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SYNTHESIS AND NOOTROPIC ACTIVITY OF NEW 3-AMINO-12-*N*-METHYLCYTISINE DERIVATIVES

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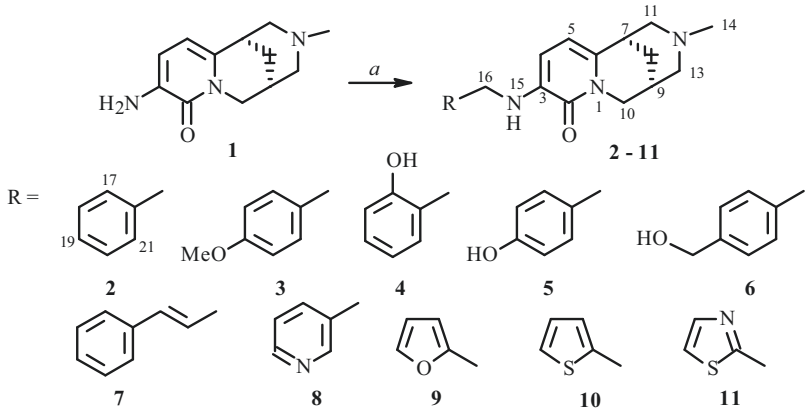
*Reductive alkylation of 3-amino-12-*N*-methylcytisine by aromatic aldehydes synthesized a series of secondary amines. The nootropic activity of the synthesized compounds was studied* in vivo *(mnestic and antihypoxic properties) and* in vitro *(antiradical properties and ability to affect transcription factor HIF-1 DNA-binding activity). The cytotoxicity of the synthesized compounds was assessed. The lead compound was identified.*

Keywords: (–)-cytisine, nootropic activity, mnestic effect, antihypoxic properties, antiradical properties, AOS, TF HIF-1.

Nootropic agents are a significant component in the therapy of neurodegenerative diseases, consequences of injuries and concussions, and brain dysfunctions caused by alcoholism and acute neural infections owing their broad spectrum of activity and proven positive clinical outcomes. Nootropic agents for human health are applied mainly to the prevention of stress and maintenance of functionality in critical situations and with heightened physical and intellectual exertion [1–4].

The mechanism of action of many nootropic agents is due to nootropic and mnestic properties themselves in addition to antioxidant and antihypoxic activity [5]. Therefore, both *in vivo* screening tests [conditioned passive-avoidance response (CPAR), normobaric hypercapnic hypoxia] and the potential protective activity of tested compounds in *in vitro* cell models for antiradical properties and the ability to influence the hypoxia-induced DNA-binding activity of transcription factor HIF-1 should be assessed [6, 7].

We demonstrated earlier that several carboxamides obtained from 3-amino-12-*N*-methylcytisine (**1**) and the corresponding isocyanates had nootropic properties that were comparable or superior to those of the reference drug piracetam [8]. A library of secondary amines **2**–**11** was prepared via one-pot syntheses using reductive alkylation of **1** with hetero- and aromatic aldehydes (the azomethines formed in the first step were reduced by $NaBH_4$ without isolation as before [9]) in order to determine nootropic activity as a function of the 2-pyridone substituent. Compounds **2**–**11** were obtained in 56–97% yields. Their structures were established by NMR (^1H, ^{13}C, ^{15}N) spectroscopy.



a. RCHO, benzene, 80°C; then $NaBH_4$, MeOH, 0÷20°C

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TABLE 1. Nootropic and Antihypoxic Activity of **2**–**11***

Compound[a]	Mnestic activity, %	Antihypoxic activity, %	Compound[a]	Mnestic activity, %	Antihypoxic activity, %
Piracetam[b]	71.7	11.2	**7**	40.3	40.1
2	72.3	–	**8**	87.4	9.0
3	71.8	9.7	**9**	35.5	11.6
4	89.9	–	**10**	50.1	–
5	99.7	–	**11**	13.3	10.2
6	38.5	–			

*Piracetam: LD_{50}, 2000 mg/kg; ED_{50}, 400; LD_{50}/ED_{50}, 26.5 mg/kg.
[a]50 μmol/kg dose; [b]400 mg/kg dose.

