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The Correlation between the Antitumor Activities and Chemical Structures of Thiocyanato Derivatives of Purines and Their Ribonucleosides[1]

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The effect of 14 thiocyanato derivatives of purines and their ribonucleosides were compared with the effects of 6-purinethiol and 2-amino-6-purinethiol against transplanted sarcoma (NF-Sarcoma) in mice and the correlation was noted between antitumor activities and substituting position of thiocyanato group and other substituent groups on the purine ring. 6-Thiocyanatopurine, 2-amino-6-thiocyanatopurines and their ribonucleosides were demonstrated as the most active of the thiocyanato derivatives.

Since some purine analogues and their nucleosides have been used for the treatment of neoplastic deseases of man, a considerable number of potential antimetabolites of the natural purine have been synthesized and their biological properties studied.

Among them the antitumor activities of 6-purinethiol (6-MP) and 2-amino-6-purinethiol (6-TG) are well known, and the 9-β-D-ribofuranosyl derivative of 6-purinethiol (6-thioinosine) are reported to show the highest therapeutic index of any purine derivatives so far studied against experimental tumor.[3]

With regard to thiocyanato derivatives of purine, some effect has been reported of 6-thiocyanatopurine against Adenocarcinoma 755 and several fungi,[4] but the subject has not been studied systematically.

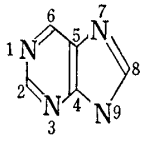
In a previous paper of this series, authors reported the synthesis and chemical properties of several thiocyanato derivatives of purines and their ribonucleosides.[5] Authors further synthesized the five other derivatives and tested the antitumor activities of 14 thiocyanato compounds in all against NF-mouse sarcoma.[6] This sarcoma was chosen because of its very rapid growth and its sensitivity to the action of nucleoside analogues.[7]

In this paper, authors report these results and discuss the relation between the antitumor activities and substituent groups on the purine ring, comparing with those of 6-purinethiol, 2-amino-6-purinethiol and their 9-β-D-ribofuranosides.

Materials and Methods

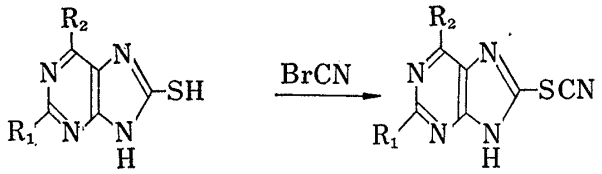
The test compounds used here were 14 kinds of thiocyanato derivatives of purines and their ribonucleosides, four thiol derivatives of purines and their ribonucleosides and two methylthio derivatives of purine and its ribonucleosides, which were as follows.

1) A part of this work was presented at the 24th Annual Meeting of the Japanese Cancer Association at Fukuoka, Oct./1965. This paper constitutes Part II of a series entitled "Synthetic Nucleosides and Nucleotides." Part I: *Chem. Pharm. Bull.* (Tokyo), **15**, 909 (1967).
2) Location: *Tsukiji 5-chome, Chuo-ku., Tokyo.*
3) R.K. Robins, *J. Med. Chem.*, **7**, 186 (1964).
4) W.T. Bradner and D.A. Clarke, *Cancer Research*, **18**, 299 (1958).
5) M. Saneyoshi and G. Chihara, *Chem. Pharm. Bull.* (Tokyo), **15**, 909 (1967).
6) F. Fukuoka and W. Nakahara, *Gann*, **42**, 55 (1951).
7) M. Saneyoshi, R. Tokuzen, and F. Fukuoka, *Gann*, **56**, 273 (1965).



6-Thiocyanatopurine (I)
6-Thiocyanato-9-(β-D-ribofuranosyl)purine (II)
2,6-Dithiocyanatopurine (III)
2-Amino-6-thiocyanatopurine (IV)
2-Amino-6-thiocyanato-9-(β-D-ribofuranosyl)purine (V)
2-Amino-6-hydroxy-8-thiocyanato-9-(β-D-ribofuranosyl)purine (VI)
6-Amino-8-thiocyanatopurine (VII)
2-Thiocyanato-6-hydroxypurine (VIII)
2,6-Dihydroxy-8-thiocyanato-9-(β-D-ribofuranosyl)purine (IX)
6,8-Dithiocyanatopurine (X)
2-Hydroxy-6-thiocyanatopurine (XI)
2-Amino-6-hydroxy-8-thiocyanatopurine (XII)
6-Hydroxy-8-thiocyanatopurine (XIII)
2,6-Dihydroxy-8-thiocyanatopurine (XIV)
6-Purinethiol (XV)[8]
2-Amino-6-purinethiol (XVI)
9-(β-D-Ribofuranosyl)-6-purinethiol (XVII)[9]
9-(β-D-Ribofuranosyl)-2-amino-6-purinethiol (XVIII)[9]
2-Amino-6-methylthiopurine (XIX)
2-Amino-6-methylthio-9-(β-D-ribofuranosyl)purine (XX)[10]

TABLE I.



No.	Starting R_1	Starting R_2	New R_2	Yield (%)	mp (°C)	UV Absorption maxima (mμ) pH 1	UV Absorption maxima (mμ) pH 11
X	H	SH	SCN	78	>240	290	258, 334
XI	OH	SH	SCN	58	>240	295	250, 340
XII	NH_2	OH	OH	38	>240	267	238, 305
XIII	H	OH	OH	77	>240	270	238, 290
XIV	OH	OH	OH	77	>240	225, 287	248, 309

No.	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
X	$C_7H_2N_6S_2$	35.99	0.85	35.99	35.68	1.03	36.34
XI	$C_6H_3ON_5S$	37.24	1.55	36.27	37.53	1.76	36.59
XII	$C_6H_4ON_6S$	34.61	1.92	40.38	34.65	2.06	40.88
XIII	$C_6H_4ON_5S$	37.24	1.55	36.27	37.39	1.66	36.81
XIV	$C_6H_2O_2N_5S$	34.61	0.96	33.65	34.82	1.09	33.11

Among the substances described above, I—IX were reported in our paper previously.[5] Compounds X—XIV were newly prepared by similar procedure described in our paper. Yield from corresponding mercaptopurines,[8-11] physical constants and analytical data were summarized in Table I. Ultraviolet

8) G.B. Elion, I. Goodman, W. Lange, and G.H. Hitchings, *J. Am. Chem. Soc.*, **81**, 1898 (1959).
9) A.G. Beaman, *J. Am. Chem. Soc.*, **76**, 5633 (1954).
10) C.W. Noell and R.K. Robins, *J. Am. Chem. Soc.*, **81**, 5997 (1959).
11) G.B. Elion and G.H. Hitchings, *J. Am. Chem. Soc.*, **77**, 1677 (1955).

absorption spectra of XIII in Fig. 1 reproduced. Compounds XVI and XIX were made available to us through the kindness of Dr. K. Nagasawa of the Biochemical Industry Co., Ltd., Tokyo. Compounds XV, XVII, XVIII and XX were synthesized in this laboratory by the method in the literatures.[11–13]

Mice were used young Swiss Albino females initially weighting about 19—20 g and five mice were used in each group.

Uniform amount of fragments of 10 day old NF–Sarcoma were implanted subcutaneously by means of trocar.

The substance to be tested was evenly suspended in saline solution and injected intraperitoneally daily for five days, beginning 24 hr after the tumor implantation.

The effect was determined by killing the mice ten days after the tumor implantation and comparing the tumor weights.

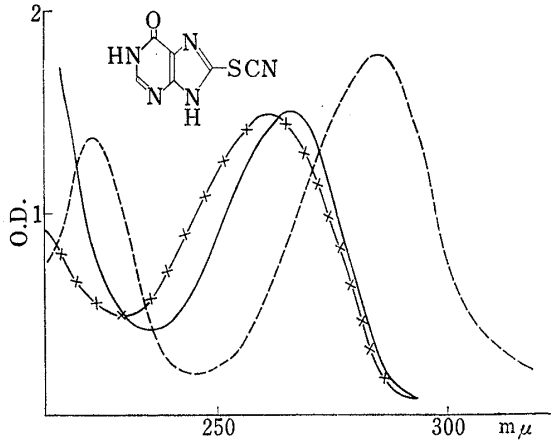


Fig. 1. Ultraviolet Absorbtion Spectrum of 6–Hydroxy–8–thiocyanatopurine (XIII)

———; pH 7 – – – – – –; pH 11
—×—×—; pH 1

Results and Discussion

The results of the entire experiments are summerized in Table II. 6–Thiocyanatopurine (I), 6–thiocyanato–9–(β–D–ribofuranosyl)purine (II), 2–amino–6–thiocyanatopurine (IV) and 2–amino–6–thiocyanato–9–(β–D–ribofuranosyl)purine (V) stand out as most effective.

In the substitution of amino, hydroxy and thiocyano groups at position 2 of 6–thiocyanatopurine, only 2–amino–6–thiocyanatopurine was as active as 2–amino–6–purinethiol (XVI) and others considered inactive.

Similarly, the substitution of thiocyano group at position 8 of 6–thiocyanatopurine decreases remarkably antitumor activities.

The introduction of ribofuranose at position 9 of 6–thiocyanatopurine derivatives which were effective, for example, 6–thiocyanato–9–(β–D–ribofuranosyl)purine (II) or 2–amino–6–thiocyanato–9–(β–D–ribofuranosyl)purine (V) did not change the antitumor activity and these results agreed with the fact that 9–β–D–ribofuranosyl derivative of 6–purinethiol or 2–amino–6–purinethiol were as active as 6–purinethiol and 2–amino–6–purinethiol.

The alkylthio or arylthio derivatives of purines and their ribonucleosides at position 6, synthesized and studied by Noell and Robins[13] and Montgomery, *et al.*[14] were less effective on the experimental tumors, and these facts, taken together with our own results with 2–amino–6–methylthiopurine (XIX) and 2–amino–6–methylthio–9–(β–D–ribofuranosyl)purine (XX), indicated that alkylthio derivatives of purine were less active than thiocyanato derivatives.

In additional experiments thiocyanato compounds I, II, IV and V, which were effective against NF–Sarcoma, were tested against Ehrlich ascites Carcinoma but all the compounds were found to be ineffective.

Authors have not given the details of the toxicity test, but thiocyanato derivatives in general were found to be more toxic than thiol derivatives. For example, 2–amino–6–thiocyanatopurine was about four times as toxic as 2–amino–6–purinethiol. The effective dose of 6–purinethiol was 30 mg/kg equimolar dose of 6–thiocyanatopurine, *i.e.* 35.1 mg/kg was nearly LD_{50}.

12) J.J. Fox, I. Wempen, A. Hampeton, and I.L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).
13) C.W. Noell and R.K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).
14) J.A. Montgomery, T.P. Johnston, A. Gollagher, C.R. Stringfellow, and F.M. Schabel Jr., *J. Med. Chem.*, **3**, 265 (1961).

Table II. Antitumor Effect of Thiocyanato Compounds

Comp. No.		Dose (mg/kg)	Survivors	Av. body wt. change (g treat/cont.)	Av. tumor wt. (g treat/cont.)	Tumor Inhibition (%)
I		35.1×1	0/5			
		7.5×5	5/5	1.74/3.62	0.26/1.22	78.7
		7.0×5	5/5	−0.10/−1.42	0.42/4.66	91.0
		7.0×5	5/5	1.80/0.80	0.15/0.96	88.9
		7.0×5	5/5	2.12/1.58	0.46/2.16	78.8
	Av.	7.0×5	5/5	1.27/0.32	0.34/2.59	86.2
		5.0×5	5/5	2.66/3.62	0.32/1.22	73.8
II		61.0×5	5/5	1.64/3.08	0.38/2.16	87.6
		61.0×5	5/5	1.70/2.18	1.10/3.20	65.6
		61.0×5	5/5	1.00/1.20	0.64/1.80	64.5
	Av.	61.0×5	5/5	1.44/2.15	0.71/2.39	72.5
III		47.0×3	2/5			
		20.0×5	5/5	2.02/2.30	3.58/3.98	10.1
IV		3.4×5	5/5	−2.85/1.98	0.06/2.18	97.3
		3.0×5	5/5	−1.20/0	0.10/2.30	95.7
		2.0×5	5/5	0.10/0	0.24/2.30	89.6
V		5.8×5	5/5	−0.80/1.20	0.32/1.80	82.3
		4.0×5	5/5	1.40/1.90	0.20/1.02	80.0
		4.0×5	5/5	0.48/0.96	0.82/3.40	75.9
	Av.	4.0×5	5/5	0.94/1.43	0.51/2.21	77.9
VI		10.0×5	5/5	2.56/3.06	2.36/1.58	−49.3
VII		38.0×3	1/5	2.20/2.56	4.70/2.74	−71.5
VIII		38.0×1	0/5			
		30.0×5	4/5	−1.64/3.20	2.14/2.18	1.9
IX		7.0×5	4/5	2.02/0.74	3.72/4.42	15.8
X		20.0×3	1/5			
		15.0×5	5/5	2.08/2.68	1.38/2.48	44.4
		9.3×5	5/5	2.50/2.56	3.48/2.74	−27.0
XI		15.0×5	5/5	1.50/2.10	2.80/1.66	−66.6
XII		15.0×5	5/5	1.90/2.12	3.14/1.66	−89.1
XIII		40.0×2	0/5			
		30.0×5	5/5	2.14/1.44	2.00/2.50	20.0
XIV		7.0×5	5/5	2.60/3.08	2.06/2.16	4.7
XV		30.0×5	5/5	1.06/1.26	0.16/2.54	93.7
		6.0×5	5/5	1.22/1.58	0.46/2.16	78.8
XVI		3.0×5	5/5	−0.90/1.00	0.30/3.40	91.2
XVII		56.0×5	5/5	3.12/2.18	0.16/3.20	95.8
XVIII		2.0×5	5/5	1.00/3.90	0.16/2.90	94.5
XIX		56.0×5	5/5	1.04/1.96	1.94/2.42	20.0
XX		3.9×5	5/5	2.88/1.28	4.08/3.92	−4.1

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