N-alkylaryl styrylcyanine dyes as fluorescent probes for nucleic acids detection

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Aim. To synthesize and characterize a series of N-alkylaryl benzothiazole styrylcyanine dyes as potential fluorescent probes for nucleic acids (NA) detection. Methods. Synthesis, absorption and fluorescence spectroscopy, gel electrophoresis. Results. The modification of N-alkyl styrylcyanine by variation of aromatic moieties insignificantly affected its inherent fluorescent properties. Weakly fluorescent in an unbound state, the dyes noticeably increased their emission upon binding to dsDNA/RNA (up to 83-fold for the derivative with N-alkylbenzylamine group (Sbt1) complexed with dsDNA: with a binding constant (Kb) of $5.0 \times 10^4$ M$^{-1}$, detection limit of dsDNA in solution of $6.2 \times 10^{-7}$ Mbp (0.4 µg)). When bound to dsDNA, styrylcyanines have moderate quantum yields (up to ~22 %). The variation of structure of the terminal aromatic group allowed to discriminate between dsDNA and RNA: the fluorescence of the Sbt2 dye with the N-alkylphenantroline group increased 14 and 55-fold, respectively. A higher discernibility of post-electrophoretic staining at low DNA concentrations (3.6 ng/lane) by the Sbt3 dye with the N-alkylpyridyl group was observed compared to the commonly used ethidium bromide. Conclusions. Due to the sensitivity of novel styrylcyanines to NA in solution and in gel electrophoresis, they could be proposed as photostable, low-toxic and inexpensive fluorescent probes for laboratory use.

Keywords: styrylcyanines, nucleic acids detection, fluorescent probes.

Introduction

Fluorescent probes for the nucleic acids (NA) detection are widely required for different biomedical purposes from wide spread routine gel electrophoresis to cell organelles visualization [1]. Mainly such fluorescent dyes converse from weakly emissive to strongly emissive state upon NA binding. The requirements to the probes include the strong affinity of their binding to a target biomolecule, sharp enhancement of the fluorescence intensity of the probe upon such binding, high molar extinction coefficient, low detection limit, photostability (for long-term monitoring of cellular processes in vitro and in vivo).
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For today the most widespread laboratory dye for DNA visualization in routine techniques (like gel electrophoresis) is low-cost dye ethidium bromide (EtBr). However, it has an inherent fluorescence in the unbound state that decreases the signal-to-background ratio and thus decreases the ability to detect very small amounts of nucleic acids [2]. Additionally, due to its high toxic and mutagenic effect [3–4], there is a search for new safer fluorescent NA-probes. In Real-Time PCR and other modern techniques for the highly sensitive detection of single stranded (ss) or double stranded (ds) nucleic acids (fluorescent immunoassays, comparative genomic hybridization and gene chips), the probes based on the cyanine dyes from SYBR family (SYBR Green, Gold) [5–6] are usually applied; however, their disadvantage for routine use is the high commercial price [7].

Styrylcyanines were earlier reported as DNA-sensitive dyes, they demonstrated considerable fluorescence intensity enhancement upon binding to DNA as well as high fluorescence quantum yield in DNA presence [8-10]. Low toxicity for the dyes belonging to styrylcyanines was shown [11]. Hence, due to these features as well as photostability, low phototoxicity (tissue damage upon the irradiation), inexpensive synthesis, styrylcyanine dyes are of interest regarding the development of fluorescent probes for NA on their base. It was shown that the binding affinity to DNA could be modified by variation of the structure of N-alkyl tail group of styrylcyanine and such modification allows retaining spectral properties of the dye chromophore (absorption, emission wavelength) [12–13].

