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Chemistry

SYNTHESIS OF DIPEPTIDES AND *IN VITRO* EVALUATION OF ANTIFUNGAL ACTIVITY

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N-Tert-butoxycarbonylglycyl-(*S*)-β-imidazolyl-α-alanine and N-tert-butoxycarbonylglycyl-(*S*)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4-triazol-1-yl]-α--alanine dipeptides, previously not described in the literature, were synthesized using the activated ester method for classical peptide synthesis. The structures of these synthesized dipeptides were confirmed through NMR spectroscopic analysis. The antifungal activity of initial non-protein amino acids and synthesized dipeptides was studied by choosing wide spread pathogenic and conditionally pathogenic fungal strains as test fungi: *Aspergillus versicolor* 12134, *A. flavus* 10567, *A. candidus* 10711, *Penicillium chrysogenum* 8190, *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *Alternaria alternata* 8126, *Ulocladium botrytis* 12027, *Aureobasidium pullulans* 8269. The antifungal activity of the initial (*S*)-β--imidazolyl-α-alanine amino acid is notable against the *P. aurantiogriseum* 12053 and *U. botrytis* 12027 fungal strains.

N-Tert-butoxycarbonylglycyl-(*S*)-β-imidazolyl-α-alanine dipeptide demonstrates a strong inhibitory effect specifically on the *P. aurantiogriseum* 12053 strain, with a similar level of inhibition observed at a concentration of 0.6 *mL* (0.331 *mmol*/*mL*). The initial amino acid exhibited comparable inhibition at a higher volume of 0.9 *mL* (0.495 *mmol*/*mL*).

In contrast, the (*S*)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine amino acid effectively suppresses the growth of multiple strains, including *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *U. botrytis* 12027, and *A. pullulans* 8269, when tested at 0.9 *mL* (0.495 *mmol*/*mL*). The corresponding N-tert-butoxycarbonylglycyl-(*S*)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine dipeptide also shows significant inhibitory effects similar to those of (*S*)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine.

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Introduction. Drug-resistant infections are increasingly posing a significant public health threat, as more pathogens are developing resistance to widely used antibiotics. This situation underscores the urgent need for new and effective antimicrobial agents to combat these infections. The rise of multidrug-resistant bacterial strains is closely associated with the overuse and misuse of antibiotics, which fosters the selection of resistant bacteria. Moreover, the pace of development new antibiotics has been sluggish, and there are currently few new antibiotics in the pipeline. Thus, the pressing need for innovative antimicrobial agents to tackle drug-resistant infections is more critical than ever [1–4].

Antimicrobial peptides (AMPs) have emerged as a promising solution to combat drug-resistant infections [5, 6]. These peptides exhibit a broad spectrum of activity against bacteria, fungi, and viruses, and they are less prone to resistance development compared to traditional antibiotics. Produced by a diverse range of organisms, including plants, animals, and microorganisms, AMPs are integral to the innate immune system's defense mechanisms. Typically small and cationic, AMPs interact with negatively charged membranes of microorganisms, causing membrane disruption and cell death. Recent advancements in AMP research have led to the discovery and characterization of numerous peptides, revolutionizing the field and offering new avenues for drug development. These peptide-based drugs have demonstrated significant potential in treating bacterial and fungal infections, as well as certain viral infections, including HIV. This progress provides renewed hope in the ongoing battle against resistant pathogens [6–14].

According to 2016 data, there are over 1000 peptides with broad-spectrum antifungal activity, including both natural and synthetic variants. Antifungal peptides can be classified based on their structure, mode of action, and origin, which includes natural, semi-synthetic, and synthetic peptides [15, 16].

In light of the structural characteristics of antifungal peptides and the well-defined efficacy ranges of azole compounds, we aimed to synthesize two specific dipeptides: N-tert-butoxycarbonyl-glycyl-(*S*)-β-(imidazolyl)-α-alanine and N-tert-butoxycar-bonylglycyl-(*S*)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4-triazol-1--yl]-α-alanine. These synthesized compounds have been evaluated *in vitro* for their antifungal activity.

We have selected a range of fungal strains for testing, including *Aspergillus versicolor* 12134, *Aspergillus flavus* 10567, *Aspergillus candidus* 10711, *Alternaria alternata* 8126, *Ulocladium botrytis* 12027, and *Aureobasidium pullulans* 8269, *Penicillium chrysogenum* 8190, *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *Alternaria alternata* 8126, *U. botrytis* 12027, *and Aureobasidium pullulans* 8269.

