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DESIGN, SYNTHESIS, MOLECULAR DOCKING, AND ANTIOXIDANT PROPERTIES OF A SERIES OF NEW S-DERIVATIVES OF ((1,2,4-TRIAZOL-3(*2H*)-YL)METHYL) THIOPYRIMIDINES

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The aim *of our work is to synthesize new S-derivatives in the series of ((1,2,4-triazol-3(2H)-yl)methyl)thiopyrimidines and to study their antioxidant activity, to identify the most promising compound using molecular docking and kinetic parameters.*

Materials and methods. *The 1H and ^{13}C NMR spectra were recorded on a Bruker AC-500 spectrometer. LC-MS was recorded on an Agilent 1260 Infinity HPLC system equipped with a diode-array detector and proton ionization. Elemental analysis (C, H, N, S) was performed on an ELEMENTAR vario EL cube. Molecular docking was performed using the AutoDock 4.2.6 program. Free radical absorption was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay.*

Results*. A series of S-acyl derivatives of 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiols were synthesized through alkylation and subsequent cyclization. The structure of the obtained compounds was confirmed by ^{1}H and ^{13}C NMR spectroscopy. Antiradical activity was evaluated using the DPPH test, with compound (6) exhibiting the highest activity, surpassing ascorbic acid. Molecular docking with cytochrome c peroxidase (PDB: 2X08) confirmed strong binding interactions, highlighting the potential of these derivatives as antioxidants.*

Conclusions*. Three compounds (1, 6, 8) exhibited higher activity than the reference drug, the natural antioxidant ascorbic acid. This high activity may be associated with the presence of pharmacophore fragments, particularly the pyrimidine skeleton and the sulfur atom linked to the 1,2,4-triazole. The IC_{50} for the most active compound was calculated as 4.458±1 μM, which is 27 times more effective than ascorbic acid. Molecular docking results showed that compounds 4 and 6 had the lowest binding energies, making them the most effective compounds in terms of antioxidant activity*

Keywords*: 1,2,4-triazole derivatives, thiopyrimidines, molecular docking, DPPH, antioxidant activity*

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1. Introduction

«The paradox of life in aerobic conditions is that oxygen is both critically necessary and harmful to the functioning of cells» [1]. Antioxidants are substances that prevent and mitigate damage caused by free radicals by donating electrons to these reactive species [2]. They also convert free radicals into waste products, which are subsequently excreted from the body. Antioxidants play a crucial role in preventing and treating many disorders by acting as free radical scavengers. It is well known that antioxidants serve as the body›s defence mechanism against harmful by-products (ROS) produced during normal cellular aerobic respiration. Therefore, the assessment of such properties remains an intriguing and valuable task, particularly in the search for promising synthetic antioxidants derived from azole heterocycles.

Pyrimidine is an aromatic heterocyclic compound containing nitrogen atoms in the 1st and 3rd positions, playing a crucial role in forming the backbone of various biologically active compounds. It serves as a structural unit of DNA and RNA and is important in various biological processes [3]. Common pyrimidines include uracil, cytosine, and thymine. Pyrimidines exhibit a range of biological activities [4], including antiviral, antitumor, antimicrobial [5], anti-inflammatory, analgesic [6], antioxidant [7], and antimalarial properties.

Pyrimidine is used as a starting material for the synthesis of a wide range of heterocyclic compounds and the development of new molecules. It has been established that pyrimidine ring complexes with various heterocyclic fragments serve as components of pharmaceutical and veterinary drugs.

The 1,2,4-triazole core is a very promising azole heterocycle [8], and the compounds obtained from its chemical transformations have various biological, pharmaceutical, and clinical applications [9, 10]. It is known that modifying azole heterocycles can enhance their effectiveness and reduce toxicity [11, 12].

Increasing solubility, enhancing known biological properties, and introducing new biological activities can be achieved by combining two pharmacophore fragments in one molecule – a pyrimidine and an azole het-

erocycle [13] – connected by a thiomethylene bridge. Therefore, we chose new S-derivatives of (1,2,4-triazol-3(2H)-yl)methylthiopyrimidines for our research. It is known that derivatives of (1,2,4-triazol-3(2H)-yl)methylthiopyrimidines exhibit anticonvulsant effects and can be used to treat disorders of the nervous system [14].

The aim of our work is to synthesize new S-derivatives in the series of (1,2,4-triazol-3(2H)-yl)methylthiopyrimidines and to study their antioxidant activity while searching for the most promising compound using molecular docking and kinetic parameters.

2. Planning (methodology) of research

The methodology of our research was as follows: new derivatives combine a pyrimidine-2-thione fragment and a variable 1,2,4-triazole fragment, which promises increased biostability, bioavailability, hydrophilicity, efficacy, selectivity of binding to target receptors, and reduced toxicity [2, 12]. To achieve this connection and obtain the target analogs of (1,2,4-triazol-3(2H)-yl)methylthiopyrimidines, we propose a publicly available synthesis method that enabled the production of 10 new compounds.

The resulting new hybrids were tested using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical test, which involves a stable free radical. When interacting with antioxidants, DPPH undergoes decolorization, and the color change can be measured spectrophotometrically. Compounds with the highest percentage of antiradical activity were identified, allowing for isolating a compound with high activity compared to the reference compound – ascorbic acid. The kinetic dependence on dilution was investigated for this compound, and the IC_{50} value was calculated.

To confirm our hypothesis, molecular docking was performed on the enzyme «EC 1.11.1.5 Cytochrome c peroxidase», which was obtained from the RCSB Protein Data Bank (crystal code 2X08). The binding affinity and the interaction potential of non-binding interactions between the investigated complexes were calculated. It is noteworthy that the difference between the results obtained from both molecular docking and experimental methods was insignificant.

3. Materials and methods of research

3. 1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-500 spectrometer (500 and 125 MHz, respectively) in DMSO-d_6, using TMS as the internal standard (Agilent Technologies, Santa Clara, California, USA). LC-MS analysis was performed on an Agilent 1260 Infinity HPLC System with a diode-array detector using proton ionization. Elemental analysis (C, H, N, S) was carried out on an ELEMENTAR vario EL cube, with sulfanilamide as the standard. Melting points were determined using the capillary method in Stanford Research Systems Melting Point Apparatus 100 (SRS, USA). The reagents used were purchased from Sigma-Aldrich (Merck).

