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HETEROCYCLIC SUBSTITUTED NON-PROTEIN AMINO ACIDS AS INHIBITORS OF *CLOSTRIDIUM HISTOLYTICUM* COLLAGENASE

A.S. SARGSYAN[1,2*], B.G. BABAYAN[2], N.S. AVETISYAN[1.2], A. G. MKRTCHYAN[1], A.M. HOVHANNISYAN[1*], N.A. HOVHANNISYAN [1,2]

[1] Chair of Pharmtechnology and Pharmacy Economics and Managements, YSU,
[2] SPC "Armbiotechnology" NAS RA
armenssargsyan@gmail.com
anhovhannisyan@ysu.am

Optically active non-protein α-amino acids have been screened for their ability to interact with collagenase of *Clostridium histolyticum*. Both structure-based drug design approach (modeling) and that of determining enzyme activity in the presence of amino acids have been used to identify low molecular weight inhibitors of collagenase. According to the docking analysis, a number of non-protein amino acids have demonstrated ability to form bounds with collagenase. Following the docking analysis, enzyme activity has been determined in the presence of investigated amino acids. The results have indicated that (S)-β-[4-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, (S)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and (S)-β-[4-allyl-3-(pyridin-3'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine inhibit collagenase activity.

Non-protein amino acid – docking – collagenase – inhibitor

Իրականացվել է *Clostridium histolyticum*-ից անջատված կոլագենազի հետ սինթետիկ ամինաթթուների փոխազդեցության հատկությունների սքրինինգ: Կոլագենազի ցածրամոլեկուլային արգելակիչների հայտնաբերման նպատակով կիրառվել է երկու մոտեցում՝ մոդելավորման եղանակով դեղերի կառուցում և ամինաթթուների առկայությամբ ֆերմենտային ակտիվության որոշում: Դոքինգ հետազոտության արդյունքների համաձայն մի շարք ամինաթթուներ կոլագենազի հետ կապվելու հատկություն են ցուցաբերել: Այնուհետև չափվել է ֆերմենտի ակտիվությունը՝ հետազոտվող ամինաթթուների առկայության պայմաններում: Արդյունքները ցույց են տվել, որ (S)-β-[4-ալիլ-3-բութիլ-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանինը, (S)-β-[4-ալիլ-3-(պիրիդին-4'-իլ)-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանինը և (S)-β-[4-ալիլ-3-(պիրիդին-3'-իլ)-5-թիօքսո-1,2,4-տրիազոլ-1-իլ]-α-ալանինը հանդիսանում են կոլագենազի արգելակիչներ:

Ոչ սպիտակուցային ամինաթթու – դոքինգ – կոլագենազ – արգելակիչ

Осуществлен скриннинг синтетических аминокислот на способность взаимодействовать с коллагеназой, выделеннной из *Clostridium histolyticum*. Для выявления низкомолекулярных ингибиторов коллагеназы были использованы два подхода: конструирование лекарств на основе структуры путем моделирования и определение активности фермента в присутствии аминокислот. Согласно данным докинг анализа ряд небелковых аминокислот проявили способность связываться с коллагеназой. Далее определялась активность фермента в присутствии исследуемых аминокислот. Результаты показали, что (S)-β-[4-аллил-3-бутил-5-тиоксо-1,2,4-триазолl-1-ил]-α-аланин, (S)-β-[4-аллил-3-(пиридин-4'-ил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин и (S)-β-[4-аллил-3-(пиридин-3'-ил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин являются ингибиторами коллагеназы.

Небелковая аминокислота – докинг – коллагеназа – ингибитор

Non-protein α-amino acids occupy a special place among optically active compounds having biological activity. The biological activity of these compounds is stipulated by their ability to interact with enzymes. Design of a number of modern antibacterial, antiviral, antitumor and other drugs, as well as food supplements is based on the property of non-protein amino acids and peptides either to inhibit or enhance the activity of relevant target enzymes [1, 2].

Matrix metalloproteases (MMPs) are a major group of enzymes that regulates cell-matrix composition. MMPs play an important role in degradation of extracellular matrix in both norm and various pathologies [3]. Due to their active participation in various physiological processes MMPs are suggested as targets for a wide range of medications, including antitumor and anti-inflammatory drugs [4, 5]. MMPs 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]. The majority of MMPs inhibitors are zinc-chelating compounds of a wide spectrum of action that do not have a specific effect. The search for new highly specific compounds able to inhibit MMPs is one of the directions in creation of drugs preventing spread of metastases [7]. Recently some low molecular weight compounds have been described as effective MMPs inhibitors [8].

