Available free online at www.medjchem.com

Mediterranean Journal of Chemistry

Mediterranean Journal of Chemistry **2025**, 15(1), 123-128

Spatial structure of the β-casomorphin-8 molecule

Leyla Namiq Agayeva, Larisa Ismail Ismailova, and Namiq Abdulavval Akhmedov *

Baku State University, Institute for Physical Problems,
Z. Khalilov Str.23, Baku, AZ-1148, Azerbaijan

Abstract: Several exogenous peptides obtained from food have opioid-like properties. These peptides were called exorphins. The first known exorphins were obtained by in vitro pepsin hydrolysis of α-casein and wheat gluten. The conformational capabilities of the β-casomorphin-8 molecule (H-Tyr1-Pro2-Phe3-Pro4-Gly5-Pro6-İle7-Pro8-OH) have been studied by theoretical conformational analysis. The potential function of the system is chosen as the sum of non-bonded, electrostatic, and torsion interactions and the energy of hydrogen bonds. Low-energy conformations of the β-casomorphin-8 molecule were found, the dihedral angles of the leading and side chains of amino acid residues included in the molecule were found, and the energy of intra- and intersubstance interactions was estimated. It has been shown that six structural types represent the spatial structure of the β-casomorphin-8 molecule. It can be assumed that the molecule performs its physiological functions in these structures. These three-dimensional structures make it possible to propose synthetic analogs for a given molecule. The results obtained can be used to elucidate the structural and structural-functional organization of casomorphin molecules.

Keywords: exorphin, β-casomorphin, opioid, structure, conformation.

1. Introduction

The regulatory peptides, first discovered in the second half of the twentieth century, are being actively studied by physiologists and pharmacologists since the area of biological activity peptides is extremely wide. The first known exorphins were obtained by in vitro pepsin hydrolysis of α-casein and wheat gluten. The resistance of exorphins to the action of pancreatic enzymes has been proven, and the opioid activity of these peptides in vivo has been confirmed. It turned out that opioid-like derivatives of casein and gluten inhibit adenylate cyclase activity in cell culture, inhibit contractions of the mouse vas deferens, and displace radiolabeled opioid receptor agonists from the binding sites on rat brain slices. Among the exorphins of animal origin, the derivatives of milk proteins are best studied. The most famous are milk exorphins (β-casomorphins-4, -5, -6, -7, -8) - products of enzymatic hydrolysis of β-casein in cow's milk. Similar peptides in the β-casein molecule were later found in the milk of other mammalian species, including humans. Exorphins have also been isolated from other milk proteins, but they are much less studied than β-casein derivatives, which mainly have an affinity for opioid receptors. The α-S1-casomorphin pentapeptide was isolated from α-casein in human milk. Interestingly, this peptide can suppress the proliferation of breast tumor cells. The physiological effect of antagonistic exorphins is still poorly understood. Some milk exorphins can not only be formed during the digestion of milk in the gastrointestinal tract but also be contained in cheeses in a "ready-made" form since the technology of making cheese is associated with enzymatic processing. Researchers have paid special attention to casomorphins because milk is the only food for young children, and it is known that some changes in the composition of milk can significantly affect the physical and mental development of infants, including causing long-term effects [1-3].

2. Materials and methods

We have investigated the structural and functional organizations of the opioid peptides enkephalins, endorphins, endomorphins, dynorphins, neoendorphins, and adrenorphins, and we are currently investigating the spatial structure of molecules of rubiscolins, soymorphins, and casomorphins. This work is a continuation of our previous research [4-19].

The molecule was calculated using theoretical conformational analysis. The potential function of the system is chosen as the sum of non-bonded, electrostatic, and torsion interactions and the energy of hydrogen bonds. Nonvalent interactions were assessed by Lennard-Jones potential. According to Coulomb's law, electrostatic interactions were calculated using partial charges on atoms in a monopole approximation. The conformational possibilities of the casomorphin molecule were studied under the conditions of the water

**Corresponding author: Namiq Abdulavval Akhmedov*
Email address: namiqabdulavval@mail.ru
DOI: http://dx.doi.org/10.13171/mjc02503121821akhmedov

Received December 26, 2024
Accepted March 10, 2025
Published March 12, 2025

environment, in connection with which the value of the dielectric constant was assumed to be 10. The energy of hydrogen bonds was estimated using the Morse potential. Our aforementioned works describe in detail the potential functions used.

