UDC 547.792'856'77+547.792'856'85]:615.015.4
DOI: 10.15587/2519-4852.2024.318160

[1,2,4]TRIAZINO[2,3-C]QUINAZOLINE HYBRIDS WITH AZOLE AND AZINE HETEROCYCLES: DESIGN, SYNTHESIS, ANTIBACTERIAL AND ANTIRADICAL ACTIVITY

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The aim. *Present paper devoted to the purposeful search of a promising biologically active compounds among heterocyclic hybrids combining in their structure [1,2,4]triazino[2,3-c]quinazoline system and a "pharmacophoric" azole or azine fragment, joined through an alkylthio linker group.*

Material and methods. *Methods of synthetic organic chemistry were used to prepare target compounds. The purity and structure of the synthesized compounds were confirmed by elemental analysis, HPLC-MS, and ^{1}H NMR spectrometry. Radical-scavenging activity was estimated using DPPH-assay, antimicrobial activity was studied by serial dilution method.*

Results. *A combinatorial library of 30 novel heterocyclic hybrids was designed and synthesized. The target compounds were obtained via the interaction of 6-chloroalkyl-3-R-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones and corresponding heterocyclic thiones in the presence of a base. The synthesized compounds were studied for their radical scavenging and antimicrobial activity. Most of the obtained compounds revealed low antimicrobial activity against studied strains. However, the heterocyclic hybrid combining triazinoquinazoline, thiadiazole, and 4-fluorophenyl moieties (compound 2.14) inhibited the growth of S. aureus, E. coli, and M. luteum. Among the obtained compounds, five heterocyclic hybrids demonstrated significant DPPH radical scavenging activity (30.41–43.53 %). The "structure-activity" correlations were evaluated and discussed. It was estimated that "linker" alkylthio-group modification resulted in the most pronounced changes in the radical scavenging activity of obtained compounds.*

Conclusions. *Triazinoquinazoline-based heterocyclic hybrids are promising objects for further screening for antimicrobial activity and pharmacological effects associated with antiradical properties*

Keywords: *heterocyclic hybrids, synthesis, radical scavenging activity, antimicrobial and antifungal activity, SAR-analysis*

How to cite:
Grytsak O., Schabelnyk K., Kinichenko A., Komarovska-Porokhnyavets O., Lubenets V., Voskoboinik O., Kovalenko S. (2024). [1,2,4]triazino[2,3-c] quinazoline hybrids with azole and azine heterocycles: design, synthesis, antibacterial and antiradical activity. ScienceRise: Pharmaceutical Science, 6 (52), 4–14. http://doi.org/10.15587/2519-4852.2024.318160

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1. Introduction

Molecular hybridization is an effective strategy for the rational design of bioactive compounds, widely used by researchers involved in drug development. This strategy is based on the combination of two or more pharmacophoric fragments in one molecule [1–3]. These fragments are, in most cases, joined by a linker group or through the formation of fused systems [2, 3]. The molecular hybridization approach is employed not only for the development of innovative multi-targeted bioactive molecules but also for improving pharmacokinetic parameters, including enhanced trans-membrane transportability, stability against biotransformation, and reductions in toxicity and drug resistance.

The majority of studies on molecular hybrids are aimed at the development of drugs for the treatment of cancer, infections, inflammatory processes, and neurodegenerative diseases (such as Parkinson's disease and Alzheimer's disease) [4–8]. For instance, this strategy has enabled the creation of novel EGFR/HER2 inhibitors and multi-kinase inhibitors (Lapatinib, Dasatinib, Sunitinib) [9], as well as DNA gyrase and topoisomerase inhibitors with antibacterial activity (Zodiflodacin, Gepotidacin, etc.) [10–13], among other drugs.

Although this strategy is quite reasonable, it has both positive and negative aspects. Among the negative aspects are the challenges of selecting a favourable combination of initial compounds, the large size of the combinatorial library for pharmacological screening, and the lack of high biological activity while preserving the properties of the initial structure. On the positive side, molecular hybridization offers the potential to create drugs with multi-target mechanisms of action, accumulate information related to structure-activity correlations, and provide data on ligand-enzyme interactions, toxicity, mechanisms of action, and more.

Previously published data revealed that hybrid structures created on the basis of existing drugs, natural compounds, or experimental bioactive compounds are promising for the development of new drugs. Heterocyclic hybrids bearing azole and azine fragments, or their condensed analogues, occupy an important place among biologically active molecular hybrids [2, 14–18].

Our previous studies revealed that the 6-thio-1,2,4-triazino[2,3-*c*]quinazoline cycle is promising pharmacophore fragment [19, 20] and being combined with dialkylamino(heterocyclyl-)alkyl moieties, resulted in agents with high anticancer and antimicrobial activity [21]. At the same time, the combination of the aforementioned tricyclic system with carcass amines yielded compounds with antiviral effects [22]. Compounds that combine a triazinoquinazoline cycle and an aminoazole fragment linked via a methylcarbonyl group demonstrated anticancer activity [23]. Hence, combining heterocyclic fragments has been proven as a reasonable approach to the purposeful search for novel bioactive compounds.

Aim of research was to create [1,2,4]-triazino[2,3-*c*]quinazoline hybrids linked to known "pharmacophoric" heterocycles through an alkylthio fragment for further preliminary study of their biological activity.

2. Planning of research

The design of promising bioactive compounds was based on the contemporary experience of applying molecular hybridization for the purposeful search for bioactive agents. The [1,2,4]triazino[2,3-*c*]quinazoline system was chosen as the core heterocyclic fragment, considering the results of our previous studies focused on the search for promising bioactive compounds among heterocyclic hybrids that combine the aforementioned tricyclic fragment and a pyrazoline moiety, joined by a thioacetate linker group [24]. Purine, benzothiazole, benzimidazole, triazole, tetrazole, and thiadiazole systems were introduced as additional heterocyclic fragments. The choice of additional heterocyclic moieties was driven by their "pharmacophoric" role and synthetic availability.

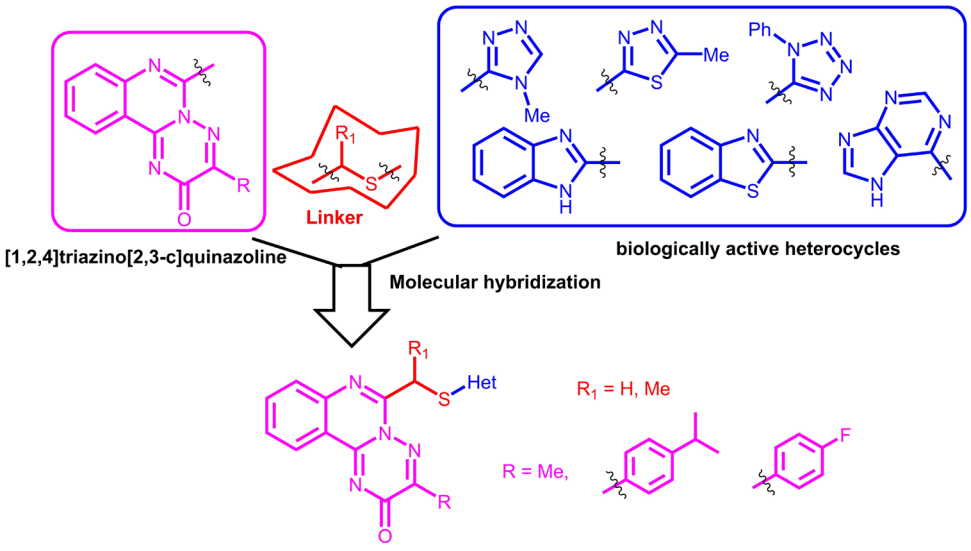

Fig. 1. Design [1,2,4]-triazino[2,3-*c*]quinazoline hybrids with azole and azine heterocycles

The merging of heterocyclic compounds was decided to be performed through a thioalkyl linker group (Fig. 1). We believe that the presence of a sulfur atom in the linker group would not only provide antioxidant properties but also enable additional binding with the molecular target. The presence of substituents with varying degrees of lipophilicity at position 3 of the core heterocycle allows for the assessment of their effect on the level of biological activity. Radical scavenging and antimicrobial activities were studied for evaluation of obtained compounds` potential as biologically active agents, especially since antiradical activity is associated with hepatoprotective, anti-ischemic and anti-inflammatory pharmacological effects.

