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Dependence of Fluorescence Properties of Compounds from Psoralen, Angelicin, and Carbazole Series on the Character of Their Terminal Substituents

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Abstract—Absorption, luminescence excitation and emission spectra of nine compounds from 4,8,4'-trimethylpsoralen, 4,4'-dimethylangelicin, and 1,2,3,4-tetrahydrocarbazole series with various terminal substituents were studied in water and 2-propanol. Proceeding from the data obtained in the present and also some previous studies we have formulated the main rules concerning a general mechanism of changes in the fluorescence characteristics of DNA-specific dyes depending on their chemical structure, substrate properties, and measurement medium.

Nowadays the application expands of artificially produced relatively low-molecular compounds capable of specific bind with a certain nucleotide sequence in a genome. Such compounds may be used as radioprotectors [1], antibacterial [2, 3], and antitumor [4–6] pharmaceuticals. When alongside a good affinity to a substrate and a high specificity the DNA-binding compound possesses also convenient measurable properties (for instance, fluorescence that sharply changes at binding with a nucleotide) the application range of the compound becomes still wider. In particular, compounds with these properties can be used for study of nucleic acids structure, enzymatic kinetics [7–10]. Besides these compounds can be applied to development of highly sensitive and specific express-analyses for diagnostics of various diseases and harmful influence based on pathology analysis from the viewpoint of changes in content and structure of nucleic acids during various physiological states of a body [11, 12].

All multitude of DNA-binding compounds known nowadays is divided by the type of interaction with the substrate in two large classes: intercalators and so-called externally binding compounds. Therewith the requirements for the structure of the latter class compound are the least stringent: in its molecule should be present at least a single heteroatom, and besides in case the compound should possess measurable spectral properties the presence in the molecule of an extended system of conjugated bonds is favorable. These molecules add to the polynucleotide from outside, not affecting the substrate structure (although therewith occurs some compression, and also local changes in allosteric conformation of the DNA double helix in the region of binding). Such substances are mainly specific to the primary and secondary organization orders of the nucleic acids structure [9, 13–16].

At the same time the intercalator molecule must contain one or several fragments of fused aromatic systems with two or more (preferably three or four) rings (preferably 5- or 6-membered), and at least one heteroatom (preferably endocyclic). These compounds build in between the complementary pairs of bases of the polynucleotide double helix (effecting local unwinding of the helix and its elongation), and they are specific mainly to the secondary structure of nucleic acids and to extent of their superspiralization [17–20].

Quite a number of DNA-fluorophores is now known and widely applied [7, 9, 16–18]. However the target of developing theoretical foundation for building up new compounds with higher sensitivity and specificity with respect to substrate is still urgent. To this end it is necessary in its turn to have an exact understanding of connection between the chemical structure of the nucleotide-specific dyes and their spectral and complexing characteristics.

In the present study were chosen as objects 9 newly synthesized derivatives of 4,8,4'-trimethyl-

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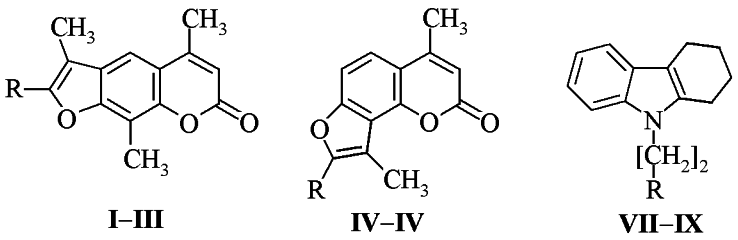
psoralen (**I–III**), 4,4'-dimethyangelicin (**IV–VI**), and 1,2,3,4-tetrahydrocarbazole (**VII–IX**) with various terminal substituents in positions *5'* (for compounds **I–VI**) and *9* (for compounds **VII–IX**) [see Scheme (1)].

We selected these compounds for both psoralens and angelicins were recently frequently used in photochemotherapy, in particular in the case of psoriasis [21, 22], for blood sterilization [3], for site-directed RNA modification [23] etc. This wide application range originates from the behavior of these compounds: The compounds of both mentioned series first reversibly intercalete into the double helix of the polynucleotide (therewith as a rule psoralens predominantly are bound with the AT-rich regions, and angelicins are nonspecific to the primary structure of nucleic acids), and then after sufficiently long (up to 1 h) irradiation in the wavelength range λ 250–370 nm are linked to substrate irreversibly with covalent bonds. Therewith the angelicins are less toxic than psoralens for the latter are more prone to cross-linking two strands of DNA; this is evidently due to shorter angular angelicin core compared to linear psoralen one.