In presenting the calculation results, we classified peptide structures by conformations, forms of the main chain, and shapes of the peptide backbone. Conformational states are entirely determined by the values of the dihedral angles of the leading and side chains of all amino acid residues included in a given molecule. A fragment's main chain forms are formed by combinations of R, B, L residues in a given sequence. Forms of the main chain of a dipeptide can be divided into two classes - folded (f) and unfolded (e) forms, which are called shapes. All conformations are grouped by backbone shape, and shapes are grouped by shape. In order to designate the conformational states of the residues, identifiers of the X_{ij} type are used, where X defines the low-energy regions of the conformational map

$\varphi-\psi: R(\varphi,\psi=-180°-0°)$,

$B(\varphi=-180^{\circ}-0^{\circ},\psi=0^{\circ}-180°), L(\varphi,\psi=0^{\circ}-180^{\circ})$ and

$P(\varphi=0°-180°,\psi=-180°-0°)$; ij ... = 11 ..., 12 ..., 13 ..., 21 ... define the position of the side chain $(\chi_1,\chi_2...)$,

with the index 1 corresponding to the angle value in the range from 0 to 120 °, 2 - from 120 ° to -120 °, and 3 - from -120 ° to 0 °. The designations and readings of the angles of rotation correspond to the IUPAC-IUB nomenclature [20].

3. Results and discussion

The spatial structures of β-casomorphin -4, -5, -6, -7 molecules were studied by theoretical conformational analysis. The spatial structure of β-casomorphin-5 molecule was studied based on the spatial structure of β-casomorphin-4, β-casomorphin-6 molecule spatial structure based on the β-casomorphin-5, β-casomorphin-7 molecule spatial structure based on the β-casomorphin-6 molecule. As mentioned, the spatial structure of β-casomorphin-7 was studied based on the low-energy conformations of β-casomorphin-6. The study of the spatial structure of the β-casomorphin-7 molecule showed that nine low-energy conformations represent the spatial structure of the molecule. Those conformations, their shapes, share, and relative energies of nonvalent, electrostatic, and torsion interaction energies are shown in Table 1. As can be seen from the table, the relative energies of the low-energy conformations of the β-casomorphin-7 molecule vary in the energy interval of 25.2 kJ/mol, and the N-side tripeptide fragment of the molecule has a rigid spatial structure. The N-side tetrapeptide fragment is represented by the two efe and eee shapes of the peptide chain, and the N-side tripeptide fragment is defined by the three BRB, BBL, and BBB forms of the main chain. The C-side tripeptide fragment of the molecule has conformational lability. The β-casomorphin-8 molecule was obtained by adding a proline amino acid residue to the β-casomorphin-7 molecule. Therefore, to study the spatial structure of the β-casomorphin-8 molecule, the starting conformations were obtained by adding the R and B conformations of the proline amino acid residue to the low-energy conformations of the β-casomorphin-7 molecule shown in Table 1.

Table 1. Energy contributions (kJ/mol) of nonvalent (U_{nv}), electrostatic (U_{el}), torsional (U_{tors}) interactions and the relative energy (U_{rel}) of the optimal conformations of the molecule casomorphine-7.

№	Shapes	Conformation	U_{nv}	U_{el}	U_{tors}	U_{rel}
1	efeeee	$B_3RB_1RLBB_{32}$	-87.4	-14.7	14.7	0
2	efeeef	$B_1RB_1RLRB_{32}$	-79.4	-13.4	8.4	2.9
3	efeffe	$B_2BL_3RRBB_{32}$	-87.8	-9.7	13.4	2.9
4	efefee	$B_3RB_2BLBB_{32}$	-78.5	-12.2	11.8	8.0
5	efefef	$B_3RB_2BLRB_{32}$	-77.3	-11.8	9.7	8.0
6	eeefef	$B_1BB_2BLRB_{32}$	-88.2	-13.4	14.7	0.8
7	eeefee	$B_1BB_2BLBB_{32}$	-75.6	-13.4	26.0	24.4
8	eeeeef	$B_1BB_1RLRB_{32}$	-75.2	-12.6	10.1	9.7
9	eeeeee	$B_1BB_1RLBB_{32}$	-70.1	-12.6	10.9	15.1

