SYNTHESIS AND BIOLOGICAL ACTIVITY OF HETERYLACETO- AND -THIOACETOHYDRAZIDE DERIVATIVES

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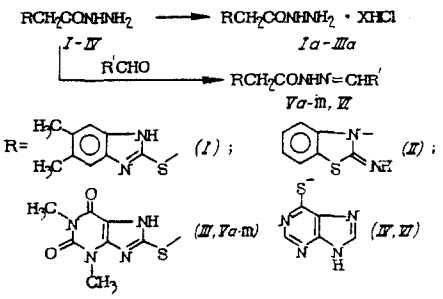
In view of the high antitumor activity of natulin (p-N'-methylhydrazino-methyl-N-isopropylbenzamide) [1], the mode of action of which differs from that of the alkylating agents, it was of interest to synthesize and examine the cytostatic activity of acid hydrazides derived from nitrogen heterocycles, namely 5,6-dimethylbenzimidazolyl-2-thioaceto- (I), 2-iminobenzothiazolyl-3-aceto- (II), and 1,3-dimethylxanthinyl-8-thioacetohydrazide (III). On heating with hydrochloric acid in ethanol, (I-III) were converted into their water-soluble mono- and dihydrochlorides (Ia-IIIa; Table 1).

Bearing in mind the importance of purines as antitumor agents (6-mercaptopurine and 6-thioguanine) [2, 4], 1,3-dimethylxanthinyl-8-thioaceto- (III) and purinyl-6-thioacetohydrazide (IV) were reacted with p-N,N-di-(2-chloroethyl)aminobenzaldehyde in ethanol at pH 2.5 to give the hydrazones (Vℓ, VI; Table 1).

Benzimidazole and its derivatives have been used as models in the study of many virological problems [10]. We have previously obtained some hydrazones of benzimidazolyl-2-thioacetic acid [3, 6, 8].



R' = 2-HOC_6H_4(Va), 4-ClC_6H_4 (Vb), 4-$O_2NC_6H_4$ (Vc), 2-HO-5-BrC_6H_3 (Vd), 4-$(CH_3)_2NC_6H_4$ (Ve), C_6H_5CH=CH (Vf), C_6H_5CH = CBr(Vg), 4-$O_2NC_6H_6CH$=CCl (Vh), 3,7-dimethyl-2,6-octadienal (Vi), 5-nitrofurfurylidene (Vj), 5-nitrofurfuryl-2-allylidene (Vk), $(ClC_2H_4)_2NC_6H_4$ (Vℓ, VI), 2,3-dihydroindolon-2-ylidene-3(Vm), X = 1 (IIIa), 2(Ia, IIa).

Continuing these investigations, (III) has been reacted with aromatic and aliphatic aldehydes and isatin to give the hydrazones (Va-k, m) (Table 1).

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained on a Perkin-Elmer-325 in KBr disks. The purity of the products was checked by thin layer chromatography on Silufol-254 plates (Czech SSR), visualized in UV or with iodine vapor. The physicochemical properties of the compounds obtained are shown in Table 1. The elemental analyses were in agreement with the calculated values.

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TABLE 1. Physicochemical Properties of Compounds Obtained

Compound	Yield, %	mp, °C	Empirical formula	IR spectrum, cm^{-1}				
				ν CO		ν NH		
Ia	90	194—5	$C_{11}H_{14}N_4OS \cdot 2HCl$			1661,	3335,	3185
II	96	178—9	$C_9H_{10}N_4OS$			1644,	3307,	3095
IIa	90	183—5	$C_9H_{10}N_4OS \cdot 2HCl$					
III	92	280—81	$C_9H_{12}N_6O_3S$	1718,	1670	1650,	3275,	3205
IIIa	95	215—7	$C_9H_{12}N_6O_3S \cdot HCl$					
Va	93	252—3	$C_{16}H_{16}N_6O_4S$	1714,	1668	1650	3209,	3120
Vb	80	269—70	$C_{16}H_{15}ClN_6O_3S$	1714,	1689	1668,	3224,	3125
Vc	81	256—7	$C_{16}H_{15}N_7O_5S \cdot 2H_2O$	1712,	1694	1663,	3212,	3115
Vd	49	259—60	$C_{16}H_{15}BrN_6O_4S$	1714,	1697	1668,	3210,	3120
Ve	90	245—6	$C_{18}H_{21}N_7O_3S$	1713,	1698	1665,	3200,	3150
Vf	96	248—9	$C_{18}H_{18}N_6O_3S$	1712,	1698	1660,	3210,	3125
Vg	88	222—3	$C_{18}H_{17}BrN_6O_3S$	1711,	1696	1666,	3190,	3120
Vh	90	245—6	$C_{18}H_{16}ClN_7O_5S$	1715,	1698	1668,	3225,	3172
Vi	49	265—6	$C_{19}H_{26}N_6O_3S$	1714,	1698	1666,	3205,	3125
Vj	90	248—9	$C_{14}H_{13}N_7O_6S \cdot H_2O$	1713,	1696	1665,	3200,	3130
Vk	96	242—3	$C_{16}H_{15}N_7O_6S$	1720,	1700	1670,	3220,	3130
Vℓ	85	122—4	$C_{20}H_{23}Cl_2N_7O_3S$	1715,	1700	1668,	3210,	3152
Vm	84	303—4	$C_{17}H_{15}N_7O_4S$	1716,	1697	1669,	3249,	3210, 3125
VI	85	218—20	$C_{18}H_{19}Cl_2N_7OS$			1686,	3308,	3080

