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SYNTHESIS AND BIOLOGICAL ACTIVITY OF *N*-BENZYL DERIVATIVES OF CYTISINE

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The reductive alkylation of cytisine by various aromatic aldehydes was studied. Preliminary pharmacological investigations of the synthesized compounds were performed.

Key words: cytisine, substituted benzaldehydes, reductive alkylation, *N*-benzylcytisine derivatives, acute toxicity, effect on H-choline receptors, hemostatic properties, spasmolytic activity.

More than 2000 derivatives of cytisine are known today. However, the reaction of cytisine with substituted benzaldehydes has not been reported.

It seemed interesting to investigate the reductive alkylation of cytisine (**1**), which contains a secondary N atom, with aromatic aldehydes.

Secondary amines are known to react with aliphatic aldehydes in a 1:2 mole ratio to form geminal diamines that give enamines during distillation [1]. The condensing agent is K_2CO_3.

Nucleophiles add slowly to aromatic aldehydes because the aromatic ring and electron-donating substitutents increase the electron density on the carbonyl and, as a rule, the reaction occurs under general base- or acid-catalysis conditions [1].

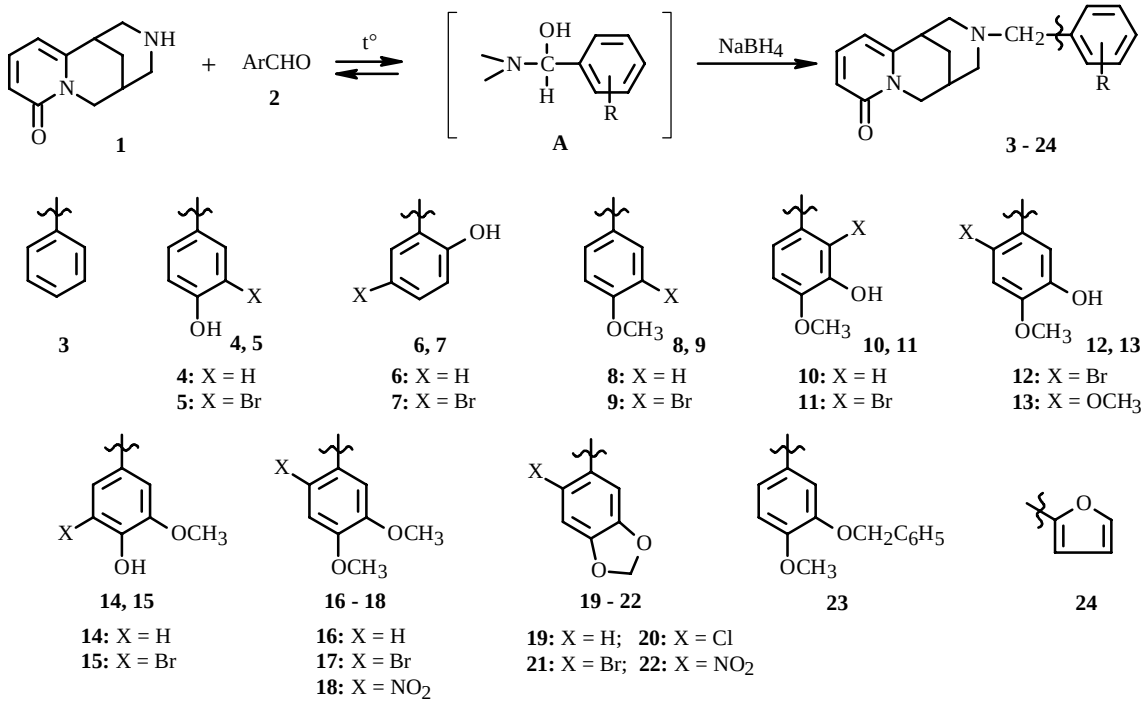

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TABLE 1. Physicochemical Properties of **3**-**24**

Compound	Formula	mp, °C	Yield, %
3	$C_{18}H_{20}N_2O$	150-151	80
4	$C_{18}H_{20}N_2O_2$	225-226	50
5	$C_{18}H_{19}N_2O_2Br$	145-146	56
6	$C_{18}H_{20}N_2O_2$	115-116	70
7	$C_{18}H_{19}N_2O_2Br$	191-192	42
8	$C_{19}H_{22}N_2O_2$	259-260	46
9	$C_{19}H_{21}N_2O_2Br$	amorph.	58
10	$C_{19}H_{22}N_2O_3$	150-151	46
11	$C_{19}H_{21}N_2O_3Br$	235-236	86
12	$C_{19}H_{21}N_2O_3Br$	180-181	74
13	$C_{20}H_{24}N_2O_4$	140-141	39
14	$C_{19}H_{22}N_2O_3$	180-183	61
15	$C_{19}H_{21}N_2O_3Br$	200-201	65
16	$C_{20}H_{24}N_2O_3$	amorph.	40
17	$C_{20}H_{23}N_2O_3Br$	170-171	65
18	$C_{20}H_{23}N_3O_5$	150-151	80
19	$C_{19}H_{20}N_2O_3$ HCl	275-276	36
20	$C_{19}H_{19}N_2O_3Cl$	amorph.	47
21	$C_{18}H_{19}N_2O_3Br$	amorph.	49
22	$C_{19}H_{19}N_3O_5$	amorph.	24
23	$C_{25}H_{28}N_2O_3$	139-140	60
24	$C_{16}H_{18}N_2O_2$	213-215	50

