Nootropic activity of *N*-(2-acetylaminoethyl)glycolurils

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The neurotropic activity of *N*-(2-acetylaminoethyl)glycoluril derivatives and their analgesic effect were studied. The nootropic activity of glycolurils was investigated for the first time, and a compound with the nootropic effect exceeding that of piracetam was revealed.

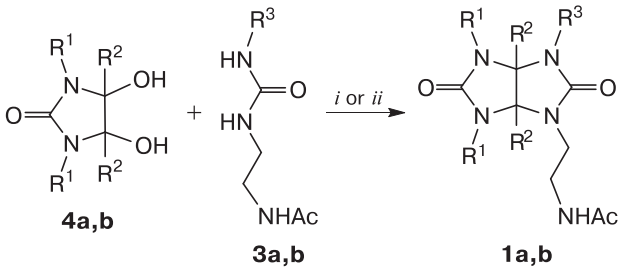
Key words: (2-acetylaminoethyl)glycolurils, neurotropic activity, the open-field test, the elevated plus maze test, nootropic activity, conditioned reflex of passive avoidance (CRPA), anxiolytic effect.

Disorders of the central nervous system (CNS), such as depression, various phobias, impaired cognitive functions of the brain, and headaches, are currently one of the most common forms of nervous diseases. The negative impact of modern lifestyle leads to physical, mental, and emotional stress, which, in turn, provokes a deterioration in the mental health of the population. In this regard, the identification of new classes of compounds with different types of neurotropic activity is of high importance. Hundreds of laboratories around the world screen chemical compounds with strictly defined types of activity, non-toxic, and effective. The main objects of scientific research of the laboratory of nitrogen-containing compounds of N. D. Zelinsky Institute of Organic Chemistry of RAS are tetrahydroimidazo[4,5-*d*]imidazole-2,5(1*H*,3*H*)-diones (glycolurils). The pharmacological potential of this class of compounds is confirmed by the introduction of Mebicar (tetramethylglycoluril) in domestic medical practice as a daily tranquilizer.[1] Other glycoluril derivatives are also under investigation.[2—13] For instance, a comparative analysis of pharmacological activity of Albicar in the forms of the racemate and (1*R*,5*R*)- and (1*S*,5*S*)-enantiomers was carried out, and it was shown that the (−)-(1*S*,5*S*)-enantiomer has a stimulating effect on the nervous system, while the (+)-(1*R*,5*R*)-enantiomer has a depressing effect.[3] Introduction of the alkoxycarbonyl group to the nitrogen atom of glycoluril molecules resulted in compounds with neuroprotective activity.[4,5] The difference in the properties and pharmacological activity was established for certain enantiomers of enantiomerically pure glycolurils comprising the (*R*)- or (*S*)-*N*-carbamoylmethionine moieties.[6] Only one of them was found to exhibit a neurotropic activity. Compounds possessing anxiolytic,[7] sedative,[8] antiproliferative, and fungicidal[9,10,14] activities were revealed among sulfur-containing analogs of glycolurils.

The present work is devoted to the study of the pharmacological activity, *viz.*, the effect on CNS, of *N*-(2-acetylaminoethyl)glycolurils with the use of the open-field and elevated plus maze tests, as well as the hot plate method, and also by observing the elaboration of the conditioned reflex of passive avoidance (CRPA). The last two approaches have not been previously applied to the studies of glycolurils.

The syntheses of glycolurils **1a**,**b** and **2** with one and two 2-acetylaminoethyl substituents were performed according to the methods developed by us earlier (Schemes 1 and 2). Compounds **1a**,**b** were obtained by α-ureido-

Scheme 1



1, 4: $R^1 = R^2 =$ H (**a**), $R^1 =$ Me, $R^2 =$ Ph (**b**); **1, 3:** $R^3 =$ Me (**a**), H (**b**)

Reagents and conditions: *i*. H_2O—Pr^iOH (2 : 3), HCl (pH 1), 80 °C, 1 h (**1a**); *ii*. MeOH, HCl (pH 1), 80 °C, 1.5 h (**1b**).

