Heterocyclizations based on *N*-(R-hydrazine-1-carbonothioyl) cycloalkancarboxamides: functionalized azoles and their antimicrobial activity

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Synthesis and structural modification of azoles remains an important area of medical chemistry and allows to obtain new compounds with a wide range of biological activity. Among the significant number of azoles, 1,3,4-thiadiazoles and 1,2,4-triazoles attract special attention, among which are known drugs, larvicides, insecticides, growth regulators, etc. Even though heterocyclizations of functionally substituted hydrazines for their synthesis are well studied, *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides, and nowadays, remain reagents with undiscovered potential. Moreover, the introduction of lipophilic "pharmacophore" fragments (cycloalkanes) in the structure of 1,3,4-thiadiazoles and 1,2,4-triazoles is a promising direction for their modification. That should provide additional intermolecular interactions with enzymes and may lead to enhancement or alteration of the biological activity vector. Thus, the synthesis of new derivatives of this class of compounds and the study of their antibacterial properties remains an urgent problem of medical and organic chemistry.

Aim. To investigate the heterocyclization of *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxa-mides, to establish the structure and antibacterial activity of the synthesized compounds.

Materials and methods. Methods of organic synthesis, physical and physical-chemical methods of analysis of organic compounds (NMR ¹H-spectroscopy, chromato-mass spectrometry, elemental analysis). The antimicrobial activity of the synthesized compounds was studied according to the generally accepted method for standard strains of microorganisms and fungi.

Results. The peculiarities of heterocyclization of *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides have been studied and the factors influencing this reaction have been elucidated. It was shown that these compounds under the conditions of the heterocyclization reaction in concentrated mineral acids form 5-R-2-amino-1,3,4-thiadiazoles. The intermediate undergoes additional hydrolysis by cleavage of the cycloalkanecarboxyl fragment. Alternative methods for the synthesis of 5-R-2-amino-1,3,4-thiadiazoles were proposed. For the first time, the original 4-cycloalkanecarbonyl-3-(amino-,phenyloxo-(thio)methyl-1,5-dihydro-4*H*-1,2,4-triazole-5-thiones were synthesized by prolonged heating of the corresponding disubstituted thiosemicarbazides. It was not possible to extend this reaction to other diacylthiosemicarbazides, the latter undergo heterocyclization in the presence of sodium hydroxide with the formation of the known 5-R-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones. ¹H NMR spectra were studied, analyzed, and regularities of splitting of characteristic protons in functionalized azoles were established. Conducted microbiological screening was showed that 5-R-2-amino-1,3,4-thiadiazoles, 4-cycloalkanecarbonyl-3-(amino-,phenyloxo-(thio)methyl-1,5-dihydro-4*H*-1,2,4-triazole-5-thiones and 5-R-2,4-dihydro-3*H*-1,2,4-triazole-3-thione were less effective antibacterial and antifungal agents (MIC 100–200 µg/ml) compared with *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides (MIC 3.125–200 µg/ml).

Conclusions. It was found that *N*-(R-hydrazine-1-carbonotioyl)cycloalkane-carboxamides, depending on the conditions of heterocyclization form 5-R-2-amino-1,3,4-thiadiazoles, 3-(phenyloxo-(thio)methyl-1,5-dihydro-4*H*-1,2,4-triazole-5-thiones or 5-R-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones. It was established that synthesized azoles were shown less effective antimicrobial and antifungal activity in comparison with *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides.

Key words: *N*-(acylhydrazine-1-carbonothioyl)cycloalkanecarboxamides, heterocyclization, 1,3,4-thiadiazoles, 1,2,4-triazoles, antimicrobial activity.

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Гетероциклізація на основі *N*-(R-гідразин-1-карбонотіоіл)циклоалканкарбоксамідів: функціоналізовані азоли та їхня протимікробна активність

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Синтез і структурна модифікація азолів залишається актуальним напрямом медичної хімії та дає можливість отримати нові сполуки з широким спектром біологічної активності. Серед чималої кількості азолів особливу увагу привертають 1,3,4-тіадіазоли та 1,2,4-тріазоли,

серед них – відомі лікарські засоби, ларвіциди, інсектициди, рістрегулятори тощо. Незважаючи на те, що гетероциклізації функціональних заміщених гідразину для їхнього синтезу добре досліджені, *N*-(R-гідразин-1-карбонотіоіл)циклоалканкарбокаміди і сьогодні залишаються реагентами з нерозкритим потенціалом. Ба більше, введення ліпофільних «фармакофорних» фрагментів (циклоалкани) у структуру 1,3,4-тіадіазолів та 1,2,4-тріазолів – перспективний напрям їхньої модифікації, забезпечуватиме додаткові міжмолекулярні взаємодії з ензимами і, можливо, призводитиме до посилення або зміни вектора біологічної активності. Отже, синтез нових похідних цього класу сполук і вивчення їхніх антибактеріальних властивостей залишається актуальною проблемою медичної та органічної хімії.

Мета роботи – дослідити гетероциклізацію *N*-(R-гідразин-1-карбонотіоіл)циклоалканкарбоксамідів, встановити структуру та антибактеріальну активність синтезованих сполук.

Матеріали та методи. Методики органічного синтезу, фізичні та фізико-хімічні методи аналізу органічних сполук (ЯМР ¹Н-спектроскопія, хромато-мас-спектрометрія, елементний аналіз). Протимікробну активність синтезованих сполук досліджували згідно з загальноприйнятим методом до стандартних штамів мікроорганізмів і грибків.

Результати. Дослідили особливості гетероциклізації *N*-(R-гідразин-1-карбонотіоіл)циклоалканкарбоксамідів і з'ясували фактори, що впливають на цю реакцію. Показано, що зазначені сполуки за умов реакції гетероциклізації в концентрованих мінеральних кислотах утворюють 5-R-2-аміно-1,3,4-тіадіазоли, тобто проміжний інтермедіат зазнає додаткового гідролізу з відщепленням циклоалканкарбоксильного фрагмента. Запропоновані альтернативні методи синтезу 5-R-2-аміно-1,3,4-тіадіазолів. Уперше синтезували оригінальні 4-циклоалканкарбоніл-3-(аміно-,фенілоксо-(тіо)метил-1,5-дигідро-4*H*-1,2,4-тріазол-5-тіони тривалим нагріванням відповідних дизаміщених тіосемикарбазидів. Розширити цю реакцію на інші діацилтіосемикарбазиди не вдалося, останні зазнають гетероциклізації у присутності натрій гідроксиду з утворенням відомих 5-R-2,4-дигідро-3*H*-1,2,4-тріазол-3-тіонів. Досліджені та проаналізовані ¹Н ЯМР-спектри, встановлені закономірності розщеплення характеристичних протонів у функціоналізованих азолів. Мікробіологічний скринінг показав, що 5-R-2-аміно-1,3,4-тіадіазоли, 4-циклоалканкарбоніл-3-(аміно-,фенілоксо-(тіо)метил-1,5-дигідро-4*H*-1,2,4-тріазол-5-тіони та 5-R-2,4-дигідро-3*H*-1,2,4-тріазол-3-тіон є менш ефективними антибактеріальними та протигрибковими агентами (МІС 100–200 мкг/мл) порівняно з *N*-(R-гідразин-1-карбонотіоіл)циклоалканкарбоксамідами (МІС 3.125–200 мкг/мл).

