

UDC 547-327:547.821:54.057

DOI: 10.15587/2519-4852.2023.286008

SYNTHESIS, DOCKING STUDY AND ANTIMICROBIAL ACTIVITY EVALUATION OF PYRIDYL AMIDES OF THIENO[2,3-D]PYRIMIDINE-4-CARBOXYLIC ACID

Sergiy Vlasov, Hanna Severina, Olena Vlasova, Oleksandr Borysov, Pavlo Shynkarenko, Olga Golovchenko, Yulian Konechnyi, Victoriya Georgiyants

The aim. The combination in one molecule of pharmacophore fragments of thieno[2,3-d]pyrimidine-4-carboxylic acids with the fragments of 2- or 4-aminopyrimidine by peptide coupling promoted acylation in order to develop the new drug-like molecules with antimicrobial activity.

Materials and methods. The molecular docking studies were performed with the AutoDock Vina and AutoDock-Tools 1.5.6 programs; TrmD *Pseudomonas aeruginosa* PDB ID – 5ZHN was used as the protein target. Synthetic methods of peptide coupling were used. ¹H and ¹³C NMR spectra were recorded with a Varian-400 spectrometer at 400 MHz and Bruker Avance DRX 500 device at 500 MHz and 125 MHz in DMSO-d₆ as a solvent, using TMS as the internal standard. LC-MS analysis of the compounds was carried out with Agilent 1100 HPLC 3 with atmospheric pressure chemical ionization (APCI). The studied derivatives were tested in vitro for their antibacterial and anti-fungal activities using agar diffusion and serial dilutions resazurin-based microdilution assays (RBMA).

Results and discussion. By the combination of the pharmacophore fragments of thieno[2,3-d]pyrimidine-4-carboxylic acids with the fragments of 2- or 4-aminopyrimidine, the combinatorial library of amides was constructed. For this library of compounds, the potential of antimicrobial activity was revealed using docking studies to the TrmD enzyme isolated from *P. aeruginosa*. The peptide coupling promoted by 1,1'-carbonyldiimidazole was found to be effective for the synthesis of pyridyl amides of thieno[2,3-d]pyrimidine-4-carboxylic acids, and it allowed to combine these pharmacophores in one molecule. The results of antimicrobial activity study revealed the broad spectrum of antimicrobial activity for N-(pyridin-4-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-carboxamide (2g), while 5,6-dimethyl-N-(6-methylpyridin-2-yl)thieno[2,3-d]pyrimidine-4-carboxamide (2c) showed the best MIC value against the reference strain of *Pseudomonas aeruginosa* ATCC 10145. N-(6-Methylpyridin-2-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-carboxamide (2h) was also found to be active against *Pseudomonas aeruginosa*.

Conclusions. An effective method for the synthesis of pyridyl amides of thieno[2,3-d]pyrimidine-4-carboxylic acid has been developed. The amides molecular docking method showed their ability to inhibit TrmD enzyme isolated from *P. aeruginosa*; the further in vitro studies of the compounds showed the rationality of the further studies of the derivatives with 2-amino-6-methylpyridine in amide substituent because this fragment favoured the selectivity against *Pseudomonas aeruginosa*.

Keywords: thieno[2,3-d]pyrimidine, amides, coupling, docking study, antimicrobials

How to cite:

Vlasov, S., Severina, H., Vlasova, O., Borysov, O., Shynkarenko, P., Golovchenko, O., Konechnyi, Y., Georgiyants, V. (2023). Synthesis, docking study and antimicrobial activity evaluation of pyridyl amides of thieno[2,3-d]pyrimidine-4-carboxylic acid. ScienceRise: Pharmaceutical Science, 5 (45), 53–62. doi: <http://doi.org/10.15587/2519-4852.2023.286008>

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1. Introduction

The antimicrobial activity of thieno[2,3-d]pyrimidines has been successfully studied in the last decade [1–3]. For many of them, the mechanism of action *via* inhibition of bacterial TrmD was either confirmed or the calculations showed its high probability [4–6]. As part of the implementation of the hybrid pharmacophore concept, our studies have identified thieno[2,3-d]pyrimidine derivatives with potent antimicrobial properties [7, 8]. In addition, some of our recent research focused mostly on the development of the efficient procedure for the synthesis of thieno[2,3-d]pyrimidine-4-carboxylic acids [9], which had been previously hardly available [10–12] regardless of their high pharmacological potential [13], revealed the antimicrobial activity of the amides of the corresponding acids' amides [9].

Pyridine is a part of many natural compounds useful for metabolism, such as nicotinic acid (B3 vitamin)

required to form NAD⁺ and NADP⁺ in the body tissue [14–16]. It is also a part of such highly active alkaloids as nicotine and anabasine [17–19]. Atropine, which is still widely prescribed [20, 21], can also be considered as a derivative of reduced pyridine.

The pharmaceutical market is also rich in synthetic medicines, which are the derivatives of pyridine. Isoniazid and ethionamide are useful antitubercular drugs with no available alternative [22]; pyridostigmine is effective against myasthenia gravis [23]; nifedipine and nilvadipine are the drugs to cure blood circulatory disorders [24, 25]; piroxicam is an effective NSAID drug mostly prescribed for therapy of arthritis [26]. Among the relatively new drugs, there are anticancer agents abiraterone and crizotinib [27, 28] and the drug for the treatment of Alzheimer's disease, tacrine [29].

Many molecules with pyridine moiety were reported as antimicrobials (Fig. 1) [30–33].

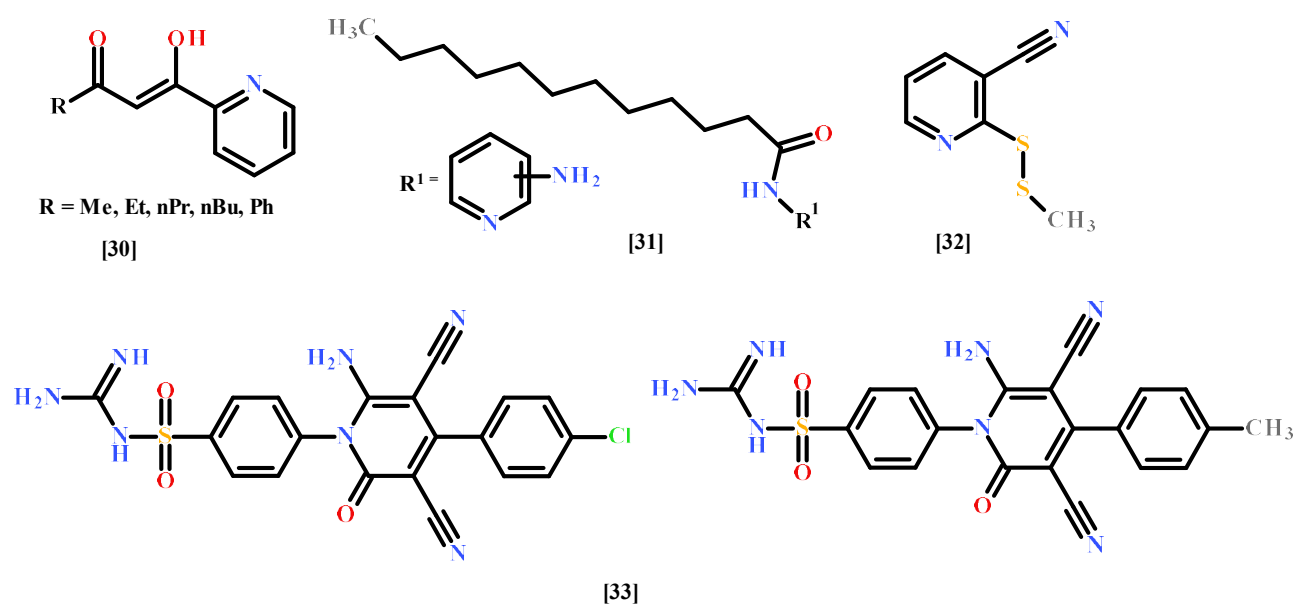


Fig. 1. Pyridine-containing molecules with antimicrobial activity

As the extension of our research in novel antimicrobials we decided to combine pyridine moiety with the previously reported thieno[2,3-d]pyrimidine-4-carboxylic acids to form amides based on 2- and 4-aminopyrimidine derivatives.

