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ORIGINAL ARTICLE / ÖZGÜN MAKALE

SYNTHESIS, PROPERTIES AND BIOLOGICAL POTENTIAL SOME CONDENSED DERIVATIVES 1,2,4-TRIAZOLE

BAZI 1,2,4-TRİAZOL YOĞUNLAŞTIRILMIŞ TÜREVLERİNİN SENTEZİ, ÖZELLİKLERI VE BİYOLOJİK POTANSİYELİ

Andrey GOTSULYA[1*], Yevhen ZAIKA[2], Tetiana BRYTANOVA[1]

[1]Zaporizhzhia State Medical University, Faculty of Pharmacy, Department of Natural Sciences for Foreign Students and Toxicological Chemistry, 69035, Zaporizhzhia, Ukraine

[2]Enamine Ltd, 02094, Kyiv, Ukraine

ABSTRACT

Objective: *The aim of the work was to develop effective methods for the synthesis of biologically active heterocyclic systems containing pyrrole, indole and 1,2,4-triazole. In this study, firstly fourteen [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles compounds requiring for this study were synthesized.*

Material and Method: *Chemical structures of synthesized compounds were characterized with elemental analysis, ^{1}H NMR, LC-MS techniques. The biological potential of the synthesized substances was estimated by the molecular docking method.*

Result and Discussion: *An optimal method for the synthesis of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles has been developed. In molecular modeling studies, the compounds were found to be similar to known drugs in some respects. Besides, the interaction of each molecule in the active site of the crystal structures of cyclooxygenase-1, lanosterol 14-α-demethylase, kinases of anaplastic lymphoma were considered as in silico.*

Keywords: *Docking, indole, pyrrole, synthesis, 1,2,4-triazole*

ÖZ

Amaç: *Çalışmanın amacı 1,2,4-triazol, indol ve pirol içeren biyolojik olarak aktif heterosiklik sistemlerin sentezi için etkili yöntemler geliştirmektir. Bu çalısmada, öncelikle çalısma için gerekli olan on üç adet [1,2,4]triazolo[3,4-b][1,3,4]tiadiazol bilesigi sentezlenmistir.*

Gereç ve Yönetim: *Sentezlenen bileşiklerin kimyasal yapıları elementel analiz, ^{1}H NMR, LC-MS teknikleri ile karakterize edildi. Sentezlenen maddelerin biyolojik potansiyelleri, moleküler yerleştirme yöntemi ile tahmin edildi.*

Sonuç ve Tartışma: *[1,2,4]triazolo[3,4-b][1,3,4]tiadiazollerin sentezi için optimal bir yöntem geliştirildi. Moleküler modelleme çalışmalarında, bileşiklerin bazı açılardan bilinen ilaçlara benzer olduğu*

* **Corresponding Author / Sorumlu Yazar:** Andrey Gotsulya
e-mail / e-posta: andrey.goculya@gmail.com, **Phone / Tel.:** +380990425382

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bulunmuştur. Ayrıca her bir molekülün siklooksijenaz-1, lanosterol 14-α-demetilaz ve anaplastik lenfoma kinazların kristal yapılarındaki aktif bölgeler ile etkileşimi in siliko olarak değerlendirildi.
Anahtar Kelimeler: *İndol, moleküler modelleme, pirol, sentez, 1,2,4-triazol*

INTRODUCTION

The search for biologically active substances among 1,2,4-triazole derivatives and the creation of a drug based on them is a promising area of research [1-5]. Synthesis of 1,2,4-triazoles fused to another heterocyclic ring has attracted wide spread attention due to their diverse applications as antibacterial [6], anticonvulsant [6], antidepressant [7], antiviral [8], anti-inflammatory [9], antinociceptive [10], anti-diabetic and antioxidant [11], anticancer [12] agents. In addition, the structure of 1,2,4-triazole is resistant to metabolic transformations and is able to participate in the formation of intermolecular chemical bonds [13]. In addition, 1,2,4-triazole derivatives have low toxicity, good pharmacokinetic and pharmacodynamic parameters [8]. The synthesis of new condensed 1,2,4-triazole derivatives can improve existing biological properties or contribute to the emergence of new valuable properties [14].

Among the great variety of derivatives of this heterocyclic system, special attention is paid to the cyclocondensation products of 4-amino-1,2,4-triazole-3-thiol derivatives with various reagents [15-22]. It is reported that many derivatives of indole and pyrrole obtained on the basis of 1,2,4-triazole as the main structure are of interest for pharmaceutical research purposes [23].

The combination of 4-amino-1,2,4-triazole-3-thiol with pyrrole or indole allows to obtain 4-amino-5-heteryl-1,2,4-triazole-3-thiols, which in the reactions of heterocyclization with aromatic carboxylic acids form [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles.

However, despite the large number of studies, the search for selective methods of obtaining triazolo[b]thiadiazole has not lost its relevance.

The aim of the work was the synthesis of heteryl derivatives [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole, study of their physicochemical properties and determination of their biological potential.

