Synthesis and properties of *S*-alkyl 4-amino-5-(5-(3-fluorophenyl)-pyrazol-3-yl)-1,2,4-triazole-3-thiol derivatives

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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

An important direction of modern pharmaceutical science is the creation of promising biologically active compounds, which in the hands of scientists can be transformed into effective medicinal products. Heterocyclic compounds are the undisputed leader in solving this problem. A well-known fact and a well-founded approach to achieving the desired pharmacological effect is the combination of different heterocyclic fragments in the structure of one molecule. And here it makes sense to focus our attention on such heterocycles as pyrazole and 1,2,4-triazole. After all, a number of well-known medicines have already been invented on their basis. Thus, the construction of a chemical tandem with heterocyclic blocks of the specified nature is an actual and promising direction of scientific work.

The aim of the work was to create a number of *S*-alkyl derivatives of 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol and study their properties, as well as preliminary selective establishment biological potential of these compounds.

Materials and methods. The synthesis of the target products of chemical transformation was successfully implemented by the step-by-step use of well-known methods of organic synthesis. Thus, the first stage was successfully implemented with the help of available reagents, the role of which was performed by diethyl oxalate and 1-(3-fluorophenyl)ethan-1-one with the participation of sodium methylate. The next stage involved hydrazinolysis. Subsequently, the corresponding potassium xanthogenate was successfully synthesized, which was subsequently transformed under the action of hydrazine hydrate into the target 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol. The next stage was *S*-alkylation. The structure of all synthesized substances was determined with IR spectrophotometry, ^{1}H NMR spectroscopy, and elemental analysis. The individuality of the compounds was confirmed by high-performance liquid chromatography-mass spectrometry. In silico studies were carried out with well-known software products, namely: AutoDock Vina, Biovia Discovery Studio, Hyper Chem 7.5, and Open Babel. Cyclooxygenase-2, lanosterol 14α-demethylase, and anaplastic lymphoma kinase were used as model enzymes.

Results. The optimal conditions for the stepwise creation of *S*-alkyl derivatives of 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol were established and the preparation of the specified compounds was carried out. The use of molecular docking made it possible to determine the perspective of further research on anti-inflammatory, antifungal, and antitumor properties in a number of synthesized structures.

Conclusions. *S*-alkyl derivatives of 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol are reasonably promising objects for the study of antifungal activity.

Key words: 1,2,4-triazole, pyrazole, properties, computer simulation.

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Синтез і властивості похідних S-алкіл 4-аміно-5-(5-(3-фторфеніл)піразол-3-іл)-1,2,4-тріазол-3-тіолу

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Важливий напрям сучасної фармацевтичної науки – створення перспективних біологічно активних сполук, які можна перетворити на ефективні лікарські засоби. Беззаперечним лідером у цьому аспекті є гетероциклічні сполуки. Відомий і цілком обґрунтований підхід до досягнення необхідного фармакологічного ефекту – поєднання в структурі однієї молекули різних гетероциклічних фрагментів. Перспективними є такі гетероцикли, як піразол і 1,2,4-тріазол, адже на їхній основі вже розроблено низку відомих ліків. Отже, побудова хімічного тандему з гетероциклічними блоками цієї природи – актуальний і перспективний напрям наукової роботи.

Мета роботи – створення ряду S-алкілпохідних 4-аміно-5-(5-(3-фторфеніл)піразол-3-іл)-1,2,4-тріазол-3-тіолу та дослідження їхніх властивостей, а також попереднє вибіркове встановлення біологічного потенціалу цих сполук.

Матеріали та методи. Синтез цільових продуктів хімічного перетворення успішно реалізовано шляхом поетапного використання відомих методів органічного синтезу. Перший етап успішно реалізовано за допомогою доступних реагентів – діетилоксалату і 1-(3-фторфеніл)етан-1-ону за участі метилату натрію. Наступний етап передбачав гідразиноліз. Згодом успішно синтезовано

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Key words: 1,2,4-triazole, pyrazole, properties, computer simulation.

