UDC 615.31:547.792].099
DOI: 10.14739/2310-1210.2023.1.266318

Study of acute toxicity of new thiophene-containing derivatives of 1,2,4-triazole

A.V. Khilkovets *B,C,D, **I. M. Bilai** A,E,F

Zaporizhzhia State Medical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Key words:
acute toxicity, rats, 1,2,4-triazole derivatives.

Zaporozhye medical journal 2023; 25 (1), 46-49

***E-mail:**
nastia010792@ukr.net

Acute toxicity studies are an integral part of preclinical studies of any new biologically active compound. It should be noted that it is this stage of research that is crucial regarding the possibility of further use of a pharmacologically active substance as a drug. This indicator also helps to determine the initial dose for clinical trials and establish a range of potentially safe doses. The data obtained will help determine the direction of new chemical syntheses, replenish the relevant libraries in silico, as well as reveal many other fundamentally important parameters that characterize the interaction in the compound – living organism system.

The aim of this research is to study the acute toxicity of 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)sodium acetate.

Materials and methods. Previously, a prediction was made using the GUSAR computer program, which helped determine the dose intervals. Acute toxicity was determined by the experimental method of Kerber in vivo using white nonlinear Wistar rats. The rats were weighed, labeled, and divided into five groups of six individuals of each.

Results. After the introduction of compound moving activity decreased, drowsiness, pupil miosis, and thirst were observed. In the fifth group, with the maximum dilution, all rats died within two hours after administration of the test compound. During the death, convulsions were observed. In the fourth group five animals died, and in the third – two rats died. In the first two groups, all the rats survived. During follow-up, the animals behaved normally. Based on the results of the research, calculations were made and the LD_{50} indicator was determined.

Conclusions. According to the results, the studied compound belonged to the V class of toxicity (almost non-toxic), and the resulting LD_{50} value was 1125 mg/kg. This indicator confirmed the prospects for further study of this compound.

Ключові слова:
гостра токсичність, щури, похідні 1,2,4-тріазолу.

Запорізький медичний журнал. 2023. Т. 25, № 1(136). С. 46-49

Вивчення гострої токсичності нових тіофен-вмісних похідних 1,2,4-тріазолу

А. В. Хільковець, І. М. Білай

Дослідження гострої токсичності – невід'ємний етап доклінічних досліджень будь-якої нової біологічно активної сполуки. Саме цей етап дослідження має вирішальне значення щодо можливості наступного використання фармакологічно активної речовини як лікарського засобу. Цей показник допомагає також визначитися з початковою дозою для клінічних досліджень і встановити діапазон потенційно безпечних доз. Результати допоможуть визначитися зі спрямованістю нових хімічних синтезів, поповнити відповідні бібліотеки in silico, а також розкрити інші принципово важливі параметри, що характеризують взаємодію в системі сполука – живий організм.

Мета роботи – дослідження гострої токсичності 2-((4-феніл-5-(тіофен-3-ілметил)-1,2,4-тріазол-3-іл)тіо)ацетат натрію.

Матеріали та методи. Попередньо здійснили прогнозування з використання комп'ютерної програми GUSAR, що допомогло визначитися з інтервалами доз. Гостру токсичність визначали за допомогою експериментального методу Кербера in vivo, використали білих нелінійних щурів лінії Вістар. Щурів зважили, промаркували та поділили на п'ять груп по 6 особин у кожній.

Результати. Після введення сполуки рухова активність щурів знижувалася, спостерігали сонливість, міоз зіниць і спрагу. В п'ятій групі (з максимальним розведенням) усі щури загинули в період близько 2 годин після введення досліджуваної сполуки, під час загибелі спостерігали судоми. В четвертій групі загинули 5 особин, у третій – 2 щури. В перших двох групах усі тварини вижили. Під час наступного спостереження щури поводили себе нормально. За результатами досліджень здійснили розрахунки, визначили показник LD_{50}.

