Synthesis, structure and properties of 7'-(4-amino-5-thio-1,2,4-triazole-3-yl)methyl)theophylline derivatives

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The combination of derivatives of 1,2,4-triazole and theophylline creates fertile soil for biologically active substances. The use of these heterocyclic systems allows the use of simple chemical modification methods and available reagents. This determines the relevance of the chosen direction of scientific research.

The aim of the work was to study synthesis methods and study the properties of heterocyclic systems containing theophylline and 1,2,4-triazole fragment in their structure, create a chemical variety that was interesting from a scientific point of view and was promising in the search for biologically active substances.

Materials and methods. Theophylline was used as the starting material. Using alkylation reactions, hydrazinolysis, interaction with a carbon disulfide followed by heterocyclization with an excess of hydrazine hydrate, 7'-(4-amino-5-thio-1,2,4-triazole-3-yl)methyl) theophylline was obtained. The following stages of the chemical conversion included alkylation reactions with haloalkanes, the formation of azomethine compounds by reaction with aromatic aldehydes, and the reaction with aromatic carboxylic acid chlorides. The structure of the obtained compounds was confirmed by data of elemental analysis, 1H NMR spectroscopy and IR-spectrophotometry. The individuality of substances was established by using high performance liquid chromatography with diode-array and mass spectrometric detection.

Results. S-alkylderivatives of 7'-(4-amino-5-thio-1,2,4-triazole-3-yl)methyl)theophylline, Schiff bases and carboxamides were synthesized, their structure was proved, and physical properties were investigated.

The synthesized compounds have been subjected to the in silico molecular docking study against the kinases of anaplastic lymphoma by using the 2XP2 ligand, lanosterol 14-α-demethylase by using the 3LD6 ligand, cyclooxygenase-2 by using the ligand 4Z0L which were downloaded from the protein data bank (PDB).

Conclusions. Molecular docking has shown the ability of the synthesized compounds to influence the kinase activity of anaplastic lymphoma, cyclooxygenase-2 and lanosterol-14-α-demethylase.
The combination of xanthine and 1,2,4-triazole fragments provides a promising direction in the search for biologically active substances. There are many examples to support this [7]. Thus, substances with analgesic, bronchodilatory and antituberculous activity were found in this class of compounds.

Methods of combining these compounds involve the formation of a 1,2,4-triazole moiety using xanthine as the starting compound. The literature describes various approaches to the conditions of this transformation [7]. But determining the optimal conditions for this process remains relevant despite the advances in research in this direction.

**Aim**

The aim of our work was to search for promising compounds from the point of biological activity in a series of derivatives that combine heterocyclic fragments of theophylline and 1,2,4-triazole.

**Materials and methods**

The implementation of the experimental part of the work has been accompanied by the use of traditional methods of organic synthesis [2,4,8]. Melting points were determined in open capillary tubes in a “MPA 100”. The elemental analysis (C, H, N) were performed through the “Elementar vario EL cube” analyzer. IR spectra (4000–400 cm$^{-1}$) were taken using “ALPHA FT-IR spectrometer”. $^1$H NMR spectra (400 MHz) were recorded at “Varian-Mercury 400” spectrometer, and $^13$C NMR spectra (100 MHz) were taken using “ALPHA FT-IR spectrometer”.

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**Chemistry**

In the primary stage, the synthesis of theophylline ester was performed using 2-chloroacetic acid, followed by hydrazinolysis and heterocyclization in excess of hydrazine. The formed thioles were used in the reactions of alkylation, synthesis of Schiff bases and carboxamides. The influence of the nature of the solvent and the duration of heating on the yield of the reaction products were investigated. Sodium salt, ester and hydrazide were prepared according to known methods [7]. Hydrazinolysis and subsequent heterocyclization carried out using traditional methods of organic synthesis. The resulting 7'-(4-amino-5-thio-1,2,4-triazole-3-yl)methyltheophylline was isolated in open capillary tubes in a “MPA 100”. The elemental analysis (C, H, N) were performed through the “Elementar vario EL cube” analyzer. IR spectra (4000–400 cm$^{-1}$) were taken using “ALPHA FT-IR spectrometer”. $^1$H NMR spectra (400 MHz) were recorded at “Varian-Mercury 400” spectrometer, and $^13$C NMR spectra (100 MHz) were taken using “ALPHA FT-IR spectrometer”.

