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Tricyclic derivatives of bispidine as AMPA receptor allosteric modulators

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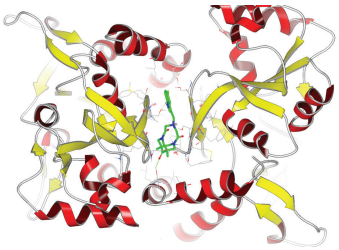
Four new tricyclic derivatives of 3,7-diazabicyclo[3.3.1]-nonane (bispidine) have been synthesized. *In vitro* patch clamp investigation has revealed for one of them the positive modulation of the kainate-induced AMPA receptor currents in a nanomolar concentration range, and for two other compounds the corresponding negative modulation has been demonstrated.



Glutamate is the most abundant excitatory neurotransmitter in the central nervous system. According to preclinical and clinical investigations, it improves cognitive functions, for example an acquisition and a collection of information as well as the consolidation and retention of data, through the activation of ionotropic glutamate receptors.[1–4]

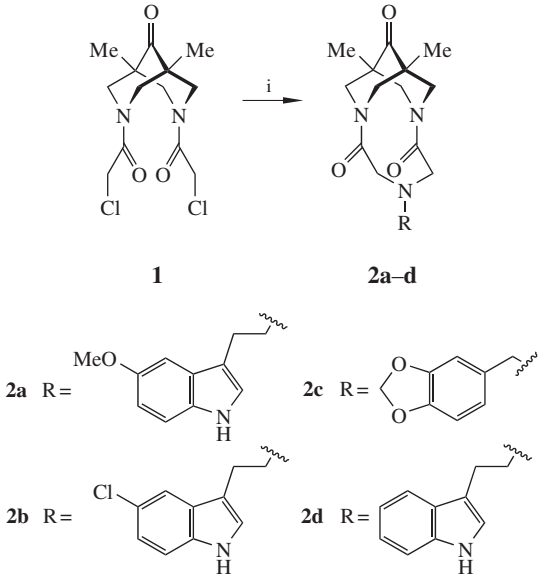
α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are a type of glutamate receptors playing a key role in the information transfer and cognitive processes as well as in the memory formation mechanism.[4–6] Receptor–channel complex of the AMPA receptor consists of four subunits with binding sites for agonists, competitive and non-competitive antagonists, as well as the allosteric sites for positive and negative modulators. The therapeutic significance of the last group has been clearly demonstrated.[7–9] In particular, the positive modulators increase an amplitude of the AMPA receptor-mediated currents, resulting in an enhancement of synaptic transmission and an improvement of cognition[10,11] as well as expression upregulation for brain-derived neurotrophic factor (BDNF),[12,13] which is known to exert neuroprotective and neurogenesis-stimulating effects. Therefore, the search for new AMPA receptor modulators is a promising scientific area.

The molecular modelling and molecular dynamics investigations oriented to the rational design of new positive allosteric modulators (PAMs) of AMPA receptors, were carried out by our group.[14] The pharmacophore hypothesis for AMPA receptor PAMs was developed and 3D QSAR Comparative Molecular Field Analysis (CoMFA) models[15] were built using the docking-based structural data for PAM–receptor complexes. As a result, we managed to find a series of new positive and negative AMPA receptor modulators and to confirm experimentally their activity in nano- and picomolar concentration ranges.[16–20] In this work, we describe new tricyclic derivatives of bispidine and report an *in vitro* assessment of their potency as AMPA receptor allosteric modulators.

Reactions of 3,7-di(chloroacetyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one **1**[21] with the corresponding amines under known conditions[16] resulted in its cyclization into compounds **2a–d** (Scheme 1).[†] Note that for ~100 g scale, precursor **1** can be obtained by a modified procedure using a careful dropwise addition of chloroacetyl chloride to an emulsion of 5,7-dimethyl-1,3-diazaadamantan-9-one in benzene–water, containing KOH and 18-crown-6, with subsequent stirring for several hours and filtration of a crude product. (1,3-Benzodioxol-5-ylmethyl)amine for the synthesis of compound **2c** was obtained from piperonylic acid *via* intermediate acylchloride and then the corresponding amide. The preparation of tricycles **2a**, **2b** and **2d** required reflux and thorough purification due to the presence of the indole moiety. Finally, compounds **2a–d** were isolated in good yields after column chromatography on silica gel. In their ^{1}H NMR spectra, two different singlets of methyl groups at bridgehead positions 1 and 11 are clearly seen, their nonequivalence originates from the fixed parallel orientation of amide carbonyl groups of the tricyclic framework.[21]