3. Materials and methods

3. 1. Synthesis

Melting points were determined in open capillary tubes in a «Mettler Toledo MP 50» apparatus and were uncorrected. The elemental analyses (C, H, N) were performed using the ELEMENTAR vario EL cube analyzer (USA). Analyses were indicated by symbols of the elements or functions within ±0.3 % of the theoretical values. ^{1}H NMR spectra (500 MHz) were recorded on a Varian-Mercury 500 (Varian Inc., Palo Alto, CA, USA) spectrometers with TMS as internal standard in DMSO-d_6 solution. LC-MS was recorded using a chromatography/mass spectrometric system, which consists of high-performance liquid chromatography «Agilent 1100 Series» (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector «Agilent LC/MSD SL» (atmospheric pressure chemical ionization – APCI). The purity of all obtained compounds was checked by ^{1}H-NMR and LC-MS.

Reagents and solvents (4-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, 1-phenyl-1,4-dihydro-5*H*-tetrazole-5-thione, 5-methyl-1,3,4-thiadiazole-2(3*H*)-thione, 1,3-dihydro-2*H*-benzo[*d*]imidazole-2-thione, benzo[*d*]thiazole-2(3H)-thione, 1,7-dihydro-6*H*-purine-6-thione) were provided by «Enamine» Ltd Ukraine).

General procedure for the synthesis of 6-(chloro(R^2)methyl)-3-R^1-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (1.1–1.6).

The 10 mmol of corresponding substituted 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2H)-one [25] was suspended in 30 ml of acetic acid and 16 mmol of chloracetyl chloride (for compounds **1.2**, **1.4, 1.5**) or 10 mmol of 2-chloropropanoyl chloride (for compounds **1.1**, **1.3**, **1.6**) was added. The formed mixture was stirred at 60 °C for 30 min (for compounds **1.2**, **1.4, 1.5**) or refluxed for 1 hour (for compounds **1.1**, **1.3**, **1.6**). After completion of the reaction, the mixture was cooled and poured in water. The formed precipitate was filtered off, dried and crystallized from acetone.

6-*(1-chloroethyl)-3-methyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**1.1**) Yield 59 %; M.p. 220–222 °C; ^{1}H NMR δ 8.58 (*d*, *J*=7.8, 1H, H-11), 7.96 (*t*, *J*=7.7, 1H, H-9), 7.85 (*d*, *J*=7.8, 1H, H-8), 7.73 (*t*, *J*=7.8, 1H, H-10), 6.02 (*q*, *J*=7.0, 1H, $\underline{CH}CH_3$), 2.46 (*s*, 3H, CH_3) 2.02 (*d*, *J*=6.9, 3H, $CH\underline{CH_3}$); LC-MS (*m/z*)=275.0 (M+1); Anal. calcd. for $C_{13}H_{11}ClN_4O$: C, 56.84; H, 4.04; N, 20.40; Found: C, 56.86; H, 4.02; N, 20.44.

6-*(Chloromethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**1.2**) Yield 89 %; M.p. 218–220 °C; ^{1}H NMR δ 8.64 (*d*, *J*=7.9 Hz, 1H, H-11), 8.35 (*d*, *J*=7.3 Hz, 2H, 3 Ph H-2,6), 8.04 (*t*, *J*=7.5 Hz, H-9), 7.94 (*d*, *J*=8.0 Hz, 1H, H-8), 7.82 (*t*, *J*=7.4 Hz, 1H, H-10), 7.69–7.43 (*m*, 3H, 3 Ph H-3,4,5), 5.19 (*s*, 2H, CH_2); LC-MS (*m/z*)=323.0 (M+1); Anal. calcd. for $C_{17}H_{11}ClN_4O$: C, 63.31; H, 3.49; N, 17.41; Found: C, 63.35; H, 3.53; N, 17.45.

6-*(1-chloroethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**1.3**) Yield 85 %; M.p. 236–238 °C; ^{1}H NMR δ 8.67 (*d*, *J*=8.3, 1H, H-11), 8.36 (*d*, *J*=7.7, 2H, 3 Ar H-2, 6), 8.02 (*t*, *J*=7.6, 1H, H-9), 7.94 (*d*, 4=7.9, 1H, H-8), 7.81 (*t*, 4=7.4, 1H, H-10), 7.64–7.48 (*m*, 3H, 3 Ar H-3,4,5), 6.07 (*q*, *J*=6.9, 1H, $CHCH_3$), 2.05 (*d*, *J*=6.5, 3H, $CHCH_3$); LC-MS (*m/z*)=337.0 (M+1); Anal. calcd. for $C_{18}H_{13}ClN_4O$: C, 64.20; H, 3.89; N, 16.64; Found: C, 64.24; H, 3.92; N, 16.68.

6-*(Chloromethyl)-3-(4-isopropylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**1.4**) Yield 83 %; M.p. 250–252 °C; ^{1}H NMR 8.66 (*d*, 1H, *J*=7.7 Hz, H-11), 8.30 (*d*, 2H, *J*=8.2 Hz, 3 Ar H-2, H-6); 8.02 (*t*, 1H, *J*=7.2 Hz, H-9), 7.92 (*d*, 1H, *J*=7.0 Hz, H-8), 7.81 (*t*, 1H, *J*=7.5 Hz, H-10), 7.39 (*d*, 2H, *J*=8.2 Hz, 2H, 3 Ar H-3, H-5), 5.16 (*s*, 2H, CH_2Cl), 3.04–2.97 (*m*, 1H, $CH(CH_3)_2$), 1.33 (*d*, 6H, *J*=6.8 Hz, $CH(CH_3)_2$); LC-MS (*m/z*)=365 (M+1); Anal. calcd. for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36; Found: C, 65.87; H, 4.71, N, 15.38.

6-*(Chloromethyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**1.5**) Yield 84 %; M.p. 250–252 °C; ^{1}H NMR 8.66 (*d*, 1H, *J*=7.7 Hz, H-11), 7.81 (*t*, 1H, *J*=7.4 Hz, H-10), 8.48 (*dd*, 2H, *J*=8.7,5.6 Hz, 3 Ar H-2, H-6), 8.03 (*t*, 1H, *J*=7.1 Hz, H-9), 7.93 (*d*, 1H, *J*=8.0 Hz, H-8), 7.29 (*t*, 1H, *J*=8.7 Hz, 3 Ar H-3, H-5), 5.18 (*s*, 2H, CH_2Cl); LC-MS (*m/z*)=341 (M+1); Anal. calcd. for $C_{17}H_{10}ClFN_4O$: C, 59.92; H, 2.96; N, 16.44; Found: C, 59.98; H, 3.01, N, 16.48.

6-*(1-chloroethyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**1.6**) Yield 92 %; M.p.230–232°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 8.67 (*d*, *J*=8.1 Hz, 1H, H-11), 8.53–8.41 (*m*, 2H, 3 Ar H-2,6), 8.09–7.97 (*m*, 1H, H-9), 7.93 (*d*, *J*=8.0 Hz, 1H, H-8), 7.80 (*t*, *J*=7.4 Hz, 1H, H-10), 7.27 (*t*, *J*=8.5 Hz, 2H, 3 Ar H-3,5), 6.06 (*q*, *J*=6.6 Hz, 1H, $CH\underline{CH_3}$), 2.05 (*d*, *J*=6.6 Hz, 3H, $\underline{CH}CH_3$); LC-MS (*m/z*)=355 (M+1); Anal. calcd. for $C_{18}H_{12}ClFN_4O$: C, 60.94; H, 3.41; N, 15.79; Found: C, 60.97; H, 3.45; N, 15.81.

General procedure for the synthesis of 6-*((hetaryl)thioalkyl)}-3-R-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (2.1-2.30).*

5 mM of the corresponding heterocyclic thiones was placed in a round bottom flask, and 2 ml of 10 % sodium hydroxide solution (5 mM) and 25 ml of propanol-2 were added, stirred and left for 2 minutes. 5 mM of the starting substance (**1.1**–**1.6**) was added to the prepared solution and refluxed for 2 hours. The reaction mixture is cooled, the precipitate formed is filtered, washed with water and alcohol. Dried. For additional purification obtained compounds were re-crystallized from DMFA-water mixture.

6-*(((4-Methyl-4H-1,2,4-triazol-3-yl)thio)methyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.1**). Yield: 88 %; M.p. 240–241 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.63 (*d*, *J*=8.1 Hz, 1H, H-11), 8.36 (s , 1H, triazole H-5), 8.29 (*d*, *J*=7.1 Hz, 2H, 3-Ar H-2,6), 7.97 (*t*, *J*=7.6 Hz, 1H, H-9), 7.82 (*d*, *J*=8.1 Hz, 1H, H-8), 7.75 (*t*, *J*=7.5 Hz, 1H, H-10), 7.61–7.48 (*m*, 3H, 3-Ar H-3,4,5), 4.91 (*s*, 2H, -$C\underline{H_2}$S-), 3.60 (*s*, 3H, -CH_3); LC-MS (m/z)=402 (M+1); Elemental analysis calculated for $C_{20}H_{15}N_7OS$ C, 59.84; H, 3.77; N, 24.42; S, 7.99; Found: C, 59.87; H, 3.79; N, 24.45; S, 8.01.