MATERIAL AND METHOD

Chemistry

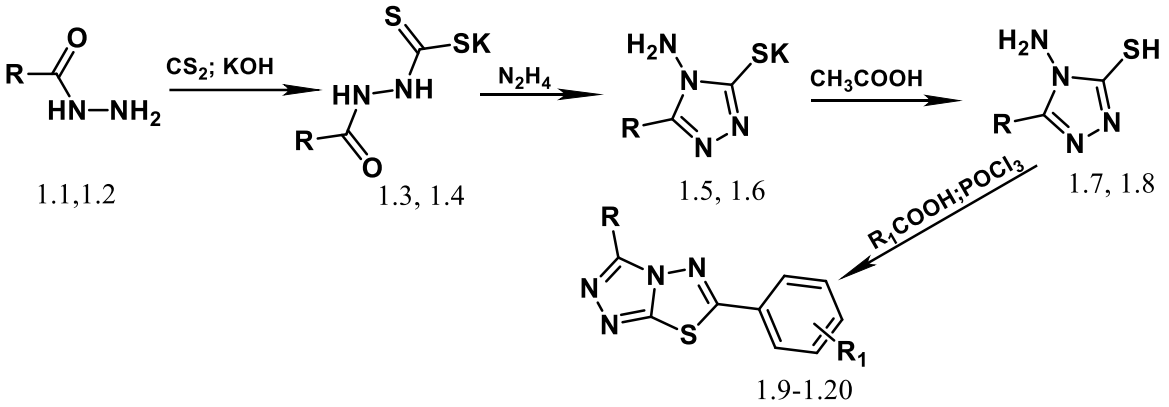
Pyrrole and indole-3-carboxylic acid were used as starting materials to obtain the target reaction products.

Stepwise conversion of pyrrole to pyrrole-2-carbohydrazide described in earlier works [31].

For the synthesis of 5-(3-(indole-3-yl)-4-amino-1,2,4-triazole-3-thiol, the reaction of the potassium salt of indole-3-carboxylic acid with bromoethane was previously carried out, which allowed to obtain the corresponding ester.

The formed 5-heteroryl derivatives of 4-amino-1,2,4-triazole-3-thiols were subsequently used in reactions with aromatic and heterocyclic carboxylic acids in the presence of phosphoryl trichloride (Figure 1).

Melting points were determined in open capillary tubes in a "Stanford Research Systems Melting Point Apparatus 100" (SRS, USA). The elemental analysis (C, H, N) were performed using the "Elementar vario EL cube" analyzer (Elementar Analysensysteme, Germany). ^{1}H NMR spectra (400 MHz) were recorded at "Varian-Mercury 400" spectrometer with SiMe4 as internal standard in DMSO-d6 solution. Chromatography-mass spectral studies were conducted on the instrument "Agilent 1260 Infinity HPLC" equipped with a mass spectrometer "Agilent 6120" (method of ionization – electrospray (ESI)).



R = pyrrole-2-yl (1.1, 1.3, 1.5, 1.7, 1.9-1.14), 3-(indole-3-yl)methyl (1.2, 1.4, 1.6, 1.8, 1.15-1.20);
R_1 = C_6H_5, C_6H_4-2-OCH_3, C_6H_4-3-OCH_3, C_6H_3-2-Br-5-OCH_3, C_6H_3-2-Br-4-F, C_6H_3-2-Cl-5-NO_2

Figure 1. The synthetic route of title compounds

Potassium 2-(pyrrole-2-carbonyl)hydrazine-1-carbodithioate (1.3). To 0.029 mol (1.6 g) of potassium hydroxide pre-dissolved in 20 ml of butan-1-ol is added 0.01 mol (1.25 g) of pyrrole-2-carbohydrazide. Stir until the precipitate dissolves, after which the flask with the solution is placed on crushed ice. Continuing stirring, 0.011 mol (0.84 g) of carbon disulfide was added dropwise. The yellow precipitate formed is continued to stir for 14 h at room temperature, then filtered and washed with diethyl ether. Yield - 85%.

Potassium 2-(indole-3-carbonyl)hydrazine-1-carbodithioate (1.4). To 0.029 mol (1.6 g) of potassium hydroxide pre-dissolved in 20 ml of butan-1-ol is added 0.01 mol (1.75 g) of indole-3-carbohydrazide. Stir until the precipitate dissolves, after which the flask with the solution is placed on

crushed ice. Continuing stirring, add dropwise 0.011 mol (0.84 g) of carbon disulfide. The yellow precipitate formed is continued to stir for 14 h at room temperature, then filtered and washed with diethyl ether and dried in vacuum. Compound 1.4 was obtained in quantitative yield. Synthesized potassium 2-(indole-3-carbonyl)hydrazine-1-carbodithioate was used for the preparation 4-amino-5-(indole-3-yl)-1,2,4-triazole-3-thiol without additional purification.

