HIGH-SENSITIVE MONOMETHINE CYANINE DYES AS FLUORESCENT PROBES FOR NUCLEIC ACID DETECTION AND VISUALIZATION

Aristova D.[1], Kosach V.[1], Chernii S.[1], Slominskii Yu.[2], Yarmoluk S.[1], Mokhir A.[3], Kovalska V.[1,4]

[1]Institute of Molecular Biology and Genetics NASU, Kyiv, Ukraine
[2]Institute of Organic Chemistry NASU, Kyiv, Ukraine
[3]Friedrich-Alexander University Erlangen-Nürnberg (FAU), Department of Chemistry and Pharmacy, Organic Chemistry Chair II, Erlangen, Germany
[4]Scientific Services Company Otava Ltd, Kyiv, Ukraine
dar.arist@gmail.com

Fluorescent dyes are small compounds that exhibit favorable optical properties, such as brightness, photostability, and narrow bandwidth relative to fluorescent proteins. They can be designed to be membrane permeable to illuminate intracellular milieu or membrane impermeable to report extracellular structures. Thus, the aim of the research is to study the new series of monomethine cyanine dyes with different substituents as fluorescent probes for nucleic acids *in vitro* detection and visualization in microscopy. Fluorescence spectroscopy, fluorescence and confocal microscopies were used to characterize spectral-luminescent properties of studied dyes.

The studied dyes possess low fluorescence intensity in the aqueous buffer. All dyes give a stronger response in the RNA presence compared to DNA. The binding to nucleic acids also results in a significant increase in fluorescence intensity of the studied dyes. We have observed that benzoxazole quinoline dye Sl-2598 and benzothiazole quinoline dye Sl-2000 possesses the most significant spectral response on nucleic acids presence.

Thus, we believe these dyes could be sensitive to organelles containing nucleic acids. To prove our suggestion, we have performed staining of live and fixed cells by fluorescence microscopy. We have found that all dyes proved to be membrane-permeant and stain the sub-cellular components in live cells. Moreover, all studied dyes penetrate the nuclear membrane and stain large structures within the nucleus, which are most probably nucleoli. We have used antibodies against Ki-67 protein for a co-localization analysis of dye Sl-2000 using confocal laser scanning microscopy (CLSM) to investigate this possibility. Thus, the co-localization data analysis indicates the localization of the benzothiazole quinoline cyanine dye Sl-2000 in nucleoli with antibodies against Ki-67 (Fig. 1). We have also studied the photostability of Sl-2000 (its photobleaching). It was shown that under direct irradiation, Sl-2000 retains fluorescent staining stability in the nucleoli for 1 minute 30 seconds.

Due to the high sensitivity to ribonucleic acid and good photostability, benzothiazole quinoline cyanine Sl-2000 is suggested as an efficient high-sensitive stain for nucleoli visualization in live and fixed cells.

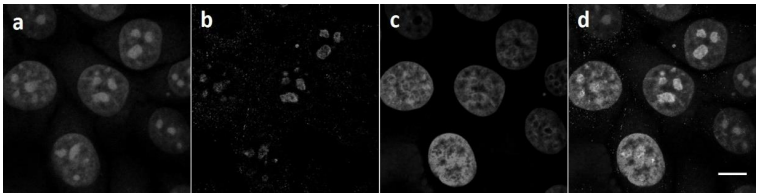

Fig. 1. CLSM images of colocalization of Sl-2000 dye with antibodies against Ki-67 protein in MCF-7 cells. (a – studied dye Sl-2000, b – antibodies against Ki-67 protein, c – Hoechst, d – merged channel of Sl-2000, antibodies against Ki-67 and Hoechst). Scale bars = 10 μm

This work was supported by the grant H2020-MSCA-RISE N872331.