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відповідний ксантогенат калію, який надалі під дією гідразингідрату перетворювали на цільовий 4-аміно-5-(5-(3-фторфеніл)піразол-3-іл)-1,2,4-тріазол-3-тіол. Наступний етап – S-алкілування. Структуру всіх синтезованих речовин визначили за допомогою ІЧ-спектрофотометрії, ЯМР-спектроскопії ^{1}H та елементного аналізу. Індивідуальність сполук підтверджено методом високоефективної рідинної хромато-мас-спектрометрії. Дослідження in silico здійснили, застосувавши відомі програмні продукти: AutoDock Vina, Biovia Discovery Studio, Hyper Chem 7.5 та Open Babel. Як модельні ферменти використовували циклооксигеназу-2, ланостерол 14α-деметилазу та кіназу анапластичної лімфоми.

Результати. Встановлено оптимальні умови поетапного створення S-алкілпохідних 4-аміно-5-(5-(3-фторфеніл)піразол-3-іл)-1,2,4-тріазол-3-тіолу, одержано ці сполуки. Застосування молекулярного докінгу дало змогу визначити перспективи наступних досліджень протизапальних, протигрибкових і протипухлинних властивостей ряду синтезованих структур.

Висновки. S-алкілпохідні 4-аміно-5-(5-(3-фторфеніл)піразол-3-іл)-1,2,4-тріазол-3-тіолу – досить перспективні об'єкти для вивчення протигрибкової активності.

Ключові слова: 1,2,4-тріазол, піразол, властивості, in silico дослідження.

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Derivatives of 1,2,4-triazole show a wide range of biological activity [1–4]. Obtaining condensed systems based on this heterocycle increases the probability of creating a promising biologically active substance [5–8]. The specified direction of research led to the successful introduction into medical practice of such drugs as triazolam, alprazolam, estazolam, brotizolam and sitagliptin. Special attention of scientists is also focused on the detection of anticancer properties of 1,2,4-triazole derivatives, including condensed systems with their participation [9–11].

In general, 1,2,4-triazole derivatives are characterized by versatile possibilities of chemical modification, which is not least due to the active use of the "hybrid-pharmacophore" approach to the construction of new molecules. This information is confirmed by numerous scientific works of domestic and foreign scientists [12–15]. Competent selection of the accompanying heterocycle makes it possible to enhance the desired properties or lead to their occurrence. And here the pyrazole fragment can come in handy, which significantly expands the synthetic possibilities and improves the pharmacological profile of the target products of chemical transformation. But despite the significant interest of the scientific community in combining the pyrazole fragment with condensed systems based on 1,2,4-triazole, the level of practical results remains insignificant.

Thus, the relevance of conducting research in the field of synthetic chemistry of condensed 1,2,4-triazole systems, which are combined with pyrazole, is undeniable.

Aim

The aim of the work was to create a number of *S*-alkyl derivatives of 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol and study their properties, as well as preliminary selective establishment biological potential of these compounds.

Materials and methods

Part of the work, which is devoted to the synthesis of target substances, is based on the use of common methods of organic synthesis. Reagents and solvents were used from known suppliers ("UKRORGSYNTEZ Ltd") and were further purified if necessary.

1-(3-Fluorophenyl)ethan-1-one, diethyl oxalate and sodium methylate were used as reagents in the first stage of chemical transformation. The interaction was carried out in the medium of methanol. The second stage involved conducting a reaction with the calculated amount of hydrazine hydrate. The third stage was based on the interaction of the synthesized hydrazide with carbon disulfide in a 9 % butan-1-ol solution. The successfully isolated xanthogenate with twice the amount of hydrazine hydrate further formed 4-amino-5-(3-(3-fluorophenyl)pyrazol-5-yl)-1,2,4-triazole-3-thiol. The next stage was carried out in an ethanol environment and involved interaction with haloalkanes (*Fig. 1*).

To confirm the structure of all synthesized compounds, proton nuclear magnetic resonance (^{1}H NMR) and infrared (IR) spectra were recorded, and elemental analysis was also performed. The individual character of the obtained substances and the degree of their purity were confirmed using the data of chromato-mass spectra.

