Synthesis and properties of some pyrazole derivatives of 1,2,4-triazole-3-thiol

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Nitrogen-containing heterocyclic compounds play an important role in the modern pharmaceutical industry. This is due to their significant biological potential. 1,2,4-Triazole and pyrazole are known pharmacophores that are responsible for the formation of a wide range of activities. The construction of the target molecule using 1,2,4-triazole and pyrazole is also interesting in terms of the availability of starting reagents and the simplicity of chemical transformations. The combination of these heterocycles in one molecule allows increasing its opportunities of participation in various biological processes.

The aim of the research was to examine the conditions for obtaining S-alkylderivatives of 5-(5-methylpyrazole)-4-ethyl-1,2,4-triazole-3-thiol and to investigate these compounds’ properties.

Materials and methods. The first phase of the research involved the use of diethyloxalate, acetone and sodium methyleate as starting substances for the synthesis of the intermediate. The obtained methyl 2,4-dioxopentanoate in the following step was converted into 5-methylpyrazole-3-carbohydrazide with the double amount of hydrazine hydrate. The subsequent modification of the molecule implied a stepwise use of the reactions of nucleophilic addition of ethyl isothiocyanate and alkaline cyclization. Thus, the obtained 5-(5-methylpyrazole)-4-ethyl-1,2,4-triazole-3-thiol became subject to an alkylation process. To establish the composition and identify the structure of the isolated substances, 1H NMR and infrared spectra were recorded, as well as qualitative and quantitative indicators of the elemental composition of the synthesized structures were obtained. The individual nature of the presence of substances and the degree of their purity were determined using high performance liquid chromatography with two types of detection: diode-matrix and mass spectrometric.

Results. The synthesis of alkylderivatives of 5-(5-methylpyrazole)-4-ethyl-1,2,4-triazole-3-thiol was carried out and the optimal conditions for the process of obtaining these substances were determined. The structure of the products of chemical transformation is confirmed and the results of the study of its physical properties are recorded.

The results of docking studies allowed to confirm the prospects of the chosen direction of synthetic transformations, which ultimately allowed to determine the biological potential of the obtained compounds. The model enzymes were kinase of the anaplastic lymphoma (code 2XPH), 14-alpha demethylase of the lanosterol (code 3LD6) and cyclooxygenase-1 (code 3N8Y), information on which was obtained from the Protein Structures Database (PDB).

Conclusions. Molecular docking resulted in obtaining data that form a concept of a certain level of probability of synthesized compounds’ influence on the activity of these enzyme structures.

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

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The search for new biologically active substances is a priority of modern pharmaceutical science [1–6].

Among the great variety of pharmacologically active molecules, special attention is drawn to the products of the processes of combining individual synths into a more complex molecule [7–9]. For example, pyrazole and 1,2,4-triazole derivatives are well studied and demonstrate a wide range of biological activity. Therefore, a combination of such fragments, which show different mechanisms of interaction with the biological target, within one molecule, is quite interesting and relevant.

**Aim**

The aim of the research was to study the conditions for obtaining and studying the properties of S-alkyl derivatives of 5-s-(5-methylpyrazole)-4-ethyl-1,2,4-triazole-3-thiol.
Materials and methods

Chemistry. Pyrazole was selected as an intermediate for chemical transformation. This five-membered heterocycle was formed using diethylxalate, acetone and sodium methanoate. Synthesized 5-methylpyrazole-3-carboxylic acid was used to form a fragment of 1,2,4-triazole. The obtained 5-(5-methylpyrazol-4-yl)-4-ethyl-1,2,4-triazole-3-thiol was used to carry out S-alkylation reactions using halogenoalkanes.

Properties (physical, chemical) of the produced organic materials have been examined in compliance with articles of the State Pharmacopoeia. The melting temperature has been defined in open capillary tubes (“MP 100 Melting Point Systems” manufactured by Mettler Toledo). Qualitative and quantitative elemental analysis was carried out with the “Vario EL cube” manufactured by Elementar Analyssysteme GmbH. Infrared spectra were obtained with the ALPHA FT-IR Spectrometer manufactured by Bruker. Nuclear magnetic resonance spectra on Hydrogen nuclei (1H NMR (δ, ppm)): 2.24 (s, 3H, CH3), 4.32 (s, 2H, NH), 6.37 (s, 1H, pyrazole, =CH-), 9.18 (s, 1H, CONH), 13.52 (s, 1H, pyrazole, NH). Analytically calculated (%): C 50.62, H 6.38, N 29.51, S 13.51. ESI-MS: m/z = 237 [M+1], 239 [M+3].