3-(4-Isopropylphenyl)-6-(((4-methyl-4H-1,2,4-triazol-3-yl)thio)methyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.2**). Yield: 82 %; M.p. 240–241 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (*d*, *J*=7.9 Hz, 1H, H-11), 8.37 (*s*, 1H, triazole H-5), 8.23 (*d*, *J*=8.1 Hz, 2H, 3-Ar H-2,6), 7.95 (*t*, *J*=7.3 Hz, 1H, H-9), 7.81 (*d*, *J*=8.1 Hz, 1H, H-8), 7.74 (*t*, *J*=7.5 Hz, 1H, H-10), 7.37 (*d*, *J*=8.1 Hz, 2H, 3-Ar H-3, 5), 4.90 (*s*, 2H, -$C\underline{H_2}$S-), 3.61 (*s*, 3H, -CH_3), 2.99–2.87 (*m*, 1H, -$C\underline{H}(CH_3)_2$), 1.31 (*d*, *J*=6.8 Hz, 6H, -$C\underline{H}(\underline{CH_3})_2$); LC-MS (m/z)=444 (M+1); Elemental analysis calculated for $C_{23}H_{21}N_7OS$ C, 62.29; H, 4.77; N, 22.11; S, 7.23; Found: C, 62.33; H, 4.82; N, 22.15; S, 7.26.

3-*(4-Fluorophenyl)-6-(((4-methyl-4H-1,2,4-triazol-3-yl)thio)methyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.3**). Yield: 78 %; M.p. 237–238 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (*d*, *J*=7.9 Hz, 1H, H-11), 8.40 (*dd*, *J*=8.6, 5.7 Hz, 2H, 3-Ar H-2,6), 8.38 (*s*, 1H, triazole H-5), 7.97 (*t*, *J*=7.4 Hz, 1H, H-9), 7.82 (*d*, *J*=8.1 Hz, 1H, H-8), 7.75 (*t*, *J*=7.6 Hz, 1H, H-10), 7.27 (*t*, *J*=8.7 Hz, 2H, 3-Ar H-3,5), 4.92 (*s*, 2H, -$C\underline{H_2}$S-), 3.61 (*s*, 3H, -CH_3). LC-MS (m/z)=420 (M+1); Elemental analysis calculated for $C_{20}H_{14}FN_7OS$ C, 57.27; H, 3.36; N, 23.38; S, 7.64; Found: C, 57.25; H, 3.31; N, 23.40; S, 7.62.

3-Methyl-6-(1-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.4**). Yield: 84 %; M.p. 210–212 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (*d*, *J*=7.7 Hz, 1H, H-11), 8.47 (*s*, 1H, triazole H-5), 7.95 (*t*, *J*=7.7 Hz, 1H, H-9), 7.80 (*d*, *J*=8.0 Hz, 1H, H-8), 7.73 (*t*, *J*=7.6 Hz, 1H, H-10), 5.57 (*q*, *J*=6.9 Hz, 1H, -$C\underline{H}(CH_3)$S-), 3.56 (*s*, 3H, 4-Me-triazole, -CH_3), 2.33 (*s*, 3H, 3-CH_3), 1.83 (*d*, *J*=6.9 Hz, 3H, -$CH(C\underline{H_3})$S-); LC-MS (m/z)=354 (M+1); Elemental analysis calculated for $C_{16}H_{15}N_7OS$ C, 54.38; H, 4.28; N, 27.74; S, 9.07; Found: C, 54.42; H, 4.30; N, 27.80; S, 9.10.

6-*(1-((4-Methyl-4H-1,2,4-triazol-3-yl)thio)ethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.5**). Yield: 86 %; M.p. 209–210 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (*d*, *J*=8.1 Hz, 1H, H-3), 8.30 (*s*, 1H, triazole H-5), 8.23 (*d*, *J*=7.2 Hz, 2H, 3-Ph H-2,6), 7.98 (*t*, *J*=8.2 Hz, 1H, H-9), 7.83 (*d*, *J*=7.9 Hz, 1H, H-8), 7.79–7.73 (*m*, 1H, H-10), 7.59–7.45 (*m*, 3H, 3-Ph H-3, 4, 5), 5.68 (*q*, *J*=6.9 Hz, 1H, -$C\underline{H}(CH_3)$S-), 3.43 (*s*, 3H, -CH_3),

1.88 (*d*, *J*=6.9 Hz, 3H, -CH(C*H*$_3$)S-); LC-MS (m/z)=416 (M+1); Elemental analysis calculated for $C_{21}H_{17}N_7OS$ C, 60.71; H, 4.12; N, 23.60; S, 7.72; Found: C, 60.70; H, 4.09; N, 23.65; S, 7.74.

3-(4-Fluorophenyl)-6-(1-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.6**). Yield: 83 %; M.p. 218–219 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (*d*, *J*=8.0 Hz, 1H, H-11), 8.45–8.19 (*m*, 3H, 3-Ar H-2,6, triazole H-5), 7.98 (*t*, *J*=8.3 Hz, 1H, H-9), 7.82 (*d*, *J*=8.0 Hz, 1H, H-8), 7.75 (*t*, *J*=8.0 Hz, 1H, H-10), 7.23 (*t*, *J*=8.7 Hz, 2H, 3-Ar H-3,5), 5.72 (*q*, *J*=6.9 Hz, 1H, -C*H*(CH$_3$)S-), 3.46 (*s*, 3H, -CH$_3$), 1.88 (*d*, *J*=6.9 Hz, 3H, -CH(C*H*$_3$)S-); LC-MS (m/z)=434 (M+1); Elemental analysis calculated for $C_{21}H_{16}FN_7OS$ C, 58.19; H, 3.72; N, 22.62; S, 7.40; Found: C, 58.23; H, 3.75; N, 22.67; S, 7.38.

3-Phenyl-6-(((1-phenyl-1H-tetrazol-5-yl)thio)methyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.7**). Yield: 75 %; M.p. 219–220 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (*d*, *J*=8.0 Hz, 1H, H-11), 8.28 (*d*, *J*=7.5 Hz, 2H, 3-Ph H-2,6), 7.96 (*t*, *J*=7.7 Hz, 1H, H-9), 7.83 (*d*, *J*=8.1 Hz, 1H, H-8), 7.75 (*t*, *J*=7.6 Hz, 1H, H-10), 7.63–7.45 (*m*, 8H, 3-Ph, H-3, 4, 5; 1-Ph-tetrazole H-2, 3, 4, 5, 6), 5.27 (s, 2H, -CH$_2$S-). LC-MS (m/z)=465 (M+1); Elemental analysis calculated for $C_{24}H_{16}N_8OS$ C, 62.06; H, 3.47; N, 24.12; S, 6.90; Found: C, 62.07; H, 3.49; N, 24.17; S, 6.91.

3-(4-Isopropylphenyl)-6-(((1-phenyl-1H-tetrazol-5-yl)thio)methyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.8**). Yield: 83 %; M.p. 179–180 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (*d*, *J*=8.0 Hz, 1H, H-11), 8.21 (*d*, *J*=8.3 Hz, 2H, 3-Ar H-2,6), 7.95 (*t*, *J*=8.3 Hz, 1H, H-9), 7.82 (*d*, *J*=8.1 Hz, 1H, H-8), 7.74 (*t*, *J*=7.6 Hz, 1H, H-10), 7.65–7.54 (*m*, 5H, 1-Ph-tetrazole, H-2, 3, 4, 5, 6), 7.36 (*d*, *J*=8.3 Hz, 2H, 3-Ar H-3,5), 5.25 (*s*, 2H, -CH$_2$S-), 3.01–2.96 (*m*, 1H, -C*H*(CH$_3$)$_2$), 1.30 (*d*, *J*=6.9 Hz, 6H, -CH(C*H*$_3$)$_2$); LC-MS (m/z)=507 (M+1); Elemental analysis calculated for $C_{27}H_{22}N_8OS$ C, 64.02; H, 4.38; N, 22.12; S, 6.33; Found: C, 64.00; H, 4.34; N, 22.17; S, 6.32.