Melting points were determined in open capillaries with "Stanford Research Systems Melting Point Apparatus 100" (SRS, USA). Analysis of the percentage content of elements (C, H, N, S) was performed with the "Elementar vario EL cube" analyzer (Elementar Analysensysteme, Germany). IR spectroscopy (spectral range 4000–400 cm^{-1}) was performed on the basis of the Bruker ALPHA FT-IR spectrometer with the ALPHA-T module (Bruker optics, Germany). ^{1}H NMR spectra were recorded on a Varian-Mercury 400 spectrometer with tetramethylsilane as an internal standard in DMSO-d_6 solution. Chromatograph "Agilent 1260 Infinity HPLC" with spectrometer "Agilent 6120" made it possible to obtain chromato-mass spectra (ionization method – electrospray (ESI)).

Molecular docking. To identify molecules with increased positive tropism to certain biological targets, the molecular docking method was implemented. Macromolecules from the Protein Data Bank (PDB) were used as biological targets, namely: fragments of cyclooxygenase-2 in complex with celecoxib, lanosterol 14α-demethylase in complex with fluconazole and anaplastic lymphoma kinase in complex with crizotinib [16–18].

The similarity of the synthesized compounds to the structure of known drugs with anti-inflammatory, antifungal and anti-cancer activity determined the choice of models of appro-

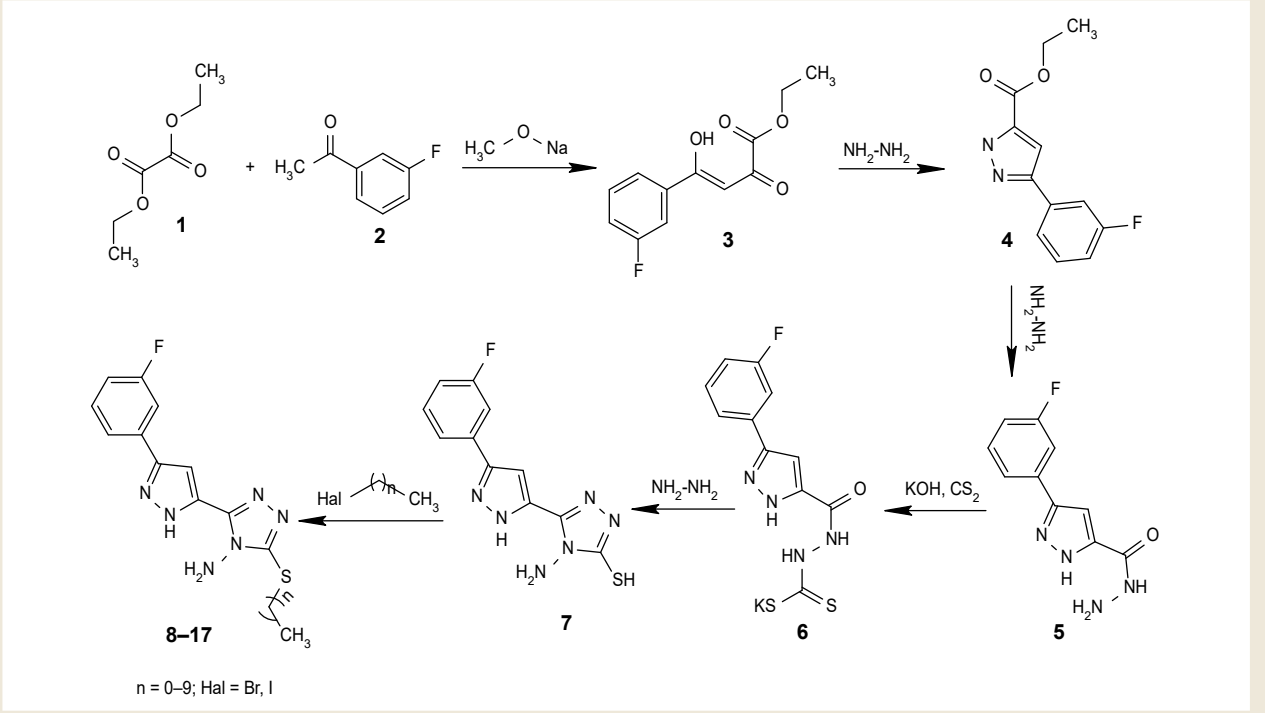

Fig. 1. Synthesis scheme of *S*-alkyl derivatives of 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol.

priate biotargets. Thus, in the structure of celecoxib, which is a selective cyclooxygenase-2 inhibitor, a pyrazole fragment is present. The well-known antifungal drugs fluconazole and voriconazole, which are inhibitors of lanosterol 14α-demethylase, are built with the participation of the 1,2,4-triazole ring. A representative of anaplastic lymphoma kinase inhibitors, crizotinib, also has a pyrazole ring in its structure.