Here, a series of the benzothiazole based styrylcyanines functionalized by aromatic moieties at N-alkyl tail group was synthesized and characterized as potential probes for NA detection. The long alkyl linkage (n-butyl) was chosen to eliminate the effects of terminal side groups on electronic transitions of chromophore. Here we studied UV-VIS absorption and fluorescent spectra of these dyes both in the absence and in the presence of NA (both dsDNA and RNA), and analyzed the effect of the terminal aryl group in the N-alkylaryl substituent. The efficiency of dyes as post-electrophoretic stains for DNA visualization was studied in comparison with ethidium bromide (EtBr).

Materials and Methods

General. dsDNA (salmon testes) and yeast total RNA were purchased from Sigma-Aldrich Co. Solvents were of analytical grade. 1H NMR spectra were recorded on Bruker ARX 400 spectrometers; chemical shifts (δ) were given in ppm relative to SiMe₄. 50mM Tris-HCl buffer (pH 7.9) was used in all assays described.

Synthesis of the dyes. General scheme of the dyes synthesis and their structures are presented at Scheme 1. At the first stage of the synthesis, dye SI-1 with iodo-alkyl substituent was obtained by condensation of the quaternary salt 2-methylbenzothiazole with p-dimethylaminobenzaldehyde in acetic anhydride [14]. Monomeric benzothiazole styryl dyes (Sbt) with positively charged tail groups were synthesized by alkylation of corresponding N-benzyldimethylamine or excess azaheterocycle by SI-1 in conditions mentioned in [14] as described below. The double excess of the heterocycle was taken to prevent the dimer formation. Referent dye with N-methyl
substituent (Ref) was synthesized as described previously [15]. The structures of the dyes and initial compound were confirmed by $^1$H NMR spectra and LC-MS and element analysis.

Method of synthesis of the dyes (Sbt1 — Sbt4) from SI-1 and their characterization

To the solution of SI-1 (59.5 mg, 0.1 mmol) in dimethylformamide (0.5 ml), N-benzyl-dimethylamine (0.1 mmol) or heterocycle (0.2 mmol) was added. The obtained mixture was heated during 8 hours on boiling water bath. Then the reaction mixture was cooled, and ethanol was added. A precipitate was filtered off, washed with ethanol and dried.

(SI-1)

Yield: 92 %. M. p. (dec.): 225–227 °C.

1H-NMR (DMSO-d6): $\delta$(ppm)= 1.94 (4H, m), 3.12 (6H, s), 4.84 (2H, t, J=6.8 Hz), 6.85 (2H, d), 7.58 (1H, d, J=14.7 Hz), 7.68 (1H, dd, J=7.8 Hz), 7.79 (1H, dd, J=7.8 Hz), 7.93 (2H, d, J=8.3), 8.08 (1H, d, J=15.2 Hz), 8.15 (1H, d, J=8.5 Hz), 8.30 (1H, d, J=7.8 Hz). LC-MS (M+): m/z (%) = 463.0 (100 %) [M – I]+.

3-(4-Benzyl-dimethylpropyl-ammonium-2-[2-(4-Dimethylamino-phenyl)-vinyl]benzothiazol-3-iium diiodide (Sbt1)

Yield: 67 %. M. p. (dec.): 226–228 °C. 1H-NMR (DMSO-d6): $\delta$(ppm)= 1.82 (2H, m), 2.04 (2H, m), 2.97 (6H, s), 3.12 (6H, s), 4.53 (2H, s), 4.83 (2H, t, J=6.8 Hz), 6.84 (2H, d, J=9.0 Hz), 7.49-7.62 (6H, m), 7.69 (1H, dd, J=7.5 Hz), 7.79 (1H, dd, J=7.5 Hz), 7.94 (2H, d, J=9.0 Hz), 8.09 (1H, d, J=15.3 Hz), 8.17 (1H, d, J=8.8 Hz), 8.31 (1H, d, J=8.2 Hz). Anal. calcd. for C30H37N3SI2: C, 49.67; H, 5.14 ; N, 5.79. Found: C, 49.55; H, 5.08; N, 5.85.

