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ADVANCED RESEARCH FOR PHYSICO-CHEMICAL PROPERTIES AND PARAMETERS OF TOXICITY PIPERAZINIUM 2-((5-(FURAN-2-YL)-4-PHENYL-4H-1,2,4-TRIAZOL-3-YL)THIO)ACETATE**Yuriy Karpenko, Yuliia Hunchak, Bohdan Gutyj, Alla Hunchak, Maryna Parchenko, Volodymyr Parchenko**

The achievements of modern domestic pharmacy clearly prove the prospects of searching for biologically active molecules among 1,2,4-triazole derivatives.

The aim of our work was to predict the safest compound, select it and unambiguously prove the structure of a new promising molecule, to investigate some parameters of its toxicity.

Materials and methods. X-ray diffraction analysis was performed in the laboratory of the Institute of Single Crystals of the National Academy of Sciences of Ukraine (Kharkiv). Computer methods were used to build "structure – toxicity" models and predict LD_{50} using already created models GUSAR, TEST. The degree of toxicity (DL_{50}) and the approximate doses for the subacute experiment were determined by studying the acute toxicity of the test chemical and the injectable solution based on it. Determination of acute toxicity parameters by intragastric administration was performed on white rats, aged 3–4 months, body weight 200–220 g.

Results. Due to the study of the toxicity of 1,2,4-triazole derivatives by non-experimental methods using GUSAR and TEST models, it was found that the test compounds could be classified as low-toxic substances.

The compound is an organic salt that exists in the crystal as a solvate with one molecule of methanol and two molecules of water. When studying the structure of the crystal, it was found that the crystal is in the pinacoidal triclinic syngony.

According to the results of the studies, it was found that after a single intragastric administration of the compound in doses of 1000, 3000 and 5000 mg/kg, all animals remained alive for 14 days. The basis of the biological action of chemical compounds is the violation of several biochemical processes. We found that the studied blood constants, on the background of the use of newly synthesized substance, underwent some changes.

Conclusions. According to the assessment of the toxicity of the drug "VPK-434" when administered intragastrically to laboratory rats, it was found that in accordance with SOU 85.2-37-736: 2011 the test substance belongs to the IV class of toxicity (low toxicity). It was found that the average lethal dose of DL_{50} of the test substance by intramuscular administration to rats is 1666.66 mg/kg body weight. It was studied that some abnormalities in the hematopoietic system (increase in the number of leukocytes, including eosinophils), liver and kidney function (increased activity of transaminases, decreased serum concentrations of total protein, urea and creatinine) and changes in mineral metabolism of experimental animals groups, on the background of receiving 10 multiple doses of the study drug, was short-term, and the restoration of the functional state of the body of rats could be said as early as 4–5 days after discontinuation of the drug into their body

Keywords: 1,2,4-triazole derivatives, structure, acute and subacute toxicity, X-ray diffraction analysis

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1. Introduction

Fragments heterocyclic compounds are part of different molecules that have great significance for industry, medicine, and veterinary medicine [1]. Heterocyclic compounds are widespread in nature, are part of important natural products: dyes of blood, plants, nucleic acids, many vitamins, antibiotics, alkaloids. Some of them are used as a variety of drugs. The achievement of modern domestic pharmacy clearly proves the prospects for the search for biologically active molecules among the derivatives of 1,2,4-triazole [2, 3]. 1,2,4-Triazole de-

rivatives have found their rightful place among a wide variety of heterocyclic compounds. The attractiveness of this heterocyclic system is due to high biological activity, low toxicity and the ability to various chemical transformations, which together create favourable conditions for scientific progress. [4]. There are many examples of successful implementation of 1,2,4-triazole derivatives in various areas of human life. The active substances of known drugs are derivatives of 1,2,4-triazole: fluconazole, itraconazole, triazolam and the like. Others are used as plant protection products – paclobutrazol. In 2017

it was registered fertilizer “Fortis Combi” (RP number A-06016 of 03.03.2017). The active ingredient which belongs to the water-soluble derivatives of 1,2,4-triazole. Chemical modelling of 1,2,4-triazole allows to purposefully create biologically active molecules [2]. Such a chemical combination of different pharmacophore fragments and 1,2,4-triazole in one molecule is popular [5]. The result of years of research is the original registered veterinary drug “Tryfuzol-NEO” (RP AV-07793-01-18 of 27.07.2018). Which came into the veterinary market in Ukraine in 2018 year and quickly became popular among synthetic immunomodulators. Also at the stage of registration is a new original antifungal veterinary drug in the form of liniment “Vetmicoderm”, the active substance of which belongs to the alkyl derivatives of 1,2,4-triazole [6, 7].

