SYNTHESIS AND ANXIOLYTIC ACTIVITY OF 4-AMINO-2,6-DIMETHYLNICOTINIC ACID ESTERS AND AMIDES

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The class of 2- and 4-aminopyridinecarboxylic acid derivatives contains compounds possessing anxiolytic, anticonvulsant, and antiamnesic activity [1 – 3]. This study was devoted to the search for new effective anxiolytics among 4-amino-2,6-dimethylnicotinic acid esters and amides.

In the first step, 4-chloro-2,6-dimethylnicotinic acid ethylate (I) [4] reacted with the corresponding amines to yield 4-amino-2,6-dimethylnicotinic acid ethyl esters (IIa – IIf, III) in the form of bases or salts. Subsequent saponification of ethylate I led to acid IV, which was converted into 2,6-dimethylnicotinic acid amide (V) under the action of thionyl chloride and ammonia. Heating compound V with amines led to amides VI.

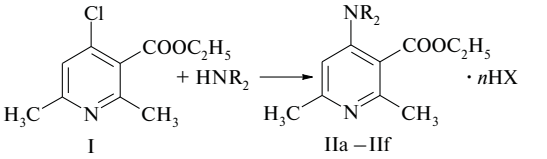
The IR and ^{1}H NMR spectra of the synthesized compounds (Table 1) agree with the proposed structures. The IR spectra if compounds IIa – IIc contain intense absorption bands due to carboethoxy groups at 1716 – 1695 cm^{-1}. In addition, the spectrum of compound IIc displays the stretching vibrations of free amino groups at 3341 and 3202 cm^{-1}.

The ^{1}H NMR spectra of compounds IIa – IIf, III, and VIb show the signals from 5-H protons of the pyridine cycle, 2- and 6-methyl groups, and substituents in position 4. The proposed structures were also confirmed by the results of elemental analyses. The synthesized compounds were characterized with respect to anxiolytic activity in the conflict situation test.

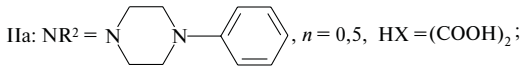
EXPERIMENTAL CHEMICAL PART

The IR spectra were recorded on a Perkin-Elmer Model 457 spectrophotometer. The ^{1}H NMR spectra were measured on a Bruker AC-250 spectrometer using TMS as the internal standard.

4-(N′-Phenylpiperazino)-2,6-dimethylnicotinic acid ethylate hemioxalate (IIa). A mixture of 4.26 g (0.02 mole) of acid I [4] and 6.48 g (0.04 mole) of 4-phenylpiperazine in 20 ml of anhydrous xylene was boiled for 12 h, evaporated in vacuum, and filtered. The residue was washed with ether and treated with anhydrous oxalic acid (1.39 g) to obtain 5.75 g of hemioxalate IIa.

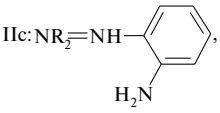


I IIa – IIf

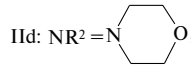


IIa: NR^2 = , n = 0,5, HX = $(COOH)_2$;

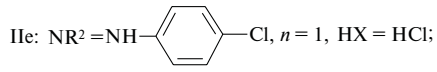
IIb: $NR^2 = NHCH_2CH_2N(CH_3)_2$, n = 2, HX = HCl;



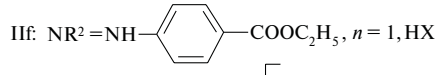
IIc: NR_2^2 = NH– , n = 1, HX = HCl;



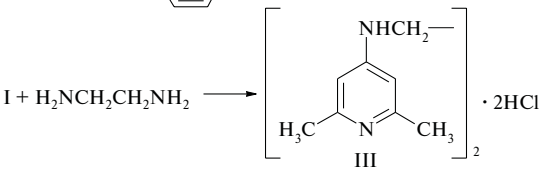
IId: NR^2 = , n = 1, HX = $(COOH)_2$;



IIe: NR^2 = NH– –Cl, n = 1, HX = HCl;