Methyl 5-methylpyrazole-3-carboxylate (4). 20 g H2N-NH2·H2O (0.4 mol) in 60 ml of ethanol was added with cooling to a solution containing 0.4 mol of methyl 2,4-dioxo-pentanoate (3) in propan-2-ol. Reaction mixture was heated for 1 hour. Then the alcohol was distilled off under vacuum. The resulting precipitate (99 %) was recrystallized from aqueous ethyl alcohol. Formed solid was dried to generate 4 with a melting point of 82–83 °C (Fig. 1).

5-Methylpyrazole-3-carboxylic acid (5). A mixture of intermediate 4 (58.4 g) and H2N-NH2·H2O (25 g) was heated at reflux for 7 hours. After cooling, the precipitate was filtered and recrystallized from H2O (Fig. 1). Yield: 88 %; melting point: 153–155 °C, IR (ν, cm-1): 3408-3237 (NH, NH2), 1625 (C=O); 1H NMR (δ, ppm): 2.24 (s, 3H, CH3), 4.32 (s, 2H, NH), 6.37 (s, 1H, pyrazole, =CH-), 9.18 (s, 1H, CONH), 13.52 (s, 1H, pyrazole, NH). Analytically calculated (%): C 48.85, H 5.75, N 39.98.

2-(5-Methylpyrazole-3-carbonyl)-N-ethylhydrazine-1-carbothioamide (6). A mixture of intermediate 5 (0.05 mol) was added and the solution was heated to reflux for 1 hour. After cooling, 100 ml of H2O was added. The resulting substances was filtered and washed with H2O and ethyl alcohol. Then it was crystallized from dimethylformamide (Fig. 1). Yield: 70 %; melting point: 263–265 °C, IR (ν, cm-1): 3230 (NH), 1648 (C=O); 1H NMR (δ, ppm): 2.26 (s, 3H, CH3), 4.65 (s, 1H, pyrazole, =CH-), 8.54 (s, 1H, CONH), 9.63 (s, 1H, NHCS), 13.28 (s, pyrazole, NH). Analytically calculated (%): C 42.28, H 5.77, N 30.81, S 14.11. Found C 42.19, H 5.78, N 30.85, S 14.07.

Results

The synthesis of the compounds 7.1–7.10 has been described in Fig. 1.

3-(5-Methylpyrazol-3-yl)-5-methylthio-4-ethyl-1,2,4-triazole (7.1). Yield: 76 %; melting point: 164–165 °C; IR (ν, cm-1): 3232 (NH), 1604 (C = N); 1H NMR (δ, ppm): 1.29 (t, J = 6.2 Hz, 3H, CH3-CH2), 2.33 (s, 3H, CH3), 2.76 (s, 3H, S-CH3), 4.35 (q, J = 6.1 Hz, 2H, CH2-CH2), 6.47 (s, 1H, pyrazole, =CH-), 11.73 (s, 1H, pyrazole, NH). Analytically calculated (%): C 48.41, H 5.87, N 31.36, S 14.36.

3-(5-Methylpyrazol-3-yl)-4-ethyl-1,2,4-triazole (7.2). Yield: 77 %; melting point: 83–84 °C; IR (ν, cm-1): 3232 (NH), 1599 (C=N); 1H NMR (δ, ppm): 1.30 (t, J = 6.1 Hz, 3H, CH3-CH2), 1.44 (t, J = 5.1 Hz, 3H, S-CH3-CH2), 2.35 (s, 3H, CH3), 3.19–3.21 (m, J = 4.8 Hz, 2H, S-CH2-CH2), 4.35 (q, J = 6.1 Hz, 2H, CH2-CH3), 6.45 (s, 1H, pyrazole, =CH-), 11.71 (s, 1H, pyrazole, NH). Analytically calculated (%): C 50.61, H 6.37, N 29.51, S 13.51. Found C 50.48, H 6.38, N 29.59, S 13.55. ESI-MS: m/z = 237 [M+1], 239 [M+3].

3-(5-Methylpyrazol-3-yl)-4-ethyl-5-propylthio-1,2,4-triazole (7.3). Yield: 78 %; melting point: 80–82 °C; IR (ν,
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Fig. 1. Synthetic pathway for the compounds 7.1–7.10.

