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Spectral Study of the Interaction of DNA with Benzothiazolyl-benz-α-chromene

V. S. Sibirtsev[1]* and A. V. Garabadzhiu[2]

[1]*Mendeleyev Russian Research Institute for Metrology, Moskovsky pr. 19, 190005 St. Petersburg, Russia; E-mail: vs1969r@mail.ru*
[2]*St. Petersburg State Technological Institute, Zagorodnyi pr. 49, 198013 St. Petersburg, Russia*

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Abstract—Absorption and luminescence excitation and emission spectra of newly synthesized 2-(4-methylphenylimino)-3-(2′-benzothiazolyl)benz-α-chromene (BCBT) have been studied in the presence of various DNA concentrations. BCBT is characterized by the existence of two different fluorescent systems, exhibiting radiationless fluorescence resonance energy transfer between them. In the range of molar ratios of polynucleotide/dye concentrations from 0 to 50, BCBT preferentially intercalates into DNA due to its benz-α-chromene fragment, whereas the 2-benzothiazolyl fragment is responsible for fluorescence.

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Key words: fluorescent DNA-specific dyes, DNA-binding ligands, spectral properties

Synthetic low molecular weight compounds that can specifically bind to certain nucleotide sequences in the genome are becoming increasingly popular research tools [1-6]. Special attention is paid to compounds that display sharply changed intensity of their fluorescence [7-12]. Such ligands may be subdivided into two large classes by the type of reversible specific interaction with the substrate. Compounds of the first class exhibit external polynucleotide binding; they are mainly specific to the first and second order structural organizations of nucleic acids. Such type of binding requires the presence of at least one heteroatom and also a system of coupled bonds (for registration of spectral properties) in the ligand molecule [9, 13-16]. The compounds of the second class are known as intercalators, which are inserted between complementary base pairs of polynucleotide double helix; they are mainly specific to the second and higher orders of structure organization of nucleic acids. Such ligand molecules should meet the following structural requirements: the presence of one or several fragments representing a condensed aromatic system with two or more rings, containing 5-6 atoms, and also at least one heteroatom (preferentially endocyclic nitrogen or oxygen atom) [7, 17-20] (Fig. 1).

However, pathways for the development of new compounds exhibiting higher sensitivity and specificity to the substrate still require better theoretical consideration and substantiation. This task is complicated by the following problem: potent selective binding to the substrate and registration usually require different structural features. One of the best ways to solve this conflict is to design complex compounds containing different functional fragments: one (or several) fragment(s) would be responsible for substrate binding, whereas the other(s) would determine spectral or any other properties suitable for registration. If a constructed complex compound consists of several subunits, which bind to the substrate, it is reasonable to expect significant increase in affinity to this substrate and specificity compared with "monomeric" constituents of such compound. Similarly, if the constructed complex compound consists of several subunits, which can, for example, increase their fluorescence after sorption on the substrate, it is reasonable to expect increased sensitivity to such substrate compared with the "monomeric" constituents. It is also clear that use of complex compounds

Abbreviations: BCBT) 2-(4-methylphenylimino)-3-(2′-benzothiazolyl)benz-α-chromene; DAPI) 4′,6-diamino-2-phenylindole; EtBr) 2,7-diamino-10-ethyl-9-phenyl-phenanthridinium bromide; FRET) fluorescence resonance energy transfer.

* To whom correspondence should be addressed.