We reacted various aldehydes **2** with **1**. The reactions were carried out in benzene with boiling. In the first step, geminal α-hydroxyamines (**A**) were formed that, as a rule, were unstable. This reaction was reversible. The course of the reaction was monitored using TLC. For most aldehydes, the product **A** was soluble in benzene (toluene). Only for 2-bromoisovanillin did the product precipitate, thereby making the process irreversible. In the second step, **A** was reduced without isolation by $NaBH_4$, which gave the expected *N*-benzyl derivatives of cytisine (**3**-**24**).

The yields of **3**-**24** in the reductive amination depended on the nature of the substituent on the aryl ring. Hydroxyls and methoxyls gave products in yields of 38-61%. The product **6** from reaction of **1** and salicylaldehyde was obtained in 70% yield. The *o*-hydroxyl group may have had an effect. As a rule, introducing Br, Cl, and NO_2 groups increased the yield. The exceptions were **7** and **22**. As expected, good yields were obtained with benzaldehyde (**3**, 80%), 6-nitroveratraldehyde (**18**, 80%), 2-bromoisovanillin (**11**, 86%), and 6-bromoisovanillin (**12**, 74%). Replacing OH and OCH_3 by a methylenedioxy decreased the yields to 24-49% (**19**-**22**) (Table 1).

The structures of the prepared compounds were confirmed by PMR spectra. Chemical shifts of the cytisine protons depended little on the nature of the substitution in the benzene ring (see Experimental).

The high analeptic activity of cytisine is well known.

Before investigating **3**-**24** for certain types of pharmacological activity, we used a computer prediction of the biological activity, PASS, performed at the V. N. Orekhovich Institute of Biomedicinal Chemistry (Moscow).

According to the PASS system, a broad spectrum of various pharmacological activities was predicted for the synthesized cytisine derivatives **3**-**24**. These included cholinomimetic, cholinolytic, spasmolytic, sedative, nootropic, pressor, hypotensive, etc.

Herein results of preliminary pharmacological investigations of the 17 *N*-benzyl cytisine derivatives are reported. These include acute toxicity of the compounds in white mice with ip administration, the effect on H-choline receptors of isolated frog stomach sphincter, and spasmolytic activity and hemostatic properties. Therefore, Table 2 lists the probability (Pa, from 0 to 1) of finding three types of activity among all those predicted by the PASS program: H-choline-stimulating, H-cholinolytic, and smasmolytic (papaverine-like).

TABLE 2. Acute Toxicity, H-Cholinolytic (on Isolated Frog Stomach Sphincter) and Spasmolytic (Rat Ileum) Activity of **3-24**

Compound	PASS data, Pa		LD_{50}, mg/kg, ip, white mice	Activity, EC_{50}, g/mL		
				cholinotropic		spasmolytic
	1	2		stimulation	blockade	
3	0.766	0.623	162 (140÷180)	-	$2.5 \cdot 10^{-6}$	$6 \cdot 10^{-6}$
4	0.692	0.581	112 (97÷128)	$5 \cdot 10^{-6}$	$5 \cdot 10^{-5}$	$7 \cdot 10^{-6}$
5	0.623	0.526				$3 \cdot 10^{-6}$
6	0.682	0.603	102 (91÷115)	$6 \cdot 10^{-5}$	-	$1.3 \cdot 10^{-5}$
7	0.612	0.533	162 (140÷180)	-	$3.3 \cdot 10^{-6}$	$6 \cdot 10^{-6}$
8	0.675	0.599	120 (90÷160)	-	$4 \cdot 10^{-5}$	$8 \cdot 10^{-6}$
10	0.627	0.546	224 (200÷250)	-	$2.5 \cdot 10^{-6}$	$1 \cdot 10^{-5}$
11	0.589	0.791	350 (270÷450)	-	$5 \cdot 10^{-5}$	$2 \cdot 10^{-6}$
12	0.640	0.862*	286 (220÷360)	10^{-6}	$1.5 \cdot 10^{-5}$	$4 \cdot 10^{-6}$
14	0.627	0.546	180 (140÷230)	$2 \cdot 10^{-6}$	-	$7 \cdot 10^{-6}$
15	0.574	0.522	224 (180÷250)	-	$1.3 \cdot 10^{-6}$	$5 \cdot 10^{-6}$
16	0.665	0.603	171 (150÷200)	-	$4 \cdot 10^{-6}$	$3 \cdot 10^{-5}$
17	0.676	0.880*				$2 \cdot 10^{-6}$
19	0.643	0.406	129 (110÷150)	-	$7 \cdot 10^{-6}$	$7 \cdot 10^{-6}$
21	0.661	0.823*				$3 \cdot 10^{-6}$
23	0.623	0.593	129 (110÷150)	-	$4 \cdot 10^{-6}$	$2 \cdot 10^{-6}$
24	0.658	0.408	282 (200÷390)	10^{-6}	$5 \cdot 10^{-6}$	$8 \cdot 10^{-6}$
Carbacholine	-	-	-	$3 \cdot 10^{-7}$		-
Tubocurarine	-	-	-	-	$6 \cdot 10^{-7}$	
Papaverine	-	-	-	-		$3 \cdot 10^{-6}$

Pa is the probability of activity (from 0 to 1); 1, nicotine receptor agonists; 2, spasmolytic activity (bladder); *predicted spasmolytic and papaverine-like activity (Pa from 0.426 to 0.480).