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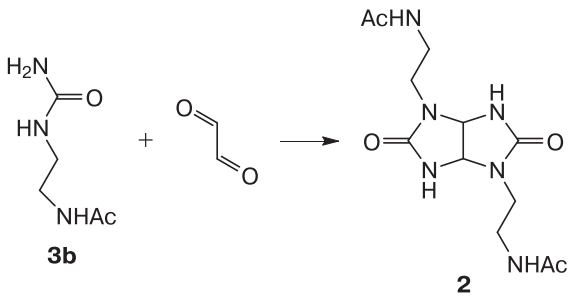
Scheme 2



Reagents and conditions: H_2O, HCl (pH 1), 80 °C, 1 h.

alkylation of 1-(2-acetylaminoethyl)-3-methylurea **3a** and 1-(2-acetylaminoethyl)urea **3b** with 4,5-dihydroxyimidazolidine-2-ones **4a**,**b**, respectively (see Scheme 1).[15] Glycoluril **2** was synthesized by the condensation of 1-(2-acetylaminoethyl)urea **3b** with glyoxal (see Scheme 2).[16]

To study the pharmacological effect of compounds **1a**,**b** and **2** on CNS, we used known methods such as the open-field and elevated plus maze tests.[17,18] Because the molecules of the synthesized glycolurils, like the nootropic drug Piracetam,[1] contain the acetylaminoethyl group, nootropic activities of compounds **1a,b** and **2** were additionally studied using the CRPA test.[19] Analgesic activity was tested with the use of the hot plate method.[20]

At the first stage, acute toxicity was determined for compounds **1a**,**b** and **2** by the described express method.[21] The studied glycolurils were found to be low toxic: the intraperitoneal introduction of these compounds to laboratory animals at the doses of 500 and 1000 mg kg^{-1} did not cause their death.

In the studies of the effects of these compounds on the locomotor and research activities, it was established that compounds **1b** and **2** have a depressive effect on the locomotion of animals, which was reflected in a proved decrease in motor activity of mice in the open-field test (Fig. 1). The anxiolytic effect was assessed by the influence of the compounds on the appearance of anxiety in mice placed in the open arms of the maze for 3 min. It was shown that compound **1a** exhibits an anxiolytic effect similar to that of Mebicar, whereas the effect of compound **1b** is superior to that of the latter.

The study of the neurotropic activity of the compounds was supplemented with experiments with the use of the hot plate method. It was found that compound **2** has some analgesic effect. The time of the appearance of the defensive reflex in 2 h after introduction of compound **2** was increased by 52% (Fig. 2).

Nootropic activity of the compounds was tested by their effects on the elaboration of the conditioned reflex of passive avoidance (CRPA). The data obtained in the study of the influence of the compounds on the phase of

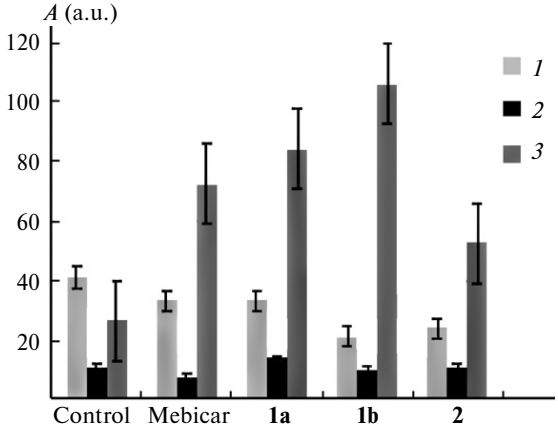


Fig. 1. Effects of compounds **1a,b** and **2** on the locomotion and research activities (*A*) of mice in the open-field and elevated plus maze tests: *1*, the number of the crossed squares; *2*, the number of the holes examined; *3*, the time (s) spent by a mouse in the open hands of the maze.

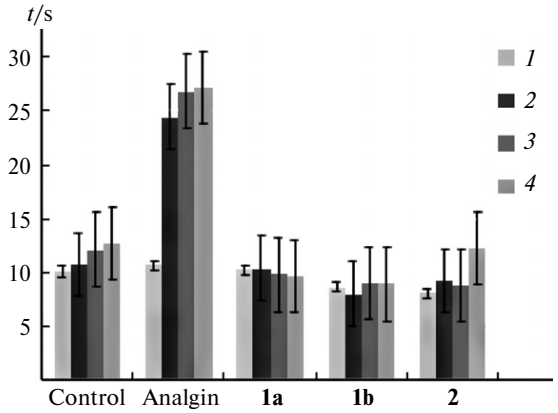


Fig. 2. Effect of compounds **1a,b** and **2** on the time of the appearance of the defensive reflex (*t*) of mice in the hot plate test: *1*, the initial state (before the compound administration), *2*, 30 min, *3*, 60 min, and *4*, 120 min after the compound administration.

