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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME ORGANO-PHOSPHORUS DERIVATIVES OF RUBIDOMYCIN WITH DI[2-CHLOROETHYL]AMINE GROUPS

L. D. Protsenko, A. B. Shapiro, V. M. Ovrutskii, V. I. Suskina, L. S. Vasil'eva, L. K. Denisova, N. I. Sharykina, and I. G. Kudryavtseva

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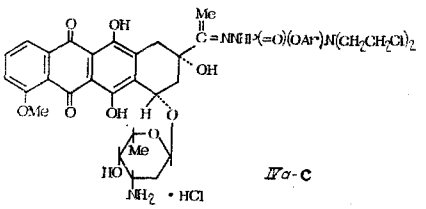
The antitumor antibiotic rubidomycin (I) is known to be effective in the treatment of acute leukosis [5]. A series of papers has recently appeared which describes attempts to chemically modify rubidomycin in order to reduce its cardiotoxicity and widen its spectrum of action [5, 9]. A group of phsophorylated chloroethylamines, known for their antiblastic activity, are the hydrochloride salts of the aryl esters of hydrazido-di(2-chloroethyl)amido-phosphoric acids (II) [1, 4].

It was of interest to study the reaction between I and II in order to obtain the phosphorylated derivatives of the hydrazones (IVa-c) containing cytotoxic groups, and to study their toxicity and antitumor action. To carry out this reaction, the hydrochloride salt II was first converted to the corresponding base (III); the reaction between I and III was conducted at room temperature in methanol—chloroform solution.



IVa: Ar = Ph; IVb: Ar = C_6H_4Br-p; IVc Ar = C_6H_4Me-p.

The hydrazones IVa-c were red crystalline substances, soluble in water and alcohols, and insoluble in benzene, ether, and petroleum ether (Table 1).

The pharmacological properties of IVa-c were compared with those of I and II. A change in toxicity was expected because the difference in the toxic parameters of I (LD_{50} 28.6 g/kg) and II (LD_{50} 500 mg/kg) is much more than an order of magnitude [3, 4]. It was also assumed that there was a possibility of an increase in selectivity of the antitumor action of IVa-c in comparison with I, a preparation with a wide spectrum of antitumor activity [3], and II which has a definite selectivity of antitumor action [4].

The test compounds displayed less toxicity, antitumor and antileukemic activity than rubidomycin and at the same time significantly inhibited leucosis La.

Institute of Chemical Physics of the Academy of Sciences of the USSR, Moscow Region. Kiev Scientific Research Institute of Pharmacology and Toxicology Ministry of Health of the Ukrainian SSR. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 19, No. 10, pp. 1199-1202, October, 1985. Original article submitted September 25, 1984.

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TABLE 1. Organophosphorus Derivatives of Rubidomycin with Di(2-chloroethyl)amine Groups IVa-c

Compound	Yield, %	mp, °C	Found, %			Empirical formula	Calculated, %			R_f	IR spectra, ν_{max}, cm^{-1}				
			Cl	N	P		Cl	N	P		NH	NH	N=C	P→O	P–OAr
IVa	73,8	175 (decomp.)	12,15	6,28	3,71	$C_{37}H_{44}Cl_3N_4O_{11}P$	12,40	6,53	3,61	0,76	3390	3420	1620	1205	1115
IVb	89,0	170 (decomp.)	11,05	5,90	3,85	$C_{37}H_{43}BrCl_3N_4O_{11}P$	11,36	5,98	3,30	0,82	3400	3480	1645	1210	1120
IVc	85,0	172 (decomp.)	12,60	6,75	3,95	$C_{38}H_{46}Cl_3N_4O_{11}P$	12,21	6,43	3,55	0,85	3410	3485	1615	1200	1110

TABLE 2. Antitumor Activity of the Hydrazones of Rubidomycins IVa and b

Compound	Dose,* mg/kg	Retardation of tumor growth, %		Relative weight of spleen, g
		sarcoma 45	Geren's carcinoma	
I	1,7 (intraperitoneally)	50,0	...	...
II	200 (subcutaneously)	Stimulation of tumor growth by 10%	18,2—79,6	0,8—1,0
IVa	20 (subcutaneously)	30,0	16.0	0,5
	30 (subcutaneously)	...	21,8	0,4—0,5
IVb	20 (subcutaneously)	4,0	14.0	0,6

*Six injections per day.

EXPERIMENTAL (CHEMICAL)

Infrared spectra were taken on a Perkin-Elmer-325 (Sweden) in the range 4000-400 cm^{-1}: samples were prepared as pellets with KBr. TLC was carried out on Silufol UV-254 plates (ChSSR) in $CHCl_3$—MeOH—water (13:6:1) solvent system.

