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Synthesis and Biological Activity of the Derivatives of 2,4,6,8,10,12-Hexaazatetracyclo[5.5.0.0^{3,11}.0^{5,9}]dodecane

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Abstract

Synthesis of the derivatives of 2,4,6,8,10,12-hexaazatetracyclo[5.5.0.0^{3,11}.0^{5,9}]dodecane is described; their biological activity is investigated.

Key words: derivatives of 2,4,6,8,10,12-hexaazatetracyclo[5.5.00^{3,11}.0^{5,9}]dodecane, anticonvulsive, anti-anxiety activity, mice

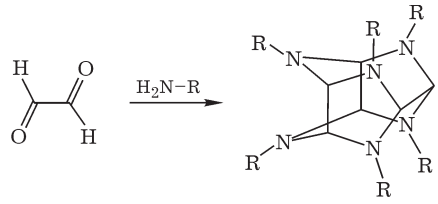
INTRODUCTION

Attention to the derivatives of 2,4,6,8,10,12-hexaazatetracyclo[5.5.0.0^{3,11}.0^{5,9}]dodecane, previously synthesized exclusively for the purpose of developing the technologies of obtaining high-energy compounds [1], is explained by their unusual structure. These compounds are frame-structured nitrogenous heterocycles, which allows one to expect their biological activity. According to the analysis carried out by us preliminarily using the PASS (Prediction of Activity Spectra for Substances, 2007, V. Poroikov, D. Filimonov *et al.*), the derivatives of 2,4,6,8,10,12-hexaazatetracyclo-[5.5.0.0^{3,11}.0^{5,9}]-dodecane may possess diverse biological activity including neurotrophic action. However, no *in vivo* studies that could confirm the predicted diversity of biological action have been carried out previously. In this connection, the goal of the present work was to study the pharmacological properties of the derivatives of 2,4,6,8,10,12- hexaazatetracyclo-[5.5.0.0^{3,11}.0^{5,9}]-dodecane.

EXPERIMENTAL

Chemistry

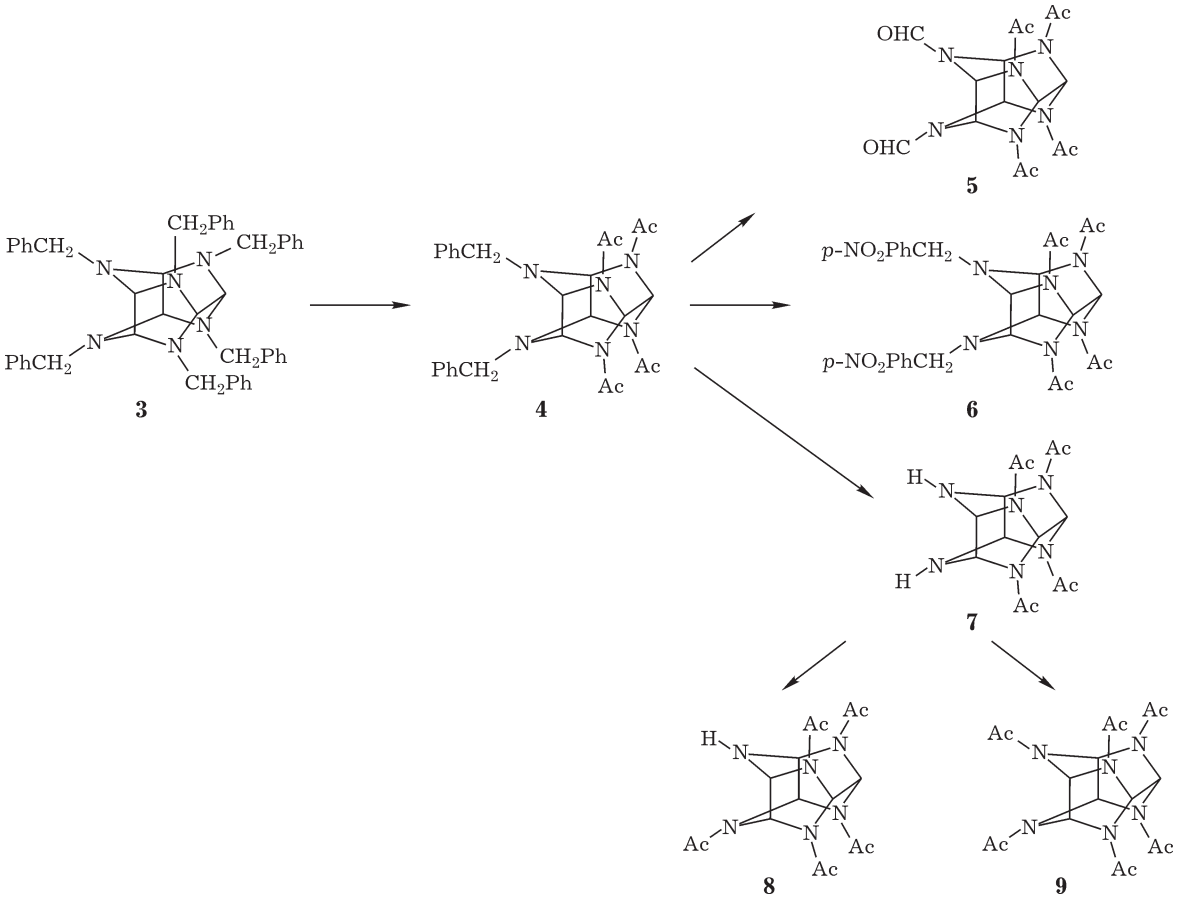
The synthesis of the derivatives of 2,4,6,8,10,12-hexaazatetracyclo-[5.5.0.0^{3,11}.0^{5,9}]-dodecane for the examination of their biological activity was carried out by means of cascade condensation of glyoxal with the corresponding amines:



R = cyanoethyl (**1**), allyl (**2**), benzyl (**3**)

and transformation of the substituents in the heterocyclic ring of the hexabenzyl derivative, in which the benzyl groups are sufficiently mobile (Scheme 1).

2,4,6,8,10,12-Hexa-(2-cyanoethyl)-2,4,6,8,10,12-hexaazatetracyclo[5.5.0.0^{3,11}.0^{5,9}]-dodecane (1). In a three-necked flask equipped



Scheme 1.

with a thermometer and a mixer, we place 7.8 g (0.11 mol) of 3-aminopropionitrile, 78 mL of acetonitrile and 0.1 mL of formic acid. At a temperature of 25 °C, an aqueous 40 % solution of glyoxal in the amount of 7.26 g (0.05 mmol) is added in portions during 30 min into the flask. Then the mixture is kept at the same temperature for 4 h. After exposure, the solvent is evaporated in vacuum, and the residue is treated with 50 % ethanol solution. The precipitate is separated by filtering, washed on the filter with 50 % ethanol solution and dried in the air. The yield of hexa(2-cyanoethyl)hexaazaisowurtzitane is 18 %. M. p. 145–148 °C. ^{1}H NMR spectra, ppm: t-2.64 (CH_2 4H), t-2.70 (CH_2 8H), t-2.84 (CH_2 8H), t-2.91 (CH_2 4H), d-4.45 (CH 4H), d-4.59 (CH 2H); ^{13}C, ppm: 16.75 (CH_2), 17.20 (CH_2), 48.73 (CH_2), 49.45 (CH_2), 62.09 (CH), 64.95 (CH), 119.12 (C).

2,4,6,8,10,12-Hexaallyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,0^{3,11}.0^{5,9}]dodecane (2). In a flask 500 mL in volume, equipped with a mixer, thermometer and a dropping funnel, we place 150 g of acetonitrile, 53 g (0.93 mol) of allyl amine and 2 g of 50 % solution of formic acid. At a temperature of 20 °C, 44.95 g (0.31 mol) of 40 % glyoxal solution is dosed to the mixture. Then the reaction mass is kept at the same temperature for 1 h. After that, the mass is kept at −18 °C for 2 days. The white precipitate is separated by filtering, washed with isopropanol on the filter and dried in the air. The yield is 49 %. M. p. 40–42 °C. ^{1}H NMR spectra, ppm: t-3.42 (CH_2 8H), t-3.53 (CH_2 4H), d-4.28 (CH 4H), d-4.42 (CH 2H), t-5.05 (CH_2 4H), t-5.18 (CH_2 8H), d-5.42 (CH 2H), 5.59 (CH 4H); ^{13}C, ppm: 54.50 (CH_2), 55.22 (CH_2), 66.90 (CH), 69.76 (CH), 117.70 (CH_2), 133.49 (CH), 135.44 (CH).