(1)

C(CH_3)=NOH (**I, IV**), C(O)CH_3 (**II, V**), CH(CH_3)OCH_3 (**III**), CH(CH_3)OH (**VI**), C≡N (**VII**), C(O)OH (**VIII**), C(O)NHNH_2 (**IX**).

A number of carbazole derivatives also exhibit significant antimicrobial [24] and antitumor [25, 26] activity.

The spectral characteristics of compounds **I–IX** were studied in two media: solutions in water and 2-propanol. As was described earlier [27, 28] in the latter solvent because of its low permittivity fluorescence was observed from the planar core proper of compound excluding the influence thereto of electrondonor or electron-acceptor effect of the terminal substituents.

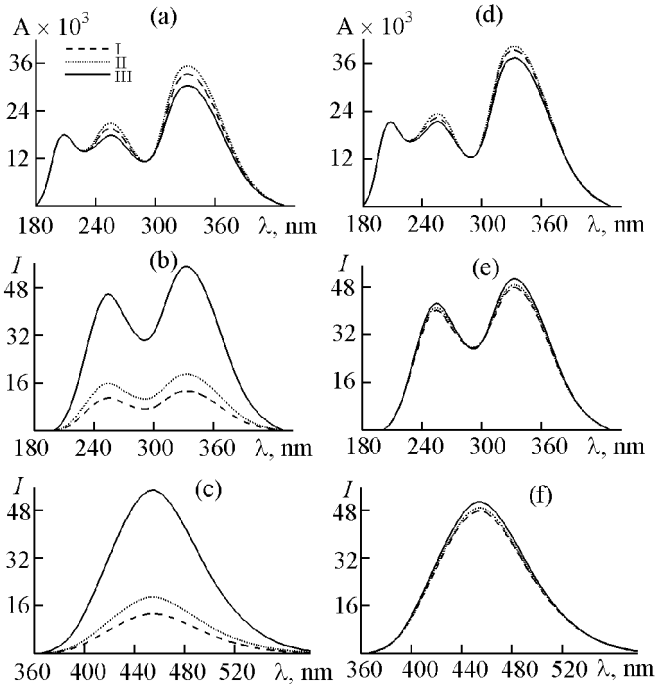


Fig. 1. Adsorption (a, d), fluorescence excitation (b, e), and emission spectra (c, f) of 4,8,4'-trimethylpsoralens (**I–III**) in aqueous buffer solution (a–c) and in 2-propanol (d–f).

As seen from Figs. 1–3 in the spectra of all compounds studied in this work the wavelengths in the absorption maxima (λ_{ab}) coincide with the wavelengths of the fluorescence excitation maxima (λ_{ex}). An exclusion is the absorption peak with a maximum at λ_{ab} 210 nm that does not appear in the fluorescence excitation spectra and evidently corresponds to electron transitions in benzene rings in the structure of the compounds under investigation [29]. The other observed absorption maxima in the spectra of compounds from furocoumarin series **I–VI** are located at λ_{ab} 255 and 330 nm regardless of the measuring conditions; in the spectra of compounds of tetrahydrocarbazole series **VII–IX** the maxima λ_{ab} in water medium are 240 and 290 nm, and in 2-propanol 255 and 355 nm respectively.

Wavelengths at the maxima of the fluorescence emission spectra (λ_{em}) for compounds of the furo-

coumarin series **I–VI** were λ_{em} 455 nm in both solvents, and for compounds **VII–IX** from the tetrahydrocarbazole series they were as follows: in water medium λ_{em} 380 nm and in 2-propanol λ_{em} 360 nm.

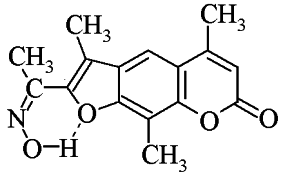
The comparison of values of fluorescence quantum efficiency for compounds **I–IX** in 2-propanol (φ_A) and in water (φ_W) shows that in all cases the former value is somewhat larger than the latter (1.2–4.2 times) (see table). Therewith the η_A values for related compounds **I–III, IV–VI**, and **VII–IX** were more alike than η_W, and decreased in the order from tetrahydrocarbazole to trimethylpsoralen to dimethylangelicin series.

Fluorescence quantum efficiency (φ) of compounds under investigation[a]

Compd. no.	φ_W	φ_A	φ_A/φ_W
I	0.096	0.293	3.05
II	0.113	0.296	2.62
III	0.193	0.301	1.56
IV	0.069	0.282	4.20
V	0.092	0.285	3.11
VI	0.131	0.289	2.20
VII	0.334	0.484	1.45
VIII	0.383	0.490	1.28
IX	0.413	0.496	1.20

[a] With indices (W) and (A) are marked the parameters measured for compounds **I–IX** in the aqueous buffer solution and 2-propanol, respectively.

