Synthesis and properties of 3-(ethylthio)-9-methyl-6-(alkylthio) pyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazines

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The combination of pyrazole and 1,2,4-triazole fragments in one structure makes it possible to achieve some success in creating potential biologically active compounds. Various factors contribute to this process. Among them, we can note the significant possibilities of chemical transformation involving these cycles, the simplicity, and reliability of methods, the creation of molecules with a certain level of bioavailability and the ability to influence a number of biochemical processes. Taking into account the presented facts, the creation of new compounds in a number of pyrazolo-triazole condensed systems is scientifically attractive with endowed features of practical significance and relevance.

The aim of the work was to identify optimal conditions for the production of 3-(ethylthio)-9-methyl-6-(alkylthio)pyrazolo[1,5-*d*][1,2,4] triazolo[3,4-*f*][1,2,4]triazines and to study the properties of the target reaction products.

Materials and methods. The chemical part of the work involved the step-by-step creation of target reaction products in the form of 3-(ethylthio)-9-methyl-6-(alkylthio)pyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazines. The first stage was aimed at conducting the interaction of diethyloxalate with acetone with the participation of sodium methylate in a methanol medium. Ethyl-2,4-dioxopentanoate was used in the conversion process to 3-methylpyrazole-5-carbohydrazide with the participation of hydrazine hydrate. Further modification of the molecule consisted of the gradual formation of the structure of 4-amino-5-(3-methylpyrazol-5-yl)-1,2,4-triazole-3-thiol. The next step involved the synthesis of 3-ethylthio-5-(3-methylpyrazol-5-yl)-1,2,4-triazole-4-amine. Further conversion included the production of potassium 3-ethylthio-9-methylpyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazine-6-thiolate and its *S*-alkyl derivatives along the triazine fragment. Cyclooxygenase-2, lanosterol-14α-demethylase and receptor tyrosine kinase were selected as model enzymes for docking, the crystal structure of which was loaded from the Protein Data Bank.

Results. The synthesis of 3-(ethylthio)-9-methyl-6-(alkylthio)-pyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazines were carried out and the optimal conditions for the production of these substances were determined. The structure of the chemical transformation products was proved and the results of the study of the main physical properties were recorded. The results of virtual studies provided an opportunity to substantiate the prospects of the selected chemical transformation vector, which ultimately made it possible to determine the biological potential of the obtained compounds.

Conclusions. Based on the results of the study, information was obtained that gives a certain idea of the possible level of influence of synthesized compounds on the activity of lanosterol-14α-demethylase, which makes it advisable to further search for substances with fungistatic and fungicidal effects.

Key words: 1,2,4-triazole, pyrazole, physico-chemical properties, molecular docking.

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Синтез та властивості 3-(етилтіо)-9-метил-6-(алкілтіо)піразоло[1,5-d][1,2,4]тріазоло-[3,4-f][1,2,4]тріазинів

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Поєднання в одній структурі фрагментів піразолу та 1,2,4-тріазолу дає змогу досягти певних успіхів у сфері створення потенційних біологічно активних сполук. Цьому процесу сприяють різні фактори, як-от суттєві можливості хімічного перетворення за участю названих циклів, простота та надійність методик, створення молекул із певним рівнем біодоступності та змогою впливати на низку біохімічних процесів. Отже, створення нових сполук у ряду піразоло-тріазолових конденсованих систем викликає науковий інтерес, має практичне значення та характеризується актуальністю.

Мета роботи – визначення оптимальних умов отримання 3-(етилтіо)-9-метил-6-(алкілтіо)піразоло[1,5-*d*][1,2,4]тріазоло[3,4-*f*][1,2,4] тріазинів і дослідити властивості цільових продуктів реакції.

Матеріали та методи. Хімічна частина роботи передбачала поетапне створення цільових продуктів реакції – 3-(етилтіо)-9-метил-6-(алкілтіо)піразоло[1,5-*d*][1,2,4]тріазоло[3,4-*f*][1,2,4]тріазинів. Перший етап мав на меті проведення взаємодії діетилоксалату з

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Key words: 1,2,4-triazole, pyrazole, physico-chemical properties, molecular docking.

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ацетоном за участю натрій метилату в середовищі метанолу. Одержаний етил-2,4-діоксопентаноат на наступному етапі застосували у процесі перетворення у 3-метилпіразол-5-карбогідразид за участю гідразин гідрату. Надалі модифікація молекули полягала в поетапному формуванні структури 4-аміно-5-(3-метилпіразол-5-іл)-1,2,4-тріазол-3-тіолу. Наступний крок передбачав синтез 3-етилтіо-5-(3-метилпіразол-5-іл)-1,2,4-тріазол-4-аміну. Дальше перетворення – одержання калій 3-етилтіо-9-метилпіразоло[1,5-*d*][1,2,4]тріазоло[3,4-*f*][1,2,4]тріазино-6-тіолату та його *S*-алкілпохідних за тріазиновим фрагментом. Як модельні ферменти для докінгу обрали циклооксигеназу-2, ланостерол-14α-деметилазу та рецепторну тирозинкіназу, кристалічну структуру яких завантажили з Protein Data Bank.

