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RESEARCH ARTICLE

The use of the docking studies with the purpose of searching Potential Antihypertensive Drugs

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ABSTRACT:

Pre-experimental studies *in silico* successfully used at various stages of the search and optimization of the structures of biologically active compounds. The purpose of present research was the search for perspective anti-hypertensive agents among 160 new synthesized N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives containing the morpholine, ethyl(propyl)morpholine, hydroxyethyl, allyl moiety in the structure and 5-substituted derivatives of 2-thio(amino)-1,3,4-thiadiazole using docking studies to two biotargets of angiotensin converting enzyme and angiotensin II receptor. Based on the results of a flexible molecular docking, a promising group of N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives containing the morpholine moiety for experimental screening for antihypertensive activity was selected. The high affinity of this series of substances is associated with the formation of bonds between the Oxygen atom of the morpholine cycle and the corresponding amino acid residue in the active site of the angiotensin converting enzyme II. According to data obtained, different lengths of alkyl chains between the Nitrogen atom of the morpholine cycle and the Nitrogen atom of 1,3-thiazole cycle of the test substances, have a significant impact on the general energy system of complexes formed during interaction of molecules of the substances under research and the enzyme specified.

KEYWORDS: Molecular docking, N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives, 5-substituted derivatives of 2-thio(amino)-1,3,4-thiadiazole, angiotensin converting enzyme inhibitors, angiotensin receptor blockers.

INTRODUCTION:

The scientific revolution in computer modeling has changed the process of searching new drugs. Experimental pharmacological screening is preceded by *in silico* studies[1-10]. *In silico* studies allow to reduce the cost of funds and the number of experimental laboratory animals. *In silico* study of the mechanisms of action of newly synthesized compounds on cellular and subcellular level is carried out by an approach to search molecules with affinity to a specific biological target[11].

The use of this approach allows to optimize structures of lead compounds; to conduct a virtual screening with the purpose of determining affinity of compounds to a particular biological target, simulate the binding of ligand-target interactions, taking into account the specificity of the interaction.

The purpose of conducting our molecular docking was study the possibility of new synthesized compounds to inhibit of angiotensin converting enzyme catalytic activity and the antagonistic properties of new synthesized compounds of angiotensin II receptor and as result – selection of promising for experimental research groups compounds.

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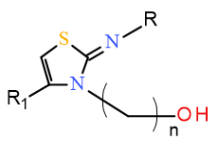
The renin-angiotensin system is one of the most important regulatory system of cardiovascular and renal function. Renin-angiotensin system blockade exerts potent antiatherosclerotic effects, which are mediated by their antihypertensive, anti-inflammatory, antiproliferative, and oxidative stress lowering properties. Inhibitors of the system, angiotensin converting enzyme inhibitors and angiotensin receptor blockers, are now first-line treatments for hypertensive target organ damage and progressive renal disease. Their effects are greater than expected by their ability to lower blood pressure alone. Angiotensin receptor blockers reduce the frequency of atrial fibrillation and stroke. Renin-angiotensin system blockade delays or avoids the onset of type 2 diabetes and prevents cardiovascular and renal events in diabetic patients. Thus, blockade of this system will remain a cornerstone of our strategies to reduce cardiovascular risk[12]. Although significant advances have been made in the therapeutic blockade of the renin-angiotensin-aldosterone system (RAAS or RAS) using angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers and non-selective aldosterone receptor antagonists, there is a clear need for both additional blocking strategies and enhancements of current therapeutic approaches[13].

