

Bichromophoric fluorescent dyes with rigid molecular structure: fluorescence ability regulation by the photoinduced intramolecular electron transfer

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Abstract

Bichromophoric 3-diethylamino-7-phenyl-7H-benzo-[4,5]-imidazo-[1,2-c]-chromeno-[3,2-e]-pyrimidines were synthesized by the condensation of 7-diethylamino-3-(2-benzimidazolyl)-iminochromene with several aromatic aldehydes. Fluorescence properties of these compounds were studied in solvents of various polarity and acidity. The excited state intramolecular electron transfer was shown as the reason for the fluorescence quenching of the studied dyes with electron donor substituents in the minor chromophoric moiety. Prospects for the usage of these compounds as fluorescence probes sensitive to polarity and pH of their surrounding were elucidated.

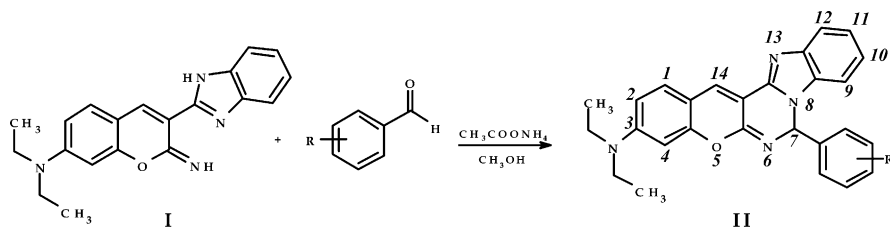
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1. Introduction

Coumarin derivatives are known by their high fluorescence ability, they are widely used as optical whitening agents [1], luminescent components of painting compositions for synthetic fibers [2], dye lasers [3], collectors of the solar energy [4], etc. During the last decade they were utilized also as fluorescent probes in biology and medicine [5]. Owing to the sensitivity of their spectral properties to the acidity of the media, coumarins were recommended as fluorescent pH indicators [6,7] and fluorescent sensors for pH estimation in biological objects on the micro-level [8].

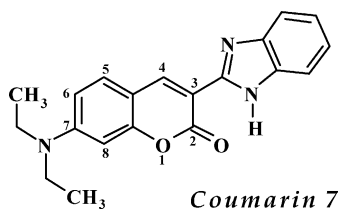
To be a good sensor, fluorescent compound must demonstrate dramatic changes in emission with changing of the micro-characteristics of its surrounding: pH, polarity, viscosity, etc. From this point of view, the products of condensation of 7-diethylamino-3-(2-benzimidazolyl)-iminochromene with aromatic aldehydes [9] (see the above scheme) are of doubtless interest. The rigid molecular structure is typical to the compounds of this group owing to the spatial fixation of benzimidazolic bicycle and C=N bond of the iminochromene moiety by the substituted methylene bridge. By the nature of their main chromophoric



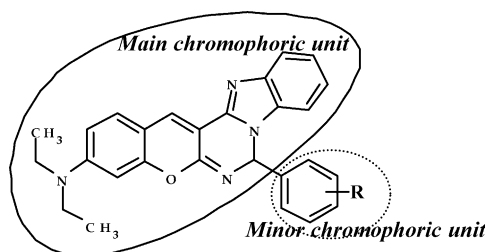
R = H (a), *p*-OCH₃ (b), *p*-OH (c), *p*-N(CH₃)₂ (d), *p*-N(C₂H₅)₂ (e),
p-Br (f), *p*-Cl (g), *o*-OCH₃ (h), *o*-OH (i), *p*-N(C₆H₅)₂ (j)

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unit, these compounds are the close analogs of the well-known dye of coumarin series—*Coumarin 7* ($\varphi_f \sim 0.87$ [10]).



Substituents in the side phenyl ring of compounds **IIa–j** (**R** on the following scheme) have low influence onto the electronic absorption spectra: absorption bands of the minor chromophoric unit should be observed in the short-wavelength region, however they are overlapped by the intensive bands of the main chromophore, therefore their correct identification was not possible generally.



In comparison with the starting iminochromene **I**, having undistinguished fluorescence efficiency ($\varphi_f \sim 0.16$ in acetonitrile and $\varphi_f \sim 0.34$ in 50% aqueous ethanol [11]), the quantum yields of the most of the synthesized dyes are quite close to 1.0 [9]. This fact reflects the decrease in radiationless losses owing to the significant reduce in probability of high-amplitude intramolecular vibrations. Formation of non-emissive TICT states, which is traditionally assumed by many authors as the reason for decreasing the fluorescence

ability of acceptor-substituted dialkylamino-derivatives [12], seems to be not important for the investigated group of compounds.

Fluorescence properties of compound **IIId** differs dramatically from those of the other molecules of the studied series: its fluorescence quantum yield in acetonitrile did not exceeded 0.1 [9]. Elucidation of the most probable reasons of such behavior is the main aim of the present communication. Also we will analyze the possibility of the usage of this effect in the monitoring of the physico-chemical parameters of the dye closest surrounding, and thus, the prospects of this compound to be a fluorescent probe. The diethyl and diphenyl- analogs of **IIId** (compounds **IIe** and **IIj**) were synthesized and studied for this purpose as well.

2. Experimental details

Compound **I** was synthesized according to [11]. Its derivatives **IIa–IIj** were obtained by the following general method.

