SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF AMINO ACID DERIVATIVES OF NICOTINIC ACID

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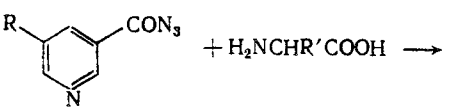
It is well known that amino derivatives of nicotinic acid possess significant neuropsychotropic properties. In particular, the nicotinoyl derivatives of glutamic and γ-aminobutyric (GABA) acids have the properties of lowering motor excitation stimulated by phenamine, promoting the action of hexenal, and lowering motor activity; in addition, these compounds have antispasmodic action according to their antagonism to korazole, and antiaggressive activity [7, 8]. The disubstituted nicotinoylamino acids in which the amino acid residue is GABA, glutamic acid, lysine, or alanine, and the substituent in position 2 is an alkoxy-, aryloxy-, and arylakoxy-group, also have psychopharmacological action of an unsatisfactory character [6]. In addition, nicotinoyl-GABA has been shown to have antiamnestic activity [4].

The aim of the present study was the synthesis of new amino acid derivatives of nicotinic, 5-bromonicotinic, and 5-hydroxynicotinic acids, where the amino acids are GABA, glutamic acid, proline, cysteine, methionine, and aspartic acid, and also a study of their nootropic activity and the influence of the substituent in position 5 on this activity.

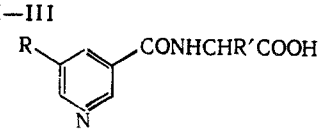
The synthesis of compounds IV-XV was brought about by the azide method, starting from the ethyl ester of nicotinic acid, which was hydrazinolyzed in aqueous or alcoholic medium to give the corresponding hydrazide. Further interaction with $NaNO_2$ in the presence of dilute HCl produced the acid azides I-III.

The azide of nicotinic acid was obtained according to [1]. The remaining azides II and III were synthesized analogously. The explosive nature of the azides obtained, especially that of the 5-hydroxynicotinic acid III should be noted.

The azides obtained (I-III) were treated with L-amino acids in aqueous basic medium to convert into the corresponding amino acid derivative of the substituted nicotinic acids IV-XV, according to the scheme:



I—III



IV—VII, IX—XIII

I, IV—VII: R = H; II, IX—XII:R = Br;
III, XIII:R = OH; IV, IX,
XIII: R′ = CH_2CH_2COOH;
V, X R′ = CH_2COOH; VI, XI: R′ = CH_2SH;
VII, XII; R′ = $CH_2CH_2SCH_3$ (DL)

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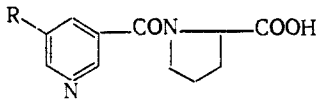
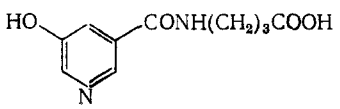


VIII, XIV



XV

VIII: R = H; :XIV:R = Br

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained in KBr with an IR-580 Perkin Elmer spectrometer, UV spectra were recorded on a 'Specord' instrument, and ^1H NMR with a Varian T-60 machine in CD_3OD-D_2SO_4 with hexamethyldisilane as internal standard. TLC was carried out on Silufol UV-254 plates in a solvent system composed of methanol: ammonia (100:1) or benzene:acetone:ethanol:acetic acid (70:5:20:5) and visualized by UV light. The experimental elemental analyses agreed with the calculated.

5-Bromonicotinic acid, ethyl 5-bromonicotinate, 5-hydroxynicotinic acid and its ethyl ester were prepared according to [10, 11]. Nicotinic acid azide was used without further purification in subsequent preparations.

5-Bromonicotinic Acid Hydrazide. A mixture of 5 g of ethyl 5-bromonicotinate and 50 ml of hydrazine hydrate was boiled for 1 h. After cooling, the resulting precipitate was filtered off and recrystallized from water to give 4.05 g (90%) of 5-bromonicotinic acid hydrazide, mp 183-184°C. $C_6H_6BrN_3O$.

