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SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMINATION PRODUCTS OF THE ALKALOID SECURININE

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A method for stereospecific synthesis of allomargaritarin and other amination products of the natural alkaloid securinine was developed. Stereoselective nucleophilic addition of an amine at the double bond of the azobicyclooctane moiety of securinin was achieved by using ytterbium triflate as the catalyst. The biological activity of securining caused by adding pharmacophores to the molecule was shown to change experimentally.

Key words: alkaloids, securinine, allomargaritarin, ytterbium triflate, stereospecific synthesis.

Securinine alkaloids form a group of tetracyclic compounds with 6-azobicyclo[3.2.1]octane as the key structure to which an α,β-unsaturated-γ-lactone moiety and a piperidine ring are fused. Such heterocycles are interesting not only due to the unique structure but also because of their biological activity. Securinine (**1**) is the most well-known alkaloid.

Securinine is a stereospecific competitive antagonist of the $GABA_A$-receptor (IC_{50} about 50 μM [1]) with proven CNS-activity in animals [2, 3]. Furthermore, this compound exhibits neuroprotective properties, enhances learning and memory [4, 5], and was used previously in medical practice [6].

Securinine and its analogs have rigidly fixed structures. Margaritarine, which was isolated from *Margaritaria indica* (Dalz.) G. L. Webster, is the only natural securinine-type alkaloid that contains an *N*-tryptamine peripheral substituent [7]. The replacement of the multiple bond by sp^3-hybridized C atoms and the presence of an additional asymmetric center imparts a definite conformational flexibility to margaritarine, which increases its ability to interact with active receptor centers. Margaritarine is a minor constituent of alkaloid mixtures and is difficultly isolated in quantities sufficient to investigate it. We attempted to develop convenient synthetic methods for this compound, to prepare other derivatives of securinine alkaloids, and to test them for certain types of biological activity.

Studies on the synthesis of margaritarine or modification of securinine alkaloids by introducing new pharmacophores have not yet been reported. The system of conjugated double bonds of the azobicyclooctane and lactone rings are most interesting for preparing modified securinine derivatives. Because this system is activated by the carbonyl group, it is readily attacked by nucleophiles. Nucleophilic addition of amines to securinin can produce the alkaloid allomargaritarine (**3**) (margaritarine epimer at C-2′) and other amino derivatives.

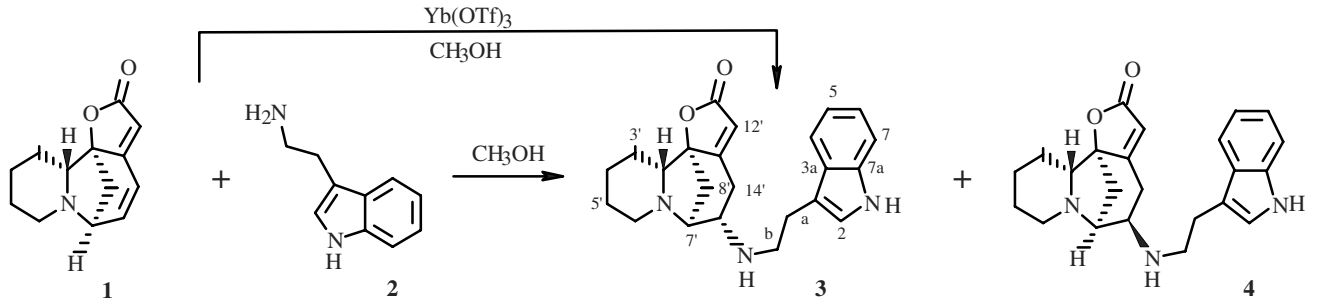

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TABLE 1. Chemical Shifts (δ, ppm) and Spin-Spin Coupling Constants (J/Hz) in PMR and ^{13}C NMR Spectra of Allomargaritarine (**3**) and its Epimer **4**