Висновки. Встановили, що *N*-(R-гідразин-1-карбонотіоіл)циклоалканкарбокаміди залежно від умов проведення гетероциклізації утворюють 5-R-2-аміно-1,3,4-тіадіазоли, 3-(фенілоксо-(тіо)метил-1,5-дигідро-4*H*-1,2,4-тріазол-5-тіони або 5-R-2,4-дигідро-3*H*-1,2,4-тріазол-3-тіони. Показали, що синтезовані азоли – менш ефективні протимікробні та протигрибкові агенти порівняно з *N*-(R-гідразин-1-карбонотіоіл)циклоалканкарбоксамідами.

Ключові слова: *N*-(ацилгідразин-1-карбонотіоіл)циклоалкан-карбоксаміди, гетероциклізація, 1,3,4-тіадіазоли, 1,2,4-тріазоли, протимікробна активність.

Гетероциклизация на основе *N*-(R-гидразин-1-карбонотиоил)циклоалканкарбоксамидов: функционализированные азолы и их противомикробная активность

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Синтез и структурная модификация азолов остается актуальным направлением медицинской химии и позволяет получить новые соединения с широким спектром биологической активности. Среди значительного количества азолов особое внимание вызывают 1,3,4-тиадиазолы и 1,2,4-триазолы, среди которых известны лекарственные средства, ларвициды, инсектициды, рострегуляторы и т. д. Несмотря на то, что гетероциклизации функциональных замещенных гидразинов для их синтеза хорошо исследованы, *N*-(R-гидразин-1-карбонотиоил)циклоалканкарбокамиды и теперь остаются реагентами с нераскрытым потенциалом. Помимо этого, введение липофильных «фармакофорных» фрагментов (циклоалканы) в структуру 1,3,4-тиадиазолов и 1,2,4-триазолов является перспективным направлением их модификации, обеспечит дополнительные межмолекулярные взаимодействия с энзимами и, возможно, приведет к усилению или изменению вектора биологической активности. Следовательно, синтез новых производных этого класса соединений и изучение их антибактериальных свойств остается актуальной проблемой медицинской и органической химии.

Цель работы – исследовать гетероциклизацию *N*-(R-гидразин-1-карбонотиоил)циклоалканкарбоксамидов, установить структуру и антибактериальную активность синтезируемых соединений.

Материалы и методы. Методики органического синтеза, физические и физико-химические методы анализа органических соединений (ЯМР ¹Н-спектроскопия, хромато-масс-спектрометрия, элементный анализ). Противомикробную активность синтезированных соединений исследовали согласно общепринятому методу к стандартным штаммам микроорганизмов и грибков.

Результаты. Исследованы особенности гетероциклизации *N*-(R-гидразин-1-карбонотиоил)циклоалканкарбоксамидов и выяснены факторы, влияющие на данную реакцию. Показано, что указанные соединения при реакции гетероциклизации в концентрированных минеральных кислотах образуют 5-R-2-амино-1,3,4-тиадиазолы, то есть промежуточный интермедиат подвергается дополнительному гидролизу с отщеплением циклоалканкарбоксильного фрагмента. Предложены альтернативные методы синтеза 5-R-2-амино-1,3,4-тиадиазолов. Впервые синтезированы оригинальные 4-циклоалканкарбонил-3-(амино,фенилоксо-(тио)метил-1,5-дигидро-4*H*-1,2,4-триазол-5-тионы продолжительным нагреванием соответствующих дизамещённых тиосемикарбазидов. Расширить данную реакцию на другие диацилтиосемикарбазиды не удалось, последние подвергаются гетероциклизации в присутствии гидроксида натрия с образованием известных 5-R-2,4-дигидро-3*H*-1,2,4-триазол-3-тионов. Исследованы и проанализированы ¹Н ЯМР-спектры, установлены закономерности расщепления характеристических протонов у функционализированных азолов. Проведённый микробиологический скрининг показал, что 5-R-2-амино-1,3,4-тиадиазолы, 4-циклоалканкарбонил-3-(амино,фенилоксо-(тио)метил-1,5-дигидро-4*H*-1,2,4-триазол-5-тионы и 5-R-2,4-дигидро-3*H*-1,2,4-триазол-3-тион являются менее эффективными антибактериальными и противогрибковыми агентами (МІС 100–200 мкг/мл) по сравнению с *N*-(R-гидразин-1-карбонотиоил)циклоалканкарбоксамидами (МІС 3.125–200 мкг/мл).

Выводы. Установлено, что *N*-(R-гидразин-1-карбонотиоил)циклоалканкарбоксамиды, в зависимости от условий проведения гетероциклизации, образуют 5-R-2-амино-1,3,4-тиадиазолы, 3-(фенилоксо-(тио)метил-1,5-дигидро-4*H*-1,2,4-триазол-5-тионы или 5-R-2,4-ди-

гидро-3*H*-1,2,4-триазол-3-тионы. Показано, что синтезированные азолы – менее эффективные противомикробные и противогрибковые агенты по сравнению с *N*-(R-гидразин-1-карбонотиоил)циклоалканкарбоксамидами.

Ключевые слова: *N*-(ацилгидразин-1-карбонотиоил)циклоалканкарбоксамиды, гетероциклизация, 1,3,4-тиадиазолы, 1,2,4-триазолы, противомикробная активность.