2.1. Condensation of **I** with aromatic aldehydes

Substituted benzaldehyde (0.01 mol) was added to suspension of 3.32 g (0.01 mol) of 7-diethylamino-3-(2-benzimidazolyl)-iminocoumarin (**I**) and 2.31 g (0.03 mol) of dry ammonium acetate in 20 ml of absolute methyl alcohol. The reaction mixture was boiled during 1 h, cooled and the formed precipitate was filtered, washed by methyl alcohol, dried and recrystallized from the appropriate solvent (see Table 1).

The main physico-chemical parameters of the synthesized compounds are presented in Table 1.

Table 1
Physico-chemical parameters of the synthesized compounds **IIa–IIj**

Compound	Yield (%)	Melting point, °C (recrystallized from)	¹ H NMR (DMSO-d ₆), δ, ppm
IIa	61	256 (benzene)	8.11 (s, 1 H, H(14)); 7.63–6.58 (m, 12 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 1.18 (t, 6 H, 2 CH ₃)
IIb	85	251 (DMF)	8.11 (s, 1 H, H(14)); 7.62–6.57 (m, 12 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 1.10 (t, 6 H, 2 CH ₃) 3.70 (s, 1 H, OCH ₃)
IIc	64	276 (ethyl alcohol)	8.10 (s, 1 H, H(14)); 7.61–6.58 (m, 12 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 1.18 (t, 6 H, 2 CH ₃) 9.48 (s, 1 H, OH)
IIId	70	264 (ethyl acetate)	8.10 (s, 1 H, H(14)); 7.60–6.58 (m, 12 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 1.12 (t, 6 H, 2 CH ₃); 2.80 (s, 6 H, 2 CH ₃)
IIe	71	246 (toluene)	8.09 (s, 1 H, H(14)); 7.62–6.56 (m, 11 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 3.35 (q, 4 H, 2 CH ₂); 1.15 (t, 6 H, 2 CH ₃); 1.05 (t, 6 H, 2 CH ₃)
IIIf	60	263 (benzene)	8.14 (s, 1 H, H(14)); 7.64–6.59 (m, 11 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 1.15 (t, 6 H, 2 CH ₃)
IIg	55	253 (benzene)	8.14 (s, 1 H, H(14)); 7.64–6.59 (m, 11 H, arom.); 6.45 (s, 1 H, H(7)); 3.45 (q, 4 H, 2 CH ₂); 1.15 (t, 6 H, 2 CH ₃)
IIh	63	263, decomp. (DMF)	8.09 (s, 1 H, H(14)); 7.60–6.55 (m, 12 H, arom.); 3.40 (q, 4 H, 2 CH ₂); 1.10 (t, 6 H, 2 CH ₃); 3.65 (s, 1 H, OCH ₃)
IIi	60	244 (benzene)	8.10 (s, 1 H, H(14)); 7.61–6.55 (m, 12 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 1.15 (t, 6 H, 2 CH ₃); 9.74 (s, 1 H, OH)
IIj	80	252 (methyl alcohol)	8.10 (s, 1 H, H(14)); 7.64–6.40 (m, 21 H, arom.); 3.45 (q, 4 H, 2 CH ₂); 1.10 (t, 6 H, 2 CH ₃)

s, t, q, m denote correspondingly to singlet, triplet, quadruplet, and multiplet. Signal of H(7) could not be identified because of its overlap with aromatic multiplets at 6.5–7.7 ppm.

^1H NMR spectra were measured on Varian Mercury Vx spectrometer (200 MHz) in DMSO- d_6 with TMS as internal standard. Electronic absorption spectra were obtained on HITACHI U-3210, fluorescence spectra—on HITACHI F-4010 spectrometers. Fluorescence quantum yields were evaluated with respect to 3-(2-benzothiazolyl)-7-diethylamino-coumarin (Coumarin-6) in ethyl alcohol ($\varphi_f = 0.89$ [10]) taking into account the difference in the refraction indices of the measured and reference solutions [13]. All the fluorimetric measurements were conducted at concentrations of the dyes, at which the excitation wavelength absorbancies were less or equal to 0.15.

Protolytic equilibrium constants in water–ethyl alcohol media (50 vol.%) were detected by spectrophotometric method with the use of standard H_2O – $\text{C}_2\text{H}_5\text{OH}$ buffer solutions on the basis of hydrochloric, acetic, malonic, and boric acids and NaCl as background electrolyte at permanent ionic force 0.05 mol/l. The pH measurements were conducted in the cell with glass and AgCl reference electrodes; the electric compensation scheme included potentiometer P-363-2 and pH-meter pH 121. Usage of water–ethyl alcohol KCl solution (0.85 mol/l) in the reference electrode half-unit allowed to increase the stability of the measurements and also to extend the available pH range for the acidic solutions down to 0.6 pH units. Calibration procedures for the above described potentiometric cell were presented in details in [11]. Activity coefficients for the charged particles were calculated by the Davis equation [14].

Fluorescence decay curves were measured on the fluorometer described in [15] and treated by the standard least-squares method [16].

Quantum–chemical calculations were conducted by PM3 method [17,18], incorporated in MOPAC 2002 [19] program package.

