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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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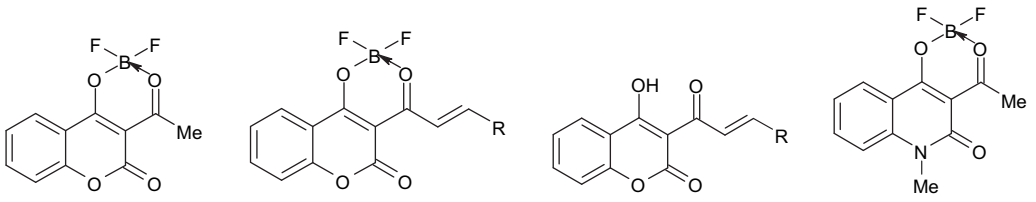


Fig. 1. General structure of parent boron difluoride complexes and previously synthesized polymethine dyes.

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.

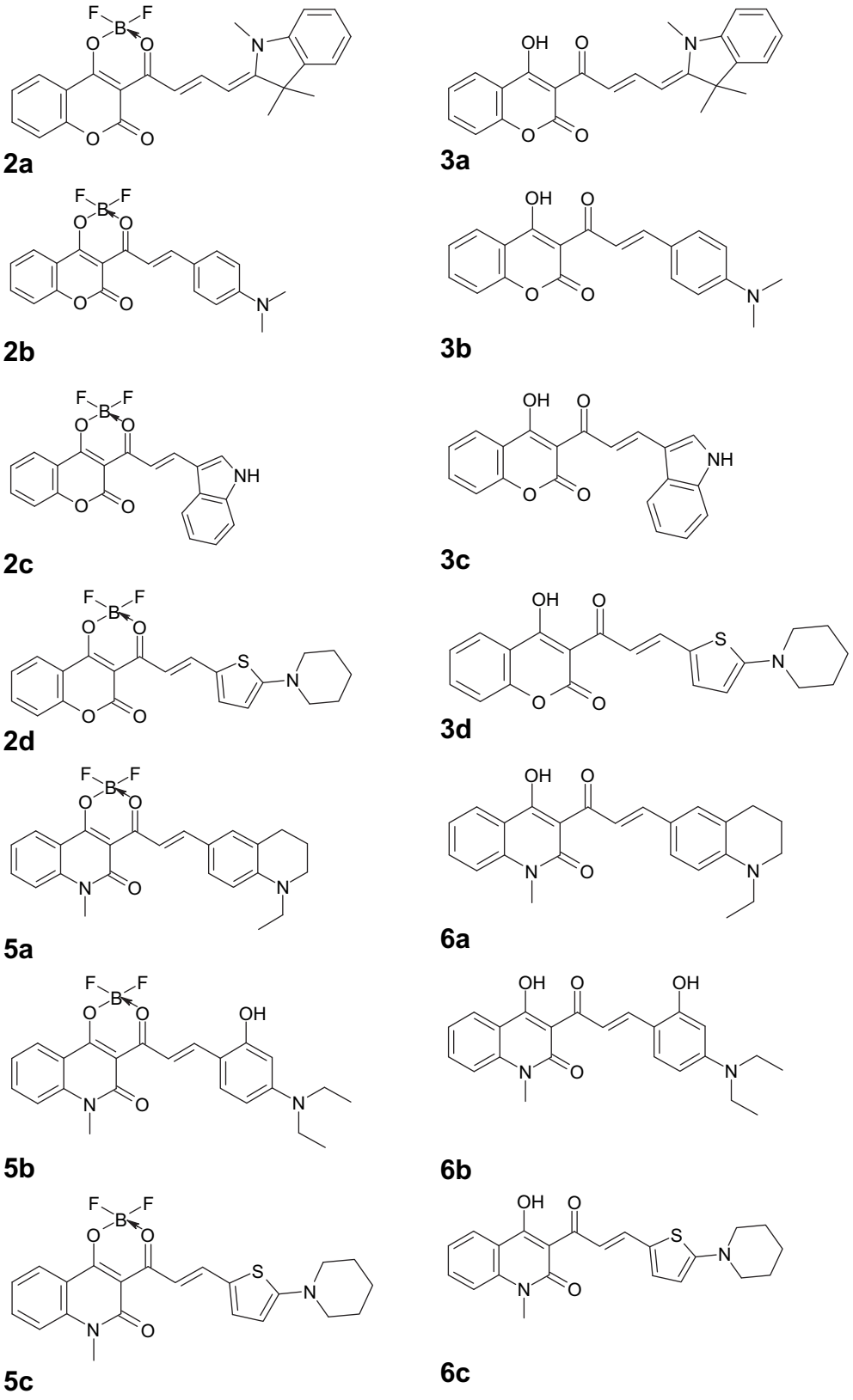


Fig. 2. Structures of studied dyes.

