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Effect of Structure and Conformation on Fluorescence Properties in Novel Coumarin-based Mannich Base Dyes

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Twelve novel coumarin-based Mannich base dyes have been synthesized via introducing functional aminomethyl group at the 8 position of coumarin ring by Mannich reaction and their chemical structures were confirmed by IR, ^{1}H NMR, MS and elemental analysis. Moreover, the fluorescence intensities and relative quantum yields of all the dyes were measured and studied. The results illustrated that the heavy atom effect was obvious in our designed system and there was a relationship between the structures, the conformations and the fluorescence spectra of the coumarins. Meanwhile, the present β-CD titration experiment illustrated that the aniline nitrogen atom was closely related to the photoinduced electron transfer (PET) course and the PET course was carried out via a conformational control mechanism.

Keywords: Coumarin-based Mannich base dyes; Conformation; Structure; Photoinduced Electron Transfer; Relative quantum yield; Synthesis.

INTRODUCTION

As an important class of organic heterocyclic dyes,[1] coumarin (2H-1-benzopyran-2-one) and its derivatives can be found in many natural or synthetic drug molecules and possess versatile bioactivities,[2] such as ani-HIV,[3] anticoagulant,[4] antibacterial,[5] anticancer,[6] anthelminthic,[7] anti-inflammatory[8] and antioxidant activities.[9] Moreover, coumarins have outstanding optical properties including high fluorescence quantum yield,[10] large Stokes shift,[11] excellent photostability and good solubility in common solvents.[12] In view of these excellent photochemical and photophysical properties, the coumarins are extensively studied, and often used as biological and chemical sensors,[13] fluorescent probes,[14] nonlinear optical chromophores,[15] and laser dyes.[16] Additionally, the absorption and fluorescence characteristics of coumarin chromophores are related to the substitution of various functional groups at different position[17] and appropriately substituted coumarins have been discovered various applications in biological, chemical and physical fields.[18] For these advantages motioned above, there remain some interests in the molecular design and synthesis of novel coumarin derivatives with excellent optical properties and greater stability.

In this paper, we have designed and synthesized twelve novel coumarin-based Mannich base dyes (**3a$_{1\text{-}3}$**-**3d$_{1\text{-}3}$**) which were developed by introducing aniline derivatives at the 8 position of coumarin ring by Mannich reaction. We proposed that aniline side chains on this core would act as switches and reversibly quench the fluorescence emission of the coumarins because of their low pK_a values, high HOMO energy levels and efficient electron-transfer ability. Then we investigated how the aniline nitrogen quenches the excited state emission of coumarin fluorophore via photoinduced electron transfer (PET). Meanwhile, the heavy atom effect on the relative compounds were researched and discussed.

EXPERIMENTAL SECTION

Reagents

All reagents of analytical grade were obtained from a commercial source and used without further purification. Twice-distilled water was used throughout the experiments. Compound **1a-1d** and **2a-2d** were obtained from our previous works.[19] All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25-mm EMD Chemical silica gel plates (60F-254) using UV-light (254 and 365 nm). Standard test solution was prepared by dilution of the stock solution. A 0.2 mol.L^{-1} NaH_2PO_4~citric acid buffer solution (pH = 7.4) was employed. All of the measurements were operated at room temperature.

Apparatus

Melting points were determined on a shanghai shenguang WRS-1A Digital Melting Point Apparatus and uncorrected. Infrared (IR) spectra in cm^{-1} were recorded on a

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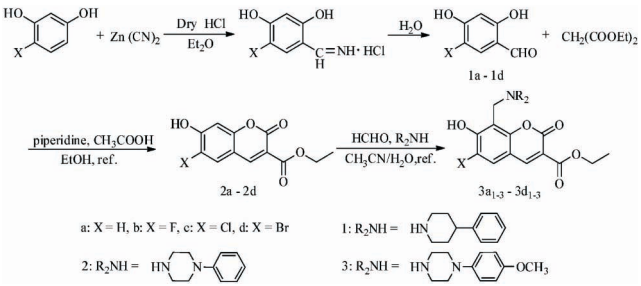
Bruker EQUIOX-55 spectrometer (Germany). NMR spectrometer was measured with Varian unity INOVA-400 spectrometer with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained with AXIMA-CFR plus MALDI-TOF Mass Spectrophotometer. Absorption spectra were determined with a Shimadzu UV-2550 spectrophotometer (Tokyo, Japan). The fluorescence spectra and relative fluorescence intensity were recorded on HITACHI spectrophotometer model F-4500 (Japan) with a 10 mm quartz cuvette. The excitation and emission wavelength bandpasses were both set at 5 nm, and the PMT Voltage (V) was set at "750".