3. Results and discussion

3.1. Fluorescence properties of **IIa–j**

The main spectral data for the synthesized compounds are presented in the Table 2. It must be emphasized, that

Table 2
Absorption and emission parameters of compounds **IIa–IIj** in acetonitrile

Compound	$\tilde{\nu}_{\text{max}}^{\text{abs}}$ (cm^{-1})	$\lambda_{\text{max}}^{\text{abs}}$ (nm)	ε ($1 \text{ mol}^{-1} \text{ cm}^{-1}$)	$\tilde{\nu}_{\text{max}}^{\text{fl}}$ (cm^{-1})	$\lambda_{\text{max}}^{\text{fl}}$ (nm)	$\Delta\tilde{\nu}_{\text{ST}}^{\text{fl}}$ (cm^{-1})	φ
IIa	22,960	436	51,200	20,080	498	2880	0.98
IIb	22,940	436	50,100	20,060	499	2880	0.96
IIc	22,900	437	51,200	20,080	498	2820	0.99
IIId	22,960	436	50,500	20,040	499	2920	0.10
IIe	22,900	437	51,500	20,060	499	2280	0.03
IIIf	22,900	437	53,500	20,000	500	2900	0.99
IIg	22,900	437	–	20,040	499	2260	0.97
IIh	23,080	433	47,600	20,120	497	2960	0.98
IIi	23,040	434	44,900	20,040	499	3000	0.97
IIj	22,920	436	54,300	20,040	499	2880	0.90

Here ν_{max} , λ_{max} , positions of the absorption and emission maxima; ε , molar extinction at long-wavelength absorption maxima; $\Delta\nu_{\text{ST}}$, Stokes shift; φ , fluorescence quantum yield. Extinction data for **IIg** is not available owing to its low solubility in acetonitrile.

Table 3
Fluorescence quantum yields of **IIb** and **IIId** in solvents of various polarity

Solvent	E_{T}^{N} (30)	φ	
		IIb (p' -OCH ₃)	IIId (p' -N(CH ₃) ₂)
Ethyl alcohol	0.654	0.99	0.13
Acetonitrile	0.460	0.96	0.10
Acetone	0.355	–	0.39
Ethyl acetate	0.228	0.99	0.99
Toluene	0.099	0.87	0.87

fluorescence quantum yields of **IIId** and **IIe** in solvents of low polarity (for example, toluene) are close to the values of the other representatives of the synthesized series (0.9–1.0).

The clear tendency in the decreasing of the fluorescence quantum yield in polar media was observed for the side-chain dimethylamino-substituted compound **IIId**, for example, in comparison with another donor-containing molecule of the studied series, **IIb** (Table 3).

The fluorescence lifetime of **IIId**, which is close to 2 ns in toluene and ethyl acetate, decreases down to 0.86 ns in acetonitrile and to 0.66 ns in ethyl alcohol. At the same time, fluorescence decay of **IIa** (R=H) and **IIb** (R=OCH₃) is insensitive to the nature of solvent and vary from 2.1 to 2.7 ns, increasing slightly in polar media. The described facts allowed us to propose the dynamic mechanism for the solvent polarity-induced fluorescence quenching of **IIId**.

Generally, our results display the absence of any correlation between the discussed fluorescence quenching and solvent ability to specific interactions (for example, formation of hydrogen bonds in alcohol solutions). Thus, we concluded this quenching has rather intramolecular than intermolecular nature.

Taking into account the bichromophoric character of **IIId** and **IIe**, the absence of any conjugation between the π -electron subsystems of these molecules, we assume the intramolecular electron transfer [20,21] from the dialkylamino-phenyl to 3-benzimidazolyl-iminochromene moieties as a possible reason of the discussed quenching.

The preliminary attempt to estimate the principal possibility of electron transfer to the excited molecule of

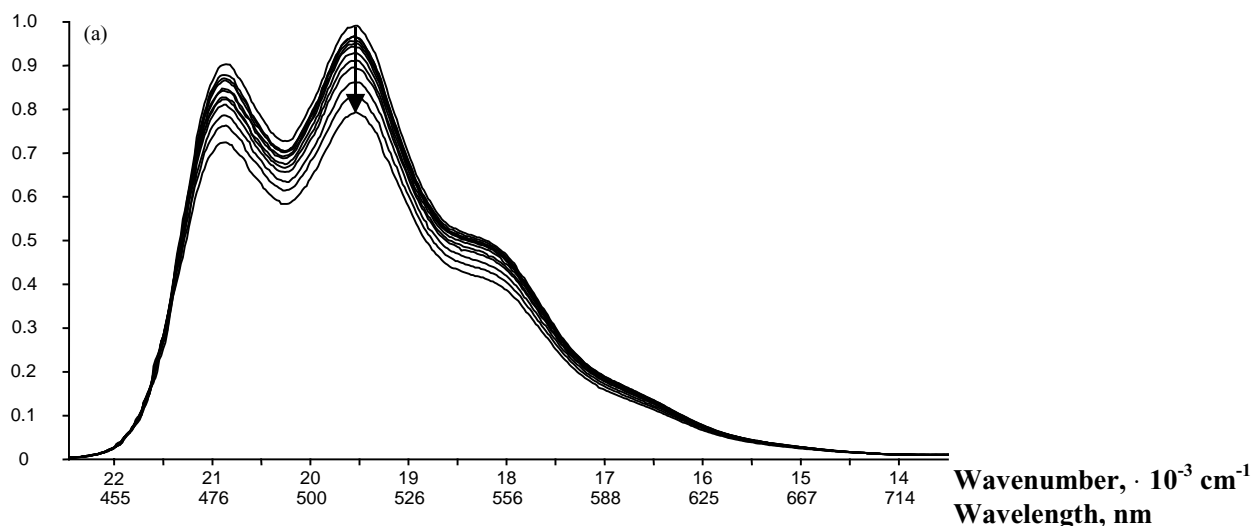
3-diethylamino-7-phenyl-7H-benzo[4,5]imidazo[1,2-c]chromeno[3,2-e]pyrimidine (**IIa**) from the ground-state dimethylaniline molecule was conducted by the model calculations with semiempirical method PM3. The ionization potential (IP) of dimethylaniline was estimated as 7.7 eV, while as the electron affinity (EA) of **IIa**, evaluated as the difference in heat of formation values for the basic molecule and its anion–radical, was found near 1.6 eV in the ground state and 5.3 eV in the excited state. These results evidenced, that in spite of the significant increase in EA of the excited **IIa** molecule, the electron transfer from the unexcited dialkylaniline is still not energetically favorable in the media of low dielectric constant (vacuum, for which these calculations were made): EA (**IIa**, S_1) < IP (*N,N*-dimethylaniline,

