SYNTHESIS AND STUDY OF PSYCHOTROPIC AND HYPOTENSIVE PROPERTIES OF NEW PICAMILON DERIVATIVES

V. M. Kopelevich,[1] L. N. Bulanova,[1] I. A. Grigor'ev,[1] S. G. Gorbunov,[1] A. V. Sabanov,[1] V. L. Adzhienko,[2] V. Ya. Il'in,[2] V. N. Perfilova,[2] I. N. Tyurenkov,[2] V. I. Petrov,[2] and V. I. Gunar[2]

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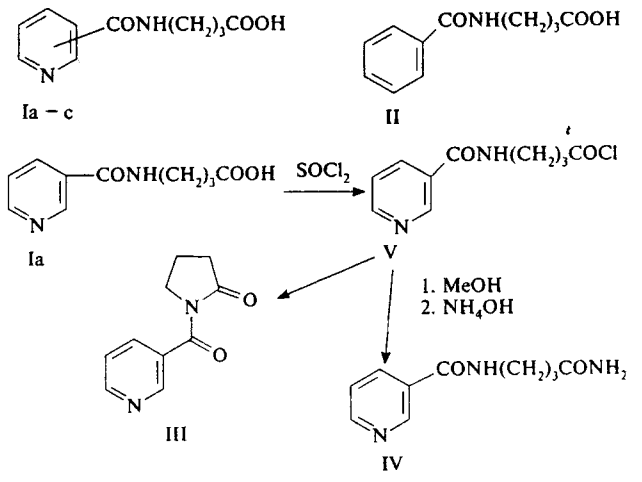
Picamilon (an original drug synthesized at the "Vitaminy" Research and Production Association [1]) is an N-nicotinoyl derivative of GABA (Ia). Experience gained in the clinical use of this drug shows that it combines pronounced vasodilatation properties with nootropic effects and tranquilizer activity [2–5]. Although the tranquilizer action of picamilon is somewhat less pronounced as compared to that of diazepam, the former has the advantages of having no myorelaxant and amnestic effects and having a weak activation component, and being able to restore or increase the physical and psychic capacity to work. Nevertheless, the needs of practical medicine stimulate the search for new psychotropic drugs possessing more pronounced nootropic and anxiolytic activity, including the further study of the series of picamilon derivatives and analogs. In particular, it was established [6] that 5-hydroxy derivatives of picamilon exhibited rather high nootropic activity, while its 2-alkoxy, 2-aryloxy, and 2-arylalkoxy derivatives possessed psychopharmacological activity of a deprivation character [7]. Previously, we have synthesized isonicotinoyl (Ib), picolinoyl (Ic), and benzoyl (II) analogs of picamilon that showed, like picamilon itself, no significant inhibiting action upon the trapping of GABA [8], although high doses of compound Ib markedly increased (unlike picamilon) the brain GABA level in mice [9]. In addition, the isonicotinoyl GABA derivative exhibited a significant anticonvulsive effect [9] and dramatically enhanced blood circulation in the brain [10].

The purpose of this work was to study variations in the pharmacological activity of picamilon in relationship with certain changes in the structure of the picamilon molecule, including (i) various positions of 3-carboxypropylaminocarbonyl group in the pyridine ring, (ii) substitution of benzene ring for pyridine, (iii) cyclization of the amino acid residue to form a pyrrolidone system, and (iv) replacement of the terminal carboxy group by carboxamide. For this purpose, we have performed experimental investigation of the psychotropic properties of N-isonicotinoyl-GABA (Ib), N-picolinoyl-GABA (Ic), N-benzoyl-GABA (II), N-nicotinoylpyrrolidin-2-one (III), and N-nicotinoyl-GABA amide (IV) in comparison with picamilon. We have also studied the hypotensive properties of the synthesized compounds.



The synthesis of compounds Ia – Ic and II was described earlier [8]. Compound III was obtained by intramolecular cyclization of N-nicotinoyl-GABA chloride (V). Study of the reaction between nicotinoyl-GABA and thionyl chloride showed that acid chloride V was susceptible to cyclization already at room temperature, the complete conversion being observed at 50°C. This observation allowed us to develop a convenient method for the synthesis of N-nicotinoyl-2-pyrrolidone, which can be used to obtain some other N-acyl derivatives of pyrrolidone as well. Until now, these derivatives were conventionally synthesized by acylating 2-pyrrolidone with the corresponding acid chlorides [11]. Amide IV was obtained by reaction of nicotinoyl-GABA methyl or ethyl ester with aqueous ammonia.

[1] "Vitaminy" Research and Production Association, Moscow, Russia.
[2] Volgograd Medical Academy, Volgograd, Russia.

