cyanine dyes were designed and synthesized. Carbazole was inserted into the structures of YO and Cy_3 to act as a bridge to link the benzoxazole and indole group. This modification resulted in a novel kind of benzoxazole indole styryl cyanine dye with a carbazole-bridged chain. The dyes were characterized by ^{1}HNMR and MS. The spectra of the novel dyes were also performed and the results showed that the maximum emission wavelength of the carbazole styryl cyanine dye was shifted red, the Stokes shift increased and the fluorescence intensity enhanced compared with those of YO and Cy_3. These results indicated that the novel dye could be used as an excellent fluorescent probe in biological labeling.

Keywords Carbazole-bridged chain · Benzoxazole · Indole · Styryl cyanine dye · Spectra properties

Introduction

Near-infrared fluorescent dyes, including cyanine dyes and styryl dyes are currently of considerable interest [1–4] due to their potential for practical applications, such as optical recording techniques [5, 6], fluorescent probes for biological research and biomedical assays, particularly for binding with nucleic acids [7–12] and proteins [13, 14], tumor imaging and photodynamic therapy [15–20].

As a fluorescent probe used in biological labeling, it is necessary that the fluorescence emission wavelength is longer and the fluorescence intensity is stronger in order to decrease the fluorescent background interference caused by the labeled organism.

The fluorescent properties of dyes mainly depend on their chemical structures, such as conjugated system, coplanarity and rigidity. With the conjugated system being longer, the fluorescence intensity becomes stronger and the fluorescent emission wavelength shifts toward the red, which causes the reduction of the background interference of the fluorescent probe. Meanwhile, the stability of the probe would become weaker with the lengthening of the conjugated system. So, to obtain a kind of dye with not only longer emission wavelength, stronger fluorescence intensity and larger Stocks shifts but also better stability is a popular research topic. In order to achieve the goal, a rigid ring can be introduced to the methine chain, such as cyclohexene [21–23], crown-ether ring [24] and squaric acid [25].

Recently we reported the synthesis and spectra of novel styryl cyanine dyes with a carbazole bridged chain in the methine chain based on thiazole orange [26]. In this paper, we designed and synthesized a series of novel benzoxazole indole styryl cyanine dyes. Based on the cyanine dyes oxazole yellow (YO) and Cy_3, carbozale ring was introducted to the styryl cyanine dye to link benzoxazole and indole, which belongs to YO and Cy_3 respectively. The spectra properties were also studied. The synthetic route was shown in Scheme 1

Materials and Methods

Materials and Instruments

Fluorescence spectra were scanned on a Cary Eclipse fluorescence spectrophotometer (Varian,American). The UV/Vis

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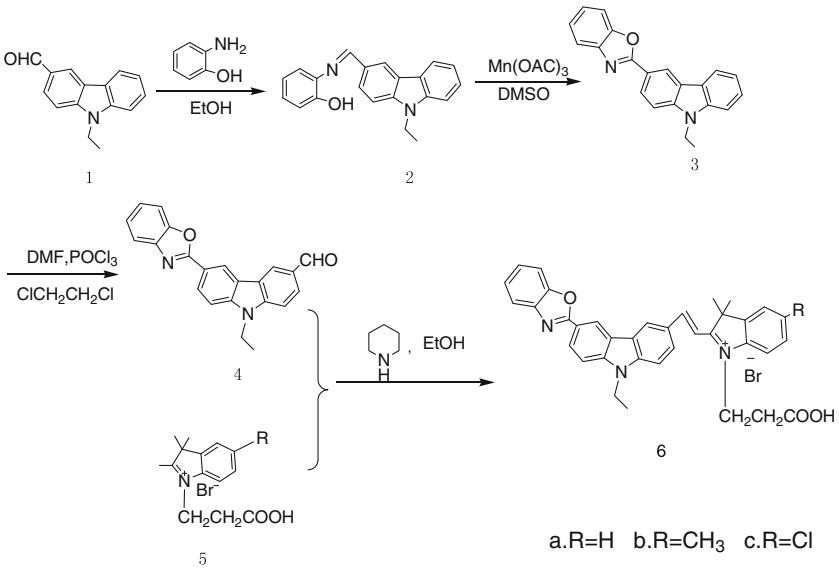
Scheme 1 Synthesis of benzoxazole indole styryl cyanine dyes



