The distribution of complexes $^{69m}ZnL^1Cl_2$, $[^{69m}Zn(L^2)_2]Cl_2$, and $[^{69m}Zn(L^2)_2]Sal_2$ (L^1 is *N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide, L^2 is 2-aminopyrimidine, Sal is a salicylate ion) *in vivo* in mice

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The distribution of complexes of thiazine and pyrimidine derivatives possessing antileukemic activity and labeled with radionuclide ^{69m}Zn in the organs of mice and its rate of excretion were studied. A solution of $ZnCl_2$ was used for comparison. An increase in the time of excretion of the drug from the body and a decrease of its penetration into the brain in the presence of a salicylate ion were observed.

Key words: thiazine and pyrimidine derivatives, ^{69m}Zn, mouse model.

The analysis of the distribution of potential pharmaceutical drugs *in vivo* is necessary for the characterization of their contribution to metabolism. Earlier,[1] inhibitors of inducible (and, in part, endothelial) NO synthases (iNOS and eNOS), possessing antitumor properties which depended on their structure, concentration, and some other parameters, were obtained and investigated. The class of 2-aminopyrimidines is the base class for the synthesis of many anticancer drugs, and 2-aminopyrimidine itself is a structural component of folic acid (vitamin B_9). Many antileukemic drugs have been developed based on 2-aminopyrimidine derivatives, which act as inhibitors of tyrosine kinase and the corresponding signaling pathways, which are excessively activated in leukemias, particularly in acute ones.

Previously, we obtained data on the antileukemic activity of *N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide (L^1) hydrochloride, which has an inhibitory effect on NO synthase (predominantly iNOS),[2] as well as 2-aminopyrimidine (L^2) hydrochloride and salicylate.[3] Salicylate ions (Sal) have been shown to increase the selectivity of drugs containing 2-aminopyrimidine toward a specific cell line. The introduction of zinc, *i.e.*, the preparation of complexes with these substances as ligands, in some cases increases the cytotoxicity with respect to healthy donor cells and affects the specificity.[4] The additional introduction of a zinc radionuclide (in our case, ^{69m}Zn) without a carrier or by isotopic exchange makes it possible to increase the cytotoxic load on cancer cells specifically.[5] However, for a further study of radiopharmaceuticals, it is necessary to understand how their distribution in the organism occurs *in vivo* and how rapidly they are eliminated. A mouse model was used for this purpose.

Experimental

Compounds. The compound $^{69m}ZnCl_2$ was used for comparison. The isotope ^{69m}Zn was obtained using the reaction with $^{71}Ga(\gamma,pn)^{69m}Zn$ (see Ref. 2). The complex ZnL^1Cl_2 (**1**) was synthesized following the earlier described procedure,[6] the complexes $[Zn(L^2)_2]Cl_2$ (**2**) and $[Zn(L^2)_2]Sal_2$ (**3**) were obtained using the method, similar to the one given in the work.[3] The radionuclide was introduced by isotopic exchange over 0.5 h. The identification of compounds was carried out by elemental analysis, ^1H NMR spectroscopy, and TLC in combination with autoradiography (ARG). The techniques are described in detail in the works.[3,4]

Measurement of radioactivity. The radioactivity of the products was measured on a Canberra GC 3020 gamma spectrometer with a semiconductor detector. The activity of ^{69m}Zn was determined using the gamma line (438.6 keV). The standard spectrometric gamma point sources were used for calibration. The irradiated samples were measured at a considerable distance from the detector (15 cm), approaching a point geometry model. When studying bulk samples, registration efficiency was modelled using GEANT 4.

Experiments *in vivo*. White laboratory mouse females (nonlinear) weighing 20 g (seven repetitions) were used. A solution of

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Table 1. The distribution of ^{69m}Zn complexes by organs in a mouse model

Compound	Organ activity (Bq)/specific organ activity[a] (%)				
	liver	kidneys	heart	lungs	brain
$^{69m}ZnCl_2$	55±10/~75	34.5±8/~19	to 10±3/~2.2	to 11±4/~3.5	1.0±0.2/~1.5
$^{69m}ZnL^1Cl_2$ (**1**)	64.7±0.9/~64	36.5±0.8/~12	—[b]	—[b]	—[b]
$[^{69m}Zn(L^2)_2]Cl_2$ (**2**)	66.7±0.8/~69.5	30.3±0.5/~22.6	to 11±3/~2.2	to 13±5/~5.2	0.7±0.1/~1.5
$[^{69m}Zn(L^2)_2]Sal_2$ (**3**)	70.1±0.8/~73.1	29.9±0.6/~20	—[b]	—[b]	0.3±0.1/~0.6

[a] Calculated per total introduced radioactivity.
[b] Activity was not measured.