4-Amino-5-(pyrrole-2-yl)-1,2,4-triazole-3-thiol (1.7). To 0.01 mol (2.39 g) of potassium 2-(pyrrole-2-carbonyl)hydrazine-1-carbodithioate, add 0.02 mol of 64 % hydrazine hydrate solution and boil for 5 h on a water heater. The solution is cooled, 10 ml of purified water is added and neutralized with acetic acid to pH = 7. The precipitate is filtered off, washed twice with 50 g of water and dried. White powder, m. p. 188-190 ºC. Yield: 52 %.

4-Amino-5-(indole-3-yl)-1,2,4-triazole-3-thiol (1.8). In a flask equipped with a mixer, refrigerator, thermometer load 0.01 mol (2.89 g) potassium 2-(indole-3-carbonyl)hydrazine-1-carbodithioate, add 0.02 mol of 64 % solution of hydrazine hydrate and boil for 5 hours on a water heater. The solution is cooled, 10 ml of purified water is added and neutralized with acetic acid to pH = 7. The precipitate is filtered off, washed twice with 50 g of water and dried. This product was recrystallized from ethanol. Light brown powder, m. p. 256-258 ºC. Yield: 86 %.

General synthetic procedure for 3,6-disubstituted-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (1.9-1.20). A mixture of corresponding 4-amino-5-substituted-1,2,4-triazole-3-thiol (1.7, 1.8) (0.001 mol) and substituted benzoic acid (0.0015 mol) in phosphorus oxychloride (15 ml) was refluxed for 5 hour. Then the mixture was slowly poured into crushed ice and neutralized with sodium hydrogen carbonate. The precipitate formed is removed by filtration and washed three times with cold water. The products were recrystallized from absolute ethanol and dried to obtain 3,6-disubstituted-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (1.9–1.20).

Molecular Docking

It is known that 1,2,4-triazole derivatives exhibit pronounced inhibitory activity against a large number of enzymes [24, 25, 26, 27]. Among these enzymes are aromatase, cholinesterase, carbonic anhydrase, xanthine reductase, methionine aminopetidase-2, lipase, 11β-hydroxysteroid dehydrogenase type 1, adenosine deaminase, thymidine phosphorylase and lipoxygenase. A well-known fact is the ability of a 1,2,4-triazole fragment to take part in the formation of intermolecular hydrogen bonds with the active center of various enzymes. The presence of other heterocyclic pharmacophores will only increase the number of such interactions. For example, pyrrole and indole.

A permanent strategy for the search for anti-inflammatory agents is to obtain new substances that can suppress cyclooxygenase, a key enzyme of the inflammation process. According to the literature, the study of the anti-inflammatory activity of various derivatives of 1,2,4-triazole and indole

demonstrates good results [26]. The results of virtual research are often confirmed by *in vivo* methods. The choice in favor of cyclooxygenase-1 against the background of the more relevant cyclooxygenase-2 is due to the possibility of assessing not only the likelihood of anti-inflammatory activity, but also the possibility of assessing the likelihood of a negative effect on a number of physiological processes (for example, protection of the gastrointestinal mucosa). The enzyme 14α-demethylase controls the conversion of lanosterol into 4,14-dimethyl ergostrienol at one of the stages of ergosterol biosynthesis (provides a barrier function and the work of enzymes associated with the membrane). This enzyme is a known target of azoles, which form complex hydrophobic interactions using side groups of nitrogen-containing rings and conserved residues of the active site of 14α-demethylase, and also form a coordination binding of the Nitrogen atom of the azole synthon with the Iron atom of the hematin group of this enzyme. These interactions lead to inactivation of 14α-demethylase. The choice of anaplastic lymphoma kinase as a model target was also dictated by a number of reasons. Firstly, derivatives of 1,2,4-triazole can act as active ligands and form a coordination interaction with the active center of the enzyme. Secondly, dovitinib is used as a tyrosine kinase inhibitor; its structure contains an indole fragment covalently linked to other nitrogen-containing heterocycles. Considering the role of cyclooxygenase-1, lanosterol 14α-demethylase and anaplastic lymphoma kinase as important pharmacological targets, and their inhibitors as the basis for the development of a number of drugs, the synthesized substances were analyzed using molecular docking.

Molecular docking was performed to obtain structural information on the interaction of the synthesized compounds and the corresponding biological structure. For this purpose, 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: cyclooxygenase-1 with diclofenac (3N8Y), lanosterol 14-α-demethylase with ketoconazole (3LD6), kinases of anaplastic lymphoma in the complex of crizotinib (2XP2) [28, 29, 30]. The ligands (diclofenac, ketoconazole, crizotinib) were previously removed from the primary structures. It was 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 free binding energy (FEB) of all compounds was calculated.

RESULT AND DISCUSSION

The synthetic route for preparation of the newly synthesized compounds has been described in Figure 1. 4-Amino-5-(pyrrole-2-yl)-1,2,4-triazole-3-thiol and 4-amino-5-(indole-3-yl)-1,2,4-triazole-3-thiol were condensed with benzoic acid, 2-, 3-methoxybenzoic acid, 2-bromo-5-methoxybenzoic acid,

2-bromo-4-fluorophenylbenzoic acid and 2-chloro-5-nitrophenylbenzoic acid in $POCl_3$, the corresponding [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles are formed.