According to the results (Table 2), the synthesized compounds are moderately toxic. These compounds can be divided according to toxicity into CNS stimulating as tremors and cramps (**4**, **12**, **14**) and sedating in combination with general muscle relaxation (**7**, **8**, **15**, **16**, **23**, **24**). With respect to the effect on stomach muscle, some of the compounds (**4**, **12**, **14**, **24**), like carbacholine, elicited contraction, i.e., were H-choline-receptor agonists. Their activity reached 10-33% of carbacholine acitivty. The other part of the compounds relieved contraction induced by carbacholine, i.e., had H-cholinolytic activity. With respect to H-cholinolytic activity, the synthesized compounds can be placed in the following order: **15** > **10** ≥ **3** > **7** > **23** ≥ **16** > **24** > **19** > **12** > **8** > **11** ≥ **4**, where their activity varies from 1 to 30% of the tubocurarine activity.

Thus, structurally similar cytisine derivatives had various activities for H-choline-receptors of isolated frog stomach sphincter. Some of them stimulated H-choline receptors and reached 33% of the carbacholine activity. The others blocked them and reached 33% of the tubocurarine activity.

The high spasmolytic activity of **11**, **12**, **17**, and **21** agrees well with the PASS data (Table 2), despite the fact that the PASS data are given for the bladder and our results were obtained for rat ileum. The experimental activities of **5** and **23** were significantly greater than that predicted.

It is known from the literature that the production and functioning of thrombocytes and the hemostasis system can be activated or suppressed depending on the stimulation or blocking of H-choline receptors [2].

The effect of the cytisine derivatives on the blood clotting system was studied in *in vitro* experiments. The time for blood clotting, the circulation time, and the amount of blood loss were studied by thromboelastography and *in vivo* in rats [3].

Table 3 lists the results of the *in vitro* experiments and shows that the cytisine derivatives containing methoxyls possessed mainly distinct hemostatic properties whereas those with one phenol hydroxyl decreased blood clotting. The presence of both substituents produced hypo- or hypercoagulation that was dependent on the dose.

TABLE 3. Effect of Cytisine Derivatives on Hemostasis *in vitro* and *in vivo* (M ± m; n = 6)

Compound	*in vitro*, 0.001 mg/mL						*in vivo*, dose 1 mg/kg, ip	
	A	thromboeleastogram parameters, mm					B	C
		R	K	R+K	MA	Ci		
K	247±22	56±4	35±2	92±6	36±2	0.4±0.02	285±32	300±27
3	180±18	57±5	27±3	85±6	36±2	0.42±0.03	205±20	200±19
4	140±15	60±5	31±3	91±8	30±2	0.3±0.02	220±22	33±32
6	150±16	20±2*	27±2*	52±4*	36±2	0.69±0.04	282±23	315±31
7	87±8.5*	20±2*	80±6	100±9	25±1	0.25±0.02	100±8.9*	15±1.2*
8	96±10*	35±3*	29±3*	64±5*	40±2	0.63±0.05	108±11*	75±8*
10	145±15	57±4	32±3	89±7	37±2	0.42±0.03	245±20	200±18
11	130±13	30±2*	25±2*	55±4*	36±2	0.66±0.05	195±13.7	100±9*
12	180±18	52±4	33±3	85±6	33±2	0.39±0.02	300±27	250±20
14	140±14	50±4	33±3	83±7	36±2	0.43±0.03	255±20	289±22
15	70±7*	57±3	48±3	105±9	29±2	0.28±0.02	76±7.0*	20±2.5
16	105±10	28±2*	22±2*	50±4*	37±2	0.74±0.05	120±9.5*	48±5*
19	160±16	32±3*	29±2*	62±5*	37±2	0.6±0.05*	270±21	280±23
23	95±10*	25±2*	22±2*	48±3*	36±2	0.76±0.04	115±11*	86±8.6*

*P ′≤ 0.01 relative to the control; K, control; A, blood clotting time, s; B, circulation time, s; C, blood loss, mg.

The most active hemostatics among the studied compounds were **7**, **8**, **15**, **16**, and **23**, which possessed H-cholinolytic activity. In *in vitro* experiments at concentrations of 0.1, 0.01, and 0.001 mg/mL, they decreased the blood clotting time by 58-73% [2, 4]. We observed in thromboelastograms a decrease of the reaction time (R) by 53-60%; of the clot formation time (K), by 20-57%; and an increase of MA from 25 ± 1 to 40 ± 2 mm and of the hypercoagulation index Ci, by 75-117%. The data indicate that the studied derivatives influence the I and II phases of blood clotting, accelerating thromboplastin formation and, probably, activating the functional activity of the thrombocytes. The vascular-thrombocytic mechanism of action of these derivatives was confirmed in *in vivo* experiments where the circulation time and amount of blood loss decreased by 58-73% [5].

