Synthesis and properties of S-derivatives of 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol

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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 various heterocyclic systems with a wide range of properties is quite expedient and is, in practice, a justified direction for obtaining biologically active substances, which ultimately forms a favorable basis for the creation of drugs. In recent decades, the attention of scientists has been closely focused on nitrogen-containing heterocyclic compounds.

Among such compounds, 1,2,4-triazole and pyrazole occupy a special place. Indeed, on the basis of these systems, a significant number of well-known drugs have been created, which are widely used at the present time.

The aim of the work was the synthesis of S-derivatives of 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol, study of their physical and chemical properties, pre-screening studies with subsequent establishment of the feasibility of further pharmacological studies.

Materials and methods. Experimental methods of organic chemistry: synthesis using microwave activation, physical and chemical methods for the analysis of organic compounds (determination of the melting point, elemental analysis, ^{1}H NMR, IR spectroscopy and chromatography-mass spectrometry). Methods for *in silico* pre-screening studies to establish the biological potential in several synthesized compounds (molecular docking).

Results. 10 new S-derivatives of 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol were synthesized. The structure of the obtained compounds was confirmed by a set of physical and chemical methods of analysis. According to the results of prescreening studies, the main directions of research of biological properties of synthesized compounds were provided.

Conclusions. The expediency of using microwave irradiation in the synthesis of a series of S-alkyl derivatives of 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol had been proved. Based on the results of *in silico* studies, the expediency of further studies of anti-inflammatory, antifungal and anticancer activities in several synthesized compounds had been substantiated.

Key words: 5-methylpyrazole, 1,2,4-triazole, synthesis, properties, molecular docking.

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

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Доцільним є поєднання різноманітних гетероциклічних систем із широким набором властивостей, оскільки це практично виправданий напрям одержання біологічно активних субстанцій, що формує підґрунтя для створення лікарських засобів. В останні десятиліття увага науковців прикута до нітрогеновмісних гетероциклічних сполук.

Особливе місце з-поміж них посідають 1,2,4-тріазол і піразол, адже на основі цих систем створено чимало відомих лікарських засобів, що нині доволі широко використовують.

Мета роботи – синтез S-похідних 4-аміно-5-(5-метилпіразол-3-іл)-1,2,4-тріазол-3-тіолу, вивчення їхніх фізико-хімічних властивостей, здійснення прескринінгових досліджень зі встановленням доцільності фармакологічних досліджень.

Матеріали та методи. Застосували експериментальні методи органічної хімії: синтез із використанням мікрохвильової активації, фізико-хімічні методи аналізу органічних сполук (визначення температури плавлення, елементний аналіз, ^{1}Н ЯМР, ІЧ-спектроскопія та хромато-мас-спектрометрія). Здійснили прескринінгові дослідження *in silico* для встановлення біологічного потенціалу в ряду синтезованих сполук (молекулярний докінґ).

Результати. Встановили оптимальні умови одержання 10 нових S-похідних 4-аміно-5-(5-метилпіразол-3-іл)-1,2,4-тріазол-3-тіолу з використанням мікрохвильового опромінення. Будову сполук підтвердили комплексом фізико-хімічних методів аналізу. За результатами прескринінгового аналізу визначили основні напрями досліджень біологічних властивостей синтезованих сполук.

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Key words: 5-methylpyrazole, 1,2,4-triazole, synthesis, properties, molecular docking.

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Висновки. Доведено доцільність застосування мікрохвильового опромінення під час синтезу ряду S-алкілпохідних 4-аміно-5-(5-метилпіразол-3-іл)-1,2,4-тріазол-3-тіолу. У результаті дослідження *in silico* обґрунтовано доцільність вивчення протизапальної, протигрибкової та протиракової активностей у ряду синтезованих сполук.

Ключові слова: 5-метилпіразол, 1,2,4-тріазол, синтез, властивості, молекулярний докінг.

Актуальні питання фармацевтичної і медичної науки та практики. 2021. Т. 14, № 3(37). С. 268–274

Синтез и свойства S-производных 4-амино-5-(5-метилпиразол-3-ил)-1,2,4-триазол-3-тиола

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Целесообразно сочетание различных гетероциклических систем с широким набором свойств, так как это оправданное на практике направление получения биологически активных субстанций. В итоге это формирует основу для создания лекарственных средств. В последние десятилетия внимание учёных приковано к азотсодержащим гетероциклическим соединениям. Особое место среди них занимают 1,2,4-триазол и пиразол, ведь на основе этих систем было создано значительное количество известных лекарственных средств, которые достаточно широко используют.