The study of the spatial structure of the β-casomorphin-8 molecule shows a sharp differentiation according to the energies of the peptide chain, the shapes of the main chain, and the conformations. When a proline amino acid residue was added to the C-side of the β-casomorphin-7 molecule, some low-energy conformations of the heptapeptide molecule became spherically infeasible. The results of the calculations show that the spatial structure of the β-casomorphin-8 molecule can be represented by the conformation of six forms of the main chain belonging to the three forms of the peptide

chain. Those shapes and the contribution of nonvalent, electrostatic, and torsional interaction energies to the total energy are shown in Table 2. The relative energies of the conformations shown in Table 2 vary in the energy interval (0 –12.6) kJ/mol. The contribution of nonvalent interaction energy to those conformations varies in the range of (-105.8) – (92.4) kJ/mol, electrostatic interaction energy is in the range of (-13.0) – (4.6) kJ/mol, torsional interaction energy is (-19.4) – (-22.8) kJ/mol. As shown in Table 2, three of the six conformations differ from the other three conformations in the conformation of proline 8. The lowest-energy conformations of all three shapes shown in Table 2 are selected, the interaction energies within and between amino acid residues in them are shown in Table 3, the values of their dihedral rotation angles are shown in Table 4, and the spatial arrangement of atoms in those conformations is shown in Figure 1.

Table 2. Energy contributions (kJ/mol) of nonvalent (U_{nv}), electrostatic (U_{el}), torsional (U_{tors}) interactions, the total (U_{tot}) and relative energy (U_{rel}) of the optimal conformations of the molecule β- casomorphin-8.

№	Shapes	Conformation	U_{nv}	U_{el}	U_{tors}	U_{rel}
1	efeeeee	$B_3RB_1RLBB_{32}B$	-94.1	-13.0	83.2	0
2	efeeeee	$B_3RB_1RLBB_{32}R$	-92.8	-12.6	81.5	0
3	eeefefe	$B_1BB_2BLRB_{22}B$	-104.2	-10.5	95.3	4.6
4	eeefefe	$B_1BB_2BLRB_{22}R$	-105.8	-10.5	95.8	3.4
5	efeffee	$B_2BL_3RRBB_{32}B$	-92.4	-4.6	83.6	10.9
6	efeffee	$B_2BL_3RRBB_{32}R$	-92.4	-4.6	83.2	10.1

The N-side tetrapeptide fragment of the β-casomorphin-8 molecule has a rigid conformation feature, the conformations of only two efe and eee shapes of the peptide chain are low-energy. The conformations of the three eeee, fefe, and ffee shapes of the C-side pentapeptide fragment were low-energy (Table 2).

Table 3. Energy inside and between residual interactions in the conformations of the molecule β-casomorphin-8: $B_3RB_1RLBB_{32}B$ (U_{rel}=0 kJ/mol, first line), $B_1BB_2BLRB_{22}R$ (U_{rel}=3.4 kJ/mol, second line), $B_2BL_3RRBB_{32}R$ (U_{rel}=10.1 kJ/mol, third line).

Tyr1	Pro2	Phe3	Pro4	Gly5	Pro6	Ile7	Pro8	
8.8 **8.0** **14.3**	-18.1 -19.7 -22.3	-30.2 -16.0 -9.7	-2.5 -3.4 -10.1	-0.8 0 -0.4	-0.4 0 -0.8	-2.5 -2.1 -2.5	-7.1 -5.0 0	Tyr1
	0.8 1.3 1.3	-8.8 -2.1 -5.9	-2.1 -3.4 -4.6	0 0 0	0 -0.4 0	0 0 0	0 -0.4 -0.4	Pro2
		4.2 -0.8 2.5	-15.5 -20.2 -14.3	-7.6 -28.1 -3.4	-0.4 -11.8 -15.1	0 -0.4 -0.8	0.4 0.4 0.4	Phe3
			1.3 1.3 1.3	-2.5 1.7 -2.9	-6.7 -4.2 -7.1	-0.4 -0.4 0	-0.4 0 0.4	Pro4
				5.9 5.9 5.9	-12.6 -12.6 -10.9	-2.9 -7.1 -2.9	-0.4 -0.8 -0.8	Gly5
					2.9 0.4 2.5	-8.4 -14.7 -8.4	-3.4 -6.7 -3.4	Pro6
						2.5 5.5 2.5	-5.5 -7.6 -3.4	Ile7
							5.9 5.9 5.9	Pro8

As can be seen from the amino acid sequence of the molecule, the amino acid proline occurs in the second, fourth, sixth, and eighth positions. As is well known, the proline amino acid itself and the preceding amino acid residue have limited conformational possibilities. For this reason, the conformations of the unfolded forms of the peptide chain are sterically possible, and only di- and tripeptide interactions between amino acid residues can occur. As can be seen from Table 3, there were no penta-, hexa-, hepta-, and octapeptide interactions between the amino acid residues that make up the molecule.

