Novel spiroderivatives of isothiourea of the 1,3-thiazine series as promising neuroprotectors*

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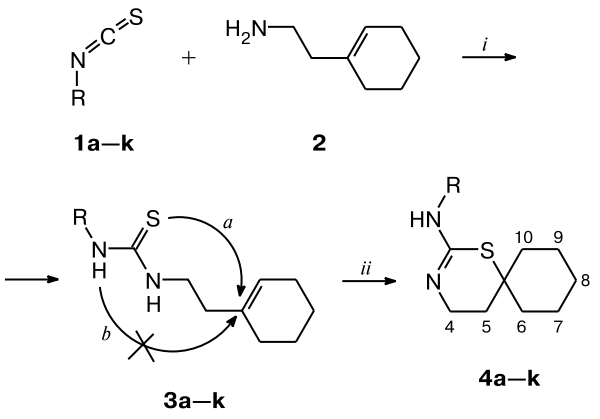
In the recent years, one of the intensively developing prospects in the search for the drugs for the treatment of the Alzheimer´s disease and relevant neurodegenerative diseases is the design of inhibitors of glutamate-stimulated Ca^{2+} ions uptake as the promising neuroprotectors.[1] Recently, it was shown that isothiourea derivatives exhibit neuroprotective and cognition-enhancing properties.[2]

We developed synthetic route towards hitherto unknown 2-aminospirothiazines, *e.g.* the cyclic derivatives of isothiourea, unsubstituted in the bicyclic moiety. The synthetic method involved intramolecular ring closure of thioureas bearing γ,δ-unsaturated fragment (Scheme 1). On the first step, a mixture of substituted isothiocyanates **1a**—**k** and γ,δ-unsaturated amine (2-(1-cyclohexenyl)-ethylamine (**2**)) in diethyl ether was stirred at ambient temperature until *N*,*N*´-substituted thioureas **3a**—**k** precipitated. The latter at reflux with 48% aqueous HBr undergo ring closure to give the corresponding *N*-substituted 1-thia-3-azaspiro[5.5]undec-2-en-2-ylamines **4b**—**k** in 60—80% total yield. Unsubstituted 1-thia-3-azaspiro[5.5]-undec-2-en-2-ylamine (**4a**) was obtained from benzoyl derivative **3a** by the removal of the benzoyl group under acidic conditions used for the ring closure.

Scheme 1



R = Bz (**1a**, **3a**), H (**4a**), Me (**b**), Et (**c**), 4-Cl—C_6H_4 (**d**), 4-CF_3—C_6H_4 (**e**), 4-Ac—C_6H_4 (**f**), Ph (**g**), 4-NH_2—C_6H_4 (**h**), 2,4,6-Me_3—C_6H_2 (**j**), 2-Me-6-Pr^i—C_6H_3 (**k**)

i. Et_2O, 20 °C, 2 h; *ii*. HBr (48%), 100 °C, 5 h.

The intramolecular cyclization of thioureas **3a**—**k** under acidic conditions proceeds *via* formation of thiazine cycle (the route *a*) rather than the pyrimidine cycle (the route *b*), which was confirmed by X-ray crystallography of the obtained spirothiazines (Fig. 1).

We studied the ability of spirothiazines **4a**—**k** to inhibit glutamate-stimulated uptake of the $^{45}Ca^{2+}$ ions in rat brain cortex synaptosomes. Analysis of the obtained results revealed that compounds **4a**—**k** affect glutamate-stimulated $^{45}Ca^{2+}$ uptake (Table 1). It is of note that the inhibiting activity depends on the substituents at exocyclic nitrogen atom. Unsubstituted spirothiazine **4a** and *N*-ethyl-substituted compound **4c** weakly inhibit $^{45}Ca^{2+}$ ions uptake, while *N*-methyl derivative **4b** exhibits stronger activity. In case of aryl derivatives, the inhibiting activity depends on the substituents in the aromatic fragment. Thus, compounds **4d**—**f** bearing electron-withdrawing groups revealed negligible activity in this test, and amino derivative **4h** even potentiates $^{45}Ca^{2+}$ uptake. However, introduction

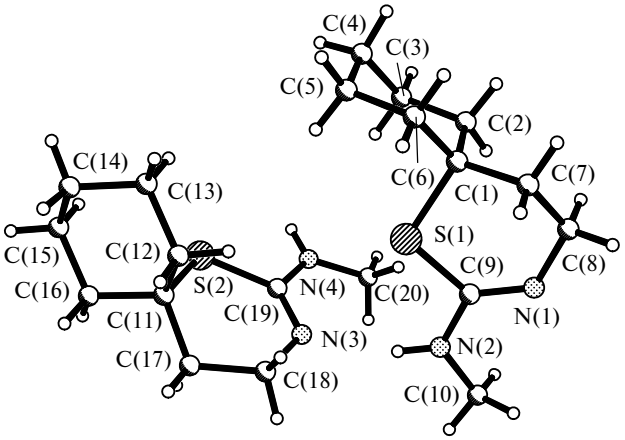


Fig. 1. Geometry of methyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (**4b**).