3-Butylythio-5-(5-methylpyrazol-3-yl)-4-ethyl-1,2,4-triazole (7.4). Yield: 67%; melting point: 74–76 °C; IR (υ, cm⁻¹): 3232 (NH), 1595 (C=N); ¹H NMR (δ, ppm): 0.84 (t, J = 5.3 Hz, 3H, S-(CH₂)₂-CΗ₂), 1.28 (t, J = 6.1 Hz, 3H, CH₂-CΗ₂), 1.37–1.40 (m, 2H, S-(CH₂)₂-CΗ₂), 1.68–1.71 (m, J = 8.16, 7.27 Hz, 2H, S-CΗ₂-(CH₂)₂-CΗ₂), 2.33 (s, 3H, CH₃), 3.18 (t, 2H, S-(CH₂)₂-(CH₂)₂, CΗ₂), 4.36 (q, J = 6.0 Hz, 2H, CH₂-CΗ₂), 4.64 (s, 1H, pyrazole, =CΗ₂), 11.75 (s, 1H, pyrazole, NH). Analytically calculated (%): C 54.31, H 7.22, N 26.39, S 12.08. Found C 54.17, H 7.20, N 26.32, S 12.11. ESI-MS: m/z = 265 [M+1], 267 [M+3].

3-(5-Methylpyrazol-3-yl)-5-pentylthio-4-ethyl-1,2,4-triazole (7.5). Yield: 74%; melting point: 71–73 °C; IR (υ, cm⁻¹): 3231 (NH), 1609 (C=N); ¹H NMR (δ, ppm): 0.85 (t, J = 5.3 Hz, 3H, S-(CH₂)₂-CΗ₂), 1.36–1.41 (m, 7H, CH₂-CΗ₂, S-(CH₂)₂-(CH₂)₂-CΗ₂), 1.66–1.69 (m, J = 7.92, 7.25 Hz, 2H, S-(CH₂)₂-(CH₂)₂-CΗ₂), 2.33 (s, 3H, CH₃), 3.15 (t, J = 5.1 Hz, 2H, S-(CH₂)₂-(CH₂)₂-CΗ₂), 4.37 (q, J = 6.2 Hz, 2H, CH₂-CΗ₂), 6.47 (s, 1H, pyrazole, =CΗ₂), 11.74 (s, 1H, pyrazole, NH). Analytically calculated (%): C 55.88, H 7.58, N 25.07, S 11.47. Found: C 56.01, H 7.56, N 25.13, S 11.44. ESI-MS: m/z = 279 [M+1], 281 [M+3].
4.37 (q, J = 6.0 Hz, 2H, CH\textsubscript{2}-CH\textsubscript{3}), 6.46 (s, 1H, pyrazole, =CH-), 11.72 (s, 1H, pyrazole, NH). Analytically calculated (%): C 60.86, H 8.71, N 20.87, S 9.56. ESI-MS: m/z = 337 [M+H\textsuperscript{+}].

3-Decylthio-5-(5-methylpyrazol-3-yl)-4-ethyl-1,2,4-triazole (7.10). Yield: 86%; melting point: 60–61 °C; IR (ν, cm\textsuperscript{-1}): 3233, 3183 (N-H), 1167, S-(CH\textsubscript{2})\textsubscript{2}, 3.09–3.13 (t, J = 6.1 Hz, 2H, CH\textsubscript{2}-CH\textsubscript{3}), 6.49 (s, 1H, pyrazole, =CH-), 11.75 (s, 1H, pyrazole, NH). Analytically calculated (%): C 61.85, H 8.94, N 20.04, S 9.17. Found C 61.68, H 8.92, N 20.09, S 9.19. ESI-MS: m/z = 349 [M+H\textsuperscript{+}], 337 [M+3].

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Table 1. Molecular docking calculation with ALK

| № | $E_{\text{min}}$, kcal/mol | № | $E_{\text{min}}$, kcal/mol | № | $E_{\text{min}}$, kcal/mol |
|---|--------------------------|---|--------------------------|---|--------------------------|
| 6 | -5.4                     | 7.4 | -7.6                     | 7.8 | -7.8                     |
| 7.1 | -6.1                   | 7.5 | -7.7                     | 7.9 | -7.7                     |
| 7.2 | -6.7                   | 7.6 | -7.4                     | 7.10 | -7.5                     |
| 7.3 | -7.5                   | 7.7 | -7.7                     | Crizotinib | -9.4                     |

$E_{\text{min}}$: the minimum interaction energy, kcal/mol.