Цель работы – синтез S-производных 4-амино-5-(5-метилпиразол-3-ил)-1,2,4-триазол-3-тиола, изучение их физико-химических свойств, проведение прескрининговых исследований с установлением целесообразности дальнейших фармакологических исследований.

Материалы и методы. Применили экспериментальные методы органической химии: синтез с использованием микроволновой активации, физико-химические методы анализа органических соединений (определение температуры плавления, элементный анализ, ^{1}Н ЯМР, ИК-спектроскопия и хромато-масс-спектрометрия). Провели прескрининговые исследования *in silico* для установления биологического потенциала в ряду синтезированных соединений (молекулярный докинг).

Результаты. Установлены оптимальные условия получения 10 новых S-производных 4-амино-5-(5-метилпиразол-3-ил)-1,2,4-триазол-3-тиола с использованием микроволнового облучения. Строение полученных соединений подтверждено комплексом физико-химических методов анализа. По результатам прескринингового анализа определены основные направления исследований биологических свойств синтезированных соединений.

Выводы. Доказана целесообразность применения микроволнового облучения при синтезе ряда S-алкилпроизводных 4-амино-5-(5-метилпиразол-3-ил)-1,2,4-триазол-3-тиола. По результатам исследования *in silico* обоснована целесообразность дальнейшего изучения противовоспалительной, противогрибковой и противораковой активностей в ряду синтезированных соединений.

Ключевые слова: 5-метилпиразол, 1,2,4-триазол, синтез, свойства, молекулярный докинг.

Актуальные вопросы фармацевтической и медицинской науки и практики. 2021. Т. 14, № 3(37). С. 268–274

Today, many research teams are studying the synthetic and biological properties of new compounds based on the nitrogen-containing system 1,2,4-triazole [1–6]. It is known that the introduction of various substituents in the structure of the nucleus of 1,2,4-triazole has a positive effect not only on the increase of existing and the emergence of new pharmacological activity but also allows offering more options for chemical transformations [4–8]. It is important to note the fact that 1,2,4-triazole derivatives, in addition to high biological activity, are mostly low-toxic or virtually non-toxic compounds. Analysis of the available literature data revealed that the combination of triazole and pyrazole fragments within one molecule has a certain level of practical significance and is interesting.

Aim

The aim of this work was to develop a preparative method for the synthesis of S-derivatives of 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol using microwave irradiation, followed by study of physical and chemical properties and establishing the biological potential of the obtained compounds.

Materials and methods

The synthetic part of the study consisted of resynthesis of 5-methylpyrazole and subsequent chemical conversion to 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol by a known method [9,10].

S-alkylation of the starting compound was performed under microwave irradiation using a microwave synthesis system Milestone FlexiWave (time – 30 min, temperature – 160 °C, pressure – 11.2 bar, power – 400 W) in propan-2-ol medium without the addition of equivalent amount of alkali, which allowed to obtain pure alkylthio derivatives with high yield (*Fig. 1*).

Synthesized haloalkanes were white crystalline substances, soluble in alcohols, insoluble in water. The structure of the obtained compounds was confirmed by a package of modern physical and chemical methods of analysis (^{1}H NMR spectroscopy, IR spectroscopy) and their individuality by chromato-mass spectrometry.

Docking studies were performed on all compounds 2.0–2.10 using the AutoDock Vina® software package. Enzyme structures for in silico studies were obtained from Protein Data Bank (PDB). Preliminary optimization of 2.0–2.10 molecules was performed using the HyperChem 7.5 program by the MM^+ molecular mechanics' method until the RMS gradient was less than 0.1 kcal/(mol·Å). The final minimization of the energies of the studied structures was carried out by the semi-empirical quantum chemical method PM3 until the RMS gradient was less than 0.01 kcal/(mol·Å).