TABLE 2. Effect of **2**–**11** on AOS and Transcription Factor HIF-1 Activity *in vitro*

Compound	AOS[a]			HIF-1, EC_{50}[b], mM
	1, μM	10, μM	100, μM	
2	121.6 ± 9.9	114 ± 8.7	165.3 ± 6.9	0.23
3	89.3 ± 5.4	100.5 ± 6.1	117.4 ± 9.9	–
4	91.9 ± 10.7	86.5 ± 8.4	93.7 ± 7.3	0.07
5	101.1 ± 7.7	95.3 ± 6.0	119.5 ± 5.4	–
6	103.84 ± 6.1	101.45 ± 2.8	104.15 ± 3.7	–
7	102.3 ± 11.2	131.2 ± 12.4	222.4 ± 16.2*	–
8	103.9 ± 9.6	78.9 ± 9.4	105.4 ± 7.1	–
9	85.2 ± 1.1	60.9 ± 12.2	69.4 ± 5.5	0.15
10	98.4 ± 5.8	95.2 ± 9.8	151.9 ± 8.9*	–
11	106.25 ± 5.6	102.95 ± 7.3	108.3 ± 1.5	–

[a]Experimental groups were compared using the Student *t*-criterion for independent sets ($n = 3$, $*p < 0.05$); [b]arithmetic averages ± standard deviation of the mean are given. The EC_{50} values (compound concentration increasing luciferase activity by 50%) were calculated using nonlinear regression of the logarithm of the compound concentrations and normalized percent luciferase activation (GraphPad Prism v.5.0, GraphPad Software Inc., USA).

The specific nootropic activity of **2**–**11** was studied previously [10–12] using a basic CPAR model that enabled the mnestic effect to be assessed. Antihypoxic activity was determined using a normobaric hypercapnic hypoxia model [10]. Table 1 presents the results.

According to the results, **6**, **7**, and **9**–**11** without oxygen-containing substituents in the aromatic amine exhibited weak mnestic activity. However, the activities of secondary amines **2** and **3**, derivatives of *p*-methoxy- and benzaldehyde, were comparable to that of the reference drug piracetam at 72.3 and 71.8%. The lead compounds in this group were secondary amines **8**, **4**, and **5**. Thus, introducing a hydroxyl into the *ortho*-position of the benzyl substituent on the 3-amine increased this parameter to 89.9%; in the *para*-position, to 99.7%. The lethal and effective median doses for lead compound **5** were LD_{50} (1800 mg/kg) and ED_{50} (9.5 mg/kg) with therapeutic index LD_{50}/ED_{50} 191.5. This exceeded significantly that for the reference drug piracetam [8].

Parallel *in vivo* screening of the antihypoxic activity showed that only amines **7** and **9**, reaction products of cinnamaldehyde and furfurol with **1**, had pronounced antihypoxic properties in the series of secondary amines **2**–**11**. Thus, the lifespan of laboratory animals under hypoxia conditions was increased relative to the control in the first case by 40%; in the second, by 11.6%, which was comparable to the antihypoxic activity of piracetam (11.2%).

The potential cytotoxicity of synthesized **2**–**11** was assessed preliminarily *in vitro* using standard MTT-assay methods [13]. As it turned out, none of these compounds exhibited cytotoxicity against the conditionally normal cell line HEK293 (because $IC_{50} > 1$ mM, data not presented).

The antiradical properties of **2**–**11** were studied using HEK293 cells under oxidative stress induced by injection of PMA (4-phorbol-12-myristate-13-acetate). Table 2 shows that the majority of the tested 12-*N*-methylcytisine derivatives (**3**–**6**, **8**, and **11**) had no effect on the intracellular active-oxygen-species (AOS) level in the HEK293 cell cytoplasm. However, secondary amines **2** and **7** and thiophene derivative **10** produced a dose-dependent increase of the AOS level, which argued in

favor of a possible pro-oxidant effect for these compounds. Compound **9** with a furan moiety probably had moderate antioxidant properties and was capable of reducing AOS accumulation stimulated by PMA.

Next, the *in vitro* effects of **2**–**11** were compared using the DNA-binding activity of TF HIF-1 and luciferase reporter constructs. The results (Table 2) showed that only a few of the experimental compounds (**4**, **9**, and **2**) exhibited moderate stimulation of HIF-1 (EC_{50} values of 0.07, 0.15, and 0.23 mM, respectively).

A comparison of results from the *in vivo* tests (CPAR, normobaric hypercapnic hypoxia model) and *in vitro* experiments (effect on AOS level and HIF-1 basal activity) showed that **9** exhibited a pronounced antihypoxic effect analogous to piracetam and also increased the DNA-binding activity of TF HIF-1. Furthermore, it reduced the accumulation of AOS under conditions modeling oxidative stress *in vitro* by demonstrating antiradical properties. It could be assumed that the antihypoxic properties of **9** were due to an increased resistance to hypoxia because of the induction of HIF-1, a dependent signal pathway, and the antioxidant activity. The positive effects of **2** and **4** on mnestic processes (assessed in the CPAR test) may have been due at least partially to activation of TF HIF-1, which requires further research.

