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SYNTHESIS AND SPECIFIC NOOTROPIC ACTIVITY OF (–)-CYTISINE DERIVATIVES WITH CARBAMIDE AND THIOCARBAMIDE MOIETIES IN THEIR STRUCTURE

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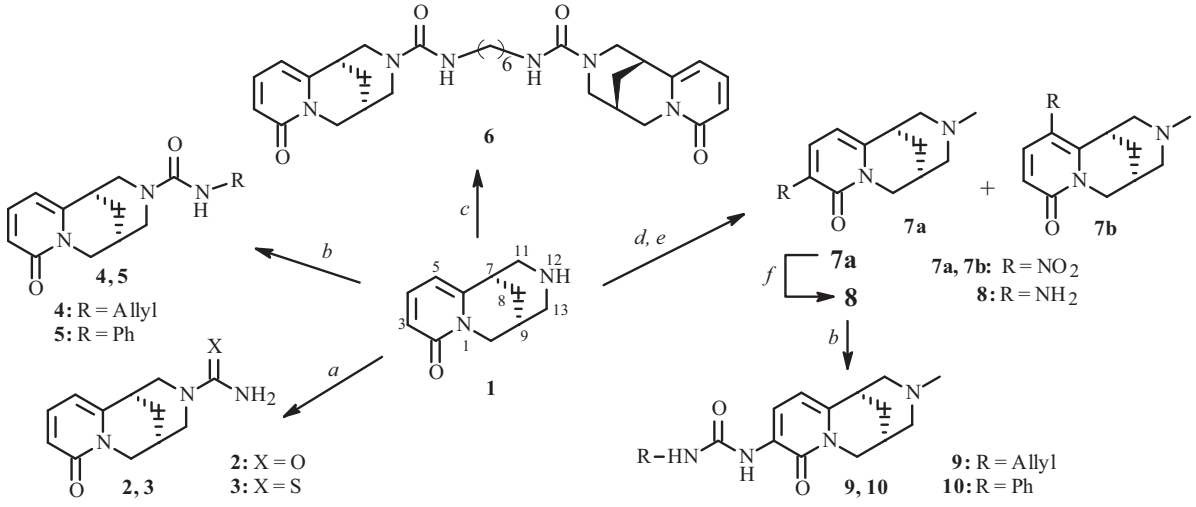
N-*(methylcytisinyl)*-N′*-substituted ureas,* N-*substituted cytisine-12-carbamides, and cytisine-12-thiocarbamide were prepared by reaction of (–)-cytisine with urea and thiourea and of (–)-cytisine and its 12*-N-*methyl-3-amino derivative with isocyanates. Their specific nootropic activity was studied* in vivo*. The therapeutic index was determined for the lead compound. Promising candidates for further pharmacological testing were found.*

Keywords: (–)-cytisine, nootropic drugs, nicotinic acetylcholine receptor.

Recent research results [1, 2] suggest that disruption of cognitive functions is currently one of the most serious medical problems in addition to cardiovascular and oncological diseases. The common prevalence of cognitive disorders increases considerably the significance of medical therapy that is constantly required by patients for treating the disease itself and accompanying pathologies and for adjusting the emotional status.

One component of combined therapy of cognitive dysfunction is represented by nootropic drugs [3–5] that are indicated for vascular diseases of the brain, ischemias, chronic cerebrovascular insufficiency (including vascular dementia), skull–brain injuries, neurodegenerative brain infections, Alzheimer's disease, disruptions of brain function caused by alcoholism, and acute neural infections.



a. $(NH_2)_2CO$, toluene, 110°C for **2**; and $(NH_2)_2CS$, pentanol, 137°C for **3**; *b*. PhNCO or AllylNCO, benzene, 20°C; *c*. $ONC(CH_2)_6CNO$, benzene, 80°C; *d*. MeI, K_2CO_3, acetone, 56°C; *e*. HNO_3, H_2SO_4, 20°C; *f*. H_2, Pd/C, MeOH, 20°C

Scheme 1

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TABLE 1. Nootropic Activity of **1**–**6** and **8**–**10** at a Dose of 50 μmol/kg and Therapeutic Index of Lead Drug **5**

Compound	Mnestic activity, %	LD_{50}, mg/kg	ED_{50}, mg/kg	LD_{50}/ED_{50}
Control	–	–	–	–
Piracetam[a]	71.7	10600	400	26.5
1	6.1	–	–	–
2	30.8	–	–	–
3	42.7	–	–	–
4	71.6			
5	92.6	3500	11.7	299.1
6	75.2	–	–	–
8	51.8	–	–	–
9	42.4	–	–	–
10	74.0	–	–	–

[a]Dose of 400 mg/kg.

Ligands of the nicotinic acetylcholine receptor (nACR) of natural origin, including the quinolizidine alkaloid (–)-cytisine are positioned as a new group of effective agents for treating cognitive disorders [6–8]. Numerous derivatives of it involving alkylation and acylation of the secondary N atom and functionalization of the 2-pyridone core were synthesized during the last decade [9–17]. The activities of (–)-cytisine itself and compounds prepared from it were tested against the nACR both *in vitro* and *in vivo* [9–18]. However, the neuropharmacological activity of (–)-cytisine derivatives with substituted urea moieties of various structures is insufficiently studied.

We synthesized *N*-(methylcytisinyl)-*N′*-substituted ureas, *N*-substituted cytisine-12-carbamides, and cytisine-12-thiocarbamide (Scheme 1) in order to discover among derivatives of the quinolizidine alkaloid (–)-cytisine compounds with nootropic activity based on their potential choline-positive action and to establish the structure–activity relationship.

Products **2** and **3** were obtained by refluxing (–)-cytisine (**1**) with urea in toluene and thiourea in amyl alcohol, respectively, in yields of 92 and 98%. Allyl-derivative **4** and phenyl-derivative **5** were synthesized by the isocyanate method in quantitative yields [19]. Bimolecular product **6** was obtained by refluxing **1** in benzene with hexamethylene-1,6-diisocyanate in 68% yield.