Note. Compounds (I-IIIa) were crystallized from ethanol, (IIIa, b, c, e, f, h, j, k, m) from DMF, (Vd, i) from aqueous DMF, (Vg) from dioxane, and (VI) from propan-2-ol.

5,6-Dimethylbenzimidazolylthioacetohydrazide Dihydrochloride (Ia). To 2.5 g (10 mmole) of 5,6-dimethylbenzimidazolyl-2-thioacetohydrazide [6] in 100 ml of 70% ethanol was added 2 ml of 36% hydrochloric acid, and the mixture boiled until all the solid had dissolved. The solvent was removed under reduced pressure, and the residue washed with ether.

2-Iminobenzothiazolyl-3-acetohydrazide Dihydrochloride (IIa). To 2.36 g (10 mmole) of ethyl 2-iminobenzothiazolin-3-acetate in 30 ml of ethanol was added 1 g of hydrazine hydrate, the mixture boiled for 5-7 min, cooled, and the solid filtered off to give 2-iminobenzothiazolyl-3-acetohydrazide (II). The salt (IIa) was obtained as for (Ia).

1,3-Dimethylxanthinyl-8-thioacetohydrazide Hydrochloride (IIIa). To a suspension of 2.8 g (10 mmole) of methyl 1,3-dimethylxanthinyl-8-thioacetate [7] in 20 ml of methanol was added 1 g of hydrazine hydrate, the mixture boiled for 7-9 min, cooled, and the solid filtered off to give 1,3-dimethylxanthinyl-8-thioacetohydrazide (III), R_f 0.26 (benzene—acetone, 65:35), visualized with iodine vapor. The salt (IIIa) was obtained as for (Ia).

Purinyl-6-thioacetohydrazide (IV) was obtained as described in [5].

1,3-Dimethylxanthinyl-8-thioacetohydrazide Hydrazones (Va-k, m). To a suspension of 3.02 g (10 mmole) of (III) in 30-50 ml of methanol (or ethanol) was added 10-11 mmole of the appropriate aldehyde or isatin, and the mixture boiled for 2-3 h (3-4 h in the case of isatin), cooled, poured into water, and the solid filtered off.

1,3-Dimethylxanthin-8-ylthioaceto-N'-[p-N,N-di-(2-chloroethyl)aminobenzylidene]-hydrazide (Vℓ). To a solution of 2.5 g (10 mmole) of P-N,N-di(2-chloroethyl)aminobenzaldehyde in 100 ml of ethanol was added a few drops of 36% HCl to pH 2.5, and 2.8 g (10 mmole) of (III). The mixture was boiled until all the solid had dissolved, cooled, diluted with 150 ml of water, and the solid filtered off and washed with petroleum ether. Compound (VI) was obtained similarly.

EXPERIMENTAL (BIOLOGY)

Antitumor activity was assessed in mongrel white mice and Wistar rats, and in mice of strain DVA/2. The models of tumor growth employed were the Pliss' lymphosarcoma, sarcoma 180, and leukemia L-1210.

The experimental conditions and results are shown in Tables 2-4.

Acute toxicities were determined in mice weighing 16-18 g. The test compounds were administered intraperitoneally, and the LD_{50} values were calculated by the method of Litchfield and Wilcoxon.