Thus, *N*-benzyl and –hetarylmethylene derivatives of 12-*N*-methylcytisine-3-amine were synthesized via reductive alkylation of 3-amino-12-*N*-methylcytisine by aromatic and heteroaromatic aldehydes. Their mnestic, antihypoxic, and antiradical properties in addition to their effects on TF HIF-1 DNA-binding activity were studied. *N*-(4-Hydroxybenzyl)-12-*N*-methylcytisine-3-amine was identified as the lead compound. Its lethal and effective median doses and therapeutic index were determined and exceeded those of the reference drug piracetam.

EXPERIMENTAL

General. Commercially available (–)-cytisine (CAS 485-35-8) and $NaBH_4$ (CAS 16940-66-2) were used as starting compounds. *N*-Methylcytisine was prepared according to the literature [14]. The course of reactions was monitored by TLC on ALUGRAM® plates. Column chromatography (CC) was performed over standard silica gel 60 (0.05–0.1 mm) (Macherey-Nagel, Germany). Melting points were determined on a Boetius apparatus. Optical rotation angles were measured on a PerkinElmer 341 LC polarimeter. PMR and ^{13}C NMR spectra were recorded on equipment at the Khimya CCU, UfIC, RAS (Bruker Avance III pulsed spectrometer at operating frequency 500.13 MHz for ^1H and 125.47 MHz for ^{13}C relative to TMS internal standard [15]).

Preparation of Substituted *N*-Benzyl-(12-*N*-methylcytisin)-3-amines (2). A mixture of **1** (260 mg, 0.9 mmol) and benzaldehyde (116 mg, 1.1 mmol) in benzene (10 mL) was refluxed. When the reaction was finished (TLC monitoring), the mixture was evaporated. The residue was dissolved in MeOH (10 mL), cooled to 0°C, treated with $NaBH_4$ (450 mg, 10.0 mmol), stirred on a magnetic stirrer for 1 h, and concentrated. The residue was dissolved in H_2O (2 mL) and extracted with $CHCl_3$ (5 × 10 mL). The extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed over SiO_2 (EtOAc eluent) to afford *N*-benzyl-(12-*N*-methylcytisine)-3-amine (**2**) (290 mg, 80% yield), $[\alpha]_D^{20}$ –28.0° (*c* 1.1, $CHCl_3$).

^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.66 (1H, dtd, ^2J = 12.7, $^3J_{8anti\text{-}7}$ = 3.2, $^3J_{8anti\text{-}9}$ = 3.2, $^4J_{8anti\text{-}10endo}$ = 1.3, H_{anti}-8), 1.78 (1H, dtt, ^2J = 12.7, $^3J_{8syn\text{-}7}$ = 3.4, $^3J_{8syn\text{-}9}$ = 3.4, $^4J_{8syn\text{-}11endo}$ = 1.7, $^4J_{8syn\text{-}13endo}$ = 1.7, H_{syn}-8), 2.08 (3H, s, H-14), 2.17 (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 = 10.5, $^3J_{13exo\text{-}7}$ = 2.4, H_{exo}-13), 2.35 (1H, m, H-9), 2.74 (1H, ddt, ^2J = 10.5, $^3J_{13endo\text{-}7}$ = 3.2, $^4J_{13endo\text{-}11endo}$ = 1.7, $^4J_{13endo\text{-}8syn}$ = 1.7, H_{endo}-13), 2.80 (1H, m, H-7), 2.85 (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), 3.94 (1H, ddd, ^2J = 15.1, $^3J_{10exo\text{-}9}$ = 7.00, $^4J_{10exo\text{-}11exo}$ = 1.0, H_{exo}-10), 4.12 (1H, dt, ^2J = 15.1, $^3J_{10endo\text{-}9}$ = 1.0, $^4J_{10endo\text{-}8anti}$ = 1.0, H_{endo}-10), 4.28 (2H, s, H-16), 5.36 (1H, s, N-H), 5.85 (1H, d, $^3J_{5\text{-}4}$ = 7.4, H-5), 6.13 (1H, d, $^3J_{4\text{-}5}$ = 7.4, H-4), 7.22 (2H, t, $^3J_{19(21)\text{-}20}$ = 7.7, $^3J_{19(21)\text{-}18(22)}$ = 7.7, H-19, 21), 7.29 (1H, d, $^3J_{20\text{-}19(21)}$ = 7.7, H-20), 7.33 (2H, d, $^3J_{18(22)\text{-}19(21)}$ = 7.7, H-18, 22). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.1 (C-8), 27.9 (C-9), 34.6 (C-7), 46.3 (C-14), 47.6 (C-16), 50.2 (C-10), 62.2 (C-11), 63.2 (C-13), 104.7 (C-5), 107.5 (C-4), 126.9 (C-20), 127.2 (C-18, 22), 128.4 (C-19, 21), 136.0 (C-3), 136.0 (C-6); 139.0 (C-17), 157.9 (C-2). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 28.9 (N12), 60.3 (N15), 167.6 (N1).

