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Novel bivalent positive allosteric AMPA receptor modulator of bis-amide series

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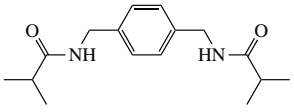
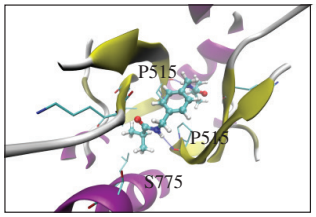
A series of bis-amides of bis(aminoalkyl)benzenes and diaminoalkanes were synthesized. Positive modulation of kainate-induced transmembrane currents was registered on Purkinje neurons for isobutyric acid derivative with 1,4-bis(aminomethyl)benzene linker (40% potentiation at 1 nM). This compound also demonstrated a neuroprotective effect at 0.1 nM in an oxidative stress model in HT-22 cells when applied 24 h before H_2O_2.



40% potentiation at 1 nM



Keywords: AMPA receptor, positive allosteric modulator, patch clamp, Purkinje neurons, carboxamides.

The glutamatergic system plays an extremely important role in the functioning of the central nervous system (CNS) in mammals.[1] AMPA receptors, a group of ionotropic glutamate receptors, are directly involved in the formation of memory and cognitive functions.[2,3] Positive allosteric modulators (PAMs) of AMPA receptors drew much attention due to their ability to improve learning and memory formation processes that makes them promising drug candidates for the treatment of cognitive disorders, including early stages of Alzheimer's disease[4,5] as well as depression and some other CNS pathologies.[6–9] Given the extremely limited range of drugs for the treatment of neurodegenerative diseases, in particular various forms of dementia, the search and development of new PAMs with high activity and specificity is an extremely urgent task.

Earlier, extensive efforts were applied to develop predictive models[10–12] based on 3D-QSAR, pharmacophore (Figure 1), and MM-PBSA approaches which allowed us to design a series of novel PAMs[13–18] including the derivative of 3,7-diazabicyclo[3.3.1]nonane (bispidine) compound **1**, which increases kainate-induced currents in 10^{-11}–10^{-7} M concentration range (more than twofold increase at 10^{-9} M), improves cognitive impairments in animal models and became one of the most active positive AMPA receptor modulators reported to date.[16,17]

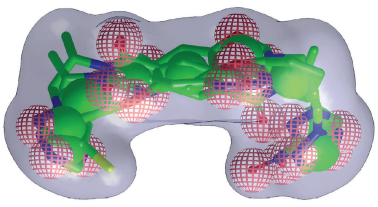


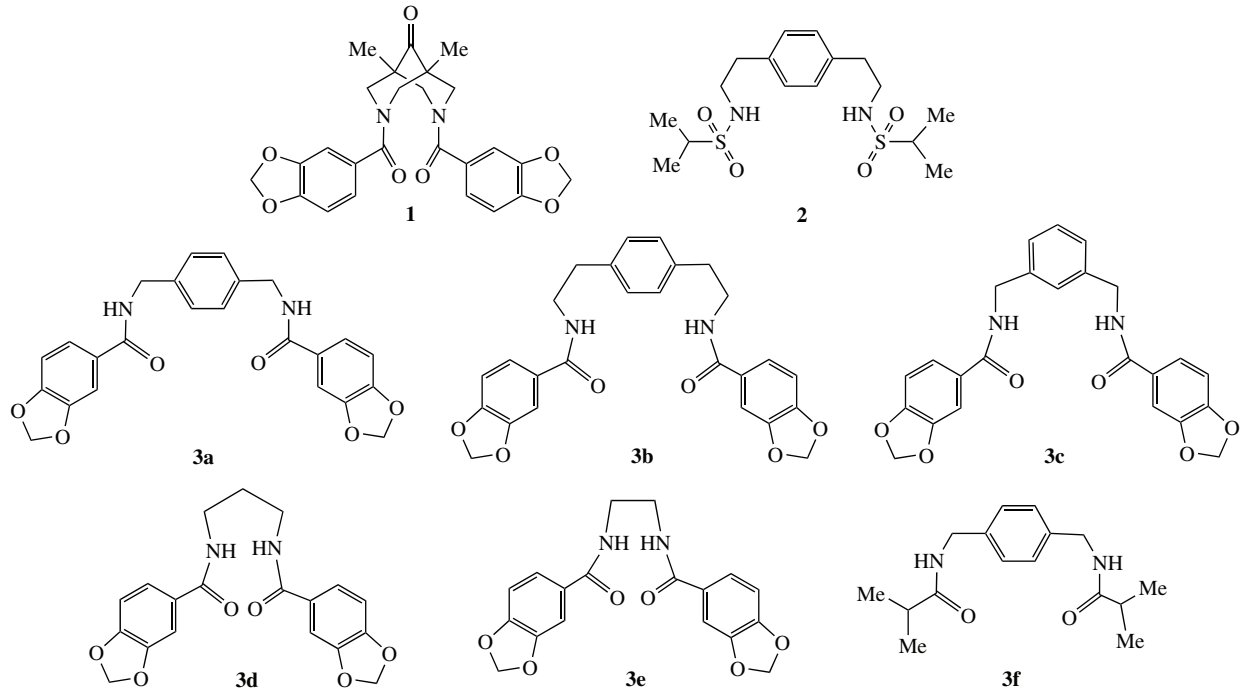
Figure 1 The ROCS pharmacophore model of positive allosteric modulators of AMPA receptor. The shape of a sterically favorable region is shown in light gray, the green balls mark the positions of the centers of ring systems, and the pink mesh balls show the positions of the hydrogen bond acceptors. Reproduced with permission from ref. 10. ©2017 Mendeleev Communications.