C atom	**3**		**4**	
	δ_C, ppm	δ_H, ppm	δ_C, ppm	δ_H, ppm
2′	62.18	2.32, (dd, J_1 = 2.7, J_2 = 11.8)	63.51	2.6 (dd, J_1 = 2.5, J_2 = 10.8)
3′	24.16	1.4 (br.s, dd, J = 11.8), 2.2 m	24.15	1.36 (br.s, dd, J = 10.8), 2.8 m
4′	21.53	1.2 m, 1.6 m	21.9	1.2 - 1.4 m (2H)
5′	26.11	1.1 - 1.2 m (2H)	26.12	1.2 - 1.4 m (2H)
6′	49.76	2.52 (br.s, d, J = 10.7); 2.95 (ddd, J_1 = J_2 = 10.7, J_3 = 3.7)	49.44	2.43 (br.s, d, J = 12.0; 2.9–3.0 m
7′	60.84	3.1 (d, J = 6.7)	59.96	3.08 (t, J_1 = 9.5, J_2 = 4.6)
8′	35.94	1.16 (d, J = 11.1); 2.58 (dd, J_1 = 11.1, J_2 = 6.7)	32.82	1.7 (d, J = 10.9); 2.75 (dd, J_1 = 10.9, J_2 = 4.6)
9′	91.68		91.85	
11′	173.92		173.77	
12′	111.75	5.5 (d, J = 2.1)	111.78	5.3 (d, J = 2.3)
13′	174.25		174.95	
14′	31.72	2.98 m, 14′α-H; 2.25 (ddd, J_1 = 2.1, J_2 = 8.9, J_3 = 16)	26.24	2.9 m, 14′α-H 2.35 (ddd, J_1 = 2.3, J_2 = 6.01, J_3 = 15)
15′	60.48	2.8 (ddd, J_1 = 8.9, J_2 = 6.5, J_3 = 1.5)	60.18	2.9 (dd, J_1 = 9.5, J_2 = 6.01)
2	122.70	6.9 (d, J = 2.1)	122.55	6.9 (d, J = 2.2)
3	113.84		113.91	
α	26.51	2.7 - 2.98 m (2H)	26.25	2.7 - 2.9 m (2H)
β	47.11	2.7 - 2.98 m (2H)	47.99	2.7 - 2.9 m (2H)
3a	127.67		127.71	
4	119.14	7.48 (br.s, d, J = 7.6)	119.18	7.53 (br.s, d, J = 7.8)
5	119.69	7.07 (ddd, J_1 = 7.6, J_2 = 7.0, J_3 = 1.0)	119.76	7.09 (ddd, J_1 = 7.8, J_2 = 7.0, J_3 = 1.0)
6	122.46	7.09 (ddd, J_1 = 8.1, J_2 = 7.0, J_3 = 1.0)	122.55	7.12 (ddd, J_1 = 7.6, J_2 = 1.3, J_3 = 1.0)
7	110.14	7.22 (dd, J = 8.1)	111.28	7.3 (dd, J = 7.6)
7a	136.97		136.91	
1-NH		8.3 br.s		8.06 br.s