***N*-(4-Methoxybenzyl)-12-*N*-methylcytisin-3-amine (3)** was prepared from **1** (260 mg), yield 300 mg (75%), $[\alpha]_D^{20}$ –13.0° (*c* 2.5, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.67 (1H, dt, ^2J = 12.7, ^3J = 3.2, ^3J = 3.2, H_{anti}-8), 1.79 (1H, dt, ^2J = 12.7, ^3J = 3.4, ^3J = 3.4, H_{syn}-8), 2.08 (3H, s, H-14), 2.16 (1H, dd, ^2J = 10.5, ^3J = 2.4, H_{exo}-13), 2.17 (1H, dd, ^2J = 10.8, ^3J = 2.5, H_{exo}-11), 2.36 (1H, m, H-9), 2.74 (1H, dd, ^2J = 10.5, ^3J = 3.2, H_{endo}-13), 2.82 (1H, m, H-7), 2.85 (1H, dd, ^2J = 10.8, ^3J = 3.3, H_{endo}-11), 3.77 (3H, s, CH_3-O), 3.92 (1H, dd, ^2J = 15.1, ^3J = 7.0, H_{exo}-10), 4.11 (1H, d, ^2J = 15.1, H_{endo}-10), 4.21

(2H, s, H-16), 5.22 (1H, s, N-H), 5.86 (1H, d, ^{3}J = 7.4, H-5), 6.16 (1H, d, ^{3}J = 7.4, H-4), 6.85 (2H, d, ^{2}J = 8.7, H-19, 21), 7.26 (2H, d, ^{2}J = 8.7, H-18, 22). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.1 (C-8), 27.9 (C-9), 34.6 (C-7), 46.3 (C-14), 47.1 (C-16), 50.1 (C-10), 55.2 (C-24), 62.3 (C-11), 63.2 (C-13), 104.7 (C-5), 107.4 (C-4), 113.9 (C-19, 21), 128.5 (C-18, 22), 131.0 (C-17), 136.0 (C-3), 136.0 (C-6), 158.0 (C-2), 158.7 (C-20). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 28.6 (N12), 62.3 (N15), 167.7 (N1).

***N*-(2-Hydroxybenzyl)-12-*N*-methylcytisin-3-amine (4)** was prepared from **1** (260 mg), yield 308 mg (80%), $[\alpha]_D^{20}$ –49.0° (*c* 3.1, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.68 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.2, ^{3}J = 3.2, H_{anti}-8), 1.79 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.4, ^{3}J = 3.4, H_{syn}-8), 2.10 (3H, s, H-14), 2.18 (1H, dd, ^{2}J = 11.7, ^{3}J = 2.5, H_{exo}-11), 2.19 (1H, dd, ^{2}J = 10.7, ^{3}J = 2.1, H_{exo}-13), 2.37 (1H, m, H-9), 2.77 (1H, dd, ^{2}J = 10.7, ^{3}J = 3.1, H_{endo}-13), 2.85 (1H, m, H-7), 2.87 (1H, dd, ^{2}J = 11.7, ^{3}J = 3.3, H_{endo}-11), 3.93 (1H, dd, ^{2}J = 15.1, ^{3}J = 7.1, H_{exo}-10), 4.12 (1H, d, ^{2}J = 15.1, H_{endo}-10), 4.29 (2H, s, H-16), 5.24 (1H, br.s, N-H), 5.90 (1H, d, ^{3}J = 7.6, H-5), 6.41 (1H, d, ^{3}J = 7.6, H-4), 6.80 (1H, td, ^{3}J = 7.6, ^{3}J = 7.6, ^{4}J = 1.5, H-21), 6.87 (dd, ^{3}J = 8.2, ^{4}J = 1.5, H-19), 7.11 (1H, dd, ^{3}J = 8.2, ^{3}J = 7.6, H-20), 7.14 (1H, dd, ^{3}J = 7.6, H-22). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 25.9 (C-8), 27.9 (C-9), 34.6 (C-7), 46.3 (C-14), 50.4 (C-16), 50.4 (C-10), 62.2 (C-11), 63.0 (C-13), 105.3 (C-5), 111.5 (C-4), 116.1 (C-19), 119.6 (C-21), 123.9 (C-18), 128.6 (C-22), 128.6 (C-20), 136.0 (C-3), 137.9 (C-6), 156.3 (C-17), 158.4 (C-2). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 29.0 (N12), 57.8 (N15), 167.9 (N1).