2-[2-(4-Dimethylamino-phenyl)-vinyl]-3-(4-[1,10]phenanthrolin-1-iium-butyl)-benzothiazol-3-iium diiodide (Sbt2)
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Yield: 78%. M. p. (dec.): 236–238°C. 1H-NMR (DMSO-d6): δ(ppm)= 2.08 (2H, m), 2.28 (2H, m), 3.14 (6H, s), 4.90 (2H, t, J=6.5 Hz), 5.92 (2H, t, J=6.5 Hz), 6.80 (2H, d, J=8.5 Hz), 7.53 (1H, d, J=15.0 Hz), 7.69–7.87 (4H, m), 7.99–8.13 (3H, m), 8.30 (1H, d, J=8.0 Hz), 8.41–8.51 (3H, m), 8.81 (1H, d, J=7.7 Hz), 9.11 (1H, dd, J=5.5 Hz), 9.38 (1H, d, J=8.3 Hz), 9.58 (1H, d, J=5.5 Hz). Anal. calcd. for C34H33N3SI2: C, 53.07; H, 4.32; N, 5.46. Found: C, 53.14; H, 4.28; N, 5.53.

3-(4-[2,2’]Bipyridinyl-1-ium-butyl)-2-[2-(4-Dimethylamino-phenyl)-vinyl]-benzothiazol-3-ium diiodide (Sbt3)

Yield: 95%. M. p. (dec.): 182–184°C. 1H-NMR (DMSO-d6): δ(ppm)= 1.93 (4H, m), 3.10 (6H, s), 4.82 (2H, t, J=8.0 Hz), 6.83 (2H, d, J=9.0 Hz), 7.44 (2H, m), 7.57 (1H, d, J=15.0 Hz), 7.67 (1H, dd, J=7.7 Hz), 7.77 (1H, dd, J=7.8 Hz), 7.93 (4H, m), 8.07 (1H, d, J=15.0 Hz), 8.14 (1H, d, J=8.5 Hz), 8.29 (1H, d, J=7.9 Hz), 8.37 (2H, m), 8.67 (2H, m). Anal. calcd. for C31H32N4SI2: C, 49.88; H, 4.32; N, 7.51. Found: C, 49.92; H, 4.27; N, 7.58.

Visible Spectrophotometer (Thermo Fisher Scientific, USA). Fluorescence excitation and emission spectra were collected on a Cary Eclipse fluorescence spectrophotometer (Varian, Australia). Fluorescence spectra were measured at excitation and emission slit widths equal to 5 nm. Spectra were acquired using standard quartz cuvettes (1 × 1 cm) at room temperature (20°C). All measurements were made at the respective excitation maxima of each dye. Each experiment was performed three times. The quantum yield values (φ) for several dyes in the presence of DNA were determined using Rhodamine 6G solution in ethanol as the reference (φ = 0.95) [16].

Preparation of the solutions. Dye stock solutions were prepared by dissolving the dyes at 2 mM concentration in DMSO. Stock solutions of dsDNA and RNA were prepared by dissolving the NA in Tris-HCl buffer (50 mM, pH 7.9) at the concentration of 6.15 mM b.p. for dsDNA and 24.6 mM b. for RNA. Working solutions of free dyes were prepared by dilution of the dye stock solutions with Tris-HCl buffer (pH 7.9) to the concentration of 2 μM. The working solutions of dye/NA mixtures were prepared by mixing a dye aliquot (1 µL) and an aliquot of DNA or RNA stock solutions (10 µL) in Tris-HCl buffer at room temperature to reach the concentration of 0.2–171 μM. The working solutions of dye/NA mixtures were prepared by mixing a dye aliquot (1 µL) and an aliquot of DNA or RNA stock solutions (10 µL) in Tris-HCl buffer at room temperature to reach the concentration of 2 μM. The working solutions of dye/NA mixtures were prepared by mixing a dye aliquot (1 µL) and an aliquot of DNA or RNA stock solutions (10 µL) in Tris-HCl buffer at room temperature to reach the concentration of 0.2–171 μM. The working solutions of dye/NA mixtures were prepared by mixing a dye aliquot (1 µL) and an aliquot of DNA or RNA stock solutions (10 µL) in Tris-HCl buffer at room temperature to reach the concentration of 0.2–171 μM.
experiment was performed three times. The titration curve is provided for the average values along with the standard deviations (SD) (Figure 4). For the calculation of binding constant, we used the points corresponding to the excess of DNA. Thus we assumed that only negligible amount of dye molecules would bind to dsDNA close to each other and affect each other’s binding. Based on this assumption, the binding of dye to DNA could be described by the following equilibrium:

\[ \text{dye} + \text{dsDNA} \leftrightarrow \text{dye-dsDNA} \quad (1) \]

the constant of this equilibrium (binding constant, \(K_b\)) can be expressed with the equation (law of mass action [17]) below:

\[
\frac{C_{bd}}{C_{fDNA} \times C_{fd}} = K_b \quad (2)
\]

where \(C_{bd}\), \(C_{fd}\) and \(C_{fDNA}\) are concentrations of a bound dye, a free dye and free dsDNA binding sites, respectively. For the DNA concentrations starting from 20 μM, that is significantly higher than that of the cyanine dye (2 μM), the concentration of free dsDNA base pairs is roughly equal to the total dsDNA concentration \(C_{DNA}\); \(C_{fDNA} \approx C_{DNA}\). Concentration of the free dye in equilibrium is \(C_{fd} = C_d - C_{bd}\) (where \(C_d\) is the total dye concentration). The measured fluorescence intensity (\(I\)) of the dye in the presence of dsDNA at the DNA concentration of \(C_{DNA}\) can be expressed with the following equation \(I = I_{max} \times C_{bd}/C_d + I_0 \times C_{fd}/C_d\), where \(I_0\) is the fluorescence of the dye (2 μM) in the absence of dsDNA and \(I_{max}\) is the fluorescence of the dye (2 μM) in presence of the indefinitely large dsDNA concentration (Figures 4). Equation (2) can be transformed into (3):

\[
I - I_0 = \frac{A \times K_b \times C_{DNA}}{1 + K_b \times C_{DNA}} \quad (3)
\]

where \(A = I_{max} - I_0\).

\(K_b\) and \(A\) values can be calculated as approximation parameters by fitting the experimentally obtained data \(I - I_0\) versus \(C_{DNA}\) by using equation (3). Fitting was performed and the values of \(K\) and \(A\) with their standard deviations were estimated by using Origin 8.0 program.

**Gel electrophoresis**

The electrophoresis of GeneRuler DNA 50bp DNA Ladder (Fermentas # SM0371) was performed in 2.5 % agarose gel in 40 mM TAE buffer, pH 8.4, according to [18]. The gel post-staining was performed in dye solution in 50 mM Tris-HCl buffer, pH 7.9, for 30 min at room temperature in the dark. An ethidium bromide stock solution (\(C = 25 \text{ mM}\)) was diluted in 2000 times. The concentration of other dyes was 5 μM. DNA concentrations and sizing are presented in Table 1. Stained agarose gel was examined under UV transillumination (\(\lambda_{max} = 312 \text{ nm}\), ECX-F20.M, Vilber Lourmat). Images of gels were obtained using the digital camera.