**Results.** Synthesized 7'-alkyl derivatives of 7'-(4-amino-5-thio-1,2,4-triazole-3-yl)methyltheophylline, the structure of which was confirmed by IR spectra, $^1$H NMR and $^13$C NMR spectra, were subjected to in silico molecular docking. The results showed that the synthesized compounds have the potential to interact with the enzyme kinase of malignant lymphoma.

**Conclusions.** The synthesized compounds can be used as potential inhibitors of the enzyme kinase of malignant lymphoma. The synthesized compounds have the potential to be used as potential inhibitors of the enzyme kinase of malignant lymphoma.
was used in S-alkylation and derivative reactions involving the amino group (Fig. 1).

7’-((4-Amino-3-thio-1,2,4-triazole-5-yl)methyl)theophylline (7). 1 g (0.02 mol) N\textsubscript{2}H\textsubscript{4}·H\textsubscript{2}O was added to a solution of 3.66 g (0.01 mol) of the potassium 2-(2-(theophylline-7-yl)acetyl)hydrazine-1-carbodithioate dissolved in 3 ml of water. The mixture was refluxed for 2 h, cooled, diluted with water and acidified with CH\textsubscript{3}COOH. The product was crystallized from ethanol and isolated as a white solid.

Alkylderivatives of 7’-((4-arylideneamino-3-thio-1,2,4-triazole-5-yl)methyl)theophylline (7.1–7.4). To dissolved in 30 ml of propan-1-ol mixture of 0.005 mol of the thiol (7) and an equivalent amount of NaOH add also an equivalent amount of halogenalkane (iodomethane, iodoethane, 1-bromopropane, 1-bromobutane). Heat for 2 h, cooled, the precipitate is filtered, washed with water and crystallized from methanol.

7’-((4-Arylideneamino-3-thio-1,2,4-triazole-5-yl)methyl)theophylline (7.5–7.7). The corresponding aldehyde (0.005 mol) and 4 drops of H\textsubscript{2}SO\textsubscript{4} concentrated were added to the compound 7 (0.005 mol) in 1,4-dioxane (50 ml). The reaction mixture was refluxed for 8 h and then diluted with 50 ml of H\textsubscript{2}O. The product was crystallized from ethanol.

\begin{align*}
N\text-(5-((theophylline-7’-yl)methyl)-3-thioxo-1,2,4-triazole-4-yl)benzamide (7.8). \text{Benzoyl chloride (0.005 mol) was added to a mixture of compound 7 (0.005 mol) and tri-ethylamine (0.7 ml, 0.005 mol) in tetrahydrofuran (50 ml). The reaction mixture was stirred for 10 h. The product was crystallized from ethanol.}
\end{align*}

\textbf{Molecular docking}

Molecular docking was performed to obtain structural information on the interaction of the synthesized compounds and the corresponding biological structure [5]. The X-ray crystal structures of the corresponding biological targets from the protein database (PDB-ID) in complex with the standard ligand were previously downloaded: kinases of anaplastic lymphoma in the complex of crizotinib (2XP2), lanosterol 14-α-demethylase with ketoconazole (3LD6), cyclooxygenase-2 with diclofenac (4Z0L). The ligands (crizotinib, ketoconazole, diclofenac) were previously removed from the primary structures. Carried out the joining of different ligands to the protein using AUTODOCK. The conformations of the ligand were analyzed in terms of energy, hydrogen bonding and hydrophobic interaction between the ligand and...
the receptor protein. A detailed analysis of the ligand-receptor interactions was performed and the final coordinates of the ligand and receptor were saved as pdb files. The binding energy (FEB) of all compounds was calculated.

**Results**

Optimal conditions were determined and 7'-((4-amino-3-thio-1,2,4-triazole-5-yl)methyl)theophylline was synthesized and its new derivatives. The alkylation reactions, synthesis of Schiff bases and carboxamides were carried out with the synthesized thiols (Fig. 1) [2,4]. The structure of the obtained compounds was confirmed by 1H NMR spectroscopy, chromatographic mass spectrometry and elemental analysis.