Azoles and their substitutes are one of the most important and well-known classes of heterocyclic compounds, which are widely represented in various natural objects and medicines [1–15]. Methods for the synthesis of azoles and their substitutes are diverse, but usually, the basis of their synthesis is the cyclization of functionally substituted hydrazine (mono- and diacylhydrazines, thiosemicarbazides, thiocarbazides, dithiocarbazates, thioacylhydrazines, dithioureas, etc.) or their interaction with cyclization reagents (phenacyl halides, chloroacetates, acid chlorides, isocyanates, isothiocyanates, dialkyl but-2-endiolates, chloroacetonitriles, bases, etc.) [1–14].

Diverse and high biologically active azoles have given them the status of "privileged" and pharmacologically attractive compounds. These heterocycles are characterized by anti-inflammatory, analgesic, antiepileptic, diuretic, antimicrobial, antiviral, antitumor, antituberculous, and many other types of activity [5–15]. However, at this stage, the synthesis and modification of azoles remains an important area of medical chemistry and allows to obtain of new compounds with a wide range of pharmacological activity. Moreover, the original *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides still remain reagents with undiscovered potential for the synthesis of functionalized azoles with lipophilic "pharmacophore" fragments (cycloalkanes), and their introduction will undoubtedly provide additional interactions with enzymes and may increase or alter the vector of biological activity.

Aim

Therefore, the aim of this work is to study the heterocyclization reactions of *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides and to establish the structure and study the antimicrobial activity of the synthesized compounds.

Materials and methods

The melting point of the compounds was determined by the capillary method on the device "Mettler Toledo MR 50". Determination of the elemental composition of the compounds was performed on an elemental analyzer "ELEMENTAR Vario EL cube". The components on the thermal conductivity detector (TCD) were quantified. The error rate was ±0.3 %. IR spectra were recorded on a Bruker Alpha spectrophotometer in the range of 7500–400 cm^{-1}, using an ATR prefix (direct injection). ^{1}H and ^{13}C NMR spectra was on a nuclear magnetic resonance spectrophotometer "Mercury 500", solvent DMSO-d_6, internal standard – TMS. Chromato-mass spectra were obtained on a high-performance liquid chromatography "Agilent 1100 Series", equipped with diode-matrix and mass-selective detector "Agilent LC/MSD SL". Ionization method – chemical ionization at atmospheric pressure (APCI). Ionization mode – simultaneous scanning of positive and negative ions in the mass range of 80–1000 m/z.

Synthetic studies were conducted according to general approaches to the search for potential biologically active substances, using reagents from Merck (Darmstadt, Germany), Sigma-Aldrich (Missouri, USA) and Enamine (Kyiv, Ukraine). Substituted *N*-(acylhydrazine-1-carbonotioyl)cycloalkanecarbocamides (**1.1–1.14**) for the synthetic part of the work were obtained by known methods with constants that correspond to the literature [16–18].

Methods for the synthesis of 5-substituted 1,3,4-thiadiazole-2-amines (2.1–2.6)

Method A. To the 0.01 mol of *N*-(acylhydrazine-1-carbonothioyl)cycloalkanecarboxamides (**1.1–1.7**) 5 ml of conc. sulfuric or phosphoric acids were added and kept at a temperature of 80 ℃ for 8 hours. Then the mixture was cooled, poured into water, neutralized by potassium acetate. The formed precipitates were filtered off. Crystallized from DMF.

Method B. 0.01 Mol of the corresponding cycloalkanecarboxylic acid (**3.1–3.6**) and 0.91 g (0.01 mol) of thiosemicarbazide were placed in a flat-bottomed flask, then 5 ml of conc. sulfuric or phosphate acids were added, mixed, and kept at a temperature of 80 °C for 8 hours. Then the mixture was cooled, poured into water, neutralized. The formed precipitates were filtered off. Crystallized from DMF.

5-Cyclopropyl-1,3,4-thiadiazol-2-amine (2.1). Yield: 41.2 % (Method A), 61.3 % (Method B); m.p. 219–221 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.12 (s, 2H, NH₂), 2.32 (qu, *J* = 8.0 Hz, 1H, Cpr H-1), 1.13–0.74 (m, 4H, Cpr H-2$_{eq}$, 3$_{eq}$, 2$_{ax}$, 3$_{ax}$); LC-MS, *m/z* = 142 (M+H); Calculated for: C$_5$H$_7$N$_3$S: C, 42.53; H, 5.00; N, 29.76; S, 22.71; Found: C, 42.57; H, 5.04; N, 29.81; S, 22.74.

5-Cyclopentyl-1,3,4-thiadiazol-2-amine (2.2). Yield: 44.3 % (Method A); 60.8 % (Method B); m.p. 232–235 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.97 (s, 2H, NH₂), 3.25 (qu, *J* = 7.9 Hz, 1H, Cpe H-1), 2.04–1.97 (m, 2H, Cpe H-5$_{eq}$, 2$_{eq}$), 1.68–1.59 (m, 6H, Cpe 5$_{ax}$, 2$_{ax}$, 3$_{eq}$, 4$_{eq}$, H-3$_{ax}$, 4$_{ax}$); LC-MS, *m/z* = 170 (M+H); Calculated for: C$_7$H$_{11}$N$_3$S: C, 49.68; H, 6.55; N, 24.83; S, 18.94; Found: C, 49.71; H, 6.59; N, 24.79; S, 18.93.

5-((Phenylthio)methyl)-1,3,4-thiadiazol-2-amine (2.3). Yield: 24.5 % (Method A); 56.6 % (Method B); m.p. 146–148 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (d, *J* = 7.8 Hz, 2H, Ph H-2,6), 7.27 (t, *J* = 7.8 Hz, 2H, Ph H-3,5), 7.20 (t, *J* = 7.8 Hz, 1H, Ph H-4), 6.93 (s, 2H, NH₂), 4.34 (s, 2H, -CH₂SPh); Calculated for: C$_9$H$_9$N$_3$S$_2$: C, 48.41; H, 4.06; N, 18.82; S, 28.71; Found: C, 48.47; H, 4.09; N, 18.84; S, 28.74.

5-(Pyridin-4-yl)-1,3,4-thiadiazol-2-amine (2.4). Yield: 21.8 % (Method A); 59.3 % (Method B); m.p. 15--154 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, J = 5.2, 2H, Py H-2, 6), 7.71 (d, J = 5.2, 2H, Py H-3, 5), 6.97 (s, 2H, NH$_2$); LC-MS, m/z = 179 (M+H); Calculated for: C$_7$H$_6$N$_4$S: C, 47.18; H, 3.39; N, 31.44; S, 17.99; Found: C, 47.21; H, 3.43; N, 31.48; S, 18.03.