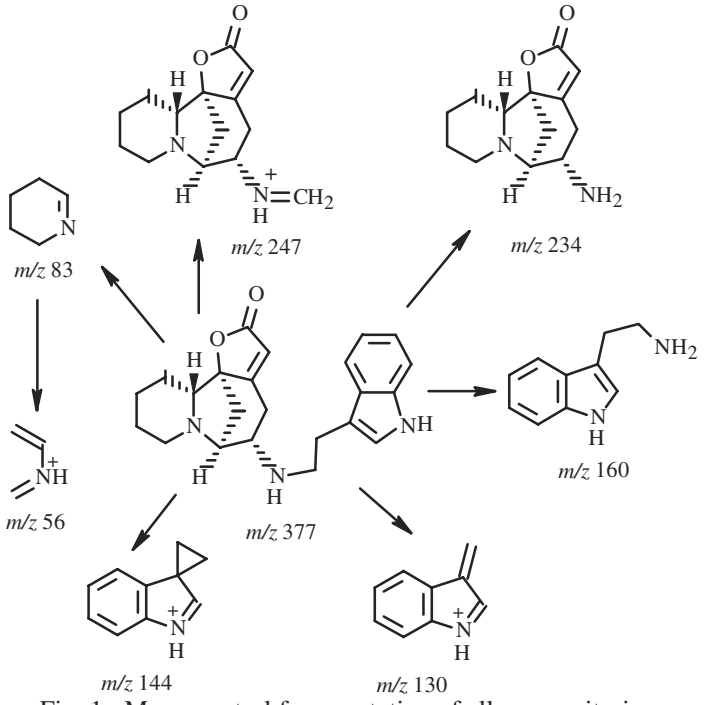


Fig. 1. Mass spectral fragmentation of allomargaritarine.

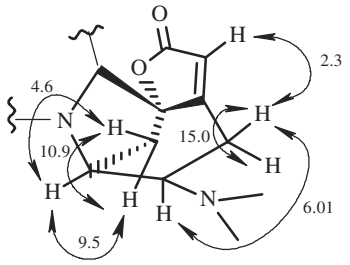


Fig. 2. Spin—spin coupling constants (Hz) of protons of the azobicyclooctane and lactone rings of allomargaritarine epimer **4**.

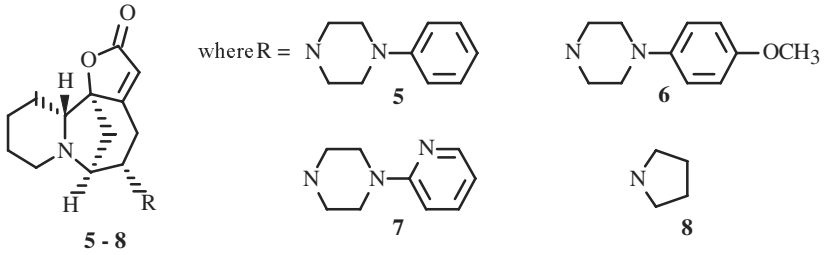
We developed a stereospecific synthetic method for allomargaritarine (**3**) from **1** and tryptamine (**2**) using ytterbium triflate as the catalyst. We also produced several products of amination of securinin by various pharmacophoric amines.

A mixture of two compounds in equal amounts that we isolated using HPLC and characterized by spectral methods was formed under non-catalytic conditions as a result of the reaction of **1** and **2**. Spectral data established that these were allomargaritarine (**3**) and its epimer **4**. The presence of an α,β-unsaturated lactone ring was confirmed by the presence in IR spectra of **3** and **4** of absorption bands at 1745 and 1630 cm^{-1} in addition to the corresponding resonances in ^{13}C NMR spectra (ppm): 173.92 and 173.77 for the carbonyl C atom and 111.75 and 111.78 for the C-12′ atom (Table 1). The PMR spectrum showed doublets for the olefinic proton on C-12′ (δ_H = 5.5, J = 2.1 Hz and 5.3 ppm, J = 2.3 Hz) that had allyl spin—spin coupling constants (SSCC) with the β-proton on C-14′ (δ_H = 2.25 and 2.35 ppm). Resonances of the last, in turn, are related to resonances of the C-15′ proton (δ_H = 2.8 and 2.9 ppm, respectively). The NMR spectra also contained resonances for protons of a β-substituted indole; the IR spectrum, absorption bands for NH vibrations at 3380 cm^{-1}. The elucidated structure **3** and the presence of tryptamine were confirmed by electron-impact fragmentation (Fig. 1).

The position and stereochemistry of adding tryptamine to the conjugated system of double bonds of securinine were determined using PMR spectra. The tryptamine moiety in **3** had the 15′α-orientation. In this instance the β-proton on C-15′ bisects the angle between the α- and β-protons on C-14′. This is evident from their SSCC (8.9 and 6.5 Hz) and the small constant with the C-7′ proton. The resonance for the C-14′ β-proton also had a constant with a well resolved resonance for the C-12′ proton.