2,4,6,8,10,12-Hexabenzyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,0^{3,11}.0^{5,9}]dodecane (3). In a flask equipped with a mixture, we place 170 mL of benzyl amine (1.56 mol), 130 mL of distilled water, 1430 mL of acetonitrile and 5.4 mL of 98 % formic acid. Then 94.25 g of 40 % aqueous solution of glyoxal is dosed into

the reaction mixture during 1 h at a temperature not higher than 20 °C. The reaction mixture is kept at room temperature for 17–20 h. The formed crystalline product is separated by filtering and washed with cold acetonitrile on the filter. The yield is 121 g (76 %, calculated for benzyl amine), m. p. 145–150 °C. For purification, the raw product is mixed in 240–250 mL of acetonitrile at6 a temperature of 50 °C for 15–20 min. Then the mass is cooled to the room temperature, filtered, washed with acetonitrile. After drying in the air, we obtain 115–118 g of the product (m. p. 150–153 °C). ^{1}H NMR spectra, ppm: d-3.59 (CH 2H), d-4.03 (CH 4H), t-4.09 (CH_2 8H), t-4.16 (CH_2 4H), d-7.22 (CH-ar 30H); ^{13}C, ppm 56.62, 57.29, 77.53, 80.94, 127.11, 128.51, 128.82, 129.67, 141.34.

4,10-Dibenzyl-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo-[5,5,0,0^{3,11}.0^{5,9}]dodecane (4). At room temperature, the autoclave 1 L in volume equipped with a mixer and a jacket to supply heat carrier is consecutively filled with 80 g of hexabenzylhexaazaisowurtzitane (0.11 mol), 16 g of 6 % palladium on sibunite (humidity 50 %) dehydrated preliminarily with dimethylformamide, 240 mL of dimethylformamide, 1.44 mL of bromobenzene. Then 120 mL of acetic anhydride (1.27 mol) is added under mixing into the mass. Then the autoclave is tightly closed, blown with argon, then with hydrogen. Heat carrier at a temperature of 50 °C is supplied into the jacket. At hydrogen pressure of 4–5 atm in the apparatus, the completeness of hydrogen absorption 12 L (120 %) is achieved within 4–4.5 h. The reaction mass is cooled to room temperature, the formed precipitate is separated by filtering together with the catalyst and washed on the filter with 50–60 mL of ethanol, thus obtaining a mixture of the catalyst and dibenzyltetraacetylhexaazaisowurtzitane. This mixture is extracted with 1 L of boiling chloroform, the catalyst is removed by filtering, the filtrate is evaporated; 46.7 g (yield 80 %) of dibenzyltetraacetylhexaazaisowurtzitane is obtained. M. p. 319–322 °C, ^{1}H NMR spectra, ppm: q-2.03 (CH_3 12H), t-4.07 (CH_2 4H), d-5.43 (CH 4H), d-6.50 (CH 2H), 7.31 (CH-ar 10H); ^{13}C, ppm: 20.73 (CH_3), 22.11 (CH_3), 56.43 (CH_2), 69.68 (CH), 70.59 (CH), 128.05 (CH-ar), 128.67 (CH-ar), 128.93 (CH-ar), 136.74 (C-ar), 168.26 (C).

4,10-Diformyl-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo-[5,5,0,0^{3,11}.0^{5,9}]dodecane (5). A mixture of 46.7 g of dibenzyltetraacetylhexaazaisowurtzitane and 8.5 g of palladium catalyst is slightly dried in the air and placed into the vessel for hydrogenation fixed on a shaker. Then 102 mL of 98 % formic acid is introduced, the vessel is blown through with hydrogen, and shaking starts. The calculated amount of hydrogen (4.5 L) is absorbed within 4–5 days. After hydrogenation is over, the catalyst is removed by filtering, washed with 20 mL of formic acid and 10 mL of water. The filtrate is evaporated dry in a rotary evaporator. The resulting resin is treated with 100 mL of ethyl acetate. The precipitated crystalline product is filtered and dried in the air; 30.5 g of diformyltetraacetylhexaazaisowurtzitane (85 %) is obtained. M. p. 293–296 °C. ^{1}H NMR spectra, ppm: m-1.55–2.30 (CH_3 12H), m-6.06–6.68 (CH 4H), s-7.25 (CH 2H), s-8.34 (CH 2H); ^{13}C ($CHCl_3$-d), δ, ppm: 20.82, 21.75 (CH_3); 60.28, 66.24, 72.13 (CH); 167.68, (CHO); 167.70 (C).

