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Spectral Study of Interactions of 4,8,4′-Trimethylpsoralen and 4,4′-Dimethylangelicin Dyes with DNA

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Abstract—Absorption and fluorescence spectra for six new synthetic dyes of 4,8,4′-trimethylpsoralen and 4,4′-dimethylangelicin derivatives containing various terminal substituents at 5′-position have been investigated in different environments using a wide range of the DNA/ligand concentrations. Various spectral and binding characteristics of the DNA–ligand systems have been determined. General principles characterizing mechanisms responsible for changes in the fluorescent properties of nucleotide-specific dyes have been proposed; they take into consideration chemical structure of the dyes, properties of the environment, and degree of sorption on substrate.

Key words: DNA, fluorescence dyes, spectral and binding properties

Artificial synthetic compounds exhibiting specific binding to certain nucleotide sequences in genomes are now attracting much attention. They may be used as radioprotector [1], antimicrobial [2, 3], antitumor [4-6] agents, etc. If such a nucleotide-binding compound besides high specificity and affinity to substrate also has properties convenient for monitoring (e.g. fluorescence which could change in intensity during interaction with polynucleotide), potential applicability of this dye is widely extended. In particular, such compounds are employed for studies of nucleic acid structure in enzyme kinetics [7-10]. This dyes may also be used for the development of highly sensitive and specific methods for rapid diagnostics of various diseases and toxic effects, based on analysis of pathological state, proceeding from change of quantity and structure of nucleic acids under different physiological states of an organism [11, 12].

All presently known compounds that reversibly (non-covalently) bind with polynucleotides can be divided mainly into two large classes by type of interaction with substrate: "polynucleotide external binding compounds" (non-intercalators) and intercalators. A non-intercalator molecule should contain at least one heteroatom; as well as for convenience of registration of spectral properties, the presence at this molecule of a well-developed system of coupled bonds is desirable. Non-intercalators bind to external parts of a polynucleotide molecule, do not alter integrity of the substrate structure (only some compression and local change of allosteric conformation of the nucleic acid double helix occur in the binding region), and are specific mainly to primary and secondary orders of nucleic acid structure [9, 13-16].

An intercalator molecule should include one or several fragments containing condensed aromatic system with two or more rings (preferentially of five or six members) with at least one or more (preferentially endocyclic) heteroatoms. Intercalators insert between complementary base pairs of polynucleotide double helix and are specific mainly to secondary and higher orders of nucleic acid structure [7, 17-20].

Many nucleotide-binding fluorophores are now widely used [7, 9, 16-18]. However, theoretical substantiation of synthetic pathways for construction of new compounds exhibiting higher sensitivity and specificity for a substrate still represents an important problem. Solution of this problem requires clear understanding of interrelation between chemical structure of nucleotide-specific dyes and their spectral and binding properties.

In the present study, we have investigated six newly synthesized compounds representing 4,8,4′-trimethyl-

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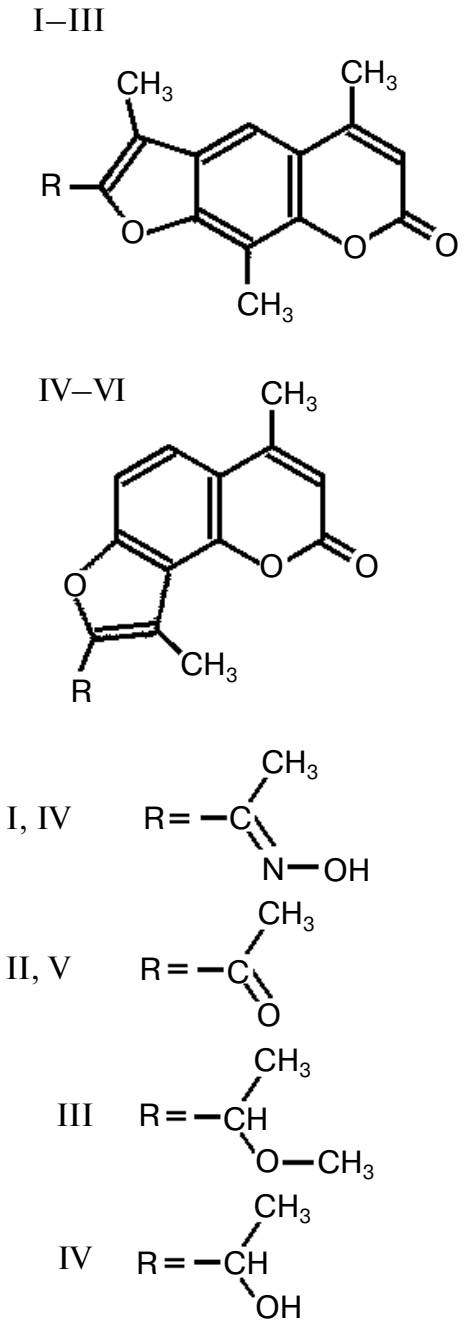
psoralen (I-III) and 4,4′-dimethylangelicin (IV-VI) derivatives containing various terminal substituents at the 5′-position (Scheme 1). Particular interest in such compounds is explained by active use of psoralens and angelicins in chemotherapy (e.g. of psoriasis [21, 22]), for blood disinfection [3], in studies of site-directed RNA modification [23], etc. Such wide applicability of these compounds is determined by that at first they reversibly intercalate into a double stranded polynucleotide (psoralens usually bind to AT-rich regions, whereas angelicin binding is not specific to primary structure of nucleic acids); and then after prolonged (up to 1 h) irradiation in the wavelength range 250-370 nm binding of these compounds to polynucleotide becomes covalently irreversible. Angelicins are less toxic than psoralens because they less effectively form cross-links between two strands of nucleic acids. This property can probably be attributed to shorter length of curved angelicin "core" compared with the length of linear psoralen "core".



Scheme 1

MATERIALS AND METHODS

Titration experiments were carried out at constant dye concentration (for absorption spectra $C_L = 4 \cdot 10^{-5}$ M, for fluorescence spectra $C_L = 4 \cdot 10^{-6}$ M) and various DNA concentrations (C_D) at 20-25°C. The following buffered solutions were employed:

1) 0.01 M NaCl + 0.01 M Na_2EDTA + 0.01 M Tris (pH 7.4);

2) buffer 1 + 4 M urea (pH 7.6);

3) 2 M NaCl + 0.1 M Na_2EDTA + 0.01 M Tris (pH 8.0).