5-Hydroxynicotinic Acid Hydrazide. A mixture of 16.7 g of ethyl 5-hydroxynicotinate and 100 ml of 50% hydrazine hydrate was boiled for 1 h. The dark solution was evaporated to dryness and the solid residue was crystallized from DMF to give 11 g (72%) of hydrazide, dec. ≈ 216°C. $C_6H_7N_3O_2$.

5-Bromonicotinic Acid Azide (II). A solution of 4.5 g (0.021 mole) if 5-bromonicotinic acid azide in a mixture of 7 ml conc. HCl and 3 ml of water was cooled to 0 to —5°C and treated dropwise with a solution of 5.3 g (0.077 mole) of $NaNO_2$ in 7 ml of water. After the $NaNO_2$ addition, the reaction mixture was kept at 0°C for 0.5 h, the resulting voluminous precipitate was filtered off, and the filtrate was treated with a cold saturated solution of $NaHCO_3$ until no further precipitation occurred. The precipitated was separated, washed with ice water and dried in air to give 4.1 g (88%) of II, dec. ≈ 87°C.

5-Hydroxynicotinic Acid Azide (III). A solution of 11 g (0.072 mole) of 5-hydroxynicotinic acid hydrazide in a mixture of 17 ml of conc. HCL and 20 ml of water was cooled to 0 to —5°C, and a solution of 11.7 g (0.18 mole) of $NaNO_2$ in 20 ml of water was added very slowly dropwise at that temperature with stirring. During the course of the reaction according to the amount of $NaNO_2$, a white voluminous precipitated occurred. After addition of the $NaNO_2$ solution, the mixture was stirred for 0.5 h at 0°C and the precipitate was filtered off. The filtrate was treated with cold saturated $NaHCO_3$ solution until no more precipitation occurred. The precipitates were combined and dried in air to give azide III, 11.5 g (97%), dec. ≈ 138°C

General Method for Obtaining Amino Acid Derivatives of Substituted Nicotinic Acids (IV-XV). To a solution of 0.01 mole of L-amino acid in 10 ml of 1 N NaOH was added in portions 0.01 mole of azide I-III, maintaining the pH at 9-9.5 by periodic addition of 5 N NaOH. After complete solution, the azide solution was stirred at 20°C for 0.5 h and acidified to pH 4-4.5 with conc. HCl. The resulting precipitate was filtered off and recrystallized. The yields and properties of the compounds obtained are presented in Table 1.

TABLE 1. Amino Acid Derivatives of Nicotinic Acid

Compound	Yield, %	mp, °C	$[\alpha]_D^{20}$ (with 5.8 % HCl)	Empirical formula	IR spectrum, cm^{-1}
IV	75	194—195 Water	+18,8°	$C_{11}H_{12}N_2O_5$	
V	50	176—177 Aqueous MeOH	—5,2°	$C_{10}H_{10}N_2O_5$	3349 (NH) 1697 (COON) 1664 (CONH)
VI	53	196—198 Aqueous Alcohol	—126°	$C_9H_{10}N_2O_3S$	3285 (NH) 1717 (COOH) 1645 (CONH)
VII[a]	61	213—214 Isopropanol	—	$C_{11}H_{14}N_2O_3S$	3294 (NH) 1716 (COOH) 1648 (CONH)
VIII	56	169—171 Alcohol	—121,9°	$C_{11}H_{12}N_2O_3$	1710 (COOH) 1625 (CON)
IX[b]	73	185—186 Water	—8,6°	$C_{11}H_{11}BrN_2O_5$	3372 (NH) 1714 (COOH) 1646 (CONH)
X	65	180—181 Alcohol	—2,12°	$C_{10}H_9BrN_2O_5$	3298 (NH) 1736 (COOH) 1642 (CONH)
XI	48	177 Dilute Alcohol	—32,4°	$C_9H_9BrN_2O_3S$	3304 (NH) 1720 (COOH) 1645 (CONH)
XII	63	209—210 Alcohol	—	$C_{11}H_{13}BrN_2O_3S$	3290 (NH) 1716 (COON) 1648 (CONH)
XIII[c]	68	224—226 Water	—12,8°	$C_{11}H_{12}N_2O_6$	3278 (NH) 1719 (COOH) 1655 (CONH)
XIV	59	157—158 Water	—87,6°	$C_{11}H_{11}BrN_2O_3$	1736 (COOH) 1642 (COH)
XV[d]	89	220—221 Water	—	$C_{10}H_{12}N_2O_4$	3304 (NH) 1639 (CONH)

