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RESPONSE OF MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* STRAINS TO ACTION OF SYNTHETIC AMINO ACIDS AND PEPTIDES

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The influence of synthetic amino acids and peptides on the growth of *Pseudomonas aeruginosa* multidrug resistant strains isolated from soil has been studied. All investigated strains were sensitive to (*S*)-β-[4-allyl-3-(furan-2-yl)-5--thioxo-1,2,4-triasol-1-yl]-α-alanine, (*S*)-β-[4-allyl-3-(2'-chlorophenyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, dipeptide N-formyl-methionyl-(*S*)-β-[4-allyl-3-(2'--chlorophenyl)-5-thioxo-1,2,4-triasol-1-yl]-α-alanine and tripeptide alanyl-glycyl-(*S*)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triasol-1-yl]-α-alanine. Dipeptide alanyl-(*S*)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine demonstrates the strongest effect on the growth of *P. aeruginosa* strains 9311 (resistant to beta-lactams) and 9211 (resistant to beta-lactams and chloramphenicol), but has no influence on the *P. aeruginosa* 5249 (resistant to all antibiotics used in this study).

***Keywords*:** multidrug resistance, *P. aeruginosa,* peptide, antimicrobials.

Introduction. *Pseudomonas aeruginosa* are widely spread gram negative bacteria. *P. aeruginosa* strains are also well known as nosocomial infection. This infection is especially dangerous for patients having suppressed immune system as it affects almost all parts of the body [1]. It is well known that the use of antibiotics leads to selection and spread of antibiotic-resistant pathogens. The emergence of multidrug-resistant *P. aeruginosa* strains creates problems in the course of diseases treatment. There are several mechanisms of bacterial resistance to antibiotics, including existence of antibiotic modifying enzymes, mutation in targets, cell efflux systems [2]. Whatever the reason is, there is a strong necessity of searching for new drugs able to overcome the problem.

Currently non-protein amino acids and peptides based thereon are widely used in biotechnology and pharmacology. Peptides composed of non-protein α-amino acids occupy a special place among active compounds. The design of many modern antibacterial, antiviral, antitumor and other drugs is based on the property of non-protein amino acids and peptides either to inhibit or enhance the activity of cell targets. Short peptides synthesized from non-protein amino acids are promising alternatives to small molecule drugs. These compounds have several advantages: high activity, high specificity, targeting capabilities, minimal drug-drug interactions, low toxicity, etc. Antibacterials

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based on synthetic peptides along with antibiotics could be a good support in battle against multidrug-resistant pathogens.

In this paper the data on the ability of new synthetic short peptides to inhibit the growth of antibiotic resistant *P. aeruginosa* strains isolated from soil are presented.

Materials and Methods. In this study (*S*)-β-[4-allyl-3-(furan-2-yl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine (**I**), alanyl-glycyl-(*S*)-β-[4-allyl-3-pyridin-3'-yl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine (**II**), alanyl-glycyl-(*S*)-β-[4-allyl-3-pyridin-4'-yl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine (**III**), alanyl-(*S*)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine (**IV**), N-formyl-methionyl-(*S*)-β-[4-allyl-3-(2'-chlorophenyl)-5-thioxo--1,2,4-triazol-1-yl]-α-alanine (**V**), N-formyl-methionyl-(*S*)-β-[4-propyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine (**VI**), (*S*)-β-[4-allyl-3-(2'-chlorophenyl)-5-thioxo-1,2,4-triazol-1-yl]--α-alanine (**VII**), (*S*)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triasol-1-yl]-α-alanine (**VIII**) are used. All synthetic amino acids and peptides have been synthesized at the SPC "Armbiotechnology" NAS RA and YSU [3, 4].

Antibiotics: Ampicillin, 50 *u*/*mL* (Ap), Cefixime, 50 *u*/*mL* (Cef), Amoxicillin, 50 *u*/*mL* (Amox), Kanamicin, 50 *u*/*mL* (Km), Chloramphenocol, 10 *u*/*mL* (Cm) and Aaugmentin (amoxicillin with clavulanic acid), 50 *u*/*mL* (Aug).

Media: LB and LA media were used for the growth of *P. aeruginosa* strains.

Strains: The following *P. aeruginosa* strains were used: strain 9211 – Ap^r, Cef^r, Cm^r, $Amox^r$, Aug^s, Km^s; strain 9311 – Ap^r, Cef^r, $Amox^r$, Aug^r, Km^s, Cm^s; strain 5249 – Ap^r, Cef^r, $Amox^r$, Aug^r, Cm^r, Km^r. The strains studied were taken from MDC of SPC "Armbiotechnology" NAS RA. All strains are of soil origin.

Antibacterial Activity Assay. Antibacterial activity of synthetic peptides was determined by diffusion method in 0.7% LA. 0.1 *mL* of *P. aeruginosa* overnight culture was mixed with 2.5 *mL* of 0.7% LA at 45°*C* and placed on the top of 1.2% LA in Petri dishes. 3 *uL* of peptide solution (10 *mM*) was dropped on the soft layer. Plates were incubated 1–2 days at 37°*C*. After incubation, the diameter of transparent (clear) zone was recorded. The action of compounds on the multidrug-resistant *P. aeruginosa* strains was tested in the LA medium, containing beta-lactam antibiotics used in this study.

