not significantly alter femoral artery resistance whereas compound 2 reduced it at a dose of 0.1 mg/kg. Total peripheral resistance was increased by these compounds. Femoral artery resistance was also significantly reduced by compound 4. Moreover, at a dose of 0.1 mg/kg it somewhat reduced total peripheral resistance whereas it was increased at a dose of 1 mg/kg. Compound 6 increased total peripheral resistance (at a dose of 0.1 mg/kg by 45%).

No significant changes in the substances' effect on systemic AP or other cardiac function parameters were observed when the oxygen in position 2 of the tetrahydropyrimidine ring was replaced by sulfur.

The acute toxicity tests of the examined compounds showed that they all have a low level of toxicity except compound 5 whose average lethal dose was 500 mg/kg. The LD_{50} for the remaining compounds was greater than 1000 mg/kg.

In comparing the effect that the tested tetrahydropyrimidine derivatives had on hemodynamic parameters to a known vasodilator from derivatives of 1,4-dihydropyridine-phenytoin, which exhibits a certain chemical affinity to the examined substances, we found that the former compounds have a less pronounced effect on system AP and flow rate in the coronary and femoral vessels and elicit a smaller significant change in the resistance of these vascular regions. Our resultant data demonstrate that the continued search for vasodilatory substances among the tetrahydropyrimidines would seem to be warranted.

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SYNTHESIS AND ANTIMONOAMINE OXIDASE ACTIVITY OF 1-(2,4-DIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-6-CARBONYL)-2-(SUBSTITUTED BENZYL)HYDRAZINES

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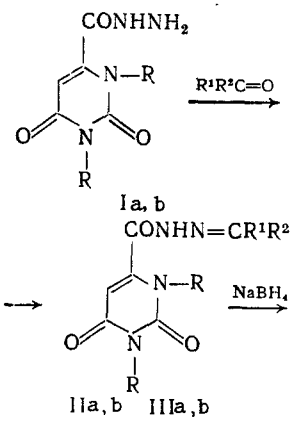
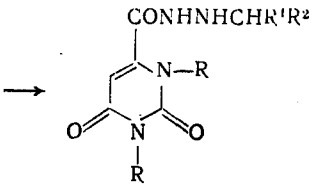
We have previously demonstrated [2] that 1-(4-pyrimidinoyl)-2-benzylhydrazines exhibit antimonoamine oxidase activity (anti-MAO activity) which depends on the nature and number of pyrimidine ring substituents. As a continuation of our search for effective monoamine oxidase inhibitors and our study of the biological activity–structure relationship, we synthesized hydrazine derivatives of orotic and 1,3-dimethylorotic acids which, in contrast to the earlier examined derivatives [2], have substituents in the benzyl fragment.



V. Kapsukas Vilnius University. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 23, No. 3, pp. 287-290, March, 1989. Original article submitted January 12, 1988.



IVa, b; Va, b

R = H (Ia—Va), Me (Ib-Vb); R^1 = H (IIa, b; IVa, b), Me (IIIa, b; Va, b)
$R^2 = C_6H_4OH$-*o* (II—V).

The acylhydrazones II and III were synthesized by the condensation of hydrazones I with salicyl aldehyde or with 2-oxyacetophenone. The hydrazines IV and V were obtained by reducing the hydrazones II and III with sodium borohydride. The experiments showed that the hydrazines IVa, b, and Vb are formed with satisfactory yields when the reaction takes place in an alkaline medium (pH 9.86) and when the reducing agent is added gradually to the reaction mixture. The hydrazines IVa and Vb were separated by acidification of the alkaline solution with conc. HCl to pH 4.0-5.0. Hydrazine IVb is readily soluble in water and does not precipitate upon acidification of the reaction mixture. After distilling off the solvent we were not able to separate IVb because it is unstable upon heating in an acid medium. We did succeed in separating hydrazine IVb by neutralizing the reaction mixture with conc. HCl followed by water distillation. During the synthesis of compounds IVa, b, and Vb, the hydrazone IIIa was not reduced to hydrazine Va. It was obtained at a satisfactory yield by reacting $NaBH_4$ with hydrazine IIIa in a mixture of DMPA and water (4:1).

