9-Methylhypoxanthine.—6-Fluoro-9-methylpurine (X, 0.15 g.) was dissolved in 5 ml. of distilled water at room temperature. After 7 days crystals formed. The product was identified as 9-methylhypoxanthine by its characteristic ultraviolet spectrum.[1b]

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0. Found: C, 47.7; H, 4.5.

The Antitumor Activity of 2-Amino-6-alkylthio-9-(β-D-ribofuranosylpurines and related Derivatives of 2-Amino-6-purinethiol (Thioguanine)[1]

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A number of 6-alkylthio-2-aminopurines and their ribosides have been prepared and tested against Adenocarcinoma 755. Alkylation of 2-amino-9-β-D-ribofuranosyl-6-purinethiol with the appropriate alkyl halide gave the desired riboside derivatives. Many of these compounds exhibit excellent activity against Adenocarcinoma 755 and significant activity against Sarcoma 180 and Leukemia 1210.

The antitumor activity of a number of 9-alkyl-2-amino-6-purinethiols[2] and 6-alkylthio-2-aminopurines[3] against Adenocarcinoma 755 suggested the synthesis of the corresponding 6-alkylthio-2-aminopurine-9-ribosides. The possibility of the increased antitumor activity of the 6-alkylthio-2-amino-9-β-D-ribofuranosylpurines was suggested by the fact that 9-β-D-ribofuranosyl-6-purinethiol possesses a therapeutic index[4] of 200 as compared to 30 for 6-mercaptopurine against Adenocarcinoma 755. 6-Mercaptopurine ribonucleoside is also inhibitory at a lower dosage[4] than 6-mercaptopurine in Adenocarcinoma 755. In addition, 2-amino-9-β-D-ribofuranosyl-6-purinethiol (thioguanosine) given orally in the clinic is more active on a

(1) This investigation was supported by Contract No. SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

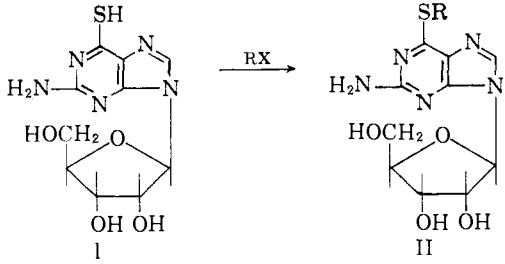
(2) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).

(3) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel, and A. G. Beaman, *J. Am. Chem. Soc.*, **82**, 2633 (1960).

(4) H. E. Skipper, J. A. Montgomery, J. R. Thomson, and F. M. Schabel, Jr., *Cancer Research*, **19**, 425 (1959).

molar basis than 2-amino-6-purinethiol.[5,6] Leukopenia was produced in patients with thioguanosine at a substantially lower oral dose than with thioguanine.[5] Thus, it was desirable to find out whether the ribosides of the active 6-alkylthio-2-aminopurines would offer further improvement in antitumor activity.

The ready availability of thioguanosine[7] greatly aided the present work. When I was treated in an ammoniacal solution with the appropriate alkyl halide, the corresponding 6-alkylthio-2-amino-9-β-D-ribofuranosylpurine (II) was prepared in good yield. The isolation and purification of the desired product presented some difficulties since it was absolutely essential that no unreacted starting material be present.



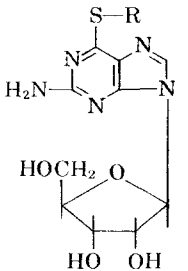
Paper chromatography was valuable in determining the purity of these derivatives. A mixture of 1-butanol saturated with water moved the alkylated products quite rapidly, while thioguanosine was characterized by very little movement. Both compounds could be readily detected with ultraviolet light. The ultraviolet absorption spectra of the products (II) are, in general, quite similar to those of the corresponding S-substituted derivatives of 2-amino-6-purinethiol.[2]

Table II shows a comparison of the activity of certain 6-alkylthio-2-aminopurines and the corresponding 9-ribosides against Adenocarcinoma 755. It can be seen by inspection of Table II that in general the 6-alkylthio-2-aminopurines possess a therapeutic index superior to the corresponding ribosides, 2-amino-6-(phenethylthio)-9-β-D-ribofuranosylpurine being the only exception. In some instances the ribosides were active at a much lower dosage than the parent purine, and in other cases the situation was reversed. There seems to be no detectable general pattern or relationship of activity between the purines and ribosides. It can be stated, however, that

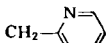
(5) I. W. Krakoff, R. R. Ellison, and C. T. C. Tan, *Proc. Am. Assoc. Cancer Research*, **3**, 34 (1959); *Cancer Research*, **21**, 1015 (1961).
(6) J. H. Burchenal and R. R. Ellison, *Clin. Pharm. and Therap.*, **2**, 537 (1961).
(7) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).