As seen from the table in the series of compounds **I–III** and **IV–VI** is also observed decrease in the ratio φ_A/φ_W evidently originating from reduction of the electron-acceptor effect with respect to the furocoumarin core in the series of groups HO–N = > O = > HO–> H_3C–O–. Obviously the hydroxyimino group is the first in this series due to both strengthening of the nitrogen electron-acceptor properties by the hydroxy group and by the possibility for this hydroxy group to form a hydrogen bond with the oxygen of the furan ring as shown in Scheme (2).



(2)

Oxo group attracts electron density from the furocoumarin core to greater extent than the hydroxy

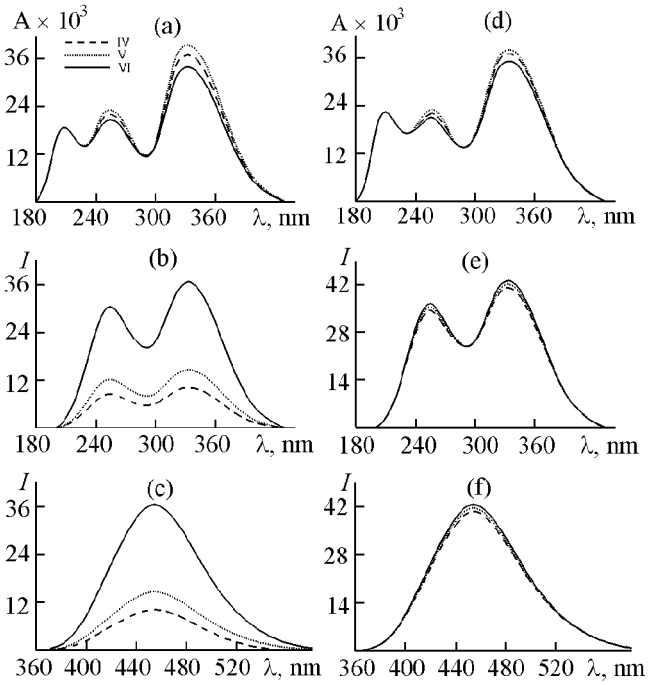


Fig. 2. Absorption (a, d), fluorescence excitation (b, e), and emission spectra (c, f) of 4,4′-dimethylangelicins (**IV–VI**) in aqueous buffer solution (a–c) and in 2-propanol (d–f).

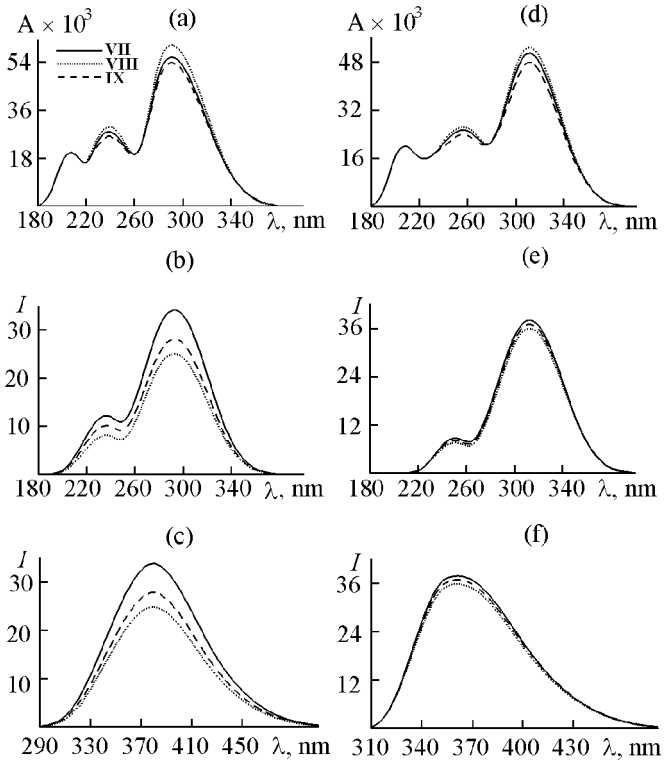


Fig. 3. Absorption (a, d), fluorescence excitation (b, e), and emission spectra (c, f) of 1,2,3,4-tetrahydrocarbazoles (**VII–IX**) in aqueous buffer solution (a–c) and in 2-propanol (d–f).

group because the former is bonded to the core by a double bond, and methoxy group is a weaker electron-acceptor than hydroxy group for its oxygen can attract electron density not only from the furocoumarin core but also from the methyl group.