1,3,4-thiadiazole-2,5-diamine (2.5). Yield: 26.9 % (Method A); m.p. 201–203 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 6.83 (s, 4H, NH$_2$); LC-MS, m/z = 117 (M+H); Calculated for: C$_2$H$_4$N$_4$S: C, 20.68; H, 3.47; N, 48.24; S, 27.60; Found: C, 20.70; H, 3.49; N, 48.28; S, 27.63.

5-Cyclobutyl-1,3,4-thiadiazol-2-amine (2.5). Yield: 41.2 % (Method B); m.p. 21–221 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 7.00 (s, 2H, NH$_2$), 3.67 (qu, J = 8.3 Hz, 1H, Cbu H-1), 2.36–2.28 (m, 2H, Cbu H-4$_{eq}$, 2$_{eq}$), 2.20–2.16 (m, 2H, Cbu 2$_{ax}$, 4$_{ax}$), 1.97–1.83 (m, 2H, Cbu H-3$_{eq}$, 3$_{ax}$); LC-MS, m/z =156 (M+H); Calculated for: C$_6$H$_9$N$_3$S: C, 46.43; H, 5.84; N, 27.07; S, 20.65; Found: C, 46.45; H, 5.89; N, 27.11; S, 20.77.

5-Cyclohexyl-1,3,4-thiadiazol-2-amine (2.6). Yield: 70.3 % (Method B); m.p. 24--244 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 6.96 (s, 2H, NH$_2$), 2.82 (qu, J = 7.9 Hz, 1H, Cy H-1), 1.95–1.92 (m, 2H, Cy H-6$_{eq}$, 2$_{eq}$), 1.79–1.68 (m, 3H, Cy H-3$_{eq}$, 5$_{eq}$, 6$_{ax}$), 1.49–1.11 (m, 5H, Cy H-2$_{ax}$, 3$_{ax}$, 5$_{ax}$, 4$_{eq}$, 4$_{ax}$); LC-MS, m/z = 184 (M+H); Calculated for: C$_8$H$_{13}$N$_3$S: C, 52.43; H, 7.15; N, 22.93; S, 17.49; Found: C, 52.44; H, 7.19; N, 22.98; S, 17.52.

Method for the synthesis of 4-cycloalkanecarbonyl-3-R-1,5-dihydro-4H-1,2,4-triazole-5-thiones (4.1–4.4). 0.01 Mol of the corresponding *N*-(2-(phenyloxy-(phenylthio-)acetyl-hydrazine-1-carbonotioyl)-cycloalkanecarboxamides (**1.4, 1.6, 1.10, 1.11**) and 2-((cyclopropanecarbonyl)carbamothioyl)hydrazine-1-carboxamide (**1.8**) were refluxed in *n*-butanol for up to 8 hours. The mixture was poured into water, the formed precipitate was filtered off, dried, and crystallized from ethanol.

4-Cyclobutanecarbonyl-3-phenoxymethyl-1,5-dihydro-4H-1,2,4-triazol-5-thione (4.1). Yield: 35.4 %; m.p. 190–192 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H, N*H*), 7.26 (t, J = 7.6 Hz, 2H, H-3,5 Ph), 6.99 (t, J = 7.6 Hz, 1H, H-4 Ph), 6.90 (d, J = 7.6 Hz, 2H, H-2,6 Ph), 5.27 (s, 2H, -OCH$_2$-), 3.59–3.43 (m, 1H, Cbu H-1), 2.29–2.05 (m, 4H, Cbu H-4$_{eq}$, 2$_{eq}$, 2$_{ax}$, 4$_{ax}$), 2.05-1.48 (m, 2H, Cbu H-3$_{eq}$, 3$_{ax}$); LC-MS, m/z = 290 (M+H); Calculated for: C$_{14}$H$_{15}$N$_3$O$_2$S: C, 58.11; H, 5.23; N, 14.52; S, 11.08; Found: C, 58.16; H, 5.29; N, 14.54; S, 11.11.

4-Cyclohexanecarbonyl-3-phenoxymethyl-1,5-dihydro-4H-1,2,4-triazol-5-thione (4.2). Yield: 48.2 %; m.p. 172–174 °C , ¹H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H, N*H*), 7.29 (t, J = 7.6 Hz, 2H, H-3,5 Ph), 7.05 (t, J = 7.6 Hz, 1H, H-4 Ph), 6.93 (d, J = 7.6 Hz, 2H, H-2,6 Ph), 5.19 (s, 2H, -OCH$_2$-), 2.63 (qu, 1H, J = 8.0 Hz, Cy H-1), 1.91–1.73 (m, 2H, Cy H-6$_{eq}$, 2$_{eq}$), 1.72–1.51 (m, 4H, Cy H-3$_{eq}$, 5$_{eq}$, H-6$_{ax}$, 2$_{ax}$), 1.50–1.44 (m, 4H, Cy 3$_{ax}$, 5$_{ax}$, 4$_{eq}$, 4$_{ax}$); LC-MS, m/z = 318 (M+H); Calculated for: C$_{16}$H$_{19}$N$_3$O$_2$S: C, 60.55; H, 6.03; N, 13.24; S, 10.10; Found: C, 60.62; H, 6.07; N, 13.27; S, 10.13.

4-Cyclopentanecarbonyl-3-(phenylthio)methyl-1,5-dihydro-4H-1,2,4-triazol-5-thione (4.3). Yield: 54.6 %; m.p. 184– 186 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 12.64 (s, 1H, N*H*), 7.38 (d, J = 7.8 Hz, 2H, H-2,6 Ph), 7.30 (t, J = 7.9 Hz, 2H, H-3,5 Ph), 7.11 (t, J = 7.8 Hz, 1H, H-4 Ph), 4.03 (s, 2H, -SCH$_2$-), 3.06 (qu, J = 8.0 Hz, 1H, Cpe H-1), 1.83–1.47 (m, 8H, Cpe H-5$_{eq}$, 2$_{eq}$, 5$_{ax}$, 2$_{ax}$, 3$_{eq}$, 4$_{eq}$, 3$_{ax}$, 4$_{ax}$); LC-MS, m/z = 320 (M+H); Calculated for: C$_{15}$H$_{17}$N$_3$OS$_2$: C, 56.40; H, 5.36; N, 13.15; S, 20.07; Found: C, 56.46; H, 5.38; N, 13.18; S, 20.12.