Результати. Здійснили синтез 3-(етилтіо)-9-метил-6-(алкілтіо)-піразоло[1,5-*d*][1,2,4]тріазоло[3,4-*f*][1,2,4]тріазинів, визначили оптимальні умови процесу одержання цих речовин. Довели структуру продуктів хімічного перетворення, зафіксували результати дослідження основних фізичних властивостей. Результати віртуальних досліджень обґрунтували перспективність обраного вектора хімічної трансформації, а отже дали змогу визначитися з біологічним потенціалом одержаних сполук.

Висновки. У результаті дослідження отримали інформацію, що формує уявлення про можливий рівень впливу синтезованих сполук на активність ланостерол-14α-деметилази, а отже доцільним є продовження пошуку речовин із фунгістатичною та фунгіцидною діями.

Ключові слова: 1,2,4-тріазол, піразол, фізико-хімічні властивості, молекулярний докінг.

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Heterocycline compounds are an inexhaustible source for obtaining promising biologically active molecules [1–5]. Therefore, the synthesis of new condensed systems with the participation of heterocycles has favorable conditions for active development, because it allows you to create molecules with different types of biological activity [6]. Among such systems, special attention is focused on those that contain Nitrogen in their structure.

Among a significant number of azageterocyclic systems, pyrazole and 1,2,4-triazole are distinguished in a certain way. The use of heterocycles in the process of creating new "drug-like" molecules is justified by the results of numerous studies in the field of prototyping innovative medicines. The formation of this phenomenon is explained by numerous factors. These are the characteristic features of the structure and significant possibilities of various chemical modifications, as well as the ability to form structures with hydrophilic properties. These principles and guidelines for targeted synthesis allowed a significant circle of scientists to obtain certain practically significant results for pharmacy and medicine [7–13]. But, despite the achievement of significant results, there is still a certain list of issues in the segment of creating condensed systems based on pyrazole and 1,2,4-triazole and studying their properties that require solving and comprehensive research.

Aim

The aim of the work was to identify optimal conditions for the production of 3-(ethylthio)-9-methyl-6-(alkylthio)pyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazines and to study the properties of the target reaction products.

Materials and methods

The chemical stage of the scientific work involved the process of its implementation and creation of target products of chemical transformation using classical methods of organic synthesis. Well-known reagents and common solvents were used (*Fig. 1*). Synthetic studies were carried out using reagents of the companies "Merck KGaA" (Germany), "Sigma-Aldrich Chemicals Ltd" (USA) with chemical qualification *purum* or *pro analysis*.

Potassium 3-(ethylthio)-9-methylpyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazine-6-thiolate (3). 0.01 Mol of 3-(ethylthio)-5-(3-methylpyrazol-5-yl)-1,2,4-triazole-4-amine was added to a solution of 0.01 mol of potassium hydroxide in 50 ml of propane-2-ol and stirred until dissolved. After complete dissolution, the mixture was placed in an ice bath and carbon disulfide was added drop by drop. This solution was stirred for 2 hours. Excess alcohol was removed under a vacuum. The resulting yellow crystalline substance was filtered and recrystallized from alcohol.

General procedure for the synthesis of 3-(ethylthio)-9-methyl-6-(alkylthio)-pyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazines (3.1–3.10). An equivalent amount of the corresponding haloalkane ($C_nH_{2n+1}Br$; n = 1–10) was added to a solution of 0.005 mol of potassium 3-(ethylthio)-9-methylpyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazine-6-thiolate in 35 ml of propane-2-ol obtained by heating. The solution then was heated for 1 hour. Excess alcohol was removed under vacuum. The resulting sediment was filtered and recrystallized.

The structure and individuality of the synthesized substances were confirmed by modern methods of analysis. Melting points were determined using open capillaries using the Stanford Research Systems Melting point Apparatus 100 (SRS, USA). Element analysis (C, H, N, S) was performed using an "Elementar vario EL cube" analyzer (Elementary Analysensysteme, Germany) with quantitative determination of components by thermal conductivity on the detector. ^{1}H NMR spectra (400 MHz) were obtained on a "Varian-Mercury 400" spectrometer (Bruker DRX 500, USA) in DMSO-d_6 medium using tetramethylsilane as an internal standard. Chromato-mass spectra were recorded on an "Agilent 1260 Infinity HPLC" liquid chromatograph system (Agilent, Germany) with an "Agilent 6120" spectrometer (Agilent, Germany) (electrospray ionization method (ESI)).

