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Research Article

Molecular docking studies of N-substituted 4-methoxy-6-oxo-1-aryl-pyridazine-3-carboxamide derivatives as potential modulators of glutamate receptors

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Abstract

Introduction: The virtual target-oriented screening is a necessary stage of modern drug-design. In the present study, the affinity of pyridazine derivatives for the most promising antiparkinsonian biotargets – I–III groups of metabotropic and ionotropic NMDA-glutamate receptors – was evaluated.

Materials and methods: Docking of the studied ligands to the active sites of biotargets – mGluR5, mGluR3, mGluR8, NMDA GluN2B – was performed using AutoDockVina. Base of the preparation of ligands and proteins – AutoDock-Tools-1.5.6. A Discovery Studio Visualizer 2017/R2 was used to visualize the interpretation of the results.

Results and discussion: A high degree of the affinity is predicted for group III of the metabotropic mGlu8 receptors – binding energy from -5.0 to -8.7 kcal/mol, compared to -6.1 kcal/mol of that of the reference drug (L-AP4), as well as for the ionotropic NMDA GluN2B receptors –binding energy from -8.7 to -11.6 kcal/mol, compared to -11.3 kcal/mol of that of ifenprodil.

Conclusion: The prospects of the searching for glutamate receptor modulators in a number of n-substituted 4-methoxy-6-oxo-1-aryl-pyridazine-3-carboxamide derivatives are proved. Some aspects of the structure-affinity relationship are discussed.

Keywords

pyridazine, antiparkinson agents, docking, mGluR, NMDA.

Introduction

Scientific achievements of the last decades concerning the mechanisms of the antiparkinsonian action, the crystal structure of target proteins and the amino acid composition of active sites of receptors, the developed arsenal of *in silico* methods for analyzing and evaluating the affinity of the ligand for the receptor makes it possible to rationalize the search for new biologically active substances, in particular, antiparkinsonian ones.

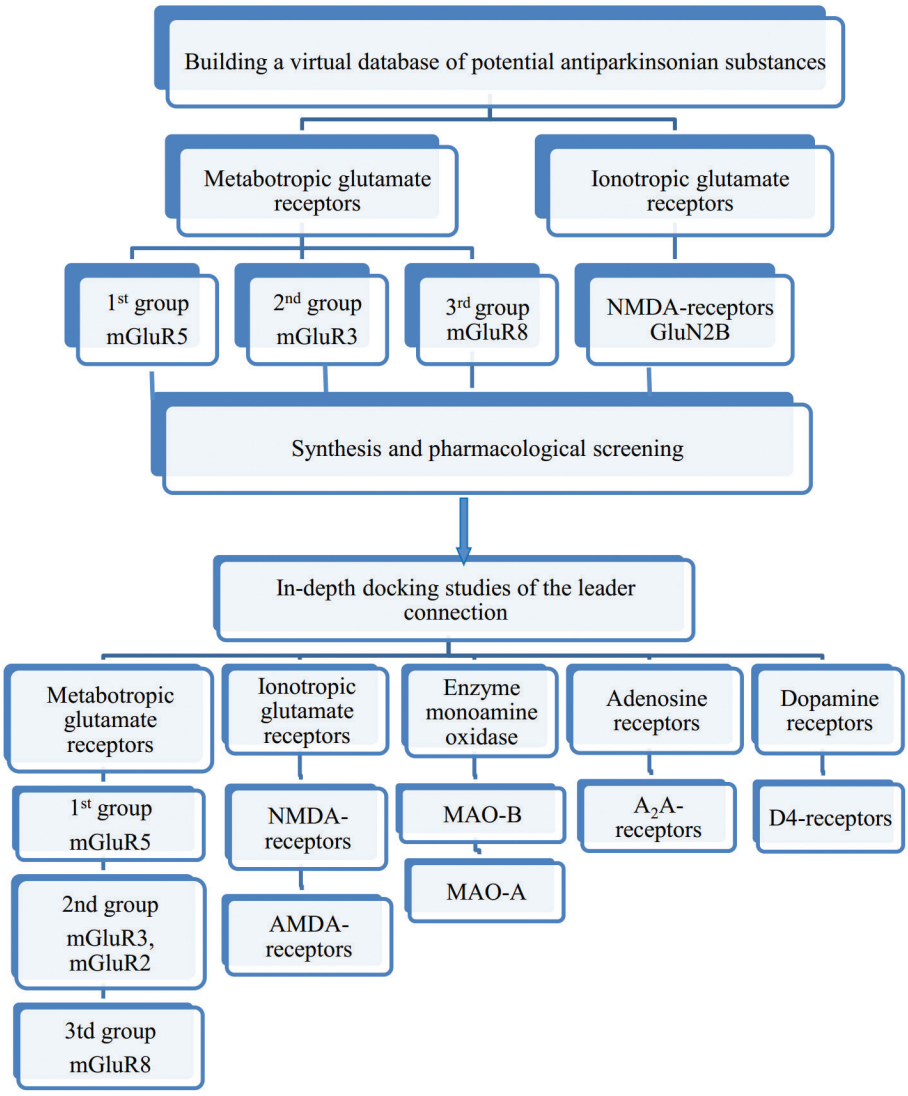
The feasibility and prospects of searching for antiparkinsonian substances among n-substituted 4-methoxy-6-oxo-1-aryl-pyridazine-3-carboxamide derivatives was evaluated using the virtual screening tools, namely, molecular docking to the active sites of the known antiparkinsonian biotargets.

In accordance with the research algorithm, the first stage of research is to assess the affinity for the metabotropic glutamate receptors (mGluR). The prospect of studying the compounds affecting mGluR as potential antiparkinsonian agents was proved in 2003 (Marino et al. 2003) and continues to be relevant nowadays (Avdeeva et al. 2017; Zhang et al. 2019).