6-Phenyl-3-(pyrrole-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.9). Yield: 82 %; brown crystals; m. p.: 212 – 214 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 8.63 (d, 1H, pyrrole, NH), 8.09 (d, *J* = 6.6 Hz, 1H, pyrrole), 7.96 – 7.88 (m, 2H, ArH), 7.61 – 7.53 (m, 3H, ArH), 7.22 (dd, *J* = 6.6, 3.7 Hz, 1H, pyrrole), 7.06 (d, *J* = 8.6 Hz, 1H, pyrrole). Anal. calcd. (%) for $C_{13}H_9N_5S$: C, 58.41; H, 3.39; N, 26.20; S, 11.99. Found: C, 58.27; H, 3.40; N, 26.14; S, 12.02.

6-(2-Methoxyphenyl)-3-(pyrrole-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.10). Yield: 71 %; brown crystals; m. p.: 205 – 207 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 8.58 (d, 1H, pyrrole, NH), 8.14 (d, *J* = 6.7 Hz, 1H, pyrrole), 8.02 (d, *J* = 7.0 Hz, 1H, ArH), 7.49-7.45 (m, 1H, ArH), 7.27 (dd, *J* = 6.7, 3.6 Hz, 1H, pyrrole), 7.19 – 7.12 (m, 2H, ArH), 7.05 (d, *J* = 8.5 Hz, 1H, pyrrole), 4.04 (s, 3H, Ar-OC$\underline{H}_3$). Anal. calcd. (%) for $C_{14}H_{11}N_5OS$: C, 56.55; H, 3.73; N, 23.55; S, 10.78. Found: C, 56.70; H, 3.72; N, 23.61; S, 10.75.

6-(3-Methoxyphenyl)-3-(pyrrole-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.11). Yield: 75 %; brown crystals; m. p.: 209 – 211 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 8.62 (d, 1H, pyrrole, NH), 8.08 (d, *J* = 6.6 Hz, 1H, pyrrole), 7.63 (d, *J* = 7.3 Hz, 1H, ArH), 7.51 – 7.46 (m, 2H, ArH), 7.27 (dd, *J* = 6.7, 3.6 Hz, 1H, pyrrole), 7.11 (d, *J* = 8.1 Hz, 1H, ArH), 7.04 (dd, *J* = 8.6, 3.6 Hz, 1H, pyrrole), 3.86 (s, 3H, Ar-OC$\underline{H}_3$). Anal. calcd. (%) for $C_{14}H_{11}N_5OS$: C, 56.55; H, 3.73; N, 23.55; S, 10.78. Found: C, 56.39; H, 3.72; N, 23.62; S, 10.81.

6-(2-Bromo-5-methoxyphenyl)-3-(pyrrole-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.12). Yield: 68 %; brown crystals; m. p.: 203 – 205 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 8.59 (d, 1H, pyrrole, NH), 8.14 (d, *J* = 6.7 Hz, 1H, pyrrole), 7.65 (d, *J* = 8.1 Hz, 1H), 7.33 (d, *J* = 2.8 Hz, 1H, ArH), 7.25 (dd, *J* = 6.7, 3.6 Hz, 1H, pyrrole), 7.06 (dd, *J* = 8.6, 3.6 Hz, 1H, pyrrole), 6.88 (d, *J* = 8.1 Hz, 1H, ArH), 3.82 (s, 3H, Ar-OC$\underline{H}_3$). Anal. calcd. (%) for $C_{14}H_{10}BrN_5OS$: C, 44.69; H, 2.68; N, 18.61; S, 8.52. Found: C, 44.79; H, 2.69; N, 18.57; S, 8.50

6-(2-Bromo-4-fluorophenyl)-3-(pyrrole-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.13). Yield: 77 %; brown crystals; m. p.: 217 – 219 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 8.62 (d, 1H, pyrrole, NH), 8.09 (d, *J* = 6.7 Hz, 2H, pyrrole), 7.68 (d, *J* = 7.8 Hz, 1H, ArH), 7.41 (d, *J* = 2.9 Hz, 1H, ArH), 7.23 (dd, *J* = 6.6, 3.7 Hz, 2H, pyrrole), 7.17 (dd, *J* = 7.8, 2.8 Hz, 1H, ArH), 7.05 (dd, *J* = 8.6, 3.6 Hz, 1H, pyrrole). Anal. calcd. (%) for $C_{13}H_7BrFN_5S$: C, 42.87; H, 1.94; N, 19.23; S, 8.80. Found: C, 42.70; H, 1.95; N, 19.28; S, 8.78

6-(2-Chloro-5-nitrophenyl)-3-(pyrrole-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.14). Yield: 82 %; brown crystals; m. p.: 224 – 226 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 8.63

(d, 1H, pyrrole, NH), 8.49 (d, *J* = 2.0 Hz, 2H, ArH), 8.26 (dd, *J* = 8.6, 2.2 Hz, 1H, ArH), 8.14 (d, *J* = 6.7, 1H, pyrrole), 7.88 (d, *J* = 8.6 Hz, 2H, ArH), 7.25 (dd, *J* = 6.7, 3.6 Hz, 2H, pyrrole), 7.06 (dd, *J* = 8.4, 3.6 Hz, 1H, pyrrole). Anal. calcd. (%) for $C_{13}H_7ClN_6O_2S$: C, 45.03; H, 2.03; N, 24.24; S, 9.25. Found: C, 44.91; H, 2.02; N, 24.30; S, 9.27.

