UDC 547.732:547.853:665.652.4:615.281.9:544.475 https://doi.org/10.24959/ophcj.21.240775

S. V. Vlasov[1], O. V. Borysov[2,3], H. I. Severina[1], S. M. Kovalenko[4], T. P. Osolodchenko[5], V. S. Vlasov[1], V. A. Georgiyants[1]

[1] National University of Pharmacy of the Ministry of Health of Ukraine, Ukraine
53, Pushkinska str., Kharkiv, 61002, Ukraine. E-mail: sergiy.vlasov@gmail.com
[2] Institute of Organic Chemistry of the National Academy of Sciences of Ukraine, Ukraine
[3] Enamine Ltd., Ukraine
[4] V. N. Karazin Kharkiv National University, Ukraine
[5] Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine, Ukraine

The synthesis, antimicrobial activity and docking studies of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones with acetamide and 1,2,4-oxadiazol-5-ylmethyl substituents

Aim. To synthesize, study the antimicrobial activity and suggest antimicrobial activity mechanism for the novel derivatives of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one.

Results and discussion. As the result of the targeted modification of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]-pyrimidin-4(3*H*)-one in position 3 with acetamide and 1,2,4-oxadiazol-5-ylmethyl substituents, the compounds, which demonstrated better antimicrobial activity in the agar well diffusion assay than the reference drug Streptomycin, were obtained. To elucidate the mechanism of action of the novel compounds, the docking studies were conducted to the active site of the 16S subunit of ribosomal RNA, the proven target for aminoglycoside antibiotics, as well as tRNA (Guanine37-N[1])-methyltransferase (TrmD), which inhibitors were considered as a new potential class of antibiotics.

Experimental part. By the interaction of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one with a series of N-arylchloroacetamides and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles in DMF in the presence of K_2CO_3 the target compounds were obtained. The antimicrobial activity was assessed by the agar well diffusion method. The concentration of microbial cells was determined by the McFarland standard; the value was 10^7 cells in 1 mL of the media. The 18–24 hour culture of microorganisms was used for tests. For the bacteria cultivation, Müller-Hinton agar was used, Sabouraud agar was applied for *C. albicans* cultivation. The compounds were tested as the DMSO solution with the concentration of 100 μg/mL; the volume of the solution was 0.3 mL, the same volume was used for Streptomycin (the concentration 30 μg/mL). The docking studies were performed using Autodock Vina. Crystallographic data for the complexes of Streptomycin with the 16S subunit of ribosomal RNA (1NTB) and its active site, as well as for tRNA (Guanine37-N[1])-methyltransferase (EC 2.1.1.228; TrmD) (5ZHN) and its active site were obtained from the Protein Data Bank.

Conclusions. It has been determined that 2-[6-(1*H*-benzimidazol-2-yl)-5-methyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl]-*N*-[4-(ethoxy)phenyl]acetamide, which is the most active as an antimicrobial agent among the compounds tested, also shows the best binding activity towards the active site of tRNA (guanine37-N[1])-methyltransferase.

Key words: thiophene; pyrimidine; alkylation; antimicrobial agents; inhibitors; molecular docking

С. В. Власов[1], О. В. Борисов[2,3], Г. І. Северіна[1], С. М. Коваленко[4], Т. П. Осолодченко[5], В. С. Власов[1], В. А. Георгіянц[1]

[1] Національний фармацевтичний університет Міністерства охорони здоров'я України, Україна
[2] Інститут органічної хімії Національної академії наук України, Україна
[3] ТОВ НВП «ЄНАМІН», Україна
[4] Харківський національний університет імені В. Н. Каразіна, Україна
[5] Інститут мікробіології та імунології імені І. І. Мечнікова Національної академії медичних наук України, Україна

Синтез, протимікробна активність та докінгові дослідження 6-(1*H*-бензімідазол-2-іл)-5-метилтієно[2,3-*d*]піримідин-4(3*H*)-онів з ацетамідними та 1,2,4-оксадіазол-5-ілметильними замісниками

Мета. Синтезувати й дослідити протимікробну активність нових похідних 6-(1*H*-бензімідазол-2-іл)-5-метилтієно[2,3-*d*]піримідин-4(3*H*)-онів та запропонувати механізм протимікробної активності.

