Synthesis and acute toxicity of new S-derivatives (1,2,4-triazole-3(2*H*)-yl)methyl) thiopyrimidines

Yu. V. Karpenko*[1,A-F], O. I. Panasenko[1,E-F], S. M. Kulish[1,B,C], A. V. Domnich[2,B,C]

[1]Zaporizhzhia State Medical and Pharmaceutical University, Ukraine, [2]Zaporizhzhia Research Expert Forensic Center of the Ministry of Internal Affairs, 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

In the literature, there is insufficient information on the synthesis of compounds in a series of pyrimidine-2-thiol derivatives containing a five-membered nitrogen-containing heterocyclic fragment; at the same time, there are a sufficient number of examples, demonstrating the synthetic and biological potential for compounds of this kind.

The relevance of the study "structure – acute toxicity" relationship in a number of newly synthesized derivatives of 1,2,4-triazole-3(2*H*)-thione with pyrimidine-2-thiol is due to the synthesis of potential low molecular weight interferon inducers and antitumor agents, the search for molecular descriptors of their structure, important for establishing "structure – acute toxicity" laws, as a system for evaluating the biological effects of compounds. Therefore, it is strategically and economically justified to conduct a study of the acute toxicity of synthesized compounds as a priority.

The aim of the work is targeted synthesis of a number of S-derivatives (1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines and the establishment of the "structure – acute toxicity" relationship.

Materials and methods. A modern set of physical-chemical research methods was used to study the compounds. The study of the acute toxicity of the synthesized compounds was performed on adult *Danio rerio*. During the experiments, the fish were kept on a diet for a test period of 96 hours, and their mortality was checked every 24, 48, 72 and 96 hours with the test compounds in each mini-aquarium containing at least 7 individuals of *Danio rerio*.

Results. Results ^{1}H NMR spectra confirm that the alkylation reaction occurs specifically on the sulfur atom. Thus, after analyzing LC_{50} data, we found that the least toxic among the studied compounds is 2-(((4-methyl-5-(octylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine with an acute toxicity value of 49.66 mg/l. The most toxic compound is 2-(((4-methyl-5-(methylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine with an LC_{50} value of 8.29 mg/l. The low toxicity of the compound 2-(((4-methyl-5-(octylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine is most likely due to the presence of an octyl substituent, which sufficiently penetrates through biological membranes and does not have a strong toxic effect on organ systems. Furthermore, it does not accumulate but is metabolized in the cell.

Conclusions. New hybrids of 1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines were obtained using the heterocyclization reaction of the intermediate carbothioamide. To reduce the indicators of acute toxicity and increase their biological activity, synthesized S-derivatives of this series were created. It was established that S-derivatives of 1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines belong to moderately toxic to low-toxic compounds according to the classification of D. R. Passino. 2-(((4-Methyl-5-(octylthio)-4*H*-1,2,4-triazole-3-yl)methyl) thio)pyrimidine has an acute toxicity value of 49.66 mg/l. The most toxic compound is 2-(((4-methyl-5-(methylthio)-4*H*-1,2,4-triazole-3-yl) methyl)thio)pyrimidine with an LC_{50} value of 8.29 mg/l.

Key words: 1,2,4-triazole, pyrimidine, acute toxicity, Danio rerio, LC_{50} concentration.

Current issues in pharmacy and medicine: science and practice, 2023. 16(2), 158-164

Синтез і гостра токсичність нових S-похідних (1,2,4-тріазол-3(2*H*)-іл)метил)тіопіримідинів

Ю. В. Карпенко, О. І. Панасенко, С. М. Куліш, А. В. Домніч

У фаховій літературі недостатньо відомостей щодо синтезу сполук у ряду похідних піримідин-2-тіолу, що містять п'ятичленний нітроген-вмісний гетероциклічний фрагмент, водночас подано достатню кількість прикладів, продемонстровано синтетичний і біологічний потенціал сполук цього типу.

Актуальність дослідження зв'язку «структура – гостра токсичність» у ряду новосинтезованих похідних 1,2,4-тріазол-3(2*H*)-тіону з піримідин-2-тіолом зумовлена синтезом потенційно низьких молекулярно-масових індукторів інтерферону й протипухлинних засобів, а також необхідністю пошуку молекулярних дескрипторів їхньої структури, важливих для встановлення закономірностей

ARTICLE INFO

http://pharmed.zsmu.edu.ua/article/view/274586

UDC 615.2.015.4.012.1:547.793
DOI: 10.14739/2409-2932.2023.2.274586

Current issues in pharmacy and medicine: science and practice, 2023. 16(2), 158-164

Key words: 1,2,4-triazole, pyrimidine, acute toxicity, Danio rerio, LC_{50} concentration.