4-Cyclohexanecarbonyl-(3-((phenylthio)methyl)-1,5-dihydro-4H-1,2,4-triazol-5-thione (4.4). Yield: 58.1 %; m.p. 164–166 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H, N*H*), 7.41 (d, J = 7.8 Hz, 2H, H-2,6 Ph), 7.32 (t, J = 7.9 Hz, 2H, H-3,5 Ph), 7.16 (t, J = 7.8 Hz, 1H, H-4 Ph), 4.09 (s, 2H, -SCH$_2$-), 2.48 (qu, 1H, J = 8.0 Hz, Cy H-1), 1.84–1.73 (m, 2H, Cy H-6$_{eq}$, 2$_{eq}$), 1.72–1.64 (m, 2H, Cy H-3$_{eg}$, 5$_{eg}$), 1.61–1.51 (m, 4H, Cy H-6$_{ax}$, 2$_{ax}$, 3$_{ax}$, 5$_{ax}$), 1.40–1.19 (m, 2H, Cy 4$_{eq}$, 4$_{ax}$); LC-MS, m/z = 334 (M+H); Calculated for: C$_{16}$H$_{19}$N$_3$OS$_2$: C, 57.63; H, 5.74; N, 12.60; S, 19.23; Found: C, 56.46; H, 5.38; N, 13.18; S, 20.12.

3-Amino-4-cyclopropanecarbonyl-1,5-dihydro-4H-1,2,4-triazol-5-thione (4.5). Yield: 38.7 %; m.p. 143–145 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H, N*H*), 6.40 (s, 2H, -NH$_2$), 2.48 (qu, J = 7.9, 4.6 Hz, 1H, Cpr H-1), 0.96-0.80 (m, 4H, Cpr H-2$_{eq}$, 3$_{eq}$, 2$_{ax}$, 3$_{ax}$); LC-MS, m/z = 185 (M+H); Calculated for: C$_6$H$_8$N$_4$OS: C, 39.12; H, 4.38; N, 30.41; S, 17.40; Found: C, 39.17; H, 4.42; N, 30.43; S, 17.45.

Method for the synthesis of 5-R-2,4-dihydro-3H-1,2,4-triazole-3-thiones (5.1–5.4). 0.01 Mol of the corresponding diacylthiosemicarbazides (**1.7, 1.10–1.14**) and 10 ml of 1 M sodium hydroxide solution were added to the flask. The mixture was refluxed for 2 hours until complete dissolution of the precipitate, then it was cooled, neutralized with hydrochloric acid to pH 4–5. The formed precipitates were filtered off and dried. If necessary, the obtained compounds were further purified by reprecipitation.

5-((Phenylthio)methyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (5.1). Yield: 54.8 %; m.p. 96–98 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H, 2-N*H*-), 10.19 (s, 1H, 4-N*H*-), 7.38 (d, J = 7.9 Hz, 2H, H-2,6 PhS-), 7.30 (t, J = 7.7 Hz, 2H, H-3,5 PhS-), 7.21 (t, J = 7.4 Hz, 1H, H-4 PhS-), 3.76 (s, 2H, PhSC*H*$_2$-); LC-MS, m/z = 224 (M+H); Calculated for: C$_9$H$_9$N$_3$S$_2$: C, 48.41; H, 4.06; N, 18.82; S, 28.71; Found: C, 48.42; H, 4.08; N, 18.84; S, 28.76.

5-Phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (5.2). Yield: 76.3 %; m.p. 13–134 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 13.24 (br.s., 1H, 2-N*H*-), 9.31 (s, 1H, 4-N*H*-), 7.71 (d, J = 7.9 Hz, 2H, H-2,6 Ph), 7.50 (t, J = 7.9 Hz, 1H, H-4 Ph), 7.44 (t, J = 7.9 Hz, 2H, H-3,5 Ph); LC-MS, m/z = 178 (M+H); Calculated for: C$_8$H$_7$N$_3$S: C, 54.22; H, 3.98; N, 23.71; S, 18.09; Found: C, 54.24; H, 4.03; N, 23.73; S, 18.11.

5-(Furan-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (5.3). Yield: 67.8 %; m.p. 158–160 °C, ¹H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H, 4-N*H*-), 7.69 (d, J = 3.6 Hz, 1H, furyl H-5), 7.18 (d, J = 3.6 Hz, 1H, furyl H-3), 6.50 (t, J = 3.6 Hz, 1H, furyl H-4); LC-MS, m/z =168 (M+H); Calculated for: C$_6$H$_5$N$_3$OS: C, 43.11; H, 3.01; N, 25.13; S, 19.18; Found: C, 43.12; H, 3.02; N, 25.17; S, 19.21.
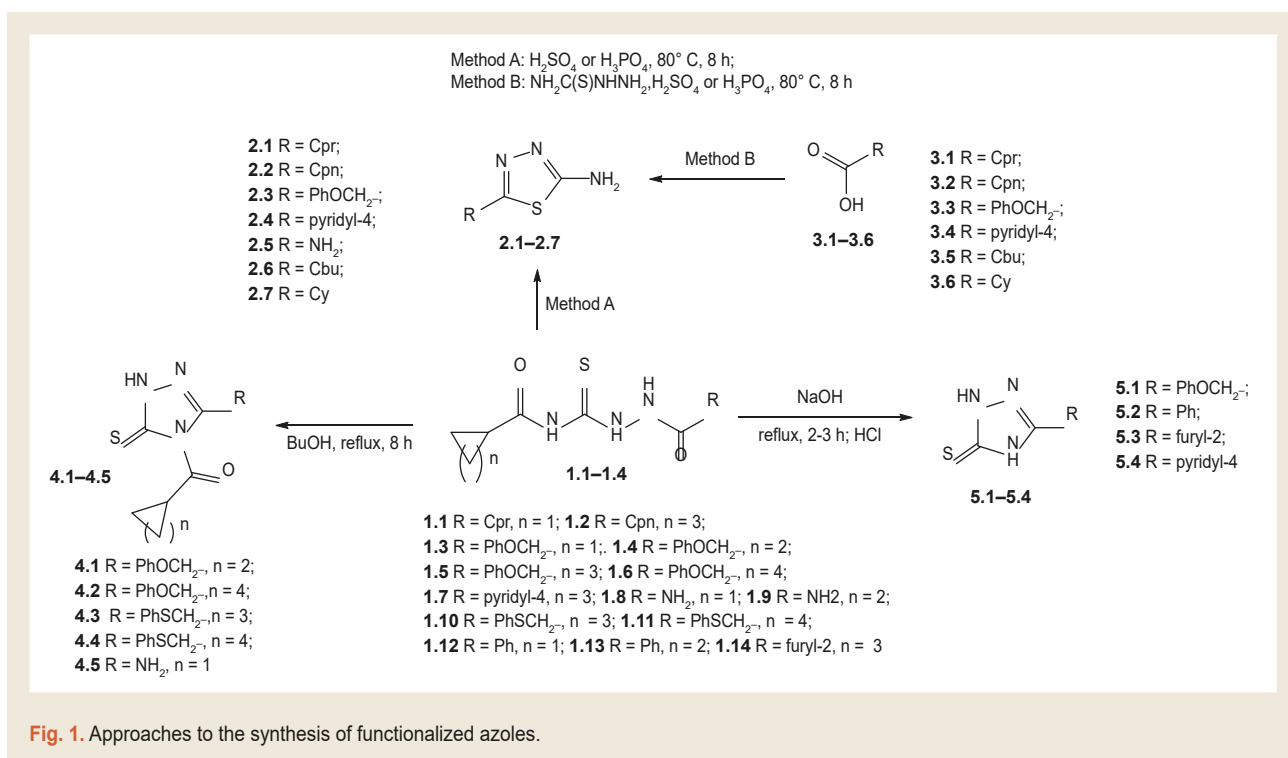
Fig. 1. Approaches to the synthesis of functionalized azoles.