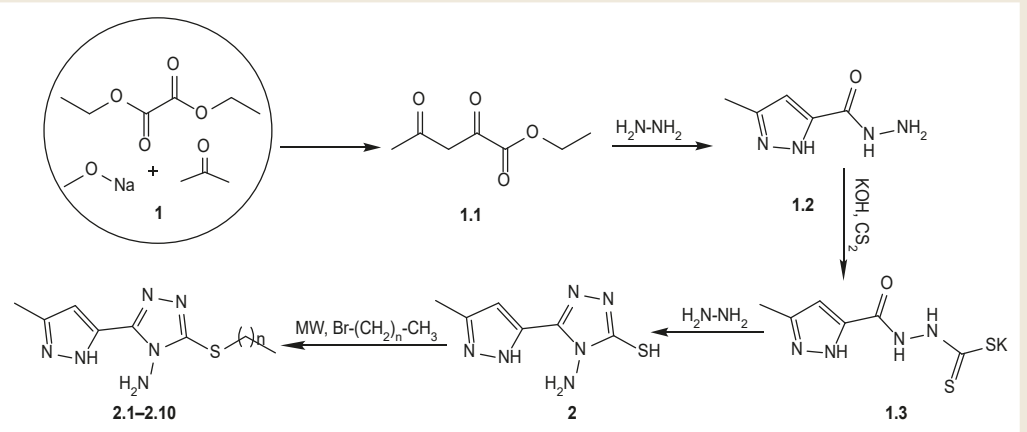

Fig. 1. Scheme of synthesis of 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol and S-alkylation products.

Results

Analysis of ^{1}H NMR spectra showed that the protons of S-alkyl fragments resonate in a strong part of the field in the form of signals with different intensities in the range 3.14–0.82 ppm. For example, singlet signals of methyl protons of the thiomethyl fragment were present in the range of 3.14–3.08 ppm. Multiple proton signals of methylene fragments were recorded in a stronger field (1.97–1.20 ppm). A gradual increase in the length of the S-alkyl chain leads to a slight shift in the signals of the protons of the methyl group in the stronger part of the field.

Proton signals of methylene moieties of S-alkyl substituents were conducted in a similar way but were difficult to differentiate because they form mostly multiproton multiplets. Exceptions were only signals of protons of the methylene group directly with the sulfur atom. In this case, there was a signal in the form of a triplet. The formation of a positive inductive effect contributed to these changes. Thus, the signal of protons of the methyl group gradually shifts to 0.83 ppm.

The IR spectrum of the synthesized thiol (2.0) was characterized by the presence of clear bands of deformation and valence oscillations of strong and medium intensity of the main fragments of the molecule: planar deformation oscillations CH in the region 1229–950 cm^{-1} (bands of low intensity at 1229–1182 cm^{-1}, 1045–1029 cm^{-1}, 1013–998 cm^{-1}, 975–960 cm^{-1}), out-of-plane deformation oscillations CH in the region 998–663 cm -1 (bands of strong intensity at 781–765 cm^{-1}, 687–672 cm^{-1}). There was the presence of a band of valence vibrations of the SH group in the range of 3298–3280 cm^{-1}. The oscillation bands of the C = N fragment in the region of 1548–1530 cm^{-1} were also recorded.

In the spectra of the synthesized alkyl derivatives (2.0–2.10) deformation oscillations of alkyl groups in the range from 645 cm^{-1} to 1390 cm^{-1} and the H-C-H fragment in the narrow frequency range 1485–1360 cm^{-1} were observed. In the spectra of the synthesized alkyl derivatives (2.1–2.10) deformation oscillations of alkyl groups in the range from 645 cm^{-1} to 1390 cm^{-1} and the H-C-H fragment in the narrow frequency range 1485–1360 cm^{-1} were observed.

4-Amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol (2.0). Yield: 71 %; m. p.: 218–216 °C; ^{1}H NMR, *d*, ppm: 13.74 (s, 1H, SH); 13.11 (1H, s, NH-pyrazole), 6.63 (s, 1H, CH-pyrazole), 5.98 (s, 2H, NH_2), 2.26 (s, 3H, CH_3-pyrazole). ESI-MS: m/z = 197 $[M+H]^+$. Analytical calculated (%) for $C_6H_8N_6S$: C, 36.72; H, 4.11; N, 42.83; S, 16.34. Found: C, 36.81; H, 4.12; N, 42.75; S, 16.32.

3-(5-Methylpyrazol-3-yl)-5-methylthio-1,2,4-triazole-4-amine (2.1). Yield: 79 %; m. p.: 196–194 °C; ^{1}H NMR, *d*, ppm: 13.07 (s, 1H, NH-pyrazole), 6.65 (s, 1H, CH-pyrazole), 6.02 (s, 2H, NH_2), 2.67 (t, 3H, S-CH_3), 2.33 (s, 3H, CH_3-pyrazole). ESI-MS: m/z = 211 $[M+H]^+$. Analytical calculated (%) for $C_7H_{10}N_6S$: C, 39.99; H, 4.79; N, 39.97; S, 15.25. Found: C, 39.89; H, 4.80; N, 39.87; S, 15.29.