***E-mail:** karpenko.y.v@gmail.com

Received: 27.02.2023 // Revised: 14.03.2023 // Accepted: 28.03.2023

зв'язку «структура – гостра токсичність» як системи оцінювання біологічних ефектів сполук. Тому нині стратегічно й економічно виправданим є першочергове дослідження гострої токсичності синтезованих сполук.

Мета роботи – цілеспрямований синтез ряду S-похідних (1,2,4-тріазол-3(2*H*)-іл)метил)тіопіримідинів, встановлення зв'язку «структура – гостра токсичність».

Матеріали та методи. Для дослідження сполук використано сучасний комплекс фізико-хімічних методів дослідження. Гостру токсичність синтезованих сполук дослідили на дорослих особинах *Danio rerio*. Під час експериментів риб утримували на дієті протягом тестового періоду тривалістю 96 годин і перевіряли їхню смертність кожні 24, 48, 72 і 96 годин у тестових комплексах у кожному міні-акваріумі з принаймні 7 особинами *Danio rerio*.

Результати. Результати спектрів ЯМР ^{1}H підтверджують проходження реакції алкілування саме на атомі Сульфуру. Отже, проаналізувавши дані LC_{50}, виявили, що найменш токсичною серед досліджуваних сполук є 2-(((4-метил-5-(октилтіо)-4*H*-1,2,4-тріазол-3-іл))метил)тіо)піримідин з показником гострої токсичності 49,66 мг/л. Найбільш токсичною сполукою є 2-(((4-метил-5-(метилтіо)-4*H*-1,2,4-тріазол-3-іл)метил)тіо)піримідин з показником LC_{50} = 8,29 мг/л. Низька токсичність сполуки 2-(((4-метил-5-(октилтіо)-4*H*-1,2,4-тріазол-3-іл)метил)тіо)піримідину, найімовірніше, зумовлена наявністю октилового замісника, який достатньою мірою проникає через біологічні мембрани і не має сильного токсичного впливу на системи органів і не накопичується, а метаболізується в клітині.

Висновки. За допомогою реакції гетероциклізації проміжного карботіоаміду отримано нові гібриди 1,2,4-тріазол-3(2*H*)-іл)метил) тіопіримідинів. Для зниження показників гострої токсичності та підвищення їхньої біологічної активності синтезовано S-похідні цього ряду. Встановлено, що S-похідні 1,2,4-тріазол-3(2*H*)-іл)метил)тіопіримідинів належать до помірно-, малотоксичних сполук, згідно з класифікацією D. R. Passino. 2-(((4-Метил-5-(октилтіо)-4*H*-1,2,4-тріазол-3-іл)метил)тіо)піримідин має значення гострої токсичності 49,66 мг/л. Найбільш токсичною сполукою є 2-(((4-метил-5-(метилтіо)-4*H*-1,2,4-тріазол-3-іл)метил)тіо)піримідин з показником LC_{50} = 8,29 мг/л.

Ключові слова: 1,2,4-тріазол, піримідин, гостра токсичність, Danio rerio, концентрація LC_{50}.

Актуальні питання фармацевтичної і медичної науки та практики. 2023. Т. 16, № 2(42). С. 158-164

Pyrimidine-2-thiol has many applications in organic chemistry. It can be used as a lactam protector in organic synthesis to prevent unwanted reactions with cyclic amides. It can also serve as an intermediate in the production of pharmaceuticals.

Pyrimidine-2-thiol also has biological activity and can be used for medicinal purposes. Some studies show that it has anti-inflammatory properties and can be used to treat certain inflammatory conditions such as arthritis. It has also been found that it may have anti-tumor activity, research in this area is ongoing [1].

1,2,4-triazole-3(2*H*)-thiol is used in the synthesis of various biologically active compounds, such as pharmaceuticals, antioxidants and others [2,3]. It can be used as a catalyst for various chemical reactions, as well as as an inhibitor for certain enzymes.

It is also known that 1,2,4-triazole-3(2*H*)-thiol has antibacterial and anti-inflammatory activity [4]. This compound may have potential medical applications in the treatment of infections and inflammation. However, more research is needed to establish the effectiveness and safety of this compound before it can be used in medicine.

In the literature, there is insufficient information on the synthesis of compounds in a series of pyrimidine-2-thiol derivatives containing a five-membered nitrogen-containing heterocyclic fragment; at the same time, there are a sufficient number of examples [5], demonstrating the synthetic and biological potential for compounds of this kind.

