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Positive and negative AMPA receptor modulators based on tricyclic bispidine derivative: minor structural change inverts the type of activity

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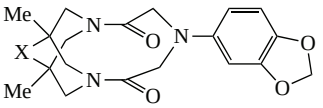
Two new potent AMPA receptor allosteric modulators, 6-(1,3-benzodioxol-5-yl)-1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione and -4,8-dione were synthesized from 1,3-benzodioxol-5-amine and the corresponding 3,7-dichloroacetyl-3,7-diazabicyclo[3.3.1]-nonanes. In a wide concentration range (10^{-12}–10^{-7} M), the 12-oxo derivative acts as a positive modulator causing the potentiation of the kainate-induced AMPA receptor currents with maximum potentiation at 1 nM (62%) while its analogue without a ketone group has significant (up to 40%) negative modulator effect. Their tentative mechanisms of action were analyzed by means of molecular modelling.



Modulation of AMPA receptor:
X = C=O – positive
X = CH_2 – negative

Keywords: 3,7-diazabicyclo[3.3.1]nonane, bispidines, heterocyclization, AMPA receptors, allosteric modulators.

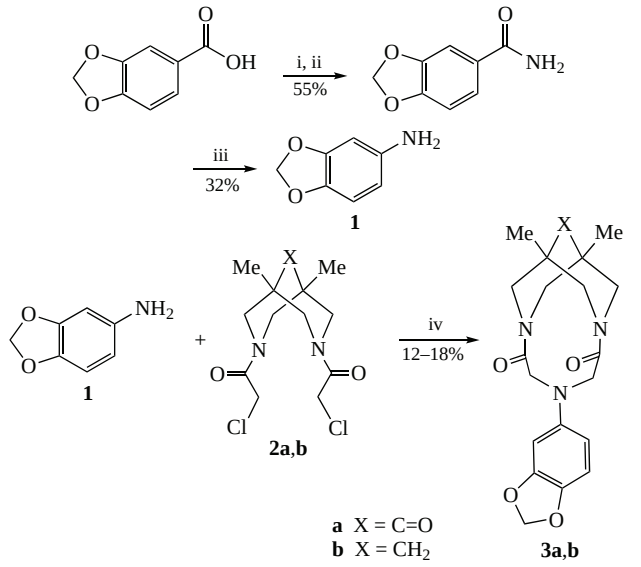
The glutamatergic receptor system is the major excitatory neurotransmitter system in the mammalian brain.[1] A subtype of ionotropic glutamate receptors selectively activated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and responsible for the fast excitatory postsynaptic signal transmission is involved in long-term potentiation processes closely related to learning and memory formation.[2,3] Among the AMPA receptor ligands, its allosteric modulators are of particular interest as potential therapeutic agents for treatment or correction of many serious neurodegenerative and psychoneurological disorders due to their better efficacy and safety profile compared to the direct agonists and antagonists.[4] Positive allosteric modulators (PAMs) facilitate synaptic plasticity and induce long-term potentiation as well as significantly increase the expression of neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which, in turn, is associated with the improvement of cognitive functions and the restoration of nerve cells.[5–11] This makes them promising candidates for the development of drugs against Alzheimer's and Parkinson's diseases, multiple sclerosis, soft cognitive disorders, age-related cognition and memory impairments, autism, depression, drug addiction, *etc*.[6,9–16] On the other hand, negative allosteric modulators (NAMs) can be used as antiepileptic drugs.[17–20] Therefore, the development of compounds acting as allosteric modulators of AMPA receptors is currently one of the most promising directions for the progress of psychopharmacology.

Earlier, we have performed molecular modelling and molecular dynamics studies for the rational design of new positive and negative modulators of AMPA receptors.[21,22] The PAM pharmacophore hypothesis as well as the 3D QSAR (CoMFA) models[23,24] based on the available X-ray structural data for PAM receptor complexes were also instrumental in the design of novel potent compounds. As a result, we were able to develop a series of novel positive and negative AMPA receptor modulators based on different scaffolds and possessing experimentally confirmed activity in nano- and picomolar range of concentrations.[25–31]

In this paper, we describe new compounds based on the tricyclic derivative of bispidine (3,7-diazabicyclo[3.3.1]nonane) as the scaffold, report the results of the *in vitro* assessment of their potency as AMPA receptor allosteric modulators, and analyze their tentative mechanisms of action by means of molecular modelling.

The target compounds **3a**,**b** were obtained by double alkylation of amine **1** with the corresponding bis(chloroacetyl) bispidine derivatives **2a**,**b** obtained by previously published method[32] (Scheme 1). In turn, amine **1** was synthesized from 1,3-benzodioxole-5-carboxylic acid as shown in Scheme 1 (for detailed synthetic procedures and spectral data, see Online Supplementary Materials). It should be noted that ^{1}H NMR spectra of compounds **3a**,**b** contained two clearly distinct singlets for methyl groups at the bridgehead positions 1 and 11. The nonequivalence of these methyl groups is caused by the fixed parallel orientation of carbonyl groups at the nitrogen atoms of the tricyclic framework (see the discussion of acyl group orientations in bicyclic analogues[32,33]).