Research *in silico* was a molecular docking method using "Vina" and "Discovery Studio" as software. This method allowed you to navigate the selection of molecules with a certain level of affinity for a certain biological target. Mac-

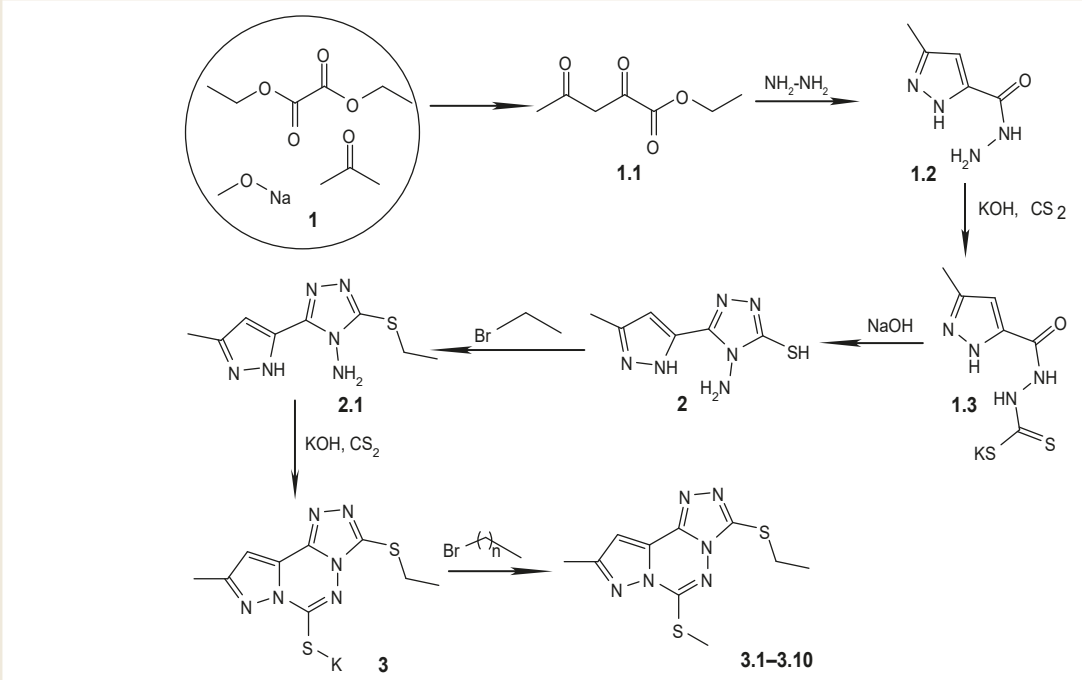

Fig. 1. Synthesis scheme of 3-(ethylthio)-9-methyl-6-(alkylthio)-pyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazines.

romolecules from the Protein Data Bank (PDB) were used as biological targets, namely: a fragment of cyclooxygenase-2 in complex with celecoxib, lanosterol-14α-dimethylase in complex with fluconazole, and receptor tyrosine kinase in complex with crizotinib.

The choice of biological targets is determined by the literature data on the mechanism of action of antifungal agents.

The research methodology consisted of the following stages:

1) ligand preparation: construction of structural formulas of compounds using the program "MarvinSketch 6.3.0" (saving in mol-format); creation of a 3D structure of formulas of compounds-molecular modeling using the program "Hyper Chem 8" based on the method of molecular mechanics MM+ and semi-empirical quantum mechanical method PM3 with the maximum number of cycles and Polak–Ribiere algorithm (saving in pdb-format); application of "AutoDockTools-1.5.6" to convert pdb files to PDBQT;

2) enzyme preparation: removing water and ligand molecules from the crystal using the "Discovery Studio 4.0" software package (saving in pdb format); adding polar Hydrogen atoms and converting the enzyme from the pdb file to PDBQT using "AutoDockTools-1.5.6";

3) molecular docking: performing docking using the "Vina" program; data visualization was performed using the "Discovery Studio 4.0" program.

Results

The stepwise formation of the structure of the Intermediate 3-ethylthio-5-(3-methylpyrazol-5-yl)-1,2,4-triazole-4-amine and the properties of all preintermediates were described in previous works [14,15]. The synthesis of potassium 3-(ethylthio)-9-methylpyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4]triazine-6-thiolate was successfully performed using carbon disulfide in an alkaline alcohol medium (*Fig. 1*).

As part of solving one of the problems that were set in this paper, alkylation for Sulfur was performed on a triazine fragment of compound 3. For this purpose, bromoalkanes were used as an alkylating agent. The reaction was carried out in a propane-2-ol medium when heated for 1 hour.

The synthesized substances are white crystalline powders, soluble in DMFA and DMSO, when heated – in 1,4-dioxane, practically insoluble in water, diethyl ether, and chloroform.

The structure of all synthesized substances was confirmed by physical-chemical methods: ^{1}H NMR spectroscopy and elemental analysis. The purity of the chemical interaction products and their identity were confirmed by chromato-mass spectra.

3.1: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.35 (s, CH, pyrazole), 3.22 (q, J = 6.2 Hz, 2H, CH_2CH_3), 2.61 (s, 3H, SCH_3), 2.54 (s, 3H, CH_3), 1.37 (t, J = 12.5 Hz, 3H, CH_2CH_3). Anal. calcd. (%) for $C_{10}H_{12}N_6S_2$ ESI-MS: m/z = 281 $[M+H]^+$. Elemental Analysis: C, 42.84; H, 4.31; N, 29.98; S, 22.87. Found: C, 42.95; H, 4.32; N, 29.90; S, 22.81.