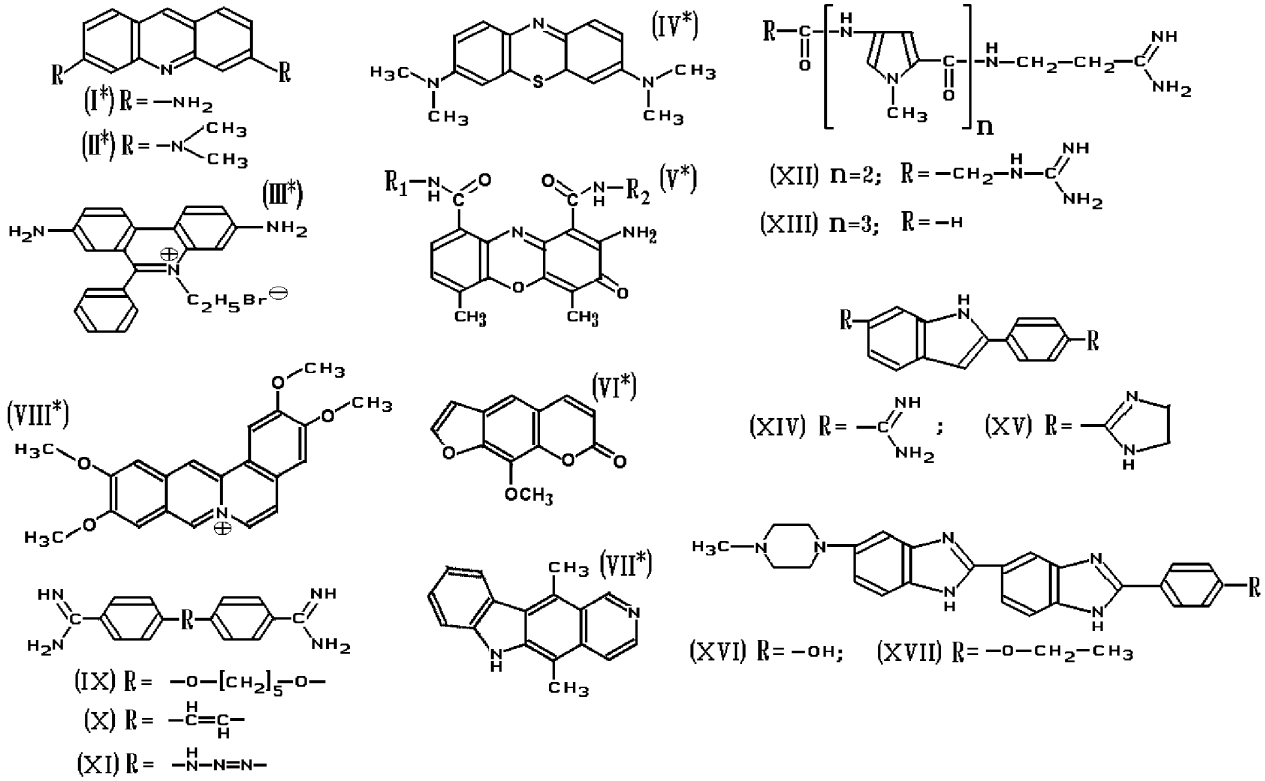

Fig. 1. Structural formulas of some "monofunctional" DNA-binding dyes: I, II) proflavin and acridine orange [17]; III) ethidium bromide [7]; IV) methylene blue [17]; V) actinomycins (chromophore is 1,9-dicarbamoyl-2-amino-3-oxo-4,6-dimethyl-β-phenoxazine) [21, 22]; VI) 8-methoxypsoralen (8-MOP) [3, 23]; VII) ellipticine [5, 24]; VIII) 8-desmethyl-coralin [25]; IX-XI) pentamidine, stilbamidine, and berenil, respectively [2, 9, 26]; XII, XIII) netropsin and distamycin A [9, 14]; XIV, XV) 4′,6-diamidino-2-phenylindole (DAPI) and 4′,6-di(2-imidazolin-2-yl)-2-phenylindole (DIPI) [10, 15, 16]; XVI, XVII) Hoechst 33258 and Hoechst 33342 [1, 16]. Asterisk (*) marks compounds exhibiting intercalation into DNA (others demonstrate external DNA binding).

containing structural fragments exhibiting different types of binding with polynucleotides can significantly increase informativeness of nucleic acid analysis.

Several complex DNA-specific compounds are known to date (Fig. 2). Some of them are bis-intercalators (e.g. TOTO (XVIII) and YOYO (XIX)) [27, 28], demonstrating affinity to DNA and increased fluorescence in response to the nucleic acid, which are higher than in any known mono-intercalator. Others (e.g. bis-netropsins (XX)) [9, 29] exhibit only external DNA binding. A third group represent a hybrid of mono-intercalator and a compound externally bound to DNA. The latter group includes:

– palmatine (XXI) [25]; (interestingly, its analog dehydrogenated by one of the aromatic cycles, 8-desmethyl-coralin (VIII), interacts with DNA only as an intercalator [25]);

– antibiotic daunomycin (XXIII) [31]; and possibly (and by analogy with it) rubomycin (XII) [30];

– the hybrid compounds like "bis-ethidium" and "ethidium–acridine" (XXIV) [32], in which only the ethidium fragment intercalates into DNA, whereas the second fragment (in spite of structural similarity with classical intercalators) exhibits external DNA binding; in the case of compound (XXIV) this is accompanied by fluorescence resonance radiationless energy transfer. There is also radiationless fluorescence resonance energy transfer in which excitation of fluorescence of the energy donor (acridine fragment having excitation and emission maximums at λ_{EX} = 470 nm and λ_{EM} = 600 nm, respectively) causes fluorescence of not only the energy donor, but also an energy acceptor (ethidium fragment, having λ_{EX} = 550 nm and λ_{EM} = 610 nm) (this phenomenon will be considered in more detail during consideration of the dye studied in this work; this dye also exhibits such type of fluorescence, see below);

– compounds of "bis-acridine" type. Their two acridine fragments intercalated into a polynucleotide [34]. Such intercalation may take place only when a linker chain joining these fragments exceeds a critical length, otherwise such compounds would exhibit interaction with the polynucleotide substrate typical for "bis-ethidium";

– hybrid compounds of "ellipticine–distamycin" type (XXV) and "netropsin–acridine", in which external DNA binding prevailed over the fragment intercalating into DNA [33], etc.

Nevertheless, only a small proportion of the actual possibilities for construction of complex DNA-specific

