A study of acute toxicity of newly synthesized compound on adult hydrobiont Danio rerio

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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

From ancient times the people used different poisons as a treatment for diseases of different genesis. To date, almost nothing has changed. One of the main conditions for the development of a new drug is its low toxicity. Literature analysis has shown that 1,2,4-triazole-3-thione derivatives are low-toxic compounds and exhibit a wide range of pharmacological activities.

The aim of this work was the investigate acute toxicity LC50 in vivo of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate on the Zebrafish (Danio rerio) aquatic model according to OECD instruction № 203.

Materials and methods. Determination of acute toxicity LC50 of the test compound was performed in vivo on a model of the aquatic organism Zebrafish (Danio rerio) according to the instruction OECD № 203 (Fish, Acute Toxicity Test) for testing chemical compounds (acute toxicity test on fish from 10.12.2009). It was used fish 2 months of age, 11.8 ± 0.1 mm long, and weighing 2.6 ± 0.2 g in the experiment. The concentration of the test compound ranged from 5.0 mg/l to 100.0 mg/l. Test water-soluble compounds were dissolved in distilled water. Each mini-aquarium with a certain dose of the compound contained at least 7 individuals of Danio rerio. During the experiments, the fish were kept on a diet for a test 96 hours and their mortality was checked every 24, 48, 72 and 96 hours. Statistical analysis of the results was performed using the program Statistica 6.

Results. According to the obtained data, graphs of the dependence of the concentration on the fish mortality percentage were constructed. Then the corresponding values of LC50, sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate were calculated. According to the acute toxicity LC50 (96 hours) of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate, which according to the classification of D. R. Passino and co-authors allowed it to be classified as a moderately toxic compound.

Conclusions. The acute toxicity LC50 in vivo of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate on the Zebrafish (Danio rerio) aquatic model was investigated. The research was conducted in accordance with the national “General Ethical Principles of Animal Experimentation” approved by the First National Congress on Bioethics and the “Bioethical Expertise of Preclinical and Other Animal Research”. The highest fish mortality occurs on the last day of observation (96 hours). The highest number of Zebrafish deaths is at a minimum concentration of the substance. The acute toxicity LC50 (96 hours) of the test substance was 4.5364 mg/l.

Key words: acute toxicity tests, 1,2,4-triazole, salts, heterocyclic compounds.

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Дослідження гострої токсичності нової сполуки на дорослому гідробіонті Zebrafish (Danio rerio)

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Від стародавніх часів люди використовували різні отрути для лікування захворювань різного ґенезу, і донині майже нічого не змінилося. Одна з основних умов створення нового лікарського засобу – його низька токсичність. Аналіз даних фахової літератури показав, що похідні 1,2,4-тріазол-3-тіону – малотоксичні сполуки, які проявляють широкий спектр фармакологічних активностей.

Мета роботи – дослідження гострої токсичності LC50 in vivo натрю 2-((4-аміно-5-((тіофен-2-ілметил)-4Н-1,2,4-тріазол-3-іл)тіо)акетату на водній моделі Zebrafish (Danio rerio) згідно з інструкцією OECD № 203.

Матеріали та методи. Гостру токсичність LC50 досліджуваної сполуки виявляли in vivo на моделі водного організму Zebrafish (Danio rerio) згідно з інструкцією OECD № 203 (Fish, Acute Toxicity Test) для випробування хімічних сполук (тест гострої токсичності на рибах від 10.12.2009 р.). В експерименті використовували риб віком 2 місяці, завдовжки 11.8 ± 0.1 мм і важію 2.6 ± 0.2 г. Концентрація досягненої сполуки становила від 5.0 мг/л до 100.0 мг/л. Водорозчинні речовини розчиняли в дистильованій воді. Кожен міні-акваріум з відповідною дозою сполуки містив щонайменше 7 індивідів Danio rerio. Під час експериментів риб тримали на дієті протягом 96 годин, а їхню смертність перевіряли кожні 24, 48, 72 і 96 годин. Статистичний аналіз результатів виконали за допомогою програми Statistica 6.

Key words: acute toxicity tests, 1,2,4-triazole, salts, heterocyclic compounds.

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From ancient times the people used different poisons as a treatment for diseases of different genesis. To date, almost nothing has changed. One of the main conditions for the development of a new drug [1–4] is its low toxicity.

The study of newly synthesized compounds for acute toxicity is a necessary component for further determination of biological activity and effective dose of the future drug [5–9].

Not only mice or rats can be used to determine acute toxicity, but it can also be an aquatic organism – Zebrafish (Danio rerio) [10,11].

**Aim**

The aim of this work was the investigate acute toxicity LC	extsubscript{50} in vivo of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)thio)acetate on the Zebrafish (Danio rerio) aquatic model according to OECD instruction № 203.

**Materials and methods**

Determination of acute toxicity LC	extsubscript{50} of the test compound was performed in vivo on a model of the aquatic organism Zebrafish (Danio rerio) according to the instruction OECD № 203 (Fish, Acute Toxicity Test) for testing chemical compounds. The experiment was performed in vivo on a model of the aquatic organism Zebrafish (Danio rerio) according to OECD instruction № 203.

**Results.** Po the obtained data, the relationship between the concentration of the test compound LC	extsubscript{50} and the percentage of fish mortality was calculated. The concentration of the test compound LC	extsubscript{50} was determined according to the OECD instruction № 203.