The compounds were synthesized using a known method [14], and the constants obtained corresponded to the literature data.

Preparation of 4-methyl-5-((pyrimidin-2-ylthio) methyl)-4H-1,2,4-triazole-3-thiol 1 (General Methods). A mixture of 10 mmol of 2-(pyrimidin-2-ylthio)acetohydrazide, 10 mmol of sodium hydroxide, and 50 mL of purified water is boiled for 2 hours. After complete cooling, 2 mL of concentrated acetic acid is added to the filtrate. The formed precipitate is filtered and washed with purified water. For analysis, the product is purified by recrystallization from DMF. The final product appears as a light yellow powder, soluble in aqueous solutions of alkali, DMF, and 1,4-dioxane.

4-Methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiol (**1**). Yield 72 %, light yellow powder, mp 266 °C (DMF). ^{1}H NMR, δ, ppm. (*J*, Hz): 3.55 (*s*, 3H, -N-CH3), 4.43 (*s*, 2H, -CH2-), 7.19 (*t*, *J*=4,4 Hz, 1H, Ar), 8.53 (*d*, *J*=4,4 Hz, 2H, Ar), 12.83 (*s*, 1H, -SH). Mass spectrum, m/z (I_{rel}, %) 240 [M+H]+ (100). Anal. calcd. for C8H9N5S2: C: 40.15 %; H: 3.79 %; N: 29.26 %; S: 26.79 %; Found: C: 40.11 %; H: 3.82 %; N: 29.35 %; S: 26.71 %.

Preparation of S-acyl Derivatives of 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiols **2-10** *(General Methods).* A mixture of 5 mmol of 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiol and 5 mmol of sodium hydroxide dissolved in 10 mL of propan-2-ol is prepared. An alkyl 2-chloroacetic acid derivative (5 mmol) or other halogen derivative is added to this mixture. The mixture is heated for 2 hours, then cooled, and the precipitate is filtered and washed with purified water. The product is crystallized from methanol for analysis. The crystalline substances (**2-10**) are yellow or brown in colour, insoluble in water, and soluble in organic solvents.

2-((4-Methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetic acid (**2**). Yield 1.16 g (78 %), white powder, mp 184 °C (MeOH). ^{1}H NMR, δ, ppm. (*J*, Hz): 3.59 (*s*, 3H, -N-CH_3), 4.08 (*s*, 2H, -CH_2-COO), 4.54 (*s*, 2H, -CH_2-), 7.20 (*t*, *J*=3.7 Hz, 1H, Ar), 8.52 (*d*, *J*=3.7 Hz, 2H, Ar), 11.36 (*s*, 1H, -COOH). Mass spectrum, m/z (I_{rel}, %) 298 $[M+H]^+$ (100). Anal. calcd. for $C_{10}H_{11}N_5O_2S_2$: C: 40.39 %; H: 3.73 %; N: 23.55 %; S: 21.56 %. Found: C: 40.32 %; H: 3.76 %; N: 23.58 %; S: 21.52 %.

Methyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**3**). Yield 1.13g (73 %), white powder, mp 169 °C (MeOH). ^{1}H NMR, δ, ppm. (*J*, Hz): 3.72 (*s*, 3H, -N-CH_3), 4.07 (*s*, 2H, -CH_2-COO), 4.54 (*s*, 2H, -CH_2-), 7.20 (*t*, *J*=3.7 Hz, 1H, Ar), 8.52 (*d*, *J*=3,7 Hz, 2H, Ar). Mass spectrum, m/z (I_{rel}, %) 312 $[M+H]^+$ (100). Anal. calcd. for $C_{11}H_{13}N_5O_2S_2$: C: 42.43 %; H: 4.21 %; N: 22.49 %; S: 20.59 %. Found: C: 42.18 %; H: 4.20 %; N: 22.54 %; S: 20.67 %.

Ethyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**4**). Yield 1.17g (72 %), yellow powder, mp 172 °C (MeOH). ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 1.23 (*t*, *J*=6.6 Hz, 3H, -CH_3), 3.59 (*s*, 3H, -N-CH_3), 4.07 (*s*, 2H, -CH_2-COO), 4.16 (*q*, *J*=6.6 Hz, 2H, -CH_2-), 4.54 (*s*, 2H, -CH_2-), 7.20 (*t*, *J*=3.7 Hz, 1H, Ar), 8.52 (*d*, *J*=3.7 Hz, 2H, Ar). Mass spectrum, m/z (I_{rel}, %) 326 $[M+H]^+$ (100). Anal. calcd. for $C_{12}H_{15}N_5O_2S_2$: C: 44.29 %; H: 4.65 %; N: 21.52 %; S: 19.70 %. Found: C: 44.37 %; H: 4.61 %; N: 21.48 %; S: 19.75 %.

Propyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**5**). Yield 1,15g (68 %), yellow powder, mp 176 °C (MeOH). ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 0.94 (*t*, *J*=8.0 Hz, 3H, -CH_3), 1.70 (*qt*, *J*=8.0, 5.7 Hz, 2H, -CH_2-), 3.59 (*s*, 3H, -N-CH_3), 4.05–4.12 (m, 4H, -CH_2-COO, -CH_2-), 4.54 (*s*, 2H, -CH_2-), 7.20 (*t*, J=3.7 Hz, 1H, Ar), 8.52 (*d*, *J*=3.7 Hz, 2H, Ar). Mass spectrum, m/z (I_{rel}, %) 340 $[M+H]^+$ (100). Anal. calcd. for $C_{13}H_{17}N_5O_2S_2$: C: 46.00 %; H: 5.05 %; N: 20.63 %; S: 18.89 %. Found: C: 46.11 %; H: 5.18 %; N: 20.63 %; S: 18.75 %.

Isopropyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**6**). Yield 1.37g (81 %), yellow powder, mp 164 °C (MeOH). ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm. (J, Hz):1.09–1.31 (*m*, 6H, -$(CH_3)_2$) 3.96 (*s*, 2H, -CH_2-COO) 4.11 (*s*, 3H, -N-CH_3) 4.58–4.65 (*m*, 2H, -CH_2-) 4.85 (*quin*, *J*=6.04 Hz, 1 H, -CH-) 7.24–7.31 (*m*, 1 H, Ar) 8.67 (*d*, *J*=4.39 Hz, 2 H, Ar). ^{13}C NMR (126 MHz, DMSO-d_6) *d* ppm 14.90 (*s*, 1 C) 14.97 (*s*, 1 C) 21.34 (*s*, 1 C) 23.75 (*s*, 1 C) 35.10 (*s*, 1 C) 68.79 (*s*, 1 C) 117.70 (*s*, 1 C) 148.62 (*s*, 1 C) 149.07 (*s*, 1 C) 152.04 (*s*, 1 C) 157.93 (*s*, 1 C) 167.54 (*s*, 1 C) 169.27 (*s*, 1 C). Mass spectrum, m/z (I_{rel}, %) 340 $[M+H]^+$ (100). Anal. calcd. for $C_{13}H_{17}N_5O_2S_2$: C: 46.00 %; H: 5.05 %; N: 20.63 %; S: 18.89 %. Found: C: 46.14 %; H: 5.01 %; N: 20.72 %; S: 18.82 %.

Butyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**7**). Yield 1,55g (88 %), white powder, mp 178 °C (MeOH). ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 0.92 (*t*, *J*=7.0 Hz, 3H, -CH_3), 1.36 (*h*, *J*=7.0 Hz, 2H, -CH_2-), 1.60 (*p*, *J*=6.8 Hz, 2H, -CH_2-), 3.59 (*s*, 2H, -N-CH_3), 4.05–4.11 (m, 4H, -CH_2-), 4.54 (*s*, 2H, -CH_2-), 7.20 (*t*, *J*=3.7 Hz, 1H, Ar), 8.52 (*d*, *J*=3.7 Hz, 2H, Ar). Mass spectrum, m/z (I_{rel}, %) 354 $[M+H]^+$ (100). Anal. calcd. for $C_{14}H_{19}N_5O_2S_2$: C: 47.57 %; H: 5.42 %; N: 19.81 %; S: 18.14 %. Found: C: 47.34 %; H: 5.38 %; N: 19.79 %; S: 18.08 %.

2-((4-Methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**8**). Yield 1,11g (75 %), white powder, mp 197 °C (MeOH). ^{1}H NMR, δ, ppm. (*J*, Hz): 3.59 (*s*, 3H, -N-CH_3), 4.01 (*s*, 2H, -CH_2-COO), 4.54 (*s*, 2H, -CH_2-), 7.05 (*s*, 2H, -NH_2), 7.20 (*t*, *J*=3.7 Hz, 1H, Ar), 8.52 (*d*, *J*=3.7 Hz, 2H, Ar). Mass spectrum, m/z (I_{rel}, %) 297 $[M+H]^+$ (100). Anal. calcd. for $C_{10}H_{12}N_6OS_2$: C: 40.53 %; H: 4.08 %; N: 28.36 %; S: 21.64 %. Found: C: 40.38 %; H: 4.16 %; N: 28.96 %; S: 21.50 %.

N-methyl-2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**9**). Yield 0.89g (58 %), white powder, mp 202 °C (MeOH). ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 2.78 (*d*, *J*=4.8 Hz, 3H, -NH-CH_3), 3.59 (*s*, 3H, -N-CH_3), 3.86 (*s*, 2H, -CH_2-COO), 4.54 (*s*, 2H, -CH_2-), 7.18 – 7.26 (*m*, 2H, NH, Ar), 8.52 (*d*, *J*=3.7 Hz, 2H, Ar). Mass spectrum, m/z (I_{rel}, %) 311 $[M+H]^+$ (100). Anal. calcd. for $C_{11}H_{14}N_6OS_2$: C: 42.57 %; H: 4.55 %; N: 27.08 %; S: 20.66 %. Found: C: 42.77 %; H: 4.34 %; N: 27.01 %; S: 20.01 %.

N,N-dimethyl-2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**10**). Yield 1.00g (62 %), white powder, mp 206 °C (MeOH). ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 2.94 (*s*, 6H, -N$(CH_3)_2$), 3.59 (*s*, 3H, -N-CH_3), 4.10 (*s*, 2H, -CH_2-COO), 4.54 (*s*, 2H, -CH_2-), 7.20 (*t*, *J*=3.7 Hz, 1H, Ar), 8.52 (*d*, *J*=3.7 Hz, 2H, Ar). Mass spectrum, m/z (I_{rel}, %) 325 $[M+H]^+$ (100). Anal. calcd. for $C_{12}H_{16}N_6OS_2$: C: 44.43 %; H: 4.97 %; N: 25.91 %; S: 19.76 %. Found: C: 44.75 %; H: 5.01 %; N: 25.87 %; S: 19.71 %.

3. 2. Molecular docking studies

Molecular docking was performed using the AutoDock 4.2.6 program [15]. The prepared ligands and receptors were docked using AutoDock Vina to calculate binding affinity and to observe the types of non-binding interactions between the investigated complexes. The screening was conducted on the crystallographic structure of the enzyme 'EC 1.11.1.5 Cytochrome c peroxidase,' obtained from the RCSB Protein Data Bank (crystal code 2X08) [16]. Visualization was carried out using the Discovery Studio Visualizer program [17]. Active sites in the processed receptors were identified using AutoDock software before docking. The grid for the binding field measured 38×34×52 Å, which was sufficiently large to cover the entire enzyme region. All the programs used were publicly available.