Synthesis and characterization of $3a_{1\text{-}3}$-$3d_{1\text{-}3}$

As shown in Scheme I, a series of coumarin-based Mannich bases were synthesized in three steps by means of formylation, Knoevenagel condensation and Mannich reaction.

Scheme I Synthesis of compound $3a_{1\text{-}3}$-$3d_{1\text{-}3}$



A solution of 1.2 mmol of paraformaldehyde and 1.2 mmol of R_2NH in 5 mL acetonitrile were refluxed for 30 minutes at 65 °C. 1 mmol of coumarins, 5 mL of acetonitrile and 3 mL of water were added to the solution, the mixture was warmed at 85 °C, and then stirred for an additional 24 h in the dark. The organic solvent was then removed from the cooled reaction in vacuo. The resulting solution was extracted with CH_2Cl_2 (3 × 50 mL), was dried over Na_2SO_4, and the solvents were evaporated. The crude product was purified by chromatography using dichloromethane/methanol (100:1, V/V) to get the desired product. The structures of the compounds were confirmed by IR, ^{1}H NMR, MS and elemental analysis.

Ethyl 7-hydroxy-8-(1-methyl-4-phenylpiperidine)-coumarin-3-carboxylate ($3a_1$)

Yellow power, yield 66%, m.p. 204~205 °C. IR (KBr, cm^{-1}): 3440, 2919, 1751, 1601, 1574, 1486, 1187. ^{1}H NMR (400 MHz, DMSO-d_6): 8.57 (s, 1H, ArH), 7.60 (d, 1H, ArH), 7.32-7.20 (m, 5H, PhH), 6.64 (d, 1H, ArH), 4.24 (q, 2H, OCH_2), 4.07 (s, 2H, NCH_2Ar), 3.41-3.34 (m, 4H, CH_2NCH_2), 2.67-2.62 (m, 1H, CH), 1.89-1.73 (m, 4H, 2 × CH_2), 1.25 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 408.64 $(M+H)^+$. Anal. calcd. for $C_{24}H_{25}NO_5$: C, 70.74; H, 6.18; N, 3.44. Found: C, 70.53; H, 6.20; N, 3.47.

Ethyl 7-hydroxy-8-(1-methyl-4-phenylpiperazine)-coumarin-3-carboxylate ($3a_2$)

Yellow power, yield 70%, m.p. 169~171 °C. IR (KBr, cm^{-1}): 3446, 2923, 1740, 1602, 1484, 1232. ^{1}H NMR (400 MHz, DMSO-d_6): 8.67 (s, 1H, ArH), 7.71 (d, 1H, ArH), 7.23-7.19 (m, 2H, PhH), 6.95-6.84 (m, 3H, PhH), 6.80 (d, 1H, ArH), 4.25 (q, 2H, OCH_2), 3.98 (s, 2H, NCH_2Ar), 3.22-3.18 (m, 4H, CH_2NCH_2), 2.76-2.73 (m, 4H, CH_2NCH_2), 1.29 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 409.79 $(M+H)^+$. Anal. calcd. for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.54; H, 5.88; N, 6.92.