spectra were recorded on a Shimazu 2550 spectrophotometer (Jap.). Mass spectra were obtained using an electrospray ionization (ESI) mass spectrometer. ^{1}HNMR spectra were recorded on a Bruker (300 MHz or 400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from TMS (tetramethylsilane), using D_2O or DMSO-d_6 as a solvent.

Synthesis

Synthesis of 3-Formyl-N-Ethyl Carbazole

The compound 1 was synthesized according to literature methods [26].

Synthesis of Schiff Base Compound 2

Glacial acetic acid (100 mL) and potassium permanganate (1.0 g) were refluxed for 3 h. The refined acetic acid was purified by reduced pressure distillation.

The refined acetic acid (50 mL) and manganese chloride tetrahydrate (5.0 g) were refluxed until the solid was dissolved. Acetic anhydride (9 mL) was added and then potassium permanganate (1.0 g) was added in batches in 20 mins. After that, the mixture was refluxed for 30 min before cooling to room temperature. Distilled water (9 mL) was added and then the mixture was kept for 12 h. The precipitation was filtered, washed with aether and dried to afford manganese(III) acetate.

3-formyl-N-ethyl carbazole (1.2 g, 5.2 mmol) and o-aminophenol (0.8 g, 8.0 mmol) were added to pure ethanol (40 mL). 5 drops of glacial acetic acid was added into the refluxing mixture and reacted for 4 h. After the reaction was finished, the mixture was poured into cool water to give yellow solid. Crude product was obtained by filteration and washing with cold ethanol.

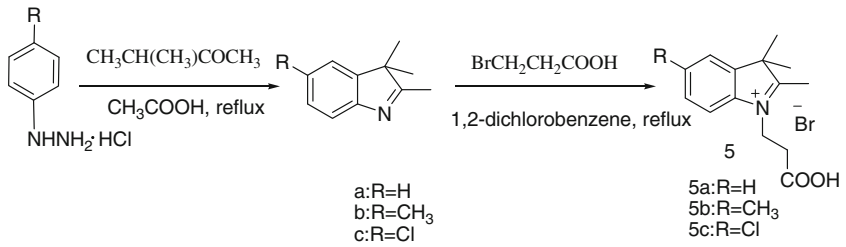
Synthesis of 3-Benzoxazole-N-Ethyl Carbazole(Compound 3)

A mixture of schiff base compound 2 (1.2 g, 3.9 mmol), manganese(III) acetate(2.1 g, 9.6 mmol) and DMSO (50 mL) was kept at 140 °C for 24 h and then cooled, poured into ice water and filtered. The precipitate was washed and chromatographed over silica gel to give compound 3. Yield: 53 %. m.p.141–143 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.43–1.48 (t, J=7.20 Hz, 3H), 4.33–4.40 (m, 2H), 7.27–7.33 (m, 3H), 7.40–7.50 (m, 3H), 7.56–7.59 (m, 1H), 7.74–7.77 (m, 1H), 8.18 (d, J=7.80 Hz, 1H), 8.32–8.35 (m, 1H), 8.97 (s, 1H). MS (EI) m/z: 313(M^++1).

Synthesis of 3-Benzoxazole-6-Formyl-N-Ethyl Carbazole (Compound 4)

$POCl_3$ (9.5 mL, 100 mmol) was added dropwise to a stirred solution of DMF (7.7 mol, 100 mmol) in a 100 mL flask under ice-cold condition. When the addition was over, the reaction was stirred for another 30 min and brought to room temperature to react for 1 h. Compound 3 (3.0 g, 10 mmol) of 1,2-dichloroethane (40 mL) was added dropwise and then stirred for 1 h, refluxed for 10 h and then cooled. The mixture was poured into cool water and extracted with CH_2Cl_2. The organic phase was washed with water, dried with anhydrous $MgSO_4$. The solvent was distilled off and the residue was chromatographed over silica gel to give yellow compound 4. Yield: 13 %. m.p.257–260 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 1.48–1.52 (t, J=7.60 Hz, 3H), 4.37–4.43

