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Synthesis of an allosteric modulator of ionotropic glutamate receptors

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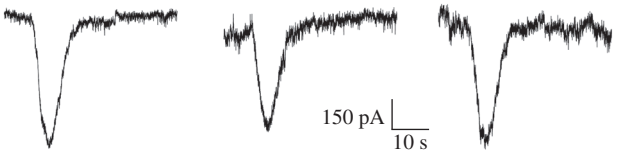
Synthesis of 6-[4-methoxy-3-(pyrrolidin-1-ylmethyl)benzyl]-1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione has been carried out and optimized. *In vitro* studies using electrophysiological patch clamp technique have revealed a negative effect of this compound on kainate-induced currents in Purkinje neurons in a wide range of concentrations from 10^{-11} to 10^{-6} M.



Keywords: 3,7-diazabicyclo[3.3.1]nonane, AMPA receptor, negative allosteric modulator, NAM, patch clamp.

Glutamatergic mediator system is the main excitatory mediator system of mammalian brain, glutamate related synapses being the major part of all excitatory synapses.[1–3] 2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptors represent a subtype of glutamate receptors. They are involved in transmission of exciting postsynaptic signals as well as participate in long-term potentiation processes, which in turn are associated with learning and memory.[4–7] Therefore, the development of new AMPA receptor ligands represents a priority goal in the creation of drugs for normalization and enhancement of cognitive function.[8–11] It is known, that AMPA receptor agonists can cause serious side effects like neurotoxicity, since they activate the receptors in a direct way. In contrast, allosteric modulators of the receptor,[8] including positive and negative ones, just enhance the receptor-mediated currents after the endogenous ligand is released, and thereby act through fine tuning of the receptor signaling.

Many researchers in this area suppose that the development of new allosteric modulators of AMPA receptor can play an important role in creation of a therapeutic strategy for treatment of CNS disorders like schizophrenia, Alzheimer's disease and adult attention deficit/hyperactivity disorder (ADHD).[12–14]

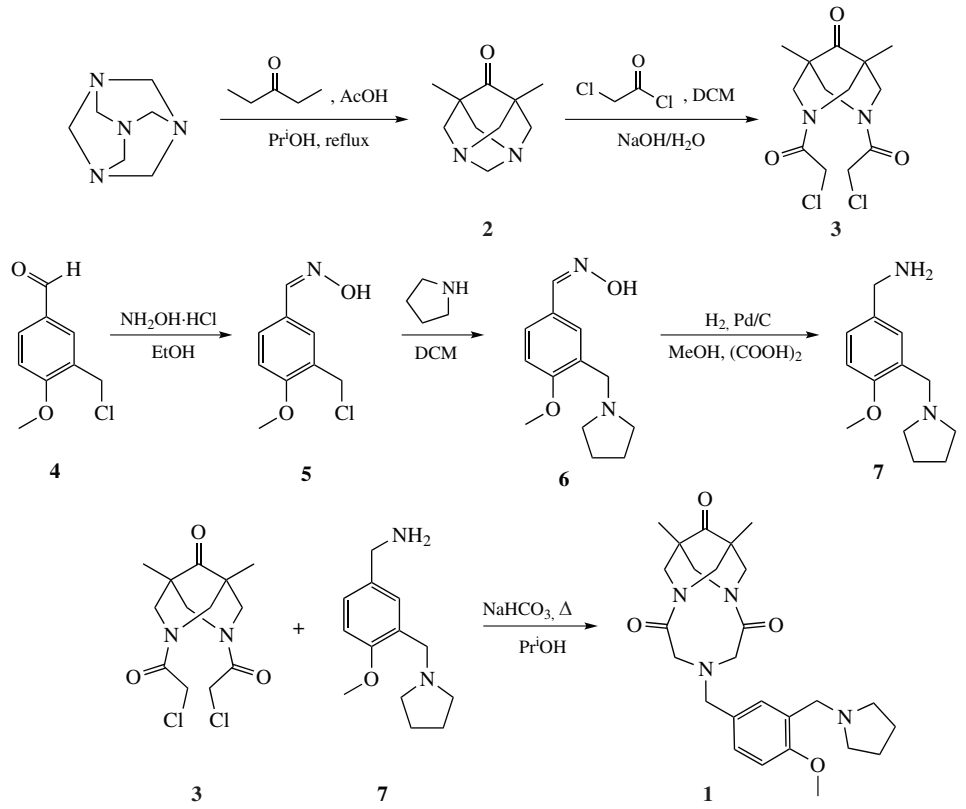
Following this trend, we developed a pharmacophore hypothesis for the AMPA receptor allosteric modulators using 3D QSAR models.[15] As a result, we managed to find new structural scaffolds, a series of compounds based on them was synthesized and their biological effect was confirmed.[16–19]

