ՀԱՅԱՍՏԱՆԻ ՀԱՆՐԱՊԵՏՈՒԹՅԱՆ ԳԻՏՈՒԹՅՈՒՆՆԵՐԻ
ԱԶԳԱՅԻՆ ԱԿԱԴԵՄԻԱ
НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК РЕСПУБЛИКИ АРМЕНИЯ
NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF ARMENIA

Հայաստանի քիմիական հանդես
Химический журнал Армении **71, №4, 2018** Chemical Journal of Armenia

TARGETED SYNTHESIS OF 9-FLUORENYLMETHYLOXYCARBONYLGLYCYL-(*S*)-β-[4-ALLYL-3-PROPYL-5-THIOXO-1,2,4-TRIAZOL-1-YL]-α-ALANINE AND STUDY OF ITS EFFECT ON COLLAGENASE ACTIVITY

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More than 15 new peptides have been constructed on the basis of (S)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine non-protein amino acid by ChemOffice software. The study of their possible interaction with collagenase enzyme was implemented by molecular docking program – AutoDockVina software. Analyzing the obtained results, 9-fluorenylmethyloxycarbonylglycyl-(S)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine dipeptide was identified by maximum values of Gibbs free energy (ΔG=8.6 *kcal/mol*) and minimum values of dissociation constant ($K_{D=}$0.497 *μmol*) of ligand-macromolecular interaction.

The synthesis of a new undescribed in the literature 9-fluorenylmethyloxycarbonylglycyl-(S)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine dipeptide has been carried out by the activated ester method.

In vitro study of the synthesized dipeptide effect on the activity of collagenase enzyme by various peptide concentrations has been carried out. The value of IC_{50} was calculated. It was 0.892 *μmol/l*:

Figs. 2, table 1, references 15.

For more than 70 years the research in the field of synthesis and study of peptides as well as the possibility of their introduction to the medical practice has been carried out [1].

Currently, there are around 60-70 approved peptide drugs in the global market, with 100-200 more in clinical trials, 400-600 more in pre-clinical studies and possibly hundreds to thousands more on the laboratory bench [2]. It should be

mentioned that most of them contain non-proteinogenic amino acid moieties [3]. There are well-known medicinal preparations created on the basis of synthetic peptides that are used in the following diseases: hypertension, type 2 diabetes, postmenopausal osteoporosis, Paget's disease, hypercalcaemia, advanced prostate cancer, acromegaly, carcinoidsyndrome, central diabetes insipidus.

It is established that the moiety of non-protein amino acid extends the process of enzyme-substrate recognition that in turn leads to retardation of the peptide bond destruction. These and other properties of peptides, containing a fragment of non-protein amino acid, enable to create on their basis physiologically and pharmacologically active drugs [4].

Matrix metalloproteases (MMPs) is a major group of enzymes that regulates cell-matrix composition. Matrix metalloproteases (MMPs) play an important role in degradation of extracellular matrix in both norm and various pathologies [5]. Metalloproteases are targets for a wide range of medications, including antitumor and anti-inflammatory drugs [6,7]. Matrix metalloproteases are responsible for many proteolytic processes that lead to tumor development. Involvement of gelatinizes (MMP-9 and MMP-2) in the process of metastases and angiogenesis formation stimulated creation of synthetic gelatinize inhibitors able to stop the development of tumors [6,7]. MMP-1 is also validated as a cancer target [8].

Unfortunately, clinical trials of gelatinize inhibitors on oncological patients so far have not revealed therapeutic effect; moreover undesirable side effects were registered. The majority of inhibitors are zinc-chelating compounds of a wide spectrum of action that did not have a specific effect. For example, calprotectin inhibits MMP by blocking zinc binding [9]. The search for new highly specific compounds able to inhibit metalloproteases is one of the directions in creation of drugs preventing spread of metastases [10]. It has been shown that some low-molecular-weight compounds are able to inhibit MMPs [11].

Taking into account the above mentioned, we aimed at constructing a new undescribed in the literature dipeptide on the basis of (*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine non-protein amino acid, implementing software research of the mentioned peptides, selecting possible active peptides to carry out their target synthesis and study the biological effect of the synthesized peptides.

In the first stage the structure-based drug design approach was used to identify potential inhibitors of enzyme. For this aim docking analysis was done for identification of substances capable of interacting with collagenase.