The problem of introducing the urea moiety into the cytisine pyridone core was solved in stages. First, **1** was converted to the 12-*N*-methyl derivative [9], which was then nitrated by a mixture of conc. HNO_3 and H_2SO_4 [16]. The yields of 3-nitro- and 5-nitro- regioisomers **7a** and **7b**, which were separated by column chromatography over SiO_2, were 70 and 10%, respectively. Catalytic hydrogenation of 3-nitro product **7a** over Pd catalyst [16] gave amine **8** in >98% yield. Subsequent reaction of **8** with allyl- and phenylisocyanate under conditions analogous to those for preparing **4** and **5** produced ureas **9** and **10**. The structures of the prepared compounds were confirmed by NMR spectroscopy, GC–MS, and comparison of physicochemical constants with the literature.

Specific nootropic activity of **2**–**6** and **8**–**10** was studied using the basic model of passive avoidance conditioned response (PACR) [20]. The reference drugs were piracetam and starting **1**. Table 1 presents the test results.

According to the results, **1** at the studied dose, in contrast with its derivatives **2**–**6** and **8**–**10** did not exhibit noticeable nootropic properties (Table 1). The mnestic activity of **1** expressed in percent was 6.1%. Replacing the O in **2** by S affected insignificantly the mnestic activity. It increased for thioamide **3** from 30.8 to 42.7%. The indices for amine **8** and allylurea **9** also turned out to be low (51.8 and 42.4%). The mnestic activities of **4**, **6**, and **10** were comparable with that of the reference piracetam and were 71.6, 75.2, and 74.0%, respectively. The index increased to 92.6% for **5**.

The median effective dose (ED_{50}), which was 11.7 mg/kg, and lethal dose (LD_{50}), 3500 mg/kg, were determined for the most promising candidate **5**. The therapeutic index of this compound that was calculated as the LD_{50}/ED_{50} ratio was significantly higher than that of piracetam and reached 299.1 vs. 26.5.

Thus, the reaction of **1** with urea and thiourea in addition to **1** and its 12-*N*-methyl-3-amino derivative with isocyanates produced *N*-(methylcytisinyl)-*N′*-substituted ureas **9** and **10**; *N*-substituted cytisine-12-carbamides **4**–**6**, and cytisine-12-thio- and –carbamide **2** and **3**.

Specific nootropic activities of the resulting derivatives were studied *in vivo* using a basic model of PACR. The therapeutic index of the lead compound was established. Promising candidates for further pharmacological testing were found.

EXPERIMENTAL

Commercially available (–)-cytisine (CAS 485-35-8), allylisocyanate (CAS 1476-23-9), phenylisocyanate (CAS 103-71-9), urea (CAS 57-13-6), thiourea (CAS 62-56-6), H_2SO_4 (98%, CAS 8014-95-7), HNO_3 (CAS 7697-37-2), Pd/C (10%, CAS 12135-22-7) were used as starting materials. *N*-Methylcytisine was prepared according to the literature [9]. The course of reactions was monitored by TLC on Alugram® plates with an aluminum backing coated by a layer (0.2 mm) of standard silica gel 60 (Macherey-Nagel, Germany). Column chromatography (CC) was performed over standard silica gel 60 (0.05–0.1 mm) (Macherey-Nagel, Germany). Melting points of crystalline compounds were determined on a Boetius apparatus (PHMK 05 VEB Wagetechnik Rapido, Radebeul). Optical rotation angles were measured on a PerkinElmer 341LC polarimeter (Na lamp, wavelength 589 nm). High-resolution mass spectra were recorded in a Thermo Finnigan MAT95XP instrument (EI, 70 eV).

PMR and ^{13}C NMR spectra were recorded on Bruker Avance III pulsed spectrometers at operating frequency 500.13 MHz (^1H) and 125.47 MHz (^{13}C) using a 5-millimeter probe with a *Z*-gradient PABBO at constant sample temperature 298 K. Chemical shifts in PMR and ^{13}C NMR spectra are given in ppm relative to TMS internal standard. 2D ^1H–^1H COSY spectra [21] were recorded in standard modes of multi-pulse sequences using the instrument software.

Specific nootropic activity was studied according to the literature [20] using a basic model of PACR. The experiments were performed on Wistar white rats (180–200 g). Tested compounds and **1** were administered perorally at a dose of 50 μmol/kg; piracetam, at a dose of 400 mg/kg. Distilled H_2O (control) was given 1 h before training in equal volumes. The test was carried out in two stages, training the routine and reproducing its retention. On the first day of the experiment, the following parameters were noted. The number of passes into the dark section was a parameter reflecting the training of the animals (the fewer the passes, the faster the training process). The latent period of the first pass was compared with the analogous parameter after training. Reproduction of the PACR routine was tested 24 h after training. The latent period of the first pass, the number of passes into the dark section, and the total time located in the dark section were recorded [22]. Mnestic activity (M_t) (improvement of memory under the influence of the tested compounds) was calculated using the formula $M_t = (t_k – t_d)/t_k \cdot 100\%$, where M_t was the mnestic activity (%), t_k, the average residence time of the control animals in the dark section; and t_d, the average residence time in the dark section of animals given the test compound [23].

Acute toxicity was determined using laboratory white mice (18–22 g) without taking into account the sex for a single administration into the stomach at doses of 100, 200, 1000, 2000, 3000, 4000, and 5000 mg/kg. Animals were observed during the acute toxicity test. The times of death, the number of deceased animals, and the clinical display of intoxication were evaluated for 14 d. Acute toxicity parameters were calculated using the Litchfield–Wilcoxon method [24].