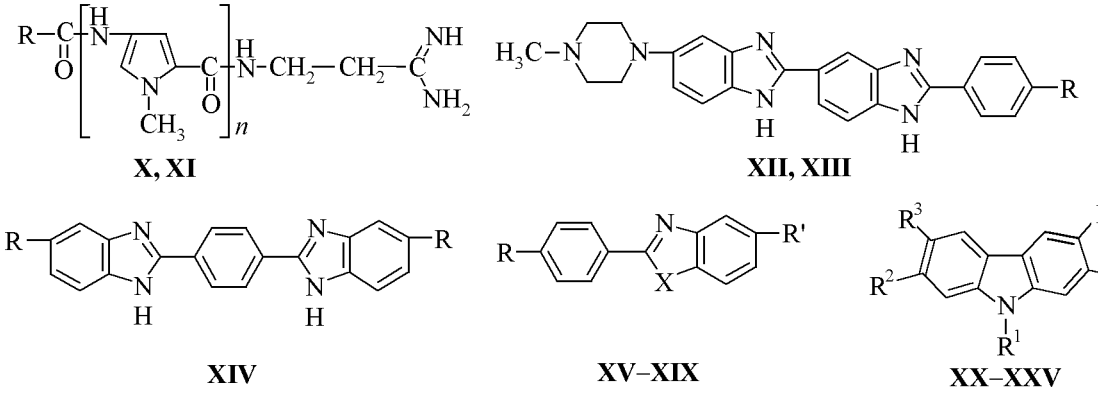
A similar decrease in the value of φ_A/φ_W was also observed in the series of compounds **VII–IX**. It was caused evidently by reduced electron-acceptor character toward the tetrahydrocarbazole core of carboxy group compared to nitrile one and of hydrazinocarbonyl group compared to carboxyl. However the range of changes was considerably smaller in compounds **VII–IX** than in compounds **I–VI** apparently due to longer distance (through two additional CH_2 groups) from the modified terminal substituents to the aromatic core.

Thus we found that for the intercalators were also valid the statements which we had formulated before [27, 28, 30] by an example of the externally bound to DNA compounds: In simulating the fluorescence properties of dyes both specific and nonspecific to nucleotides the factors listed below should be considered.

(1) Active fluorescence can be produced only from the structures with developed electronic conjugation system involving π-electrons of multiple (double and triple) bonds or unshared *n*-electrons of heteroatoms.

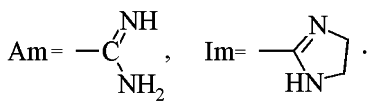
(2) This conjugation is fulfilled only when this electron system possesses sufficiently rigid coplanar structure (that is, all fragments involved thereto are located in the same plane).

The latter condition is as a rule met by intercalators with a core that as was already mentioned should by definition be built of several fused aromatic rings. However for compounds externally binding to DNA, e.g., netropsin, distamycin A [9], Hoechst-33258, Hoechst-33258 [27] [see compounds **X–XIII** in Scheme (3)] etc. the stabilization of the planar molecular structure preventing reciprocal rotation of separate building blocks of the molecule and therewith increasing the overall quantum efficiency of fluorescence can be achieved only in very viscous media (e.g., in saccharose solution [30]), by insertion of certain substituents (for instance, 3-dimethylaminopropylcarbamoyl, as in compound **XIV**, Scheme 3 [27, 30]), or at specific interaction with a substrate.



X, XI **XII, XIII** **XIV** **XV–XIX** **XX–XXV** (3)

R = $CH_2NHC(NH_2)=NH$, *n* = 2 (**X**); R = H, *n* = 3 (**XI**); R = OH (**XII**); OCH_2CH_3 (**XIII**); $C(O)NH[CH_2]_3N(CH_3)_2$ (**XIV**); R = H, R' = NH_2, X = O (**XV**); R = R' = NH_2, X = O (**XVI**); R = R' = NH_2, X = S (**XVII**); R = COONa, R' = NH_2, X = S (**XVIII**); R = COONa, R' = $N(CH_3)_2$, X = S (XIX); R^1 = CH_3, R^2 = Im, R^3 = H (XX); R^1 = CH_3, R^2 = H, R^3 = Am (**XXI**); R^1 = H, R^2 = Im, R^3 = H (**XXII**); R^1 = H, R^2 = Am, R^3 = H (**XXIII**); R^1 = H, R^2 = H, R^3 = Im (**XXIV**); R^1 = H, R^2 = H, R^3 = Am (**XXV**);



(3) When the core of a molecule possesses a sufficiently developed and rigid electronic conjugated structure (as, for instance, in compounds **I–IX** under consideration in this study) the quantum efficiency of fluorescence (φ) of this molecule in nonbound state in polar solvent can be significantly reduced by introduction of substituents electron-acceptor with respect to the core (or the quantum efficiency would be increased if the introduced substituent would be an electron-donor toward the core). On the contrary in

the media of low permittivity (as, e.g., 2-propanol) or at specific interaction with a substrate the above mentioned effect of the substituents on the core of the ligand is considerably reduced due to the changed character of the microenvironment of the molecule, and as a result the overall quantum efficiency of fluorescence (φ) becomes close in value to φ of the core of compound proper (i.e. without any substituents).