Structurally, mGluR belong to the C-class of G-protein-coupled receptors. According to the functional activity, structure homology, and the list of selective ligands, all mGlu receptors are divided into 3 groups, which in turn are subdivided into 8 more subtypes (Kunishima et al. 2000; Kravchenko et al. 2016; Avdeeva et al. 2018).

All glutamate receptors have a modular architecture (Jin and Ma 2017), wherein each of the subunits is made up of 4 main parts: an amino-terminal domain (ATD) located in the extracellular space, a ligand-binding domain (LBD) also located in the extracellular space, a transmembrane domain (TD) located inside the membrane, and a C-terminal domain (CTD) located in the intracellular space.

Objective of the study: To conduct the docking and to evaluate the affinity of the virtual ligand base among



Flowchart 1. The algorithm of the searching for the antiparkinsonian agents.

n-substituted 4-methoxy-6-oxo-1-aryl-pyridazine-3-carboxamide derivatives for the active sites of the most promising antiparkinsonian biotargets – the metabotropic receptors of groups I-III and ionotropic NMDA receptors of glutamate.

Materials and methods

The affinity for a biological target was studied using flexible molecular docking. For this purpose, the AutoDock Vina program was used, which gives a good correlation indicators between the calculated and experimentally obtained data (Wang et al. 2016). The ability of the used docking algorithm to reproduce the experimental data was evaluated by the reference docking of the native ligands. Macromolecules (proteins) from the Protein Data Bank (PDB) were used as the biological targets, which are freely available: metabotropic glutamate receptors: mGlu5 – PDB ID 6FFH, mGlu3 – PDBID 4XAR, mGlu8 – PDBID 6BT5, and ionotropic NMDAgluN2B receptors of glutamate – PDBID 3QEL.

Preparation of the ligands

The substance structures were obtained using MarvinSketch 18.23 and saved in mol format. After that, they were optimized by Chem3D, using the molecular mechanics (MM2) algorithm and saved as pdb files. Using AutoDockTools-1.5.6, pdb files were converted to PDBQT (Trott and Olson 2010).

Preparation of the proteins

PDB files were downloaded from The Protein Data Bank. Discovery Studio Visualizer 2017/R2 was used to remove water and ligand molecules from the crystal. Protein structures were saved as pdb files. In AutoDockTools-1.5.6, polar hydrogens were added to the protein structure and stored as PDBQT. Gridboxes were established relative to native ligands. AutoDock Vina was used for docking (Trott and Olson 2010). Discovery Studio V17.2. 0.16349 was used to visualize the docking results.

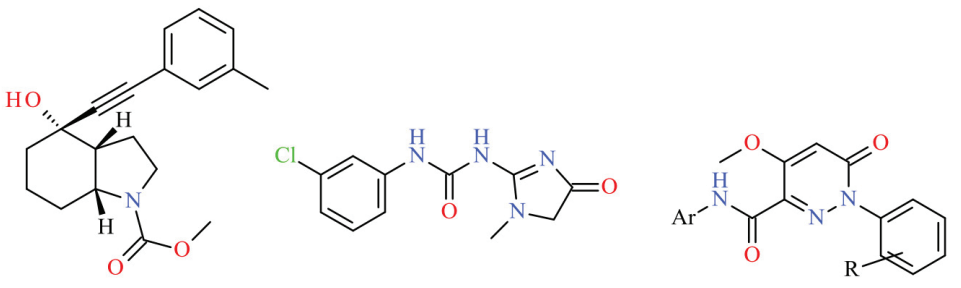
Results and discussion

Stage I of the study: the assessment of the compounds affinity for metabotropic glutamate receptors of Group I

The affinity of the virtual data base of compounds for Group I of the metabotropic glutamate receptors was evaluated by docking of the mGlu5 receptor into a active site. The crystal structure of the transmembrane domain of mGlu5 receptor in a closed conformation with the negative allosteric modulator mavoglurant in the active site was established in 2014 (Doré et al. 2014). Despite the fact that mavoglurant passed the second phase of the clinical trials for the treatment of Levodopa-induced dyskinesia, the affinity of potential antiparkinson agents for the active site of the transmembrane domain of mGlu5 (PDB 6FFH) was evaluated in comparison with fenobam – an mGluR5-selective negative allosteric modulator (Christopher et al. 2019). The choice of fenobam as a reference drug for assessing the affinity for the mGluR5 receptor is due to some structural similarity with the substances to be synthesized (Fig. 1).

The transmembrane domain of the mglu5 receptor consists of seven transmembrane α-helices (Fig. 2A). The configuration of the helical bundle together with extracellular loops strongly restricts the entrance to the allosteric pocket, which results in its relatively small size – the entrance radius is about ~7 A°. In addition, the allosteric site itself is located at a distance of about 8 A° from the receptor surface and is a fairly narrow hydrophobic space. This size of the pocket entry and the location of the active site condition a small size of the ligand and can impair the scoring functions during docking.

According to the literature data (Doré et al. 2014, Christopher et al. 2019), the main hydrophobic pocket of the active site is formed by residues of the following amino acids: valine (Val806), methionine (Met802), phenylalanine (Phe788), tryptophan (Trp785), leucine (Leu744), isoleucine (Ile651), proline (Pro655), asparagine (Asn747), and glycine (Gly652). The main ”shrinkage” of the ligand occurs between alanine (Ala810) and proline (Pro 655), bordered by isoleucine (Ile625), glycine (Gly624), serine (Ser654 and 658) on one side, and by tyrosine (Tyr659) – on the other. Hydrophobic interac-



Mavoglurant Phenobam Potential antiparkinsonic substances

Figure 1. The structure of the mGluR5 negative allosteric modulators in comparison with the ligands under study.