**Conclusions.** The obtained acute toxicity LC	extsubscript{50} of the test compound indicated its low toxicity. The results of the experiment confirmed the reliability of the experimental data and the appropriateness of the methods used.

**Key words:** 1,2,4-triazole, acute toxicity, soli, gteroцикличные соединения.

Aim: The aim of this work was to investigate acute toxicity LC	extsubscript{50} in vivo of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate on the Zebrafish (Danio rerio) aquatic model according to OECD instruction № 203.
(pH = 7.3 ± 0.3; 95 % Cl) at a temperature of 26.5 ºC. This oxygen-enriched water was used for experiments. Prior to the experiments, the fish were acclimatized, with a mortality rate of no more than 1 in 500 individuals. Test water-soluble compounds were dissolved in distilled water. Each mini-aquarium with a certain dose of the compound contained at least 7 individuals of Danio rerio. During the experiments, the fish were kept on a diet for a test 96 hours and their mortality was checked every 24, 48, 72, and 96 hours.

Statistical analysis of the results was performed using the program Statistica 6.

The degree of toxicity of the tested compounds was determined according to the classification of D. R. Passino [11].

The research was conducted in accordance with the national “General Ethical Principles of Animal Experimentation” approved by the First National Congress on Bioethics and the “Bioethical Expertise of Preclinical and Other Animal Research”. These principles have been developed in accordance with the basic principles of bioethics and bioethical expertise in the interests of human protection and the entire biological diversity of the world. They are in line with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes”.

Fish are considered dead if there are no visible movements (for example, gill movements) and if touching the tail stalk does not cause a reaction. Dead fish were removed by observation and mortality was recorded.

Results

As a result, the acute toxicity LC$_{50}$ in vivo of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate on the Zebrafish (Danio rerio) aquatic model was investigated.

The acute toxicity LC$_{50}$ (96 hours) of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate was 4.5364 mg/l. It can be concluded that the compound belongs to the class of moderately toxic substances.

Discussion

Every 24, 48, 72, and 96 hours, mortality of the Zebrafish was entered in the Table 1.

After examining the mortality of animals at appropriate intervals, it is possible to draw conclusions about the relationship between “mortality – a period of time”.

Table 1 shows that the highest fish mortality occurs on the last day of observation (96 hours). This can be explained by the accumulation of the compound in the body of animals (Fig. 1).

After 48 hours, acute poisoning occurs, as evidenced by the mortality of animals at high concentrations.

According to the obtained data, graphs of the dependence of the concentration on the fish mortality percentage were constructed (Fig. 2, 3, 4). Then the corresponding values of LC$_{50}$ sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate were calculated.

Table 1. The dependence of the mortality of the Zebrafish on the substance concentration

| Concentration, mg/l | 24 hours | 48 hours | 72 hours | 96 hours |
|--------------------|----------|----------|----------|----------|
| 5                  | 0        | 2/29     | 2/29     | 3/42     |
| 10                 | 0        | 1/14     | 0        |          |
| 18                 | 0        | 0        | 0        | 2/29     |
| 34                 | 0        | 0        | 1/14     | 2/29     |
| 65                 | 0        | 1/14     | 0        | 1/14     |
| 100                | 0        | 1/14     | 0        | 2/29     |

After analyzing the initial data, it can be concluded that the acute toxicity LC$_{50}$ (48 hours) of the test compound was 4.1129 mg/l.

Based on the above data, it can be concluded that the acute toxicity of LC$_{50}$ (72 hours) of the test compound was 8.4888 mg/l.

Acute toxicity LC$_{50}$ (96 hours) was 4.5364 mg/l.

The equation for calculating mortality taking into account a certain concentration of the compound at 96 hours:

\[
\text{Mortality} \% = \frac{7,8506 + \frac{91,1372 - 7,8506}{1 + (\text{concentration, mg/l})^{11,3741}}}{4,5364}
\]

According to the acute toxicity of LC50 (96 hours) of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate, which according to the classification of D. R. Passino and co-authors allowed it to be classified as a moderately toxic compound.

At low concentrations (5–10 mg/l) of the test substance – exophthalmos was observed (swelling in the orbital fossae, which leads to convexity of one or both eyes). In some cases, there was an increase in spontaneous activity.

Clinical signs in the middle range of concentrations (18–65 mg/l) were marked by the appearance of petechiae (spots the size of the head). Hematomas (area of blood) due to intradermal or submucosal bleeding in fish, as well as excessive mucus production, was observed in some individuals.
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The effect of high concentrations (100 mg/l) on the aquatic organism Zebrafish (Danio rerio) caused abdominal edema due to fluid accumulation.

Conclusions

The acute toxicity LC$_{50}$ in vivo of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate on the Zebrafish (Danio rerio) aquatic model was investigated. The research was conducted in accordance with the national “General Ethical Principles of Animal Experimentation” approved by the First National Congress on Bioethics and the “Bioethical Expertise of Preclinical and Other Animal Research”.

The highest fish mortality occurred on the last day of observation (96 hours).

The highest number of deaths was at minimum concentration of sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-yl)thio)acetate.

The acute toxicity LC$_{50}$ (96 hours) of the test substance was 4.5364 mg/l. It can be concluded that the compound belongs to the class of moderately toxic substances.

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Fig. 2. Acute toxicity of compounds within 48 hours.

Fig. 3. Acute toxicity of compounds within 72 hours.

Fig. 4. Acute toxicity of compounds within 96 hours.
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