MOLECULAR DOCKING STUDIES OF SOME N-AMIDOALKYLATED DERIVATIVES OF 2-AMINO-1,3,4-OXADIAZOLE AS POTENTIAL INHIBITORS OF GLYCOGEN SYNTHASE KINASE-3β

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Abstract: Alzheimer's disease is a neurodegenerative disease characterized by pathological features of neurofibrillary tangles and β-amyloid plaques in the cerebral cortex. In Alzheimer's disease, tau protein undergoes excess phosphorylation, due to which its threads begin to merge and form neurofibrillary tangles within nerve cells. It has been shown that glycogen synthase kinase-3β is a key factor in the phosphorylation of tau protein, its increased activity leading to pathologies of neurofibrillary tangles and, consequently, to neurodegenerative changes in the brain. In this connection, the search for effective inhibitors of GSK-3β is a very important and urgent task, for their further use in the treatment of Alzheimer's disease.

Aim of research. The aim of this study is to search new inhibitors of GSK-3β among N-amidoalkylated derivatives of 2-amino-1,3,4-oxadiazole by molecular docking methods.

Materials and methods. We have carried out geometry optimization of analyzed structures within PM3 semi-empirical method, and GSK-3β molecular docking using software ArgusLab 4.0.1. The three-dimensional crystal structure of co-crystallizer GSK-3β and inhibitor has been loaded from the data bank of protein molecules (PDB ID: 3F7Z).

Results. In this study it has been shown that the structures being studied mainly form stronger complexes with the enzyme compared to the known inhibitor. Based on the results of molecular docking, the compounds leaders N-(((5-(2-bromophenyl)-1,3,4-oxadiazol-2-yl)amino)methyl)benzamide and 2,4-dichloro-N-(2,2,2-trichloro-1-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)amino)ethyl)benzamide have been chosen. The structures of the compounds leaders have been tested for compliance with Lipinski criteria.

Conclusions. Proposed compounds leaders can be recommended for further studies in the treatment of Alzheimer's disease. Despite the good results obtained in silico analysis, it is mandatory to perform biological tests in vitro and in vivo.

Keywords: Alzheimer's disease, 1,3,4-oxadiazole, docking, GSK-3β, inhibitors, synthesis, in silico, ArgusLab, N-amidoalkylated, RMSD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by pathological features of neurofibrillary tangles (tau pathology) and β-amyloid plaques in the cerebral cortex. In this case, pathology of β-amyloid plaques varies considerably, and neurofibrillary tangles are closely correlated with symptoms of the disease and its progression [1]. Pathology of neurofibrillary tangles is associated with abnormal aggregation of tau protein. Tau protein is associated with microtubules and stabilizes them after phosphorylation. Microtubules are fulfilling the role of the cytoskeleton of the neuron. They are involved in the transport of vital substances from the cell center towards the end of the axon and back. In Alzheimer's disease, tau protein undergoes excess phosphorylation, due to which its threads begin to merge and form neurofibrillary tangles within nerve cells. This causes the destruction of microtubules and the collapse of the transport system within the neuron [2, 3]. Initially, this leads to disruption of the biochemical signals transmission between cells, and then to their death [4].

It has been shown that GSK-3β (glycogen synthase kinase-3β) is a key factor in the phosphorylation of tau protein [5], its increased activity leading to pathologies of neurofibrillary tangles and, consequently, to neurodegenerative changes in the brain [6, 7]. In this connection, the search for effective inhibitors of GSK-3β is a very important and urgent task, for their further use in the treatment of Alzheimer's disease. In recent years, an increasing number of works devoted to this subject has appeared [8, 9].

As promising inhibitors of GSK-3β of 2-mercapto-1,3,4-oxadiazole derivatives (**Fig. 1, *a***) have been proposed [10].

In this paper, we have proposed *N*-amidoalkylated derivatives of 2-amino-1,3,4-oxadiazole as potential inhibitors of GSK-3β (**Fig. 1, *b***). The search for compounds leaders has been based on the results of the molecular docking [12]. The structures of the compounds for the prediction have been taken from our virtual library, their general synthesis methodology having been developed by us earlier [11, 13, 14].

