The comparative study of antimicrobial activity for 4-methylthieno[2,3-*d*]pyrimidine and their 4-oxo analogues

Sergiy Vlasov [1], Hanna Severina [1], Olena Vlasova [1], Oleksandr Borysov [2, 3], Pavlo Shynkarenko [3], Victoriya Georgiyants [1]

[1] Department of Pharmaceutical Chemistry, National University of Pharmacy, 53 Pushkinska str., 61002 Kharkiv, Ukraine

[2] Institute of Organic Chemistry of the NASU, 5 Academician Kukhar str., 02660 Kyiv, Ukraine

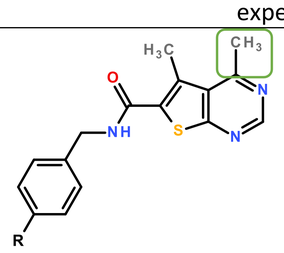
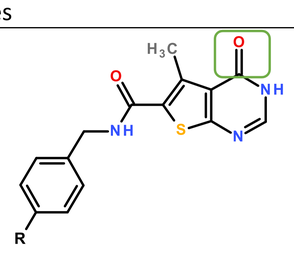
[3] Enamine Ltd., 78 Chervonotkatska str., 02094 Kyiv, Ukraine

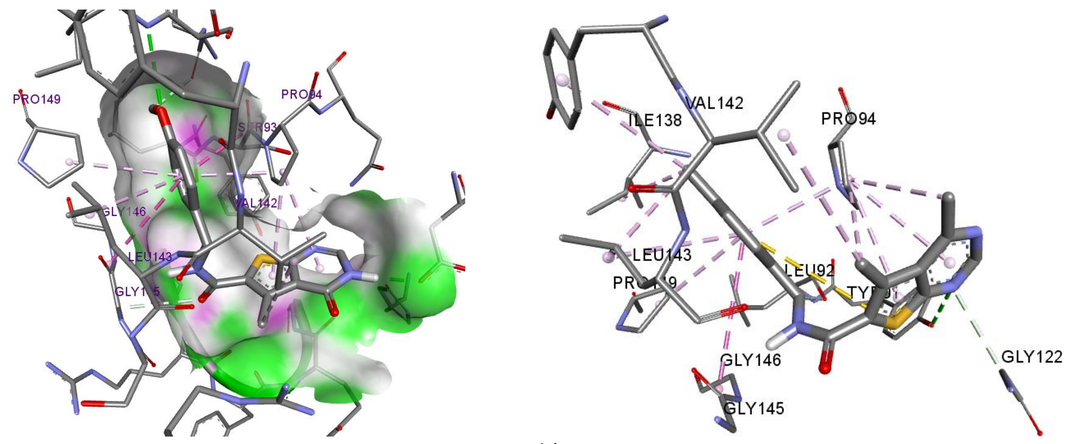
Introduction. Drug discovery of antibacterials is in a challenging situation for decades [1,2] regardless the great number of the possible targets known [3] not many new antibiotics appear [4] and they are still from the same legacy classes [5]. In recent years the research in TrmD inhibitors showed a progress producing inhibitors with in vitro antibacterial activity and the structure of the proteins was disclosed . The most effective small molecular inhibitors of TrmD with confirmed in vivo antibacterial activity contain either 3-indolyl of thieno[2,3-d]pyrimidine moiety. Recently we have reported the antimicrobial activity of the synthetically available 4-methylthieno[2,3-d]pyrimidine-6-carboxamides with benzyl substituents at the primary amide fragment as effective antimicrobials with the predicted affinity to TrmD isolated from P. aeruginosa [7]. It was also reported that 4-oxothieno[2,3-d]pyrimidine derivatives were more effective TrmD inhibitors rather than their 4-alkoxy analogues with aromatic pyrimidine fragment [6]. Therefore we decided it was reasonable to compare decided it was reasonable to carry out the comparative study of 4-methylthieno[2,3-d]pyrimidine-6-carboxamides with their 4-oxo analogues.

Materials and methods. The benzyl amides of were prepared in reaction of commercially available benzyl amines with either 4,5-dimethylthieno[2,3-*d*]pyrimidine-6-carboxylic acid [7] or 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid promoted by 1,1'-carbonylidiimidazole. To confirm the structure of the target compound the set of instrumental methods was used (^{1}H, ^{13}C NMR, LCMS). For the docking studies AutoDockTools 1.5.6rc3 and AutoDock Vina, BIOVIA Draw 2021, Chem3D software, and Discovery Studio Client 2021 were used. The complex of tRNA (Guanine37-N^1)-methyltransferase with the native ligand was downloaded from Protein Data Bank; the structure ID code is 5ZHN. The size of the Grid box and its center were determined by the native ligand of subunit A: TrmD (PDB ID 5ZHN): x = 40.04, y = 107.23, z = -3.40; size x = 18, y = 22, z = 20. Reference ligand – *N*-(4-((octylamino)methyl)benzyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-5-carboxamide. The study of the antimicrobial activity of the synthesized compounds was carried out on the basis of the Laboratory of Biochemistry of Microorganisms and Nutrient Media of the Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine (Kharkiv) under the supervision of Candidate of biological sciences, senior researcher T. P. Osolodchenko. The antimicrobial activity of the obtained compounds was evaluated in accordance with WHO recommendations on test strains of *Staphylococcus aureus* ATCC 25923 (S.a.), *Pseudomonas aeruginosa* ATCC 27853 (P.a.), *Bacillus subtilis* ATCC 6633 (B.s.).

