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Dedicated to the memory of Prof. L. I. Bagal on the 100th anniversary of his birth

Effect of Heteroatom on the Spectral Properties of Benzazoles

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

[*] *Central Research Roentgenoradiological Institute, Ministry of Health Protection of the Russian Federation, St. Petersburg, Russia*

[**] *St. Petersburg State Institute of Technology, Moskovskii pr. 26, St. Petersburg, 198013 Russia; fax: (812) 112 7791*

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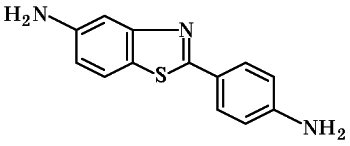
Abstract—Electron absorption and fluorescence spectra of a series of 2-phenylbenzazoles in water and alcohol have been analyzed. The results obtained suggest that the indole fragment can be regarded as an optimal constituent for newly synthesized potential DNA-binding fluorophores.

Artificial low-molecular-weight compounds capable of being specifically bound to definite nucleotide sequences in a genome currently receive extending applications. Such compounds can be used directly as radioprotectors and antitumor, antibacterial and antiviral preparations [1, 2]. When a DNA-binding compound, apart from high specificity, possesses properties facilitating its registration (e.g., when its fluorescence parameters sharply change upon binding with a polynucleotide), the field of its application considerably extends. In particular, such compounds can be used as DNA-tropic probes for fast estimation of biological contamination of water. In addition, highly sensitive and specific methods for express diagnostics of hereditary, malignant, and infectional diseases and radiation damages can be developed on this basis [3, 4].

It should be noted that the majority of DNA-sensitive fluorophores contain one or more heterocyclic fragments which both endow them with fluorescent properties and ensure specificity of their interaction with polynucleotides. For instance, DNA-binding compounds should necessarily contain fragments consisting of one or two fused aromatic rings, one of which should be five-membered and include at least one heteroatom [5]. A molecule of potential intercalator should contain one or several fused aromatic systems consisting of three or more rings, one of them should necessarily contain a heteroatom [6].



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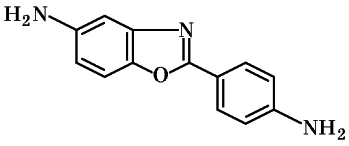

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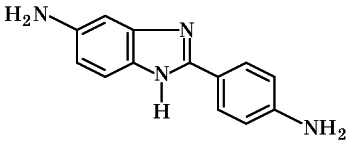

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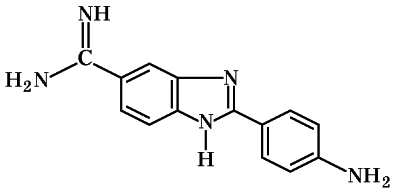

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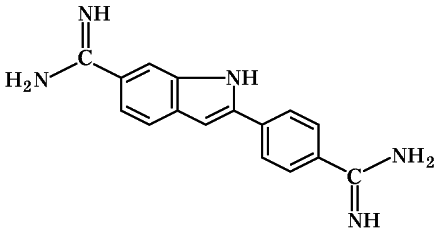