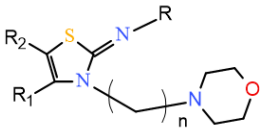
MATERIALS AND METHODS:

For the receptor-based flexible docking the software package Autodock 4.2.6 was used[14]. Docking studies for 160 substances N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives and 5-substituted derivatives of 2-thio(amino)-1,3,4-thiadiazole were performed. The synthesis of test compounds was described in articles[15,16]. Test compound were divided into 6 main groups on the basis of chemical structure:



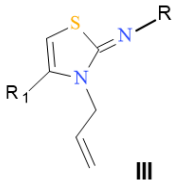
I

n = 2, 3;
R = Ar;
R_1 = Ar, Ad



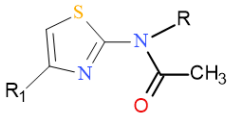
II

n = 0, 2, 3;
R = Ar;
R_1 = Ar, Alk;
R_2 = H, Alk



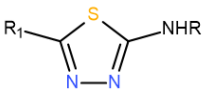
III

R = Ar;
R_1 = Ar



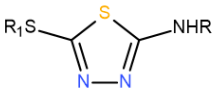
IV

R = Ar;
R_1 = Ar



V

R = H, Alk, Ar, SO_2Ar,
COR_2 (R_2 = Alk, Ar, Het);
R_1 = Ar, Alk, Het



VI

R = Ar, COR_2 (R_2 = Ar, Het, SO_2Ar);
R_1 = H, $CH_2CONR_3R_4$
(R_3 = H, Alk; R_4=Alk, Ar);
CH_2COR_5 (R_5 = Alk, Ar, Het)

Preparation of ligands was performed using such programs as Vega ZZ (command line) and MGL Tools 1.5.6[17]. For calculations in Autodock 4.2.6 program the input data for the receptor and ligands were converted in a special format PDBQT. The PDBQT file creation, calculation of torsion angles and removal of hydrogen atomsin non-polar atoms for the ligands studied were performed using Vega ZZ program (command line).

As a biological target for docking the active site of the macromolecule from Protein Data Bank (PDB) of angiotensin converting enzyme (PDB ID: 1R4L, 3NXQ, 4BZR) and angiotensin receptor II (PDB ID: 3R8A) were chosen[18-21]. The choice of a biological target was stipulate by the literature data concerning the mechanism of action of known antihypertensive drugs[22]. The receptor maps were prepared in MGL Tools and Auto Grid programs.

From PDB file ID: 1R4L, 3NXQ, 4BZR, 3R8A were removed. The following parameters of docking were determined: the translational motion step was equal to 2 A, the quaternion angle – 50°, the torsion angle – 50°. The torsion degree of freedom and the coefficient were 2 and0.274, respectively; the cluster tolerance – 2 Å; the external lattice energy– 1000, the maximal initial energy – 0, the maximal number of attempts – 10 000; the number of structures in the population – 300, the maximal number of stages of energy evaluation – 850 000, the maximal number of generations – 27 000, the number of structures, which pass to the next generation – 1, the level of gene mutation – 0.02,the level of

crossover – 0.8, the way of crossover – arithmetic. The α-parameter of Gaussian distribution was equal to 0, and β-parameter of Gaussian distribution – 1; the number of iterations of the Lamarckian genetic algorithm – 50 for eachligand. Visual analysis of the complexes of compounds from the active site of angiotensin converting enzyme (PDB ID: 1R4L, 3NXQ, 4BZR) and angiotensin receptor was performed using Discovery Studio Visualizer 4.0 program[23].

RESULT AND DISCUSSION:

As a result of the conducted molecular docking, the scoring function values for the investigated substances were calculated. Scoring function values allows us to assess the stability of the complexes formed between the ligands and the corresponding receptors, predict the ability of the compounds to inhibit the catalytic activity of the angiotensin converting enzyme and block the angiotensin receptor II.

The best affinity was observed in the case of binding molecules with crystallographic models of angiotensin-converting enzyme (PDBID: 1R4L) angiotensin receptor II (PDB ID: 3R8A) (Table 1).