Scheme 2 Synthesis of N- propionic acid-2,3, 3-trimethyl-3H-indole



(m, 2H), 7.37–7.40 (m, 2H), 7.47–7.52 (m, 2H), 7.60 (d, J=9.60 Hz, 1H), 7.79 (d, J=9.60 Hz, 1H), 8.04 (d, J=8.80 Hz, 1H), 8.39 (d, J=8.80 Hz, 1H), 8.63 (s, 1H), 8.79 (s, 1H), 10.10 (s, 1H CHO). MS (EI) m/z: 341(M^++1).

Synthesis of N- Propionic Acid-2,3,3-Trimethyl-3H-Indole (Compound 5)

The synthetic route of derivatives of N-propionic acid-2,3,3-trimethyl-3H-indole (5a–5c) was illustrated in Scheme 2.

One mole Phenylhydrazinium chloride and 150 mL glacial acetic acid were added to a 3-neck flask under a flow of nitrogen. The solution was stirred and refluxed in the dark and 44 mL 3-methyl-2-butanone was added to the refluxed solution for another 8 h. After the solvent was removed using a rotary evaporator, the residue was washed with saturated sodium bicarbonate solution to adjust the pH value to a final pH of about 7. The crude product was extracted with chloroform. The organic layer was then dried with anhydrous magnesium sulfate overnight. After the solvent was evaporated, the remaining solid was purified by column chromatography with eluent of dichloromethane/petroleum ether=1:3 (v/v) to afford final product.

The intermediates 5a–5c were synthesized according to the procedure as follows. 2,3,3-trimethylindole derivative synthesized above (0.02 mol) and 3-bromopropanoic acid (0.03 mol) were refluxed in 1,2-dichlorobenzene for 12 h. The mixture was cooled to room temperature and poured into ethyl acetate. The precipitate was collected by filtration, washed with acetone and dried under vacuum to yield compound 5.

5a (300 MHz, DMSO-d_6) δ: 1.53(s, 6H), 2.95(s, 3H), 2.95–3.00(t, J=6.90 Hz, 2H), 4.62–4.66(t, J=6.90 Hz, 2H), 7.58–7.61(m, 2H), 7.80–7.83(m, 1H), 7.95–7.98 (m, 1H). ESI-MS(m/z): 232.1[M^+], 233.2 [M^++1].

5b (300 MHz, DMSO-d_6) δ: 1.50(s, 6H), 2.50(s, 3H), 2.81(s, 3H), 2.93–2.97(t, J=7.05 Hz, 2H), 4.58–4.63(t, J=6.90 Hz, 2H), 7.40(d, J=8.40 Hz, 1H), 7.62(s, 1H), 7.84(d, J=8.40 Hz, 1H). ESI-MS(m/z): 246.0[M^+], 247.1 [M^++1].

5c (300 MHz, DMSO-d_6) δ: 2.84(s, 3H), 1.53(s, 6H), 2.93–2.97(t, J=6.75 Hz, 2H), 4.60–4.64(t, J=6.75 Hz, 2H), 7.67–7.71(m, 1H), 8.00–8.04(m, 2H). ESI-MS(m/z): 266.0[M^+], 268.1 [M^++2].