3. 3. Antiradical Activity

Free radical scavenging was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical test [18]. An exact mass of the substance (0.001 M) was placed in a 25.00 mL volumetric flask, dissolved in DMSO, and diluted to the mark before mixing. Then, 1.00 mL of this solution was transferred to a 10.00 mL volumetric flask (0.0001 M) filled to the mark with DMSO and mixed. Next, 2.00 mL of the resulting solution was placed in a test tube, combined with 2.00 mL of a 0.1 mM DPPH solution in methanol, mixed, and tightly closed. The tubes were vigorously shaken and left for 30 minutes at room temperature in the dark. Absorbance was measured at 516 nm. The control was a 2.00 mL of 0.1 mM DPPH solution in the presence of 2.00 mL of methanol, while ascorbic acid served as the standard. The free radical scavenging activity was expressed as a percentage of inhibition and calculated according to the formula (1):

$$\%\ \text{antiradial activity} = \frac{(A_0 - A_1)}{A_0} \times 100, \qquad (1)$$

where A_0 is the absorbance of the control sample, and A_1 is the absorbance of the test sample. The absorbance of the studied solutions was measured in aqueous-organic solutions, with the absorption maximum at 516 nm recorded using a SPECORD 250 spectrophotometer.

4. Result

One of the well-known methods for synthesizing 5-substituted-1,2,4-triazole-3(2H)-thiones involves the formation of intermediate carbothioamides, followed by heterocyclization in an alkaline environment [19]. The starting pyrimidine-2-thione was synthesized through [3+3] cyclization of thiourea with 1,1,3,3-tetraethoxypro-

pane, and ethyl 2-(pyrimidin-2-ylthio)acetate was obtained by an alkylation reaction in acetone in the presence of K_2CO_3 (Fig. 1).

Subsequently, the ester undergoes hydrazinolysis, and the resulting hydrazide reacts with methyl isothiocyanate in ethanol to form the carbothioamide intermediate. Further cyclization was achieved by stirring the mixture with an aqueous solution of sodium hydroxide for 2 hours on a magnetic stirrer. The resulting solution was then acidified with glacial acetic acid, precipitating 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4*H*-1,2,4-triazole-3-thiole (**1**). It is known that the presence of substituents at the sulfur atom in 1,2,4-triazole-3(2*H*)-thiones enhances their biological activity. Therefore, it was considered appropriate to synthesize S-derivatives of (1,2,4-triazol-3(2*H*)-yl)methyl)thiopyrimidines. Acyl derivatives (**2-10**) were obtained by reacting the original compound (**1**) with the corresponding halogen derivative in a polar solvent (ethanol), with the addition of an equimolar amount of sodium hydroxide.

Analyzing the ^{1}H NMR spectra of the synthesized compounds, certain conclusions can be drawn: multiplets and quintets characteristic of the isopropyl residue are observed at 1.09–1.31 ppm and 4.85 ppm, respectively, confirming the presence of this residue in the compound. The aromatic pyrimidine nucleus resonates as doublets and multiplets in the corresponding region. The signals for the protons of ethanoic acid appear as a singlet at 3.96 ppm (Fig. 2).

Additionally, the formation of the 1,2,4-triazole nucleus is confirmed by the ^{13}C NMR spectrum of compound (**6**), which shows characteristic signals for two sp^2-hybridized carbon atoms at 148.62 and 149.07 ppm. Signals for the methylene groups are registered at 21.34 and 35.10 ppm (Fig. 3). The signals for the aromatic pyrimidine nucleus are observed in the 117.70–167.54 ppm range.

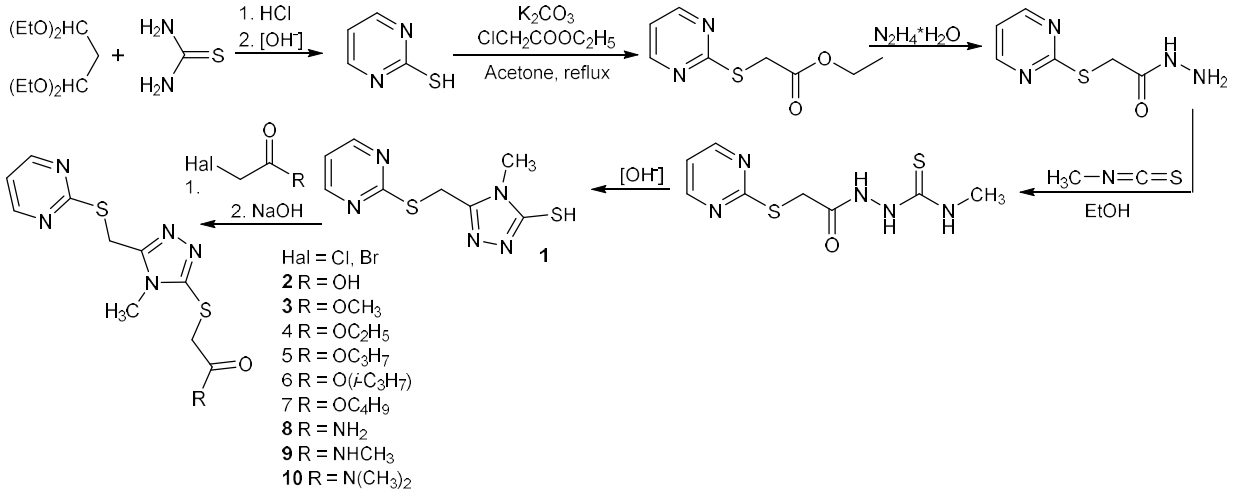

Fig. 1. Synthesis of derivative hybrids of two pharmacophore fragments – pyrimidine-2-thione and 1,2.4-triazole