4,10-Di-(*para*-nitrobenzyl-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazaisowurtzitane (6). In a three-necked flask equipped with a mixer and a thermometer, we place 30 mL of 98 % nitric acid, and then the mixture is cooled with liquid nitrogen to the temperature of −33 °C. After that, 6 g of dibenzyltetraacetylhexaazaisowurtzitane is added. The reaction mixture is kept at a temperature of −28...−32 °C for 1 h, and then it is diluted with ice cold water to the volume of 500 mL. The precipitate is washed with ice cold water (decantation), filtered, washed with water on the filter. The precipitate dried in the air is then treated with 40 mL of acetonitrile at a temperature of 40–45 °C. The product is separated by filtering and washed on the filter with 8 mL of acetonitrile. After drying in the air, 3.6 g of 4,10-di-(*para*-nitrobenzyl-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazaisowurtzitane is obtained. After concentrating the filtrate under vacuum, 0.9 g of the product is also isolated. M. p. 262–272 °C (from acetonitrile). Found, %: C 55.45, H 5.16, N 18.39. $C_{28}H_{30}N_8O_8$. Calculated, %: C 55.44, H 4.98, N 18.47. ^{1}H NMR spectra (DMSO d_6), ppm: m-1.82 (CH_3 12H), t-4.08 (CH_2 4H,), d-5.42 (CH 4H), d-6.45 (CH 2H), 7.78 (CH-ar 4H), 8.26 (CH-ar 4H). ^{13}C (ДМСО-d_6, δ, ppm): 20.70, 21.98 (CH_3); 54.14,

54.75 (CH_2); 68.14, 69.75, 70.72, 72.67 (CH); 123.38, 129.36, 129.89 (CH-ar); 146.78, 146.92 (C-ar); 167.04, 167.99 (C).

2,6,8,12-Tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,$0^{3,11}$.$0^{5,9}$]dodecane (7). A mixture of 46.7 g of dibenzyltetraacetylhexaazaisowurtzitane with 8.5 g of palladium catalyst is slightly dried in the air and placed into the vessel for hydrogenation fixed on a shaker. Then 102 mL of acetic acid is added, the vessel is blown through with hydrogen, and shaking starts. The calculated amount of hydrogen (4.5 L) is absorbed within 7–8 days. After hydrogenation is over, the catalyst is separated by filtering, washed with 20 mL of acetic acid and 10 mL of water. The filtrate is evaporated dry using the rotary evaporator. The resulting resin is treated with 100 mL of ethanol. The precipitated crystalline product is separated by filtering and dried in the air; 30.5 g of tetraacetylhexaazaisowurtzitane is obtained. M. p. 360 °C (with decomposition). ^{1}H NMR spectra, ppm: m-1.80–2.14 (CH_3 12H), m-4.02–4.25 (NH 2H), m-5.20–5.30 (CH 4H), m-6.00–6.50 (CH 2H); ^{13}C ($CDCl_3$): 20.43, 25.48 (CH_3); 47.5, 56.2, 61.3, 67.9 (CH); 161.0, 166.0 (C).

2,6,8,10,12-Pentaacetyl-2,4,6,8,10,12-hexaazaisowurtzitane (8). In a three-necked flask equipped with a mixer and a thermometer, we place 6 g of 2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazaisowurtzitane, 90 mL of glacial acetic acid and 60 mL of acetic anhydride. The mixture is kept at a temperature of 55–60 °C for 12 h. Then the reaction mixture is evaporated under vacuum, the residue is treated with 120 mL of ethyl acetate (boiling for 30 min) and kept 12 h under mixing. The precipitate is separated by filtering, washed with ethyl acetate, and dried in the air. The mass of the product was 6.1 g. The product was once more treated with 60 mL of ethyl acetate; 5.6 g of 2,6,8,10,12-pentaacetyl-2,4,6,8,10,12-hexaazaisowurtzitane with the major product content of 98 % is obtained. M. p. 305–306 °C. ^{1}H NMR spectra (DMSO d_6), ppm: q-2.04 (CH_3 12H), q-2.41 (CH_3 3H,), m-6.04-7.10 (CH 6H), 8.31 (CH 1H), 7.78 (CH-ar 4H), 8.26 (CH-ar 4H); ^{13}C (DMSO d_6, δ, ppm): 19.20, 20.0, 20.2, 58.7, 59.5, 64.7, 64.9, 70.5, 159.8, 166.0, 166.5, 168.1.