3. Results and discussion

The synthesis of several dyes starting with boron difluoride complex **4** is shown in Scheme 1. Condensations of **4** with aldehydes in acetic anhydride undergo with high yields and provide boron difluoride complexes **5a–c** of polymethines **6a–c**. Reported yields are given for analytically pure compounds, as shown by good results of elemental analysis. The pure dyes were also characterized by ^{1}H NMR spectral method. The large values of the coupling constants between protons of the methine chain (J = 12–15 Hz) are indicative of trans-configuration.

3.1. Characterization of fluorescent properties of free chalcone dyes in buffer

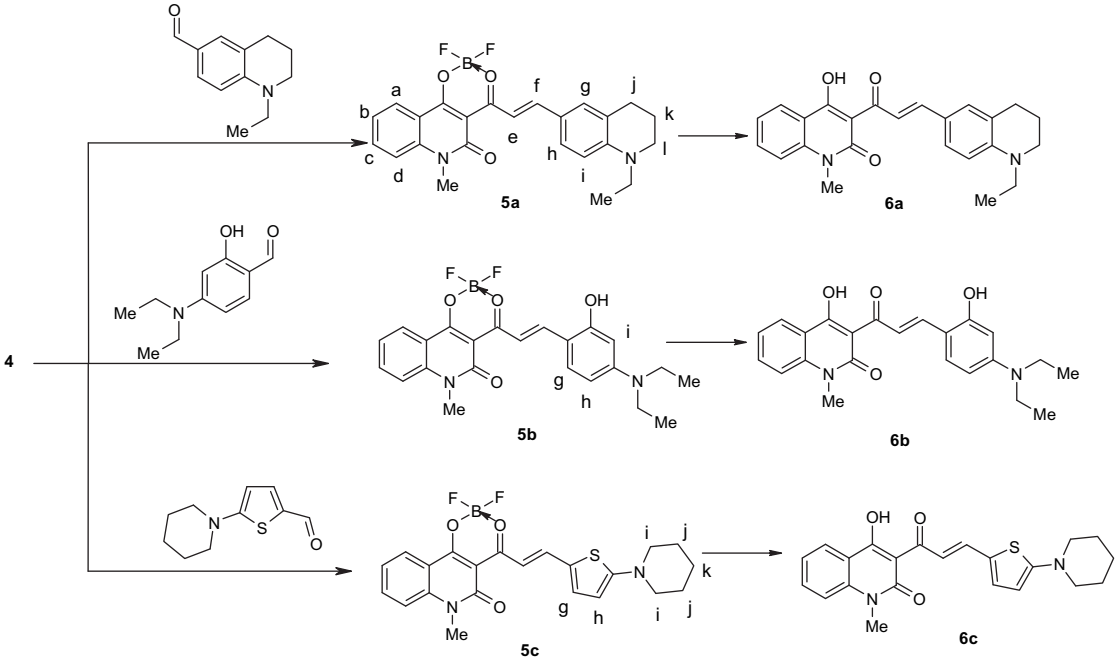
Selected characteristics of fourteen polymethine dyes in buffer are presented in Table 1. In fluorescence excitation spectra of majority of hydroxyquinolone dyes single band, situated between 472 and 581 nm is observed, exception is the dye **6b**, that has two maxima on 451 nm and 549 nm. The same situation is in the fluorescence emission spectra of hydroxyquinolone dyes, which have single emission band with the maxima in the range 549–630 nm. In the case of hydroxyquinolone dye **6b**, two emission bands with maxima on 543 nm and 593 nm are observed. The dyes demonstrate from medium to large values of the Stokes shifts, which lay in the range 44–137 nm. Very low intensity of intrinsic fluorescence of all dyes should be noted (0.6–4.4 a.u.).

In excitation spectra of all coumarin dyes two bands are presented, except these of **2a** and **2c**, where only long-wavelength maxima are observed. The maxima of short-wavelength bands are located between 404 and 470 nm, and these of long-wavelength ones are between 470 and 585 nm. Emission maxima of the dyes are situated in the range 462–556 nm for the short-wavelength maxima and 522–712 nm for the long-wavelength bands. The studied coumarins have from medium to large Stokes shifts values from 26 nm to 134 nm. Low intrinsic fluorescence is observed for the coumarins (0.2–41 a.u.), for these dyes having two bands in the spectra, the short-wavelength band being more intensive than long-wavelength one. Generally it should be noted that emission of the dyes containing BF_2-ether group is less intensive than that of their unsubstituted analogues.

