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New 1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione derivative as an allosteric modulator of the glutamatergic system

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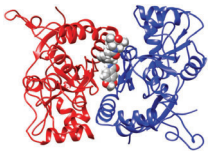
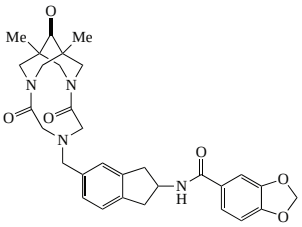
An optimized synthesis of *N*-{5-[(1,11-dimethyl-4,8,12-trioxo-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradec-6-yl)methyl]-indan-2-yl}-1,3-benzodioxole-5-carboxamide has been carried out from indan-2-one oxime in seven steps. *In vitro* studies using electrophysiological patch clamp technique have revealed a positive modulation effect of this compound on kainate induced currents in Purkinje neurons in a wide range of concentrations from 10^{-12} to 10^{-8} M.





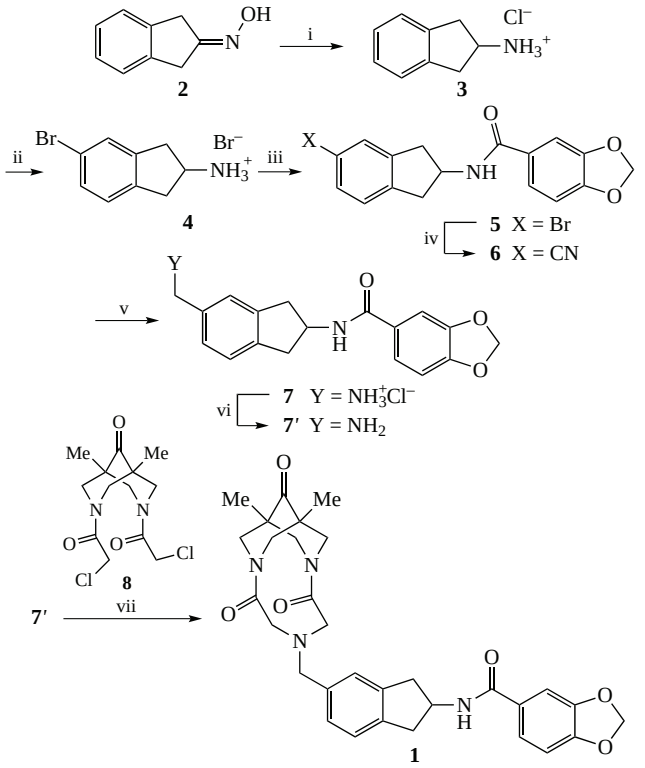
Keywords: 3,7-diazabicyclo[3.3.1]nonanes, indane derivatives, piperonylic acid, cross-coupling, nitriles, AMPA receptor, allosteric modulators, PAMs, patch clamp.

L-Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system (CNS) that plays a fundamental role in the control of motor function, cognition, and mood. The physiological effects of glutamate are mediated by two functionally distinct families of receptors. Activation of metabotropic (G-protein-coupled) glutamate receptors leads to modulation of neuronal excitability, while ionotropic glutamate receptors (ligand-gated ion channels) are responsible for mediating a rapid synaptic response to extracellular glutamate. Ionotropic glutamate receptors are divided into three subclasses based on molecular and pharmacological differences and are named after the agonists that selectively activate them: AMPA (*R*-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid), NMDA (*N*-methyl-D-aspartate), and kainate (2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine).[1,2] AMPA receptors are ubiquitous in the CNS and mediate much of the rapid neurotransmission. They are also critical for synaptic plasticity and long-term potentiation (LTP) induction, which in turn has a direct impact on memory-forming processes.[3–9] Currently, a number of researchers believe that positive allosteric modulators of AMPA receptors fine-tune the glutamatergic system, since they do not cause any effects in the absence of a natural ligand in the synapse. In particular, in the case of direct agonists, overdose can cause overstimulation and neurotoxicity, leading to uncontrolled brain damage.[10] In contrast, allosteric AMPA receptor modulators have relatively few side effects at therapeutically significant doses.[11] In addition, it was shown that positive allosteric modulators of the AMPA receptor can be used to protect nerve cells from neurotoxic effects.[12] These molecules have been shown to enhance synaptic transmission and LTP and increase the expression of neurotrophic factors. Thus, the potential therapeutic application of these molecules may have a significant positive impact in the treatment of neurodegenerative diseases such as schizophrenia, depression, Alzheimer's disease, Parkinson's disease, ADHD and respiratory depression.[13–17]

Earlier, we have performed molecular modelling and molecular dynamics studies for the rational design of new positive and negative modulators of AMPA receptors.[18,19] The PAM pharmacophore hypothesis as well as the 3D QSAR (CoMFA) models[20,21] 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.[22–25]

In this work, we have synthesized new *N*-{5-[(1,11-dimethyl-4,8,12-trioxo-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradec-6-yl)-methyl]indan-2-yl}-1,3-benzodioxole-5-carboxamide **1**[†] as a