2,4,6,8,10,12-Hexaacetyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,$0^{3,11}$.$0^{5,9}$]dodecane (9). In a three-necked flask 250 mL in volume, equipped with a mixer and a backflow condenser, we place 10 g (0.03 mol) of 2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo-[5,5,0,$0^{3,11}$.$0^{5,9}$]-dodecane and 100 mL of acetonitrile. Acetyl chloride in the amount of 21 mL (0.3 mol) is added to the suspension, and then the mixture is heated to 50 °C and kept at this temperature for 24 h. The resulting solution is evaporated dry under vacuum. The residue, which is a resin-like mass with dark brown colour, is treated with 50 mL of acetone. The formed precipitate is separated by filtering, washed on the filter with acetone and dried in the air. The raw product is recrystallized from acetonitrile. The yield of the purified product with m. p. 310–311 °C is 70 % of the theoretical value. ^{1}H NMR spectra, ppm: q-2.01 (CH_3 6H), q-2.05 (CH_3 12H), d-5.06 (CH 2H), d-5.67 (CH 2H), d-5.85 (CH 2H); ^{13}C, ppm: 22.67 (CH_3), 22.85 (CH_3), 23.23 (CH_3), 56.17 (CH), 60.77 (CH), 154.73 (C), 156.64 (C). Elemental analysis, calculated, %: C 67.92, H 3.77, N 13.21. Found, %: C 68.12, H 3.01, N 13.69.

Pharmacology

Investigation of the effect of agents on the central nervous system (CNS) was performed with white outbred mice with body mass 20–25 g using standard screening tests [2]. All the compounds under examination were dissolved in water with Tween-80 and introduced once intragastrically in the dose of 10 mg/kg (0.2 mL per 10 g of the body mass). Tests of the pharmacological activity were performed 1 h after the introduction of the agents. The animals of the reference group obtained equivalent volumes of the solvent.

Investigation of the effect of the agents on the motor and emotional activity of the animals was performed in the Coulbourn Instruments TruScan system. The animals were placed in the centre of the photosensor setup TruScan in which the parameters of vertical and horizontal activity were recorded for 2 min.

Corazol toxicity was caused by introducing corazol (80 mg/kg, intraperitoneally). The percentage of deaths of the animals in each group was estimated. The result was presented as the change of lethality percentage in comparison with the reference group.

Chloral hydrate sleep was reproduced by introducing chloral hydrate (350 mg/kg, intraperitoneally); the action of the somniferous preparation was estimated from the duration of the side position of the animals, from the loss and recovery of the overturn reflex.

RESULTS AND DISCUSSION

It was established as a result of the investigation of the effect of agents on the motor and emotional behaviour of the animals that neither of the compounds under examination affects the horizontal motor activity (Table 1). It should be noted that only agents **4**, **5** and **7** in the dose of 10 mg/kg reliably suppress the vertical activity decreasing the number of vertical stands and the time spent in them, which can be an indirect evidence of the general decrease in the anxiety of the animals. Agent **1** increases the time spent in stands without changing the number of stands, which is likely to point to the prevalence of the so-called long-term grooming and can bet he evidence of enhancement of the emotional status of the animals. This is also evidenced by a decrease in the investigative reaction of animals in this group. Other agents have no pronounced effect on the motor and emotional activity of the animals.

The next test allowing one to estimate the action of the substances on the CNS, namely the action of agents on the GABA-ergic system, is corazol toxicity. The data on the effect of agents **1–9** on the toxic action of corazol (lethality of the animals in per cent with respect to the reference group) are presented below:

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
−24	−16	−16	−50	−74	+50	−100	−16	−50

Here signs. "−" and "+" mean a decrease or an increase in lethality in comparison with the reference, respectively. One can see that agent **7** demonstrated the high anticonvulsive activity completely preventing blockage of GABA receptors and exhibiting the properties of GABA mimetics. Also rather clearly exhibited anticonvulsive activity in this test was exhibited by agents **4**, **5** and **9**. Compound **6** acted as a GABA-lytic and enhanced the toxic action of corazol by a factor of 1.5. Other agents did not exhibit any pronounced activity in the test of corazol toxicity.