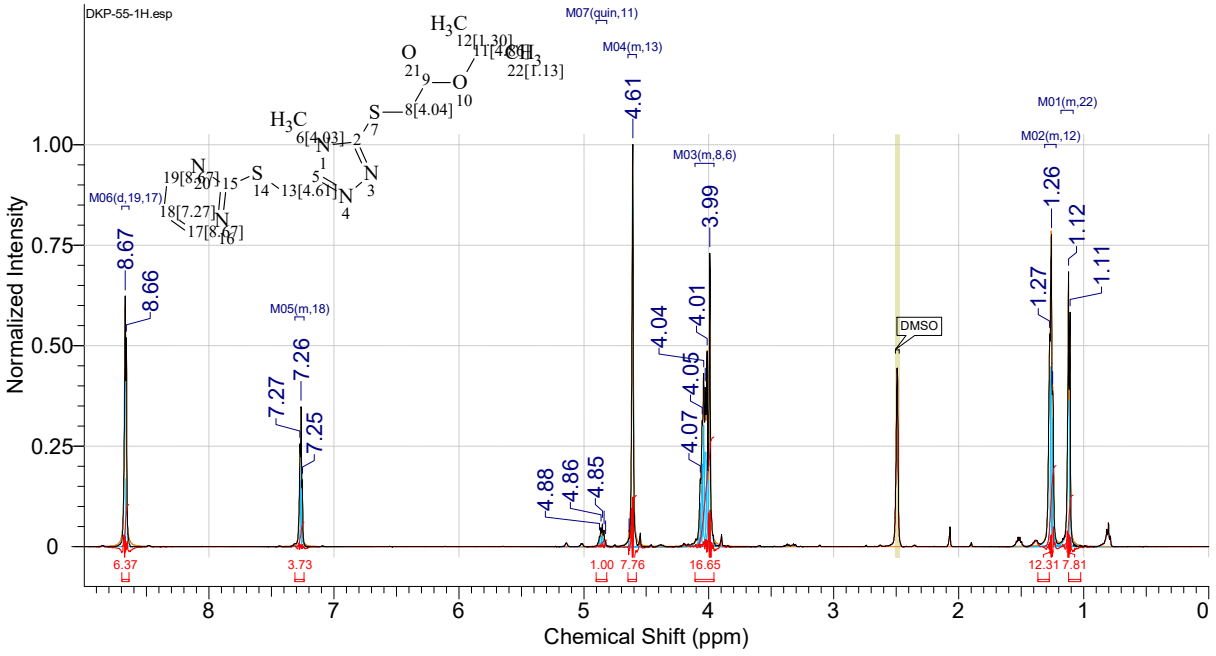

Fig. 2. Fragment of the ^{1}H NMR spectrum of isopropyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate **(6)**

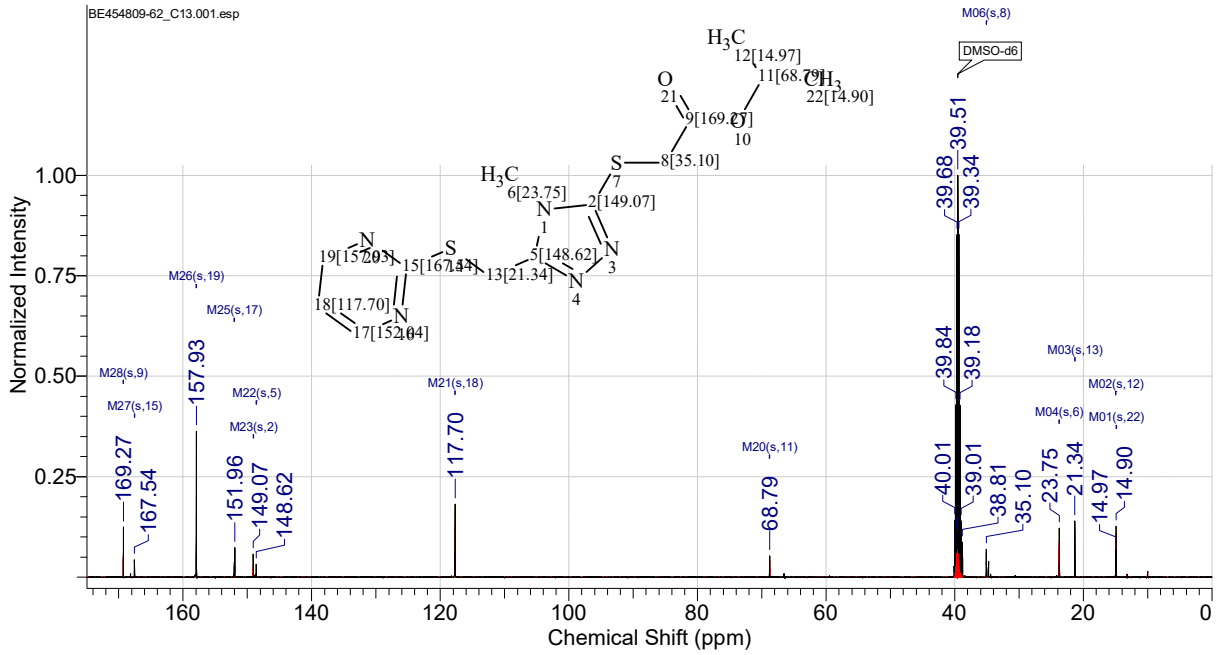

Fig. 3. Fragment of the ^{13}C NMR spectrum of isopropyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate **(6)**

Among the various groups of antioxidants with different mechanisms of action, the most significant role is played by antiradical antioxidants—substances that interact with free radicals to form products that cannot continue oxidation chain reactions or reduce the reaction rate. The antiradical activity of the synthesized compounds, assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical test, demonstrates high levels of activity (Table 1).

Table 1

Antiradical activity and absorption coefficients of 1,2,4-triazole derivatives

Compounds	Absorption coefficient, A	% antiradical activity
Control	0.4305	–
Ascorbic acid	0.2959	31.26
1	0.1864	56.70
2	0.3812	9.66
3	0.4304	0.023
4	0.3804	9.05
5	0.3678	14.56
6	0.0681	83.86
7	0.4087	5.06
8	0.2572	39.05
9	0.3124	27.43
10	0.3578	16.88

It is worth noting that three compounds (**1, 6, 8**) exhibit higher activity than the comparison drug, the natural antioxidant ascorbic acid. Such high activity may be attributed to the presence of pharmacophore fragments, particularly the pyrimidine skeleton and the sulfur atom connected to the 1,2,4-triazole. A more detailed analysis of the compounds indicates a relationship between antiradical activity and structure, where the increased activity can be linked to the presence of free proton donors in the compounds, particularly in the protonated atoms of the pyrimidine ring, as well as proton donors in the carboxylic acid, methine substituent, and amide residues.

The absorption spectra of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with the compounds are shown in Fig. 4, with absorbance values at 516 nm.