[a]Lit., [12] mp 213-214°C

[b]UV λ_{max} = 276 nm.

[c]UV λ_{max} = 289 nm. ^1H NMR, δ, ppm; 2.0-2.7 (4H, m, CH_2CH_2COOH); 4.60 (1H, m, CH(COOH)); 8.52 (1H, d, pyridine-2H); 8.80 (1H, d, pyridine-6H).

[d]UV λ_{max} = 288 nm. ^1H NMR, δ ppm: 1.95 (2H, m, CH_2); 2.40 (2H, m, CH_2CH_2); 3.43 (2H, m, CH_2COOH); 8.52 (1H, pyridine-2H); 8.40 (1H, q, pyridine-4H); 8.78 (1H, d, pyridine-6H).

TABLE 2. Pharmacological Activity of Amino Acid Derivatives of Nicotinic Acids

Compound	Dose, mg/kg	Antiamnestic effect: time passed in light compartmental chamber during reproduction, seconds	Antihypoxic effect: Prolongation of the life of the animal (in % compared to the control)		Orientational behavior carried out in open field	
			beaker hypoxia	hemic hypoxia	total result	degree of changes of result compared to control
IV		Control without amnesia			Control	
		103±10,6			11,8±2,5	1
		Control with amnesia				
		35±10,6				
	50	56,8±12,6	107±8,0	103±6,6	9,8±2,2	
	100		115±10,0	123±10,5*		0,8
V		Control without amnesia			Control	
		109±12,6			3,7±0,7	1
		Control with amnesia				
		57±5,4				
	50	80±7,9**	110±2,2**	108±17,3	7,1±1,7*	1,9
VI		Control without amnesia			Control	
		101±1,5			5,2±2,5	1
		Control with amnesia				
		65,8±16,7				
	50	72,8±12	130±4,4**	110±13,6*	6,3±4,2	1,2
VII		Control without amnesia			Control	
		118±1,8			7±1,4	1
		Control with amnesia				
		40,3±9,7				
	50	66±15*	68,7±4,4	94,8±4,2	15,4±1,8*	2,2
VIII		Control without amnesia			Control	
		101±1,5			5,2±2,5	1
		Control with amnesia				
		65,8±16,7				
	50	61,4±16,6	150±3,1**	89,5±8,9	9±3,8	1,7
IX		Control without amnesia			Control	
		103±10,6			11,8±2,5	1
		Control w. amnesia				
		35±10,6				
	50	46,4±12	96±8,2	92,8±8,9	8,4±1,9	0,7
X		Control without amnesia			Control	
		109±12,6			7±1,4	1
		Control with amnesia				
		57±5,4				
	50	81,4±12*	130±10,9	120±6,6	12,6±2,4*	1,8
XI		Control without amnesia			Control	
		120±0			5,2±2,5	1
		Control with amnesia				
		68±15				
	50	76±12	110±2,7*	100±4	5,2±3,7	1

TABLE 2 (continued)

