SYNTHESIS OF 6-β-HYDROXYALKYL(ARALKYL, HETERYL)THIOPURINES AND THEIR INFLUENCE ON CERTAIN IMMUNOGENESIS REACTIONS

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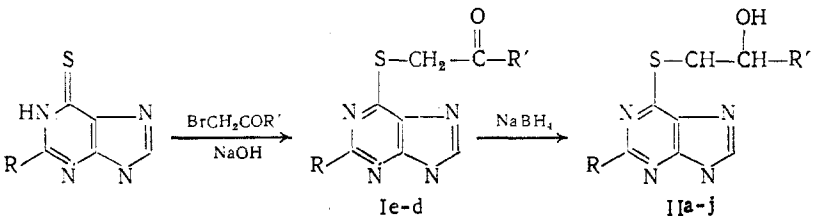
UDC 615.4:54:547.857:543

In [4, 5] two hydroxyalkyl derivatives of 6-thiopurine are described, which were obtained by the reaction of the latter compound with β-chloroalcohols in the presence of alkalies. The compounds exhibit various biological actions.

Our attempt to extend this method to the synthesis of 6-β-hydroxyaralkylthiopurines was unsuccessful, since when 6-thiopurine was treated by styrene or p-nitrostyrene chlorohydrin in the presence of NaOH, instead of obtaining S-alkylation, HCl was split off and the corresponding oxides were formed.

Therefore, to obtain 6-β-hydroxyalkyl(aralkyl, heteryl)thiopurines (IIa-j, Table 1), we carried out the reduction of known 6-acylmethylthiopurines [2] and acylmethylthioguanines (Ia-d, see Table 1) described in the present work, using $NaBH_4$. The process proceeds readily in aqueous ethanol at room temperature and leads to the formation of IIa-j in a 80-94% yield.

The initial Ia-d were synthesized by alkylation of thioguanine by the corresponding α-bromoketones according to [2].



Ie-d IIa-j

Ia: R = NH_2, R′ = Ph; Ib: R=NH_2, R′ = 4-$CH_3OC_6H_4$; Ic: R = NH_2, R′ = 4-$NO_2C_6H_4$; Id: R = NH_2, R′ = 4-BrC_6H_4; IIa: R = H, R′ = Bu-*t*; IIb: R = H, R′ = Ph; IIc: R = = H, R′ = 4-$CH_3C_6H_4$; IId: R = H, R′ = 4-$O_2NC_6H_4$; IIe: R = H, R′ = 4-BrC_6H_4; IIf: R = H, R′ = α-thienyl; IIg: R=NH_2, R′ = Ph; IIh: R = NH_2, R′ = 4-$CH_3OC_6H_4$; IIi: R = NH_2, R′ = 4-$O_2NC_6H_4$; IIj: R = NH_2, R′=4-BrC_6H_4.

The structure of compounds IIa-j was confirmed by IR spectra, in which, in contrast to those of the initial ketones, the absorption bands of the CO group in the 1724-1670 cm^{-1} region [2] are absent, and combined characteristic absorption bands of the OH, NH or NH_2 groups (in the case of thioguanine derivatives) are observed in the 3460-3100 cm^{-1} region.

EXPERIMENTAL (CHEMICAL SECTION)

The IR spectra were run on a UR-20 spectrophotometer (GDR) in KBr tablets. The purity of the reaction products was controlled by TLC on Silufol UV-254 plates. For the analysis of Ia,b the compounds were purified by crystallization from ethanol, and Ic,d from aqueous DMFA.

6-β-Hydroxyalkyl(aralkyl, heteryl)thiopurines (IIa-j). A solution of 0.76 g (0.15 mole) of $NaBH_4$ in 6-7 ml of water is added dropwise to a finely divided suspension of 0.01 mole of 6-acylmethylthiopurine in 15-20 ml of ethanol. The mixture is vigorously stirred for 3 h at 20-22°C, neutralized by 2 N H_2SO_4 to pH 6.0-7.0, diluted with water (40-50 ml), and the precipitate is filtered, washed with water, and dried. For the analysis, the compounds are purified by crystallization from methanol (IIa, IIf-h), ethanol (IIb-d, IIj), or DMFA (IIi).

The Zaporozhe Medicinal Institute. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 20, No. 10, pp. 1198-1202, October, 1986. Original article submitted May 22, 1985.