The compositions of buffers 2 and 3 were determined by the well-known [13, 24, 25] fact that solutions with a high ionic strength attenuate electrostatic (especially ionic) bonds in the DNA–ligand system; the presence of 4 M urea at low ionic strength of solution attenuates hydrogen bonds between nucleic acid and dye; and 2 M NaCl or 4 M urea do not induce significant conformational rearrangements in the polynucleotide. The low value of C_L was selected, that first provide reliable dissolution of all compounds in aqueous medium, and second, rule out formation of dye aggregates in the system. The latter was proved by the linear dependence of fluorescence intensity and absorbance capacity on concentration of the studied compounds at constant shape of the spectrum.

The investigated values C_D/C_L were 3-100 mol/mol. To avoid dilution effects 0.02 ml aliquots containing buffer, certain dye concentration and DNA in concentration provided maximal C_D/C_L ratio used in this study were added to "nil" solution containing buffer with the same dye concentration. We also recorded spectra of compounds I-VI in the absence of DNA in aqueous buffers 1-3 and also in 2-propanol.

Compounds I-VI were synthesized and purified as described [26, 27]. Calf thymus DNA was used in the study. This was characterized by 58% AT-pairs, average molecular mass of one nucleotide of 326 daltons, and the absorbance coefficient $\varepsilon_{260} = 6600$ $M^{-1} \cdot cm^{-1}$. Dry preparation purchased from Serva (Germany) was initially dissolved in distilled water and then sonicated (for homogenization and reduction of light scattering) using a UZDN-2 disintegrator (Russia) for 15 sec at 0.3 A and 22 kHz. Commercially available Na_2EDTA (ethylenedi-

amine tetraacetate, disodium salt) and Tris (2-amino-2-hydroxymethyl-1,3-propane diol) were also purchased from Serva. Other chemicals were of chemically pure grade.

Absorbance spectra were registered using a Beckman model 35 spectrophotometer (Austria). Fluorescence was analyzed using a Hitachi model 850 spectrofluorimeter (Japan). Fluorescent spectra were recorded at monochromator slit for excitation and emission of 3 nm, using scan rate of 120 nm/min, response time of 2 sec, and normal photoelectric amplitude. Excitation and emission spectra were recorded at 455 and 330 nm, respectively. Fluorescent spectra were corrected following the spectrofluorimeter manual and using a quantum counter based on standard alcohol solution of rhodamine B. All measurements were carried out in standard 1 cm cuvettes.

Quantum yield of dye fluorescence (φ) was determined by comparing fluorescence of compounds studied with that of standard solution of quinine sulfate in 1 M sulfuric acid (φ = 0.55). All concentrations are given as molar concentrations. All fluorescence spectral characteristics and binding parameters were detected at the excitation wavelength of 330 nm.

Relative contribution of ionic (*E*) and hydrogen (*H*) bonding in formation of the first type DNA–ligand complex was determined using the method described in [28]:

$$E = (\Phi_2 - 1)/(\Phi_1 - 1)\cdot 100\%,$$

$$H = (\Phi_3 - 1)/(\Phi_1 - 1)\cdot 100\%, \qquad (1)$$

where Φ_1-Φ_3 are ratios of quantum yield of dye fluorescence at $C_D/C_L = 20$ and in the absence of DNA in buffers 1-3, respectively.

Binding parameters were determined by change in fluorescence intensity of solutions of compounds I-VI during their titration with DNA using the Scatchard model [16, 29]. To avoid the influence of effects that are not taken into consideration by this model and are observed at ligand excess compared with DNA (cooperative, statistic, etc. [16]), data for calculations have been taken at C_D/C_L values exceeding 1. The method of calculation is given below.

Initially we selected ranges of C_L/C_D values at which parameters *K* (adsorption constant representing the inverse value of free ligand concentration in the system at half saturation of potential binding sites) and *n* (value corresponding to maximally possible number of ligand molecules that can be bound to one DNA molecule per base pair) presumably remained constant (i.e. only one type of binding predominates). For compounds used in this study, we found three such ranges in buffer 1 and two ranges in buffers 2 and 3.

Within the selected range we determined concentration of bound ligand in solution ($C^b_{L,i}$) for the set of experimentally determined concentration ratios $(C_L/C_D)_i$ and corresponding values of maximal fluorescence intensity (I_i):

$$C^b_{L,i} = C_L\cdot(I_i - I_0)/(I_m - I_0). \qquad (2)$$

For the first (in reduced order of C_L/C_D value in the system) C_L/C_D range the fluorescence intensity of a dye in the absence of DNA was used as the I_0 value, whereas the I_m value was determined by extrapolation of the extreme linear part of the dependence of fluorescence intensity on the ratio of C_L/C_D to value $C_L/C_D = 0$ as described in [16]. For the second C_L/C_D range the I_m value was also determined by extrapolation of the dependence of fluorescence intensity of the DNA–dye system on the C_L/C_D ratio in this system to value $C_L/C_D = 0$ (this extrapolation also involved the linear part only). Under these conditions, if fluorescence intensity of the DNA–dye system increased with decrease in C_L/C_D value, the I_0 value was the same as in the first C_L/C_D range; and if fluorescence intensity decreased with the decrease in C_L/C_D, the I_0 value was determined as equal to I_m value of the previous C_L/C_D range. For the third C_L/C_D range, the values I_0 and I_m were determined as in the case of the second C_L/C_D range when fluorescence intensity decreased with the decrease in C_L/C_D (Fig. 1a).

Using the general Scatchard equation,

$$Y_i = K\,n - K\,X_i, \qquad (3)$$

where

$$X_i = r_i = C^b_{L,i}/C_{D,i},\ Y_i = r_i/m_i = X_i/(C_L - C^b_{L,i}), \qquad 4)$$

K and *n* values characterizing interactions of a particular compound with DNA (Fig. 1b) were found for each C_L/C_D range using the following formulas:

$$K = \frac{Q\,\Sigma(X_i\,Y_i) - \Sigma X_i\,\Sigma Y_i}{(\Sigma X_i)^2 - Q\,\Sigma X_i^2},\quad n = \frac{\Sigma Y_i + K\,\Sigma X_i}{Q\,K} \qquad (5)$$

(where *Q* is total number of approximated points in the selected range).