The aim of research. In view of the high activity of thieno[2,3-d]pyrimidine-4-carboxylic acids amides and compounds with the fragment of pyridine as antimicrobials, we decided to obtain previously unknown pyridyl amides of thieno[2,3-d]pyrimidine-4-carboxylic acid and test them for antimicrobial activity using *in silico* and *in vitro* methods.

2. Planning (methodology) of research

In our previous study, which was mostly devoted to the development of an effective procedure for the synthesis of thieno[2,3-d]pyrimidine-4-carboxylic acids (Fig. 2) [9], we revealed promising antimicrobial activity of some benzylic and simply substituted aromatic amides.

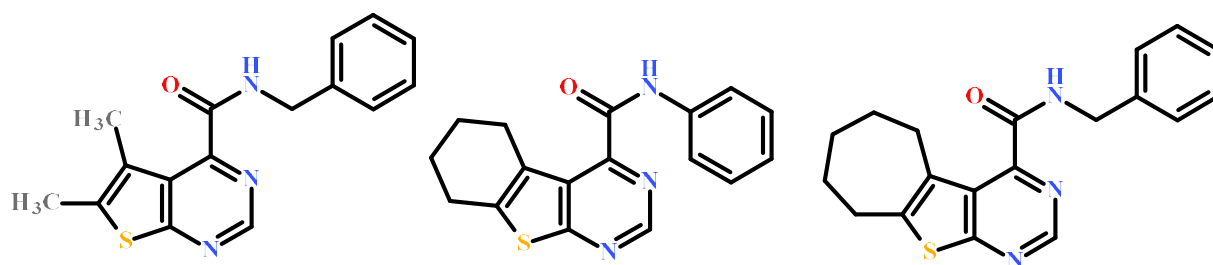
The developed procedure made available some thieno[2,3-d]pyrimidine-4-carboxylic acids, and their modification with pyridines as the amide part substituent was found to be a good strategy for the construction of novel antimicrobials.

In order to check whether pyridyl fragments could be beneficial as an amide substituent for potentially antimicrobial thieno[2,3-d]pyrimidine-4-carboxylic acids' amides, we decided to construct the virtual library of the molecules based on the readily available acids and the series of 2- or 4-aminopyrimidines and predict the possibility of TrmD inhibitory activity for these molecules. The next step was to obtain the series of pyridyl amides of thieno[2,3-d]pyrimidine-4-carboxylic acid and test their antimicrobial activity. The methodology of antibacterial activity screening included not only standard strains but also the clinical strains of the microorganisms.

3. Materials and methods

3. 1. Molecular docking studies

Molecular docking studies were conducted at the Department of Pharmaceutical Chemistry of the National University of Pharmacy (20.04.2023-15.05.2023). (Guanine37-N1)-methyltransferase TrmD isolated from *Pseudomonas aeruginosa* (PDB ID – 5ZHN) as a complex with its native inhibitor N-(4-((octylamino)methyl)benzyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-5-carboxamide was used as a target protein of the docking studies. The model deposited from the Protein Data Bank was taken [6].

Fig. 2. Previously reported amides of thieno[2,3-d]pyrimidine-4-carboxylic acids with activity against *Proteus vulgaris* and *Pseudomonas aeruginosa*

Preparation of the ligands. The structures of the compounds were drawn with BIOVIADraw 2021 program and stored in .mol format. The structures were further optimized with Chem3D, using the quantum-mechanical algorithm MM2 and stored as .pdb. AutoDockTools-1.5.6 program was used for the conversion of .pdb files into .pdbqt, the number of active rotatable bonds was set as default.

Preparation of the protein. Removal of water molecules and ligands was performed with Discovery Studio Visualizer 2021. The protein structure was stored as .pdb. The AutoDockTools-1.5.6 program was used to add polar hydrogens and to store the protein model in .pdbqt format. The coordinates of Grid box and its center were determined from the native ligand position; the redocking methodology was used for the validation of the docking methodology as it was reported earlier [7]. The reproducibility of the positions of all the bonds was achieved in the active site in correspondence to the experimental results reported earlier [6].

Vina was used to carry docking. For visualization, Discovery Studio 2021 was used.

3. 2. Chemistry

¹H and ¹³C NMR spectra were recorded on a Bruker 170 Avance 500 spectrometer (500 and 125 MHz, respectively) in DMSO-*d*₆, with TMS as the internal standard at room temperature. Mass spectra were recorded on an Applied Biosystems apparatus (Shimadzu 10-AV LC, Gilson-215, automatic sample feed, API 150EX mass spectrometer, UV (215 and 254 nm) and ELS detectors, Luna-C18 column, Phenomenex, 5 cm×2 mm, electrospray ionization). Elemental analysis was done using a Euro Vector EA-3000 CHNS analyzer. Melting points were determined on a Kofler hot bench. All reagents were obtained from commercial sources and used without further purification.

The starting acids 1 were prepared according to the previously reported procedure [9]:

1. 5,6-Dimethyl-N-(pyridin-2-yl)thieno[2,3-*d*]pyrimidine-4-carboxamide (2a).

Yield: 76 %, white crystals. M.p. 199–200 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.29 (*br.s*, 1H, NH), 9.06 (*s*, 1H, CH), 8.39 (*d*, *J*=4.8 Hz, 1H), 8.23 (*d*, *J*=8.3 Hz, 1H), 7.91 (*t*, *J*=8.0 Hz, 1H), 7.23 (*t*, *J*=6.2 Hz, 1H), 2.54 (*s*, 3H, thiophCH₃), 2.25 (*s*, 3H, thiophCH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 167.7, 164.6, 153.9, 151.4, 151.1, 148.4, 138.5, 136.5, 126.9, 124.0, 120.5, 114.3, 13.7, 12.0. LC-MS *m/z* (ES⁺) 285.0 (MH⁺). Anal. calcd. for C₁₄H₁₂N₄OS (284,34): C, 59.14; H, 4.25; N, 19.70. Found: C, 59.21; H, 4.30; N, 19.09.

2. 5,6-dimethyl-N-(pyridin-4-yl)thieno[2,3-*d*]pyrimidine-4-carboxamide (2b).

Yield: 82 %, white crystals. M.p. 187–188 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.32 (*br.s*, 1H, NH), 9.10 (*s*, 1H, CH), 8.66–8.44 (*m*, 2H), 7.70 (*dt*, *J*=5.5, 2.7 Hz, 2H), 2.54 (*s*, 3H, thiophCH₃), 2.21 (*s*, 3H, thiophCH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 168.1, 164.7, 153.3, 151.5, 150.7, 144.8, 137.2, 126.9, 124.0, 113.7, 13.8, 12.1. LC-MS *m/z* (ES⁺) 285.0 (MH⁺). Anal. calcd. for C₁₄H₁₂N₄OS (284,34): C, 59.14; H, 4.25; N, 19.70. Found: C, 59.19; H, 4.33; N, 19.80.