[†] *N-{5-[(1,11-Dimethyl-4,8,12-trioxo-3,6,9-triazatricyclo[7.3.1.1^{3,11}]-tetradec-6-yl)methyl]indan-2-yl}-1,3-benzodioxole-5-carboxamide* **1**. A 20% aqueous solution of KOH was added to a suspension of *N*-[5-(aminomethyl)indan-2-yl]-1,3-benzodioxole-5-carboxamide hydrochloride **7** (86 mg, 0.248 mmol) in distilled water (pH 10). The resulting mixture was extracted with CH_2Cl_2, the combined organic phases were dried over anhydrous Na_2SO_4, then filtered and the solvent was distilled off. The product was dissolved in NMP (15 ml), then K_2CO_3 (319 mg, 2.31 mmol) and 3,7-bis(chloroacetyl)-1,5-dimethyl-3,7-diazabicyclo-[3.3.1]nonan-9-one **8** (88 mg, 0.273 mmol) were added. The mixture was stirred under heating at 75 °C for 15 h. Then the reaction mixture was



Scheme 1 *Reagents and conditions*: i, H_2, Pd/C, HCl, MeOH; ii, Br_2, H_2O, then HBr; iii, KOH, H_2O, then piperonylic acid, CDI, MeCN; iv, $Zn(CN)_2$, $Pd(OAc)_2$, dppf, NMP/H_2O, PMHS; v, H_2, Pd/C, HCl, EtOH; vi, KOH, H_2O; vii, **8**, K_2CO_3, NMP.

new representative of our focused compound library with a tricyclic scaffold (Scheme 1, more detailed scheme is given in Online Supplementary Materials) and evaluated its biological activity *in vitro*.

For the synthesis of required indan-2-one oxime **2** from the preceding ketone, a known method[26,27] was modified so that addition of pyridine was avoided thus facilitating the isolation of product **2**. The synthesis was carried out by mixing a solution of indan-2-one and hydroxylamine hydrochloride in the EtOH/H_2O system (1 : 1). For synthesis of indan-2-ylamine hydrochloride **3** with a 91% yield, another known method[26,27] was also modified. It was shown that the addition of $PdCl_2$ was not required, and conducting the reaction at a hydrogen pressure of 5 atm in an autoclave increased the yield, reduced the reaction time and prevented the formation of byproducts. The crude material did not need an additional purification.

Selective bromination of indan-2-ylamine hydrochloride **3** presents certain difficulties, since the rates of mono- and dibromination in an aqueous solution are similar. Optimization revealed that the key factor was the amount of water. The optimal H_2O/indan-2-ylamine hydrochloride ratio of 4.5 ml per 1 g allows the reaction to be carried out selectively with a good yield because the monobromo derivative **4** precipitates and does not undergo the second bromination. Cyano derivative **6** was obtained from the corresponding amide **5** upon a Pd-catalyzed cross-coupling reaction using dppf as a ligand, $Zn(CN)_2$ as a source of $CN^–$, and polymethylhydrosiloxane (PMHS) as the reducing additive.

Amine **7** was obtained by the reduction of the corresponding nitrile **6**. The key problem herein is to ensure the selectivity in the presence of the amide group, which makes the use of classical complex hydrides impossible. In this study, heterogeneous hydrogenation of nitrile **6** was carried out in an autoclave using 10% Pd/C as a catalyst. By varying the reaction conditions (time, pressure, and concentration of HCl), it was found that the key factor for the reaction was the addition of 1.5 equiv. concentrated HCl, which provided preparation of amine **7** in the form of hydrochloride with high yield.

The key tricyclic compound **1** was synthesized by double alkylation of amine **7′** with dichloro-derivative **8**. Compound **8** was obtained using a modified method for the acylation of 5,7-dimethyl-1,3-diazaadamantan-6-one in a two-phase $CHCl_3$/H_2O system, which makes it easier to isolate the product and increase the yield.[23] Varying the base, solvent and temperature, the alkylation was optimized. Both K_2CO_3 and Cs_2CO_3 as bases gave the best results, so finally K_2CO_3 was used in the reaction. NMP was used as a solvent because it both dissolved the starting amines (unlike acetonitrile) and had rather high boiling point to keep the necessary reaction temperature.

In vitro electrophysiological experiments were carried out using a patch clamp technique with local fixation of potential. We used freshly isolated single Purkinje neurons from the cerebellum of 12–16 day old Wistar rats[28] as a test system. Transmembrane currents were induced by the activation of AMPA receptors with a solution of their partial agonist, namely kainic acid, using fast superfusion of solutions, where 30 μl of the agonist buffer were added to the neuron washing buffer at a constant rate, 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 in a whole-cell configuration with an EPC-9 device from HEKA, Germany. The data were processed by a Pulsfit program from HEKA, Germany. Kainate-induced binding of compound **1** demonstrated positive modulation (Figure 1) of the AMPA receptor in the range of concentrations from $10^{–12}$ to $10^{–7}$ M with a maximum (current amplitude 165 ± 5%) at $10^{–9}$ M concentration (Table 1).

