SYNTHESIS AND PHARMACOLOGICAL INVESTIGATION OF 1-SUBSTITUTED 2-CYANIMINOPYRROLIDINES THAT ARE STRUCTURALLY RELATED TO PIRACETAM

D. B. Nilov, A. V. Kadushkin, V. G. Granik,
N. P. Solov'eva, V. V. Asnina, R. B. Parimbetova,
V. A. Parshin, and M. D. Mashkovskii

UDC 615.214: 547.745].012.1.07

One of the efficient ways of the search for compounds with high nootropic and antihypoxic activities is structural modification of the most important nootropic agent, piracetam [7]. Up to now there have been prepared many compounds that have the mentioned biological activity and differ from piracetam in the structure of fragments, mainly at positions 1 and 2 (see, for example, [3-6, 9-12]). In this investigation, during the search for new effective nootropic agents, we have studied a series of 2-cyaniminopyrrolidines that are structurally related to piracetam and its analogs and differ by the presence at position 2 of the pyrrolidine ring of a cyanimino group (instead of oxo [4, 11, 12] and substituted alkyl- or arylimino [10] or thio [3, 6] groups), that is, a group with a small volume that has distinct electron-accepting properties. In this case it is also important that the amidine fragment can relative easily been hydrolyzed [2], which, in the present case, in the organism may lead to transformation of the amindines under investigation to derivatives of pyrrolidone-2, to which belong piractam and a series of its most active analogs [13-15].

In our investigation, as starting compounds we have selected N-cyaniminopyrrolidine (I), which is easily prepared by reacting O-methylbutyrolactim with cyanamide [1], and the product of its alkylation with ethyl chloroacetate at the endocyclic NH group, 1-ethoxycarbonyl-2-cyaniminopyrrolidine (III), which we have described earlier [8].

The N-cyanimino analog of piracetam, 1-carbamidomethyl-2-cyaniminopyrrolidine (IV), is smoothly formed by reacting compound III with ammonia in methanol at room temperature. Obtained carbamide IV reacts easily with the diethyl acetals of dimethylformamide and dimethylacetamide with formation of the corresponding acylamidines (Va, b), in which the amidine group under mild conditions is converted to a formyl group by means of hydrolysis in aqueous acetic acid with formation of formyl derivative (VI). It should be noted that hydrolysis of the acylamidine group proceeds selectively and does not affect the N-cyanimino group. Investigation of reactions of acylamidines Va, b with arylhydrazines has shown that they proceed according to the same scheme as for the corresponding derivatives of pyrrolidone-2 [12]: reaction with phenylhydrazine does not stop at the first stage but goes with subsequent cyclization with formation of derivatives of 1,2,4-triazole (VIIIa, b). The first step in the process is transamination, which follows from the structure of product (VIII) from the reaction of acylamidine Va with the significantly less basic 2,4-dinitrophenylhydrazine. Cyclization of derivative VIII to the corresponding triazole failed.

It follows from comparison of some data on N-cyanimino derivatives obtained in the present work with corresponding results for pyrrolidones-2 that transformation of the lactim carbonyl to the N-cyanimino group leads to noticeable increase in reactivity of functional substituents at position 1 of the ring. Thus, condensation of piracetam with dimethylformamide diethyl acetal requires heating for 3-4 h in refluxing xylene [12] whereas its 2-cyanimino analog IV reacts under considerably milder conditions and much

---

Central Chemical Laboratory Buildings. All-Union Scientific Research Institute of the Chemical and Pharmaceutical Industry, Moscow. Translated from Khimko-Farmatsevticheskii Zhurnal, Vol. 27, No. 1, pp. 35-38, January, 1993. Original article submitted March 6, 1992.