These strains include both pathogenic and opportunistic fungi affecting humans and animals. For instance, *Aspergillus niger* is commonly associated with otomycosis, while *A. flavus* can infect the paranasal sinuses and produce aflatoxins, which have mutagenic, carcinogenic, and allergic properties [17–19].

Materials and Methods.

Materials. All reagents were obtained from commercial sources and used without further purification. Thin-layer chromatography (TLC) was carried out on Merck aluminium foil backed sheets pre-coated with 0.2 *mm* Kielselgel 60 F254.

Melting points (*mp*) were determined by Electrothermal. ^{1}H NMR spectra were recorded on Varian Mercury 30000 300 *MHz* spectrometer using TMS as internal standard. The NMR spectra were calibrated by solvent at 7.27 ($CDCl_3$), 3.31 (CD_3OD), 4.79 (D_2O), 2.50 ($(CD_3)_2SO$) for ^{1}H.

Methods. Synthesis of N-Tert-Butoxycarbonylglycine. 0.66 *g* (0.0066 *mol*) of glycine was added to 0.5 *M* aqueous solution of sodium hydroxide (0.7 *mL*) in a flat-bottomed flask. To this mixture was added 0.37 *g* (0.0044 *mol*) of sodium bicarbonate dissolved in 5 *mL* of water. The mixture was stirred with a magnetic stirrer at room temperature until glycine was fully dissolved. Then, 1.44 *g* (0.0066 *mol*) of di-tert-butylpyrocarbonate, dissolved in 6.6 *mL* of isopropanol, was added. The reaction was stirred at room temperature for 2 *h*.

The progress of the reaction was monitored by TLC, which indicated that the reaction was complete after 2 *h*. The reaction mixture was then diluted to 50 *mL* and the excess reagent was extracted with ethyl acetate (2×20 *mL*). Subsequently, 6 *mL* of 10% citric acid solution was added to the aqueous layer, and re-extraction was performed with ethyl acetate (2×20 *mL*). The combined organic extracts were washed with saturated aqueous sodium chloride solution and then dried over anhydrous sodium sulfate.

After decanting, the organic solvents were removed under vacuum at 50–60°C. The resulting product was recrystallized from an ethyl acetate–hexane mixture (1:3), filtered, and dried under vacuum at 50–60°C. Obtaining of N-tert-butyloxycarbonylglycine was carried out by the method of [20]. TLC analysis was in the chloroform–ethyl acetate–methanol system (2:4:1). Yield of N-tert-butyloxycarbonylglycine – 70%, *mp* – 89–92°C.

Synthesis of N-Tert-Butoxycarbonylglycyl-N-Hydroxysuccinimide Ester. 0.0046 *mol* of N-tert-butoxycarbonylglycine and 0.579 *g* (0.005 *mol*) of N-hydroxysuccinimide were placed into a flat-bottomed flask. The compounds were dissolved in a mixture of 5.8 *mL* of dioxane and 1.56 *mL* of methylene chloride, using a magnetic stirrer at room temperature. To prevent moisture ingress, a calcium chloride tube was attached to the flask. Once dissolution was complete, the reaction mixture was cooled to 0°C. 1.004 *g* (0.00487 *mol*) of dicyclohexylcarbodiimide, dissolved in 1.5 *mL* of dioxane, was added in small portions over 15 *min* with 5-minute intervals. During this addition, the reaction mixture turned from transparent to white, indicating the formation of dicyclohexylurea and progress of the reaction. The mixture was stirred for an additional hour at –2 to 0°C, after which the cooling was removed, and stirring continued for another hour at room temperature. The reaction progress was monitored by TLC using a chloroform–methanol–ethyl acetate (4:2:1) solvent system. Upon completion, the precipitated dicyclohexylurea was filtered out, and the resulting solution was immediately used for the subsequent synthesis of the dipeptide.