Table 2. Molecular docking calculation with COX-1

| № | $E_{\text{min}}$, kcal/mol | № | $E_{\text{min}}$, kcal/mol | № | $E_{\text{min}}$, kcal/mol |
|---|--------------------------|---|--------------------------|---|--------------------------|
| 6 | -8.8                     | 7.4 | -6.2                     | 7.8 | -6.9                     |
| 7.1 | -5.3                   | 7.5 | -6.2                     | 7.9 | -7.3                     |
| 7.2 | -5.4                   | 7.6 | -5.3                     | 7.10 | -7.4                     |
| 7.3 | -5.6                   | 7.7 | -6.5                     | Diclofenac | -19.9                     |

$E_{\text{min}}$: the minimum interaction energy, kcal/mol.

Table 3. Molecular docking calculation with lanosterol 14α-demethylase

| № | $E_{\text{min}}$, kcal/mol | № | $E_{\text{min}}$, kcal/mol | № | $E_{\text{min}}$, kcal/mol |
|---|--------------------------|---|--------------------------|---|--------------------------|
| 6 | -6.0                     | 7.4 | -8.5                     | 7.8 | -7.8                     |
| 7.1 | -6.1                   | 7.5 | -8.5                     | 7.9 | -9.3                     |
| 7.2 | -6.4                   | 7.6 | -7.4                     | 7.10 | -9.2                     |
| 7.3 | -8.5                   | 7.7 | -8.5                     | Ketoconazole | -10.1                     |

$E_{\text{min}}$: the minimum interaction energy, kcal/mol.

It is known about the manifestation of anti-inflammatory activity, which is associated with the combination of triazole and pyrazole fragments within one molecule. In addition, it has been established which aminoacid residues of cyclodehydrogenases are directly responsible for the formation of hydrogen intermolecular bonds with substances that demonstrate biological activity. Thus, this area of research involving hydrogen vibrations of alkyl groups form bands in the area of 3075–2845 cm\textsuperscript{-1}. Valence vibrations of bonds of C-H alkyl groups form bands in the area of 3075–2845 cm\textsuperscript{-1}. In the mass spectrum, there is a peak of the molecular ion and peaks of fragment ions, which confirm this structure. The methodology for rational drug development involves the use of molecular docking. Docking experiments of synthesized compounds (7, 7.1–7.8) with the 2XP2 (anaplastic lymphoma receptor tyrosine kinase) receptor revealed that compound 7.8 is the most active with a calculated binding energy of 8.1 kcal/mol (Table 1) [6,8–10]. The effect of 1,2,4-triazole derivatives on the activity of lanosterol 14-alpha demethylase is a proven fact. It is established that 1 and 2 Nitrogen atoms of 1,2,4-triazole and pyrazole fragments within one molecule. In addition, it has been established which aminoacid residues of cyclo-oxygenases are directly responsible for the formation of hydrogen intermolecular bonds with substances that demonstrate biological activity. Thus, this area of research involving S-alkyl derivatives of 5-(5-methylpyrazol-3-yl)-4-ethyl-1,2,4-triazole-3-thiol is quite interesting. Cyclooxygenase-1 was chosen as the model enzyme (Table 2). The effect of 1,2,4-triazole derivatives on the activity of lanosterol 14-alpha demethylase is a proven fact. It is established that 1 and 2 Nitrogen atoms of 1,2,4-triazole fragment are responsible for the formation of $\pi$-$\pi$-interaction with the active center of the specified enzyme.
study of the effect of synthesized compounds on this enzyme was considered relevant and was carried out (Table 3).

Discussion

According to the docking results, the synthesized compounds show different levels of binding to the aminocid residues of anaplastic lymphoma kinase, cyclooxygenase-1 and lanosterol-14α-demethylase.

The transition from thiol to its alkyl derivatives in a number of synthesized compounds leads to an increase in the level of binding to the active centers of anaplastic lymphoma kinase and lanosterol-14α-demethylase.

Docking to cyclooxygenase-1 revealed a decrease in the interaction energy with the specified enzyme of the synthesized alkyl derivatives in comparison with the original thiol. The most relevant for further studies was 3-(5-methylpyrazol-3-yl)-5-octythio-4-ethyl-1,2,4-triazole.

Conclusions

1. The optimal conditions for obtaining S-alkylderivatives of 5-(5-methylpyrazole)-4-ethyl-1,2,4-triazole-3-thiol were determined. It was found that the highest yield of products of the alkylation reaction of 5-(5-methylpyrazole-3-yl)-4-ethyl-1,2,4-triazole-3-thiol was observed when methanol and propan-2-ol were used as solvents.

2. As a result of the molecular docking of the synthesized compounds, promising structures for further studies of anti-inflammatory and antifungal activity were identified. The most promising object for further research is 3-(5-methylpyrazol-3-yl)-5-octythio-4-ethyl-1,2,4-triazole.

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

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