V

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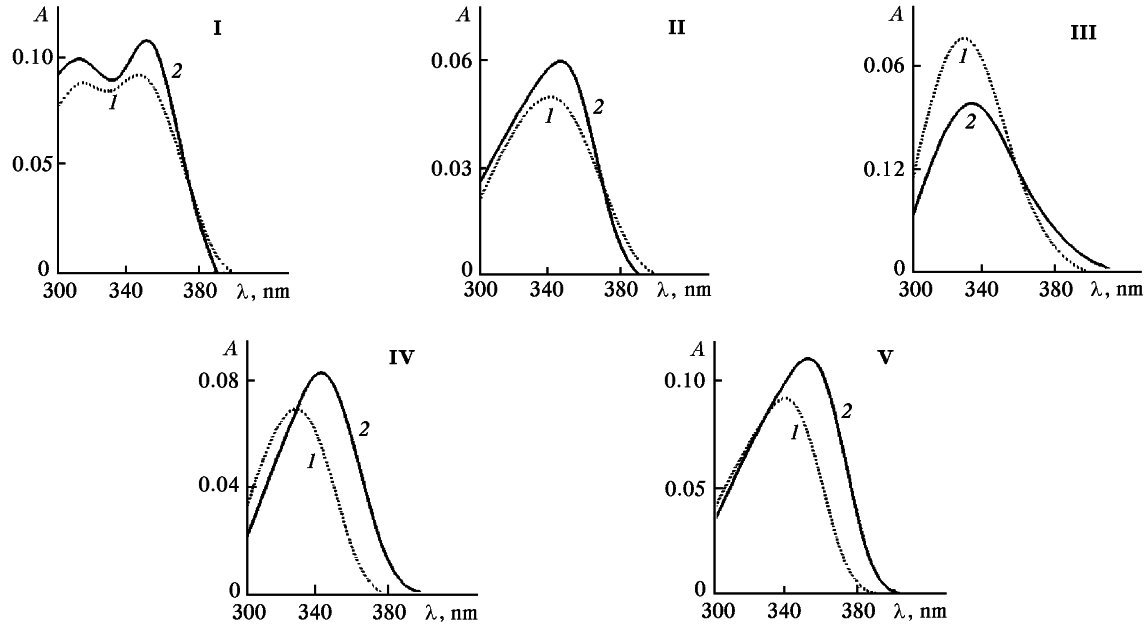

Fig. 1. Electron absorption spectra of compounds **I**–**V** in (*1*) water and (*2*) 2-propanol; concentration 4.0×10^{-6} M.

In view of the above stated, for development of theoretical principles for creation of new potential DNA-sensitive fluorophores it was of interest to perform a quantitative study of the effect of heteroatom in benzazoles on their spectral properties. It is known [7–9] that benzazole ring system is a key structural fragment of many DNA-binding ligands synthesized in recent time.

We chose five compounds: 5-amino-2-(4-aminophenyl)benzothiazole (**I**), 5-amino-2-(4-aminophenyl)-benzoxazole (**II**), 5(6)-amino-2-(4-aminophenyl)benzimidazole (**III**), 5(6)-amidino-2-(4-aminophenyl)benzimiazole (**IV**), and 6-amidino-2-(4-amidinophenyl)-indole (**V**).

The electron absorption and fluorescence emission spectra of compounds **I**–**V** in water and 2-propanol (as was shown in [8–10], 2-propanol simulates microenvironment of a dye molecule interacting with DNA) are given in table and Figs. 1 and 2. It is seen that the spectra become more structuralized as the weight of the heteroatom in the benzazole ring rises. In this respect, the fluorescence emission spectra of compounds **I**–**III** are more sensitive than their electron absorption spectra. Thus, only compound **I** shows two absorption maxima at above 300 nm, whereas two fluorescence emission maxima are observed for both compound **I** and **II**. The intensity ratio of the short-wave maximum to the long-wave maximum (I_{sw}/I_{lw}) is higher for compound **I**. Also, the I_{sw}/I_{lw} ratio for compounds **I** and **II** increases in going from water to 2-propanol. Probably, these compounds form hydrogen bonds with alcohol molecules more readily than with water. According to the data of Ivanov *et al.* [7], the long-wave maximum in the fluorescence emission spectra of **I** and **II** arises from an H-complex formed by two molecules of the dye, whereas the short-wave maximum corresponds to a single molecule.

The fluorescence quantum yields (both in water and in 2-propanol, see table) increase in the series of compounds **I**–**III**, in parallel with increasing structuralization of the absorption and fluorescence spectra. Hence, the fluorescence properties enhance in the series benzothiazole–benzoxazole–benzimidazole. A possible reason is the "internal heavy atom effect" [7, 11]. The fluorescence quantum yield of compound **IV** in water was much lower than that for compound **III**. Probably, the amidino group in **IV**, being a stronger electron acceptor than the amino group in **III**, inhibits fluorescence properties.