It is possible to build a series of substituents in the order of decreasing electron-donor (and consequently increasing electron-acceptor) activity with respect to the core of the fluorophore (as we have for instance done in [29, 30]) proceeding from certain simple principles [28, 31] defined below:

– The electrnegativity of heteroatoms with respect to hydrocarbon moiety linked thereto decreases in the series O > N > S > P;

– π-Electrons are capable to shift to the more electronegative atom to a greater extent than *n*-electrons, and the latter shift better than σ-electrons;

– A heteroatom linked to an aromatic ring with an ordinary bond shows with respect to the ring electron-donor properties owing to its unshared electron pairs, and the more the less is the electronegativity of the heteroatom;

– A heteroatom linked to a neutral nonaromatic hydrocarbon moiety shows toward the latter electron-acceptor properties, and the more the higher is the proper electronegativity of the heteroatom;

– The more a heteroatom withdraws the electron density from one of groups bonded thereto, the less it is prone to attract it from the other groups linked to it;

– Positively charged substituents are electron-acceptors with respect to a neutral aromatic core bonded thereto, and negatively charged substituents play part of electron-donors;

– therewith the greater delocalization of the charge, the less is the influence of this substituen, etc., and from the already known Hammett's and Taft's constants [28, 32].

It is logic to assume that in the same order as decrease the electron-donor properties of substituents would decrease the relative values of fluorescence quantum efficiency of compounds having such terminal substituents measured in water medium (at high permittivity) in the absence of substrates capable of specific interaction with these compounds, and also at similar character of their core. On the contrary the ratio of quantum efficiency of fluorescence measured in alcohol and in water (φ_A/φ_W) (or in water in the presence of DNA and in the absence of the polynucleotide) would increase in the above indicated order.

(4) However the pattern often is even more complicated. That is, sufficiently often the compounds in question can be present in solution in several equilibrium forms (distinguished by extent of ionization of the core and substituents, by the number of intramolecular hydrogen bonds, etc.), and moreover, the relative content of these forms can change depending on pH and the solvent character.

For instance, taking phenylbenzazoles **XV**–**XIX** (see Scheme 3) described in [33], it was found that although nonionized NH_2 group would have showed electron-donor effect with respect to the aromatic core, the fluorescence quantum efficiency in aqueous buffer solution with no DNA was considerably smaller for compound **XVI** (φ_W 0.12) than for compound **XV** (φ_W 0.87) which was distinguished from dye **XVI** only by the absence of a terminal amino group at the benzoxazole fragment of its molecule. It means that under the described conditions the amino group was present predominantly in the ionized H_3N^+-form that behaved with respect to the bound thereto benzoxazole core of compound **XVI** as electron-acceptor. Similarly nonionized carboxy group should be an electron-acceptor toward the aromatic fragment linked to it, and in ionized $^-$OOC-form the character of the influence on the neutral core of the compound becomes electron-donating. As a result at replacement of amino group of compound **XVII** by a carboxy group in compound **XVIII** the φ_W value of the latter considerably grew (from 0.07 in **XVII** to 0.55 in **XVIII**). Finally, for dyes **XVIII** and **XIX** evidently the properties of the terminal dimethylamino group in compound **XIX** were decisive: in ionized state it was a weaker acceptor, and in nonionized form a stronger donor with respect to the phenylbenzothiazole core than the corresponding amino group in compound **XVIII** in the respective state. Apparently this was the reason of higher φ_W for dye **XIX** than for compound **XVIII** (φ_W 0.66 and 0.55 respectively).

In the same way the quantum efficiencies of carbazoles **XX**–**XXV** [34, 35] (see Scheme 3) in water medium with no DNA may be compared using the ratio $\varphi_2/\varphi_1 = (I_2A_1)/(I_1A_2)$ [27] [where I_1, I_2 and A_1, A_2 are intensities of fluorescence and optical density of compounds (1) and (2) at wavelengths corresponding to the maxima on their absorption and emission spectra reduced to unit dye concentration]. After that it is possible to establish that the derivatives with a methyl group in *9* position possess smaller φ_W value than analogous compounds with only a hydrogen in the same position. Similarly the derivatives with amidine (AM) groups in positions *2, 7* or *3, 6* have

smaller φ_W value than analogous compounds with imidazoline (Im) substituents. This happens although obviously in a neutral form of the compound the methyl group is a stronger electron donor for the carbazole core than single hydrogen, and Am groups are stronger acceptors compared to Im groups. Thus the observed pattern is apparently due to the presence in the water medium at pH ~7 (see the measuring conditions in [34, 35]) alongside neutral forms of compounds under study also positively-charged ones. Since the basicity of nitrogen is known to decrease in the series $R_3N > R_2NH > RNH_2 > NH_3$ [31] the relative equilibrium ratio of ionized and nonionized forms should be larger for H_3C-N-derivatives than for analogous HN-derivatives, and for Im-derivatives larger than for Am-derivatives. We believe that this situation is reflected in the overall values (summing up all the forms) of φ_W compounds under consideration.