3-(4-Fluorophenyl)-6-(((1-phenyl-1H-tetrazol-5-yl)thio)methyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.9**). Yield: 75 %; M.p. 217–218 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (*d*, *J*=7.9 Hz, 1H, H-11), 8.49–8.30 (*m*, 2H, 3-Ar H-2,6), 7.96 (*t*, *J*=7.4 Hz, 1H, H-9), 7.82 (*d*, *J*=8.0 Hz, 1H, H-8), 7.75 (*t*, *J*=7.4 Hz, 1H, H-10), 7.68–7.53 (m, 5H, 1-Ph-tetrazole, H-2, 3, 4, 5, 6), 7.27 (*t*, *J*=8.5 Hz, 2H, 3-Ar H-3,5), 5.27 (*s*, 2H, -CH$_2$S-); LC-MS (m/z)=483 (M+1); Elemental analysis calculated for $C_{24}H_{15}FN_8OS$ C, 59.74; H, 3.13; N, 23.22; S, 6.64; Found , C, 59.72; H, 3.17; N, 23.25; S, 6.66.

3-Phenyl-6-(1-((1-phenyl-1H-tetrazol-5-yl)thio)ethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.10**). Yield: 85 %; M.p. 193–194 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (*d*, *J*=8.9 Hz, 1H, H-11), 8.20 (*d*, *J*=7.2 Hz, 2H, 3-Ph H-2,6), 7.97 (*t*, *J*=8.3 Hz, 1H, H-9), 7.82 (*d*, *J*=8.1 Hz, 1H, H-8), 7.75 (*t*, *J*=7.2 Hz, 1H, H-10), 7.56 (*t*, *J*=7.3 Hz, 1H, 3-Ph H-4), 7.51–7.38 (*m*, 7H, 3-Ar H-3,5, 1-Ph-tetrazole H-2, 3, 4, 5, 6), 6.04 (*q*, *J*=6.9 Hz, 1H, -C*H*(CH$_3$)S-), 2.01 (*d*, *J*=6.9 Hz, 3H. -CH(C*H*$_3$)S-); LC-MS (m/z)=479 (M+1); Elemental analysis calculated for $C_{25}H_{18}N_8OS$ C, 62.75; H, 3.79; N, 23.42; S, 6.70; Found: C, 62.76; H, 3.81; N, 23.46; S, 6.70.

3-(4-Fluorophenyl)-6-(1-(((1-phenyl-1H-tetrazol-5-yl)thio)ethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.11**). Yield: 86 %; M.p. 204–206 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59 (d, *J*=7.8 Hz, 1H, H-11), 8.33 (*dd*, *J*=8.5, 5.6 Hz, 2H, 3-Ar H-2,6), 7.97 (*t*, *J*=7.3 Hz, 1H, H-9), 7.82 (*d*, *J*=8.0 Hz, 1H, H-8), 7.75 (*t*, *J*=7.5 Hz, 1H, H-10), 7.56–7.36 (*m*, 5H, 1-Ph-tetrazole, H-2, 3, 4, 5, 6), 7.24 (*t*, *J*=8.6 Hz, 2H, 3-Ar H-3,5), 6.06 (*q*, *J*=6.6 Hz, 1H, -C*H*(CH$_3$)S-), 2.00 (*d*, *J*=6.8 Hz, 3H, -CH(C*H*$_3$)S-); LC-MS (m/z)=497 (M+1); Elemental analysis calculated for $C_{25}H_{17}FN_8OS$ C, 60.48; H, 3.45; N, 22.57; S, 6.46; Found: C, 60.45; H, 3.43; N, 22.62; S, 6.48.

6-(((5-Methyl-1,3,4-thiadiazol-2-yl)thio)methyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.12**). Yield: 86 %; M.p. 210–212 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (*d*, *J*=8.0 Hz, 1H, H-11), 8.28 (*d*, *J*=7.1 Hz, 2H, 3-Ar H-2,6), 7.97 (*t*, *J*=7.6 Hz, 1H, H-9), 7.86 (*d*, *J*=8.1 Hz, 1H, H-8), 7.75 (*t*, *J*=7.6 Hz, 1H, H-10), 7.56 (*t*, *J*=7.2 Hz, 1H, 3-Ar H-4), 7.51 (*t*, *J*=7.3 Hz, 2H, 3-Ar H-3,5), 5.14 (*s*, 2H, -CH$_2$S-), 2.69 (*s*, 3H, 5-CH$_3$). LC-MS (m/z)=419 (M+1); Elemental analysis calculated for $C_{20}H_{14}N_6OS_2$ C, 57.40; H, 3.37; N, 20.08; S, 15.32; Found C, 57.38; H, 3.34; N, 20.11; S, 15.35.

3-(4-Isopropylphenyl)-6-(((5-methyl-1,3,4-thiadiazol-2-yl)thio)methyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.13**). Yield: 86 %; M.p. 214–215 °C; 1H NMR (500 MHz, DMSO-d6) δ 8.62 (*d*, *J*=7.9 Hz, 1H, H-11), 8.22 (*d*, *J*=8.0 Hz, 2H, 3-Ar H-2,6), 7.96 (*t*, *J*=7.3 Hz, 1H, H-10), 7.85 (*d*, *J*=8.0 Hz, 1H, H-8), 7.74 (*t*, *J*=7.4 Hz, 1H, H-10), 7.35 (*d*, *J*=8.0 Hz, 2H, 3-Ar H-3,5), 5.12 (*s*, 2H, -CH$_2$S-), 3.00 – 2.96 (*m*, 1H, -C*H*(CH$_3$)$_2$), 2.69 (s, 3H, 5-CH$_3$), 1.30 (*d*, *J*=6.8 Hz, 6H, -CH(C*H*$_3$)$_2$); LC-MS (m/z)=461 (M+1); Elemental analysis calculated for $C_{23}H_{20}N_6OS_2$: C, 59.98; H, 4.38; N, 18.25; S, 13.92; Found: C, 59.95; H, 4.40; N, 18.28; S, 13.90.

3-(4-Fluorophenyl)-6-(((5-methyl-1,3,4-thiadiazol-2-yl)thio)methyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.14**). Yield: 73 %; M.p. 228–229 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.63 (*d*, *J*=7.9 Hz, 1H, H-11), 8.40 (*dd*, *J*=8.5, 5.7 Hz, 2H, 3-Ar H-2,6), 7.97 (*t*, *J*=7.7 Hz, 1H, H-9), 7.86 (*d*, *J*=8.1 Hz, 1H, H-8), 7.75 (*t*, *J*=7.6 Hz, 1H, H-10), 7.26 (*t*, *J*=8.7 Hz, 2H, 3-Ar H-3,5), 5.15 (*s*, 2H, -CH$_2$S-), 2.69 (*s*, 3H, 5-CH$_3$). LC-MS (m/z)=437 (M+1); Elemental analysis calculated for $C_{20}H_{13}FN_6OS_2$ C, 55.04; H, 3.00; N, 19.25; S, 14.69; Found: C, 55.06; H, 3.01; N, 19.28; S, 14.72.

3-Methyl-6-(1-((5-methyl-1,3,4-thiadiazol-2-yl)thio)ethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.15**). Yield: 84 %; M.p. 210–212 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59 (*d*, *J*=7.9 Hz, 1H, H-11), 7.95 (*t*, *J*=7.7 Hz, 1H, H-9), 7.81 (*d*, *J*=8.1 Hz, 1H, H-8), 7.72 (*t*, *J*=7.6 Hz, 1H, H-10), 5.88 (*q*, *J*=6.9 Hz, 1H, , -C*H*(CH$_3$)S-), 2.71 (*s*, 3H, 5-CH$_3$), 2.36 (*s*, 3H, 3-CH$_3$), 1.91 (*d*, *J*=6.9 Hz, -CH(C*H*$_3$)S-); LC-MS (m/z)=471 (M+1); Elemental analysis calculated for $C_{16}H_{14}N_6OS_2$ C, 51.88; H, 3.81; N, 22.69; S, 17.31; Found: C, 51.92; H, 3.85; N, 22.74; S, 17.30.

6-(1-((5-Methyl-1,3,4-thiadiazol-2-yl)thio)ethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.16**). Yield: 70 %; M.p. 121–122 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (*d*, *J*=7.4 Hz, 1H, H-11), 8.20 (*d*, *J*=7.4 Hz,

2H, 3-Ar H-2,6), 7.98 (*t*, *J*=7.0 Hz, 1H, H-9), 7.85 (*d*, *J*=8.0 Hz, 1H, H-8), 7.76 (*t*, *J*=7.5 Hz, 1H, H-10), 7.53 (*t*, *J*=7.3 Hz, 1H, 3-Ar H-4), 7.45 (*t*, *J*=7.6 Hz, 2H, 3-Ar H-3,5), 6.01 (*q*, *J*=6.8 Hz, 1H, , -C*H*(CH$_3$)S-), 2.66 (s, 3H, thiadiazole 5-CH$_3$), 1.98 (*d*, *J*=6.9 Hz, 3H, -CH(C*H*$_3$)S-); LC-MS (m/z)=433 (M+1); Elemental analysis calculated for $C_{21}H_{16}N_6OS_2$ C, 58.32; H, 3.73; N, 19.43; S, 14.82; Found: C, 58.30; H, 3.70; N, 19.47; S, 14.80.