3. 5,6-dimethyl-N-(6-methylpyridin-2-yl)thieno[2,3-*d*]pyrimidine-4-carboxamide (2c).

Yield: 67 %, white crystals. M.p. 169–170 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.24 (*br.s*, 1H, NH), 9.02 (*s*, 1H, CH), 8.02 (*dd*, *J*=8.4, 3.9 Hz, 1H), 7.89–7.67 (*m*, 1H), 7.07 (*dd*, *J*=7.8, 4.0 Hz, 1H), 2.52 (*s*, 3H, thiophCH₃), 2.40 (*s*, 3H, CH₃), 2.22 (*s*, 3H, thiophCH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 164.5, 157.0, 154.0, 151.4, 150.4, 138.8, 136.3, 129.1, 126.9, 124.0, 119.7, 111.1, 23.6, 13.7, 12.0. LC-MS *m/z* (ES⁺) 299.0 (MH⁺). Anal. calcd. for C₁₅H₁₄N₄OS (298,37): C, 60.38; H, 4.73; N, 18.78. Found: C, 60.46; H, 4.78; N, 18.78.

4. 5,6-dimethyl-N-(4-methylpyridin-2-yl)thieno[2,3-*d*]pyrimidine-4-carboxamide (2d).

Yield: 79 %, white crystals. M.p. 191–192 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.21 (*br.s*, 1H, NH), 9.04 (*s*, 1H, CH), 8.21 (*d*, *J*=11.4 Hz, 1H), 8.07 (*d*, *J*=8.9 Hz, 1H), 7.05 (*s*, 1H), 2.53 (*s*, 3H, thiophCH₃), 2.38 (*s*, 3H, CH₃), 2.23 (*s*, 3H, thiophCH₃). ¹³C NMR (151 MHz, dmso) δ 168.2, 165.0, 154.4, 151.9, 151.6, 149.8, 148.4, 136.9, 127.4, 124.5, 121.9, 115.0, 21.3, 14.1, 12.4. LC-MS *m/z* (ES⁺) 299.0 (MH⁺). Anal. calcd. for C₁₅H₁₄N₄OS (298,37): C, 60.38; H, 4.73; N, 18.78. Found: C, 60.45; H, 4.83; N, 18.84.

5. 5,6-dimethyl-N-(3-methylpyridin-2-yl)thieno[2,3-*d*]pyrimidine-4-carboxamide (2e).

Yield: 57 %, white crystals. M.p. 130–131 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.21 (*br.s*, 1H, NH), 9.03 (*s*, 1H, CH), 8.20 (*t*, *J*=2.8 Hz, 1H), 8.10 (*dd*, *J*=8.6, 3.1 Hz, 1H), 7.71 (*dd*, *J*=8.3, 2.7 Hz, 1H), 2.52 (*s*, 3H, thiophCH₃), 2.27 (*s*, 3H, CH₃), 2.22 (*s*, 3H, thiophCH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 164.4, 154.0, 151.5, 148.9, 148.2, 138.8, 136.4, 129.5, 126.9, 124.0, 113.8, 17.3, 13.7, 11.9. LC-MS *m/z* (ES⁺) 299.0 (MH⁺). Anal. calcd. for C₁₅H₁₄N₄OS (298,37): C, 60.38; H, 4.73; N, 18.78. Found: C, 60.44; H, 4.81; N, 18.82.

6. N-(pyridin-2-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine-4-carboxamide (2f).

Yield: 85 %, white crystals. M.p. 156–157 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.24 (*br.s*, 1H, NH), 9.06 (*s*, 1H, CH), 8.39 (*d*, *J*=4.9 Hz, 1H), 8.22 (*d*, *J*=8.3 Hz, 1H), 7.91 (*d*, *J*=7.4 Hz, 1H), 7.22 (*t*, *J*=6.2 Hz, 1H), 2.91 (*d*, *J*=6.3 Hz, 2H), 2.68 (*d*, *J*=6.1 Hz, 2H), 1.92–1.67 (*m*, 4H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 168.5, 164.5, 153.4, 151.5, 151.1, 148.4, 139.5, 138.5, 126.2, 126.1, 120.4, 114.2, 25.4, 24.1, 22.2, 21.6. LC-MS *m/z* (ES⁺) 311.0 (MH⁺). Anal. calcd. for C₁₆H₁₄N₄OS (310,38): C, 61.92; H, 4.55; N, 18.05. Found: C, 61.96; H, 4.59; N, 18.10.

7. N-(pyridin-4-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine-4-carboxamide (2g).

Yield: 83 %, white crystals. M.p. 179–180 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.28 (*br.s*, 1H, NH), 9.10 (*s*, 1H, CH), 8.58–8.44 (*m*, 2H), 7.81–7.60 (*m*, 2H), 2.91 (*d*, *J*=6.3 Hz, 2H), 2.65 (*t*, *J*=6.2 Hz, 2H), 1.94–1.66 (*m*, 4H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 168.8, 164.7, 152.8, 151.6, 150.7, 144.8, 140.2, 126.2, 126.1, 113.7, 25.4, 24.3, 22.1, 21.6. LC-MS *m/z* (ES⁺) 311.0 (MH⁺). Anal. calcd. for C₁₆H₁₄N₄OS (310,38): C, 61.92; H, 4.55; N, 18.05. Found: C, 61.99; H, 4.60; N, 18.02.

8. N-(6-methylpyridin-2-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-carboxamide (2h).

Yield: 71 %, white crystals. M.p. 178–179 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.18 (*br.s*, 1H, NH), 8.01 (*s*, 1H, CH), 7.77 (*t*, $J=7.8$ Hz, 1H), 7.07 (*d*, $J=7.5$ Hz, 1H), 2.90 (*d*, $J=5.9$ Hz, 2H), 2.65 (*d*, $J=5.6$ Hz, 2H), 2.41 (*s*, 3H, CH_3), 1.90–1.68 (*m*, 4H). ^{13}C NMR (126 MHz, DMSO) δ 168.4, 164.5, 157.0, 151.5, 139.4, 138.8, 133.7, 126.2, 119.7, 111.1, 110.4, 103.3, 25.4, 24.1, 23.6, 22.2, 21.6. LC-MS m/z (ES $^+$) 325.0 (MH $^+$). Anal. calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_4\text{OS}$ (324.41): C, 62.94; H, 4.97; N, 17.27. Found: C, 62.92; H, 5.05; N, 17.36.

9. N-(4-methylpyridin-2-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-carboxamide (2i).

Yield: 68 %, white crystals. M.p. 176–177 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.16 (*br.s*, 1H, NH), 9.04 (*s*, 1H, CH), 8.22 (*d*, $J=4.7$ Hz, 1H), 8.06 (*s*, 1H), 7.04 (*d*, $J=5.2$ Hz, 1H), 2.90 (*d*, $J=5.9$ Hz, 2H), 2.66 (*d*, $J=5.7$ Hz, 2H), 2.37 (*s*, 3H, CH_3), 1.88–1.68 (*m*, 4H). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 168.4, 164.5, 153.4, 151.5, 151.2, 149.3, 148.0, 139.5, 126.2, 126.1, 121.4, 114.5, 25.4, 24.1, 22.2, 21.6, 20.9. LC-MS m/z (ES $^+$) 325.2 (MH $^+$). Anal. calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_4\text{OS}$ (324.41): C, 62.94; H, 4.97; N, 17.27. Found: C, 63.02; H, 4.98; N, 17.37.