DMA, S_0). Our calculations clarify the absence of the emission quenching of **IId–e** in non-polar surrounding. At the same time, polar solvents stabilize the ionic structures formed as a result of the electron transfer and thus they must increase the probability of this process [22].

3.2. Intermolecular fluorescence quenching experiments

For the experimental confirmation of these theoretical findings we studied the intermolecular fluorescence quenching of **IIa** by in two solvents, significantly differ by their polarity—toluene and methyl alcohol (Fig. 1). Both cases demonstrated the decrease of emission intensity with dimethylaniline content in solution, however, the efficiency

Fluorescence intensity, arb.units



Fluorescence intensity, arb.units

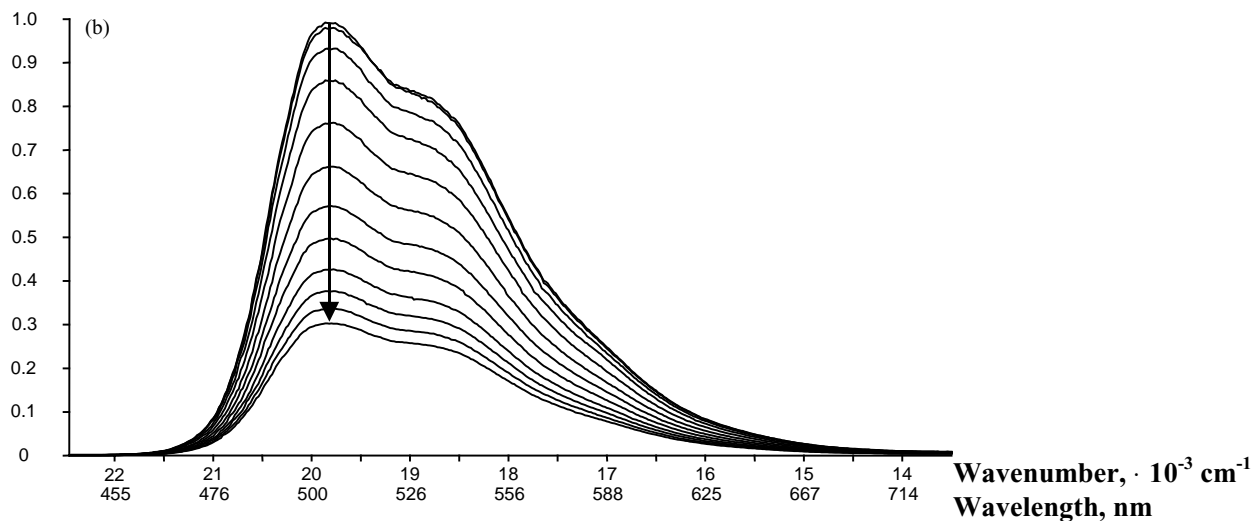


Fig. 1. Fluorescence spectra of **IIa** in (a) toluene and (b) methyl alcohol with the increasing concentration of *N,N*-dimethylaniline: a, 0–0.3 mol/l; b, 0–0.2 mol/l (corrected on the excitation wavelength absorption changing due to the dilution at adding of the quencher).

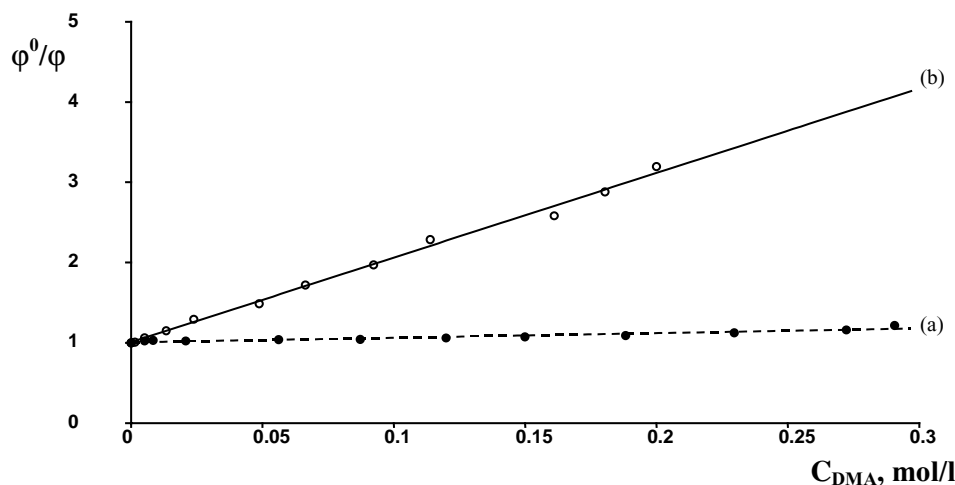


Fig. 2. Stern–Vollmer plots for the fluorescence quenching of **IIa** by dimethylaniline in (a) toluene and (b) methyl alcohol.

of the observed quenching was significantly different for the studied solvents.