Cytisine-12-carbamide (2). A mixture of **1** (1 g, 5.2 mmol) and urea (0.63 g, 10.5 mmol) in toluene was refluxed until **1** disappeared completely (TLC monitoring) and concentrated at reduced pressure. The solid was chromatographed over SiO_2 with elution by EtOH:MeOH (9:1) to afford **2** (1.1 g, 92%), mp 203–205°C, $[\alpha]_D^{20}$ –256.0° (MeOH), HR-MS (EI, *m*/*z*): calcd for $C_{12}H_{15}N_3O_2$, 233.1159 $[M]^+$; found, 233.1160.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.02 (1H, m, H_{anti}-8); 2.03 (1H, m, H_{syn}-8); 2.50 (1H, m, H-9); 3.11 (1H, ddd, ^2J = 13.0, $^3J_{11exo-9}$ = 2.5, $^4J_{11exo-10exo}$ = 1.3, H_{exo}-11); 3.13 (1H, dd, ^2J = 12.4, $^3J_{13exo-7}$ = 2.1, H_{exo}-13); 3.17 (1H, m, H-7); 3.86 (1H, ddd, ^2J = 15.6, $^3J_{10exo-9}$ = 6.5, $^4J_{10exo-11exo}$ = 1.3, H_{exo}-10); 4.07 (1H, ddt, ^2J = 12.4, $^3J_{13endo-7}$ = 4.2, $^4J_{13endo-11endo}$ = 1.9, $^4J_{13endo-8syn}$ = 1.9, H_{endo}-13); 4.13 (1H, dt, ^2J = 15.6, $^3J_{10endo-9}$ = 0.8, $^4J_{10endo-8anti}$ = 0.8, H_{endo}-10); 4.15 (1H, ddt, ^2J = 13.0, $^3J_{11endo-9}$ = 3.8, $^4J_{11endo-8syn}$ = 1.9, $^4J_{11endo-13endo}$ = 1.9, H_{endo}-11); 6.34 (1H, dd, $^3J_{5-4}$ = 7.0, $^4J_{5-3}$ = 1.3, H-5); 6.41 (1H, dd, $^3J_{3-4}$ = 8.9, $^4J_{3-5}$ = 1.3, H-3); 7.45 (1H, dd, $^3J_{4-3}$ = 8.9, $^3J_{4-5}$ = 7.0, H-4).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.37 (C-8); 28.88 (C-9); 35.95 (C-7); 50.62 (C-10); 51.15 (C-11); 52.00 (C-13); 108.54 (C-5); 117.12 (C-3); 141.38 (C-4); 151.52 (C-6); 161.01 (C-14); 165.56 (C-2).

Cytisine-12-thiocarbamide (3). A mixture of **1** (1 g, 5.2 mmol) and thiourea (0.79 g, 10.5 mmol) in amyl alcohol was refluxed until **1** disappeared completely (TLC monitoring). The resulting crystalline precipitate was filtered off to afford **3** (1.3 g, 98%), mp 249–251°C, $[\alpha]_D^{20}$ –292.0° (DMSO), HR-MS (EI, *m*/*z*): calcd for $C_{12}H_{15}N_3OS$, 249.0930 $[M]^+$; found, 249.0923.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.86 (1H, dtt, ^2J = 13.3, $^3J_{8syn-7}$ = 3.4, $^3J_{8syn-9}$ = 3.4, $^4J_{8syn-11endo}$ = 1.7, $^4J_{8syn-13endo}$ = 1.7, H_{syn}-8); 1.93 (1H, dtd, ^2J = 13.3, $^3J_{8anti-7}$ = 3.3, $^3J_{8anti-9}$ = 3.3, $^4J_{8anti-10endo}$ = 1.0, H_{anti}-8); 2.49 (1H, m, H-9); 3.09 (m, H-11); 3.15 (1H, m, H-7); 3.16 (m, H-13); 3.66 (1H, ddd, ^2J = 15.3, $^3J_{10exo-9}$ = 6.7, $^4J_{10exo-11exo}$ = 1.0, H_{exo}-10); 3.97 (1H, dt, ^2J =15.3, $^3J_{10endo-9}$ = 1.0, $^4J_{10endo-8anti}$ = 1.0, H_{endo}-10); 6.14 (1H, dd, $^3J_{5-4}$ = 7.0, $^4J_{5-3}$ = 1.2, H-5); 6.21 (1H, dd, $^3J_{3-4}$ = 9.0, $^4J_{3-5}$ = 1.2, H-3); 7.32 (1H, dd, $^3J_{4-3}$ = 9.0, $^3J_{4-5}$ = 7.0, H-4).

^{13}C NMR spectrum (DMSO-d_6, δ, ppm): 24.80 (C-8); 27.24 (C-9); 34.08 (C-7); 48.05 (C-10); 52.49 (C-11); 53.50 (C-13); 104.81 (C-5); 115.86 (C-3); 138.67 (C-4); 149.28 (C-6); 162.06 (C-2); 181.90 (C-14).