3.2: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.36 (s, CH, pyrazole), 3.22 (q, J = 6.2 Hz, 2H, CH_2CH_3), 3.13 (q, J = 5.5 Hz, 2H, SCH_2CH_3), 2.54 (s, 3H, CH_3), 1.38 – 1.31 (m, 6H, $2CH_2CH_3$). Anal. calcd. (%) for $C_{11}H_{14}N_6S_2$ ESI-MS: m/z = 295 $[M+H]^+$. Elemental Analysis: C, 44.88; H, 4.79; N, 28.55; S, 21.78. Found: C, 44.76; H, 4.78; N, 28.48; S, 21.84.

3.3: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.35 (s, CH, pyrazole), 3.22 (q, J = 6.2 Hz, 2H, CH_2CH_3), 3.14 (t, J = 4.6 Hz, 2H,

$SCH_2CH_2CH_3$), 2.54 (s, 3H, CH_3), 1.77 – 1.71 (m, 2H, $SCH_2CH_2CH_3$), 1.37 (t, J = 6.2 Hz, 3H, CH_2CH_3), 1.07 (t, J = 7.2 Hz, 3H, $S(CH_2)_2CH_3$). Anal. calcd. (%) for $C_{12}H_{16}N_6S_2$ ESI-MS: m/z = 309 $[M+H]^+$. Elemental Analysis: C, 46.73; H, 5.23; N, 27.25; S, 20.79. Found: C, 46.86; H, 5.24; N, 27.17; S, 20.73.

3.4: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.37 (s, CH, pyrazole), 3.12 (t, J = 4.6 Hz, 2H, $SCH_2(CH_2)_2CH_3$), 3.21 (q, J = 5.8 Hz, 2H, CH_2CH_3), 2.54 (s, 3H, CH_3), 1.69 – 1.61 (m, 2H, $SCH_2CH_2CH_2CH_2CH_3$), 1.39 – 1.33 (m, 4H, CH_2CH_3, $S(CH_2)_2CH_2CH_3$), 0.92 (t, J = 7.1 Hz, 3H, $S(CH_2)_3CH_3$). Anal. calcd. (%) for $C_{13}H_{18}N_6S_2$ ESI-MS: m/z = 323 $[M+H]^+$. Elemental Analysis: C, 48.42; H, 5.63; N, 26.06; S, 19.89. Found: C, 48.29; H, 5.62; N, 26.13; S, 19.94.

3.5: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.36 (s, CH, pyrazole), 3.27 – 3.18 (m, 4H, CH_2CH_3, $SCH_2(CH_2)_3CH_3$), 2.54 (s, 3H, CH_3), 1.78 – 1.70 (m, 2H, $SCH_2CH_2(CH_2)_2CH_3$), 1.41 – 1.33 (m, 7H, CH_2CH_3, $SCH_2CH_2(CH_2)_2CH_3$), 0.89 (t, J = 7.1 Hz, 3H, $S(CH_2)_4CH_3$). Anal. calcd. (%) for $C_{14}H_{20}N_6S_2$ ESI-MS: m/z = 337 $[M+H]^+$. Elemental Analysis: C, 49.98; H, 5.99; N, 24.98; S, 19.06. Found: C, 49.83; H, 6.00; N, 25.05; S, 19.01.

3.6: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.36 (s, CH, pyrazole), 3.26 – 3.17 (m, 4H, CH_2CH_3, $SCH_2(CH_2)_4CH_3$), 2.54 (s, 3H, CH_3), 1.65 – 1.57 (m, 2H, $SCH_2CH_2(CH_2)_3CH_3$), 1.42 – 1.32 (m, 9H, CH_2CH_3, $S(CH_2)_2(CH_2)_3CH_3$), 0.94 – 0.84 (m, 3H, $S(CH_2)_5CH_3$). Anal. calcd. (%) for $C_{15}H_{22}N_6S_2$ ESI-MS: m/z = 351 $[M+H]^+$. Elemental Analysis: C, 51.40; H, 6.33; N, 23.98; S, 18.29. Found: C, 51.53; H, 6.34; N, 23.92; S, 18.24.

3.7: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.35 (s, CH, pyrazole), 3.27 – 3.20 (m, 4H, CH_2CH_3, $SCH_2(CH_2)_5CH_3$), 2.55 (s, 3H, CH_3), 1.65 – 1.57 (m, 2H, $SCH_2CH_2(CH_2)_4CH_3$), 1.37 – 1.25 (m, 11H, CH_2CH_3, $S(CH_2)_2(CH_2)_4CH_3$), 0.92 – 0.86 (m, 3H, $S(CH_2)_6CH_3$). Anal. calcd. (%) for $C_{16}H_{24}N_6S_2$ ESI-MS: m/z = 365 $[M+H]^+$. Elemental Analysis: C, 52.72; H, 6.64; N, 23.05; S, 17.59. Found: C, 52.57; H, 6.63; N, 22.99; S, 17.64.

3.8: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.37 (s, CH, pyrazole), 3.27 – 3.19 (m, 4H, CH_2CH_3, $SCH_2(CH_2)_6CH_3$), 2.55 (s, 3H, CH_3), 1.65 – 1.57 (m, 2H, $SCH_2CH_2(CH_2)_5CH_3$), 1.39 – 1.28 (m, 13H, CH_2CH_3, $S(CH_2)_2(CH_2)_5CH_3$), 0.94 – 0.85 (m, 3H, $S(CH_2)_7CH_3$). Anal. calcd. (%) for $C_{17}H_{26}N_6S_2$ ESI-MS: m/z = 379 $[M+H]^+$. Elemental Analysis: C, 53.94; H, 6.92; N, 22.20; S, 16.94. Found: C, 54.09; H, 6.91; N, 22.14; S, 16.99.