Table 1. Nootropic effects of the compounds (the CRPA test)

Compound	Dose/mg kg^{-1}	*N* (%)*	*p*
Control	—	20	—
Mebicar	250	90	0.05
Nootropil	400	90	0.05
1a	50	90	0.05
1a	75	90	0.05
1a	100	90	0.05
1a	250	90	0.05
1b	100	20	0.5
2	100	50	0.1

* The percentage of mice that did not enter the dark compartment of the dark-light chamber on the second day.

the memory consolidation in the development of CRPA are shown in Table 1.

Thus, a detailed study of the effects of *N*-(acetylaminoethyl)glycolurils on CNS was carried out, which showed that compound **1a** is the most promising one. This compound is non-toxic, has no depressive effect on CNS and exhibits a definite nootropic activity. In the whole range of the used doses (from 50 to 250 mg kg^{-1}), this compound stimulated memorizing processes in 90% of mice. This effect exceeds the efficiency of the reference drug Mebicar and the known nootropic drug Piracetam (Nootropil).

Experimental

Compounds **3a,b** were synthesized by *N*-carbamoylation of *N*-acetylethylenediamine with potassium cyanate and methyl isocyanate, respectively.[15,16] *N*-Acetylethylenediamine was prepared from ethylenediamine and ethyl acetate.[22] Dihydroxyimidazolidinones **4a,b** were obtained according to known procedures by the reaction of glyoxal or benzil with urea or 1,3-dimethylurea.[23,24] The yields, melting points, and physicochemical characteristics of glycolurils **1a**,**b**[15] and **2**[16] and starting compounds **3a,b**[15,16] and **4a,b**[23,24] correspond to those reported earlier.

^{1}H NMR spectra were recorded using a Bruker AM-250 spectrometer (250.13 MHz) in DMSO-d_6, chemical shifts are given relative to Me_4Si used as an internal standard. Melting points were measured using a GALLENKAMP apparatus (Sanyo).

Synthesis of *N*-{2-(hexahydro-1-methyl-2,5-dioxoimidazo-[4,5-*d*]imidazol-3(6a*H*)-yl)ethyl}acetamide (1a) and *N*-{2-(hexahydro-1,3-dimethyl-2,5-dioxo-3a,6a-diphenylimidazo[4,5-*d*]-imidazol-6(6a*H*)-yl)ethyl}acetamide (1b) (general procedure). Concentrated HCl was added dropwise (to pH 1) under stirring and heating at 50 °C to a solution of compound **4a** or **4b** (10.1 mmol) and corresponding urea **3a** or **3b** (10 mmol) in water—propan-2-ol mixture (10 mL, 2 : 3) (for **1a**) or in methanol (20 mL, for **1b**), and stirring was continued at 80 °C for 1 h (for **1a**) or 1.5 h (for **1b**). Solvent was evaporated using a rotary evaporator; the obtained oily residue was triturated with propan-2-ol (for**1a**) or water (for **1b**). The formed precipitates of compounds **1a** and **1b** were filtered and recrystallized from dioxane and acetone.

Compound 1a · H_2O. Yield 45% (*cf.* Ref. 15: yield 43—45%), m.p. 128 °C (*cf.* Ref. 15: m.p. 126—128 °C). ^{1}H NMR, δ: 1.77 (s, 3 H, COMe); 2.63 (s, 3 H, NMe); 2.93—3.11 (m, 2 H, CH_2); 3.12—3.32 (m, 2 H, CH_2); 5.10 (d, 1 H, CH, J = 8.1 Hz); 5.27 (d, 1 H, CH, J = 8.1 Hz); 7.49 (br.s, 1 H, NH); 7.56 (br.s, 1 H, NH); 7.86 (t, 1 H, NH, J = 5.1 Hz) (*cf.* Ref. 15).