***N*-Allylcytisine-12-carbamide (4).** Compound **4** was prepared according to the literature [19] in 98% yield, mp 137–139°C, $[\alpha]_D^{20}$ –235.0° ($CHCl_3$). HR-MS (EI, *m/z*): calcd for $C_{15}H_{19}N_3O_2$, 273.1472 $[M]^+$; found, 273.1480.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.94 (1H, dtd, ^2J = 12.8, $^3J_{8anti\text{-}7}$ = 3.0, $^3J_{8anti\text{-}9}$ = 3.0, $^4J_{8anti\text{-}10endo}$ = 1.0, H_{anti}-8); 2.01 (1H, dtt, ^2J = 12.8, $^3J_{8syn\text{-}7}$ = 3.3, $^3J_{8syn\text{-}9}$ = 3.3, $^4J_{8syn\text{-}11endo}$ = 1.6, $^4J_{8syn\text{-}13endo}$ = 1.6, H_{syn}-8); 2.52 (1H, m, H-9); 3.05 (1H, dd, ^2J = 11.4, $^3J_{13exo\text{-}7}$ = 2.3, H_{exo}-13); 3.08 (1H, m, H-7); 3.10 (1H, ddd, ^2J = 12.8, $^3J_{11exo\text{-}9}$ = 2.4, $^4J_{11exo\text{-}10exo}$ = 1.2, H_{exo}-11); 3.64 (1H, dtt, ^2J = 15.7, $^3J_{16A\text{-}15}$ = 5.6, $^3J_{16A\text{-}17}$ = 5.6, $^4J_{16A\text{-}18trans}$ = 1.6, $^4J_{16A\text{-}18cis}$ = 1.6, H_A-16); 3.75 (1H, dtt, ^2J = 15.7, $^3J_{16B\text{-}15}$ = 5.6, $^3J_{16B\text{-}17}$ = 5.6, $^4J_{16B\text{-}18trans}$ = 1.6, $^4J_{16B\text{-}18cis}$ = 1.6, H_B-16); 3.87 (1H, ddd, ^2J = 15.7, $^3J_{10exo\text{-}9}$ = 6.8, $^4J_{10exo\text{-}11exo}$ = 1.2, H_{exo}-10); 4.13 (1H, ddt, ^2J = 11.4, $^3J_{13endo\text{-}7}$ = 3.4, $^4J_{13endo\text{-}11endo}$ = 1.6, $^4J_{13endo\text{-}8syn}$ = 1.6, H_{endo}-13); 4.14 (1H, ddt, ^2J = 12.8, $^3J_{11endo\text{-}9}$ = 3.4, $^4J_{11endo\text{-}13endo}$ = 1.6, $^4J_{11endo\text{-}8syn}$ = 1.6, H_{endo}-11); 4.20 (1H, dt, ^2J = 15.7, $^3J_{10endo\text{-}9}$ = 0.9, $^4J_{10endo\text{-}8anti}$ = 0.9, H_{endo}-10); 4.97 (1H, dq, $^3J_{18cis\text{-}17}$ = 10.3, $^2J_{18cis\text{-}18trans}$ = 1.6, $^4J_{18cis\text{-}16A}$ = 1.6, $^4J_{18cis\text{-}16B}$ = 1.6, H_{cis}-18); 5.00 (1H, dq, $^3J_{18trans\text{-}17}$ = 17.1, $^2J_{18trans\text{-}18cis}$ = 1.6, $^4J_{18trans\text{-}16A}$ = 1.6, $^4J_{18trans\text{-}16B}$ = 1.6, H_{trans}-18); 5.31 (1H, t, $^3J_{15\text{-}16A}$ = 5.6, $^3J_{15\text{-}16B}$ = 5.6, H-N15); 5.72 (1H, ddt, $^3J_{17\text{-}18trans}$ = 17.1, $^3J_{17\text{-}18cis}$ = 10.3, $^3J_{17\text{-}16A}$ = 5.6, $^3J_{17\text{-}16B}$ = 5.6, H-17); 6.08 (1H, dd, $^3J_{5\text{-}4}$ =7.0, $^4J_{5\text{-}3}$ = 1.3, H-5); 6.40 (1H, dd, $^3J_{3\text{-}4}$ = 9.1, $^4J_{3\text{-}5}$ = 1.3, H-3); 7.28 (1H, dd, $^3J_{4\text{-}5}$ = 7.0, $^3J_{4\text{-}3}$ = 9.1, H-4).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 25.85 (C-8); 27.38 (C-9); 34.59 (C-7); 43.15 (C-16); 49.10 (C-10); 50.38 (C-11); 50.99 (C-13); 105.60 (C-5); 114.97 (C-18); 116.82 (C-3); 135.69 (C-17); 139.00 (C-4); 149.65 (C-6); 157.96 (C-14); 163.43 (C-2).

***N*-Phenylcytisine-12-carbamide (5).** Compound **5** was prepared according to the literature [19] in 98% yield, mp 119–121°C, $[\alpha]_D^{20}$ –242° ($CHCl_3$), HR-MS (EI, *m/z*): calcd for $C_{18}H_{19}N_3O_2$, 309.1472 $[M]^+$; found, 309.1470.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.87 (1H, dddd, ^2J = 12.8, $^3J_{8anti\text{-}7}$ = 3.6, $^3J_{8anti\text{-}9}$ = 2.8, $^4J_{8anti\text{-}10endo}$ = 1.1, H_{anti}-8); 1.94 (1H, dtt, ^2J = 12.8, $^3J_{8syn\text{-}7}$ = 3.4, $^3J_{8syn\text{-}9}$ = 3.4, $^4J_{8syn\text{-}11endo}$ = 1.7, $^4J_{8syn\text{-}13endo}$ = 1.7, H_{syn}-8); 2.40 (1H, m, H-9); 3.10 (1H, m, H-7); 3.05 (1H, dd, ^2J = 12.3, $^3J_{13exo\text{-}7}$ = 2.0, H_{exo}-13); 3.09 (1H, ddd, ^2J = 11.0, $^3J_{11exo\text{-}9}$ = 2.4, $^4J_{11exo\text{-}10exo}$ = 1.2, H_{exo}-11); 3.83 (1H, ddd, ^2J = 15.6, $^3J_{10exo\text{-}9}$ = 6.7, $^4J_{10exo\text{-}11exo}$ = 1.2, H_{exo}-10); 4.17 (1H, ddt, ^2J = 12.3, $^3J_{13endo\text{-}7}$ = 3.5, $^4J_{13endo\text{-}11endo}$ = 1.7, $^4J_{13endo\text{-}8syn}$ = 1.7, H_{endo}-13); 4.23 (1H, ddt, ^2J = 11.0, $^3J_{11endo\text{-}9}$ = 3.4, $^4J_{11endo\text{-}8syn}$ = 1.7, $^4J_{11endo\text{-}13endo}$ = 1.7, H_{endo}-11); 4.23 (1H, dt, ^2J = 15.6, $^3J_{10endo\text{-}9}$ = 1.1, $^4J_{10endo\text{-}8anti}$ = 1.1, H_{endo}-10); 6.03 (1H, dd, $^3J_{5\text{-}4}$ = 6.9, $^4J_{5\text{-}3}$ = 1.1, H-5); 6.36 (1H, dd, $^3J_{3\text{-}4}$ = 9.1, $^4J_{3\text{-}5}$ = 1.1, H-3); 6.94 (1H, tt, $^3J_{19\text{-}18(20)}$ = 7.2, $^4J_{19\text{-}17(21)}$ = 1.5, H-19); 7.16 (2H, dd, $^3J_{18(20)\text{-}17(21)}$ = 7.4, $^3J_{18(20)\text{-}19}$ = 7.2, H-18(20)); 7.23 (1H, dd, $^3J_{4\text{-}3}$ = 9.1, $^3J_{4\text{-}5}$ = 6.9, H-4); 7.24 (2H, dd, $^3J_{17(21)\text{-}18(20)}$ = 8.4, $^4J_{17(21)\text{-}19}$ = 1.5, H-17(21)); 7.52 (1H, br.s, H-15).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 25.79 (C-8); 27.43 (C-9); 34.59 (C-7); 49.09 (C-10); 50.89 (C-11); 51.35 (C-13); 105.66 (C-5); 117.12 (C-3); 120.49 (C-17(21)); 123.02 (C-19); 128.65 (C-18(20)); 139.12 (C-4); 149.37 (C-6); 156.18 (C-14); 163.50 (C-2).