3.9: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.37 (s, CH, pyrazole), 3.28 – 3.20 (m, 4H, CH_2CH_3, $SCH_2(CH_2)_6CH_3$), 2.54 (s, 3H, CH_3), 1.65 – 1.57 (m, 2H, $SCH_2CH_2(CH_2)_6CH_3$), 1.40 – 1.33 (m, 15H, CH_2CH_3, $S(CH_2)_2(CH_2)_6CH_3$), 0.94 – 0.85 (m, 3H, $S(CH_2)_8CH_3$). Anal. calcd. (%) for $C_{18}H_{28}N_6S_2$ ESI-MS: m/z = 393 $[M+H]^+$. Elemental Analysis: C, 55.07; H, 7.19; N, 21.41; S, 16.33. Found: C, 54.93; H, 7.18; N, 21.46; S, 16.37.

3.10: ^{1}H NMR, *δ (ppm), J (Hz)*: 7.36 (s, CH, pyrazole), 3.27 – 3.18 (m, 4H, CH_2CH_3, $SCH_2(CH_2)_7CH_3$), 2.56 (s, 3H, CH_3), 1.63 – 1.57 (m, 2H, $SCH_2CH_2(CH_2)_7CH_3$), 1.40 – 1.27 (m, 17H, CH_2CH_3, $S(CH_2)_2(CH_2)_7CH_3$), 0.94 – 0.85 (m, 3H, $S(CH_2)_9CH_3$). ESI-MS: m/z = 407 $[M+H]^+$. Analytical calculated (%) for $C_{19}H_{30}N_6S_2$: C, 56.12; H, 7.44; N, 20.67; S, 15.77. Found: C, 56.27; H, 7.45; N, 20.61; S, 15.73.

Methods for creating innovative drugs ("drug-design") include molecular docking, molecular modeling, and molecular dynamics to elucidate the activity of promising molecules, establish biological targets for ligand binding, and rationally create potential drug substance candidates.

It should be noted that the synthesized structures have some similarities with the corresponding drugs, which exhibit anti-inflammatory (celecoxib), antifungal (fluconazole), and anti-cancer (crizotinib) activity. At the same time, these drugs have a wide range of side effects. Therefore, the creation of new medicines with such types of activities is relevant.

Complexes of synthesized compounds with cyclooxygenase-2, lanosterol-14α-demethylase, and receptor tyrosine kinase were analyzed by the ligand-receptor binding energy and evaluated visually [16–18].

Analysis of the results of docking studies demonstrates a wide range of amino acid residues of the active site of cyclooxygenase-2, which can take part in the formation of bonds with synthesized ligands (*Table 1*).

The completeness of the idea of the possibility of interaction of synthesized structures with the active COX-2 center was supplemented by quantitative indicators (*Table 2*).

Among antifungal drugs, a significant percentage are built on the basis of various heterocyclic fragments. Among such fragments, nitrogen-containing compounds occupy an important place. This interaction leads to a violation of the functions of lanosterol-14α-demethylase, which leads to blocking the synthesis of ergosterol, which is an important structural component of the fungal cell membrane and ensures the implementation of its important functions.

The nature of amino acid fragments of lanosterol-14α-demethylase, which were probably responsible for interaction with synthesized ligands, has been established (*Table 3*).

The obtained energy indicators of intermolecular interactions confirm a significant probability of detecting antifungal properties in a number of synthesized compounds (*Table 4*).

The obtained compounds were also docked relative to the receptor tyrosine kinase, which made it possible to establish the nature of the actual amino acid fragments (*Table 5*).

The obtained energy values of intermolecular interactions indicate a diverse level of probability of detecting compounds with the ability to actively influence the activity of receptor tyrosine kinase (*Table 6*).

Discussion

^{1}H NMR spectra of all synthesized compounds were characterized by the presence of singlet signals due to the Hydrogen of the pyrazole fragment (7.37–7.35 ppm) and the methyl group associated with this heterocycle (2.56–2.54 ppm). The signal intensity in this case corresponds to 1 and 3 protons, respectively. The spectra of the obtained compounds also contain thioethyl substitute signals in the form of a two-proton quadruplet (3.22 ppm) and a three-proton triplet (1.37 ppm). But in most cases, the signals of this fragment resonate together with the protons of methylene groups of

Table 1. Amino acid residues of the active COX-2 center involved in interaction with the studied compounds