Висновки. Встановили, що досліджувана сполука належить до V класу токсичності (практично нетоксичні), показник LD_{50} становить 1125 мг/кг. Це обґрунтовує перспективність продовження вивчення цієї сполуки.

The accelerated development of the chemical industry is explained by the desire to compensate for the lack of natural materials that are traditionally used. Also, the rapid development is explained by the need to create new synthetic substances that are superior to natural compounds in consumer properties or differ in a wider range of applications. In today's completely natural conditions, continuous population growth has its downside, namely, an increase in the degree of negative impact on the environment. Currently, a significant part of humanity is more or less exposed to various chemical compounds. They enter the body with air, water, and food, and of course in the form of medicines [1,2]. An obvious consequence of this effect was an increase in the incidence rate, especially associated with drug poisoning, impaired immune status, and so on. Therefore, the most important requirement for newly synthesized compounds is their low, acceptable degree of toxicity.

Taking into account the work of scientists at our university and foreign scientists, it can be concluded that derivatives of 1,2,4-triazole are low-toxic or practically non-toxic

compounds [3,4]. This fact is undoubtedly convincing when choosing a "molecular framework" for obtaining new promising heterocyclic compounds. To date, a considerable number of these derivatives have been obtained, which are used in numerous industries, pharmacies, and veterinary medicine [5]. A striking example is morpholinium-3-methyl-1,2,4-triazolyl-5-thioacetate, which later became known as Thiotriazoline. To date, Thiotriazoline has a wide spectrum of use in the treatment of diseases in neurology, surgery, cardiology, ophthalmology, gynecology, and urology [6].

A team of Ukrainian scientists continues to study Thiotriazoline as a means of combating today's current disease – COVID-19 [7]. Another significant scientific discovery among 1,2,4-triazole derivatives is the drug Angiolin ((S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate) which has high prospects in ophthalmology and cardiology [8,9]. It should be noted that the compounds mentioned above belong to the V class of toxicity (almost non-toxic substances). But the search continues in the future because science is progressing.

Traditionally, the detection of the degree of toxicity is carried out experimentally on laboratory animals. Toxicological testing is a long-term process, an alternative to which can be non-experimental screening using expert systems. These systems are based on previously identified quantitative structure-toxicity patterns for already known compounds and allow predicting toxic properties for new, not yet synthesized chemical compounds. Taking into account all the possibilities of the present and biotic norms, we analyzed a number of compounds and selected one for the study of acute toxicity in vivo.

Aim

The aim of our work is to determine acute toxicity in vivo for the previously obtained compound XPI I-28, namely 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl) thio)sodium acetate.

Materials and methods

The studied compound was obtained at the Department of Natural Sciences for Foreign Students and Toxicological Chemistry of Zaporizhzhia State Medical University (ZSMU) as part of the planned thesis work under the supervision of Professor V. V. Parchenko (*Fig. 1*).

The research was conducted in accordance with the national "general ethical principles of animal experiments" (Ukraine, 2001), which is consistent with the provisions of the "European Convention for the protection of vertebrates used for experiments and other scientific purposes" (Strasbourg, France, 1985), as well as in accordance with the EU Council Directive [10].

To determine dose levels, the GUSAR (General unlimited Structure-Activity Relationships) program performed computer prediction of acute toxicity. The specified GUSAR computer program is designed to build models of quantitative dependencies between the structure and various properties of organic molecules. GUSAR uses QNA (Quantitative neighbors of Atoms) descriptors, some physical-chemical descriptors, and (as additional parameters) the results of predicting

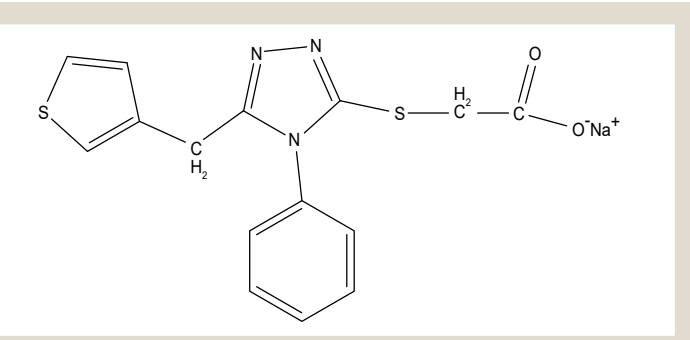

Fig. 1. Sodium 2-((4-phenyl-5-(thiophen-3-ylmethyl)-1,2,4-triazole-3-yl)thio)acetate.