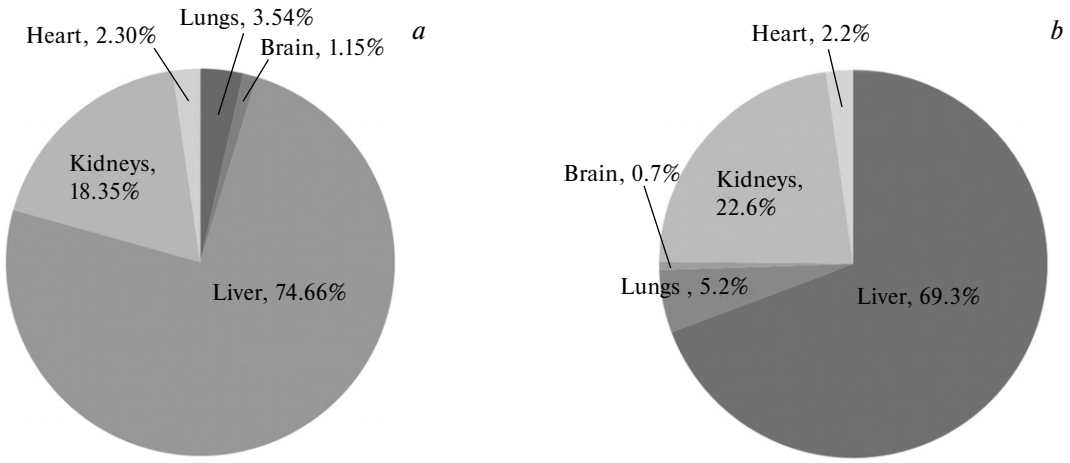

Fig. 1. The distribution of the specific activity of the samples $^{69m}ZnCl_2$ (*a*) and $[^{69m}Zn(L^2)_2]Cl_2$ (**2**) (*b*) by mouse organs in one of the experiments.

the complex (200 μL), containing the radionuclide (in each case, the concentration of L^1 or L^2 ligand was 7 mg mL^{-1}), was administered through the tail vein. The initial activity of the drug was 430 Bq. In the control experiment, 200 μL of a $^{69m}ZnCl_2$ solution (four repetitions) was administered. After 1 hour, the animals were sacrificed, and, after dissection, the radioactivity of the organs was measured on a γ-spectrometer (438.6 keV line), recalculating the activity in accordance with the half-life.

Results and Discussion

The results of the radioactivity and specific radioactivity distribution in some organs of mice after the administration of zinc pyrimidine and thiazine complexes labeled with ^{69m}Zn are given in Table 1.

It can be seen that 1 h after administration, in all the considered cases, most of the radioactivity (*A*) became distributed between the liver and the kidneys. The ratio *A*(liver) : *A*(kidneys), which is on average close to 70 : 30, decreased in the order **3** > **2** > **1** = $ZnCl_2$. For now, we can only assume that the pharmacokinetic properties of complex **3** differ from the others; complex **3** was characterized by a somewhat slower rate of excretion, probably due to the fact that the sample contained a salicylate ion. However, this requires further study. Some differences were also evident for specific activity of drugs in the brain (Fig. 1). It is possible that the presence of salicylate reduced the permeability of the complex in the brain, while the permeabilities of the chloride-containing complex **2** and $ZnCl_2$ into the brain were the same.

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References

1. S. Huerta, S. Chilka, B. M. Bonavida, *Int. J. Oncol.*, 2008, **33**, 909.
2. M. A. Orlova, T. P. Trofimova, R. A. Aliev, A. P. Orlov, S. V. Nikulin, A. N. Proshin, S. N. Kalmykov, *J. Radioanal. Nucl. Chem.,* 2017, **311**, 1177.
3. A. P. Orlov, M. A. Orlova, T. P. Trofimova, E. Yu. Osipova, A. N. Proshin, *Russ. Chem. Bull.*, 2016, **65**, 1879.
4. A. P. Orlov, T. P. Trofimova, E. Yu. Osipova, A. N. Proshin, M. A. Orlova, *Russ. Chem. Bull.*, 2017, **66**, 1860.
5. A. P. Orlov, M. A. Orlova, T. P. Trofimova, S. N. Kalmykov, D. A. Kuznetsov, *J. Biol. Inorg. Chem.,* 2018; doi: 10.1007/s00775-018-1545-9.
6. T. P. Trofimova, M. A. Orlova, A. V. Severin, E. S. Shalamova, A. N. Proshin, A. P. Orlov, *Russ. Chem. Bull.*, 2018, **67**, 768.

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