The tryptamine in **4** had the 15′β-orientation. This was confirmed by analyzing the PMR spectra. The main difference in the spectra of the two epimers was seen in the position and shape of the resonance for the C-7′ proton. Although a slightly broadened doublet for this proton was observed for **3** at 3.1 ppm, the resonance for the C-7′ proton of **4** was a triplet with coupling constants with the α-protons on C-14′ and C-15′. The value of the last constant was much greater than for allomargaritarin and reached 9.5 Hz (Fig. 2).

We used the method developed for tryptamine to prepare amination products of securinin. Compounds **5**-**8** were formed in good yields by the reactions of securinin with pharmacophoric arylpiperazines and pyrrolidine.



The structures of the prepared compounds were elucidated using PMR and mass spectrometry.

Compounds **3** and **5** in addition to securinine (**1**) were tested using the patch-clamp method for the ability to modify currents in rat brain Purkinje neurons.

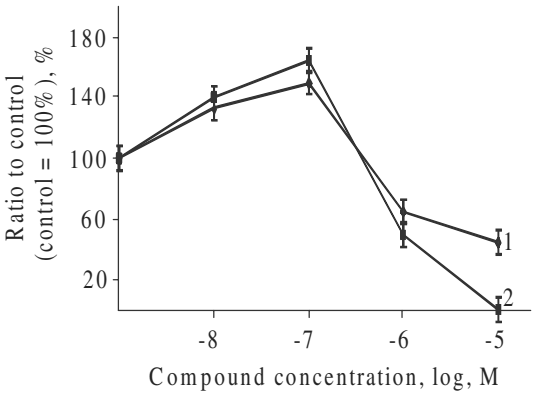


Fig. 3

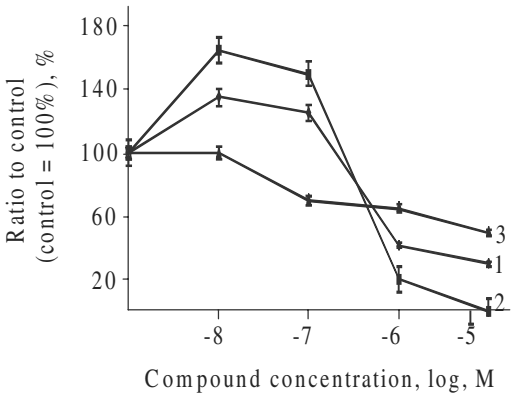


Fig. 4

Fig. 3. Activity of securinine (**1**) and **5** toward cainate-induced currents in Purkinje neurons of rat cerebellum; securinine (1) and **5** (2).

Fig. 4. Activity of securinine (**1**), **5**, and allomargaritarine (**3**) toward GABA-activated currents in Purkinje neurons of rat cerebellum; securinine (1), **5** (2), and allomargaritarine (3).

The electrohysiological investigations showed that the biological activity of securinin and its derivatives changed after adding the new fragments to the molecule. For example, **1** had a substantial effect on currents in rat cerebellum Purkinje neurons that were due to cainic acid (CA) (Fig. 3). Low doses typically increased currents whereas large ones blocked them. Thus, currents at a concentration of 10 nM were 133 ± 7% of the control; at 100 nM, 150 ± 20%; at 1-10 μM, 65 ± 7 and 45 ± 6%, respectively. Compound **5** was even more active for CA-induced currents. Thus, at a concentration of 10 nM the currents were 140 ± 7% of the control; at 100 nM, 165 ± 8%; at 1 μM, 50 ± 4%; and at 10 μM, 0%. Compound **3** had no effect on the amplitude of CA-induced currents in the studied (from 10 nM to 10 μM) dose range.