3,663 types of activity by the PASS program. When predicting the LD_{50} value for the analyzed compound, it is estimated that it falls within the scope of the QSAR model. Computer prediction of acute toxicity was carried out according to the structural formula in the Internet version of the GUSAR-online program. According to the received online prediction data, the average lethal dose of LD_{50} for the tested compound XPI I-28 had the following values: with intraperitoneal administration – 681.6 mg/kg, intravenously – 183.3 mg/kg, orally – 752.7 mg/kg, and subcutaneously – 468.7 mg/kg.

The study of acute toxicity of a water-soluble compound was carried out in accordance with the recommendations of O. V. Stefanov [11]. The determination of acute toxicity parameters (LD_{50}) was calculated by the Kerber method in modifications of A. O. Loyt and M. F. Savchenkov. The research was carried out jointly with the staff of the ESMLC with the vivarium of ZSMU.

For this, six groups were formed, one of which was a control group. Each group consisted of six non-linear white rats. The rats were weighed and labeled. An aqueous solution of the test compound was administered to animals on an empty stomach intraperitoneally.

During the experiments, feeding and maintenance conditions were maintained, and water intake was not restricted. Rats were monitored for 14 days from the moment of administration of the substance. During the observation, their behavior, general condition, the clinical picture of poisoning, attitude to food, the condition of the coat, and visible mucous membranes were taken into account. Body weight control was performed on the day of administration of the substance, as well as on days 3, 7, and 14.

Results

According to the results of the study, it was found that the compound XPI I-26 when administered intraperitoneally caused the death of rats. General information on the dose, weight, and death of animals is given below (*Table 1*).

During the experiment, it was found that the highest dose of 3000 mg/kg caused the death of all rats within two hours after administration. Abdominal breathing, drowsiness, loss of pain reflex, atypical ways of movement (convulsions), and prostration were noted during clinical observations of animals. The death of animals was accompanied by tonic-clonic convulsions lasting up to one minute.

A dose of 1500 mg/kg also caused suppression of the general condition of the rats, as well as a decrease in appetite and motor activity. Diaphragmatic breathing and

Table 1. Characteristics of the material under study

Dose, mg/kg	Cipher	Weight, g	Volume, ml	Time of death
200	DS-I	302	3.0	
	DS-II	277	2.8	
	DS-III	280	2.8	
	F-I	292	2.9	
	F-II	271	2.7	
	F-III	264	2.6	
500	DS-I	348	3.5	
	DS-II	243	2.4	
	DS-III	331	3.3	
	F-I	212	2.1	
	F-II	313	3.1	
	F-III	276	2.8	
700	DS-I	270	2.7	
	DS-II	250	2.5	
	DS-III	238	2.4	10:10
	F-I	197	1.9	
	F-II	240	2.4	
	F-III	390	3.9	12:34
1500	DS-I	282	2.8	10:45
	DS-II	324	3.4	12:30
	DS-III	282	2.8	
	F-I	228	2.3	11:10
	F-II	259	2.6	10:30
	F-III	255	2.5	11:11
3000	DS-I	260	2.6	10:50
	DS-II	301	3.0	11:30
	DS-III	219	2.2	10:55
	F-I	240	2.4	10:57
	F-II	197	2.0	11:01
	F-III	219	2.2	10:55

Table 2. Toxicity results

Dose, mg/kg	n_1 alive pieces	n_2 died pieces	n pieces	e_1 interval mg/kg	$n \times e_1$	LD_{100}, mg/kg	LD_{50}, mg/kg
200	6	0	0	200	0	3000	1125
500	6	0	0	300	0		
700	4	2	1	200	200		
1500	1	5	3.5	800	2800		
3000	0	6	5.5	1500	8250		