Compound	Dose, mg/kg	Antiamnestic effect: time passed in light compartmental chamber during reproduction, seconds	Antihypoxic effect: prolongation of the life of the animal (in % compared to the control)		Orientational behavior carried out in open field	
			beaker hypoxia	hemic hypoxia	total result	degree of changes of result compared to control
XII		Control without amnesia			Control	
		92,8±6,0			3,7±0,7	1
		Control with amnesia 55±10,5				
	50	85,7±7,5	110±4,4	97±3,1	7,7±1,9*	2
XIII		Control without amnesia			Control	
		112,8±4.8			11,8±2,5	1
		Control with amnesia 43,8±11,3				
	50	110±6,1*	105±11,8	120±21,6	16,7±3,06	1,4
	100		121±6,6	127±16,1*		
XIV		Control without amnesia			Control	
		111±3,5			8,9±3,5	1
		Control with amnesia 41±7,5				
	50	56,8±9,9	116±7,8	70±8,8	7,6±2,6	0,8
XV		Control without amnesia			Control	
		112.8±4,8			11,8±2,5	1
		Control with amnesia 44,3±10,7				
	50	82,3±18,7			3,7±1,06*	0,3
	100		175±10,9*	134±19,9*		
Piracetam		Control without amnesia 87,0±10,5				
		17,5±6,9				
	300	80±13,9				
	500		130±30,2	115±15,4		
Deanol Aceglumate		Control without amnesia 106±7,1				
		Control with amnesia 54,6±17				
	50	81±13,1*				
	100		98±6,2			
	400			113±19		
Pikamilon (Nicotinoyl GABA)		Control without amnesia			Control	
		112,5±8,4			14,6±3,1	1
		Control with amnesia 45±11,3				
	50	58,8±5,9			13,5±3,2	0,9
	100		100±8,3	71,7±6,8		
Meclofenoxate		Control without amnesia				
		87±10,5				
		Control with amnesia 17,5±6,9				
	50	68,4±8,4*	108±7,2	122±16		

Notes. The asterisks indicated the significance of the results of the antiamnestic and an antihypoxic tests according to Wilcoxon—Mann—Whitney: one = $p \leq 0.05$; two = $p \leq 0.01$; for the Open Field by Student's method: one = $p \leq 0.05$ and two = $p \leq 0.01$.

EXPERIMENTAL (PHARMACOLOGY)

Pharmacological studies of the materials were carried out on non-hybrid white mice weighing 16-20 g. The materials were introduced intraperitoneally 30-60 min before the beginning of the experiment. Antiamnestic activity was studied under passive avoidance reaction conditions (PAR) [4]. The amnesia-inducing factor was a 50 mA electric shock of 0.2 s duration, which was administered through corneal electrodes directly after the training session. Regeneration of PAR was followed for 24 h after training. Antihypoxic properties were studied on two types of hypoxia: hemic and hypercapnia in a sealed chamber [9]. Hypoxia by hypercapnia in a sealed chamber was produced with the animals in 200 ml beakers with hermetically-closed lids. Hemic hypoxia was produced in the mice by subcutaneous injection of a 300 mg/kg dose of $NaNO_2$. The prolongation of survival of the animals was recorded. Orientational studies were carried out by the behavior of the animals tested under open field conditions, and myorelaxant effects by use of the rotating rod test [3]. Piracetam ("Polfa," PNR), meklofenoksat ("Germed," GDR, meclofenoxate), and demanol atseglyumat ("Merck-Clevent," deanol aceglumate) were used as comparative preparations.

Statistical workup of the results was carried out by calculation of the arithmetical means and their confidence intervals by the method of Student. For evaluation of the significance of the results also was used nonparametric criteria by the method of Wilcoxon—Mann—Whitney [5].

With regard to the antiamnestic properties of these amino acid derivatives of nicotinic acid, it was established that the highest capability of preventing PAR amnesia induced by electroshock, and of reliably increasing the time of residence of the mice in the light compartments during reproduction was characteristic of compounds in which the amino acid substituents were glutamic acid (XIII), GABA (XV, pikamilon) and aspartic acid (V, X). Compounds containing methionine (VII, XII) showed less activity in the dosages used, and the nicotinoyl derivatives of proline (VIII, XIV) and cystine (VI, XI) showed no antiamnestic properties (Table 2).

A study of the antihypoxic effects of the materials on the two types of hypoxia allowed us to establish that the most significant protective properties under the conditions of the beaker or hemic hypoxia was shown by compounds with aspartic acid (V, X), cysteine (VI, XI), glutamic acid (IV, VIII) or GABA (XV). The remaining materials showed no antihypoxic properties at the dosages used.