3-(Indole-3-yl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.15). Yield: 81 %; yellow crystals; m. p.: 233 – 235 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 11.65 (s, 1H, NH indole), 8.26 (d, *J* = 9.2 Hz, 1H, Indole-H), 8.03 (d, *J* = 8.4 Hz, 1H, Indole-H), 7.96 – 7.89 (m, 2H, ArH), 7.58 – 7.46 (m, 3H, ArH), 7.38 (d, *J* = 7.9 Hz, 1H, Indole-H), 7.25 (t, *J* = 7.3, 6.9 Hz, 1H, Indole-H), 7.19 (t, *J* = 7.8, 7.4 Hz, 1H, Indole-H). Anal. calcd. (%) for $C_{17}H_{11}N_5S$: C, 64.34; H, 3.49; N, 22.07; S, 10.10. Found: C, 64.16; H, 3.50; N, 22.01; S, 10.13

3-(Indole-3-yl)-6-(2-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.16). Yield: 67 %; brown crystals; m. p.: 222 – 224 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 11.66 (s, 1H, NH indole), 8.29 (d, *J* = 9.4 Hz, 1H, Indole-H), 8.05 – 7.99 (m, 2H, ArH, Indole-H), 7.51 – 7.46 (m, 1H, ArH), 7.39 (d, *J* = 7.8 Hz, 1H, Indole-H), 7.25 (t, *J* = 7.3, 6.9 Hz, 1H, Indole-H), 7.18 – 7.13 (m, 3H, ArH, Indole-H), 4.09 (s, 3H, Ar-OC$\underline{H}_3$). Anal. calcd. (%) for $C_{18}H_{13}N_5OS$: C, 62.23; H, 3.77; N, 20.16; S, 9.23. Found: C, 62.07; H, 3.76; N, 20.21; S, 9.25

3-(Indole-3-yl)-6-(3-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.17). Yield: 72 %; brown crystals; m. p.: 235 – 237 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 11.66 (s, 1H, NH indole), 8.29 (d, *J* = 9.4 Hz, 1H, Indole-H), 8.01 (d, 1H *J* = 8.6 Hz, 1H, Indole-H), 7.57 (d, *J* = 7.2 Hz, 1H, ArH), 7.49 – 7.43 (m, 2H, ArH), 7.38 (d, *J* = 7.8 Hz, 1H, Indole-H), 7.25 (t, *J* = 7.3, 6.9 Hz, 1H, Indole-H), 7.16 (t, *J* = 7.2, **7.7** Hz, 1H, Indole-H), 7.10 (d, *J* = 8.1 Hz, 1H, ArH), 3.93 (s, 3H, Ar-OC$\underline{H}_3$). Anal. calcd. (%) for $C_{18}H_{13}N_5OS$: C, 62.23; H, 3.77; N, 20.16; S, 9.23. Found: C, 62.39; H, 3.78; N, 20.11; S, 9.21

6-(2-Bromo-5-methoxyphenyl)-3-(indole-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.18). Yield: 77 %; brown crystals; m. p.: 241 – 243 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 11.63 (s, 1H, NH indole), 8.31 (d, *J* = 9.3 Hz, 1H, Indole-H), 7.94 (d, *J* = 7.2 Hz, 1H, Indole-H), 7.70 (d, *J* = 8.1 Hz, 1H, ArH), 7.41 (d, *J* = 7.2 Hz, 1H, Indole-H), 7.33 (d, *J* = 3.2 Hz, 1H, ArH), 7.25 (t, *J* = 7.3, 6.8 Hz, 1H, Indole-H), 7.17 (t, *J* = 7.2, 7.6 Hz, 1H Indole-H), 6.83 (d, *J* = 7.8 Hz, 1H, ArH), 3.87 (s, 3H, Ar-OC$\underline{H}_3$). Anal. calcd. (%) for $C_{18}H_{12}BrN_5OS$: C, 50.72; H, 2.84; N, 16.43; S, 7.52. Found: C, 50.84; H, 2.83; N, 16.47; S, 7.50.