3.2. Characterization of fluorescent properties of chalcone dyes in the presence of BSA

Characteristics of emission and excitation spectra of the dyes and their boron difluoride complexes in presence of BSA are given in Table 1. It should be noted that only for one hydroxyquinolone dye (**6c**) positions of maxima of excitation and emission spectra were close to these in buffer. In spectra of other dyes in BSA complexes rather significant shifts took place or even new bands appeared. Maxima of excitation spectra of studied hydroxyquinolone dyes in complexes with BSA are situated in the 495–596 nm region. In the spectra of these dyes single excitation and emission bands were observed. Emission maxima of the dyes are located between 569 and 626 nm. Stokes shifts values for these dyes vary between small (10 nm for **5c**) and large (85 nm for **6a**) ones. The increase in emission intensity for the studied hydroxyquinolone dyes in the presence of BSA (I_{BSA}/I_0) reaches 900 times (**5a**). Particularly, for the most bright dye–BSA complexes (**5b**) the high emission enhancement (in 470 times) is observed.

Interaction of coumarins **2a**, **3a**, **2b** and **2c** with BSA is accompanied by significant shift of the bands in excitation and/or emission spectra, or with appearing of the new ones, as compared to the dyes spectra in buffer. For other coumarins corresponding maxima positions were close to these observed in buffer. Thus in the excitation/emission spectra of coumarins two bands were observed, with exceptions of dyes **2a**, **3b** and **3d**, having single band. Fluorescence excitation maxima of studied dyes are located between 402 nm and 576 nm and emission maxima are situated in the range 480–614 nm. Observed Stokes shifts values are between 14 and 104 nm. All coumarins increase their fluorescence intensity in presence of BSA but demonstrate from low to moderate intensity level of fluorescence. The highest intensity increase and the



Scheme 1. Scheme of dyes synthesis from boron difluoride complex **4**.

Table 1
Characteristics of fluorescence excitation/emission spectra of studied chalcone dyes (5×10^{-6} M) solutions in buffer, as well as in the presence of SDS (0.05%), BSA (0.2 mg/ml) and BSA/SDS mixture (0.2 mg/ml and 0.05%, respectively).

Dye	In buffer			In SDS presence			In BSA presence			In BSA/SDS presence		
	λex, nm	λem, nm	I, a.u.	λex, nm	λem, nm	I, a.u.	λex, nm	λem, nm	I, a.u.	λex, nm	λem, nm	I, a.u.
5a	472	549	0.6									
				504	586	3				507	588	145
							585	626	537	578	629	81
6a	479	616	1.2	490	595	23.7	495	580	171	505	587	770
5b	546	594	4.4	544	599	9.6	578	614	2082	576	620	174
6b	451	543	2.5									
				505	573	27.4	507	569	675	506	569	1273
	549	593	1.7									
5c	581	630	0.75	560	615	1.6	596	606	19	606	618	34
6c	545	594	1.5	537	595	25	550	594	36	540	591	414
2a	585	712	3.7	574	586	11.5	576	590	138	572	588	192
3a	453	525	41				462	520	1173			
	496	588	19.5									
				540	564	100.5	523	555	545	540	562	3659
2b	413	462	1.6	400	458	6	432	524	2.3	405	467	4
							574	606	67	540	680	2
3b	418	552	5.6	435	551	8	429	533	225			
	520	560	0.6	520	588	9.2				511	578	45
2c							402	480	14.4			
	517	539	1.1	515	550	8.4	518	552	4	471	537	61.1
3c	404	493	6				407	481	35	408	510	40
	470	522	0.8	472	505	24.7	464	514	4	471	516	148
2d	470	556	0.4				470	552	15			
	564	610	0.2	560	607	1.1	574	614	3	603	611	13
3d	464	548	6	523	591	8.7	471	552	377			
	547	586	0.8	576	596	13.5				558	589	265

λex (λem) – maximum wavelength of fluorescence excitation (emission) spectrum, I – emission intensity, a.u. – arbitrary units.

brightest emission in BSA complex were observed respectively for the unsubstituted dyes **3d** (about 63 times) and **3a** (1173 a.u.).