 0091-150X/86/2010-0722 $12.50

TABLE 1. 2-Amino-6-acylmethylthiopurines (Ia-d) and 6-β-Hydroxyalkyl(aralkyl, heteryl) thiopurines (IIa-j)

Compound	Yield, %	mp, °C	R_f[a]	Found, %				Empirical formula	Calculated, %			
				C	H	N	S		C	H	N	S
Ia	85	189—91	0,77	55,0	3,9	24,3	11,1	$C_{13}H_{11}N_5OS$	54,7	3,9	24,6	11,2
Ib	88	180—81	0,54	53,7	4,6	21,9	10,4	$C_{14}H_{13}N_5O_2S$	53,3	4,2	22,2	10,2
Ic	90	210—11	0,73	46,3	3,4	20,6	9,2	$C_{13}H_{10}N_6O_3 \cdot H_2O$	46,7	3,6	20,9	9,6
Id	89	178—80	0,76	42,9	2,9	19,3	8,2	$C_{13}H_{10}BrN_5OS$[b]	42,9	2,8	19,2	8,6
IIa	88	189—91	0,80	52,4	6,2	22,2	12,6	$C_{11}H_{16}N_4OS$	52,3	6,4	22,4	12,7
IIb	91	198—200	0,84	57,8	4,8	20,2	11,7	$C_{13}H_{14}N_4OS$	57,4	4,6	20,5	11,7
IIc	90	205—7	0,74	55,4	4,4	18,6	10,3	$C_{14}H_{14}N_4O_2S$	55,6	4,7	18,5	10,6
IId	87	196—8	0,77	49,2	3,7	22,0	10,1	$C_{13}H_{11}N_5O_3S$	49,2	3,5	22,1	10,0
IIe	94	>320	0,79	44,0	3,0	15,8	9,0	$C_{13}H_{11}BrN_4OS$[c]	44,5	3,2	16,0	9,1
IIf	80	179—81	0,59	47,4	3,9	21,0	22,9	$C_{11}H_{10}N_4OS_2$	47,5	3,6	21,1	23,0
IIg	92	119—21	0,65	54,7	5,1	24,9	11,7	$C_{13}H_{13}N_5OS$	54,3	4,7	24,4	11,3
IIh	92	218—20	0,72	. . .	. . .	22,5	10,3	$C_{14}H_{15}N_5O_2S$	. . .	. . .	22,1	10,0
IIi	89	222—4	0,68	. . .	. . .	25,6	9,8	$C_{13}H_{12}N_6O_3S$	. . .	. . .	25,3	9,6
IIj	94	180—82	0,79	. . .	. . .	19,4	9,0	$C_{13}H_{12}BrN_5OS$[d]	. . .	. . .	19,1	8,8

TABLE 2. Change in the Number (in %) of Granulocytes, Lymphocytes, and Monocytes in Blood of Rats in the Course of 21 Days after Administration of the Compounds

Compound	Initial				After 10 days	
	neutrophils	eosinophils	lymphocytes	monocytes	neutrophils	eosinophils
Control	43,7±6,9	2,7±0,9	52,5±6,7	1,7±0,35	34,7±5,7	1,8±0,4
Azathioprine	44,4±6,9	1,8±0,4	52,2±6,6	1,6±0,21	26,6±4,3	0
IIb	31,0±1,6	3,3±0,9	64,3±2,3	1,3±0,17	30,3±4,1	0
IId	34,4±5,4	4,6±0,9	59,2±8,1	1,8±0,21	32,8±6,4	0,4±0,2*
IIe	42,5±6,0	1,7±0,4	52,2±4,6	1,8±0,71	33,3±3,0	0,2±0,2*

Compound	After 10 days		After 21 days			
	lymphocytes	monocytes	neutrophils	eosinophils	lymphocytes	monocytes
Control	57,7±6,6	5,8±1,06	25,7±3,1	1,7±0,3	70,72±2,1	1,8±0,84
Azathioprine	85,6±4,1	4,4±1,07	7,4±4,3*	0,6±0,2*	90,2±4,5	1,8±0,64
IIb	65,8±4,6	3,8±0,89	16,8±3,9	0,5±0,2	82,0±3,9	0,7±0,35*
IId	61,4±7,3	5,4±1,29	22,0±4,1	0	77,0±4,1	1,0±0,43
IIe	60,0±3,0	6,5±2,48	36,2±7,8	0	62,7±7,3	1,2±0,53

Note. Here and in Table 3, the statistically reliable results are marked by an asterisk.