6-*(((1H-Benzo[d]imidazol-2-yl)thio)methyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.17**). Yield: 70 %; M.p. 253–254 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.42 (s, 1H, benzimidazole NH), 8.63 (*d*, *J*=8.1 Hz, 1H, H-11), 8.26 (*d*, *J*=8.1 Hz, 2H, 3-Phr H-2,6), 7.94 (*t*, *J*=7.6 Hz, 1H, H-9), 7.84 (*d*, *J*=8.2 Hz, 1H, H-8), 7.73 (t, *J*=7.6 Hz, 1H, H-10), 7.54–7.45 (m, 2H, 3-Ph H-4, benzimidazole H-4), 7.37 (*t*, *J*=7.6 Hz, 2H, 3-Ph H-3,5), 7.33–7.25 (m, 1H, benzimidazole H-7), 7.16 – 6.99 (m, 2H, benzimidazole H-5,6), 5.20 (*s*, 2H, -CH$_2$S-); LC-MS (m/z)=437 (M+1); Elemental analysis calculated for $C_{24}H_{16}N_6OS$ C, 66.04; H, 3.69; N, 19.25; S, 7.34; Found: C, 66.09; H, 3.72; N, 19.29; S, 7.37.

6-*(((1H-Benzo[d]imidazol-2-yl)thio)methyl)-3-(4-isopropylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.18**). Yield: 84 %; M.p. 239–240 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.47 (s, 1H, benzimidazole NH), 8.70–8.54 (*m*, 1H, H-11), 8.16 (*d*, *J*=8.3 Hz, 2H, 3-Ar H-2,6), 7.93 (*t*, *J*=7.2 Hz, 1H, H-9), 7.82 (*d*, *J*=8.1 Hz, 1H, H-8), 7.72 (*t*, *J*=7.6 Hz, 1H, H-10), 7.55–7.21 (*m*, 2H, benzimidazole H-4,7), 7.15 (*d*, *J*=8.4 Hz, 2H, 3-Ar H-3,5), 7.11–6.97 (*m*, 2H, , benzimidazole H-5,6), 5.19 (*s*, 2H, -CH$_2$S-), 2.97–2.85 (*m*, 1H, -C*H*(CH$_3$)$_2$), 1.25 (*d*, *J*=6.9 Hz, 6H, -CH(C*H*$_3$)$_2$); LC-MS (m/z)=479 (M+1); Elemental analysis calculated for $C_{27}H_{22}N_6OS$ C, 67.76; H, 4.63; N, 17.56; S, 6.70; Found: C, 67.82; H, 4.63; N, 17.64; S, 6.67.

6-*(((1H-Benzo[d]imidazol-2-yl)thio)methyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.19**). Yield: 79 %; M.p. 253–255 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.44 (s, 1H, benzimidazole NH), 8.62 (*d*, *J*=7.6 Hz, 1H, H-11), 8.38–8.26 (*m*, 2H, 3-Ar H-2,6), 7.95 (*t*, *J*=8.3 Hz, 1H, H-9), 7.84 (*d*, *J*=8.4 Hz, 1H, H-8), 7.74 (*t*, *J*=8.3 Hz, 1H, H-10), 7.54–7.42 (*m*, 1H, benzimidazole H-4), 7.41–7.21 (*m*, 1H, benzimidazole H-7), 7.13–6.88 (*m*, 4H, 3-Ar H-3,5, benzimidazole H-5,6), 5.20 (*s*, 2H, -CH$_2$S-); LC-MS (m/z)=455 (M+1); Elemental analysis calculated for $C_{24}H_{15}FN_6OS$ C, 63.43; H, 3.33; N, 18.49; S, 7.05; Found: C, 63.45; H, 3.35; N, 18.52; S, 7.08.

6-*(1-((1H-Benzo[d]imidazol-2-yl)thio)ethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.20**). Yield: 95 %; M.p. 229–230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.42 (*s*, 1H, benzimidazole NH), 8.63 (*d*, *J*=7.9 Hz, 1H, H-11), 8.13 (*d*, *J*=7.6 Hz, 2H, 3-Ph H-2,6), 7.95 (*t*, *J*=7.5 Hz, 1H, H-9), 7.84 (*d*, *J*=8.1 Hz, 1H, H-8), 7.74 (*t*, *J*=7.6 Hz, 1H, H-10), 7.53 (*d*, 2H, benzimidazole H-4, 7), 7.35 (*t*, *J*=7.3 Hz, 1H, 3-Ph H-4), 7.22–6.98 (*m*, 4H, 3-Ph H-3,5, benzimidazole H-5,6), 6.21 (*q*, *J*=6.7 Hz, 1H, -C*H*(CH$_3$)S-), 2.03 (*d*, *J*=6.8 Hz, 3H, -CH(C*H*$_3$)S-); LC-MS (m/z)=451 (M+1); Elemental analysis calculated for $C_{25}H_{18}N_6OS$ C, 66.65; H, 4.03; N, 18.65; S, 7.12; Found: C, 66.67; H, 4.03; N, 18.70; S, 7.14.

6-*(1-((1H-Benzo[d]imidazol-2-yl)thio)ethyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino-[2,3-c]quinazolin-2-one* (**2.21**). Yield: 71 %; M.p. 194–196 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.44 (*s*, 1H, benzimidazole NH), 8.63 (*d*, *J*=8.0 Hz, 1H, H-11), 8.20 (*dd*, *J*=8.4, 5.7 Hz, 2H, 3-Ar H-2,6), 7.96 (*t*, *J*=7.6 Hz, 1H, H-9), 7.85 (*d*, *J*=8.1 Hz, 1H, H-8), 7.74 (*t*, *J*=7.6 Hz, 1H, H-10), 7.52 (*d*, *J*=7.0 Hz, 1H, benzimidazole H-4), 7.28 (*d*, *J*=7.9 Hz, 1H, benzimidazole H-7), 7.17–7.04 (m, 2H, benzimidazole H-5,6), 6.75 (*t*, *J*=8.5 Hz, 2H, 3-Ar H-3,5), 6.22 (*q*, *J*=6.8 Hz, 1H, -C*H*(CH$_3$)S-), 2.04 (*d*, *J*=6.9 Hz, 3H, -CH(C*H*$_3$)S-); LC-MS (m/z)=469 (M+1); Elemental analysis calculated for $C_{25}H_{17}FN_6OS$ C, 64.09; H, 3.66; N, 17.94; S, 6.84; Found: C, 64.07; H, 3.64; N, 17.98; S, 6.81.

6-*((Benzo[d]thiazol-2-ylthio)methyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.22**). Yield: 73 %; M.p. 231–232 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.63 (*d*, *J*=7.9 Hz, 1H, H-11), 8.27 (*d*, *J*=8.2 Hz, 2H, 3-Ar H-2,6), 7.96 (*t*, *J*=7.7 Hz, 1H, H-9), 7.91–7.80 (*m*, 3H, H-8, benzothiazole H-4, 7), 7.75 (*t*, *J*=7.6 Hz, 1H, H-10), 7.51 (*t*, *J*=7.4 Hz, 1H, 3-Ar H-4), 7.47–7.37 (*m*, 3H, 3-Ar H-3,5, benzothiazole H-5), 7.32 (*t*, *J*=7.6 Hz, 1H, benzothiazole H-6), 5.29 (*s*, 2H, -CH$_2$S-); LC-MS (m/z)=454 (M+1); Elemental analysis calculated for $C_{24}H_{15}N_5OS_2$ C, 63.56; H, 3.33; N, 15.44; S, 14.14; Found: C, 63.59; H, 3.37; N, 15.45; S, 14.16.

6-*((Benzo[d]thiazol-2-ylthio)methyl)-3-(4-isopropylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.23**). Yield: 83 %; M.p. 198–199 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (d, *J*=8.0 Hz, 1H, H-11), 8.19 (*d*, *J*=8.3 Hz, 2H, 3-Ar H-2,6), 7.95 (*t*, *J*=7.6 Hz, 1H, H-9), 7.89–7.81 (*m*, 3H, H-8, benzthiazole H-4,7), 7.73 (*t*, *J*=7.6 Hz, 1H, H-10), 7.43 (*t*, *J*=7.6 Hz, 1H, benzthiazole H-5), 7.32 (*t*, *J*=7.6 Hz, 1H, benzthiazole H-6), 7.22 (*d*, *J*=8.3 Hz, 2H, 3-Ar H-3,5), 5.28 (s, 2H, -CH$_2$S-), 2.94 (dt, *J*=13.3, 6.6 Hz, 1H, -C*H*(CH$_3$)$_2$), 1.26 (*d*, *J*=6.9 Hz, 6H, -CH(C*H*$_3$)$_2$).; LC-MS (m/z)=496 (M+1); Elemental analysis calculated for $C_{27}H_{22}N_6OS$ C, 65.43; H, 4.27; N, 14.13; S, 12.94; Found: C, 65.40; H, 4.24; N, 14.16; S, 12.99.