Synthesis of Benzoxazole Indole Styryl Cyanine dye (Compounds 6a–6c)

The typical procedure for synthesis of 6a–6c was: aldehyde 4 (0.28 mmol) in 30 mL CH_2CH_3OH and salt 5 (0.42 mmol) in 20 mL CH_2CH_3OH were added to a 100 mL flask, followed by catalytic piperdine (1–3 drop). The resulting

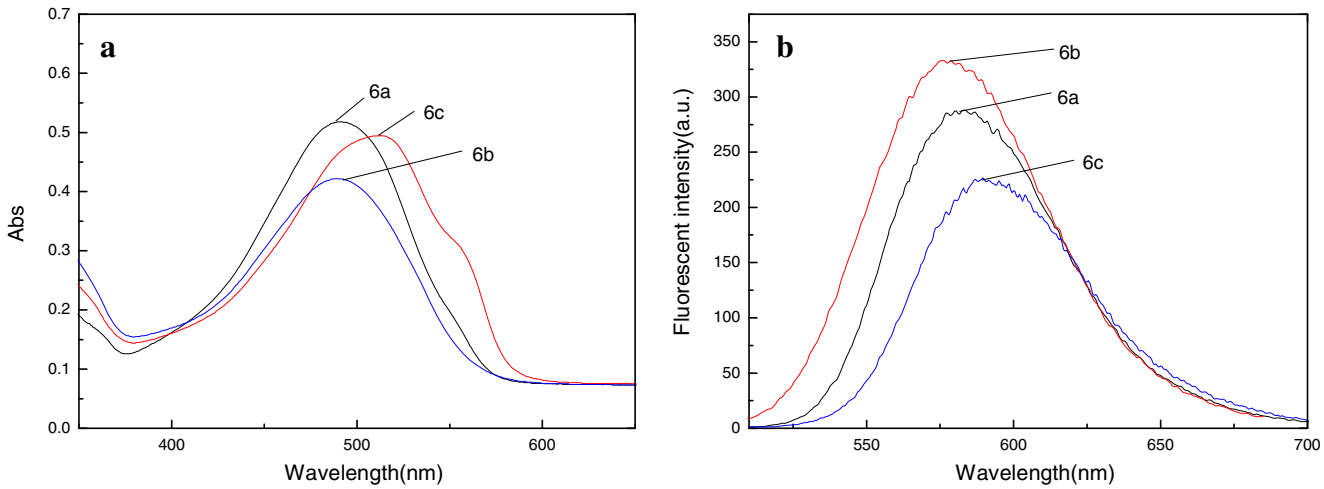


Fig. 1 Absorption and fluorescence spetra of carzabole styryl cyanine dyes. **a** Absorption sptecra of carzabole styryl cyanine dyes. **b** Fluorescence spetra of carzabole styryl cyanine dyes

Table 1 Spectra data of carzabole styryl cyanine dyes

Dye	Absorption wavelength (nm)	Emission wavelength (nm)	Stokes shift (nm)	$\varepsilon(10^5 \cdot mol \cdot L^{-1} \cdot cm^{-1})$
6a	491.00	584.00	93	1.41010
6b	489.00	580.00	91	0.93240
6c	512.00	591.00	79	1.84030

mixture was refluxed and stirred for 12 h. When it was cooled to room temperature, aether was added to give a red solid. The crude product was filtered, washed and the residue was chromatographed over silica gel to give red compound 6.

6a. ^{1}HNMR (300 MHz, DMSO-d_6) δ: 1.42 (t, J=5.20 Hz, 3H), 1.83 (s, 6H), 2.92–2.95 (t, J=4.50 Hz 2H), 4.59–4.63 (t, J=6.00 Hz, 2H), 4.90–4.87 (t, J=4.50 Hz, 2H), 7.43 (d, J=6.00 Hz, 2H), 7.56–7.60 (m, 2H), 7.81–7.95 (m, 7H), 8.37 (d, J=6.30 Hz, 2H), 8.60 (d, J=11.70 Hz, 1H), 9.10 (s, 1H), 9.31 (s, 1H). ESI-MS (m/z):554[M^+], 555[M^++1].

6b. ^{1}HNMR (300 MHz, DMSO-d_6) δ: 1.42 (t, J=5.00 Hz, 3H), 1.85 (s, 6H), 2.47(s, 3H), 2.94–2.98 (t, J=6.90 Hz, 2H), 4.60–4.62 (m, 2H), 4.92–4.94 (m, 2H), 7.43–7.45 (m, 3H), 7.72 (s, 1H), 7.80–7.96 (m, 6H), 8.37–8.43 (m, 2H), 8.64 (d, J=12.00 Hz, 1H), 9.10(s, 1H), 9.34 (s, 1H). ESI-MS (m/z):568[M^+], 569[M^++1].