3-Ethylthio-5-(5-methypyrazol-3-yl)-1,2,4-triazole-4-amine (2.2). Yield: 82 %; m. p.: 193–191 °C; ^{1}H NMR, *d*, ppm: 13.10 (s, 1H, NH-pyrazole), 6.61 (s, 1H, CH-pyrazole), 6.05 (s, 2H, NH_2), 3.13 (t, 2H, S-CH_2-CH_3), 2.30 (s, 3H, CH_3-pyrazole), 1.36 (t, 3H, S-CH_2-CH_3). ESI-MS: m/z = 225 $[M+H]^+$. Analytical calculated (%) for $C_8H_{12}N_6S$: C, 42.84; H, 5.39; N, 37.47; S, 14.29. Found: C, 42.74; H, 5.38; N, 37.56; S, 14.32.

3-(5-Methylpyrazol-3-yl)-5-propylthio-1,2,4-triazole-4-amine (2.3). Yield: 76 %; m. p.: 186–184 °C; ^{1}H NMR, *d*, ppm: 13.05 (s, 1H, NH-pyrazole), 6.67 (s, 1H, CH-pyrazole), 6.01 (s, 2H, NH_2), 3.11 (t, 2H, S-CH_2-CH_3), 2.35 (s, 3H, CH_3-pyrazole), 1.92–1.56 (m, 2H, S-CH_2-CH_2-CH_3), 1.05 (s, 3H, S-$(CH_2)_2$-CH_3). ESI-MS: m/z = 239 $[M+H]^+$. Analytical calculated (%) for $C_9H_{14}N_6S$: C, 45.36; H, 5.92; N, 35.27; S, 13.45. Found: C, 45.47; H, 5.91; N, 35.18; S, 13.41.

3-Butylthio-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-4-amine (2.4). Yield: 71 %; m. p.: 182–180 °C; ^{1}H NMR, *d*, ppm: 13.08 (s, 1H, NH-pyrazole), 6.63 (s, 1H, CH-pyrazole), 6.04 (s, 2H, NH_2), 3.14 (t, 2H, S-CH_2-$(CH_2)_2$-CH_3), 2.30 (s, 3H, CH_3-pyrazole), 1.84 – 1.50 (m, 2H, S-CH_2-CH_2-CH_2-CH_3), 1.46–1.33 (m, 2H, S-$(CH_2)_2$-CH_2-CH_3), 0.92 (t, 3H, S-$(CH_2)_3$-CH_3). ESI-MS: m/z = 253 $[M+H]^+$. Analytical calculated (%) for $C_{10}H_{16}N_6S$: C, 47.60; H, 6.39; N, 33.31; S, 12.71. Found: C, 47.49; H, 6.40; N, 33.23; S, 12.74.

3-(5-Methylpyrazol-3-yl)-5-pentylthio-1,2,4-triazole-4-amine (2.5). Yield: 74 %; m. p.: 172–174 °C; ^{1}H NMR, *d*, ppm: 13.04 (s, 1H, NH-pyrazole), 6.57 (s, 1H, CH-pyrazole), 6.07 (s, 2H, NH_2), 3.10 (t, 2H, S-CH_2-$(CH_2)_3$-CH_3), 2.33 (s, 3H, CH_3-pyrazole), 1.87 – 1.55 (m, 2H, (m, 2H, S-CH_2-CH_2-$(CH_2)_2$-CH_3), 1.49 1.25 (m, 4H, S-$(CH_2)_2$-$(CH_2)_2$-CH_3), 0.83

