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2-Quinolone and coumarin polymethines for the detection of proteins using fluorescence

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ABSTRACT

A series of novel, polymethine chalcone dye – 2-quinolone derivatives and their boron difluoride complexes were synthesized. The spectral-luminescence properties of a series of 2-quinolones and their coumarin analogues were characterized in the presence of a denaturing agent (sodium dodecyl sulfate), native bovine serum albumin as well as a combination of serum albumin and sodium dodecyl sulfate. A study of the influence of the BF_2-ether group on the sensitivity of the polymethine dyes to proteins revealed that three of the dyes, namely two hydroxyquinoline dyes containing a 4-diethylamino-2-hydroxyphenyl substituent and a coumarine dye that contained an indolenine substituent, displayed high emission and bright fluorescence (quantum yield ≤ 0.27) and thus offer promise for use in protein detection.

1. Introduction

Optical markers for biomolecules are widely used in biochemical and medical studies [1,2]. Detection based upon fluorescence has received much attention and notable progress has been made in both fluorescence instrumentation and the synthesis of novel fluorophores [2–4]. Polymethine dyes are well known to be sensitive fluorescent probes for the non-covalent labeling of proteins [5–8].

Novel polymethines can be obtained from boron difluoride complexes of hydroxyl(acyl)arenes and heteroarenes. The incorporation of the boron atom greatly increases the reactivity of the acetyl function in ortho-hydroxyacetophenones, benzoylacetones and acetylnaphthols [9–11]. The methyl group in such complexes undergoes a facile condensation with carbonyl compounds and equivalents. The recent research group recently developed a similar procedure for the synthesis of polymethine – heteroarene derivatives [12–15]. For example, complex **1** was found to be useful starting substrate for the synthesis of 4-hydroxy-3-cinnamoylcoumarins both as the BF_2-complex **2** and the free base **3** (Fig. 1).

This paper concerns the synthesis of novel polymethine dyes (Fig. 2) derived from the boron difluoride complex of 3-acetyl-4-hydroxy-1-methyl-2-quinolone **4** (Fig. 1). In addition, the synthesized 2-quinolones and their coumarin analogues were evaluated as fluorescent dyes for the detection of native proteins using bovine serum albumin (BSA) as model protein and as probes for the nonspecific detection of proteins using a BSA/sodium dodecyl sulfate (SDS) mixture. The influence of dye structure upon selectivity towards certain protein was studied.

2. Experimental

The ^1H NMR spectra (400 MHz) were recorded on a Bruker WP-400 SY spectrometer in DMSO-d_6 or $CDCl_3$ with TMS as an internal reference. The mass spectra were recorded on a MAT-112 spectrometer operating at 80 eV. M.p.'s (Pyrex capillary) are not corrected.

2.1. Materials

Methanol, anhydrous dimethylformamide (DMF) distilled under reduced pressure and 0.05 M Tris–HCl buffer (pH 8.0) were used as solvents. Bovine serum albumin (BSA) and sodium dodecyl sulfate (SDS) were purchased from "Sigma" (USA). Boron difluoride complex **4** was obtained by the reaction of the 4-hydroxy-3-acetylquinolon-2 with boron trifluoride etherate, as described previously [6]. The dyes of coumarin series **2a** [12], **2b**, **2c**, **3a**, **3b**, **3c** [13], **2d**, and **3d** [14] were obtained according to the known

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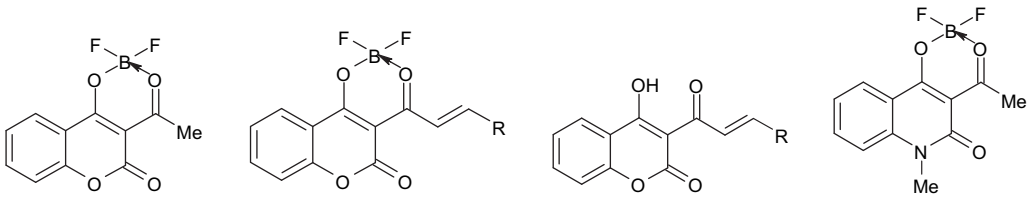

Fig. 1. General structure of parent boron difluoride complexes and previously synthesized polymethine dyes.

procedures, their melting temperatures were in agreement with the literature data.