The implementation of *in silico* studies involved the following algorithm:

1) working with the ligand: forming structural formulas of compounds with the MarvinSketch 6.3.0 program and saving them in mol format; preparation of the 3D structure of compounds – molecular modeling (Hyper Chem 8 program using the MM+ molecular mechanics method and the semi-empirical quantum mechanical method PM3 with the maximum number of cycles and the Polak-Ribiere algorithm and saving in PDB file format); conversion of PDB- to PDBQT-files using the software product AutoDockTools-1.5.6;

2) working with the enzyme: elimination of water molecules and ligands from the file with the Discovery Studio 4.0 software package and saving the enzyme in PDB format; converting enzyme from PDB to PDBQT file using AutoDockTools-1.5.6;

3) molecular docking: with the "Vina" program; creation of visual objects with the Discovery Studio 4.0 software tool.

Results

Ethyl 4-(3-fluorophenyl)-2,4-dioxobutanoate (3). Sodium hydride (0.2 mol) was added to a mixture of 1-(3-fluorophenyl)ethan-1-one (0.10 mol) and diethyl oxalate (0.15 mol) in 60 ml of anhydrous toluene. The resulting mixture was stirred at 30 °C for 8 hours. The solvent was removed under a vacuum. The crude mixture was poured into 100 ml of ice water and acidified with dilute hydrochloric acid. The resulting crystalline precipitate was filtered and washed with water. It was crystallized from methanol. Yellow crystalline substance. Yield – 83 %. M. p.: 96–98 °C. IR (cm^{-1}): 1736 (C=O, ester), 1687 (C=O, ketone), 1445 (C=C). ^{1}H NMR, *δ (ppm), J (Hz)*: 15.10 (s, 1H, OH), 7.62–7.36 (m, 4H, 3-F-C_6H_4), 6.45 (s, 1H, =CH), 4.27 (q, J = 6.9 Hz, 2H, OCH_2CH_3), 1.18 (t, 3H, OCH_2CH_3). Analytical calculated (%) for ($C_{12}H_{11}FO_4$) C, 60.51; H, 4.65; found C, 60.35; H, 4.66.

Ethyl 5-(3-fluorophenyl)pyrazole-3-carboxylate (4). A mixture of ethyl 4-hydroxy-4-(3-fluorophenyl)-2-oxobut-3-ethanoate (0.1 mol) and hydrazine hydrate (0.5 mol) in 40 ml of propan-1-ole was heated to boiling for 8 hours After removing the alcohol, an oily liquid was obtained, which was poured onto crushed ice. A white crystalline substance was formed. Yield: 79 %. M. p.: 135–137 °C. IR (ν, cm^{-1}): 1734, 1725 (C=O, ester), 1438 (C=C). ^{1}H NMR, *δ (ppm), J (Hz)*: 12.45 (s, 1H, NH, pyrazole), 7.86 (s, 1H, CH, pyrazole), 7.48–7.19 (m, 4H, 3-F-C_6H_4), 4.34 (q, J = 6.4 Гц, 2H, OCH_2CH_3), 1.22 (t, 3H, OCH_2CH_3). Analytical calculated (%) for ($C_{12}H_{11}FN_2O_2$) C, 61.53; H, 4.73; N, 11.96; found C, 61.36; H, 4.72; N, 11.99.