Results. We have prepared N-(benzyl)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides as the close analogues of the 4-methyl substituted molecules. The series of 4-oxo derivatives turned out to be active *in vitro* against the strains of *S. aureus* and *B. subtilis* and only moderately active against *P. aeruginosa* strain (Table 1). The activity of 4-methyl analogues was higher; especially such substituents in the amide as 4-flouro or 4-methoxy were beneficial for the activity against *B. subtilis* and *P. aeruginosa* strains. Despite the high synthetic availability of the 4-oxo derivatives it seems that the change of this group with methyl, which can be done by chlorination with subsequent Suzuki coupling [7], can be a good structural modification to increase antimicrobial activity. It is especially important that 4-methyl derivatives are active against *P. aeruginosa,* which is one that can form highly antibiotic resistant strains [8] and the new compound that can inhibit the growth of these bacteria are always interesting.

Table 1. The results of in vitro antimicrobial activity screening

The average value of growth inhibition zone, mm (agar well diffusion method), experiments repeated 3 times						
						
	B.s.	P.a.	S.a.	B.s.	P.a.	S.a.
R = H	21	20	17	24	20	24
R = F	25	23	21	22	20	21
R =OCH_3	26	24	20	23	20	22



a) b)

Fig. 1 3D visualization of the interaction of 4-oxo- (a) and 4-methyl- (b) ligands with the amino acid residues of the active site of the TrmD inhibitor P. aeruginosa

For studied 4-oxo derivatives, a high degree of affinity to the site of the TrmD inhibitor was calculated, which exceeds the affinity of the native reference inhibitor : -9.4, -10.1, -10.4 kcal/mol relative to -8.2 kcal/mol, respectively. However, that despite the good values of the scoring functions, the conformational analysis of the ligands' poses in the active site revealed the ability of 4-oxo compounds for only partial inhibition of TrmD of *P. aeruginosa*. None of the derivatives interact with glutamic acid residues Gly145, 146 and do not interact with glutamine Gln95, which in the experiment is the site of fixation of the methionine fragment of S-adenosylmethionine (SAM) - the cofactor of the TrmD enzyme. This indicates that the investigated ligands cannot be deeply located in the cavity of the active site and enter into competition with SAM, exhibiting inhibitory activity against bacterial TrmD. According to our studies for 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid benzyl amides oxo-group does not interact with any amino acid of the active site of TrmD (fig. 1a), while methyl group at position 4 forms the hydrophobic bond with pyrrolidne cycle of proline (Pro94), which is one of the amino acids of the active site (fig. 1b). It is obvious that introduction of methyl group at position 4 is beneficial for TrmD inhibitory properties and it was confirmed by the docking studies. On the other hand it showed the effectiveness of the docking methodology in TrmD inhibitors design, which can be a first virtual screening strep of antimicrobial drug discovery.

Conclusion. The results of *in vitro* studies for the novel derivatives of *N*-benzyl-5-dimethylthieno[2,3-*d*]pyrimidine-6-carboxamide revealed that 4-methyl substituted derivatives are more active rather than their 4-oxo analogues against *P. aeruginosa* and *B. subtilis* strains, On the other hand 4-oxo derivatives were more active against *S. aureus. In silico* studies confirmed the suggested mechanism of antimicrobial activity *via* inhibition of bacterial TrmD. The importance of the alkyl substituent at position 4 of theno[2,3-*d*]pyrimidine fragment introduction was showed to be reasonable as it played the role of the additional center for binding into the active site of TrmD. The obtained results are rather inspiring because they confirms the assumption that the small molecules with benzylic carboxamide thieno[2,3-d]pyrimidine core can be effective TrmD inhibitors and have prospects of becoming of the new group of innovative antibiotics with possible bactericidal activity. The more studies for sure are required in the directions of synthetic methodology improvement in order to make introduction of variety of alkyl substituents at position 4 of thieno[2,3-d]pyrimidine more effective as well as the profound studies of the already obtained compounds for the effective lead-search are needed. It is also rational to try more differently substituted benzyl amines for the search of the leader. The more microbiological experiments are required for the similar compounds to be tested against the antibiotic resistant strains of *P. aeruginosa* for the development of novel antibiotics with the mechanism of action based on inhibition of bacterial TrmDs.

Funding. This 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" (State registration number: 0121U109472; Order of the Ministry of Health of Ukraine of November 17, 2020, № 2651).

Acknowledgments. The authors acknowledge Enamine Ltd. for the measurement of ^{1}H, ^{13}C NMR and LCMS spectra of the obtained substances.

References. [1] Payne D. J., Gwynn M. N., Holmes D. J., Pompliano D. L. Nat. Rev. Drug Discov. 2007, 6, 29–40. [2] Butler M. S., Blaskovich M. A., Cooper M. A. J. Antibiot. 2013, 66, 571–591. [3] Tommasi R., Brown D., Walkup G. et al. Nat. Rev. Drug Discov. 2015, 14, 529–542. [4] Brown DG, Wobst HJ. J. Med. Chem. 2021, 64, 2312-2338. [5] Malasala S, Polomoni A, Ahmad MdN et al. J. Molec. Struct., 2021; 1234, 130168. [6] Zhong W, Pasunooti KK, Balamkundu S et al. J Med Chem. 2019, 62, 7788-7805. [7] Vlasov S, Krolenko K, Severina H, Vlasova O, Borysov O, Shynkarenko P, Vlasov V, Georgiyants V. J. Appl. Pharm. Sci., 2023, 13, 105–113. [8] Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Biotechnol Adv. 2019, 37, 177-192.