Synthesis of Dipeptides. 0.6 *mmol* of the non-protein amino acid was placed into a flat-bottomed flask with a magnetic stirrer and heated to 60°C. It was dissolved in 1.63 *mL* of 0.5 *M* NaOH, and then 0.26 *mmol* of sodium bicarbonate was added. Subsequently, 0.816 *mmol* of N-tert-butoxycarbonyl-(*S*)-alanine-N-hydroxysuccinimide ester, dissolved in 2 *mL* of dioxane, was added at room temperature. The reaction mixture was stirred for 3 *h* at room temperature and then transferred to

a refrigerator at 5°C overnight. The next day, 5 *mL* of ethyl acetate, 3 *mL* of 10% citric acid solution, and 0.2 *g* of sodium chloride were added to the mixture and stirred for 15 *min*. The organic layer was separated, dried over sodium sulfate, and the solvent was removed under vacuum at 50°C. The residue was recrystallized from an ethyl acetate–hexane mixture (1:3). The reaction progress was monitored by TLC using chloroform as the solvent system.

N-Tert-Butoxycarbonylglycyl-(S)-β-Imidazolyl-α-Alanine (6a). ^{1}H NMR spectrum, *δ*, *ppm*, *Hz*: 1.42 s (3H, 9H), 3.56 dd (*J* = 16.8, 5.9, CH_2NH), 3.63 dd (*J* = 16.8, 5.9, CH_2NH), 4.28–4.45 m (3H, CH_2CH), 6.80 br. (1H, NCH), 6.85 br.t (1H, *J* = 5.9, $NHCH_2$), 7.00 br. (1H, NCH), 7.55 br. (1H, NCHN), 7.62 br.d (1H, *J* = 6.2, NHCH).

13C: 46.5 (CH); 46.9 (NCH_2); 49.2 (NCH_2); 51.9 (NCH); 65.9 (OCH_2); 117.3 (CH); 119.22 (CH); 119.24 (CH); 121.8; 122.9 (CH); 124.9 (CH); 125.0 (CH); 126.5 (2.CH); 126.91 (CH); 126.94 (CH); 130.9 (CH); 135.2 (CH); 140.4; 140.5; 143.3, 143.5; 147.3; 148.4 (CH); 150.8 (CH); 155.3; 167.9; 170.4.

N-Tert-butoxycarbonylglycyl-(S)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine (6b). ^{1}H NMR spectrum, *δ*, *ppm*, *Hz*: 1.42 s (9H, Me_3), 1.67–1.77 m (2H, $CH_2CH_2CH_2OH$) 2.49 br.t (2H, *J* = 7.3, $CH_2CH_2CH_2OH$), 3.45 dd (*J* = 16.5, 5.7, CH_2NH), 3.63 dd (*J* = 16.5, 5.7, CH_2NH), 3.41 t (2H, *J* = 6.0, CH_2OH), 4.30–4.64 m (3H, CH_2CH), 6.50 br.d (1H, *J* = 8.0, NCH), 7.33–7.39 m (2H), 7.48–7.60 m (3H, C_6H_5).

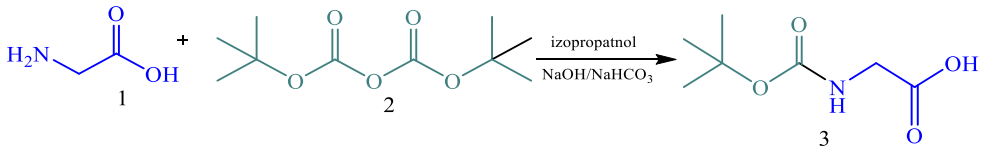
13C: 21.7 (CH_2); 28.0 (Me_3); 28.3 (CH_2); 49.0 (NCH_2); 51.6 (CH); 59.2 (OCH_2); 77.9 (CMe_3); 119.22 (CH); 119.24 (CH); 127.8 (2.CH); 128.9 (2.CH); 128.9; 134.1; 150.3; 154.5, 167.7; 170.8.

