The synthesis, antimicrobial activity and docking studies of 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-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-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-one.

Results and discussion. As the result of the targeted modification of 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-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-N3)-methyltransferase (TrmD), which inhibitors were considered as a new potential class of antibiotics.

Experimental part. By the interaction of 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-one with a series of N-arylchloroacetamides and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles in DMF in the presence of K2CO3, 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 106 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-N3)-methyltransferase (EC 2.1.1.228; TrmD), which inhibitors were considered as a new potential class of antibiotics, 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-N3)-methyltransferase (TrmD), which inhibitors were considered as a new potential class of antibiotics.

Conclusions. It has been determined that 2-[6-(1H-benzimidazol-2-yl)-5-methyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-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-N3)-methyltransferase (TrmD) and its active site were obtained from the Protein Data Bank.

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

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**Introduction**

Derivatives of 6-(1H-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(3H)-one bearing in position 6 the fragment of 1H-benzimidazole the construction of them starting from 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-one was planned.

**Results and discussion**

Taking into account the positive results of our previous research on the regioselectivity of the alkylation of 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-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(3H)-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 medium 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 1H NMR spectra of compounds 2 the signals of the acetamide CH 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 microorganisms in the experiment. The best result was demonstrated by 2-[6-(1H-benzimidazol-2-yl)-5-methyl-4-oxothieno[2,3-d]pyrimidin-3(4H)-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-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-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 amino-glycoside 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
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-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-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-N1)-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-N1)-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

| 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, H₂O solution (the concentration 30 μg/mL).
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.

Table 2

Experimental part

Chemical part

All solvents and conventional reagents were obtained from the commercial sources or prepared by the well-known methods. 1H 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-(1H-Benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-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-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-ones 2 and 3

To the suspension of 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-one (1) (0.15 g, 0.531 mmol) in DMF an alkylation 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$_{20}$O$_5$N$_8$: C 65.63; H 5.07; N 15.31. Found, %: C 65.78; H 5.05; N 15.43. 1H NMR (200 MHz, DMSO-$_d_6$), δ, ppm: 1.15 (6H, d, J = 7.0 Hz, CH$_2$); 2.82–2.87 (1H, m, CHCH$_3$); 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-oxothieno[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$_{25}$H$_{21}$O$_9$N$_8$: C 62.73; H 4.61; N 15.24. Found, %: C 62.86; H 4.68; N 15.27. 1H NMR (200 MHz, DMSO-$_d_6$), δ, ppm: 1.28 (3H, t, J = 7.0 Hz, OCCH$_3$); 2.83 (3H, s, CH$_3$); 3.96 (2H, q, J = 7.0 Hz, OCH$_2$); 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-oxothieno[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$_{26}$H$_{18}$ClFN$_2$O$_8$S: C 56.47; H 3.23; N 14.97. Found, %: C 56.28; H 3.35; N 14.95. 1H 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-oxothieno[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. Calcld. for C_{38}H_{38}N_{10}O_{9}S; %: 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\(_6\)), \(\delta\), ppm: 3.28 (3H, s, CH\(_3\)); 6.23 (2H, s, CH\(_2\)); 6.23 (2H, d, \(J = 2.1\) 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, 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. Calcld. for C_{38}H_{38}N_{10}O_{9}S; %: 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\(_6\)), \(\delta\), ppm: 2.88 (3H, s, CH\(_3\)); 7.17 – 7.29 (2H, m, ArH); 7.51 – 7.71 (4H, m, ArH); 7.96 (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. Calcld. for C_{38}H_{38}N_{10}O_{9}S; %: C 63.17; H 3.18; N 17.78. Found, %: C 63.42; H 3.99; N 18.49. Synthesis and the antimicrobial activity study of the novel derivatives of 4-oxo- and 4-thio-5-methylthieno[2,3-d]pyrimidines.

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\(^2\) cells in 1 mL of the media. The 18–24 hour culture of microorganisms was used for tests. For the bacteria cultivation, Miller-Hinton agar was used, Sambourlag 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 three times. 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\(^3\))-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-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-one in the conditions similar to the previously reported for benzyl chlorides has been proven. In this manner the series of novel 3-(3-arylaminocarbonyl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-ones and its biological activity were obtained from the Protein Data Bank.

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

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