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Table 1. Inhibiting activity of spirotiazines **4a—k**

4	R	$K_{43/21}$ (%)*	IC_{50} /μmol L^{-1}
a	H	91.4±9.8	—
b	Me	134.2±13.2	—
c	Et	65.3±12.1	—
d	4-Cl—C_6H_4	12.7±7.4	35.5
e	4-CF_3—C_6H_4	42.1±5.3	77.6
f	4-Ac—C_6H_4	45.1±6.3	81.3
g	Ph	33.2±6.3	63.1
h	4-NH_2—C_6H_4	114.7±14.5	—
j	2,4,6-Me_3—C_6H_2	2.6±2.5	9.3
k	2-Me-6-Pr^i—C_6H_3	5.4±5.4	20.9

*K** is percentage of $^{45}Ca^{2+}$ uptake in rat brain synaptosomes to control (control — 100%).

of the electron-releasing substituents in the *ortho*- and *para*-positions of the benzene ring (compounds **4j**,**k**) resulted in sharp increase in the inhibiting activity.

Thus, it is possible to conclude that 1-thia-3-azaspiro[5.5]undec-2-en-2-ylamines synthesized actively regulate glutamate-stimulated uptake of $^{45}Ca^{2+}$ ions, which makes promising to search for new neurocorrectors in this class of compounds.

Experimental

^{1}H NMR spectra were recorded on Bruker CXP-200 instrument (Germany) in $CDCl_3$, the chemical shifts are given in the δ scale relative to Me_4Si. Melting points were determined on a Boetius apparatus and were uncorrected. The solvents were removed using rotary evaporator under water pump vacuum.

***N*-Substituted 1-thia-3-azaspiro[5.5]undec-2-en-2-ylamines 4a—k.** To a stirred solution of 2-(1-cyclohexenyl)ethylamine **2** (1.25 g, 0.01 mol) in diethyl ether (20 mL), a solution of arylisothiocyanate (0.01 mol) in diethyl ether (20 mL) was added dropwise. Then the reaction mixture was stirred for 2—5 h at ambient temperature until formation of precipitate. The precipitate of thiourea **3** was filtered off, dried, suspended in 48% aqueous HBr (10 mL), and refluxed for 5 h; the precipitate dissolved during the reaction. After the completion of the reaction, the mixture was cooled to ambient temperature, diluted with water (20 mL) and dichloromethane (50 mL), and then saturated aqueous $NaHCO_3$ was carefully added until the solution was basic. The organic layer was separated and dried with Na_2SO_4. The drying agent was filtered off and the solvent was removed. The residue was recrystallized from propane-2-ol to give *N*-substituted 1-thia-3-azaspiro[5.5]undec-2-en-2-ylamine (**4**).

1-Thia-3-azaspiro[5.5]undec-2-en-2-ylamine (4a). Light gray crystals, m.p. 104—106 °C, yield 67%. ^{1}H NMR, δ: 1.27 (m, 1 H, C(8)HH); 1.56 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.67 (t, 2 H, $C(5)H_2$, J = 5.9 Hz); 1.85 (m, 2 H, C(6)HH, C(10)HH); 3.59 (t, 2 H, $C(4)H_2$, J = 5.8 Hz); 3.66 (br.s, 2 H, NH_2).

Methyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4b). Colorless crystals, m.p. 72—73 °C, yield 72%. ^{1}H NMR, δ: 1.30 (m, 1 H, C(8)HH); 1.58 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.71 (t, 2 H, $C(5)H_2$, J = 5.8 Hz); 1.88 (m, 2 H, C(6)HH, C(10)HH); 2.81 (s, 3 H, CH_3); 3.66 (t, 2 H, $C(4)H_2$, J = 5.8 Hz); 3.88 (br.s, 1 H, NH).

Ethyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4c). Light gray crystals, m.p. 107—108 °C, yield 62%. ^{1}H NMR, δ: 1.16 (t, 3 H, CH_3, J = 7.2 Hz); 1.32 (m, 1 H, C(8)HH); 1.58 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.72 (t, 2 H, $C(5)H_2$, J = 5.8 Hz); 1.88 (m, 2 H, C(6)HH, C(10)HH); 3.28 (kq, 2 H, NHCH$_2$, J = 7.2 Hz); 3.61 (br.s, 1 H, NH); 3.64 (t, 2 H, $C(4)H_2$, J = 5.8 Hz).