6-*((Benzo[d]thiazol-2-ylthio)methyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.24**). Yield: 89 %; M.p. 231–232 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (*d*, 1H, *J*=7.6 Hz, H-11), 8.48–8.26 (*m*, 2H, 3-Ar H-2,6), 7.96 (*t*, *J*=7.6 Hz, 1H, H-9), 7.87–7.79 (*m*, 3H, H-8, benzothiazole H-4, 7), 7.74 (*t*, *J*=7.5 Hz, 1H, H-10), 7.43 (*t*, *J*=7.7 Hz, 1H, benzthiazole H-5), 7.33 (*t*, *J*=7.6 Hz, 1H, benzthiazole H-6), 7.12 (*t*, *J*=8.7 Hz, 2H, 3-Ar H-3,5), 5.29 (*s*, 2H, -CH$_2$S-); LC-MS (m/z)=472 (M+1); Elemental analysis calculated for $C_{24}H_{14}FN_5OS_2$ C, 61.13; H, 2.99; N, 14.85; S, 13.60; Found: C, 61.11; H, 2.97; N, 14.86; S, 13.65.

6-*(1-(Benzo[d]thiazol-2-ylthio)ethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.25**). Yield: 95 %; M.p. 183–184 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (*d*, *J*=8.0 Hz, 1H, H-11), 8.12 (*d*, *J*=7.8 Hz, 2H, 3-Ar H-2,6), 7.98 (*t*, *J*=7.6 Hz, 1H, H-9), 7.90–7.83 (m, 2H, benzthiazole H-4,7), 7.81 (d, *J*=8.1 Hz, 1H, H-8), 7.76 (*t*, *J*=7.6 Hz, 1H, H-10), 7.45 (*t*, *J*=7.6 Hz, 1H, 3-Ar H-4), 7.38–7.28 (*m*, 2H, benzimidazole H-5,6), 7.12 (*t*, *J*=7.3 Hz, 2H, 3-Ar H-3,5), 6.36 (*q*, *J*=6.3 Hz, 1H,

-C<u>H</u>(CH_3)S-), 2.07 (*d*, *J*=6.6 Hz, 3H, -CH(C<u>H</u>$_3$)S-); LC-MS (m/z)=468 (M+1); Elemental analysis calculated for $C_{25}H_{17}N_5OS_2$ C, 64.22; H, 3.66; N, 14.98; S, 13.71; Found: C, 64.22; H, 3.66; N, 14.98; S, 13.71.

6-*(1-(Benzo[d]thiazol-2-ylthio)ethyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.26**). Yield: 92 %; M.p. 235–236 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (*d*, *J*=7.7 Hz, 1H, H-11), 8.32–8.14 (*m*, 2H, 3-Ar H-2,6), 7.98 (*t*, *J*=7.6 Hz, 1H, H-9), 7.92–7.84 (*m*, 2H, benzothiazole H-4, 7), 7.83 (*d*, *J*=8.0 Hz, 1H, H-8), 7.80–7.69 (*m*, 1H, 10), 7.55–7.43 (*m*, 2H, benzothiazole H-5), 7.40–7.29 (*m*, 1H, benzothiazole H-6), 6.79 (*t*, *J*=7.8 Hz, 2H, 3-Ar H-3,5), 6.35 (*q*, *J*=6.8 Hz, 1H, -C<u>H</u>(CH_3)S-), 2.07 (*d*, *J*=6.7 Hz, 3H, -CH(C<u>H</u>$_3$)S-); LC-MS (m/z)=486 (M+1); Elemental analysis calculated for $C_{25}H_{16}FN_5OS_2$ C, 61.84; H, 3.32; N, 14.42; S, 13.21; Found: C, 61.86; H, 3.35; N, 14.45; S, 13.22.

6-*(((7H-Purin-6-yl)thio)methyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.27**). Yield: 72 %; M.p. 284–285 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.41 (*s*, 1H, purine NH), 8.65–8.61 (*m*, 2H, H-11, purine H-2), 8.28 (*d*, *J*=7.4 Hz, 2H, 3-Ar H-2,6), 8.22 (*s*, 1H, purine H-8), 7.94 (*t*, *J*=8.2 Hz, 1H, H-9), 7.84 (*d*, *J*=8.0 Hz, 1H, H-8), 7.72 (*t*, *J*=7.7 Hz, 1H, H-10), 7.53 (*t*, *J*=7.3 Hz, 1H, 3-Ar H-4), 7.45 (*t*, *J*=7.6 Hz, 2H, 3-Ar H-3,5), 5.34 (*s*, 2H, -CH_2S-); LC-MS (m/z)=439 (M+1); Elemental analysis calculated for $C_{22}H_{14}N_8OS$ C, 60.26; H, 3.22; N, 25.56; S, 7.31; Found , 60.31; H, 3.25; N, 25.61; S, 7.35.

6-*(((7H-Purin-6-yl)thio)methyl)-3-(4-isopropylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.28**). Yield: 85 %; M.p. 282–283 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.43 (*s*, 1H, purine NH), 8.75–8.42 (*m*, 2H, H-11, purine H-2), 8.53–8.11 (*m*, 3H, 3-Ar H-2, 6, purine H-8), 8.04–7.88 (*m*, 1H, H-9), 7.83 (*d*, *J*=7.0 Hz, 1H, H-8), 7.80–7.58 (*m*, 1H, H-10), 7.30 (*d*, *J*=6.7 Hz, 2H, H3-Ar H-3,5), 5.32 (*s*, 2H, CH_2), 2.93–2.78 (*m*, 1H, -C<u>H</u>(CH_3)$_2$), 1.45–0.94 (*m*, 6H, CH(C<u>H</u>$_3$)$_2$); LC-MS (m/z)=481 (M+1); Elemental analysis calculated for $C_{25}H_{20}N_8OS$ C, 62.49; H, 4.20; N, 23.32; S, 6.67; Found: C, 62.46; H, 4.18; N, 23.36; S, 6.65.

6-*(((7H-Purin-6-yl)thio)methyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.29**). Yield: 67 %; M.p. 299–301 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.44 (s, 1H, purine NH), 8.64 (s, 1H, purine H-2), 8.62 (*d*, *J*=9.1 Hz, 1H, H-11), 8.39 (*dd*, *J*=8.4, 6.6 Hz, 2H, 3-Ar H-2,6), 8.22 (*s*, 1H, purine H-8), 7.99–7.91 (*m*, 1H, H-9), 7.85 (*d*, *J*=8.0 Hz, 1H, H-8), 7.77.66 (*m*, 1H, H-10), 7.32–7.10 (*m*, 2H, 3-Ar H-3,5), 5.34 (*s*, 2H, CH_2). LC-MS (m/z)=457 (M+1); Elemental analysis calculated for $C_{22}H_{13}FN_8OS$ C, 57.89; H, 2.87; N, 24.55; S, 7.02; Found: C, 57.92; H, 2.85; N, 24.57; S, 7.03.

6-*(1-((7H-Purin-6-yl)thio)ethyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one* (**2.30**). Yield: 71 %; M.p. 283–284 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.44 (*s*, 1H, purine NH), 8.67 (*s*, 1H, purine H-2), 8.63 (*d*, *J*=7.6 Hz, 1H, H-11), 8.22 (*s*, 1H, purine H-8), 8.22–8.17 (*m*, 2H, 3-Ar H-2,6), 7.98 (*t*, *J*=7.6 Hz, 1H, H-9), 7.90 (*d*, *J*=8.0 Hz, 1H, H-8), 7.74 (*t*, *J*=7.4 Hz, 1H, H-10), 7.01 (*t*, *J*=8.5 Hz, 2H, 3-Ar H-3, 5), 6.68 (*q*, *J*=6.6 Hz, 1H, -C<u>H</u>(CH_3)S-), 1.99 (*d*, *J*=6.9 Hz, 3H, -CH(C<u>H</u>$_3$)S-); LC-MS (m/z)=471 (M+1); Elemental analysis calculated for $C_{23}H_{15}FN_8OS$ C, 58.72; H, 3.21; N, 23.82; S, 6.81; Found: C, 58.76; H, 3.23; N, 23.87; S, 6.82.

