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SYNTHESIS OF N-TERT-BUTYLOXYCARBONYLGLYCYL-(*S*)-β-[4-ALLYL-3-PROPYL-5-THIOXO-1,2,4-TRIAZOL-1-YL]-α-ALANINE DIPEPTIDE AND STUDY OF ITS ANTIFUNGAL EFFECT

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A new undescribed in the literature N-t-butyloxycarbonylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine dipeptide has been synthesized by the method of activated esters.

To obtain comparative data on antifungal effect, the synthesized dipeptide and the initial amino acids (*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and glycine were studied *in vitro*. 3 Fungi strains were selected for the study: *Aspergillus fumigatus* MDC 8403, *Aspergillus candidus* MDC 10556, *Penicillium chrysogenum* MDC 8281. They were provided by the Microbial Depository Center of the Scinetific and Production Center "Armbiotechnology" of NAS RA.

The study showed that the initial protein and non-protein amino acids did not exhibit antifungal effect, while the synthesized dipeptide suppressed the growth of the selected strains. Concentration-dependent subsequent experiments showed that with the increase in peptide concentration the inhibitory effect enhanced.

Figs. 3, references 6.

Despite the fact that peptides have been studied in various fields of chemistry and medicine for decades, interest in peptides remains topical today.

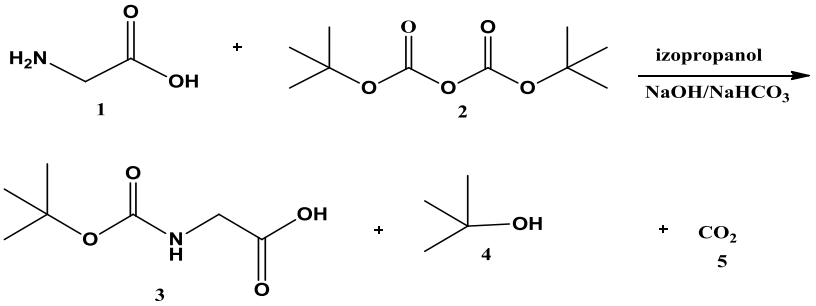
Peptides are pharmacologically active compounds used in the treatment of various diseases starting from diabetes to tumors [1-2].

It is worth mentioning that the number of peptides containing non-protein amino acids is high among both well-known drugs and new tested compounds [3].

However, there are almost no data on peptide-nature drugs with antifungal effect. Thus, taking into account the efficacy [4] of the triazole ring containing compounds among antifungal drugs, a new undescribed in the literature N-t-butyloxycarbonylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine dipeptide, which contains a triazole ring in its structure, has been synthesized by us. We have also studied the antifungal effect of the synthesized dipeptide.

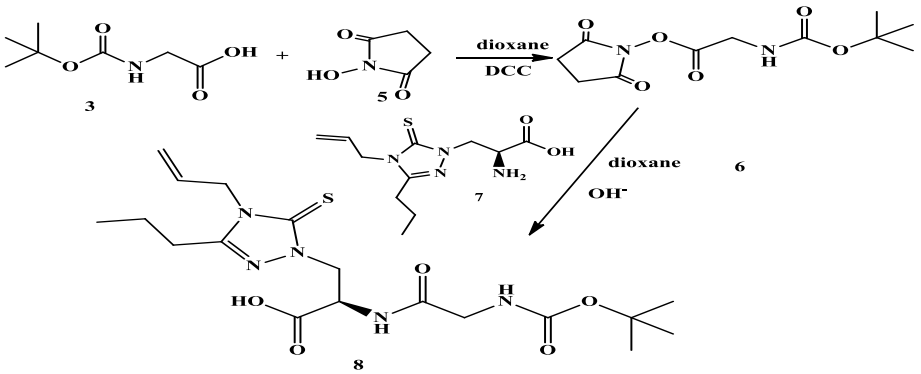
The peptide synthesis was carried out by the method of activated esters in a solution. The method is distinguished by its simplicity and possibility to obtain final products with good yields and high purity [5]. At the first stage, N-t-butyloxycarbonylglycine was obtained using di-tert-butyl pyrocarbonate in an alkaline aqueous-organic medium (Scheme 1).