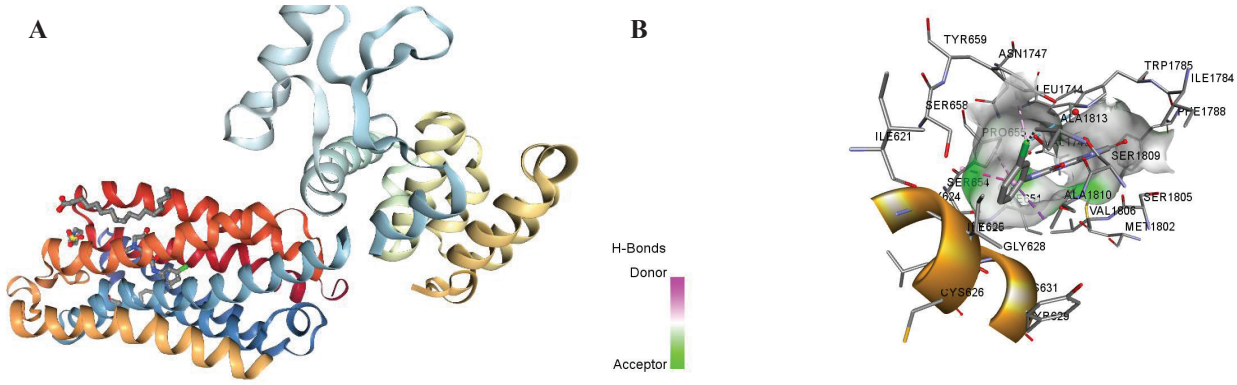


Figure 2. A. 3D structure of mGlu5 with a negative allosteric modulator fenobam in the active site **B.** 3D image of the fenobam conformation in the mGluR5 active site.

tion occurs with tyrosine (Tyr659), serial (Ser 809) valine (Val806), and proline (Prp 655).

Despite the location of the allosteric site and its narrow size, when evaluating the reproducibility of the docking technique in mGluR5 of the native ligand, it was possible to achieve a suitable conformation and a satisfactory evaluation function, which was -8.7 kcal/mol for fenobam. As seen in Fig. 2B, fenobam is completely immersed in a narrow hydrophobic cleft of the active site of the receptor, forming a stable conformation by entering a hydrophobic interaction; this conformation is further stabilized by the hydrogen bonds, in particular with the hydroxyl groups of serine and tyrosine (Ser809, Tyr659), which is consistent with the literature data (Christopher et al. 2019).

At the stage of the selecting compounds for synthesis, no detailed analysis of conformational placement was carried out, with only the binding energy (the scoring function) being taken into account in comparison with the native ligand. The results of the docking of the compounds with the active site of the mGluR5 receptor are presented in Table 1.

As can be seen from the results of the docking, a satisfactory level of affinity for the active site of the mGlu5 receptor is predicted for pyridazine derivatives: the binding energy was from -11.2 to -5.2 kcal/mol, versus -8.7 kcal/mol for the native fenobam ligand. The highest(unfavorable) binding energy level was demonstrated by the compound with the N-(4-diethylamino)phenyl substituent (0182), and the lowest – with the diphenylpropyl in the carboxamide fragment (0131), which may be explained by the conformational mobility of the radical and by the additional centers of the hydrophobic interaction. The compounds with a benzyl amide fragment have a higher affinity than the substances with a phenylamide radical. A decrease in the affinity is predicted for all the compounds that have a substituted NH-group of the carboxamide residue (for example, 0129, 0265, 0249), which is probably due to the ability of the NH-group to form hydrogen bonds to stabilize the ligand in the cavity of the active site.

Stage 2 of the study: the evaluation of the compounds affinity for the metabotropic glutamate receptors of Group II

At the next stage, the virtual database of compounds was docked into the active site of the metabotropic glutamate receptor of Group II subtype 2 – mGluR3 (PDB ID 4XAR). A strong allosteric agonist of the mglur2/3 receptor (1S, 2S, 5R, 6S)–2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740)) was used as a reference ligand (Schoepp et al. 2003, Linden et al. 2005, Menezes et al. 2013).

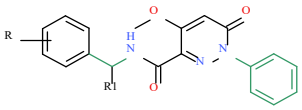
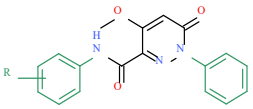
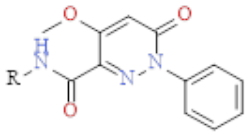
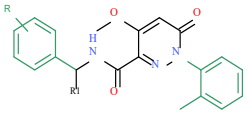
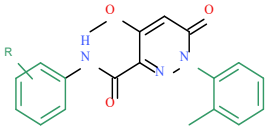
The recombinant protein of the mGlu3 amino-terminal domain in the conformation with Ly354740, from the macroscopic point of view, is a closed topology (Monn et al. 2015), where the upper (LB1) and lower (LB 2) lobes of the protein are closely related to the ligand in the hinge region (Fig. 3A). The binding of the agonist induces the process of joining the protein lobes, which in turn leads to the opening of the channel and the activation of the receptor.

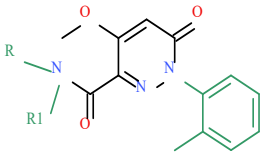
The visualization of the experimentally established interaction and the image of the ligand-binding pocket are shown in Figure 3B. Describing the binding site, it should be noted that there is a fairly spacious entrance to the binding pocket, the predominant majority of hydrogen interactions, including the tetrahedral network of ligand amino group bonds with the carboxyl groups of alanine and asparagine (Ala172, Asp301), and the threonine hydroxyl (Thr 1744), participation in the interaction of both protein lobes (LB1 and LB2), and the possibility of the ligand conformational mobility.

The amino acids of the binding site, according to the literature data, are (Fig. 4A):

- the upper lobe (LB 1) – arginine (Arg68), lysine (Lys 389), alanine (Ala172), threonine (Thr174), serine (Ser 151), which participate in the formation of hydrophilic bonds; tyrosine (Tyr222) – 4-hydroxyphenyl radical enter into hydrophobic interaction (Fig. 5A);
- the lower lobe (LB2) – aspartic acid (Asp301) and tyrosine (Tyr 150).