10. N-(3-methylpyridin-2-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-carboxamide (2j).

Yield: 62 %, white crystals. M.p. 140–141 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.15 (*br.s*, 1H, NH), 9.03 (*s*, 1H, CH), 8.20 (*t*, $J=3.1$ Hz, 1H), 8.10 (*dd*, $J=8.4$, 3.8 Hz, 1H), 7.70 (*dt*, $J=6.8$, 3.1 Hz, 1H), 2.90 (*d*, $J=5.7$ Hz, 2H), 2.65 (*d*, $J=5.7$ Hz, 2H), 2.27 (*s*, 3H, CH_3), 1.92–1.58 (*m*, 4H). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 168.4, 164.3, 153.5, 151.5, 148.9, 148.1, 139.4, 138.8, 129.5, 126.2, 126.1, 113.8, 25.4, 24.1, 22.2, 21.6, 17.3. LC-MS m/z (ES $^+$) 325.2 (MH $^+$). Anal. calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_4\text{OS}$ (324.41): C, 62.94; H, 4.97; N, 17.27. Found: C, 62.98; H, 5.03; N, 17.36.

3.3. Antimicrobial Activity

The studied derivatives were tested *in vitro* for their antibacterial and anti-fungal activities using agar diffusion and serial dilutions resazurin-based microdilution as-

says (RBMA) [34–36]. For this purpose, 100 μL (1 mg/mL) of the tested compound was placed in an agar well with a diameter of 5.5 mm. The diameter of the growth retardation was measured using a micrometre with an error of 0.1 mm. Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) was used as a controls. Pure DMSO was used as a solvent due to the poor solubility of the test compound in dilute DMSO. In addition, Mueller-Hinton agar (Sigma-Aldrich, St. Louis, MO, USA) and Saburo agar (for fungi) (Sigma-Aldrich, St. Louis, MO, USA) were used, and Petri dishes were incubated at 37 °C for 24 h for bacteria and at 25 °C for 24–48 h for fungi. The RBMA method involved the introduction of a 96-well plate of 50 μL of nutrient medium (Mueller-Hinton broth or glucose broth), 50 μL of a suspension of the microorganism (McFarland 1.5–2.0), and 100 μL of the tested compound, with the addition of 15 μL of 0.02 % resazurin in each well.

Eighteen reference and clinical microbial and fungal strains were used (Table 1), previously identified by the MALDI TOF system (Bruker, Bremen, Germany) and 16S rRNA gene sequences. All clinical strains were multi-drug-resistant or extensively drug-resistant with different antibiotic resistance patterns. Clinical strains were isolated from a patient with healthcare-associated infections (respiratory tract, blood, urine) from Lviv regional hospitals. All testing was repeated triplicate. All biofilm-forming strains had the *pox* gene, which is responsible for the formation of biofilms.

4. Result

In order to check the assumption about the rationality of the combination of thieno[2,3-d]pyrimidine-4-carboxylic acids with the fragments of aminopyridine in the form of amide substituent, we carried out the docking studies for the generated library of the virtual molecules. We studied the opportunity for the compound generated from the available to us 2- or 4-aminopyridines and substituted thieno[2,3-d]pyrimidine-4-carboxylic acids to have antimicrobial activity due to inhibition of TrmD. The constructed compounds are listed in Fig. 3.

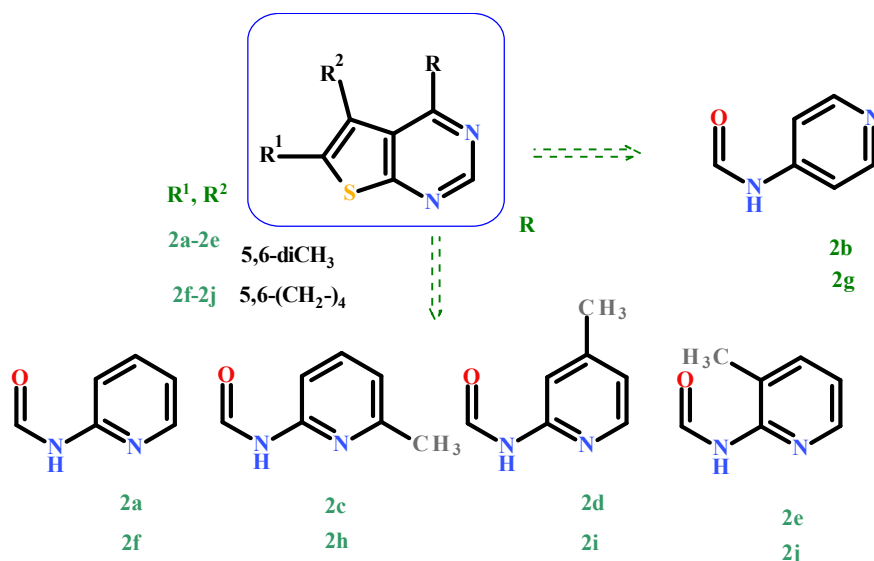


Fig. 3. The designed structures of pyridyl amides of thieno[2,3-d]pyrimidine-4-carboxylic acid

The molecular docking studies were performed for the library of compounds to the active site of selective TrmD inhibitors, for the enzyme isolated from *P. aeruginosa*. The inhibitory activity of the constructed ligands was estimated by the affinity of the enzyme in relation to the reference ligand, the conformation analysis of the ligand's pose, and its interaction with the amino acids of the active site (Table 2). All the ligands, according to their affinity, were at the same level or showed even better affinity in comparison to the reference ligand. Their binding energy was in the region -8.2 to -9.3 kcal/mole (Table 1). The affinity for tetrahydrobenzothieno[2,3-*d*]pyrimidine derivatives 2f-2j was the highest in the experiment.

In order to combine the pyrimidine fragment with thieno[2,3-*d*]pyrimidine-4-carboxylic acids we used the reaction of acylation of 2- or 4-aminopyrimidines with the corresponding acids 1. The reaction was performed using 1,1'-carbonyldiimidazole as a coupling reagent in the anhydrous dimethylformamide Fig. 4.

It is very likely that the position of the carboxyl group at the pyrimidine ring due to the electron-withdrawing ef-

fect of two nitrogen atoms makes intermediate imidazolide very reactive. The reaction gives high yields of products 2, which needs to be just purified by crystallization.

In the spectra of amides 2a-e, the signals of the corresponding substituents at the thiophene ring are observed in the form of two singlets in the regions 2.52–2.54 and 2.21–2.25 ppm. For the compounds 2f-j the signals of tetrahydrobenzo substituent are observed as three groups of signals; they are in the form of two doublets of methylene groups at positions near the thiophene ring 2.90–2.92 (2H) and 2.65–2.68 (2H), the signals if more distant from thiophene methylene groups are observed in the region 1.58–1.94 (*m*, 4H).

For all of the amides 2, the typical signal of NH proton is observed in the region 11.15–11.32 ppm; among all of the compounds 2, this signal is observed the most downfield for 4-pyridyl amides 2b and 2g. The signal of CH group at position 2 of thieno[2,3-*d*]pyrimidine ring system is observed as a singlet in the region 8.01–9.10 ppm. The signals of pyrimidine ring protons are observed in the region of aromatic proton resonance. Methyl groups for aminopicolines have signals in the region 2.27–2.41 ppm.