Ethyl 7-hydroxy-8-(1-(4-methoxyphenyl)-4-methyl-piperazine)-coumarin-3-carboxylate ($3a_3$)

Yellow power, yield 61%, m.p. 138~139 °C. IR (KBr, cm^{-1}): 3494, 2821, 1754, 1606, 1511, 1234. ^{1}H NMR (400 MHz, DMSO-d_6): 8.66 (s, 1H, ArH), 7.70 (d, 1H, ArH), 6.91-6.80 (m, 4H, PhH), 6.79 (d, 1H, ArH), 4.26 (q, 2H, OCH_2), 3.97 (s, 2H, NCH_2Ar), 3.67 (s, 3H, OCH_3), 3.08-3.05 (m, 4H, CH_2NCH_2), 2.75-2.67 (m, 4H, CH_2NCH_2), 1.29 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 439.18 $(M+H)^+$. Anal. calcd. for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.49; H, 5.91; N, 6.34.

Ethyl 6-fluoro-7-hydroxy-8-(1-methyl-4-phenylpiperidine)-coumarin-3-carboxylate ($3b_1$)

Yellow power, yield 74%, m.p. 221~222 °C. IR (KBr, cm^{-1}): 3455, 2919, 1741, 1624, 1502, 1228, 1110. ^{1}H NMR (400 MHz, DMSO-d_6): 8.38 (s, 1H, ArH), 7.36 (d, 1H, ArH), 7.32-7.22 (m, 5H, PhH), 4.25 (s, 2H, NCH_2Ar), 4.19 (q, 2H, OCH_2), 3.19-3.14 (m, 4H, CH_2NCH_2), 2.83-2.79 (m, 1H, CH), 1.97-1.93 (m, 4H, 2 × CH_2), 1.25 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 426.55 $(M+H)^+$. Anal. calcd. for $C_{24}H_{24}FNO_5$: C, 67.75; H, 5.69; N, 3.29. Found: C, 67.54; H, 5.67; N, 3.25.

Ethyl 6-fluoro-7-hydroxy-8-(1-methyl-4-phenylpiperazine)-coumarin-3-carboxylate ($3b_2$)

Yellow power, yield 72%, m.p. 218~219 °C. IR (KBr, cm^{-1}): 3434, 2925, 1756, 1588, 1502, 1232, 1105. ^{1}H NMR (400 MHz, DMSO-d_6): 8.44 (s, 1H, ArH), 7.45 (d, 1H, ArH), 7.24 (t, 2H, PhH), 6.97 (d, 2H, PhH), 6.84 (t, 1H, PhH), 4.25 (s, 2H, NCH_2Ar), 4.20 (q, 2H, OCH_2), 3,37-3.34 (m, 4H, CH_2NCH_2), 3.19-3.16 (m, 4H, CH_2NCH_2), 1.26 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 427.47

$(M+H)^+$. Anal. calcd. for $C_{23}H_{23}FN_2O_5$: C, 64.78; H, 5.44; N, 6.57. Found: C, 64.57; H, 5.46; N, 6.53.

Ethyl 6-fluoro-7-hydroxy-8-(1-(4-methoxyphenyl)-4-methylpiperazine)-coumarin-3-carboxylate ($3b_3$)

Yellow power, yield 67%, m.p. 206~207 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 8.43 (s, 1H, ArH), 7.44 (d, 1H, ArH), 6.93-6.82 (m, 4H, PhH), 4.26 (s, 2H, NCH_2Ar), 4.20 (q, 2H, OCH_2), 3.64 (s, 3H, OCH_3), 3.21-3.17 (m, 8H, 2 × NCH_2CH_2N), 1.26 (t, 3H, CH_3). IR (KBr, cm^{-1}): 3449, 2968, 1757, 1583, 1510, 1229, 1099. MS (MALDI-TOF): *m/z* = 457.57 $(M+H)^+$. Anal. calcd. for $C_{24}H_{25}FN_2O_6$: C, 63.15; H, 5.52; N, 6.14. Found: C, 63.27; H, 5.48; N, 6.20.