***N*-(4-Hydroxybenzyl)-12-*N*-methylcytisin-3-amine (5)** was prepared from **1** (260 mg), yield 310 mg (80%), $[\alpha]_D^{20}$ –48.0° (*c* 1.71, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.71 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.2, ^{3}J = 3.2, H_{anti}-8), 1.82 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.4, ^{3}J = 3.4, H_{syn}-8), 2.11 (3H, s, H-14), 2.20 (1H, dd, ^{2}J = 11.1, ^{3}J = 2.5, H_{exo}-11), 2.21 (1H, dd, ^{2}J = 10.5, ^{3}J = 2.4, H_{exo}-13), 2.39 (1H, m, H-9), 2.81 (1H, dd, ^{2}J = 10.5, ^{3}J = 3.2, H_{endo}-13), 2.89 (1H, m, H-7), 2.91 (1H, dd, ^{2}J = 11.1, ^{3}J = 3.3, H_{endo}-11), 3.98 (1H, dd, ^{2}J = 15.1, ^{3}J = 7.0, H_{exo}-10), 4.13 (2H, s, H-16), 4.18 (1H, d, ^{2}J = 15.1, H_{endo}-10), 5.20 (1H, br.s, N-H), 6.01 (1H, d, ^{3}J = 7.4, H-5), 6.29 (1H, d, ^{3}J = 7.4, H-4), 6.81 (2H, d, ^{3}J = 8.6, H-19, 21), 7.09 (2H, d, ^{3}J = 8.6, H-18, 22). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.0 (C-8), 27.8 (C-9), 34.5 (C-7), 46.3 (C-14), 47.1 (C-16), 50.4 (C-10), 62.0 (C-11), 63.0 (C-13), 106.3 (C-5), 108.5 (C-4), 115.6 (C-19, 21), 128.7 (C18, 22), 129.2 (C-17), 135.7 (C-6), 136.1 (C-3), 156.3 (C-20), 157.9 (C-2). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 29.7 (N12), 64.1 (N15), 169.5 (N1).

***N*-(4-Hydroxymethylbenzyl)-12-*N*-methylcytisin-3-amine (6)** was prepared from **1** (260 mg), yield 225 mg (56%), $[\alpha]_D^{20}$ –22.0° (*c* 2.49, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.67 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.2, ^{3}J = 3.2, H_{anti}-8), 1.78 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.4, ^{3}J = 3.4, H_{syn}-8), 2.08 (3H, s, H-14), 2.16 (1H, dd, ^{2}J = 10.7, ^{3}J = 2.1, H_{exo}-13), 2.18 (1H, dd, ^{2}J = 11.7, ^{3}J = 2.5, H_{exo}-11), 2.35 (1H, m, H-9), 2.74 (1H, dd, ^{2}J = 10.7, ^{3}J = 3.1, H_{endo}-13), 2.82 (1H, m, H-7), 2.85 (1H, dd, ^{2}J = 11.7, ^{3}J = 3.3, H_{endo}-11), 3.36 (1H, br.s, OH), 3.90 (1H, dd, ^{2}J = 15.1, ^{3}J = 7.00, H_{exo}-10), 4.09 (1H, d, ^{2}J = 15.1, H_{endo}-10), 4.23 (2H, m, H-16), 4.60 (2H, m, H-23), 5.20 (1H, s, N-H), 5.86 (1H, d, ^{3}J = 7.4, H-5), 6.12 (1H, d, ^{3}J = 7.4, H-4), 7.27 (2H, m, H-18, 22), 7.28 (2H, m, H-19, 21). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.0 (C-8), 27.9 (C-9), 34.6 (C-7), 46.3 (C-14), 47.3 (C-16), 50.2 (C-10), 62.2 (C-11), 63.2 (C-13), 64.7 (C-23), 105.1 (C-5), 107.8 (C-4), 127.2 (C-18, 22), 127.2 (C-19, 21), 136.0 (C-3), 136.0 (C-6), 138.0 (C-17), 140.3 (C-20), 158.0 (C-2). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 28.9 (N12), 60.4 (N15), 168.1 (N1).