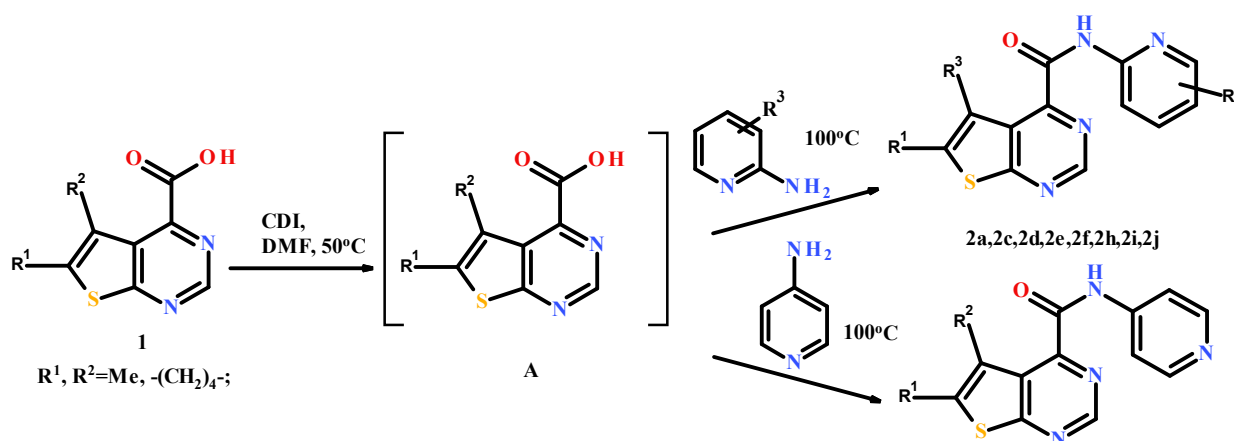


Fig. 4. Synthesis of pyridyl amides of thieno[2,3-*d*]pyrimidine-4-carboxylic acid

Table 1

The results of docking studies of pyridyl amides of thieno[2,3-*d*]pyrimidine-4-carboxylic acid

Ligand	Binding energy, kcal/mol	Hydrophobic interaction	Hydrogen interaction	Other interaction
Reference ligand	–8.2	Tyr141, Ser93(2), Pro94 (4), Pro149(2), Tyr120, Ile138, Leu143, Gly 145, Gly146	Leu143, Gln95, Glu121, Gly139, Asp182	–
2a	–8.5	Gly145, Gly146, Pro94(3), His185*, Pro149	leu92*	Glu121 (Electrostatic)
2b	–8.2	Pro94(2), Val142*, Ile138, Leu143, Pro149	Tyr91*, Ile138, Gly145, Leu92*	–
2c	–8.9	Pro94(2), Val142*, Ile138 (2), Leu143, Tyr141, Leu143, Pro149	Tyr91*, Leu92*	–
2d	–9.2	Pro94 (4), Leu143(2), His185*, Pro149, Gly145, Gly146	Leu92*	Glu121 (Electrostatic)
2e	–8.3	Pro94 (4), Val142*, Leu143(3), Ile138, Pro149(2)	–	–
2f	–9.0	Ser93, His185*, Pro94 (2)	Tyr91(2)*, Gln95(2)*, Pro94, Asp182	
2g	–9.0	Ser93, Pro94(5), Val142*, Leu143, Pro149	Tyr91*, Gly145(2), Tyr141	
2h	–9.1	Ser93, Pro94(5), Val142*, Ile138, Pro149(2), Leu143	Tyr91*, Gly145, Leu92*	
2i	–9.0	Ser93, Pro94 (6), Val142*, Ile138, Pro149, Leu143, Pro149	Tyr91*, Gly145	
2j	–9.3	Ser93, Pro94 (6), Val142 (2)*, Leu143 (2), Pro149	Tyr91*, Gly145, Leu92 (2)*	

Note: () – the number of bonds is given in brackets; * – the amino acids which do not interact with reference ligand in the experiment

All the ^{13}C NMR spectra show a good correlation between the number of the signals in the spectra and the number of nonequivalent Carbon atoms in the molecules of amides 2. For the LC-MS spectra of amides 2, the correspondence between the quasimolecular ion m/z value and the suggested structure is observed.

The amides 2 were tested for antimicrobial activity *in vitro*. From the ten compounds 2 tested (Table 3), the compound 2g showed the best antimicrobial effect against Gram-positive microorganisms, including clinical biofilm-forming strains of staphylococci. Com-

pounds 2h, 2c also showed activity against the reference strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

No significant activity against fungi was observed, but later maturation of *Aspergillus* spores (blackening), compared to the control, may indicate a potential sporidical effect. But this requires a more detailed study.

A minimum inhibitory concentration was determined for the three hit compounds, and the lowest MIC was for compound 2c against the reference strain of *Pseudomonas aeruginosa*.

Table 2

In vitro antimicrobial activity (zone of growth inhibition at conc. 1 mg/mL after 24–48 h)

Type of species		Species of bacteria and fungi	Zone of growth inhibition (mm \pm SE)										
			2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	
Gram-neg- ative bacteria	Reference strains	<i>Pseudomonas aeruginosa</i> ATCC 10145	00	00	12.1 \pm ± 0.3	00	00	00	8.7 \pm ± 0.3	11.3 \pm ± 0.3	7.1 \pm ± 0.3	00	00
	Clinical strains	<i>Pseudomonas aeruginosa</i> N197	00	00	00	00	00	00	00	00	00	00	00
		<i>Klebsiella pneumoniae</i> N218	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2	10.0 \pm ± 0.2
		<i>Escherichia coli</i> N221	00	00	00	00	00	00	00	00	00	00	00
Gram-pos- itive bacteria	Reference strain	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 biofilm-forming	00	00	7.1 \pm ± 0.3	00	00	00	11.6 \pm ± 0.3	7.3 \pm ± 0.3	00	00	00
		<i>Staphylococcus epidermidis</i> ATCC 12228 non-biofilm forming	00	00	00	00	00	00	13.5 \pm ± 0.3	00	00	00	00
	Clinical strain	<i>Staphylococcus aureus</i> N 221	00	00	00	00	00	00	00	00	00	00	00
		<i>Staphylococcus delphini</i> N 223	00	00	00	00	00	00	7.0 \pm ± 0.2	00	00	00	00
		<i>Enterococcus</i> spp. N 222	00	00	00	00	00	00	00	00	00	00	00
		<i>Staphylococcus aureus</i> bio-film-forming N2	00	00	00	00	00	00	10.1 \pm ± 0.2	00	00	00	00
		<i>Staphylococcus aureus</i> bio-film-forming N3	00	00	00	00	00	00	7.0 \pm ± 0.2	00	00	00	00
		<i>Staphylococcus aureus</i> bio-film-forming N5	00	00	00	00	00	00	00	00	00	00	00
		<i>Staphylococcus aureus</i> bio-film-forming N12	00	00	00	00	00	00	11.3 \pm ± 0.2	7.0 \pm ± 0.2	00	00	00
		<i>Staphylococcus aureus</i> bio-film-forming N19	00	00	00	00	00	00	16.6 \pm ± 0.2	7.0 \pm ± 0.2	00	00	00
Fungi	Reference strain	<i>Candida. albicans</i> ATCC 885-653	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4
		<i>Aspergillus niger</i> *	9.0 \pm ± 0.2	9.0 \pm ± 0.2	13.0 \pm ± 0.4	9.0 \pm ± 0.2	9.0 \pm ± 0.2	9.0 \pm ± 0.2	13.0 \pm ± 0.4	13.0 \pm ± 0.4	9.0 \pm ± 0.2	9.0 \pm ± 0.2	9.0 \pm ± 0.2
	Clinical strain	<i>Candida lusitanae</i> N 89	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4	13.0 \pm ± 0.4

Note: *Maturation of *Aspergillus* spores (blackening) was observed later, compared to the control