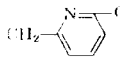
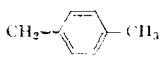
TABLE I

6-ALKYLTHIO-2-AMINO-9-β-D-RIBOFURANOSYLPURINES



R	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Prepn.	Recrysta. solvent	Yield, %	MeOH λ_{max} mμ	MeOH ε	Alkyl halide
CH_3	182	$C_{11}H_{15}N_5O_4S$	42.1	42.4	4.8	4.8	22.3	22.2	A	EtOAc	30	221 245 310	15,300 14,400 11,000	[a]
C_2H_5	[b]	$C_{12}H_{17}N_5O_4S \cdot 0.5H_2O$	42.9	43.1	5.4	5.3	20.8	21.0	A	EtOAc–pet. ether (60–110°)	49	221 246 311	18,800 16,800 13,400	I
n-C_3H_7	[b]	$C_{13}H_{19}N_5O_4S \cdot 0.5H_2O$	44.5	44.4	5.7	5.5	20.0	19.7	A	EtOAc–pet. ether (60–110°)	63	221 246 311	19,000 16,600 13,300	I
iso-C_3H_7	[b]	$C_{13}H_{19}N_5O_4S \cdot 0.5H_2O$	44.5	44.4	5.7	5.8	20.0	20.0	A	EtOAc–pet. ether (60–110°)	60	221 246 312	18,600 15,800 13,400	I
n-C_4H_9	[c]	$C_{14}H_{21}N_5O_4S \cdot 0.5H_2O$	46.2	45.9	6.1	5.6	19.2	19.3	A	EtOAc–pet. ether (60–110°)	57	221 246 311	19,600 16,900 13,800	I
iso-C_4H_9		$C_{14}H_{21}N_5O_4S \cdot 0.5H_2O$	46.2	46.3	6.1	5.9	19.2	18.8	A	EtOAc–pet. ether (60–110°)	57	221 246 311	19,600 16,400 13,800	I
sec-C_4H_9		$C_{14}H_{21}N_5O_4S \cdot 0.5H_2O$	46.2	46.8	6.1	6.1	19.2	18.8	A	EtOAc–pet. ether (60–110°)	48	221 247 312	18,900 16,400 13,800	Br

$CH_2CH=CH_2$		$C_{13}H_{17}N_5O_4S \cdot 0.5H_2O$	44.8	44.9	5.2	4.9	20.1	20.5	A	EtOAc–pet. ether (60–110°)	33	221 246 312	18,800 16,000 13,200	Br
$CH_2C_6H_5$	101–103	$C_{17}H_{19}N_5O_4S \cdot H_2O$	50.0	50.5	5.2	5.1	17.2	17.2	A	Pet. ether (60–110°)–acetone	69	219 247 312	24,800 16,200 14,200	Cl
$CH_2C_6H_4$-Br-*p*	147–148	$C_{17}H_{18}BrN_5O_4S \cdot H_2O$	41.9	42.2	4.1	4.0	14.4	14.4	B	Pet. ether (60–110°)–acetone	89	222 245 312	31,600 18,400 14,800	Br
$CH_2C_6H_4$-Cl-*o*	115–117	$C_{17}H_{18}ClN_5O_4S \cdot H_2O$	46.2	46.5	4.5	4.4	15.9	16.2	B	Pet. ether (60–110°)–acetone	77	219 246 312	27,900 17,200 15,000	Cl
$CH_2C_6H_4$-Cl-*p*	162	$C_{17}H_{18}ClN_5O_4S \cdot H_2O$	46.2	46.3	4.5	4.8	15.9	15.9	B	Pet. ether (60–110°)–acetone	74	222 246 312	31,400 17,600 15,000	Cl
$CH_2C_6H_4$-F-*m*	102–105	$C_{17}H_{18}FN_5O_4S \cdot H_2O$	48.0	48.6	4.7	4.7	16.5	16.7	B	Pet. ether (60–110°)–acetone	54	219 246 313	23,800 16,300 14,200	Cl
$CH_2C_6H_4$-NO_2-*p*	200–201	$C_{17}H_{18}N_6O_6S$	47.0	46.7	4.2	4.4	19.4	18.9	C	Pet. ether (60–110°)–acetone	86	219 246 312	23,500 18,200 16,100	Cl
$CH_2C_6H_4$-NO_2-*o*	156–158	$C_{17}H_{18}N_6O_6S$	47.0	47.1	4.2	4.2	19.4	18.8	C	Pet. ether (60–110°)–acetone	83	219 245 312	25,500 19,100 14,600	Cl
$CH_2COC_6H_5$	186	$C_{18}H_{19}N_5O_5S$	51.9	52.1	4.6	4.4	16.8	16.5	C	EtOAc–MeOH	69	223 243 311	26,600 26,300 14,800	Cl
$CH_2COC_6H_4Cl$-*p*	124–125	$C_{18}H_{18}ClN_5O_5S$	47.8	47.5	4.0	4.2	15.5	15.7	C	EtOAc–MeOH	83	221 246 311	24,400 28,000 14,000	Br
	210–211	$C_{16}H_{18}N_6O_5S$	49.3	48.7	4.6	4.7	21.5	21.3	D	EtOH	92	221 246 312	23,800 18,300 14,600	Cl
	175	$C_{16}H_{18}N_6O_4S$	49.3	49.0	4.6	4.8	21.5	21.6	D	H_2O	74	220 246 313	24,200 18,000 14,200	Cl