***N*-(3-Phenylprop-2-en-1-yl)-12-*N*-methylcytisin-3-amine (7)** was prepared from **1** (260 mg), yield 278 mg (70%), $[\alpha]_D^{20}$ –36.0° (*c* 0.78, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.68 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.2, ^{3}J = 3.2, H_{anti}-8), 1.80 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.4, ^{3}J = 3.4, H_{syn}-8), 2.10 (3H, s, H-14), 2.18 (1H, dd, ^{2}J = 10.7, ^{3}J = 2.1, H_{exo}-13), 2.18 (1H, dd, ^{2}J = 11.7, ^{3}J = 2.5, H_{exo}-11), 2.37 (1H, m, H-9), 2.77 (1H, dd, ^{2}J = 10.7, ^{3}J = 3.1, H_{endo}-13), 2.84 (1H, m, H-7), 2.88 (1H, dd, ^{2}J = 11.7, ^{3}J = 3.3, H_{endo}-11), 3.89 (2H, dd, ^{3}J = 5.5, ^{4}J = 1.8, H-16), 3.94 (1H, dd, ^{2}J = 15.1, ^{3}J = 7.00, H_{exo}-10), 4.12 (1H, d, ^{2}J = 15.1, H_{endo}-10), 5.09 (1H, s, N-H), 5.92 (1H, d, ^{3}J = 7.4, H-5), 6.26 (1H, d, ^{3}J = 7.4, H-4), 6.27 (1H, dt, ^{3}J = 15.9, ^{3}J = 5.6, H-17), 6.59 (1H, dt, ^{3}J = 15.9, ^{4}J = 1.8, H-18), 7.20 (1H, d, ^{3}J = 7.7, H-22), 7.29 (2H, t, ^{3}J = 7.7, H-21, 23), 7.35 (2H, d, ^{3}J = 7.7, H-20, 24). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.1 (C-8), 27.9 (C-9), 34.6 (C-7), 45.5 (C-16). 46.3 (C-14), 50.2 (C-10), 62.2 (C-11), 63.2 (C-13), 104.9 (C-5), 107.7 (C-4), 126.3 (C-20, 24), 126.7 (C-17), 127.3 (C-22), 128.5 (C-21, 23), 131.0 (C-18), 136.0 (C-3), 136.0 (C-6), 136.9 (C-19), 158.1 (C-2). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 29.2 (N12), 59.2 (N15), 167.8 (N1).

***N*-(Pyridin-3-ylmethyl)-12-*N*-methylcytisin-3-amine (8)** was prepared from **1** (260 mg), yield 353 mg (96%), $[\alpha]_D^{20}$ –35.5° (*c* 1.3, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.75 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.3, ^{3}J = 3.3, H_{anti}-8), 1.80 (1H, dt, ^{2}J = 12.7, ^{3}J = 3.4, ^{3}J = 3.4, H_{syn}-8), 2.10 (3H, s, H-14), 2.25 (1H, dd, ^{2}J = 11.3, ^{3}J = 2.5, H_{exo}-11), 2.25 (1H, dd, ^{2}J = 10.8, ^{3}J = 2.5, H_{exo}-13), 2.40 (1H, m, H-9), 2.79 (1H, dd, ^{2}J = 10.8, ^{3}J = 3.1, H_{endo}-13). 2.92 (1H, dd, ^{2}J = 11.3, ^{3}J = 3.3, H_{endo}-11), 2.93 (1H, m, H-7), 3.92 (1H, dd, ^{2}J = 15.3, ^{3}J = 6.5, H_{exo}-10), 4.08 (1H, d, ^{2}J = 15.3, H_{endo}-10), 4.40 (2H, s, H-16), 6.06 (1H, d, ^{3}J = 7.6, H-5), 6.28 (1H, d, ^{3}J = 7.6, H-4), 7.36 (1H, dd, ^{3}J = 7.8, ^{3}J = 4.9, H-21), 7.80 (1H, dt, ^{3}J = 7.8, ^{4}J = 1.7, ^{4}J = 1.7, H-22), 8.40 (1H, dd, ^{3}J = 4.9, ^{4}J = 1.7, H-20), 8.53 (1H, d, ^{4}J = 1.7, H-18). ^{13}C NMR spectrum (MeOD, δ, ppm): 26.6

(C-8), 29.1 (C-9), 35.5 (C-7), 45.2 (C-16), 46.6 (C-14), 51.4 (C-10), 63.0 (C-11), 64.1 (C-13), 107.3 (C-5), 110.5 (C-4), 125.1 (C-21), 136.6 (C-3), 137.1 (C-22), 137.1 (C-17), 137.7 (C-6), 148.5 (C-20), 149.1 (C-18), 159.1 (C-2). ^{15}N NMR spectrum (MeOD, δ, ppm): 29.2 (N12), 56.6 (N15), 169.6 (N1), 300.1 (N19).