***N*,*N*-Hexane-1,6-diyl-bis(cytisinecarbamide) (6).** A solution of **1** (0.5 g, 2.6 mmol) in benzene was treated with hexamethylene-1,6-diisocyanate (0.78 g, 1.4 mmol), refluxed until **1** disappeared completely (TLC monitoring), and concentrated at reduced pressure. The solid was chromatographed over SiO_2 with elution by benzene:MeOH (9:1) to afford **6** (1.1 g, 68%), mp 120–123°C, $[\alpha]_D^{20}$ –168.0° (MeOH), HR-MS (EI, *m/z*): calcd for $C_{30}H_{70}N_6O_4$, 548.3106 $[M]^+$; found, 548.3080.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.99 (1H, m, H_A-18); 1.06 (1H, m, H_B-18); 1.19 (2H, m, H-17);1.95 (1H, dtd, ^2J = 12.7, $^3J_{8anti\text{-}7}$ = 3.0, $^3J_{8anti\text{-}9}$ = 3.0, $^4J_{8anti\text{-}10endo}$ = 1.0, H_{anti}-8); 2.00 (1H, dtt, ^2J = 12.7, $^3J_{8syn\text{-}7}$ = 3.3, $^3J_{8syn\text{-}9}$ = 3.3, $^4J_{8syn\text{-}11endo}$ = 1.6, $^4J_{8syn\text{-}13endo}$ = 1.6, H_{syn}-8); 2.51 (1H, m, H-9); 2.92 (1H, dq, ^2J = 12.0, $^3J_{16A\text{-}17}$ = 12.0, $^3J_{16A\text{-}15}$ = 5.0, H_A-16); 3.01 (1H, dd, ^2J = 13.0, $^3J_{13exo\text{-}7}$ = 2.2, H_{exo}-13); 3.08 (1H, ddd, ^2J = 11.0, $^3J_{11exo\text{-}9}$ = 2.4, $^4J_{11exo\text{-}10exo}$ = 1.2, H_{exo}-11); 3.09 (1H, m, H-7); 3.09 (1H, dq, ^2J = 12.0, $^3J_{16B\text{-}17}$ = 12.0, $^3J_{16B\text{-}15}$ = 5.0, H_B-16); 3.88 (1H, ddd, ^2J = 15.5, $^3J_{10exo\text{-}9}$ = 6.9, $^4J_{10exo\text{-}11exo}$ = 1.2, H_{exo}-10); 4.26 (1H, ddt, ^2J = 13.0, $^3J_{13endo\text{-}7}$ = 3.4, $^4J_{13endo\text{-}11endo}$ = 1.6, $^4J_{13endo\text{-}8syn}$ = 1.6, H_{endo}-13); 4.28 (1H, ddt, ^2J = 11.0, $^3J_{11endo\text{-}9}$ = 3.4, $^4J_{11endo\text{-}13endo}$ = 1.6, $^4J_{11endo\text{-}8syn}$ = 1.6, H_{endo}-11); 4.30 (1H, dt, ^2J = 15.5, $^3J_{10endo\text{-}9}$ = 0.9, $^4J_{10endo\text{-}8anti}$ = 0.9, H_{endo}-10); 5.93 (1H, t, $^3J_{15\text{-}16A}$ = 5.0, $^3J_{15\text{-}16B}$ = 5.0, H-15); 6.10 (1H, dd, $^3J_{5\text{-}4}$ =7.1, $^4J_{5\text{-}3}$ = 1.0, H-5); 6.36 (1H, dd, $^3J_{3\text{-}4}$ = 9.0, $^4J_{3\text{-}5}$ = 1.0, H-3); 7.28 (1H, dd, $^3J_{4\text{-}5}$ = 7.1, $^3J_{4\text{-}3}$ = 9.0, H-4).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 25.92 (C-18); 25.98 (C-8); 27.52 (C-9); 29.74 (C-16); 34.70 (C-7); 40.29 (C-17); 49.17 (C-10); 50.42 (C-11); 50.76 (C-13); 105.93 (C-3); 116.63 (C-5); 139.28 (C-4); 149.90 (C-6); 158.21 (C-14); 163.50 (C-2).

12-Methyl-3-nitrocytisine (7a) and 12-Methyl-5-nitrocytisine (7b). Regioisomers **7a** and **7b** were prepared according to the literature [16] in yields of 70 and 10%, respectively.