Ethyl 6-chloro-7-hydroxy-8-(1-methyl-4-phenylpiperidine)-coumarin-3-carboxylate ($3c_1$)

Yellow power, yield 70%, m.p. 222.5~222.9 °C. IR (KBr, cm^{-1}): 3448, 2919, 1741, 1606, 1574, 1490, 1226, 751. ^{1}H NMR (400 MHz, DMSO-d_6): 8.39 (s, 1H, ArH), 7.72 (s, 1H, ArH), 7.32 (t, 2H, PhH), 7.25-7.20 (m, 3H, PhH), 4.24 (s, 2H, NCH_2Ar), 4.19 (q, 2H, OCH_2), 3.17-3.10 (m, 4H, CH_2NCH_2), 2.84-2.80 (m, 1H, CH), 1.97-1.92 (m, 4H, 2 × CH_2), 1.25 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 442.53 $(M+H)^+$. Anal. calcd. for $C_{24}H_{24}ClNO_5$: C, 65.23; H, 5.47; N, 3.17. Found: C, 65.11; H, 5.42; N, 3.14.

Ethyl 6-chloro-7-hydroxy-8-(1-methyl-4-phenylpiperazine)-coumarin-3-carboxylate ($3c_2$)

Yellow power, yield 71%, m.p. 196~198 °C. IR (KBr, cm^{-1}): 3442, 2937, 1763, 1581, 1498, 1228, 761. ^{1}H NMR (400 MHz, DMSO-d_6): 8.43 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.24 (t, 2H, PhH), 6.97 (d, 2H, PhH), 6.83 (t, 1H, PhH), 4.27 (s, 2H, NCH_2Ar), 4.21 (q, 2H, OCH_2), 3.39-3.37 (m, 4H, CH_2NCH_2), 3.27-3.24 (m, 4H, CH_2NCH_2), 1.25 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 443.51 $(M+H)^+$. Anal. calcd. for $C_{23}H_{23}ClN_2O_5$: C, 62.37; H, 5.23; N, 6.33. Found: C, 62.11; H, 5.27; N, 6.28.

Ethyl 6-chloro-7-hydroxy-8-(1-(4-methoxyphenyl)-4-methylpiperazine)-coumarin-3-carboxylate ($3c_3$)

Yellow power, yield 68%, m.p. 224~225 °C. IR (KBr, cm^{-1}): 3504, 2958, 1758, 1577, 1506, 1224, 784. ^{1}H NMR (400 MHz, DMSO-d_6): 8.44 (s, 1H, ArH), 7.95 (s, 1H, ArH), 6.94-6.83 (m, 4H, PhH), 4.29 (s, 2H, NCH_2Ar), 4.20 (q, 2H, OCH_2), 3.68 (s, 3H, OCH_3), 3.32-3.25 (m, 8H, 2 × NCH_2CH_2N), 1.26 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 473.43 $(M+H)^+$. Anal. calcd. for $C_{24}H_{25}ClN_2O_6$: C, 60.95; H, 5.23; N, 5.92. Found: C, 60.81; H, 5.18; N, 5.77.

Ethyl 6-bromo-7-hydroxy-8-(1-methyl-4-phenylpiperidine)-coumarin-3-carboxylate ($3d_1$)

Yellow power, yield 73%, m.p. 226~228 °C. IR (KBr, cm^{-1}): 3446, 2923, 1743, 1574, 1457, 1224, 563. ^{1}H NMR (400 MHz, DMSO-d_6): 8.39 (s, 1H, ArH), 7.37 (s, 1H, ArH), 7.35-7.24 (m, 5H, ArH), 4.25 (s, 2H, NCH_2Ar), 4.20 (q, 2H, OCH_2), 3.28 (t, 4H, CH_2NCH_2), 3.19-3.15 (m, 1H, CH), 1.97-1.94 (m, 4H, 2 × CH_2), 1.25 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 486.38 $(M+H)^+$. Anal. calcd. for $C_{24}H_{24}BrNO_5$: C, 59.27; H, 4.97; N, 2.88. Found: C, 59.08; H, 4.78; N, 2.93.