The test of chloral hydrate sleep allows one to evaluate the effect of the agents on the som-

TABLE 1

Effect of the derivatives of 2,4,6,8,10,12-hexaazatetracyclo[5.5.0.0^{3,11}0^{5,9}]dodecane on the parameters of motor and emotional activity of animals

Agents	A	B	C	D	E	F	G	H	I
Reference	13.6±1.2	98.0±3.1	350.5±36.0	2.9±0.3	21.5±3.1	12.3±1.8	22.0±2.8	5.5±1.1	7.4±0.9
1	14.1±1.0	100.8±2	325.2±28.9	2.7±0.2	19.3±2.0	13.6±2.4	33.2±4.0*	3.3±0.7	3.9±0.7**
6	14.3±0.8	101.9±1.4	281.4±17.2	2.3±0.1	18.1±1.4	10.0±0.9	18.9±1.9	5.4±0.7	7.6±1.5
8	11.1±0.8	103.3±1.8	370.7±22.4	3.1±0.2	16.8±1.8	15.4±1.7	29.3±2.5	4.5±0.6	5.4±0.9
Reference	11.5±1.5	103.3±2.4	308.5±33.2	2.53±0.3	16.8±2.4	13.9±1.8	25.6±2.5	6.75±0.9	7.88±1.2
4	10.75±0.5	104.5±1.0	270.9±16.9	2.45±0.3	15.5±1.0	7.3±1.2**	12.6±3.4**	6.1±1.0	6.9±1.1
5	9.9±1.0	105.9±2.1	281.1±17.7	2.29±0.15	14.1±2.1	5.8±1.6**	9.0±3.6**	5.9±0.8	6.5±1.1
7	13.0±1.1	100.8±2.7	272.0±18.6	2.21±0.2	19.3±2.7	7.6±1.1**	13.0±2.1**	7.0±0.7	8.63±1.0
Reference	11.4±1.2	105.9±1.7	284.1±24.3	2.3±0.2	12.9±1.3	8.9±1.7	16.0±2.7	6.1±0.9	8.3±1.5
2	11.3±1.4	106±1.9	275.7±30.7	2.2±0.3	14.0±1.9	10.5±2.0	14.8±3.1	7.3±0.8	10.1±1.6
3	14±1.2	101.0±2.0	316.9±27.4	2.6±0.2	19.0±2.0*	14.3±2.0	22.6±3.9	6.3±1.2	7.9±1.6
9	13.1±1.7	102.8±2.8	296.2±26.4	2.4±0.2	14.7±1.4	13.4±2.0	19.3±3.2	6.4±0.7	6.6±1.1

Note. A − total motor activity; B − time of activity, s; C − travelled distance, cm; D − velocity of movement, cm/s; E − immobile time, s; F − number of vertical stands; G − time spent in stands, s; H − number of holes investigated; I − time of investigative reaction, s.

*$p < 0.05$, **$p < 0.01$ with respect to the reference.

TABLE 2

Effect of the derivatives of 2,4,6,8,10,12-hexaazatetracyclo[5.5.0.0^{3,11}0^{5,9}]dodecane on the duration of chloral hydrate sleep

Agents	Latent time to fall asleep, min	Sleep duration, min
Reference	4.16±0.66	62.0±9.3
1	3.34±0.1	96.5±14.5
2	1.86±0.15**	85.5±8.6
3	4.83±0.23	73.1±5.5
4	3.29±0.09	100.5±10.4*
5	3.64±0.51	60.5±6.1
6	3.44±0.27	106.3±10.5**
7	3.11±0.27	86.6±10.0
8	3.68±0.3	108.8±18.1*
9	3.23±0.41	72.6±11.8

*$p < 0.05$, **$p < 0.01$ with respect to the reference.

niferous action of barbiturates. Results of the investigation (Table 2) show that agents **4**, **6** and **8** cause an increase in the duration of chloral hydrate action (by 62, 71,5 and 75.5 %, respectively) acting similarly to imipramine-like preparations, while agent **2** decreases the time of falling asleep without any effect on the duration of sleep itself.

CONCLUSION

Thus, as a result of screening of the effect of agents on the CNS, we revealed the most active compounds that are promising for further investigation. It was established that agent **9** is promising as an anticonvulsive agent; agents **5** and **7** are interesting as anticonvulsive means with anti-anxiety activity, while agent **4** exhibits anxyolytic activity along with anticonvulsive action. Agent **8** can be considered as the means potentiating the action of somniferous preparations, and agent **6** is distinguished by allosteric stimulation of the CNS affecting at the same time the GABA-ergic system and the cerebral cortex.

For the compounds that exhibited the highest activity, acute toxicity after single intragastric introduction was determined for mice. It was established that the LD_{50} value for agents **4**, **7** exceeds 2000 mg/kg, while for agents **5**, **6**, **8**, **9** it is higher than 1000 mg/kg. All the compounds belong to the 3rd (moderate toxic) class.

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