It is shown that for studied chalcone dyes pair (i.e. boron difluoride complexes dye and non-substituted one) emission intensity in BSA presence is higher for non-substituted compounds, the exception being **5a**–**6a**, and **5b**–**6b** dyes pairs. The highest emission increasing and fluorescence intensity in the BSA presence are observed for the hydroxyquinoline dyes **5a**, **5b**, **6b** and coumarine dyes **3a**, **3d**. These dyes will be further studied for their sensitivity to various proteins.

3.3. Characterization of fluorescent properties of chalcone dyes in presence of SDS

Characteristics of excitation and emission spectra of studied polymethine dyes in presence of SDS are given in Table 1. Despite the used concentration of SDS (0.05%) was lower than its critical micelle concentration (0.24%), spectral changes were observed for all studied dyes upon addition of SDS. Thus from small to medium increasing of emission (up to 31 times) was recorded for the polymethines, but because of low intrinsic fluorescence of studied dyes, the fluorescence intensity of chalcones in SDS presence is quite insignificant. In spectra of hydroxyquinoline dyes **5a**, **6b** and coumarine dyes **2a**, **3a** and **3d** in the presence of SDS the band appeared which was not observed in spectra of free dyes in buffer. For other dyes shift of excitation and emission bands on up to 28 nm occurred upon addition of SDS. For the coumarine dyes **3b** and **3d** having two bands in intrinsic excitation and emission spectra, considerable redistribution of the bands intensities was noted in the presence of SDS.

3.4. Characterization of fluorescent properties of chalcone dyes in the presence of BSA/SDS mixture

Fluorescent properties of chalcone dyes and their boron difluoride complexes in presence of BSA/SDS mixture are given in Table 1. For majority of studied hydroxyquinoline dyes in the presence of BSA/SDS mixture in excitation and emission spectra single band is observed, while in corresponding spectra of the dye **5a** two bands exist. Fluorescence excitation maxima for the hydroxyquinoline dyes are located between 505 and 606 nm, emission maxima for these dyes being situated between 569 and 629 nm. Only for the dye **6c** positions of excitation/emission maxima are similar to these in buffer. For all the dyes positions of the bands in excitation/emission spectra in the presence of BSA/SDS mixture are quite close to these in presence of BSA (only in the case of **5a** additional band appears). Stokes shifts values for all hydroxyquinoline dyes in BSA/SDS complexes are between 12 and 82 nm. Fluorescence enhancement up to hundreds times upon addition of BSA/SDS mixture is observed for these dyes, fluorescence intensity reaching 1273 a.u. (Fig. 3) for the dye **6b** (quantum yield for **6b** in presence of BSA/SDS mixture is about 0.23). The noticeable fluorescence is also observed for dyes **6a** and **6c**.

Emission intensity of hydroxyquinolines in the presence of BSA/SDS mixture is higher comparing with "pure" SDS presence in 16–50 times. The excitation and emission bands of the dyes observed in BSA/SDS mixture are close to these recorded in SDS presence, except **5a** where additional band appears and **5b** where the 32 nm excitation and 21 nm emission spectra shifts took place. It was shown that emission intensity of unsubstituted hydroxyquinolines in the presence of BSA/SDS mixture is higher than for corresponding boron difluoride complexes analogues.

In spectra of coumarin dyes **2b** and **3c** in BSA/SDS mixture presence two bands in excitation and emission spectra are observed, while other coumarin dyes have single bands in corresponding spectra. Excitation maxima of coumarines are situated between 405 and 603 nm, maxima of fluorescence emission spectra being located in the range 467–680 nm. For the dyes **2a**, **3a**, **3c**, and **2d** in BSA/SDS mixture presence in emission spectra the mostly pronounced bands have similar positions to those in BSA presence. Stokes shifts values for coumarines are between 8 and 102 nm. The lowest increasing of the dye fluorescence upon