In 2-propanol, possessing a low dielectric permittivity (as well as upon interaction with DNA), the electron systems of the terminal groups and aromatic core of the dye partially fall out of conjugation; as a result, its fluorescence properties are restored. Though the fluorescence quantum yields in water and alcohol (φ_{wat} and φ_{alc}, respectively) for compound **III** are higher than those for **IV**, the $\varphi_{alc}/\varphi_{wat}$ ratio for compound **IV** is much greater.

On the other hand, both absolute (φ_{wat} and φ_{alc}) and relative ($\varphi_{alc}/\varphi_{wat}$) fluorescence quantum yields

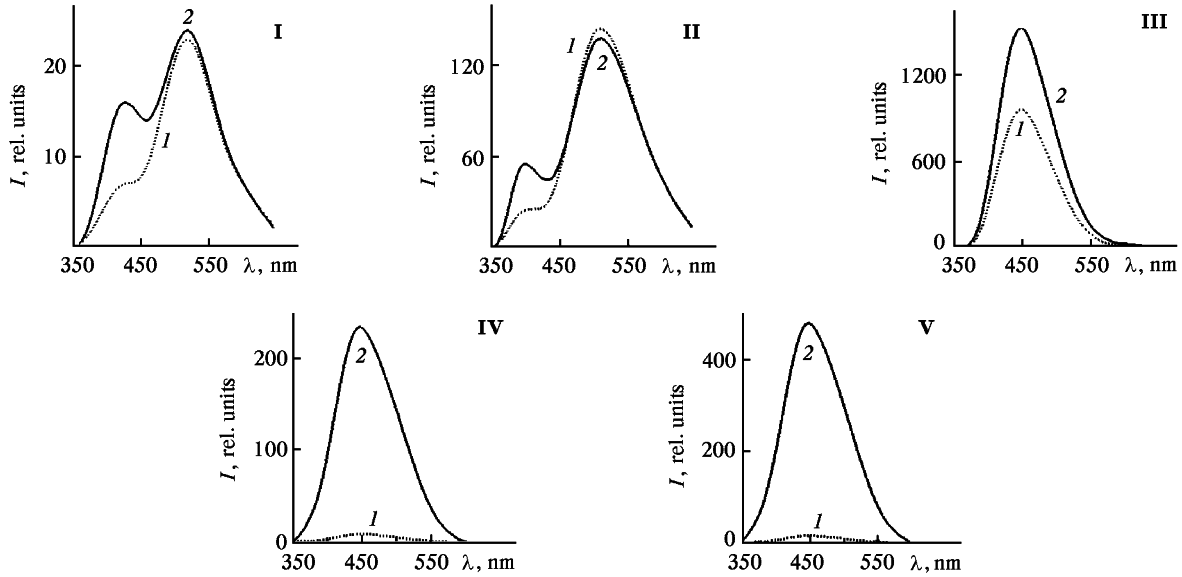

Fig. 2. Fluorescence emission spectra of compounds **I**–**V** in (*1*) water and (*2*) 2-propanol; concentration 4.0×10^{-6} M.

for compound **V** possessing two terminal amidino groups are higher than those for compound **IV**. Obviously, π-excessive indole ring in **V** ensures more intense and stable fluorescence, as compared with π-amphoteric benzimidazole ring in **IV** [12].

We can conclude that fluorescence properties enhance in the series: benzothiazole–benzoxazole–benzimidazole–indole. A similar pattern was observed for the activity of these heterocycles in binding with DNA [7]. Thus, benzimidazole or indole fragment may be regarded as an optimal structural unit for potential DNA-binding fluorophore.

EXPERIMENTAL

The synthesis of compounds **I**–**III** was reported previously [13]. Compound **V** (commercial fluorophore DAPI) was purchased from Serva (Germany).