On the other hand, H-cholinomimetics suppress thrombocytopoeia and elicit hypocoagulation. Only **4** of the H-cholinomimetics studied by us exhibited mild hypocoagulation. Thus, the R+K index increased by 20%. The hypercoagulation index Ci decreased by 25%.

The most promising compounds as hemostatic agents were **7**, **8**, **15**, **16**, and **23**; as anticoagulants, **4**.

EXPERIMENTAL

General Comments. Column chromatography used KSK silica gel; TLC, silica gel of the same grade and $CHCl_3$:CH_3OH (4:1). The developer was iodine and Dragendorff's reagent.

PMR spectra were recorded on a Tesla BS-567A spectrometer at working frequency 100 MHz in DMSO-d_6, $CDCl_3$, and C_5D_5N-d_5.

Condensation of Cytisine and Aromatic Aldehydes. A mixture of cytisine (0.01 mol) and the appropriate aldehyde (0.012 mol) in benzene (30 mL) was refluxed for 4-6 h. The solvent was distilled off. The solid was treated with CH_3OH (20 mL) and reduced with $NaBH_4$ (5 g) with cooling over 1 h. The solvent was distilled off. The solid was dissolved in water (20 mL) and extracted exhaustively with $CHCl_3$. The crude product was purified over a silica-gel column using $CHCl_3$:CH_3OH (100:0-100:10). The products were crystallized from acetone or methanol.

***N*-Benzylcytisine (3).** Prepared from cytisine (1.9 g) and benzaldehyde (1.2 g), R_f 0.78.

PMR spectrum (CCl_4, δ, ppm, J/Hz): 7.15 (1H, dd, J = 9, 7, H-4), 7.10-6.94 (5H, m, Ar-H), 6.25 (1H, d, J = 9, H-3), 5.59 (1H, d, J = 7, H-5), 3.88 (1H, d, J = 15.7, H_{eq}-10), 3.62 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.38 (2H, s, N–CH_2), 2.88 (1H, m, H-7), 2.80 (1H, d, J = 13, H_{eq}-11), 2.71 (1H, d, J = 13, H_{eq}-13), 2.22 (1H, m, H-9), 2.20-2.12 (2H, d, H_{ax}-11, H_{ax}-13), 1.69 (2H, m, H-8).

***N*-(4-Hydroxybenzyl)cytisine (4).** Prepared from cytisine (1.9 g) and *p*-hydroxybenzaldehyde (1.4 g), R_f 0.6.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 7.06 (1H, dd, J = 6.8, 9, H-4), 6.40 (4H, br.s, H-2′, H-3′, H-5′, H-6′), 6.41 (1H, dd, J = 9, 1.6, H-3), 5.70 (1H, dd, J = 6.8, 1.6, H-5), 4.09 (1H, d, J = 15.7, H_{eq}-10), 3.74 (1H, ddd, J = 15.7, 6.4, 1.0, H_{ax}-10), 3.16 (2H, s, N–CH_2), 2.73, 2.61 (3H each, H-7, H_{eq}-11, H_{eq}-13), 2.03 (2H, H_{ax}-11, H_{ax}-13), 1.93 (1H, m, H-9), 1.40 (2H, m, H-8).

***N*-(3-Bromo-4-hydroxybenzyl)cytisine (5).** Prepared from cytisine (1.9 g) and 3-bromo-4-hydroxybenzaldehyde (2.1 g) [6], R_f 0.78.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 9.90 (1H, s, OH-4′), 7.26 (1H, dd, J = 9, 7, H-4), 6.99 (1H, d, J = 5.3, H-5′), 6.71 (2H, s, H-6′, H-2′), 6.18 (1H, dd, J = 9, 1.4, H-3), 5.94 (1H, dd, J = 7, 1.4, H-5), 3.81 (1H, d, J = 15.7, H_{eq}-10), 3.69 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.26 (2H, d, J = 5, N–CH_2), 2.90 (1H, m, H-7), 2.75 (1H, d, J = 12, H_{eq}-11), 2.59 (1H, d, J = 13, H_{eq}-13), 2.29 (1H, m, H-9), 2.19 (1H, d, J = 12, H_{ax}-13), 2.08 (1H, d, J = 13, H_{ax}-11), 1.70 (1H, m, H-8).

***N*-(2-Hydroxybenzyl)cytisine (6).** Prepared analogously from cytisine (1.9 g) and salicylaldehyde (1.18 g), R_f 0.6.

Mass spectrum (*m*/*z*, I_{rel}, %): 296 (10) $[M]^+$, 279 (11), 233 (11), 232 (48), 218 (50), 217 (100), 191 (10), 190 (45), 167 (21), 158 (41), 146 (57), 145 (81), 145 (98), 122 (88), 107 (37), 95 (21), 94 (28), 92 (33), 86 (87), 69 (64).