In obedience to the IR spectroscopic data of the compounds 7, 7.5–7.8 the observation of C=N stretching bands at 1203–1217 cm⁻¹. Valence vibrations of bonds of C=H alkyl groups form bands in area 2935–2850 cm⁻¹. The synthesized compounds are also characterized by valence vibrations of the C=C bond of the aromatic rings at 1468–1453 cm⁻¹.

In the 1H NMR spectra of compounds (7.1–7.4) protons of the S-alkyl fragments resonate in a strong field as a singlet, a triplet or a multiplet in area 3.17–0.97 ppm. Proton of the N=CH fragment forms a signal in the form of the singlet at 8.82–8.71 ppm. The signal in the spectrum of compound 7.8 at 8.09 ppm corresponds to the proton of the CONH fragment and resonates in the form of a singlet.

In the chromatographic mass spectra, individual peaks of the molecular ion and peaks of the fragment ions are recorded, which have a high intensity, which confirms the structure and identity of the compounds 7, 7.1–7.8.

7'-((4-Amino-3-thiao-1,2,4-triazole-5-yl)methyl)theophylline (7). Yield: 86 %, m. p. 214–216 °C; IR (cm⁻¹): 3312, 3183 (N-H); 2865 (C-H aliphatic); 1694, 1653 (C=O); 1469, 1455 (C=C, C=N); 1317 (C=S); 1H NMR, δ (ppm): 13.52 (s, 1H, N=H, triazole), 8.01 (d, J = 10.6 Hz, 2H, NH₂), 7.93 (s, 1H, CH), 5.15 (s, 2H, NH), 3.56 (s, 3H, N=CH), 3.39 (s, 3H, N=CH), Anal. calcd. for C₁₇H₁₈N₁₀O₅S: C, 40.88; H, 3.92; N, 36.43; S, 10.37.

7'-((4-Amino-3-thio-1,2,4-triazole-5-yl)methyl)theophylline (7.1). Yield: 68 %, m. p. 214–216 °C; IR (cm⁻¹): 3312, 3183 (N-H); 2865 (C-H aliphatic); 1694, 1653 (C=O); 1472, 1455 (C=C, C=N); 1H NMR, δ (ppm): 7.96 (s, 1H, C=H theophylline), 5.02 (s, 2H, NH), 5.34 (s, 2H, NH₂), 3.54 (s, 3H, N=CH), 3.45 (s, 3H, N=C=H), 2.68 (s, 3H, CH₃). Anal. calcd. for C₁₇H₁₈N₁₀O₅S: C, 40.99; H, 4.38; N, 34.76; S, 9.95. Found: C, 40.88; H, 4.37; N, 34.85; S, 9.98.

7'-((4-Amino-ethylthio-1,2,4-triazole-5-yl)methyl)theophylline (7.2). Yield: 81 %, m. p. 214–216 °C; IR (cm⁻¹): 3317, 3177 (N-H); 2865 (C-H aliphatic); 1697, 1648 (C=O); 1461, 1453 (C=C, C=N); 1H NMR, δ (ppm): 7.98 (s, 1H, C=H theophylline), 5.27 (s, 2H, NH), 5.03 (s, 2H, NH₂), 3.52 (s, 3H, N=CH), 3.47 (s, 3H, N=CH), 3.17 (m, 2H, S=CH₂-CH), 1.34 (t, J = 5.3 Hz, 3H, S-CH₃). Anal. calcd. for C₁₇H₂₁N₁₀O₅S: C, 42.85; H, 4.79; N, 33.31; S, 9.53. Found: C, 42.97; H, 4.78; N, 33.39; S, 9.50.
(t, J = 7.5 Hz, 1H, H-4, C₆H₅), 7.45 (t, J = 7.4 Hz, 2H, H-3,5, C₆H₅), 5.01 (s, 2H, N⁺CH₂), 3.41 (s, 3H, N⁺CH₃), 3.22 (s, 3H, N⁺CH₃). Anal. calcd. for C₁₇H₁₆N₈O₃S: C, 49.51; H, 3.91; N, 27.17; S, 7.77. Found: C, 49.37; H, 3.92; N, 27.09; S, 7.79.

The methodology for rational drug development involves the use of molecular docking. Docking experiments of synthesized compounds (7, 7.1–7.8) with the 2XP2 (ALK tyrosine kinase receptor) receptor revealed that compound 7.8 is the most active with a calculated binding energy of 8.1 kcal/mol (Table 1) [3,6,8–10].