Accuracy of calculations was evaluated using relative *K* and *n* errors:

$$\varepsilon_K = 100\cdot\Delta K/K,\ \varepsilon_n = 100\cdot\Delta n/n, \qquad (6)$$

relative error of approximation (calculated by Eq. (3)):

$$\varepsilon_S = 100\cdot\Sigma(Y_{T,i} - Y_{E,i})/(Q\,Y_{E,i}), \qquad (7)$$

and the criterion characterizing adequacy of behavior of the DNA–dye system within a given C_D/C_L range described by the selected binding model:

$$F_p = \Sigma(Y_{\text{т},i})^2/(Q\,S_a^2) > F_\text{т}. \quad (8)$$

Here

$$\Delta K = t_\text{т}\,S_a \cdot [\Sigma X_i^2 - (\Sigma X_i)^2/Q],$$

$$\Delta n = t_\text{т} \cdot (S_a/K) \cdot [Q - (\Sigma X_i)^2/\Sigma X_i^2] + (\Delta K\, n)/K,$$

$$S_a = [\Sigma(Y_{\text{т},i} - Y_{\text{E},i})^2/(Q-2)]^{1/2}$$

(where $t_\text{т}$ is the table mean of Student's criterion corresponding to the 0.05 significance level and $Q-2$ number of freedom degrees, S_a is adequacy dispersion of Eq. (3), $Y_{\text{E},i}$ are experimental values of parameter Y calculated by formulas (4), $Y_{\text{т},i}$ are theoretical values of parameter Y calculated by Eq. (3), $F_\text{т}$ is the table mean of Fisher's criterion corresponding to the 0.05 significance level and 2 and $Q-2$ numbers of degrees of freedom).

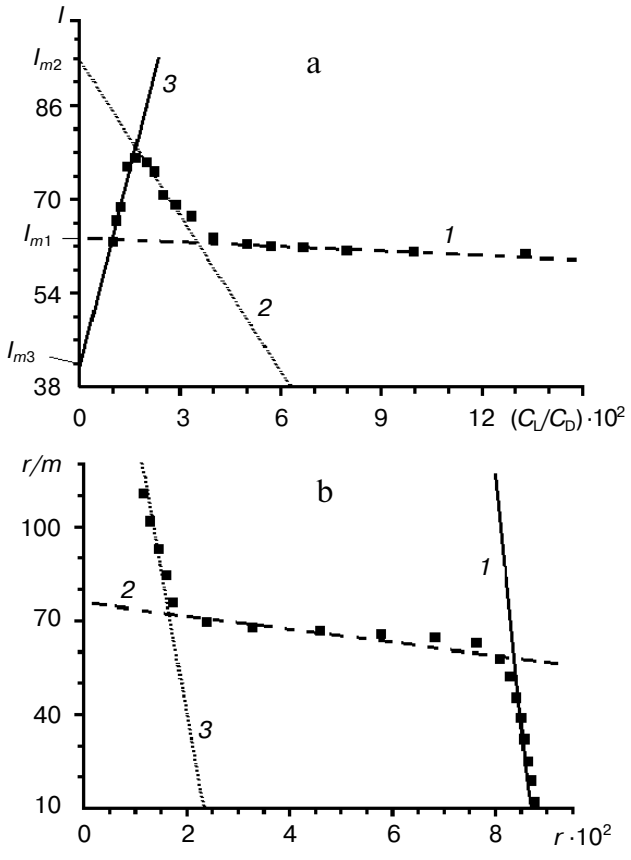


Fig. 1. Calculation of binding parameters for compound III and DNA in buffer 1 using Scatchard's model (see "Materials and Methods"). Dots show experimental data; lines *1-3* show calculated dependencies for corresponding types of dye–DNA complexes; I_{m1}, I_{m2}, and I_{m3} are maximally possible values of fluorescence intensity for corresponding types of dye–DNA complexes; $I_{03} = I_{m2}$ is "nil" fluorescence intensity for the third type of dye–DNA complex; C_L and C_D are molar concentrations of dye and DNA, respectively.

RESULTS AND DISCUSSION

Figures 2 and 3 show that both in aqueous media and in 2-propanol absorption (λ_{AB}) and emission (λ_{EM}) maxima are $\lambda_{AB,1}$ = 210 (it corresponds to electronic transitions in aromatic rings included in structure of molecules [30]), $\lambda_{AB,2}$ = 255, $\lambda_{AB,3}$ = 330, and λ_{EM} = 455 nm. Fluorescence excitation spectra of compounds I-VI were characterized by the presence of peaks with maxima at 255 and 330 nm only.

Increasing the DNA/ligand concentration ratio (C_D/C_L) in buffer 1, used in this study as the standard, absorbance (A) of compounds I-VI in the visible (VB) region of spectrum (300-700 nm) decreased by 10-15%. In the UV range (200-300 nm) absorbance of ligands was not registered due to masking effect of DNA. However, on increase in C_D/C_L ratio in buffer 1 and fluorescence excitation both in UV and VB regions of the spectrum the emission intensity (I) of compounds I-VI increased only up to C_D/C_L ~ 65, then began to decrease down to C_D/C_L = 100 (the maximal ratio used in this study) (Figs. 4 and 5).

In buffers 2 and 3 which attenuate hydrogen and ionic bonds respectively in the DNA–ligand system (see "Materials and Methods") absorbance of compounds I-VI in the VB spectral region decreased with the increase of C_D/C_L value in a lesser degree than in buffer 1. The fluorescence intensity of these dyes increased with the increase in C_D/C_L value in buffer 3 over the whole C_D/C_L range and in buffer 2 only up to C_D/C_L ~ 20 (then I began to decrease with the increase in C_D/C_L) (Fig. 5). Moreover, data of Fig. 5 show that in the absence of DNA fluorescence intensity of compounds I-VI in buffers 2 and 3 was a bit higher than in buffer 1, whereas absorbance of these dyes remained unchanged.

Thus, within the investigated C_D/C_L range (from 0 up to 100) compounds I-VI are characterized by at least three types of DNA–ligand binding in buffer 1.