PMR spectrum (300 MHz, DMSO-d_6 + CCl_4, δ, ppm, J/Hz): 8.96 (1H, s, OH-4′), 7.22 (1H, dd, J = 9, 6.8, H-4), 6.95* (1H, dd, J = 8, 1.2, H-6′), 6.81* (1H, d, J = 8, H-3′), 6.60 (2H, dd, J = 8, 1.2, H-4′, H-5′), 6.24 (1H, d, J = 9, H-3), 5.91 (1H, d, J = 6.8, H-5), 3.85 (1H, d, J = 15.7, H_{eq}-10), 3.71 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.56 (2H, s, N–CH_2), 3.06** (1H, d, J = 13, H_{eq}-13), 3.00 (1H, s, H-7), 2.95** (1H, d, H_{eq}-11), 2.49 (H-9), 2.37, 2.35 (1H each, d, J = 13, H_{ax}-11, H_{ax}-13), 1.85 (2H, m, H-8).

***N*-(2-Hydroxy-5-bromobenzyl)cytisine (7).** Prepared from cytisine (1.9 g) and 5-bromosalicylaldehyde (2.1 g) [7], R_f 0.76.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 7.14 (1H, dd, J = 9, 7, H-4), 7.08 (1H, s, H-6′), 7.06 (1H, d, J = 8, H-3′), 6.65 (1H, d, J = 8, H-4′), 6.50 (1H, dd, J = 9, 1.4, H-3), 5.75 (1H, dd, J = 7, 1.4, H-4), 4.16 (1H, d, J = 15.7, H_{eq}-10), 3.76 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.36 (2H, d, J = 5, N–CH_2), 2.77 (1H, m, H-7), 2.69 (2H, m, H_{eq}-11, H_{eq}-13), 2.02-2.13 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.45 (1H, m, H-8).

***N*-(4-Methoxybenzyl)cytisine (8).** Synthesized from cytisine (1.73 g) and anisaldehyde (1.3 g), R_f 0.8.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.26 (1H, dd, J = 9, 6.8, H-4), 6.85* (2H, d, J = 8.2, H-2′, H-6′), 6.69* (2H, d, J = 8.3, H-3′, H-5′), 6.18 (1H, d, J = 9, H-3), 5.96 (1H, d, J = 6.8, H-5), 3.63 (3H, s, OCH_3-4′), 3.65 (2H, m, 2H-10), 3.28 (2H, s, N–CH_2), 2.91 (1H, m, H-7), 2.74 (1H, m, H_{eq}-11), 2.65 (1H, m, H_{eq}-13), 2.44 (1H, m, H_{ax}-13), 2.23 (1H, m, H-9), 2.12 (1H, m, H_{ax}-11), 1.68 (2H, m, H-8).

***N*-(3-Bromo-4-methoxybenzyl)cytisine (9).** Prepared from cytisine (2 g) and 3-bromo-4-methoxybenzaldehyde (2.26 g) [6], R_f 0.76.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.23 (1H, dd, J = 9, 7, H-4), 7.02 (1H, s, H-5′), 6.87 (1H, s, H-2′), 6.76 (1H, d, J = 8, H-6′), 6.19 (1H, d, J = 9, H-3), 5.93 (1H, d, J = 7, H-5), 4.36 (2H, d, J = 15.7, H_{eq}-10, H_{ax}-10), 3.67 (3H, s, OCH_3-4′), 3.26 (2H, s, NCH_2), 2.89, 2.76, 2.62 (3H, m, H-7, H_{eq}-11, H_{eq}-13), 2.29, 2.19, 2.10 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.69 (2H, m, H-8).

***N*-(3-Hydroxy-4-methoxybenzyl)cytisine (10).** Prepared from cytisine (1.9 g) and isovanillin (1.7 g) [8], R_f 0.76.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 7.09 (1H, d, J = 8, H-6′), 7.07 (1H, dd, J = 9, 7, H-4), 6.82 (1H, br.s, H-2′), 6.70 (1H, d, J = 8, H-5′), 6.46 (1H, d, J = 9, H-3), 5.69 (1H, d, J = 6.8, H-5), 4.13 (1H, d, J = 15.7, H_{eq}-10), 3.76 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.55 (3H, d, J = 1, OCH_3-4′), 3.17 (2H, s, N–CH_2), 2.62 (3H, m, H-7, H_{eq}-11, H_{eq}-13), 2.00 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.42 (2H, m, H-8).

***N*-(3-Hydroxy-4-methoxy-2-bromobenzyl)cytisine (11).** Prepared from cytisine (1.9 g) and 2-bromoisovanillin (2.6 g) [9], R_f 0.8.

Mass spectrum (*m*/*z*, I_{rel}, %): 405 (8) $[M]^+$, 325 (22), 214 (33), 190 (12), 189 (32), 158 (12), 146 (16), 145 (29), 137 (100), 95 (13), 93 (16), 84 (20), 69 (28).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.25 (1H, dd, J = 8.9, 6.8, H-4), 6.67 (1H, d, J = 8.4, H-2′), 6.63 (1H, d, J = 8.4, H-3′), 6.16 (1H, dd, J = 8.9, 1.4, H-3), 5.96 (1H, dd, J = 6.8, 1.4, H-5), 3.70 (5H, br.s, 2H-10, OCH_3-4′), 3.29 (2H, s, N–CH_2), 2.86 (1H, m, H-7), 2.76, 2.70 (1H each, m, H_{eq}-11, H_{eq}-13), 2.31, 2.21 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.66 (2H, m, H-8).