Analysis of the fauna of ruminant helminthiasis, both wild and domestic, inhabiting certain regions, shows its commonality for different species. In the conditions of concrete regions or separate hunting farms no more than 25–30 kinds of helminths come to light [8]. The interests of industrial development of deer farms require the breeding of animals free of parasites, which indicates the need for further research on the development of effective antiparasitic drugs [9, 10]. Veterinary medicine has a significant arsenal of anthelmintics. The most suitable at the present stage are derivatives of benzimidazole, isoquinisole, imidazothiazole, salicylamide, thiocyanate, dinothiazine, macrocyclic compounds and the like. However, most anthelmintic drugs cause side effects, and antiparasitic activity is not always characteristic and depends on the species of animals and the stage of development of the parasite, the nature of the lesions (mono- and mixinvazil) and so on. Therefore, the need to develop new effective tools and individual active substances that would provide a wide range of antiparasitic action, in particular in deer, have a direct impact on helminths, do not damage animal organs and tissues and are rapidly excreted from the body is particularly important and requires a comprehensive solution [5].

One of the important conditions for the development and trade of new veterinary drugs is to ensure their quality by implementing the principles and rules of good manufacturing practice (GMP). According to them, at the initial stage primary toxicological research and pharmacological assessment of potential medicines, or their separate components, various forms of veterinary drugs which define not only successful development of experimental, clinical research and practical developments, but also have decisive influence on possibility of creation are carried out. competitive, low-toxic, environmentally friendly drug that is not able to cause side effects and long-term consequences [11]. The route of drug adminis-

tration depends on the toxicity indicators, which in turn affects the development of the final dosage form of the drug. Also, a key stage in the implementation of promising molecules in practice is the study of the structure of molecules, understanding the presence of different bonds, which, of course, also affect the overall mechanism of action of biologically active compounds.

Thus, further studies of the structure of new molecules substituted 1,2,4 – triazole and parameters of toxicity are relevant and have theoretical and practical significance.

The aim of our work was to predict the safest compound, select it and unambiguously prove the structure of a new promising molecule and investigate some parameters of its toxicity.

2. Planning (methodology) of the research

This scientific study is based on the use of computer methods to find the least toxic compound, to study the peculiarities of its structure and changes in the biochemical parameters of the organism. The experiment is designed to reduce the number of experimental animals and allows for a comprehensive study of the toxicity parameters of the compound (Fig. 1).

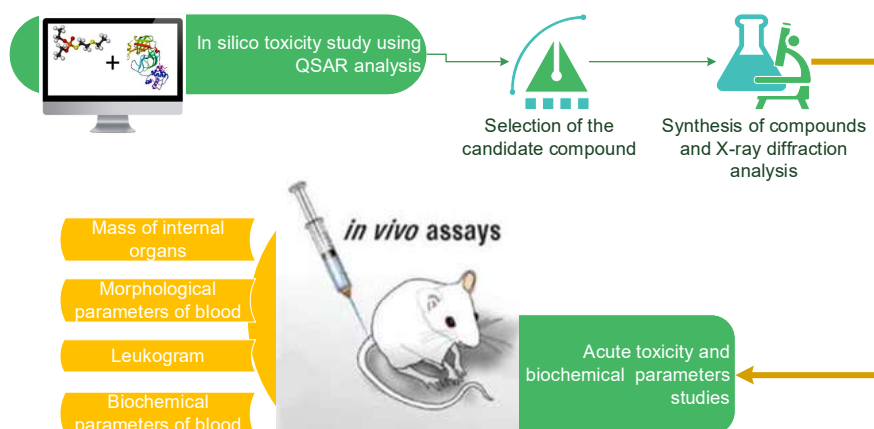


Fig. 1. Block diagram of the study of the parameters of toxicity of compounds

3. Materials and methods of the research

Research on X-ray analysis was conducted in the laboratory “Institute for Single Crystals” NAS of Ukraine (Kharkiv). The structure of the connection was deciphered by the direct method according to the SHELXTL software package [12]. Experiments on laboratory animals were conducted in the vivarium of the State Research Control Institute of Veterinary Drugs and Feed Additives (Lviv) from 2020 to 2021.

Construct models of “structure – toxicity” and forecasting LD_{50} using existing object models GUSAR (Germany). GUSAR allows you to create QSAR models based on predicted biological activity profiles of chemical compounds. Each chemical compound is presented as a list of MNA descriptors used as input parameters [5] to predict the biological profile of activity.

Experimental studies were performed according to the methods and techniques described in the monograph “Preclinical studies of veterinary drugs” (2006). Toxicological evaluation of the drug was determined according to the

guidelines “Toxicological control of new animal protection products” [13]. Animal experiments were performed in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes (ETS No. 123. Strasbourg, 1986) and the decision of the First National Congress on Bioethics (Kyiv, 2001) [14]. Conclusion of the commission on bioethical examination of scientific research of Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhytsky, protocol No. 9 dated 22.10.2020. According to the study investigated the acute toxicity of chemical substances and established based on injectable solution measured toxicity (DL_{50}) and the estimated dose of subacute experiment. Determination of the parameters of acute toxicity by intragastric administration was performed on white rats, aged 3–4 months, body weight 200–220 g. The drug was injected intragastrically, once.