Table 1. Results of docking of N-substituted 4-methoxy-6-oxo-1-arylpyridazine-3-carboxamides with the glutamate receptors.

Ligand	R/R1	Receptors			
		mGluR5 (6FFH)	mGlu3 (4XAR)	mGlu8 (6BT5)	NMDA Glu N2B (3QEL)
		Binding energy (kcal/mol)			
Native reference ligand		-8.7 (fenobam)	-8.2 (Ly235)	-6.1 (L-AP4)	-11.3(Ifenprodil)
					
0057	4-Me/H	-7.5	-6.7	-7.5	-10.4
0077	4-F/H	-9.2	-6.1	-7.9	-10.3
0098	4-Cl/H	-8.9	-6.5	-7.4	-9.4
0102	2-OMe/H	-8.9	-7.2	-7.9	-8.9
0128	H/Me	-8.0	-6.5	-8.2	-10.0
0129	H/Et	-6.1	-5.2	-6.7	-8.4
					
0058	2-Me,5-Cl	-8.2	-5.5	-7.9	-10.1
0066	3,5-diMe	-8.7	-6.6	-8.1	-10.1
0067	2,6-diMe	-7.2	-6.6	-6.0	-10.3
0070	4,5-diMe	-9.4	-5.1	-8.6	-10.7
			-6.7	-6.7	-10.2
			-5.8	-6.7	-10.5
			-6.7	-6.9	-10.2
0095	3-Me,6-OMe	-7.5	-5.3	-7.4	-9.9
0101	2-Me,3-Cl	-8.6	-5.3	-6.6	-10.4
0105	2-Me, 4-Br	-8.7	-5.5	-6.2	-10.6
					
0060	$CH(Ph)_2$	-7.5	-5.7	-8.7	-9.7
0126	$CH(CH_2)_2(Ph)_2$	-5.6	-6.7	-6.8	-10.2
0131	$(CH_2)_2$ $CH(Ph)_2$	-11.2	-5.1	-8.8	-12.3
					
0001	4-Cl/H	-6.7	-3.9	-8.3	-11.1
0175	H/H	-8.7	-6.0	-8.0	-9.5
0176	4-OMe/H	-8.6	-5.8	-7.2	-9.4
0197	4-Me/H	-8.7	-6.1	-7.3	-9.9
0233	2-Cl/H	-7.9	-7.3	-8.1	-9.5
0241	3-OMe/H	-8.3	-7.1	-7.4	-9.7
0244	4-I/H	-7.4	-3.9	-6.4	-9.4
0258	H/Me	-6.7	-6.7	-7.2	-10.0
0265	H/H/ NMe	-6.4	-6.0	-6.0	-9.6
					
0181	H	-8.3	-7.0	-6.2	-10.1
0182	$4\text{-}N(Et)_2$	-5.2	-4.8	-5.8	-9.6

Ligand	R/R1	Receptors			
		mGluR5 (6FFH)	mGlu3 (4XAR)	mGlu8 (6BT5)	NMDA Glu N2B (3QEL)
		Binding energy (kcal/mol)			
0184	3-OMe	-8.1	-5.4	-6.7	-9.6
0185	4-OMe	-7.8	-5.1	-6.4	-9.6
0190	3-$COCH_3$	-8.3	-6.0	-7.2	-9.7
0192	2-COOMe	-5.7	-5.9	-6.4	-8.7
0198	2-Me, 4-Cl	-6.2	-4.8	-6.6	-9.9
0199	2,5-diCOOMe	-5.4	-4.4	-5.0	-9.3
0201	2-OH	-8.0	-7.0	-6.6	-9.7
0205	4-OEt	-6.7	-5.6	-6.5	-9.2
0207	2,6-diMe	-6.4	-7.0	-5.4	-10.7
0208	2-OEt	-6.1	-6.6	-5.7	-8.7
0209	3,4-diMe	-6.1	-6.7	-6.5	-8.7
0210	3-Cl	-7.8	-7.6	-7.4	-9.6
0213	4-Et	-6.3	-5.8	-6.5	-9.4
0215	2-Et	-6.3	-6.6	-5.9	-8.7
0216	2,4,6-triMe	-5.6	-7.2	-4.8	-8.5
0218	4-Bu	-6.2	-5.3	-6.5	-9.6
0219	2-naphtyl	-8.6	-5.4	-7.6	-10.4
0220	3-Me	-9.1	-5.0	-6.8	-10.4
0222	2,3-diMe	-6.9	-7.5	-6.7	-11.1
0224	2,4-diMe	-7.0	-7.6	-6.7	-10.9
0226	2-OMe, 5-Me	-6.1	-7.2	-6.9	-9.3
0228	4-Br	-7.1	-4.6	-6.2	-9.8
0230	3-Cl, 4-Me	-7.6	-7.3	-7.3	-9.6
0231	2-Me, 3-Cl	-6.3	-5.2	-6.3	-10.3
0234	3,5-diCF_3	-8.0	-5.8	-8.1	-10.9
0235	2-Me,4-Br	-6.5	-5.8	-6.9	-9.5
0239	3,5-diCl	-6.9	-5.6	-6.6	-10.9
0245	4-Me	-7.8	-5.8	-6.2	-9.6
0246	3-Br	-8.9	-6.0	-6.5	-9.9
0248	2-OMe, 5-Cl	-5.7	-5.2	-6.5	-9.7
0260	4-O-Ph	-7.6	-6.3	-7.3	-11.0
0268	2-$CONH_2$	-6.2	-6.7	-6.7	-9.3
					