Table 3. Dynamics of rat body mass (g), V ± m

Animal groups	Observation period			
	Outgoing data	3rd day	7th day	14th day
Intact control	273.00 ± 20.83	274.00 ± 20.38	274.00 ± 20.26	274.00 ± 20.64
200 mg/kg	281.00 ± 13.91	276.00 ± 14.81*	278.00 ± 14.89*	279.00 ± 15.13
500 mg/kg	287.1 ± 52.9	281.00 ± 55.00*	284.00 ± 52.68*	284.00 ± 53.12*
700 mg/kg	264.00 ± 32.5	210.00 ± 20.07	219.00 ± 20.30	220.00 ± 21.07
1500 mg/kg	271.00 ± 32.5	–	–	–
3000 mg/kg		–	–	–

*: reliability relative to the initial data, according to the Student's t-criterion, P ≥ 0.05.

prostration (abdominal position) were observed in animals in this group. Miosis, narrowing of the pupils regardless of the presence of light, was also detected. In this group, five animals out of six died, during the death of which convulsions were also observed.

Among the animals that received a dose of 700 mg/kg in the first hours after administration, one animal died, during the death of which convulsions were not observed. The rats were depressed and sedentary, and their appetite was reduced during the first day of observation.

As a result of monitoring animals that received a dose of 500 mg/kg and 200 mg/kg, signs of intoxication and animal death were not been. The general condition of the rats was satisfactory, behavior changes were not detected, the rats' appetite and thirst did not change, and seizures were not observed.

Based on the data obtained during the experiment, the average annual dose was calculated by the method of Kerber in the modification of A. O. Loyt and M. F. Savchenkov, according to the formula:

$$LD_{50} = LD_{100} - \frac{\sum \bar{n} \times e}{n}$$

According to calculations (*Table 2*), the compound XPI I-28 belonged to the V Class of toxicity. It was practically non-toxic since LD_{50} was equal to 1125 mg/kg. It was in the range of 1001–3000 mg/kg when administered intraperitoneally. The dynamics of weight in surviving rats is shown in *Table 3*. Rat body weight changed most significantly at a dose of 700 mg/kg on days 3, 7, and 14 (20.45 %, 17.04 %, and 16.60 % relative to the initial data). To a lesser extent, changes were observed at a dose of 500 mg/kg on all days of follow-up. In the group of rats that were given a dose of 200 mg/kg, the body weight of rats did not change significantly, namely within 1 % relative to the initial data.

The picture of acute poisoning of animals that received a toxic dose of the test substance during intraperitoneal administration was characterized by lethargy, passivity, and polydipsia in all rats. After a few minutes, Cheyne-Stokes breathing and inactivity worsened. After 2 hours, breathing became barely noticeable, and weak, movements were not been. Animals, that received intermediate doses of the test substance and survived up to 14 days, experienced lethargy, passivity, and polydipsia for 12 hours. Cheyne-Stokes breathing occurred due to a violation of blood supply and a decrease in excitability of the respiratory center. After 36 hours, the symptoms gradually began to subside, until they completely disappeared. The rats breathing returned to normal, motor activity resumed, and a healthy appetite appeared after 72 hours later on the drug was administered.

It was pronounced fullness of the peritoneal vessels, full-blooded liver, light brown color, soft consistency, and rounded edges of the liver after an anatomical autopsy of dead rats from all groups that were intragastrical injected with the test substance. The gastric mucosa was hyperemic and swollen. On the cut was a shiny fabric. Portal tracts are swollen, and it showed the fullness of the central interlobular veins. Brain-no visible changes, small hemorrhages, vessels of the cerebellum and trunk are dilated, full-blooded. The thymus was moderately flabby, pale pink. The bladder was full. The small intestine was filled with contents, the descending colon was swollen. Kidneys with visible external did not change. In the heart of dead animals, the ventricles were dense in the stage of contraction, the blood vessels of the arterial and venous bed were full-blooded, and there was a small accumulation of blood in the pericardial space.