6-(2-Bromo-4-fluorophenyl)-3-(indole-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.19). Yield: 72 %; brown crystals; m. p.: 229 – 231 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 11.60 (s, 1H, NH indole), 8.30 (d, *J* = 9.3 Hz, 1H, Indole-H), 7.93 (d, *J* = 7.2 Hz, 1H, Indole-H), 7.69 (d, *J* = 8.0 Hz, 1H, ArH), 7.46 (d, *J* = 2.8 Hz, 1H, ArH), 7.40 (d, *J* = 7.2 Hz, 1H, Indole-H), 7.23 (t, *J* =

7.3, 6.8 Hz, 1H, Indole-H), 7.19 – 7.13 (m, 2H, ArH, Indole-H). Anal. calcd. (%) for $C_{17}H_9BrFN_5S$: C, 49.29; H, 2.19; N, 16.91; S, 7.74. Found: C, 49.42; H, 2.18; N, 16.96; S, 7.72

6-(2-Chloro-5-nitrophenyl)-3-(indole-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1.20). Yield: 69 %; brown crystals; m. p.: 247 – 249 °C; ^{1}H NMR (400 MHz, dimethyl sulfoxide d_6, ppm): 11.61 (s, 1H, NH indole), 8.48 (d, *J* = 2.8 Hz, 1H, ArH), 8.35 – 8.27 (m, 2H, ArH, Indole-H), 7.94 (d, *J* = 7.2, Hz, 1H, Indole-H), 7.87 (d, *J* = 8.6 Hz, 1H, ArH), 7.40 (d, *J* = 8.1 Hz, 1H, Indole-H), 7.25 (t, *J* = 7.3, 6.8 Hz, 1H, Indole-H), 7.16 (t, *J* = 7.8, 7.4 Hz, 1H, Indole-H). Anal. calcd. (%) for $C_{17}H_9ClN_6O_2S$: C, 51.46; H, 2.29; N, 21.18; S, 8.08. Found: C, 51.34; H, 2.28; N, 21.23; S, 8.10.

The product structures (1.9-1.20) were confirmed by elemental analysis and ^{1}H NMR spectra that showed expected signals. The signals of the protons of the pyrrole fragment form doublet (positions 3 and 5) and also form doublet of doublets (position 4). The signal from the NH-group of pyrrole forms a pronounced singlet at 8.63-8.55 ppm.

In the ^{1}H NMR spectra of the indole fragment the protons at the 4 and 7 position appear as doublets and at the 5 and 6 position as triplets. Also in the spectra of the synthesized compounds 1.15-1.20, extended signals of protons of the NH-acid center in the form of a singlet in the low magnetic field are observed. Proton signal at the 2 position of the indole synthon appear as doublets. The presence of aryl fragments determines the formation of a number of multiplets in the "aromatic" region. The appearance of electron-withdrawing substituents in the phenyl fragment leads to an insignificant shift of the signals of aromatic protons towards the weak field and simplification of their multiplicity.

In order to investigate the probability of detection of molecules with molecules with anti-inflammatory activity, the interaction parameters with the active center of cyclooxygenase-1 (COX-1) were studied (Table 1). Visualization of the interaction of the most active compounds with the active center of COX-1 made it possible to establish the presence of hydrogen intermolecular chemical bonds with such amino acid residues as: ARG A: 185, SER A: 403.

It should also be noted the presence of π-alkylhydrophobic interactions with such amino acid residues as ALA A: 443, ILE A: 442, LYS A: 405, PHE A: 404, PRO A: 441 (Figure 2).

Table 1. Energy values of the intermolecular interactions of the studied compounds with COX-1 (3N8Y)

N	E_{min}, kcal/mol	N	E_{min}, kcal/mol	N	E_{min}, kcal/mol
1.5	-7.1	**1.11**	-5.7	**1.16**	-6.7
1.6	-5.6	**1.12**	-5.5	**1.17**	-5.9
1.9	-5.5	**1.13**	-5.6	**1.18**	-6.9
1.10	-5.5	**1.14**	-5.8	**1.19**	-6.4
Diclofenac	-10.4	**1.15**	-6.0	**1.20**	-6.9

*E_{min} - The minimum energy of complex formation, kcal/mol.