No.	Nature of the amino acid residue
3.1	ALA A: 517, ALA A: 528, HIS A: 90, LEU A: 353, LEU A: 532, PHE A: 519, SER A: 354, TRP A: 388, TYR A: 386, VAL A: 350, VAL A: 524
3.2	ALA A: 517, ALA A: 528, HIS A: 90, LEU A: 385, LEU A: 532, PHE A: 519, SER A: 354, TRP A: 388, TYR A: 386, VAL A: 350, VAL A: 524
3.3	ALA A: 517, ALA A: 528, ARG A: 514, HIS A: 90, LEU A: 353, LEU A: 532, PHE A: 519, SER A: 354, TYR A: 356, VAL A: 117, VAL A: 350, VAL A: 524
3.4	ALA A: 517, ALA A: 528, ARG A: 514, LEU A: 353, LEU A: 532, PHE A: 519, SER A: 354, TYR A: 356, VAL A: 117, VAL A: 350, VAL A: 524
3.5	ALA A: 517, ALA A: 528, ARG A: 514, LEU A: 353, LEU A: 532, PHE A: 519, SER A: 354, TYR A: 356, VAL A: 117, VAL A: 350, VAL A: 524
3.6	ALA A: 528, ARG A: 121, ILE A: 113, LEU A: 93, LEU A: 353, PRO A: 529, TRP A: 100, TYR A: 116, TYR A: 349, TYR A: 356, VAL A: 89, VAL A: 117, VAL A: 350
3.7	ALA A: 528, ARG A: 121, ILE A: 113, LEU A: 93, LEU A: 353, PRO A: 529, TRP A: 100, TYR A: 116, TYR A: 349, TYR A: 356, TYR A: 386, VAL A: 89, VAL A: 117, VAL A: 350
3.8	ALA A: 528, ARG A: 121, ILE A: 113, LEU A: 93, LEU A: 353, PRO A: 529, TRP A: 100, TRP A: 388, TYR A: 116, TYR A: 349, TYR A: 356, TYR A: 386, VAL A: 350
3.9	ALA A: 528, HIS A: 90, ILE A: 113, LEU A: 93, LEU A: 353, LEU A: 535, PHE A: 206, TYR A: 116, TYR A: 349, TYR A: 356, VAL A: 117, VAL A: 345, VAL A: 350, VAL A: 524
3.10	ALA A: 528, ILE A: 113, LEU A: 93, LEU A: 353, LEU A: 535, PHE A: 206, SER A: 531, TYR A: 116, TYR A: 349, TYR A: 356, VAL A: 117, VAL A: 345, VAL A: 350, VAL A: 524

Table 2. Energy value of intermolecular interactions of the studied compounds with cyclooxygenase-2

No.	E_{min}	N	E_{min}	N	E_{min}
3.1	-6.9	3.4	-4.9	3.8	-3.8
3.2	-6.4	3.5	-5.0	3.9	-4.3
3.3	-5.8	3.6	-3.9	3.10	-4.7
Celecoxib	-13.4	3.7	-3.7		

E_{min}: minimum complexation energy, kcal/mol.

Table 3. Amino acid residues of the active lanosterol-14α-demethylase center, which are involved in interaction with the studied compounds

No.	Nature of the amino acid residue
3.1	ALA A: 256, ALA A: 400, CYS A: 394, GLY A: 396, LEU A: 100, LEU A: 152, PHE A: 387, PRO A: 320, VAL A: 395
3.2	ALA A: 256, ALA A: 400, ARG A: 96, CYS A: 394, GLY A: 396, LEU A: 100, LEU A: 152, PHE A: 387, PRO A: 320, SER A: 261, VAL A: 395
3.3	ALA A: 256, CYS A: 394, LEU A: 100, LEU A: 321, LEU A: 324, LEU A: 315, PHE A: 387, PRO A: 320, PRO A: 349
3.4	ALA A: 256, ALA A: 400, CYS A: 394, LEU A: 315, LEU A: 321, LEU A: 324, PHE A: 83, PHE A: 387, PRO A: 320
3.5	ALA A: 256, ALA A: 400, CYS A: 394, ILE A: 404, LEU A: 315, LEU A: 324, PHE A: 387
3.6	ALA A: 400, CYS A: 394, ILE A: 404, LEU A: 311, LEU A: 315, LEU A: 321, LEU A: 324, PHE A: 83, PHE A: 387
3.7	ALA A: 400, CYS A: 394, ILE A: 404, LEU A: 311, LEU A: 315, LEU A: 321, LEU A: 324, PHE A: 83, PHE A: 387
3.8	ARG A: 96, HIS A: 392, ILE A: 323, LEU A: 321, MET A: 79, MET A: 433, PHE A: 78, PHE A: 83, TYR A: 76, VAL A: 395
3.9	ARG A: 96, HIS A: 392, ILE A: 323, LEU A: 321, MET A: 79, MET A: 433, PHE A: 78, TYR A: 76, VAL A: 395
3.10	ALA A: 400, CYS A: 394, LEU A: 321, LEU A: 324, MET A: 79, PHE A: 83, TYR A: 76

Table 4. Energy values of intermolecular interactions of the studied compounds with lanosterol-14α-demethylase (3LD6)

No.	E_{min}	N	E_{min}	N	E_{min}
3.1	-7.2	3.4	-8.0	3.8	-7.9
3.2	-7.6	3.5	-8.1	3.9	-8.0
3.3	-7.7	3.6	-7.7	3.10	-5.4
Fluconazole	-8.4	3.7	-7.6		

E_{min}: minimum complexation energy, kcal/mol.