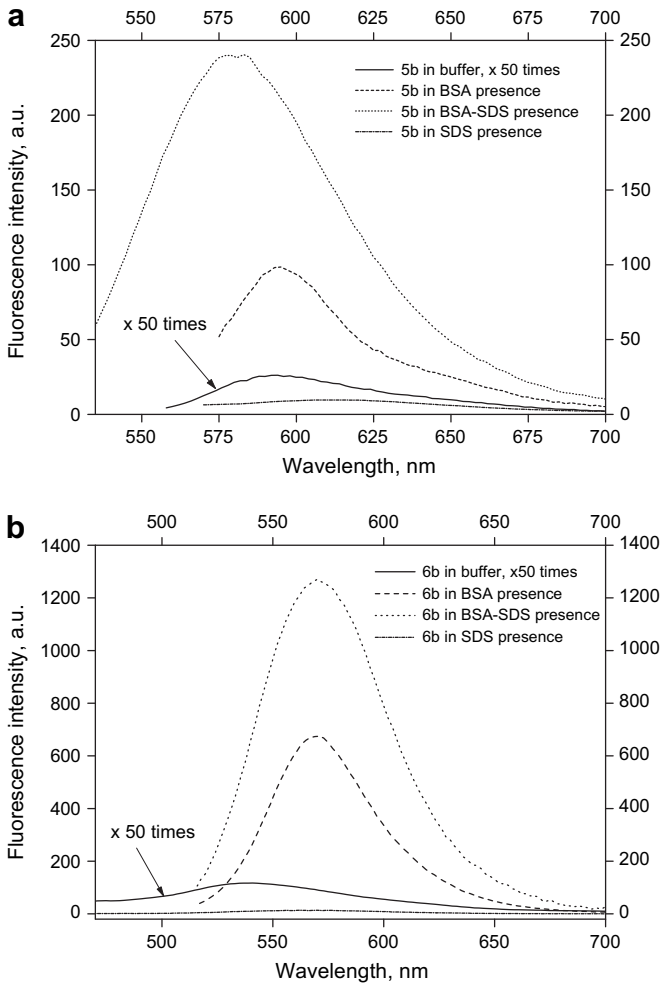


Fig. 3. Emission spectra of hydroxyquinoline dyes **5b** (a) and **6b** (b) (5×10^{-6} M) solutions in buffer, as well as in the presence of SDS (0.05%), BSA (0.2 mg/ml) and BSA/SDS mixture (0.2 mg/ml and 0.05%, respectively).

BSA/SDS addition was observed for the dye **2b** (in 2.5 times). The highest fluorescence enhancement was observed for the dye **3d** (in 330 times), while the brightest complex with BSA/SDS was formed by the dye **3a**, its intensity reaching 3659 a.u. and quantum yield being about 0.27. Despite of noticeable emission enhancement value, fluorescence intensity of other coumarin dyes in presence of BSA/SDS mixture could be characterized as either low or moderate.

Emission intensities of coumarine dyes (except **2b**) in complexes with BSA/SDS increased comparing with that in detergent presence in 6–35 times. In the excitation/emission spectra of the dyes **3b** and **3d** in SDS presence two bands were observed, while in complexes with BSA/SDS mixture single bands were recorded. For the dyes **2b** and **3c**, contrarily, in the presence of BSA/SDS new maxima appear which were absent in the spectra of dyes in SDS presence. Positions of spectral bands for the dyes **2a** and **3a** in complexes with BSA/SDS mixture and in presence of SDS were the similar.

Two of chalcone dyes, namely hydroxyquinoline **6b** and coumarine **3a** in BSA/SDS presence demonstrate bright emission and high emission increasing value, and also these dyes have rather high value of quantum yield and thus could be proposed for using as probes for nonspecific detection of proteins.

4. Conclusions

A series of novel chalcones derived from the boron difluoride complex of 3-acetyl-4-hydroxy-1-methyl-2-quinolone was synthesized. The 2-quinolones, together with the previously reported coumarin analogues, were studied in the presence of SDS, native BSA and BSA/SDS mixture using fluorescence spectroscopy. The dyes displayed low intrinsic fluorescence intensity. The emission intensity of the non-substituted chalcones in buffer, as well as in the presence of SDS, BSA and BSA/SDS mixture was generally higher than that of the corresponding boron difluoride complex. The interaction of the dyes with BSA and BSA/SDS mixture was accompanied by changes in excitation/emission spectra. The addition of SDS enhanced fluorescence intensity by 30 times. The fluorescence response of the dyes in the presence of both BSA or BSA/SDS mixture was more pronounced and the emission enhancement was several hundred times.

The hydroxyquinoline dye **5b** gave strong emission enhancement (470 times) and provided the brightest complex with native BSA. In the cases of the hydroxyquinoline **6b** and coumarin **3a**, very high fluorescence intensity in the presence of BSA and BSA/SDS mixture (quantum yields reaches 0.23 and 0.27 correspondingly) together with noticeable emission enhancement (one hundreds times) were observed. Thus the presence of diethylaminophenyl and dimethyl indolenyl increased the affinity of the chalcones to both native and denaturized proteins. Dyes **5b**, **6b** and **3a** are proposed for further study as fluorescent probes for protein detection.

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