It should be noted that materials having an hydroxyl radical in position 5 of the pyridine ring (XIII, XV) have the most significant antiamnestic and antihypoxic properties. Conversely, the introduction of bromine into the molecule leads to a weakening of these properties (Table 2).

Compounds containing aspartic acid (V, X) and methionine (VII, XII) at doses of 50 mg/kg stimulated orientational-explorational behavior and motor activity under the 'open field' test, significantly increasing the change over the control by a factor of 1.9-2.2. The hydroxynicotinic acid derivative of GABA (XV), on the other hand, showed a corresponding lessening of the orientational-explorational behavior. All of the studied materials at the dosages used did not show myorelaxant effects in the rotating rod test. These studies permit the conclusion that the series of amino acid derivatives of nicotinic, 5-bromonicotinic, and 5-hydroxynicotinic acids contains substances possessing antiamnesic activity based on results from shock amnesia and PAR, and antiantihypoxic properties in hemic and beaker hypoxia, as well as the capacity of influencing orientational-explorational behavior and motor activity. The value and relationship of the antiamnestic and antihypoxic effects in the spectrum of pharmacological activity of these materials depends to a significant degree on the amino acid substituted on the molecule and their characteristics are determined to a large extent by the substituent in position 5 of the pyridine ring.

The highest antiamnestic and antihypoxic activity was shown by the hydroxynicotinoyl derivatives XII and XV [2]. According to their pharmacological profile, these materials are similar to the known nootropic preparations piracetam, deanol-aceglumenate, and others, and possess parallel advantages. Compound XIII also is significantly better in activity and latitude of antiamnestic and antihypoxic effects than piracetam and meclofenoxate. In contrast to the structure of the similar preparations nicotinoyl GABA (pikamilon) and deanol-aceglumate, XIII, incorporating a glutamic acid residue, has a wide spectrum of antihypoxic activity. These results indicate the potential for a search for materials with nootropic for materials with nootropic activity in the amino acid derivatives of nicotinic acid.

LITERATURE CITED

1. Inventor's Certificate No. 388554 (USSR). Otkrytiya, Izobret., Prom. Obraztsy, Tovarnye Znaki, No. 46 (1975).
2. Inventor's Certificate No. 1368314 (USSR). Otkrytiya, Izobret., Prom. Obraztsy, Tovarnye Znaki, No. 3, (1988).
3. T. A. Voronina, Yu. I. Vikhlyaev, L. I. Nerobkova, et al., Fenazepam [in Russian], Kiev (1982), pp. 87-169.
4. T. A. Voronina, T. L. Garibova, I. V. Khromova, and U. M. Tilekeeva, Farmakol. Toksikol., No. 3, 21-23 (1987).
5. E. V. Gubler, Computational Methods of Analysis and Recognition of Pathological Processes [in Russian], Moscow (1978), pp. 72-75.
6. L. A. Zhmurenko, S. A. Borisenko, O. M. Glozman, et al., Khim.-farm. Zh., No. 9, 49-55 (1980).
7. V. M. Kopelevich, I. A. Sytinskii, and V. I. Gunar, ibid., No. 5, 27-39 (1981).
8. G. I. Kuz'menko, L. E. Gotsulyakh, A. I. Gaidenko, et al., Farmakol. Toksikol., No. 1, 12-14 (1988).
9. U. M. Tilekeeva, T. A. Voronina, V. I. Kuz'min, et al., ibid., No. 1, 74 (1987).
10. J. B. Bachman and D. D. Micucci, J. Am. Chem. Soc., **70**, 2381 (1948).
11. M. Kawazu, T. Kanno, S. Saito, and M. Takaki, J. Med. Chem., **15**, 914 (1972).
12. K. U. M. Prasad, B. H. Gyer, and M. Sirsi, J. Indian Chem. Soc., **42**, 9 (1965).