0091-150X/93/2701-0043$12.50

TABLE 1. PMR Spectra of the Prepared Compounds*

Compound	Chemical shifts, δ, ppm				
	3-CH_2 (m)	4-CH_2 (quint.)	5-CH_2 (m)	N—CH_2 (s)	other signals
III	2,88	2,07	3,63	4,20	1,22 (t, CH_3—CH_2), 4,14 (q, CH_3 CH_2)
IV	2,85	2,04	3,61	3,94	7,23; 7,50 (NH_2)
VIa	2,96	2,18	3,76	4,42	9,10 (s, CH); 11,39 (s, NH)
VIIb	2,84	2,08	3,73	4,84	2,34 (s, CH_3); 7,62—7,64 (m, Ph)
VIII	2,90	2,09	3,66	4,25	7,70 (1H, 6-CH, $^3J_{6\text{-CH},5\text{-CH}}$=9,7 Hz); 8,38 (1H, d, s. with growth, 5-CH, $^4J_{3\text{-CH},5\text{-CH}}$=2,7 Hz); 8,85 (1H, s with growth 3-CH); 9,10 (1H, d, CH— NH, $J_{CH,NH}$=9,5 Hz); 11,25 (1H, d, HCNHNHAr); 11,60 (1H, s, NHNHAr).

*Solvent DMSO-d_6 for III, IV, VIIa, and VIII; DMF-d_7 for VIIb.

TABLE 2. Physico-Chemical Properties of the Prepared Compounds

Compound	Yield, %	MP, °C (solvent)	Empirical formula
IIa	51	163—6, propanol-2	$C_{12}H_{11}N_3O$
IIb	35	109—11, propanol-2	$C_{13}H_{13}N_3O_2$
IV	90	209—12, water	$C_7H_{10}N_4O$
Va	97	120—31, toluene	$C_{10}H_{15}N_5O$
Vb	89	99—101, tolune	$C_{11}H_{17}N_5O$
VIa	81	160—3, propanol-2	$C_8H_{10}N_4O_2$
VIb	60	151—3, propanol-2	$C_9H_{12}N_4O_2$
VIIa	87	137—41, benzene	$C_{14}H_{14}N_6$
VIIb	93	138—41, benzene	$C_{15}H_{16}N_6$
VIII	87	223—6, DMF	$C_{14}H_{14}N_8O_5$

faster (refluxing benzene, [10]). The cause of such a promotion of the condensation process is not entirely clear because the electron-accepting groups (C=O and =NCN) are substantially removed from the reaction center. At the same time also another reaction, transamination of acylamidines Va, be with arylhydrazines, confirm the grater reactivity of N-cyanimino derivatives. Comparison with acylamidines of the pyrrolidone series show that the latter react more difficultly with p-nitrophenylhydrazine than N-cyaniminoacylamidine Va with less basic 2,4-dinitrophenylhydrazine*.

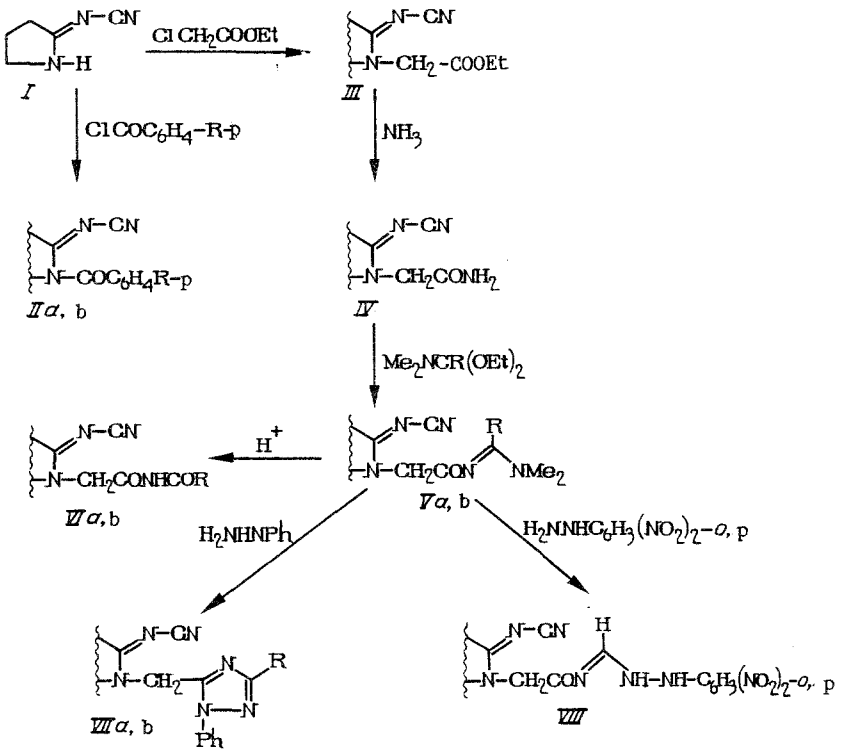
By acylation of the sodium salt of 2-cyaniminopyrrolidine I with benzoyl and p-methoxybenzoyl chlorides we have obtained corresponding acyl derivatives (Ia, b), which ar analogs of the nootropic preparation aniracetam [13].