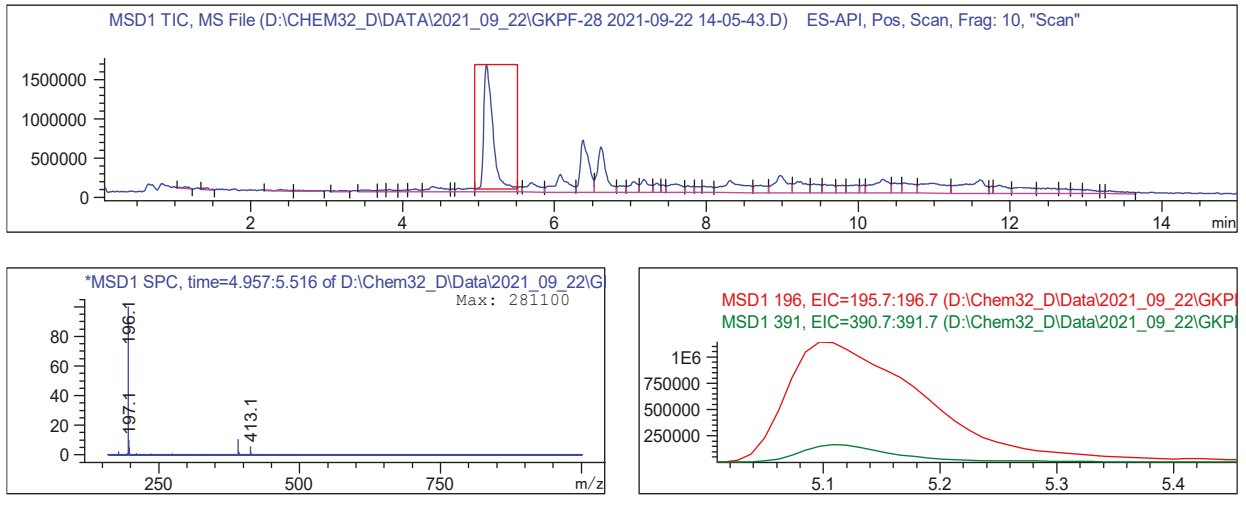

Fig. 2. Chromato-mass spectrum of compound 2.0.

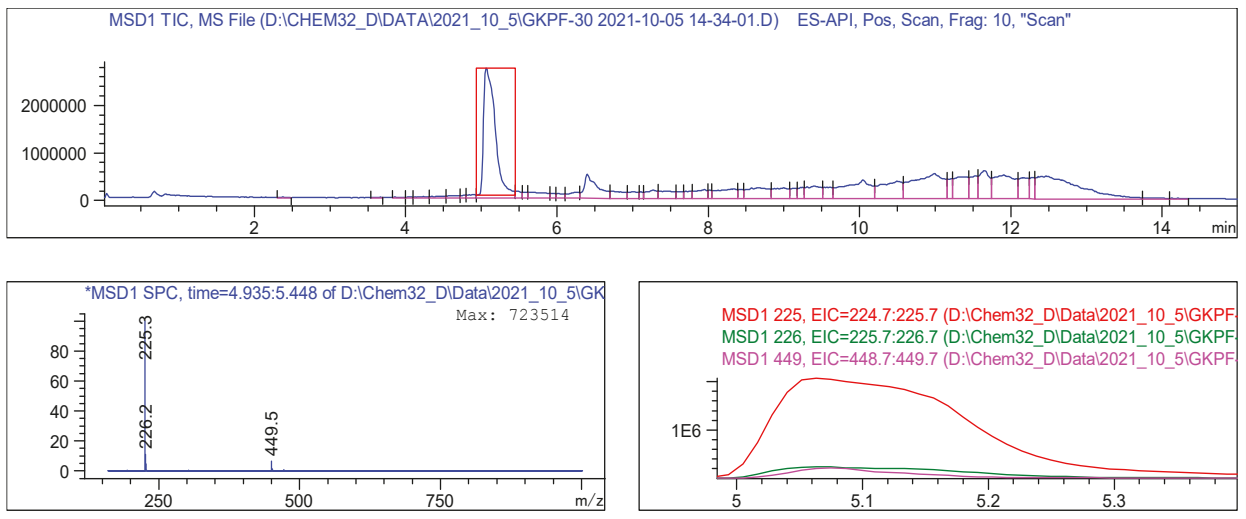

Fig. 3. Chromato-mass spectrum of compound 2.2.

(t, 3H, S-$(CH_2)_4$-CH_3). ESI-MS: m/z = 267 $[M+H]^+$. Analytical calculated (%) for $C_{11}H_{18}N_6S$: C, 49.60; H, 6.81; N, 31.55; S, 12.04. Found: C, 49.69; H, 6.82; N, 31.47; S, 12.01.

3-Hexylthio-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-4-amine (2.6). Yield: 78 %; m. p.: 177–179 °C; ^{1}H NMR, *d*, ppm: 13.08 (s, 1H, NH-pyrazole), 6.59 (s, 1H, CH-pyrazole), 6.05 (s, 2H, NH_2), 3.12 (t, 2H, S-CH_2-$(CH_2)_4$-CH_3), 2.34 (s, 3H, CH_3-pyrazole), 1.69–1.51 (m, 2H, S-CH_2-CH_2-$(CH_2)_3$-CH_3), 1.39 – 1.24 (m, 6H, S-$(CH_2)_2$-$(CH_2)_3$-CH_3), 0.84 (t, 3H, S-$(CH_2)_5$-CH_3). ESI-MS: m/z = 281 $[M+H]^+$. Analytical calculated (%) for $C_{12}H_{20}N_6S$: C, 51.40; H, 7.19; N, 29.97; S, 11.43. Found: C, 51.30; H, 7.18; N, 29.91; S, 11.45.