Results and Discussion. Antibiotic resistance of *P. aeruginosa* strains to beta-lactam antibiotics (Ap, Amox and Cef), aminoglicoside antibiotic Km and Cm has been studied. *P. aeruginosa* strains demonstrated a wide range of antibiotic resistance. Three multidrug-resistant *P. aeruginosa* strains were selected for further investigation: strain 9211 – Ap^r, Cef^r, Cm^r, $Amox^r$, Aug^s, Kms; strain 9311 – Ap^r, Cef^r, $Amox^r$, Aug^r, Km^s, Cm^s; strain 5249 – Ap^r, Cef^r, $Amox^r$, Aug^r, Cm^r, Km^r. The mechanisms of investigated multidrug-resistant *P. aeuginosa* strains have not been examined yet.

Optically active heterocycle substituted non-α-amino acids and peptides based thereon have been screened for their ability to inhibit the growth of multidrug-resistant *P. aeruginosa* strains. The influence of compounds on the multidrug-resistant *P. areuginosa* strains was tested in the LA medium, containing beta-lactam antibiotics used in this study. The data on responses of *P. aeruginosa* strains 9211, 9311 and 5249 to the action of synthetic amino acids and peptides are presented in Table.

More than 40 heterocycle substituted amino acids and peptides based thereon have been used for screening in order to reveal antibacterial compounds able to influence multidrug-resistant *P. aeuginosa* strains growth. Results concerning compounds inhibiting the growth of these strains are presented in this paper.

All investigated strains appeared to be sensitive to **I**, **III**, **V** and **VII**, but resistant to aminoacid **VIII** (data not presented).

*The influence of synthetic amino acids and peptides on the growth of P. aeuginosa strains 9211 (**1**), 9311 (**2**), 5249 (**3**)*

Compound (10 *mM*)	Diameter of clear zone (growth inhibition), *mm*														
	LA			LA, Ap			LA, Amox			LA, Cef			LA, Aug		
	1	***2***	***3***	***1***	***2***	***3***	***1***	***2***	***3***	***1***	***2***	***3***	***1***	***2***	***3***
I. (*S*)-β-[4-allyl-3-(furan-2-yl)-5--thioxo-1,2,4-triazol-1-yl]-α-alanine	3	5	5	5	5	5	5	3	5	3	6	2	–	3	5
II. alanyl-glycyl-(*S*)-β-[4-allyl-3--pyridin-3'-yl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine	–	5	–	4	4	–	5	4	–	2	4	–	–	4	–
III. alanyl-glycyl-(*S*)-β-[4-allyl-3--pyridin-4'-yl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine	5	5	5	4	5	3	4	6	3	2	6	2	–	6	3
IV. alanyl-(*S*)-β-[4-allyl-3-(pyridin-4'--yl)-5-thioxo-1,2,4-triazol-1-yl]--α-alanine	12	14	–	10	9	–	10	14	–	10	8	–	–	14	–
V. N-formyl-methionyl-(*S*)-β-[4-allyl--3-(2'-chlorophenyl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine	5	10	3	3	8	3	3	8	3	2	8	3	–	8	3
VI. N-formyl-methionyl-(*S*)-β-[4--propyl-3-butyl-5-thioxo-1,2,4--triazol-1-yl]-α-alanine	–	3	4	3	3	4	4	3	4	3	2	4	–	4	4
VII. (*S*)-β-[4-allyl-3-(2'-chlorophenyl)--5-thioxo-1,2,4- triazol-1-yl]--α-alanine	6	3	2	3	3	2	3	2	2	6	3	2	–	2	2

P. aeruginosa 9311 demonstrated sensitivity to all the compounds listed in Table.

P. aeuginosa 5249 was resistant to **II** and **IV**.

P. aeruginosa 9211 was sensitive to augmentin, but resistant to amoxicillin. Augmentin consists of amoxicillin and clavulonic acid, which is known as beta-lactamase inhibitor [5]. Thus, it may be supposed that *P. aeruginosa* 9211 posses beta-lactamase similar to TEM1 beta-lactamase. Tripeptide **III** and dipeptide **VI** inhibited the growth of *P. aeruginosa* 9211 in the medium containing ampicillin or amoxicillin. However, in the antibiotic free medium these peptides did not have influence on bacterial growth. Taking into account these results it may be assumed that these peptides are inhibitors of bacterial beta-lactamase.

According to the data, **IV** demonstrate the strongest effect on the growth of strains 9311 (resistant to beta-lactams) and 9211 (resistant to beta-lactams and chloramphenicol), but does not have influence on strain 5249 (resistant to all antibiotics used in this study).

Thus, multidrug resistance of *P. aeuginosa* strains has been overcome by using **I–VII** synthetic amino acids and peptides as antimicrobials.

Previously it was shown that alanyl-glycyl-(*S*)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triasol-1-yl]-α-alanine also had the ability to inhibit collagenase (metallo-protease) activity (IC50 = 11.5 *mM*) [6]. It is known that *P. aeruginosa* proteases facilitate inflammation (excessive tissue destruction) in wounds by activating host matrix metalloproteases [7]. Further investigation is necessary to understand, if this tripeptide can be useful in wounds treatment.

Thus multidrug-resistant *P. aeruginosa* strains respond to the action of synthetic peptides differently possibly because of the different mechanisms of antibiotic resistance. Antibacterial properties depend on both the growth conditions and peptide structure.

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