The first type of binding is characterized by the highest affinity (which is determined by K_i values given in Table 1), the lowest specificity to DNA (which here represents the inverse n_i value from Table 1), presence of ionic and hydrogen bonds between DNA and ligand and increase in fluorescence intensity of substrate-bound dye compared with free dye. This type of binding predominates at C_D/C_L = 3-20.

The second type of binding is characterized by the lowest affinity to substrate, increase in specificity to polynucleotide compared with the first type of binding, the existence of only hydrogen bonds between DNA and ligand (this type of DNA–ligand binding was observed in buffers 1 and 3 but not in buffer 2) and continued increase in fluorescence intensity during dye binding. This type of binding predominated at C_D/C_L = 25-65.

The third type of binding is characterized by the highest specificity to polynucleotide, increased affinity to

substrate compared with the second type of binding, the existence of only ionic bonds between DNA and ligand (this type of DNA–ligand binding was observed in buffers 1 and 2 but not in buffer 3) and decrease in fluorescence intensity during dye binding. This type of binding predominated at C_D/C_L = 65-100.

It should be noted that binding of trimethylpsoralen 5′-derivatives to DNA in buffer 1 (considered in this study as standard) exhibited two times lower affinity to substrate and twofold higher specificity for DNA than dimethylangelicins.

Comparison of fluorescence quantum yield (φ) of compounds I-VI shows that all these dyes are characterized by some (1.3-4-fold) increase in φ both in the presence of DNA and in 2-propanol (see Table 2; for comparison Table 3 shows data similar to those given in Tables

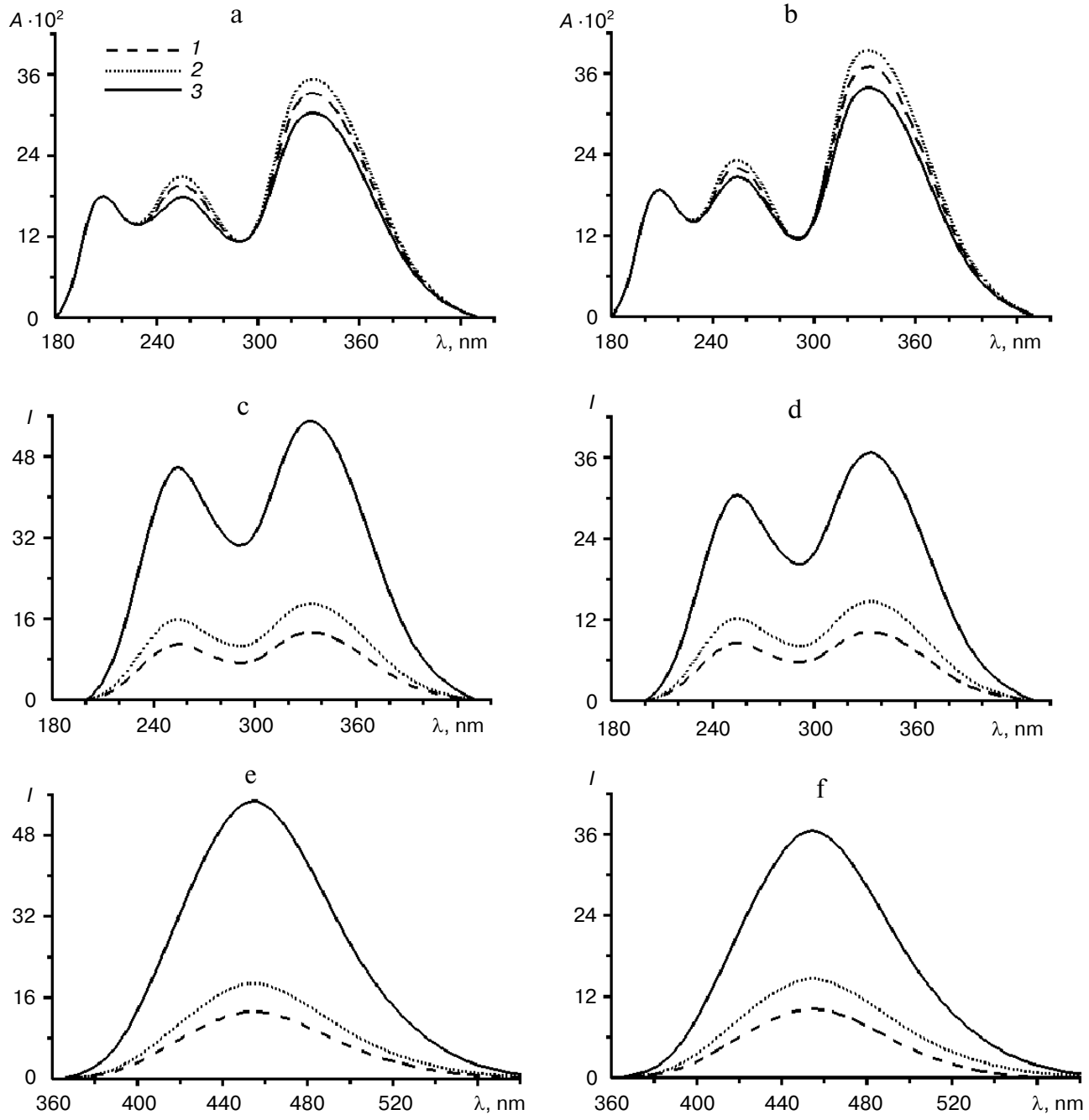


Fig. 2. Spectra of absorbance (a, b), fluorescence excitation (c, d) and emission (e, f) of psoralen (a, c, e) and angelicin (b, d, f) compounds in buffer 1. Curves *1-3* correspond to compounds I-III (a, c, e) or IV-VI (b, d, f). All details are given in the "Materials and Methods".