3-Heptylthio-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-4-amine (2.7). Yield: 70 %; m. p.: 170–172 °C; ^{1}H NMR, *d*, ppm: 13.09 (s, 1H, NH-pyrazole), 6.55 (s, 1H, CH-pyrazole), 6.07 (s, 2H, NH_2), 3.08 (t, 2H, S-CH_2-$(CH_2)_5$-CH_3), 2.32 (s, 3H, CH_3-pyrazole), 1.74–1.63 (m, 2H, S-CH_2-CH_2-$(CH_2)_4$-CH_3), 1.31–1.22 (m, 8H, S-$(CH_2)_2$-$(CH_2)_4$-CH_3), 0.82 (t, 3H, S-$(CH_2)_6$-CH_3). ESI-MS: m/z = 295 $[M+H]^+$. Analytical calculated (%) for $C_{13}H_{22}N_6S$: C, 53.03; H, 7.53; N, 28.54; S, 10.89. Found: C, 53.12; H, 7.54; N, 28.49; S, 10.87.

3-(5-Methylpyrazol-3-yl)-5-octylthio-1,2,4-triazole-4-amine (2.8). Yield: 74 %; m. p.: 162–164 °C; ^{1}H NMR, *d*, ppm: 13.06 (s, 1H, NH-pyrazole), 6.59 (s, 1H, CH-pyrazole), 6.06 (s, 2H, NH_2), 3.10 (t, 2H, S-CH_2-$(CH_2)_6$-CH_3), 2.33 (s, 3H, CH_3-pyrazole), 1.82–1.56 (m, 2H, S-CH_2-CH_2-$(CH_2)_5$-CH_3), 1.43–1.22 (m, 10H, S-$(CH_2)_2$-$(CH_2)_5$-CH_3), 0.86 (t, 3H, S-$(CH_2)_7$-CH_3). ESI-MS: m/z = 309 $[M+H]^+$. Analytical calculated (%) for $C_{14}H_{24}N_6S$: C, 54.52; H, 7.84; N, 27.25; S, 10.39. Found: C, 54.62; H, 7.83; N, 27.20; S, 10.41.

3-(5-Methylpyrazol-3-yl)-5-nonylthio-1,2,4-triazole-4-amine (2.9). Yield: 70 %; m. p.: 166–168 °C; ^{1}H NMR, *d*, ppm: 13.11 (s, 1H, NH-pyrazole), 6.56 (s, 1H, CH-pyrazole), 6.05 (s, 2H, NH_2), 3.12 (t, 2H, S-CH_2-$(CH_2)_7$-CH_3), 2.31 (s, 3H, CH_3-pyrazole), 1.70–1.66 (m, 2H, S-CH_2-CH_2-$(CH_2)_6$-CH_3), 1.30–1.21 (m, 12H, S-$(CH_2)_2$-$(CH_2)_6$-CH_3), 0.84 (t, 3H, S-$(CH_2)_8$-CH_3).

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
2.0	-4.9	**2.4**	-5.7	**2.8**	-7.0
2.1	-4.5	**2.5**	-6.1	**2.9**	-7.3
2.2	-5.0	**2.6**	-5.5	**2.10**	-7.0
2.3	-5.3	**2.7**	-6.6	*Diclofenac*	-6.2

E_{min}: the minimum energy of complex formation.

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
2.0	-4.9	**2.4**	-7.5	**2.8**	-8.4
2.1	-6.5	**2.5**	-7.7	**2.9**	-8.9
2.2	-6.6	**2.6**	-8.1	**2.10**	-9.4
2.3	-6.8	**2.7**	-8.1	*Ketoconazole*	-8.1

E_{min}: the minimum energy of complex formation.

ESI-MS: m/z = 323 $[M+H]^+$. Analytical calculated (%) for $C_{15}H_{26}N_6S$: C, 55.87; H, 8.13; N, 26.06; S, 9.94. Found: C, 55.76; H, 8.12; N, 26.11; S, 9.96.

3-Decylthio-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-4-amine (2.10). Yield: 68 %; m. p.: 158–160 °C; ^{1}H NMR, *d*, ppm: 13.11 (s, 1H, NH-pyrazole), 6.53 (s, 1H, CH-pyrazole), 6.05 (s, 2H, NH_2), 3.10 (t, 2H, S-CH_2-$(CH_2)_8$-CH_3), 2.33 (s, 3H, CH_3-pyrazole), 1.74–1.63 (m, 2H, S-CH_2-CH_2-$(CH_2)_7$-CH_3), 1.41–1.30 (m, 2H, S-$(CH_2)_2$-CH_2-$(CH_2)_6$-CH_3), 1.34–1.20 (m, 12H, S-$(CH_2)_3$-$(CH_2)_6$-CH_3), 0.82 (t, 3H, S-$(CH_2)_9$-CH_3). ESI-MS: m/z = 337 $[M+H]^+$. Analytical calculated (%) for $C_{16}H_{28}N_6S$: C, 57.11; H, 8.39; N, 24.98; S, 9.53. Found: C, 57.21; H, 8.40; N, 24.93; S, 9.50.