Table 5. Amino acid residues of the active center of the receptor tyrosine kinase, which are involved in interaction with the studied compounds

No.	Nature of amino acid recidue
3.1	ALA A: 1148, LEU A: 1122, LEU A: 1256, VAL A: 1130
3.2	ALA A: 1148, LEU A: 1122, LEU A: 1256, VAL A: 1130
3.3	ALA A: 1148, LEU A: 1122, LEU A: 1196, LEU A: 1256, VAL A: 1130
3.4	ALA A: 1148, ARG A: 1253, LEU A: 1122, LEU A: 1256, VAL A: 1130
3.5	ALA A: 1148, ALA A: 1200, LEU A: 1122, LEU A: 1196, LEU A: 1198, LEU A: 1256, VAL A: 1130
3.6	LEU A: 1122, LEU A: 1196, LEU A: 1198, LEU A: 1256, LYS A: 1150, VAL A: 1130
3.7	LEU A: 1122, LEU A: 1196, LEU A: 1198, LEU A: 1256, LYS A: 1150, VAL A: 1130
3.8	ALA A: 1130, ALA A: 1148, LEU A: 1122, LEU A: 1196, LEU A: 1256, MET A: 1199, VAL A: 1130
3.9	ALA A: 1200, ARG A: 1253, LEU A: 1122, LEU A: 1196, LEU A: 1198, LEU A: 1256, VAL A: 1130
3.10	LEU A: 1122, LEU A: 1196, LEU A: 1256, VAL A: 1130

Table 6. Energy value of intermolecular interactions of the studied compounds with receptor tyrosine kinase

No.	E_{min}	N	E_{min}	N	E_{min}
3.1	-5.6	3.4	-7.0	3.8	-5.9
3.2	-6.5	3.5	-7.4	3.9	-8.5
3.3	-5.8	3.6	-7.8	3.10	-5.9
Krizotonib	-10.8	3.7	-6.0		

E_{min}: minimum complexation energy, kcal/mol.

another thioalkyl substitute, forming a complex multiproton multiplet (1.42–1.25 ppm).

In the ^{1}H NMR spectra of compounds 3.3 and 3.4, proton signals of the methylene fragment directly bound to sulfur in the triazine fragment are observed. The signal is formed in the form of a two-proton quadruplet and is fixed at 3.22–3.21 ppm in compounds 3.5–3.10, this methylene fragment, together with the proton signals of a methylene fragment of another thioalkyl fragment, forms a multiplet at 3.28–3.17 ppm also in compounds 3.3–3.10, the signals of the next methylene group of the thioalkyl substitute with a triazine fragment are recorded in a separate multiplet. It should be noted that an increase in the length of the thioalkyl fragment is accompanied by a gradual shift of the signal to a stronger-field section of the spectrum. So, in the spectrum of compound 3.3, this multiplet is prescribed in the strong field region at 1.77–1.71 ppm, for compound 3.10 – at 1.63–1.57 ppm.

Protons of the methyl group, which is part of the thioalkyl substituent structure in the triazine fragment, form a signal in the form of a singlet (3.1), triplet (3.3–3.5) or multiplet (3.2, 3.6–3.10). These signals are recorded at 2.61 ppm (3.1), 1.38–1.31 ppm (3.2), 1.07–0.89 ppm (3.3–3.5) and 0.94–0.85 ppm (3.6–3.10), respectively.

The chromato-mass spectra of the synthesized compounds contain a signal of the quasi-molecular ion [M+1], which, according to the m/z value, corresponds to the molecular masses of the presented substances.

Visualization of the interaction of structures with the active site of cyclooxygenase-2 showed that they have a significant spectrum of interactions with a significant number of amino acid residues.

For example, the hydrophobic alkyl interaction of compound 3.1 was realized with the participation of the thiomethyl fragment of the ligand and the amino acid residue TYR A: 386 and the thioethyl fragment of the specified ligand and the amino acid residues ALA A: 517 and HIS A: 90. Among the hydrophobic interactions, a π-alkyl can also be noted. This interaction was provided by a triazine fragment with residues ALA A: 528 and LEU A: 350. The amino acid residue ALA A: 528 also provided a π-alkyl interaction with the triazole fragment. Coordination at the active COX-2 site of compound 3.1 was also determined by the π-th interaction, which was realized with the participation of the VAL A: 524 residue and the triazine fragment. Carbon-Hydrogen stacking with the participation of the SER A: 354 residue enhances the presented interaction.

Elongation of the alkyl fragment was accompanied by the formation of additional interactions, namely, intermolecular hydrogen bonds and Van der Waals forces appear. The first type of interaction occurred with the participation of the TYR A: 356 residue. Here, the hydrogen bond of the ligand was formed with the participation of electronegative atoms of the triazine fragment of nitrogen and the Sulfur of the thiopropyl substitute. The second type of interaction was formed with the participation of the SER A: 354 residue. It promoted the orientation of compound 3.3 at the active site and was enhanced by amide-π stacking with the participation of the LEU A: 353 residue. These were alkyl, π-alkyl, and π-Sulphur interactions. The first type of interaction was re-

alized using residues ALA A: 528, LEU A: 532, VAL A: 117 (with a thiopropyl fragment). The second was using ALA A: 517, ARG A: 514, HIS A: 90 (with a thioethyl substitute). The third was realized with the participation of the PHE A: 519 residue in contact with thioethyl substituent sulfur.