5-(3-Fluorophenyl)pyrazole-3-carbohydrazide (5). A mixture of ethyl 5-(3-fluorophenyl)pyrazole-3-carboxylate (0.1 mol) and hydrazine hydrate (0.2 mol) was heated to boiling in 40 ml of ethanol for 6 hours. After cooling, the precipitate was filtered and recrystallized from water (*Fig. 1*). Yield: 80 %. M. p.: 173–175 °C; IR (ν, cm^{-1}): 3390–3245 (NH, NH_2),

1608 (C=O); ^{1}H NMR, *δ (ppm), J (Hz)*: 12.48 (s, 1H, NH, pyrazole), 9.24 (t, *J* = 4.6 Hz, 1H, N*H*NH$_2$), 7.88 (s, 1H, CH, pyrazole), 7.46–7.21 (m, 4H, 3-F-C$_6$*H*$_4$), 4.31 (d, *J* = 4.8 Hz, 2H, NHN*H*$_2$). Analytical calculated (%) for ($C_{10}H_9FN_4O$) C, 54.54; H, 4.12; N, 25.44; found C, 54.37; H, 4.13; N, 25.51.

Potassium 2-(5-(3-fluorophenyl)pyrazole-3-carbonyl) hydrazine-1-carbo-dithioate (6). To carry out the reaction, weigh (0.2 mol) 5-(3-fluorophenyl)-pyrazole-3-carbohydrazide and potassium hydroxide equivalent (0.2 mol). A 9 % solution in butan-1-ole was prepared from potassium hydroxide, in which 5-(3-fluorophenyl)pyrazole-3-carbohydrazide was dissolved. After the complete dissolution of the substance, stirring was performed, during which 0.3 mol of CS_2 was added dropwise. After the addition of carbon disulfide, the mixture was stirred for another 0.5 hours. As a result of the chemical process, the formation of a yellow crystalline substance was observed.

4-Amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol (7). The calculated amount of potassium 2-(5-(3-fluorophenyl)pyrazole-3-carbonyl)hydrazine-1-carbodithioate (0.2 mol) was dissolved in 180 ml of purified water and the calculated 5-fold excess of hydrazine hydrate was added. In this form, the mixture was boiled for 6 hours. At the end of the heating, the solution was cooled and gradually, with constant stirring, concentrated hydrochloric acid was added to it until the medium becomes acidic (pH = 1–2) and a white precipitate of thiol began to form. The reaction was very violent. The formed precipitate was filtered and dried. Yield: 81 %. M. p.: 277–279 °C; IR (ν, cm^{-1}): 3390–3245 (NH, NH$_2$), 1608 (C=O); ^{1}H NMR, *δ (ppm), J (Hz)*: δ 13.87 (s, 1H, SH) 11.45 (s, 1H, NH, pyrazole), 8.05 (s, 1H, CH, pyrazole),7.58–7.48 (m, 4H, 3-F-C$_6$H$_4$), 6.18 (s, 2H NH$_2$). Analytical calculated (%) for ($C_{11}H_9FN_6S$) C, 47.82; H, 3.28; N, 30.42; S, 11.60; found C, 47.69; H, 3.27; N, 30.50; S, 11.63.

S-alkyl derivatives of 4-amino-5-(5-(3-fluorophenyl) pyrazol-3-yl)-1,2,4-triazole-3-thiol (8-17). To a mixture of 0.005 mol of 4-amino-5-(5-(3-fluoro-phenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol and 0.005 mol of sodium hydroxide dissolved in 40 ml of ethanol, 0.005 mol of haloalkane (iodomethane, bromoethane, 1-bromopropane, 1-bromobutane, 1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane) were added. This mixture was heated for 2 hours, cooled, filtered the sediment, and washed with purified water. For analysis, it was crystallized from methanol. These were white substances, insoluble in water, soluble in organic solvents.

^{1}H NMR spectra of the synthesized compounds contain signals of protons of aromatic fragments, which are fixed in the form of doublets and multiplets (aryl derivatives) and singlet (pyrazole fragment). For example, in the spectrum of compound **17**, there are multiplet signals in the "aromatic" zone at 7.58–7.51 ppm, which are formed with the help of hydrogens in the second, fifth, and sixth positions of the 3-fluorophenyl substituent. At the same time, the signal of the aromatic proton in the fourth position of this substituent has the form of a doublet of doublets with a chemical shift value of 7.20–7.15 ppm.

