Synthesis and properties of some 3-(5-(4-methoxyphenyl)pyrazol-3-yl)-6-R-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles

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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

Pyrazole and 1,2,4-triazole derivatives play an important strategic role in modern medicine and pharmacy. This fact is due to the significant possibilities of chemical modification and significant pharmacological potential among the derivatives of these heterocycles. The introduction of 1,2,4-triazole and pyrazole fragments into the structure of new substances allows to influence the formation of a certain type of activity. The structural combination of these heterocycles in one molecule increases the likelihood of interaction with various biological targets. At the same time, the creation of condensed systems involving 1,2,4-triazole is undoubtedly scientifically attractive and promising.

The aim of the research was to study the conditions for obtaining 3-(5-(4-methoxyphenyl)pyrazol-3-yl)-6-R-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles and studying the properties of these compounds.

Materials and methods. The first stage of the synthetic part of the work involved the use of diethyl oxalate and 1-(4-methoxyphenyl)ethan-1-one with the participation of sodium hydride in toluene. The obtained ethyl 4-hydroxy-4-(4-methoxyphenyl)-2-oxobut-3-ethanoate in the next step was used in the process of conversion into ethyl 5-(4-methoxyphenyl)pyrazole-3-carboxylate with the participation of hydrazine hydrate. Further modification of the molecule was the stepwise formation of the structure of 4-amino-5-(5-(4-methoxyphenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol. The next stage of the work involved the interaction with carboxylic acids in the environment of phosphorus oxychloride. To determine the composition and identify the structure of the isolated substances, ^{1}H 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.

Results. Synthesis of 3-(5-(4-methoxyphenyl)pyrazol-3-yl)-6-R-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles was performed and optimal conditions were determined the process of obtaining these substances. The structure of the products of chemical transformation was confirmed and the results of the study of physical properties were 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 enzyme was 14-α-demethylase lanosterol (code 3LD6), information on which was used from the database of the Protein Structures Database (PDB).

Conclusions. As a result of the molecular docking, data were obtained that form an idea of a certain level of probability of the effect of synthesized compounds on the activity of 14α-demethylase lanosterol, which justified the need for further study of antifungal activity.

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

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Синтез і властивості деяких 3-(5-(4-метоксифеніл)піразол-3-іл)-6-R-[1,2,4]тріазоло[3,4-*b*][1,3,4]тіадіазолів

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Похідні піразолу та 1,2,4-тріазолу відіграють важливу стратегічну роль у сучасній медицині та фармації. Цей факт зумовлений істотними можливостями хімічної модифікації та значним фармакологічним потенціалом серед похідних цих гетероциклів. Введення в структуру нових речовин фрагментів 1,2,4-тріазолу та піразолу дає змогу вплинути на формування певного типу активності. Структурне поєднання цих гетероциклів в одній молекулі підвищує імовірність взаємодії з різними біологічними мішенями. Створення конденсованих систем за участю 1,2,4-тріазолу, безперечно, є науково привабливим і перспективним.

Мета роботи – дослідження умов одержання 3-(5-(4-метоксифеніл)піразол-3-іл)-6-R-[1,2,4]тріазоло[3,4-*b*][1,3,4]тіадіазолів і вивчення властивостей цих сполук.

Матеріали та методи. Перший етап синтетичної частини роботи передбачав використання діетилоксалату та 1-(4-метоксифеніл) етан-1-ону за участю натрій гідриду в середовищі толуену. Одержаний етил-4-гідрокси-4-(4-метоксифеніл)-2-оксобут-3-етаноат на

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

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наступному етапі застосували в процесі перетворення на етил-5-(4-метоксифеніл)піразол-3-карбоксилат за участю гідразин гідрату. Модифікація молекули надалі полягала в поетапному формуванню структури 4-аміно-5-(5-(4-метоксифеніл)піразол-3-іл)-1,2,4-тріазол-3-тіолу. Наступний етап роботи передбачав взаємодію з карбоновими кислотами в середовищі фосфор оксихлориду. Для встановлення складу й ідентифікації структури виділених речовин записали ^{1}Н ЯМР та інфрачервоні спектри, а також одержали якісні та кількісні показники елементного складу синтезованих структур. Індивідуальний характер наявності речовин і ступінь їхньої чистоти визначили, використавши високоефективну рідинну хроматографію.