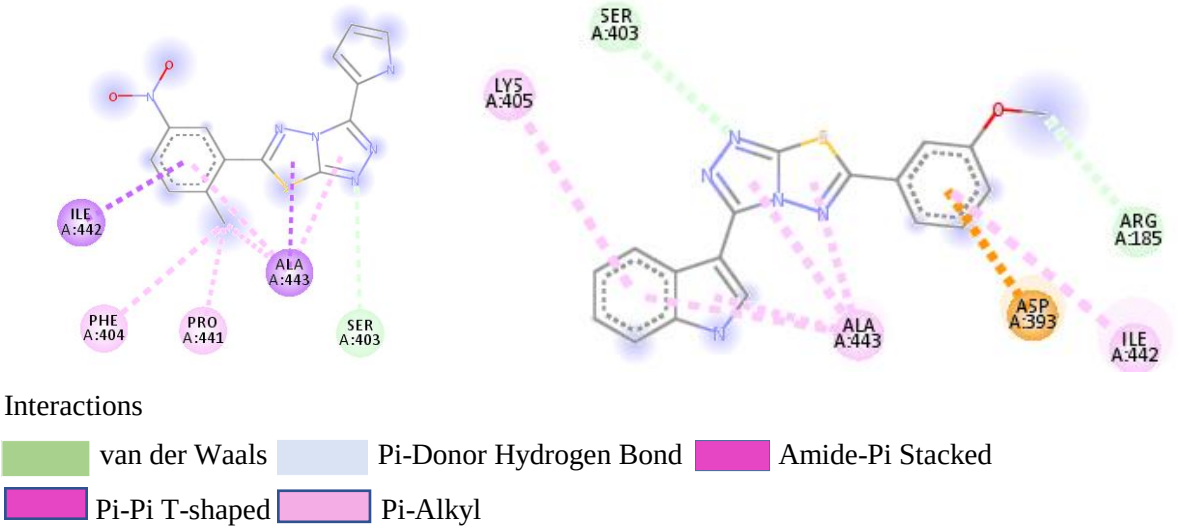


Figure 2. Visualization of the affinity of the COX-1 with compound 1.14 and 1.17

The free energy of binding of the synthesized substances of their lowest energy positions with lanosterol-14α-demethylase was calculated (Table 2). Compounds 1.16-1.20 showed a good range of binding energies from – 10.1 to – 10.6 kcal/mol. Visualization of the interaction of active structures with the active site of lanosterol-14α-demethylase revealed that they have chemical bonds with the following amino acid residues: ALA A: 311, CYS A: 449, GLY A: 307, HIS A: 314, ILE A: 377, ILE A: 379, ILE A: 450, LEU A: 159, LEU A: 308, MET A: 304, MET A: 487, TYR A: 145. In addition, you can note the interaction with LYS A 79, TYR A 131, PRO A 81, MET A 378, GLU A 83, PHE A 84, SER A 80 (Figure 3).

Table 2. Energy values of the intermolecular interactions of the studied compounds with lanosterol-14α-demethylase (3LD6)

N	E_{min}, kcal/mol	N	E_{min}, kcal/mol	N	E_{min}, kcal/mol
1.5	-5.7	**1.11**	-8.6	**1.16**	-10.2
1.6	-7.5	**1.12**	-8.1	**1.17**	-10.5
1.9	-8.3	**1.13**	-8.5	**1.18**	-10.1
1.10	-8.5	**1.14**	-8.7	**1.19**	-10.2
Ketoconazole	-9.9	**1.15**	-9.6	**1.20**	-10.6

*E_{min} - The minimum energy of complex formation, kcal/mol.

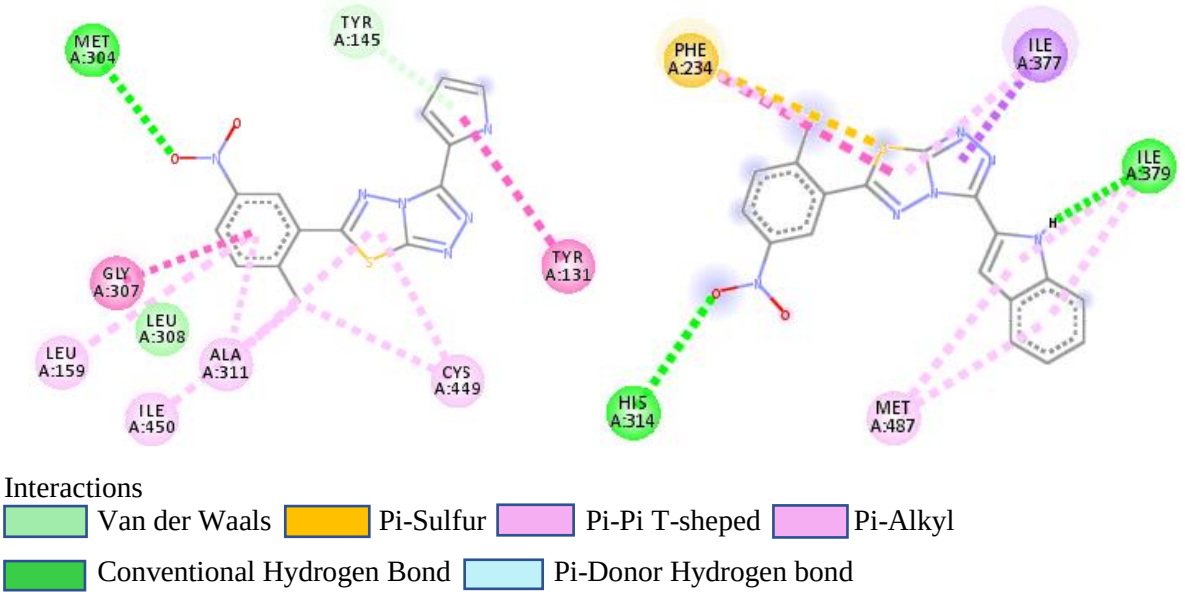


Figure 3. Visualization of the affinity of the lanosterol-14α-demethylase with compound 1.14 and 1.20

The free binding energy calculations performed demonstrate that most of synthesized compounds have good binding affinity for the anaplastic lymphoma kinase (Table 3). Among synthesized compounds, substances 1.14 and 1.20 bind the most strongly at the target site 3LD6. In addition, for these compounds, the free energy of binding was established, which was – 8.3 kcal/mol and – 9.5 kcal/mol, respectively (Table 3).