6c. ^{1}HNMR (300 MHz, DMSO-d_6) δ: 1.45 (t, J=5.60 Hz, 3H), 1.85 (s, 6H), 2.93–2.98 (t, J=6.45 Hz, 2H), 4.57–4.61 (t, J=6.00 Hz, 2H), 4.84–4.88 (t, J=7.50 Hz, 2H), 7.43–7.46(m, 2H), 7.65–7.70(m, 1H), 7.96–7.79 (m, 6H), 8.08 (s, 1H), 8.36–8.41(m, 2H), 8.61 (t, J=7.80 Hz, 1H), 9.11 (s, 1H), 9.34 (s, 1H). ESI-MS (m/z):588[M^+], 589[M^++1].

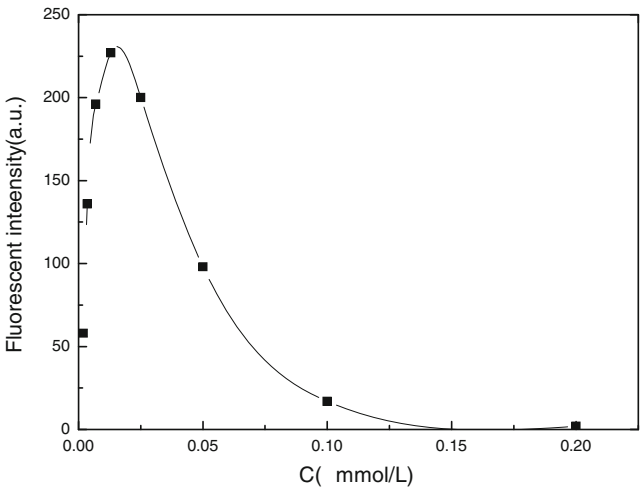


Fig. 2 Fluorescent spectra of 6a with different concentrations

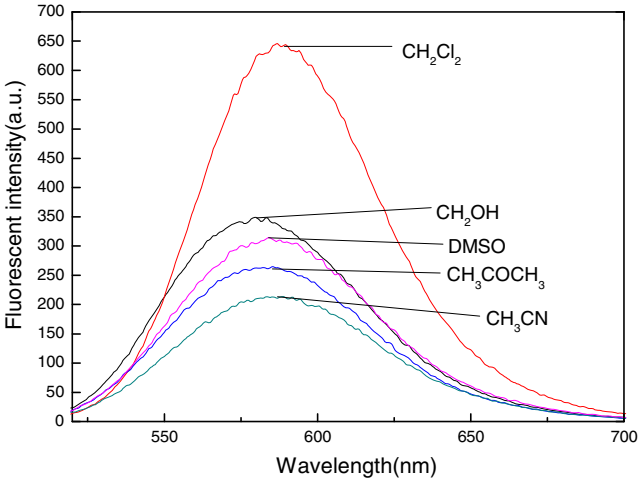


Fig. 3 Fluorescent spectra of 6a in different solvents

Results and Discussion

Spectral Properties of Cyanine Dyes (6a–6c) with Carbazole Bridged Chain

In order to study the effect of substitutional groups of indole ring on absorption and fluorescent properties, spectra of three dyes with the same concentration of 10^{-5}mol/L in CH_3OH were performed and studied results were shown in Fig. 1 and Table 1. With different substitutional groups, the absorption wavelength and emission wavelength changed and the order is 6c > 6a > 6b. Regarding the compounds with similar structure, the dyes with electron-donating groups possessed stronger fluorescence intensities than those with electron-withdrawing groups. The nonbonding electron n on electron-donating groups -CH_3 was in parallel with the π orbital of aromatic ring, which produces the n-π conjugated system and hence enhances the conjugation degree, resulting in the increase of fluorescence intensity. There were also a nonbonding electron n on the electron-withdrawing group -Cl, but as the n electron was not in parallel with the π orbital of the aromatic ring so the n-π conjugated system did not exit. Since the n-π*transition was a kind of forbidden transition, its molar absorptivity was low enough to lead to the fluorescence intensity decreased.