IIf: NR^2 = NH– –$COOC_2H_5$, n = 1, HX = HCl



I + $H_2NCH_2CH_2NH_2$ → · 2HCl

III

4-(4-Carboethoxyanilino)-2,6-dimethylnicotinic acid ethylate hydrochloride (IIf). A mixture of 2.55 g (0.012 mole) of acid I and 1.65 g (0.01 mole) of anesthesine in 20 ml of anhydrous toluene was boiled for 15 h in the presence of 2.21 g (0.022 mole) triethylamine. Then 20 ml of anhydrous DMF was added and the homogeneous reaction

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TABLE 1. Yields and Physicochemical Characteristics of the Synthesized Compounds

Compound	Yield, %	M.p., °C (solvent)	Empirical formula	IR spectrum: ν_{max}, cm^{-1}	^1H NMR spectrum in DMSO-d_6: δ, ppm
IIa	66.9	187 – 188 anhydr. ethanol	$C_{20}H_{25}N_3O_2 \cdot 0.5(COOH)_2$	1716(C=O) 1654(C=O) 1613	1.32 (t, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.37 (q, 2H, CH_2), 5.2 (ms, 1H, COOH), 6.8 – 7.5 (m, 5H, C_6H_5)
IIb	50.0	238 – 239 anhydr. ethanol	$C_{14}H_{23}N_3O_2 \cdot 2HCl$	1702(C=O) 1665(C=O)	1.30 (t, 3H, CH_3), 2.42 [2s, 6H, 2, 6-$(CH_3)_2$], 2.68 [s, H, $N(CH_3)_2$], 2.92 – 3.50 (m, 4H, CH_2CH_2), 4.35 (q, 2H, CH_2), 7.05 (s, 1H, 5-H)
IIc	97.0	171 – 172 acetone	$C_{16}H_{19}N_3O_2 \cdot HCl$	3341 (NH_2) 3202 1695(C=O) 1652(N–H) 1637(N–H)	1.42 (t, 3H, CH_3), 2.45 (s, 3H, 6-CH_3), 2.78 (s, 3H, 2-CH_3), 4.42 (q, 2H, CH_2), 6.35 (s, 1H, 5-H pyridine), 6.45 – 7.2 (m, 4H, C_6H_4), 9.5 (bs, 1H, NH)
IId	34.7	173 – 174 ethanol	$C_{14}H_{20}N_2O_3 \cdot (COOH)_2$	…	1.32 (t, 3H, CH_3), 2.44, 2.45 [2s, 6H, 2, 6-$(CH_3)_2$], 3.37, 3.69 (2m, 8H, morpholine), 4.32 (q, 2H, CH_2), 5.06 (bs, 1H, COOH), 7.06 (s, 1H, 5-H pyridine)
IIe	94.0	200 – 201 2-propanol	$C_{16}H_{17}ClN_2O_2 \cdot HCl$	…	
IIf	94.0	194 – 195 2-propanol	$C_{19}H_{22}N_2O_4 \cdot HCl$	…	1.25, 1.38 (2t, 6H, 2 · CH_3), 2.51 (s, 3H, 6-CH_3), 2.65 (s, 3H, 2-CH_3), 4.32 (q, 4H, 2 · CH_2), 7.05 (s, 1H, 5-H pyridine), 7.45, 8.05 (m, 4H, C_6H_4), 10.3 (s, 1H, NH)
III	36.0	> 290 anhydr. ethanol	$C_{22}H_{30}N_4O_4 \cdot 2HCl$	…	[in CCl_4]: 1.37 (t, 6H, 2 · CH_3), 2.37 (s, 6H, 2 · CH_3), 2.58 (2s, 6H, 2 · CH_3), 3.44 (m, 4H, CH_2CH_2), 4.35 (q, 4H, 2 · CH_2-ethyl), 6.24 (s, 2H, 2 · 5-H pyridine); 7.92 (bs, 2H, 2 · NH)
V	59.4	192 – 193 acetone	$C_8H_9ClN_2O$	…	…
VIa	62.7	170 – 171 ethyl acetate	$C_{12}H_{17}N_3O_2$	…	…
VIb	56.0	279 – 280 (decomp.) (ethanol – acetone)	$C_{18}H_{22}N_4O \cdot 2HCl$	3428(NH_2) 3363 (NH_2) 1676(C–O) 1643	2.55 [s, 6H, 2, 6-$(CH_3)_2$], 3.52, 4.00 (2m, 8H, 2 · CH_2CH_2), 5.60 (bs, 1H, COOH), 7.25 – 7.40 (m, 5H, C_6H_5), 7.25 (s, 1H, 5-H pyridine), 8.10 (s, 1H, NH), 8.45 (s, 1H, NH)
VIc	50.0	191 – 192 anhydr. ethanol–acetone	$C_{15}H_{17}N_3O \cdot HCl$	…	…