5-(Pyridin-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5.4). Yield: 81.0 %; m.p. 162–164 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H, 4-N*H*-), 8.60 (d, *J* = 7.4 Hz, 2H, H-3,5 Py), 7.68 (t, *J* = 7.4 Hz, 2H, H-2,6 Py); LC-MS, *m/z* = 168 (M+H); Calculated for: $C_7H_6N_4S$: C, 47.18; H, 3.39; N, 31.44; S, 17.99; Found: C, 47.21; H, 3.43; N, 31.49; S, 18.03.

Antimicrobial test. The sensitivity of the microorganisms to the synthesized compounds was evaluated according to the described methods [19]. The assay was conducted on Mueller-Hinton agar by two-fold serial dilution of the compound in 1 ml. After which, 0.1 ml of microbial seeding (106 cells/ml) was added. Minimal inhibition concentration of the compound was determined by the absence of visual growth in the test tube with a minimal concentration of the substance. Minimal bactericide/fungicide concentration was determined by the absence of growth on agar medium after inoculation of the microorganism from the transparent test tubes. DMSO was used as a solvent, initial solution concentration was 1 mg/ml. For preliminary screening of the abovementioned standard test, cultures were used: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 885-653.

All test strains were received from the bacteriological laboratory in Zaporizhzhia Regional Laboratory Center of State Sanitary and Epidemiological Service of Ukraine. Nitrofural ((*E*)-2-((5-nitrofuran-2-yl)methylene)hydrazine-1-carboxamide) and Ketoconazole (1-(4-(4-[(2-((1*H*-imidazol-1-yl)methyl)-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)ethan-1-one) were used as reference compounds with proved antibacterial/antifungal activity. Additional quality control of the culture media and solvents was conducted by commonly used methods [19].

Results

Synthesis of substituted 1,3,4-thiadiazoles (**2**) was carried out by heterocyclization of *N*-(acylhydrazine-1-carbamothioyl)cycloalkanecarboxamides (**1.1–1.7**) and 2-(cycloalkanecarbonyl)carbamothioyl)hydrazine-1-carboxamides (**1.8, 1.9**) or alternative synthesis by cyclization of carboxylic acids (**3.1–3.6**) with thiosemicarbazide in concentrated mineral acids (*Fig.1*).

Synthesis of the series of 4-cycloalkanecarbonyl-3-R-1,5-dihydro-4*H*-1,2,4-triazole-5-thiones (**4.1–4.5**) was carried out by prolonged refluxing of N-(R-hydrazine-1-carbonothioyl)-cycloalkanecarboxamides (**1.4, 1.6, 1.8, 1.10, 1.11**) in butanol (scheme). Heterocyclization of diacylthiosemicarbazides (**1.7, 1.10–1.14**) by the conventional method, namely in the presence of alkalis [11], leads to the formation of known triazole-5-thiones (**5.1–5.4**, *Fig.1.*).

The results of the microbiological screening were showed (*Table 1*) that diacylthiosemicarbazides (**1**) in contrast to azoles (**2, 4,** and **5**) had a higher antimicrobial and fungicidal effect. Thus, compounds **1.1, 1.12,** and **1.13** were active against *E. coli* (MIC 3.125–25 µg/ml, MBC 6.2–50 µg/ml), while azoles (**2, 4,** and **5**) inhibited the growth of this bacterium in MIC 100–200 µg/ml. A similar picture was typical for *S. aureus*, namely compounds **1.1, 1.3, 1.5–1.14** inhibit growth in MIC 6.25–50.0 µg/ml, and azoles **2, 4,** and **5** – in MIC 100–200 µg/ml. Unfortunately, all test compounds had moderate activity against *P. aeruginosa* (MIC 50.0–100.00 µg/ml, MBC 100.0–200.0 µg/ml). High rates of fungicidal activity of diacylthiosemicarbazides (**1**), in comparison with azoles (**2, 4,** and **5**), were also obtained with respect to *C. albicans* (*Table 1*). Thus, compounds **1** had an inhibitory effect at a concentration of 12-50 µg/ml, and azoles (**2, 4** and **5**) – 100–200 µg/ml.

Table 1. Antibacterial and fungicidal activity of synthesized compounds

Compounds	Strains							
	E. coli		*S. aureus*		*P. aeruginosa*		*C. albicans*	
	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MFC, µg/ml
1.1	3.125	6.25	6.25	12.5	50	100	12.5	12.5
1.3	50	100	25	50	50	100	25	50
1.5	50	100	12.5	25	100	200	25	50
1.6	200	>200	25	100	100	200	50	50
1.7	50	100	50	200	50	100	50	50
1.8	50	100	12.5	25	50	100	50	50
1.9	25	50	12.5	25	50	100	50	50
1.10	100	200	25	100	50	100	25	25
1.11	100	>200	12.5	50	100	200	25	50
1.12	6.25	12.5	6.25	12.5	50	100	25	50
1.13	12.5	25	12.5	25	100	200	25	50
1.14	100	200	25	50	50	100	50	50
Nitrofural	1.5	–	6.25	–	6.25	–	–	–
Ketoconazole	–	–	–	–	–	–	25	50

For compound **1.4, 2.1–.2.7, 4.1–4.5, 5.1–5.4** MIC, MBC and MFC is 100–200 µg/ml against all strains of microorganisms and fungi.