1 and 2 for four commercially available phenylindole and benzimidazole compounds widely used for DNA analysis). In 2-propanol, fluorescence quantum yields of the studied compounds (φ_A) were higher than in aqueous medium in the presence of DNA (φ_D). Besides this φ_A and φ_D were closer to each other by values obtained for related compounds I-III and IV-VI compared with the fluorescence quantum yields of these dyes in the aqueous medium in the absence of DNA (φ_W). This may be attributed to the previously noted fact [28, 33] that due to low dielectric permeability of alcohol medium fluorescence involves only the aromatic planar "core" of the molecules, excepting electron donor or electron acceptor effects of terminal substituents. Table 2 also shows that fluorescence quantum yields (φ) for trimethylpsoralens I-III were somewhat more than φ obtained for dimethylan-

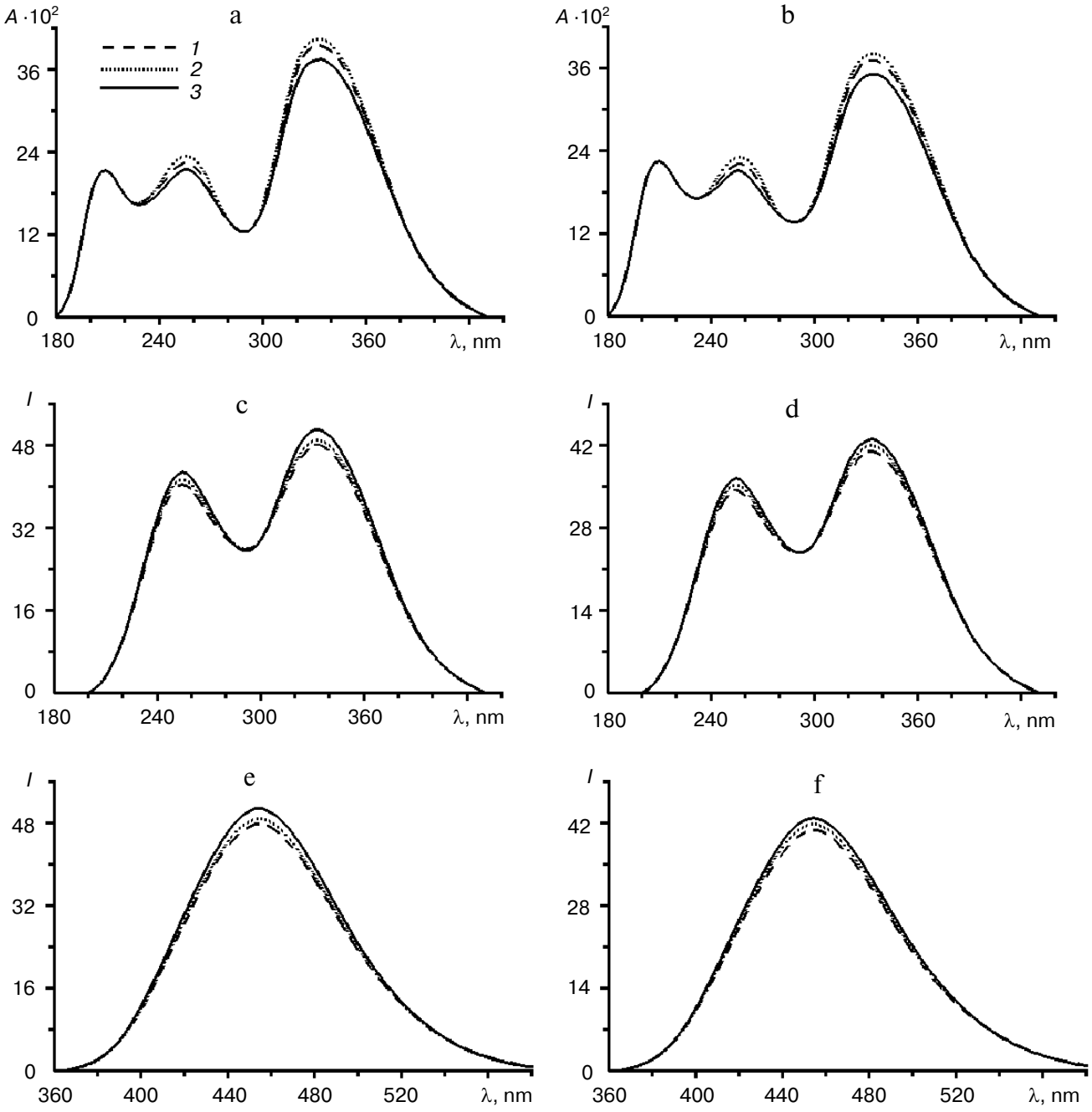


Fig. 3. Spectra of absorbance (a, b), fluorescence excitation (c, d) and emission (e, f) of psoralen (a, c, e) and angelicin (b, d, f) compounds in 2-propanol. All other designations are the same as in Fig. 2.

gelicins IV-VI. This difference may be due to distinct configuration of the "core" of psoralens and angelicins molecules or presence additional electron donor methyl terminal substituents in compounds I-III.

Data of Table 2 also show reduction in φ_A/φ_W and φ_D/φ_W ratios for compounds I-III and IV-VI. This may be attributed to reduction of electron acceptor properties with respect to the furocoumarin "core" in the following order of functional groups: HO–N= > O= > HO– > H_3C–O–. The first position of the hydroxyimino group in this order is determined by both increase in electron acceptor properties of the nitrogen atom and possible formation of an intramolecular hydrogen bond between this group and the oxygen atom of the furocoumarin ring