Ethyl 6-bromo-7-hydroxy-8-(1-methyl-4-phenylpiperazine)-coumarin-3-carboxylate ($3d_2$)

Yellow power, yield 73%, m.p. 208~209 °C. IR (KBr, cm^{-1}): 3449, 2935, 1764, 1579, 1496, 1228, 557. ^{1}H NMR (400 MHz, DMSO-d_6): 8.43 (s, 1H, ArH), 7.94 (s, 1H, ArH), 7.24 (t, 2H, PhH), 6.97 (d, 2H, PhH), 6.84 (t, 1H, PhH), 4.27 (s, 2H, NCH_2Ar), 4.23 (q, 2H, OCH_2), 3.39-3.36 (m, 4H, CH_2NCH_2), 3.24-3.21 (m, 4H, CH_2NCH_2), 1.26 (t, 3H, CH_3). MS (MALDI-TOF): *m/z* = 487.32 $(M+H)^+$. Anal. calcd. for $C_{23}H_{23}BrN_2O_5$: C, 56.68; H, 4.76; N, 5.75. Found: C, 56.42; H, 4.73; N, 5.81.

Ethyl 6-bromo-7-hydroxy-8-(1-(4-methoxyphenyl)-4-methylpiperazine)-coumarin-3-carboxylate ($3d_3$)

Yellow power, yield 67%, m.p. 214~215 °C. IR (KBr, cm^{-1}): 3513, 2946, 1766, 1606, 1579, 1193, 557. ^{1}H NMR (400 MHz, DMSO-d_6): 8.43 (s, 1H, ArH), 7.95 (s, 1H, ArH), 6.93 (d, 2H, PhH), 6.84 (d, 2H, PhH), 4.25 (q, 2H, OCH_2), 4.19 (s, 2H, NCH_2Ar), 3.68 (s, 3H, OCH_3), 3.34-3.25 (m, 8H, 2 × NCH_2CH_2N), 1.26 (t, 3H, CH_3). MS: *m/z* = 517.13 $(M+H)^+$. Anal. calcd. for $C_{24}H_{25}BrN_2O_6$: C, 55.72; H, 4.87; N, 5.41. Found: C, 55.54; H, 4.81; N, 5.47.

Procedure

Quantum Yield Measurements

The fluorescence quantum yields of all compounds were estimated by comparison with quinine sulfate (φ_f = 0.55) in 0.05 M sulfuric acid as the standard reference [20]. All the coumarin-based dyes in DMSO and methanol (1.0×10^{-3}mol·L^{-1}) were diluted to 1.5×10^{-5}mol·L^{-1} with methanol and twice-distilled water. The measurements were performed in 10 mL volumetric tube. To a test tube containing 1.0 mL of sample solution (1.5×10^{-5}mol·L^{-1}), 1.0 mL of buffer solution (pH 7.4) was added and the solution was diluted to 10 mL with twice-distilled water. The result solution was allowed to stand at room temperature for 10 min, and then the absorption spectra and emission spectra were recorded. For fluorescence intensity measurement, the excitation wavelength was set at 366 nm.

Cyclodextrin Titration

Solutions of $3a_3$ (final concentration: 5 μM) in pH 7.4

phosphate buffer were prepared containing 1 mL of β-cyclodextrin (final concentration: 5 mM). To test the effect of a competitive inhibitor, 1 mL of 1-(4-methoxyphenyl)-piperazine (final concentration: 5 mM) was added to a 5 μM $3a_3$ solution contain β-cyclodextrin solution.[21] Samples were excited at 366 nm, and integrated emission spectra were compared using Origin Pro software.

RESULT AND DISCUSSION

To determinate the changes in fluorescence properties after introducing functional aminomethyl group, we calculated the relative quantum yields of compounds **3a$_{1-3}$**-**3d$_{1-3}$** and collected all the fluorescent data in Table 1. Compounds **3a$_1$**-**3d$_1$** showed high quantum yields (φ_{3a1} = 0.713, φ_{3b1} = 0.765, φ_{3c1} = 0.619, φ_{3d1} = 0.502), compounds **3a$_2$**-**3d$_2$** (φ_{3a2} = 0.223 φ_{3b2} = 0.252, $\varphi 3c_2$ = 0.198, φ_{3d2} = 0.146) and **3a$_3$**-**3d$_3$** (φ_{3a3} = 0.116, φ_{3b3} = 0.151, φ_{3c3} = 0.096, φ_{3d3} = 0.083) showed relative lower quantum yields and fluorescent intensity. The results were consistent with the PET quenching mechanism (Figure 1) and proved that the aniline nitrogen atom acted as the electron-donating groups to the coumarin ring.