Discussion

The data obtained on the acute toxicity of 2-((4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)sodium acetate did not contradict the data of other researchers. Thus, the study of the acute toxicity of the drug Thiotriazoline showed that it had low toxicity with different routes of administration to four animal species, and the drug belonged to the V class of toxicity (almost non-toxic substances). When administered to mice, its LD_{50} was 6500 mg/kg with intragastric and 2350 mg/kg with intramuscular administration.

In rats, LD_{50} with intragastric administration – 10300 mg/kg, intravenous – 890 mg/kg and intramuscular – 5150 mg/kg. In dogs and rabbits, LD_{50} with intravenous administration is more than 2000 mg/kg, and with intramuscular administration is 2500 mg/kg. This is due to the fact that the structure of 1,2,4-triazole was closely related to some nuclear transcription factors capable of regulating gene expression.

1,2,4-Triazoles were involved in energy and protein metabolism [12]. We attempted to modify the Thiotriazoline molecule by replacing substituents in the 5 positions of methyl with thiophene-3-ilmethyl and in the 4 position by changing hydrogen to a phenyl group in order to increase the safety profile of a potential medicinal substance. Obtaining a new molecule led to a decrease in toxicity compared to Thiotriazoline but did not exceed the safety parameters of Angiolin. Thus, after addition of the aromatic heterocycle of thiophene in the fifth position significantly reduced the acute toxicity of the compound.

Conclusions

According to the results, the studied compound belonged to the V class of toxicity (almost non-toxic), and the resulting LD_{50} value was 1125 mg/kg. This indicator confirmed the prospects for further study of this compound.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Надійшла до редакції / Received: 28.10.2022
Після доопрацювання / Revised: 01.12.2022
Прийнято до друку / Accepted: 07.12.2022

Information about authors:
Khilkovets A. V., PhD-student of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Assistant of the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0001-7401-9458
Bilai I. M., MD, PhD, DSc, Professor, Head of the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0002-7574-4093

Відомості про авторів:
Хільковець А. В., очний аспірант каф. природничих дисциплін для іноземних студентів та токсикологічної хімії, асистент каф. клінічної фармації, фармакотерапії, фармакогнозії та фармацевтичної хімії, Запорізький державний медичний університет, Україна.
Білай І. М., д-р мед. наук, професор, зав. каф. клінічної фармації, фармакотерапії, фармакогнозії та фармацевтичної хімії, Запорізький державний медичний університет, Україна.

References

[1] Liu, Z., Dang, K., Gao, J., Fan, P., Li, C., Wang, H., Li, H., Deng, X., Gao, Y., & Qian, A. (2022). Toxicity prediction of 1,2,4-triazoles compounds by QSTR and interspecies QSTTR models. *Ecotoxicology and environmental safety*, *242*, 113839. https://doi.org/10.1016/j.ecoenv.2022.113839

[2] Opsomer, T., & Dehaen, W. (2022). 1,2,4-Triazoles. In *Comprehensive Heterocyclic Chemistry IV* (pp. 78-121). Elsevier. https://doi.org/10.1016/b978-0-12-409547-2.14854-1

[3] Shcherbyna, R. O., Danilchenko, D. M., Parchenko, V. V., Panasenko, O. I., Knysh, E. H., Hromyh, N. A., & Lyholat, Y. V. (2017). Studying Of 2-((5-R-4-R-1-4H-1,2,4-Triazole-3-Yl)Thio)Acetic Acid Salts Influence On Growth And Progress Of Blackberries (KIOWA Variety) Propagules. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, *8*(3), 975-979.