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0168		-6.2	-5.3	-7.5	-9.9
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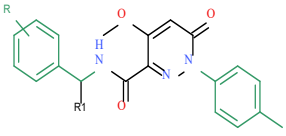
0243		-6.4	-3.6	-5.6	-9.7
0249	Ph/Bn	-6.7	-4.4	-7.5	-9.1
0256	Bn/Bn	-7.5	-3.2	-7.0	-9.8
0194		-8.1	-6.9	-6.5	-9.6
					
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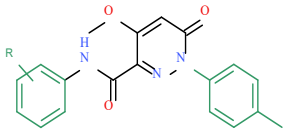
0325	4-Me/H	-9.4	-5.0	-7.5	-9.8
0357	4-Cl/H	-9.1	-5.0	-7.4	-9.7
0360	2-OMe	-9.2	-5.8	-7.6	-9.8
0386	H/Me	-8.2	-5.4	-7.9	-10.7
0387	H/H/NMe	-7.2	-5.1	-6.2	-9.1

Ligand	R/R1	Receptors			
		mGluR5 (6FFH)	mGlu3 (4XAR)	mGlu8 (6BT5)	NMDA Glu N2B (3QEL)
		Binding energy (kcal/mol)			
					
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0335	2,6-diMe	-7.0	-5.6	-6.6	-10.5
0337	3,4-diMe	-9.3	-5.5	-7.8	-11.3
0338	3-Cl	-8.7	-7.4	-6.6	-10.8
0339	2-Cl	-8.0	-6.5	-6.5	-11.3
0348	3-Me	-8.1	-5.3	-7.1	-10.6
0349	2-Me	-8.0	-5.5	-6.7	-11.6
0354	2-OMe, 5-Me	-7.2	-5.6	-6.0	-9.6
0355	4-Me	-8.0	-6.4	-6.7	-10.7
0358	3-Cl,4-Me	-9.1	-5.5	-7.0	-11.2
0359	3-Cl,2-Me	-8.4	-5.4	-6.7	-10.7
0373	4-Me	-8.5	-5.5	-6.7	-10.7
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	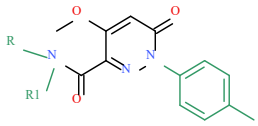				
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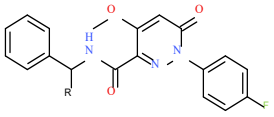
0377	Ph/Bn	-6.7	-4.9	-7.5	-9.3
					
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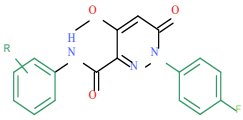
0484	Me	-8.4	-6.1	-8.0	-10.6
					
0435	2-Me,5-Cl	-7.7	-6.3	-7.2	-10.3
0455	2-Me	-7.9	-5.3	-6.9	-10.9
0458	4-Cl	-8.6	-5.5	-6.9	-10.2

The ability of the docking algorithm used in the study to reproduce the experimental data in the case of the mGlu3 receptor is demonstrated in Figure 4B. The location of the ligand and all the interactions agree with the experimental data, except the missing hydrogen bond with threonine (Thr174), but its close and correct location indicates the correct location of the ligand in the active site. The success of the method is also confirmed by the low binding energy of the native ligand -8.2 kcal/mol.

No compound exceeded the affinity index of the native ligand, demonstrating higher binding energy values from -3.2 to -7.4 kcal/mol in the result of the virtual database of compounds docking to the active binding site of the allosteric agonist of mGlu3. No clear patterns of dependence of the affinity level on structural features were identified. The lowest affinity is predicted for the N,N-dibenzylcarboxamide derivative with a 2-methylphenyl radical in the 1st position (0256), and the highest for the N-3-Chlorobenzyl-substituted with a 4-methylphenyl radical in the 1st position.

Stage 3 of the study: the evaluation of the compounds affinity for Group III of the metabotropic glutamate receptors

The first ligand that exhibits a strong selective agonism to mGluIII group receptors, but at the same time not being

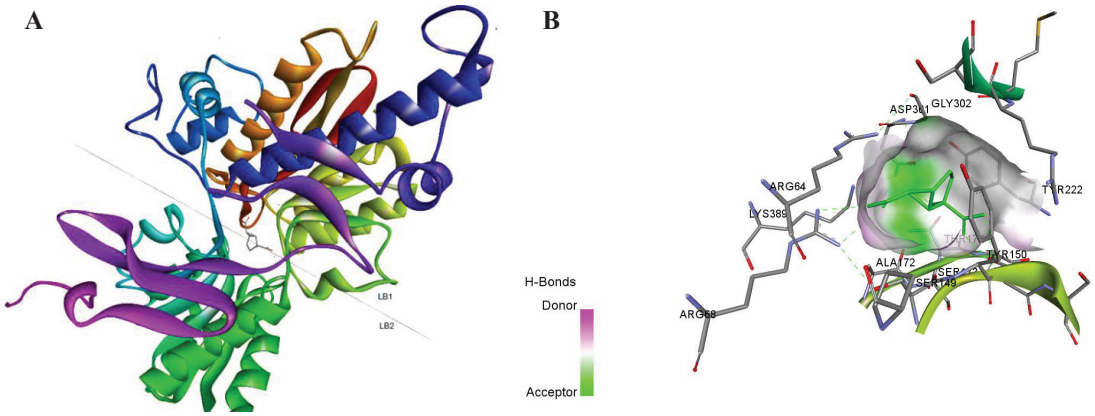


Figure 3. A. 3D macroscopic structure of mGluR3 with allosteric agonist of mGlu2/3 receptors – LY354740 **B.** 3D molecular structure of the active mGluR3 site with the LY354740 ligand (green-colored molecule).