Результати та їх обговорення. У результаті цілеспрямованої модифікації положення 3 6-(1*H*-бензімідазол-2-іл)-5-метилтієно[2,3-*d*]піримідин-4(3*H*)-ону ацетамідним та 1,2,4-оксадіазол-5-ілметильним замісниками було одержано сполуки з визначеною методом дифузії в агар протимікробною активністю, що є більшою за активність препарату порівняння Стрептоміцину. З метою з'ясування механізму дії синтезованих сполук було проведено докінгові дослідження щодо активного сайту субодиниці 16S рибосомальної РНК, яка є підтвердженою мішенню для аміноглікозидних антибіотиків, а також тРНК (Гуанін-37-N[1])-метилтрансферази (TrmD), інгібітори якої розглядаються як новий потенційний клас антибіотиків.

Експериментальна частина. Шляхом взаємодії 6-(1*H*-бензімідазол-2-іл)-5-метилтієно[2,3-*d*]піримідин-4(3*H*)-ону з рядом N-арилхлороацетамідів та 3-арил-5-(хлорометил)-1,2,4-оксадіазолів в умовах ДМФА-K_2CO_3 було одержано цільові сполуки. Антимікробну активність визначали методом дифузії в агар. Концентрацію мікробних клітин визначали за МакФарландом; мікробне навантаження склало 10^7 мікробних одиниць в 1 мл середовища. Для тестів використовували 18–24 годинну культуру мікроорганізмів. Для культивування бактерій використовували агар Мюллера-Гінтона; для культивування *C. albicans* використовували агар Сабуро. Сполуки вводили методом дифузії в агар (лунками) у вигляді розчину у ДМСО в концентрації 100 мкг/мл в об'ємі 0,3 мл; аналогічний об'єм використовували для Стрептоміцину (конц. 30 мкг/мл). Докінгові дослідження проводили за допомогою програми Autodock Vina. Кристалографічні дані для комплексів стрептоміцину з 16S субодиницею рибосомальної РНК (1NTB) та її активного сайту і для тРНК (Гуанін-37-N^1)-метилтрансферази (EC 2.1.1.228; TrmD) (5ZHN) та її активного сайту було отримано з Protein Data Bank.

Висновки. Виявлено, що сполука 2-[6-(1*H*-бензімідазол-2-іл)-5-метил-4-оксотієно[2,3-*d*]піримідин-3(4*H*)-іл]-*N*-[4-(етокси)феніл]ацетамід, яка характеризується найбільшою протимікробною активністю, у докінгових розрахунках є також найбільш ефективним інгібітором тРНК (Гуанін-37-N^1)-метилтрансферази.

Ключові слова: тіофен; піримідин; алкілування; протимікробні засоби; інгібітори; молекулярний докінг

Introduction

Derivatives of 6-(1*H*-benzimidazol-2-yl)thieno-[2,3-*d*]pyrimidines attract attention of researchers as promising biologically active compounds. Earlier different preparation methods for these compounds were reported [1–3], and the selectivity of their alkylation with benzyl chlorides was studied [3]. The works published in recent years has also shown the positive impact of the acetamide or isoxadiazole substituent in position 3 of thieno[2,3-*d*]pyrimidine on the antimicrobial activity [4, 5]. The substitution of other positions of the core heterocyclic structure with 1,2,4-oxadiazole was also effective for improving the antimicrobial activity [6–9]. Therefore, with the aim of studying the impact of both acetamide and 1,2,4-oxadiazol-5-ylmethyl substituents on the antimicrobial activity of thieno[2,3-*d*]pyrimidin-4(3*H*)-one bearing in position 6 the fragment of 1*H*-benzimidazole the construction of them starting from 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one was planned.

