PHENYL- AND HETARYL SUBSTITUTED TRIMETHINE CYANINE DYES AS ALBUMIN-SENSITIVE PROBES FOR NONSPECIFIC PROTEIN DETECTION

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Fluorescent detection and visualization are widely used for various biomedical applications, such as gel electrophoresis, real-time PCR, fluorescence microscopy imaging, etc. These methods are based on the ability of low-fluorescent compounds to bind noncovalently with biomolecule with a significant increase in their fluorescence emission intensity. Cyanine dyes are broadly used for biological research due to their spectral characteristics: because of the minimum photodamage to biosamples, minimum interference from autofluorescence of biomolecules, and deep tissue penetration, those dyes were excellent platforms for fluorescent probes design. Further, the quaternary ammonium salt moiety provided sufficient water solubility for biological experiments.

Here the spectral-luminescent properties of the series of phenyl- and hetaryl-substituted trimethine cyanine dyes free and in the presence of biomolecules were examined (dyes Y1-Y5). The general structure of the studied dyes is shown in Fig. 1.

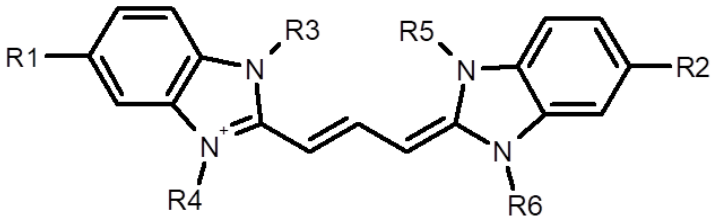


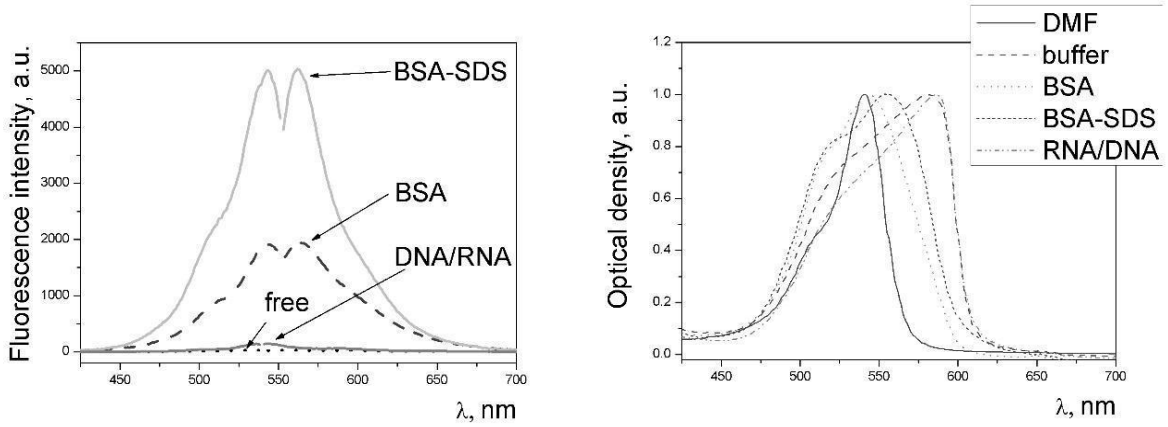
Fig. 1 General structure of phenyl- and hetaryl-substituted trimethine cyanine dyes Y1-Y5. R3-R6: phenyl, ethyl, 2-hydroxyethyl and R1, R2 – metoxy, benzoxazole, benzothiazole, quinoline substituents.

The absorption maxima of the dyes solutions in DMF are located in the range of 520-545 nm, and the values of molar extinction coefficient were in the range from moderate $(0.48\text{-}1.0)\times10^5 M^{-1}cm^{-1}$ to high $(1.84\text{-}1.95)\times10^5 M^{-1}cm^{-1}$. We observe a shift of the absorption band to the long-wavelength region for all dyes in an aqueous solution compared to one in DMF. The studied dyes are characterized by moderate to high fluorescence in a free state. Their fluorescent emission maxima are located at the range 553–621 nm with a Stokes shift up to 67 nm. The dyes Y3 and Y4 were shown to be fluorescently sensitive to the BSA-SDS system with an emission intensity increase of 155 and 5.6 times respectively. Thus, we suggest the dyes Y3 and Y4 could be proposed as probes for nonspecific detection of proteins in gel electrophoresis (usually in the presence of denatured agent sodium dodecyl sulfate, SDS). In addition, the dye Y3 is also sensitive to BSA in its native conformation (in Tris-HCl buffer pH 7.9) with an increase of up to 60-fold.

Fig. 2a,b represents the absorption and fluorescence spectra of the most promising dye Y3 free and in the presence of biomolecules. The dye is insensitive to nucleic acids (both dsDNA and RNA), while strongly enhancing its fluorescence intensity in the presence of BSA and the BSA-SDS system. The increase in fluorescence intensity is also accompanied by a decrease in the Stokes shift from 60 to 20 nm.

Absorption spectra of the dye Y3(Fig. 2b) demonstrate, that the shape of the spectrum strongly depends on the medium and the presence of biological molecules. Namely, instead of a sharp band for the dye solution in DMF, the broad band appears in buffer, shifted by 46 nm to the

long-wavelength region compared to the corresponding spectrum in DMF. The presence of RNA/DNA practically does not change the spectrum's shape and the maximum's location, that points to only weak interaction of Y3 with these biopolymers. On the other hand, in the presence of BSA or BSA-SDS, there is a short-wavelength shift of the absorption maximum towards that for DMF. This shift points to the strong binding of the dye Y3 to the native or denatured serum albumin with a high increase in fluorescence emission.



a *b*

Fig. 2 (a) Fluorescence emission (right) and excitation (left) spectra of cyanine dye Y3 in an unbound state and the presence of serum albumin, BSA-SDS system, DNA/RNA in 50 mM Tris–HCl (pH 7.9) buffer. (b) The Vis absorption spectra of the cyanine dye Y3 in DMF, buffer in an unbound state and in the presence of BSA, BSA-SDS, RNA/DNA. The optical density of the dye was normalized to unity at corresponding maximum wavelengths of the dye spectra. Dye concentration 5 μM.

Due to the specificity of studied trimethine cyanine dyes to serum albumin and albumin-SDS system, they could be considered promising fluorescent dyes for applications demanding noncovalent labeling of proteins, proteins detection, and quantification in solution, gel electrophoresis, or for protein analysis.

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2-OXOIMIDAZOLIDIN-4-YLIDENEPIPERIDINESULFONILAMIDES AS POTENTIAL ANTIBACTERIALS AGAINST MULTIDRUG-RESISTANT MICROBIAL PATHOGENS

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With the emergence of multidrug-resistant bacterial strains, there is an urgent need to find antibacterial agents directed at alternative molecular targets. It is known that imidazolones are important scaffolds and biologically active compounds. Functionalized derivatives of imidazolones were recognized due to their significant antibacterial and antifungal properties, including antituberculosis activity, anticancer, anti-inflammatory, antihistamine, antihypertensive, antiparkinsonian and anthelmintic activities [1,2]. It was found that 2-thioxo-4-imidazolidinone derivatives which involved various substituents exhibited moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and significant antifungal activities toward *Candida albicans* and *Aspergillus niger* [3].