The procedure of evaluating an overall electron-donor or electron-acceptor effect of any substituent on the core of fluorophore which is present in solution in N different equilibrium form we have treated before [36]. In the most general case the combined influence on the fluorescence of the compound in question of all above mentioned factors should be taken into account.

EXPERIMENTAL

Compounds **I**–**VI** were synthesized by A. Yu. Tolmachev, and compounds **VII**–**IX** were prepared by A. Yu. Suslov at the Chair of Organic Chemistry, Mendeleev Russian Chemical Technological University. ^{1}H NMR spectra were registered on spectrometer Bruker AC-200 at operating frequency 200 MHz from solutions in deuterochloroform (internal reference TMS) or from solutions in dimethyl sulfoxide. Mass spectra were measured on SSQ-710 instrument (Finnigan-MAT) at ionizing electrons energy 70 eV. The reaction progress was monitored by TLC on Silufol UV-254 plates (eluent chloroform). The chromatographic separation was carried out on silica gel(eluent chloroform).

All photometric and fluorometric measurements were carried out at concentration of compounds **I**–**IX** equal to **IX**) $C_L = 4 \times 10^{-6}$ M and at temperature 20–25°C in an aqueous buffer solution of the following composition: 0.01 M NaCl, 0.01 M Na_2EDTA, and 0.01 M Tris (pH 7.4), and also in 2-propanol. As a substrate was used calf tymus DNA (58% AT-pairs; average molecular weight per one nucleotide 326Da, molar extinction χ_{260} = 6600 M^{-1}–cm^{-1}). The substrate solution was prepared by dissolving a dry substance (Serva, Germany) in distilled water followed by treating with ultrasound (for homogenization and reduction of light scattering) on UZDN-2 device (Russia) for 15 s at 0.3 A current and with resonance frequency 22 kHz. After the treatment the average molecular weight of DNA was 3500 Da. The sodium ethylenediaminetetraacetate and Tris (2-amino-2-hydroxymethyl-1,3-propandiol) were also commercial products from Serva, Germany. The other reagents used were of "chemically pure" grade.

The absorption spectra of solutions under study were recorded on spectrophotometer Beckman Model 35 (Austria). The fluorescence was measured on a spectrofluorimeter Hitachi model 850 (Japan). In registering the spectra on fluorimeter the slots of monochromators of excitation and emission were set at 3 nm, rate of scanning 120 nm min^{-1}, response time 2 s, normal amplification of photomultiplier. The excitation spectra were registered at wavelengths corresponding to the maxima of the most longwave among the registered excitation peaks of compound under study. All the spectra were registered with subtraction of background. The fluorescence spectra were corrected using quantum counter basing on alcoholic rhodamine B solution in keeping with the instruction manual supplied with the spectrofluorimeter. The spectral characteristics were measured with the use of standard cells of square cross-section 1 cm thick.

Quantum efficiency of fluorescence (φ) of dyes were determined by comparison with quinine sulfate 1 M solution in sulfuric acid taken as reference (φ 0.55).

8-Acetyl-4,9-dimethyl-2*H*-furo[2,3-h]chromen-2-one (I) and 2-acetyl-3,5,9-trimethyl-7*H*-furo[3,2-*g*]-chromen-7-one (II). To a boiling solution of an appropriate *o*-acetyl-7-hydroxycoumarin (2.3 mmol) and potassium carbonate (1.58 g, 0.01 mmol) in anhydrous acetonitrile (40 ml) was added dropwise at vigorous stirring a solution of chloroacetone (0.24 g, 2.6 mmol) in anhydrous acetonitrile (15 ml). The reaction mixture was stirred at reflux for 3–4 h. Then the mixture was cooled to room temperature, acetonitrile was evaporated in a vacuum. To the residue 10% water solution of hydrochloric acid was added to neutralize potassium carbonate till pH 2–3. The precipitate was filtered off,washed with water, and dried in air. Then it was recrystallized from 95% ethanol.