**Results and Discussion**

**Absorption characteristics of the dyes**

UV-Vis spectra of the studied dyes **Sbt1 — Sbt4** were acquired in a free state (in either ethanol or aqueous buffer solution) and in a mixture with DNA (aqueous buffer solution). The data are provided in Table 2 and Figures 1–2.
The absorption maxima of the styrylcyanines in ethanol are located in the range of 517–534 nm, the molar extinction values were moderate in the range (3.7–9.8)×10^4 M^−1 cm^−1. The most long-wavelength maximum (534 nm) and highest extinction coefficient (9.8×10^4 M^−1 cm^−1) were observed for Sbt3 dye with N-alkylidipyridyl group. In the aqueous buffer solution, the absorption bands of the dyes had spectral shape similar to those of ethanol solutions, close (for Sbt2) or lower (up to 1.6 times for Sbt3) extinction values, and were slightly blue-shifted (up to 19 nm for Sbt3, except for Sbt2 where the red shift is observed; Table 2) (Figure 1). The hypsochromic shifts evidence to the negative solvatochromism [19] of the dye molecules due to an increase of the solvent polarity from ethanol to aqueous buffer solution.

### Table 1. DNA fragment size and quantities in gel

<table>
<thead>
<tr>
<th>Band №</th>
<th>Fragment length, base pairs</th>
<th>Quantity per band, ng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left lane</td>
<td>Right lane</td>
</tr>
<tr>
<td>1</td>
<td>1031</td>
<td>73.3</td>
</tr>
<tr>
<td>2</td>
<td>900</td>
<td>63.9</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>56.9</td>
</tr>
<tr>
<td>4</td>
<td>700</td>
<td>50.0</td>
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<td>5</td>
<td>600</td>
<td>42.7</td>
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<tr>
<td>6</td>
<td>500</td>
<td>71.0</td>
</tr>
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<td>400</td>
<td>28.4</td>
</tr>
<tr>
<td>8</td>
<td>300</td>
<td>21.3</td>
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<tr>
<td>9</td>
<td>250</td>
<td>17.8</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>28.5</td>
</tr>
<tr>
<td>11</td>
<td>150</td>
<td>10.7</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>21.3</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
<td>14.2</td>
</tr>
</tbody>
</table>

### Table 2. The characteristics of the dyes’ UV-Vis absorption spectra.

<table>
<thead>
<tr>
<th>Name</th>
<th>Free dye*</th>
<th>In buffer solution</th>
<th>In EtOH</th>
<th>With DNA**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ_{max}, nm</td>
<td>ε, 10^4 M^−1 cm^−1</td>
<td>λ_{max}, nm</td>
<td>ε, 10^4 M^−1 cm^−1</td>
</tr>
<tr>
<td>Sbt1</td>
<td>524</td>
<td>3.8</td>
<td>534</td>
<td>6.3</td>
</tr>
<tr>
<td>Sbt2</td>
<td>533</td>
<td>4.2</td>
<td>526</td>
<td>4.4</td>
</tr>
<tr>
<td>Sbt3</td>
<td>515</td>
<td>6.0</td>
<td>534</td>
<td>9.8</td>
</tr>
<tr>
<td>Sbt4</td>
<td>515</td>
<td>5.2</td>
<td>517</td>
<td>6.4</td>
</tr>
<tr>
<td>Ref</td>
<td>512</td>
<td>2.6</td>
<td>515</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*C_{dye} = 10 µM; ** C_{dye} = 2 µM; C_{DNA} = 61.5 µM b.p; λ_{max} – absorption peak maximum; ε – molar extinction coefficient.
Upon NA addition, the spectral bands of the dyes mostly shifted to the long-wavelength range (up to 27 nm for Sbt1) and the extinction coefficients slightly decreased (Figure 1, Table 2). The long-wavelength shift of the absorption maxima could be due to the change in the dye molecule nearest environment when bound to DNA [20].

Thus, the introduction of the aromatic moiety in N-alkyl substituent of styrylcyanines leads to some changes in their absorption properties in both buffer and ethanol solutions, these changes are mostly pronounced for dyes Sbt1 and Sbt2. The referent dye absorption maximum was blue-shifted and slightly less intensive comparing to the maxima of N-alkylaryl substituted dyes (Table 2).