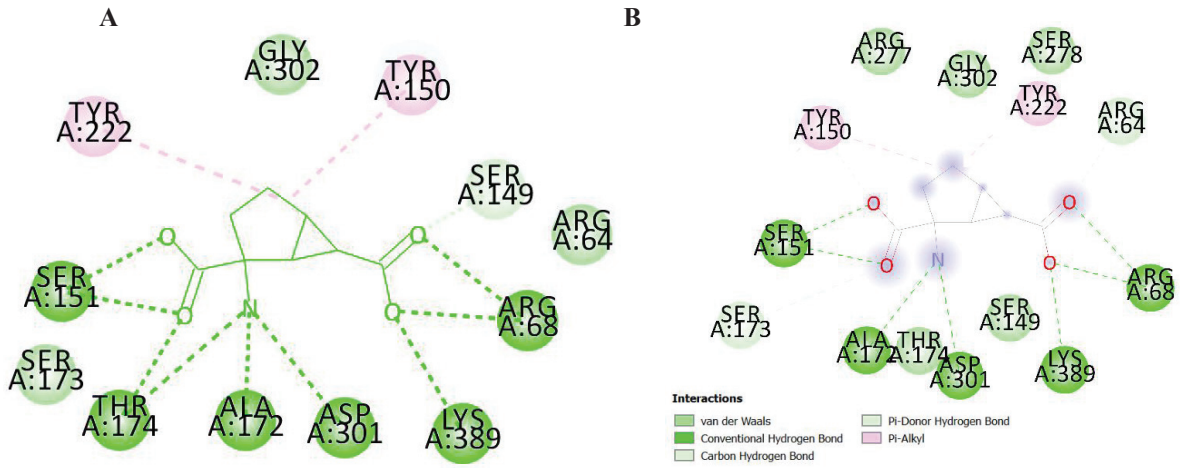


Figure 4. A. Experimentally established interaction of LY354747 with the amino acids in the active mGluR3 site. **B.** Reference interaction of LY354747 with amino acids in the active site of the mGlu3 receptor.

selective of certain subtypes of Group III mGlu receptors, is L-2-amino-4-phosphonobutyric acid (L-AP4), described back in 1997 (Thomsen 1997). The crystal structure of the recombinant human amino-terminal domain mGlu8 in combination with the selective l-AP4 agonist was isolated and described only in 2018 (PDBID 6BT5) (Avdeeva et al. 2019). Unfortunately, at the moment, the crystal structure of the closed conformations with agonists of other subtypes of Group III mGlu receptors – mGlu4, mGlu6 or mGlu7 – has not been established. However, there is evidence of a significant similarity of the amino acid sequence of active sites of Group III mGlu receptors, which, accordingly, allows predicting to some extent the affinity for all receptors in this group.

The mGlu8 amino-terminal domain consists of two asymmetric protomers that form a homodimer (Fig. 5A) (Schkeryantz et al. 2018). The binding center of the selective LAP 4 agonist is the hinge region of the protein, located between the complementary globules (LB1/LB2), which are connected to each other by three short loops.

The following amino acid residues are the experimentally established binding site of the L-AP4 agonist (Fig. 5B): two molecules of alanine (Ala 155 and 177), serine (Ser156), threonine (Thr179) from the upper lobe of the protomer (LB1), tyrosine (Tyr227), and aspartic acid (Asp309) from the lower lobe (LB2). The important interactions which may be responsible for the selectivity of L-AP4 are postulated to be ionic interactions of phosphate with the formation of salt bridges with lysine 71 and 401, arginine 75 (LB1), and its bidentant hydrogen interaction with lysine 314 (LB2).

In this study, the binding energy was -6.1 kcal/mol, when docking the reference ligand l-AP4 into the active site of the mGlu8 amino-terminal domain. The visualization of the docking results (Fig. 6A, B) demonstrates almost a complete compliance of the obtained conformation of l-AP4/mGlu8 with the experimentally established data: a characteristic tetrahedral network of hydrogen bonds between the amino group of the ligand and the threonine hydroxyl (Thr179), the alanine carbonyl (Ala 155), and the carboxyl group of aspartic acid (Asp309), as well as all salt bridges between the phosphate. The exception was the hydrogen bond with a water molecule, since the automatic docking methodology involves removing water molecules from the protein globule.

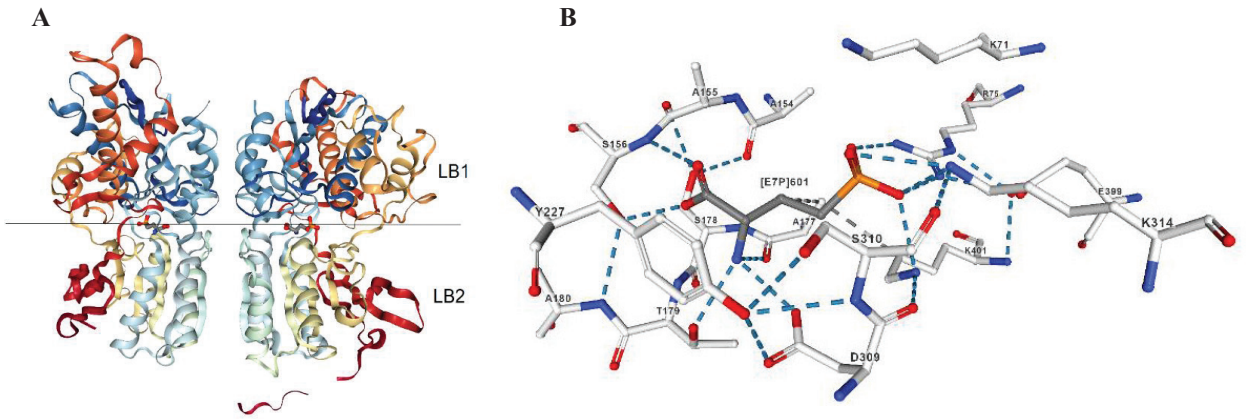


Figure 5. Spatial structure of the mGlu8 receptor with an L-AP4 agonist at the binding site **A.** 3D MGlu8 structure with an L-AP4 agonist in the binding site **B.** Interaction of L-AP4 with amino acid residues of the binding site. Hydrophilic bonds are indicated by blue lines, hydrophobic bonds are indicated by gray lines.

