SYNTHESIS AND ANTITUMOR ACTIVITY OF 6-DIETHYLENIMIDOPHOSPHAMIDO- AND 6-TRIETHYLENIMINOPHOSPHAZOPURINES

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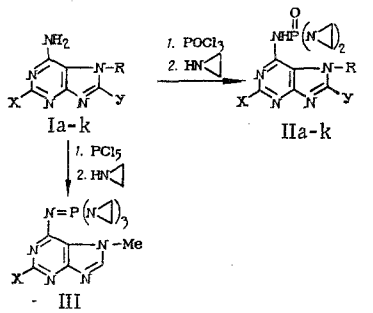
As a result of a directed search for diethylenimidophosphamidopurines with antitumor properties, carried out at the All-Union Scientific-Research Institute of Pharmaceutical Chemistry, a number of compounds were found which at moderate toxicity had a significant antitumor action on different types of transplanted tumors of mice and rats. The most active of these compounds were found to be the purine derivatives (Ia-k), with a diethylenimidophosphamide group in the 6-position, and, in particular, 2-dimethylamino-6-diethylenimidophosphamido-7-methylpurine (IIa) ("Fopurin") [1]. In the course of the investigation, it was found experimentally that the antitumor effect of IIa is due to both the alkylating action of the preparation itself and the antimetabolic effect of its degradation product in the organism – 2-dimethylamino-6-amino-7-methylpurine (Ia).

In order to find new antitumor agents, and also to study the effect of the character of substituents in the purine nucleus on the antitumor activity of these compounds, we synthesized a number of new analogs of IIa which can be cleaved at the N–P bond and which possess a twofold mechanism of antitumor action.

We studied 7-methyl- and 7-benzyl-6-diethylenimidophosphamidopurines with amino groups (mainly from saturated heterocyclic amines) in the 2-position of the purine ring. Moreover, since dealkylation takes place readily in the organism, we decided to synthesize the corresponding compounds with no substituents at the purine N-7; these compounds can be considered to be potential metabolytes of 7-substituted purines, among them IIa. Finally, for biological study, analogs of IIa, containing a triethyleniminophosphazo group in the 6-position (IIIa-c), were prepared. These compounds were synthesized from the corresponding 6-aminopurines (I) by the following reaction scheme:



a: **X = NMe_2**; б: b: X-morpholino; c: X-piperidino

I, II	X	R	Y
a	NMe_2	Me	H
b	pyrrolidino	Me	H
c	2,2-dimethylpyrrolidino	Me	H
d	NMe_2	CH_2Ph	H
e	morpholino	CH_2Ph	H
f	piperidino	CH_2Ph	H
g	NMe_2	H	H
h	morpholino	H	H
i	piperidino	H	H
j	NMe_2	Me	Cl
k	NMe_2	Me	morpholino

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A study of the biological activity of these compounds (Table 1) shows that, in any case, they are less toxic than IIa. Thus, for compounds IIb-i, the maximum tolerable dose (MED) varied from 60 mg/kg (IIg) to 150 mg/kg (IIe), while for IIa the MED was 40 mg/kg. Practically all the compounds were moderately effective against sarcoma 45 in rats and sarcoma 180 in mice. The data obtained gave some indications of the effect of substituents in the molecule on the antitumor activity of the purine derivatives. In particular, removal of the methyl group at the 7-position of IIa, i.e., conversion to IIg, led to some increase in activity against sarcoma 45 in rats and sarcoma 180 in mice, and at the same time to loss of activity against Jensen's sarcoma in rats. A sharp decrease in antitumor action was observed when compounds IIa-k were converted to the 6-triethyleniminophosphazo analogs (IIIa-c). Increasing the lypophilic character of the purine molecule by introducing a benzyl group to the 7-position (compounds IId-f) lowered the antitumor activity of these compounds considerably. Of the compounds studied, IIa, which has the most lypophilic substituents at the 2- and 7-positions, showed the most promise, and would be a suitable subject for future work on antitumor agents and the elucidation of the relation between the antitumor action and physicochemical properties of compounds.