The action of compounds **3a**,**b** on AMPA receptors was assayed in the electrophysiological experiments using the patch clamp technique on freshly isolated Purkinje neurons as



Scheme 1 *Reagents and conditions*: i, $SOCl_2$, Py; ii, NH_3/H_2O; iii, Br_2, $NaOH/H_2O$; iv, K_2CO_3, DMF, 70–75 °C.

described earlier[28,30] (for details, see Online Supplementary Materials). Surprisingly, despite very similar chemical structures, these compounds exert radically different influence on the kainate-induced currents (Table 1). For compound **3a**, the potentiation of the kainate-induced AMPA receptor currents is observed in a wide concentration range (10^{-12}–10^{-7} M) and has a bell-shaped concentration dependence with maximum potentiation at 1 nM [62%, Figure 1(*a*)]. Conversely, compound **3b** in a wide concentration range (10^{-12}–10^{-7} M) demonstrates

Table 1 Effects of various concentrations of compounds on the kainate-induced AMPA receptor currents in rat cerebellum Purkinje cells.

Compound	Number of neurons	Compound concentration (M), current amplitude (% to control, ±SD)						Ref.
		10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	
3a	7	111±4	141±5	152±5	162±6	148±5	135±4	
3b	7	89±3	87±3	84±4	75±4	68±5	61±6	
4a	3	–	65±7	75±5	80±4	83±3	87±3	26
4b	3–6[a]	143±22	129±15	117±22	122±7	109±6	110±1	27
4c	4	–	81±12	78±13	66±11	91±2	95±4	29

[a] Different number of neurons was used for different concentrations.

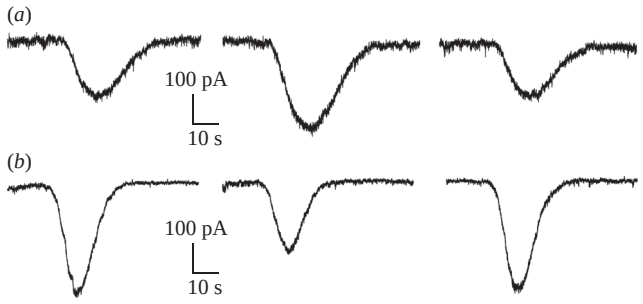
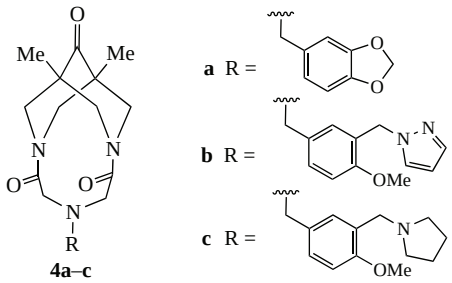


Figure 1 Kainate-induced currents for (*a*) compound **3a** at 10^{-9} M and (*b*) for compound **3b** at 10^{-7} M concentration (left – control, center – after application of the compound, right – after wash-out).



significant (up to 40%) blocking of the AMPA receptor [Figure 1(*b*)]. In other words, the removal of the carbonyl oxygen leads to a complete inversion of the receptor modulation. Similar activity cliffs have been observed previously for some related compounds based on the tricyclic bispidine scaffold[26,27,29] **4a–c** as well as for the acylated bis(aminoalkyl)benzenes.[30,31] However, those phenomena have not yet been explained.

In order to elucidate the probable mechanism of action of the allosteric modulators **3a**,**b**, their interactions with the GluA2 AMPA receptor were modelled by means of molecular docking using AutoDock Vina 1.1.2 software[34] and molecular dynamics simulations using the CHARMM36/CGenFF 4.4 force field[35,36] in the GROMACS 2021.2 software[37] (for detailed computational workflow and additional data, see Online Supplementary Materials). The binding mode of compound **3a** in the PAM binding site at the interface between the dimeric ligand-binding domains is stable over the entire course of the simulation [200 ns, see Figure S1(*a*)]. Similar to other medium-sized modulators,[38] the **3a** molecule occupies a symmetrical position in the central subpocket of the symmetrical PAM binding site [Figure 2(*a*),(*b*)].