Correlation of Quencher HOMO Level to Fluorescence Intensity

We chose compounds **3a$_1$**, **3a$_2$** and **3a$_3$** as embodiments to study the PET quenching mechanism herein. Compound **3a$_1$**, lacking aniline nitrogen atom, showed a quantum yield (φ = 0.713) close to that of quinine sulfate, however compound **3a$_2$**, possessing aniline nitrogen atom, showed a lower quantum yield (φ = 0.223). The results revealed that compound **3a$_2$** or its congeners could be used as fluorescent compounds for optical visualization if an applicable method for unquenching can be found. The significant role that the aniline nitrogen atom played in the PET quenching course was confirmed by the comparison of the fluorescence changes of compound **3a$_1$** and **3a$_2$**. This is consistent with the widely accepted PET quenching mechanism based on coupling of the electrons of the aniline system to the relaxation of the excited coumarin-chromophore.

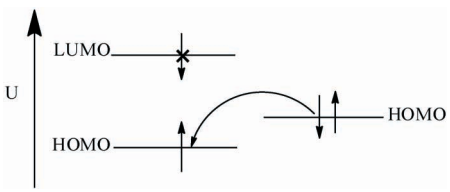


Fig. 1. The PET quenching mechanism.

Table 1. The fluorescent data of all coumarin derivatives

Sample	$\lambda_{ex\text{-}max}$	$\lambda_{em\text{-}max}$	Quantum Yield	Fluorescent Intensity
	402	440	0.713	2211
3a$_{1-3}$	402	440	0.223	588
	402	440	0.116	271
	405	442	0.765	2349
3b$_{1-3}$	405	442	0.252	600
	406	442	0.151	477
	407	443	0.619	1609
3c$_{1-3}$	407	443	0.198	403
	407	443	0.096	257
	407	444	0.502	1012
3d$_{1-3}$	408	444	0.146	383
	408	444	0.083	211

To further confirm the PET phenomenon in the experiment, we designed *p*-methoxyaniline derivative **3a$_3$** and hypothesized that it should have a lower quantum yield. The relative quantum yield of compound **3a$_3$** was found to be 0.116, and we thought it was due to the higher HOMO level of the aniline system which caused by the electron-donating nature of the methoxy group. The result reminded us that the electron-donating group could promote the quenching course and suggested compound **3a$_3$** had great potential as a sensitive fluorogenic molecule. In other words, the fluorescent signal of compound **3a$_3$** would be increased dramatically if the PET quenching course can be broke down.

The fluorescence intensities of all compounds were collected to illustrate the effect of different substituent groups in the PET quenching course. The changes of fluorescence intensities were consistent with the changes of

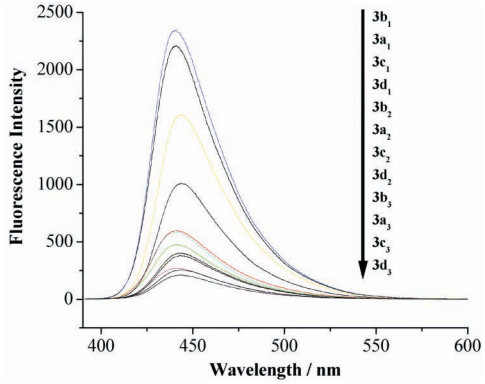


Fig. 2. The fluorescence spectra of all coumarins (1.5×10^{-6} mol·L^{-1}) in water at pH 7.4 (Na_2HPO_4-citric acid). Excitation wavelength was set at 366 nm.

relative quantum yields (Table 1, Figure 2). The fluorescent intensities of all the compounds changed from 2349 to 211 (Figure 2), which confirmed that the functional aminomethyl groups led to different influences on the fluorescent properties of coumarin.