The most evident mechanism of this quenching is the electron transfer from the DMA to the excited fluorescent dye. The bimolecular quenching constants, evaluated from the Stern–Vollmer plots (Fig. 2) were the following: $2.7 \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$ for toluene and $4.0 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$ for methyl alcohol. The last value is close to the diffusion-controlled rate constant in methanol, therefore we concluded that every collision of dimethylaniline molecule with the excited **IIa** molecule leads to the radiationless deactivation of the latter. The probability of radiationless deactivation in the corresponding collision complex is close to 1.0. More than that, the efficient fluorescence quenching should be typical also to the intramolecular analogs of the above described collision complex of **IIa** and dimethylaniline—the bichromophoric molecules with non-conjugated chromophoric units **IIc** and **IIe**.

The additional proof in favor of the proposed electron transfer quenching mechanism of **IIc** and **IIe** in polar surrounding was obtained on the example of compounds **IIc** and **IIi**, which contains the hydroxy-group in the minor chromophoric unit of their molecules. In alkaline media at pH 10–11 the dissociation of OH-group with the formation of electron rich phenolate moiety takes place. It is characterized by the low ionization potential and therefore quenches the fluorescence emission of **IIc** and **IIi** by the analogous mechanism, via the electron transfer between the non-conjugated main and minor chromophoric moieties of these molecules.

3.3. Media polarity sensing with **IIc** and **IIe**

The above discussed changing of the emission properties could be utilized in the monitoring of the media polarity [23] with the usage of **IIc** and **IIe** as fluorescent probes. The fluorescence quenching of these compounds has dynamic char-

acter: it results in decreasing of both their fluorescence quantum yields and lifetimes. Thus, we consider the discussed compounds as prospective fluorescence probes, which lifetime could serve as indicator of the polarity of their surrounding.

The investigation of the emission properties of **IIc** (Table 4, Fig. 3) in the wide series of solvents of different polarity have shown this probe is sensitive exactly to the polarity of the media, while as various specific interactions are less important for this molecule. This thesis is supported by the better correspondence of the reversed lifetime of **IIc** and Lippert solvatochromic function ($L = ((\epsilon - 1)/(\epsilon + 1)) - ((n^2 - 1)/(n^2 + 1))$) [24], which takes into account only universal interactions, rather than empirical Reichardt polarity parameter $E_T^N(30)$ [25], which includes both specific and universal interactions between

Table 4
Fluorescence lifetimes of **IIc** in solvents of different polarity

Solvent	ϵ	n	L	$E_T^N(30)$	τ (ns)
Octane	1.95	1.3974	−0.0003	0.012	2.37
Dioxane	2.21	1.4233	0.0202	0.164	2.40
Carbon tetrachloride	2.23	1.4601	0.0102	0.052	1.94
Toluene	2.38	1.4961	0.0135	0.099	2.13
Glycerol triacetate	6.0	1.4305	0.1792	0.299	2.54
Ethyl acetate	6.02	1.3723	0.1997	0.228	2.08
1,2-Dichlorobenzene	9.93	1.5515	0.1861	0.225	2.32
1,2-Dichloroethane	10.4	1.4448	0.2211	0.327	2.42
n-Butyl alcohol	17.1	1.3993	0.2625	0.586	1.49
Propanol-2	18.3	1.3747	0.2739	0.546	1.16
Ethylmethylketone	18.5	1.3788	0.2729	0.327	1.44
Acetone	20.7	1.3588	0.2843	0.355	1.09
Ethyl alcohol	24.3	1.3611	0.2886	0.654	0.66
DMF	36.7	1.4303	0.2744	0.386	0.39
Acetonitrile	37.4	1.3437	0.3055	0.460	0.86

ϵ , dielectric constants; n , refraction indices of the solvents; L , Lippert solvent polarity [24]; $E_T^N(30)$, Reichardt solvent polarity [25] functions; τ , fluorescence lifetime.