mass was boiled for 35 h, cooled, and diluted with water. The precipitated oil was extracted with benzene, and the extract was dried over $MgSO_4$ and filtered. Finally, the filtrate was treated with HCl-saturated ether to obtain 1.75 g of compound IIf.

4-(2-Dimethylamionoethylamino)-2,6-dimethylnicotinic acid ethylate dihydrochloride (IIb). A mixture of 2.13 g (0.01 mole) of acid I and 0.88 g (0.01 mole) of N,N-dimethylethylenediamine in 10 ml of butanol was boiled for 17 h. The precipitate was washed with ether and converted into dihydrochloride to obtain 1.7 g of compound IIb.

4-(2-Amionoanilino)-2,6-dimethylnicotinic acid ethylate hydrochloride (IIc). Compound IIc was synthesized using a procedure analogous to that described above for compound IIb.

4-Morpholino-2,6-dimethylnicotinic acid ethylate oxalate (IId). Compound IId was synthesized using a procedure analogous to that described above for compound IIa.

4-(4-Chloroanilino)-2,6-dimethylnicotinic acid ethylate hydrochloride (IIe). Compound IIe was synthesized using a procedure analogous to that described above for compound IIb.

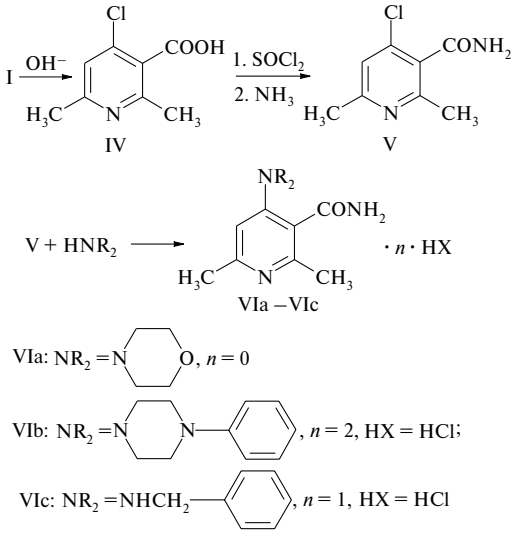
1,2-Bis-(2,6-dimethyl-3-carboethoxypyridinyl-4-amino)ethane dihydrochloride (III). A mixture of 2.13 g (0.01 mole) of acid I and 0.6 g (0.01 mole) of ethylenediamine in 15 ml of butanol was boiled for 15 h, cooled, diluted with ether, and treated with HCl-saturated ether to obtain 1.75 g of compound III.

4-Chloro-2,6-dimethylnicotinic acid amide (V). A mixture of 10 g (0.054 mole) of 4-chloro-2,6-dimethylnicotinic acid and 70 ml of $SOCl_2$ was boiled for 1 h and evaporated in vacuum. The residue was diluted with 50 ml of anhydrous benzene and distilled in vacuum. The residue was dissolved in 250 ml of anhydrous benzene and the solution was bubbled with gaseous NH_3 for 2 h. To this solution was added 43 ml of concentrated NH_4OH solution and the mixture was allowed to stand overnight. The precipitate was separated by filtration to obtain 0.4 g of compound V; 5.9 g of compound V was isolated from benzene solution.

4-Morpholino-2,6-dimethylnicotinic acid amide (VIa). A mixture of 1.0 g (0.0054 mole) of amide V and 2 g of morpholine was heated to ~ 100°C for 9 – 10 h, cooled, and diluted with 100 ml of ether, The precipitate was separated by filtration and dissolved in water. The solution was saturated with solid NaCl. Then the product was extracted with chloroform, the extract was dried over $MgSO_4$ and filtered, and the filtrate was evaporated in vacuum to obtain 0.8 g of compound VIa.