Результати. Здійснили синтез 3-(5-(4-метоксифеніл)піразол-3-іл)-6-R-[1,2,4]тріазоло[3,4-*b*][1,3,4]тіадіазолів і визначили оптимальні умови процесу одержання цих речовин. Будова продуктів хімічного перетворення підтверджена, результати дослідження фізичних властивостей зафіксовано. Результати докінгових досліджень дали підстави для підтвердження перспективності обраного напряму синтетичних перетворень, це врешті дало змогу визначитись із біологічним потенціалом одержаних сполук. Модельний фермент –14-α-деметилаза ланостеролу (код 3LD6), інформація щодо неї використана з бази Банку даних білкових структур (PDB).

Висновки. У результаті молекулярного докінгу отримали дані, що формують уявлення про певний рівень імовірності впливу синтезованих сполук на активність 14-α-деметилази ланостеролу. Це обґрунтовує необхідність продовження дослідження протигрибкової активності.

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

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The combination of different heterocyclic fragments with pharmacophore properties in one molecule makes it possible to use in practice the basic principles of the hybrid pharmacophore method for constructing molecules that are promising from the point of view of Pharmacology [1–9]. This approach allows you to achieve the necessary pharmacological effect, influence the indicators of toxicity and selectivity of action [10–15]. Recently, there has been an increased interest in the study of condensed heterocyclic systems. This fact is explained by the high probability of detecting highly active molecules among them [10,11].

An interesting area of work that allows us to obtain this result is the condensed system of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole in combination with pyrazole [10]. Thus, the preliminary results of the study of biological properties in a number of derivatives of this condensed heterocyclic system allow us to speak about the prospects and validity of the chosen direction of scientific research [10]. Among the confirmed types of activity are antitumor, antimicrobial, antidepressant, anticonvulsant, antiviral and others.

Aim

The aim of the work was to synthesize compounds in the series 3-(5-(4-methoxyphenyl)pyrazole-3-yl)-6-R-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles.

Materials and methods

The synthetic part of the work is a step-by-step formation of target products of chemical transformation based on well-known methods of organic synthesis using available reagents and solvents with their additional purification.

The structure of all synthesized substances was proved using physical-chemical analysis methods. Modern analysis methods were used to establish the structure and confirm the purity of the obtained compounds. Melting points were set in open capillaries using "Stanford Research Systems Melting point Apparatus 100" (SRS, USA). Elemental analysis (C, H, N, S) was performed using an "Elementary vario EL cube" analyzer (Elementary Analysensysteme, Germany). IR spectra (in the frequency range 4000–400 cm^{-1}) were obtained on the ALPHA-T module of the Bruker ALPHA FT-IR spectrometer (Bruker optics, Germany). 1H NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 spectrometer using tetramethylsilane as an internal standard in a DMSO-d_6 solution. Chromato-mass spectra were obtained using an Agilent 1260 Infinity HPLC liquid chromatograph equipped with an Agilent 6120 spectrometer (electrospray ionization method (ESI)).

Molecular docking

The next stage of this work was the preliminary determination of substances with possible antifungal activity. For this purpose, molecular docking was performed [16–18]. The use of *in silico* methods makes it possible to preserve laboratory animals in the case of low affinity of the studied compounds to potential biotargets.

The molecular docking method is based on an approach to detecting molecules with affinity for a specific biological target and allows you to save laboratory animals. Macromolecules from the Protein Data Bank (PDB), namely a fragment of lanosterol-14α-dimethylase (CYP51) in complex with fluconazole, were used as biological targets.