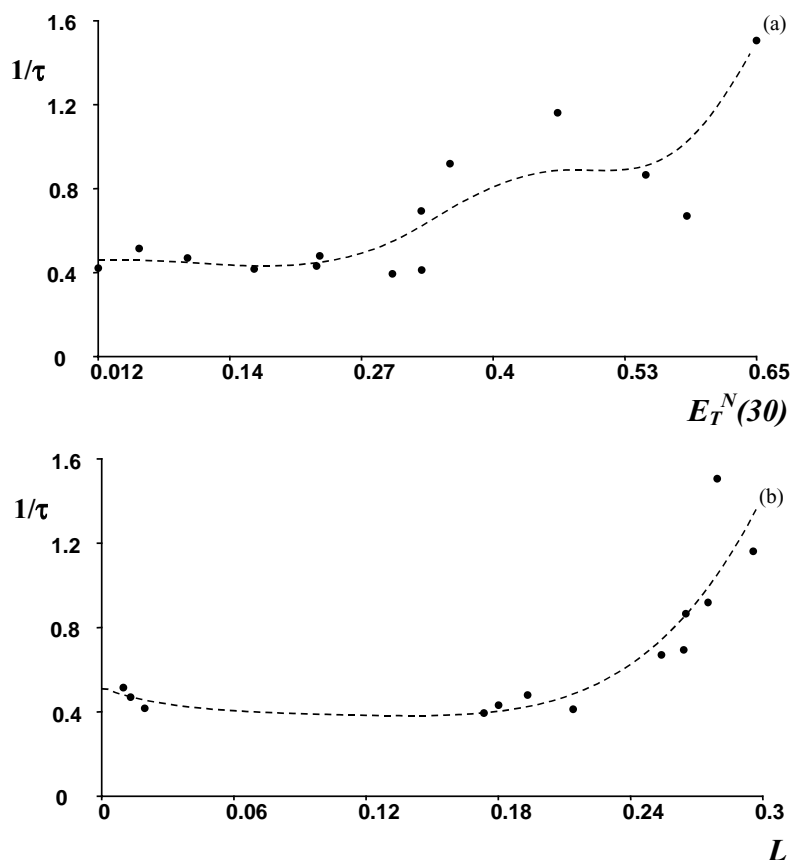


Fig. 3. Plots of the reversed fluorescence lifetimes of **II d** ($1/\tau$, ns^{-1}) in different solvents vs. the solvent polarity functions of (a) Reichardt and (b) Lippert. Data were interpolated by the smoothing spline functions (dash lines).

the solvent and solute (Fig. 3). Completing the discussion of the spectral properties of bichromophoric derivatives of **II**, we did like to note, that these series of compounds allow to shift the sensitivity range to media polarity by the directed modification of their molecular structure. The most prospects have changing of the nature of the alkyl/aryl substituents at nitrogen atoms in the main (in position 7 of the iminocoumarinic unit) and minor chromophoric moieties. This would allow to vary the donor ionization potential and the acceptor electron affinity in wide

ranges and thus, to develop a series of new probes with the “emission jump” in the pre-defined ranges of solvent polarity.

3.4. pH sensing

The potential sensitivity of several derivatives of **II** not only to polar, but also to the acid–base properties of the media owing to the participation of substituents in the main and in the minor chromophoric units of their molecules in

Table 5
Spectral and protolytic properties of compounds **II a–e, i** in 50% aqueous ethyl alcohol

Compound	<i>R</i>	Uncharged base				Monoprotonated cationic form		
		$\tilde{\nu}_{\text{max}}^{\text{abs}}$	ϵ	$\tilde{\nu}_{\text{max}}^{\text{fl}}$	φ	$\lambda_{\text{max}}^{\text{abs}}$	ϵ	$\text{p}K_{\text{a}}$
II a	H	21,220	51,900	19,680	0.92	19,720	72,900	3.36 ± 0.01
II b	<i>n</i> -OCH ₃	21,220	48,800	19,660	0.96	19,700	71,700	3.31 ± 0.01
II c	<i>n</i> -OH	21,240	49,700	19,560	0.97	19,700	73,200	3.46 ± 0.01
II d	<i>n</i> -N(CH ₃) ₂	21,180	49,100	19,640	0.04	19,660	72,400	3.42 ± 0.02
II e	<i>n</i> -N(C ₂ H ₅) ₂	21,180	49,500	19,660	0.03	19,700	75,300	2.69 ± 0.02
II i	<i>o</i> -OH	22,420	44,900	19,700	0.90	19,960	63,200	3.78 ± 0.01

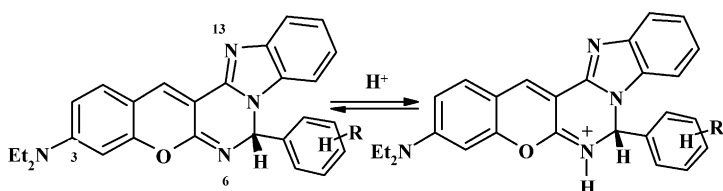
R is the substituent in the minor chromophoric moiety; ν_{max} , correspondent positions of the long-wavelength absorption and emission maxima; ϵ , extinction coefficient; $\text{p}K_{\text{a}}$, ionization constant.

protolytic interactions, impels us to study their spectral behavior in the wide pH range. The 50% aqueous ethyl alcohol was chosen as a media for this investigations, because it allows to control pH potentiometrically and most of the studied compounds have quite satisfactory solubility in this mixed solvent system.

The spectrophotometric titration of compounds **IIa–j** in 50% aqueous ethyl alcohol displays the existence of only one protolytic equilibrium in pH interval from 1 to 9 units (Table 5). OH groups dissociation of the hydroxy-compounds **IIc** and **IIi** has practically no influence onto their absorption spectra and manifests itself only in decrease of their emission ability in highly alkaline media (see below).