Amino acids and peptides structures were built by ChemBioOffice 2010 (ChemBio3D Ultra12.0). Ligand free energy was minimized using MM2 force field and truncated Newton–Raphson method. Crystallographic structure of collagenase was taken from http://www.rcsb.org website (PDB-ID: 1NQJ). Docking of ligand to enzyme was done by AutoGrid 4, AutoDock Vina software [12]. AutoDock used the Lamarckian genetic algorithm by alternating local search with selection and crossover [13]. The ligands were ranked using an energy-based scoring function and

a grid-based protein–ligand interaction was used to speed up the score calculation. Dissociation constant was calculated by using the following formula:

$K_D = \exp((\Delta G \times 1000)/(Rcal \times TK))$

Rcal=1.98719 cal/(*mol* × K) (gas constant)

TK = 298.15 *K* (room temperature by Kelvin)

The data of enzyme-peptide interaction are presented in Table.

Table

Data of molecular modeling

Experimental dipeptides	Gibbs free energy (ΔG) *kcal/mol*	Dissociation constant (K_D) *μmol*
N-formyl-(*S*)-methionyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.8	56.05
(*S*)-methionyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.1	33.78
9-Fluorenylmethyloxycarbonyl-(*S*)-alanyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α- alanine	-7.7	2.27
(*S*)-alanyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.2	28.53
N-tretbutoxycarbonyl-(*S*)-alanyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α- alanine	-6.7	12.27
N-tretbutoxycarbonylalanylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.6	14.53
N-tretbutoxycarbonyl-(*S*)-β-phenyl-alanyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.9	8.75
(*S*)-β-phenyl-alanyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-7.2	5.28
9-Fluorenylmethyloxycarbonylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α- alanine	-8.6	0.497
Glycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.1	33.78

The negative value of ΔG proves that the complex has been generated. According to Table, the value of ΔG is negative for all compounds, which proves that all dipeptides are interacting with the enzyme. Based on the results obtained, we aimed to perform further research on a compound with a maximum value of the Gibbs free energy, which turned out to be 9-fluorenylmethyloxycarbonylglycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine.

The docking data are presented in the Figure, where the fragments of ligand-collagenase interaction are shown.

