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Novel positive allosteric modulator of AMPA-receptors based on tricyclic scaffold

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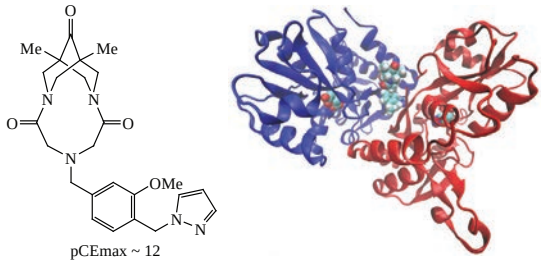
Molecular modeling, synthesis, and activity evaluation of a novel positive allosteric modulator of the AMPA receptor, 6-[4-methoxy-3-(1*H*-pyrazol-1-ylmethyl)benzyl]-1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione, are presented. The main point of the design was to fill the interdomain cavity of GluA2 ligand binding domains based on the cyclothiazide binding mode. Electrophysiological studies showed high potentiation of the kainate-induced currents (pECmax ~ 12).

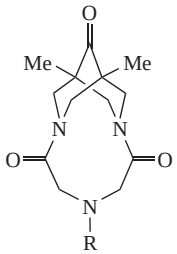


Glutamatergic system plays an essential role in the functioning of the mammalian central nervous system (CNS).[1] Two different families of glutamate receptors, *viz.* ionotropic (ligand-gated ion channels, iGluRs) and metabotropic (G-protein coupled) ones, are widely distributed in the CNS.[2] In turn, ionotropic glutamate receptors are divided into three types based on their sensitivity to the selective agonists: *N*-methyl-D-aspartic acid (NMDA-receptors), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA receptors), and kainic acid (KA, kainate receptors).[3]

In recent years, a special interest has been attracted to positive allosteric modulators (PAMs) of AMPA receptors. Their therapeutic value has been the subject of deep discussions in reviews.[4] As compared to the direct AMPA receptor agonists, positive allosteric modulators perform the fine-tuning of glutamatergic system in a better way since they do not cause any effects in the absence of the natural ligand in a synapse. In particular, in the case of direct agonists, an overdose can cause the hyperstimulation of CNS glutamatergic system and neurotoxicity leading to the uncontrolled brain damage.[5] In contrast, positive allosteric AMPA receptor modulators have relatively few side effects in therapeutically effective doses.[6] Moreover, PAMs are neuroprotective against lesions induced by NMDA agonists.[7]

The dysfunction of glutamatergic neurotransmission is a primary reason of a number of neurological and psychiatric diseases.[8] Enhancement of AMPA signals by allosteric modulators can be useful in the treatment of neurological disorders such as depression,[9] schizophrenia,[10] Parkinson's disease,[11] Alzheimer's disease,[12] attention deficit/hyperactivity disorder, and mood disorders.[13] This therapeutic effect is associated, at least in part, with the property of AMPA receptor PAMs to significantly increase the expression of neurotrophic factors – nerve growth factor and brain-derived neurotrophic factor,[14] which, in turn, is the most powerful mechanism of differentiation of neural stem cells.

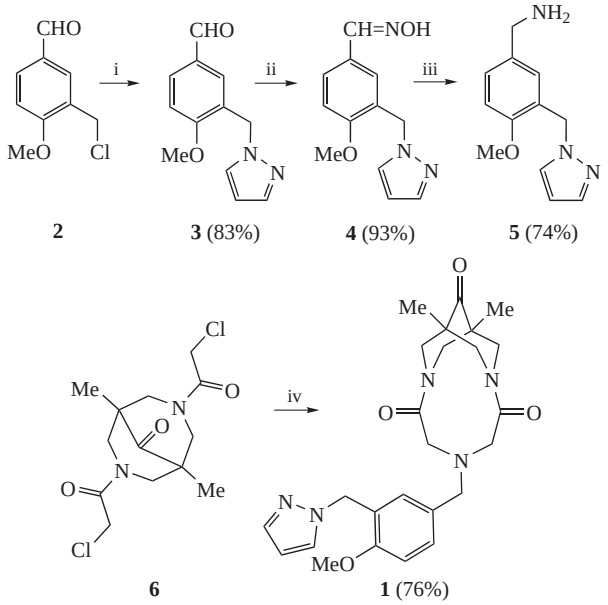
Earlier, we performed a series of molecular modeling studies of various PAMs in complexes with the dimer of AMPA receptor glutamate-binding domains, the synthesis of promising structures and their biological tests.[15–17] For the rational design of new PAMs, a pharmacophore hypotheses were also suggested.[18,19] As a result, we designed a new scaffold of general formula **A** and a series of compounds was synthesized on its basis. In this communication, we present 6-[4-methoxy-3-(1*H*-pyrazol-1-ylmethyl)benzyl]-1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]-tetradecane-4,8,12-trione **1** (Scheme 1) from this series which exhibited the most significant activity as a positive allosteric modulator of AMPA receptor.



A

The results of the docking study demonstrate the cyclothiazide-like binding mode of compound **1** on the contact surface between GluA2 ligand binding domains (flip form). The docking procedure[20] was carried out using Rosetta 3.5 (10 000 launches of the search algorithm, the best pose was selected). Captions were made using VIDA 4.3.0[21] and Grapheme TK.[22] Binding is maintained by hydrogen bonds between the ligand and Ser518 (Figure 1).