Table 1: The range of calculated scoring function values for potential antihypertensives

PDB ID	1R4L	3NXQ	4BZR	3R8A
The number of group	**The range of calculated scoring function values, EDoc, kcal/mol**			
I	-6.10 – -8.72	-5.54 – -8.90	-5.16 – -7.50	-5.72 – -9.38
II	-5.03 – -10.15	-5.29 – -10.05	-6.17 – -8.52	-7.46 – -10.81
III	-6.61 – -8.06	-6.28 – -8.67	-6.98 – -7.92	-8.39 – -9.29
IV	-7.16 – -8.21	-6.87 – -7.78	-7.16 – -8.20	-8.66 – -9.50
V	-5.33 – -8.77	-5.13 – -8.51	-5.05 – -8.90	-4.03 – -9.68
VI	-4.66 – -9.73	-6.06 – -8.44	-6.36 – -9,11	-5.55 – -10.19

Complexes of almost all test compounds with angiotensin receptor II (PDB ID: 3R8A) give high absolute values of the scoring function, which is evidence of a higher thermodynamic probability of blocking activity of compounds precisely to this target. It should be noted that the highest absolute values of the scoring function in the case of complexes of substances with angiotensin converting enzyme (PDB ID: 1R4L) have substances of ***II*** group, namely N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives, containing a morpholine moiety in the structure – N-[4-methyl(aryl)-2-(R-phenylimino)thiazol-3-yl]-morpholine, 4-(R-phenyl)-3-[2-(morpholin-4-yl)ethyl]-N-phenyl-1,4-(R-phenyl)-3-[2-(morpholin-4-yl)-2-(R-phenylimino)thiazol-3-yl]-morpholine, 3-thiazol-2(3*H*)-imine, 4-aryl(methyl)-3-[3-(morpholin-4-yl)propyl]-N-phenyl-1,3-thiazol-2(3*H*)-imine, 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholin-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethan-1-one derivatives. In addition, the obtained data may reveal the structural and pharmacological regularity of the test compounds of this group in the presence of different lengths of the alkyl chain between the Nitrogen atom of the thiazole cycle and the Nitrogen atom of morpholine cycle.

During the analysis of the geometric location of the molecules of the tested substances of the ***II*** group in the active sites of the angiotensin converting enzyme and the angiotensin II receptor, it was found that it is precisely in molecules with an ethymorpholine fragment the Oxygen atom of morpholine is coupled to bio targets by means of hydrogen bonds, intermolecular electrostatic and donor-acceptor interactions.

The highest affinity (EDoc = -10,15 and -9.11 kcal/mol) in the case of binding angiotensin-converting enzyme (PDB ID: 1R4L) and lead compounds of 3-[2-(morpholin-4-yl)ethyl]-N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives was observed. Substances form complexes with angiotensin converting enzyme due to the unfavorable acceptor acceptor and hydrogen bond between the Oxygen atom of the morpholine fragment and the residues of Asp269 and Cys361. Pi-Cationic and Pi-anionic interactions are formed between the phenyl fragments of the molecules and the residues of Glu145 and Arg273. Pi-Alk and Alk interactions arise with the participation of aromatic and morpholine fragments of molecules with aminoacid residues of Leu144, Pro346, Trp271, Phe274, Arg273, Lys363 and Met360. Additional stabilization of complexes occurs as a result of Pi-Pi interaction of the phenyl and thiazole fragments of the molecules with residues Phe274 and His345 respectively (Fig. 1, 2).

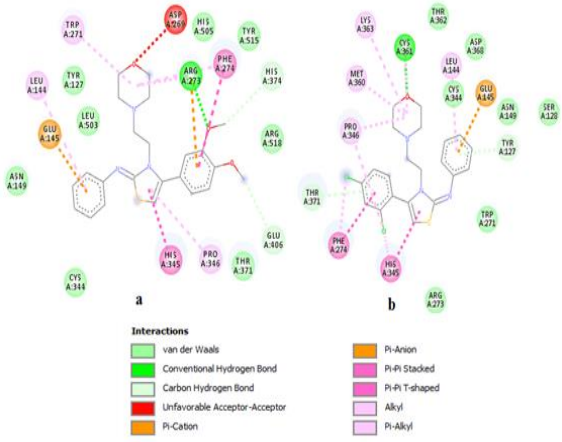


Fig.1: Diagrams of interaction of ligands in complexes with angiotensin converting enzyme (PDB ID: 1R4L) for lead compounds of 3-[2-(morpholin-4-yl) ethyl]-N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives

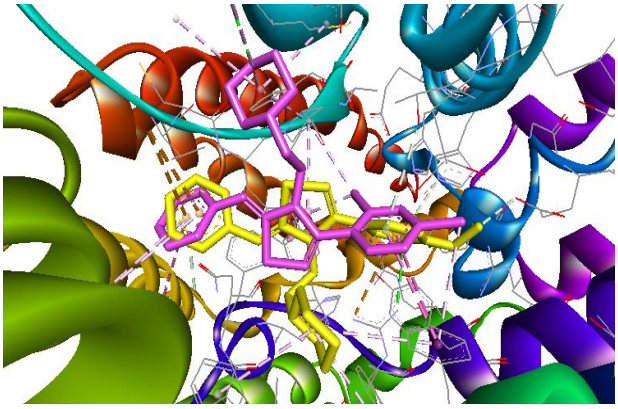


Fig. 2: Superposition of molecules a (yellow), b (lilac) in the active site of angiotensin converting enzyme (PDB ID: 1R4L)

The Oxygen atom of morpholine fragments does not participate in the formation of complexes of lead compounds of N-[4-methyl(aryl)-2-(R-phenylimino) thiazol-3-yl]-morpholine and 3-[3-(morpholin-4-yl)propyl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazole-2-imine derivatives (EDoc = -9,92 and -8,38 kcal/mol) with angiotensin converting enzyme. This is possible and explains the decrease in affinity for this target. Complexes by Pi-S interaction of Sulfur atoms of thiazole cycles of molecules with residues of His345, Trp271 and Pi-anionic interaction of phenyl fragments with residues Glu145 and Arg273 are formed. The presence of acetyl fragments in the position 5 of the thiazole cycle leads to the formation of a hydrogen bond between the Oxygen atom and the residue of tyrosine Tyr127. Pi-Alk and Alk interactions arise with the participation of alkyl and morpholine fragments of molecules with residues of Leu144, Leu503, Cys344, Pro346, Phe274, Arg273, His345, His505. Complexes of 3-[morpholin-4-yl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazol-2-imine derivatives with angiotensin converting enzyme have additional stabilization due to Pi-H and Pi-Pi interactions between thiazole and phenyl fragments of molecules with amino acid residues of Trp273 and Tyr127, respectively. In contrast to the 3-[3-(morpholin-4-yl)propyl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazole-2-imine derivatives, which do not have additional stabilization, but have additional carbon hydrogen bonds between morpholine cycle and residue of His345 (Fig. 3, 4).

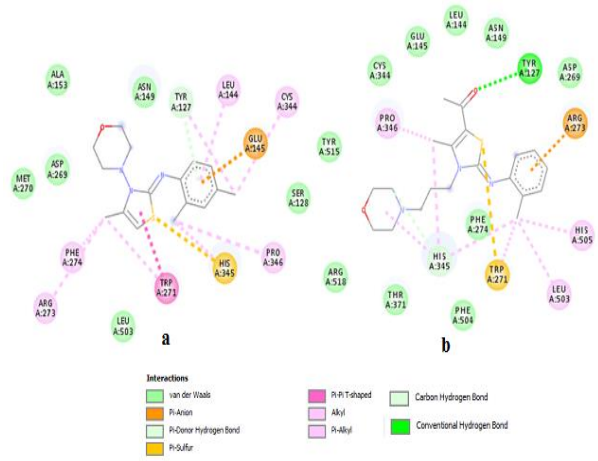


Fig. 3: Diagrams of interaction of ligands in complexes with angiotensin converting enzyme (PDB ID: 1R4L) for lead compounds of N-[4-methyl(aryl)-2-(R-phenylimino)thiazol-3-yl]-morpholine and 3-[3-(morpholin-4-yl)propyl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazole-2-imine derivatives