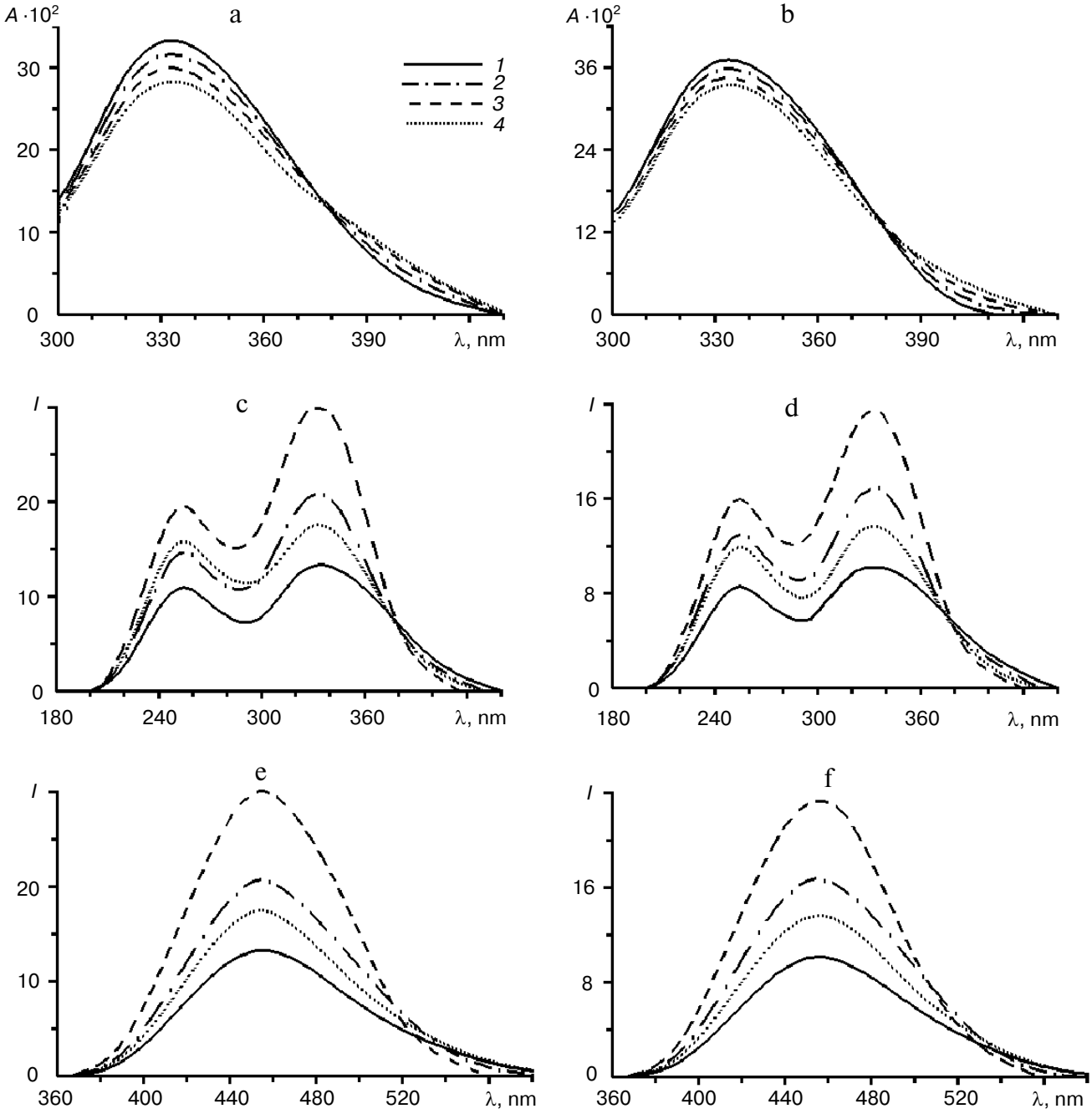


Fig. 4. Spectra of absorbance (a, b), fluorescence excitation (c, d) and emission (e, f) of compounds I (a, c, e) and IV (b, d, f) (these are typical representatives of psoralen and angelicin compounds studied in the present work) in buffer 1 in the presence of various DNA concentrations. Curves *1-4* correspond to DNA/ligand ratios: 0, 5, 25, 65-100 (in absorption spectra) and 0, 25, 65, 100 (in fluorescence spectra). All other details are given in the "Materials and Methods".

(Scheme 2). The oxo group is more active than a hydroxyl group in attracting electron density of the furan ring due to higher conjugation of its bond with the "core". The methoxy group exhibits the lowest electron acceptor properties because its electronegative (with respect to the carbon atom) oxygen atom can attract to itself electron density not only of the furocoumarin "core" but also of the methyl group.

Thus, both for intercalators and for non-intercalators (which we studied earlier [28, 33, 34]) on modeling of fluorescent properties of nucleotide-specific (and other) dyes it is necessary to take into account the following factors.