In the special case of Sbt2 dye bearing N-alkylphenanthroline group, the short-wavelength shift of the absorption maximum to λ = 508 nm and the changes of the peak shape (Figure 2, Table 2) were shown to take place upon DNA addition. This short-wavelength shift could point to the aggregation of the dye in DNA presence [21]. The shoulder of Sbt2 absorption band at 545 nm, which rises with the DNA concentration, could correspond to the absorption of monomer dye.

Study of the dependence of dye Sbt2 absorption spectrum on the DNA concentration (Fig. 2) evidences that this dye could form the aggregates on DNA, whereas other dyes bind to DNA as monomers. This could be explained as follows. Typically, planar aromatic molecules interact with dsDNA by intercalation into the base pair stack [22–26]. Thus it could be suggested that planar phenanthrolinium fragment (intercalator) strongly intercalates between base pairs, in which case styrylcyanine moieties of the dyes are mostly placed in DNA groove. Due to the “high density” of styrylcyanine moieties on DNA, they could associate with each other.

Characterization of the dyes' fluorescent properties in free state and in complexes with NA

The characteristics of fluorescence spectra of free styrylcyanine dyes and in the presence of dsDNA or RNA are provided in Table 3. The impact of the aromatic moiety on the dyes properties was estimated comparing with the referent N-methyl substituted styrylcyanine (Ref). For free dyes, the aromatic moiety in the N-alkyl tail group results in the slight bathochromic shift (up to 6 nm) of the excitation and emission maxima (situated at 533–535 nm and 596–597 nm respectively). The typical for styrylcyanines comparably large [12] Stokes shift values (61–64 nm) and low [27] intrinsic fluorescence intensities (up to 20 a.u.) were observed for these dyes. In comparison to the intrinsic signal of Ref (27 a.u.), the
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N-alkylaryl derivatives decrease their emission with the rise of the aromatic rings number (from 20 a.u. for Sbt1 with N-alkylbenzylamine group to 6 a.u. for Sbt2 with N-alkylphenanthroline group). It is suggested that this signal intensity decrease could be caused by nonplanar distortion of the excited chromophore framework by more bulk substituents.

The presence of the NA resulted in the strong increase of the fluorescent emission (up to 83 times for Sbt1 dye) and shifts of the maxima of all studied dyes that pointed to the dyes interaction with the NA (Figure 3, Table 3). The more pronounced red-shifts of excitation maxima (up to 38 nm) comparing to emission one (up to 13 nm) led to the Stokes shifts decrease after NA addition (Table 3).

The quantum yields of the dyes in their complexes with dsDNA were estimated as moderate (2.8–21.8 %, Table 3) that was less than for the referent dye-dsDNA complex (24.7 %).

It was shown that the chemical kind of N-substituent could determine the ability of the dyes to discriminate the NA types by the intensity of fluorescent signal. While for the referent dye and dyes with one or two aromatic rings (Ref, Sbt1, Sbt3, Sbt4), fluorescence intensity values did not differ strongly in the presence of dsDNA and RNA at the

**Table 3. Fluorescence intensity of the dyes in free form in buffer solution and in the presence of DNA or RNA in the same buffer at room temperature.**

<table>
<thead>
<tr>
<th></th>
<th>Free*</th>
<th>In the DNA presence</th>
<th>In the RNA presence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ&lt;sub&gt;ex&lt;/sub&gt;</td>
<td>λ&lt;sub&gt;em&lt;/sub&gt;</td>
<td>I</td>
</tr>
<tr>
<td>Sbt1</td>
<td>535</td>
<td>596</td>
<td>20</td>
</tr>
<tr>
<td>Sbt2</td>
<td>533</td>
<td>596</td>
<td>6</td>
</tr>
<tr>
<td>Sbt3</td>
<td>532</td>
<td>596</td>
<td>15</td>
</tr>
<tr>
<td>Sbt4</td>
<td>535</td>
<td>597</td>
<td>11</td>
</tr>
<tr>
<td>Ref</td>
<td>529</td>
<td>593</td>
<td>27</td>
</tr>
</tbody>
</table>