Compound **I** was prepared from 8-acetyl-7-hydroxy-4-methylcoumarin. Yield 0.38 g (65%),

mp 231–232°C (ethanol). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.51 d (3H, C_4CH_3, *J* 1.14 Hz), 2.92 s (3H, C^9CH_3), 2.63 s (3H, $COCH_3$), 6.30 d (1H, C^3H, *J* 1.14 Hz), 7.43 d (1H, C^6H, *J* 8.84 Hz), 7.67 d (1H, C^5H, *J* 8.86 Hz). Mass spectrum, *m/z* (I_{rel}, %): 256 (100) $[M]^+$. Found, %: C 70.26; H 4.68. $C_{15}H_{12}O_4$. Calculated, %: C 70.31; H 4.72; O 24.97.

Compound **II** was prepared from 6-acetyl-7-hydroxy-4,8-dimethylcoumarin. Yield 0.35 g (56%), mp 239–240°C (ethanol). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.53 d (3H, C^5CH_3, *J* 1.16 Hz), 2.63 s (3H, C^9CH_3), 2.64 s (3H, $COCH_3$), 2.65 s (3H, C^3CH_3), 6.30 d (1H, C^6H, *J* 1.16 Hz), 7.69 s (1H, C^4H). Mass spectrum, *m/z* (I_{rel}, %): 270 (100) $[M]^+$. Found, %: C 71.16; H 5.23. $C_{16}H_{14}O_4$. Calculated, %: C 71.10; H 5.22; O 23.68.

8-Acetyl-4,9-dimethyl-2H-furo[2,3-h]chromen-2-one ketoxime (III) and 2-acetyl-3,5,9-trimethyl-7*H*-furo[3,2-*g*]chromen-7-one ketoxime (IV). To a solution of an appropriate acetylfurocoumarin (2.3 mmol) in a mixture of ethanol (30 ml) and pyridine (10 ml) was added hydroxylamine hydrochloride (0.24 g, 3.5 mmol), and the mixture obtained was boiled for 3–4 h. Then the hot reaction mixture was poured into ice water (150 ml). The separated precipitate was filtered off, washed first with 10% solution of hydrochloric acid and then with water, and dried in air.

For compound **III** yield was 93%, mp 234–235°C (methanol). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.26 s (3H, $CH_3C{=}NOH$), 2.54 (3H, C^4CH_3, *J* 1.10 Hz), 2.70 s (3H, C^9CH_3), 6.33 d (1H, C^3H, *J* 1.10 Hz), 7.52 d (1H, C^6H, *J* 8.80 Hz), 7.72 d (1H, C^5H, *J* 8.80 Hz), 11.65 s (1H, OH). Mass spectrum, *m/z* (I_{rel} , %): 271 (80) $[M]^+$, 254 (100) $[M–OH]^+$. Found, %: C 66.38; H 4.82; N 5.14. $C_{15}H_{13}NO_4$. Calculated, %: C 66.41; H 4.83; N 5.16; O 23.59.

For compound **IV** yield was 92%, mp 234–235°C (methanol). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 2.21 s (3H, $CH_3C{=}NOH$), 2.45 s (3H, C^3CH_3), 2.50 (3H, C^5CH_3, *J* 1.16 Hz), 2.55 s (3H, C^9CH_3), 6.23 d (1H, C^6H, *J* 1.16 Hz), 7.69 s (1H, C^4H), 11.42 s (1H, OH). Mass spectrum, *m/z* (I_{rel}, %): 285 (33) $[M]^+$, 269 (100) $[M–H–CH_3]^+$. Found, %: C 67.32; H 5.32; N 4.90. $C_{16}H_{15}NO_4$. Calculated, %: C 67.36; H 5.30; N 4.91; O 22.43.

8-(1-Hydroxyethyl)-4,9-dimethyl-2*H*-furo[2,3-h]-chromen-2-one (V). A suspension of 8-acetyl-4,9-dimethylangelicin (0.51 g, 2.0 mmol) and sodium borohydride (0.1 g, 2.6 mmol) in ethanol (40 ml) was stirred at room temperature for 6–8 h. On completion of reaction the mixture was poured into ice water (150 ml). The separated precipitate was filtered off, washed with water, and dried in air. Then it was recrystallized from methanol. Yield of alcohol V was 0.39 g (78%), mp 160–161°C (methanol). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.65 d (3H, CH_3CH, *J* 6.64 Hz), 2.12 br.s (1H, OH), 2.46 (3H, C^4CH_3, *J* 1.18 Hz), 2.55 s (3H, C^9CH_3), 5.12 q (1H, CH_3CH, *J* 6.64 Hz), 6.21 d (1H, C^3H, *J* 1.18 Hz), 7.32 d (1H, C^6H, *J* 8.68 Hz), 7.43 d (1H, C^5H, *J* 8.68 Hz). Mass spectrum, *m/z* (I_{rel}, %): 258 (46) $[M]^+$, 243 (100) $[M–CH_3]^+$. Found, %: C 69.72; H 5.44. $C_{15}H_{14}O_4$. Calculated, %: C 69.76; H 5.46.