Hydrazines IV and V are colorless crystalline substances which are more readily soluble than the corresponding hydrazones in ordinary organic solvents and water. The PMR-spectra of compounds IVa and b do not have a methine group proton signal in the 8.48-8.58 ppm region which is characteristic of the starting hydrazones IIa and b, but they have a new signal of methylene protons in the 4.16-4.41 ppm region. The PMR-spectra of the hydrazones IIIa and b were found to have a methyl proton signal located next to a double bond in the 2.40-2.59 ppm region. A new methine proton signal appears in the spectra of the hydrazines Va, b in the 4.29-4.78 ppm region that divides into a quartet because of the spin-spin reaction with the methyl protons, but the signal of the methyl protons (1.19-1.51 ppm) breaks up into a doublet and shifts toward the stronger fields in comparison to the methyl proton signals in the spectra of the starter hydrazones IIIa, b.

EXPERIMENTAL CHEMICAL

The progress of the reactions and the purity of the resultant compounds were controlled by TLC on Silufol UV-254 plates in a EA-MeOH system (10:1). Detection was by UV-light and iodine. PMR-spectra were recorded on a BS487C Tesla spectrometer (80 MHz, Czechoslovakia) at 33°C. The solvent for IIa-Va was d_6-DMSO, and CF_3COOH for compounds IIb-Vb. HMDS was the standard. The found element analysis values corresponded to the calculated values.

Orotic hydrazide (Ia) was synthesized by method [6], and 1,3-dimethylorotic hydrazide (Ib) was synthesized by method [2].

Orotic 2-Oxybenzylidene Hydrazide (IIa). A 1.32 g (0.012 mole) portion of salicyclic aldehyde was added to a solution of 1.7 g (0.01 mole) of hydrazide Ia in 120 ml of DMPA heated to 80°C. The mixture was stirred for 4 h at that temperature and then cooled. The precipitate was filtered off, the filtrate was concentrated in a vacuum, and the additional amount of IIa was separated. The combined precipitates were recrystallized.

1,3-Dimethylorotic 2-Oxybenzylidene Hydrazide (IIb). A 1.98 g (0.01 mole) portion of hydrazide Ib was dissolved in 40 ml of boiling ethanol to which 1.32 g (0.012 mole) of salicylic aldehyde was added. The solution was boiled for 6 h and left in a refrigerator for 8 h. The precipitate was filtered off and recrystallized.

α-Methyl-2-oxybenzylidene Hydrazines of Orotic and 1,3-Dimethyl Orotic Acids (IIIa, b). A 0.01 mole portion of hydrazine Ia, b was dissolved in boiling water and 1.63 g (0.012 mole) of 2-oxyacetophenone was added upon stirring. The mixture was boiled for 6 h, then cooled. The precipitate was filtered off and recrystallized.

TABLE 1. Characteristics of the Synthesized Compounds

Comp.	Yield, %	mp, °C	Empirical formula
IIa	70	<300(decomp.)	$C_{12}H_{10}N_4O_4$
IIb	95	250–1	$C_{14}H_{14}N_4O_4$
IIIa	56	300 (decomp.)	$C_{13}H_{12}N_4O_4$
IIIb	54	230—1	$C_{15}H_{16}N_4O_4$
IVa	51	246—7	$C_{12}H_{12}N_4O_4$
IVb	50	268—9	$C_{14}H_{16}N_4O_4$
Va	55	230—1	$C_{13}H_{14}N_4O_4$
Vb	47	170—1	$C_{15}H_{18}N_4O_4$

*Compounds recrystallized: IIa from DMPA; IIIa from DMPA–ethanol; IIb, IIIb, IVb, and Vb from ethanol; Va from 50% ethanol.

TABLE 2. Anti-MAO Activity of Tested Compounds

Compound	In vivo activity upon reaction with L-dopa in mice		Inhibition of rat liver MAO in vitro		
	dose, mg/kg	inhibition, %	dose of inhibitor	inhibition of oxidative deamination, %	
				tyramine	serotonin
IVa	20	25,0±2,6	$1,10^{-3}$	22,9±1,8	25,0±5,2
	50	48,0±3,5	$1,10^{-4}$	16,0±2,2	22,2±5,2
	60	30,0±1,77	$1,10^{-5}$	12,0±1,8	5,7±2,3
			$1,10^{-6}$	8,2±2,2	—
IVb	50	22,5±2,6	$1,10^{-3}$	22,0±3,1	29,3±4,9
	60	38,0±5,3	$1,10^{-4}$	15,2±2,7	9,7±0,3
	100	32,0±2,8	$1,10^{-5}$	13,3±2,2	6,12±1,9
			$1,10^{-6}$	9,35±0,8	—
Va	50	20,0±2,6	$1,10^{-3}$	15,8±2,0	31,5±4,3
	60	30,0±0	$1,10^{-4}$	7,8±1,8	18,2±5,3
	100	27,0±3,2	$1,10^{-5}$	5,0±1,8	15,7±4,5
			$1,10^{-6}$	2,6±0,2	10,8±0
Vb	50	23,3±1,77	$1,10^{-3}$	19,6±3,1	35,32±6,0
	60	30,0±0	$1,10^{-4}$	15,0±5,3	11,4±1,0
	100	27,0±1,08	$1,10^{-5}$	9,2±2,2	3,6±1,7
			$1,10^{-6}$	7,4±2,6	—
Nialamide	30	67,0±2,6	$1,10^{-4}$	46,4±5,3	—
	50	80,0±1,06	$1,10^{-5}$	17,0±0,08	—
	60	70,0±0	$1,10^{-6}$	2,0±0,14	—