The relevance of the study of the "structure – acute toxicity" relationship in a number of newly synthesized derivatives of 1,2,4-triazole-3(2*H*)-thione with pyrimidine-2-thiol is due to the synthesis of potential low molecular weight interferon inducers and antitumor agents, as well as the search for molecular descriptors of their structure, important for establishing "structure – acute toxicity" laws as a system for evaluating the biological effects of compounds. Therefore, it is strategically and economically justified to conduct a study of the acute toxicity of synthesized compounds as a priority.

Aim

The purpose of the work is targeted synthesis of a number of S -derivatives (1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines and the establishment of the "structure – acute toxicity" relationship.

Materials and methods

The ^{1}H spectra were recorded on a Bruker AC-400 spectrometer (400 MHz, respectively) in DMSO-d6, the internal standard was TMS. LC-MS were recorded on a high-performance liquid chromatograph Agilent 1260 Infinity HPLC System and with the help of a diode array detector with proton ionization. Elemental analysis (C, H, N, S) was made on ELEMENTAR vario EL cube (standard – sulfanilamide). The melting points were determined by the capillary method in "Stanford Research Systems Melting Point Apparatus 100" (SRS, USA). The used reagents were purchased from Sigma-Aldrich (Merck).

Compounds 1.1, 1.2, 1.3 were synthesized using the well-known method [6, 7] with constants corresponding to literature data.

N-methyl-2-(2-(pyrimidin-2-ylthio)acetyl)hydrazine-1-carbothioamide (1.4). Yield 92 %, yellow powder, Mp 278 °C (DMF). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 2.93 (d, J = 1.2 Hz, 3H), 3.86 (s, 2H), 7.21 (t, J = 4.4 Hz, 1H), 7.50–7.54 (m, 1H), 8.56 (d, J = 4.4 Hz, 2H), 9.55 (d, J = 5.2 Hz, 1H),

9.77 (d, J = 5.2 Hz, 1H),. Mass spectrum, m/z (I_{rel}, %) 258 $[M+H]^+$ (100). Found, %: C 37.31; H 4.33; N 27.20; S 24.95. $C_8H_{11}N_5OS_2$. Calculated, %: C 37.34; H 4.31; N 27.22; S 24.92.

Obtaining of 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4*H*-1,2,4-triazole-3-thiol 1.5 (general methods). A mixture of 0.01 mol of 2-(pyrimidin-2-ylthio)acetohydrazide, 0.01 mol of sodium hydroxide, and 50 ml of purified water is boiled for 2 hours. After complete cooling, 2 ml of concentrated ethanoic acid is added to the filtrate. The resulting precipitate is filtered and washed with purified water. For analysis, it was purified by recrystallization from DMF. Light yellow powder, soluble in aqueous solutions of alkalis, DMF and 1,4-dioxane.

4-methyl-5-((pyrimidin-2-ylthio)methyl)-4*H*-1,2,4-triazole-3-thiol (1.5). Yield 72 %, light yellow powder, Mp 266 °C (DMF). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 3.55 (s, 3H), 4.43 (s, 2H), 7.19 (t, J = 4.4 Hz, 1H), 8.53 (d, J = 4.4 Hz, 2H), 12.83 (s, 1H). Mass spectrum, m/z (I_{rel}, %) 240 $[M+H]^+$ (100). Found, %: C 40.11; H 3.82; N 29.35; S 26.71. $C_8H_9N_5S_2$. Calculated, %: C 40.15; H 3.79; N 29.26; S 26.79.

Obtaining of S-alkyl derivatives 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4*H*-1,2,4-triazole-3-thiols 1.6–1.15 (general methods). A mixture of 0.005 mol of 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4*H*-1,2,4-triazole-3-thiol and 0.005 mol of sodium hydroxide dissolved in 50 ml of propan-2-ol is prepared. Then, add 0.005 mole of haloalkane (iodomethane, bromoethane, 1-bromopropane, 1-bromobutane, 1-bromopentane, 1 bromohexane, 1bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane). Heat the mixture for 2 hours, cool, filter the sediment, and wash it with purified water. For analysis, crystallize the compounds from methanol. The obtained crystalline substances (1.6–1.15) are yellow or brown in color, insoluble in water, and soluble in organic solvents.