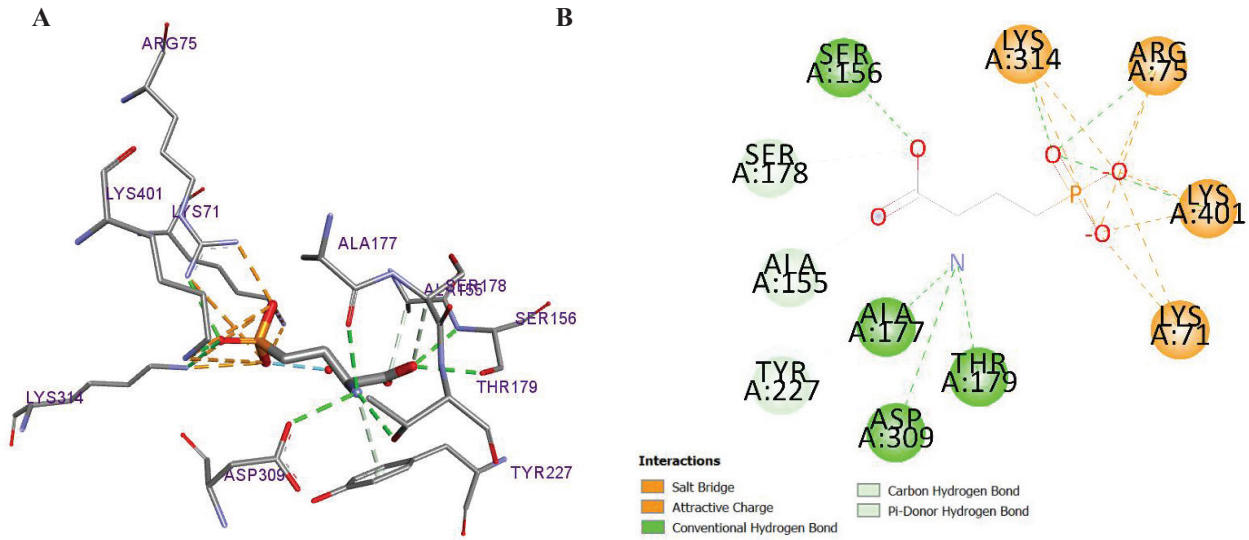


Figure 6. 3D (**A**) and 2D (**B**) interactions of L-AP4 with the amino acids of the mGlu8 binding site.

The binding energy of the native ligand was -6.1 kcal/mol. For derivatives of N-substituted 4-methoxy-6-oxo-1-aryl-pyridazine-3-carboxamides, a higher degree of affinity for the active site of the mGlu8 receptor is predicted: the binding energy of all the compounds that were selected for synthesis is either at or above the reference drug value (from -5.0 to -8.7 kcal/mol). The lowest affinity (binding energy -5.0 kcal/mol) is predicted for dimethyl 2 - [[4-methoxy-1-(2-methylphenyl)-6-oxo-pyridazine-3-carbonyl]amino]benzene-1,4-dicarboxylate (0199). It can also be noted that benzylamine derivatives have some of the best indicators of the affinity in comparison with the phenylsubstituted carboxamide derivatives.

Stage 4 of the study: the evaluation of the affinity of the compounds for NMDA glutamate receptors

The next stage of the research was to study the affinity of the virtual database of the compounds for the allosteric site of ionotropic NMDA (N-methyl-D-aspartate) glutamate receptors. The ionotropic NMDA glutamate receptor is a heterotetramer of two subunits – N1R and N2R. Each of the subunits is made up of 4 parts: an amino-terminal domain (ATD), a ligand-binding domain (LBD), a transmembrane domain (TD), and a C-terminal domain (CTD). The activity of the NMDA receptors in the ion channels is regulated by the allosteric binding of small molecules to the amino-terminal domain, or the ligand-binding domain, in a subtype-specific manner: in particular, after the simultaneous binding of glycine and glutamate to the GluN1 and GluN2 subunits, respectively (Mothet et al. 2015). Ifenprodyl-4 - [(1R, 2S) - 2 - (4-benzylpiperazine-1-yl)-1-hydroxypropyl phenol is considered to be such a molecule, specifically inhibiting the NMDA receptor of the GluN1 and GluN2 subtypes (Williams 1993; Gallagher et al. 1996). The negative allosteric modulation of the NMDA receptors occurs by binding ifenprodil to active sites of the amino-terminal domain.

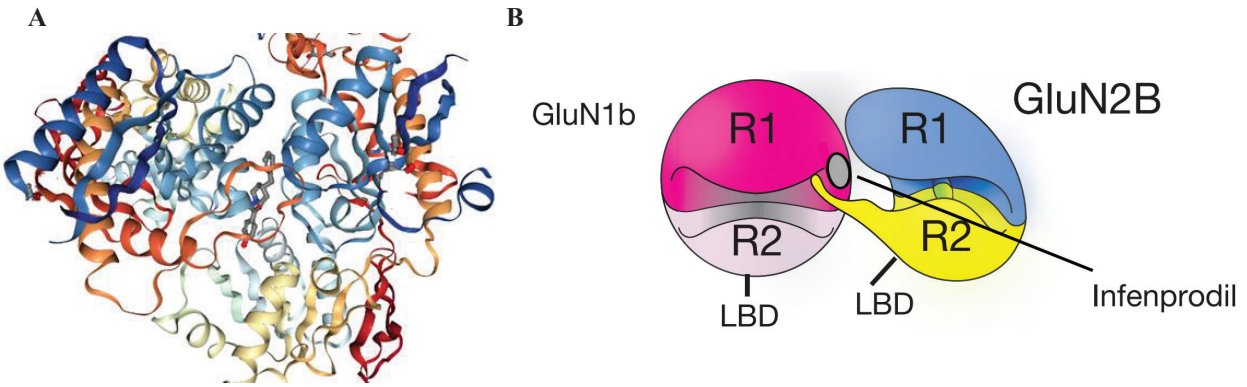


Figure 7. Macroscopic 3D (**A**) and schematic (**B**) images of the structure of the amino-terminal domain of the NMDA receptor with a negative allosteric negative modifier in the active site.

