SYNTHESIS AND ANTIAGGREGATIONAL ACTIVITY FOR THROMBOCYTES OF NITRILES AND AMIDES OF 6-METHYLNICOTINIC ACIDS

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During work previously undertaken [1, 2] to search for biologically active substances among nitriles and amides of 2-arylamino-5-carboxy(carbethoxy)-6-methylnicotinic acids, we have synthesized new compounds in this series and studied their acute toxicity, and antiinflammatory and antiaggregational activity.

Synthesis of 2-aryl(Ar)amino-5-carbethoxy-6-methylnicotinonitriles (Ia-d, Table 1) was carried out by heating the corresponding amine in alcohol with 5-carbethoxy-6-methyl-2-chloronicotinonitrile. Final products of this reaction are obtained in good yields after 4-6 h of refluxing, while compounds Ia, b may be obtained following incubation of the starting solution for 24 h at room temperature.

2-Aryl(Ar)amino-5-carboxy-6-methylnicotinamides (IIa-d) are formed by reacting nitriles Ia-g with alcoholic potassium hydroxide. In this case, under gentle conditions there occurs the simultaneous saponification of the ester and nitrile groups. To study antiaggregational activity, we prepared sodium salts of 2-arylamino-5-carboxy-6-methylnicotinamides (IIIa-d) by reacting compounds IIa-d with aqueous sodium hydroxide.

Structures of compounds synthesized were confirmed by IR and PMR spectral data.

EXPERIMENTAL (CHEMICAL)

IR spectra were taken on a UR-20 instrument from pastes in vaseline mull (compound I) and in CCl_4 (for compound II); PMR spectra were obtained on a PYa-2310 (60 MHz), internal standard HMDS, solvent—DMSO-d_6. TLC was performed on Silufol UV-254 plates in the system benzene—chloroform—acetone (10:9:1) for compound I, and in ethyl acetate for compound II. Elemental analysis data for C, H, N, Br corresponded to values calculated.

2-Aryl(Ar)amino-5-carbethoxy-6-methylnicotinonitriles(Ia-d). A solution of 2.25 g (10 mmoles) 5-carbethoxy-6-methyl-2-chloronicotinonitrile and 15 mmoles arylamine in 25 ml of ethanol was refluxed for 4-6 h, or left for 24 h at 20°C (for Ia, b). The precipitate was filtered and crystallized from aqueous ethanol. IR spectrum, ν_{max}, cm^{-1}: 1690-1710 (C = O, COOEt), 2230-2250 (CN), 3340-3400 (NH). PMR spectra, δ, ppm: 7.58-9.32 s (1H, NH), 8.16-8.42 S [1H, C(4)], 6.89-7.41 m (aromatic protons), 4.15-4.28 q (2H, CH_2 in COOEt), 2.42-2.75 s [3H, Me at C(6)], 2.10-2.52 s (3H, Me in R), 1.22-1.35 t (3H, Me in COOEt).

TABLE 1. Characteristics of Synthesized Compounds

Compound	Mp, °C	Yield, %	*Rf*	Empirical formula
Ia	171—172	64	0,70	$C_{16}H_{15}N_3O_2$
Ib	149—150	73	0,77	$C_{17}H_{17}N_3O_2$
Ic	120—121	62	0,74	$C_{18}H_{19}N_3O_2$
Id	193—194	83	0,69	$C_{16}H_{14}BrN_3O_2$
IIa	273—275	65	0,72	$C_{14}H_{13}N_3O_3$
IIb	269—270	67	0,75	$C_{15}H_{14}N_3O_3$
IIc	281—282	58	0,78	$C_{16}H_{16}N_3O_3$
IId	270—271	88	0,84	$C_{14}H_{12}BrN_3O_3$

Notes. Ar = Ph (a), 4-MeC_6H_4 (b), 2,4-$Me_2C_6H_3$ (c), 3-BrC_6H_4 (d).

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TABLE 2. Pharmacologic Properties of Compounds IIIa-d

Compound	Acute toxicity, LD_{50}, mg/kg	Conc. of compound, mg/ml	Thrombocyte aggregation, %	Inhibition of aggregation, %
IIIa	899 (866—934)	2,25	50,4±2,27 $P<0,01$	49,2
IIIb	400 (383—417)	1,00	56,7±3,21 $P<0,01$	42,9
IIIc	268 (248—292)	0,67	38,3±2,93 $P<0,01$	61,3
IIId	347 (315—383)	0,87	40,0±3,16 $P<0,01$	59,6
Papaverine hydrochloride	27 (24—31)	0,07	70,5±0,32 $P<0,01$	29,1
Control: NaCl	—	0,60	99,6±0,41	—

Note. In parentheses — limits of variation.