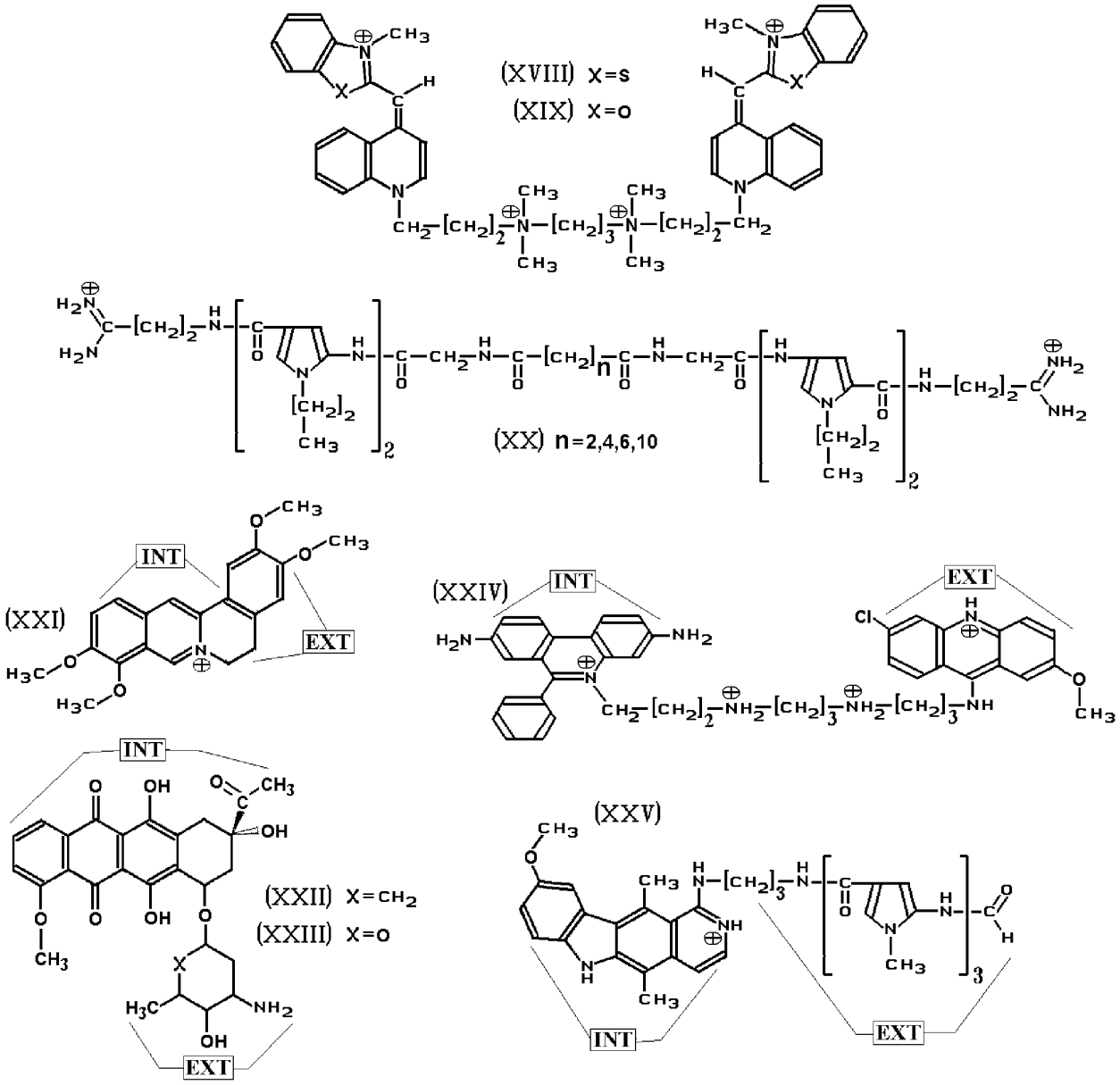

Fig. 2. Structural formulas of some "bifunctional" DNA-binding dyes: XVIII, XIX) TOTO and YOYO [27, 28]; XX) bis-netropsins (synthesized and investigated for interaction with polynucleotides; these include ligands with the other mode of attachment of netropsin residues, not shown on the figure) [9, 29]; XXI) palmatine [25]; XXII, XXIII) rubomycin [30] and daunomycin [31]; XXIV) EtAc (hybrid "ethidium–acridine") [32]; XXV) ElDs (hybrid ellipticine–distamycin) [33]; for compounds XXI-XXV, the fragments intercalating into polynucleotide and exhibiting external binding are shown as "INT" and "EXT", respectively.

compounds has been realized. Thus, in this study we have used the newly synthesized "bifunctional" DNA-binding dye, 2-(4-methylphenylimino)-3-(2′-benzothiazolyl)benz-α-chromene (BCBT) (Fig. 3).

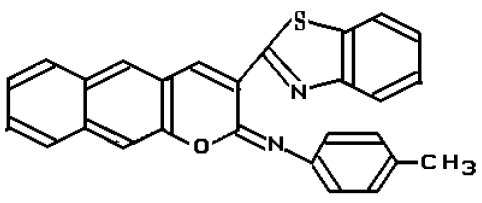

Fig. 3. Structural formula of the compound used in the present study (BCBT).

MATERIALS AND METHODS

Absorption and fluorescence spectra were recorded as described in [35] using Beckman-35 (Austria) and

Hitachi-850 (Japan) instruments, respectively. All measurements were carried out at the constant dye concentration (C_L), of $2.8 \cdot 10^{-5}$ M and various concentrations of DNA calculated per nucleotide pair (C_D) at 20-25°C using standard aqueous buffer containing 0.01 M NaCl, 0.01 M Na_2EDTA, 0.01 M Tris (pH 7.4). Commercially available fluorophores DAPI (4′,6-diamidino-2-phenylindole) (XIV) and EtBr (2,7-diamino-10-ethyl-9-phenyl-phenanthridinium bromide) (III) (see Fig. 1) and also Na_2EDTA and Tris were purchased from Serva (Germany). The newly synthesized compound BCBT was kindly presented by the Department of Molecular Biotechnology (St. Petersburg Technological Institute). Chicken erythrocyte DNA (60% AT base pairs; mean molecular mass of 326 daltons per nucleotide; molar absorbance coefficient $\varepsilon_{260} = 6600\ M^{-1} \cdot cm^{-1}$) was used as the substrate. The dry preparation obtained from Serva was dissolved in distilled water and sonicated (for homogenization and reduction of light scattering) using a UZDN-2 instrument (Russia) for 15 sec at 0.3 A and 22 kHz. This results in formation of DNA fragments of 35,000 daltons. Other chemicals were of chemically pure grade.

Since overlapping of absorption and fluorescence spectra was absent in the compounds studied, the ratio of quantum yields was determined by the following formula:

$$\varphi_2/\varphi_1 = (I_2A_1)/(I_1A_2), \quad (1)$$

where A and I are the values of optical density and fluorescence intensity of the compound studied at the wavelengths corresponding to maximums at the spectra of its fluorescence excitation and emission. The sensitivity coefficients (η_S), reflecting the value of fluorescence augmentation of the dye studied in response to the increase in DNA concentration by 1 mol/liter and the ratio of concentrations $C_D/C_L = S$, was calculated by the formula:

$$\eta_S = (I_{S+1} - I_{S-1})/(2C_L), \quad (2)$$

where I_{S+1} and I_{S-1} are maximal fluorescence intensities of the studied dye at ratios of molar concentrations $C_D/C_L = S + 1$ and $C_D/C_L = S - 1$, respectively. The relative contribution of hydrogen bonds (*Hb*) to formation of active fluorescent complex of the dyes under study and a polynucleotide is determined as follows:

$$Hb = 100\ (\Phi_{Ib} - 1)/(\Phi_{St} - 1), \quad (3)$$

where Φ_{St} and Φ_{Ib} are relative values of quantum yields of the compound in the presence of saturating concentration of DNA and in the absence of DNA in the standard aqueous buffer (*St*) and in the buffer containing 2 M NaCl, 0.01 M Na_2EDTA, 0.01 M Tris (pH 8.0) (*Ib*), respectively.