[†] *6-[2-(5-Chloro-1*H*-indol-3-yl)ethyl]-1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione* **2b**. To a suspension of 2-(5-chloro-1*H*-indol-3-yl)ethanamine (0.19 g, 1 mmol) and K_2CO_3 (1.10 g, 8 mmol) in anhydrous MeCN, a solution of 3,7-di(chloroacetyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one **1** (0.30 g, 1 mmol) in anhydrous MeCN was added. The mixture was refluxed until the complete consumption of starting material as monitored by TLC. Then K_2CO_3 was filtered off and washed several times with anhydrous MeCN. The solvent from combined organic filtrates was evaporated *in vacuo* affording a crude product, which was further purified by column chromatography on silica gel with $CHCl_3$→$CHCl_3$–EtOH (80 : 1, 50 : 1 and finally 20 : 1). Yield 0.28 g (65%), white solid, mp 174–176 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.55 (s, 3H, Me–C^{1,11}<), 0.87 (s, 3H, Me–C^{1,11}<), 2.76 and 4.06 (d, 4H, bispidine), 2.91–3.00 (m, 8H, CH_2CH_2N and bispidine), 4.57 and 4.73 (d, 4H, CO–CH_2–N<, *J* 13 Hz), 7.05, 7.35, 7.68 and 11.07 (5H, arom.). ^{13}C NMR (100.4 MHz, DMSO-d_6) δ: 15.0, 16.0, 21.4, 44.6, 45.2, 53.6, 54.7, 57.1, 60.2, 112.1, 112.9, 123.0, 124.7, 128.3, 134.8, 168.1, 211.2. HRMS (ESI), *m*/*z*: 443.1832 (calc. for $C_{23}H_{27}N_4O_3Cl$ [M+H]$^+$, *m*/*z*: 443.1844).

For the synthesis of compounds **2a, 2c** and **2d** as well as original ^{1}H and ^{13}C NMR spectra of compounds **2a–d**, see Online Supplementary Materials.



Scheme 1 *Reagents and conditions*: i, RNH_2, K_2CO_3, MeCN, reflux or 50 °C, 15 h.

In vitro tests were carried out by an electrophysiological method with fresh single Purkinje neurons isolated from the cerebellum of 12–15 day old Wistar rats.[17] Transmembrane currents were induced by activation of the AMPA receptors with kainic acid as a partial agonist, using fast superfusion of solutions, where 30 μl of the agonist buffer were added to the neuron washing buffer at a constant speed, and the agonist concentration was varied in the range of 10^{-6}–10^{-4} M. The transmembrane currents for individual neurons were recorded using 2.5–5.5 MΩ borosilicate microelectrodes by the patch clamp local potential fixation method in a whole cell configuration using an EPC-9 device, the data were processed by the Pulsfit program.

When kainic acid was applied, the incoming currents with concentration dependent amplitude were recorded due to the activation of AMPA receptors located on the neurons,[17] the amplitude of these currents was concentration dependent. After addition of compounds **2a–d** to the neuron washing solution, the amplitude of these currents increased for the positive modulators, decreased for the negative modulators or remained unchanged. These effects are collected in Table 1.

The results of the electrophysiological experiments revealed, that among the four synthesized tricycles, compound **2b** invoked a concentration dependent potentiation of the AMPA receptor currents in a wide range of concentrations from 10^{-11} to 10^{-7} M, after activation by kainic acid as a partial agonist. With a further increase in concentration, a weaker action occurred, *i.e.* the effect of tricycle **2b** on the AMPA receptor currents was roughly bell-shaped, which is common to various positive modulators.

Table 1 Effects of various concentrations of compounds **2a–d** on the kainate-induced AMPA receptor currents in the rat cerebellum Purkinje neurons.

Compound	Number of neurons	Compound concentration/M, current amplitude (% to control, ± SD)				
		10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}
2a	3	100±2	100±4	100±3	100±2	100±5
2b	6	118±2.4	124±2.9	132±4.8	125±3.6	119±2.7
2c	3	65±6.8	75±4.6	80±4	83±3.4	87±2.8
2d	4	86±3.5	81±4.1	79±3.8	76±5	71±5.2