Compound 1b. Yield 67% (*cf.* Ref. 15: yield 65—67%), m.p. 267 °C (*cf.* Ref. 15: m.p. 265—267 °C). ^{1}H NMR, δ: 1.74 (s, 3 H, COMe); 2.61 (s, 3 H, NMe); 2.86 (s, 3 H, NMe); 2.77—2.91 (m, 1 H, CH_2); 3.21—3.38 (m, 3 H, CH_2); 6.79 (m, 2 H, Ph); 6.89 (m, 2 H, Ph); 7.09 (m, 6 H, Ph); 7.95 (br.s, 1 H, NH); 8.52 (s, 1 H, NH) (*cf.* Ref. 15).

Synthesis of 1,4-di(2-acetylaminoethyl)tetrahydroimidazo-[4,5-*d*]imidazole-2,5(1*H*,3*H*)-dione (2). (2-Acetylaminoethyl)-urea (**3b**) (20 mmol) and concentrated HCl (to pH 1) were added to a 40% aqueous solution of glyoxal (d = 1.265 g mL^{-1}, 1.15 mL, 10 mmol) in water (10 mL), and the mixture was heated at 80 °C for 1 h. The solvent was evaporated *in vacuo*. The formed oily residue was treated with propan-2-ol and methanol. The formed precipitate of compound **2** was filtered. Yield 51% (*cf.* Ref. 16: yield 51%), m.p. 257 °C (*cf.* Ref. 16: m.p. 255—257 °C). ^{1}H NMR, δ: 1.78 (s, 6 H, 2 Ac); 2.94—2.98 (m, 2 H, NCH_2); 3.05—3.09 (m, 2 H, NCH_2); 3.20—3.28 (m, 4 H, 2 NCH_2); 5.25 (s, 2 H, 2 CH); 7.49 (s, 2 H, 2 NH); 7.83 (t, 2 H, 2 NHAc, J = 4.9 Hz) (*cf.* Ref. 16).

All experiments with animals were carried out in accordance with the EU Directive (86/609/EEC), the experimental protocol was approved by the IPAC RAS Bioethics Commission. Outbred white mice (males, 1.5—2 months, 20—25 g) were obtained from the nursery of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences (Pushchino) and were quarantined for five days before the start of the experiment. The animals were kept in ventilated plastic cages at 2—22 °C with a 12-hour day/night cycle, 40—70% humidity, and free access to water and food.

The experiments were carried out strictly from 11.00 to 13.00 h, 10 mice per day were tested. Compounds were administered intraperitoneally in physiological saline with additions of tween-80. The effects were observed in 1 h after administration the compounds.

Acute toxicity[21] of compounds **1a,b** and **2** was measured after their intraperitoneal administration at the doses of 500 and 1000 mg kg^{-1}.

The open-field test.[17,18] The effect of the compounds under study on the motor activity was assessed by the number of squares crossed by a mouse for 3 min. The effect of the compounds on the research activity was determined by the number of holes examined in 3 min. The data were compared with those obtained for the control group.

The elevated plus maze test.[17,18] The degree of anxiety was determined by the time spent by a mouse in the open hands of the maze during 3 min of the experiment. The data were compared with those obtained for the control group.

The hot plate method.[20] A mouse was placed on a plate warmed to 55 °C, and the time of the appearance of the defensive reflex consisting in licking the hind foot was measured. The results were assessed by increasing the time of the onset of the defensive reflex compared with the initial data. Statistical data processing was carried out according to Student's test. The effect was considered significant at $p \leqslant 0.05$.[25]

The CRPA test.[19] The compounds were administered immediately after mice received a 50 V electric shock in the dark compartment of a dark-light chamber. Each group of mice consisted of 10 animals. The elaboration of the conditioned reflex was checked in 24 h. The percentage of mice that did not enter the dark compartment for 3 min on the second day of the study was calculated. Statistical data processing was carried out according to Pearson's χ^2 test.[25]

References

1. M. D. Mashkovsky, in *Lekarstvennye sredstva* [*Medicines*], Novaya Volna, Moscow, 2016, Vol. **1**, p. 89 (in Russian).
2. A. N. Kravchenko, V. V. Baranov, G. A. Gazieva, *Russ. Chem. Rev.*, 2018, **87**, 89.
3. L. V. Anikina, Y. B. Vikharev, O. R. Malyshev, V. V. Baranov, A. N. Kravchenko, *Mendeleev Commun.*, 2018, **28**, 317.