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

The research methodology consisted of the following stages:

1) ligand preparation: construction of structural formulas of compounds using the program MarvinSketch 6.3.0 and their storage in mol format; generation of 3D structure of formulas of compounds-molecular modeling (Hyper Chem 8 program using the method of molecular mechanics MM+ and semi-empirical quantum mechanical method PM3 with the maximum number of cycles and Polak-Ribiere algorithm and saving molecules in PDB files); use AutoDockTools-1.5.6 to convert PDB to pdbqt files;

2) preparation of enzymes: removing water and ligand molecules from a file using the discovery Studio 4.0 software package and saving the enzyme in PDB format; using AutoDockTools-1.5.6 to convert the PDB enzyme to PDBQT files;

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

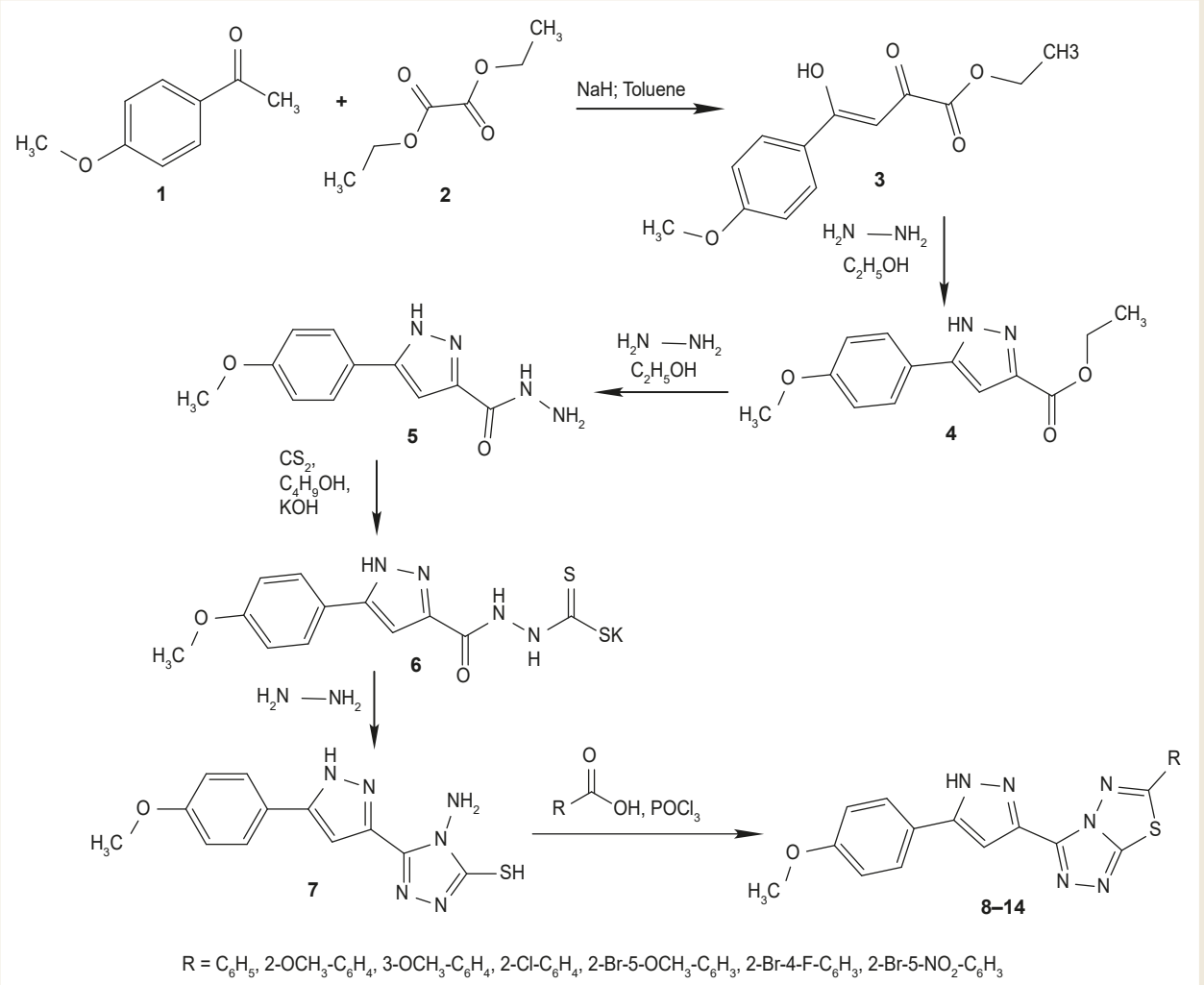


Fig. 1. Scheme of synthesis of 3-(5-(4-methoxyphenyl)pyrazole-3-yl)-6-R-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles.