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The proposed structures were checked and the synthesized compounds were identified by TLC, IR and ^{1}H NMR spectroscopies, and elemental analyses. The IR spectrum of compound III showed intense absorption bands at 1670 cm^{-1} (amide I) due to C=O bonds in the side group and the lactam bands at 1740 cm^{-1}. Note that introduction of an isonicotinoyl group into 2-pyrrolidone increases the frequency of carbonyl vibrations by 35 cm^{-1} above the initial value.

EXPERIMENTAL CHEMICAL PART

N-Nicotinoylpyrrolidin-2-one (III). A mixture of 1.04 g (0.005 mole) of N-nicotinoyl-GABA (Ia) and 2 ml of $SOCl_2$ in 30 ml benzene was heated for 3 h at 50°C and then the solvent was distilled off in vacuum. The residue was triply treated with 40 ml of benzene, each time followed by the solvent evaporation. To the final residue was added 40 ml of a 10% $NaHCO_3$ solution, the mixture was extracted with chloroform (3 × 60 ml), and the organic layer was separated and dried over Na_2SO_4. Then the solvent was distilled off in vacuum and the residue was recrystallized from an ethanol – petroleum ether mixture (1 : 1) to obtain 0.59 g (62.4%) of compound III; m.p., 103 – 104°C; IR spectrum, nujol mull (ν_{max}, cm^{-1}): 1740 (C=O), 1670 (amide I), 1590, 1575 (C=C, C=N); ^{1}H NMR spectrum, D_2O (δ, ppm): 2.19 (m, CH_2-β), 2.73 (t, CH_2CO), 4.02 (t, NCH_2), 7.48 – 8.94 (m, 4H, Py).

4-(Nicotinoylamino)butanoic acid amide (IV). A mixture of 11.8 g (0.053 mole) of N-nicotinoyl-GABA methyl ester (obtained from compound V and MeOH), 30 ml of 25% S aqueous ammonia, and 0.8 g NH_4Cl was stirred for 28 h at 20°C and then the resulting solution was partly evaporated in vacuum. To the residue was added 15 ml of ethanol, the mixture was filtered, and the solvent distilled off in vacuum. The residue was recrystallized from ethyl acetate to obtain 7.76 g (74.8%) of compound IV; m.p., 135 – 136°C (reported m.p., 137°C [12]).

EXPERIMENTAL PHARMACOLOGICAL PART

The psychotropic activity of compounds Ia – Ic and II – IV was studied in experiments on a group of white male mongrel rats weighing 150 – 200 g. The synthesized compounds were injected intraperitoneally at a dose of 10 mg/kg 1 h prior to testing the animals.

The spontaneous motor activity, orientation-exploratory behavior, and emotional response of test rats were studied in the "open-field" test [13] which allowed us to assess the psychostimulation (or psychodeprivation) effect of injected drugs (horizontal motor activity and orientation-exploratory activity) and their action upon the emotional state of animals (brief grooming).

The antidepressant activity of the synthesized compounds was studied in the forced swimming test [14] and evaluated by a decrease in the total duration of the immobilization period and an increase in the number of jumps.

In order to determine the tranquilizer (antiphobic) activity of the compounds, the animals were subjected to the elevated plus-labyrinth test [15] in which we measured the time spent in open and blind alleys of the elevated maze. An increase in the time spent in the open compartments was interpreted as manifestation of the tranquilizer drug activity.

The effect of compounds on the state of short-term memory and cognitive functions was assessed using the crossed labyrinth test [16], whereby a sequence of walks in the labyrinth alleys under free choice conditions was monitored. The data were processed on a computer using an original program that analyzed eight parameters characterizing the ability of test animals to periodic (purposeful) walks into blind alleys and, hence, the degree of retained rational brain function and short-term memory.

Effects of the synthesized compounds on the long-term memory and learned behavior of the test animals were studied using the commonly accepted models based on the passive avoidance conditioned reflex (PACR) [17] and active avoidance conditioned reflex (AACR) [18].