Results and discussion

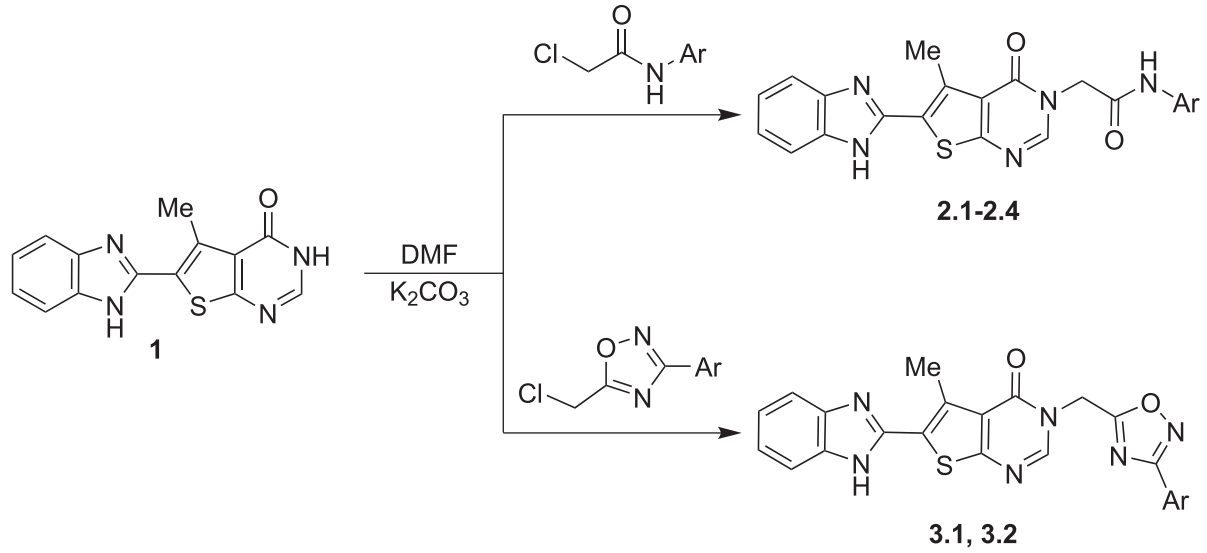
Taking into account the positive results of our previous research on the regioselectivity of the alkylation of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]-pyrimidin-4(3*H*)-one with benzyl chlorides [3], as well as some successful experience of the transfer of the method developed for benzyl chlorides to chloroacetamides for the similar systems with the fragment of 5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one [4] we decided to do the same for benzimidazole containing derivatives. The starting compound **1** obtained according to the method previously reported [3] was treated with either N-arylchloroacetamides or 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles in the DMF media using the equimolar amount of potassium carbonate to promote the reaction (Scheme).

The reaction was carried out for 5–8 hours at 60°C. After cooling the reaction mixture was quenched with water, and the crystalline products were filtered. The properties of compounds **2** and **3** synthesized are given in the Experimental part. If required, products **2** and **3** can be additionally purified by boiling in ethanol.

In the ^{1}H NMR spectra of compounds **2** the signals of the acetamide CH_2 group protons were observed in the region of 4.83–4.86 ppm, while for compounds **3** the signals of CH_2 were in the region of 5.61–5.63 ppm. All compounds **2** and **3** had the signal of benzimidazole NH at 12.63–12.68 ppm; for compounds **2** the signal of the acetamide NH proton was observed in the region of 10.33–10.70 ppm. The signal of the methyl group in position 5 of the thieno[2,3-*d*]pyrimidine system was found as a singlet at 2.86–2.88 ppm (Table 1).

Most of the compounds from **2** and **3** series moderately inhibited the growth of the strains of the microorganisms in the experiment. The best result was demonstrated by 2-[6-(1*H*-benzimidazol-2-yl)-5-methyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl]-*N*-[4-(ethoxy)-phenyl]acetamide (**2.2**), which appeared to be the most active against the bacterial strains (Table 1).

To elucidate the mechanism of action of the 3-(*N*-arylacetamido/1,2,4-oxadiazol-5-ylmethyl)-6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones synthesized the docking studies were conducted. For the antimicrobial activity screening experiment Streptomycin was used as a reference drug. It is widely used in clinics and can effectively tackle many dangerous strains of pathogenic bacteria [10–12]. On the other hand, for now there are many resistant strains towards this antibiotic [13], and it encourages the search for new antimicrobials with a possibly similar mechanism of action. As for most of aminoglycoside antibiotics [14, 15] the complexes of Streptomycin with its molecular target 16S subunit of ribosomal RNA were isolated and studied in details [16]. The structures of these complexes are available as pdb files (1NTB and 1NTA), which represent the structures with different metal cations. For our calculations we chose the model containing a Magnesium cation (1NTB). The results of the docking studies showed that none



2.1: Ar = 4-*i*Pr-C_6H_4; **2.2:** Ar = 4-EtO-C_6H_4; **2.3:** Ar = 4-F-3-Cl-C_6H_3; **2.4:** Ar = 3,5-*di*MeO-C_6H_3
3.1: Ar = 4-Me-C_6H_4; **3.2:** Ar = 4-Cl-C_6H_4

Scheme. Alkylation of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one with a series of N-arylchloroacetamides and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles

of the target molecules appeared to be suitable as a ligand for the active site. According to the docking results it is very unlikely for 3-(*N*-arylacetamido/1,2,4-oxadiazol-5-ylmethyl)-6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones to have the mechanism of the antimicrobial action similar to aminoglycoside antibiotics.

Among the recently discovered molecular targets, which can be applied for the search of new antibiotics, there is tRNA (Guanine37-N^1)-methyltransferase (TrmD) known as the enzyme important for survival of different bacteria under stress [17]. It has been proven that its inhibitors are effective antimicrobials [17–20]. Therefore, we tried this protein for the docking calculations with compounds **2** and **3** as ligands.