EXPERIMENTAL (BIOLOGICAL)

The toxicity and antitumor activity of 12 analogs of IIa were studied in tests on animals; noninbred mice weighing 20-22 g and rats weighing 100-120 g were used. The test substances were administered mainly by intraperitoneal injection of solutions or suspensions of the compound in a physiological solution or in distilled water. A few compounds were administered orally in vegetable (linseed) oil. Before conducting therapeutic tests, for each compound determinations were made of the approximate LDL_{100} for mice using a single injection and of the MED for mice and rats using daily injections for 6 days (MED_6) and 8 days (MED_8). The antitumor effect was studied for solid tumors: sarcoma 180 in mice, sarcoma 45 in rats, and Jensen's sarcoma in rats. Injections of the test substances were started 4-6 days after transplantation of the tumor and continued daily for 6 days (mice) and for 8 days (rats). Observations were continued for 12-17 days from the moment of transplantation of the tumor. The antitumor effect was assessed by the percentage inhibition of tumor growth in comparison with a control. The overall effect of the compounds on the animals was evaluated from observations of the state of the animals, and also from the growth index (K_p in %), which reflects the change in the body weight of the animal during the test in comparison with a control. Numerical data was treated statistically by the Fisher-Student method. The experimental data is presented in Table 1.

EXPERIMENTAL (CHEMICAL)

Yields, constants, and elemental analysis data for the compounds are given in Table 2.

TABLE 1. Antiblastic Activity and Toxicity of Analogs of IIa

Compound	Method of administration	Toxicity, mg/kg		Number of tests	Antitumor effect		
		MED_6 (mice)	MED_8 (rats)		sarcoma 45 of rats	sarcoma 180 of mice	Jensen's sarcoma of rats
IIa	Intraperitoneally	100—125	40	18	+++	+	++++
IIb	"	<25	60—100	9	+	+	++
IIc	"	—	60—100	6	+	+	—
IId	Orally (oil)	125	25—50	7	0	+	—
IIe	Intraperitoneally	125	150	9	+	0	—
IIf	"	125	50—100	11	+	+	—
IIg	"	125	~60	20	++	++	—
IIh	"	225	100	5	+	+	—
IIi	"	150	100	5	+	+	+++
IIIa	"	25	25	5	++	+	—
IIIb	"	>50	>100	6	±	0	—
IIIc	"	>50	~100	8	0	0	—

Notes. Inhibition of tumor growth: 0, no inhibition; --, not studied; ±, less than 30%; +, 30-59%; ++, 60-79; +++, 80-94%; ++++, 95-100%.

TABLE 2. Physicochemical Properties of Substituted 6-Amino- and 6-Diethylenimidophosphamidopurines