In our previous publications it was supposed[16] that in the molecule of compound **1** the bispidine core acts as a spacer, while the piperonyloyl substituents serve as the fragments which bind symmetrically to the side parts of the binding site. However, the binding mode of compound **1** has not been investigated experimentally while the molecular modeling results also indicate an alternative non-symmetric pose[17] where the bispidine fragment is bound in a side part of the binding site and maintains a number of interactions critical for binding. It should be noted that another well-known highly active compound **2** with 1,4-bis(2-aminoethyl)benzene linker (pEC_{50} = 7.3) demonstrates the symmetric binding mode in the crystal structure[19] (PDB: 3RNN). In order to elucidate the importance of the linker nature in dipiperonyloyl derivatives, we replaced a bispidine spacer with 1,4- or 1,3-bis(aminomethyl) benzene (compounds **3a**,**c**) or 1,4-bis(2-aminoethyl)benzene (compound **3b**) fragments and also with two linear aliphatic diamines (compounds **3d**,**e**).

The docking results of compounds **3a**–**e** did not yield poses filling two symmetric pockets[10–12] where all known high affinity PAMs bind. However, the replacement of piperonyloyl substituents in compound **3a** having 1,4-bis(aminomethyl)-benzene linker by smaller acyl ones (compound **3f**) resulted in better binding pose with two hydrogen bonds with Pro515 (which were observed for a number of recently reported high affinity PAMs[19]) and without too close contacts.

Thus, a series of compounds **3a**–**f** have been synthesized by the standard acylation of diamines (for details, see the Online Supplementary Materials).

The electrophysiological experiments for the evaluation of the effect of compounds **3a**–**f** on the AMPA receptors were carried out using the patch clamp technique on freshly isolated



Purkinje neurons, as described earlier.[16,17] Influence of compounds **3a–f** on the kainate-induced currents is shown in Table 1. It can be seen that the replacement of the bispidine spacer in compound **1** (dipiperonyloyl derivative) with 1,4- or 1,3-bis(aminomethyl)benzene, 1,4-bis(2-aminoethyl)benzene, and with two linear aliphatic diamines (compounds **3a–e**) results in the complete loss of activity. Thus, the inactivity of compounds **3a–e** confirms the importance of the linker nature for the activity of compound **1**.

However, among the studied compounds, derivative **3f** with smaller isobutyryl substituents demonstrated a potentiation of the kainate-induced AMPA receptor currents in a wide concentration range (10^{-10}–10^{-6} M, a bell-shaped dependence). The maximum potentiation was observed at 10^{-9} M, however with further increase in concentration compound **3f** shows a noticeably weaker effect on the currents of Purkinje neurons. For comparison, the data for well-known AMPA receptor PAM cyclothiazide (CTZ) obtained under the same conditions are also given in Table 1.

The neuroprotective effect of compound **3f** has been studied in an oxidative stress model in a culture of mouse hippocampal neuronal HT-22 cells.[20–22] Compound **3f** was introduced into HT-22 cell culture as an aqueous solution with the addition of 3% DMSO. All wells, except for the intact control, were supplemented with 3% DMSO (final concentration of DMSO was 0.03%, 100-fold dilution). DMSO did not affect the viability of HT-22 cells. The introduction of hydrogen peroxide led to a reliable decrease in cell viability. In the first series of experiments, compound **3f** protected cells from damage at a final concentration of 10^{-8} M when applied 24 h before H_2O_2 [Figure 2(*a*)]. In the next series of experiments, the final concentrations of 10^{-8}–10^{-11} M were investigated and it was shown that compound **3f** protected cells from damage at final concentrations as low as 10^{-10} M [Figure 2(*b*)]. In another series of experiments, when compound **3f** was applied immediately after damaging the cells with hydrogen peroxide, the protective effect was observed only at a final concentration of 10^{-6} M [Figure 2(*c*)]. It is important to note that the neuroprotective effect of compound **3f** was observed in a wide range of concentrations from 10^{-8} to 10^{-10} M, which is

Table 1 The effect of various concentrations of compounds **3a–f** on the kainate-induced AMPA receptor currents of rat cerebellum Purkinje cells.