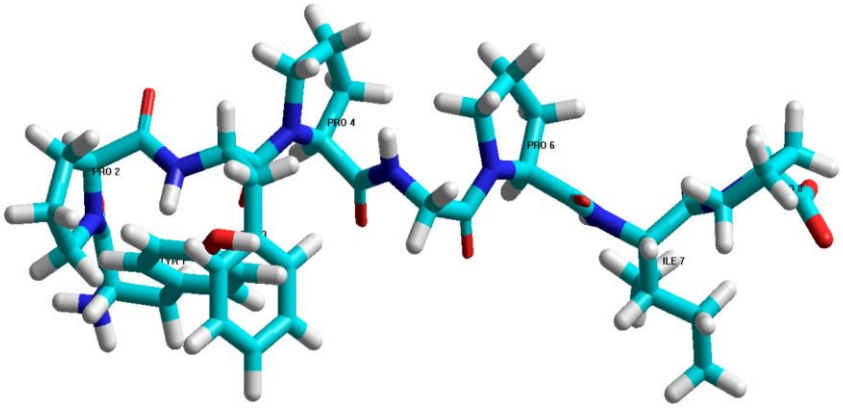
The global conformation of the β-casomorphin-8 molecule is the $B_3RB_1RLBB_{32}B$ conformation of the efeeeee shape. In this conformation, only the Pro2-Phe3 dipeptide fragment is folded, while all the remaining amino acid residues form the fully unfolded form of the peptide chain. Conformation stabilization is contributed by nonvalent interaction energy (-94.1) kJ/mol, electrostatic interaction energy (-13.0) kJ/mol, and torsional interaction energy (83.2) kJ/mol. The interaction of the Tyr1 amino acid residue with the following Pro2-Phe3 dipeptide fragment contributes up to (47.9) kJ/mol, and the interaction of the Pro6 amino acid residue with the following Ile7-Pro8 dipeptide fragment contributes to the total energy (-12.2) kJ/mol.

Table 4. Geometric parameters (in degrees) of low energy conformations of the molecule β-casomorphine-8.

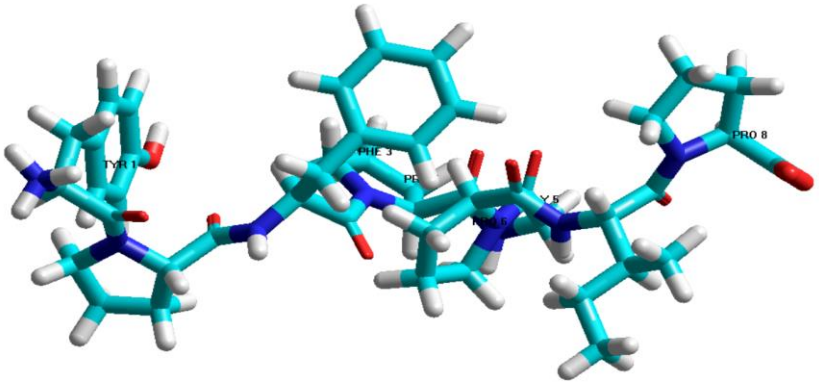
Residue	$B_3RB_1RLBB_{32}B$	$B_1BB_2BLRB_{22}R$	$B_2BL_3RRBB_{32}R$
Tyr1	-65 154 170 -72 106 0	-77 149 175 65 92 0	-91 127 179 179 83 0
Pro2	-60 -37 177	-60 113 -170	-60 130 -173
Phe3	-88 149 178 57 82	-94 130 177 -175 88	53 70 -179 -50 97
Pro4	-60 -60 -179	-60 115 -172	-60 -62 175
Gly5	63 69 -179	63 70 -174	-71 -68 -171
Pro6	60 158 177	-60 -24 173	-60 156 177
Ile7	-105 132 176 -51 -170 176 -172	-99 136 169 -163 -175 176 -179	-106 133 176 -53 -179 175 -172
Pro8	-60 112 -	-60 -63 -	-60 -62 -
ΔU (kJ/mol)	0	3.4	10.1

Note: The values of dihedral angles are given in the sequence φ, ψ, ω, χ^1, χ^2, ...