The structure of the prepared compounds was confirmed by IR and PMR spectral data (Table 1). It should be noted that in the IR spectra of all the prepared N-cyanimino derivatives (when the spectrum is taken in paraffin oil) there is observed a doubling of the absorption band of the CN group, which disappears when the spectra are recorded in chloroform solution. In the NMR spectra only one set of signals is observed and thus the found doubling is probably caused by either the presence of two crystalline modifications or by the presence of two geometric isomers relative to the C=N bond in the solid state, which are easily interconvertible in solutions. (See scheme on following page.)

EXPERIMENTAL (CHEMICAL)

Mass spectra of the prepared compounds were taken on a Variant MAT-112 spectrometer, ionization voltage 50 eV, temperature of the ionization chamber 140°C. PMR spectra were recorded on a Varian XL-200 spectrometer with TMS as internal standard. Melting points were

*We note that acylamidine derivatives of the piracetam series generally failed to enter into a transamination reaction with 2,4-dinitrophenylhydrazine [12].



R=H(IIa, Va, VIa, VIIa); Me(Vb, VIb, VIIb); OMe(IIb)

determined on a Boetius hot stage. IR spectra were taken from dispersions in paraffin oil on a Perkin-Elmer 457 spectrometer.

Data of elemental analysis corresponded with calculated values. Physico-chemical properties of the prepared compounds are given in Table 2.

1-Benzoyl-2-cyaniminopyrrolidine (IIa). To a suspension of 0.5 g (22 mmole) of sodium in 30 ml of dry toluene is added 2.2 g (20 mmole) of N-cyanamidine I, the mixture is refluxed for 30 min, 3 ml of ethanol is added, refluxing is continued for 30 min, and solvent is distilled from the reaction mixture until the vapor temperature reaches 110°C. The mixture is cooled to 0°C, 2.8 g (20 mmole) of benzoyl chloride is added dropwise at such a rate that the temperature does not exceed 5°C, the mixture is stirred for 2 h, and allowed to stand overnight. The mixture is filtered, the filtrate is evaporated, the residue is triturated with isopropanol, and compound IIa is filtered off, M^+ 213.

1-(p-Methoxybenzoyl)-2-cyaniminopyrrolidine (IIb). Prepared in much the same way as compound IIa from cyanimidine I and p-metoxybenzoyl chloride.

1-Carbamidomethyl-2-cyaniminopyrrolidine (IV). Through a solution of 4.9 g (25 mmole) of compound III in 60 ml of methanol is bubbled in a stream of ammonia for 2 h, the mixture is cooled to 0-5°C, compound IV is filtered off, and washed with methanol. IR spectrum, ν_{max}: 3150, 3300 (NH_2), 2150, 2170 (CN), 1680 (CO).

1-[(N,N-Dimethylaminomethylene)carbamidomethyl]-2-cyaniminopyrrolidine (Va). To a suspension of 5.5 g (33 mmole) of IV in 50 ml of dry benzene is added at reflux temperature 6 ml (35 mmole) of dimethylformamide diethyl acetal and refluxing is continued till complete solution of the starting compound (about 10 min). The reaction mixture is evaporated under vacuum, the residue is triturated with hexane, and compound Va is filtered off. IR spectrum, ν_{max}: 2140, 2160 (CN), 1650 (CO).