The calculation performed showed that compared to the known inhibitors the molecules of derivatives **2** and **3** were unable to interact with all of the amino acids of the active site. The best binding results were obtained for compound **2.2**, which also showed the best result in the antimicrobial activity assay against *P. vulgaris* (ATCC 4636) and *P. aeruginosa* (ATCC 27853) (Table 2). Its activity was even higher than that one for the reference drug Streptomycin. The obvious correlation between the ability to bind the active site of tRNA (Guanine37-N^1)-methyltransferase and the antimicrobial activity screening results can be the evidence for the possible impact of compounds **2** and **3** on the activity of the enzyme. On the other hand, the results of the docking studies were shown no

Table 1

The results of the antimicrobial activity screening for 3-(*N*-arylacetamido/1,2,4-oxadiazol-5-ylmethyl)-6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones **2** and **3** (the concentration 100 μg/mL)

Cmp	Diameter of the growth inhibition zone, mm					
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*P. vulgaris* ATCC 4636	*P. aeruginosa* ATCC 27853	*B. subtilis* ATCC 6633	*C. albicans* ATCC 653/885
2.1	16, 17, 16	16, 16, 16	14, 14, 15	14, 15, 15	17 17, 18	growth
2.2	16, 17, 18	16, 16, 16	14, 15, 15	14, 14, 14	18, 17, 18	growth
2.3	17, 17, 18	16, 15, 17	14, 15, 14	15, 15, 14	17, 17, 17	growth
2.4	16, 16, 16	16, 16, 16	14, 15, 14	15, 15, 15	17, 17, 18	growth
3.1	15, 14, 15	14, 13, 14	growth	growth	16, 17, 16	growth
3.2	15,14, 15	13, 14, 13	growth	growth	15, 16, 16	growth
Strep.*	15, 16, 15	15, 16, 17	growth	growth	17, 16, 17	growth

Note: * – Streptomycin, H_2O solution (the concentration 30 μg/mL).

Table 2

The results of the computer docking study of the interaction of compounds **2** and **3** with the active site of PaTrmDc

Cmp	Affinity, kcal/mol	Ligand binding with the active site (+/–), amino acids of the active site interacting with the ligand*
2.1	–9.5	+/– VAL142; **ASP178**; GLY179; **LEU180**; **LEU181**; **ASP182**
2.2	–9.7	+ **LEU92**; PRO94; ARG119; **TYR120**; **ILE138**; **TYR141**; VAL142; **LEU143**; **GLY145**; PRO149; LEU228
2.3	–9.8	+/- TYR91; PRO94; GLN95; ARG119; **TYR120**; GLY122; VAL142; **ASP178**; GLY179; **LEU180**; ASP182; HIS185
2.4	–10.1	+/- TYR91; GLN95; ARG119; **TYR120**; ASP140; VAL142; GLN101; ALA102; ARG105; **ASP178**; **LEU180**; **ASP182**; HIS185
3.1	–10.5	+/- GLY118; ARG119; **TYR120**; VAL142; **ASP178**; GLY179; **LEU180**
3.2	–10.4	+/- GLY118; ARG119; **TYR120**; VAL142; GLN101; **ASP178**; GLY179; **LEU180**

Notes: + – almost complete binding; +/- – partial binding; - – no binding observed. * – amino acids binding to the known inhibitor are provided in bold.

evidence for them to have the antimicrobial activity mechanism being similar to Streptomycin.

Experimental part

Chemical part

All solvents and conventional reagents were obtained from the commercial sources or prepared by the well known methods. ^{1}H NMR spectra were recorded on a Varian Mercury-200 device (200 MHz) in DMSO-d_6 solution; TMS was used as an internal standard; the spectral chemical shift scale was presented as δ (ppm). The elemental analysis was performed on a EuroVector EA-3000 instrument. Melting points were determined on a Kofler bench.

6-(1*H*-Benzimidazol-2-yl)-5-methylthieno-[2,3-*d*]pyrimidin-4(3*H*)-one (1) was obtained according to the method previously reported [3].

The general method for preparation of 3-(*N*-arylacetamido/1,2,4-oxadiazol-5-ylmethyl)-6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]-pyrimidin-4(3*H*)-ones 2 and 3

To the suspension of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**1**) (0.15 g, 0.531 mmol) in DMF an alkylating agent (0.531 mmol) and potassium carbonate (0.074 g, 0.531 mmol) are added. The mixture is stirred at 60°C for 5–8 hours. After the reaction mixture is cooled, it is quenched with water, then the precipitate formed is filtered and dried at 70°C. If required, compounds **2** and **3** can be purified by boiling in ethanol.