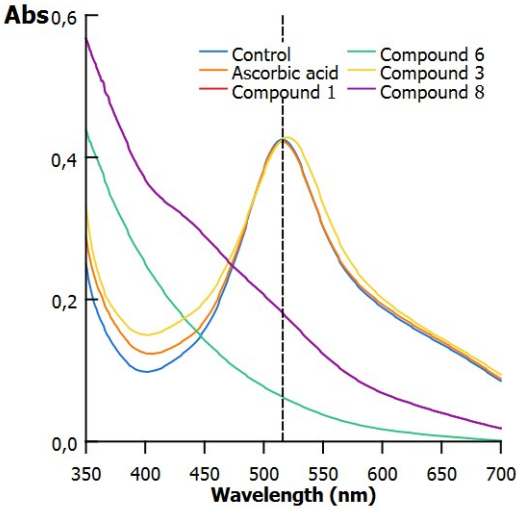

Fig. 4. Absorption spectra of compound solutions with 1,1-diphenyl-2-picrylhydrazyl (DPPH)

IC_{50} was calculated for the most active compound (Fig. 5). When compared with known literature sources [20], the IC_{50} of ascorbic acid is 41.25 μg/mL. In comparison, compound (**6**) has an IC_{50} of 1.51 μg/mL, exceeding the effectiveness of ascorbic acid by 27 times.

Subsequently, we performed molecular docking to validate our data and hypothesis regarding the en-

zyme «EC 1.11.1.5 Cytochrome c peroxidase» (PDB: 2X08) (Table 2).

The docking results in Table 2 indicate that compounds 4 and 6 exhibited the lowest binding energies of –8.7866 and –8.9324 kcal/mol, respectively. This allows them to be considered the most effective compounds with antioxidant activity, interacting with the same six amino acids (LEU, MET, TRP, SER, HID, ARG) during the docking process.

It was established that the atoms of the isopropyl substituent of ethanoic acid at the 5-position of the 1,2,4-triazole nucleus of compound **6** participate in interactions through hydrophobic interactions with the amino acid residues LEU 171, LEU 269, MET 172, PHE 266, PHE 262, ALA 174, LEU 232, PRO 145, ALA 147, PRO 80, and TRP 51. The pyrimidine core and 1,2,4-triazole were primarily connected through hydrophobic interactions, as well as π-π and π-cation bonds (Fig. 6).

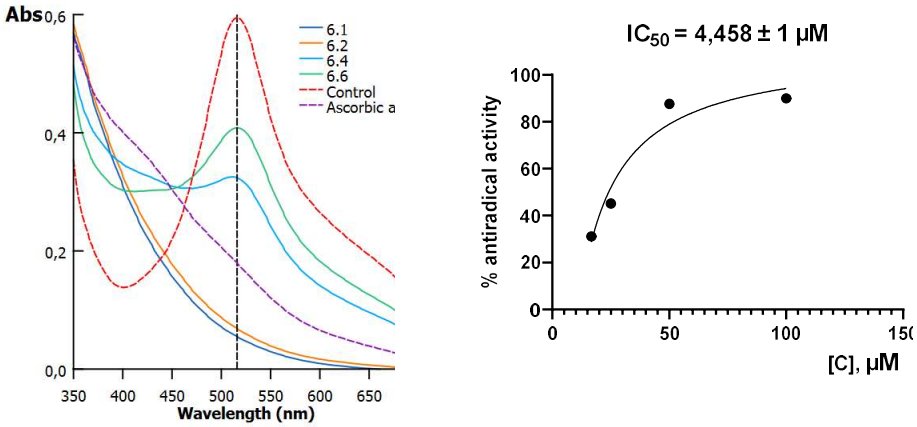

Fig. 5. Study of the kinetic parameters of the reaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH) and calculation of the IC_{50} value

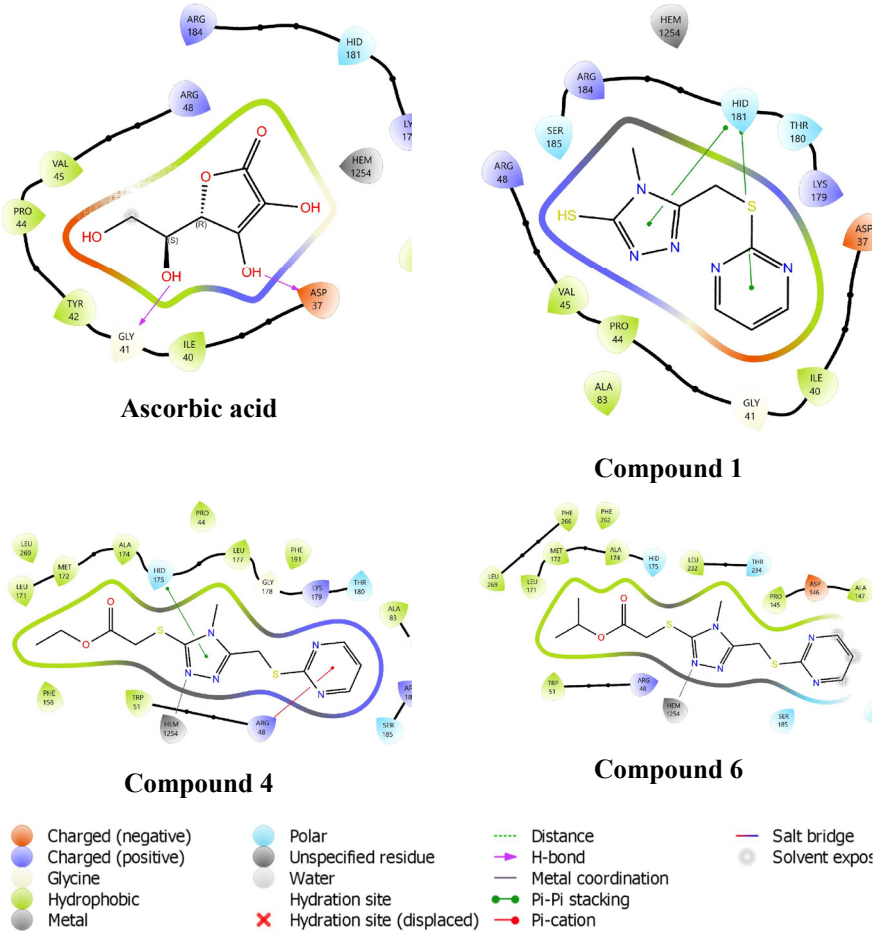

Fig. 6. Interaction network between the enzyme «EC 1.11.1.5 Cytochrome c peroxidase» (PDB: 2X08) and ascorbic acid and compounds **1, 4, 6**. Negatively charged residues are shown in red, polar residues in blue, and hydrophobic residues in green