3. 2. Biological studies

Radical scavenging activity. A 2 ml solution of the studied substances in DMSO at concentrations of 0.2 mM was mixed with 2 ml of a 0.1 mM methanol solution of 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazin-1-y (DPPH). The formed mixture was incubated for 30 min at ambient temperature, and its absorption was measured (AD). The optical density of the mixture containing 2 ml of a 0.1 mM methanol solution of DPPH and 2 ml of DMSO (ADPPH) was measured as well [26]. Antiradical activity (ARA %) was calculated using the formula:

$$ARA\ \% = \frac{ADPPH - AD}{ADPPH} * 100\ \%.$$

Antimicrobial test. The sensitivity of microorganisms to synthesized compounds was evaluated according to described methods [27]. An assay was conducted on Mueller-Hinton medium by two-fold serial dilution of the compound in 1 ml; after that, 0.1 ml of microbial seeding (10^6 cells/ml) was added. Minimal inhibit concentration of the compound was determined by the absence of visual growth in a test tube with a minimal concentration of a substance, and minimal bactericide/fungicide concentration was determined by the absence of growth on agar after inoculation of microorganisms from transparent test tubes. Dimethylsulfoxide was used as a solvent, and the initial solution concentration was 1 mg/ml. Preliminary screening was performed on *Staphylococcus aureus 209-p, Escherichia coli B-906, Mycobacterium luteum BKM B-868, Candida tenuis BKM Y-70, Aspergillus niger BKM F-1119* standard test cultures. All test strains were received from the bacteriological laboratory at the Institute of Chemistry and Chemical Technologies, National University "Lviv Polytechnic» of Ukraine. Trimethoprim was used as a reference compound with proven antibacterial/antifungal activity. The choice of reference compound is caused by its synthetic nature, presence of the pyrimidine cycle and mechanism of antimicrobial activity. Additional quality control of culture medium and solvents was conducted by commonly used methods. The experiment was repeated three times to obtain valid data. The results were considered reliable if identical values were obtained in three experiments. Evaluated minimal inhibition concentrations (MIC), minimal bactericidal concentrations (MBC) and minimal fungicidal concentrations (MFC) were presented as a single value [28].

4. Results

4. 1. Chemistry assay

Target heterocyclic hybrids (**2.1**–**2.30**) were obtained reaction of heterocyclic thiones with 6-(chloro(R^2)methyl)-3-R^1-2H-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**1.1**–**1.5**), which were obtained according to known procedure [29].

The structure of synthesized compounds **2.1**–**2.30** was confirmed using ^{1}H NMR and LC-MS spectra. The

molecular ion peaks observed in the mass spectra for the *m/z* values of the synthesized compounds in the positive ionization mode corresponded to M+1, which confirmed the formation of the derivatives **2.1**–**2.30**.

In ^{1}H NMR spectra of compounds **2.1**–**2.30** signals of protons of triazinoquinazoline cycle were observed as typical ABCD systems that correspond to the sequentially located four signals of H-8-H-11 protons. Signals of H-11 and H-8 were registered as doublets at 8.63–8.62 ppm and 7.82–7.81 ppm properly; signals of H-9 and H-10 were observed at 7.97–7.95 ppm and 7.75–7.74 ppm correspondingly.

Characteristic signals of linker SCH_2-groups protons (compounds **2.1**–**2.3**, **2.7**–**2.9**, **2.12**–**2.14**, **2.17**–**2.19**, **2.22**–**2.24**, **2.27**–**2.29**) were registered as singlet at the 5.34-4.90 ppm. At the same time, signals of -S-CH(CH_3)-group`s protons (compounds **2.4**–**2.6**, **2.10**, **2.11**, **2.15**, **2.16**, **2.20, 2.21**, **2.25**, **2.26**, **2.30**) formed much more complex ^{1}H NMR spectral pattern. Thus, signals of methyne proton were observed as quintet at the 6.68–5.57 ppm (spin-spin interaction constant 6.3–6.9 Hz), and signals of methyl group were registered as doublets at the 2.07–1.83 ppm (spin-spin interaction constant 6.6–6.9 Hz).

In ^{1}H NMR spectra signals of azole substituents in 6th position in most of cases were associated with their functional groups. Thus, ^{1}H NMR spectra of compounds **2.1**–**2.6** were characterized by signals of methyl group in 4th position of triazole cycle that were observed as singlet at the 3.61–3.56 ppm. At the same time, signals of the methyl group in the 5th position of the thiadiazol fragment (compounds **2.12**–**2.16**) were registered as singlets in the higher field at 2.71–2.69 ppm. Compounds **2.7**–**2.11** with 1-phenyltetrazole fragment additionally characterized by signals of aromatic protons that were observed as wide multiplet at the 7.68–7.38 ppm, in some cases (**2.7**, **2.10**) together with protons of H-3, H-4 and H-5 of aromatic moiety in 3rd position.

^{1}H NMR spectra of compounds with benzimidazole fragment (**2.17**–**2.21**) characterized by the signal of NH-proton that was registered as singlet at the 12.47–12.42 ppm and signals of the aromatic protons (H-4 and H-7, H-5 and H-6) as multiplets (except compounds **2.20**, **2.21**) at the 7.55–7.21 ppm (H-4 and H-7) and 7.17–6.88 ppm (H-5 and H-6), in some cases together with protons of substituents in position 3. In case of benzothiazole fragment (**2.22**–**2.26**) signals of aromatic fragments` protons were observed as multiplets at the 7.91–7.79 ppm (H-4 and H-7), triplets at the 7.45–7.43 ppm (H-5), triplets at the 7.33–7.32 ppm (H-6) or complex multiplets. ^{1}H NMR spectra of compounds bearing purine moiety were characterized by the singlet of NH-proton at the 13.44–13.41 ppm, signal of H-2 proton that was observed as singlet at the 8.67–8.64 ppm or multiplet (overlapped on H-11) at the 8.75–8.42 ppm, signal of H-8 that was registered as singlet at the 8.22 ppm or multiplet (overlapped on H-2,6 of aryl moiety in 3rd position) at the 8.53 – 8.11 ppm.

Synthesized compounds are additionally characterized by signals of protons of substituents at the 3rd position of the triazinoquinazolines ring, the chemical shift and multiplicity of which are determined by the nature of the substituent [22–24].

4. 2. Biological assay

It is commonly known [30] that oxidative stress is a consequence of an excess of free radicals caused by the insufficient function of the cell's antioxidant protection system. This excess of free radicals leads to severe damage to biological systems, including molecular injuries. The damage to biomolecules (nucleic acids, lipids, and proteins), as well as the induction of secondary free radical formation, results in cell death. Ultimately, oxidative stress is associated with ageing processes and a significant number of diseases, including cancer, diabetes, heart disease, and neurodegenerative disorders [31]. Therefore, the search for compounds that can act as free radical scavengers and enhance the enzymatic antioxidant protection of cells is a topical task in current medicinal chemistry [32, 33]. The conducted study allowed to evaluate radical scavenging activity of obtained compounds. The results of this study are presented in Fig. 3.