In this work the screening of heterocycle substituted non-protein amino acids as *Clostridium histolyticum* collagenase (MMP-1) inhibitors has been carried out. This metalloprotease has a high level of homology with mammals collagenases [9]. The structure-based drug design approach was used to identify small inhibitors of enzyme. Enzyme activity in the presence of investigated compounds was determined as well. Some of the investigated non-protein α-amino acids were characterized as *Clostridium histolyticum* collagenase inhibitors.

Materials and methods. The heterocyclic substituted non-nprotein amino acids studied in this work were synthesized at Scientific and Production Center "Armbiotechnology" NAS RA and the Institute of Pharmacy of Yerevan State University [10]. Collagenase from *Closridium histolyticum* (EC 3.4.24.3), and miscellaneous reagents were purchased from Sigma (USA).

***Collagenase activity*.** The screening of investigated compounds on the ability to influence enzyme activity was carried by using 0.6% agarose gel containing 5 mg/ml gelatin and 0.05 M HEPES buffer, pH 7.2. The mixture of $CaCl_2$ activated collagenase and amino acid was placed on agarose surface and incubated at 37^0C. The diameter of cleared spots was measured after 2-3 hours.

Collagenase activity was determined by measuring free amino groups according to o-phthalaldehyde (OPA) method [11]. 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 $CaCl_2$).

Modeling. Modeling was done according to the procedure described previously [12]. Docking of ligand to enzyme was done by AutoGrid 4, AutoDock Vina software [13]. Crystallographic structures of collagenase were taken from http://www.rcsb.org website (PDB-ID: 1NQD, 1NQJ). The both structures of collagen-binding domain with (1NQD) and without Ca^{2+} ions (1NQJ) were considered.

Results and Discussion. *Selection of collagenase inhibitors by docking analysis (modeling).* The interaction of collagenase with 50 nonprotein amino acids was investigated by using AutoGrid 4, AutoDock Vina software aimed to select enzyme inhibitors. ΔG and K_I values were calculated. Based on values of ΔG (free energy of binding) the compounds were selected which are able to make complexes with enzyme. Comparing the results of docking analysis on two models it was found out that Ca^{++} free 1NQJ collagenase model formed stronger complexes with amino acids compared with Ca^{++} 1NQD. In this work both results were considered. It should be mentioned that as

the most suitable molecules which are able to make complexes with enzyme we consider those for which values of ΔG are <-5.5 (K_I<0.093mM) determined by docking analysis on 1NQD model, and ΔG are <-6.2 (K_I<0.025mM) determined by docking analysis on 1NQJ model (tab. 1). Moreover, attention was paid (in terms of inhibition) to molecules, which bind enzyme not at the active center. The number, position and length of hydrogen bonds in protease-inhibitor complexes were calculated (data not shown).

Calculation results suggest that the hydrogen bonds play the main role in this interaction. According to the calculated values of ΔG (K_I) collagenase forms the most stable complexes with compounds presented in tab. 1. These synthetic amino acids bind collagenase at sites, which are not involved in the active center of enzyme.

Table 1. Interaction of non-protein amino acids with collagenase

Non-protein amino acids	1NQD model		1NQJ model	
	ΔG (kcal/mol)	**K_I (uM)**	**ΔG (kcal/mol)**	**K_I (uM)**
S)-β-[4-phenyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.6	0.07855	-7.3	0.00446
S)-β-[4-phenyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.5	0.09299	-6.9	0.00875
S)-β-[4-allyl-3-benzyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.2	0.02853	-7.1	0.00625
S)-β-[4-allyl-3-(furan-2-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.8	0.05605	-6.6	0.01453
S)-β-[4-allyl-3-(2'-chlorophenyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.4	0.02036	-7.6	0.00269
S)-β-[4-(furan-2-yl-methyl)-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.1	0.03378	-6.3	0.0241
S)-β-[4-methyl-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.9	0.04734	-6.5	0.0172
S)-β-[4-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.0	0.03999	-6.3	0.0241
S)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.6	0.07855	-6.3	0.0241
S)-β-[4-allyl-3-(pyridin-3'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.6	0.07855	-7.0	0.00739

The effect of non-protein amino acids on the activity of collagenase. The preliminary screening of amino acids listed in tab. 1 on the ability to influence collagenase activity was carried out on agarose gel containing gelatin, as described in section *Materials and methods*. The results revealed three non-protein amino acids, which inhibited collagenase activity: (*S*)-β-[4-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, (S)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and (*S*)-β-[4-allyl-3-(pyridin-3'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine. Then the activity of collagenase was determined in the presence of these non-protein amino acids. According to obtained results collagenase was inhibited by (*S*)-β-[4-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, (*S*)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and (*S*)-β-[4-allyl-3-(pyridin-3'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine. The results are presented in Table 2. The rest of investigated compounds had no influence on enzyme activity.