Compound **1** also affected GABA-induced currents in rat cerebellum Purkinje neurons (Fig. 4). Like for CA-induced currents, low doses of the compound increased the currents whereas large ones blocked them. This agreed well with the literature [8]. Thus, currents at a concentration of 10 nM were 135 ± 7% of the control; at 100 nM, 125 ± 7%; at 1-10 μM, 42 ± 3 and 30 ± 3%, respectively. Compound **5** was more active toward GABA-induced currents than **1**. Thus, the currents at a concentration of 10 nM were 165 ± 12%; at 100 nM, 150 ± 8%; at 1 μM, 20%; at 10 μM, 0% of the control. Compound **3** at low doses had no effect on the amplitude of GABA-induced currents. It decreased them at higher concentrations of 100 nM to 70%; 1 μM, 65%; and 10 μM, 50% of the control.

The investigations conducted by us confirmed that **1** was active toward the rat CNS. The amination products of **1** were also CNS-active but differed from the starting alkaloid.

Thus, a method of stereoselective addition of amines to the natural alkaloid securinin using ytterbium triflate as catalyst was developed. Several new addition products of securinin and secondary and tertiary amines were prepared and characterized. It was shown that adding new pharmacophoric fragments to the rigid securinin molecule is a promising route for synthesizing new biologically active compounds.

EXPERIMENTAL

NMR spectra were recorded on a Bruker DPX-200 spectrometer at operating frequency 200 MHz (Table 1). Mass spectra were recorded in a Finnigan 4021 GC—MS at ionizing potential 70 eV; IR spectra (KBr disks); on a Bruker ZFS-113v instrument.

Chromatographic analysis and preparative isolation of the compounds were performed on a Gilson chromatograph with UV detection (280 nm) using an analytical column (4 × 100 mm) with Diasorb 130 C16/T (5 μm) and a preparative column (16 × 250 mm) with the same stationary phase (BioChemMack, Russia). The mobile phase was a gradient of CH_3CN and distilled water with added trifluoroacetic acid (0.1%) from 5 to 75%. The flow rate was 1.5 mL/min for the analytical column; 5 mL/min for the preparative column. The sample volume was 20 μL for the analytical column; 1 mL for the preparative

column. Alkaloid **1** was supplied by InterBioScreen (Moscow). Elemental analysis of all newly prepared compounds agreed with those calculated.

3-{*N*-[(2β,15α)-14,15-Dihydro-11-oxosecurinan-15-yl]-2-aminoethyl}1*H*-indole (3) and 3-{*N*-[(2β,15β)-14,15-Dihydro-11-oxosecurinan-15-yl]-2-aminoethyl}1*H*-indole (4). A mixture of **1** (0.217 g, 1 mmol) and tryptamine (**2**, 0.24 g, 1.5 mmol) in methanol (5 mL) was stirred at room temperature. The course of the reaction was monitored by TLC on Silufol UV 254 plates with elution by acetone:benzene (1:3). After the spot of the starting lactone disappeared (after several days), the reaction mixture was evaporated in vacuo to form an oil that was dissolved in CH_3CN and analyzed by reversed-phase HPLC. The pure components were separated by preparative HPLC. Fractions were evaporated in vacuo to afford **3** and **4** as amorphous powders (~1:1 ratio), mp 65-67°C (**3**), 75-77°C (**4**), yields 30% (for both). Table 1 gives the NMR spectra.

Compound 3. Mass spectrum (*m/z*, *I*, %): 377 (5) $[M]^+$, 333 (8) $[M - CO_2]^+$, 247 (22), 234 (6), 218 (14), 203 (29), 187 (6), 174 (44), 160 (40), 144 (52), 130 (78), 108 (80), 83 (100), 77 (70), 56 (55), 43 (27). IR spectrum (cm^{-1}): 3335 br. (NH), 2922 m, 2845 m, 1745 vs (C=O), 1636 m, 1455 m, 1351 w, 1200 m, 1069 m, 970 w, 737 m.