In this work, we have synthesized 6-[4-methoxy-3-(pyrrolidin-1-ylmethyl)benzyl]-1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione **1** as a new representative of our focused compound library with a tricyclic scaffold (Scheme 1) and evaluated its *in vitro* biological activity. Molecular modeling of this type of structures had already been performed in our earlier studies.[17]

Scheme 1 demonstrates preparation of compound **1**.[†] Opening of diazaadamantanone **2**[20] with chloroacetyl chloride resulted in the bis-chloroacetyl derivative of 1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one **3**. Amination of chloromethylanisaldehyde **4** and alkylation of pyrrolidine by the resulting oxime **5** followed by reduction of the obtained *N*-hydroxy-4-methoxy-3-(pyrrolidin-1-ylmethyl)benzylideneamine **6** using hydrogenation on a Pd/C catalyst (5 wt%) led to 4-methoxy-3-(pyrrolidin-1-ylmethyl)benzylamine **7**. The final step was the dialkylation of amine **7** by 3,7-bis(chloroacetyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one **3**.

[†] *6-[4-Methoxy-3-(pyrrolidin-1-ylmethyl)benzyl]-1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione* **1**. A mixture of amine **7** (11.44 g, 51.8 mmol), isopropanol (115 ml), sodium hydrogen carbonate (17.47 g, 208 mmol) and compound **3** (16.62 g, 51.8 mmol) was heated to boiling and held for 6 h, then cooled to 2 °C. The inorganic precipitate was filtered off and washed on a filter with isopropanol (30 ml). The filtrate was collected, solvent evaporated and the residue recrystallized from ethyl acetate (150 ml). The precipitate formed was filtered and washed on a filter with cold ethyl acetate (20 ml). Yield 20.66 g (85%), mp 115–117 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.99 (s, 3H), 1.11 (s, 3H), 1.78 (br. s, 4H), 2.54 (br. s, 4H), 2.73 (d, 2H, *J* 13.3 Hz), 3.00 (d, 2H, *J* 13.3 Hz), 3.18 (d, 2H, *J* 13.3 Hz), 3.54 (s, 2H), 3.63 (s, 2H), 3.76 (d, 2H, *J* 13.3 Hz), 3.84 (s, 3H), 4.91–4.95 (m, 4H), 6.85 (d, 1H, *J* 8.4 Hz), 7.14 (d, 1H, *J* 8.4 Hz), 7.36 (s, 1H). ^{13}C NMR (100.4 MHz, $CDCl_3$) δ: 15.5, 16.3, 23.7, 45.4, 46.2, 53.7, 54.5, 55.9, 60.3, 62.1, 110.4, 127.7, 128.5, 129.5, 131.9, 157.4, 168.7, 211.3. HRMS (ESI), *m/z*: 469.2809 $[M+H]^+$ (calc. for $C_{26}H_{37}N_4O_4^+$, *m/z*: 469.2809).

For the synthesis of compounds **2**, **3** and **5**–**7** as well as the original ^{1}H and ^{13}C NMR spectra for compounds **1**–**3** and **5**–**7**, see Online Supplementary Materials.



Scheme 1

Table 1 Hydrogenation of oxime **6** on Pd/C (5 wt%).

