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Synthesis, study of biological activity, and hemocompatibility of potential antitumor compounds of thiazolopyrimidinium systems

Olga S. Shemchuk,*[a,b] Boris V. Paponov,*[a] Danil A. Rakitianskii,[c] Dmitrii N. Kalyuzhny,[d] Andrey M. Rumyantsev,[e] Elena V. Sambuk,[e] Iliya M. Bublik,[a] Polina V. Khomenko,[a] Pavel A. Andoskin,[a,b] Oleg E. Molchanov,[b] Dmitrii N. Maistrenko,[b] Konstantin N. Semenov[a,b] and Vladimir V. Sharoyko[a,b]

[a] *I. P. Pavlov First St. Petersburg Medical University, 197002 St. Petersburg, Russian Federation. E-mail: olja.shemchuk17@gmail.com, paponov.orgchem@gmail.com*
[b] *A. M. Granov Russian Research Center for Radiology and Surgical Technologies, 197758 St. Petersburg, Russian Federation*
[c] *V. I. Chuikov Moscow South-Eastern School, 109457 Moscow, Russian Federation*
[d] *V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation*
[e] *Department of Genetics and Biotechnology, St. Petersburg State University, 199034 St. Petersburg, Russian Federation*

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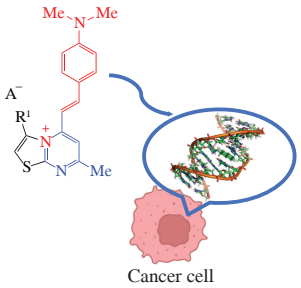
Four novel antitumor agents, representatives of (*E*)-5-(4-dimethylaminostyryl)-7-methylthiazolo[3,2-*a*]pyrimidin-4-ium salts, were synthesized by sequential reactions of the corresponding aminothiazoles with acetylacetone and 4-dimethylaminobenzaldehyde. Cytotoxicity was assessed on five different cell lines (HeLa, PANC-1, A549, MCF-7, and ECV304). The results indicate that the salts have significant potential for further development as anticancer drugs.



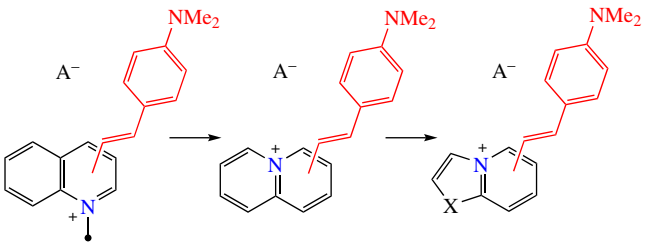
Keywords: heterocycles, thiazolo[3,2-*a*]pyrimidin-4-ium salts, quaternized nitrogen, DNA, genotoxicity, hemolysis, cytotoxic activity.

Nowadays, DNA molecules of tumor cells can be considered as priority targets, and the creation of new selective DNA-tropic drugs is one of the urgent tasks of pharmacotherapy of oncological diseases.[1] Today, DNA-tropic antitumor and antibacterial drugs, widely used in practical medicine, are *de facto* represented by three classes of compounds. These are antitumor antibiotics of the anthracycline series [the doxorubicin (Dox) group];[2] a number of semisynthetic derivatives of natural camptothecin, such as topotecan and irinotecan;[3] and platinum complexes, such as cisplatin.[4] All of these compounds were developed in the second half of the 20th century. Thus, we can state a certain stagnation that has arisen in the search and use of new antitumor antibiotics.[5]

Cyanine dyes consisting of a heterocyclic core containing a quaternized nitrogen atom and a 4-dimethylaminostyrene moiety have been known since the beginning of the 20th century, and 2-(4-dimethylaminostyryl)-1-methylpyridinium iodide (DASPMI) is a polar sensitive dye that measures the membrane potential of mitochondria in living cells.[6–10] Antitumor and cytostatic activities for such compounds are also known and studied to date.[11–16] Heterocycles containing a bridged, quaternized nitrogen atom and one or two styryl substituents have been less studied. Cyanine dyes containing a quinazolinium core and 4-dimethylaminostyrene moieties can act as markers of mitochondrial DNA and probes for G-quadruplexes.[17–20] However, there are no data on the cytotoxic and antitumor activity of these compounds.