	246–248	$C_{11}H_{20}N_6O_4S$	50.5	50.5	5.0	5.7	20.8	20.5	D	EtOH	61	220 246 312	21,000 15,600 13,800	Cl
	108–110	$C_{18}H_{21}N_5O_6S$	53.7	53.8	5.2	5.8	17.4	17.6	B	Pet. ether (60–110°)–acetone	83	221 247 312	27,200 16,100 14,900	Br
	234–236 dec.	$C_{11}H_{16}N_6O_6S \cdot H_2O$	38.0	38.1	4.1	4.1	25.3	25.5	D	H_2O	78	223 311	29,100 11,600	Cl

[a] Dimethyl sulfate. [b] These compounds did not melt sharply but gradually turned into a glassy melt.

TABLE II

COMPARISON OF THE CHEMOTHERAPEUTIC INDICES OF CERTAIN S-SUBSTITUTED DERIVATIVES OF 6-THIOGUANINE AND THE CORRESPONDING 9-RIBOSIDE AGAINST ADENOCARCINOMA 755

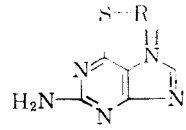
Compound	Inhibition T/C at MTD	MTD[a] mg./kg./day	MED[b] mg./kg./day	CI MTD/MED	Ratio[c] CI_R/CI_P
2-Amino-6-(isopropylthio)-purine	0.07	100	1	100	0.16
2-Amino-6-(isopropylthio)-9-β-D-ribofuranosylpurine	0.00	6.25	0.38	16	
2-Amino-6-(*n*-butylthio)purine	0.00	125	2	63	0.13
2-Amino-6-(*n*-butylthio)-9-β-D-ribofuranosylpurine	0.00	31.25	3.9	8	
2-Amino-6-(isobutylthio)purine	0.04	64	2	32	1.00
2-Amino-6-(isobutylthio)-9-β-D-ribofuranosylpurine	0.02	150	4.65	32	
2-Amino-6-(*o*-chlorobenzylthio)purine	0.00	36	0.56	64	0.45