In order to elucidate the probable mechanism of action of the allosteric modulator **1**, its interactions with the GluA2 AMPA receptor were modelled by means of molecular docking using AutoDock Vina 1.1.2 software[29] and molecular dynamics simulations using the CHARMM36/CGenFF 4.6 force field[30,31] in the GROMACS 2021.2 software[32] (for detailed computational workflow and additional data, see Online

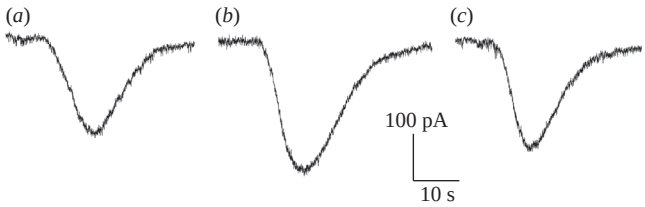


Figure 1 Kainate-induced currents: (*a*) control, (*b*) after application of 0.01 nM compound **1**, (*c*) after washout.

Table 1 Effects of various concentrations of compound **1** on the kainate-induced AMPA receptor currents in rat cerebellum Purkinje cells (the number of neurons is 7).

C/mol dm$^{–3}$	$10^{–12}$	$10^{–11}$	$10^{–10}$	$10^{–9}$	$10^{–8}$	$10^{–7}$
Current amplitude (% to control ± SD)	111 ± 3	145 ± 4	152 ± 5	165 ± 5	120 ± 3	96 ± 3

cooled to room temperature and filtered, the solvent was distilled off with an oil pump. The solid residue was treated with Et_2O and filtered off. The resulting product was purified by column chromatography [eluents $CHCl_3$, $CHCl_3$/EtOH (100 : 1) and $CHCl_3$/EtOH (50 : 1)]. This yielded 80 mg (0.143 mmol, 58%) of pure title compound **1** as a white crystalline product. Mp 173–174 °C.

For the physico-chemical characterization of the final and intermediate products, synthetic procedures and the original ^{1}H and ^{13}C NMR spectra, see Online Supplementary Materials.

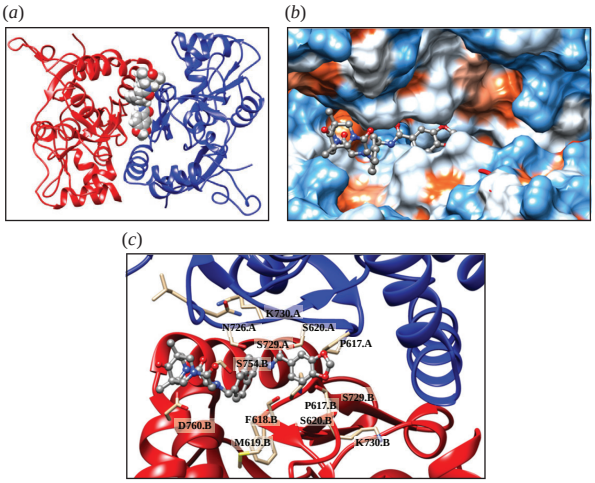


Figure 2 Possible binding mode of the modulator **1** in the PAM binding site refined using molecular dynamics simulation. (*a*) General view of the dimeric ligand-binding domain of AMPA receptor (GluA2) and location of the binding site. (*b*) Binding pockets in the protein molecular surface colored by local hydrophobicity (brown for hydrophobic and blue for hydrophilic). (*c*) Detailed view of the binding site. The ligand is represented by grey ball-and-stick model, the amino acid residues located within 3 Å of it are represented by beige stick models.

Supplementary Materials). The binding mode of compound **1** in the PAM binding site at the interface between the dimeric ligand-binding domains is slightly adjusted compared to the docked pose and then remains stable over the entire course of the simulation (100 ns, see Figure S1). Similar to other larger-sized modulators,[33,24] the molecule **1** occupies the central and side subpockets of the symmetrical PAM binding site [Figure 2(*a*),(*b*)]. The binding is primarily stabilized by hydrophobic interactions and steric fit [Figure 2(*b*),(*c*)]. The binding free energy estimated over the stable portion (last 20 ns) of the trajectory using the MM/GBSA approach implemented in the gmx_MMPBSA 1.4.3 software[34,35] is –38.6 ± 0.3 kcal mol^{-1}. Overall, these results indicate that compound **1** can indeed act as a positive AMPA receptor modulator which binds in the validated PAM binding site.

In summary, it can be concluded that compound **1** exhibits a pronounced effect characteristic of allosteric AMPA receptor modulators. Further optimization of the structure of compound **1** can lead to the creation of broad-spectrum drugs for the treatment and prevention of diseases associated with dysfunction of the central nervous system. We hope that further *in vitro* and *in vivo* studies will allow us to develop 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.2023.01.022.

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