Compound	Yield, %	mp, °C	Recrystallization solvent	Found, %					Empirical formula	Calculated, %				
				C	H	Cl	N	P		C	H	Cl	N	P
Ia	75,5	280—284	Ethanol	49,65	6,01	—	43,78	—	$C_8H_{12}N_6$	50,00	6,25	—	43,75	—
Ib	59,5	303—305	Same	55,18	6,60	—	38,41	—	$C_{10}H_{14}N_6$	55,05	6,42	—	38,53	—
Ic	60,5	256—257	"	58,80	7,25	—	33,91	—	$C_{12}H_{18}N_6$	58,54	7,32	—	34,14	—
Id	74,6	295—296	DMFA	62,77	6,06	—	31,63	—	$C_{14}H_{16}N_6$	62,68	5,97	—	31,34	—
Ie	80,3	268—270 with decomp.	DMFA	62,28	5,91	—	26,84	—	$C_{16}H_{18}N_6O$	61,93	5,81	—	27,10	—
If	70,7	285—287	DMFA	66,48	6,50	—	27,06	—	$C_{17}H_{20}N_6$	66,25	6,49	—	27,27	—
Ig	70,5	346—348	DMFA	49,05	5,61	—	38,56	—	$C_9H_{12}N_6O$	49,09	5,49	—	38,36	—
Ih	69,2	281—282	DMFA	54,94	6,39	—	38,83	—	$C_{10}H_{14}N_6$	55,04	6,42	—	38,53	—
Ii	77,5	285—287	Ethanol	—	—	15,95	37,04	—	$C_8H_{11}ClN_6$	—	—	15,70	37,09	—
Ij	78,0	274 with decomp.	Aqueous ethanol	52,08	6,92	—	35,29	—	$C_{12}H_{18}N_7O$	51,98	6,86	—	35,38	—
Ih·HCl	71,8	287—288	Water	—	—	13,77	32,68	—	$C_9H_{13}ClN_6O$	—	—	13,88	32,87	—
Ii·HCl	70,9	262—264	"	—	—	14,03	33,23	—	$C_{10}H_{15}ClN_6$	—	—	13,95	33,01	—
IIb	57,3	~272 with decomp.	Toluene	48,10	6,26	—	31,96	8,70	$C_{14}H_{21}N_6OP$	48,28	6,03	—	32,18	8,92
IIc	47,5	~250 with decomp.	Ethyl acetate	51,06	6,65	—	29,79	8,24	$C_{16}H_{25}N_6OP$	50,90	6,65	—	29,68	8,14
IId	47,1	~270 with decomp.	Benzene	54,20	5,75	—	27,93	7,73	$C_{18}H_{23}N_6OP$	54,27	5,78	—	28,10	7,76
IIe	52,8	~275 with decomp.	Absolute ethanol	54,31	5,50	—	25,27	6,80	$C_{20}H_{25}N_6O_2P$	54,54	5,68	—	25,45	7,05
IIf	57,6	~290 with decomp.	Same Same	57,64	6,40	—	25,46	7,20	$C_{21}H_{27}N_6OP$	57,53	6,16	—	25,57	7,07
IIg	47,7	~250 with decomp.	" "	42,74	5,42	—	36,04	9,92	$C_{11}H_{17}N_6OP$	42,86	5,52	—	36,40	10,05
IIh	39,1	~265 with decomp.	" "	44,46	5,60	—	31,91	8,95	$C_{13}H_{19}N_6O_2P$	44,57	5,42	—	32,00	8,85
IIi	81,0	~260 with decomp.	Ethanol—ethyl acetate	48,12	6,33	—	31,99	9,01	$C_{14}H_{21}N_6OP$	48,27	6,03	—	32,18	8,90
IIj	61,0	~285 with decomp.	Ethyl acetate	40,59	4,96	9,92	31,45	8,60	$C_{12}H_{16}ClN_6OP$	40,39	5,04	9,95	31,42	8,69
IIk	63,2	~275 with decomp.	Toluene—ether	47,03	6,31	—	31,17	7,83	$C_{16}H_{26}N_9O_2P$	47,17	6,39	—	30,96	7,61
IIIa	54,2	160 with decomp.	Ethyl acetate	48,41	6,34	—	36,31	8,93	$C_{14}H_{22}N_9P$	48,10	6,04	—	36,06	8,62
IIIb	48,7	150 with decomp.	"	49,36	6,17	—	32,38	7,96	$C_{16}H_2N_9OP$	49,43	6,28	—	32,68	7,94
IIIc	61,3	145 with decomp.	Ethyl acetate—ethanol	52,70	6,70	—	32,56	8,00	$C_{17}H_{26}N_9P$	52,80	6,94	—	32,48	7,80

2-Pyrrolidino-6-amino-7-methylpurine (Ib). A mixture of 7 g of 2-chloro-6-amino-7-methylpurine, 16 ml of pyrrolidine, and 140 ml of water was heated for 3 h at 125-130°. After cooling, the precipitate was filtered off, washed with water, dried, and recrystallized from alcohol. A yield of 4.95 g of Ib was obtained.