1-[(N,N-Dimethylaminoethylidene-1)carbamidomethyl]-2-cyaniminopyrrolidine (Vb). Prepared in much the same way as compound Va from compound IV and dimethylacetamide diethyl acetal IR spectrum, ν_{max}: 2150, 2170 (CN), 1600-1640 (CO, C=C).

1[(N-Formylcarbamido)methyl-2-cyaniminopyrrolidine (VIa). A solution of 3.5 g (16 mmole) of Va in 20 ml of 70% acetic acid is stored at 20°C for 10 h, evaporated under vacuum, 10 ml of water and NaOH solution is added till pH 7, the precipitate is filtered off, and washed with water and isopropanol. M^+ 194. IR spectrum, ν_{max}: 3150 (NH), 2150, 2170 (CN).

TABLE 3. Pharmacological Activity of Piracetam Derivatives with Respect to Parameters of Anticonvulsive and Nootropic Activities

Compound	Dose, mg/kg by mount	Effect on convulsive activity						Effect on exposure to CRPM		Effect on hypoxic hypoxia	
		corazole		TSC (20 mg/kg)		bicuculline		latent period of entering the dark chamber, sec	time of residence in the dark chamber, sec	life time	
		latent period, min		latent period, min		mg/kg, iv	mg/kg, iv			in min	in %
		convulsion	deaths	convulsion	deaths	convulsion	deaths				
Control	—	2,7—0,3	5,2—0,3	62—1,3	73—3,0	10/10	10/10	123—19,5	51—7,0	27,3—0,9	—
IIa	50	2,2±0,2	8,0±0,7	60±1,5	90±4,0	—	—	158±13,0	10±2,4	—	
	100	3,0±0,2	7,2±0,4	65±1,3	80±3,0	6/12	6/10	145±13,2	12±3,0	24,3±1,6	0
IIb	50	2,5±0,3	7,0±0,8	60±1,3	96±4,0	—	—	164±12,0	17±4,2	—	
	100	4,0±0,8	9,2±0,5	64±1,3	82±1,5	6/10	7/10	153±10,0	22±4,0	32±2,0	18
III	50	2,3±0,1	5,0±0,3	62±1,5	80±1,5	—	—	168±11,0	6,0±2,2	—	
	100	3,2±0,3	6,6±0,7	70±2,0	100±5,0	6/10	6/12	162±12,0	10±2,3	27,4±1,3	0
IV	50	3,1±0,4	9,4±1,0	62±1,5	92±3,8	—	—	174±12,0	6,0±2,0	—	
	100	2,2±0,3	17,2±2,3	64±1,3	79±3,0	5/10	7/10	155±13,0	11±2,3	27,3±0,3	0
Va	50	3,1±1,2	8,1±1,6	68±1,3	95±4,0	—	—	117±16,0	63±10,0	—	
	100	3,1±1,0	9,3±1,3	85±1,6	112±4,0	2/10	8/10	153±10,0	24±6,5	34,0±1,8	26
Vb	50	2,1±0,2	4,7±0,4	72±1,6	85±5,0	—	—	115±14,0	36±6,0	—	
	100	3,7±0,4	7,5±0,4	56±2,0	112±4,0	10/10	10/10	150±10,0	22±4,3	28,0±1,2	0
VIa	50	4,3±1,2	9,7±0,5	74±1,7	87±5,3	—	—	114±17,0	40±9,6	30,0±1,3	11
	100	2,4±0,1	15,4±0,8	57±2,2	115±4,0	3/10	9/10	138±15,0	49±10,0	32,0±1,8	18
VIb	50	2,2±0,2	6,0±0,6	70±1,6	90±2,0	—	—	120±18,0	42±8,2	—	
	100	2,2±0,2	6,3±0,5	82±1,5	110±5,0	4/10	9/10	112±12,0	46±11,0	26,0±1,3	0
VIIa	50	3,7±0,3	6,6±0,5	66±1,5	63±1,5	—	—	70±33,0	35±12,0	—	
	100	4,5±0,3	7,2±0,7	52±1,2	72±1,0	9/10	9/10	87±20,0	32±5,2	31,6±3,2	19
VIIb	50	3,3±0,4	6,2±0,7	68±1,5	65±1,3	—	—	80±23,0	40±12,0	—	
	100	4,2±0,8	6,0±0,8	54±1,3	76±2,0	10/10	10/10	95±20,0	32±6,6	31,0±1,7	15
V	50	4,0±0,4	7,0±0,8	68±1,7	80±5,0	—	—	126±20,0	74±10,0	—	
Piracetam	100	4,2±0,4	8,5±0,8	55±1,2	72±5,0	8/10	8/10	118±16,0	48±9,0	24,0±0,7	0
	300	2,4±0,6	5,5±0,3	64±1,5	78±4,0	10/10	10/10	178±13,2	13±3,6	31,0±4,0	15