Individual peaks of molecular ions [M+1] were recorded in the chromato-mass spectra, which had a high intensity, which confirms the structure and individuality of the compounds (*Fig 2, 3*).

Molecular docking. It is noteworthy that a significant number of antifungal drugs contain a fragment of 1,2,4-triazole (fluconazole, itraconazole, voriconazole, posaconazole). Triazole-containing anastrozole and letrozole were also quite effective anticancer drugs. On the other hand, the presence of pyrazole enzyme indicates the feasibility of testing for anti-inflammatory activity.

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) [11–13]. The use of cyclooxygenase-1 as a model enzyme is dictated by the need to determine the possible impact on the complex of processes, and not only on the inflammatory response. At the same time, a few highly effective antifungal agents have a 1,2,4-triazole fragment in their structure, so the choice in favor of 14-α-demethylase as a model enzyme is obvious. The choice of aplastic lymphoma kinase to determine the affinity of the synthesized substances to its active center is due to the use of known anticancer drugs created based on nitrogen-containing heterocycles.

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 [14–17].

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*). These studies have found that synthesized compounds form chemical bonds with the following amino acid residues: ASN B: 68, TYR B: 38, TYR B: 39. At the same time, the presence of π-alkyl hydrophobic interactions with such amino acid residues as LYS B: 4668, PRO B: 35, PRO B: 40, PRO B: 434, TYR B: 38, TYR B: 55.

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: GLY A: 310, HIS A: 381, ILE A: 139, LEU A: 380, MET A: 509, PHE A: 134, PHE A: 241, PHE A: 384, TYR A: 126, VAL A: 311 (*Fig. 4*). The estimated free energy of binding of the synthesized substances of their lowest energy positions with lanosterol-14α-demethylase was calculated (*Table 2*). Synthesized substances 2.6–2.10 showed a good range of binding energies from -8.1 to -9.4 kcal/mol.

The obtained substances were stabilized in the active center of anaplastic lymphoma kinase due to intermolecular hydrogen chemical bond with MET A: 1199, alkyl hydrophobic

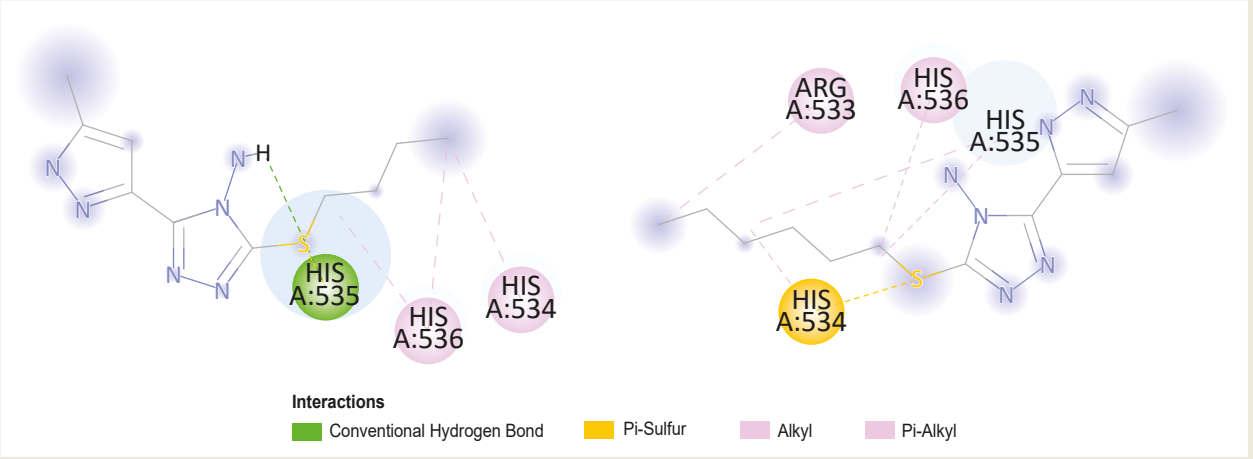

Fig. 4. Visualization of the affinity of the lanosterol-14α-demethylase with compound 2.10.