The analysis of ^{1}H NMR spectra also demonstrates that the signals of protons of S-alkyl fragments (**8–17**) are observed in the strong part of the field in the region of 3.23–0.82 ppm. For example, singlet signals of methyl protons S-CH$_3$ of fragment (**8**) are present at 1.84 ppm. S-C$_2$H$_5$ protons of group (**9**) form a triplet at 1.37 ppm and a quadruple at 3.13 ppm. The gradual transition from the S-CH$_3$ to the S-(CH$_2$)$_9$-CH$_3$ fragment leads to a slight chemical shift of the proton signals of the CH$_3$ group to the region of stronger fields (from 2.66 to 0.82 ppm). Multiplet signals of protons of (CH$_2$)$_n$-fragments are also recorded in the high-field part of the spectrum (1.78–1.19 ppm) and are difficult to differentiate. The specified spectral features are determined by the electron-donating properties of the alkyl substituent and the positive inductive effect, which increases with the elongation of the alkyl substituent. In the spectra of the synthesized compounds, there are also singlet signals of protons of the amino group at 5.75–5.77 ppm.

In order to substantiate further studies, the results of molecular docking with the active centers of COX-2, lanosterol 14α-demethylase, and anaplastic lymphoma kinase were visualized.

Visualization of the interaction of the synthesized thiol (**7**) with the active center of COX-2 made it possible to establish its multifaceted nature. Thus, among the interactions, the following can be noted: intermolecular hydrogen chemical bonds, which are realized with the help of the hydrogen of the amino group and the amino acid residue of methionine (MET A: 523), π-S – formed with the participation of the thiol group and the amino acid fragment of tryptophan (TRP A: 388), π-σ – formed by the π-chemical bond of the 3-fluorophenyl substituent and fragments of alanine (ALA A: 528) and leucine (LEU A: 360). Among other interactions, amide-π stacking can be noted – a triazole fragment and a glycine residue (GLY A: 527) are involved, π-alkyl – valine fragments are involved (VAL A: 117, VAL A: 524, VAL A: 532). The transition to the *S*-methyl derivative (**8**) changes in a certain way the pattern of interactions with the active center of COX-2. The disappearance of π-S and intermolecular hydrogen chemical bonds is observed, i.e., the participation of covalently bound Sulfur and the amino group is eliminated, which may be associated with a change in the spatial configuration of the molecule in the zone of the active center. Instead, new forms of interactions appear in the form of a π-donor hydrogen bond involving pyrazole and triazole fragments and a tyrosine residue (TYR A: 356) and in the form of alkyl interactions with the methyl group of valine residues (VAL A: 89) and tyrosine (TYR A: 116). The coordination of the specified ligand in the active center is strengthened by the already discussed π-alkyl interactions, which are formed during interaction with the amino acids VAL A: 350, VAL A: 524 and LEU A: 353. The elongation of the alkyl substituent is expected to be accompanied by an increase in alkyl interactions. For example, the ethyl substituent of compound **9** binds to the active center of the enzyme already with the help of 4 amino acid residues: VAL A: 89, VAL A: 117, LEU A: 93, TYR A: 116. It is also necessary to

Table 1. The value of the energy of intermolecular interactions of the studied compounds with COX-2 (3LN1)

N	E_{min}*	N	E_{min}	N	E_{min}	N	E_{min}
7	-7.2	9	-5.9	12	-2.5	15	-5.3
8	-6.8	10	-4.7	13	-3.1	16	-8.1
Celecoxib	-8.4	11	-2.6	14	-1.8	17	-8.1

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

note the active assistance of the Fluorine atom in the possible interaction: this atom actively supports the formation of an intermolecular hydrogen chemical bond with the residue TYR A: 386 (9). And these are not all possible intermolecular hydrogen bonds: the TYR A: 356 residue also joins the coordination of this compound (9), which binds to the triazole fragment (π-donor hydrogen bond). π-S interaction, which disappeared in the case of the ethyl substituent (9) and was not formed by the pentyl substituent (12), has a tendency to stable formation with the participation of all other alkyl substituents (10, 11, 13–17). The enzyme-ligand interactions of celecoxib with COX-2 are characterized by the following types: π-σ (phenyl fragment with VAL A: 524), amide-π stacking (phenyl fragment with GLY A: 527), alkyl (methyl substituent of the 4-methylphenyl fragment with LEU A: 385, TRP A: 388, TYR A: 386, *tert*-butyl substituent from LEU A: 353, LEU A: 360, VAL A: 117), π-alkyl (phenyl moiety from ALA A: 528, LEU A: 353; pyrazole moiety with ALA A: 528, VAL A: 350), C-H (ARG A: 514, HIS A: 90), intermolecular hydrogen bond (N and O of sulfonamide moiety with GLN A: 193, PHE A: 519, SER A: 354). Thus, the number of interactions with the active center of COX-2, which can form synthesized substances in comparison with the standard ligand, is an order of magnitude smaller. Although the nature of the docking interactions of the synthesized ligands is similar to celecoxib. The results of calculating the affinity of the synthesized ligands to COX-2 are shown in *Table 1*.