Table 2

Calculation score, amino acid residue and types of non-bonding interactions

No.	Binding energy (kcal/mol)	Amino acid residues and non-binding interactions
Ascorbic acid	–6.8324	Hydrophobic: VAL 45, PRO 44, TYR 42, ILE 40, LEU 177; polar: HID 181; ionized: positive – ARG 48, ARG 184, LYS 179; negative – ASP 37
1	–7.7361	Hydrophobic: VAL 45, PRO 44, ILE 40, ALA 83; polar: HID 181, THR 180, SER 185; ionized: positive – ARG 48, ARG 184, LYS 179; negative – ASP 37
2	–8.2501	–
3	–8.0477	
4	–8.7866	Hydrophobic: LEU 171, LEU 269, MET 172, ALA 174, LEU 177, PHE 191, TRP 51, VAL 45, PRO 44, ILE 40, ALA 83; polar: HID 175, THR 180, SER 185; ionized: positive – ARG 48, ARG 184, LYS 179
5	–8.1199	–
6	–8.9324	Hydrophobic: LEU 171, LEU 269, MET 172, PHE 266, PHE 262, ALA 174, LEU 232, PRO 145, ALA 147, PRO 80, TRP 51; polar: SER 81, SER 185, HID 175, THR 234; ionized: positive – ARG 48; negative – ASP 146
7	–8.4042	–
8	–8.4233	
9	–8.4209	
10	–8.3062	

5. Discussion

The results of our study align with and expand upon previous findings regarding the potential of the 1,2,4-triazole scaffold in the design of bioactive compounds with diverse pharmacological applications [10]. For instance, earlier research has demonstrated the efficacy of 1,2,4-triazole derivatives in antioxidant applications through mechanisms involving enzyme inhibition and radical scavenging [4, 5]. In comparison, the synthesized compounds in this study exhibit promising antioxidant activity, as evidenced by their docking performance with cytochrome c peroxidase (PDB: 2X08) and the detailed geometric positioning, which indicates potential interactions via peroxidation mechanisms.

Furthermore, the low antiradical activity observed in some compounds, particularly those with the ethyl ester group of acetic acid, corresponds to findings by [7], who reported that electronic configurations significantly influence radical dissociation processes. Similarly, [13] highlighted that overlapping absorption spectra could interfere with accurate measurements of antiradical activity, which supports our hypothesis regarding the interaction of absorption bands in the DPPH assay.

The structure of the synthesized compounds was confirmed using modern physico-chemical analysis methods, including elemental analysis, ^{1}H and ^{13}C NMR spectroscopy, and their purity was verified by chromatography-mass spectrometry. This allows us to conclude the successful formation of the target products and their isolation in pure form. The array of calculated docking values and the detailed analysis of the geometric positions of the synthesized compounds on the enzyme 'EC 1.11.1.5 Cytochrome c peroxidase' (PDB: 2X08) suggest that all the studied molecules will contribute to antioxidant activity to varying degrees, through peroxidation mechanisms.

The very low value of antiradical activity (%) can be explained by two factors: the similarity between the absorption spectra of the substance and DPPH, which causes overlap of the absorption bands, or an increase in compound degradation, leading to the release of free radicals. This is evident in the case of the compound with the ethyl ester group of acetic acid at the 5-position of 1,2,4-triazole-3(2H)-thione and may be due to a suboptimal electronic configuration that hinders the reduction of free radicals through dissociation.

Practical relevance. The obtained results may indicate preliminary findings of APIs with potential antioxidant effects. The proposed studies on derivatives of (1,2,4-triazol-3(2*H*)-yl)methyl)thiopyrimidines could be utilized for synthesizing a wider range of substances in this series and for optimizing the molecular docking procedure in accordance with the chosen direction.

Research limitations. The proposed synthesis methods are multi-stage, resulting in insufficient yields of the target products. A limitation also applies to the free radical method, where the obtained spectra of the compounds may overlap with DPPH.

Prospects for further research. The developed methods for synthesizing substituted (1,2,4-triazol-3(2H)-yl) methyl)thiopyrimidines can be applied in the development of new potential antioxidants. The incorporation of this 1,2,4-triazole pharmacophore into the molecule may enhance activity, potentiate the desired antioxidant effect, and reduce toxicity. Docking studies demonstrated the potential of the obtained derivatives as antioxidants, thus recommending them for further investigation.

6. Conclusions

Three compounds (**1, 6, 8**) exhibited higher activity than the reference drug, the natural antioxidant ascorbic acid. This high activity may be associated with the presence of pharmacophore fragments, particularly the pyrimidine skeleton and the sulfur atom linked to the 1,2,4-triazole. The IC_{50} for the most active compound was calculated as 4.458±1 μM, which is 27 times more effective than ascorbic acid. Molecular docking results showed that compounds 4 and 6 had the lowest binding energies (–8.7866 to –8.9324 kcal/mol), making them the most

effective compounds in terms of antioxidant activity, as they interacted with six key amino acids (LEU, MET, TRP, SER, HIS, ARG) during the docking process.

Conflict of interests

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.

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Data availability

Data will be made available at a reasonable request.