1-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidine-6-carbonyl)-2-(2-oxybenzyl)hydrazine(IVa, b) and 1-(1,3-Dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-6-carbonyl)-2-(α-methyl-2-oxybenzyl)-hydrazine (Vb). A 0.3 g (0.008 mole) portion of $NaBH_4$ was added in small amounts over a period of 3 h to 0.002 mole of hydrazone IIa, b and IIIb in 80 ml (60 ml in the case of IIb and IIIb) of a buffer solution [0.1 N $Na_2B_4O_7 \cdot 10H_2O$–0.1 N NaOH (1:1), pH 9.86] with stirring while maintaining a temperature of 15-20°C. The mixture was stirred for 4 h and left to stand for 12 h. The mixture was acidified with conc. HCl to pH 5.0 (neutralized to pH 7.0 in the case of IVb and Vb) and cooled. The hydrazine IVa precipitate was filtered off and recrystallized. In the case of IVa and Vb the precipitate was vacuum-evaporated and the dry residue was treated with abs. ethanol and the ethanol solution was concentrated in a vacuum and cooled. The precipitate was filtered off and recrystallized.

1-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidine-6-carbonyl)-2-(α-methyl-2-oxybenzyl)hydrazine (Va). A 0.3 g (0.008 mole) portion of $NaBH_4$ was added over a period of 1.5 h in small portions with stirring to a suspension of 0.58 g (0.002 mole) of hydrazone IIIa in 60 ml of a DMPA–H_2O (4:1) mixture while maintaining a temperature of 15-20°C. The mixture was stirred for 4 h and left to stand for 12 h. The precipitate was filtered off, the filtrate was neutralized with conc. HCl, and the solvent was vacuum-distilled. The dry residue was recrystallized.

Data on the compounds are given in Table 1.

EXPERIMENTAL PHARMACOLOGICAL

The anti-MAO activity of compounds IV and V was tested in vivo and in vitro. In the first case that activity was tested by reacting L-dopa with the test substances in mice [5]. Four hours after the oral administration of the compounds to white mice weighing 20-25 g, L-dopa was administered ip at a dose of 200 mg/kg. The compounds' anti-MAO activity was judged by the mice's response to L-dopa. Nialamide was used as the standard of comparison.

In the second case a homogenate of rat liver tissue was used as the source of the enzyme. Tyramine and serotonin were used as the substrate. MAO activity in the homogenates was judged by the amount of ammonia formed in the course of the enzyme reaction. Probe incubation, fixation, and ammonia assay with the aid of Nessler's reagent was carried out by method [3]. The isothermic distillation of ammonia was performed by a modified Conway method [4]. The test compounds were used at concentrations of $1 \cdot 10^{-3}$-$1 \cdot 10^{-6}$ M in a probe. MAO inhibition was presented in percent of the control experiments (without an inhibitor).

Acute toxicity of the examined compounds was tested on white mice weighing 18-25 g upon a single oral administration of the preparations in the form of a suspension with a 1% starch mucilage. LD_{50} was calculated by the Litchfield and Wilcoxon method as modified by Ross [1]. All of the compounds were found to be comparatively low in toxicity (LD_{50} > 1000 mg/kg).

As can be seen from Table 2, all of the examined compounds IV and V exhibit activity both in the in vitro and in vivo experiments. The compounds have about the same in vitro activity as Nialamide, although they are less active than the latter in the in vivo experiments. There was practically no change in their antimonoamine oxidase activity when the structure of the tested compounds' benzyl fragment was altered by introducing an oxy group in position 2 of the benzene ring.

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