2-Amino-6-(*o*-chlorobenzylthio)-9-β-D-ribofuranosylpurine	0.03	50	1.7	29	0.25
2-Amino-6-(*p*-nitrobenzylthio)-purine	0.06	1000	62.5	16	
2-Amino-6-(*p*-nitrobenzylthio)-9-β-D-ribofuranosylpurine	0.08	75	>18.75	>4	
2-Amino-6-(*p*-methylbenzylthio)-purine	0.07	400	50	8	0.25
2-Amino-6-(*p*-methylbenzylthio)-9-β-D-ribofuranosylpurine	0.00	100	50	2	
2-Amino-6-(phenethylthio)purine	0.11	500	125	4	2.00
2-Amino-6-(phenethylthio)-9-β-D-ribofuranosylpurine	0.00	300	37.5	8	
2-Amino-6-(6-methyl-2-pyridylmethylthio)purine	0.00	62.5	3.9	16	0.67
2-Amino-6-(6-methyl-2-pyridylmethylthio)-9-β-D-ribofuranosylpurine	0.03	400	37.5	10.7	
2-(2-Amino-6-purinylthio)acetophenone	0.02	240	3.75	65	0.12
2-(2-Amino-9-β-D-ribofuranosylpurin-6-ylthio)acetophenone	0.00	300	>37.5	>8	
2-Amino-6-(methylthio)-9-β-D-ribofuranosylpurine	0.00	225	33	3.4	
2-Amino-6-(1 methyl-4-nitro-5-imidazolylthio)-9-β-D-ribofuranosylpurine	0.01	7	0.11	64	

[a] Defined as the maximum tolerated dose with no less than $^{7}/_{10}$ survivors. [b] Defined as the minimum effective dose that inhibits tumor growth to 50% of untreated controls. [c] Chemotherapeutic Index of Riboside/Chemotherapeutic Index of the Simple Purine.

TABLE III

ANTITUMOR ACTIVITY OF SOME SELECTED DERIVATIVES OF 2-AMINO-6-PURINETHIOL (THIOGUANINE)



R	Dose, mg./kg.	Survivors	Wt. change, test/control	Tumor wt., test/control	T/C
		Ca-755			
iso-C_3H_7	100	10/10	−0.9/0.4	107/1417	0.07
	50	8/10	−3.0/2.1	6/1577	0.00
	25	8/10	−2.5/2.1	0/1577	0.00
	12.5	10/10	−2.4/2.1	25/1577	0.01
	6.25	9/10	−1.7/2.1	44/1577	0.02
	3.1	10/10	−1.4/2.1	185/1577	0.11
	1.6	10/10	0.3/2.1	267/1577	0.16
		S-180			
	50	5/6	−2.0/0.1	238/358	0.35
		L-1210			
	75	6/6	−0.6/0.1	13.3/9.8	1.35
	50	6/6	−1.0/0.5	12.3/9.5	1.62
	33	6/6	−0.2/0.1	14.5/9.8	1.47
	22	6/6	−0.1/0.1	13.5/9.8	1.37
		Dunning Leukemia			
	50	6/6	17.0/41.0	22.0/15.0	1.46
	10	6/6	17.0/14.0	19.5/15.0	1.30
		Ca-755			
iso-C_4H_9	64	9/10	−1.9/0.9	72/1526	0.04
	32	10/10	−2.3/0.9	80/1526	0.05
	16	10/10	−2.1/0.9	105/1526	0.06
	8	10/10	−0.5/0.9	162/1526	0.10
	4	10/10	0.3/0.9	471/1526	0.30
	2	8/10	0.7/0.9	708/1526	0.46
		Ca-755			
iso-C_5H_{12}	250	9/10	−2.9/2.2	49/908	0.05
	200	8/10	−3.3/1.6	0/1286	0.00
	100	8/10	−2.3/2.6	46/1182	0.03
	50	10/10	−0.4/0.2	20/587	0.03
	25	10/10	−1.5/0.1	45/854	0.05
	12.5	9/10	−0.5/1.0	444/1119	0.39
	3.1	8/10	−1.1/1.0	350/1119	0.31
		S-180			
	300	6/6	−4.5/−3.7	267/1414	0.18
	200	5/6	−6.0/−3.7	250/1414	0.17
	89	4/6	−4.7/−3.7	650/1414	0.45

		Ca-755			
cyclo-C_6H_{11}	160	8/10	−1.9/0.8	0/1526	0.00
	80	10/10	−1.1/3.8	10/1965	0.00
	40	10/10	−1.2/0.8	18/1526	0.01
	20	10/10	−0.9/0.8	76/1526	0.04
		Ca-755			
$CH_2C_6H_4Cl$-*o*	36	8/10	−2.6/0.3	0/1004	0.00
	25	10/10	−2.0/2.6	35/1809	0.01
	18	8/10	−2.2/−0.3	6/1120	0.00
	9	10/10	−2.6/0.3	0/1004	0.00
	4.5	9/10	−1.4/0.3	181/1004	0.18
	2.25	7/10	−1.2/−0.3	521/1120	0.46
		Ca-755			
$CH_2C_6H_4NO_2$-*p*	1000	10/10	−1.3/3.4	113/1862	0.06
	800	10/10	−0.2/2.5	144/1732	0.08
	600	10/10	−2.6/0.0	116/1101	0.10
	500	8/10	−1.8/0.6	25/1141	0.02
	250	9/10	−1.8/0.5	333/996	0.33
	125	9/10	−1.4/0.5	406/996	0.40
	62.5	10/10	−0.5/0.4	530/1106	0.47