Compound	*n* (number of neurons)	Compound concentration/M					
		10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
		Current amplitude M ± m relative to control (%)					
3a	5	–	100 ± 2	100 ± 2	100 ± 3	100 ± 2	100 ± 3
3b	5	–	100 ± 2	100 ± 3	100 ± 3	100 ± 2	100 ± 4
3c	5	–	100 ± 1	100 ± 3	100 ± 2	100 ± 2	100 ± 3
3d	7	–	100 ± 1	100 ± 3	100 ± 2	100 ± 2	100 ± 3
3e	5	–	100 ± 2	100 ± 2	100 ± 2	100 ± 2	100 ± 4
3f	5	106 ± 6	118 ± 7	140 ± 8	128 ± 5	119 ± 4	119 ± 3
CTZ	8	–	–	–	–	100 ± 3	145 ± 11

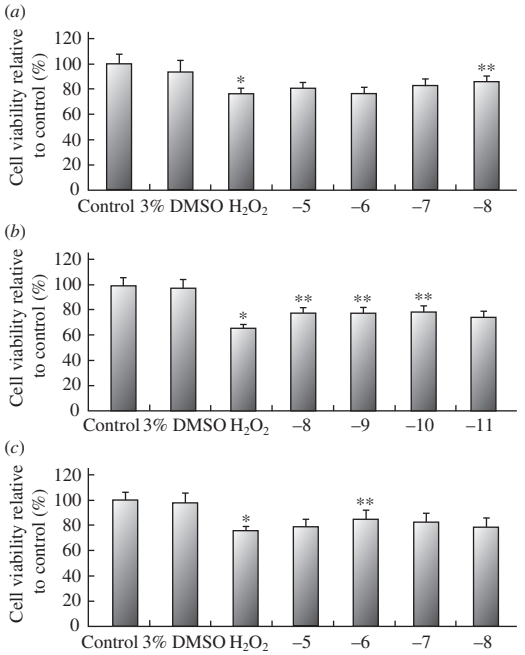


Figure 2 Influence of compound **3f** (log*C*) on the viability of HT-22 cells in an oxidative stress model. (*a*), (*b*) Application 24 h before H_2O_2, (*c*) application after H_2O_2. MTT tests results are shown. Note: significance of differences $p \leqslant 0.05$ from control (*), from H_2O_2 (**). Kruskal–Wallis test followed by Dunn's test (ANOVA).

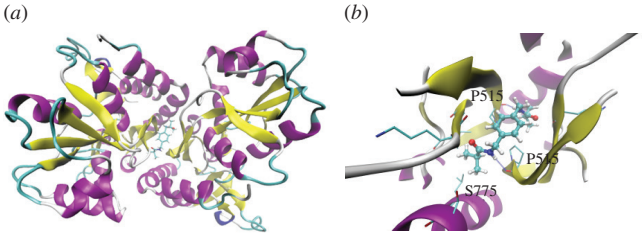


Figure 3 Possible binding mode of compound **3f** between two ligand binding domains of GluA2 receptor: (*a*) general view, (*b*) amino acid residues surrounding compound **3f**.

a good advantage for the development of pharmacological agents.

The docking study was performed using OpenEye FRED software[23] with high dock resolution into the GluA2 receptor model prepared from X-ray structure[19] (PDB: 3RNN). Conformations for docking were generated with OpenEye OMEGA2 program.[24] The results were visualized with VMD program.[25] Figure 3 shows a possible binding mode of positive allosteric modulator **3f** between two ligand-binding domains of GluA2 receptor. For steric reasons, a single **3f** molecule can fit into the binding site, unlike two smaller molecules of well-known AMPA receptor PAM cyclothiazide (CTZ), which bind there symmetrically.[26] A 3D alignment of nanomolar positive modulator **2** bound with the receptor in a crystal structure[19] and a docking pose of compound **3f** in the same receptor structure is shown in Figure S1 (Online Supplementary Materials).

Thus, it has been found that isobutyric acid derivative with a 1,4-bis(aminomethyl)benzene linker **3f** in patch clamp experiments demonstrates a concentration-dependent potentiation of the kainate-induced AMPA receptor currents of Purkinje neurons in a wide concentration range (10^{-10}–10^{-6} M) with a maximum of 40% at 10^{-9} M. It also reveals a neuroprotective effect in an oxidative stress model in HT-22 cells, where it protects cells from damage at a final concentration as low as 10^{-10} M when applied 24 h before the application of H_2O_2. We hope that further structural optimization of compound **3f** obtained by simple synthetic procedures from commercially available starting compounds as well as further *in vitro* and *in vivo* studies will make it possible to develop novel highly potent and safe AMPA receptor modulators applicable as drug candidates.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.03.023.

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