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7-Benzyl-1,5-dimethyl-3-piperonyloyl-3,7-diazabicyclo[3.3.1]nonan-9-one as an allosteric modulator of glutamatergic system

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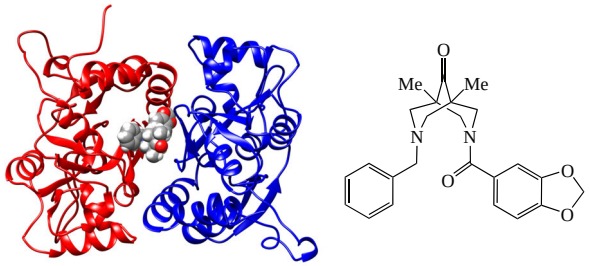
The title compound has been synthesized and assessed *in vitro* by means of electrophysiological patch clamp technique revealing a positive modulatory effect on the kainate-induced currents in Purkinje neurons in a wide concentration range from 10^{-12} to 10^{-6} M. Molecular docking and molecular dynamics simulation revealed a putative binding mode of this compound in the binding site of positive allosteric modulators of AMPA receptors.



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The development of novel approaches leading to new or improved treatments for central nervous system (CNS) diseases is currently of great interest in medicinal chemistry research.[1,2] There exist thousands of compounds of different classes targeting CNS *via* different mechanisms of action.[3] Among them, positive allosteric modulators (PAMs) of the α-amino-β-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) type of glutamate receptors are of particular interest as therapeutic candidates in preclinical and clinical studies for the management of a broad spectrum of neurodegenerative diseases and neuroprotection.[4–6] Being one of the most highly expressed receptors in the brain, AMPA receptor is actively involved in the operation of the CNS glutamatergic system.[7–11]

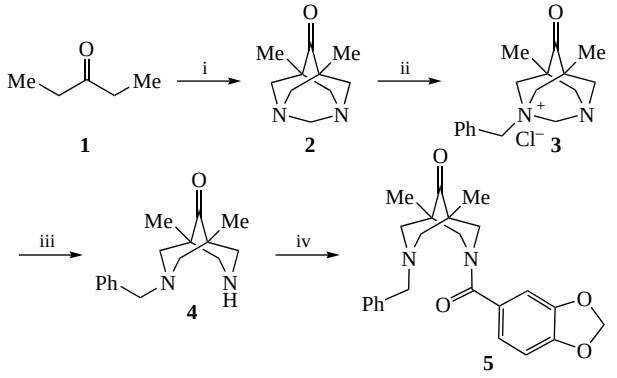
A relatively large number of allosteric modulators of AMPA receptor which belong to rather diverse structural classes have been proposed; however, their potency, safety and pharmacokinetic profiles often make them unfit for the therapeutic use.[12,13] In particular, many PAMs may induce excitotoxicity at higher concentrations.[8,14] In addition, the type and strength of activity of the AMPA receptor allosteric modulators may change dramatically even for fairly similar structures while the factors affecting the activity and the causes for such activity cliffs are not yet fully understood.[12]

Earlier,[15–17] we have reported that *N*,*N'*-substituted compounds based on the bispidine (3,7-diazabicyclo[3.3.1]-nonane) scaffold exhibit modulatory activity due to the binding to an allosteric site of AMPA receptor. Although several promising derivatives have been synthesized and studied in the *in vitro* and *in vivo* assays,[18] the definitive structure–activity relationships (SAR) are yet to be established.[19]

In this work, we have synthesized (Scheme 1) a novel bispidine derivative, 7-benzyl-1,5-dimethyl-3-piperonyloyl-3,7-diazabicyclo[3.3.1]nonan-9-one, possessing AMPA receptor modulatory activity, and performed its *in vitro* and *in silico* evaluation. The target compound **5** was synthesized in four steps. For the final step, the initially attempted approach was the coupling of amine **4** with piperonylic acid activated by *N*,*N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt). Although the reaction proceeded with high conversion, we found it impossible to completely separate compound **5** from 1,3-dicyclohexylurea (DCU) byproduct due to their very close R_f values. Therefore, for the *N*-acylation the Schotten–Baumann method was employed.[†]

^{1}H NMR spectrum of compound **5** presented difficulties for the assignment of the particular protons. Further experiments employing 2D NMR (see Online Supplementary Materials)