Parameters of the complex formation between BCBT and DNA were determined as follows. Let us introduce the following designations: $m = C_L^f$ is molar concentration of a free ligand in solution; $r = C_L^b/C_D$ is amount of a bound ligand per unit of substrate concentration (here C_D is total molar concentration of nucleotides in the system, C_L^b is molar concentration of a bound ligand in solution); $n = r_{max}$ is maximally possible number of ligand molecules that can bind to one DNA molecule divided by the total number of nucleotide pairs in it. In this case at $n = 1$ the considered system is consistent with the Langmuir isotherm equation [36]:

$$K = r/(m - mr). \quad (4)$$

At $n < 1$ (this is typical for real DNA–ligand systems), Scatchard [37] proposed transformation of Eq. (4) into the following equation:

$$r/m = Kn - Kr\,. \quad (5)$$

In this case "r" and "$n - r$" can be interpreted as proportions of occupied and vacant binding sites at the considered polymer (the length of each site is $h = C_D^b/C_L^b \neq 1/n$ nucleotide pairs, where C_D^b is molar concentration of nucleotides bound to the ligand); K is an empiric constant; its value is the inverse of the concentration of a free ligand in the system under conditions when it occupies half of potential binding sites on the substrate.

To exclude possible effects ignored by the selected model and observed only at excess of the ligand over the polynucleotide (cooperative, statistical, etc. [35]) data used for calculation in this study were taken at ratios of molar concentrations $C_D/C_L >> 1$. The method of calculation consisted of the following steps.

Initially, using the values of fluorescent intensity of the dye in the presence of given amount of DNA (I_i), we calculated the value of maximally possible fluorescence intensity of the fluorescent dye in the studied system (I_{max}). This value was determined by extrapolation of the linear part of the curve on the plots of dependence of I versus ratio of concentrations C_L/C_D, obtained during fluorescence titration of the studied dye to $C_L/C_D = 0$, as shown at Fig. 4. However, it should be noted that this plot serves only as the illustration and selection of number of points (in this case $Q = 4$) in the upper part of the curve $Y = f(X)$ (where $Y = I$, and $X = C_L/C_D$) for their subsequent approximation by the least square method [38] by the following dependence:

$$Y = a_0 + a_1X,$$

where

$$a_1 = [\Sigma X_i \Sigma Y_i - Q\Sigma(X_iY_i)]/[(\Sigma X_i)^2 - Q\Sigma X_i^2],$$

$$a_0 = (\Sigma Y_i - a_1\Sigma X_i)/Q,\ i = 1 - Q \quad (6)$$

(in this case accuracy of the value I_{max} is significantly higher than that obtained by graphic determination; this also influences accuracy of parameters of complex formation determined using such approach).

After selection of the required number of points for approximation, we calculated $C^{b}_{L,i}$ for corresponding values of $C_{D,i}$:

$$C^{b}_{L,i} = C_L(I_{max} - I_i)/(I_{max} - I_0), \quad (7)$$

where I_0 is fluorescence intensity of the dye in the absence of DNA. Assuming $X_i = r_i = C^{b}_{L,i}/C_{D,i}$ at $Y_i = r_i/m_i = X_i/(C_L - C^{b}_{L,i})$ and then calculating a_0 and a_1 using Eqs. (6), we determined $K = -a_1$ and $n = -a_0/a_1$. Figure 5 illustrates this step.

The accuracy of these calculations was evaluated using values of relative errors for K and n, which were determined by formulas:

$$\varepsilon_K = 100\ \Delta K/K, \quad (8)$$

and

$$\varepsilon_n = 100\ \Delta n/n, \quad (9)$$

where

$$\Delta K = t_T S_a \cdot [\Sigma X_i^2 - (\Sigma X_i)^2/Q], \quad (10)$$

$$\Delta n = t_T \cdot (S_a/K) \cdot [Q - (\Sigma X_i)^2/\Sigma X_i^2] + (\Delta K n)/K, \quad (11)$$

t_T is the table value of Student's criterion at the significance level of "0.05" and number of degrees of freedom "$Q - 2$"; $S_a = [\Sigma(Y_{T,i} - Y_{E,i})^2/(Q - 2)]^{1/2}$ is the adequateness dispersion of Eq. (5); $Y_{T,i}$ and $Y_{E,i}$ are theoretical and experimental values of the parameter of relative approximation error: $\varepsilon_S = 100\Sigma(Y_{T,i} - Y_{E,i})/(QY_{E,i})$ and criterion $F_P = \Sigma(Y_{T,i})^2/(QS_a^2) > F_T$ (where F_T is the table value of Fisher's criterion at the significance level of "0.05" and number and degrees of freedom "2" and "$Q - 2$", respectively), characterizing whether the selected model adequately describes the behavior of the DNA–dye system in the given range of ratios C_D/C_L. For all compounds used in this study we obtained $\varepsilon_S < 3\%$, $\varepsilon_K < 10\%$, and $\varepsilon_n < 10\%$.

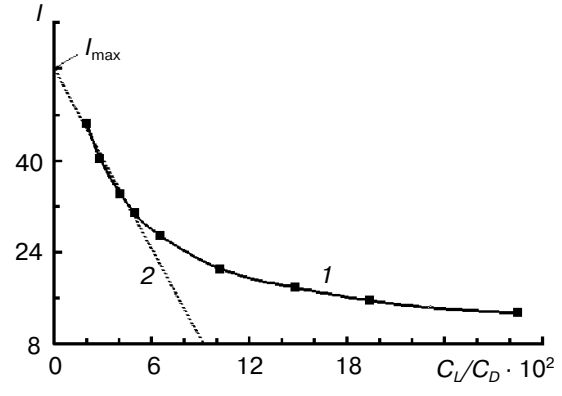

Fig. 4. Determination of maximally possible fluorescence intensity (I_{max}) of a dye in the presence of DNA using the first fluorescent system BCBT as an example: *1*) dependence obtained after global cubic spline interpolation of experimental data (shown as dots); *2*) linear extrapolation of the upper part of curve *1* to $C_L/C_D = 0$ (where C_D and C_L are molar concentrations of DNA and the ligand in the system).

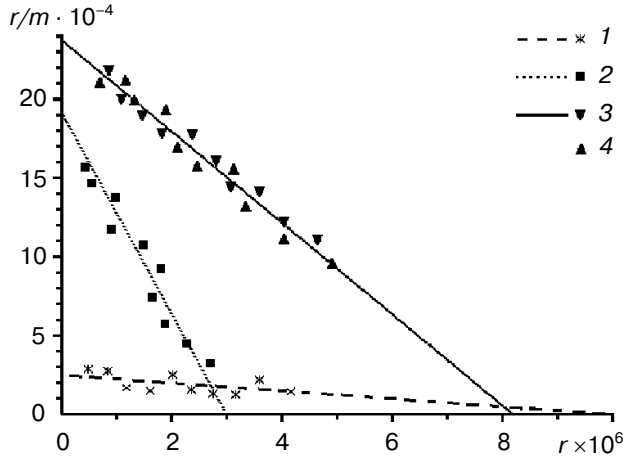

Fig. 5. Calculation of parameters of complex formation between fluorophores and DNA. Lines *1-3* correspond to a characteristic equation of Scatchard's model (5) for binding of calf thymus DNA with EtBr, DAPI, and BCBT, respectively. Symbols *1-4* designate experimental data obtained for EtBr, DAPI, and also for the first and the second fluorescent systems of BCBT, respectively, in the presence of various DNA concentrations.