2-Aryl(Ar)amino-5-carboxy-6-methylnicotinamides (IIa-d). A soluton of 10 mmoles of compounds Ia-d and 2.8 g (50 mmoles KOH) in 20 ml ethanol was refluxed 10-20 min, and left for 15 h at 20°C. It was then poured into 100 ml water, neutralized with 50% AcOH, and the precipitate was filtered and crystallized from a mixture of DMFA—H_2O. IR spectrum, ν_{max}, cm^{-1}: 1635-1655 (C = O, $CONH_2$), 1660-1685 (C = O, COOH), 3180-3220 and 3380-3420 (NH_2, $CONH_2$), 3340-3380 (NH), 3550-3600 (OH). PMR spectrum, δ, ppm: 8.88-11.32 s (1H, OH), 7.75-8.58 s (1H, NH), 8.05-8.42 [1H, C(4)], 7.62-8.28 br s (2H, $CONH_2$), 6.93-7.25 m (aromatic protons), 2.52-2.68 m [3H, Me at C(6)], 2-18-2.55 s (3H, Me in R).

Sodium Salts of 2-Arylamino-5-carboxy-6-methylnicotinamides (IIIa-d) were prepared by dissolving 10 mmoles of the corresponding acid in 20 ml of an aqueous solution of 0.4 g (10 mmoles) NaOH. The solution was filtered and evaporated.

EXPERIMENTAL (PHARMACOLOGICAL)

The biologic activity of compounds IIIa-d was assessed from data on acute toxicity, and antiaggregational activity toward thrombocytes.

Acute toxicity was determined in white mice of both sexes weighing 16-20 g, by intravenous administration [3]. Antiaggregational activity was studied using the photometric method of Born [4] with platelets from dog plasma, and was expressed as percent decrease in optical density. Thrombocyte aggregation was induced by ADP at a dose of 0.05 mg/ml plasma. As a standard from comparison, we used a known drug having antiaggregational activity, papaverine hydrochloride [5]. All compounds were tested at concentrations equal to $^1/_{10}\ LD_{50}$, calculated according to the weight of the animal.

Acute toxicities showed that the mean lethal does (LD_{50}) of compounds IIIa-d varies considerably, from 268 (IIIc) to 899 mg/kg (IIIa), and is 10 or more times greater than for papaverine (27 mg/kg). When studying the effects of compounds on the aggregational properties of thrombocytes, it was found that all displayed high antiaggregational activity, suppressing aggregation by 42.9-61.3% (Table 2) and exceeding that of the standard by 1.5-2.1 fold.

Because there has recently been increased interest in compounds having both antiaggretional and antiinflammatory activity, we decided to study compounds IIIa-d, as well as previously obtained compounds of this series [1, 2], for antiinflammatory activity. We also studied the antiinflammatory activity of compounds Ia-d. This activity was tested in white rats weighing 160-220 g using the model of acute inflammatory edema, induced by subplantar injection into a hind paw of 0.1 ml of a 1% aqueous carrageenan solution. The volume of the inflamed foot was measured oncometrically 4 h following injection of the phlogogen [6]. As a comparison standard for antiinflammatory activity, we chose orthophen. All compounds studied were injected intraabdominally at a dose of 50 mg/kg, and orthophen — at 10 kg/kg, 1 h prior to administration of carrageenan. Control animals were given an equivolume amount of 2% starch gel.

It was found, as a result of the studies, that compounds Ia-d and IIIa-d do not possess antiinflammatory activity. Among compounds previously synthesized, significant antiinflammatory activity was found only for 2-(4'-bromoanilino)5-carboxy-6-methylnicotinamide, which decreased swelling of the inflamed paw by 39.3% at a dose of 50 mg/kg, but was inferior to orthophen in potency. It has previously been reported that the sodium salt of this compound has antiplatelet aggregational activity, inhibiting aggregation by 39.3% and having low toxicity (LD_{50} equal to 448 mg/kg) [1].

Thus, from a search among 2-arylamino-5-carboxy-6-methylnicotinamides, compounds were found that have high antiaggregational activity and low toxicity. In addition, one of the compounds tested showed both antiaggregational and antiinflammatory activity. All this indicates the promise of further work with 2-arylamino-5-carboxynicotinamides for use in medical practice.

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SYNTHESIS AND IMMUNOTROPIC ACTIVITY OF DERIVATIVES OF PYRIMIDINES

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The production of immunomodulating agents has important significance for modern immunology and practical medicine [9], since it reveals the possibility of the correction of the immunity in a series of pathological states of man and animals [4, 8]. In recent years, a whole series of natural and synthetic compounds possessing immunomodulating properties has been isolated and investigated. In spite of the large number of agents able to show an immunomodulating influence, the search for preparations with the purposeful immunostimulating or immunosuppressing effect is continuing [13, 16, 23].

Derivatives of pyrimidine pertain to promising classes of immunomodulators, whereby the presence of antioxidant properties in some of them (methyluracil, oxymetacil) is considered to be an important link in the mechanism of their immunomodulating action [6].

Among 22 studies derivatives of pyrimidine [5], the most active stimulators of the phagocytic activity of leukocytes and macrophages in vivo and in vitro proved to be deriva-

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