2-[6-(1H-Benzimidazol-2-yl)-5-methyl-4-oxothieno-[2,3-d]pyrimidin-3(4H)-yl]-N-(4-isopropylphenyl)-acetamide (**2.1**)

Yield – 0.155 g (64%), a white solid. M. p. > 300°C. Anal. Calcd. for $C_{25}H_{23}N_5O_2S$, %: C 65.63; H 5.07; N 15.31. Found, %: C 65.78; H 5.05; N 15.43. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 1.15 (6H, d, *J* = 7.0 Hz, CH(C<u>H</u>$_3$)$_2$); 2.82–2.87 (1H, m, C<u>H</u>(CH$_3$)$_2$); 2.88 (3H, s, CH$_3$); 4.84 (2H, s, CH$_2$); 7.12–7.29 (4H, m, ArH); 7.43–7.71 (4H, m, ArH); 8.45 (1H, s, CH); 10.39 (1H, s, NH); 12.65 (1H, s, NH).

2-[6-(1H-Benzimidazol-2-yl)-5-methyl-4-oxo-thieno[2,3-d]pyrimidin-3(4H)-yl]-N-(4-ethoxyphenyl)-acetamide (**2.2**)

Yield – 0.190 g (78%), a grey solid. M. p. > 300°C. Anal. Calcd. for $C_{24}H_{21}N_5O_3S$, %: C 62.73; H 4.61; N 15.24. Found, %: C 62.86; H 4.68; N 15.27. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 1.28 (3H, t, *J* = 7.0 Hz, OCH$_2$C<u>H</u>$_3$); 2.88 (3H, s, CH$_3$); 3.96 (2H, q, *J* = 7.0 Hz, OC<u>H</u>$_2$CH$_3$); 4.83 (2H, s, CH$_2$); 6.86 (2H, d, *J* = 8.8 Hz, Ar 2'-H + 6'-H); 7.16–7.28 (2H, m, ArH); 7.54 (2H, d, *J* = 8.8 Hz, Ar 3'-H + 5'-H); 7.54–7.69 (2H, m, ArH); 8.45 (1H, s, CH); 10.33 (1H, s, NH); 12.65 (1H, s, NH).

2-[6-(1H-Benzimidazol-2-yl)-5-methyl-4-oxo-thieno[2,3-d]pyrimidin-3(4H)-yl]-N-(3-chloro-4-fluoro-phenyl)acetamide (**2.3**)

Yield – 0.206 g (83%), a white solid. M. p. 288–289°C. Anal. Calcd. for $C_{22}H_{15}ClFN_5O_2S$, %: C 56.47; H 3.23; N 14.97. Found: C 56.28; H 3.35; N 14.95. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 2.88 (3H, s, CH$_3$); 4.86 (2H, s, CH$_2$); 6.86 (2H, m, ArH); 7.33–7.71 (4H, m, ArH); 7.85–7.92 (1H, m, ArH); 8.45 (1H, s, CH); 10.70 (1H, s, NH); 12.66 (1H, s, NH).

2-[6-(1H-Benzimidazol-2-yl)-5-methyl-4-oxo-thieno[2,3-d]pyrimidin-3(4H)-yl]-N-(3,5-dimethoxy-phenyl)acetamide (**2.4**)

Yield – 0.156 g (62%), a beige solid. M. p. 266–267°C. Anal. Calcd. for $C_{24}H_{21}N_5O_4S$, %: C 60.62; H 4.45; N 14.73. Found, %: C 60.70; H 4.49; N 14.82. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 2.88 (3H, s, CH_3); 3.69 (6H, s, 2 × OCH_3); 4.84 (2H, s, CH_2); 6.23 (1H, t, *J* = 2.1 Hz, Ar 4'-H); 6.83 (2H, d, *J* = 2.1 Hz, Ar 2'-H + 6'-H); 7.17–7.27 (2H, m, ArH); 7.54–7.66 (2H, m, ArH); 8.45 (1H, s, CH); 10.44 (1H, s, NH); 12.63 (1H, s, NH).

6-(1H-Benzimidazol-2-yl)-5-methyl-3-{[3-(4-methylphenyl)-1,2,4-oxadiazol-5-yl]methyl}thieno[2,3-d]pyrimidin-4(3H)-one (**3.1**)

Yield – 0.205 g (85%), a white solid. M. p. > 300°C. Anal. Calcd. for $C_{24}H_{18}N_6O_2S$, %: C 63.42; H 3.99; N 18.49. Found, %: C 63.38; H 4.12; N 18.57. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 2.35 (3H, s, CH_3); 2.86 (3H, s, CH_3); 5.61 (2H, s, CH_2); 7.17–7.27 (2H, m, ArH); 7.33 (2H, d, *J* = 7.9 Hz, ArH); 7.51-7.71 (2H, m, ArH); 7.84 (2H, d, *J* = 6.4 Hz, ArH); 8.69 (1H, s, CH); 12.68 (1H, s, NH).