***Biological Activity*.** Investigation of untifungal activity. As objects of study *A. versicolor* 12134, *A. flavus* 10567, *A. candidus* 10711, *P. chrysogenum* 8190, *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *A. alternata* 8126, *U. botrytis* 12027, *A. pullulans* 8269 from the National Culture Collection of microorganisms of Armenia fungal strains were selected. The effect of the synthesized compounds on the growth activity of the above mentioned fungi was investigated. In order to investigate the antifungal activity, the investigated compounds were dissolved in dimethylsulfoxide by obtaining 0.1 *M* solutions, which at volumes of 0.3 *mL*, 0.6 *mL*, and 0.9 *mL* were added to the Czapek medium in the amount of 90 *mL* each, with the following composition: sucrose – 30.0 *mg*, sodium nitrate – 2.0 *mg*, potassium hydrogen phosphate – 1.0 *mg*, magnesium sulfate – 0.5 *mg*, potassium chloride – 0.5 *mg*, iron sulfate – 0.01 *mg*, then the obtained mass was evenly distributed in 9 Petri dishes, three strains in each dish and three replicates. The dishes were incubated at a temperature of 28°C for 5–7 days. The experiment was repeated three times. Upon completion of the test, the dishes were examined with the naked eye and under a binocular magnifier, after which the antifungal activity of various concentrations of the samples was assessed by the intensity of growth and sporulation of fungi. The growth of fungi without the addition of the synthesized compounds and the addition of the corresponding milliliters of DMSO 0.3 *mL*, 0.6 *mL*, 0.9 *mL* served as the control. The results of the study were compared with the control and the intensity of the growth of the fungi was observed by eye and a magnifying glass.

Results and Discussion.

***Chemistry*.** For the synthesis of dipeptides containing non-protein amino acids, the process involves several key steps.

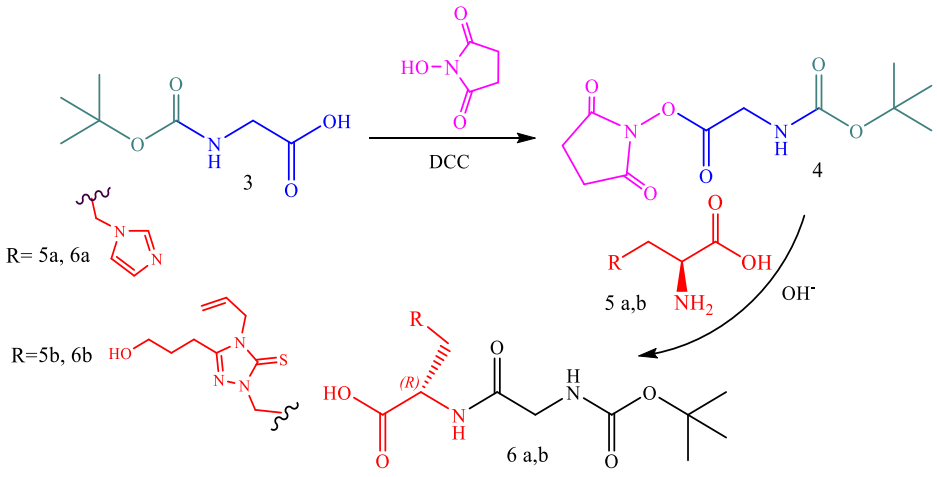
Synthesis of N-Tert-Butoxycarbonylglycine (N-t-boc-gly). Initially, N-tert--butoxycarbonyl-glycine (3) (Scheme 1) is synthesized.



Scheme 1.

Conversion to Succinimide Ester. N-t-boc-gly (3) is then converted to the succinimide ester form. This reaction utilizes N-hydroxysuccinimide ester as an activator (HOSu) and dicyclohexylcarbodiimide (DCC) as a water-splitting agent, with dioxane and methylene chloride serving as solvents. Following this reaction, stable N-tert-butoxycarbonyl-(*S*)-alanyl-N-hydroxysuccinimide ester (4) is obtained according to a previously established method (Scheme 2).

Condensation Reaction. In the final stage, the activated ester of N-t-boc-gly (3) reacts with non-protein amino acids (5 a,b) in the presence of 0.5 *N* aqueous NaOH in a dioxane medium at room temperature. This condensation reaction yields the dipeptides N-tert-butoxycarbonylglycyl-(*S*)-β-imidazolyl-α-alanine (6a) and N-tert-butoxycarbonylglycyl-(*S*)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine (6b) (Scheme 2).



Scheme 2.

***Biological Activity, Antifungal Activity*.** The results are shown in Table. Upon addition of initial amino acids and dipeptides, test-fungi in comparison with the control showed evident inhibition of sporulation and mycelium growth, which enhanced with the increase in the concentration of the studied substances in the nutrient medium (see Table).

The antifungal activity of the initial 5a amino acid is notable against the *P. aurantiogriseum* 12053 and *U. botrytis* 12027 fungal strains. The 6a dipeptide containing this amino acid demonstrates a strong inhibitory effect specifically on the *P. aurantiogriseum* 12053 strain, with a similar level of inhibition observed at a concentration of 0.6 *mL* (0.331 *mmol*/*mL*). The initial amino acid exhibited comparable inhibition at a higher volume of 0.9 *mL* (0.495 *mmol*/*mL*).