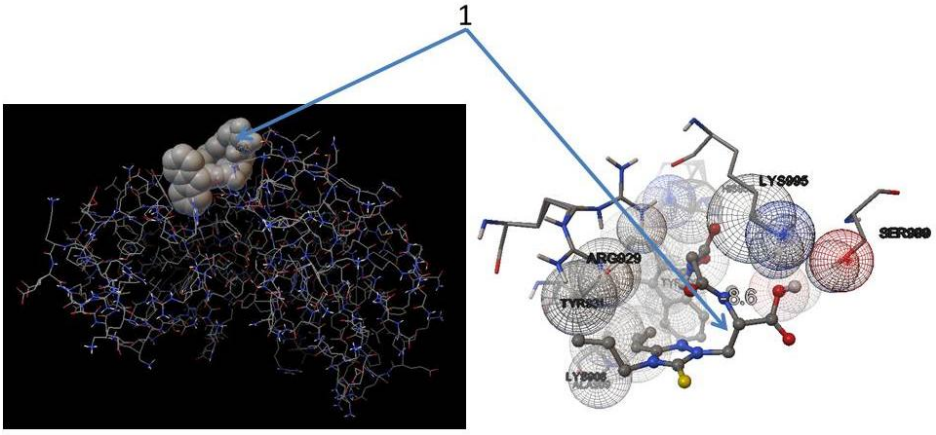


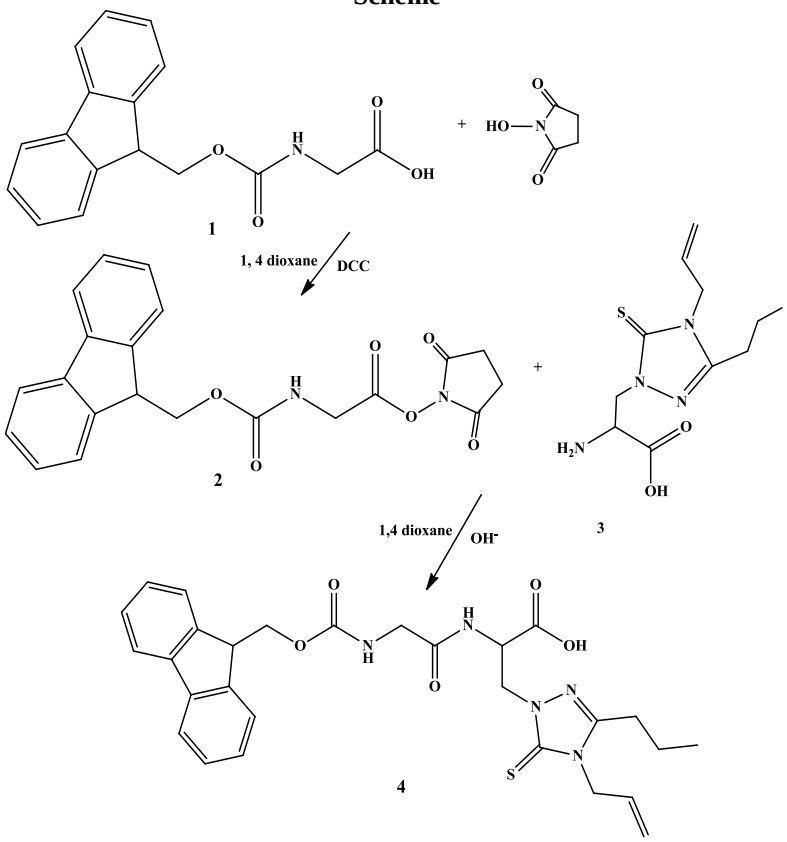
Fig. 1. The bond of Fmoc-glycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine with collagenase enzyme by software.

Taking into account the data of software modeling, Fmoc-glycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine dipeptide was selected from the mentioned range for further research, that is for carrying out the peptide synthesis followed by studying the synthesized peptide effect on the activity of collagenase enzyme.

The synthesis of peptide was carried out by the method of activated esters in a solution. The method is distinguished by its simplicity and makes it possible to obtain final products in good yields and high purity [14].

At the first stage with the help of dicyclohexylcarbodiimide from 9-fluorenylmethyloxycarbonyl-glycyl (1) was obtained its succinimide ether **(2)**, transformed by condensation with (*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine non-protein amino acid in alkaline aqueous-organic medium in the corresponding dipeptide Fmoc-glycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine (4) (Scheme).

Scheme



Experimental Part

^{1}H NMR spectra were recorded on a "Varian Mercury 300VX" device with an operating frequency of 300.08 *MHz* in a solution of DMSO-D6/CCl4 1/3 using the method of double resonance. TLC was conducted on "Silufol UV-254" plates in a mixture of chloroform-ethyl acetate-methanol (4:4:1), developer – chlorotoluidine. Elemental analysis was performed on elemental analyzer CNS-O "Euro EA3000".

Synthesis of N-9-fluorenylmethyloxycarbonylglycine succinimide ester (2). 0.218 *g* (1.06 *mmol*) of dicyclohexylcarbodiimide, preliminary dissolved in 3 *ml* of dioxane was added at 0^{o}C to 0.29 *g* (1.0 *mmol*) of N-9-fluorenylmethyloxycarbonyl-glycine **(2)** and 0.127 *g* (1.1 *mmol*) of N-hydroxysuccinimide in a mixture of 6 *ml* of dioxane and 3 *ml* of methylene chloride. The reaction mixture was stirred for ~ 2 h at 0^{o}C and left overnight in a refrigerator.

The analysis was performed by TLC [SiO_2, $CHCl_3$/ethyl acetate/CH_3OH (4:2:1), developer - chlorotoluidine]. The precipitate formed was filtered off, the solvent distilled off on a rotary evaporator, and the precipitate crystallized from a mixture of ethyl acetate hexane (1:2).

Yield 0.25 g (67.5%). Mp = 175 °C [14].