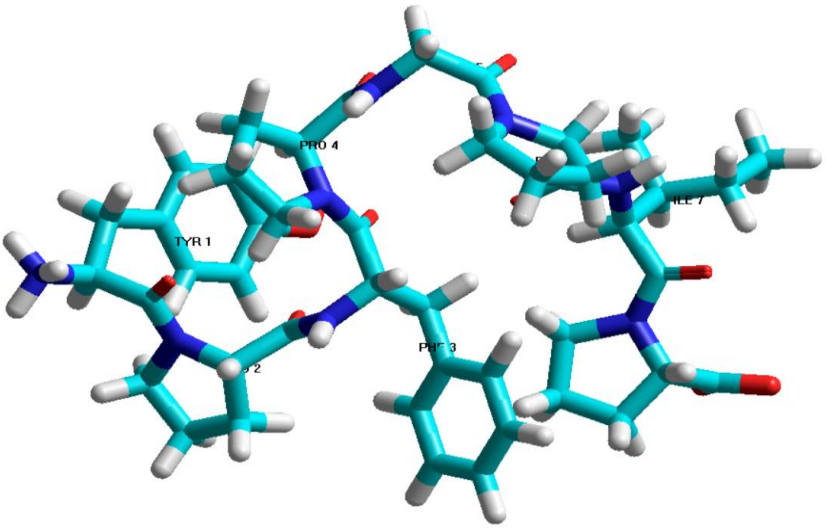
The relative energy of the $B_1BB_2BLRB_{22}R$ conformation of the β-casomorphin-8 molecule is 3.4 kJ/mol. Conformation stabilization is contributed by nonvalent interaction energy (105.8) kJ/mol, electrostatic interaction energy (-10.5) kJ/mol, and torsional interaction energy (95.8) kJ/mol. As can be seen, the contribution of nonvalent interaction energy to the stabilization of the conformation is the largest (Table 2). In this conformation, the Tyr1 amino acid interacts favorably with the Pro2-Phe3-Pro4 tripeptide fragment that follows it and contributes to the total energy (39.1) kJ/mol, the interaction of the Gly5 amino acid residue with the Pro6-Ile7 dipeptide fragment that follows it (-19.7) kJ /mol, the Pro6 amino acid residue interacts favorably with the following Ile7-Pro8 dipeptide fragment and contributes to the total energy (-21.4) kJ/mol and stabilizes it (Table 3).



a) $B_3RB_1RLBB_{32}B$



b) $B_1BB_2BLRB_{22}R$



c) $B_2BL_3RRBB_{32}R$

Figure 1. Atomic model of the spatial structure of the β-casomorphine-8 molecule a), b) and c) corresponded to the structures with the relative energies 0 kJ/mol, 3.4 kJ/mol, and 10.1 kJ/mol, respectively.

Another low-energy conformation of the β-casomorphin-8 molecule is B2BL3RRBB32R, belonging to the efeffee shape, with a relative energy of (10.1) kJ/mol. Nonvalent interaction energy (92.4) kJ/mol, electrostatic interaction energy (-4.6) kJ/mol, and torsional interaction energy (83.2) kJ/mol contribute to the stabilization of the conformation. In this conformation, Pro2-Phe3 and Pro4-Gly5-Pro6 are

in a folded form of the main chain (Table 2). The amino acid residue Tyr1 makes a favorable interaction with the tripeptide fragment Pro2-Phe3-Pro4, contributing to the total energy (-42.0) kJ/mol, the amino acid residue Phe3 makes a favorable interaction with the tripeptide fragment Pro4-Gly5-Pro6, contributing to the total energy (-32.8) kJ/mol, Gly5 and Pro6-Ile7 contributed to the total energy (18.9) kJ/mol, and the interaction between Pro6 and Ile7-Pro8 (12.2) kJ/mol made it low-energy (Table 3).

4. Conclusion

The study of the spatial structure of the β-casomorphin-8 molecule shows a sharp differentiation in terms of the shapes of the molecule, the shapes of the main chain, and the energies of the conformations. It is shown that the three-dimensional spatial structure of the molecule can be represented by six low-energy conformations of the three forms of the peptide chain. Geometrical and energy parameters of low-energy conformations interaction energies that stabilize them have been determined. The study of the spatial structure of the β-casomorphin-8 molecule shows that the molecule has such a set of spatial structures that it can perform various biological functions. The results obtained from studying the spatial structure of the β-casomorphin-8 molecule can be used to study other casomorphin molecules' spatial structures and structure-function relationships.