Table 3

MIC value (μM) of compounds against bacterial species

Species of bacteria	μM		
	2g	2h	2c
<i>Staphylococcus aureus</i> subsp. <i>Aureus</i> ATCC 25923 biofilm-forming	805.5	770.6	1675.8
<i>Pseudomonas aeruginosa</i> ATCC 10145	402.7	385.3	209.5

5. Discussion

The results obtained in the research showed that modification of the readily available for preparation according to the previously reported procedure [9] thieno[2,3-*d*]pyrimidine-4-carboxylic acids by amidation with the derivatives of aminopyridines promoted by 1,1'-carbonyldiimidazole as a peptide coupling reagent produces the yields of the amides which are no less than for anilines and benzyl amines [9]. The microbiological research on the variety of bacterial strains more than the standard number revealed the selectivity of antimicrobial action of pyridyl amides of thieno[2,3-*d*]pyrimidine-4-carboxylic acids against *Pseudomonas aeruginosa* ATCC 10145, *Klebsiella pneumoniae* N218 and some biofilm-forming strains of *Staphylococcus aureus*. The antifungal activity, which was typical for similar amides with carbocycles in the amide fragment [9], was also disclosed for the pyridyl analogues 2 presented in the current research. The molecular docking study of the obtained pyridyl carboxamides 2, which contains carboxamide group at position 4, in comparison with the native ligand containing carboxamide group at position 5 [6] to TrmD isolated from *P. aeruginosa* showed the opportunity for 4-carboxamides to bind with the active site of the enzyme. The docking studies gave a deeper insight into the possible mechanism of antibacterial action of pyridyl amides of thieno[2,3-*d*]pyrimidine-4-carboxylic acids.

The virtual library of pyridyl amides thieno[2,3-*d*]pyrimidine-4-carboxylic acids constructed for the study of the antimicrobial activity and the influence of the substituents in the pyridine cycle at this type of activity was tested using the docking studies at the active site of (guanine37-*N*¹)-methyltransferase (TrmD) isolated from *Pseudomonas aeruginosa*. The docking study revealed a high probability of antimicrobial activity by inhibition of bacterial TrmD at this stage of research.

The detailed study of the library members with the peptide fragments evidently shows the similarity in the interaction pattern for all of the compounds 2 and their strong fixation in the pocket by the branched network of hydrophobic interactions. The additional stabilization of the ligand-enzyme conformation is achieved by interaction with glycine Gly 145 and glutamine Gln95 for most of the compounds, with the exception of amide 2e (Fig. 5, *a*). These interactions and the bond with leucine (Leu92) confirm the opportunity for the deep immersion into the active site because it is the same as for S-Adenosyl methionine, which is the main cofactor of TrmD. It should also be noticed that for all of the picolines (2c-e, 2h-g) the hydrophobic interaction of methyl group is typical with Val42, Ile138, Leu143.

The comparison of the studied ligands' conformations with the reference ligand conformation shows the same spatial pose of thienopyrimidine cycles for instance (Fig. 5, *b*). On the other hand the molecules of the ligands 2a-2j are shorter than the molecule of the reference ligand and the hydrophobic pocket occupied with the alkyl chain of the native ligand remains empty. As a result, no hydrophobic interactions with the residue of tyrosine (Tyr120, 141) are observed.

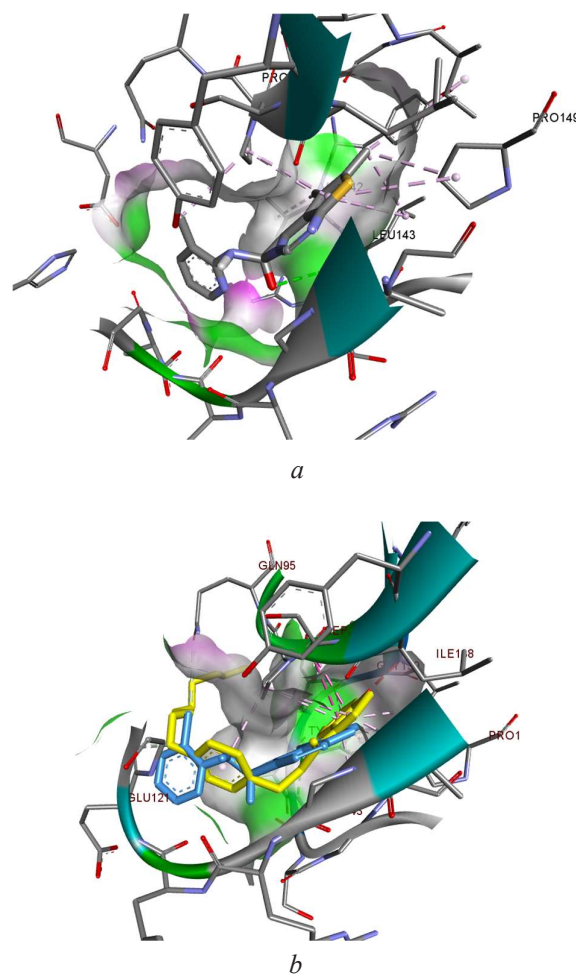


Fig. 5. 3D visualization: *a* – interaction of the ligand 2e with amino acid residues of the active site of TrmD inhibitors; *b* – conformations of native inhibitor (yellow molecule) and ligand 2e (blue molecule)

The results of docking studies showed a high probability of antimicrobial activity for amides 2 via inhibition of TrmD.

For the synthesis of the library we used the reaction between thieno[2,3-*d*]pyrimidine-4-carboxylic acids and substituted aminopyridines promoted by 1,1'-carbonyldiimidazole as peptide coupling reagent. The suggested procedure for pyridyl amides thieno[2,3-*d*]pyrimidine-4-carboxylic acids synthesis starting from readily available thieno[2,3-*d*]pyrimidine-4-carboxylic acids was found to be highly effective and produced us the target molecules with high yields and high purity. The results of the antimicrobial activity *in vitro* screening for the amides 2 revealed that N-(pyridin-4-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine-4-carboxamide (2g) showed a wide range of antimicrobial activity while 5,6-dimethyl-N-(6-methylpyridin-2-yl)thieno[2,3-*d*]pyrimidine-4-carboxamide (2c) had the best MIC value against the reference strain of *Pseudomonas aeruginosa* ATCC 10145. N-(6-Methylpyridin-2-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine-4-carboxamide (2h) was also found to be active against *Pseudomonas aeruginosa* with high selectivity to this bacteria.

Study limitations. The study of antimicrobial properties of pyridyl amides thieno[2,3-*d*]pyrimidine-4-carboxylic acids is limited by the availability of the starting acids as well as the availability of a collection of *Pseudomonas aeruginosa* strains. The limitations are also in the use of restricted substituents, which are known to increase the toxicity of the molecules for humans. The docking method for study is also limited by the differences in software, and thus, the calculated docking parameters may vary. To make the calculations more accurate, validation of methodology depending on the native reference ligand should be performed for every particular calculation.

Prospects for further research. The suggested methods for synthesis of pyridyl amides thieno[2,3-*d*]pyrimidine-4-carboxylic acids and the docking to TrmD can be applied for the construction of more potent antimicrobials selective to TrmD of *Pseudomonas aeruginosa*. The docking studies can be applied for the preliminary *in silico* screening of the virtual libraries of amides of thieno[2,3-*d*]pyrimidine-4-carboxylic acids. The result of the antimicrobial screening *in vitro* showed the prospects of additional study of the compound 2g for anti-biofilm activity. Compounds 2g; 2j; 2i; 2h are interesting objects for study regarding sporidical activity.