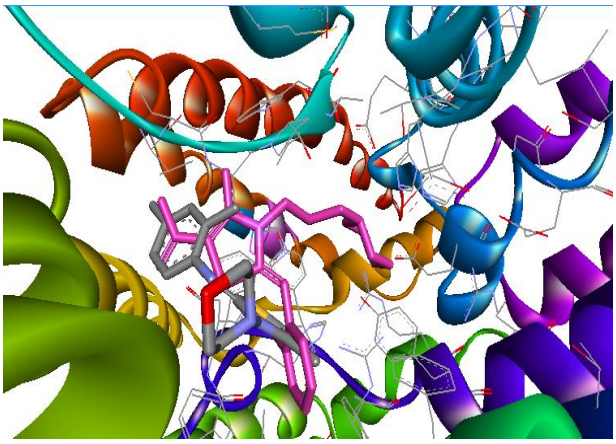


Fig. 4: Superposition of molecules a (gray), b (lilac) in the active site of angiotensin converting enzyme (PDB ID: 1R4L)

Complexes of the lead compounds of 3-[2-(morpholin-4-yl)ethyl]-N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives with angiotensin receptor II (EDoc= -10,81 and -10,27 kcal/mol) are formed with the participation of Oxygen atoms of the morpholine fragment with His449 residue by means of a Pi-donor hydrogen bond. Pi-S and Pi-sigma bonds arise between the aromatic systems of the molecules with aminoacid residues of Met348, Ile341 and Ala292, respectively. Pi-Alk and Alk interactions are formed between phenyl fragments, morpholine and thiazole cycles of amino acid residues Ile326, Ile341, Il281, Met348, Met364, Phe363, Cys285, His449, Arg288 and Leu330. The presence of carbon hydrogen bonds between the alkyl and morpholine fragments of the molecules with the residues of Cys285 and Ser289, respectively, also contribute to the formation of complexes (Fig. 5,6).

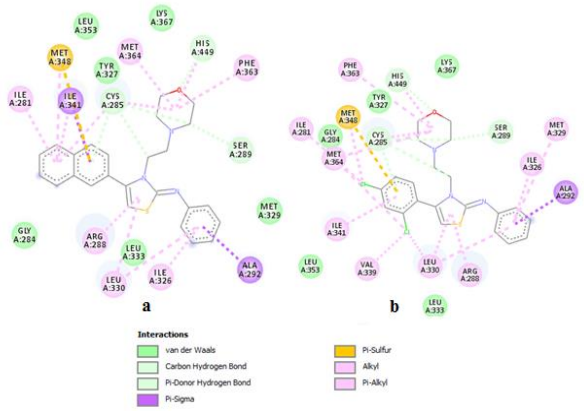


Fig. 5: Diagrams of interaction of ligands in complexes with angiotensin converting enzyme II (PDB ID: 3R8A) for lead compounds of 3-[2-(morpholin-4-yl)ethyl]-N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives

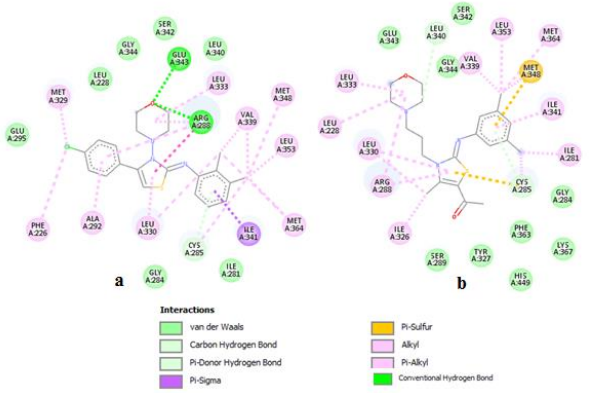


Fig. 7: Diagrams of interaction of ligands in complexes with angiotensin converting enzyme II (PDB ID: 3R8A) for lead compounds of N-[4-methyl(aryl)-2-(R-phenylimino)thiazol-3-yl]-morpholine and 3-[3-(morpholin-4-yl)propyl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazole-2-imine derivatives