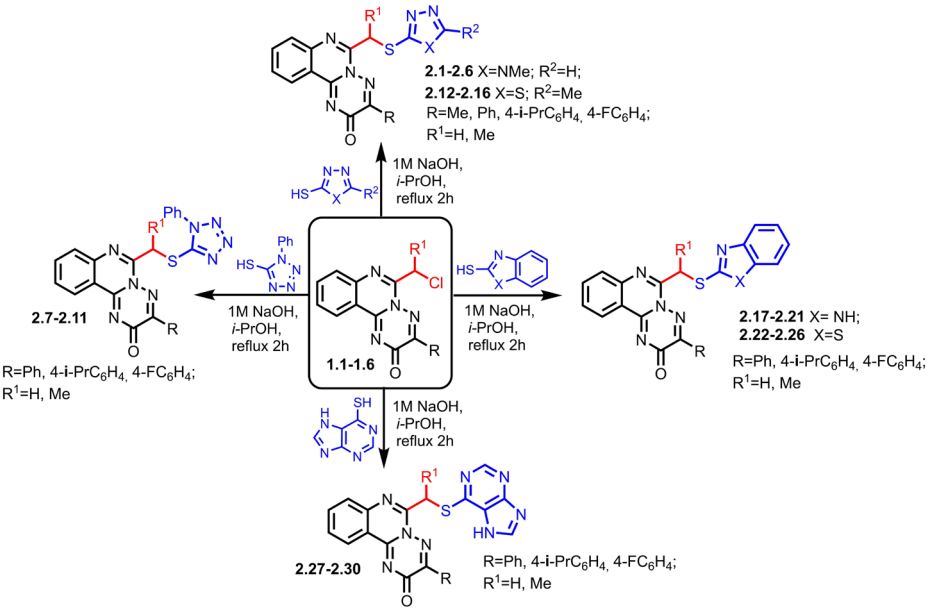

Fig. 2. Synthesis of target heterocyclic hybrids

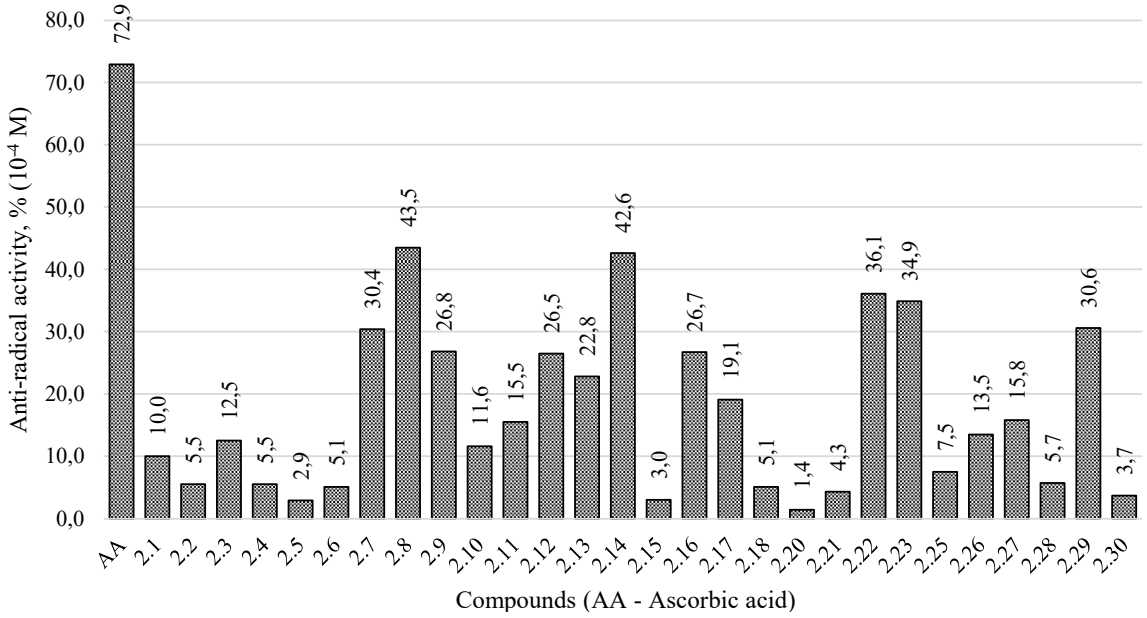

Fig. 3. Antiradical activity of synthesized compounds

Considering the relatively moderate radical-scavenging activity of heterocyclic hybrids **2.1–2.30**, it was decided to study their antimicrobial effects. This is particularly important given that antibiotic resistance and its rapid development have led to a decrease in the effectiveness of most antibiotics against "priority pathogens" – 12 bacterial families that pose the highest threat to human health [34]. The results of antimicrobial activity screening are shown in Table 1.

Table 1

Indicators of the MIC and MB(F)C of synthesized compounds

Comp.*	*E. coli*		*S. aureus*		*M. luteu*m		*C. tenuis*		*A. niger*	
	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MFC, μg/ml	MIC, μg/ml	MFC, μg/ml
2.1	125.0	250.0	–	–	–	–	250.0	500.0	62.5	125.0
2.3	–	–	–	–	–	–	250.0	500.0	250.0	500.0
2.9	–	–	–	–	–	–	250.0	500.0	250.0	500.0
2.13	–	–	–	–	500.0	500.0	–	–	125.0	250.0
2.14	62.5	125.0	62.5	125.0	31.2	125.0	500.0	500.0	125.0	250.0
Trimethoprim	50.0	50.0	31.2	62.5	62.5	125	62.5	125	62.5	125

*Note: * – compounds **2.2, 2.4–2.8, 2.10–2.12, 2.15–2.30** in studied concentration (MIC 500 μg/ml) do not inhibit the growth of the microorganism.*

5. Discussion

Alkylation of heterocyclic thiones by 6-(chloro(R^2) methyl)-3-R^1-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**1.1**–**1.5**) proceeded without features in propanol-2 in the presence of an equimolar quantity of base (Fig. 2) with satisfactory to high yields of the target compounds (67–95 %). Obtained compounds are heterocyclic hybrids in molecules of which [1,2,4]triazino[2,3-*c*]quinazoline fragment combined with various nitrogen-containing heterocyclic moieties. It should be noted that similar structures, heterocyclic hybrids are described as compounds with antimicrobial and radical scavenging activity [24].

It was found that compounds **2.7, 2.8, 2.14, 2.22, 2.23** and **2.29** exhibit DPPH scavenging activity in the range of 30.4–43.5 % (Fig. 3). These compounds, in addition to their core heterocycle, contain tetrazole (**2.7, 2.8**), thiadiazole (**2.14**), benzothiazole (**2.22, 2.23**), and purine (**2.29**) cycles. The lowest activities (APA=2.9–12.5 %) were observed for compounds **2.1–2.6** and **2.17–2.21**, which are triazinoquinazoline-triazole and triazinoquinazoline-benzimidazole hybrids, respectively. It should be noted that there is a certain dependence of the antiradical activity on the nature of the substituent at position 3 of the triazinoquinazoline cycle in each of the classes of synthesized hybrids. Namely, a certain increase in activity with the introduction of a 4-fluorophenyl substituent (compounds **2.3**, **2.9**, **2.11**, **2.14**, **2.26**, **2.29**). However, the most pronounced "structure-radical scavenging activity" correlation was observed in the case of the "linker" alkylthio-group modification. Thus, compounds **2.1–2.3, 2.7–2.9, 2.12–2.14, 2.22, 2.23** and **2.29**, which combine methylthio groups in their structures, demonstrate significantly higher radical-scavenger activity compared to substances with an ethylthio linker group (Fig. 3).

It was established (Table 1) that molecular hybridization, in this case, was not effective in the design of antibacterial agents. The antibacterial activity of molecular hybrids **2.1–2.30** against four strains of bacteria (*S. aureus*, *E. coli*, *M. luteum*) and two strains of fungi (*C. tenuis*, *A. niger*) was, in most cases, quite low (MIC≥500 μg/ml). At the same time compound **2.14** (3-(4-fluorophenyl)-6-(((5-methyl-1,3,4-thiadiazol-2-yl)thio)methyl)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one) efficiently inhibits *S. aureus* (MIC 62.5 μg/ml), *E. coli* (MIC 62.5 μg/ml), *M. luteum* (MIC 31.2 μg/ml)

strains showing competitive activity with the reference compound "Trimetoprim". A comparison of the results obtained with previously published data revealed that the molecular design we proposed appeared to be a reasonable approach for the development of novel agents with antimicrobial and antiradical activity. Obtained compounds equal or slightly lagged behind quinazoline-based heterocyclic hybrids described by other authors in terms of biological activity [35, 36].

Practical importance. Elaborated synthetic procedures can be used to synthesize related heterocyclic hybrids and further study their biological effects.

Study limitation. Present study is limited by the bounded set of available heterocyclic thiones. Studied heterocyclic thiones do not contain functional group that play a roles of additional pharmacophore fragments or can be modified for improvement of pharmacokinetics and pharmaco-technological properties.

Prospects for further research. The increasing of heterocyclic thiones diversity would allow obtaining of larger combinatorial library of compounds with wider spectra of biological activity.

6. Conclusions

The present article describes the synthesis of 30 novel 6-[{(azolyz-(azinyl-)thio}methyl)-3-R-2H-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones by the alkylation of a wide range of heterocyclic thiones with 6-(chloro(R^2)methyl)-3-R^1-2H-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones in propanol-2 in the presence of a base. Among the synthesized substances, compound **2.14** was identified as an effective growth inhibitor of *S. aureus* (MIC 62.5 μg/ml), *E. coli* (MIC 62.5 μg/ml), and *M. luteum* (MIC 31.2 μg/ml), showing competitive activity with the reference compound "Trimetoprim". In addition, five compounds demonstrated DPPH radical scavenging activity (30.4–43.5 %), which justifies their further study for biological activities associated with anti-radical mechanisms. It has been shown that the presence of tetrazole, thiadiazole, and benzothiazole moieties, as well as a methylthio linker group, is essential for the radical scavenging activity of the obtained compounds.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

Funding

The study was performed without financial support.

Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Acknowledgement

The authors thank all the Defenders of Ukraine who made the performance of this study possible.

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Received 02.10.2024
Received in revised form 28.11.2024
Accepted 19.12.2024
Published 30.12.2024

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