2.2. Preparation of stock solutions

Dye stock solutions were prepared by dissolving the dyes (2×10^{-3} M) in DMF. Stock solutions of BSA (0.2 mg/ml) and SDS (0.05%) as well as BSA/SDS were prepared by their dissolution in 0.05 M Tris–HCl buffer (pH 8.0).

2.3. Preparation of working solutions

All working solutions were prepared immediately before experimentation. Working solutions of free dyes were prepared by dilution of the dye stock solution in Tris–HCl buffer (pH 8.0).

Working solutions of the dyes in the presence of SDS, BSA and BSA/SDS were prepared by dilution of the dye stock solution using SDS, BSA or BSA/SDS stock solution, respectively. The concentrations of dye, BSA and SDS in the working solutions were 5×10^{-6} M, 0.2 mg/ml and 0.05%, respectively.

2.4. Spectroscopic measurements

Absorption spectra were recorded on Specord M-40 spectrophotometer (Carl Zeiss, Germany). Fluorescence excitation and emission spectra were registered on Cary Eclipse fluorescence spectrophotometer (Varian, Australia). Fluorescence emission was excited at the maximum of the fluorescence excitation spectrum. Both excitation and emission slits had the width equal to 5 nm. Fluorescence measurements were performed using the quartz cell (1×1 cm). The quantum yield value for **3a** and **6b** in presence of BSA/SDS mixture was determined using Rhodamine 6G solution in ethanol as the reference (quantum yield value 0.95). All the measurements were performed at room temperature.

2.5. Dyes ***5a–5c***

A solution of **4** (1.06 g, 4 mmol) in acetic anhydride (10 ml) was added to a solution of aldehyde (4 mmol) in acetic anhydride (2 ml) at 60 °C. The mixture was heated at 90 °C for 30 min, and then cooled. The resultant precipitate was filtered off and crystallized from glacial acetic acid.

2.5.1. 3-[(E)3-(1-ethyl-1,2,3,4-tetrahydroquinoline-6-yl)propyl-2-enoyl]-4-difluoroboronoxy-1-methylquinone-2 (***5a***)

Yield 82%. M.p. 294–295 °C. ^{1}H NMR (DMSO-d_6): δ 1.20 (t, 3H, Me); 1.93 (m, 2H, H_k); 2.80 (t, 2H, H_j); 3.52 (m, 4H, H_l, NCH_2); 3.66 (s, 3H, MeN); 6.87 (d, $^3J_{H,H} = 9.0$ Hz, 1H, H_i); 7.39 (t, 1H, H_c); 7.54–7.71 (m, 3H, H_d, H_g, H_h); 7.84 (t, 1H, H_b); 8.13 (d, $^3J_{H,H} = 8.3$ Hz, 1H, H_a,); 8.32 (d, $^3J_{H,H} = 14.8$ Hz, 1H, H_f); 8.41 (d, $^3J_{H,H} = 14.8$ Hz, 1H, H_e). Anal. calcd. for $C_{24}H_{23}BF_2N_2O_3$: C, 66.08; H, 5.31; N, 6.42. Found: C, 66.04; H, 5.27; N, 6.49.

2.5.2. 3-{(E)3-[2-hydroxy-4-(N,N-diethyaminol)-phenyl]prop-2-enoyl}-4-difluoroboronoxy-1-methylquinone-2 (***5b***)

Yield 73%. M.p. 257–258 °C. ^{1}H NMR ($CDCl_3$): δ 1.21 (m, 6H, Me); 3.45 (m, 4H, NCH_2); 3.65 (s, 3H, MeN); 6.41 (s, 1H, H_i); 6.58 (d, 1H, H_h, $J = 9.2$ Hz); 7.24–7.32 (m, 2H, H_c, H_d); 7.68–7.81 (m, 2H, H_b, H_g); 8.37 (d, $^3J_{H,H} = 8.2$ Hz, 1H, H_a); 8.41 (d, $^3J_{H,H} = 14.6$ Hz, 1H, H_f); 8.50 (d, $^3J_{H,H} = 14.6$ Hz, 1H, H_e). Anal. calcd. for $C_{23}H_{23}BF_2N_2O_4$: C, 62.75; H, 5.27; N, 6.36. Found: C, 62.81; H, 5.29; N, 6.43.