***N*-(2-Furylmethyl)-12-*N*-methylcytisin-3-amine (9)** was prepared from **1** (260 mg), yield 308 mg (87%), $[\alpha]_D^{20}$ –39.7° (*c* 3.18, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.66 (1H, dt, ^2J = 12.7, ^3J = 3.2, ^3J = 3.2, H_{anti}-8), 1.77 (1H, dt, ^2J = 12.7, ^3J = 3.4, ^3J = 3.4, H_{syn}-8), 2.06 (3H, s, H-14), 2.14 (1H, dd, ^2J = 13.2, ^3J = 2.4, H_{exo}-13), 2.15 (1H, dd, ^2J = 11.4, ^3J = 2.5, H_{exo}-11), 2.34 (1H, m, H-9), 2.73 (1H, dd, ^2J = 13.2, ^3J = 3.2, H_{endo}-13), 2.82 (1H, m, H-7), 2.83 (1H, dd, ^2J = 11.4, ^3J = 3.3, H_{endo}-11), 3.89 (1H, dd, ^2J = 15.1, ^3J = 7.00, H_{exo}-10), 4.07 (1H, d, ^2J = 15.1, H_{endo}-10), 4.24 (2H, s, H-16), 5.17 (1H, s, N-H), 5.89 (1H, d, ^3J = 7.4, H-5), 6.19 (1H, d, ^3J = 3.1, H-18), 6.26 (1H, d, ^3J = 7.4, H-4), 6.27 (1H, dd, ^3J = 3.1, ^3J = 1.8, H-19), 7.31 (1H, d, ^3J = 1.8, H-20). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.0 (C-8), 27.9 (C-9), 34.6 (C-7), 40.8 (C-16), 46.3 (C-14), 50.15 (C-10), 62.2 (C-11), 63.2 (C-13), 104.6 (C-5), 106.8 (C-18), 107.7 (C-4), 110.2 (C-19), 135.6 (C-3), 136.5 (C-6), 141.7 (C-20), 152.5 (C-17), 158.0 (C-2). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 29.0 (N12), 57.8 (N15), 167.9 (N1).

***N*-(2-Thienylmethyl)-12-*N*-methylcytisin-3-amine (10)** was prepared from **1** (260 mg), yield 360 mg (97%), $[\alpha]_D^{20}$ –90.0° (*c* 1.09, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.67 (1H, dt, ^2J = 12.7, ^3J = 3.2, ^3J = 3.2, H_{anti}-8), 1.79 (1H, dd, ^2J = 12.7, ^3J = 3.4, ^3J = 3.4, H_{syn}-8), 2.08 (3H, s, H-14), 2.16 (1H, dd, ^2J = 11.3, ^3J = 2.5, H_{exo}-11), 2.16 (1H, dd, ^2J = 10.3, ^3J = 2.4, H_{exo}-13), 2.35 (1H, m, H-9), 2.75 (1H, dd, ^2J = 10.3, ^3J = 3.2, H_{endo}-13), 2.83 (1H, m, H-7), 2.85 (1H, dd, ^2J = 11.3, ^3J = 3.3, H_{endo}-11), 3.90 (1H, dd, ^2J = 15.1, ^3J = 7.00, H_{exo}-10), 4.10 (1H, d, ^2J = 15.1, H_{endo}-10), 4.45 (1H, d, ^3J = 5.5, H-16), 5.29 (1H, t, ^3J = 5.5, N-H), 5.89 (1H, d, ^3J = 7.4, H-5), 6.26 (1H, d, ^3J = 7.4, H-4), 6.93 (1H, dd, ^3J = 5.0, ^3J = 3.5, H-20), 6.98 (1H, d, ^3J = 3.5, H-21), 7.17 (1H, d, ^3J = 5.0, H-19). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.0 (C-8), 27.9 (C-9), 34.6 (C-7), 42.9 (C-16), 46.3 (C-14), 50.1 (C-10), 62.2 (C-11), 63.1 (C-13), 104.6 (C-5), 108.0 (C-4), 124.3 (C-19), 124.7 (C-21), 126.7 (C-20), 135.6 (C-3), 136.6 (C-6), 142.8 (C-17), 157.9 (C-2). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 28.6 (N12), 63.1 (N15), 167.5 (N1).