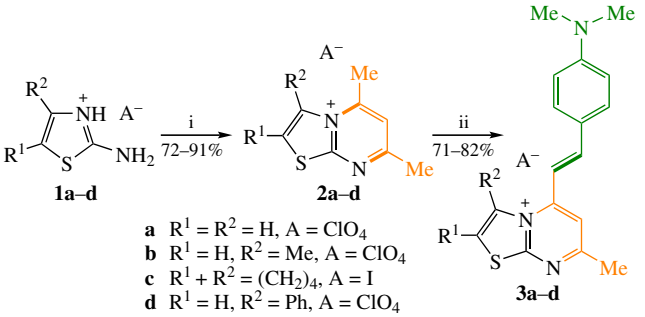
Cyanine dyes containing an azoloazinium core with a quaternized nitrogen atom and a 4-dimethylaminostyryl fragment have been practically not studied, despite the fact that the synthetic approach to them has been known for 50 years.[21] Neither antitumor activity nor the ability to interact with DNA has been reported for these compounds (Figure 1).

Herein, four heterocyclic thiazolo[3,2-*a*]pyrimidin-4-ium salts containing a bridged quaternized nitrogen atom and one 5-positioned styryl moiety were synthesized in a two-step



	quinolinium salts	quinolizin-5-ium salts	azoloazinium salts
cytotoxic	+	–	in this paper
cell staining	+	+	unknown

Figure 1 Evolution of cyanine dyes consisting of a heterocyclic core with a quaternized nitrogen atom and a 4-dimethylaminostyryl substituent and their cytotoxic and cell staining properties.



Scheme 1 *Reagents and conditions*: i, $MeC(O)CH_2C(O)Me$ (1.5 equiv.), neat, 150 °C, 1 h; ii, $4\text{-}Me_2NC_6H_4CHO$, DMF or $(EtCO)_2O$, Δ (<140 °C), 1 h.

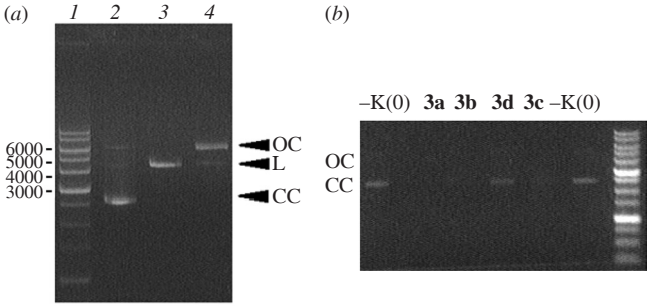


Figure 2 (*a*) Results of electrophoresis of pBR322 plasmid DNA: (*1*) DNA length marker 1kB (Evrogen, Russia); (*2*) native plasmid pBR322; (*3*) plasmid pBR322 treated with BamHI restriction enzyme, which, due to the introduction of a double-strand break, transforms the molecules into a linear form (L); and (*4*) plasmid pBR322 treated with Nb.BssSI nickase, which, due to the introduction of a single-strand break, transforms the molecules into a relaxed conformation (OC). (*b*) Results of electrophoresis of pBR322 plasmid DNA after treatment with substances **3a**–**d**.

procedure from the corresponding 2-aminothiazole salts **1a**–**d** (Scheme 1). At the first stage, salts **2a**–**d** were obtained by the solvent-free condensation of aminothiazols **1a**–**d** with acetylacetone in accordance with the known procedure.[17] Then the thus obtained 5,7-dimethylthiazolo[3,2-*a*]pyrimidin-4-ium salts **2a**–**d** were reacted with an equimolar amount of 4-dimethylaminobenzaldehyde. The reaction was carried out in DMF or propionic anhydride at temperatures not higher than 140 °C to avoid the formation of the condensation product of the aldehyde at both methyl groups of salts **2**. In this way, 5-(4-dimethylaminostyryl)-7-methylthiazolo[3,2-*a*]pyrimidin-4-ium salts **3a**–**d** were prepared. It should be noted that the influence of the quaternized bridged nitrogen atom ensures high regioselectivity of the reaction. The formation of 7-styryl isomers was not observed even in trace amounts. The synthesized compounds were identified using a complex of modern physicochemical methods of analysis (for synthetic procedures and identification data, see Online Supplementary Materials).