The interaction of synthesized substances with the active site of lanosterol 14α-demethylase occurs with the participation of amino acid residues of various natures. For example, thiol (7) uses aryl and pyrazole fragments to implement a π-alkyl interaction with the residues ALA A: 400, CYS A: 394 and PRO A: 320. The π-donor hydrogen bond strengthens the coordination in the enzyme binding site of these fragments, which is formed with the direct participation of threonine (THR A: 260). In addition, the active participation of the Fluorine atom, which forms an intermolecular hydrogen bond with the THR A: 264 residue, is monitored. The triazole fragment does not stand aside from contact with the active center. Thus, this heterocycle is involved in interaction with residues ALA A: 256 (π-σ interaction) and LEU A: 100 (π-alkyl interaction). This fragment is assisted by a thiol group. With its help, an intermolecular hydrogen chemical bond is formed with the LEU A: 100 residue.

The appearance of an alkyl substituent on the Sulfur atom made it possible to determine characteristic changes in the interaction with the active center of lanosterol 14α-demethylase. For example, molecules with methyl (8), ethyl (9) and propyl (10) substituents in the process of coordination in the active center form a relatively significant number of intermolecular hydrogen chemical bonds. This interaction is formed due to the Nitrogen atoms of the triazole ring (from CYS A: 394 and THR A: 260), with the participation of the Hydrogen atom conjugated with the Nitrogen atom of the pyrrole type of the pyrazole ring (from PRO A: 386) and in contact with the Fluorine atom of 3-fluorophenyl substitute (from ARG A: 326).

Among other interactions that create the possibility of forming stable complexes with the active center of the enzyme, it is necessary to note: π-alkyl interaction involving the phenyl fragment and the LEU A: 324 residue, the pyrazole ring and the CYS A: 394 residue, the 1,2,4-triazole skeleton and the PRO A residue: 320. Hydrogen bonds formed with the participation of the triazole fragment and the THR A: 260 residue increase the probability of binding to the active site of the π-donor protein. In addition, all synthesized compounds are able to form alkyl interactions with S-$(CH_2)_nCH_3$ with the participation of ALA A: 400, ILE A: 404, LEU A: 324, TYR A: 76, MET A: 79, PHE A: 83. It is also possible to note the π-S interaction between the pyrazole fragment and the CYS A residue: 394 (17) or between the Sulfur atom of the synthesized ligand and PHE A: 83 (14). Visualization of standard ligand docking in the active site of lanosterol 14α-demethylase, demonstrating the formation of bonds involving the 2,4-difluorophenyl substituent with ARG A: 96 (hydrogen bond), LEU A: 321, MET A: 79 (π-alkyl interaction), TYR A: 76 (π-π T-like interaction) and involving triazole fragments with ALA A: 256, LEU A: 100, LEU A: 321 (π-alkyl interaction), PHE A: 83 (π-π T-like interaction).

Thus, not only a certain similarity in the structure of the synthesized ligands to fluconazole is observed, but also a certain similarity in the nature of the amino acid residues that take part in the location of these ligands in the active center of the enzyme.

The calculation of the interaction energies of the obtained ligands with the active site of lanosterol 14α-demethylase demonstrates the ability of a number of compounds to influence the activity of this enzyme (*Table 2*).