Results

The first stage of work was to gradually form a pyrazole fragment. As a starting material, 1-(4-methoxyphenyl) ethane-1-one was used, which in the reaction of interaction with diethyloxalate with the participation of sodium hydride in toluene formed ethyl-4-hydroxy-4-(4-methoxyphenyl)-2-oxobut-3-ethanoate. The resulting diketoester was used in the reaction of interaction with hydrazine hydrate in an ethanol medium. As a result of this chemical interaction, ethyl-5-(4-methoxyphenyl)pyrazole-3-carboxylate was obtained. The next stage of work again involved hydrazinolysis. The resulting hydrazide then interacted with carbon disulfide in a 9 % potassium hydroxide solution in butane-1-ole. As a result, the structure of 4-amino-5-(5-(4-methoxyphenyl) pyrazole-3-yl)-1,2,4-triazole-3-thiol was formed.

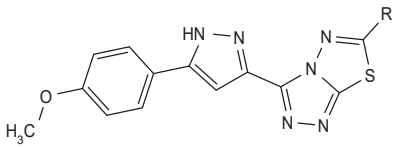
The next stage of chemical transformation occurred with the participation of synthesized thiol and aromatic carboxylic acids in a phosphorus oxychloride medium, which made it possible to obtain 3-(5-(4-methoxyphenyl)pyrazole-3-yl)-6-R-[1,2,4]triazolo-[3,4-*b*][1,3,4]thiadiazoles (*Fig. 1*).

The structure and individuality of the obtained compounds were proved using physical-chemical methods of analysis: elemental analysis (*Table 1*), IR sectrophotometry, ^{1}H NMR spectroscopy, chromato-mass spectrometry.

Ethyl-4-hydroxy-4-(4-methoxyphenyl)-2-oxobut-3-ethanoate (3). Sodium hydride (0.2 mol) was added to a mixture of 1-(4-methoxyphenyl)ethane-1-one (0.10 mol) and diethyloxalate (0.15 mol) in 60 ml of anhydrous toluene.

The resulting mixture was stirred at 30 °C for 8 hours. The solvent was distilled off under vacuum. The crude mixture was poured into 100 ml of ice water and acidified with dilute hydrochloric acid. The resulting crystalline precipitate was filtered off and washed with water. Crystallized from methanol. Yellow crystalline substance. Yield: 86 %. M. p.: 96–98 °C. IR, ν (cm^{-1}): 1735 (C = O, ester), 1685 (C = O, keton), 1447 (C = C). ^{1}H NMR, δ *(ppm), J (Hz)*: 14.76 (s, 1H, OH), 7.94–7.88 (m, 2H, H-2,6, 4-OCH_3-C_6H_4), 7.25–7.31 (m, 2H, H-3,5, 4-OCH_3-C_6H_4), 6.43 (s, 1H, =CH), 4.25 (q, J = 6.8 Hz, 2H, OCH_2CH_3), 3.74 (s, 3H, 4-OCH_3-C_6H_4), 1.17 (t, 3H, OCH_2CH_3).

Table 1. Physical and chemical properties of synthesized compounds



Compound	R	M. p., °C	Empirical formula	Yield, %	Calculated, %				Found, %			
					C	H	N	S	C	H	N	S
8	C_6H_5	205–207	$C_{19}H_{14}N_6OS$	79	60.95	3.77	22.45	8.56	60.78	3.76	22.51	8.58
9	2-OCH_3-C_6H_4	189–191	$C_{20}H_{16}N_6O_2S$	63	59.39	3.99	20.78	7.93	59.23	4.00	20.72	7.91
10	3-OCH_3-C_6H_4	194–196	$C_{20}H_{16}N_6O_2S$	65	59.39	3.99	20.78	7.93	59.55	3.98	20.82	7.95
11	2-Cl-C_6H_4	202–204	$C_{19}H_{13}ClN_6OS$		55.82	3.20	20.56	7.84	55.96	3.19	20.60	7.82
12	2-Br-5-OCH_3-C_6H_3	185–187	$C_{20}H_{15}BrN_6O2S$	73	49.70	3.13	17.39	6.63	49.56	3.14	17.42	6.61
13	2-Br-4-F-C_6H_3	176–178	$C_{19}H_{12}BrFN_6OS$	61	48.42	2.57	17.83	6.80	48.56	2.58	17.79	6.78
14	2-Br-5-NO_2-C_6H_3	172–174	$C_{19}H_{12}BrN_7O_3S$	69	45.80	2.43	19.68	6.43	45.69	2.42	19.71	6.45