***N*-(1,3-Thiazol-2-ylmethyl)-12-*N*-methylcytisin-3-amine (11)** was prepared from **1** (260 mg), yield 330 mg (88%), $[\alpha]_D^{20}$ –17.5° (*c* 1.53, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.70 (1H, dt, ^2J = 12.7, ^3J = 3.2, ^3J = 3.2, H_{anti}-8), 1.80 (1H, dd, ^2J = 12.7, ^3J = 3.4, ^3J = 3.4, H_{syn}-8), 2.10 (3H, s, H-14), 2.16 (1H, dd, ^2J = 10.4, ^3J = 2.2, H_{exo}-13), 2.19 (1H, dd, ^2J = 11.3, ^3J = 2.5, H_{exo}-11), 2.39 (1H, m, H-9), 2.76 (1H, dd, ^2J = 10.4, ^3J = 3.5, H_{endo}-13), 2.84 (1H, m, H-7), 2.88 (1H, dd, ^2J = 11.3, ^3J = 3.3, H_{endo}-11), 3.95 (1H, dd, ^2J = 15.1, ^3J = 7.00, H_{exo}-10), 4.12 (1H, d, ^2J = 15.1, H_{endo}-10), 4.64 (2H, d, ^3J = 6.0, H-16), 5.64 (1H, t, ^3J = 6.0, N-H), 5.87 (1H, d, ^3J = 7.4, H-5), 6.24 (1H, d, ^3J = 7.4, H-4), 7.25 (1H, d, ^3J = 3.3, H-20), 7.72 (1H, d, ^3J = 3.3, H-19). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 26.0 (C-8), 27.9 (C-9), 34.6 (C-7), 46.1 (C-16), 46.3 (C-14), 50.2 (C-10), 62.2 (C-11), 63.1 (C-13), 104.5 (C-5), 108.7 (C-4), 119.2 (C-20), 135.2 (C-3), 137.4 (C-6), 142.5 (C-19). 158.0 (C-2), 171.8 (C-17). ^{15}N NMR spectrum ($CDCl_3$, δ, ppm): 28.6 (N12), 58.0 (N15), 168.1 (N1), 310.5 (N21).

Specific nootropic activity of the synthesized compounds was studied using a basic CPAR model [10] and Wistar white rats (180–200 g). Tested compounds were administered orally at a dose of 50 μmol/kg; piracetam, 400 mg/kg; distilled H_2O (control group), various volumes 1 h before training. The test protocol was described by us earlier [8]. Mnestic activity (M_t) (memory improvement under the influence of the tested compounds) was calculated using the formula $M_t = [(t_c - t_t)/t_c]\cdot 100\%$, where M_t is the mnestic activity (%), t_c, the average residence time in the dark section of control animals; t_t, the average residence time in the dark section of test animals [11, 12].

Antihypoxic properties of the synthesized compounds were studied using laboratory white mice of both sexes (20–25 g). Distilled H_2O (control group) and tested compounds were administered orally at a dose of 50 μmol/kg 1 h before the start of the experiment. Hypercapnic hypoxia was modeled as before [10]. The lifespan of an animal under hypoxia was recorded in minutes. The efficacy of the tested compounds was assessed using the formula $[(t_t - t_c)/t_k]\cdot 100\%$, where t_t is the average lifespan of test animals; t_c, the average lifespan of control animals [16].

Acute toxicity was determined using laboratory white mice (18–22 g) of both sexes with a single i.p. injection at doses of 100, 200, 1000, 2000, 3000, 4000, and 5000 mg/kg. During the acute toxicity study, the animals were observed, times of death were estimated, number of dead animals was recorded, and the clinical intoxication condition was noted for 14 d. The acute toxicity parameters were calculated using the Litchfield–Wilcoxon method [17].

***In vitro* experiments** used cell line HEK293 (Russian Collection of Cell Cultures, Institute of Pathology, Russian Academy of Sciences, St. Petersburg) that was cultivated at 37°C with 5% CO_2 in DMEM medium containing fetal bovine serum (10%), L-glutamine (2 mM), and gentamicin sulfate (50 μg/mL). Stock solutions of the compounds (1 M in DMSO) were diluted in complete growth medium immediately before the experiments to final concentrations of active ingredients 1, 10, 100, and 1000 μM. The final DMSO concentration in the growth medium was 0.1%.

Cytotoxic properties of the compounds were studied using the MTT-assay according to the manufacturer's protocol [13].

Antiradical Properties. The intracellular level of AOS in HEK293 cell cytoplasm was measured by the fluorescent probe 2,7-dichlorodihydrofluorescein diacetate (CM-H2DCFDA, Invitrogen, USA) according to the manufacturer's protocol. HEK293 cells were seeded into 96-well plates in DMEM growth medium in order to evaluate the effects of the compounds on the AOS level. After 24 h, they were treated with **2**–**11** (final concentrations 1, 10, and 100 μM). Oxidative stress was induced by adding PMA (Sigma, USA) to a final concentration of 1 μM. Measurements were made on a 2300 EnSpire® Multimode Plate Reader (PerkinElmer, USA) at 488 and 530 nm.

Quantitative assessment and comparison of the abilities of the 12-*N*-methylcytisine derivatives **2**–**11** to regulate TF HIF-1 activity used a luciferase reporter construct containing the HIF-1 binding site [18]. Cells were incubated in the presence of the compounds (final concentrations 1, 10, 100, and 1000 μM) for 24 h. Cells treated with solvent (DMSO, 0.1%) served as the controls. Luciferase activity in cell lysates was detected using a Dual-Luciferase Reporter Assay System kit and a 2300 EnSpire® Multimode Plate Reader.

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