whenever a given 6-alkylthio-2-aminopurine exhibited significant antitumor activity against Adenocarcinoma 755, the corresponding riboside also showed similar activity in the same test system. All the compounds listed in Table I showed significant activity at least at one dosage level. Table III gives the general testing data for a few selected derivatives.

The testing was done under the auspices of the Cancer Chemotherapy National Service Center, and the testing procedures have been adequately described previously.[8,9]

Of particular interest is 2-amino-6-(isopropylthio)purine which possesses a therapeutic index of 100 (Table II). 2-Amino-6-(*n*-butylthio)purine and 2-amino-6-(*o*-chlorobenzylthio)purine show a therapeutic index of 63 and 64, respectively, against Adenocarcinoma 755.

It is of interest to compare the present work with a similar study by Montgomery, *et al.*,[10] on the 6-alkylthiopurines and their ribonucleosides. These investigators found that none of the 6-alkylthio-9-β-D-ribofuranosyl-purines were significantly more effective against

(8) J. Leiter, A. R. Bourke, D. B. Fitzgerald, S. A. Schepartz, and I. Wodinsky, *Cancer Research*, **22**, 221, part 2 (1962).

(9) J. Leiter, A. R. Bourke, S. A. Schepartz, and I. Wodinsky, *ibid.*, **20**, 734, part 2 (1960).

(10) J. A. Montgomery, T. P. Johnston, A. Gallagher, C. R. Stringfellow, and R. M. Schabel, Jr., *J. Med. Pharm. Chem.*, **3**, 265 (1961).

Andenocarcinoma 755 than the simple 6-alkylthiopurines. In all cases studied by Montgomery[10] the 6-alkylthiopurine appeared more active per given dosage of drug than the corresponding 6-alkylthio-9-β-D-ribofuranosylpurine. In contrast the present study shows that in three cases studied the 6-alkylthio-2-aminopurine riboside was more active at a given dosage level than the corresponding 6-alkylthio-2-aminopurine. This suggests a possible difference in the mechanism of action of the 6-alkylthiopurine ribosides and the 6-alkylthio-2-aminopurine ribosides against Adenocarcinoma 755. This seems quite reasonable since recent findings indicate that a different mechanism of action exists for 6-mercaptopurine and 6-thioguanine. The work of Bieber, *et al.*,[11] has shown that a 6-mercaptopurine-resistant strain of Ca-755 incorporates as much or more 6-mercaptopurine into DNA as does the sensitive parent strain. These workers have shown there is no correlation of the amount of incorporation of 6-mercaptopurine and tumor inhibition. LePage and Jones[12] on the other hand have shown that the tumor-inhibitory response of 6-thioguanine in Ca-755 and several other tumors can be correlated readily with the incorporation of this analog into the nucleic acids of the tumor.

In view of these findings, as previously suggested by Noell and Robins,[2] it would seem that the use of more than one purine derivative at one time in the clinic could be quite rewarding. Because of different biological mechanisms of action by similar drugs, one might well expect a synergistic response.

Several of the 6-alkylthio-2-aminopurines have been previously studied for activity against Sarcoma 180 by Clarke, *et al.*[13] Comparison of the data obtained by these investigators on the activity of 2-amino-6-(*o*-chlorobenzylthio)purine against Sarcoma 180 and the present study of the corresponding 2-amino-6-(*o*-chlorobenzylthio)-9-β-D-ribofuranosylpurine (Table IV) is of considerable interest. The parent purine is inactive at 500 mg./kg., and the riboside gives excellent inhibition at 125 mg./kg. against the same tumor. Further testing of the 6-alkylthio-2-aminopurine ribosides against Sarcoma 180 and other tumors is required, however, before the superiority of the riboside can be definitely established. It would be of considerable interest to examine the 6-alkylthio-2-amino-9-β-D-ribofuranosylpurines (Table IV) for possible cross-resistance or synergism with the simple purine derivatives presently employed by the clinician. The

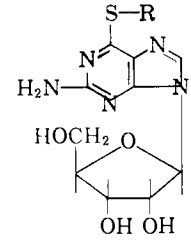
(11) S. Bieber and R. Pomales, *Proc. Am. Assoc. Cancer Research*, **3**, 304 (1962); S. Bieber, L. S. Deitrich, G. B. Elion, G. H. Hitchings, and D. S. Martin, *Cancer Research*, **21**, 228 (1961).