Hydrazone Hydrochloride from the Phenyl Ester of Hydrazido-N,N-di(2-chloroethyl)amidophosphoric Acid and Rubidomycin (IVa). The hydrochloride of the phenyl ester of hydrazido-di(2-chloroethyl)amidophosphoric acid (1.7 g; 0.004 mole) was suspended in 20 ml of $CHCl_3$, and from a micropipette was added dropwise with mixing, 0.51 g (0.005 moles) of Et_3N. To the reaction mixture was then added 100 ml of ether, the precipitated $Et_3N \cdot HCl$ filtered off, and the solution evaporated to dryness at 35°C. The oily residue was dissolved in 50 ml of MeOH, and a solution of 2.25 g (0.004 mole) of rubidomycin in 75 ml of MeOH added, followed by 0.2 ml of AcOH. Formation of the hydrazone was checked by TLC. After 4 days, the MeOH was evaporated to 3/4 volume in vacuo at 35°C. To the concentrated solution of IVa was added 100 ml of ether to yield a red oil, which crystallized on standing. The finely-divided precipitate was filtered off and washed with ether to give 2.25 g of IVa as a red powder. The mother liquor was evaporated in vacuo at 35°C, the oily residue dissolved in 15 ml of MeOH, and 60 ml of ether added to give an additional 0.35 g of IVa. Total yield of IVa, 2.6 g (73.8%).

Compounds IVb and c were prepared in the same way.

EXPERIMENTAL (BIOLOGICAL)

Tests were carried out using 30 white nonpedigree mice, 130 rats, 100 DBA/2 mice, and 50 C57Bl/6 mice of both sexes, bred at the Central Nursery of the Academy of Medical Sciences of the USSR. The mice weighed 20-30 g, the rats, 100-120 g. The animals were maintained on a standard food ration, and slaughtered by breaking the neck while narcotized with ether.

The toxicities of the hydrazones of the rubidomycins IVa-c were determined using nonpedigree mice; solutions of the compound in physiological NaCl solution were injected subcutaneously and intraperitoneally. The LD_{50} was determined by the method described in [2, 10]. The antitumor activity of the compounds was studied using the following tumors: melanoma B-16, sarcoma 45, and Geren's carcinoma (the percentage retardation in tumor growth was used to assess activity). The antileukosis activity was studied using hemocytoblastoma La, lymphoid leukemia L-1210, and lymphocytic leukemia P-388 (the average life-span was used as a measure of activity; the coefficient of retardation of tumor growth was used as a kinetic criterion κ* and indicates how much the leukosis process was retarded compared to the control [7, 8].

The structural modification of I decreased its toxicity on subcutaneous injection by 3 and 4 times (IVb and IVa, respectively) and by 12-28 times on intraperitoneal injection. The test compounds were 4-5 times more toxic than the hydrochloride II. The LD_{50} (in mg/kg) of compounds I, II, and IVa-c are respectively: 28, 6 [3], 500, 125, and 98 (subcutaneously), and 5, 6, 140, 75 and 121 (intraperitoneally).

The antitumor activity of compounds IVa and b was less than that of I (sarcoma 45) and the hydrochlorides II (Geren's carcinoma) (Table 2). The retardation of tumor growth was

TABLE 3. Antileukemic Effect of the Hydrazones IVa-c

Tumor	Preparation	Dose, mg/kg	Increase in average life-span of animals in comparison with control, %	Retardation of growth, %
La	IVa	50	125	0.65
	IVb	25	100	0,35
	IVc	50	98	...
	I	1.4	18	0.5
L-1210	IVa	50	20	...
	IVb	25	15	...
	I	0,8	47,5	...
P-388	IVa	50	6	...
	IVb	25	18	...
	III	0.8	61,7	...

Note. Compounds I and IVa-c were injected daily for 7 days starting one day after transplantation of the tumor. Compound I was injected on the day of transplantation and after 4, 8, and 12 days.

less than the criterion of significance (50%) [7]. Both substances, as well as rubidomycin, were inactive against melanoma B-16. Moreover, compounds IVa and b, together with compound I, have the ability to depress the process of blood-cell production; the spleens of treated animals weighed 0.4-0.6 g, compared with 0.8-1.0 g for animals treated with compound II.

Against models of leukosis L-1210 and P-388, compounds IVa and b were much less active than compound I; life-span was increased by 25% (Table 3).

In a kinetic study of the development of hemocytoblastoma La, compounds IVa and c exhibited greater antileukemia activity than rubidomycin (Table 3).

Thus, modification of rubidomycin led to a decrease in the toxicity of its derivatives. However, there was also a decrease in antitumor and antileukemic activity, except for leukosis La, against which the activity of the modified rubidomycin exceeded that of the original compound.

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