1[(N-Acetylcarbamido)methyl]-2-cyaniminopyrrolidine (VIb). Prepared in much the same way as VIa and Va.

1-[(1-Phenyl-1,2,4-triazolyl-5)methyl]-2-cyaniminopyrrolidine (VIIa). A mixture of 4.2 g (20 mmoles) of compound Va, 2.5 g (22 mmole) of phenylhydrazine, and 20 ml of AcOH is stirred at 20°C for 30 min, evaporated under vacuum, the residue is triturated with water, and compound VIIa is filtered off. M^+ 266.

1-[(1-Phenyl-3-methyl-1,2,4-triazolyl-5)methyl]-2-cyaniminopyrrolidine (VIIb). Prepared in much the same way as compound VIIa from compound Vb.

N-(2,4-Dinitrophenylamino)-N′-(2-cyaniminopyrrolidinyl-1-acetyl)formamidine (VIII). A mixture of 1.1 g (5 mmole) of compound Va, 1 g (6 mmole) of 2,4-dinitrophenylhydrazine, and filtered, and washed with isopropanol. M^+ 374.

EXPERIMENTAL (PHARMACOLOGICAL)

Experiments were carried out in white mongrel male mice weighing 18-20 g with respect to parameters that characterize nootropic, antihypoxic, and anticonvulsive activities.

The anticonvulsive activity was assessed by the effect on convulsions evoked by bicuculline (0.45 and 0.9 mg/kg, iv), corazole (125 mg/kg, sc), strychnine (3 mg/kg, sc), and thiosemicarbazide (TSC, 20 mg/kg, sc). The presence of anticonvulsant activity was judged by lengthening of the latent period of an onset of convulsions and the time of death of the animals.

The antihypoxic activity was studied with the model of hypoxic hypoxia in mice. The animals were placed in hermetically sealed vessels with a volume of 250 ml. In parallel experiments with a control group we determined the lifetime of the animals in minutes.

The nootropic properties of the compounds were studied in experiments in mice with the output of the conditional reflex of passive movement (CRPM) by the method proposed by Bures and Buresova (1963). For a period of 180 sec we recorded the residence time in light and dark chambers. At the end of the exposure time the animal received a single shock with an electric stream via an electrode floor in the dark chamber. The conditional reflex of passive movement produced in this way was repeated by testing after 24 h.

The acute toxicity (LD_{50}) in the case of oral administration was studied in experiments with mice with 10 animals in each group.

The compounds under investigation were administered orally at doses of 1/10 and 1/20 of the LD_{50}. Pharmacological studies were carried out in comparison with piracetam. Obtained data are listed in Table 3.

It can be seen from Table that piracetam in case of oral administration at a dose of 300 mg/kg improves the development and keeping of CRPM in mice, has medium antihypoxic activity, and has anticonvulsive properties.

Its most closely related analog, cyanimino derivative IV, approaches it in activity. At doses of 50 and 100 mg/kg the compound prolongs the latent period of entering into the dark chamber (LPEDC) by 30%, has a tendency of reducing corazole and bincuculline convulsions, and odes not show antihypoxic activity (see Table 3).