[†] *Procedure for the synthesis of compound* **5**. To piperonylic acid (100 mg, 0.6 mmol), $SOCl_2$ (3.6 mmol, 260 μl, 6 equiv.) and pyridine (20 μl) were added. The mixture was refluxed for 6 h until dissolution of the acid. The excess $SOCl_2$ was evaporated *in vacuo* to quantitatively yield the corresponding acid chloride. The obtained acid chloride (111 mg, 0.6 mmol) in chloroform (5 ml) was added dropwise to a solution of compound **4** (155 mg, 0.6 mmol, 1 equiv.) in chloroform (10 ml). The reaction mixture was stirred for 4 h at room temperature until reaction completion (TLC monitoring), then quenched with saturated solution of $KHCO_3$ (5 ml) and stirred for 1 h. The organic layer was separated and dried over Na_2SO_4. After evaporation of chloroform *in vacuo*, the crude product was purified by column chromatography on silica gel, eluting with chloroform (for characterization data, see Online Supplementary Materials).



Scheme 1 *Reagents and conditions*: i, hexamethylenetetramine, AcOH, Pr^iOH, 4 h, reflux (ref. 20); ii, $PhCH_2Cl$, PhH, reflux, 4 h (ref. 21); iii, KOH, $CHCl_3$–H_2O, 1 h (ref. 21); iv, piperonyloyl chloride, $CHCl_3$, room temperature, 4 h, then $KHCO_3$, 1 h.

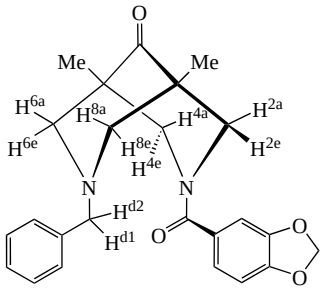


Figure 1 Conformation of compound **5** indicating non-equivalent protons.

made it possible to assign all signals and suggest a *chair–chair* conformation of the bicyclic moiety (Figure 1).

The aliphatic part of ^{1}H NMR spectrum of compound **5** contains a set of ten doublets corresponding to five pairs of diastereotopic protons (Figure 2) and two singlets for methyl groups in positions 1 and 5. The existence of eight geminal couplings of the bispidine moiety instead of usual four is characteristic for the unsymmetrically substituted bispidines.

In vitro electrophysiological experiments were carried out using fresh single Purkinje neurons isolated from the cerebellum of 12–15 days old Wistar rats. Transmembrane currents were induced by the activation of AMPA receptors with their partial agonist kainic acid using fast superfusion of solutions, where 30 μl of the agonist buffer were added to the neuron washing buffer at a constant speed, the agonist concentration varying in the range 10^{-6}–10^{-4} M.‡ Compound **5** demonstrated the positive

Table 1 Effects of various concentrations of compound **5** on the kainate-induced AMPA receptors currents in cerebellum Purkinje neurons (the number of neurons is 5).

C/M	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Current amplitude (% to control ±SD)	148 ± 10	197 ± 25	209 ± 17	151 ± 23	134 ± 10	132 ± 21	110 ± 19

modulation of kainate-induced currents (Figure 3, Table 1) of the AMPA receptors in a broad range of concentrations from 10^{-12} to 10^{-6} M with a maximum current amplitude 209 ± 17% (+109% potentiation) at 10^{-10} M.

The interaction of the positive modulator **5** with the GluA2 AMPA receptor was modelled by means of molecular docking using AutoDock Vina 1.1.2 software[22] and molecular dynamics simulations using the CHARMM36/CGenFF 4.6 force field[23,24] in the GROMACS 2023.0 software[25] following the previously published[19] computational workflow. In the non-protonated form, the ligand shifted from the initial docking-based pose, and then its binding mode in the PAM binding site at the interface of the dimeric ligand-binding domains remained stable over the entire course of the simulation [100 ns, see root mean squared deviation (RMSD) plots in Figure S1]. In general agreement with the results for symmetric modulators based on the 3,7-diazabicyclo[3.3.1]nonane scaffold,[11,18] the modulator molecule occupied a slightly off-center position in the central subpocket of the symmetrical PAM binding site (Figure 4). The benzyl moiety anchors the molecule to the binding site wall while the piperonyl group is immersed deeper inside the pocket. The binding is primarily stabilized by steric fit and hydrophobic interactions. The binding free energy calculated using the MM/GBSA (molecular mechanics, generalized Born, surface area) method was –21.1 ± 0.4 kcal mol^{-1}. Interestingly, the form with protonated benzylamine moiety, although predominant at physiological pH, is apparently unable to attain a stable binding mode, instead undergoing quick dissociation during the molecular dynamics simulations.