7a: mp 105–107°C, $[\alpha]_D^{20}$ –245° (MeOH), HR-MS (EI, *m/z*): calcd for $C_{12}H_{15}N_3O_3$, 249.1108 $[M]^+$; found, 249.1121.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.81 (1H, dtd, ^2J = 13.0, $^3J_{8anti\text{-}7}$ = 3.1, $^3J_{8anti\text{-}9}$ = 3.1, $^4J_{8anti\text{-}10endo}$ = 1.1, H_{anti}-8); 1.91 (1H, dtt, ^2J = 13.0, $^3J_{8syn\text{-}7}$ = 3.3, $^3J_{8syn\text{-}9}$ = 3.3, $^4J_{8syn\text{-}11endo}$ = 1.6, $^4J_{8syn\text{-}13endo}$ = 1.6, H_{syn}-8); 2.12 (3H, s, 3H-14); 2.27 (1H, ddd, ^2J = 11.4, $^3J_{11exo\text{-}9}$ = 2.6, $^4J_{11exo\text{-}10exo}$ = 1.3, H_{exo}-11); 2.35 (1H, dd, ^2J = 11.3, $^3J_{13exo\text{-}7}$ = 2.3, H_{exo}-13); 2.51 (1H, m, H-9); 2.88 (1H, ddt, ^2J = 11.3, $^3J_{13endo\text{-}7}$ = 3.5, $^4J_{13endo\text{-}11endo}$ = 1.6, $4J_{13endo\text{-}8syn}$ = 1.6, H_{endo}-13); 2.89 (1H, ddt, ^2J = 11.4, $^3J_{11endo\text{-}9}$ = 3.3, $^4J_{11endo\text{-}13endo}$ = 1.6, $^4J_{11endo\text{-}8syn}$ = 1.6, H_{endo}-11); 3.12 (1H, m, H-7); 3.97 (1H, ddd, ^2J = 15.8, $^3J_{10exo\text{-}9}$ = 6.5, $^4J_{10exo\text{-}11exo}$ = 1.3, H_{exo}-10); 4.13 (1H, dt, 2J = 15.8, $^3J_{10endo\text{-}9}$ = 1.1, $^4J_{10endo\text{-}8anti}$ = 1.1, H_{endo}-10); 6.16 (1H, d, $^3J_{5\text{-}4}$ = 8.1, H-5); 8.32 (1H, d, $^3J_{4\text{-}3}$ = 8.1, H-4).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 24.57 (C-8); 27.49 (C-9); 36.27 (C-7); 45.89 (C-14); 51.24 (C-10); 61.48 (C-13); 61.80 (C-11); 102.87 (C-5); 134.46 (C-3); 137.85 (C-4); 155.26 (C-6); 161.01 (C-2).

7b: mp 138–141°C, $[\alpha]_D^{20}$ –350.0° (MeOH), HR-MS (EI, *m/z*): calcd for $C_{12}H_{15}N_3O_3$, 249.1108 $[M]^+$; found, 249.1122.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.83 (2H, t, $^3J_{8\text{-}7(9)}$ = 3.2, 2H-8); 2.17 (3H, s, 3H-14); 2.26 (1H, ddd, ^2J = 11.1, $^3J_{11exo\text{-}9}$ = 3.3, $^4J_{11exo\text{-}10exo}$ = 1.2, H_{exo}-11); 2.34 (1H, dd, ^2J = 11.4, $^3J_{13exo\text{-}7}$ = 2.6, H_{exo}-13); 2.48 (1H, m, H-9); 2.90 (1H, ddt, ^2J = 11.1, $^3J_{11endo\text{-}9}$ = 3.3, $^4J_{11endo\text{-}13endo}$ = 1.6, $^4J_{11endo\text{-}8syn}$ = 1.6, H_{endo}-11); 3.28 (1H, ddt, ^2J = 11.4, $^3J_{13endo\text{-}7}$ = 3.3, $^4J_{13endo\text{-}11endo}$ = 1.6, $^4J_{13endo\text{-}8syn}$ = 1.6, H_{endo}-13); 3.98 (1H, ddd, ^2J = 15.7, $^3J_{10exo\text{-}9}$ = 6.2, $^4J_{10exo\text{-}11exo}$ = 1.2, H_{exo}-10); 4.02 (1H, m, H-7); 4.05 (1H, dt, ^2J = 15.7, $^3J_{10endo\text{-}9}$ = 1.0, $^4J_{10endo\text{-}8anti}$ = 1.0, H_{endo}-10); 6.45 (1H, d, $^3J_{3\text{-}4}$ = 10.0, H-3); 8.13 (1H, d, $^3J_{4\text{-}3}$ =10.0, H-4).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 24.97 (C-8); 27.15 (C-9); 31.75 (C-7); 46.05 (C-14); 51.79 (C-10); 60.12 (C-13); 62.00 (C-11); 115.86 (C-3); 128.36 (C-5); 134.97 (C-4); 155.33 (C-6); 162.33 (C-2).

3-Amino-12-methylcytisine (8). Compound **8** was prepared from **7a** according to the literature [16] in 98% yield, mp 169–172°C, $[\alpha]_D^{20}$ –113.0° (MeOH), HR-MS (EI, *m/z*): calcd for $C_{12}H_{17}N_3O$, 219.1366 $[M]^+$; found, 219.1365.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.68 (1H, dtd, ^2J = 12.5, $^3J_{8anti\text{-}7}$ = 3.2, $^3J_{8anti\text{-}9}$ = 3.2, $^4J_{8anti\text{-}10endo}$ = 1.3, H_{anti}-8); 1.80 (1H, dtt, ^2J = 12.5, $^3J_{8syn\text{-}7}$ = 3.4, $^3J_{8syn\text{-}9}$ = 3.4, $^4J_{8syn\text{-}11endo}$ = 1.7, $^4J_{8syn\text{-}13endo}$ = 1.7, H_{syn}-8); 2.09 (3H, s, 3H-14); 2.17 (1H, ddd, ^2J = 11.1, $^3J_{11exo\text{-}9}$ = 2.4, $^4J_{11exo\text{-}10exo}$ = 1.2, H_{exo}-11); 2.18 (1H, dd, ^2J = 10.5, $^3J_{13exo\text{-}7}$ = 2.2, H_{exo}-13); 2.37 (1H, m, H-9); 2.76 (1H, ddt, ^2J = 10.5, $^3J_{13endo\text{-}7}$ = 3.4, $^4J_{13endo\text{-}11endo}$ = 1.7, $^4J_{13endo\text{-}8syn}$ = 1.7, H_{endo}-13); 2.84 (1H, m, H-7); 2.85 (1H, ddt, ^2J = 11.1, $^3J_{11endo\text{-}9}$ = 3.4, $^4J_{11endo\text{-}13endo}$ = 1.7, $^4J_{11endo\text{-}8syn}$ = 1.7, H_{endo}-11); 3.91 (1H, ddd, ^2J = 15.1, $^3J_{10exo\text{-}9}$ = 6.8, $^4J_{10exo\text{-}11exo}$ = 1.3, H_{exo}-10); 4.09 (1H, dt, ^2J = 15.1, $^3J_{10endo\text{-}9}$ = 1.0, $^4J_{10endo\text{-}8anti}$ = 1.0, H_{endo}-10); 5.85 (1H, d, $^3J_{5\text{-}4}$ = 7.2, H-5); 6.53 (1H, d, $^3J_{4\text{-}5}$ = 7.2, H-4).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.01 (C-8); 28.00 (C-9); 34.79 (C-7); 46.27 (C-14); 50.18 (C-10); 62.31 (C-11); 63.19 (C-13); 104.29 (C-5); 113.03 (C-4); 134.67 (C-3); 138.50 (C-6); 158.30 (C-2).