(12) G. A. LePage and M. Jones, *ibid.*, **21**, 1590 (1961).

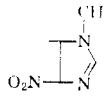
(13) D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *ibid.*, **18**, 445 (1958).

TABLE IV

ANTITUMOR ACTIVITY OF SOME SELECTED DERIVATIVES OF 2-AMINO-9-β-D-RIBOFURANOSYL-6-PURINETHIOL



R	Dose, mg./kg.	Survivors	Wt. change, test/control	Tumor wt., test/control	T/C
		Ca-755			
CH_3	225	10/10	−1.6/1.9	5/1102	0.00
	112.5	8/10	−1.0/1.7	31/683	0.04
	66	10/10	−0.3/1.0	112/1146	0.09
	33	10/10	0.1/1.0	236/1146	0.20
	16.5	9/10	0.3/1.0	616/1146	0.53
		Ca-755			
n-C_4H_9	62.5	6/10	−3.8/−0.9	0/574	toxic
	31.25	10/10	−3.4/1.3	20/1691	0.01
	15.62	10/10	−2.7/1.3	110/1691	0.06
	7.81	9/10	−1.9/1.3	331/1691	0.19
	3.9	10/10	−0.9/1.3	860/1691	0.50
		Ca-755			
iso-C_4H_9	150	9/10	−2.1/0.0	39/1412	0.02
	75	9/10	−3.3/0.0	39/1412	0.02
	37.5	9/10	−2.7/0.0	44/1412	0.03
	18.75	10/10	−2.6/0.0	180/1412	0.12
	9.3	10/10	−1.2/0.4	450/1778	0.25
	4.65	9/10	−0.2/0.4	663/1778	0.37
		Ca-755			
$CH_2C_6H_4Cl$-o	50	7/10	−3.3/0.7	44/1352	0.03
	27	10/10	−1.6/2.0	35/1649	0.02
	13.5	9/10	−0.9/2.0	47/1649	0.02
	6.75	10/10	−0.9/2.4	84/1815	0.04
	3.4	9/10	−0.2/2.4	481/1815	0.26
	1.7	7/10	0.7/2.4	415/1815	0.22
		S-180			
	125	6/6	−2.8/−0.7	102/1126	0.09
	45	5/6	−2.7/−0.7	228/621	0.36
		Ca-755			
$C_2H_4C_6H_5$	300	8/10	−4.2/−0.9	0/574	0.00
	150	8/10	−2.4/−0.9	6/574	0.01
	75	10/10	−1.8/−0.9	20/574	0.03
	37.5	9/10	−1.9/−0.9	50/574	0.08
	18.75	8/10	0.1/1.3	1150/1691	0.68



7	7/10	−3.1/2.0	20/1649	0.01
3.5	9/10	−1.2/2.0	37/1649	0.02
1.75	10/10	−1.0/2.0	66/1649	0.04
0.44	10/10	−0.6/2.9	37/1830	0.02
.22	10/10	0.4/2.4	16/1587	0.01
.11	10/10	0.5/2.4	231/1587	0.14
.06	10/10	1.6/2.4	1218/1587	0.76
	S-180			
11	6/6	−2.5/−0.8	118/641	0.18
7.5	6/6	1.2/2.5	229/1100	0.20
5	6/6	0.4/−0.8	411/641	0.64
	L-1210			
7.5	6/6	−2.2/0.2	11.5/8.5	1.35
5	6/6	−1.4/0.2	12.6/8.5	1.48
3.3	6/6	−0.8/1.1	15.5/9.1	1.70
0.94	6/6	−1.6/0.7	11.5/9.5	1.21

relationship of the 9-alkyl-6-alkythio-2-aminopurines[2] and 9-alkyl-2-amino-6-purinethiols[2] to the compounds here described remains to be clarified. It is interesting that all these related derivatives possess a very high order of antitumor activity.