Discussion

It was shown 5-substituted 2-amino-1,3,4-thiadiazoles (**2.1–2.5**, scheme) by the result of heterocyclization of compounds **1.1–1.8** in concentrated sulfuric or phosphate acids, according to chromato-mass spectra. This heterocyclization was realized by a known mechanism, namely through the nucleophilic attack of the electron pair of the sulfur atom on the carbonyl group of the semicarbazide fragment, followed by dehydration of the resulted intermediate and intramolecular proton migration to form an aromatic system [20]. Unfortunately, the cycloalkane-urea fragment of the molecule was additionally hydrolyzed to form an amino group in the process of cyclization, regardless of its size. In addition, this method is not preparative due to the formation of a significant number of side products, and crystallization leads to final products with low yields (2–30 %). It was carried out their counter-synthesis by cyclization of carboxylic acids (**3.1–3.6**) with thiosemicarbazide under these conditions to prove the structure of compounds **2**. The reaction was carried out by the above mentioned mechanism through the stage of formation of intermediate, 2-(cycloalkanecarbonyl) hydrazine-1-carbothioamides, followed by the formation of 5-R-2-amino-1,3,4-thiadiazoles (**2.1–2.7**) with a yield of 41–70 % (scheme) [20].

It was also found that diacyl hydrazides with phenoxy- (**1.4, 1.6**), phenylthio- (**1.10, 1.11**) acetylhydrazide or semicarbazide (**1.8**) fragments in the molecule undergo spontaneous heterocyclization refluxing prolonged heating in butanol (scheme). However, for compounds with benzoyl- (**1.12, 1.13**) and heteroyl- (**1.7, 1.14**) hydrazide fragments, this transformation was not characteristic [11]. It can probably be explained either

by their lower solubility or by the effect on the redistribution of electron density in the molecule (hydrazide-hydrazone tautomerism) and, therefore, a decrease in the electrophilicity of the carbonyl group. It was important that the yield of final products in this reaction is not significant (35–58 %).

There is no doubt that the heterocyclization of diacyl-thiosemicarbazides (**1.7, 1.1–1.14**) in the presence of alkalis was rate through intermediate 4-cycloalkanecarbonyl-tri-azole-3-thiones, which under these conditions are hydrolyzed to the target compounds **5** (scheme).

The structure and individuality of synthesized compounds were confirmed by elemental analysis, chromato-mass, and ¹H NMR spectrometric. A quasimolecular ion [M+1] was registered in the chromato-mass spectra of azoles, which confirms their structure and individuality. ¹H NMR spectra also was indicated their unambiguous formation. Thus, in the ¹H NMR spectra of 5-R-1,3,4-thiadiazol-2-amines (**2**) the two-proton singlet of the amino group was characteristic, which was registered at 7.12–6.83 ppm. Whereas, in the ¹H NMR spectra of compounds **4**, single-proton singlet of protons at the 1ˢᵗ position of the heterocycle were observed, which resonate at 12.75–12.43 ppm. In compound **4.5**, this proton undergoes a significant diamagnetic shift (11.93 ppm) due to the presence of an amino group at the 3ʳᵈ position (positive mesomeric effect). The ¹H NMR spectra of compounds **5** were also characterized by weak-field singlet protons of the 2ⁿᵈ and 4ᵗʰ positions of the heterocycle at 13.24–12.28 and 10.19–9.14 ppm, respectively. Significant weak field shifts of these protons in compounds **4** and **5**, clearly indicate their existence in the form of thions. Additionally, signals of axial and equatorial protons of methylene and methine groups of the cycloalkyl

fragment were observed in the ^{1}H NMR spectra of compounds **2.1, 2.2, 2.6** and **2.7** in a strong magnetic field, the chemical shift of which depends on the conformational stress in the cycle [20]. For example, methine protons resonate as quadruplets with J = 7.9–8.3 Hz at 2.32 ppm for cyclopropane at 3.67 ppm for cyclobutane at 3.25 ppm for cyclopentane and 2.82 ppm for cyclohexane. A similar cleavage pattern for methine protons of cycloalkyl substituents was also characteristic for compounds **4.4-4.1**, but they undergo a slight diamagnetic shift (up to 0.19 ppm) due to the influence of the carbonyl group. Characteristic shift and proton multiplicity of phenoxy-(phenyl-thio-)methyl fragments in molecules were also characteristic for compounds **2.3, 4.1–4.4, 5.1**. Thus, a two-proton singlet of the methylene group of the PhOCH$_2$ fragment at 5.47–5.19 ppm and the PhSCH$_2$ group at 4.09–3.76 ppm was observed in the spectra, along with the "classical" set of aromatic proton signals. In addition, there were signals of protons of phenyl, furan, and pyridine fragments of the molecule with characteristic cleavage and chemical shift in the spectra of compounds **2.4, 5.2–5.4** in the "aromatic" region [21].

SAR analysis showed that structural modification of diacylthiosemicarbazides with the formation of substituted 1,3,4-thiadiazoles or 1,2,4-triazoles leads to loss of antimicrobial and fungicidal activity. More interesting were compounds **1.1** and **1.12**, which inhibit the growth of *S. aureus* in MIC 6.25 µg/ml and compete with the reference compound Nitrofural (MIC 6.25 µg/ml) and require further study.

Conclusions

1. It was found that *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides under the conditions of the heterocyclization reaction in concentrated mineral acids form 5-R-2-amino-1,3,4-thiadiazoles. Namely the intermediate was undergone additional hydrolysis with the cleavage of the cycloalkane fragment. Alternative methods of their synthesis were offered.

2. Prolonged refluxing of the corresponding diacylthiosemicarbazides in butanol resulted in the formation of the novel 4-cycloalkanecarbonyl-3-(amino-, phenyloxo-(thio)methyl-1,5-dihydro-4*H*-1,2,4-triazole-5-thiones. This reaction with diacylthiosemicarbazides was failed because the latter undergo heterocyclization in the presence of sodium hydroxide with the formation of known 5-R-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones.

3. ^{1}H NMR spectra were studied and patterns of splitting of characteristic protons were established for the synthesized azoles.

4. It was found that 5-R-2-amino-1,3,4-thiadiazoles, 4-cycloalkanecarbonyl-3-(amino-, phenyloxo-(thio)methyl-1,5-dihydro-4*H*-1,2,4-triazole-5-thiones and 5-R-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones were less effective antimicrobial and antifungal agents compared to *N*-(R-hydrazine-1-carbonothioyl)cycloalkanecarboxamides.