Doses of 1000, 3000 and 5000 mg/kg body weight of the animal were taken to determine the toxicity of the compound solution to white rats. 6 laboratory animals were used for each dose. A dose of 5000 mg/kg body weight of the animal was administered to twice the number of animals. In the study of acute toxicity in white rats by intramuscular injection in an approximate experiment ($n=3$), the drug was administered in doses of 500, 1500, 2500 and 5000 mg/kg. It was found that a solution of the compound administered at a dose of 5000 mg/kg body weight caused 100 % death. Therefore, in the detailed experiment ($n=6$) the drug was administered in a dose range of 500, 1000, 1500, 2000, 2500 mg/kg body weight of the animal. After administration of the compound, laboratory animals were observed for 14 days. The following indicators were considered: appearance, behaviour of animals, condition of fur, visible mucous membranes, relation to food, rhythm, respiration rate, time of occurrence and nature of intoxication, its severity, course, time of death of animals or their recovery.

Determining the effect of drugs is the main and most responsible for the analysis and generalization of the results in terms of establishing possible side and long-term effects, maximum permissible levels, as well as environmental impacts. In order to study the effect of the compound on the body of animals with multiple receipts, a control and two experimental groups of animals were formed, 6 rats in each. The animals in the control group daily for 3 days intramuscularly injected water for injections and research – investigated in the compound in the therapeutic dose – 1 ml/10 kg of b. w. (group D1) and 10 ml/10 kg of b. w. (group D2). The clinical condition and behaviour of the animals were observed during the experiment.

The next day, after administration of the drug, laboratory animals were decapitated under light ether anesthesia, blood samples were taken, and morphological and biochemical studies were performed according to generally accepted methods. After autopsy, the animals were determined by the coefficients of mass of their or-

gans, compared with analogues of the control group. DL_{50} drug for white mice and rats was determined by the method of G. Kerber and calculated by the formula:

$$DL_{50}=DL_{100}-\Sigma(z \cdot d)/m,$$

where DL_{100} – the dose from which all animals died;

Σ – sum symbol;

z is half of the total number of animals that died from the next two doses;

d is the difference between the next two doses;

m is the number of animals in the group for each dose.

4. Result

As a result of studying the toxicity of 1,2,4-triazole derivatives by non-experimental methods using the GUSAR (Germany) and TEST (USA) models, it was found that the test compounds can be classified as low-toxic substances (Table 1). It studied acute toxicity dependent on the availability of fragments studied molecular alkyl and/or heterocyclic moiety as well. It was found that the toxicity of the compounds decreases with the introduction of both a furan heterocyclic relative to the pyrimidine heterocyclic (1.5 times) and a phenyl substituent in the nucleus of 1,2,4-triazole. The results of the tests indicate that the test substances did not differ significantly in toxicity, which is determined primarily by 1,2,4-triazole fragment of the compound. Perspective of using computer-QSAR analysis method for the study of acute toxicity to reduce the number of dead animals. Compound 1, which proved to be the least toxic, was selected for further studies.

The compound represents an organic salt, which exists as a crystal solvate with one molecule of methanol and two molecules of water (Fig. 2).

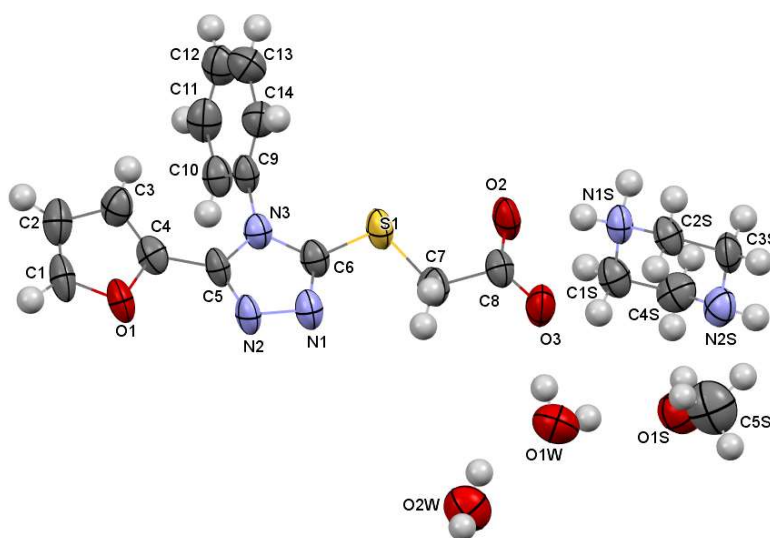


Fig. 2. Molecular structure of the compound

The positive charge of the cation in localized ground in protonated to atom of nitrogen piperazine, as evidenced diagnosed with difference synthesis of electron density in the two hydrogen atoms and atom N1S extension connection C2S-N1S 1.51 (1) Å, compared to an average of [15] 1,469 Å. Only one hydrogen atom and the bond lengths of N2S-C3S 1.47 (1) Å and N2S-C4S

1.44 (1) Å were found on the second nitrogen atom of the piperazine molecule in comparison with the average value. The piperazine molecule is in the chair conformation with folding parameters $S=1.19$, $\Theta=3.18^\circ$, $\Psi=10.35^\circ$. Carbon atoms lie in the plane of the cycle with an accuracy of 0.002 Å, and the deviations of N1S and N2S atoms are -0.64 Å and 0.70 Å, respectively [16].