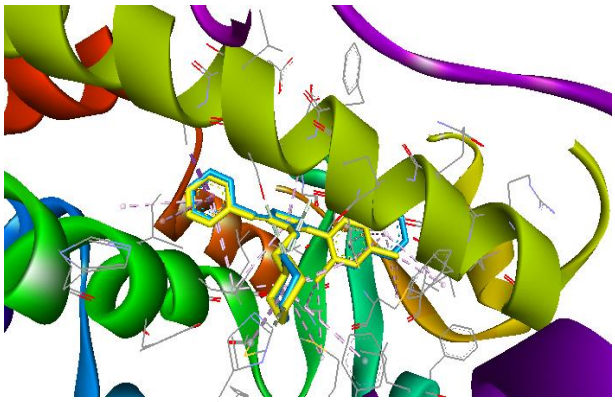


Fig. 6: Superposition of molecules a (blue), b (yellow) in the active site of angiotensin converting enzyme II (PDB ID: 3R8A)

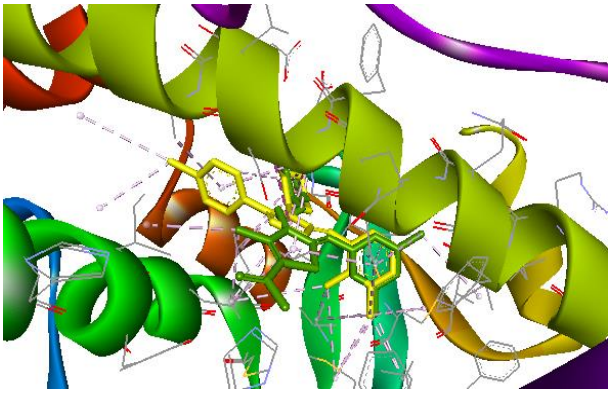


Fig. 8: Superposition of molecules a (yellow), b (green) in the active site of angiotensin converting enzyme II (PDB ID: 3R8A)

For lead compounds N-[4-methyl(aryl)-2-(R-phenylimino)thiazol-3-yl]-morpholine and 3-[3-(morpholin-4-yl)propyl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazole-2-imine derivatives (EDoc = -9,98 and -9,79 kcal/mol) Pi-donor hydrogen bonds formed between the phenyl fragment of the molecules and the residue of Cys285 contribute to the formation of complexes with the angiotensin II receptor. The formation of complexes promotes Pi-Alk and Alk interactions between phenyl fragment, morpholine and thiazole cycles with residues of aminoacids Ile341, Ala292, Arg288, Leu330, Leu333 and Leu228. The presence of a hydrogen bond between the Oxygen atom of the morpholine cycle with the residues of Glu343 and Arg288, the Pi-sigma bond between the phenyl fragment and the Ile341 residue is characteristic for the formation of complexes of 3-[morpholin-4-yl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazole-2-imine derivatives with an angiotensin II receptor. The presence of Pi-S bonds, arising between the phenyl and thiazole fragments with the residues of Met348 and Cys285, respectively, for the 3-[3-(morpholin-4-yl)propyl]-N-(R-phenyl)-2,3-dihydro-1,3-thiazole-2-imine derivatives is characterized (Fig. 7,8).

Thus, the inhibitory activity of the test compounds in relation to the angiotensin converting enzyme and the angiotensin II receptor by forming a complex between them may be realized. The stability of those complexes mainly due to the energetically favorable geometric location of the ligands in the active centers of these acceptors, the formation of hydrogen bonds between them, intermolecular electrostatic and donor-acceptor interactions was provided. As a result, the thermodynamic probability of this binding was confirmed by negative values scoring function.

CONCLUSION:

Based on the results of a flexible molecular docking, a promising group – N-R-phenyl-2,3-dihydro-1,3-thiazole-2-imine derivatives containing the morpholine fragment (group ***II***) for experimental screening for antihypertensive activity is selected. The high affinity of this series of substances is associated with the formation of bonds between the Oxygen atom of the morpholine cycle and the corresponding aminoacid residue in the active site of the angiotensin converting enzyme II.

According to data obtained, different lengths of alkyl chains between the Nitrogen atom of the morpholine cycle and the Nitrogen atom of 1,3-thiazole cycle of the test substances, have a significant impact on the general energy system of complexes formed during interaction of molecules of the substances under research and the enzyme specified.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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