Computational evaluation of the physicochemical, ADMET, and PAINS profiles for compound **5** included the predictions of its lipophilicity and aqueous solubility,[26] human intestinal absorption,[27] blood–brain barrier permeability,[28] and hERG-mediated cardiac toxicity risk,[29] as well as calculation of quantitative estimate of drug-likeness[30] and pan-assay interference compounds (PAINS) alert check using RDKit version 2020.03.4 software.[31] The results (Table 2) were quite acceptable for a potential lead compound at the early drug

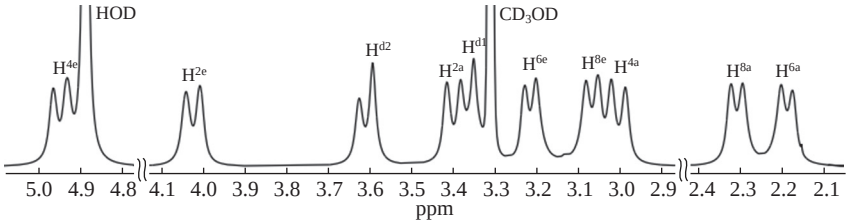


Figure 2 Upfield region of ^{1}H NMR spectrum of compound **5**.

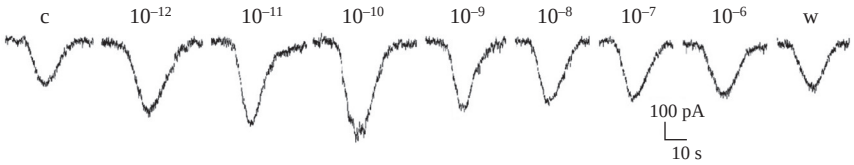


Figure 3 Kainate-induced currents: control (c), after adding compound **5** at different concentrations, and after washout (w).

‡ The transmembrane currents for individual neurons were recorded with 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 (HEKA, Germany) and Pulsfit program (HEKA, Germany) for signal processing.

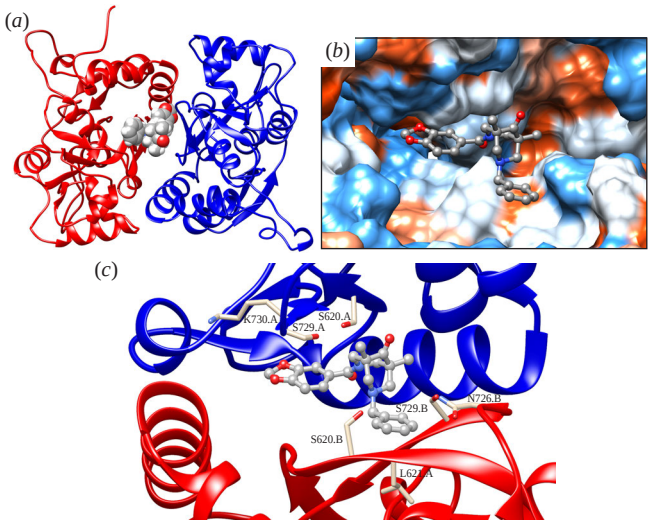


Figure 4 Binding mode of the PAM **5**, refined using molecular dynamics simulation (100 ns). (*a*) General view of the dimeric ligand binding domain of AMPA receptor (GluA2) and location of the binding site. (*b*) Binding pockets in the protein molecular surface colored by local hydrophobicity (brown for hydrophobic and blue for hydrophilic). (*c*) Detailed view of the binding site. The ligand is represented by a grey ball-and-stick model, the amino acid residues located within 3 Å of it are represented by beige stick models.

Table 2 Predicted physicochemical and ADMET profiles of compound **5**.[a]

$LogP_{ow}$	pS_{aq}	LogBB	HIA	hERG pK_i	hERG pIC_{50}	QED
2.73	3.36	–0.50	100	7.27	5.22	0.78

[a] $LogP_{ow}$ is octanol–water partition coefficient, pS_{aq} is aqueous solubility [−log(M)], LogBB is blood–brain barrier permeability, HIA is human intestinal absorption (%), hERG pK_i is hERG potassium-channel affinity [−log(M)], hERG pIC_{50} is hERG potassium channel inhibitory activity [−log(M)], QED is quantitative estimate of drug-likeness.

development stages, although additional checks and structure optimization would likely be required.

To conclude, compound **5** has demonstrated a pronounced positive modulatory activity at subnanomolar concentrations with respect to AMPA receptor in electrophysiological experiments *in vitro*. Molecular docking and molecular dynamics simulation have shown that a putative binding mode of this compound is located in the binding site of positive allosteric modulators of AMPA receptors. Further modification of this compound may lead to the development of broad-spectrum drugs for the prevention and treatment of neurodegenerative and psychoneurological diseases.

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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.2024.04.014.

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