2-(((4-methyl-5-(methylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.6). Yield 86 %, yellow powder, Mp 251 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 2.71 (s, 3H), 3.55 (s, 3H), 4.51 (s, 2H), 7.21 (t, J = 7.5 Hz, 1H), 8.59 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 254 $[M+H]^+$ (100). Found, %: C 42.63; H 4.29; N 27.67; S 25.38. $C_9H_{11}N_5S_2$. Calculated, %: C 42.67; H 4.38; N 27.64; S 25.31.

2-(((5-(ethylthio)-4-methyl-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.7). Yield 93 %, yellow powder, Mp 246 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 1.39 (t, J = 8.0 Hz, 3H), 3.16 (q, J = 8.0 Hz, 2H), 3.55 (s, 3H), 4.51 (s, 2H), 7.21 (t, J = 7.5 Hz, 1H), 8.60 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 268 $[M+H]^+$ (100). Found, %: C 44.87; H 4.98; N 26.17; S 24.05. $C_{10}H_{13}N_5S_2$. Calculated, %: C 44.92; H 4.90; N 26.19; S 23.98.

2-(((4-methyl-5-(propylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.8). Yield 82 %, yellow powder, Mp 245 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 1.05 (t, J = 8.0 Hz, 3H), 1.72–1.76 (m, 2H), 3.13 (t, J = 7.1 Hz, 2H), 3.55 (s, 3H), 4.51 (s, 2H), 7.21 (t, J = 7.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 282 $[M+H]^+$ (100). Found, %: C 46.91; H 5.41; N 24.82; S 22.81. $C_{11}H_{15}N_5S_2$. Calculated, %: C 46.95; H 5.37; N 24.89; S 22.79.

2-(((5-(butylthio)-4-methyl-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.9). Yield 91 %, yellow powder, Mp 241 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 0.94 (t, J = 8.0 Hz, 3H), 1.36–1.50 (m, 2H), 1.64 – 1.76 (m, 2H), 3.10 (t, J = 7.1 Hz, 2H), 3.56 (s, 3H), 4.51 (s, 2H), 7.21 (t, J = 7.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 296 $[M+H]^+$ (100). Found, %: C 48.81; H 5.78; N 23.76; S 21.65. $C_{12}H_{17}N_5S_2$. Calculated, %: C 48.79; H 5.80; N 23.71; S 21.70.

2-(((4-methyl-5-(pentylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.10). Yield 94 %, yellow powder, Mp 238 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 0.83 – 0.94 (m, 3H), 1.29–1.39 (m, 1H), 1.34 – 1.45 (m, 3H), 1.67 – 1.78 (m, 2H), 3.18 (t, J = 7.1 Hz, 2H), 3.58 (s, 3H), 4.51 (s, 2H), 7.21 (t, J = 7.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 2H),. Mass spectrum, m/z (I_{rel}, %) 310 $[M+H]^+$ (100). Found, %: C 50.43; H 6.14; N 22.69; S 20.76. $C_{13}H_{19}N_5S_2$. Calculated, %: C 50.46; H 6.19; N 22.63; S 20.72.

2-(((5-(hexylthio)-4-methyl-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.11). Yield 88 %, yellow powder, Mp 237 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 0.84 – 0.95 (m, 3H), 1.24–1.36 (m, 6H), 1.65 – 1.76 (m, 2H), 3.15 (t, J = 7.1 Hz, 2H), 3.60 (s, 3H), 4.51 (s, 2H), 7.21 (t, J = 7.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 254 $[M+H]^+$ (100). Found, %: C 51.95; H 6.58; N 21.66; S 19.80. $C_{14}H_{21}N_5S_2$. Calculated, %: C 51.98; H 6.54; N 21.65; S 19.82.

2-(((5-(heptylthio)-4-methyl-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.12). Yield 78 %, yellow powder, Mp 232 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 0.84 – 0.95 (m, 3H), 1.23–1.38 (m, 8H), 1.65 – 1.76 (m, 2H), 3.16 (t, J = 7.1 Hz, 2H), 3.61 (s, 3H), 4.54 (s, 2H), 7.21 (t, J = 7.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 338 $[M+H]^+$ (100). Found, %: C 53.46; H 6.82; N 20.81; S 18.91. $C_{15}H_{23}N_5S_2$. Calculated, %: C 53.38; H 6.87; N 20.75; S 19.00.

2-(((4-methyl-5-(octylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.13). Yield 82 %, light brown powder, Mp 228 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 0.83 – 0.96 (m, 3H), 1.21–1.46 (m, 10H), 1.65 – 1.76 (m, 2H), 3.16 (t, J = 7.1 Hz, 2H), 3.62 (s, 3H), 4.54 (s, 2H), 7.20 (t, J = 7.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 352 $[M+H]^+$ (100). Found, %: C 54.73; H 7.12; N 19.97; S 18.18. $C_{16}H_{25}N_5S_2$. Calculated, %: C 54.67; H 7.17; N 19.92; S 18.24.