Scheme 1



At the next stage, from N-t-butyloxycarbonylglycine (3) using dicyclohexylcarbodiimide, succinimide ester (6) was obtained, which by condensation with a non-protein amino acid in an alkaline aqueous-organic medium was converted to the corresponding dipeptide – N-t-butyloxycarbonylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine (8) (Scheme 2).

Scheme 2



The next stage related to study of the effects of the initial amino acids, including glycine, (*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and that of the synthesized dipeptide N-t-butyloxycarbonylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine.

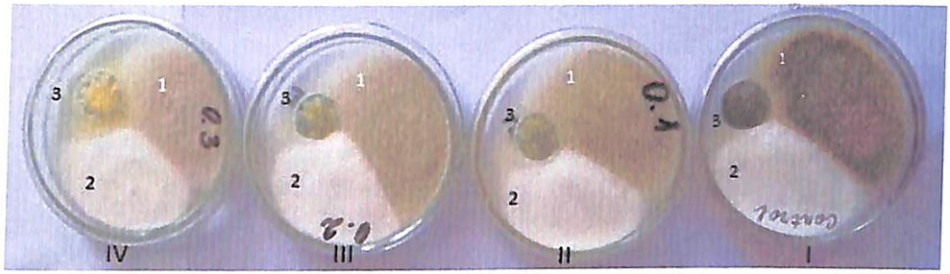


Fig. 1. I-control, II-0.1 ml, III-0.2 ml, IV-0.2 ml solution of N-t-butyloxycarbonylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine. 1- *Aspergillus fumigatus* MDC 8403, 2- *Aspergillus candidus* MDC 10556, 3- *Penicillium chrysogenum* MDC 8281.

The objects of the study were 3 strains of fungi from the National Culture Collection of Microorganisms of MDC: *Aspergillus fumigatus* MDC 8403, *Aspergillus candidus* MDC 10556, *Penicillium chrysogenum* MDC 8281.

The study has shown that the synthesized dipeptide suppresses the growth of strains, whereas the initial amino acids do not affect the growth of strains.

At the next stage, different concentrations of the synthesized dipeptide were tested, the results are shown in Fig. 1.

As follows from the Figure, when adding the studied dipeptide to the nutrient medium, suppression of sporulation and partial growth are observed in test fungi compared with the control, strengthening with the increase in concentration of dipeptide.

Experimental Part

^{1}H NMR spectra were recorded on Varian "Mercury 300VX" with an operating frequency of 300.08 *MHz* in a DMSO-D_6/CCl_4 1/3 solution using the method of double resonance. TLC was performed on "Silufol UV-254" plates in a mixture of chloroform-ethyl acetate-methanol (4:4:1), and the developer was chlorotoluidine. The elemental analysis was performed on an elemental CNS-O "Euro EA3000" analyzer.

HPLC analysis of the dipeptide was carried out on a "Waters 2695 Separations Module" liquid chromatographer (USA) with a "Waters 2487" ultraviolet detector using a stationary phase "AltimaC 18", 5 μm, 250×4.6 *mm*; elution was performed in an isocratic mode; as a mobile phase A: 0.15% TFA + H2O; B: 0.13% TFA + MeCN was used, the flow rate was 1 ml/min, detection was carried out at a wavelength of 210 *nm*, column temperature was 25°C, injection volume was 10 μl. Chemicals and eluents from "Sigma-Aldrich" were used with a purity of > 99.9% (gradient grade for HPLC).

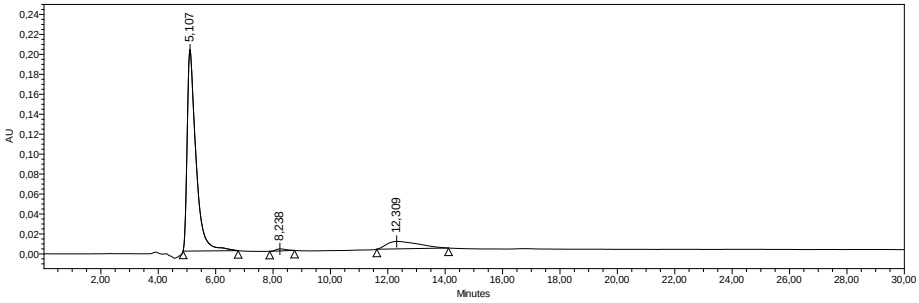
An optically pure non-protein amino acid was provided by the researchers of the Laboratory of Asymmetric Synthesis.