The Influence of Heavy Atom Effect

In addition, we illustrated the heavy atom effect on the relative compounds. Taken $\mathbf{3a_1}$, $\mathbf{3b_1}$, $\mathbf{3c_1}$, $\mathbf{3d_1}$ as examples, the changes of fluorescence intensities were followed in Figure 3. The fluorescent intensities of compound $\mathbf{3c_1}$ and $\mathbf{3d_1}$ had distinct changes. The results indicated the heavy atom had definite influence in the system.

Conformational Analysis

The designed experiments explained the effect of the aniline nitrogen atom in PET quenching mechanism. Meanwhile, we referred to previous literature[22] which described the correlation between the structure and fluorescence nature of quenched chromophores to research the effect of structure and conformation on the quenching phenomenon.

It was known that the quenching efficiency is in proportion to $e^{-\beta d}$ (d = distance between the HOMO electrons of the quencher and the chromophore to be quenched). Herein compound $\mathbf{3a_3}$ was chosen as an example. If the piperazine ring assume a chair conformation (Scheme II, i) in which the methylene group was in equatorial position, the aniline nitrogen atom appeared to be too far apart from the coumarin ring to effectively quench fluorescence through space effect by the PET mechanism. In another case, the aniline nitrogen atoms should be proximal to the xanthene ring of the coumarin fluorophore and promote the phenylpiperazine moieties to effectively quench the fluorescence through space effect by the PET mechanism (Scheme II, ii).

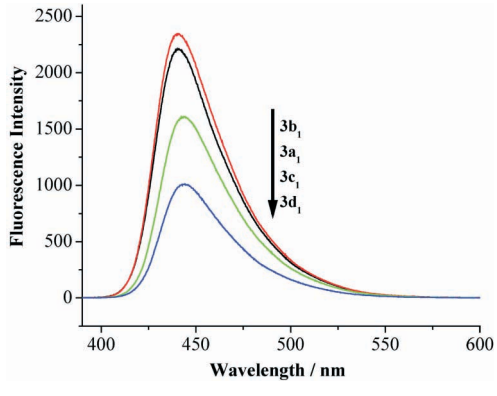
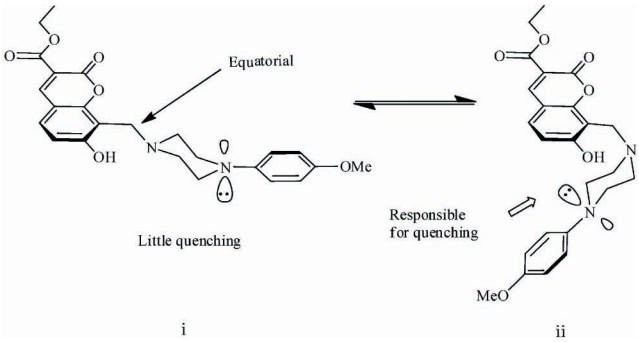


Fig. 3. The fluorescence spectra of compounds $\mathbf{3a_1}$, $\mathbf{3b_1}$, $\mathbf{3c_1}$ and $\mathbf{3d_1}$ on the heavy atom effect. The measurements were proceeded in water at pH 7.4 (Na_2HPO_4-citric acid) and the final concentration was diluted to 1.5×10^{-6} mol·L^{-1}. The excitation wavelength was set at 366 nm.

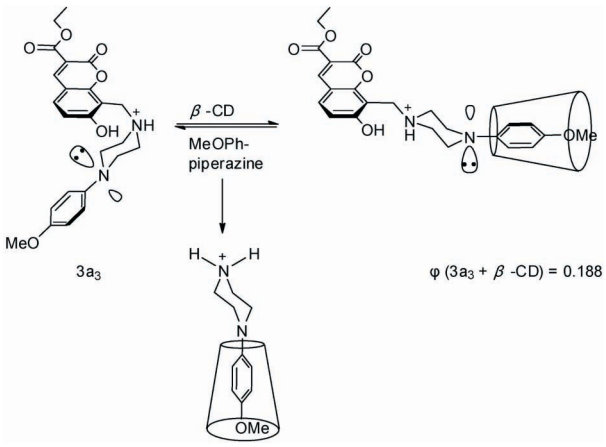
Scheme II Conformational analysis of compound $\mathbf{3a_3}$