Organic anion negative charge localized ground on deprotonated carboxyl group, as evidenced by the lack of electron density peaks near O2 and O3 atoms that could be interpreted as hydrogen atoms and aligned lengths links C8 O2 1.24 (1) Å and C8 O3 1.24 (1) Å. Deputy in the atom of sulfur is sp⁻ position relative to endocyclic tion connection N1-C6, and deprotonated of old carboxyl group anti-periplanar communication C6-S1 (torsion angles N1-C6-S1-C7-1.6 (9)°, C6-S1-C7-C8-173.2 (6)°). Communication between furan and triazole cycles slightly raised (torsion angle N2-C5-C4-O1-7 (1)°), and the phenyl moiety deployed almost perpendicular to the plane of the triazole cycle (torsion angle C5-N3-C9-C14 98 (1)°), which, apparently, due erased steric voltage between the vicinal substituents (shortened intramolecular contacts ... C3 C9 H3 3.20 Å and 2.73 Å ... C9 with the amount Van der Waals radii of 3.42 Å and 2.87 Å, respectively) [17].

In the crystal, the cation and the anion are linked by the hydrogen bond N1S-H ... O2 (Table 1). In addition, the presence of strong proton donors and acceptors strong proton in cations and anions, and the presence of methanol and water molecules causes the formation of a series of hydrogen bonds (Table 2).

Analysis of the detected hydrogen bonds allows to isolate the tetramer of two molecules of the anion and two molecules of the cation formed by the hydrogen bonds N1S-H1Sa ... O2 'and N1S-H1Sb ... O2'. Tetramers are interconnected through bridging molecules of methanol and water (Fig. 3).

Table 2
Geometric characteristics of intermolecular hydrogen bonds in the compound crystal

In about daily communication	Operation with a measuring	Geometer and CSSR characteristics	
		H... A, Å	D-H... A, l c.
H1S-H1Sa... O2	–	1.92	168
H1S-H1Sb... O2	2-x, 1-y, 1-z	2.32	132
H1C-X1C6... H1	1+x, y, z	2.27	127
N2S-H2S... O2w	2-x, 1-y, 2-z	2.31	177
O1S-H1S... N2S	–	2.04	162
O1w-H1wa... O3	–	1.82	165
O1w-H1wb... O1S	–	2.02	149
O2w-H2wa... O1w	–	2.12	142
O2w-H2wb... O1w	1-x, 1-y, 2-z	2.03	157

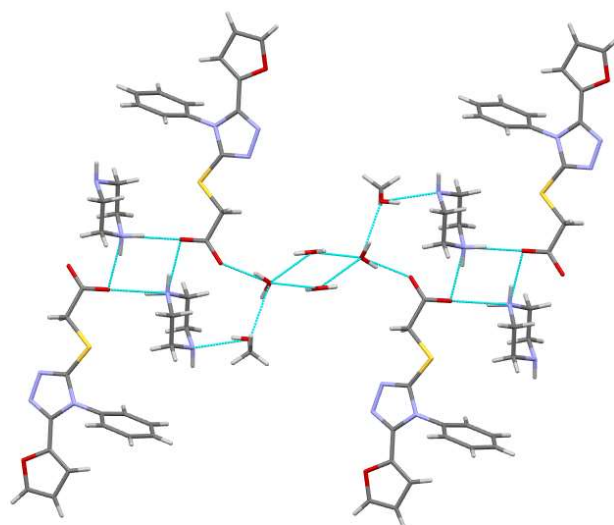


Fig. 3. Tetramers of two cations and two anions bound by hydrogen bonds

Table 1
Acute toxicity of compounds predicted by computer QSAR analysis

No.	Structure	Predicted toxicity (GUSAR, TEST)
1		Intraperitoneal route of administration LD ₅₀ (mg/kg) – 958.1 (GUSAR); Oral route of administration LD ₅₀ (mg/kg) – 1219.53 (TEST)
2		Intraperitoneal route of administration LD ₅₀ (mg/kg) – 387.5 (GUSAR); Oral route of administration LD ₅₀ (mg/kg) – 726.84 (TEST)
3		Intraperitoneal route of administration LD ₅₀ (mg/kg) – 911.3 (GUSAR); Oral route of administration LD ₅₀ (mg/kg) – 845.56 (TEST)

When investigated the crystal structure was found that the crystal is in pinacoid triclinic syngony (Fig. 4). The crystalline triclinic system is unique in that it has only one center of symmetry. Minerals that crystallize in this system have a lower symmetry than each of the other six systems.