Ethyl-5-(4-methoxyphenyl)pyrazole-3-carboxylate (4). A mixture of ethyl-4-hydroxy-4-(4-methoxyphenyl)-2-oxobut-3-ethanoate (0.1 mol) and hydrazine hydrate (0.5 mol) in 40 ml of propane-1-ol was heated to a boil for 8 hours. After removing the alcohol, an oily liquid was obtained, which was poured on the crushed ice. A crystalline substance was formed. The yield is 76 %. M. p.: 166–168 °C. IR, *ν* (cm^{-1}): 1721 (C = O, ester), 1435 (C = C). ^{1}H NMR, *δ (ppm), J (Hz)*: 13.04 (s, 1H, NH, pyrazole), 8.66 (s, 1H, CH, pyrazole), 7.59–7.54 (m, 2H, H-2,6, 4-OCH_3-C_6H_4), 7.04–6.98 (m, 2H, H-3,5, 4-OCH_3-C_6H_4), 4.31 (q, *J* = 6.2 Hz, 2H, OCH_2CH_3), 3.77 (s, 3H, 4-OCH_3-C_6H_4), 1.15 (t, 3H, OCH_2CH_3).

5-(4-Methoxyphenyl)pyrazole-3-carbohydrazide (5). A mixture of ethyl-5-(4-methoxyphenyl)pyrazole-3-carboxylate (0.1 mol) and hydrazine hydrate (0.2 mol) was heated with 40 ml of ethanol to a boil for 6 hours. After cooling, the sediment was filtered and recrystallized from the water (*Fig. 1*). Yield: 84 %; m. p.: 153–155 °C; IR, *ν* (cm^{-1}): 3404–3233 (NH, NH_2), 1615 (C = O); ^{1}H NMR, *δ (ppm), J (Hz)*: 13.12 (s, 1H, NH, pyrazole), 9.21 (t, *J* = 4.6 Hz, 1H, N*H*NH_2), 8.27 (s, 1H, CH, pyrazole), 7.57–7.52 (m, 2H, H-2,6, 4-OCH_3-C_6H_4), 7.03–6.97 (m, 2H, H-3,5, 4-OCH_3-C_6H_4), 4.29 (d, *J* = 4.6 Hz, 2H, $NHNH_2$), 3.79 (s, 3H, 4-OCH_3-C_6H_4). Elemental analysis (%): C 56.89, H 5.21, N 24.12. Found: C 56.73, H 5.22, N 24.06.

Potassium 2-(5-(4-methoxyphenyl)pyrazole-3-carbonyl) hydrazine-1-carbo-dithioate (6). For the reaction, it was weighed (0.2 mol) 5-(4-methoxyphenyl)pyrazole-3-carbohydrazide and potassium hydroxide equivalently (0.2 mol). From potassium hydroxide, a 9 % solution for butan-1-ol was prepared, in which 5-(4-methoxyphenyl)pyrazole-3-carbohydrazide was dissolved. After the substance was completely dissolved, a mechanical agitator was used and 0.3 mol of carbon disulfide was added drop by drop. During the reaction, a yellow precipitate of potassium salt was formed.

At the end of adding carbon disulfide, the mixture was stirred for another 30 minutes, filtered, dried and prepared for the next stage.

4-Amino-5-(5-(4-methoxyphenyl)-pyrazole-3-yl)-1,2,4-triazole-3-thiol (7). Potassium 2-(5-(4-methoxyphenyl)pyrazole-3-carbonyl)hydrazine-1-carbo-dithioate was weighed and placed in a heat-resistant flask, dissolved in 150–200 ml of purified water and added five times the excess hydrazine hydrate. In this form, the mixture was boiled for 6 hours. At the end of 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 thiol precipitate begins to form. The reaction was very violent. The resulting sediment was filtered and dried.