Compound 4. Mass spectrum (*m/z*, *I*, %): 377 (4) $[M]^+$, 333 (8) $[M - CO_2]^+$, 247 (4), 217 (11), 203 (8), 175 (15), 160 (27), 143 (15), 130 (100), 108 (56), 83 (95), 56 (55), 43 (31). IR spectrum (cm^{-1}): 3300 br.s (NH), 2930 m, 2849 m, 2362 w, 1748 vs (C=O), 1640 m, 1455 m, 1339 m, 1250 m, 1200 m, 1073 m, 907 w, 741 s.

Stereospecific Amination of Securinine (general method). A mixture of **1** (1 mmol) and the amine (1.5 mmol) in methanol (5 mL) with ytterbium(III) trifluoromethanesulfonate hydrate (0.1 mmol) was stirred at room temperature. The course of the reaction was monitored by TLC (Silufol UV 254; benzene:acetone, 3:1; R_f 0.55, **1**; R_f ~0.30, amine derivative) and by the disappearance of resonances of olefinic protons on 14′C and 15′C in the PMR spectrum. After the reaction was complete (after several days), the mixture was evaporated in a rotary evaporator. The dry solid was dissolved in benzene and passed over a column of neutral Al_2O_3 (5 g) to remove ytterbium salts. The product was purified by column chromatography over SiO_2 (benzene:acetone, 10:1).

The following compounds were prepared by this method.

3-{*N*-[(2β,15α)-14,15-Dihydro-11-oxosecurinan-15-yl]-2-aminoethyl}1*H*-indole (3). Yield 80%, mp 65-67°C.

(2β,15α)-14,15-Dihydro-15-(4-phenylpiperazin-1-yl)securinan-11-one (5). Yield 62%, mp 227-228°C. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.07 (2H, d, J = 10.2, 8′-H), 3.18 (4H, m, piperazine), 3.48 (1H, d, J = 6.5, H-7′), 5.66 (1H, d, J = 2.0, H-12′), 6.85 (2H, m, *o*-H), 6.95 (2H, m, *m*-H), 7.30 (2H, m, *p*-H). Mass spectrum (*m/z*, *I*, %): 379 (6) $[M]^+$, 335 (11), 218 (8), 175 (100), 160 (38), 132 (64), 120 (85), 106 (45), 83 (62), 70 (55), 56 (54), 43 (38). IR spectrum (cm^{-1}): 2934 m, 2841 m, 1745 vs (C=O), 1640 m, 1598 m, 1501 m, 1447 m, 1231 s, 1200 m, 1150 m, 1069 m, 907 m, 752 m, 686 w.

(2β,15α)-14,15-Dihydro-15-[(4-(4-methoxyphenyl)piperazin-1-yl)]securinan-11-one (6). Yield 44%, mp 152-154°C. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.97 (2H, d, J = 10.0, 8′-H), 3.06 (4H, m, piperazine), 3.48 (1H, d, J = 6.8, H-7′), 3.75 (3H, s, OMe), 5.61 (1H, d, J = 2.0, H-12′), 6.83 (4H, m, Ph). Mass spectrum (*m/z*, *I*, %): 409 (11) $[M]^+$, 365 (6), 288 (4), 217 (15), 205 (91), 192 (63), 174 (15), 162 (31), 150 (100), 134 (29), 120 (35), 106 (42), 83 (85), 70 (95), 55 (74), 45 (45), 43 (44). IR spectrum (cm^{-1}): 2930 m, 2814 m, 1745 vs (C=O), 1636 m, 1509 vs, 1451 m, 1246 s, 1030 m, 907 m, 822 m.