Obtaining of N-t-butyloxycarbonylglycine (3) was carried out by the method of **[6]**. TLC analysis was in the chloroform-ethyl acetate-methanol system – 2:4:1. Yield of product **3** – 70%, mp – 95-96^{o}C.

Synthesis of N-t-butyloxycarbonylglycyl-(S)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine dipeptide (8). 0.48 *g* (2.32 *mmol*) of dicyclohexyl-carbodiimide, previously dissolved in 32 *ml* of dioxane was added to a solution of 0.35 *g* (2 *mmol*) of N-t-butyloxy-carbonylglycine and 0.25 *g* (2.2 *mmol*) of N-hydroxysuccinimide in a mixture of 5.4 *ml* of dioxane and 2 *ml* of methylene chloride at 0°C. The reaction mixture was stirred for ~2 *hrs* at 0°C and left in the refrigerator overnight. TLC analysis [SiO_2, $CHCl_3$/ethyl acetate/CH_3OH (2:4:1), developer chlorotoluidine]. The residue formed was filtered off, the solvent was distilled off on a rotary evaporator, and the precipitate was crystallized from isopropyl alcohol. Yield 0.38 *g* (71%). The obtained succinimide ester was used at the next stage for the synthesis of N-t butyloxycarbonyl tripeptide.

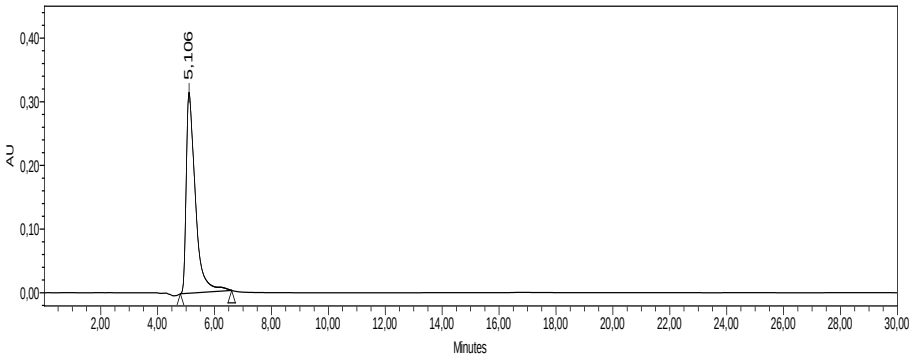
In a flat-bottomed flask with a magnetic stirrer, 0.381 *g* (1.41 *mmol*) of (*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, 2.8 *ml* (1.4 *mmol*) of 0.5M sodium hydroxide solution (NaOH) and 0.089 *g* (1.07 *mmol*) of baking soda ($NaHCO_3$) were mixed. At room temperature, 0.365 *g* (1.34 *mmol*) of N-t-butyloxycarbonylglycine succinimide in 4 ml of dioxane was added, the reaction mixture was stirred for 6 *hrs* and left overnight. The next day, 10 *ml* of ethyl acetate and 3 *ml* of 10% citric acid were added to the contents of the flask. After vigorous stirring, the organic layer was separated, and the aqueous layer was twice extracted with ethyl acetate (6 *ml* each). The organic layer was dried with anhydrous sodium sulfate, then the solvent was evaporated to dryness. The viscous residue was dissolved by heating in a mixture of 10 *ml* of hexane and 3 *ml* of ethyl acetate and left overnight. The white precipitate was filtered off on a nutsche filter, washed successively with 2 *ml* of ethyl acetate, after which the peptide was dried at a temperature of 65°C. TLC analysis [SiO_2, $CHCl_3$/ethyl acetate/CH_3OH (2:4:1), developer – chlorotoluidine]. Product yield 0.4 g (70%). Found, %: C 50.11; H 6.75; N 16.31. $C_{18}H_{29}N_5O_6S$. Calculated, %: C 50.57; H 6.84; N 16.38. ^{1}H NMR Spectrum, δ, ppm Hz: 1.00 (3H, t, J=7.4, $\underline{CH_3}$), 1.41 (9H, s, Me_3), 1.73 (2H, sx, J=7.4, $\underline{CH_2}CH_3$), 2.57 (2H, t, J=7.4, $\underline{CH_2}C_2H_5$), 3.54 (2H, br.d, J=5.5, $\underline{CH_2}NH$), 4.30 (1H, dd, J=13.6, 8.3, $\underline{CH_2}CH$), 4.52 (1H, dd, J=13.6, 4.8, $\underline{CH_2}CH$), 4.62 (2H, dt, J=5.1, 1.5, CH_2All), 4.72 (1H, td, J=8.3, 4.8, $\underline{CH}CH_2$), 5.08 (1H, dtd, J=17.2, 1.5, 1.0, $=CH_2$), 5.19 (1H, dtd, J=10.5 1.5, 1.0, $=CH_2$), 5.86 (1H, ddt, J=17.2 10.5, 5.1, =CH), 6.28 (1H, br.t, J=5.5, $NHCH_2$), 7.79 (1H, br.d, J=8.3, NHCH).