2.5.3. 3-[(E)3-(5-piperidinyle-1-thien-2-yl)prop-2-enoyl]-4-difluoroboronoxy-1-methylquinone-2 (***5c***)

Yield 75%. M.p. 296–297 °C. ^{1}H NMR (DMSO-d_6): δ 1.71 (m, 6H, H_j, H_k); 3.61 (s, 3H, MeN); 3.78 (m, 4H, H_i); 7.01 (d, $^3J_{H,H} = 5.1$ Hz, 1H, H_h); 7.32 (t, 1H, H_c); 7.55 (d, $^3J_{H,H} = 8.3$ Hz, 1H, H_d); 7.67–7.77 (m, 2H, H_b, H_f); 8.04–8.11 (m, 2H, H_a, H_g); 8.31 (d, $^3J_{H,H} = 12.9$ Hz, 1H, H_e). Anal. calcd. for $C_{22}H_{21}BF_2N_2O_3S$: C, 59.74; H, 4.79; N, 6.33. Found: C, 59.81; H, 4.72; N, 6.40.

2.6 Dyes ***6a–6c***

A solution of **5a–c** (3 mmol) in 60% ethanol (10 ml) was treated with 1.59 g (15 mmol) Na_2CO_3. Then the mixture was boiled for 6–10 h. The decomposition of BF_2-complex was controlled by TLC; the resultant precipitate was filtered off, dried and crystallized from 2-propanol.

2.6.1. 4-Hydroxy-3-[(E)3-(1-ethyl-1,2,3,4-tetrahydroquinoline-6-yl)prop-2-enoyl]-1-methylquinone-2 (***6a***)

Yield 75%. M.p. 198–199 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H, Me); 1.96 (m, 2H, H_k); 2.76 (t, 2H, H_j); 3.36 (m 4H, H_l, NCH_2); 3.65 (s, 3H, MeN); 6.55 (d, $^3J_{H,H} = 8.7$ Hz, 1H, H_i); 7.19–7.41 (m, 4H, H_c, H_d, H_g, H_h); 7.63 (t, 1H, H_b); 8.04 (d, $^3J_{H,H} = 15.4$ Hz, 1H, H_f); 8.24 (d, $^3J_{H,H} = 8.2$ Hz, 1H, H_a); 8.48 (d, $^3J_{H,H} = 15.4$ Hz, 1H, H_e); 18.82 (s, 1H, OH). Anal. calcd. for $C_{24}H_{24}N_2O_3$: C, 74.21; H, 6.23; N, 7.21. Found: C, 74.28; H, 6.22; N, 7.15.

2.6.2. 4-Hydroxy-3-{(E)3-[2-hydroxy-4-(N,N-diethylamino)-phenyl]prop-2-enoyl}-1-methylquinone-2 (***6b***)

Yield 80%. M.p. 183–184 °C. ^{1}H NMR ($CDCl_3$): δ 1.17 (m, 6H, Me); 3.34 (m, 4H, NCH_2); 3.65 (s, 3H, MeN); 6.13–6.28 (m, 2H, H_i, H_h,); 7.20–7.31 (m, 2H, H_c, H_d); 7.50–7.69 (m, 2H, H_b, H_g); 8.24–8.33 (m, 2H, H_a, H_f); 8.62 (d, $^3J_{H,H} = 15.4$ Hz, 1H, H_e); 18.86 (s, 1H, OH). Anal. calcd. for $C_{23}H_{24}N_2O_4$: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.44; H, 6.19; N, 7.12.

2.6.3. 4-Hydroxy-3-[(E)3-(5-piperidinyle-1-thiene-2-yl)prop-2-enoyl]-1-methylquinone-2 (***6c***)

Yield 86%. M.p. 202–203 °C. ^{1}H NMR (DMSO-d_6): δ 1.65 (m, 6H, H_j, H_k); 3.61 (s, 3H, MeN); 3.77 (m, 4H, H_i); 6.37 (d, $^3J_{H,H} = 4.2$ Hz, 1H, H_h); 7.28 (t, 1H, H_c); 7.48–7.54 (m, 2H, H_d, H_g); 7.74 (t, 1H, H_b); 7.93 (d, $^3J_{H,H} = 14.8$ Hz, 1H, H_f); 8.08–8.16 (m, 2H, H_a, H_e). Anal. calcd. for $C_{22}H_{22}N_2O_3S$: C, 66.98; H, 5.62; N, 7.10. Found: C, 67.02; H, 5.59; N, 7.14.