(2β,15α)-14,15-Dihydro-15-[4-(2-pyridyl)piperazin-1-yl)]securinan-11-one (7). Yield 89%, mp 267-268°C. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.99 (2H, d, J = 10.4, H-8′), 3.33 (2H, m, H-4′), 3.45 (4H, m, piperazine), 5.60 (1H, d, J = 1.8, H-12′), 6.56 (1H, t, J_1 = 5.5, J_2 = 7.2, β-H), 6.67 (1H, d, J = 9.2, β'-H), 7.44 (1H, ddd, J_1 = 2.0, J_2 = 7.23, J_3 = 9.2, γ-H), 8.15 (H, dd, J_1 - 2.0, J_2 = 5.5, α-H). Mass spectrum (*m/z*, *I*, %): 380 (9) $[M]^+$, 336 (11), 308 (4), 218 (14), 176 (100), 163 (18), 147 (70), 133 (26), 121 (78), 107 (69), 95 (67), 83 (74), 77 (60), 55 (56), 43 (42). IR spectrum (cm^{-1}): 2930 m, 2837 m, 1745 vs (C=O), 1640 m, 1590 s, 1482 s, 1439 s, 1200 m, 1154 m, 976 m, 907 m, 829 m, 771 s.

(2β,15α)-14,15-Dihydro-15-(pyrrolidin-1-yl)securinan-11-one (8). Yield 20%, viscous yellow oil. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.99 (2H, d, J = 10.4, H-8′), 2.87 (4H, m, pyrrolidine), 3.36 (1H, d, J = 6.5, H-14′β), 3.46 (1H, d, J = 6.5, H-14′α), 3.76 (1H, t, J_1 = J_2 = 7.0, 2′-H), 4.27 (1H, dd, J_1 = 4.0, J_2 = 6.5, H-15′), 5.73 (1H, d, J = 1.2, H-12′). Mass spectrum (*m/z*, *I*, %): 288 (3) $[M]^+$, 233 (44), 218 (45), 204 (38), 172 (35), 160 (42), 134 (45), 120 (37), 84 (100), 55 (57), 43 (31). IR spectrum (cm^{-1}): 2945 m, 2856 w, 2250 s, 1752 vs (C=O), 1632 w, 1454 w, 1250 w, 1901 w.

Biological activity of the compounds was investigated by an electrophysiological method on freshly isolated Purkinje neurons from cerebellum of rats that were 12-15 days old. A modified method was used for the isolation. Slices of cerebellum 400-600 μm thick were placed in a 10-mL thermostatted chamber. The isolation solution contained (mM) NaCl (150.0), KCl (5.0), $CaCl_2$ (2.0), $MgSO_4 \cdot 7H_2O$ (2.0), HEPES (10.0), and glucose (15.0) at pH 7.42. Slices were incubated in the solution for

60 min. Then, the solution was replaced by an analogous solution containing pronase (2 mg/mL, Sigma) and collagenase (1 mg/mL, Sigma) and incubated for 70 min. After rinsing with the initial solution for 20 min, slices were placed in Petri dishes and joined mechanically using Pasteur pipettes. Solutions were continuously purged with O_2 (100%) at 34°C. Purkinje neurons were placed in the working chamber (0.6 mL) that contained (mM) NaCl (150.0), KCl (5.0), $CaCl_2$ (2.6), $MgSO_4 \cdot 7H_2O$ (2.0), HEPES (10.0), and glucose (15.0) at pH 7.36.

Transmembrane currents were induced by activation of AMPA receptors through application of solutions of their agonist cainic acid (CA). GABA-activated currents were induced by application of 5 or 10 μM γ-aminobutyric acid using fast superfusion. Currents were recorded using borosilicate microelectrodes (resistance 2.0-4.5 mΩ) filled with a solution containing (mM) KCl (100.0), EGTA (11.0), $CaCl_2$ (1.0), $MgCl_2$ (1.0), HEPES (10.0), and ATP (5.0) at pH 7.2.

The studied compounds at various concentrations were placed into the experimental chamber. Then, the corresponding receptor agonists were applied and transmembrane currents were recorded. Mediators were applied repeatedly at a given concentration in order to obtain a steady response. An EPC-9 instrument (HEKA, Germany) was used for the recording. Currents were plotted using the Pulse program (HEKA, Germany). Results were processed by the Pulsefit program (HEKA, Germany). The reliability of the differences was calculated using the Student criterion.

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