[4] Kaplaushenko, A. H., Parchenko, V. V., Panasenko, O. I., Knysh, Ye. H., & Darii, K. V. (2008). Syntez i hostra toksychnist solei 2-[(5-R-4-R1-1,2,4-triazol-3-iltio)-2-(karboksymetyltio)karbonotioil] hidrazynoatsetatnykh kyslot [Synthesis and acute toxicity of salts of 2-[(5-R-4-R_1-1,2,4-triazole-3-ylthio)-2-(carboxymethylthio)-carbonothioyl]hydrazinoacetate acids]. *Current issues of pharmaceutical and medical science and practice, 21*, (Vol. 2, pp. 87-92). [in Ukrainian].

[5] Bigdan, O. (2021). Toxicity of Substance BKP-115 on Rats and Mice of Both Sexes at Long Term Intragastric Introduction. *Archives of Pharmacy Practice*, *12*(2), 6-11. https://doi.org/10.51847/v8mvj5iqgt

[6] Bielenichev, I. F., Vizir, V. A., Mamchur, V. Yo., & Kuriata, O. V. (2019). Mesto tiotriazolina v galeree sovremennykh metabolitotropnykh lekarstvennykh sredstv [Place of tiotriazoline in the gallery of modern metabolitotropic medicines]. *Zaporozhye medical journal, 21*(1), 119-128. [in Russian]. https://doi.org/10.14739/2310-1210.2019.1.155856

[7] Belenichev, I., Kucherenko, L., Pavlov, S., Bukhtiyarova, N., Popazova, O., Derevianko, N., & Nimenko, G. (2022). Therapy of post-COVID-19 syndrome: improving the efficiency and safety of basic metabolic drug treatment with tiazotic acid (thiotriazoline). *Pharmacia*, *69*(2), 509-516. https://doi.org/10.3897/pharmacia.69.e82596

[8] Belenichev, I., Kucherenko, L., Nagornaya, E., Mazur, I., Bidnenko, A., Bukhtiyarova, N., & Avramenko, N. (2015). Functional nitric oxide conjugate systems state/restored heart thiols of rats in modeling isadrine-pituitrin's myocardial infarction using metabolite-tropic cardioprotector "Angiolin". *International Journal of Basic & Clinical Pharmacology*, *4*(1), 15-21. https://doi.org/10.5455/2319-2003.ijbcp20150238

[9] Belenichev, I. F., Aliyeva, E. G., Kamyshny, O. M., Bukhtiyarova, N. V., Ryzhenko, V. P., & Gorchakova, N. O. (2022). Pharmacological Modulation of Endogenous Neuroprotection after Experimental Prenatal Hypoxia. *Neurochemical Journal*, *16*(1), 68-75. https://doi.org/10.1134/s1819712422010044

[10] (1999). Direktiva Soveta ES o sblizhenii zakonov, postanovlenii i administrirovanie polozhenii gosudarstv ES po voprosam zashchity zhivotnykh, ispol'zuemykh dlya eksperimental'nykh i drugikh nauchnykh tselei (86/609/EES) [Directive of the Council of the EU on the convergence of laws, decrees and administrations of the EU states on the protection of animals used for experimental and other scientific purposes (86/609/EEC)]. In N. A. Lyapunov, V. A. Zagorii, V. P. Georgievskii, & E. P. Bezuglaya (Eds.), *Nadlezhashchaya proizvodstvennaya praktika lekarstvennykh sredstv* [Appropriate production practice of medicinal products] (pp. 508-545). Morion. [in Russian].

[11] Stefanov, O. V. (Ed.). (2001). *Doklinichni doslidzhennia likarskykh zasobiv* [Preclinical studies of medicinal products: methodical recommendations]. Kyiv: Avicena. [in Ukrainian].

[12] Bak, P. G., Belenichev, I. F., Kucherenko, L. I., Abramov, A. V., & Khromylova, O. V. (2021). Morpho-functional indicators changes of rats' myocardium in experimental doxorubicin-induced chronic heart failure and its pharmacological modulation with new 4-amino-1,2,4-triazole derivative. *Pharmacia*, *68*(4), 919-925. https://doi.org/10.3897/pharmacia.68.e75298