As to the absorption spectra, as the electron-withdrawing group -Cl is a auxochrome, the conjugation's length was

Table 2 Spectra data of 6a–6c in different solvents

Dye	CH_2Cl_2	CH_3COCH_3	CH_3OH	CH_3CN	DMSO
6a	589 nm	585 nm	583 nm	588 nm	587 nm
6b	587 nm	582 nm	581 nm	594 nm	586 nm
6c	590 nm	591 nm	591 nm	593 nm	594 nm

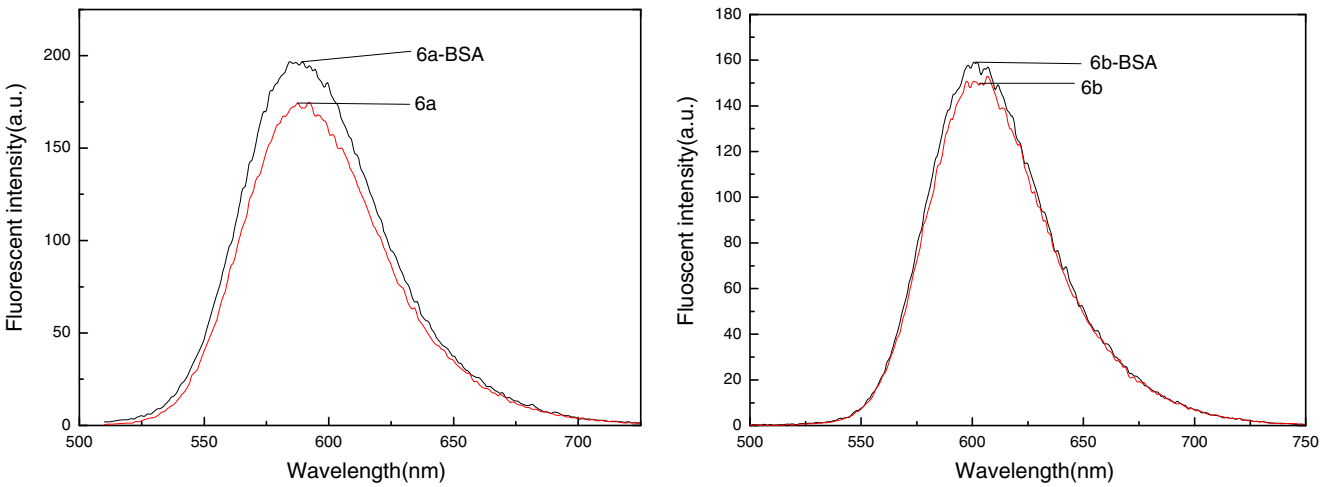


Fig. 4 Fluorescent spectra of 6a and 6b labeled with BSA. **a** Fluorescent spectra of 6a labeled with BSA, **b** Fluorescent intensities of 6b labeled with BSA concentrations

added, causing the wavelength becomes the longest together with bigger molar extinction coefficient (ε).

Effect of Concentration on the Fluorescence Properties

Concentration was another factor that would affect the fluorescence properties. In this paper, the fluorescent spectra of a series of 6a samples in CH_3OH solvent were scanned and the results were shown in Fig. 2.

The maximum emission wavelength and peak shape of samples 6a with different concentrations were of little difference. The fluorescent intensity increased with the increasing of concentration during the range of 2×10^{-3} mmol/L–1.5×10^{-2} mmol/L, but decreased when the concentration exceeded 1.5×10^{-2} mol/L. With the increasing of the concentration, the absorption, excited quantum number and emissive quantum number increased, leading to the enhancement of the fluorescent intensity. When the fluorescence intensity reached to the maximum, it decreased with the continued increasing of the concentration, which may be because of the fluorescence self-quenching and inner filter effect. The fluorescent properties of 6b and 6c are the same as that of 6a. The strongest fluorescence intensity of 6b and 6c occurs at the concentration of 0.013 mmol/L and 0.025 mmol/L respectively.