TABLE 1. Effect of Compounds Ia – Ic and II – IV on the Behavior of Animals in Various Tests

Compound	Open-field			Plus-maze	Forced swimming	PACR	Pain tolerance
	HA	EA	GR	TOA	IT	LP	VTH
Control	35.8 ± 4.9	9.1 ± 1.6	5.5 ± 0.9	24.8 ± 5.5	182.6 ± 22.2	42.8 ± 18.7	37.9 ± 4.3
Ia	41.9 ± 3.5	8.4 ± 1.3	4.5 ± 0.7	50.4 ± 7.3*	200.1 ± 11.4	103.0 ± 27.8*	38.8 ± 2.1
II	16.5 ± 5.6*	3.8 ± 1.0*	1.8 ± 0.6*	19.3 ± 8.2	200.5 ± 13.3	56.8 ± 25.3	42.5 ± 1.3
III	17.4 ± 4.1*	3.9 ± 0.4*	2.6 ± 0.6*	20.3 ± 8.4	199.3 ± 10.7	65.3 ± 24.4	39.4 ± 1.4
Control	23.7 ± 1.9	4.4 ± 1.9	2.8 ± 0.4	13.1 ± 2.7	161.5 ± 16.8	42.8 ± 18.7	30.6 ± 2.1
Ib	21.4 ± 6.0	2.4 ± 1.2	0.8 ± 0.5*	51.1 ± 9.3*	187.8 ± 18.7	99.1 ± 19.3*	27.9 ± 2.4
Ic	29.9 ± 4.5	4.9 ± 0.9	1.4 ± 0.8	16.1 ± 5.8	102.5 ± 18.7*	116.8 ± 28.9*	36.3 ± 1.2
IV	18.9 ± 3.8	2.4 ± 1.3	2.8 ± 0.6	10.9 ± 4.9	183.1 ± 12.8	77.6 ± 28.3	30.0 ± 1.3

Notes. HA = horizontal motor activity (number of squares crossed); EA = exploratory activity (number of stands on hind paws); GR = grooming (number of acts); TOA = time spent in open alleys (sec); IT = immobilization time (sec); LP = latent period before visiting dark compartments (sec); VTH = vocalization threshold (V).

* Difference against control statistically reliable for $p < 0.05$ (the Wilcoxon – Whitney – Mann criterion).

TABLE 2. Effect of Compounds Ia – Ic on the Parameters of Short-Term Memory and Cognitive Functions According to the Crossed Maze Model

Compound	FCD	DRV	TNC	NSR	NDV
Control (physiological solution)	5.4 ± 0.7	2.6 ± 0.9	2.0 ± 0.1	1.4 ± 0.3	0.4 ± 0.3
Ia	5.8 ± 1.0	2.3 ± 0.5	2.1 ± 0.7	1.5 ± 0.5	0.3 ± 0.03
Ib	4.7 ± 0.8	3.0 ± 1.0	2.1 ± 0.4	1.3 ± 0.4	0.4 ± 0.02
Ic	4.0 ± 0.6	2.5 ± 0.9	2.8 ± 0.2*	0.3 ± 0.01*	0

Notes. FCD = first cycle duration in maze (sec); DRV = number of delayed repeated walks into alleys; TNC = total number of cycles in maze; STR = number of stereotype reactions; NDV = number of direct repeated walks into alleys.

* Difference against control statistically reliable for $p < 0.05$ (the Wilcoxon – Whitney – Mann criterion).

TABLE 3. Effect of Compounds Ia – Ic on the Parameters of Rational Brain Function in the Extrapolated Escape Test

Compound	LPD	IT	NJ
Control (physiological solution)	78.5 ± 12.4	39.2 ± 17.8	1.8 ± 0.9
Ia	40.0 ± 12.5*	11.9 ± 6.6	2.4 ± 1.1
Ib	59.9 ± 13.5	20.8 ± 9.0	2.4 ± 0.6
Ic	45.9 ± 13.2*	13.8 ± 9.1	3.7 ± 1.3

Notes. LPD = latent period for diving (sec); IT = immobilization time (sec); NJ = number of jumps.

* Difference against control statistically reliable for $p < 0.05$ (the Wilcoxon – Whitney – Mann criterion).

The action of compounds on the rational brain function was evaluated by the time required to solve the task of avoiding the aversive medium in the extrapolated escape test [19].

The analgesic activity of the synthesized compounds was evaluated by the tolerance threshold of electric-pain irritation detected in the animal vocalization test.

The hypotensive action of compounds Ia – Ic and II – IV was studied by acute injections to white mongrel rats narcotized with pentobarbital sodium (40 mg/kg, i.p.). The test compounds were dissolved in a 10% aqueous ethanol and injected into the external jugular vein. Animals in the control group were intravenously injected with the solvent. The systemic pressure was monitored with a mercury manometer in the carotid artery 1 – 60 min after drug introduction.

The experimental results were statistically processed using the Wilcoxon – Whitney – Mann method. The effect of compounds studied on the AACR learning dynamics in rats was assessed by the method of two-factor dispersion analysis (ANOVA) using a nonparametric Fisher criterion [20].