Quantitative indicators of the minimum free binding energy of synthesized compounds to COX-2 were in the range of -3.8 ... -6.9 kcal/mol, which indicates a low probability of detecting substances with anti-inflammatory activity in a number of synthesized compounds (**3.1–3.10**) (*Table 2*).

The position of the obtained ligands in the active site of lanosterol-14α-demethylase was provided by a number of known interactions, among which we can note: alkyl, π-alkyl, π-σ and π-sulfur, as well as intermolecular hydrogen chemical bonds. For example, the alkyl interaction of compound **3.1** was realized with the participation of amino acid residues ALA A: 256, LEU A: 100, LEU A: 152, which were coordinated with the methyl group of the pyrazole fragment, VAL A: 395 – with the methyl group of the thiomethyl substitute, ALA A: 400, PRO A: 320 – with the methyl group of the thioethyl substitute. The π-alkyl interaction of compound **3.1** was realized by alanine residues ALA A: 256 and ALA A: 400 in coordination with fragments of triazole and triazine, respectively. The π-σ-interaction, which occurred with the participation of the GLY A: 396 residue and the triazine fragment, can also have a positive effect on the possible activity of this compound. Docking also demonstrated the formation of the π-Sulphur interaction, which for compound **3.1** was realized with the active participation of PHE A: 386 and the thioethyl substitute and with the participation of CYS A: 394 and the triazine fragment. An increase in the length of the alkyl residue was first accompanied by an increase in the number of intermolecular hydrogen chemical bonds (**3.2**) and then their disappearance. But sometimes this type of bond was formed again, for example, in compounds **3.5** and **3.10** (involving CYS A: 394 and the triazine fragment).

Taking into account the calculated values of the free binding energy for complexes formed by 3-(ethylthio)-9-methyl-6-(alkylthio)-pyrazolo[1,5-*d*][1,2,4]triazolo[3,4-*f*][1,2,4] triazines and lanosterol-14α-demethylase receptors, it can be assumed that this hydrogen bond may be associated with the activity of compound **3.5** (*Table 4*). Compounds **3.4** and **3.9** can also be considered promising for further studies of antifungal activity (*Table 4*).

Analysis of docking with the active site of the receptor tyrosine kinase allows us to state a certain level of uniformity of interactions. These include alkyl, π-alkyl and π-σ interactions. And only for compound **3.8**, stabilization in the active center of this enzyme was additionally provided by an intermolecular hydrogen bond. This interaction was provided by the residue MET A: 1199. If we tell about other types of interactions that were defined for this compound, then we can note the alkyl interaction here. This type of coordination was due to the residues ALA A: 1148, LEU A: 1196, VAL A: 1130 (with a thioethyl substitute), LEU A: 1122 (with the methyl group of the pyrazole fragment). At the same time, VAL A: 1130 provided an alkyl type of interaction with a thiooctile substitute. It was also necessary to mention the π-alkyl interaction provided by the residues ALA A: 1148, LEU A: 1122 (with a triazole fragment), LEU A: 1196, LEU A: 1122, VAL A: 1130 (with a triazine fragment). Changing the length of the carbon chain of a thioalkyl fragment was not possible it showed a significant effect on the nature and amount of amino acid residues in the active center of tyrosine kinase.

The minimum free binding energy of the synthesized compounds (**3.1–3.10**) to the receptor tyrosine kinase was in the range from -5.8 to -8.5 kcal/mol (*Table 6*). Compound **3.9** was the most promising for further research.

Thus, the obtained results of molecular docking allow us to justify the choice of haloalkanes for the design of target products of alkylation reactions in order to create promising biologically active molecules. In a number of synthesized compounds, the biological potential can vary significantly, which may be due to changes in the position of the ligand in the active center. This, in turn, affects the number of chemical bonds that form between the amino acid residues of the enzyme and the structure of the synthesized substance. After evaluating the possible effect on the activity of the three enzymes, it was necessary to pay attention to the length of the thioalkyl substitute combined with the triazine fragment. Thus, in the case of cyclooxygenase-2, the active ligand may be a compound with a short alkyl substitute, for example, methyl or ethyl. If it was lanosterol-14α-demethylase or receptor tyrosine kinase, then alkyl substituents with the number of Carbon atoms from 5 to 9 contribute to the interaction with these enzymes.

Conclusions

1. A simple construction of a poly heterocyclic structure was presented, which successfully combines fragments of pyrazole, 1,2,4-triazole, and 1,2,4-triazine and acts as the initial compound for obtaining a range of promising alkyl derivatives.

2. The conducted molecular docking made it possible to determine compounds with a high potential of antifungal activity in a number of synthesized substances.

3. The ability to influence the activity of the receptor tyrosine kinase 3-(ethyltio)-9-methyl-6-(nonyltio)-pyrazolo[1,5-*d*][1,2,4]-triazolo[3,4-*f*][1,2,4]triazine was demonstrated.

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