4-(N′-phenylpiperazino)-2,6-dimethylnicotinic acid dihydrochloride (VIb). A mixture of 1.85 g (0.01 mole) of amide V and 3.24 g (0.02 mole) N-phenylpiperazine in 10 ml of anhydrous DMF was heated to 100°C for 17 h, diluted with ~150 ml of water, and filtered. The filtrate was washed with 50 ml of ether and the precipitated base was converted into dihydrochloride VIb.

4-Benzylamino-2,6-dimethylnicotinic acid amide hydrochloride (VIc). Compound VIc was synthesized proceeding from amide V and benzylamine using a procedure analogous to that described above for compound VIa.



EXPERIMENTAL BIOLOGICAL PART

The anxiolytic activity was studied using a model conflict between drinking motivation and electric-pain irritation [5 – 7]. The experiments were performed during three days on white mongrel male rats weighing 200 – 250 g. On the first day, the test animals were deprived of water. On the next day, the reflex of taking water from a feeder was elaborated by placing each test animal into experimental boxes (27.5 × 87.5 × 40 cm) with stainless steel feeder fountains and metal electrode floor connected to a stabilized current source. The animals investigated the chamber for some time, found the feeder, and began to drink. At this instant, a weak current (~50 μA, which is below the sensitivity threshold) was passed through the feeder – floor circuit. Thus, taking water was not punished and the number of takes was determined by the drinking motivation. On the third day, the trained animals were placed into the experimental chamber again, but an increased voltage corresponding to a dc current of 1 mA was applied between feeder and floor 10 sec after the first water take, so that each next water take was punished. The anxiolytic effect was evaluated by a reliable increase in the punished water takes in the test group relative to the untreated control. The reference drug was tranquilizers medazepam and phenazepam.

TABLE 2. Anxiolytic Activity of 4-Amino-2,6-dimethylnicotinic Acid Esters and Amides

Compound	Dose, mg/kg	Number of punished water takes, $M \pm m$
Control	–	15.96 ± 5.28
IIa	5	15.75 ± 6.4
	10	17.15 ± 9.53
	20	21.86 ± 18.18
	40	11.43 ± 5.53
IIb	20	33.58 ± 14.59*
	40	27.67 ± 21.62
IIc	–	–
IId	–	–
IIe	40	60.25 ± 38.42*
III	10	18.00 ± 12.10
	20	17.5 ± 11.45
VIa	–	–
VIb	10	22.00 ± 15.68
	20	29.4 ± 16.43
	40	22.5 ± 20.25
VIc	–	–
Medazepam	10	39.42 ± 9.19*
Phenazepam	1	114.72 ± 28.45**

Notes. Difference from control reliable for * $P < 0.05$, ** $P < 0.01$.

The experimental results (Table 2) showed that most of the studied substances produced anxiolytic action comparable to that of medazepam but inferior to the effect of phenazepam.

REFERENCES

1. O. M. Glozman, L. A. Zhmurenko, S. A. Borisenko, et al., *Fiziol. Aktivn. Veshch.*, No. 8, 89 – 92 (1976).
2. R. A. Akhundov, L. A. Zhmurenko, O. M. Glozman, et al., *Khim.-Farm. Zh.*, **20**(1), 48 – 50 (1986).
3. R. A. Akhundov, O. M. Glozman, L. A. Zhmurenko, et al., *Abstracts of Papers. All-Union Symp. "Chemistry, Pharmacology, and Clinical Use of Neuroleptics"* [in Russian], Tallinn (1986), pp. 23 – 24.
4. A. Michaelis, *Liebigs Ann. Chem.*, **366**, 324 – 407 (1909).
5. J. R. Vogel, D. Beer, and D. E. Clody, *Psychopharmacology*, **21**, 1 – 7 (1971).
6. J. A. Engel, P. Egbe, S. Liljeguist, and B. Soderpalm, *Pharmacol. Toxicol.*, **64**, 429 – 433 (1989).
7. G. M. Molodavkin and T. A. Voronina, *Eksp. Klin. Farmakol.*, **58**(2), 54 – 56 (1995).