2-(((4-methyl-5-(nonylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.14). Yield 91 %, light brown powder, Mp 227 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 0.83 – 0.96 (m, 3H), 1.20–1.46 (m, 12H), 1.65 – 1.76 (m, 2H), 3.14 (t, J = 7.1 Hz, 2H), 3.64 (s, 3H), 4.53 (s, 2H), 7.20 (t, J = 7.5 Hz, 1H), 8.61 (d, J = 7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 366 $[M+H]^+$ (100). Found, %: C 55.91; H 7.40; N 19.11; S 17.59. $C_{17}H_{27}N_5S_2$. Calculated, %: C 55.86; H 7.45; N 19.16; S 17.54.

2-(((5-(decylthio)-4-methyl-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.15). Yield 91 %, brown powder, Mp 225 °C (MeOH). ^{1}H NMR spectrum, δ, ppm. (J, Hz): 0.84–0.95 (m, 3H), 1.20–1.32 (m, 10H), 1.28 – 1.40 (m, 2H), 1.35–1.47 (m, 2H), 1.70 – 1.74 (m, 2H), 3.14 (t, J = 7.1 Hz, 2H), 3.67 (s, 3H), 4.53 (s, 2H), 7.18 (t, J = 7.5 Hz, 1H). 8.61 (d, J =

7.5 Hz, 2H). Mass spectrum, m/z (I_{rel}, %) 380 $[M+H]^+$ (100). Found, %: C 56.92; H 7.74; N 18.52; S 16.82. $C_{18}H_{29}N_5S_2$. Calculated, %: C 56.96; H 7.70; N 18.45; S 16.89.

Study of acute toxicity of synthesized compounds on adult of *Danio rerio*. The experiment used 2-months-old fish, 11.8 ± 0.1 mm in length and 2.6 ± 0.2 g in weight.

The concentration of the studied compound ranged from 5.0 to 100.0 mg/l. *Danio rerio* adults were kept in aerated aquariums with carbon-filtered tap water (pH = 7.3 ± 0.3; 95 % Cl) at a temperature of 26.5 °C. Oxygen-enriched water was also used for experiments. Before setting up the experiments, the fish were acclimatized, with a mortality rate of no more than 1 in 500 individuals. The studied newly synthesized compounds were first emulsified in ERCASORB 2080 and then dissolved in distilled water. Each mini-aquarium with a specific dose of the compound contained at least 7 individuals of *Danio rerio*. During the experiments, the fish were kept on a diet for the 96-hour test period, and their mortality was checked every 24, 48, 72 and 96 hours.

Fish are considered dead if there is no visible movement (e.g., gill movements) and if the caudal peduncle does not elicit a reaction. Dead fish were removed during observation and mortality was recorded.

Statistical analysis of the obtained results was carried out using the STATISTICA 6 program. The degree of toxicity of the studied compounds was determined according to the classification D. R. Passino [8].

Results

One of the well-known methods of synthesis of 5-substituted-1,2,4-triazole-3(2*H*)-thiones consists in the synthesis of intermediate carbothioamides followed by heterocyclization in an alkaline medium [9,10]. Therefore, the previously obtained hydrazide **1.3** was reacted with methyl isothiocyanate in an ethanol medium to form the intermediate product carbothioamide **1.4**, and further cyclization was carried out under the influence of an aqueous solution of sodium hydroxide for 2 hours with stirring on a magnetic stirrer. The obtained solution was acidified with glacial acetic acid, resulting in the precipitation of compound **1.5** (*Fig. 1*).

It is known that the presence of alkyl substituents at the sulfur atom in 1,2,4-triazole-3(2*H*)-thiones increases their biological activity. For this reason, it was expedient to obtain S-derivatives of (1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines.

Alkyl derivatives (**1.6–1.15**) were obtained by the reaction of the starting thione 1.5 and the corresponding haloalkane in the medium of a polar solvent – ethanol with the addition of an equimolar amount of sodium hydroxide.

Subsequently, the compounds were tested for acute toxicity on *Danio rerio* hydrobionts for 96 hours, and lethal concentrations were calculated. Four compounds were selected for the study.

Mortality every 24, 48, 72 and 96 hours was tabulated for each concentration / percentage respectively (*Table 1*).