Compounds Ia, and Ic-f were prepared in the same way.

2-Dimethylamino-6-aminopurine [2] (Ig). The hydrochloride of Id (12 g) in 180 ml of water was hydrogenated at 85-90° using 10 g of 5% Pd/C. The reaction mixture was filtered, the filtrate evaporated to 1/3 of its original volume, cooled, and the hydrochloride of Ig isolated. The latter was dissolved in water (1:10) and made alkaline to pH 8.0 with 40% NaOH; compound Ig was separated at 5°. UV spectrum λ_{max} nm: 256 and 293.

Compounds Ih and i were prepared in the same way.

2-Dimethylamino-6-amino-7-methyl-8-chloropurine (Ij). A. To 100 ml of SO_2Cl_2 was added gradually, at 0-5°, 23.5 g of Ia, and the mixture left at 20° for 90 h. The precipitated material was separated and treated with ice-water; the suspension was made alkaline to pH 7.0, filtered, and dried. A yield of 21.5 g of Ij was obtained. B. Chlorine was passed through a suspension of 32 g of Ia in 140 ml of dry chlorofrom at 40-50° for 1.5 h. The mixture was then cooled and the precipitate filtered off, dried, and dissolved in water; the solution was treated with charcoal, filtered, and made alkaline to pH 3.0-9.0. The precipitate was filtered off and washed with water to give 26.65 g of Ij.

2-Dimethylamino-6-amino-7-methyl-8-morpholinopurine (Ik). A mixture of 11 g of Ij and 45 ml of morpholine was stirred and heated at 100° for 2 h. It was allowed to cool and the precipitate filtered off, washed with water, and dried, giving 10.5 g of Ik.

2-Pyrrolidino-6-diethylenimidophosphamido-7-methylpurine (IIb). A mixture of 3 g of Ib and 15 ml of phosphorus oxychloride was refluxed for 4.5 h, cooled, the precipitate separated, washed with petroleum ether, dried, and suspended in 100 ml of chloroform. To this suspension was added a solution of 2 ml of ethylenimine and 2 g of K_2CO_3 in 10 ml of water at a temperature below 10°. The mixture was stirred for 2 h, and the chloroform layer separated, dried, and concentrated. The residue was filtered with ether and recrystallized to give 2.75 g of IIb.

Compounds IIc-f, j, and k were prepared in the same way.

2-Dimethylamino-6-diethylenimidophosphamidopurine (IIg). A mixture of 2 g of Ig and 40 ml of phosphorus oxychloride was refluxed for 15 h, the excess phosphorus oxychloride distilled off in vacuum, and the residue triturated with petroleum ether. The precipitated material was filtered off, dried, and added to a solution of 5 ml of ethylenimine in 60 ml of water at 5-8°. After stirring for 2 h at 20-22°, the solution was extracted with chloroform to yield 1.65 g of IIg.

Compounds IIh and i were prepared in the same way.

2-Dimethylamino-6-triethyleniminophosphazo-7-methylpurine (IIIa). A mixture of 4 g of Ia and 4.3 g of PCl_5 in 40 ml of dry chloroform was refluxed for 7 h, cooled, and filtered; the filtrate was evaporated to dryness. The residue was dissolved in 40 ml of dry chloroform, and the solution slowly added at 0.5° to a mixture of 10 ml of triethylamine, 3.6 ml of ethylenimine, and 10 ml of dry chloroform. This was stirred for 2 h at 20° and twice its volume of benzene was added. The triethylamine hydrochloride was filtered off; the filtrate washed with ice-water until no chloride ions remained, dried, and evaporated. The residue was recrystallized to give 2.7 g of IIIa.

Compounds IIIb and c were obtained in the same way.

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