4. Yu. B. Vikharev, L. V. Anikina, I. E. Tchikunov, A. S. Sigachev, A. N. Kravchenko, Yu. V. Shklyaev, N. N. Makhova, *Vopr. Biol., Med. i Farmacevt. Khimii* [*Quest. Biol., Med. Pharm. Chem.*], 2006, No. 2, 12 (in Russian).
5. R. Kamburg, US Pat. 227838, *Chem. Abstr.*, 2008, **149**, 347537.
6. A. N. Kravchenko, V. V. Baranov, L. V. Anikina, Yu. B. Vikharev, I. S. Bushmarinov, Yu. V. Nelyubina, *Russ. J. Bioorg. Chem.*, 2012, **38**, 550.
7. V. V. Baranov, G. A. Gazieva, Yu. V. Nelyubina, A. N. Kravchenko, Н. Н. Махова, *Russ. J. Org. Chem.*, 2011, **47**, 1564.
8. G. A. Gazieva, Yu. B. Vikharev, L. V. Anikina, T. B. Karpova, A. N. Kravchenko, E. A. Permyakov, I. V. Svitanko, *Mendeleev Commun.*, 2013, **23**, 202.
9. G. A. Gazieva, L. V. Anikina, T. V. Nechaeva, S. A. Pukhov, T. B. Karpova, S. V. Popkov, Yu. V. Nelyubina, N. G. Kolotyrkina, A. N. Kravchenko, *Eur. J. Med. Chem.*, 2017, **140**, 141.
10. G. A. Gazieva, L. V. Anikina, S. A. Pukhov, T. B. Karpova, Yu. V. Nelyubina, A. N. Kravchenko, *Mol. Divers.*, 2016, **20**, 837.
11. A. S. Berlyand, A. A. Prokopov, *Pharm. Chem. J.*, 2014, **48**, 347.
12. A. S. Berlyand, O. V. Lebedev, A. A. Prokopov, *Pharm. Chem. J.*, 2013, **47**, 176.
13. A. A. Prokopov, A. S. Berlyand, O. N. Kazantseva, *Pharm. Chem. J.*, 2003, **37**, 132.
14. G. A. Gazieva, T. V. Nechaeva, N. N. Kostikova, N. V. Sigay, S. A. Serkov, S. V. Popkov, *Russ. Chem. Bull.*, 2018, **67**, 1059.
15. G. A. Gazieva, P. V. Lozhkin, V. V. Baranov, A. N. Kravchenko, N. N. Makhova, *Russ. Chem. Bull.*, 2010, **59**, 642.
16. A. N. Kravchenko, V. V. Baranov, G. A. Gazieva, I. E. Chikunov, Yu. V. Nelyubina, *Russ. Chem. Bull.*, 2014, **63**, 416.
17. I. R. Bossier, P. Simon, I. M. Zwolf, *Therapie*, 1964, **19**, 571.
18. D. Perals, A. S. Griffin, I. Bartomeus, D. Sol, *Animal Behaviour*, 2017, **123**, 69.
19. *Rukovodstvo po eksperimental'nomu (doklinicheskomu) izucheniyu novykh farmakologicheskikh veshchestv* [*Manual on Experimental (Preclinical) Study of Pharmacological Substances*], Ed. R. U. Khabriev, Meditsina, Moscow, 2005, 832 pp. (in Russian).
20. N. B. Eddy, D. Leimbach, *J. Pharmacol. Exp. Ther.*, 1953, **107**, 385.
21. V. B. Prosorovsky, M. P. Prozorovskaya, V. M. Demchenko, *Farmacologiya i Toxikologiya* [*Pharmacology and Toxicology*], 1978, **41**, 497 (in Russian).
22. S. R. Aspinall, *J. Am. Chem. Soc.*, 1941, **63**, 852.
23. R. Neville, *J. Org. Chem.*, 1958, **23**, 158.
24. S. Vail, R. Barker, P. Mennitt, *J. Org. Chem.*, 1965, **30**, 2179.
25. M. L. Belen´kiy, *Elementy Kolichestvennoy otsenki pharmacologicheskogo effecta* [*Elements of Qantitative Evaluation of the Pharmacological Effect*], 2d ed., Medgiz, Leningrad, 1963, 146 pp. (in Russian).

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