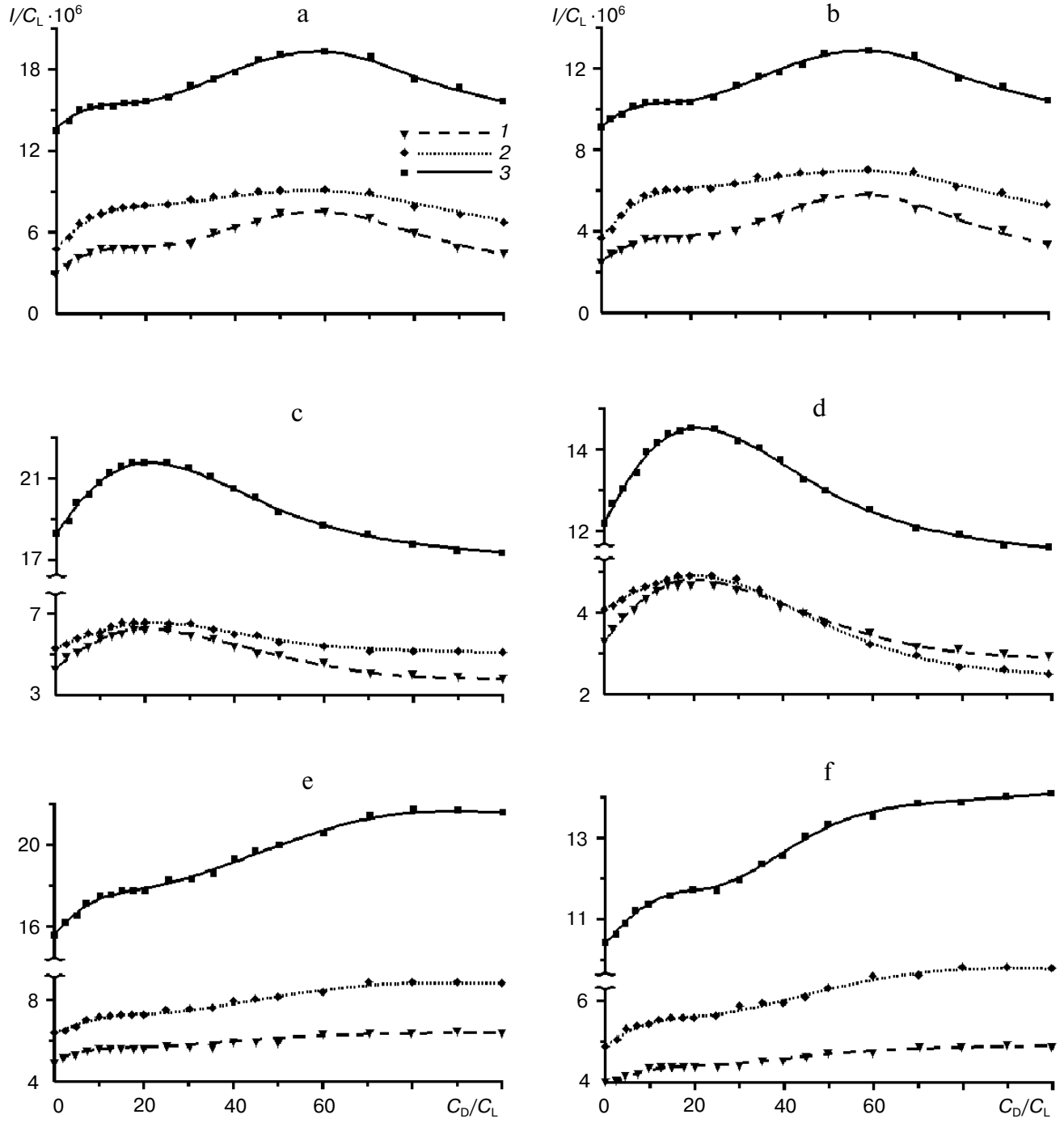


Fig. 5. Dependence of fluorescence intensity on DNA concentration obtained using procedure of global cubic spline interpolation of experimental data [31, 32] and data normalization by dye concentration in aqueous buffers 1 (a, b), 2 (c, d), and 3 (e, f). Curves *1-3* correspond to compounds I-III (a, c, e) or IV-VI (b, d, f). Points *1-3* indicate experimental data for compounds I-III (a, c, e) or IV-VI (b, d, f).

Table 1. Binding parameters of compounds I-VI with DNA in buffer 1 (defined in this study as standard)

Compound	H	E	$K_1 \cdot 10^{-3}$	$n_1 \cdot 10^2$	$K_2 \cdot 10^{-3}$	$n_2 \cdot 10^2$	$K_3 \cdot 10^{-3}$	$n_3 \cdot 10^2$
I	79	16	34.50	11.50	4.50	3.85	18.80	2.84
II	46	49	31.10	10.90	3.89	3.66	17.90	2.71
III	64	32	16.90	9.85	2.12	3.47	9.27	2.44
IV	71	25	67.20	21.30	8.78	7.50	36.70	5.28
V	37	60	63.70	23.50	7.98	8.28	35.00	5.82
VI	55	40	43.90	25.60	5.51	9.02	24.10	6.34

Note: H and E (%) are relative values showing contribution of hydrogen and ionic bonds, respectively, into formation of the first type of DNA–ligand complexes; K_1-K_3 (M^{-1}) are Scatchard constants (representing inverse value of free ligand concentration in the system at half saturation of potential binding sites); n_1-n_3 are values corresponding to maximally possible number of ligand molecules that can be bound to one DNA molecule per base pair; indexes "$_1$" - "$_3$" indicate parameters for corresponding types of complexes dominating at ratios of molar concentrations DNA/ligand (C_D/C_L = 3-20, 20-65, and 65-100, respectively); relative error of parameter determination at p = 95% was 1-2%.

Table 2. Fluorescence quantum yield (φ) of compounds I-VI in aqueous media (in the presence and absence of DNA) and in 2-propanol

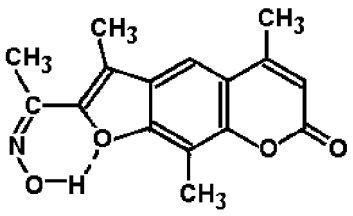
Compound	φ_A	φ_{W1}	φ_{W2}	φ_{W3}	φ_A/φ_{W1}	$\varphi_{D1}/\varphi_{W1}$	$\varphi_{D2}/\varphi_{W2}$	$\varphi_{D3}/\varphi_{W3}$
I	0.293	0.096	0.119	0.135	3.05	2.43	1.17	1.71
II	0.296	0.113	0.126	0.142	2.62	2.08	1.36	1.50
III	0.301	0.193	0.224	0.206	1.56	1.26	1.15	1.17
IV	0.282	0.069	0.093	0.106	4.10	2.87	1.35	2.33
V	0.285	0.092	0.105	0.121	3.11	2.39	1.60	1.52
VI	0.289	0.131	0.165	0.143	2.20	1.74	1.18	1.41

Note: Index "$_A$" shows parameters measured for the compounds in 2-propanol; indexes "$_{W1}$" - "$_{W3}$" show parameters measured in aqueous buffers 1-3 in the absence of DNA (C_D/C_L = 0); indexes "$_{D1}$" - "$_{D3}$" show parameters measured in aqueous buffers 1-3 at C_D/C_L providing maximal dye quantum yield in the presence of DNA (C_D/C_L ~ 65, 20, and 100 for buffer solutions 1-3, respectively). Relative error of parameter determination at p = 95% did not exceed 3%.

Table 3. Spectral and binding parameters of commercially available phenylindole and benzimidazole compounds [16]

Compound	H, %	E, %	$K_1 \cdot 10^{-3}$, M^{-1}	$n_1 \cdot 10^2$	$\varphi_{D1}/\varphi_{W1}$
XVII	37	56	4900	2.60	27.1
XVIII	90	6	8420	0.69	35.4
IX	51	43	8900	0.46	41.6
X	48	45	9030	0.46	39.6

Note: Numbers XVII - X designate the following compounds: 4′,6-diamidino-2-phenylindole (DAPI, XVII), 6-(2-imidazolin-2-yl)-2-[4-(2-imidazolin-2-yl)phenyl]indole (DIPI, XVIII), 2-[2-(4-hydroxyphenyl)benzimidazol-5(6)-yl]-5(6)-(4-methylpiperazin-1-yl)benzimidazole (Hoechst-33258, IX), 2-[2-(4-ethoxyphenyl)benzimidazol-5(6)-yl]-5(6)-(4-methylpiperazin-1-yl)benzimidazole (Hoechst-33342, X); structural formulas are given in Scheme 3. Other designations are the same as in Tables 1 and 2.