The system has no axes of rotation of symmetry and no mirror planes. Crystals of the pinacoid class are limited only by pinacoids (two parallel faces), which we see in the figure. On the structure of the crystal, the axes have different lengths and intersect at different oblique angles. The mineral of the test compound is like microscopic strips, has a flat structure corresponding to triclinic syngony.

According to the results conducted, research found that after a single intragastric administration of the compound at doses of 1,000, 3,000 and 5,000 mg/kg all animals remained alive for 14 days. It was noted that the functional condition of the rats on the experimental group was satis-

factory, they actively ate food and moved. Reflex activity was preserved, no deviations of behavioural reactions were noted. According to general clinical indicators, the condition of the outer mucous membranes and the function of the gastrointestinal tract, the urinary system of the rats of the experimental group did not differ from the control animals. For acute toxicity study in a tentative experiment found that intramuscular application of the compounds at doses of 2500 and 5000 mg/kg of b. w. caused 100 % killing of laboratory rats. Therefore, in the detailed experiment, the compound was administered in the dose range of 500, 1000, 1500, 2000, 2500 mg/kg of body weight of the animal. The research results are shown in Table 3.

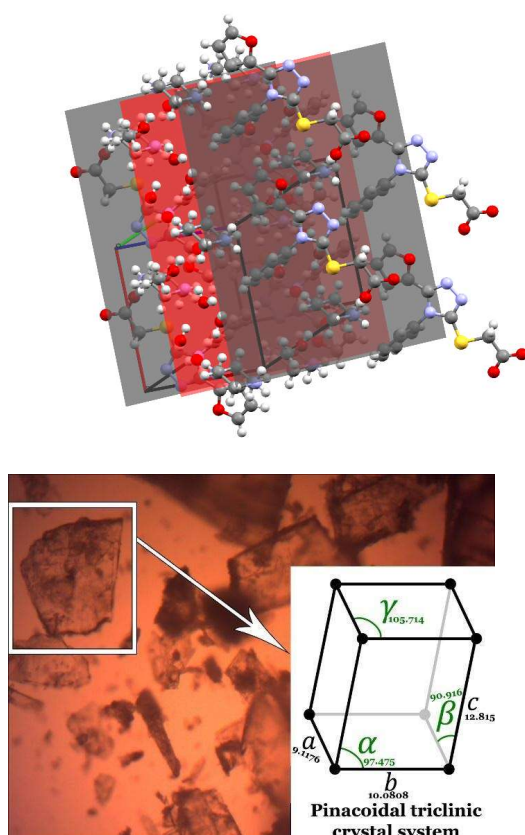


Fig. 4. Crystal structure of the compound

Table 3
Acute toxicity compounds by intramuscular administration to white rats

Number of animals in the group	Dose, mg/kg of b. w.	The number of dead animals	
		total	total
6	500	0	0
6	1,000	1	16.7
6	1,500	2	33.3
6	2,000	4	66.6
6	2,500	6	100

It was found that the intramuscular administration of the compound to animals at a dose of 2500 mg/kg body weight resulted in the death of all 6 animals of this experimental group. Intake of laboratory rats of the lowest test dose (500 mg/kg of b. w.) did not cause fatalities.

In Table 4 there are moderately fatal dose of the compound that served as the basis for calculating the DL_{50} .

Therefore, $DL_{50} = 2,500 - (5,000:6) = 2,500 - 833.33 = 1,666.666$ mg/kg of b. w. According to JMA 85.2-37-736: 2011 [18] a compound belonging to the I V class of toxicity (low-toxic substance) was investigated. During the experiment to study the subacute toxicity of the death of laboratory rats was not found. The course of metabolic processes in the body of laboratory rats, on the background of 3-day intramuscular administration of the compound indicates the body weight of animals of the experimental group and their individual organs (Table 5). Thus, the body weight of animals of the second group who received 10 times the therapeutic dose of the study drug was 9.4 % less than in the control. At the same time, the weights of internal organs also tended to change. Thus, against the background of a slight increase in this indicator in the liver (by 10.4 %), the mass indices of the spleen, heart and kidneys decreased.

Table 4
Mean lethal doses of the compound in white rats by intramuscular injection ($n=6$)

Dose, (mg/kg body weight)	500	1,000	1,500	2,000	2,500
Survived	6	5	4	2	0
Died	0	1	2	4	6
Z	0.5 1.5 3 5				
d	500 500 500 500				
zd	250 750 1500 2500				

Table 5
Coefficients of internal organs of laboratory rats after application of the compound ($M \pm m$, $n=6$)

Internal	A group of animals		
	control	1 experimental	2 experimental
Liver	28.7±1.98	30.9±1.89	31.7±1.98
Spleen	5.22±1.21	5.04±0.63	4.50±0.47
Heart	3.7±0.07	3.53±0.17	3.49±0.08
Lungs	7.08±0.38	8.35±0.94	7.98±0.69
Kidney right	3.82±0.28	3.24±0.09	3.48±0.11
Kidney left	3.51±0.27	3.21±0.09	3.4±0.13
Body weight	251.0±3.98	243.6±7.03	227.3±7.44 *