Visualization of docking results of the synthesized thiol (7) with respect to anaplastic lymphoma kinase allowed us to determine the following types of interactions with the active site of the protein. This is, above all, the π-alkyl interaction of phenyl, pyrazole and triazole fragments with LEU A: 1122, LEU A: 1256, and VAL A: 1130. Additionally, the connection between the ligand and the target protein is strengthened by the Fluorine atom, which coordinates with the GLY residue A: 1269 and the Hydrogen atom of pyrrole

Table 2. The value of the energy of intermolecular interactions of the studied compounds with lanosterol 14α-demethylase (3LD6)

N	E_{min}*	N	E_{min}	N	E_{min}	N	E_{min}
7	-8.5	9	-9.0	12	-8.7	15	-7.0
8	-8.8	10	-8.5	13	-8.3	16	-6.8
Fluconazole	-10.1	11	-8.5	14	-7.3	17	-4.2

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

Table 3. Energetic values of intermolecular interactions of the studied compounds with anaplastic lymphoma kinase (2XP2)

N	E_{min}*	N	E_{min}	N	E_{min}	N	E_{min}
7	-7.4	9	-7.7	12	-8.5	15	-7.9
8	-7.6	10	-8.2	13	-8.4	16	-6.6
Crizotinib	-9.4	11	-8.4	14	-6.5	17	-7.7

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

Nitrogen of pyrazole (intermolecular hydrogen bond with MET: 1199). The appearance of methyl (8) and ethyl (9) substituents does not have a significant effect on the nature of the interaction with the specified enzyme. The increase of up to three Carbon atoms in the structure of the *S*-alkyl substituent (10) additionally contributes to the appearance of alkyl interactions, which are realized with the participation of LEU A: 1196 and LYS A: 1150. The S-butyl derivative (11) is additionally stabilized by the C-H bond, which is formed between the pyrazole fragment and the GLY A residue: 1202. Further increase in the length of the alkyl fragment (12–16) did not have a significant effect on the nature of active interactions with the active site of the protein. Only in 3-(decylthio)-5-(3-(3-fluorophenyl)pyrazol-5-yl)-1,2,4-triazole-4-amine (17) the overall interaction of the ligand with the enzyme is improved due to two intermolecular hydrogen chemical bond, which is formed with the participation of the amino group of the molecule and the residues ALA A: 1200 and MET A: 1199. The specified analysis allows us to preliminarily assert the low probability of the effect of the synthesized compounds on the activity of the kinase of anaplastic lymphoma. The effective location in the active center of the kinase of anaplastic lymphoma of crizotinib is provided by the following amino acid residues: ALA A: 1148 (π-alkyl interaction), ASN A: 1254 (intermolecular bond involving the Fluorine atom of the 2,6-dichloro-3-fluorophenyl substituent), GLU A: 1197 (intermolecular hydrogen bond formed with the participation of the Nitrogen atom of the amino group), GLY A: 1269 (intermolecular halogen bonds with the participation of the Fluorine and Chlorine atoms of the 2,6-dichloro-3-fluorophenyl substituent), LEU A: 1122 (alkyl- and π-alkyl interaction with piperidine and pyrazole fragments, respectively), LEU A: 1256 (π-σ interaction with pyridine and phenyl fragments), MET A: 1199 (intermolecular hydrogen bond involving the Nitrogen atom of the pyridine fragment). Quantitative indicators of the energy of intermolecular interactions demonstrate that most of the synthesized compounds have affinity for anaplastic lymphoma kinase (*Table 3*). But none of the synthesized substances exceeds the value of crizotinib (*Table 3*).

Thus, the indicated study made it possible to more precisely and specifically determine the prospects of further research on the mentioned class of compounds.

Conclusions

1. Simple and preparatively convenient methods for the synthesis of 4-amino-5-(5-(3-fluorophenyl)-pyrazol-3-yl)-1,2,4-triazol-3-thiol and its S-alkyl derivatives have been developed.

2. The structure and individuality of the synthesized compounds were confirmed by a complex of physical-chemical methods (IR spectrophotometry, ^{1}H-NMR spectroscopy, elemental analysis, chromatography-mass spectrometry).

3. It was established by the method of molecular docking that the further study of antifungal activity is a priority in a number of synthesized compounds. Among the synthesized substances, 3-(ethylthio)-5-(3-(3-fluorophenyl)pyrazol-5-yl)-1,2,4-triazol-4-amine was recommended for the purpose of extended research on antifungal activity.

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