For all compounds used in this study we also determined the coefficient of specificity of a dye to tertiary structure of DNA:

$$Sd = 100\ (I_Z/I_N - 1), \quad (12)$$

where I_Z is maximal fluorescence intensity at the ratio of molar concentrations $C_D/C_L = 50$ for the complex formed by dye and DNA sonicated using the above-described method to average mass of one molecule of 35,000 daltons, and I_N is maximal fluorescence intensity at $C_D/C_L = 50$ for the complex formed by the dye and untreated DNA.

RESULTS AND DISCUSSION

The table and Fig. 6 show data on spectral and complex forming properties of BCBT in aqueous buffer in the presence of various concentrations of the polynucleotide and also similar characteristics of such commercially available DNA fluorophores as DAPI (XIV) and EtBr (III) (Fig. 1).

In the case of BCBT within the range of wavelengths λ = 200-700 nm there are four peaks with maximums at

Spectral and complex formation properties of the dyes studied in various media and in the absence and in the presence of DNA

Dye	BCBT-1	BCBT-2	DAPI	EtBr
λ^0_{AB}	365	520	340	480
λ^{50}_{AB}	365	520	360	520
λ^0_{EM}	450	615	450	595
λ^{50}_{EM}	450	615	455	605
$\varepsilon \cdot 10^{-3}$	1.5	0.43	19	5.6
$\mu \cdot 10^{-5}$	3.2	0.62	51	3.0
φ^{50}/φ^0	5.2	4.1	21	10
$\eta^1 \cdot 10^{-5}$	1.0	0.71	56	4.8
$\eta^{10} \cdot 10^{-5}$	0.42	0.33	28	2.9
$\eta^{50} \cdot 10^{-5}$	0.22	0.12	11	0.54
Hb	15	15	40	30
Sd	104	104	6.3	54
$K \cdot 10^{-6}$	2.9	2.9	6.4	0.25
$n \cdot 10^2$	8.2	8.2	3.0	10

Note: BCBT-1 and BCBT-2 designate first and second fluorescent systems of BCBT; EtBr and DAPI designate compounds III and XIV from Fig.1; λ_{AB} and λ_{EM} (nm) designate wavelength maximums in absorption and fluorescence emission spectra of dyes, respectively; ε ($M^{-1}\cdot cm^{-1}$) and μ ($M^{-1}\cdot cm^{-1}$) designate molar absorption and fluorescence coefficients in the absence of DNA; φ is fluorescence quantum yield; η (M^{-1}) is coefficient of fluorescent sensitivity (see "Materials and Methods"); *Hb* (%) is relative contribution of hydrogen bonds to formation of active fluorescent complex of the dye and a polynucleotide; *Sd* (%) is coefficient of specificity of a dye to tertiary structure of DNA (see "Materials and Methods"); K (M^{-1}) and n designate parameters of binding of the dye with DNA calculated on Scatchard's model (see "Materials and Methods"); indices "0", "1", "10", and "50" designate parameters which were measured at the ratios of molar DNA and dye concentrations (C_D/C_L): 0, 1, 10, and 50, respectively. Maximum peaks of absorption and excitation of fluorescence in the visible area of a spectrum at all specified dyes coincided. And also in absorption spectra of these dyes there were peaks with maxima at wavelength of 210 and 270 ($\pm$10) nm, the first of which in excitation spectra was not duplicated.

λ_{AB} = 210, 265, 365, and 520 nm (the peaks located in UV region at 210 and 265 nm are not shown at Fig. 6 due to the masking effect of DNA). Intensity of the third peak of BCBT absorbance (λ_{AB} = 365 nm) increased with the increase in molar ratios DNA/dye (C_D/C_L) from 0 to 50, but the wavelength of maximum and one isosbestic point at λ_{i1} = 385 nm remained unchanged, whereas intensity of the fourth peak (at λ_{AB} = 520 nm) decreased at constant wavelength and λ_{i2} = 555 nm.

The absorption maximum in the shortest wavelength region was not duplicated in the fluorescence excitation spectra of BCBT. Use of excitation light with wavelengths corresponding to the third and fourth absorbance maximums (λ_{EX} = 265 and 365 nm) there were two emission peaks with maximums at λ_{EM} = 450 and 615 nm. In the case of BCBT excitation with light of the wavelengths corresponding to the fourth absorption maximum (λ_{EX} = 520 nm) one emission peak with maximum at λ_{EM} = 615 nm was observed.

In each of the above-described cases, the increase in the ratio of C_D/C_L concentrations in the system was accompanied by the increase in fluorescence intensity. The excitation spectrum of BCBT recorded at λ_{EM} = 450 nm yielded two peaks with maximums at λ_{EX} = 265 and 365 nm (Fig. 6c). On the excitation spectrum of BCBT recorded at λ_{EM} = 615 nm there were three peaks with maximums at λ_{EX} = 265, 365, and 520 nm (Fig. 6d).

It has been reported [39] that 2-phenylbenzimidazole (DAPI chromophore; Fig. 1 and table) is characterized by the presence of three absorption peaks with maximums at λ_{AB} = 207, 241, and 301 nm. The first band is related to electron transitions in benzene rings, the second one is determined by electron transitions in the heterocycle, whereas the third band corresponds to electron transition from the benzene orbital to the free orbital of the HC=N-group [40]. Taking into consideration these data we can suggest that in the compound studied the absorption band with maximum at λ_{AB} = 210 nm corresponds to electron transitions in benzene rings of BCBT molecule, whereas the peak with maximum at λ_{AB} = 265 nm corresponds to electron transitions in oxine and thiazole fragments of BCBT molecules.