* C<sub>dye</sub> = 2 μM; C<sub>DNA</sub> = 61.5 μM b.p.; C<sub>RNA</sub> = 246 μM b.; λ<sub>ex</sub>, λ<sub>em</sub> — excitation, emission maxima wavelengths, nm; I — emission intensity, arbitrary units (a.u.); the standard deviations of the I were within 15 % range from the average values that are provided in the Table; Q<sub>DNA</sub> — quantum yield of the dye in the presence of dsDNA; I<sub>DNA/RNA/I_0</sub> — emission intensity increase in the presence of DNA/RNA.
studied concentrations, for Sbt2 dye with planar positively charged phenanthrolinium residue, the fluorescent response to RNA was pronouncedly higher than to dsDNA (increase by 55 and 14 times, respectively, Table 3). This decreased response of Sbt2 to dsDNA is most possibly connected with the self-association of this dye when bound to dsDNA (observed in the corresponding absorption spectra, Fig. 2) resulting in the formation of non-fluorescent dye aggregates.

**Determination of NA detection sensitivity and binding constant**

For the most NA-sensitive dye from the studied series Sbt1, the NA-detection sensitivity was determined by the titration of this dye solution with increasing amounts of dsDNA (Figure 4). The fluorescent intensity of dye increases pronouncedly with arising concentration of dsDNA (Figure 4A). The limit of dsDNA detection in our assay was 6.2×10⁻⁷ M b.p. (0.4 µg), i.e. dsDNA concentration at which the fluorescence of the dye (2 µM) in the DNA presence exceeds that of free dye by 3×SD. The SD is a standard deviation of the blank signal, where the blank signal is the fluorescence of the dye solution (2 µM) in the aqueous buffer in the absence of dsDNA [28]. The binding constant (K_b) for the dye was estimated by using the approximation of the fluorescent titrations with the equation (3) (Figure 4B).

The average K_b obtained for Sbt1 was 5.0×10⁴ M⁻¹. This value is typical for intercalating dyes: the usual range is 10⁴–10⁶ M⁻¹. In contrast, groove binders have higher binding constants: typical values are in the range of 10⁵–10⁹ M⁻¹ [29]. This is consistent with the reference data [30–31] that point out the preferable intercalation binding mode in the case of planar aromatic molecules, whereas binding within dsDNA groove is typical for the elongated crescent shape molecules containing functional groups, which are able to participate in H-bonding [31]. The average K_b for Ref dye was about three times lower (1.6×10⁴ M⁻¹) than that for Sbt1, that points to the changing of
dye affinity due to the incorporation of terminal aromatic fragment.

**Post-electrophoretic staining of DNA**

The dyes with the highest quantum yields **Sbt1** (with N-alkyldimethylbenzyl group) and **Sbt3** (with N-alkyldipyridyl group) were explored for their efficiency as stains for post-electrophoretic visualization of DNA. The commonly used DNA dye EtBr was taken for the reference. The dyes applicability for post gel electrophoresis DNA staining, i.e. visualizing of DNA fragments in the range of 50-1031 b.p. in agarose gel under UV-transilluminator (312 nm) was shown. The DNA ladder was taken in two concentration ranges (10.7–73.3 ng/lane and 2.14–14.66 ng/lane). The images of stained gels are presented in Figure 5, and the sizing of DNA fragments and masses of the lanes are presented in Table 1.

At high DNA concentrations, a brightness of DNA staining was similar for all studied dyes (Figure 5). At low DNA concentration (3.6 ng/lane, right lane N°9, Figure 5) a higher DNA discernibility by the dye **Sbt3** in comparison with EtBr was observed. The dyes do not demonstrate bleaching upon repeating UV-irradiation during ~30 min that points out their sufficient photostability for using in the gel electrophoresis post staining experiments.