The chemical purity of the synthesized dipeptide was also studied by HPLC. The chromatograms are shown below in Figures 3, 4.



Name	Retention Time	Area	% Area	Height
N-t-Boc-Gly-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-Ala	5,107	4277543	87,15	202276
N-t-Boc-Gly-OSu	8,238	51141	1,04	2090
N-t-Boc-Gly	12,309	579505	11,81	7456

Fig. 2. Chromatogram of N-t-Boc-Gly-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-Ala dipeptide after crystallization.



Name	Retention Time	Area	% Area	Height
N-t-Boc-Gly-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-Ala	5,106	7151078	100,00	315864

Fig. 3. Chromatogram of N-t-Boc-Gly-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-Ala dipeptide after final crystallization.

As follows from the graphs, the dipeptide synthesized at the first stage after crystallization contained side compounds, the peaks of which corresponded to N-t-butyloxy-carbonylglycine and N-t-BOC-glycyl-succinimide ester peaks. This was proved by the HPLC analysis of the mentioned compounds. Subsequent recrystallization made it possible to purify the target peptide.

Study of antifungal effect. The objects of the study were 3 strains of fungi from the National Culture Collection of Microorganisms of MDC: *Aspergillus fumigatus* MDC 8403, *Aspergillus candidus* MDC 10556, *Penicillium chrysogenum* MDC 8281.

For the study, an aqueous suspension of fungal spores, obtained after 14 days of growth, was used. Suspensions were added to a 40°C Chapek agar medium and poured into Petri dishes.

0.1M solution of the dipeptide dissolved in DMSO was added per 0.1, 0.2, 0.3 *ml* to 20 *ml* Chapek agar medium cooled to 37-38^{o}C and poured into Petri dishes. After cooling, test fungi were inoculated with an injection. The control was fungi inoculated on a dipeptide-free Chapek agar medium in the presence of DMSO.

To evaluate antifungal activity, the studied compound was applied to a solid nutrient Chapek medium with a fungi culture. Dishes were incubated at a temperature of 28°C for 5-7 days.

The research results were expressed by visual assessment of the inhibition of fungal growth by amino acids. The control was the growth of fungi without adding amino acids.

This study was supported by the ISTC A-2209.