We can also conclude that within a range of ratios C_D/C_L BCBT is characterized by a single type of binding with the polynucleotide and two fluorescent systems. In the visible region of the spectrum the first fluorescent sys-

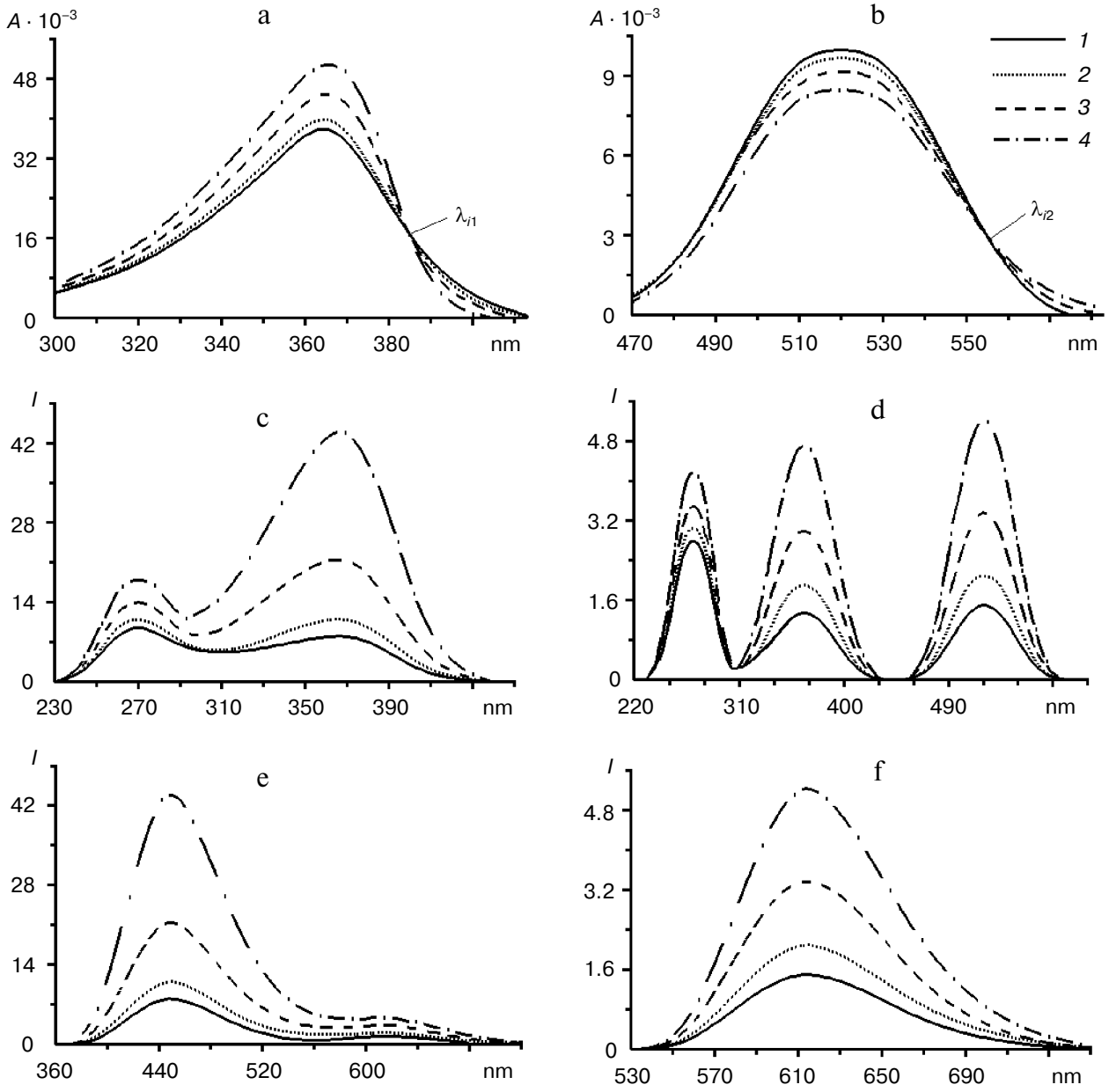

Fig. 6. Spectra of absorbance (a, b), fluorescence excitation (c, d), and emission (e, f) in the presence of various concentrations of DNA. Spectra were recorded at the emission wavelengths of 450 (c) and 615 nm (d) and fluorescence excitation wavelengths of 365 (e) and 520 nm (f), respectively. Curves *1-4* correspond to the ratios of molar concentrations of DNA and dye (C_D/C_L): 0, 1, 10, and 50; λ_{i1} and λ_{i2} indicate the positions of isosbestic points registered on the absorption spectra.

tem has maximums of excitation and emission at λ_{EX} = 365 nm and λ_{EM} = 450 nm, respectively. These characteristics are close to those in phenyl-benzothiazoles [41] and DAPI (table); therefore, it is reasonable to suggest that the benzothiazole fragment is responsible for the existence of the first fluorescence system in BCBT.

The second fluorescence system is characterized by fluorescence intensity that is 5-8 times less than that of the first fluorescence system, and maximums of excitation and emission in the visible region of the spectrum at λ_{EX} = 520 nm and λ_{EM} = 615 nm, respectively. These characteristics are close to those of EtBr (see table). It is also known that the dye possessing more developed system of coupled bonds is characterized by longer wavelengths of its absorbance and emission [40]. So, we conclude that the second fluorescence system of BCBT is apparently determined by its benz-α-chromene fragment.

Thus, there is radiationless fluorescence resonance energy transfer between the two fluorescence systems of

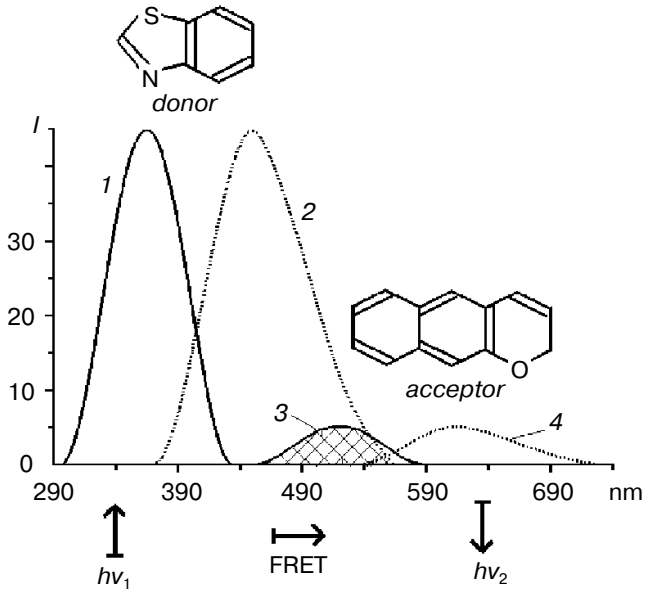

Fig. 7. Scheme of fluorescence resonance energy transfer (FRET) between benzothiazole (donor) and benz-α-chromene (acceptor) fragments of the BCBT molecule at the ratio of molar concentrations of DNA and dye $C_D/C_L = 50$. Curves *1* and *2* represent fluorescence excitation and emission spectra of a "donor", curves *3* and *4* represent fluorescent excitation and emission spectra of an "acceptor"; "$h\nu_1$" and "$h\nu_2$" are light quanta absorbed by the "donor" and emitted by the "acceptor", respectively.

BCBT (Fig. 7). This phenomenon takes place when the region of wavelengths of fluorescence emission of an energy donor (in our case the fluorescence system associated with the benzothiazole fragment of BCBT) is localized within the wavelength region of fluorescence excitation of fluorescence energy acceptor (in our case the fluorescence system associated with the benz-α-chromene fragment of BCBT). In such case when a primarily excited energy donor is involved into weak dipole–dipole interaction with an energy acceptor located at a distance, which is shorter than the wavelength of its emission, additional probability for transition of the donor into electron-oscillation state with lower energy appears simultaneously with transition of acceptor into the state with higher energy.

Efficacy of such resonance energy transfer (E, determined as the ratio of numbers of energy quanta transmitted from donor to acceptor to total number of quanta emitted by donor during its transition from the excited state to the ground state under the same conditions in the absence of the acceptor) depends on the distance between donor and acceptor (f) as:

$$E = f_0^l/(f_0^l + f^l), \qquad (13)$$

where f_0 is Forster's critical radius (such f, at which probability of transition of the donor from the excited state into the ground state due to energy transfer to the acceptor is equal to the sum of probabilities of all other processes, resulting in transition of the donor from the excited state into the ground state under the same conditions in the absence of the acceptor), and $l = 2$, 4, or 6 depending on the type of dipole–dipole interaction between donor and acceptor [42-44].

It should also be noted that the phenomenon when excitation of donor fluorescence is accompanied by emission not only of the donor but also the acceptor can be observed even at distances exceeding the wavelength of the donor emission. However, this involves a different mechanism for energy transfer (the acceptor reabsorbs quanta emitted by the donor) and the dependence of E on f differs from that described by the expression (13).

Study of absorbance and fluorescence spectra of BCBT in the presence of various concentrations of DNA has shown that BCBT is not a very active fluorophore; such characteristics as φ^{50}/φ^0 (ratio of quantum yields of a dye in the presence and in the absence of DNA) and η (coefficient of fluorescent sensitivity, reflecting augmentation of fluorescence intensity of the dye during the increase in DNA concentration in solution by 1 mol/liter at various ratios C_D/C_L) are lower than those for DAPI and EtBr (table). This was quite expectable because earlier it was shown [41, 45] that electron donor and proton donor activity of heterocycles with respect to their substituents as well as affinity to DNA decreased in the order "indole > imidazole > thiazole > oxazole".

Besides spectral properties, we also investigated parameters of BCBT binding with DNA; they were basically the same for both fluorescence systems (within experimental error of ±9%). These experiments have shown that BCBT demonstrates higher affinity and specificity to the polynucleotide substrate than EtBr, but lower affinity and specificity than DAPI (table).

The relative contribution of hydrogen bonds to formation of the active fluorescence complex of BCBT with DNA was 15%. This suggests that the BCBT–DNA system is more sensitive to changes in ionic strength of a solution than the DAPI–DNA and EtBr–DNA systems (table).

Finally, we have determined coefficient of dye sensitivity to tertiary structure of DNA, which was 6% for DAPI, 54% for EtBr, and 104% for BCBT. Since compounds exhibiting external binding are much less sensitive to higher orders of nucleic acid organization than intercalators (this is also confirmed by the above-mentioned data for DAPI and EtBr), one can conclude that binding to the substrate preferentially occurs via intercalation. Bound BCBT was characterized by following differences compared with free BCBT: first, total positive charge of the thiazole cycle decreased; second, there was uncoupling of electron systems of benzothiazole, 4-methylphenylimine, and benz-α-chromene fragments (this decreased electron acceptor influence of benzothia-

zole and 4-methylphenylimine on the benz-α-chromene fragment). This causes the increase in fluorescence of both benzothiazole and benz-α-chromene fragments of the dye during its binding to DNA.