Note: * – $p < 0.05$ regarding control; ** – $p < 0.01$ regarding control; *** – $p < 0.001$ regarding control

The blood system is one of the most mobile systems that responds quickly to changes in the body's homeostasis due to toxic damage [11]. We found that the intramuscular investigational second compound in the therapeutic (group D1) and 10 times the dose (group D2) is not accompanied by a significant alteration of hematological parameters compared with the control. It was noted that in the blood of rats of the first experimental group the hemoglobin content was higher compared to the control by 7.9 % (Table 6).

When determining the number of leukocytes, it was found that their content in the blood of animals of the experimental groups increased (Table 7). Thus, the number of white blood cells in the blood of rats of group D1 and D2 exceeded that of animals in the control group by 12.0

and 23.7 %, respectively. At the same time, according to the analysis of the leukogram, a probable increase in the percentage of eosinophils (by 66.6 %) in the blood of animals of the second experimental group was noted against the background of a decrease in the level of lymphocytes.

The basis of the biological action of chemical compounds is the violation of several biochemical processes. We found that the studied blood constants, against the background of the use of newly synthesized substance, underwent some changes (Table 8).

Table 6
Morphological parameters of the blood of white rats after application of the compound ($M \pm m$, $n=6$)

Indicator	A group of animals		
	control	1 experimental	2 experimental
Hemoglobin, g/l	146.8±6.21	158.4±4.64	144.2±4.25
Erythrocytes, T/l	6.96±0.35	7.07±0.21	7.03±0.25***
Hematocrit, %	36.04±1.23	39.0±1.04	37.4±0.97
Colour indicator	0.948±0.014	0.992±0.018	0.958±0.021
Platelets	634.6±127.9	657.6±46.33	751.6±68.16*
MSN, pg	21.14±0.29	22.4±0.56	20.5±0.46
MSNS, g/dl	40.5±0.46	40.6±0.16**	38.6±0.32
MCV, μm^3	52.02±1.16	55.3±1.51	53.4±1.21

Note: * – $p < 0.05$ regarding control; ** – $p < 0.01$ regarding control; *** – $p < 0.001$ regarding control

Table 7
Leukogram of rat blood under the action of the compound ($M \pm m$, $n=6$)

Indicator	A group of animals		
	control	1 experimental	2 experimental
Leukocytes, g/l	9.2±1.98	10.3±1.04	11.38±1.68
Eosinophils, %	2.4±0.4	3.2±0.5	4.00±1.1 *
Lymphocytes, %	60.0±2.76	60.8±1.91	54.8±1.85
Neutrophils, %	32.8±2.49	30.5±1.71	35.2±2.42
Monocytes, %	4.8±0.8	5.5±0.5	6.0±0.49

Note: * – $p < 0.05$ regarding control; ** – $p < 0.01$ regarding control; *** – $p < 0.001$ regarding control

Table 8
Biochemical parameters of the blood of white rats after application compounds ($M \pm m$, $n=6$)

Indexes	A group of animals		
	control	1 experimental	2 experimental
Total protein, g/l	71.77±2.07	71.5±2.75	69.3±2.13
Urea, mmol/l	8.63±0.72	8.13±0.51	7.78±0.83
Creatinine, $\mu\text{mol/l}$	74.6±1.39	67.7±1.28***	63.7±1.23**
AST, U/l	215.2±9.72	246.9±19.4	336.12±8.57
ALAT, Od/l	71.7±1.87	86.9±3.33	153.2±2.49**
LF, Od/l	280.95±36.8	305.8±25.04	372.3±17.9
Ca, mmol/l	2.6±0.04	2.43±0.07	2.35±0.07*
Phosphorus, mmol/l	2.53±0.09	2.37±0.13	2.11±0.06***
Magnesium, mmol/l	1.01±0.05	1.72±0.07**	1.51±0.09**
Total cholestr., mmol/l	1.27±1.11	1.16±0.07	1.53±0.23
TAG, mmol/l	0.94±0.09	1.17±0.09	0.98±0.08

Note: * – $p < 0.05$ regarding control; ** – $p < 0.01$ regarding control; *** – $p < 0.001$ regarding control

5. Discussion

Thus, it was found that the content of total protein and urea in the serum of animals of the first and second experimental groups tended to decrease. Moreover, in animals of group D2, which were injected intramuscularly with the drug in 10 times the therapeutic dose, the studied parameters were lower by 3.4 and 9.8 % than in the control. The probable violation of the functional state of the kidneys, under these conditions, was indicated by a probable decrease in the blood of animals of the experimental groups of the concentration of creatinine (by 9.2 and 14.6 %, $P < 0.01$). Therefore, this important factor is also discussed in the development of drugs, which is noted in leading publications and common kidney damage caused by drugs [19]. Given that enzymes play a crucial role in ensuring normal metabolism, which is crucial in maintaining homeostasis, we studied their activity in the body of laboratory animals under the action of the test substance [20]. Transaminase activity has been shown to increase the serum levels of the compounds in test rats. Moreover, in the animals of the second experimental group, ALT activity was probably more than 2 times higher ($P < 0.01$), while AST – by 56.2 %. Alkaline phosphatase activity also increased significantly (by 8.8 and 32.5 %) and depended on the dose of the compound used.