6-(1H-Benzimidazol-2-yl)-3-{[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]methyl}-5-methylthieno-[2,3-d]pyrimidin-4(3H)-one (**3.2**)

Yield – 0.194 g (77%), a white solid. M. p. 291–292°C. Anal. Calcd. for $C_{23}H_{15}ClN_6O_2S$, %: C 58.17; H 3.18; N 17.70. Found, %: C 58.18; H 3.24; N 17.78. ^{1}H NMR (200 MHz, DMSO-d_6), δ, ppm: 2.86 (3H, s, CH_3); 5.63 (2H, s, CH_2); 7.17–7.29 (2H, m, ArH); 7.51–7.71 (4H, m, ArH); 7.96 (2H, d, *J* = 8.2 Hz, ArH); 8.69 (1H, s, CH); 12.68 (1H, s, NH).

Biological and *in silico* studies

The *study of the antimicrobial activity* of compounds **2** and **3** was performed by the agar well diffusion method [21, 22]. The concentration of microbial cells was determined by the McFarland standard [23]; the value was 10^7 cells in 1 mL of the media. The 18–24 hour culture of microorganisms was used for tests. For the bacteria cultivation, Müller-Hinton agar was used, Sabouraud agar was applied for *C. albicans* cultivation. The compounds were tested as the DMSO solution with the concentration of 100 μg/mL; the volume of the solution was 0.3 mL, the same volume was used for Streptomycin (the concentration 30 μg/mL). Each experiment was repeated thrice. The antibacterial activity was estimated by the growth inhibition zone diameter for each microorganism.

The docking studies were performed using Autodock Vina [24]. They were performed for flexible ligands and rigid models of proteins. Crystallographic data for complexes of Streptomycin with the 16S subunit of ribosomal RNA (1NTB) with its active site [25] and tRNA (Guanine37-N^1)-methyltransferase (EC 2.1.1.228; TrmD) (5ZHN) with its active site [26] were obtained from the Protein Data Bank.

Conclusions

The possibility to use aromatic chloroacetamides and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles for alkylation of position 3 of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one in the conditions similar to the previously reported for benzyl chlorides has been proven. In this manner the series of novel 3-(*N*-arylcetamido/1,2,4-oxadiazol-5-ylmethyl)-6-(1*H*-benzimidazol-2-yl)-5-methylthieno-[2,3-*d*]pyrimidin-4(3*H*)-ones have been obtained.

It has been determined that 2-[6-(1*H*-benzimidazol-2-yl)-5-methyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl]-*N*-[4-(ethoxy)phenyl]acetamide, which is the most active as an antimicrobial agent among the compounds tested, also shows the best binding activity towards the active site of tRNA (Guanine37-N^1)-methyltransferase.

Conflict of interests: the authors have no conflict of interests to declare.