General scheme of synthesis of 3-(5-(4-methoxyphenyl)pyrazole-3-yl)-6-R-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (8–14)

To 0.01 mol of the initial 4-amino-5-(5-(4-methoxyphenyl) pyrazole-3-yl)-1,2,4-triazole-3-thiol, 0.01 mol of the corresponding carboxylic acid (benzoic, 2-methoxy-, 3-methoxybenzoic, 2-chlorobenzoic, 2-bromo-5-methoxybenzoic, 2-bromo-4-fluorobenzoic, 2-bromo-5-nitrobenzoic) and 15 ml of $POCl_3$ was added and heated for 3 hours at the temperature of 80 °C. After cooling, the solution was poured onto the ice. The resulting sediment was filtered and washed with water.

The compounds crystallized from concentrated ethanoic acid. The substances were crystalline white (8-11), yellow (12, 14), and bright yellow (13). These substances were practically insoluble in water, soluble in organic solvents.

8. ^{1}H NMR, *δ (ppm), J (Hz)*: 13.07 (s, 1H, NH, pyrazole), 8.70 (s, 1H, CH, pyrazole), 7.97–7.90 (m, 2H, H-2,6, C_6H_5), 7.56–7.45 (m, 5H, H-3,4,5, C_6H_5, H-2,6, 4-OCH_3-C_6H_4), 7.03–6.97 (m, 2H, H-3,5, 4-OCH_3-C_6H_4), 3.82 (s, 3H, 4-OCH_3-C_6H_4).

9. ^{1}H NMR, *δ (ppm), J (Hz)*: 13.05 (s, 1H, NH, pyrazole), 8.72 (s, 1H, CH, pyrazole), 7.79 (dd, *J* = 6.8, 1.7 Hz, 1H, H-6, 2-OCH_3-C_6H_4), 7.56–7.51 (m, 2H, H-2,6, 4-OCH_3-C_6H_4), 7.36 (t, 1H, H-4, 2-OCH_3-C_6H_4), 7.21 (t, 1H, H-5, 2-OCH_3-C_6H_4), 7.04–6.97 (m, 3H, H-3,5, 4-OCH_3-C_6H_4, H-5, 2-OCH_3-C_6H_4), 3.96 (s, 3H, 2-OCH_3-C_6H_4), 3.80 (s, 3H, 4-OCH_3-C_6H_4).

10. ^{1}H NMR, *δ (ppm), J (Hz)*: 13.07 (s, 1H, NH, pyrazole), 8.71 (s, 1H, CH, pyrazole), 7.73 (dd, *J* = 6.9, 2.1 Hz, 1H, H-6, 3-OCH$_3$-C$_6H_4$), 7.58–7.53 (m, 2H, H-2,6, 4-OCH$_3$-C$_6H_4$), 7.37 (t, 1H, H-5, 3-OCH$_3$-C$_6H_4$), 7.05–6.98 (m, 2H, H-3,5, 4-OCH$_3$-C$_6H_4$), 6.85 (dd, *J* = 8.1, 1.9 Hz, 1H, H-4, 3-OCH$_3$-C$_6H_4$), 3.88 (s, 3H, 3-OC*H*$_3$-C$_6$H$_4$), 3.79 (s, 3H, 4-OC*H*$_3$-C$_6$H$_4$).

11. ^{1}H NMR, *δ (ppm), J (Hz)*: 13.07 (s, 1H, NH, pyrazole), 8.70 (s, 1H, CH, pyrazole), 7.81 (d, *J* = 7.3 Hz, 1H, H-6, 2-Cl-C$_6H_4$), 7.59–7.52 (m, 3H, H-3, 2-Cl-C$_6H_4$, H-2,6, 4-OCH$_3$-C$_6H_4$), 7.41 – 7.32 (m, 2H, H-4,5, 2-Cl-C$_6H_4$), 7.04–6.97 (m, 2H, H-3,5, 4-OCH$_3$-C$_6H_4$), 3.80 (s, 3H, 4-OC*H*$_3$-C$_6$H$_4$).