According to the data obtained some amino acids despite their ability to interact with collagenase do not have influence on enzyme activity. Only (S)-β-[4-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, (S)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and (S)-β-[4-allyl-3-(pyridin-3'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine have demonstrated inhibition effect on collagenase. Thus the results indicate that inhibition of collagenase depends not only on ability to interact with enzyme, but also on the structure of amino acid molecule. Amino acids that inhibited collagenase have distinct substituted moieties such as -3-butyl, 3-(pyridin-4'-yl) and -3-(pyridi-3'-yl).

Table 2. Inhibition effect of nonprotein amino acids on collagenase activity

Non-protein amino acids	ΔG (kcal/mol)	KI (uM)	IC50 (uM)
(S)-β-[4-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6,3	0,0241	2,67
(S)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6,3	0,0241	2,1
(S)-β-[4-allyl-3-(pyridin-3'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-7	0,00739	2,2
(S)-β-[4-phenyl-3-(3'-hydroxypropyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5,6	0.07855	-
(S)-β-[4-phenyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.5	0.09299	-
(S)-β-[4-allyl-3-benzyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.2	0.02853	-
(S)-β-[4-allyl-3-(furan-2-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.8	0.05605	-
(S)-β-[4-allyl-3-(2'-chlorophenyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.4	0.02036	-
(S)-β-[4-(furan-2-yl-methyl)-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-6.1	0.03378	-
(S)-β-[4-methyl-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine	-5.9	0.04734	-

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REFERENCES

1. *Goulet M.T.* Synthesis and structure—activity relations of thieno-[2,3 a-]-pyridine-2,4-dione derivatives as potent GnRH receptor antagonists. Annu. Rep. Med. Chem., *30*, 169-177, 1995.
2. *Van Der Baan J., Barnik J., Bickelhaupt F.* Antibiotic A 19009. Structural investigation and synthesis. Antibiotics, *36*, 784-790, 1983.
3. *Close D.R.* Matrix metalloproteinase inhibitors in rheumatic diseases. Ann Rheum Dis. 60 Suppl. 3:iii62-7, 2001.
4. *Migliaccio A., Castoria G., Giovannelli P., Auricchio F.* Cross talk between epidermal growth factor (EGF) receptor and extra nuclear steroid receptors in cell lines. Mol. Cell. Endocrinol., *327*, 1-2, 19-24, .2010.
5. *McCawley L.J., Matrisian L.M.* Matrix metalloproteinases: they're not just for matrix anymore! Curr. Opin. Cell. Biol., *13*, 534-540, 2001.
6. *Krüger A., Arlt M.J., Gerg M., Kopitz C., Bernardo M.M., Chang M., Mobashery S., Fridman R.* Antimetastatic activity of a novel mechanism-based gelatinase inhibitor. Cancer Res., *65*, 9, 3523-6, 2005.
7. *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.* Dehydroandrographolide inhibits oral cancer cell migration and invasion through NF-κB-, AP-1-, and SP-1-modulated matrix metalloproteinase-2 inhibition. Biochem Pharmacol., *17*, 2952, 30041-2, 2017.
8. *Bannikov G.A., Lakritz J., Premanandan C., Mattoon J.S., Abrahamsen E.J.* Kinetics of inhibition of purified bovine neutrophil matrix metalloproteinase 9 by low-molecular-weight inhibitors. Am. J. Vet. Res. *70*, 5, 633-9, 2009.
9. *Edkins T.J., Koller-Eichhorn R., Alhadeff J.A., Mayer U., Faust H., Del Tito B.J.* Assessment of potential cross-reactivity of human endogenous matrix metalloproteinases with collagenase Clostridium histolyticum antibodies in human sera obtained from patients with Dupuytren's contracture. Clin Vaccine Immunol., *19*, 4, 562-9, 2012.
10. Ashot S. Saghyan, Peter Langer. Asymmetric synthesis of non-proteinogenic amino acids. John Wiley and Sons Ltd., UK, 2016.
11. *Gade W., Brown J.* Purification, characterization and possible function of alpha-N-acylamino acid hydrolase from bovine liver. Biochemica and Biophysica Acta, *13*, 86-93, 1981.
12. *Hovhannisyan N., Harutyunyan Sh., Hovhannisyan A., Hambardzumyan A., Chitchyan M., Melkumyan M., Oganezova G., Avetisyan N.* The novel inhibitors of serine proteases. Amino Acids., *37*, 3, 531-6, 2009.
13. *Trott O., Olson A.J.* Software News and Update AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem., *31*, 455-461, 2010.

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