Obviously, the intake of laboratory animals 10 times the therapeutic dose of the compound had a negative impact on mineral metabolism in their body. The calcium-phosphorus ratio in the blood of animals of this experimental group underwent significant deviations, as the concentration of calcium ions probably decreased by 9.6 % ($P < 0.05$) and phosphorus – by 16.6 % ($P < 0.01$). Regarding magnesium, its level exceeded that of animals in the control group by 49.5 % ($P < 0.01$).

It is known that along with the indicators of biological activity of the molecule, it is necessary to consider the class of its toxicity [21]. These data are often related and usually directly proportional. Therefore, before conducting any preclinical studies of new potential drugs, it is advisable to begin an experiment to establish toxicity. From a practical point of view, the expediency of the above pathway is to further use the DL_{50} values to obtain a potential active pharmaceutical moiety from the targeted antibacterial and anthelmintic agent [22, 23].

Study limitations. The study limited the use of a number of laboratory animals and failed to investigate all compounds from these groups.

Prospects for future research. For the development of new drugs, it is advisable to determine the acute toxicity with long-term use of the compound. This study is in the field of practical research in contrast to theoretical and allows a comprehensive assessment of the impact of the compound on the health of the animal and to determine the relationship “chemical structure-toxicity”.

6. Conclusions

1. According to the assessment of the toxicity of the drug “VPK-434” when administered intragastrically to laboratory rats, it was found that in accordance with

SOU 85.2-37-736: 2011 the test substance belongs to the IV class of toxicity (low toxicity).

2. It was found that the average lethal dose of DL50 of the test substance by intramuscular administration to rats is 1,666.66 mg/kg body weight.

3. It was studied that some abnormalities in the hematopoietic system (increase in the number of leukocytes, including eosinophils), liver and kidney function (increased activity of transaminases, decreased serum concentrations of total protein, urea and creatinine) and changes in mineral metabolism of experimental animals groups, on the back-

ground of receiving 10 multiple doses of the study drug, was short-term, and the restoration of the functional state of the body of rats could be said as early as 4–5 days after discontinuation of the drug into their body.

Conflict of interests

The authors declare that they have no conflict of interests.