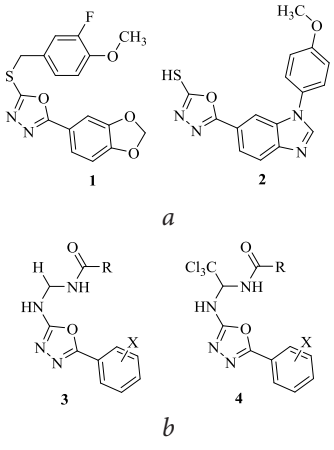


Fig. 1. The structures of some inhibitors of GSK-3β: *a*– 2-mercapto-1,3,4-oxadiazole derivatives [10]; *b* – structures of *N*-amidoalkylated derivatives of 2-amino-1,3,4-oxadiazole [11]

2. Materials and methods

We have carried out geometry optimization of analyzed structures within PM3 semi-empirical method, and GSK-3β molecular docking using software ArgusLab4.0.1 [15]. Previously, this software package was successfully used to solve similar problems [16, 17].

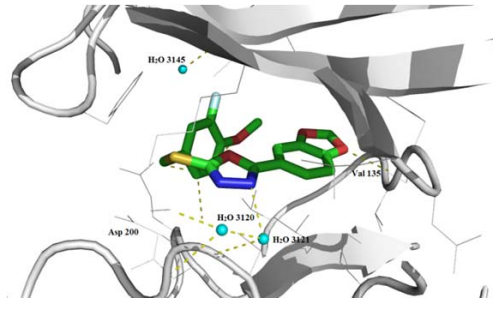
The three-dimensional crystal structure of co-crystallizer GSK-3β and 2-(benzo[*d*][1,3]dioxol-5-yl)-5-((3-fluoro-4-methoxybenzyl)thio)-1,3,4-oxadiazole (**1**) (PDB ID: 3F7Z) has been loaded in PDB format from the data bank of protein molecules (http://www.rcsb.org). The protein molecule is symmetrical and contains two active sites, and consequently, co-crystallizer contains two molecules of inhibitor (**1**). To be able to assess the root-mean-square deviation of atomic positions (RMSD) one molecule from (**1**) was previously removed. Crystalline inclusions PTR 216 and PTR 560 were also removed for ease of operation. Before the molecular docking, the hydrogen atoms were added throughout the protein structure. The molecules of crystal water were not removed from the binding site since they were involved in the binding of the inhibitor by means of hydrogen bonds [10].

On the basis of remaining inhibitor molecule (**1**) (code in co-crystallizer 3000 340), we have created ligand group named Ligand_X-ray. A three-dimensional model of the binding site has been created on the basis of this group, its dimensions being

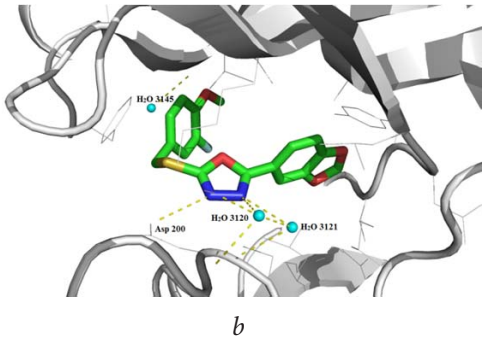
calculated automatically and being along the X-axis – 19.967, Y-axis – 15.898 and the Z-axis – 17.203 Å. Docking has been done with a flexible ligand. A semi-empirical AScore function has been used to the scoring procedure, based on the XScore function [18]. The cell resolution has been set at 0.250 Å. The calculation type has been Dock; Docking Engine – ArgusLab. Visualization of the results has been carried out using program PyMOL [19].

3. Results

The active GSK-3β site is a large lipophilic pocket with small polar regions at opposite ends. In these areas, there are molecules of crystalline water, which take an active part in ligand-protein interactions. In carrying out molecular docking, we have used 2-(benzo[*d*][1,3]dioxol-5-yl)-5-((3-fluoro-4-methoxybenzyl) thio)-1,3,4-oxadiazole (**1**) as a reference. According to X-ray diffraction analysis, the molecule of compound (**1**) interacts additionally with the active site of GSK-3β due to the formation of a complex system of intermolecular hydrogen bonds involving water molecules H_2O 3121 and H_2O 3120 and the Nitrogen atom of pyridine type of N(4) 1,3,4-oxadiazole rings. The Oxygen atom of the benzodioxole cycle and the amino acid valine 135 also form the hydrogen bond, the bond length is 2.887 Å. The calculated energy of inhibitor binding (**1**) with the active site of the enzyme is -9.8475 kcal/mol, the calculation time – 5 seconds. The calculated position of the inhibitor in the active site of the enzyme is similar to the results obtained by X-ray analysis (**Fig. 2**), root-mean-square deviation of atomic positions (RMSD) is 1.4 Å. According to molecular docking data, the molecule of compound (**1**) interacts additionally with the active site of GSK-*3β* due to the formation of a complex system of intermolecular hydrogen bonds involving water molecules H_2O 3121 and H_2O 3120 and the Nitrogen atom of pyridine type of N(4) 1,3,4-oxadiazole rings. The Nitrogen atom of pyridine type of N(3) 1,3,4-oxadiazole rings and the amino acid asparagine 200 also form the hydrogen bond, the bond length is 3.316 Å.