Assessment of the antifungal activity

Amino acids and peptides	*mL*	*mmol*/*mL*	Names and numbers of strains according to MDC								
			A. versicolor 12134	*A. flavus* 10567	*A. candidus* 10711	*P. chrysogenum* 8190	*P. aurantiogriseum* 12053	*P. funiculosum* 8258	*A. alternata* 8126	*U. botrytis* 12027	*A. pullulans* 8269
(*S*)-β-Imidazolyl-α-alanine (5a)	0.3	0.166	+	+	+	++	+	++	–	+	–
	0.6	0.331	+	++	++	++	++	++	++	++	–
	0.9	0.495	+	++	++	++	+++	++	++	+++	++
N-Tert-butoxycarbonylglycyl-(*S*)-β--imidazolyl-α-alanine (6a)	0.3	0.166	+	+	+	+	++	++	+	+	–
	0.6	0.331	+	++	++	++	+++	++	+	+	–
	0.9	0.495	++	++	++	++	+++	++	++	++	–
(*S*)-[4-allyl-3-(3'-hydroxypropyl)-5--thioxo-1,2,4-triazol-1-yl]-α-alaninie (5b)	0.3	0.166	+	–	–	+	+	+	–	+	+
	0.6	0.331	+	+	+	+	++	++	+	++	++
	0.9	0.495	+	++	+	++	+++	++	+	+++	+++
N-Tert-butoxycarbonylglycyl-(*S*)-[4--allyl-3-(3'-hydroxypropyl)-5-thioxo--1,2,4-triazol-1-yl]-α-alaninie (6b)	0.3	0.166	+	+	+	+	+	+	–	+	+
	0.6	0.331	+	+	+	+	++	++	+	++	++
	0.9	0.495	+	++	++	+	+++	+++	+	+++	+++

Note: +++ – active suppression of mycelial growth and sporulation; ++ – moderate suppression of mycelial growth and sporulation; + – suppression of sporulation; − – lack of antifungal action.

In contrast, the 5b amino acid effectively suppresses the growth of multiple strains, including *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *U. botrytis* 12027, and *A. pullulans* 8269, when tested at 0.9 *mL* (0.495 *mmol*/*mL*). The corresponding 6b dipeptide also shows significant inhibitory effects similar to those of 5b.

Overall, the derivatives of imidazole, triazole, and the dipeptides based on these compounds present promising potential as active agents in controlling various pathogenic and opportunistic fungal strains.

Conclusion. N-Tert-butoxycarbonylglycyl-(*S*)-β-imidazolyl-α-alanine and N-tert-butoxycarbonylglycyl-(*S*)-[4-allyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine dipeptides, previously not described in the literature, were synthesized using the activated ester method for classical peptide synthesis. The structures of these synthesized dipeptides were confirmed through NMR spectroscopic analysis.

The antifungal activity of the initial amino acids and their corresponding dipeptides was evaluated. The results demonstrated that both the non-protein amino acids and the synthesized peptides inhibited the growth of selected fungal strains with varying effectiveness. Notably, the compounds exhibited significant growth suppression of *P. chrysogenum* 8190, *P. aurantiogriseum* 12053, and *P. funiculosum* 8258.

While some fungal strains showed no growth inhibition at lower concentrations, increased amounts of the active substances resulted in observable inhibition zones. Overall, most of the tested compounds displayed pronounced antifungal activity, suggesting their potential as active agents in combating various pathogenic and opportunistic fungi.

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ԴԻՊԵՊՏԻԴՆԵՐԻ ՍԻՆԹԵԶ ԵՎ ՀԱԿԱՍՆԿԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ *IN VITRO* ԳՆԱՀԱՏՈՒՄ

Ակտիվացված էսթերների եղանակի կիրառմամբ իրականացվել է գրականության մեջ չնկարագրված N-t-բուտօքսիկարբոնիլգլիցիլ-(*S*)-β-իմիդոզիլ-α-ալանին և N-t-բուտօքսիկարբոնիլգլիցիլ-(*S*)-[4-ալիլ-3-(3'-հիդրօքսիպրոպիլ)-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանին դիպեպտիդների սինթեզ: Սինթեզված դիպեպտիդների կառուցվածքները հաստատվել են ՄՄՌ սպեկտրոդիտական անալիզի եղանակով:

Ուսումնասիրվել է ելային ոչպիտակուցային ամինաթթուների և սինթեզված դիպեպտիդների հակասնկային ակտիվությունը, սնկային տարբեր ախտածին և պայմանական ախտածին շտամների վրա՝ *Aspergillus versicolor* 12134, *A. flavus* 10567, *A. candidus* 10711, *Penicillium chrysogenum* 8190, *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *Alternaria alternata* 8126, *Ulocladium botrytis* 12027 և *Aureobasidium pullulans* 8269, որի արդյունքում ելային (*S*)-β-իմիդոզիլ-α-ալանին ամինաթթվի հակասնկային ազդեցությունը ցայտուն արտահայտվել է *Penicillium aurantiogriseum* 12053 և *Ulocladium*

botrytis 12027 սնկային շտամների վրա, այն պարունակող դիպեպտիդը բարձր արգելակիչ ազդեցություն է ցուցաբերել *P. aurantiogriseum* 12053 շտամի դեպքում՝ ակտիվությունը ընկճման նույն աստիճան ցուցաբերելով 0,6 *մլ* դիպեպտիդ պարունակող լուծույթի դեպքում, իսկ ելային ամինաթթուն նույն աստիճանի ընկճման է բերում 0,9 *մլ*-ի դեպքում:

(*S*)-[4-Ալիլ-3-(3'-հիդրօքսիպրոպիլ)-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանին ամինաթթուն առավել ցայտուն ընկճում է *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *U. botrytis* 12027, *Aureobasidium pullulans* 8269 սնկային շտամների աճը 0,9 *մլ* 0,495 *մմոլ/մլ* լուծույթի դեպքում, այն պարունակող դիպեպտիդը ցուցաբերել է գրեթե նույն ակտիվությունը նույն կոնցենտրացիայում:

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СИНТЕЗ ДИПЕПТИДОВ И ОЦЕНКА ПРОТИВОГРИБКОВОЙ АКТИВНОСТИ *IN VITRO*

N-Трет-бутоксикарбонилглицил-(*S*)-β-имидозил-α-аланин и N-трет-бутоксикарбонилглицил-(*S*)-[4-аллил-3-(3'-гидроксипропил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин – дипептиды, ранее не описанные в литературе, были синтезированы с использованием метода активированного эфира для классического синтеза пептидов. Структуры этих синтезированных дипептидов были подтверждены с помощью ЯМР-спектроскопического анализа. Антифунгальная активность исходных небелковых аминокислот и синтезированных дипептидов была изучена на различных патогенных и условно патогенных грибковых штаммах: *Aspergillus versicolor* 12134, *A. flavus* 10567, *A. candidus* 10711, *Penicillium chrysogenum* 8190, *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *Alternaria alternata* 8126, *Ulocladium botrytis* 12027 и *Aureobasidium pullulans* 8269. Антифунгальная активность (*S*)-β-имидозил-α-аланин аминокислоты особенно заметна против штаммов *P. aurantiogriseum* 12053 и *U. botrytis* 12027.

Дипептид N-трет-бутоксикарбонилглицил-(*S*)-β-имидозил-α-аланин демонстрирует сильный ингибирующий эффект в отношении штамма *Penicillium aurantiogriseum* 12053 с аналогичным уровнем ингибирования, наблюдаемым при концентрации 0,6 *мл* (0,331 *ммоль/мл*). Начальная аминокислота показала сопоставимое ингибирование при более высокой дозе 0,9 *мл* (0,495 *ммоль/мл*).

В отличие от этого, аминокислота (*S*)-[4-аллил-3-(3'-гидроксипропил)-5--тиоксо-1,2,4-триазол-1-ил]-α-аланин эффективно подавляет рост нескольких штаммов, включая *P. aurantiogriseum* 12053, *P. funiculosum* 8258, *U. botrytis* 12027 и *A. pullulans* 8269, при тестировании на 0,9 *мл* (0,495 *ммоль/мл*). Соответствующий дипептид N-трет-бутоксикарбонилглицил-(*S*)-[4-аллил-3-(3'-гидроксипропил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин также демонстрирует значительные ингибирующие эффекты, аналогичные тем, что наблюдаются для (*S*)-[4-аллил-3-(3'-гидроксипропил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланина.