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Pyrazolotriazines: a new class of tumour-inhibitory agents

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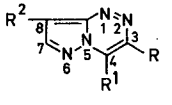
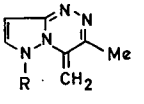
A series of pyrazolo-*as*-triazines have been screened for tumour-inhibitory activity against sarcoma S 180 in mice and a methylcholanthrene-induced tumour in rats. The antitumour activities of ethyl 4-aminopyrazolo[3,2-*c*]-*as*-triazine-3-carboxylate (Ig), 6-acetyl- and 6-iodoacetyl-3-methyl-4-methylenepyrazolo[3,2-*c*]-*as*-triazine (IIa and IIb respectively) against sarcoma S 180 exceed the inhibitory activity of 6-mercaptopurine against the same tumour.

INVESTIGATIONS of structural analogues of naturally occurring purines have provided a fruitful source of compounds showing inhibitory activity against a range of experimental animal neoplasms. Thus the antitumour activities and structure-activity relationships of derivatives of purine, pyrazolo[3,4-*d*]pyrimidine and *v*-triazolo[*d*]pyrimidine have received widespread interest in the search for cancer chemotherapeutic agents (Robins, 1964). Pyrazolo[3,2-*c*]-*as*-triazine (I; R = R^1 = R^2 = H) is isosteric with purine and the synthesis of compounds based on this ring-system has already been described (Partridge & Stevens, 1966; Bedford, Partridge & Stevens, 1966). This communication reports on studies of the tumour-inhibitory properties of the compounds listed in the Tables.



I



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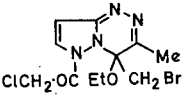

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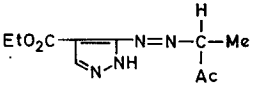

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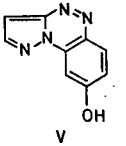

V

Experimental

METHODS

The procedure for testing compounds against mouse sarcoma S 180 was based on the protocol of the United States Cancer Chemotherapy Service Centre (1962). Tumour (7 to 10 days old) was taken under aseptic

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conditions from donor mice and fragments (2–4 mm in average diameter) were implanted subcutaneously using a 9 gauge trocar into stock albino mice (Schofield; 25–30 g weight).

Soluble compounds were administered in physiological saline. Insoluble compounds were suspended by homogenisation in 0·5% carboxymethyl cellulose in physiological saline.

Treatment was begun 24 hr after tumour implantation and compounds were administered daily by intraperitoneal injection for 9 days (days 1–9). Mice were weighed and killed (day 10) and the tumours excised and weighed. The ratio of the mean weights of tumours in treated mice to that in controls (T/C × 100%) was recorded together with body weight changes.

A limited number of tests were also made with a methylcholanthrene-induced rat sarcoma (Baldwin, 1955). This tumour was carried by subcutaneous implantation in female rats of an inbred Wistar strain, and was used between the 65th and 103rd transfer generations. In these tests, tumour fragments (5–10 mm in average diameter) were implanted subcutaneously using a 15 gauge trocar, and compounds were administered daily by intraperitoneal injection for 17 days (days 1–17). Rats were then killed 24 hr after the last treatment and inhibition determined from the average weights of tumour in test and control animals.

Results and discussion

Derivatives of pyrazolo[3,2-*c*]-*as*-triazine (I) with substituents in the 3-, 4- and 6- positions differed in their abilities to inhibit the growth of sarcoma S 180 (Table 1). The monomethyltriazine (Ia) was inactive, whereas its dimethyl analogue (Ib) showed activity compared to the positive controls; these were treated with 6-mercaptopurine and *N*-methylformamide. Introduction of a bromine atom into the pyrazole ring abolished activity (Ic); similar deactivation resulted from the replacement of methyl by phenyl (Id). The dyschemotherapeutic effect of a substituted 3-carbonyl (Ie and If) was offset by a 4-amino-group (Ig and Ih), the ester (Ig) being somewhat more active than the corresponding acid (Ih). The chemical reactivity of the 4-amino-group of (Ig) was exploited to synthesise a compound combining the structural features of a purine analogue and a nitrogen mustard (Partridge & Stevens, 1966); the resulting 4-di-β-chloroethylaminopyrazolotriazine (Ii) was however inactive. Sarcoma S 180 is known to be resistant to biological alkylating agents. Moreover, the biological activities of nitrogen mustards are related to the chemical reactivity of their halogen atoms (Ross, 1962). The ethoxycarbonyl group in (Ii) adjacent to the di-β-chloroethylamino-group would deactivate the chlorine atoms to nucleophilic attack.

The 6-acetyltriazine (IIa) was tumour inhibitory. The chemical reactivity of 6-acyl derivatives towards nucleophilic reagents to yield 3,4-dimethylpyrazolo[3,2-*c*]-*as*-triazine (Ib) (Partridge & Stevens, 1966) implied the possibility that the 6-acetyl derivative might exert its activity after *in vivo* metabolism to (Ib). The feasibility of exploiting such metabolism by the incorporation of a biologically active side-chain at the

6-position of (Ib) in order to potentiate cytotoxicity was investigated. Two derivatives, 6-iodoacetyl- and 6-fluoroacetyl-3-methyl-4-methylene-pyrazolo[3,2-*c*]-*as*-triazine (IIb and IIc respectively) were significantly more toxic to the mouse than the corresponding 6-acetyltriazine (IIa); this suggested that *in vivo* (IIb and IIc) were degraded to the dimethyltriazine (Ib) and iodo- and fluoroacetic acids respectively. The iodoacetyltriazine (IIb) was a more potent inhibitor of sarcoma S 180 than

TABLE 1. INHIBITION OF SARCOMA S 180 BY PYRAZOLOTRIAZINES

Compound	Substituents R	R¹	R²	Dosage (mg/kg/day)	Survivors	Average body-weight change, test/control (g)	Mean tumour weights, test/control (g)	Tumour inhibition, mean tumour weights, test/control (%)
6-Mercapto-purine				30	5/6	+1·4/+5·9	0·66/0·95	69
N-Methyl-formamide				200	6/6	+1·5/+8·2	0·62/1·42	44
				200	6/6	+0·2/+7·0	0·38/1·33	29
Ia	H	Me	H	200	6/6	+2·8/+6·2	1·1 /0·92	120
Ib	Me	Me	H	200	6/6	+2·5/+3·5	0·68/1·03	66
				200	6/6	+1·0/+6·2	0·76/0·92	83
Ic	Me	Me	Br	50	6/6	+2·5/+3·2	0·95/0·60	158
Id	Ph	Ph	H	100	6/6	+5·8/+6·2	3·21/2·07	155
Ie	Ac	Me	H	50	6/6	+1·7/+1·0	0·70/0·64	109
If	CO_2Et	Me	H	50	5/6	+2·4/+4·8	0·99/1·01	98
Ig	CO_2Et	NH_2	H	25	5/6	+2·8/+4·2	0·72/1·39	52
				25	5/6	+0·8/+4·8	0·65/1·48	44
Ih	CO_2H	NH_2	H	10	6/6	+3·2/+6·2	1·47/2·07	72
Ii	CO_2Et	$N([CH_2]_2Cl)_2$	H	25	6/6	+2·5/+2·7	0·40/0·32	125
IIa	Ac			200	6/6	+1·3/+0·3	0·17/0·46	37
				200	6/6	+6·0/+5·8	0·63/1·01	62
				200	6/6	+2·5/+1·0	0·43/0·66	68
IIb	$ICH_2·CO$			5	6/6	+0·5/+2·0	0·75/1·30	58
				7·5	6/6	+0·3/+6·5	0·48/0·87	55
				10	5/6	+0·4/+3·7	0·33/0·93	35
IIc	$FCH_2·CO$			10	6/6	−1·0/+2·5	0·39/0·40	98
IId	$ClCH_2·CO$			50	5/6	+1·2/+3·3	0·88/0·93	95
				100	6/6	−5·0/+0·0	0·45/0·89	50
IIe	$Cl_2CH·CO$			200	6/6	+0·3/+4·2	1·00/1·39	72
IIf	$Ph·CO$			100	6/6	+2·7/+4·7	1·07/0·74	144
IIg	$Ph·SO_2$			200	6/6	+0·0/+1·2	1·25/1·32	94
IIh	*p*-$Ac·NH·C_6H_4·SO_2$			100	6/6	+2·5/+1·3	0·58/0·83	70
IIi	phthalimidoacetyl			100	6/6	+0·8/+2·0	0·96/1·30	74
IIj	4-$NC_5H_4·CO$			100	6/6	+3·7/+4·7	0·41/0·32	128
III				100	5/6	+0·0/+6·5	0·63/0·89	72
IV				100	6/6	+1·8/+1·3	0·52/0·83	63
				100	6/6	+1·2/+2·0	0·92/1·30	71
V				100	6/6	+5·2/+4·8	0·64/0·63	102

6-mercaptopurine at the three dose levels tested, but surprisingly the fluoroacetyl derivative (IIc) was inactive. The monochloroacetyltriazine (IId) and its dichloro analogue (IIe) were tumour-inhibitory: however the inhibitory dose of the monochloro derivative produced excessive body-weight loss in the test animals. Of the remaining 6-substituted compounds (IIf-j, and III), three triazines (IIh, IIi and III) showed inhibitory properties; the activity of compound (III) can possibly be attributed to its metabolic degradation to 3,4-dimethylpyrazolo[3,2-*c*]-*as*-triazine (Ib), since compound (III) is labile to acid and alkali (Bedford, Partridge & Stevens, 1966). The pyrazole-azobutanone (IV) displayed similar activity to 6-mercaptopurine but the pyrazolobenzotriazine (V) was inactive.

Both the dimethyltriazine (Ib) and its acetyl derivative (IIa) which inhibit sarcoma S 180, inhibit a methylcholanthrene-induced tumour in rats (Table 2). The inactivity of the ester (If) against sarcoma S 180 was paralleled by its inactivity against the methylcholanthrene tumour, but the amine (Ig) was less active against this tumour than against S 180.

TABLE 2. INHIBITION OF A METHYLCHOLANTHRENE-INDUCED TUMOUR BY PYRAZOLOTRIAZINES

Compound	Substituents R	R^1	R^3	Dosage (mg/kg/day)	Survivors	Average body-weight change, test/control (g)	Mean tumour weights, test/control (g)	Tumour inhibition, mean tumour weights, test/control (%)
Ib*	Me	Me	H	100	4/4	+14·3/+25·8	5·57/14·77	38
				200	5/6	+5·4/+12·5	1·89/ 5·12	37
If	CO_2Et	Me	H	50	6/6	+14·0/+10·0	6·93/ 4·86	143
Ig	CO_2Et	NH_2	H	25	6/6	+2·3/+11·3	3·96/ 4·86	82
IIa†	Ac			200	6/6	−2·9/+0·8	1·88/ 7·89	24
				200	6/6	−5·0/+10·0	2·96/ 4·86	61

* This compound (dose, 160 mg/kg/day) also inhibited the Walker carcinoma carried in male Wistar rats (T/C = 66).
† The acetyltriazine (dose, 200 mg/kg/day) inhibited growth of the Walker carcinoma (T/C = 64)

Although too few pyrazolo[3,2-*c*]-*as*-triazines have been studied to draw any significant structure-activity conclusions, the preliminary screening reported in this paper indicates that several derivatives show anti-tumour properties.

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