4-Chlorophenyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4d). Colorless crystals, m.p. 156—158 °C, yield 65%. ^{1}H NMR, δ: 1.27 (m, 1 H, C(8)HH); 1.57 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.91 (t, 4 H, C(6)HH, C(10)HH, $C(5)H_2$, J = 5.9 Hz); 3.59 (t, 2 H, $C(4)H_2$, J = 5.9 Hz); 6.24 (br.s, 1 H, NH); 7.06 (d, 2 H, H_{aryl}, J = 8.6 Hz); 7.38 (d, 2 H, H_{aryl}, J = 8.6 Hz).

4-Trifluoromethylphenyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4e). Colorless crystals, m.p. 150—152 °C, yield 75%. ^{1}H NMR, δ: 1.29 (m, 1 H, C(8)HH); 1.58 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.85 (t, 4 H, C(6)HH, C(10)HH, $C(5)H_2$, J = 5.9 Hz); 3.60 (t, 2 H, $C(4)H_2$, J = 5.9 Hz); 6.11 (br.s, 1 H, NH); 7.25 (d, 2 H, H_{aryl}, J = 8.4 Hz); 7.49 (d, 2 H, H_{aryl}, J = 8.4 Hz).

4-Acetylphenyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4f). Cream-colored crystals, m.p. 162—164 °C, yield 69%. ^{1}H NMR, δ: 1.29 (m, 1 H, C(8)HH); 1.56 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.85 (t, 2 H, $C(5)H_2$, J = 5.9 Hz); 1.88 (m, 2 H, C(6)HH, C(10)HH); 3.61 (t, 2 H, $C(4)H_2$, J = 5.9 Hz); 2.55 (s, 3 H, $C(O)CH_3$); 6.25 (br.s, 1 H, NH); 7.24 (d, 2 H, H_{aryl}, J = 8.6 Hz); 7.87 (d, 2 H, H_{aryl}, J = 8.6 Hz).

Phenyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4g). Light gray crystals, m.p. 105—107 °C, yield 79%. ^{1}H NMR, δ: 1.22 (m, 1 H, C(8)HH); 1.50 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.78 (m, 4 H, $C(5)H_2$, C(6)HH, C(10)HH); 3.55 (m, 2 H, $C(4)H_2$); 6.20 (br.s, 1 H, NH); 6.90 (m, 1 H, H_{aryl}); 7.19 (m, 4 H, H_{aryl}).

4-Aminophenyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4h). Gray brownish crystals, m.p. 160—162 °C, yield 67%. ^{1}H NMR, δ: 1.31 (m, 1 H, C(8)HH); 1.59 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.89 (m, 4 H, $C(5)H_2$, C(6)HH, C(10)HH); 3.59 (m, 2 H, $C(4)H_2$); 4.94 (br.s, 2 H, NH_2); 6.20 (br.s, 1 H, NH); 6.64 (d, 2 H, H_{aryl}, J = 8.6 Hz); 6.99 (d, 2 H, H_{aryl}, J = 8.6 Hz).

2,4,6-Trimethylphenyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4j). Light yellow crystals, m.p. 124—126 °C, yield 76%. ^{1}H NMR, δ: 1.33 (m, 1 H, C(8)HH); 1.55 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.83 (m, 2 H, C(6)HH, C(10)HH); 2.17 (s, 6 H, 2 CH_3); 1.93 (t, 2 H, $C(5)H_2$, J = 5.7 Hz); 3.52 (t, 2 H, $C(4)H_2$, J = 5.7 Hz); 2.29 (s, 3 H, CH_3); 6.21 (br.s, 1 H, NH); 6.86 (s, 2 H, H_{aryl}).

2-Methyl-6-isopropylphenyl(1-thia-3-azaspiro[5.5]undec-2-en-2-yl)amine (4k). Yellow brownish crystals, m.p. 96—98 °C, yield 75%. ^{1}H NMR, δ: 1.22 (d, 6 H, CH(CH_3)$_2$, J = 6.8 Hz); 1.31 (m, 1 H, C(8)HH); 1.56 (m, 7 H, C(6)HH, $C(7)H_2$, C(8)HH, $C(9)H_2$, C(10)HH); 1.85 (m, 2 H, C(6)HH, C(10)HH); 2.01 (t, 2 H, $C(5)H_2$, J = 5.7 Hz); 2.23 (s, 3 H, CH_3); 3.11

(m, 1 H, C<u>H</u>$(CH_3)_2$); 3.54 (m, 2 H, C(4)H_2); 6.25 (br.s, 1 H, NH); 7.11 (m, 3 H, H_{aryl}).

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