All the studied compounds have several potential protonation centers, thus our first task was the elucidation of the main reactive group in protolytic interactions of **IIa–e, i**. With this purpose, we made a series of quantum–chemical calculations of **IIa, II d**, and **IIe** molecules in the semiempirical PM3 scheme with the purpose to analyze the electron density distribution on their potential protonation centers (nitrogen atoms). For the first calculations in vacuo the minor chromophoric group nitrogen atom (compounds **II d** and **IIe**) was the most negatively charged. It followed by the nitrogen atom of diethylaminogroup in the iminochromenic unit (position 3) and the least negatively charged were the nitrogen atoms in positions 6 and 13. The media polarity effects were not taken into account in such calculations, therefore they were repeated in the dielectric continuum model (COSMO [26]) with $\epsilon = 37$ (arbitrary modeling the aqueous ethyl alcohol surrounding). In this more polar media the most negative charge was localized on the nitrogen atom in position 6, a little bit less—on the minor chromophore dialkylamino groups, the iminochromenic dialkylamino nitrogen and nitrogen atom in position 13 were the last in this sequence (Table 6). Surely, such calculations could not reflect all the peculiarities of the protolytic interactions in the real solutions, however, they are able to display definite tendencies in the changing of the electron density redistribution under the influence of the polar solvent.

In accordance with the experimental data [11] and our calculations we consider the following equilibrium as the first step in the protolytic interactions of **IIa–e, i** in polar surrounding.



The protolytic behavior of compounds **IIc** and **IIi** seemed to us somewhat unusual. It was logical to expect for them in the alkaline media the products for their hydroxy-groups

dissociation [11,27]. Probably, changes in their absorption spectra caused by dissociation of OH-groups in the minor chromophoric moiety are hidden by the intensive absorption of the main chromophore. Another possible reason for the absence of changes in the absorption spectra is the bichromophoric nature of the discussed compounds and low interaction between their chromophoric units. That is why, the ionization of the minor chromophore reactive groups could not be trustworthy detected by the spectrophotometric methods. At the same time, the intramolecular fluorescence quenching at OH-group dissociation makes it possible to detect this process by the change in fluorescence intensity versus pH.

Fluorimetric titration of compound **IIc** (pH 8–12) and **IIi** (pH 6–12) in 50% aqueous ethyl alcohol displays the decrease of its emission without changing of the shape of the spectra and fluorescence lifetime. The dissociation constants calculated from the obtained classical sigmoid titration curves were the following: 10.53 ± 0.01 (**IIc**) and 10.73 ± 0.01 (**IIi**).

3.5. Different mechanisms of **II d** and **IIe** protolytic interactions in the acidic media

The polarity-induced fluorescence quenching, discussed above for compounds **II d** and **IIe** allows us to assume their spectral properties are affected by pH as well. The dialkylamino groups in the minor chromophoric unit of these molecules act as the additional protolytic centers, which, according to our calculations (Table 6), are protonated nearly in the same pH range as the nitrogen atom in position 6.

The fluorescence spectra of these compounds, normalized to their total quantum yield, are presented in Fig. 4. Both band shape and fluorescence quantum yield of **II d** remained unchanged at decreasing pH from 11 to 5.6. Progressive increase of emission intensity (first arrow, Fig. 4a) and starting from pH 4.2—also the changes in the shape of the spectrum (second arrow) were observed down to pH 2–1.5, and then definite decrease in intensity took place (third arrow). At titration of **IIe** the quantum yield increasing was started at pH 6.6 (first arrow, Fig. 4b), while as changing in the shape of the fluorescence spectrum was detected only at pH 3.4 (second arrow), when the emission intensity reaches its maximal value. The final emission bands of **II d** and **IIe** were quite similar by their shape, this allowed us to suppose

they belong to the analogous products of protolytic interactions, possessing protonated nitrogen atom in position 6 and protonated dialkylamino group in the minor chromophoric

Table 6

Calculated charges on the potential centers of protolytic interactions at different polarity of the media (dielectric constant, ϵ)

Compound	R	ϵ	$q_{N(6)}$	$q_{N(13)}$	$q_{N(NEt2)}$	$q_{N(R)}$
IIa	H	1	-0.210	-0.132	<i>-0.247</i>	–
II d	<i>n</i> -N(CH ₃) ₂	1	-0.208	-0.133	<i>-0.246</i>	-0.259
II e	<i>n</i> -N(C ₂ H ₅) ₂	1	-0.207	-0.133	<i>-0.248</i>	-0.288
IIa	H	37	-0.325	-0.250	-0.262	–
II d	<i>n</i> -N(CH ₃) ₂	37	<i>-0.325</i>	-0.251	-0.262	-0.270
II e	<i>n</i> -N(C ₂ H ₅) ₂	37	<i>-0.324</i>	-0.251	-0.262	-0.280

See the scheme below for the atoms numbering. The most negative values are presented in italics.

unit. However, the reaction pathways from the starting to the final equilibrium forms were quite different in these two compounds.

Protolytic equilibria constants, calculated from the fluorimetric titration data at the assumption of the static mechanism of the observed two-step processes (“static” means ground-state protolytic interactions determine all the changes in fluorescence spectra), were the following: 3.53 ± 0.02 and 2.23 ± 0.04 (**II d**); 4.49 ± 0.02 and 2.54 ± 0.02 (**II e**). Comparing these values with the results of our spectrophotometric titration (Table 5), we can conclude, that despite the insignificant deviations in the numerical data, protolytic equilibria with pK 3.53 for **II d** and 2.54 for **II e** correspond to the analogous interactions, which were observed in the absorption spectra as well (protonation of

the nitrogen atom in position 6 of the main chromophoric moiety). The other two constants correspond to the protonation of dialkylamino groups in the minor chromophoric unit, which regulate the possibility of intramolecular electron transfer and the emission efficiency of compounds **II d** and **II e** in polar surrounding.