RESULTS AND DISCUSSION

It was established that compounds II and III decrease the indices of general motor and exploratory activity of rats in the open-field test (Table 1). Compounds Ia and Ib increased the number of visits to the central zone of labyrinth and decreased the number of brief grooming acts, thus suggesting the presence of tranquilizer properties in these substances. This assumption was confirmed by results obtained using the elevated plus-labyrinth model.

As for the forced swimming test, it was only compound Ic that reliably reduced the duration of immobilization in rats that was evidence of the antidepressant activity.

In the whole group of drugs studied, only compounds Ia – Ic (introduced 1 h before the PACR learning followed by the electroshock irritation) produced an antiamnestic (nootropic) effect manifested by a reliable increase in the latent period before visiting dark compartments and a decrease in the time spent there (as compared to the control group).

None of the substances studied affected the sensitivity and tolerance thresholds of the electric-pain irritation, that is, these compounds exhibited no analgesic properties.

A thorough analysis of the antiamnestic activity of compounds Ia – Ic showed that only compound Ic at a dose of 10 mg/kg reliably increased the characteristics of cognitive functions and the short-term memory according to the crossed labyrinth test (Table 2).

Data of the extrapolated escape test show that animals treated with compounds Ia and Ic (but not Ib) have a reliably shorter latent period of diving compared to that in the control group, which indicates improved ability of solve the task of avoiding aversive situation (Table 3).

TABLE 4. Effects of New Derivatives of GABA and α-Pyrrolidone on Systemic Arterial Pressure (AP) in Narcotized Rats

Compound	Dose, mg/kg (i.p.)	Initial AP, Torr	AP dynamics (% of initial level) after (min): 1	5	10	15	20	25	30	45	60
Control	–	120.0 ± 11.0	3.30 ± 0.85	– 2.77 ± 1.08	– 2.73 ± 0.38	– 4.3 ± 1.3	– 7.13 ± 0.64	– 8.83 ± 0.52	– 10.53 ± 0.48	– 10.51 ± 1.12	– 10.70 ± 5.30
Ib	10	128.0 ± 4.62	0 ± 0.7	– 2.70 ± 1.15	– 5.83 ± 1.29	– 7.37 ± 1.53	– 8.68 ± 1.60	– 11.07 ± 1.97	– 12.60 ± 1.36	– 17.30 ± 1.53*	– 19.88 ± 1.74
Ic	10	117.3 ± 10.41	2.30 ± 0.57	– 2.40 ± 0.80	– 5.87 ± 1.09	– 8.00 ± 3.46	– 11.37 ± 1.22*	– 13.77 ± 1.07*	– 15.40 ± 1.81	– 20.30 ± 1.28*	– 21.80 ± 2.98
II	10	136.7 ± 8.82	– 3.00 ± 0.87	– 2.90 ± 0.76	– 7.70 ± 0.81*	– 10.53 ± 1.47*	– 13.20 ± 1.94*	– 15.80 ± 2.87	– 16.70 ± 2.78	– 18.23 ± 2.89*	– 22.70 ± 2.67
III	10	113.3 ± 8.11	– 1.27 ± 0.21	– 2.83 ± 1.56	– 6.70 ± 1.15*	– 9.80 ± 1.95	– 12.10 ± 2.07	– 12.60 ± 2.35	– 13.47 ± 2.79	– 16.27 ± 3.80	– 16.00 ± 3.07
IV	10	124.7 ± 5.46	1.03 ± 0.31	– 1.53 ± 0.90	– 3.58 ± 1.86	– 5.17 ± 2.75	– 6.27 ± 2.63	– 7.20 ± 3.73	– 7.73 ± 4.13	– 12.03 ± 3.37	– 15.30 ± 2.42

* Difference against control statistically reliable for $p < 0.05$.

An analysis of the influence of compounds upon the AACR development revealed that compounds Ia and Ic (but not Ib) at a dose of 10 mg / kg improve the memory trace formation as manifested by a more rapid increase (daily dynamics) in the number of correct walks ($F_A(9; 240) = 42.9$, $p < 0.01$; $F_B(2; 240) = 12.3$, $p < 0.01$).

Pronounced hypotensive activity in the series of drugs studied was observed only for compounds Ic and II (Table 4).

According to the data available in the literature, GABA produces a pronounced hypotensive effect only at a dose of 200 mg / kg [21]. Apparently, the higher hypotensive activity of some compounds in the series studied is due to higher ability to penetrate through the brain – blood barrier and act upon the GABAergic processes of vasomotor regulation.

Thus, the group of picamilon derivatives and analogs studied in this work contains compounds possessing a high psychotropic activity. The most interesting candidate for the further preclinical characterization is picolinoyl-GABA, combining nootropic activity with pronounced antidepressant and hypotensive properties, and isonicotinoyl-GABA showing a high tranquilizer activity.

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