Various amino acids residues are involved in this process: ALA A: 1148, LEU A: 1122, LEU A: 1256, MET A: 1199, VAL A: 1130 (1.14, 1.20) and LYS A: 1150 (1.20) (Figure 4).

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

N	*E_{min}, kcal/mol	N	*E_{min}, kcal/mol	N	*E_{min}, kcal/mol
1.5	-5.4	**1.11**	-8.2	**1.16**	-9.0
1.6	-7.2	**1.12**	-7.5	**1.17**	-9.0
1.9	-7.7	**1.13**	-7.3	**1.18**	-8.6
1.10	-8.1	**1.14**	-8.3	**1.19**	-8.9
Crizotinib	-10.2	**1.15**	-8.5	**1.20**	-9.5

*E_{min} - The minimum energy of complex formation, kcal/mol.

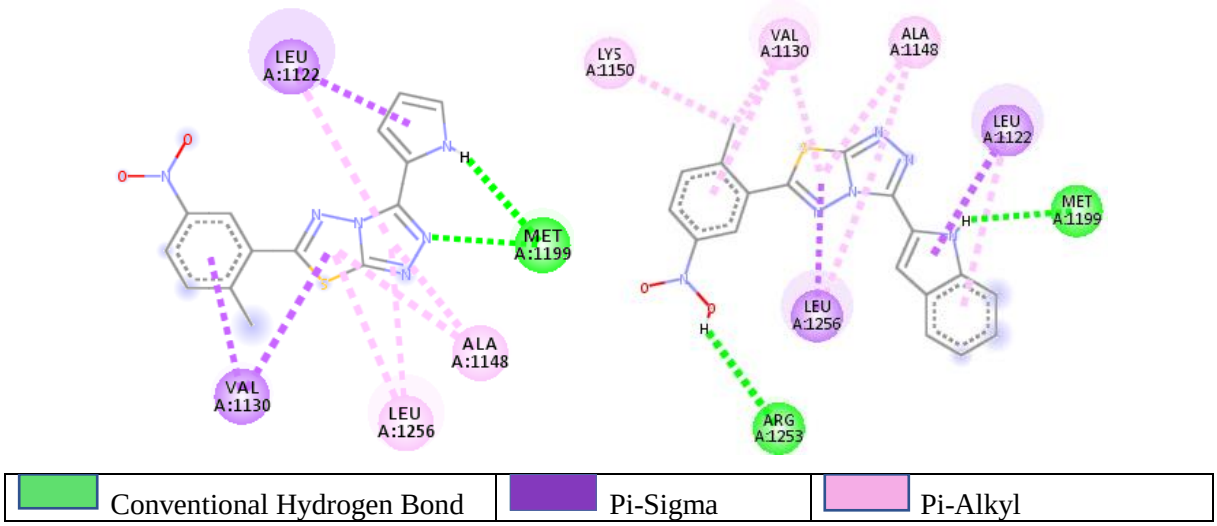


Figure 4. Visualization of the affinity of the anaplastic lymphoma kinase with compound 1.14 and 1.20

In docking analysis, the interaction of active structures with the active site of anaplastic lymphoma kinase π-π-interaction and hydrogen bond were observed in the potent ligand docking complex. Moreover, triazolo[3,4-*b*][1,3,4]thiadiazole fragment formed the number π-interactions such as π-alkyl and π-σ interactions with ALA A: 1148, LEU A: 1256, VAL A: 1130 (Figure 4). The anaplastic lymphoma kinase affinity of compounds 1.14 and 1.20 was also associated with the presence of hydrogen bonds with the corresponding amino acid residues (MET A: 1199).

A series of novel [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles have been successfully synthesized. The structure and individuality of the synthesized compounds were confirmed by ^{1}H NMR, and LC-MS spectra, elemental analysis. Molecular docking studies have shown the ability of the synthesized compounds to integrate into the active centers of enzymes. According to docking results, compounds 1.14 and 1.20 are of interest for further research. These compounds showed good binding energy to specific proteins (COX-1, lanosterol-14α-demethylase, anaplastic lymphoma kinase).

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AUTHOR CONTRIBUTIONS

Conception: *A. G., Y. Z., T. B.;* Design: *A. G., Y. Z.;* Supervision: *A. G., Y. Z.;* Resources: *A. G., Y. Z., T. B.;* Materials: *A. G., Y. Z.;* Data collection and/or processing: *A. G., Y. Z., T. B.;* Analysis and/or

interpretation: *A. G.;* Literature search: *A. G.;* Writing manuscript: *A. G., Y. Z., T. B.;* Critical review: *A. G., Y. Z.;* Other: -

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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