***N*-(12-Methylcytisin-3-yl)-*N′*-allylurea (9).** Compound **9** was prepared according to the literature [19] in 98% yield, mp 176–179°C, $[\alpha]_D^{20}$ –63.3° ($CHCl_3$), HR-MS (EI, *m/z*): calcd for $C_{16}H_{22}N_4O_2$, 302.1737 $[M]^+$; found, 302.1711.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.73 (1H, dtd, ^2J = 13.3, $^3J_{8anti\text{-}7}$ = 3.4, $^3J_{8anti\text{-}9}$ = 3.4, $^4J_{8anti\text{-}10endo}$ = 1.3, H_{anti}-8); 1.83 (1H, ddt, ^2J = 13.3, $^3J_{8syn\text{-}7}$ = 3.4, $^3J_{8syn\text{-}9}$ = 3.4, $^4J_{8syn\text{-}11endo}$ = 1.7, $^4J_{8syn\text{-}13endo}$ = 1.7, H_{syn}-8); 2.09 (3H, s, H-14); 2.21 (1H, ddd, ^2J = 11.3, $^3J_{11exo\text{-}9}$ = 2.5, $^4J_{11exo\text{-}10exo}$ = 1.0, H_{exo}-11); 2.22 (1H, dd, ^2J = 11.2, $^3J_{13exo\text{-}7}$ = 2.4, H_{exo}-13); 2.36 (1H, m, H-9); 2.80 (1H, ddt, ^2J = 11.2, $^3J_{13endo\text{-}7}$ = 3.1, $^4J_{13endo\text{-}11endo}$ = 1.7, $^4J_{13endo\text{-}8syn}$ = 1.7, H_{endo}-13); 2.83 (1H, ddt, ^2J = 11.3, $^3J_{11endo\text{-}9}$ = 3.3, $^4J_{11endo\text{-}13endo}$ = 1.7, $^4J_{11endo\text{-}8syn}$ = 1.7, H_{endo}-11); 2.94 (1H, m, H-7); 3.85 (1H, ddd, ^2J = 15.3, $^3J_{10exo\text{-}9}$ = 6.6, $^4J_{10exo\text{-}11exo}$ = 1.0, H_{exo}-10); 3.95 (2H, m, H-18); 3.98 (1H, dt, ^2J = 15.3, $^3J_{10endo\text{-}9}$ = 1.0, $^4J_{10endo\text{-}8anti}$ = 1.0, H_{endo}-10); 5.08 (1H, dq, $^3J_{20cis\text{-}19}$ = 10.2, $^4J_{20cis\text{-}18}$ = 1.7, H_{cis}-20); 5.24 (1H, dq, $^3J_{20trans\text{-}19}$ = 17.0, $^4J_{20trans\text{-}18}$ = 1.9, H_{trans}-20); 5.93 (1H, ddt, $^3J_{19\text{-}20trans}$ = 17.1, $^3J_{19\text{-}20cis}$ = 10.2, $^3J_{19\text{-}18}$= 5.9, H-19); 6.11 (1H, d, $^3J_{5\text{-}4}$ = 7.8, H-5); 7.52 (1H, t, $^3J_{17\text{-}18}$ = 5.5, H-17); 8.30 (1H, d, $^3J_{4\text{-}5}$ = 7.8, H-4); 8.90 (1H, s, H-15).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 25.50 (C-8); 27.70 (C-9); 34.61 (C-7); 42.20 (C-18); 46.08 (C-14); 50.76 (C-10); 61.96 (C-11); 62.62 (C-13); 106.18 (C-5); 114.81 (C-20); 120.54 (C-4); 128.28 (C-3); 136.37 (C-19); 140.72 (C-6); 156.26 (C-16); 157.70 (C-2).

***N*-(12-Methylcytisin-3-yl)-*N′*-phenylurea (10).** Compound **10** was prepared according to the literature [19] in 98% yield, mp 109–112°C, $[\alpha]_D^{20}$ –34.5° ($CHCl_3$), HR-MS (EI, *m/z*): calcd for $C_{19}H_{22}N_3O_2$, 338.1737 $[M]^+$; found, 338.1716.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.66 (1H, dtd, ^2J = 13.3, $^3J_{8anti\text{-}7}$ = 3.4, $^3J_{8anti\text{-}9}$ = 3.4, $^4J_{8anti\text{-}10endo}$ = 1.3, H_{anti}-8); 1.79 (1H, ddt, ^2J = 13.3, $^3J_{8syn\text{-}7}$ = 3.4, $^3J_{8syn\text{-}9}$ = 3.4, $^4J_{8syn\text{-}11endo}$ = 1.7, $^4J_{8syn\text{-}13endo}$ = 1.7, H_{syn}-8); 2.04 (3H, s, H-14); 2.12 (1H, ddd, ^2J = 11.3, $^3J_{11exo\text{-}9}$ = 2.5, $^4J_{11exo\text{-}10exo}$ = 1.0, H_{exo}-11); 2.16 (1H, dd, ^2J = 11.2, $^3J_{13exo\text{-}7}$ = 2.4, H_{exo}-13); 2.38 (1H, m, H-9); 2.72 (1H, ddt, ^2J = 11.2, $^3J_{13endo\text{-}7}$ = 3.3, $^4J_{13endo\text{-}11endo}$ = 1.7, $^4J_{13endo\text{-}8syn}$ = 1.7, H_{endo}-13); 2.86 (1H, ddt, ^2J = 11.3, $^3J_{11endo\text{-}9}$ = 3.3, $^4J_{11endo\text{-}13endo}$ = 1.7, $^4J_{11endo\text{-}8syn}$ = 1.7, H_{endo}-11); 2.98 (1H, m, H-7); 3.82 (1H, ddd, ^2J = 15.3,