Based on the obtained data, graphs of the dependence of concentration on fish mortality percentage were constructed, and the corresponding LC_{50} values of the newly synthesized compound were calculated.

Discussion

The ^{1}H NMR spectra of the compound are characterized by a singlet signal of the protons of the -S-CH_2 group at the pyrimidine ring at 4.51 ppm, and in the case of the 1,2,4-triazole nucleus, it is manifested by a singlet at 3.16 ppm. The presence of these pixels confirms the passage of the alkylation reaction precisely on the sulfur atom. The proton signal of the thiol group was registered in the spectrum of compound **1.5** at 12.83 ppm. in the form of a singlet. All S-derivatives (1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines are characterized by the signals of 3 protons of the pyrimidine ring at 7.21 ppm and 8.59 ppm in the form of doublets and triplets with a characteristic coupling constant for aromatic rings.

It is known that along with indicators of biological activity of molecule, its toxicity class should also be taken into

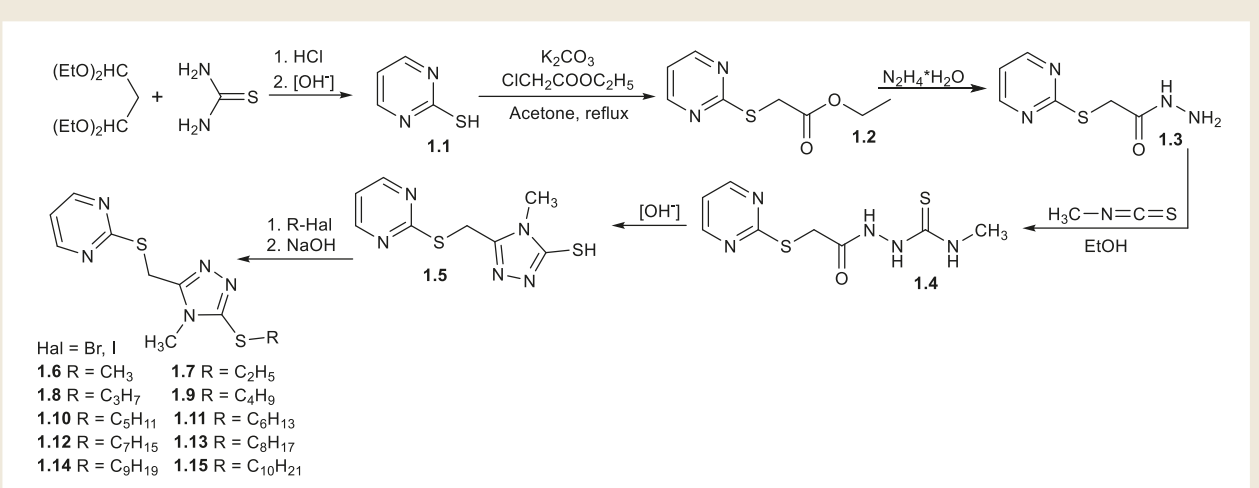

Fig. 1. Synthesis of molecular hybrids of 1,2,4-triazole and pyrimidine-2-thiole as potential multifunctional agents with low toxicity.

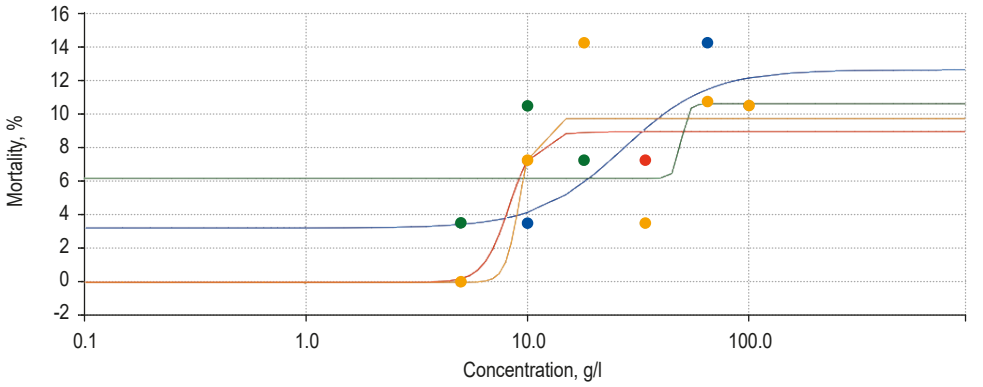

Fig. 2. Graph of dependence of concentration on % mortality of fish.