***N*-(3-Hydroxy-4-methoxy-6-bromobenzyl)cytisine (12).** Prepared from cytisine (1.9 g) and 3-hydroxy-4-methoxy-6-bromobenzaldehyde (2.6 g) [10], R_f 0.63.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 8.94 (1H, s, OH-3′), 7.22 (1H, dd, J = 9, 7, H-4), 6.92 (1H, s, H-5′), 6.44 (1H, s, H-2′), 6.14 (1H, dd, J = 9, 1.4, H-3), 5.94 (1H, dd, J = 1.4, 7, H-5), 3.67 (4H, br.s, 2H-10, N–CH_2), 2.38 (3H, OCH_3-4′), 2.92, 2.77-2.69 (3H, H-7, H_{eq}-11, H_{eq}-13), 2.20-2.28 (3H, H-9, H_{ax}-11, H_{ax}-13), 1.68 (2H, m, H-8).

Preparation of 6-Methoxyisovanillin. Sodium methoxide (70 mL absolute CH_3OH, 4.5 g metallic Na) was treated with freshly dried $CuCl_2$ (anhydrous, 0.75 g), 6-bromoisovanillin (13 g), and dry DMF (56 mL). The reddish-brown suspension was stirred at room temperature and treated dropwise with HCl solution (31 mL, 20%) until the pH was 2-3. When the pH of the suspension reached 7, it became a dark red solution. The resulting solution was extracted with $CHCl_3$ (3 × 70 mL). The $CHCl_3$ extract was washed with KOH (4%, 50 mL, 5 × 25 mL). The alkaline solution was acidified with conc. HCl (with cooling). The resulting yellow precipitate was filtered off to afford a mixture of three compounds (7.02 g). The mother liquor was extracted with ether and $CHCl_3$. Recrystallization from benzene produced the compound (5 g), mp 139-141°C.

***N*-(3-Hydroxy-4,6-dimethoxybenzyl)cytisine (13).** Prepared from cytisine (1.7 g) and 6-methoxyisovanillin (1.8 g), R_f 0.5.

Mass spectrum (*m*/*z*, I_{rel}, %): 355 (6) $[M]^+$, 320 (17), 319 (77), 288 (9), 231 (13), 217 (24), 190 (69), 168 (22), 167 (56), 160 (36), 146 (50), 145 (83), 145 (100), 117 (32), 96 (12), 95 (14), 88 (24).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 8.94 (1H, s, OH-3′), 7.22 (1H, dd, J = 9, 7, H-4), 6.92 (1H, s, H-5′), 6.44 (1H, s, H-2′), 6.13 (1H, dd, J = 9, 1.2, H-3), 5.95 (1H, dd, J = 7, 1.2, H-5), 3.68 (4H, br.s, 2H-10, N–CH_2), 3.27 (6H, s, OCH_3-4′, OCH_3-6′), 2.69-2.93 (3H, m, H-7, H_{eq}-11, H_{eq}-13), 2.28-2.44 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.69 (2H, m, H-8).

***N*-(3-Methoxy-4-hydroxybenzyl)cytisine (14).** Prepared from cytisine (1.9 g) and vanillin (1.7 g), R_f 0.76.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 8.64 (1H, s, OH), 7.28 (1H, dd, J = 9, 7, H-7), 6.56 (1H, d, J = 8, H-5), 6.42 (1H, s, H-2′), 6.27 (1H, dd, J = 8, 1.2, H-6′), 6.17 (1H, dd, J = 9, 1.4, H-3), 5.96 (1H, dd, J = 7, 1.4, H-5), 3.84, 3.64 (2H, dd, J = 15.7, 6.4, 2H-10), 3.50 (3H, s, OCH_3-3′), 3.29 (2H, s, N–CH_2), 2.90 (1H, m, H-7), 2.76 (1H, m, H_{eq}-11), 2.65 (1H, m, H_{eq}-13), 2.22 (2H, m, H-9, H_{ax}-13), 2.10 (1H, m, H_{ax}-11), 1.68 (1H, m, H-8).

***N*-(3-Methoxy-4-hydroxy-5-bromobenzyl)cytisine (15).** Prepared from cytisine (1.9 g) and 5-bromovanillin (2.6 g) [11], R_f 0.6.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 9.4 (1H, s, OH-4′), 7.27 (1H, dd, J = 9, 7, H-4), 6.65* (1H, d, J = 1.2, H-2′), 6.45* (1H, d, J = 1.2, H-6′), 6.16 (1H, dd, J = 1.2, 9, H-3), 5.97 (1H, dd, J = 7, 1.2, H-5), 3.87 (1H, d, J = 15.7, H_{eq}-10), 3.71 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.56 (3H, s, OCH_3-3′), 3.29 (2H, s, N–CH_2), 2.93 (1H, m, H-7), 2.76, 2.64 (1H each, m, H_{eq}-11, H_{eq}-13), 2.28, 2.21, 2.11 (3H, m, H-9, H_{ax}-11, H_{ax}-13).