The biological activity, biocompatibility (hemolysis, platelet aggregation) and toxicity of the target compounds were studied in *in vitro* models and included cyto- and genotoxicity. Spontaneous hemolysis was studied using the described method.[22,23] The study of hemolysis was carried out by measuring the optical density of supernatants at a wavelength $\lambda = 520$ nm. The test mixture with a volume of 0.4 ml was prepared from 200 μl of a solution of substances **3a**–**d** ($C = 0.025$–150 μM) and 200 μl of a suspension of erythrocytes in physiological solution. Substances **3a**–**d** can be considered safe in the concentration range of 0.03–150 μM (see Online Supplementary Materials, Figure S9). Platelet aggregation in platelet-rich plasma (PRP) was studied in the presence of the aggregation inducer adenosine diphosphate (ADP) ($C = 0.005$ g dm^{-3}).[20] As a result, weak antiplatelet activity of the substances in the concentration range of 1.16–150 μM was revealed in relation to ADP-induced platelet aggregation (Table S1).

The genotoxic effect of substances **3a**–**d** on DNA was evaluated *in vitro* using the pBR322 plasmid. Concentration of substances **3a**–**d** was 200 μM; samples were incubated for 15 h at 37 °C and analyzed by electrophoresis in 1% agarose gel.[24] This approach is widely used to assess the genotoxicity of various compounds. Depending on the conformation, plasmid DNA molecules move differently during electrophoresis in agarose gel. Molecules that are in a supercoiled state (coiled coil, CC) pass the farthest; molecules containing a single-strand gap, which are in a relaxed state (open coil, OC), migrate a shorter distance; in the middle, linear molecules move, resulting from a double-strand break (linear, L) [Figure 2(*a*)]. After incubation of plasmid DNA with the test compound, changes in its conformation were analyzed [Figure 2(*b*)]. It was shown that substance **3d** did not cause breaks in plasmid DNA under the studied conditions. But at the same time, substances **3a**–**c** cause significant DNA cleavage.

Table 1 IC_{50} values (μM) for substances **3a**–**d** and Dox.

Substance	Cell line				
	HeLa	PANC-1	ECV304	MCF-7	A549
3a	1.2	37.9	5.5	–	3.5
3b	25.1	50.6	7.7	2.1	3.4
3c	0.3	7.9	44.5	0.05	1.5
3d	0.4	25.6	56.4	6.2	3.5
Dox	1.5	46.3	0.3	1.3	0.1

The MTT assay [colorimetric test with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was performed on pancreatic adenocarcinoma (PANC-1), cervical carcinoma (HeLa), breast cancer (MCF-7), and adenocarcinomic human alveolar basal epithelial (A549) cell lines; the human umbilical vein endothelial cell line (ECV304) was used as a control; Dox (0.025–150 μM) was used as a control substance. For each cell line, the half-maximal inhibition concentrations (IC_{50}) of the substances were determined. The obtained values were compared with the IC_{50} of Dox. Analysis of the data obtained on the cytotoxicity of substances **3a**–**d** show a dose-dependent decrease in the survival of HeLa, PANC-1, MCF-7, A549 and ECV304 cell lines (Figure S10). Table 1 provides a comparison of IC_{50} values for substances **3a**–**d** and Dox. It can be seen that substance **3c** showed the highest activity on all tumor cell lines, while on healthy cells (ECV304) this substance was less toxic. Moreover, the activity of the compounds is much greater than that of Dox, the activity of substance **3c** was 5–6 times higher on the HeLa and PANC-1 cell lines.

As a result, we can conclude the following: (i) a number of biologically active thiazolo[3,2-*a*]pyrimidin-4-ium compounds have been synthesized; (ii) the studied compounds showed significant antitumor activity against a number of tumor cell lines (HeLa, PANC-1, ECV304, MCF-7, and A549); (iii) hemocompatibility analysis demonstrated that these compounds have an acceptable safety profile when interacting with blood cells, which is an important aspect for further clinical use; and (iv) taken together, the data obtained confirm the promise of the studied compounds as candidates for the development of new anticancer drugs.

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synthetic antitumor antibiotics, including heterocyclic systems with a quaternized nitrogen atom and styryl fragments in the form of conjugates with targeted delivery vectors to the tumor microenvironment' EGISU: 1023022200055-4-3.2.21;3.1.3).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7540.

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