References

1. E.A. Chesnokova, N.Y. Sarycheva, V.A. Dubynin, A.A. Kamensky. Food-Derived Opioid Peptides and Their Neurological Impact, Advances in Physiological Sciences, **2015**, 46, 22-46.
2. Swati Garg, Kulmira Nurgali, Vijay Kumar Mishra, Food Proteins as Source of Opioid Peptides-A Review, Curr.Med.Chem, **2016**, 23, 893-910. DOI: 10.2174/0929867323666160219115226
3. V.A. Dubynin, Exorphins: possible biological and clinical significance, Psychiatry. **2010**, 45, 65-73.
4. N.A. Akhmedov, Theoretical conformation analysis of β- cazomorphin, valmuceptin and morphiceptin molecules, Molecular Biol., **1989**, 23, 240–248.
5. N.A. Akhmedov, N.M. Godjaev, E.V. Suleymanova, et al. Structural organization of the [Met] encephalin and endorphins molecules, J. Bioorganic. Chem., **1990**, 16, 649–667.
6. N.A. Akhmedov, L.I. Ismailova, R.M. Abbasli, et al., Spatial Structure of Octarphin molecule, IOSR. J Appl Phys (IOSR-JAP), **2016**, 8, 66–70.
7. N.A. Akhmedov, R.M. Abbasli, L.N. Agayeva, et al. Three-dimensional structure of exorpin B5 molecule. Conference proceedings Modern Trends in Physics, **2019**, 201–204.
8. N.A. Akhmedov, L.N. Agayeva, G.A. Akverdieva et al. Spatial structure of the ACTH-(6-9)-PGP molecule, J.Chem.Soc.Pak., **2021,** 43, 500–504. https://jcsp.org.pk/
9. N.A. Akhmedov, L.N. Agayeva, S.R. Akhmedova, et al. Spatial structure of the β- Casomorphin-7 molecule. IOSR J Appl Phys (IOSR-JAP) E-ISSN: 2278-4861. **2021**, 13, 62–67. DOI: 10.9790/4861-1305026267
10. L.N. Agayeva, A.A. Abdinova, S.R. Akhmedova, et al. The spatial structure of the ACTH-(7–10) molecule, Biophysics, **2021**, 66, 531–534. doi: 10.1134/S0006350921040023
11. Sh.N. Gadjieva, N.A. Akhmedov, E.A. Masimov, et al. Spatial structure of Thr-Pro-Ala-Glu-Asp-Phe-Met-Arg-Phe-NH2 molecule, Biophysics, **2013**, 58, 587–590. doi: 10.1134/S0006350913040052
12. N.A. Akhmedov, L.N. Agayeva, R.M. Abbasli, et al. Spatial structure of casoxin a molecule. Topical issues of biological physics and chemistry, Russ J Biol Phys & Chem., **2021,** 6, 62–68.
13. N.A. Akhmedov, L.N. Agayeva, R.M. Abbasli, L.I. Ismailova, Computer modeling of low-energy spatial structures of casoxin G molecule. Proceedings of the 8 th International Conference on control and optimization with industrial applications applications, **2022**, I, 63-65.
14. L.N. Agayeva, A.A. Abdinova, S.R. Akhmedova, et al. Spatial structure of Soymorphin-6 molecule, Biophyics, **2023**, 68, 1122–1127. doi: 10.1134/S0006350923060027
15. L.I. Ismailova, G.A. Akverdieva, S.D. Demukhamedova, et al. Molecular modelling of Pro-Gly gliproline and its complexes, Mosc Univ Phys Bull, **2023**,78, 668–680. doi: 10. 3103/S0027134923050077
16. N.A. Akhmedov, L.N. Agayeva, R.M. Abbasli, et al. Spatial structure of Casoxin C Molecule, Biophyics, **2024,** 69, 1122–1127.
17. L.N. Agayeva, L.I. Ismailova, N.A. Akhmedov, Spatial structure of the exorphin B4 molecule, Baku State University Journal of Physics & Space Sciences, **2024**, 1, 30-36.
18. L.I. Ismailova, N.A. Akhmedov, Spatial structure of the heptapeptide analog of Nociceptin molecule, Mediterranean Journal of Chemistry, **2024,** 14, 105-111.
19. L.N.Agayeva, N.A. Akhmedov, G.T. Imanova, Spatial Structure of the Casoxin D Molecule, Materials Research Innovations, Journal homepage: www.tandfonline.com/journals, **2024**, DOI:10.1080/14328917.2024.2342013, To link to this article: https://doi.org/10.1080/14328917.2024.2342013 Published online. .
20. IUPAC-IUB. Quantities, Units and Symbols in Physical Chemistry, **1993**, Blackwell Scientific Publications, Oxford.