Spectral properties of 2-phenylbenzazoles in water and 2-propanol

Parameter[a]	**I**	**II**	**III**	**IV**	**V**	Parameter[a]	**I**	**II**	**III**	**IV**	**V**
λ_{ab}^{wat}, nm	315	–	–	–	–	$(I_{sw}/I_{lw})_{wat}$	0.30	0.16	–	–	–
	350	340	330	330	340	$(I_{sw}/I_{lw})_{alc}$	0.67	0.40	–	–	–
λ_{ab}^{alk}, nm	315	–	–	–	–	φ_{wat}^{sw}	0.015	0.021	0.45	0.014	0.026
	355	345	335	340	350	φ_{wat}^{lw}	0.051	0.13	–	–	–
λ_{em}, nm	430	400	450	450	450	φ_{alc}^{sw}	0.032	0.045	0.98	0.21	0.50
	520	510	–	–	–	φ_{alc}^{lw}	0.046	0.10	–	–	–
$\varepsilon\times10^{3}$, l mol^{-1} cm^{-1}	22.7[b]	12.3	34.2	17.3	23.0	$\varphi_{alc}^{sw}/\varphi_{wat}^{sw}$	2.12	2.14	2.18	21.5	25.1
$\mu\times10^{-6}$, l mol^{-1} cm^{-1}	5.8[c]	36.1[c]	240.1	2.3	4.0	$\varphi_{alc}^{lw}/\varphi_{wat}^{lw}$	0.90	0.80	–	–	–

[a] λ_{ab}^{wat} and λ_{ab}^{alc} are wavelengths of the absorption maxima in water and alcohol, respectively (for compounds **I**–**V**, these values coincided with the fluorescence excitation maxima); λ_{em} is the fluorescence emission maximum; ε is the molar absorption coefficient in water at λ_{ab}^{wat}; μ is the molar fluorescence coefficient in water at λ_{em} (for conditions, see Experimental); $(I_{sw}/I_{lw})_{wat}$ and $(I_{sw}/I_{lw})_{alc}$ are the intensity ratios of the short- and long-wave fluorescence emission maxima in water and 2-propanol, respectively; φ_{wat}^{sw} and φ_{wat}^{lw} are fluorescence quantum yields in water at the short-wave and long-wave emission maxima, respectively; and φ_{alc}^{sw} and φ_{alc}^{lw} are fluorescence quantum yields in 2-propanol at the short-wave and long-wave emission maxima, respectively.

[b] At λ_{ab} 350 nm; at λ_{ab} 315 nm, ε 22 (in water).

[c] At the long-wave emission maxima of compounds **I** and **II**.

Compound **IV** was synthesized by the procedure described in [14]. All measurements were made with a constant concentration of dyes, $c = 4.0 \times 10^{-6}$ M, at 20–25°C. The electron absorption spectra were recorded on a Beckman Model 35 spectrophotometer (Austria). The fluorescence emission spectra were recorded on a Hitachi Model 850 spectrofluorimeter (Japan). The slits of excitation and emission monochromators were set at 3 nm, scan rate 120 nm/min, response time 2 s, normal magnification on the photoelectron multiplier. The fluorescence emission spectra were recorded at excitation maxima which coincided with the absorption maxima; the molar fluorescence coefficients were determined as $\mu = I/c$, where I is the fluorescence intensity recorded under the above conditions in a standard 1-cm^2 cell. The fluorescence quantum yields were determined with the use of a solution of quinine sulfate in 1 M sulfuric acid (φ 0.55) as reference [15]. Since the absorption and fluorescence emission spectra of compounds **I**–**V** did not overlap, the relative quantum yields were calculated from the absorption and fluorescence maxima by the following equation: $\varphi_2/j_1 = I_2 A_1 I_1^{-1} A_2^{-1}$ [9], where I_2, I_1 and A_2, A_1 are, respectively, the fluorescence intensities and optical densities of solutions of a compound in states 2 and 1. The fluorescence spectra were corrected with the use of a quantum counter on the basis of a standard alcoholic solution of Rodamin B (as described in the Manual for Hitachi-850 spectrofluorimeter).

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