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References

[1] Bedane, K. G., Singh, G. S. (2015). Reactivity and diverse synthetic applications of acyl isothiocyanates. *ARKIVOC*, 206-245. http://dx.doi.org/10.3998/ark.5550190.p009.052

[2] Moharana, A. K., Dash, R. N., & Subudhi, B. B. (2020). Thiosemicarbazides: Updates on Antivirals Strategy. *Mini reviews in medicinal chemistry*, *20*(20), 2135-2152. https://doi.org/10.2174/1389557520666200818212408

[3] Metwally, M. A., Bondock, S., El-Azap, H., & Kandeel, E. E. M. (2011). Thiosemicarbazides: Synthesis and reactions. *Journal of Sulfur Chemistry*, *32*(5), 489-519. https://doi.org/10.1080/17415993.2011.601869

[4] Vincent-Rocan, J. F., & Beauchemin, A. M. (2016). N-Isocyanates, N-Isothiocyanates and Their Masked/Blocked Derivatives: Synthesis and Reactivity. *Synthesis, 48*(21), 3625-3645. https://doi.org/10.1055/s-0036-1588066

[5] Nora De Souza, M. V. (2005). Synthesis and biological activity of natural thiazoles: An important class of heterocyclic compounds. *Journal of Sulfur Chemistry, 26*(4-5), 429-449. https://doi.org/10.1080/17415990500322792

[6] Jain A. K., Sharma S., Vaidya A., Ravichandran V., & Agrawal, R. K. (2013). 1,3,4-Thiadiazole and its Derivatives: A Review on Recent Progress in Biological Activities. *Chemical Biology & Drug Design*, *81*(5), 557-576. https://doi.org/10.1111/cbdd.12125

[7] Hu Y., Li C.-Y., Wang X.-M., Yang Y.-H., Zhu H.-L. (2014). 1,3,4-Thiadiazole: Synthesis, Reactions, and Applications in Medicinal Agricultural and Materials Chemistry. *Chemical Reviews*, *114*(10), 5572-5610. https://doi.org/10.1021/cr400131u

[8] Sukinah, H. A., & Abdelwahed, R. S. (2020). Review of the synthesis and biological activity of thiazoles, *Synthetic Communications*, *51*(5), 670-700. https://doi.org/10.1080/00397911.2020.1854787

[9] Sahiba, N., Sethiya, A., Soni, J., Agarwal, D. K., & Agarwal, S. (2020). Saturated Five-Membered Thiazolidines and Their Derivatives: From Synthesis to Biological Applications. *Topics in Current Chemistry, 378,* 34. https://doi.org/10.1007/s41061-020-0298-4

[10] Sathish Kumar, S., & P. Kavitha, H. (2013). Synthesis and Biological Applications of Triazole Derivatives – A Review. *Mini-Reviews in Organic Chemistry*, *10*(1), 40-65. https://doi.org/10.2174/1570193x11310010004

[11] Maddila, S., Pagadala, R., & Jonnalagadda, S. (2013). 1,2,4-Triazoles: A Review of Synthetic Approaches and the Biological Activity. *Letters in Organic Chemistry*, *10*(10), 693-714. https://doi.org/10.2174/157017861010131126115448

[12] Gümüş, M., Yakan, M., & Koca, İ. (2019). Recent advances of thiazole hybrids in biological applications. *Future Medicinal Chemistry*, *11*(15), 1979-1998. https://doi.org/10.4155/fmc-2018-0196

[13] Matysiak, J. (2015). Biological and pharmacological activities of 1,3,4-thiadiazole based compounds. *Mini reviews in medicinal chemistry*, *15*(9), 762-775. https://doi.org/10.2174/1389557515666 150519104057

[14] Sahu, J. K., Ganguly, S., & Kaushik, A. (2013). Triazoles: a valuable insight into recent developments and biological activities. *Chinese journal of natural medicines*, *11*(5), 456-465. https://doi.org/10.1016/S1875-5364(13)60084-9

[15] *DrugBank Online* : [database]. https://go.drugbank.com/drugs

[16] Antypenko, L., Meyer, F., Kholodniak, O., Sadykova, Z., Jirásková, T., Troianova, A., Buhaiova, V., Cao, S., Kovalenko, S., Garbe, L. A., & Steffens, K. G. (2019). Novel acyl thiourea derivatives: Synthesis, antifungal activity, gene toxicity, drug-like and molecular docking screening. *Archiv der Pharmazie*, *352*(2), e1800275. https://doi.org/10.1002/ardp.201800275

[17] Kholodniak, O. V., Sokolova, K. V., Kovalenko, S. I., & Pidpletnya, O. A. (2020). Directed search for compounds that affect the excretory function of rat kidneys, among new cycloalkylcarbonyl thioureas and thiosemicarbazides derivatives. *Medychna ta klinichna khimiia – Medical and Clinical Chemistry*, (2), 5-16. https://doi.org/10.11603/mcch.2410-681X.2020.v.i2.11351

[18] Kholodniak, O. V., Stavytskyi, V. V., Kazunin, M. S., Bukhtiayrova, N. V., Berest, G. G., Belenichev, I. F., & Kovalenko, S. I. (2021). Design, synthesis and anticonvulsant activity of new Diacylthiosemicarbazides. *Biopolymers and Cell*, 37(2), 125-142. https://doi.org/10.7124/bc.000A46

[19] Clinical and Laboratory Standards Institute. (2006). Performance standards for antimicrobial disk susceptibility tests, (9th ed) CLSI standard M2-A9. Wayne, PA: Clinical and Laboratory Standards Institute.

[20] Barbosa, G. A. D., & de Aguiar, A. P. (2019). Synthesis of 1,3,4-Thiadiazole Derivatives and Microbiological Activities: A Review. *Revista Virtual de Química*, *11*(3), 806-848. https://doi.org/10.21577/1984-6835.20190058

[21] Baranac-Stojanović, M., & Stojanović, M. (2013). 1H NMR chemical shifts of cyclopropane and cyclobutane: a theoretical study. *The Journal of organic chemistry*, *78*(4), 1504-1507. https://doi.org/10.1021/jo3025863

[22] Breitmaier E. (2002). *Structure elucidation by NMR in organic chemistry: a practical guide* (3rd ed). Wiley. https://doi.org/10.1002/0470853069