REFERENCES

1. Denison, L., Haigh, A., D'Cunha, G., and Martin, R. F. (1992) *Int. J. Radiat. Biol.*, **61**, 69-81.
2. Gazzard, B. G. (1989) *J. Antimicrob. Chemother.*, **23**, 67-75.
3. Van Iperen, H. P., and van Henegouwen, G. M. J. B. (1997) *J. Photochem. Photobiol. B: Biol.*, **39**, 99-109.
4. Baguley, B. C. (1982) *Mol. Cell. Biochem.*, **43**, 167-181.
5. Osashi, M., and Oki, T. (1996) *Expert. Opin. Therp. Patents*, **6**, 1285-1294.
6. Bartulewicz, D., Markowska, A., Wolczynski, S., Dabrowska, M., and Rozanski, A. (2000) *Acta Biochim. Polonica*, **47**, 23-35.
7. Morgan, A. R., Lee, J. S., Pulleyblank, D. E., Murray, N. L., and Evans, D. H. (1979) *Nucleic Acids Res.*, **7**, 547-571.
8. Darzynkiewicz, Z., Traganos, F., Kapuscinski, J., Staiano-Coico, L., and Melamed, M. R. (1984) *Cytometry*, **5**, 355-363.
9. Zimmer, C., and Wahnert, U. (1986) *Progr. Mol. Biol.*, **47**, 31-112.
10. Campejohn, R. S., Macarthey, J. C., and Morris, R. W. (1989) *Cytometry*, **10**, 410-416.
11. Ivanov, S. D. (1992) *Postradiation Reaction of Blood Leukocyte DNA Nucleotides. Detection*, *Consistency*, *Diagnostic and Prognostic Importance*: Doctoral dissertation [in Russian], Leningrad, Central Rentgenoradiological Institute.
12. Ivanov, S. D., Korytova, L. I., Yamshanov, V. A., Ilyn, N. V., and Sibirtsev, V. S. (1997) *J. Exp. Clin. Cancer Res.*, **16**, 183-188.
13. Pjura, P. E., Grzeskowiuk, K., and Dickerson, R. E. (1987) *J. Mol. Biol.*, **197**, 257-271.
14. Pelton, J. C., and Wemmer, D. E. (1990) *J. Am. Chem. Soc.*, **112**, 1393-1399.
15. Eriksson, S., Kim, S. K., Kubista, M., and Norden, B. (1993) *Biochemistry*, **32**, 2987-2998.
16. Sibirtsev, V. S., Garabadzhiu, A. V., and Ivanov, S. D. (2001) *Russ. J. Bioorg. Chem.*, **27**, 57-65.
17. Muller, W., and Grothers, D. M. (1975) *Eur. J. Biochem.*, **54**, 267-277.
18. Muller, W., Bunemann, H., and Dattagupta, N. (1975) *Eur. J. Biochem.*, **54**, 279-291.
19. Miller, K. J., and Newlin, D. D. (1982) *Biopolymers*, **21**, 633-652.
20. Tomosaka, H., Omata, S., Hasegawa, E., and Anzai, K. (1997) *Biosci. Biotech. Biochem.*, **61**, 1121-1125.
21. Muller, W., and Grothers, D. M. (1968) *J. Mol. Biol.*, **35**, 251-290.
22. Mosher, C. W., Kuhlmann, K. F., Kleid, D. G., and Henry, D. W. (1977) *J. Med. Chem.*, **20**, 1055-1059.
23. Gia, O., Anselmo, A., Consoni, M. T., Antonello, C., Uriarte, E., and Caffieri, S. (1996) *J. Med. Chem.*, **39**, 4489-4496.
24. Ismail, M. A., Sanders, K. J., Fendell, G. C., Latham, H. C., Wormell, P., and Rodger, A. (1998) *Biopolymers*, **46**, 127-143.
25. Pilch, D. S., Yu, Ch., Makhey, D., La Voie, E. J., Srinivasan, A. R., Olson, W. K., Sauers, R. R., Breslauer, K. J., Geacintov, N. E., and Lin, L. F. (1997) *Biochemistry*, **36**, 12542-12553.
26. Edwards, K. J., Jenkins, T. C., and Neidle, S. (1992) *Biochemistry*, **31**, 7104-7109.
27. Rye, H. S., Dabora, J. M., Quesada, M. A., Mathiens, R. A., and Glazer, A. N. (1993) *Analyt. Biochem.*, **208**, 144-150.
28. Larsson, A., Carlsson, Ch., and Jonsson, M. (1995) *Biopolymers*, **36**, 153-167.
29. Surovaya, A. N., Grokhovsky, S. L., Pis'mensky, V. F., Burkhardt, G., Zimmer, K., and Gursky, G. V. (1999) *Mol. Biol. (Moscow)*, **33**, 611-619.
30. Egudina, S. V., Baranov, E. P., and Bogush, T. A. (1991) *VII Conf. on Biopolymer Spectroscopy. Book of Abstracts*, Kharkov, pp. 93-94.
31. Iton, R. J., Veselkov, D. A., Pakhomov, V. I., Baranovsky, S. F., Bolotin, P. A., Osetrov, S. G., Dymant, L. N., Davis, D. B., and Veselkov, A. N. (1999) *Mol. Biol. (Moscow)*, **33**, 803-813.
32. Gaugain, B., Barbet, J., Oberlin, R., Roques, B. P., and Le Pecq, J. B. (1991) *Biochemistry*, **17**, 5071-5088.
33. Bourdouxhe, C., Colson, P., Houssier, C., Sun, J. S., Garestier, M. T., Helene, C., Rivalle, C., Bisagni, E., Waring, M. J., Henichart, J. P., and Bailly, C. (1992) *Biochemistry*, **31**, 12385-12396.
34. Le Pecq, J. B., Le Bret, M., Barbet, J., and Roques, B. P. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 2915-2922.
35. Sibirtsev, V. S., Tolmachev, A. Yu., Kovaleva, M. V., Garabadzhiu, A. V., and Traven', V. F. (2005) *Biochemistry (Moscow)*, **70**, 822-832.
36. Zasedatelev, A. S., Gursky, G. V., and Volkenstein, M. V. (1971) *Mol. Biol. (Moscow)*, **5**, 245-251.
37. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.*, **51**, 660-672.
38. Sibirtsev, V. S. (2006) *Biochemistry (Moscow)*, **71**, 90-98.
39. Mishra, A. K., and Dogra, S. K. (1983) *Spectrochim. Acta*, **39a**, 609-611.
40. Krasovitsky, B. M., and Bolotin, B. M. (1984) *Organic Luminophores* [in Russian], Khimiya, Moscow.
41. Ivanov, S. D., Kvitko, I. Ya., Rtischev, N. I., Fomina, E. I., and Nagorskaya, L. P. (1989) *Russ. J. Bioorg. Chem.*, **15**, 608-615.
42. Ermolaev, V. L., Bodunov, E. N., Sveshnikova, E. B., and Shakhverdov, T. A. (1977) *Nonradiative Transfer of Electron Excitement Energy* [in Russian], Nauka, Leningrad.
43. Speiser, S. (1996) *Chem. Rev.*, **96**, 1953-1976.
44. Lankiewicz, L., Malika, J., and Wiczk, W. (1997) *Acta Biochim. Polonica*, **44**, 477-490.
45. Sibirtsev, V. S., and Garabadzhiu, A. V. (1997) *Russ. J. Org. Chem.*, **33**, 1810-1813.