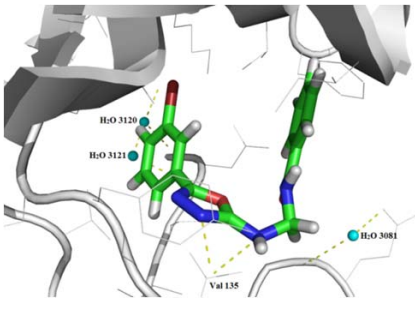
a



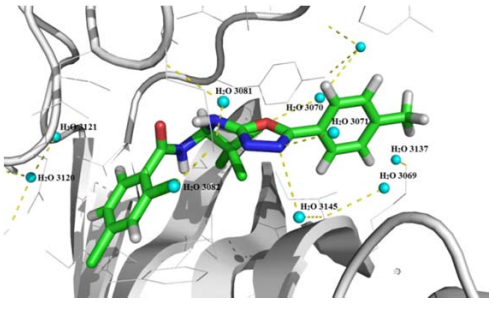
b

Fig. 2. Position of compound (**1**) in active site of GSK-3β: *a* – according to X-ray analysis data [10]; *b* –according to molecular docking

According to the molecular docking, compounds (**3**), (**4**) have formed the most stable complexes with GSK-3β. *N*-(((5-(2-Bromophenyl)-1,3,4-oxadiazol-2-yl)amino)methyl)benzamide (**3**) effectively binds to GSK-3β by the formation of two intermolecular hydrogen bonds (**Fig. 3, *a***). Hydrogen bonds are formed by 1) The Nitrogen atom N(3) of the 1,3,4-oxadiazole cycle and -NH group of valine (135), bond length 2.988 Å; 2) The Hydrogen atom of the amine moiety and the Oxygen atom of valine (135), bond length 2.853 Å. The molecule of 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)amino) ethyl)benzamide (**4**) is additionally fixed in the active center of InhA enzyme due to the complex system of intermolecular hydrogen bonds involving the molecules of crystalline water – H_2O 3070, H_2O 3071 and H_2O 3145 (**Fig. 3, *b***).



a



b

Fig. 3. Position of *N*-amidoalkylated derivatives of 2-amino-1,3,4-oxadiazolein GSK-3β active site according to the results of molecular docking: *a* – *N*-(((5-(2-bromophenyl)-1,3,4-oxadiazol-2-yl)amino) methyl)benzamide (**3**); *b* – 2,4-dichloro-*N*-(2,2,2-tric hloro-1-((5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)amino)ethyl) benzamide (**4**)

The compounds leaders have been tested for compliance with Lipinski criteria using Molinspiration web resource (http://www.molinspiration.com/cgi-bin/properties). The compound (**3**) corresponds to them, while the compound (**4**) does not, since it has lipophilicity (log P) above 5.0.

4. Discussion

The above results showed that the novel *N*-amidoalkylated derivatives of 2-amino-1,3,4-oxadiazole has excellent potential as inhibitors of GSK-3β. They can be recommended for further studies in the treatment of Alzheimer's disease. Their potential activity may be attributed with the significant interactions towards the inhibitor binding cavity of GSK-3β [20]. Also the molecules of the compounds leaders are additionally stabilized in the GSK-3β active center due to the formation of intermolecular hydrogen bonds [12]. Despite

the good results obtained *in silico* analysis, it is mandatory to perform biological tests *in vitro* and *in vivo*. It should be noted that in addition to the compounds analyzed, similar series of 1,3,4-thiadiazole and 1,2,4-triazole derivatives should be studied, since their derivatives also have activity against Alzheimer's disease [21, 22].

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