Use of artificial intelligence

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

References

1. Singh, S. (2024). Antioxidant nanozymes as next-generation therapeutics to free radical-mediated inflammatory diseases: A comprehensive review. International Journal of Biological Macromolecules, 260, 129374. https://doi.org/10.1016/j.ijbiomac.2024.129374

2. Olfat, N., Ashoori, M., Saedisomeolia, A. (2022). Riboflavin is an antioxidant: a review update. British Journal of Nutrition, 128 (10), 1887–1895. https://doi.org/10.1017/s0007114521005031

3. Ungor, D., Gombár, G., Juhász, Á., Samu, G. F., Csapó, E. (2023). Promising Bioactivity of Vitamin B1-Au Nanocluster: Structure, Enhanced Antioxidant Behavior, and Serum Protein Interaction. Antioxidants, 12 (4), 874. https://doi.org/10.3390/antiox12040874

4. Bhatnagar, A., Pemawat, G. (2023). Functionalized Pyrimidines: Synthetic Approaches and Biological Activities. A Review. Organic Preparations and Procedures International, 56 (1), 1–18. https://doi.org/10.1080/00304948.2023.2225385

5. Alamshany, Z. M., Nossier, E. S. (2024). New thiazole derivatives linked to pyridine, fused pyridine, pyrimidine and thiazolopyrimidine scaffolds with potential dual anticancer and antimicrobial activities: Design, synthesis and docking simulation. Journal of Molecular Structure, 1316, 138973. https://doi.org/10.1016/j.molstruc.2024.138973

6. Bafail, R. S. M., Samman, W. A. (2024). Anti-parkinsonian, anti-inflammatory, anti-microbial, analgesic, anti-hyperglycemic and anticancer activities of poly-fused ring pyrimidine derivatives. Tropical Journal of Pharmaceutical Research, 23 (1), 67–75. https://doi.org/10.4314/tjpr.v23i1.9

7. Myriagkou, M., Papakonstantinou, E., Deligiannidou, G.-E., Patsilinakos, A., Kontogiorgis, C., Pontiki, E. (2023). Novel Pyrimidine Derivatives as Antioxidant and Anticancer Agents: Design, Synthesis and Molecular Modeling Studies. Molecules, 28 (9), 3913. https://doi.org/10.3390/molecules28093913

8. El-Naggar, M., Hasan, K., Khanfar, M., Shehadi, I. A., El-Awady, R., El-Dein, A. N. et al. (2024). Synthesis, biological assessment and molecular docking study of new sulfur-linked 1,2,4-triazole and 1,2,3-triazole hybrid derivatives as potential DNA gyrase inhibitors. Zeitschrift Für Naturforschung B, 79 (7), 419–429. https://doi.org/10.1515/znb-2024-0012

9. Miedviedieva, K. P., Prytula, R. L., Shmatenko, O. P., Bushuieva, I. V., Parchenko, V. V., Kucherenko, L. I., Vasiuk, S. O. (2024). Express quantitative spectrophotometric determination of 2-(((3-(2-fluorophenyl)- 5-thio-4H-1,2,4-triazol-4-yl)imino)methyl) phenol as an active substance of a medicinal product for the treatment of mycoses. Zaporozhye Medical Journal, 26 (1), 59–65. https://doi.org/10.14739/2310-1210.2024.1.291449

10. Karpenko, Y., Kusdemir, G., Parchenko, V., Tüzün, B., Taslimi, P., Karatas, O. F. et al. (2023). A biochemistry-oriented drug design: synthesis, anticancer activity, enzymes inhibition, molecular docking studies of novel 1,2,4-triazole derivatives. Journal of Biomolecular Structure and Dynamics, 42 (3), 1220–1236. https://doi.org/10.1080/07391102.2023.2253906

11. Bitounis, D., Jacquinet, E., Rogers, M. A., Amiji, M. M. (2024). Strategies to reduce the risks of mRNA drug and vaccine toxicity. Nature Reviews Drug Discovery, 23 (4), 281–300. https://doi.org/10.1038/s41573-023-00859-3

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

13. Pachuta-Stec, A. (2022). Antioxidant Activity of 1,2,4-Triazole and its Derivatives: A Mini-Review. Mini-Reviews in Medicinal Chemistry, 22 (7), 1081–1094. https://doi.org/10.2174/1389557521666210401091802

14. Karpenko, Yu. V., Panasenko, O. I., Kulish, S. M., Domnich, A. V. (2023). Synthesis and acute toxicity of new S-derivatives (1,2,4-triazole-3(2H)-yl)methyl) thiopyrimidines. Current Issues in Pharmacy and Medicine: Science and Practice, 16 (2), 158–164. https://doi.org/10.14739/2409-2932.2023.2.274586

15. Pham, Q. M., Le, T. T. H., Pham, T. H. M., Tran, Q. T., Do, T. L., Vu, T. T. L., Pham, Q. L. (2022). Molecular docking tutorial using AutoDock 4.2.6 on SARS-CoV-2 main protease for beginner. Vietnam Journal of Science and Technology, 60 (6), 929–947. https://doi.org/10.15625/2525-2518/16459

16. Murphy, E. J., Metcalfe, C. L., Basran, J., Moody, P. C. E., Raven, E. L. (2008). Engineering the Substrate Specificity and Reactivity of a Heme Protein: Creation of an Ascorbate Binding Site in Cytochrome c Peroxidase. Biochemistry, 47 (52), 13933–13941. https://doi.org/10.1021/bi801480r

17. Aallaei, M., Molaakbari, E., Mostafavi, P., Salarizadeh, N., Maleksah, R. E., & Afzali, D. (2022). Investigation of Cu metal nanoparticles with different morphologies to inhibit SARS-CoV-2 main protease and spike glycoprotein using Molecular Docking and Dynamics Simulation. Journal of Molecular Structure, 1253, 132301. https://doi.org/10.1016/j.molstruc.2021.132301

18. Gulcin, İ., Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. Processes, 11 (8), 2248. https://doi.org/10.3390/pr11082248

19. Gotsulya, A., Fedotov, S., Zinych, O., Trofimova, T., Brytanova, T. (2023). Synthesis and properties of s-alkyl 4-(4-chlorophenyl)-5-(pyrrole-2-yl)-1,2,4-triazole-3-thiol derivatives. Journal of Faculty of Pharmacy of Ankara University, 47 (3), 1020–1032. https://doi.org/10.33483/jfpau.1280492

20. Matuszewska, A., Jaszek, M., Stefaniuk, D., Ciszewski, T., Matuszewski, Ł. (2018). Anticancer, antioxidant, and antibacterial activities of low molecular weight bioactive subfractions isolated from cultures of wood degrading fungus Cerrena unicolor. PLOS ONE, 13 (6), e0197044. https://doi.org/10.1371/journal.pone.0197044

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