EXPERIMENTAL (BIOLOGICAL SECTION)

The study of acute toxicity of the compounds obtained, carried out on mice weighing 18-20 g each by the Litchfield and Wilkoxon method [1], revealed its relatively low level, compared with the widely used immunodepressants, 6-mercaptopurine (6-MP) and 6-(1-methyl-4-nitro-5-imidazolyl)mercaptopurine (azathioprine) [3, 6]. The LD_{50} of the compounds studied is 1630 ± 240 for IIb; 1780 ± 300 for IId; and 1400 ± 250 for IIe.

To evaluate the influence of the synthesized compounds on the structure and function of the immunocompetent lymphoid system, the following parameters were selected: number of lymphocytes, monocytes, granulocytes — neutrophilic and eosinophilic — in peripheral blood; change in the mass coefficients of the immunocompetent organs (thymus, spleen); phagocytic activity of blood, tested according to phagocytic factor and phagocytic index.

The experiments were carried out on female white rats of the Wistar line, weighing 160-180 g each. Azathioprine was chosen as the standard preparation. Compounds IIb, IId, IIe were administered subcutaneously, daily for 3 weeks in a dose of 1/10 of LD_{50} in the form of a fine aqueous suspension, stabilized by Tween-80. The control group animals were administered with physiological solution. To study the dynamics of the leucocytic formula before the beginning of the experiment, on the 10th and 21st day, blood was taken from the tail artery of the animals, and then they were killed under ether narcosis, and the organs were isolated and weighed. The blood was used for the determination of the phagocytic activity.

Table 2 shows that azathioprine and compounds IIb, IId, and IIe significantly changed the leucocytic formula of blood. The changes were in the same direction, but their degrees of expression were unequal.

Azathioprine causes the most substantial neutropenia towards the 21st day. This effect was less pronounced in IIb and IId, and IIe practically did not influence the level of neutrophils. All the compounds studied caused a considerable eosinopenia, IId and IIe being more active than azathioprine. With respect to lymphocytes, the effect was less pronounced and consisted in a tendency to increase in their number towards the 21st day after administration. Shifts in the content of monocytes differed inappreciably from the control.

TABLE 3. Influence of Compounds Studied on Phagocytic Activity Parameters of Neutrophils and Mass Coefficients of Immunocompetent Organs

Parameter	Azathioprine	Control	Compound		
			IIb	IId	IIe
Phagocytic factor, %	52,8±21,4	61,5±2,7	29,3±4,1*	51,6±7,7	47,0±6,9
Phagocytic index	6,4±2,9	9,8±1,0	12,6±4,2	6,1±0,4*	5,9±0,9*
Mass coefficient:					
of thymus	0,08±0,014*	0,18±0,014	0,18±0,025	0,18±0,012	0,20±0,019
of spleen	0,43±0,014	0,38±0,39	0,45±0,053	0,52±0,057	0,58±0,101

*Indicates statistically reliable results.

The analysis of the phagocytic function of neutrophils (Table 3) showed that azathioprine, IId, and IIe lower the phagocytic factor only in the form of tendency, while IIb lowers it statistically substantially (by 52%). The calculation of the phagocytic index showed an opposite dependence.

The calculation of mass coefficients of the immunocompetent organs (see Table 3) showed that azathioprine substantially decreases the weight of thymus, while IIb, IId, and IIe practically do not change this parameter. All the compounds studied have a tendency to increase the mass coefficient of spleen.

Thus, the results of our investigations showed that the 6-β-hydroxyaralkylthiopurines studied have pronounced immunodepressant activity. This is indicated by the same direction of change in the immunity parameters when the standard preparation and compounds IIb, IId, and IIe are introduced. However, the effectiveness of their influence on the individual parameters was different. They caused a less substantial decrease, compared with azathioprine, in the number of neutrophils in blood, but caused a more considerable eosinopenia. The effect of the suppression of the phagocytic function of blood was most pronounced in compound IIb, which is almost twice as active as the standard preparation. At the same time, the compounds studied practically did not lead to a decrease in the mass coefficient of the thymus gland, while azathioprine decreased this parameter by a factor greater than 2. It is probable that the differences in the degree of expression of the effects at their unequivocal, in most cases, directivity, indicates that the compounds synthesized inhibit different parts of chain processes, ensuring an integral reaction of the organism, i.e., the immunity.

The results obtained showed that, compared with azathioprine, 6-β-hydroxyaralkylthiopurines have a more pronounced action on individual immunogenesis reactions (phagocytosis) at relatively low toxicity levels.

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