TABLE 2. Toxicity Data for Hydrazides Derived from Benzimidazole, Benzothiazole, and Purine

Compound	Solvent	LD_{50} (mice), mg/kg
Ia	0.9 % soln. NaCl	290 (219—382,8)
IIa	The same	1500 (1171—1920)
IIIa	DMSO	2350(1600—3400)
Vℓ	The same	2820(2400—3200)
VI	» »	1290(1100—1500)

TABLE 3. Effects of Benzimidazole and Benzothiazole Hydrazides on the Growth of Experimental Tumors

Compound	No. of animals in group	Dose, mg/kg	% change in tumor growth	% primary recovery	% deaths
		Pliss' lymphosarcoma			
Ia	7	100	0	0	0
	7	80	19,8	0	0
	7	64	+16,5*	14	0
IIa	7	400	0	0	0
	7	275	41,9	0	0
	7	200	0	0	0
		Sarcoma 180			
Ia	7	100	+53,0*	0	43
	7	80	+17,0*	0	0
IIa	8	500	+65,8*	0	62
	8	350	0	0	25

*Stimulation of tumor growth.

Compounds (Va-i) were tested for bacteriostatic and mycostatic activity by standard methods [9] using serial dilutions in a liquid nutrient medium on five strains of bacteria and fungi: Staphylococcus, E. coli, anthracoid, and yeastlike fungus. Antiviral activity was examined against the viruses influenza A_2 (NK) 68, parainfluenza type 3, arbovirus, ECHO type 6, adeno type 3, herpes L_2, pox virus, and coliphages f_2 and T_2. The tests with influenza virus were carried out using living fragments of chick embryo chorioallantoic membrane, with the adeno-, parainfluenza, and ECHO viruses in passivated human embryonic dermomuscular cells (titration of the adenovirus was carried out in an initially trypsinized culture of human kidney embryo), with arbovirus, pox virus, and herpes virus in an initially trypsinized culture of chick embryo fibroblasts, and with coliphages in E. coli bacteria. The viruses were cultured in the presence of nontoxic doses of the compounds, and these samples were then titrated and compared with the control (virus reproducing in the absence of the compound). The effects of the influenza virus were assessed by hemagglutination, of parainfluenza by hemadsorption, and of arbovirus, ECHO, adeno-, herpes, and pox viruses by their cytostatic effects. The effects of coliphages were assessed by the suppression of platelet formation by the compounds diffusing into agar.

The toxicity and effects of (Ia-IIIa), (Vℓ), and (VI) on tumor growth were examined in biological tests.

Of the compounds tested, only (Ia) was of moderate toxicity (LD_{50} 500 mg/kg), the remainder being of low toxicity (LD_{50} between 1290 and 2820 mg/kg; Table 2).

The results, shown in Table 3, indicate that the benzimidazole and benzothiazole compounds (Ia-IIa) inhibit tumor growth in Pliss' lymphosarcoma by 19.8 and 41.9% in doses of 80 and 270 mg/kg, respectively. With sarcoma 180, when the doses were increased (to 100 and 500 mg/kg), these compounds behaved as purine metabolites, showing a clear stimulant effect on tumor growth (+53.0 and +65.8%).

TABLE 4. Effects of (IIIa), (Vℓ), and (VI) on the Lifespan of Mice with Leukemia L-1210

Compound	Dose, mg/kg	Mean lifespan		% change in lifespan
		control	test	
IIIa	1083	7,2±0,01	3,4±0,04	—52,7
	650	7,2±0,01	7,4±0,97	0
Vℓ	312	7,2±0,01	7,4±0,97	0
	187	7,2±0,01	6,4±1,15	—11,1
VI	343	7,2±0,01	7,0±1,15	0
	206	7,2±0,01	3,8±0,96	—47,2

Note. A minus sign indicates a decrease in lifespan.

In doses of 650, 312, and 343 mg/kg, respectively, (IIIa), (Vℓ), and (VI) had little effect on the development of leukemia L-1210, and failed to increase the lifespan of the animals as compared with the controls (untreated animals with leukemia L-1210; Table 4). In some tests, there was a decrease in the lifespan of the animals, possibly as a result of toxic effects of (IIIa) and (VI) on the body, and their stimulant effects on the leukemic process.

The antimicrobial and antiviral activity of (Va-m) were examined. It was found that only (Vj, k) had any bacteriostatic and mycostatic activity against Staphylococcus aureus, anthracoid, and yeast-like fungus, having MBC and MFC concentrations of 31.2, 31.2, 15.6 and 15.6, 15.6, 7.8 μg/ml, respectively.

Comparison of the antiviral activity of previously-synthesized benzimidazolyl-2-thioacetohydrazide hydrazones [3, 6, 8] with that of (Va-m), which contain the common fragment $-SCH_2SONHN{=}CHR$, shows that the letter have lost the ability to suppress the growth of the test viruses.

Only one of the compounds, (Vm), which contains the isatin residue, showed weak activity against coliphage f_2 (activity is indicated by a single + sign).

In general, the test compounds may be regarded as being active in various ways on the experimental tumors, depending on the dose and metabolic profile of the tumor. The tests for antiviral and antimicrobial activity failed to reveal any compounds with appreciable activity.

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