In the synthesis of compound **1** (see Scheme 1), amine **5** was prepared[24,25] by N-alkylation of pyrazole with 3-chloromethyl-4-methoxybenzaldehyde **2**, transformation of aldehyde **3** into the



Scheme 1 *Reagents and conditions*: i, pyrazole, 18-crown-6/C_6H_6/KOH/H_2O, 80 °C; ii, NH_2OH·HCl/pyridine/EtOH; iii, $LiAlH_4$/THF; iv, amine **5**, K_2CO_3/MeCN, 60 °C.

corresponding oxime **4** and its subsequent reduction. Synthesis of bis(chloroacetamide) **6** was performed according to the published procedures.[26] Final stage involved the bis-alkylation of amine **5**.

In vitro studies of compound **1** were conducted by electrophysiological technique on freshly isolated Purkinje neurons from the cerebellum of the rat brain slices (Wistar males, 12–15 days old). Isolation of single neurons was facilitated by an enzymatic

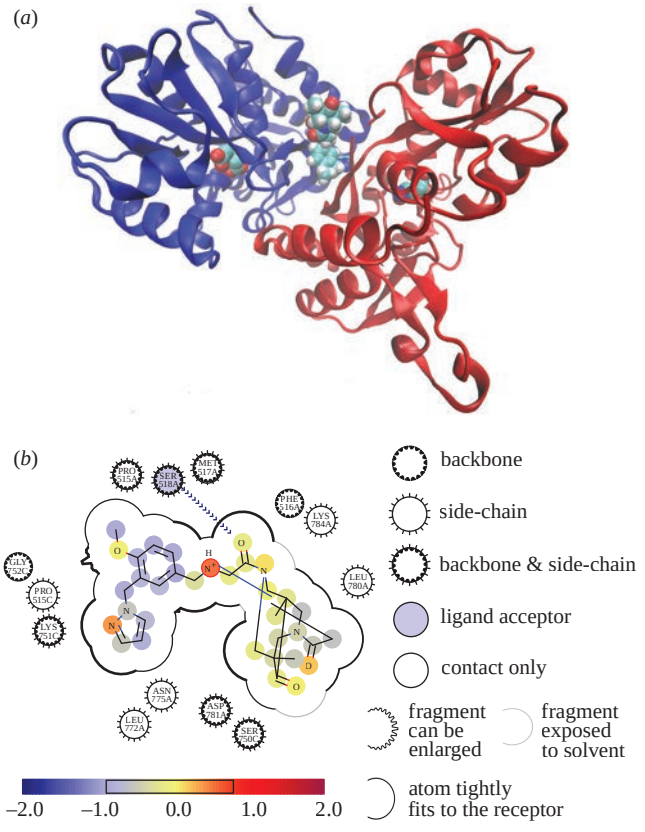


Figure 1 The binding pose of compound **1**: (*a*) 3D structure of the complex of compound **1** and GluA2 LBD homodimer; (*b*) a schematic representation of the binding site with the colour-coded atomic contributions of Chemgauss4 score[23] (the colour-codes are shown on the scale under the figure, the negative contributions increase binding).

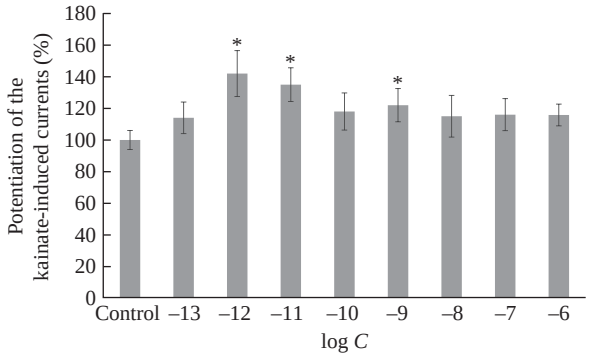


Figure 2 Results of *in vitro* study of compound **1** by the patch-clamp technique. The vertical axis demonstrates the increase in kainate-induced currents relative to the control (M ± SD). Asterisks mark the reliable differences from the control ($p < 0.05$).

treatment with subsequent mechanical dissociation by Pasteur pipettes.[27] The transmembrane currents of individual nerve cells were recorded by the local potential fixation method (patch-clamp) in a whole-cell configuration using the EPC-9 device. To obtain AMPA cell responses to mediator application, KA in a concentration of 2×10^{-4} to 5×10^{-4} mol dm^{-3} dissolved in physiological solution was used.

Figure 2 shows the final results for the entire range of compound **1** concentrations (n = 22, the number of neurons): the increase of the KA-induced AMPA receptor currents relative to the control *vs.* the concentration of compound **1** (n = 3–7 experiments for each column).

The data obtained demonstrate that compound **1** enhances AMPA receptor responses in isolated Purkinje neurons in a wide range of concentrations from 10^{-13} to 10^{-6} mol dm^{-3}, while the maximum potentiating effect is achieved at the concentration of 10^{-12} mol dm^{-3}. The decay of potentiation at higher concentrations possibly could be explained by the binding of compound **1** also to one of the antagonist, negative modulator or channel blocker binding sites despite the low affinity to it.

In summary, it can be concluded that compound **1** exhibits a pronounced effect characteristic of allosteric AMPA receptor modulators. This gives a hope that after further optimization of the structure it will be possible to develop a drug for the treatment and prevention of neurodegenerative and neuropsychiatric diseases, as well as for the improvement of memory and cognitive functions.

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