6. Conclusions

The results of the study in the series of pyridyl amides of thieno[2,3-*d*]pyrimidine-4-carboxylic acids revealed their antimicrobial activity with a special impact on the inhibition of *Pseudomonas aeruginosa*. The effective coupling method for preparation of the amides using peptide coupling procedures of thieno[2,3-*d*]pyrimidine-4-carboxylic acids with unsubstituted 2- and

4-aminopyridines as well as their picoline analogs has been developed.

The *in vitro* studies of antimicrobial activity confirmed the wide range of antimicrobial activity for N-(pyridin-4-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine-4-carboxamide (2g). The selectivity and the lowest MIC value against the reference strain of *Pseudomonas aeruginosa* ATCC 10145 in the series was found for 5,6-dimethyl-N-(6-methylpyridin-2-yl)thieno[2,3-*d*]pyrimidine-4-carboxamide (2c). For the compound 2g, the potential antibiofilm activity was determined.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

Funding

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

Data availability

Data will be made available on reasonable request.

Acknowledgement

The authors acknowledge Enamine Ltd. for the measurement of ¹H, ¹³C NMR and LC-MS spectra of the obtained substances.

References

1. Malasala, S., Polomoni, A., Ahmad, Md. N., Shukla, M., Kaul, G., Dasgupta, A. et al. (2021). Structure based design, synthesis and evaluation of new thienopyrimidine derivatives as anti-bacterial agents. *Journal of Molecular Structure*, 1234, 130168. doi: <https://doi.org/10.1016/j.molstruc.2021.130168>
2. Badiger, N. P., Gonkar, S. L., Shetty, N. S. (2015). Synthesis of some new thienopyrimidines and triazole fused thienopyrimidines and their antimicrobial activities. *International Journal of Chemical and Pharmaceutical Sciences*, 6 (1), 58–62.
3. Triloknadh, S., Venkata Rao, C., Nagaraju, K., Hari Krishna, N., Venkata Ramaiah, C. et al. (2018). Design, synthesis, neuro-protective, antibacterial activities and docking studies of novel thieno[2,3-*d*]pyrimidine-alkyne Mannich base and oxadiazole hybrids. *Bioorganic & Medicinal Chemistry Letters*, 28 (9), 1663–1669. doi: <https://doi.org/10.1016/j.bmcl.2018.03.030>
4. Hill, P. J., Abibi, A., Albert, R., Andrews, B., Gagnon, M. M., Gao, N. et al. (2013). Selective Inhibitors of Bacterial t-RNA-(N1G37) Methyltransferase (TrmD) That Demonstrate Novel Ordering of the Lid Domain. *Journal of Medicinal Chemistry*, 56 (18), 7278–7288. doi: <https://doi.org/10.1021/jm400718n>
5. Zhong, W., Koay, A., Ngo, A., Li, Y., Nah, Q., Wong, Y. H. et al. (2019). Targeting the Bacterial Epitranscriptome for Antibiotic Development: Discovery of Novel tRNA-(N1G37) Methyltransferase (TrmD) Inhibitors. *ACS Infectious Diseases*, 5 (3), 326–335. doi: <https://doi.org/10.1021/acsinfecdis.8b00275>
6. Zhong, W., Pasunooti, K. K., Balamkundu, S., Wong, Y. H., Nah, Q., Gadi, V. et al. (2019). Thienopyrimidinone Derivatives That Inhibit Bacterial tRNA (Guanine37-N1)-Methyltransferase (TrmD) by Restructuring the Active Site with a Tyrosine-Flipping Mechanism. *Journal of Medicinal Chemistry*, 62 (17), 7788–7805. doi: <https://doi.org/10.1021/acs.jmedchem.9b00582>
7. Vlasov, S. V., Vlasova, O. D., Severina, H. I., Krolenko, K. Yu., Borysov, O. V., Abu Sharkh, A. I. M. et al. (2021). Design, Synthesis and In Vitro Antimicrobial Activity of 6-(1H-Benzimidazol-2-yl)-3,5-dimethyl-4-oxo-2-thio-3,4-dihydrothieno[2,3-*d*]pyrimidines. *Scientia Pharmaceutica*, 89 (4), 49. doi: <https://doi.org/10.3390/scipharm89040049>
8. Vlasov, S., Krolenko, K., Severina, H., Vlasova, O., Borysov, O., Shynkarenko, P. (2023). Novel 4-methylthienopyrimidines as antimicrobial agents: synthesis, docking study and in vitro evaluation. *Journal of Applied Pharmaceutical Science*, 13 (4), 105–113. doi: <https://doi.org/10.7324/japs.2023.102631>