***N*-(3,4-Dimethoxybenzyl)cytisine (16).** Prepared from cytisine (1.9 g) and 3,4-dimethoxybenzaldehyde (1.8 g), R_f 0.8.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.26 (1H, dd, J = 8.9, 6.8, H-4), 6.83 (1H, d, J = 7, H-5′), 6.67 (1H, s, H-2′), 6.49 (1H, dd, J = 7, 1.9, H-6′), 6.18 (1H, dd, J = 1.5, 8.9, H-3), 5.97 (1H, dd, J = 1.5, 6.8, H-5), 3.70 (1H, d, J = 15.6, H_{eq}-10), 3.63 (1H, dd, J = 15.6, 6.4, H_{ax}-10), 3.67 (3H, s, OCH_3), 3.63 (3H, s, OCH_3), 3.49 (2H, s, N–CH_2), 2.75, 2.67 (1H each, m, H_{eq}-11, H_{eq}-13), 2.92 (1H, H-7), 2.12 (2H, m, H-9, H_{ax}-11, H_{ax}-13), 1.68 (2H, m, H-8).

***N*-(3,4-Dimethoxy-6-bromobenzyl)cytisine (17).** Prepared from cytisine (1.9 g) and 3,4-dimethoxy-6-bromobenzaldehyde (2.6 g) [6], R_f 0.33.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 7.11 (1H, dd, J = 9, 7, H-4), 7.02 (1H, s, H-5′), 6.66 (1H, s, H-2′), 6.45 (1H, dd, J = 9, 1.2, H-3), 5.77 (1H, dd, J = 7, 1.2, H-5), 4.12 (1H, d, J = 15.7, H_{eq}-10), 3.76 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.58 (6H, s, OCH_3-3′, OCH_3-4′), 3.38 (2H, s, N–CH_2), 2.62-2.71 (1H, m, H-7, H_{eq}-11, H_{eq}-13), 2.00-2.18 (1H, m, H-9, H_{ax}-11, H_{ax}-13), 1.45 (2H, m, H-8).

***N*-(3,4-Dimethoxy-6-nitrobenzyl)cytisine (18).** Prepared from cytisine (1.5 g) and 3,4-dimethoxy-6-nitrobenzaldehyde (1.64 g) [12], R_f 0.57.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 7.52 (1H, s, H-5′), 7.14 (1H, dd, J = 9, 6.8, H-4), 6.80 (1H, s, H-2′), 6.44 (1H, dd, J = 9, 1.6, H-3), 5.78 (1H, dd, J = 7, 1.6, H-5), 4.13 (1H, d, J = 15.6, H_{eq}-10), 3.81 (1H, dd, J = 15.6, 6.4, H_{ax}-10), 3.65 (3H, s, OCH_3-3′ or OCH_3-4′), 3.62 (5H, s, OCH_3-4′ or OCH_3-3′ and N–CH_2), 2.66 (3H, m, H-7, H_{eq}-11, H_{eq}-13), 2.09 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.42 (2H, br.s, H-8).

***N*-(3,4-Methylenedioxybenzyl)cytisine (19).** Prepared from cytisine (1.9 g) and 3,4-methylenedioxybenzaldehyde (1.7 g), R_f 0.85.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 7.16 (1H, dd, J = 9, 7, H-4), 6.64 (1H, d, J = 8, H-5′), 6.47 (1H, d, J = 8, H-6′), 6.50 (1H, s, H-2′), 6.44 (1H, dd, J = 9, 1.2, H-3), 5.77 (2H, s, OCH_2O), 5.74 (1H, dd, J = 7, 1.2, H-5), 4.12 (1H, d, J = 15.7, H_{eq}-10), 3.77 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.14 (2H, s, N–CH_2), 2.70 (1H, m, H-7), 2.63 (2H, br.s, H_{eq}-11, H_{eq}-13), 2.08 (1H, d, J = 13, H_{ax}-13), 2.01 (1H, d, J = 13, H_{ax}-11), 1.90 (1H, m, H-9), 1.42 (2H, m, H-8).

***N*-(3,4-Methylenedioxy-6-chlorobenzyl)cytisine (20).** Prepared from cytisine (1.9 g) and 3,4-methylenedioxy-6-chlorobenzaldehyde (2.0 g) [13], R_f 0.6.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.25 (1H, dd, J = 9, 7, H-4), 6.87 (1H, s, H-5′), 6.34 (1H, s, H-2′), 6.19 (1H, dd, J = 9, 1.4, H-3), 5.93 (2H, s, OCH_2O), 5.95 (1H, dd, J = 7, 1.4, H-5), 3.82 (1H, d, J = 15.7, H_{eq}-10), 3.60 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.30 (2H, s, N–CH_2), 2.93 (1H, m, H-7), 2.97 (1H, d, J = 13, H_{eq}-11), 2.65 (1H, d, J = 13, H_{eq}-13), 2.20-2.37 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.72 (2H, m, H-8).