12. ^{1}H NMR, *δ (ppm), J (Hz)*: 13.05 (s, 1H, NH, pyrazole), 8.70 (s, 1H, CH, pyrazole), 7.67 (d, *J* = 8.5 Hz, 1H, H-3, 2-Br-5-OCH$_3$-C$_6H_3$), 7.55–7.51 (m, 2H, H-2,6, 4-OCH$_3$-C$_6H_4$), 7.26 (d, *J* = 7.3 Гц, 1H, H-6, 2-Br-5-OCH$_3$-C$_6H_3$), 7.03–6.97 (m, 2H, H-3,5, 4-OCH$_3$-C$_6H_4$), 6.82 (dd, *J* = 8.4, 2.8 Hz, 1H, H-4, 2-Br-5-OCH$_3$-C$_6H_3$), 3.86 (s, 3H, 2-Br-5-OC*H*$_3$-C$_6$H$_3$), 3.81 (s, 3H, 4-OC*H*$_3$-C$_6$H$_4$).

13. ^{1}H NMR, *δ (ppm), J (Hz)*: 13.07 (s, 1H, NH, pyrazole), 8.71 (s, 1H, CH, pyrazole), 7.72 (dd, *J* = 7.8, 4.9 Hz, 1H, H-6, 2-Br-4-F-C$_6H_3$), 7.57–7.52 (m, 2H, H-2,6, 4-OCH$_3$-C$_6H_4$), 7.48 (dd, *J* = 11.6, 2.2 Hz, 1H, H-3, 2-Br-4-F-C$_6H_3$), 7.19–7.12 (m, 1H, H-5, 2-Br-4-F-C$_6H_3$), 7.02 – 6.97 (m, 2H, H-3,5, 4-OCH$_3$-C$_6H_4$), 3.80 (s, 3H, 4-OC*H*$_3$-C$_6$H$_4$).

14. ^{1}H NMR, *δ (ppm), J (Hz)*: 13.07 (s, 1H, NH, pyrazole), 8.70 (s, 1H, CH, pyrazole), 8.61 (d, *J* = 2.1 Hz, 1H, H-6, 2-Br-5-NO$_2$-C$_6H_3$), 8.19 (dd, *J* = 8.6, 2.2 Hz, 1H, H-4, 2-Br-5-NO$_2$-C$_6H_3$), 7.96 (d, *J* = 8.5 Hz, 1H, H-3, 2-Br-5-NO$_2$-C$_6H_3$), 7.59–7.54 (m, 2H, H-2,6, 4-OCH$_3$-C$_6H_4$), 7.03–6.97 (m, 2H, H-3,5, 4-OCH$_3$-C$_6H_4$), 3.78 (s, 3H, 4-OC*H*$_3$-C$_6$H$_4$).

Molecular docking

A thorough analysis of the results of doping studies demonstrated excellent affinity values for the obtained ligands to lanosterol-14α-demethylase. This fact was confirmed by the calculated values of the free binding energy for complexes formed by 3-(5-(4-methoxyphenyl)pyrazole-3-yl)-6-R-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles and the lanosterol-14α-demethylase receptor (*Table 2*).

Visualization of the interaction of structures with the active site of lanosterol-14α-demethylase showed that they had a significant spectrum of interactions with a significant number of amino acid residues.

Discussion

The structure and individuality of the synthesized substances were confirmed by modern physical-chemical methods of analysis.

^{1}H NMR spectra of synthesized compounds (**8–14**) were recorded in accordance with the proposed structures. Thus, the signal of protons of the NH group of the pyrazole cycle was located at 13.05–13.07 ppm in the form of a singlet. In the weak part of the magnetic field, singlets were recorded that form protons of the methyl group of the pyrazole fragment at 8.70–8.72 ppm. The 4-methoxyphenyl substitute in most cases (compounds **10, 12–14**) had a multiplet signal of *ortho*-protons in the region of 7.51–7.59 ppm and a multiplet signal of *meta*-protons in the region of 6.97–7.05 ppm in the spectra of other compounds (**8, 9, 11**) in the "aromatic" part of the spectrum, more complex multiplets were observed, the formation of which was due to the presence in the structure of synthesized compounds of the order 4-methoxyphenyl substitute phenyl (**8**), 2-methoxyphenyl (**9**) and 2-chlorophenyl (**11**).

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

N	E_{min}	N	E_{min}	N	E_{min}
8	-10.6	10	-10.6	13	-10.1
9	-9.5	11	-10.3	14	-11.3
Fluconazole	-8.4	12	-10.6	–	–

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

For example, in the spectrum of compound **7**, signals of *meta*- and *para*-protons of the phenyl substituent and signals of *ortho*-protons of the 4-methoxyphenyl substituent gave a five-proton multiplet in the spectrum at 7.45–7.56 ppm.