$^3J_{10exo-9}$ = 6.7, $^4J_{10exo-11exo}$ = 1.0, H_{exo}-10); 3.93 (1H, dt, ^2J = 15.3, $^3J_{10endo-9}$ = 1.0, $^4J_{10endo-8anti}$ = 1.0, H_{endo}-10); 6.12 (1H, d, $^3J_{5-4}$ = 7.8, H-5); 6.98 (1H, tt, $^3J_{para-meta}$ = 9.0, $^4J_{para-ortho}$ = 1.1, H_{para}-Ph); 7.27 (1H, dd, $^3J_{meta-para}$ = 9.0, $^3J_{20meta-ortho}$ = 7.3, H_{meta}-Ph); 7.45 (1H, dd, $^3J_{ortho-meta}$ = 7.3, $^4J_{ortho-para}$ = 1.1, H_{ortho}-Ph); 8.02 (1H, d, $^3J_{4-5}$ = 7.8, H-4); 8.51 (1H, s, H-15); 9.52 (1H, s, H-17).

^{13}C NMR spectrum (DMSO-d_6, δ, ppm): 24.88 (C-8); 27.00 (C-9); 33.80 (C-7); 45.76 (C-14); 50.23 (C-10); 61.60 (C-11); 62.46 (C-13); 103.51 (C-5); 117.69 (C-19); 118.96 (C-4); 121.63 (C-21); 126.62 (C-3); 128.71 (C-20); 139.67 (C-18); 142.15 (C-6); 152.42 (C-16); 156.81(C-2).

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REFERENCES

1. http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf
2. R. M. Sousa, C. P. Ferri, D. Acosta, E. Albanese, M. Guerra, Y. Huang, K. S. Jacob, A. T. Jotheeswaran, J. J. Llibre Rodriguez, G. Rodriguez Pichardo, M. Calvo Rodriguez, A. Salas, A. L. Sosa, J. Williams, T. Zuniga, and M. Prince, *Lancet*, **374**, 1821 (2009).
3. P. K. Fischhof, B. Saletu, E. Ruther, G. Litschauer, R. Moslinger-Gehmayr, and W. M. Herrmann, *Neuropsychobiology*, **26**, 65 (1992).
4. L. G. Neyens, W. C. Alpherts, and A. P. Aldenkamp, *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **19**, 411 (1995).
5. B. Winblad, *CNS Drug Rev.*, **11**, 169 (2005).
6. M. Romanelli and P. Gratteri, *Med. Res. Rev.*, **23**, 393 (2003).
7. M. Romanelli, P. Gratteri, L. Guandalini, E. Martini, C. Bonnacini, and F. Gualtieri, *Chem. Med. Chem.*, **2**, 746 (2007).
8. P. M. Joyner and R. H. Cichewicz, *Nat. Prod. Rep.*, **28**, 26 (2011).
9. C. Canu Boido and F. Sparatore, *Farmaco*, **54**, 438 (1999).
10. O. Nicolotti, C. Canu Boido, F. Sparatore, and A. Carotti, *Farmaco*, **57**, 469 (2002).
11. C. Canu Boido, B. Tasso, V. Boido, and F. Sparatore, *Farmaco*, **58**, 265 (2003).
12. G. Bombieri, F. Meneghetti, R. Artali, B. Tasso, C. Canu Boido, and F. Sparatore, *Chem. Biodiversity*, **5**, 1867 (2008).
13. B. Tasso, C. Canu Boido, E. Terranova, C. Gotti, L. Riganti, F. Clementi, R. Artali, G. Bombieri, F. Meneghetti, and F. Sparatore, *J. Med. Chem.*, **52**, 4345 (2009).
14. P. Imming, P. Klaperski, M. T. Stubbs, G. Seitz, and D. Gundisch, *Eur. J. Med. Chem.*, **36**, 375 (2001).
15. N. Houllier, J. Copisetti, P. Lestage, M.-C. Lasne, and J. Rouden, *Bioorg. Med. Chem. Lett.*, **20**, 6667 (2010).
16. E. Marrire, J. Rouden, V. Tadino, and M.-C. Lasne, *Org. Lett.*, **2**(8), 1121 (2000).
17. J. Abin-Carriquiri, G. Costa, J. Urbanavicius, B. Cassels, M. Rebolledo-Fuentes, S. Wonnacott, and F. Dajas, *Eur. J. Pharmacol.*, **589**, 80 (2008).
18. V. N. Tikhonov, I. V. Komissarov, and V. V. Volobuev, *Neirofiziologiya*, **35**, 26 (2003).
19. F. Moll and G. Luputiu, *Arch. Pharm.*, **305**, 771 (1972).
20. *Handbook of Experimental (Preclinical) Study of New Drugs* [in Russian], Moscow, 2005.
21. K. Nagayama, A. Kumar, K. Wuthrich, and R. R. Ernst, *J. Magn. Reson.*, **40**, 321 (1980).
22. Ya. Buresh, O. Bureshova, and D. Houston, in: *Methods and Principal Experiments for Studying the Brain and Behavior* [in Russian], Vysshaya Shkola, Moscow, 1991.
23. T. A. Gudasheva, R. U. Ostrovskaya, S. S. Trofimov, M. Yu. Kosoi, F. V. Ienkina, Yu. V. Burov, and A. P. Skoldinov, *Khim.-farm. Zh.*, **11**, 1322 (1985).
24. M. L. Belen′kii, *Elements of Quantitative Assessment of the Pharmacological Effect* [in Russian], Medgiz, Leningrad, 1963.