The sequence of the starting two stages of protolytic interactions in the acidic media is different for the discussed two molecules: nitrogen atom in position 6 was initially protonated for **II d**, then proton was attached to dialkylamino group in the minor chromophoric moiety. **II e** displays the opposite behavior, when the dialkylamino group in minor chromophore reacted first and the main chromophoric unit was protonated only at the next stage.

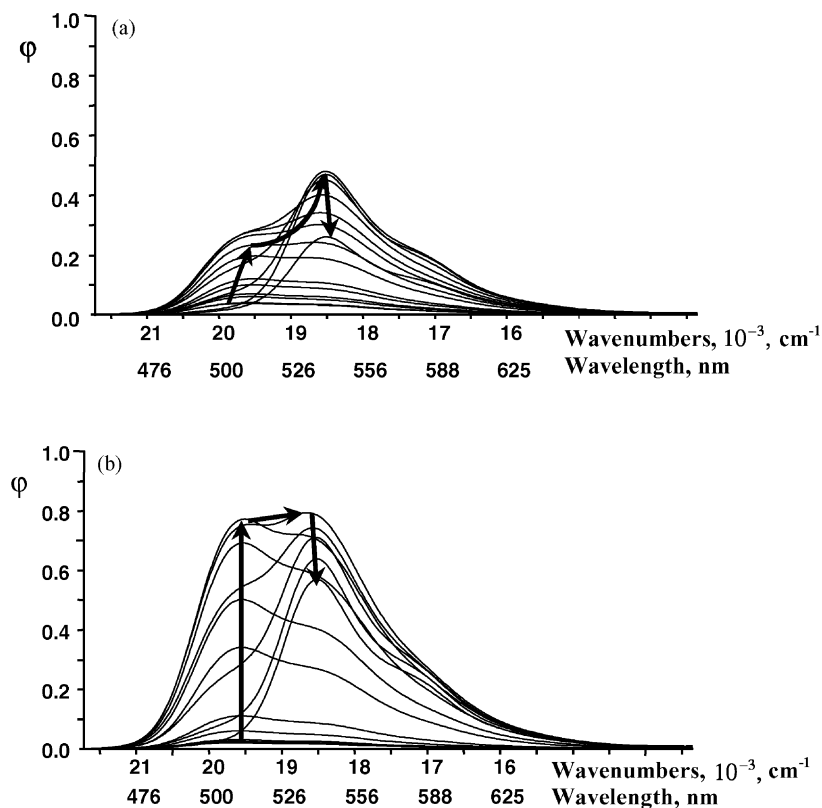
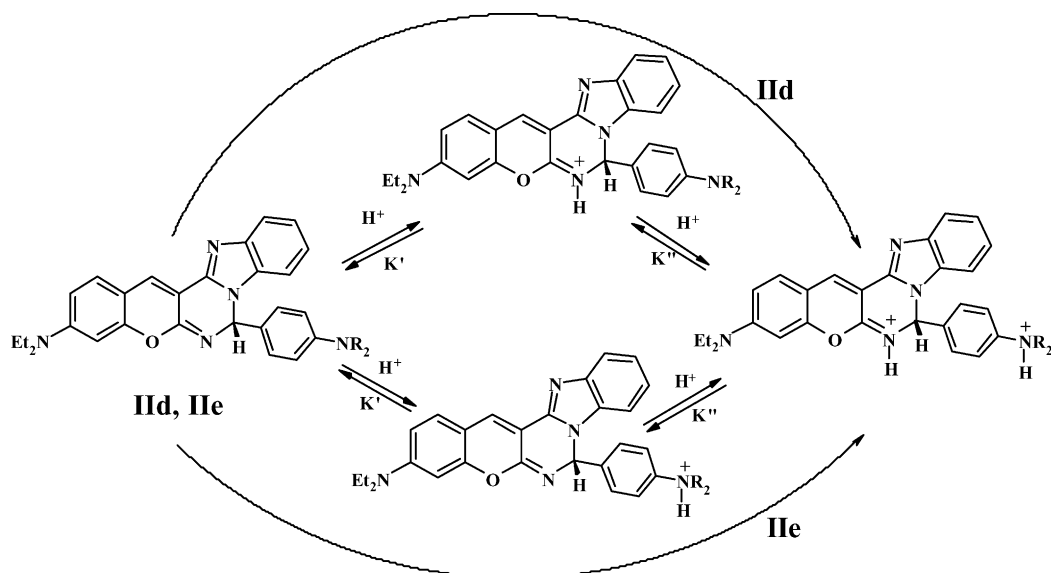


Fig. 4. Fluorimetric titration of compounds (a) **II d** and (b) **II e** in 50% aqueous ethyl alcohol, normalized to the total quantum yield. Changes in the emission spectra with the decrease of pH from 11 down to 1.5 units are shown by arrows.



4. Conclusion

The excited state intramolecular electron transfer was shown to be the reason for the fluorescence quenching of the bichromophoric derivatives with electron donor substituents in the minor chromophoric moiety—3-diethylamino-7-phenyl-7H-benzo-[4,5]-imidazo-[1,2-c]-chromeno-[3,2-e]-pyrimidines, synthesized by the condensation of 7-diethylamino-3-(2-benzimidazolyl)-iminochromene with aromatic aldehydes. Prospects for the usage of these compounds as fluorescence probes sensitive to polarity and pH of their surrounding were outlined.

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