Table 1. Mortality results obtained at 24, 48, 72 and 96 hour intervals are given respectively as the number of deaths / percent of total (%) of some S-derivatives of (1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines

The concentration, mg/l	Time	1.5	1.6	1.13	1.15
5	24 hours	0	0	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
	96 hours	1 / 14	0	1 / 14	0
10	24 hours	0	0	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
	96 hours	1 / 14	2 / 29	3 / 42	2 / 29
18	24 hours	0	0	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
	96 hours	2 / 29	2 / 29	2 / 29	4 / 57
34	24 hours	0	0	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
	96 hours	2 / 29	2 / 29	1 / 14	1 / 14
65	24 hours	0	0	0	0
	48 hours	0	0	2 / 29	0
	72 hours	2 / 29	2 / 29	0	2 / 29
	96 hours	4 / 57	1 / 14	1 / 14	1 / 14
100	24 hours	0	0	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
	96 hours	3 / 42	3 / 42	3 / 42	3 / 42

Table 2. Acute toxicity of compounds of some S-derivatives of (1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines

Parameter	1.5	1.6	1.13	1.15
LC_{50}, mg/l	27.07	8.29	49.66	9.24
Relative toxicity	Slightly toxic	Moderately toxic	Slightly toxic	Moderately toxic

account. These data are often related to each other and are usually directly proportional. Therefore, before conducting any preclinical studies of new potential drugs, it is advisable to start an experiment to establish indicators of acute toxicity. From a practical point of view, the expediency of the above-mentioned method consists in the further use of LC_{50} values to calculate the doses of the studied substances when administered in subsequent experiments to determine biological activity (*Fig. 2*).

Thus, after analyzing LC_{50} data (*Table 2*), we found that the least toxic among the studied compounds is 2-(((4-methyl-5-(octylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (1.13) with an acute toxicity value of 49.66 mg/l. The most toxic compound is 2-(((4-methyl-5-(methylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (**1.6**). with the LC_{50} value of 8.29 mg/l.

The low toxicity of the compound 2-(((4-methyl-5-(octylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (**1.13**) is most likely caused by the presence of an octyl substituent, which sufficiently penetrates through biological membranes and does not have a strong toxic effect on organ systems and does not accumulate, but is metabolized in the cell.

According to the acute toxicity LC_{50} (96 hours) of the compounds, as classified by D. R. Passino and co-authors [8], they can be assigned to the classes of moderately toxic to low-toxic compounds.

The following clinical signs were observed in experimental animals: at low concentrations (5–10 mg/l), exophthalmia (swelling in the orbital pits, which leads to bulging of one or both eyes) and increased spontaneous activity were observed. The middle range of concentrations (18–65 mg/l) was marked by the appearance of petechiae (small spots) or hematoma (area of blood) due to intradermal or submucosal bleeding in fish, as well as excessive mucus production. At high concentrations (100 mg/l), abdominal swelling due to fluid accumulation was observed, which may cause bulging scales and/or a crack in the abdominal wall.

Conclusions

1. New hybrids of 1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines were obtained using the heterocyclization reaction of the intermediate carbothioamide. To reduce the indicators of acute toxicity and increase their biological activity, synthesized S-derivatives of this series.

2. It was established that S-derivatives of 1,2,4-triazole-3(2*H*)-yl)methyl)thiopyrimidines belong to moderately toxic to low-toxic compounds according to the classification of D. R. Passino.

3. 2-(((4-Methyl-5-(octylthio)-4*H*-1,2,4-triazole-3-yl) methyl)thio)pyrimidine (**1.13**) with an acute toxicity value of 49.66 mg/l. The most toxic compound is 2-(((4-methyl-5-(methylthio)-4*H*-1,2,4-triazole-3-yl)methyl)thio)pyrimidine (**1.6**). with the LC_{50} value of 8.29 mg/l.

Prospects for further research. The results of the work made it possible to plan further pharmacological studies based on the parameters of acute toxicity and to identify the most toxic pharmacophores in the compound. To supplement the virtual library of 1,2,4-triazole derivatives as promising biologically active compounds.

Funding
The research is carried out within the SRW of Zaporizhzhia State Medical University "Synthesis, modification and study of the properties of 1,2,4-triazole derivatives for the purpose of antimicrobial drug production", state registration No. 0120U101649 (2020–2022).