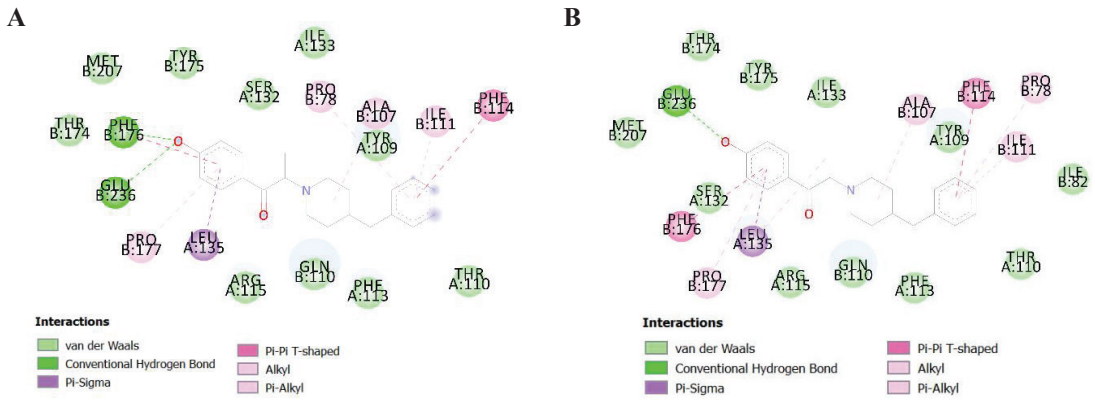


Figure 8. Experimentally established (**A**) and reference interaction (**B**) of ifenprodil with amino acids in the active site of the NMDA glutamate receptor.

It is the complex of amino-terminal domains GluN1/GluN2B in a closed conformation with ifenprodil (PDB ID 3QEL) that were used to dock the virtual database of the compounds (Fig. 7A, B).

It was experimentally established that the hydrophobic pocket where ifenprodil is immersed consists of the following amino acid residues of both subunits:

- GluN1b subunit – threonine (Thr 110), tyrosine (Tyr109), phenylalanine (Phe 113), serine (Ser132), and leucine (Leu135);
- GluN2B subunit – (Pro 177), isoleucine (Ile 111), glutamine (Gln110), alanine (Ala107), glutamic acid (Glu 236), and phenylalanine (Phe176).

Next to the binding pocket is an empty space that is surrounded by hydrophobic residues, including alanine (Ala75) of the GluN1b subunit and isoleucine (Ile 82), phenylalanine (Phe114) of the GluN2B subunit.

The accuracy of the docking methodology and the ability to reproduce the parameters of the experimental data were confirmed when evaluating the affinity of the native ifenprodil ligand for the active site of the amino-terminal domain of the NMDA glutamate receptor.

Comparing the experimental literature data and the results of the reference docking (Fig. 8A, B), the reproducibility of the method becomes obvious: all hydrophobic and hydrophilic bonds are present, and the locations of ifenprodyl fragments relative to amino acid residues are comparable.

The difference in the reference docking is the absence of a single hydrogen bond between phenylalanine (Phe176) and ifenprodyl hydroxyl, whereas the hydrophobic interaction is preserved. The binding energy of the native ifenprodil ligand was -11.3 kcal/mol, which demonstrates a high affinity for the receptor.

As for the studied derivatives of N-substituted 4-methoxy-6-oxo-1-aryl-pyridazine-3-carboxamides, they demonstrate a high affinity for the NMDA glutamate receptor: the binding energy ranges from -8.7 to-11.6 kcal/mol. When docked to this receptor, it is substituted phenyl carboxamide derivatives that show more stable results, whereas benzyl-substituted ligands show a bit worse results.

Conclusion

- According to the results of docking, a high degree of affinity of 4-methoxy-6-oxo-1-arylpyridazine-3-car-

boxamide derivatives is predicted for the following glutamate receptors:
  - metabotropic mGluR8 (Group III) – binding energy from -5.0 to -8.7 kcal/mol, versus -6.1 kcal/mol in the reference drug (L-AP4);
  - ionotropic NMDA of the GluN2 subtype – the binding energy from -8.7 to -11.6 kcal/mol, compared to -11.3 kcal/mol in the native ifenprodyl ligand.
- A satisfactory level of the affinity is predicted with the active mGluR5 site (Group I): binding energy 11.2–5.2 kcal/mol versus -8.7 kcal/mol in the reference ligand fenobam.
- The results of the docking to the active mGluR3 site (Group II) were less satisfying. No compound exceeded the affinity of the native ligand: binding energies from -3.2 to -7.4 kcal/mol compared to -8.2 kcal/mol, respectively.
- SAR analysis of the docking results shows that the N-benzyl-substituted derivatives have better affinity, compared to that of the N-phenyl-substituted carboxamide derivatives. Substitution of hydrogen of the NH-group of the carboxamide residue with a methyl or ethyl radical leads to a decrease in the affinity, which is probably due to the ability of the NH-group to form hydrogen bonds to stabilize the ligand conformation in the cavity of the active site. There is no clear relationship between the substituent in the phenyl ring and the affinity level.
- According to the binding energy values for metabotropic and ionotropic glutamate receptors, 96 substances were selected for synthesis, with the best affinity predicted for at least two of the four types of the glutamate receptors.

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