Analysis of complexes of synthesized compounds with anaplastic lymphoma kinase showed the participation of the following amino acid residues: A: ASP 1203, A: ALA 1148, A: ARG 1253, A: VAL 1130, A: LEU 1122, A: LEU 1198, A: LEU 1256 (Fig. 2).

The next stage is reaching at the base of the specified disparity of synthesizing compounds to the site of the enzyme’s link cyclooxygenase-2 (COX-2) (Table 2) [9]. Visualization of the interaction of the most active compound (7) with the center of COX-2 allowed to establish that it has a hydrogen bond with the amino acid residue D: TYR 3355, in addition, three pi-alkylhydrophobic interactions with D: LEU 3531, D: 3523, D: 3352.

Docking of 1,2,4-triazole-3-thiol derivatives and reference compound (ketoconazole) against the generated homology model for lanosterol-14α-demethylase was carried out (Table 3) [9].

Analysis of the complex of the most active compound with lanosterol-14α-demethylase showed interactions with the following amino acid residues: B: His 447, B: TYR 131, B: ILE 377, B: ILE 379, B: PRO 376, B: MET 487.

### Table 1. Energy values of the intermolecular interactions of the studied compounds with anaplastic lymphoma kinase (2XP2)

| N | E_{min}^{\text{val}} kcal/mol | N | E_{min}^{\text{val}} kcal/mol | N | E_{min}^{\text{val}} kcal/mol |
|---|---|---|---|---|---|
| 7 | -6.8 | 7.3 | -7.1 | 7.6 | -8.0 |
| 7.1 | -6.7 | 7.4 | -7.8 | 7.7 | -7.9 |
| 7.2 | -7.0 | 7.5 | -7.9 | 7.8 | -8.1 |
| Crizotinib | -9.4 | | | | |

*E_{\text{min}}^{\text{val}}*: The minimum energy of complex formation, kcal/mol.

### Table 2. Energy values of the intermolecular interactions of the studied compounds with COX-2 (4Z0L)

| N | E_{min}^{\text{val}} kcal/mol | N | E_{min}^{\text{val}} kcal/mol | N | E_{min}^{\text{val}} kcal/mol |
|---|---|---|---|---|---|
| 7 | -7.3 | 7.3 | -3.6 | 7.6 | -1.6 |
| 7.1 | -5.1 | 7.4 | -6.0 | 7.7 | -2.7 |
| 7.2 | -4.9 | 7.5 | -2.3 | 7.8 | -0.4 |
| Diclofenac | -6.6 | | | | |

### Table 3. Energy values of the intermolecular interactions of the studied compounds with lanosterol-14α-demethylase (3LD6)

| N | E_{min}^{\text{val}} kcal/mol | N | E_{min}^{\text{val}} kcal/mol | N | E_{min}^{\text{val}} kcal/mol |
|---|---|---|---|---|---|
| 7 | -7.4 | 7.3 | -8.5 | 7.6 | -9.8 |
| 7.1 | -7.5 | 7.4 | -8.7 | 7.7 | -9.5 |
| 7.2 | -8.0 | 7.5 | -9.4 | 7.8 | -9.6 |
| Ketoconazole | -10.1 | | | | |

Fig. 2. Visualization of affinity according to the docking: a – compound 7.1 with COX-2 (4Z0L); b – compound 7.5 with COX-2 (4Z0L) [1].
Synthesis, structure and properties of 7-[(4-amino-5-thio-1,2,4-triazole-3-yl)methyl]-theophylline derivatives

Discussion
The results of molecular docking using three classes of substances demonstrate the different nature of the interactions of synthesized substances with amino acid residues anaplastic lymphoma kinase (2XP2), COX-2 (4Z0L) and lanosterol-14α-demethylase (3LD6).

Amino acid modification of the starting molecule led to an increase in the number of hydrogen chemical bonds and hydrophobic interactions with anaplastic lymphoma kinase (2XP2) and lanosterol-14α-demethylase (3LD6).

The appearance of an alkyl substituent for Sulfur of synthesized substances may be justified in planning further studies related to the search for inhibitors COX-2.

Conclusions
1. An universal method for the preparation of S-alkyl-derivatives of 7-[(4-amino-5-thio-1,2,4-triazole-3-yl)methyl] theophylline was developed. It was found that the highest yield of the products of this chemical transformation was observed in propan-1-ol medium and heated for two hours.