***N*-(3,4-Methylenedioxy-6-bromobenzyl)cytisine (21).** Synthesized from cytisine (1.9 g) and 3,4-methylenedioxy-6-bromobenzaldehyde (2.5 g) [6], R_f 0.8.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.25 (1H, dd, J = 9, 7, H-4), 7.00 (1H, s, H-5′), 6.34 (1H, s, H-2′), 6.18 (1H, dd, J = 9, 1.4, H-3), 5.95 (1H, dd, J = 7, 1.4, H-5), 5.93 (2H, s, OCH_2O), 3.81 (1H, d, J = 15.6, H_{eq}-10), 3.55 (1H, dd, J = 15.6, 6.4, H_{ax}-10), 3,27 (2H, d, J = 5, N–CH_2), 2.91 (1H, m, H-7), 2.75 (1H, br.s, H_{eq}-11), 2.63 (1H, br.s, H_{eq}-13), 2.20-2.35 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.71 (2H, m, H-8).

***N*-(3,4-Methylenedioxy-6-nitrobenzyl)cytisine (22).** Prepared from cytisine (1.7 g) and 3,4-methylenedioxy-6-nitrobenzaldehyde (1.9 g) [14], R_f 0.7.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.26 (1H, dd, J = 9, 7, H-4), 6.62 (1H, s, H-5′), 6.38 (1H, s, H-2′), 6.19 (1H, dd, J = 9, 1.4, H-3), 5.94 (1H, dd, J = 7, 1.4, H-5), 5.86 (2H, s, OCH_2O), 3.81 (1H, d, J = 15.7, H_{eq}-10), 3.61 (1H, dd, J = 15.7, 6.4, H_{ax}-10), 3.24 (2H, s, N–CH_2), 2.90 (1H, m, H-7), 2.75 (1H, d, H_{eq}-11), 2.63 (1H, d, H_{eq}-13), 2.24 (2H, H-9, H_{ax}-13), 2.11 (1H, H_{ax}-11), 1.67 (2H, m, H-8).

***N*-(4-Methoxy-3-benzyloxybenzyl)cytisine (23).** Prepared from cytisine (1.9 g) and 3-benzyloxy-4-methoxybenzaldehyde (2.64 g) [15], R_f 0.7.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 7.34-7.15 (7H, m, H-5′, H-6′), 7.26 (1H, dd, J = 9, 7, H-4), 6.83 (1H, s, H-2′), 6.16 (1H, dd, J = 9, 1.4, H-3), 5.96 (1H, dd, J = 7, 1.4, H-5), 4.35 (2H, d, 2H-10), 3.67 (3H, s, OCH_3), 3.64 (2H, s, OCH_2), 3.36 (2H, s, N–CH_2), 2.69-2.88 (3H, H-7, H_{eq}-11, H_{eq}-13), 2.06-2.19 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.65 (2H, m, H-8).

***N*-Furfurylcytisine (24).** Prepared from cytisine (1.9 g) and furfurol (1.15 g), R_f 0.8.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 7.18 (1H, dd, J = 7, 8.5, H-3′), 7.16 (1H, dd, J = 9, 7, H-4), 6.49* (1H, d, J = 8.5, H-2′), 6.41* (1H, dd, J = 9, 1.4, H-3), 5.88 (1H, d, J = 7, H-4′), 5.81 (1H, dd, J = 7, 1.4, H-5), 4.12 (1H, d, J = 15.7, H_{eq}-10), 3.77 (3H, dd, J = 15.7, 6.4, H_{ax}-10, N–CH_2), 2.5-2.8 (3H, m, H-7, H_{eq}-11, H_{eq}-13), 2.02-2.4 (3H, m, H-9, H_{ax}-11, H_{ax}-13), 1.40 (2H, m, H-8).

Acute toxicity of the studied compounds was determined in white mongrel mice of mass 20 ± 2 g of both sexes with ip injection. The Litchfield and Wilcoxson method was used to determine the acute toxicity parameters [16].

The influence of the synthesized compounds on blood clotting was investigated on a Tromb-2 thromboelastograph. Blood was drained dropwise into a cuvette from a rabbit ear vein. The cuvette was charged beforehand with the preparation at a final concentration of 0.1, 0.01, and 0.001 mg/mL (1:9).

The following parameters were considered on the thromboelastograms: R, blood reaction time, which characterizes phases I and II of blood clotting; K, clot formation time or thromboelastographic constant of thrombin, which depends on the concentration of the thrombin formed and the amount of fibrinogen; R + K, coagulation constant that expresses the overall duration of blood clotting; MA, maximum amplitude, which is influenced by the fibrinogen concentration, the amount and quality of thrombocytes; and Ci, index of hypercoagulation [3].

The effect of the preparations on hemostasis was studied in *in vivo* experiments for the screening model "circulation time and blood-loss value" [5]. Rats of mass 130 ± 10 g were administered once ip preparations at a dose of 1 mg/kg. The circulation time and blood-loss value were studied 60 min after administering the preparations.

The effect of the compounds on H-choline receptors was studied using isolated frog stomach sphincter by the De Elio method [17].

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