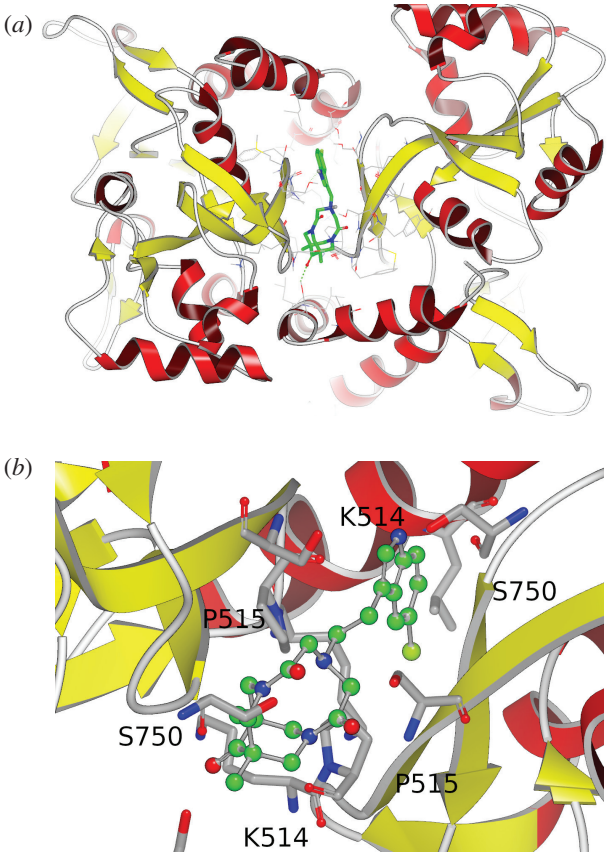


Figure 1 Possible binding mode of compound **2b** between two ligand binding domains of GluA2 receptor: (*a*) general view, (*b*) enlarged view with amino acid residues K514, P515 and S750 of the two ligand binding domains surrounding a molecule of tricycle **2b**.

Compounds **2c** and **2d** demonstrated a somewhat blocking effect on the AMPA receptor currents, most likely due to a negative allosteric modulation. Tricycle **2a** had virtually no effect on the AMPA receptor currents.

A docking investigation was carried out for compounds **2a–d** using an OpenEye Scientific FRED program[22] with high dock resolution into the GluA2 receptor model, which was prepared from X-ray structure data[23] with PDB code 3RNN. Conformations for the docking were generated by OMEGA2 software[24] and the results were visualized with Visual Molecular Dynamics (VMD) program.[25] Figure 1 shows a possible binding mode of compound **2b** between two ligand binding domains of GluA2 receptor, which resembles the known one for the positive allosteric modulator cyclothiazide (CTZ).[26] For steric reasons, only one molecule of tricycle **2b** can fit into the binding site, contrary to CTZ, where two smaller molecules fit there symmetrically.[26]

Figure 2 demonstrates the preferable binding modes for the remaining three compounds. The tricyclic cage moiety of all four products **2a–d** has similar binding, while the orientation of indole and 1,3-benzodioxole parts is somewhat different. Note that it is rather difficult to evaluate positive or negative modulating effect as well as its absence merely from docking results. However, the virtual screening allowed us to concentrate our efforts on four compounds of many dozens of analogues and find three active compounds from the four ones tested *in vitro*.

In summary, compound **2b** has been found to exhibit a pronounced effect of positive allosteric AMPA receptor modulation. Note, that the corresponding negative modulation found for compounds **2c** and **2d** can also find application, for example, the review[27] lists a number of beneficial effects for the negative modulators, including anticonvulsant activity. Thus, further optimization of the structures described here can lead to

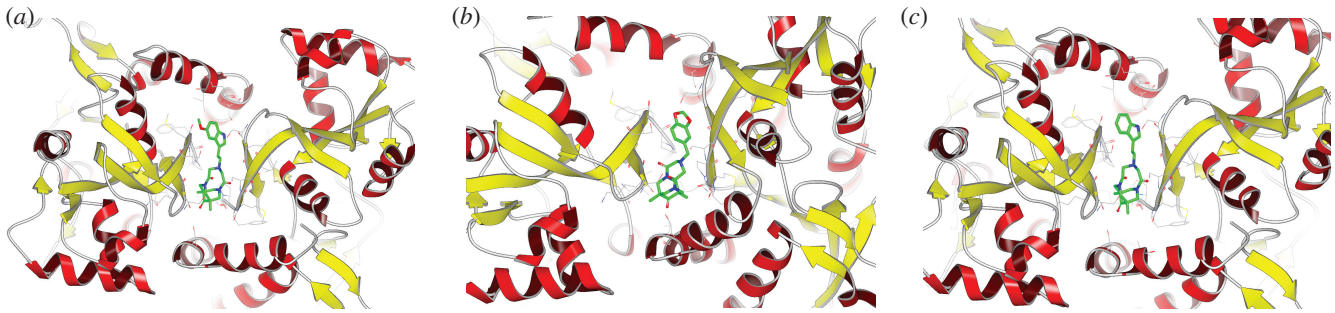


Figure 2 Possible binding modes of compounds (*a*) **2a**, (*b*) **2c** and (*c*) **2d** between two ligand binding domains of GluA2 receptor.

broad-spectrum drugs for the treatment and prevention of neurodegenerative diseases and neuropsychiatric conditions as well as for improvement of human cognitive function and memory.

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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.2019.11.004.

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