**Conclusions**

A series of the benzothiazole styrylcyanines with different N-arylalkyl substituents was firstly synthesized and characterized as potential fluorescent probes for nucleic acids detection. Weakly fluorescent in unbound state, the studied styrylcyanines noticeably increase their emission intensity upon the binding to dsDNA/RNA (up to 83 times for the derivative with N-alkyldimethylamine group **Sbt1** complexed with dsDNA) and shift excitation/emission bands (up to 38 nm). The binding constant for the **Sbt1**-dsDNA complex formation (K_b) has moderate value (5.0×10^4 M^-1), which is typical for intercalating molecules; the in-solution detection limit of dsDNA by **Sbt1** is about 6.2×10^-7 M b.p. (0.4 µg). The dyes in complex with dsDNA have moderate quantum yields (up to 21.8 %).

The ability of styrylcyanines to visualize DNA in gel electrophoresis tracks under UV-

**Fig. 5.** Photos of agarose DNA gel post-stained by Ethidium bromide (EtBr) and the styrylcyanine dyes; fluorescence excited by UV light (312 nm). DNA fragment size and quantities, corresponding to separate bands, are presented in Table 1. For each lane, upper band is marked as band N° 1 in Table 1.
illumination was shown. It was observed a higher discernibility of staining at low DNA concentrations (3.6 ng/lane) by the **Sbt3** dye with N-alkylidipyridyl group comparing to commonly used EtBr. Good photostability of the studied dyes upon UV-irradiation was established.

The kind of N-alkylaryl substituent could provide to the dye molecule the ability to discriminate between NA types (dsDNA/RNA) by sharp difference in the fluorescent response intensity. Such selectivity was observed for the case of **Sbt2** dye with N-alkylphenanthroline group, emission increase was 55 and 14 times in RNA and dsDNA presence, respectively.

Thus, the variation of N-alkykaryl terminal substituents in styrylcyanine molecule is suggested as affordable chemical approach for the directed design of fluorescent dyes with required properties.

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N-alkylaryl styrylcyanine dyes as fluorescent probes for nucleic acids detection


на його флуоресцентні властивості. Барвники, що мають слабку флюоресценцію у вільному стані, значно підвищують емісію при зв’язуванні з ддДНК / РНК (до 83 раз при зв’язуванні Sbt1 з N-алкілбензиламінною групою з ДНК, його константа зв’язування (Kb) — 5.0×10⁴ M⁻¹, межа визначення ддДНК в розчині — 6.2×10⁻⁷ М п.о. (0.4 µg)). У комплексі з ДНК квантові виходи стирилціанінів є середніми (до ~22 %). Структура термінальної ароматичної групи в N-заміснику може визначати здатність барвника відрізняти ддДНК від РНК, наприклад у Sbt2 з N-алкіл-фенантрософіліновою групою емісія зростає в 14 і 55 рази відповідно. Показано, що пост-електрофоретичне забарвлення низької концентрації ДНК (3.6 нг/лінія) за допомогою барвника Sbt3 з N-алкілдіпірідіольною групою дає більш чітку візуалізацію у порівнянні з широко використовуваним бромистим етідієм.

Висновки. Завдяки чутливості нових стирилціанінів до НК в розчині і при гель-електрофорезі, вони можуть бути запропоновані як нові зонди для рутинних лабораторних експериментів.

Ключові слова: стирилціанінові барвники, детекція нуклеїнових кислот, флуоресцентні зонди.

N-алкіларил бензотиазольні стирилціанинів, які вперше були синтезовані нашими науковцями, можуть бути застосовані у багатьох діагностичних процесах, таких як визначення концентрації ДНК і РНК в розчині, а також в інших біологічних системах. Вони мають значну потенційність для використання в ядерно-магнітних резонансних спектроскопічних вимірюваннях, які дозволяють унікально детектувати та ідентифікувати біологічні молекули.

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