2-(1-Methoxyethyl)-3,5,9-trimethyl-7*H*-furo-[3,2-g]chromen-7-one (VI). To a solution of compound **II** (0.54 g, 2 mmol) in a mixture of dichloromethane (70 ml) and methanol (10 ml) was added sodium borohydride (0.1 g, 2.6 mmol). The reaction mixture was vigorously stirred at room temperature for 6–8 h (TLC monitoring). On completion of reduction to the reaction mixture was added concn. HCl (3 ml), and the mixture was stirred for 3 min more. Then to the reaction mixture 200 ml of water was added, and it was transferred into a separatory funnel. The organic layer was separated, and the water layer was extracted with dichloromethane (2 × 5 ml). The organic solutions were combined and dried with anhydrous sodium sulfate. The solvent was evaporated in a vacuum, and the residue was subjected to column chromatography on silica gel (eluent chloroform). Yield of ether **VI** was 0.41 g (71%), mp 128–129°C (methanol). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.62 d (3H, CH_3CH, *J* 6.62 Hz), 2.31 s (3H, C^3CH_3), 2.52 d (3H, C^5CH_3, J 1.12 Hz), 2.60 s (3H, C^9CH_3), 3.30 s (3H, OCH_3), 4.58 m (1H, CH_3CH), 6.24 d (1H, C^6H, *J* 1.12 Hz), 7.50 s (1H, C^4H). Mass spectrum, *m/z* (I_{rel}, %): 286 (20) $[M]^+$, 271 (54) $[M–CH_3]^+$, 255 (100) $[M–OCH_3]^+$. Found, %: C 71.27; H 6.32. $C_{17}H_{18}O_4$. Calculated, %: C 71.31; H 6.34.

3-(1,2,3,4-Tetrahydro-9*H*-carbazol-9-yl)propionitrile (VIII). To acrylonitrile (28.12 g, 0.53 mol) at 40–45°C was added dropwise phenylhydrazine (54.07 g, 0.5 mol) and Triton B (5 drops). Then the mixture was heated at 100°C for 1.5 h. On cooling cyclohexanone (53.97 g, 0.55 mol) an ethanol (100 ml) was added. The mixture was heated again, and into the boiling reaction mixture was added dropwise concn. HCl (50 ml). Then the mixture was boiled for 30 min, cooled, the separated precipitate was filtered off, washed with water, dried, and recrystallized from alcohol. Yield of compound **VIII**

was 95.33 g (85%), fine colorless crystals, mp117–119°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.8–2.0 m (4H), 2.6–2.7 m (4H), 2.7 t (2H), 4.3 t (2H), 7.0–7.5 m (4H).

3-(1,2,3,4-Tetrahydro-9*H*-carbazol-9-yl)propionic acid (VII). Compound **VIII** (67.23 g, 0.3 mol) was dissolved in boiling concn. HCl, and the solution was boiled for 2 h more. On cooling to room temperature it was diluted with water (300 ml), the separated precipitate was filtered off, washed with water, and dried. Yield of compound **VII** was 58.39 g (80%), fine colorless crystals, mp 119–120°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.8–2.0 m (4H), 2.6–2.7 m (4H), 2.7 t (2H), 4.3 t (2H), 7.0–7.5 m (4H), 9.1 © (1H).

3-(1,2,3,4-Tetrahydro-9*H*-carbazol-9-yl)-propanoylhydrazide (IX). A mixture of compound **VII** (48.66 g, 0.2 mol), ethanol (100ml), toluene (150 ml), and toluenesulfonic acid (0.2 g) was boiled for 15 h distilling off the water–toluene azeotrope. Then the reaction mixture was washed with water, 10% solution of sodium carbonate, and again with water. Then mixture was evaporated at reduced pressure, and the residue was distilled in a vacuum. Thus was obtained ethyl 3-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)propionate (**XXVI**) in 54.27 g (85%) yield, n_D^{20} 1.568, bp 228–230°C (15 mm Hg). Then a mixture of compound **XXVI** (13.56 g, 0.05 mol), hydrazine hydrate (40 ml), and ethanol (150 ml) was heated to 100°C for 3 h. Then the mixture was evaporated at reduced pressure and left standing for 10 h for crystallization. The separated precipitate was filtered off, washed with water, with alcohol, and dried. Yield of compound **IX** was 11.83 g (92%), mp 142–143°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.8–2.0 m (4H), 2.4 t (2H), 2.6–2.7 m (4H), 3.7 s (2H), 4.3 t (2H), 6.7 s (1H), 7.0–7.4 m (4H).

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