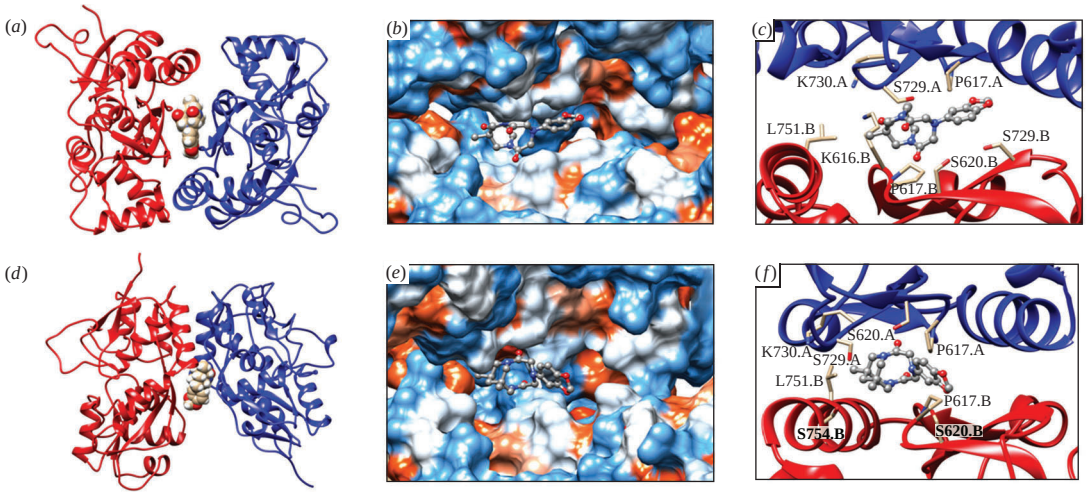


Figure 2 Possible binding modes of (*a*–*c*) modulator **3a** and (*d*–*f*) modulator **3b** in the PAM binding site refined using molecular dynamics simulation. (*a*, *d*) General view of the dimeric ligand-binding domain of AMPA receptor (GluA2) and location of the binding site. (*b*, *e*) Binding pockets in the protein molecular surface colored by local hydrophobicity (brown for hydrophobic and blue for hydrophilic). (*c*, *f*) Detailed view of the binding site. The ligand is represented by grey ball-and-stick model, the amino acid residues located within 3 Å of it are represented by beige stick models.

The binding is primarily stabilized by hydrophobic interactions and steric fit with additional contribution from the electrostatic interaction between the ligand's carbonyl group and the positively charged Lys616 residue [Figure 2(*b*),(*c*)]. On the other hand, the binding stability of compound **3b** is much lower, it undergoes partial dissociation [Figure S1(*b*)] and eventually attains a loosely anchored position in the cleft between the ligand-binding domains [Figure 2(*d*)–(*f*)]. The binding free energy estimated over the stable portion (last 20 ns) of the trajectories using the MM/GBSA approach implemented in the gmx_MMPBSA 1.4.3 software[39,40] is -28.4 ± 0.3 and -24.0 ± 0.2 kcal mol^{-1} for compounds **3a** and **3b**, respectively. Overall, these results indicate that compound **3a** can indeed act as a positive AMPA receptor modulator, which binds in the validated PAM binding site while compound **3b** can be expected to have much weaker PAM activity.

For both of the compounds **3a** and **3b**, the binding in the negative allosteric modulator (NAM) binding site at the interface between ligand-binding (LBD) and transmembrane (TMD) domains, similar to perampanel,[19,41] is also possible according to the molecular docking results. The molecular dynamics simulations of the complexes containing a tetrameric LBD–TMD fragment and four modulator molecules indicate that some of the modulator molecules are stably bound while others can undergo significant displacement or partial dissociation (Figures S3, S4). At this time, no significant differences in the behavior of the compounds can be conclusively detected. Nevertheless, these results can suggest that the NAM activity of compound **3b** can be mediated by its action on the NAM binding sites at the LBD–TMD interface that might be less pronounced in the case of compound **3a**.

In conclusion, we have synthesized two compounds based on the tricyclic 3,7-diazabicyclo[3.3.1]nonane derivative scaffold. In the *in vitro* assessment of their potency as AMPA receptor allosteric modulators, these compounds, despite very similar chemical structures, demonstrate radically different activity profiles. In a wide concentration range (10^{-12}–10^{-7} M), compound **3a** acts as a positive modulator causing the potentiation of the kainate-induced AMPA receptor currents with maximum potentiation at 1 nM (62%) while compound **3b** exhibits a significant (up to 40%) negative modulator effect. The removal of the ketone oxygen leads to a complete inversion of the receptor modulation, representing an activity cliff. Molecular modelling suggests that compound **3a** can interact with the validated PAM binding site at the interface between the dimeric LBDs while compound **3b** can be tentatively expected to act *via* the NAM binding site at the LBD–TMD interface. We hope that more detailed analysis of their binding and interactions coupled with further exploration of this scaffold and subsequent *in vitro* and *in vivo* investigations will allow one to develop more potent and safe positive and negative AMPA receptor modulators with a wide range of potential psychopharmacological applications.

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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.2022.05.023.

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