2. The synthesis was substantiated and the peculiarities of the formation of reactions were established for the Schiff bases and carboxamides based on 7-[(4-amino-5-thio-1,2,4-triazole-3-yl)methyl]theophylline. The most suitable solvent for synthesis of the Schiff bases was 1,4-dioxane, for carboxamides – tetrahydrofuran.

3. The structure and individuality of the synthesized compounds were confirmed by 1H NMR, IR and LC-MS spectra, elemental analysis.

4. The prospect of studying the antifungal activity of the synthesized compounds based on the use of molecular docking has been shown.

Prospects for further research. According to the research results it is planned to expand classes of compounds to identify promising biologically active compounds among them.

Funding
The research is carried out within the RDW of Zaporizhzhia State Medical University “Synthesis, physical-chemical and biological properties of 3,4-disubstituted 3(5)-thio-1,2,4-triazole with antioxidant, antihypoxic, antimicrobial, cardio and hepatoprotective action” State registration number 0118U007143 (2018–2022).

Conflicts of interest: authors have no conflict of interest to declare.

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References
[1] Biovia. (2019). Discovery Studio Visualizer, v 19.1.0.18287 [Software]. http://www.3dsbiovia.com/
[2] Boraei, A. T. A., El Ashry, E. S. H., & Duerkop, A. (2016). Regioselectivity of the alkylation of S-substituted 1,2,4-triazoles with dihaloalkanes. Chemistry Central Journal, 10(1). 22. https://doi.org/10.1186/s13065-016-0165-0
[3] ChemAxon. (2015). MarvinSketch, Version 6.3.0. [Software]. http://www.chemaxon.com
[4] El-Shaieb, K. M., Mohamed, A. H., & Abdel-latif, F. F. (2019). Investigation of the reactivity of 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thio towards some selected carbonyl compounds: synthesis of novel triazolotriazine-, triazolotetrazine-, and triazolophthalazine derivatives. Zeitschrift für Naturforschung B, 74(11-12), 847-855. https://doi.org/10.1515/znb-2019-0140
[5] Backer, M. M. E., McSweeney, S., Lindley, P. F., & Hough, E. (2004). Ligand-binding and metal-exchange crystallographic studies on shrimp alkaline phosphatase. Acta Crystallographica Section D-Structural Biology, 60, 1555-1561. https://doi.org/10.1107/s0907444904016028
[6] Kaur, R., Devi, A., Kumar, B., Kumar, V. (Recent, 2016). Developments on 1,2,4-triazole nucleus in anticancer compounds. Anti-Cancer Agents in Medicinal Chemistry, 16(4), 465-489. https://doi.org/10.2174/1871520615666150819121106
[7] Gotsulya, A. S., Pansanenko, O. I., Knysh, Ye. G., Knyazevich, P. S. (2015). Synthesis and physical-chemical research of 7-[(4-thio-3-R-4H-1,2,4-triazole-5-yl)methyl]theophylline carbonyl derivatives. Zaporozhye Medical Journal, (3), 103-107. https://doi.org/10.14739/2310-1210.2015.3.44510
[8] El-Shenief, H. A. M., Youssif, B. G. M., Abbas Bukhari, S. N., Abdel-Aziz, M., & Abdel-Rahman, H. M. (2018). Synthesis, anticancer activity and molecular modeling studies of 1,2,4-triazole derivatives as EGFR inhibitors. European Journal of Medicinal Chemistry, 156, 774-789. https://doi.org/10.1016/j.ejmech.2018.07.024
[9] Worldwide Protein Data Bank. (n.d.). Protein Data Bank (PDB) [Database]. http://www.pdb.org
[10] Filimonov, D. A., Druzhilovsky, D. S., Lagunin, A. A., Gloriosoza, T. A., Rudik, A. V., Dmitriev, A. V., Pogodin P. V., & Paroikov, V. V. (2018). Komp’yuternoe prognozirovanie spektrov biologicheski aktivnykh khr-micheskikh soedinenii: vozmoznosti i ogranicheniya [Computer-aided prediction of biological activity spectra for chemical compounds: opportunities and limitations]. Biomedical Chemistry: Research and Methods, 1(1), e00004. [in Russian]. https://doi.org/10.18097/bcmcr00004