Experimental[14]

2-Amino-6-methylthio-9-β-D-ribofuranosylpurine. Method A.—Ten grams of 2-amino-9-β-D-ribofuranosyl-6-purinethiol monohydrate[7] (0.0316 mole) was stirred in 20 ml. of water and 20 ml. of 28% ammonium hydroxide, and then 4.0 g. of dimethyl sulfate (0.0328 mole) in 10 ml. of *p*-dioxane was added. This mixture was stirred at 45–50° for 2.5 hr. The cooled mixture was extracted 8 times with 50-ml. portions of ethyl acetate, and the combined extracts were washed with 20 ml. of water. The ethyl acetate extract was dried over 20 g. of anhydrous sodium sulfate and filtered. The excess ethyl acetate was removed *in vacuo* using a water bath as the source of heat. The crystalline mass was dissolved in 100 ml. of boiling methanol, treated with Norite, and filtered. The filtrate was again reduced to dryness *in vacuo*, and the crystalline mass was triturated in 50 ml. of petroleum ether (30–60°) and finally dried at 60° to give the desired product (see Table I).

Method B.—Five grams of 2-amino-9-β-D-ribofuranosyl-6-purinethiol monohydrate[7] (0.0158 mole) was dissolved in 100 ml. of water and 20 ml. of 28% ammonium hydroxide. Then 0.0165 mole of alkyl halide, dissolved in 30 ml. of *p*-dioxane, was added and the mixture stirred at 55–60° for 3 hr. The condenser was removed, and the temperature was increased to a gentle boil with stirring until the volume was reduced to approximately three-fourths the original. Ammonium hydroxide (28%, 30 ml.) was added, and the mixture was allowed to cool and then decanted from the gummy precipitate. The gummy material was dissolved in 100 ml. of boiling methanol, treated with Norite, and filtered. The filtrate was reduced to dryness *in vacuo* with a water bath as the source of heat,

(14) All melting points were taken on a Fisher–Johns melting point apparatus and are uncorrected unless otherwise indicated.

and the product was redissolved in methanol and reduced to dryness. This process was repeated 2 or 3 times until a completely crystalline mass was obtained. The crystals were triturated in 50 ml. of warm petroleum ether (30–60°), filtered, and dried at 60° to give the desired product (see Table I).

Method C.—This method is identical to method A except that the crude reaction mixture was cooled and filtered and the precipitate washed with water and dried. The crude product was finely pulverized and triturated with 100 ml. of petroleum ether, filtered, and dried at 60° to give the desired product.

Method D.—Method D is identical to method C except that no dioxane was employed.

2-Amino-6-[(6-methyl-2-pyridyl)methylthio]purine.—To 10.0 g. of 2-amino-6-purinethiol,[3] dissolved in 150 ml. of concd. ammonium hydroxide, was added with stirring and heating at 40° 11.0 g. of 6-methyl-2-picolyl chloride hydrochloride. The reaction temperature was maintained for one additional hr., then the product was filtered, washed with water, and recrystallized twice from ethanol. The yield was 11.2 g., m.p. 236–237°.

Anal. Calcd. for $C_{12}H_{12}N_6S$: C, 53.1; H, 4.4; N, 30.9. Found: C, 53.6; H, 4.5; N, 30.6.

Pyrimidines. IX. 4- and 5-(Substituted-anilino)pyrimidines[1,2]

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A number of 4- and 5-(substituted-anilino)pyrimidines have been synthesized as potential riboflavin antagonists. These compounds represent four general types of the uncyclized isoalloxazine ring. A new general synthetic method for the synthesis of 5-anilinopyrimidines has been devised.

Interference of the biosynthesis of important enzymes and cofactors, such as DPN, TPN, FAD (flavin-adenine-dinucleotide) and coenzyme A, has generally been accepted as one of the best methods to block cellular growth.[3] This interference has also been postulated[4]

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(2) Presented in part before the Div. of Med. Chem., 139th Meeting of the American Chemical Society, St. Louis, Missouri, March, 1961.

(3) F. Bergel, "Chemistry of Enzymes in Cancer," Charles C Thomas, Springfield, Ill., 1961, pp. 47–80.

(4) M. R. Atkinson, J. F. Jackson and R. K. Morton, *Nature*, **192**, 946 (1961), and references listed therein.