9. Vlasova, O. D., Krolenko, K. Yu., Nechayev, M. A., Shynkarenko, P. E., Kabachnyy, V. I., Vlasov, S. V. (2019). Efficient method for the synthesis of novel substituted thieno[2,3-d]pyrimidine-4-carboxylic acids, their derivatization, and antimicrobial activity. *Chemistry of Heterocyclic Compounds*, 55 (2), 184–188. doi: <https://doi.org/10.1007/s10593-019-02437-1>
10. Ono, N., Hironaga, H., Ono, K., Kaneko, S., Murashima, T., Ueda, T. et al. (1996). A new synthesis of pyrroles and porphyrins fused with aromatic rings. *Journal of the Chemical Society, Perkin Transactions 1*, 5, 417. doi: <https://doi.org/10.1039/p19960000417>
11. Murashima, T., Fujita, K., Ono, K., Ogawa, T., Uno, H., Ono, N. (1996). A new facet of the reaction of nitro heteroaromatic compounds with ethyl isocyanoacetate. *Journal of the Chemical Society, Perkin Transactions 1*, 12, 1403–1407. doi: <https://doi.org/10.1039/p19960001403>
12. Dang, Q., Carruli, E., Tian, F., Dang, F. W., Gibson, T., Li, W. et al. (2009). A tandem decarboxylation and inverse electron-demand Diels–Alder reaction of amino-thiophenecarboxylic acids with 1,3,5-triazines. *Tetrahedron Letters*, 50 (24), 2874–2876. doi: <https://doi.org/10.1016/j.tetlet.2009.03.180>
13. Goto, T., Kondo, Y., Takawa, T., Honjo, E., Hamaguchi, W., Hoshii, H., Shiraishi, N. (2015). Sulfur-containing bicyclic compound. Pat. 2015111876 U.S.
14. Ahmed, I. A.; Watson, R. R., Preedy, V. R. (Ed.) (2019). Major dietary interventions for the management of liver disease. *Dietary interventions in liver disease*. Academic Press, 205–212. doi: <https://doi.org/10.1016/b978-0-12-814466-4.00017-3>
15. Nakhaee, S., Mehrpour, O. (2022). Niacin. *Reference Module in Biomedical Sciences*, Elsevier, 755–761. doi: <https://doi.org/10.1016/B978-0-12-824315-2.00113-5>
16. Sledge, C. L., Morgan, B. W.; Wexler, P. (Ed.) (2014). Niacin. *Encyclopedia of Toxicology*. Academic Press, 504–505. doi: <https://doi.org/10.1016/B978-0-12-386454-3.00760-0>
17. WHO Report on the Global Tobacco Epidemic (2017). WHO, 135. Available at: <http://apps.who.int/iris/bitstream/handle/10665/255874/9789241512824-eng.pdf;jsessionid=2C22C4F8E9201354D0A22AF1950AD744?sequence=1>
18. Kivell, B. M., Danielson, K.; Preedy, V. R. (Ed.) (2016). Neurological effects of nicotine, tobacco, and particulate matter. *Neuropathology of drug addictions and substance misuse*. Academic Press, 115–122. doi: <https://doi.org/10.1016/B978-0-12-800213-1.00011-0>
19. Feldhammer, M., Ritchie, J. C. (2017). Anabasine Is a Poor Marker for Determining Smoking Status of Transplant Patients. *Clinical Chemistry*, 63 (2), 604–606. doi: <https://doi.org/10.1373/clinchem.2016.265546>
20. Smulyan, H. (2018). The Beat Goes On: The Story of Five Ageless Cardiac Drugs. *The American Journal of the Medical Sciences*, 356 (5), 441–450. doi: <https://doi.org/10.1016/j.amjms.2018.04.011>
21. Wu, P.-C., Chuang, M.-N., Choi, J., Chen, H., Wu, G., Ohno-Matsui, K. et al. (2018). Update in myopia and treatment strategy of atropine use in myopia control. *Eye*, 33 (1), 3–13. doi: <https://doi.org/10.1038/s41433-018-0139-7>
22. Diacon, A., Miyahara, S., Dawson, R., Sun, X., Hogg, E., Donahue, K. et al. (2020). Assessing whether isoniazid is essential during the first 14 days of tuberculosis therapy: a phase 2a, open-label, randomised controlled trial. *The Lancet Microbe*, 1 (2), e84–e92. doi: [https://doi.org/10.1016/s2666-5247\(20\)30011-2](https://doi.org/10.1016/s2666-5247(20)30011-2)
23. Remijn-Nelissen, L., Verschuuren, J. J. G. M., Tannemaat, M. R. (2022). The effectiveness and side effects of pyridostigmine in the treatment of myasthenia gravis: a cross-sectional study. *Neuromuscular Disorders*, 32 (10), 790–799. doi: <https://doi.org/10.1016/j.nmd.2022.09.002>
24. Nuzum, D., Snider, Veverka, A. (2008). Long-acting nifedipine in the management of the hypertensive patient. *Vascular Health and Risk Management*, 4 (6), 1249–1257. doi: <https://doi.org/10.2147/vhrm.s3661>
25. de Heus, R. A. A., Donders, R., Santoso, A. M. M., Olde Rikkert, M. G. M., Lawlor, B. A., Claassen, J. A. H. R. et al. (2019). Blood Pressure Lowering With Nilvadipine in Patients With Mild-to-Moderate Alzheimer Disease Does Not Increase the Prevalence of Orthostatic Hypotension. *Journal of the American Heart Association*, 8 (10). doi: <https://doi.org/10.1161/jaha.119.011938>
26. Calvo, A., Zupelari-Gonçalves, P., Dionísio, T., Brozoski, D., Faria, F. A., Santos, C. (2017). Efficacy of piroxicam for postoperative pain after lower third molar surgery associated with CYP2C8 and CYP2C9. *Journal of Pain Research*, 10, 1581–1589. doi: <https://doi.org/10.2147/jpr.s138147>
27. Procopio, G., Chiuri, V. E., Giordano, M., Mantini, G., Maisano, R., Bordonaro, R. et al. (2020). Effectiveness of abiraterone acetate plus prednisone in chemotherapy-naïve patients with metastatic castration-resistant prostate cancer in a large prospective real-world cohort: the ABItude study. *Therapeutic Advances in Medical Oncology*, 12. doi: <https://doi.org/10.1177/1758835920968725>
28. Blackhall, F., Cappuzzo, F. (2016). Crizotinib: from discovery to accelerated development to front-line treatment. *Annals of Oncology*, 27, iii35–iii41. doi: <https://doi.org/10.1093/annonc/mdw304>
29. Mitra, S., Muni, M., Shawon, N. J., Das, R., Emran, T. B., Sharma, R. et al. (2022). Tacrine Derivatives in Neurological Disorders: Focus on Molecular Mechanisms and Neurotherapeutic Potential. *Oxidative Medicine and Cellular Longevity*, 2022, 1–22. doi: <https://doi.org/10.1155/2022/7252882>
30. Riahi, A., Wurster, M., Lalk, M., Lindequist, U., Langer, P. (2009). Synthesis and antimicrobial activity of 4-hydroxy-4-(pyridyl)alk-3-en-2-ones. *Bioorganic & Medicinal Chemistry*, 17 (13), 4323–4326. doi: <https://doi.org/10.1016/j.bmc.2009.05.023>
31. Sarova, D., Kapoor, A., Narang, R., Judge, V., Narasimhan, B. (2010). Dodecanoic acid derivatives: Synthesis, antimicrobial evaluation and development of one-target and multi-target QSAR models. *Medicinal Chemistry Research*, 20 (6), 769–781. doi: <https://doi.org/10.1007/s00044-010-9383-5>
32. Karunanidhi, A., Ghaznavi-Rad, E., Jeevajothi Nathan, J., Joseph, N., Chigurupati, S., Mohd Fauzi, F. et al. (2019). Bioactive 2-(Methyldithio)Pyridine-3-Carbonitrile from Persian Shallot (*Allium stipitatum* Regel.) Exerts Broad-Spectrum Antimicrobial Activity. *Molecules*, 24 (6), 1003. doi: <https://doi.org/10.3390/molecules24061003>

33. Ragab, A., Fouad, S. A., Ali, O. A. A., Ahmed, E. M., Ali, A. M., Askar, A. A., Ammar, Y. A. (2021). Sulfaguanidine Hybrid with Some New Pyridine-2-One Derivatives: Design, Synthesis, and Antimicrobial Activity against Multidrug-Resistant Bacteria as Dual DNA Gyrase and DHFR Inhibitors. *Antibiotics*, 10 (2), 162. doi: <https://doi.org/10.3390/antibiotics10020162>
34. Antimicrobial susceptibility testing EUCAST disk diffusion method version 8.0 (2020). EUCAST, 22. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2020_manuals/Manual_v_8.0_EUCAST_Disk_Test_2020.pdf
35. Balouiri, M., Sadiki, M., Ibensouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6 (2), 71–79. doi: <https://doi.org/10.1016/j.jpha.2015.11.005>
36. Konechnyi, Y., Lozynskyi, A., Ivasechko, I., Dumych, T., Paryzhak, S., Hrushka, O., Partyka, U., Pasichnyuk, I., Khylyuk, D., Lesyk, R. (2023). 3-[5-(1H-Indol-3-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl]-propionic Acid as a Potential Polypharmacological Agent. *Scientia Pharmaceutica*, 91 (1), 13. doi: <https://doi.org/10.3390/scipharm91010013>

Received date 21.09.2023

Accepted date 26.10.2023

Published date 31.10.2023

Sergiy Vlasov, Doctor of Pharmaceutical Sciences, Professor, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Hanna Severina*, Doctor of Pharmaceutical Science, Professor, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Olena Vlasova, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Oleksandr Borysov, PhD, Senior Researcher, Department of Medicinal Chemistry, Institute of Organic Chemistry of National Academy of Sciences of Ukraine, Akademika Kukharia str., 5, Kyiv, Ukraine, 02660, Enamine Ltd., Chervonotkatska str., 78, Kyiv, Ukraine, 02094

Pavlo Shynkarenko, PhD, Enamine Ltd., Chervonotkatska str., 78, Kyiv, Ukraine, 02094

Olga Golovchenko, PhD, Associate Professor, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Yulian Konechnyi, PhD, Department of Microbiology, Danylo Halytsky Lviv National Medical University, Pekarska str., 69, Lviv, Ukraine, 79010

Victoriya Georgiyants, Doctor of Pharmaceutical Sciences, Professor, Head of the Department, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

**Corresponding author: Hanna Severina, e-mail: severina.ai@ukr.net*