Proton signals at positions 4 and 5 of the 2-methoxyphenyl substitute compound **9** appeared as triplets with an intensity of 1H at 7.36 ppm and 7.21 ppm, respectively. Also, in the spectrum of compound **10**, the signal of a proton in the 5th position of the 3-methoxyphenyl substitute forms a triplet at 7.37 ppm at the same time, a proton in the 4th position of the specified substitute of compound **10** turned out to be a doublet of doublets at 6.85 ppm.

Proton signals of the 2-bromo-5-methoxyphenyl substituent of compound **12** gave doublets (H-3 and H-6) in the spectrum at 7.67 ppm and 7.26 ppm, respectively, doublet of doublets (H-4) at 6.82 ppm a similar picture of proton signals was formed by 2-bromo-4-fluorophenyl and 2-bromo-5-nitrophenyl substituents. But there were also expected features. For example, the presence of a nitro-group in the structure of the aromatic substitute of compound **14** contributes to the shift of proton signals to a weaker field, which was due to the pronounced "–I" effect of this group. Thus, a proton in the 6th position of the 2-bromo-5-nitrophenyl substitute forms a signal in the form of a doublet at 8.61 ppm, in the 4th position – in the form of a doublet of doublets at 8.19 ppm, and in the 3rd position – in the form of a doublet at 7.96 ppm.

In the strong part of the magnetic field, the proton signals of the methoxygroup of 2-, 3-, and 4-methoxyphenyl substituents appeared as a singlet at 3.96 ppm, 3.88 ppm, and 3.79–3.82 ppm, respectively.

Significant potential for antifungal activity was confirmed by molecular docking.

The synthesized ligands were characterized by a significant variety of interactions with the active site of lanosterol-14α-demethylase, including alkyl, π-alkyl, π-σ, π-sulfur, and amide-π stacking interactions. These types of interactions were enhanced by intermolecular hydrogen chemical bonding, Van der Waals intermolecular interaction forces, and halogen and Carbon-Hydrogen interactions. For example, the hydrophobic alkyl interaction of compound **12** was realized with the participation of the methoxy-group of the ligand and the amino acid ALA A: 400 residue. The π-alkyl

interaction was formed using the 4-methoxyphenyl fragment and the PRO A: 400 residue, the 1,2,4-triazole fragment and the LEU A: 324 residue, the 2-bromo-4-fluorophenyl substitute, and ARG A: 96. The π-σ interaction occured using the 2-bromo-4-fluorophenyl and pyrazole fragments and VAL A: 395 and LEU A: 321 residues, respectively. The amide-π stacking interaction was characteristic of this compound and contributes to the formation of a complex involving the PHE A: 387 residue and the pyrazole fragment. The sulfur of the 1,3,4-thiadiazine fragment and the pyrrole-like Nitrogen of the pyrazole fragment additionally interact with GLN A: 72 and PRO A: 386 residues due to intermolecular hydrogen chemical bonds. Also characteristic was the interaction involving Fluorine, which interacts with LEU A: 100. The resulting complex with the active site of the enzyme was further enhanced by the Carbon-Hydrogen interaction involving the methyl fragment of the methoxy-substitute and the SER A: 261 residue and Van der Waals forces involving GLY A: 388.

Conclusions

1. A convenient formation of a pyrazole-containing pharmacophore was proposed, followed by a combination with a 1,2,4-triazole synton, which eventually made it possible to create a convenient framework for obtaining 3-(5-(4-methoxyphenyl) pyrazole-3-yl)-6-R-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles.

2. The method of molecular docking established a significant potential of antifungal activity in a number of synthesized substances. The high affinity of the synthesized compounds to the lanosterol-14α-demethylase enzyme was demonstrated, which was provided by alkyl, π-alkyl, π-σ, π-sulfur, and amide-π stacking interactions, as well as intermolecular hydrogen chemical bonding forces, Van der Waals forces, and halogen interactions.

3. The energy value of intermolecular interactions of the studied compounds confirmed the high probability of antifungal activity in a number of synthesized compounds.

Conflicts of interest: authors have no conflict of interest to declare.
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