Financing

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References

1. Zazharskyia, V., Parchenko, M., Parchenko, V., Davydenko, P., Kulishenko, O., Zazharsk, N. (2020). Physicochemical properties of new s-derivatives of 5- (5-bromofuran-2-yl) -4-methyl-1,2,4-triazol-3-thiols. *Voprosy Khimii i Khimicheskoi Tekhnologii*, 6, 50–58. doi: <http://doi.org/10.32434/0321-4095-2020-133-6-50-58>
2. Karpun, E. O., Parchenko, V. V. (2020). Synthesis, physicochemical properties and antigypoxic activity of some S-derivatives of 4-alkyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol. *Farmatsevtichnyi Zhurnal*, 6, 56–64. doi: <http://doi.org/10.32352/0367-3057.6.20.06>
3. Griffin, B. R., Faubel, S., Edelstein, C. L. (2019). Biomarkers of Drug-Induced Kidney Toxicity. *Therapeutic Drug Monitoring*, 41 (2), 213–226. doi: <http://doi.org/10.1097/ftd.0000000000000589>
4. Linciano, P., Gianquinto, E., Montanari, M., Maso, L., Bellio, P., Cebrián-Sastre, E. et. al. (2020). 4-Amino-1,2,4-triazole-3-thione as a Promising Scaffold for the Inhibition of Serine and Metallo- β -Lactamases. *Pharmaceuticals*, 13 (3), 52. doi: <http://doi.org/10.3390/ph13030052>
5. Bigdan, O. A., Parchenko, V. V., Kyrychko, B. P. (2020). Test of antimicrobial activity of morpholine 2- (5- (3-fluorophenyl) -4-amino-1,2,4-triazol-3-ylthio) acetate (BKP-115) by experimental model of pancreatitis in rats. *Ukrainian Journal of Ecology*, 10 (3), 201–207.
6. Yu, X.-Y., Xiao, W.-J., Chen, J.-R. (2019). Synthesis of Trisubstituted 1,2,4-Triazoles from Azlactones and Aryldiazonium Salts by a Cycloaddition/Decarboxylation Cascade. *European Journal of Organic Chemistry*, 2019 (41), 6994–6998. doi: <http://doi.org/10.1002/ejoc.201901467>
7. Huang, T., Jiang, H., Zhao, Y., He, J., Cheng, H., Martyniuk, C. J. (2022). A comprehensive review of 1,2,4-triazole fungicide toxicity in zebrafish (*Danio rerio*): A mitochondrial and metabolic perspective. *Science of The Total Environment*, 809, 151177. doi: <http://doi.org/10.1016/j.scitotenv.2021.151177>
8. Govorka, J., Maklakova, L. P., Mitukh, J. et. al. (1988). Helminths of wild ungulates of Eastern Europe. Moscow: Nauka, 208.
9. Ten Doesschate, S. J., Pomroy, W. E., Tapia-Escárate, D., Scott, I., Wilson, P. R. (2017). Establishment rate of cattle gastrointestinal nematodes in farmed red deer (*Cervus elaphus*). *Veterinary Parasitology*, 243, 105–108. doi: <http://doi.org/10.1016/j.vetpar.2017.06.016>
10. Hora, F. S., Genchi, C., Ferrari, N., Morariu, S., Mederle, N., Dărăbuș, G. (2017). Frequency of gastrointestinal and pulmonary helminth infections in wild deer from western Romania. *Veterinary Parasitology: Regional Studies and Reports*, 8, 75–77. doi: <http://doi.org/10.1016/j.vprsr.2016.12.009>
11. Kosenko, M. V., Malik, O. H., Kotsyumbas, I. Ya., Paterega, I. P., Chura, D. O. (1997). Toxicological control of new means of animal protection. Kyiv, 34.
12. Sheldrick, G. M. (2007). A short history of SHELX. *Acta Crystallographica Section A Foundations of Crystallography*, 64 (1), 112–122. doi: <http://doi.org/10.1107/s0108767307004390>
13. Kotsyumbas, I. et. al. (Eds.) (2006). Preclinical studies of veterinary drugs. Lviv: Triada Plus, 360.
14. Burgi, H.-B., Dunitz, J. D. (1994). Structure correlation. Vol. 2. VCH. Weinheim, 741–784.
15. Zefirov, N. S., Palyulin, V. A., Dashevskaya, E. E. (1990). Stereochemical studies. XXXIV. Quantitative description of ring puckering via torsional angles. The case of six-membered rings. *Journal of Physical Organic Chemistry*, 3 (3), 147–158. doi: <http://doi.org/10.1002/poc.610030304>
16. Zefirov, Iu. V. (1997). Sokrashchennye mezhmolekuliarnye kontakty i spetsificheskie vzaimodeistviia v molekuliarnykh kristallakh. *Kristallografiia*, 42 (5), 936–958.
17. Prozorovsky, V. B. (2007). Statistical processing of results of pharmacological researches. *Psychopharmacology and biological narcology*, 7 (3-4), 2090–2120.
18. Stefanov, O. V. (2001). Preclinical studies of medicinal drugs. Kyiv: Avicenna, 528.
19. Qiao, K., Fu, W., Jiang, Y., Chen, L., Li, S., Ye, Q., Gui, W. (2020). QSAR models for the acute toxicity of 1,2,4-triazole fungicides to zebrafish (*Danio rerio*) embryos. *Environmental Pollution*, 265, 114837. doi: <http://doi.org/10.1016/j.envpol.2020.114837>
20. Jabli, D., Milad, R., Abderrabba, M., Efrat, M. L. (2019). Synthesis, Antibacterial Activity and DFT Calculation of Naphtopyrano, Furo and Pyrazolo [3,2,e] [1,2,4]Triazolo-[1,5-c]Pyrimidine Derivatives. *Chemistry Africa*, 2 (4), 597–613. doi: <http://doi.org/10.1007/s42250-019-00081-y>
21. Zazharskyi, V., Parchenko, M., Parchenko, V., Davydenko, P., Kulishenko, O., Zazharska, N. (2020). Physicochemical properties of new S-derivatives of 5-(5-bromofuran-2-yl)-4-methyl-1,2,4-triazol-3-thiols. *Voprosy Khimii i Khimicheskoi Tekhnologii*, 6, 50–58. doi: <http://doi.org/10.32434/0321-4095-2020-133-6-50-58>

22. Kaproń, B., Czarnomysy, R., Wysokiński, M., Andrys, R., Musilek, K., Angeli, A. et. al. (2020). 1,2,4-Triazole-based anti-convulsant agents with additional ROS scavenging activity are effective in a model of pharmacoresistant epilepsy. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 35 (1), 993–1002. doi: <http://doi.org/10.1080/14756366.2020.1748026>
23. Wu, H., Sun, Q., Sun, Y., Zhou, Y., Wang, J., Hou, C. et. al. (2019). Co-metabolic enhancement of 1H-1,2,4-triazole biodegradation through nitrification. *Bioresource Technology*, 271, 236–243. doi: <http://doi.org/10.1016/j.biortech.2018.09.112>

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