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

Information about authors:
Karpenko Yu. V., PhD, Senior Lecturer of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-4390-9949
Panasenko O. I., PhD, DSc, Professor, Head of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-6102-3455
Kulish S. M., PhD, Associate Professor of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0003-0545-993X
Domnich A. V., forensic expert, Zaporizhzhia Research Expert Forensic Center of the Ministry of Internal Affairs, Ukraine.
ORCID ID: 0000-0001-6076-8386

Відомості про авторів:
Карпенко Ю. В., канд. хім. наук, ст. викладач каф. природничих дисциплін для іноземних студентів та токсикологічної хімії, Запорізький державний медико-фармацевтичний університет, Україна.
Панасенко О. І., д-р фарм. наук, професор, зав. каф. природничих дисциплін для іноземних студентів та токсикологічної хімії, Запорізький державний медико-фармацевтичний університет, Україна.
Куліш С. М., канд. фарм. наук, доцент каф. природничих дисциплін для іноземних студентів та токсикологічної хімії, Запорізький державний медико-фармацевтичний університет, Україна.
Домніч А. В., судовий експерт, Запорізький науково-дослідний експертно-криміналістичний центр МВС, Україна.

References

[1] Karpenko, Yu. V., Panasenko, O. I., & Knysh, Ye. H. (2020). Biolohichno oriientovanyi syntez likiv (BIODS) na osnovi heterylpokhidnykh 2,5 dyzamishchenykh 1,3,4-oksadiazoliv (Chastyna 1) [Biologically oriented synthesis of medicines (BIODS) based on heterylpoxid 2,5-disubstituted 1,3,4-oxadiazoles (Part 1)]. *Current issues in pharmacy and medicine: science and practice, 13*(2), 390-398. [in Ukrainian]. https://doi.org/10.14739/2409-2932.2020.2.207211

[2] Varynskyi, B., Parchenko, V., Kaplaushenko, A., Panasenko O., & Knysh, Ye. Development and Validation of a LC-ESI-MS Method for Detection of Piperidin-1-IUM {[5-(2-Furyl)-4-Phenyl-4H-1,2,4-Triazol-3-YL]Thio}acetate Residues in Poultry Eggs. *Ankara Üniversitesi Eczacılık Fakültesi Dergisi, 2016, 40*(3), 29-40. https://doi.org/10.1501/Eczfak_0000000586

[3] Karpenko, Yu. V., & Panasenko, O. I. (2021). Search for antibacterial activity in a number of new S-derivatives (1,2,4-triazole-3(2H)-yl) methyl)thiopyrimidines. *Current issues in pharmacy and medicine: science and practice, 14*(2), 173-178. https://doi.org/10.14739/2409-2932.2021.2.234565

[4] 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. https://doi.org/10.32434/0321-4095-2020-133-6-50-58

[5] Gotsulya, A., & Brytanova, T. (2022). Synthesis, properties and biological potential some condensed derivatives 1,2,4-triazole. *Journal of Faculty of Pharmacy of Ankara University, 46*(2), 308-321. https://doi.org/10.33483/jfpau.971602

[6] Kaplancıklı, Z., Yurttas, L., Turan-Zitouni, G., Özdemir, A., Göger, G., Demirci, F., Mohsen, A. U. (2013). Synthesis and Antimicrobial Activity of New Pyrimidine-Hydrazones. *Letters in Drug Design & Discovery, 11*(1), 76-81. https://doi.org/10.2174/15701808113109990037

[7] Karpenko, Y., Hunchak, Y., Gutyj, B., Hunchak, A., Parchenko, M., & Parchenko, V. (2022). 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. *ScienceRise: Pharmaceutical Science*, (2), 18-25. https://doi.org/10.15587/2519-4852.2022.255848

[8] Passino, D. R. M., & Smith, S. B. (1987). Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. *Environmental Toxicology and Chemistry, 6*(11), 901-907. https://doi.org/10.1002/etc.5620061111

[9] Khilkovets, A., Karpenko, Y., Bigdan, O., Parchenko, M., & Parchenko, V. (2022). Synthetic and Biological Aspects of Studying the Properties of 1,2,4-Triazole Derivatives. *Scientific Journal of Polonia University, 51*(2), 324-331. https://doi.org/10.23856/5138

[10] Zozulynets, D. M., Kaplaushenko, A. G., & Korzhova, A. S. (2021). Syntez ta vzaiemodiia z aldehidamy 4-amino-5-(khinolin-2-il)-4H-1,2,4-tryazol-3-tiolu [The synthesis of 4-amino-5-(quinolin-2-yl)-4H-1,2,4-triazole-3-thiol and its interaction with aldehydes]. *Zhurnal orhanichnoi ta farmatsevtychnoi khimii, 19*(1), 48-52. [in Ukrainian]. https://doi.org/10.24959/ophcj.21.188137