Among the tested compounds the most expressed effect on the development of CRPM is shown by acyl derivatives of 2-cyaniminopyrrolidine, which are analogs of aniracetam (compounds IIa, b) and the carbethoxymethyl derivative (compound III). At doses of 50 and 100 mg/kg by mount these compounds increase on average by 28% the latent period of getting into the dark chamber and shorten by 73% the residence time in the dark chamber (see Table 3). Compounds IIa, b and III, and also IV show anticonvulsive activity and decrease by 40-60% the number of animals with convulsions and death from bicuculline and increase the latent period of death in the case of administration of TSC (see Table 3).

These properties, but to a considerably lesser degree, are noticed in amidine derivatives (compounds Va, b).

Derivatives of 2-cyaniminopyrrolidine having a formyl moiety in the molecule (compounds VIa, b) differ mainly with respect to the effect on convulsions evoked by bicuculline and TSC, and in triazole-containing compounds VIIa, b the antihypoxic activity is more pronounced (see Table 3).

The investigations that we have carried out have shown that all the compounds are of low toxicity; their LD_{50} is mote than 1000 mg/kg in the case of oral administration.

The investigations have also shown that 2-cyanimino derivatives of pyrrolidine, being structural analogs of piracetam and aniracetam, have elements of nootropic activity and, moreover, antihypoxic and anticonvulsive activities.

Modification of the structure of piracetam by means of substituting 2-cyaniminopyrrolidine for pyrrolidone-2 and subsequent substitution at the nitrogen atom of the pyrrolidine ring led to a small but certain shift in the activity spectrum of the piracetam derivatives to the side of anticonvulsive properties.

The obtained result make it possible to conclude that research in the series of derivatives of piracetam and its imino derivatives is advisable and promising.

LITERATURE CITED

1. E. N. Dozorova, N. P. Solov'eva, V. V. Chistyakov, et al., Khim.-Farm. Zh., No. 10, 1187-1193 (1987).
2. V. G. Granik, Usp. Khim., 52, No. 4, 669-703 (1983).
3. V. G. Granik, T. V. Golovko, R. G. Glushkov, et al., Khim.-Farm. Zh., No. 10. 1186-1193 (1989).
4. T. A. Gudasheva, R. U. Ostrovskaya, S. S. Trofimov, et al., Khim.-Farm. Zh., No. 11, 1322-1329 (1985).
5. A. V. Kadushkin, T. V. Golovko, V. G. Granik, et al., Khim.-Farm. Zh., No. 10, 1193-1197 (1989).
6. A. V. Kadushkin, T. V. Goloko, V. G. Granik, et al., Khim.-Farm. Zh., No. 11, 1301 (1989).
7. M. D. Mashkovskii, Drugs [in Russian], Moscow (1988).
8. D. B. Nilov, A. V. Kadushkin, S. G. Kalistratov, et al., Khim.-Farm. Zh., No. 9-10, 63-68 (1922).
9. R. Ya. Popova, T. A. Gudasheva, S. S. Trofimov, et al., Khim.-Farm. Zh., No. 12, 1439-1445 (1983).
10. T. V. Stezhko, V. G. Granik, R. G. Glushkov, et al., Khim.-Farm. Zh., No. 3, 2990-297 (1984).
11. T. V. Stezhko, V. G. Granik, R. G. Glushkov, et al., Khim.-Farm. Zh., No. 7, 823-828 (1984).

12. T. V. Stezhko, V. G. Granik, A. V. Kadushkin, et al., Khim.-Farm. Zh., No. 10, 119801203 (1984).
13. V. I. De Noble, S. I. Repetti, L. W. Gelpke, et al., Pharmacol. Biochem. Behav., 24, No. 4, 1123-1135 (1986).
14. C. L. Murray and H. C. Fibiger, Psycopharmacology, 89, No. 3, 378-381 (1986).
15. B. I. R. Nicolaus, Drug Develop. Res., 2, No. 5, 463-474 (1982).