Correlation of Conformation to Fluorescence

To demonstrate the reversibility of the PET quenching switch, we turned to β-cyclodextrin (β-CD). We reasoned that β-CD, possessing millimolar affinities for benzene and substituted aromatic systems,[23] would bind to the anisole groups of $\mathbf{3a_3}$ (Scheme III) to prevent the N-lone pair electrons from quenching the fluorescence in the excited state. This would force compound $\mathbf{3a_3}$ to assume an open conformation, reducing the PET rate as described above and enhancing the fluorescence intensity. The resulting fluorescence intensity enhanced from 945 to 1528 (Figure 4), and the relative quantum yield increased up to 0.188 when the final concentration of β-CD was 5 mM. As shown in Scheme III, the PET course between the aniline nitrogen atom and coumarin ring was interrupted because of β-CD

Scheme III The reversible quenching-unquenching switch system



bounding to the *p*-methoxyphenyl group of **3a$_3$**. This interaction and resulting conformational change suggested that the unquenching of **3a$_3$** occurred because the aniline nitrogen atom of the bound 1-(4-methoxyphenyl)piperazine moiety was too distant from the coumarin ring for efficient PET quenching. In other words, the fluorescence must recover partly if a suitable method for unquenching could be found.

To verify that the β-CD was specifically binding to the switching moiety, 1-(4-methoxyphenyl)piperazine (5×10^{-3}mol·L^{-1}) was added to the mixture of **3a$_3$** and β-CD as a competitor for β-CD binding (Scheme III). As shown in Figure 5, the fluorescence intensity decreased from 1434 to 943. This result can be explained by the competition binding between **3a$_3$** and 1-(4-methoxyphenyl)piperazine toward β-CD.

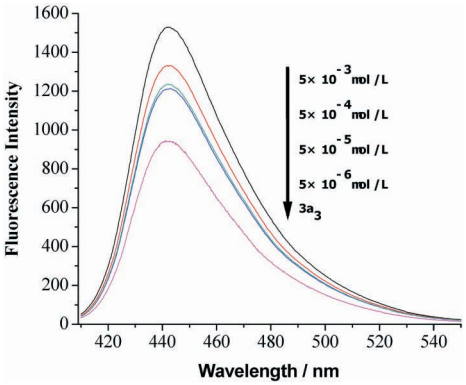


Fig. 4. The fluorescence spectra of β-CD titration**.** The final concentration of β-CD were 5×10^{-3}mol·L^{-1}, 5×10^{-4}mol·L^{-1}, 5×10^{-5}mol·L^{-1} and 5×10^{-6}mol·L^{-1} respectively, and the excitation wavelength was set at 366 nm.

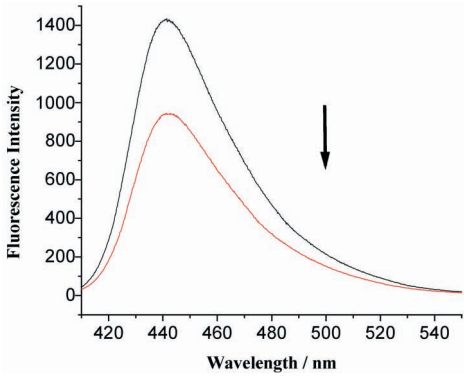


Fig. 5. The fluorescence spectra resulting from the competition between **3a$_3$** and 1-(4-methoxyphenyl)piperazine toward β-CD, and the fluorescence intensity decreased from 1434 to 943.

CONCLUSION

In summary, a novel series of coumarin-based Mannich base dyes have been designed, synthesized and characterized. The quenching efficiency of the aniline nitrogen atom is relative to the structure and conformation of the relative compounds in PET quenching phenomenon. The effect of heavy atom was obvious to the compounds in the experiment. Moreover, the present β-CD titration experiment proved the aniline nitrogen atom or donating group has crucial effects in PET course, which correlated the fluorescence intensity to the conformation of coumarin derivative and illustrated how the quenching course carried out via a conformational control mechanism.

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