Synthesis of N-9-fluorenylmethyloxycarbonylglycyl-(S)-β-(3-propyl-4-allyl-5-thioxo-1,2,4-triazol-1-yl)-α-alanine (4). The resulting succinimide ether **2** was used in the next stage of dipeptide synthesis. In a flat-bottomed flask with a magnetic stirrer, 0.18 *g* (0.66 *mmol*) of (*S*)-β-(3-propyl-4-allyl-5-thioxo-1,2,4-triazol-1-yl)-α-alanine, 1.25 *ml* (0.63 *mmol*) of 0.5*M* sodium hydroxide solution and 0.016 (0.19 *mmol*) of baking soda were placed. At room temperature, 0.24 *g* (0.6 *mmol*) of N-9-fluorenylmethyloxycarbonylglycine succinimide ester (**2**) was added to 2 *ml* of dioxane, and the reaction mixture was stirred for 3 *h*. The next day, 5 *ml* of ethyl acetate and 1.45 *ml* of 10% citric acid were added to the flask contents. After vigorous stirring, the organic layer was separated, and the aqueous layer was extracted twice with ethyl acetate (5 *ml* each). The organic layer was dried with anhydrous sodium sulfate, then the solvent was evaporated to dryness.

The product was isolated by column chromatography using SiO_2 L-40/100 silica gel. Analysis by TLC [SiO_2, $CHCl_3$/ethyl acetate/CH_3OH (4: 2: 1), the developer – chloro-toluidine]. The product yield per succinimide ester 72.8%, Mp 99-100^oC. Found, %: C, 61.25; H, 5.61; N, 12.71. $C_{28}H_{31}N_5O_5S$ Calc., %: C, 61.19; H, 5.68; N, 12.74. ^1H NMR (DMSO, δ, ppm) 0.9 (m, 3H, CH_3-$\underline{CH_2}$); 1.44 (m, 2H, CH_3-$\underline{CH_2}$); 1.5 (m, 2H, CH_3-CH_2-$\underline{CH_2}$); 3.65 (m, 2H, $NHCH_2$), 3.8 (dd, 1H, J_1=13.6,J_2=8.2, NH-CH-$\underline{CH_2}$); 4.06 (dd, 1H, J_1=13.6,J_2=5.1, NH-CH-$\underline{CH_2}$); 4.46 (m, 1H, OCH_2-CH); 4.7 (ddd, 1H, J_1=8.2,J_2=8.1, J_3=5.1, NH-$\underline{CH}$-CH_2); 4.7 (m, 2H, O-CH_2-$\underline{CH}$);5.19 (dq, 1H, J_1=17.2, J_2~J_3=1.5, CH_2-CH=$\underline{CH_2}$); 5.22 (dq, 1H, J_1=10.4, J_2~J_3=1.5, CH_2-CH=$\underline{CH_2}$); 5.87 (ddt, 1H, J_1=17.2, J_3=10.4, J_3=4.9, CH_2-$\underline{CH}$=CH_2);7.28-7.87 (m, 8H, fluorenyl); 8.03 (t, 1H, ^3J=8.1, $\underline{NH}$-CH-CH_2); 8.03 (m, 1H, $\underline{NH}$-CH_2);11 (br, 1H, COOH):

Collagenase activity. Collagenase activity was determined by measuring free amino groups according to o-phthalaldehyde (OPA) method [15].

The reaction mixture contained 0.05 M HEPES buffer, pH 7.2, 10 *mg*/*ml* gelatin and 0.025 *mg*/*ml* collagenase (activated by 0.36 M $CaCl_2$). The concentration of investigated compounds in the reaction mixture was 5*mM*. The aliquot (50 *ul*) was taken and the remaining mixture was incubated at 37^oC. Every 30 *min* the aliquot was picked up and the reaction was stopped by adding 10 ul of 30% trichloroacetic acid. The concentration of free amino groups in the reaction mixture was determined by OPA reagent containing 0.2 *M* borate buffer, pH 9.7, 0.1667 *mg*/*ml* OPA and 1.25 *mM* mercaptoethanol. The reaction mixture (50 *ul*) was added to OPA reagent (1.5 *ml*) and H_2O (1.5 *ml*). A340 was recorded after 5 *min* incubation at RT.

Peptides have been tested in different concentrations in order to link the concentration and effect. The dependence curve of the inhibition percentage dependent on concentration is presented in Fig. 2.