Entry	Pressure/atm	Temperature/°C	Time/h	Yield of amine **7** (%)
1	9	25	0.5	Traces
2	7	25	1	5
3	5	25	1	15
4	3	25	1.5	30
5	2	25	1.5	50
6	1	0	10	62
7	1	25	2	72
8	1	35	1.5	69
9	1	45	1	55

For the synthesis of compound **3**, we developed a technique, which as distinct from our known method[21] avoided several manipulations during isolation, namely extraction, recrystallization and column chromatography. Also, during the preparation of diazaadamantanone **2**, isopropanol was used as a solvent instead of *n*-butanol in the known procedure,[20] resulting in a simplified isolation of the product.

Note that earlier we used $LiAlH_4$ to reduce oxime **6** to amine **7**, however the unsatisfactory product yields of 40–55%, poor reproducibility and the need to use column chromatography for this synthetic step stimulated us to search for alternative reducing agents. The hydrogenation on Raney nickel under a pressure of 10–15 atm was found to be unsuccessful, whereas the hydrogenation using Pd/C (5 wt%) allowed us to significantly increase the yield up to 70% and to simplify the isolation of product **7**. The optimization results are collected in Table 1.

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[22] 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. Cyclothiazide (CTZ) as a well-known positive allosteric modulator of AMPA receptors was used as a reference ligand. The experimental results are presented in Table 2.

When kainic acid was applied, the incoming currents with concentration dependent amplitude were recorded (Figure 1). After addition of compound **1**, a concave bell-shaped dependence was observed in a wide range of concentrations from 10^{-11} to 10^{-6} M. The maximum blocking was detected at 0.1 to 1 nM. At higher concentrations, the activity of compound **1** decreased and at 100 nM and more it became indistinguishable from the control level. For the CTZ reference the results were consistent with known data on its EC_{50} value.[18,23]

Table 2 The effect of compound **1** and CTZ[18] on the AMPA receptor currents in rat cerebellum Purkinje cells.

Compound	Number of neurons	Current amplitude (% to control, ±SD) for various compound concentrations						
		10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
1	4	81±12	78±13	66±11	91±2	95±4	93±3	–
CTZ	4	–	–	–	–	100±3	145±11	235±18

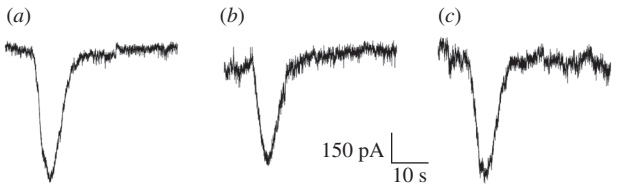


Figure 1 Kainate-induced currents: (*a*) control, (*b*) after application of 0.1 nM compound **1**, (*c*) after washout.

In summary, it has been shown that compound **1** is capable of exerting a blocking effect on the currents induced by kainic acid in Purkinje neurons of rat cerebellum, *i.e.* most likely tricycle **1** is a negative allosteric modulator of the AMPA receptor. Note that compound **1** differs from the positive modulator described[17] only by the presence of pyrrolidin-1-yl substituent in its structure rather than 1*H*-pyrazol-1-yl one, however the reason for the large discrepancy in activity is not quite clear. Moreover, it is impossible to draw a more detailed conclusion about the mechanism of the effect from the data available, since kainic acid is capable of activating both AMPA and kainate receptors. In particular, there is a wide variety of AMPA and kainate receptor subtypes, which differ in the composition of their subunits as well as in their post-translational modification, and therefore the detailed composition of receptors expressed on the surface of Purkinje rat cerebellum neurons still remains unknown.

We plan to investigate modulation of GluA1/GluA2 and GluA1/GluA4 ion channels of the AMPA receptor expressed in HEK293 cells in attempt to answer a question whether the pattern observed in the *in vitro* patch clamp experiments originates from a negative modulation of the AMPA receptor or it is the result of interaction with a different biological target. We expect that with further structural optimization, it will be possible to create a series of broad-spectrum drugs for the treatment and prevention of neurodegenerative and neuropsychiatric diseases as well as for an 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.2020.03.008.

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