Scheme 2

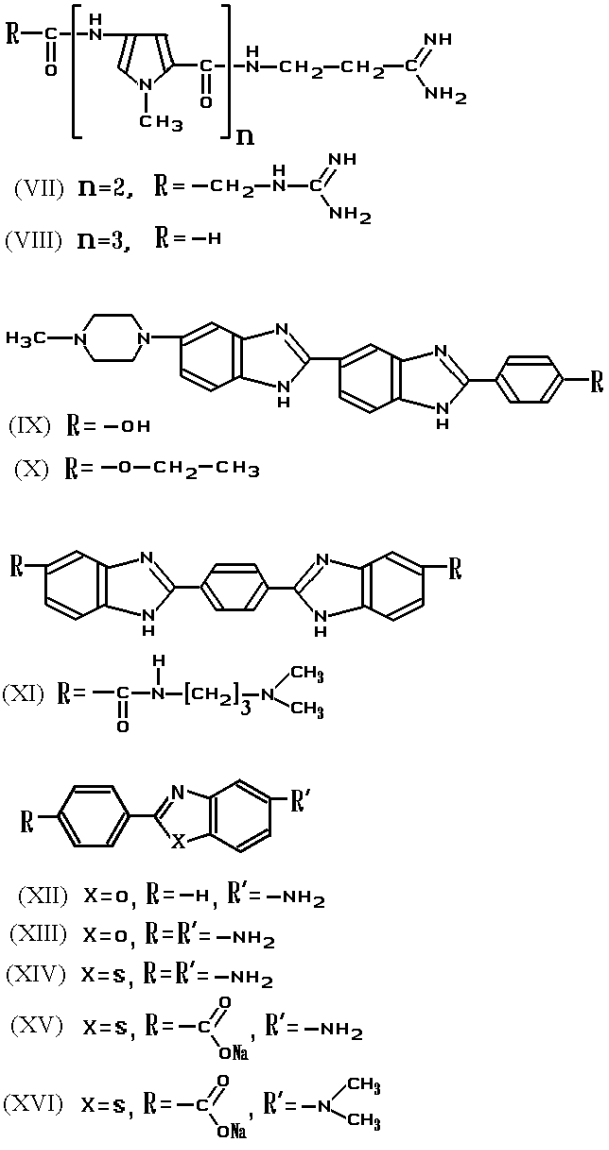
1. Only structures with well-developed conjugated electron system including π-electrons of double or triple bonds or non-bonding electron pairs of heteroatoms can actively fluoresce.

2. Such conjugation can be achieved only when the abovementioned electron system has rather rigid co-planar structure (i.e. all its fragments are positioned relative to each other in one plane). However, the latter precondition is usually observed only for intercalators, which must consist of several condensed aromatic rings. And the majority of non-intercalators (e.g. netropsin, distamycin A [9], Hoechst-33258, Hoechst-33342 [28]; see compounds VII-X in Scheme 3) for intensive fluorescence requires the presence of factors providing additional stabilization of planar structure of the dye molecule (this reduces the possibility of mutual rotation of separate fragments, which together make a dye molecule, and increases total quantum yield of fluorescence). Such factors may be: significant viscosity of dye environment (as for example in case of a 50% sucrose solution [34]); the presence of certain substituents at the "core" of dye molecule (e.g. 3-dimethylaminopropylcarbamoyl substituents as in compound XI [28, 34] in Scheme 3); specific dye interaction with substrate, etc.

3. Even if the "core" of a molecule possesses a rather developed and rigid conjugated electron structure (e.g. compounds I-VI) the fluorescence quantum yield of such a molecule in the unbound state in polar solvent can be reduced by introducing electron acceptor (with respect to the molecular "core") substituents or increased by introduction of substituents exhibiting electron donor properties. However, in media with low dielectric permeability (e.g. 2-propanol) or during specific interaction with polynucleotide the above described effects of substituents on the molecular "core" are significantly attenuated due to altered microenvironment of the dye molecule. As a result total fluorescence quantum yield (φ) becomes closer to the φ value of the molecular "core" of such a compound observed in the absence of any substituents.

Thus, if we put substituents in decreasing order by their electron donor activity (or increasing order by their electron acceptor activity, respectively) with respect to the fluorophore "core", it is relevant to suggest that the φ values of derivatives, which carry corresponding terminal substituents, will be reduced in the same order in aqueous medium (characterized by high dielectric permeability). On the contrary, values of fluorescence quantum yield ratios for such compounds in aqueous medium in the presence and absence of DNA (φ_D/φ_W), and also in alcohol versus aqueous medium (φ_A/φ_W), should be increased in the same order.

4. The above-described considerations have generally been confirmed by results of the present and previous studies [33, 34]. However, rather often a compound can exist in solution as a mixture of several equilibrium forms (differing by ionization degree of the molecular "core" and its substituents, number of intramolecular hydrogen bonds, etc.); the ratio between which may differ depending on pH, nature of the solvent, etc. It in addition complicates the general picture.



Scheme 3

As an example, it is possible to compare properties of phenylbenzenes XII-XVI (Scheme 3) described in [35]. In particular, in the non-ionized state the H_2N-group should exhibit electron donor properties with respect to the aromatic molecular "core". However, the fluorescence quantum yield of compound XIII ($\varphi_W = 0.12$) in aqueous buffer was significantly lower than in compound XII ($\varphi_W = 0.87$); which differed from compound XIII by the absence of a terminal amino group only. This suggests that under conditions, which were described in [35], protonated H_3N^+ form, which exhibits electron acceptor properties with respect to the molecular "core" of compound (XIII), evidently predominated. Similarly, although a non-ionized carboxyl group should demonstrate clear electron acceptor properties with respect to the aromatic fragment, which is connected from it; whereas in the ionized ^-OOC form this group causes an opposite effect on the molecular "core" of the compound. This may explain why change in amino group (in compound XIV) for carboxyl group (in compound XV) was accompanied by significant increase in φ_W value from 0.07 to 0.55. Finally, the terminal dimethylamino group of compound XVI probably in the ionized form is a weaker acceptor and in the non-ionized form is a stronger donor of electron density to phenylbenzothiazole "core" than the similar amino group of compound XV in corresponding forms. As a result, φ_W value of compound XVI was higher than in compound XV (0.66 and 0.55, respectively).

The method for determination of total electron donor or electron acceptor effect of any substituent on a molecular "core" of fluorophore existing in solution as several equilibrium forms we considered earlier [36]. And in the most common case one should take into consideration the possibility of mutual influence of all the above-considered factors on fluorescence of compounds considered in this work.

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