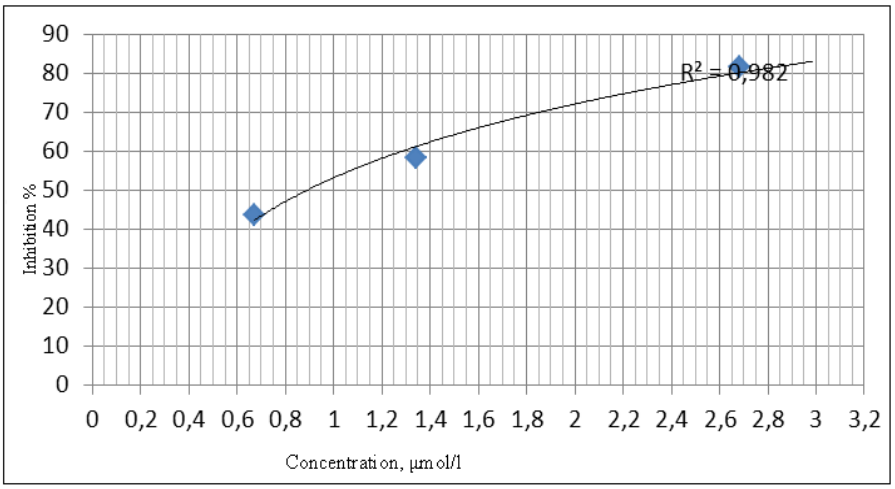


Fig. 2. The effect of various concentrations of Fmoc-glycyl-(S)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine on the activity of collagenase enzyme.

According to the data obtained, in case of Fmoc-glycyl-(*S*)-β-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine IC_{50} is 0.892 *μmol/l*:

9-ՖԼՈՒՈՐԵՆԻԼՄԵԹԻԼՕՔՍԻԿԱՐԲՈՆԻԼԳԼԻՑԻԼ-(S)-β-[4-ԱԼԻԼ-3-ՊՐՈՊԻԼ-5-ԹԻՕՔՍՈ-1,2,4-ՏՐԻԱԶՈԼ-1-ԻԼ]-α-ԱԼԱՆԻՆ ԴԻՊԵՊՏԻԴԻ ՆՊԱՏԱԿԱՅԻՆ ՍԻՆԹԵԶԸ ԵՎ ԿՈԼԱԳԵՆԱԶ ՖԵՐՄԵՆՏԻ ԱԿՏԻՎՈՒԹՅԱՆ ՎՐԱ ԱԶԴԵՑՈՒԹՅԱՆ ՀԵՏԱԶՈՏՈՒՄԸ

Յու. Մ. ԴԱՆՂՅԱՆ, Տ.Հ. ՍԱՐԳՍՅԱՆ, Ս. Մ. ՋԱՄԳԱՐՅԱՆ, Ա. Ս. ՍԱՐԳՍՅԱՆ, Է. Ա. ԳՅՈՒԼՈՒՄՅԱՆ, Հ. Ա. ՓԱՆՈՍՅԱՆ, Ն. Ա. ՀՈՎՀԱՆՆԻՍՅԱՆ, Ա. Մ. ՀՈՎՀԱՆՆԻՍՅԱՆ, Ա. Հ. ԾԱՏՈՒՐՅԱՆ և Ա. Ս. ՍԱՂՅԱՆ

(S)-β-[4-Ալիլ-3-պրոպիլ-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանին ոչ սպիտակուցային ամինաթթվի հենքի վրա ChemOffice software ծրագրի կիրառմամբ կառուցվել են ավելի քան 15 նոր գրականության մեջ չնկարագրված դիպեպտիդներ:

Իրականացվել է կառուցված պեպտիդների և կոլագենազ ֆերմենտի հնարավոր փոխազդեցության մոդելավորում AutoDockVina software համակարգչային ծրագրի կիրառմամբ:

Ստացված տվյալների վերլուծության արդյունքում՝ ընտրվել է 9-ֆլուորենիլմեթիլ-օքսիկարբոնիլգլիցիլ-(S)-β-[4-ալիլ-3-պրոպիլ-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանին դիպեպտիդը, որը ունեցել է Գիբսի ազատ էներգիայի (ΔG=8.6 կկալ/մոլ) առավելագույն և դիսոցման հաստատունի (KD=0.497 մկմոլ) նվազագույն արժեքներ:

Նոր գրականության մեջ չնկարագրված 9-ֆլուորենիլմեթիլօքսիկարբոնիլգլիցիլ-(S)-β-[4-ալիլ-3-պրոպիլ-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանին դիպեպտիդի սինթեզը իրականացվել է՝ ակտիվացված էսթերների մեթոդի կիրառմամբ:

Կատարվել է սինթեզված դիպեպտիդի ազդեցության in vitro հետազոտում կոլագենազ ֆերմենտի ակտիվության վրա՝ պեպտիդի տարբեր կոնցենտրացիաների կիրառմամբ: Հաշվարկվել է IC50-ի արժեքը, որը ստացվել է 0.892 մկմոլ/լ:

ЦЕЛЕНАПРАВЛЕННЫЙ СИНТЕЗ 9-ФЛУОРЕНИЛМЕТИЛОКСИКАРБОНИЛГЛИЦИЛ-(*S*)-β-[4-АЛЛИЛ-3-ПРОПИЛ-5-ТИОКСО-1,2,4-ТРИАЗОЛ-1-ИЛ]-α-АЛАНИН ДИПЕПТИДА И ИССЛЕДОВАНИЕ ЕГО ДЕЙСТВИЯ НА АКТИВНОСТЬ КОЛЛАГЕНАЗЫ

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С помощью программы ChemOffice software были построены структуры 15 новых не описанных в литературе дипептидов, содержащих небелковую аминокислоту (*S*)-β-[4-аллил-3-пропил-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин.

С использованием программы AutoDockVina software было проведено моделирование вероятного взаимодействия дипептидов с ферментом колагеназ. На основании полученных данных был выбран 9-флуоренилметилоксикарбонилглицил-(*S*)-β-[4-аллил-3-пропил-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин, который имеет наибольшую свободного энергию (*ΔG=8.6 ккал/мол*) и минимальное значение константы диссоциации ($K_{D=}$0.497 *мкмол*).

Синтез 9-флуоренилметилоксикарбонилглицил-(*S*)-β-[4-аллил-3-пропил-5-тиоксо-1,2,4-триазол-1-ил]-α-аланина осуществлен методом активированных эфиров.

Проведено *in vitro* исследование влияния синтезированого дипептида на активность фермента колагеназ при различных концентрациях пептида. Рассчитано значение IC_{50} -0.892 *мкмол/л*.

REFERENCES

[1] *Gregory A.* Grant Synthetic peptides Oxford University press, second edition, 2002.
[2] *Sun L.* // Mod. Chem. Appl. 2013; 1: e103. doi: 10.4172/2329-6798.1000E103.
[3] *Vlieghe P., Lisowski V., Martinez J., Khrestchatisky M.* // Drug Discov Today, 2010 Jan. 15(1-2), p. 40.
[4] *Hughes A.* // WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. v. 4 Protection Reactions, Medicinal Chemistry & Combinatoral Synthesys, 2011, v. 4, p. 538.
[5] *Baker A.B.* // A. Baker, D. Edwards, G. Murphy // J. Cell Science, 2002, v. 115, p. 3719.
[6] *Hsieh M.J., Chen J.C., Yang W.E., Chien S.Y., Chen M.K., Lo Y.S., His Y.T., Chuang Y.C., Lin C.C., Yang S.F.* // Biochem Pharmacol., 2017, 2952(17), p. 30041-2,
[7] *Krüger A., Arlt M.J., Gerg M., Kopitz C., Bernardo M.M., Chang M., Mobashery S., Fridman R.* // Cancer Res., 2005, 65(9), p. 3523.
[8] *Overall CM, Kleifeld O.* // 2006, Nat. Rev. Cancer, 6(3), p. 227.
[9] *Isaksen B., Fagerhol M.K.* // J.Clin pathol: Mol pathol., 2001, v. 54, p. 289.
[10] *Hsieh M.J., Chen J.C., Yang W.E., Chien S.Y., Chen M.K., Lo Y.S., His Y.T., Chuang Y.C., Lin C.C., Yang S.F.* // Biochem Pharmacol., 2017, 2952(17), p. 30041.
[11] *Bannikov G.A., Lakritz J., Premanandan C., Mattoon J.S., Abrahamsen E.J.* // Am J Vet Res. 2009; 70(5):633-9.
[12] *Trott O., Olson A.J.* // J. Comput. Chem., 2010, v. 31, p. 455.
[13] *Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A.J.* Automated Docking. // J.Comput.Chem., 1998, v. 19, p. 1639.
[14] *Anderson G., Zimmerman J., Callahan F.* // J. Am. Chem. Soc., 1964, v. 86, issue. 9, p. 1839.
[15] *Gade W., Brown J.* // Biochemica and Biophysica Acta 1981, v. 13, p. 86.