N-ՏՐԵՏԲՈՒՏՕՔՍԻԿԱՐԲՈՆԻԼԳԼԻՑԻԼ-(S)-β-[4-ԱԼԻԼ-3-ՊՐՈՊԻԼ-5-ԹԻՕՔՍՈ-1,2,4-ՏՐԻԱԶՈԼ-1-ԻԼ]-α-ԱԼԱՆԻՆԻ ԴԻՊԵՊՏԻԴԻ ՍԻՆԹԵԶԸ ԵՎ ՀԱԿԱՄԱՆԿԱՅԻՆ ԱԶԴԵՑՈՒԹՅԱՆ ՀԵՏԱԶՈՏՈՒՄԸ

Տ. Հ. ՍԱՐԳՍՅԱՆ, Յու. Մ. ԴԱՆՂՅԱՆ, Ս. Մ. ՋԱՄԳԱՐՅԱՆ, Է. Ա. ԳՅՈՒԼՈՒՄՅԱՆ, Ն. Ս. ԽԱՉԱՏՈՒՐՅԱՆ, Ս. Ա. ԳԵՎՈՐԳԱՅԱՆ, Հ. Ի. ՀԱԿՈԲՅԱՆ, Զ. Զ. ՄԱՐԴԻՅԱՆ և Ա. Ս. ՍԱՂՅԱՆ

Սինթեզվել է N-տրետբուտօքսիկարբոնիլգլիցիլ-(S)-β-[4-ալիլ-3-պրոպիլ-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանին նոր, գրականության մեջ չնկարագրված, դիպեպտիդ՝ կիրառելով ակտիվացված էսթերների եղանակը:

Իրականցվել է սինթեզված դիպեպտիդի և համեմատական տվյալներ ստանալու ակնկալիքով ելային ամինաթթուների (S)-β-[4-ալիլ-3-պրոպիլ-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանինի և գլիցինի հակասնկային ազդեցության in vitro հետազոտում: Ուսումնասիրման համար ընտրվել են 3 սնկային շտամները՝ Aspergillus fumigatus MDC 8403, Aspergillus candidus MDC 10556, Penicillium chrysogenum MDC 8281, որոնք ձեռք են բերվել Հայաստանի մանրէների ավանդադրման կենտրոնից:

Հետազոտության արդյունքում բացահայտվել է, որ ելային ամինաթթուները չեն ցուցաբերել հակասնկային ազդեցություն, իսկ սինթեզված դիպեպտիդը ճնշել է ընտրված շտամերի աճը: Հետագա փորձարկումները կախված կոնցենտրացիայից ցույց են տվել, որ պեպտիդի կոնցենտրացիայի մեծացումը բերում է արգելակիչ ազդեցության մեծացմանը:

СИНТЕЗ N-ТРЕТБУТИЛОКСИКАРБОНИЛ-(S)-β-[4-АЛЛИЛ-3-ПРОПИЛ-5-ТИОКСО-1,2,4-ТРИАЗОЛ-1-ИЛ]-α-АЛАНИН ДИПЕПТИДА И ИССЛЕДОВАНИЕ ЕГО АНТИГРИБКОВОГО ДЕЙСТВИЯ

Т. О. САРГСЯН, Ю. М. ДАНГЯН, С. М. ДЖАМГАРЯН, Э. А. ГЮЛУМЯН, Н. А. ХАЧАТУРЯН, С. А. ГЕВОРГЯН, Е. И АКОПЯН, З.З. МАРДИЯН и А. С. САГЯН

Методом активированных эфиров синтезирован не описанный в литературе дипептид N-трет-бутилоксикарбонилглицил-(*S*)-β-[4-аллил-3-пропил-5-токсо-1,2,4-триазол-1-ил]-α-аланина.

Осуществлено исследование *in vitro* антигрибкового действия синтезированого дипептида и исходных аминокислот (*S*)-β-[4-аллил-3-пропил-5-токсо-1,2,4-триазол-1-ил]-α-аланина и глицина.

Объектами исследования служили 3 штамма грибов из Национальной коллекции культур микроорганизмов Армении; *Aspergillus fumigatus* MDC 8403, *Aspergillus candidus* MDC 10556, *Penicillium chrysogenum* MDC 8281.

Результаты исследования выявили, что исходные аминокислоты не проявляют антигрибкового действия, а синтезированный дипептид подавляет рост выделенных штаммов по сравнению с контролем. Было также отмечено подавление спороношения и частично роста грибков, усиливающееся с повышением концентрации дипептида в питательной среде.

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