Table 3 Data of carzabole styryl cyanine dye labeled with BSA

Dye	Fluorescence wavelength (nm)	Fluorescence intensity (a.u.)
6a	590.0	171.0
6a-BSA	588.0	196.0
6b	600.5	149.5
6b-BSA	598.0	159.0

Solvent Effect on the Fluorescent Spectra

Solution could obviously affects the fluorescent properties. The fluorescent spectra of 6a in different solutions were shown in Fig. 3.

The strongest fluorescence intensity of 6a was observed in the solvent of $CHCl_3$ and the weakest was observed in CH_3CN. The dye had similar maximum fluorescent emission wavelength in different solvents, which indicated that the polarity of the solvent affected little on the maximum fluorescent emission wavelength of the dye. The spectra data were shown in Table 2.

Spectra of Dyes Labeled with BSA

BSA (Bovine albumin) stock solution was prepared by dissolving 25 mg BSA in 50 ml NaH_2PO_4-Na_2HPO_4 buffer (pH=7.7).

0.2 mmol/L stock solutions of free dyes 6a and 6b were prepared respectively. The working solutions of free dyes or dye-BSA complexes with the concentration of 0.003 mmol/L were prepared by diluting 2 ml of every dye stock solutionin in distilled water or BSA stock solution respectively. The fluorescence spectra were shown in Fig. 4 and Table 3. The emission wavelengths and fluorescence intensities of

Table 4 Spectra data of dye 6a,YO and Cy_3

Dye	Emission wavelength (nm)	Stokes shift (nm)	Fluorescence intensity (a.u.)
6a	584	104	286
YO	530	20	9
Cy_3	563	20	71

8a-BSA and 8b-BSA were similar to their corresponding free dyes, which suggested that the dyes could be used as a label of BSA. The fluorescence intensity keep strong and did not decrease like that of Cy_3, which may be resulting in the sensitivity raised.

Fluorescence of 6a Compared with YO and Cy_3

In order to compare the fluorescent properties of dye 6a, YO and Cy_3, the solutions of them with the same concentration of 0.03 mmol/L in CH_3OH were prepared and the fluorescent specra were studied and the results were shown in Table 4. The fluorescent wavelength of 6a was shifted toward the red of over 80 nm compared to those of YO and Cy_3, and the corresponding fluorescence intensity also increased significantly. When 6a was used for biological applications, due to the reduction of the overlap between the excitation and emission wavelength, the background interference would be decreased, leading to the higher sensitivity.

In comparison with the structure of YO and Cy_3, the novel carbazole styryl dyes have extended conjugated system. Simultaneously, as the π electrons are easier to be excited, these dye possess stronger fluorescent intensity and the maximum emission wavelengths are shifted toward the red.

These novel carbazole styryl dyes also have large rigidity plane, which makes the reciprocity and conjugation of the π electrons increasing. Consequently, the wavelength of the novel carbazole styryl dyes is red-shifted to the near-infrared region with increasing fluorescent intensity and larger Stocks shift. All these advantages jointly make these novel dyes potentially be used as an excellent cyanine dye probe for biological labeling.

Conclusion

Based on the structure of the excellent cyanine dye, YO and Cy_3, carbazole was inserted into the methylidyne structure of YO and Cy_3 as a bridge to afford a series of novel carbazole styryl cyanine dyes. The compounds were characterized by ^{1}HNMR and MS. Fluorescent results showed that the fluorescent wavelength of the novel carbzole styryl cyanine dye was shifted toward the red up to 50 nm compared with that of YO, together with Stokes shift increasing 60 nm and fluorescent intensity enhancing by 30 times. When the novel dye was labeled by BSA, both its fluorescent wavelength and intensity are nearly not changed. As these fluorescent dyes possess better performance than YO and Cy_3, such as enhanced fluorescent intensity and larger Stock shift, they can be used as an excellent cyanine dye probe in biological labeling.

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