References

1. Sherif, M. H.; Amr, A. E-G. E.; Assy, M. G.; Ramadan, Z. M. Synthesis of some new thienopyrimidine with benzoxazine, quinazoline and azole moieties. *Afinidad* **2008**, *65* (535), 243–248.
2. Tumkevicius, S.; Kaminskas, A.; Bucinskaite, V.; Labanauskas, L. Synthesis of 4,6-disubstituted thieno[2,3-*d*]pyrimidines from 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde. *Heterocycl. Commun.* **2003,** *9* (1), 89–94. https://doi.org/10.1515/HC.2003.9.1.89.
3. Vlasov, S. V.; Kovalenko, S. M.; Chernykh, V. P.; Krolenko K. Yu. Synthesis and alkylation of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]-pyrimidin-4(3*H*)-ones. *Journal of Organic and Pharmaceutical Chemistry* **2015**, *13* (2), 30–34. https://doi.org/10.24959/ophcj.15.822.
4. Vlasov, S. V.; Kovalenko, S. N.; Osolodchenko, T. P.; Lenitskaya, E. B.; Chernykh, V. P. Synthesis and biological activity of 6-(1,3-benzoxazol-2-yl)-5-methylthieno-[2,3-*d*]pyrimidines. *Pharm. Chem. J.* **2018**, *52* (6), 510–514. https://doi.org/10.1007/s11094-018-1850-1.
5. Aruna Kumari, M.; Triloknadh, S.; Harikrishna, N.; Vijjulatha, M.; Venkata Rao, C. Synthesis, Antibacterial Activity, and Docking Studies of 1,2,3-triazole-tagged Thieno[2,3-d]pyrimidinone Derivatives. *J. Heterocycl. Chem.* **2017,** *54* (6), 3672-3681. https://doi.org/10.1002/jhet.2995.
6. Vlasov, S. V.; Zaremba, O. V.; Kovalenko, S. M.; Fedosov, A. I.; Chernykh, V. P. Synthesis of 5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives modified in position 6 with 1,2,4- and 1,3,4-oxadiazoles and their biological activity. *Journal of Organic and Pharmaceutical Chemistry* **2011**, *9* (4), 24–30.
7. Vlasov, S. V.; Kovalenko, S. M.; Fedosov, A. I.; Chernykh, V. P. Synthesis of novel 3-substituted 1-alkyl-5-methyl-6-(3-aryl-1,2,4-oxadiazole-5-yl)-thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and their antimicrobial activity. *Journal of Organic and Pharmaceutical Chemistry* **2011**, *9* (3), 51–55.
8. Vlasov, S. V.; Chernykh, V. P. Synthesis, antiinflammatory and antimicrobial activity of 6-(1*H*-benzimidazol-2-yl)-5-methyl-4-(alkylthio)-thieno[2,3-*d*]pyrimidines. *News of Pharmacy* **2016**, 3, 9–16. https://doi.org/10.24959/nphj.16.2112.
9. Vlasov, S. V.; Kovalenko, S. M.; Chernykh, V. P. Synthesis and the antimicrobial activity study of the novel derivatives of 4-oxo- and 4-thio-5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines. *Journal of Chemical and Pharmaceutical Research* **2015**, *7* (4), 1043–1048.
10. Ahmed, Z.; Saeed Khan, S.; Khan, M. *In vitro* trials of some antimicrobial combinations against Staphylococcus aureus and Pseudomonas aeruginosa. *Saudi Journal of Biological Sciences* **2013,** *20* (1), 79–83. https://doi.org/10.1016/j.sjbs.2012.10.005.
11. Araújo, S. G.; Alves, L. F.; Pinto, M. E. A.; Oliveira, G. T.; Siqueira, E. P.; Ribeiro, R. I. M. A.; Ferreira, J. M. S.; Lima, L. A. R. S. Volatile compounds of Lamiaceae exhibit a synergistic antibacterial activity with streptomycin. *Braz. J. Microbiol.* **2014**, *45* (4), 1341–1347. https://doi.org/10.1590/S1517-83822014000400026.