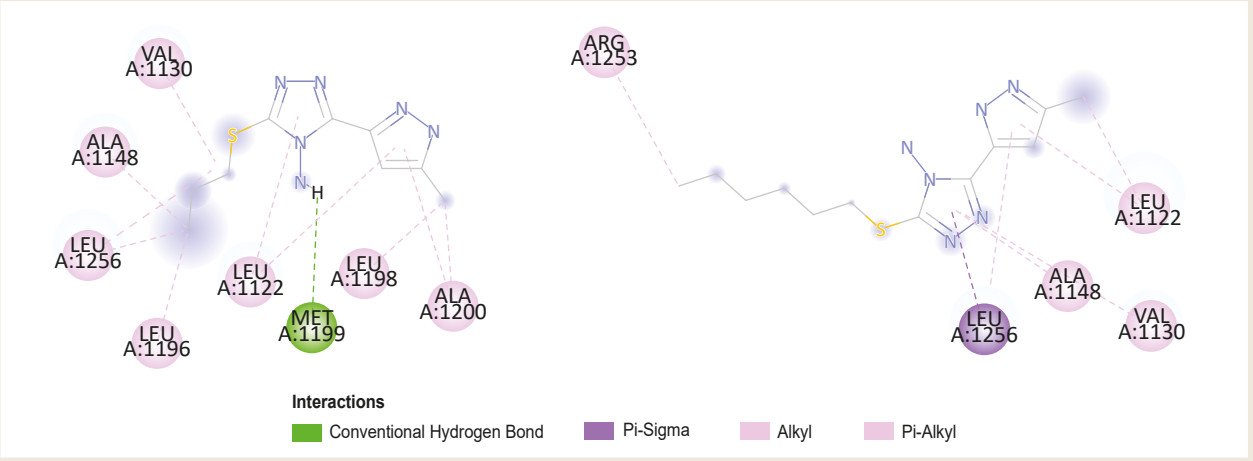

Fig. 5. Visualization of the affinity of the anaplastic lymphoma kinase with compounds 2.5 and 2.10.

with ALA A: 1200, LEU A: 1122, LEU A: 1196, LEU A: 1198, LEU A: 1256, LYS A: 1150 (*Fig. 5*). Moreover, attention is drawn to the presence of a certain amount of π-alkyl and π-anion hydrophobic interactions with the active site of the enzyme (with ALA A: 1200, LEU A: 1122 and GLU A: 1122), which had an immediate effect on the stability of a particular biologically active substance in the active site (*Fig. 5*).

Calculations of the free binding energy showed that an increase in the length of the S-alkyl fragment of the synthesized substances can have a positive effect on the affinity with the active site of the enzyme (*Table 3*). The most significant level of interaction with the active center of the enzyme were demonstrated by substances 2.8 and 2.10 with values of the free energy of interaction -8.0 kcal/mol and -8.4 kcal/mol accordingly (*Table 3*).

Discussion

The performed docking studies suggest that the synthesized S-alkyl derivatives exhibit the ability to bind to the active sites of COX-1, lanosterol 14-α-demethylase, and anaplastic lymphoma kinase.

It is also necessary to emphasize the participation of all fragments of molecules of new substances in interactions with the active site of enzymes.

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
2.0	-5.5	**2.4**	-6.7	**2.8**	-8.0
2.1	-5.2	**2.5**	-6.5	**2.9**	-7.9
2.2	-6.0	**2.6**	-6.8	**2.10**	-8.4
2.3	-6.4	**2.7**	-7.4	*Crizotinib*	-7.6

E_{min}: the minimum energy of complex formation.

Comparison of the calculated E_{min} values in the series of synthesized substances made it possible to establish the effect of the length of the S-alkyl fragment on the affinity with the active site of the enzymes under consideration. Moreover, the transition from a methyl substituent to a decyl substituent was accompanied by an increase in this affinity. Alkyl hydrophobic interactions of the synthesized substances with amino acid residues of the corresponding enzymes had a significant influence on the formation of this dependence.

Conclusions

1. Using the appropriate bromalkanes as alkylating agents (bromopropane, bromobutane, bromopentane, bromhexane, bromoheptane, bromoctane, bromonan, bromodecane), the reaction of nucleophilic substitution 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol was investigated. 10 new compounds were obtained. The structure was confirmed by complex modern physical and chemical methods of analysis (elemental analysis, PMR spectroscopy, chromatographic mass spectrometry), and their individuality was chromatographic.

2. The performed docking studied suggest that an increase in the length of the S-alkyl fragment increases the likelihood of anti-inflammatory, antifungal, and anticancer activity. Moreover, in molecules with an even number of carbon atoms in the alkyl substituent, this probability will only increase.

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

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