12. Rodríguez-García, Á.; Mares-Alejandre, R. E.; Muñoz-Muñoz, P. L. A.; Ruvalcaba-Ruiz, S.; González-Sánchez, R. A.; Bernáldez-Sarabia, J.; Meléndez-López, S. G.; Licea-Navarro, A. F.; Ramos-Ibarra, M. A. Molecular Analysis of Streptomycin Resistance Genes in Clinical Strains of Mycobacterium tuberculosis and Biocomputational Analysis of the MtGidB L101F Variant. *Antibiotics* **2021,** *10* (7), 807. https://doi.org/10.3390/antibiotics10070807.
13. Ward, H.; Perron, G. G.; Maclean, R. C. The cost of multiple drug resistance in Pseudomonas aeruginosa. *Journal of Evolutionary Biology* **2009,** *22* (5), 997–1003. https://doi.org/10.1111/j.1420-9101.2009.01712.x.
14. Jiang, L.; Patel, D. J. Solution structure of the tobramycin–RNA aptamer complex. *Nature Structural Biology* **1998,** *5* (9), 769–774. https://doi.org/10.1038/1804.
15. Jiang, L.; Majumdar, A.; Hu, W.; Jaishree, T. J.; Xu, W.; Patel, D. J. Saccharide-RNA recognition in a complex formed between neomycin B and an RNA aptamer. *Structure* **1999,** *7* (7), 817–827. https://doi.org/10.1016/S0969-2126(99)80105-1.
16. Tereshko, V.; Skripkin, E.; Patel, D. J. Encapsulating Streptomycin within a Small 40-mer RNA. *Chemistry & Biology* **2003,** *10* (2), 175–187. https://doi.org/10.1016/S1074-5521(03)00024-3.
17. Zhong, W.; Pasunooti, K. K.; Balamkundu, S.; Wong, Y. H.; Nah, Q.; Gadi, V.; Gnanakalai, S.; Chionh, Y. H.; McBee, M. E.; Gopal, P.; Lim, S. H.; Olivier, N.; Buurman, E. T.; Dick, T.; Liu, C. F.; Lescar, J.; Dedon, P. C. Thienopyrimidinone Derivatives That Inhibit Bacterial tRNA (Guanine37-N1)-Methyltransferase (TrmD) by Restructuring the Active Site with a Tyrosine-Flipping Mechanism. *J. Med. Chem.* **2019,** *62* (17), 7788–7805. https://doi.org/10.1021/acs.jmedchem.9b00582.
18. Li, Y.; Zhong, W.; Koay, A. Z.; Ng, H. Q.; Koh-Stenta, X.; Nah, Q.; Lim, S. H.; Larsson, A.; Lescar, J.; Hill, J.; Dedon, P. C.; Kang, C. Backbone resonance assignment for the N-terminal region of bacterial tRNA-(N1G37) methyltransferase. *Biomolecular NMR Assignments* **2019,** *13* (1), 49–53. https://doi.org/10.1007/s12104-018-9849-9.
19. Zhong, W.; Koay, A.; Ngo, A.; Li, Y.; Nah, Q.; Wong, Y. H.; Chionh, Y. H.; Ng, H. Q.; Koh-Stenta, X.; Poulsen, A.; Foo, K.; McBee, M.; Choong, M. L.; El Sahili, A.; Kang, C.; Matter, A.; Lescar, J.; Hill, J.; Dedon, P. Targeting the Bacterial Epitranscriptome for Antibiotic Development: Discovery of Novel tRNA-(N1G37) Methyltransferase (TrmD) Inhibitors. *ACS Infectious Diseases* **2019,** *5* (3), 326–335. https://doi.org/10.1021/acsinfecdis.8b00275.
20. Jaroensuk, J; Wong, Y. H.; Zhong, W; Liew, C. W.; Maenpuen, S.; Sahili, A. E.; Atichartpongkul, S.; Chionh, Y. H.; Nah, Q.; Thongdee, N.; McBee, M. E.; Prestwich, E. G.; DeMott, M. S.; Chaiyen, P.; Mongkolsuk, S.; Dedon, P. C.; Lescar, J.; Fuangthong, M. Crystal structure and catalytic mechanism of the essential m1G37 tRNA methyltransferase TrmD from Pseudomonas aeruginosa. *RNA* **2019**, *25* (11), 1481–1496. https://doi.org/10.1261/rna.066746.118.
21. Pro zatverdzhennia metodychnykh vkazivok "Vyznachennia chutlyvosti mikroorhanizmiv do antybakterialnykh preparativ" [Methodological Instructions "Determination of the sensitivity of microorganisms to antibacterial drugs"]. https://zakon.rada.gov.ua/rada/show/v0167282-07#Text (accessed June 11, 2021), Ministry of health of Ukraine, Order №167, 05.04.2007 (*in Ukrainian*).
22. *Bakteriolohichnyi kontrol pozhyvnykh seredovyshch* [Bacteriological control of nutrient media]; Ministry of Health of Ukraine information letter No. 05.4.1/1670; Kyiv, 2001 (*in Ukrainian*).
23. McFarland, J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association* **1907,** *XLIX* (14), 1176–1178. https://doi.org/10.1001/jama.1907.25320140022001f.
24. Trott, O.; Olson, A. J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010,** *31* (2), 455–461. https://doi.org/10.1002/jcc.21334.
25. Tereshko, V.; Skripkin, E.; Patel, D. J. 2.9 Å crystal structure of Streptomycin RNA-aptamer complex. https://www.wwpdb.org/pdb?id=pdb_00001ntb (accessed May 21, 2021), https://doi.org/10.2210/pdb1NTB/pdb.
26. Zhong, W.; Pasunooti, K. K.; Balamkundu, S.; Wong, Y. W.; Nah, Q.; Liu, C. F.; Lescar, J.; Dedon, P. C. Crystal structure of TrmD from Pseudomonas aeruginosa in complex with active-site inhibitor. https://www.wwpdb.org/pdb?id=pdb_00005zhm (accessed May 21, 2021), https://doi.org/10.2210/pdb5zhm/pdb.

Received: 05. 07. 2021

Revised: 23. 08. 2021

Accepted: 27. 08. 2021

The research was funded by the Ministry of Health of Ukraine at the expense of the State Budget in the framework # 2301020 "Scientific and scientific-technical activity in the field of health protection" on the topic "Synthesis and study of new thienopyrimidines for detection of the antimicrobial and related types of pharmacological activity" (Order of the Ministry of Health of Ukraine of November 17, 2020 No. 2651).