40.4 g. (0.58 mole) of hydroxylamine hydrochloride, 100 ml. of ethanol, and 200 ml. of 20% aqueous sodium hydroxide was heated under reflux for 4 hr. Most of the ethanol was distilled under reduced pressure, water was added, and the mixture was acidified with hydrochloric acid. The solid was collected, giving 67.5 g. of material, m.p. 140–143°. This was recrystallized several times from ethyl acetate and twice from water, yielding 49.4 g. (56%) of crystals, m.p. 153° dec.

Anal. Calcd. for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.65; H, 4.89; N, 7.67.

3,4-Diacetoxy-5-methoxybenzonitrile.—A mixture of 39.8 g. (0.218 mole) of 3,4-dihydroxy-5-methoxybenzaldoxime and 75 g. of acetic anhydride was heated until a vigorous reaction started. After the reaction subsided the mixture was again heated under reflux with stirring for 1 hr. The dark mixture was poured into ice-water, giving a dark solid, wt. 44.5 g. A sample was recrystallized from ethanol with the aid of Darco, giving crystals, m.p. 119.5–121°.

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.82; H, 4.05; N, 5.80.

Acknowledgments.—The authors wish to thank the following people who contributed to this work: our Department of Physical and Analytical Chemistry for analytical and spectral data; Dr. George Slomp and Mr. Forrest MacKellar for n.m.r. spectra, Mr. William Veldkamp, Dr. Margaret Greig, and associates for biological data, Dr. R. V. Heinzelman for guidance, and Mr. R. F. Tripp and Mr. A. Koning for technical assistance.

Antitumor Activity and Structural Relationships of Purine Derivatives and Related Compounds against Neoplasms in Experimental Animals

ROLAND K. ROBINS[1]

Department of Chemistry, Arizona State University, Tempe, Arizona

Received August 9, 1963

A study of the various purines exhibiting antitumor activity has been made with special attention given to those compounds prepared in the author's laboratory over the past ten years. The antitumor activity of a number of purines is presented for the first time. Previously noted structure–activity relationships have been discussed and extended in view of possible binding sites on the purine molecule. Certain biochemical studies on the mode of action of the purines and purine analogs have been reviewed and several suggestions made for future synthetic work. Greater specificity of drug action and further biochemical studies on mechanisms of inhibition are needed.

The limited but encouraging success of certain simple purine derivatives and related compounds in the clinic[2a–d] against neoplastic diseases in man has stimulated the synthesis and study of a considerable number of potential purine antagonists in recent years. In our own laboratory over the past ten-year period approximately 1300 compounds (purines or related derivatives) have been prepared and evaluated under the auspices of the Cancer Chemotherapy National Service Center[2e] against various induced rodent tumors. It is now quite possible to classify most of the active compounds in various groups and to make certain general statements concerning structure and antitumor activity. In most instances preliminary screening was accomplished against Adenocarcinoma 755 since this tumor has been shown to be especially sensitive to inhibition by purines.[3] Adenocarcinoma 755 had earlier proved to be especially useful in the evaluation of structure–activity relationships of certain 4-aminopyrazolo[3,4-*d*]pyrimidines shown to possess antitumor activity in experimental mice.[4] Several previous studies of the antitumor activity of certain purine derivatives against this tumor have already appeared[3,5–7] and will be referred to often in the present report. In addition various structural derivatives of 6-purinethiol have been evaluated against Sarcoma 180 in mice.[8] Although there is certainly grave danger in extrapolating antitumor activity and structural relationships from one tumor to another, certain useful comparisons can be made. In the present work attention will be focused on functional group changes on the purine ring. The antitumor activity of purine nucleosides will only be referred to in the sense that D-ribose may be considered a 9-substituent. The antitumor activity of the various purine nucleoside-type antibiotics and active compounds prepared by changes in the carbohydrate moiety are beyond the scope of the present study.

From the point of view of the organic chemist the synthetic program was begun with the following goals. (1) Beginning with a lead "active" purine it was proposed to prepare a series of structurally related purines which could be evaluated to determine the maximum therapeutic index in experimental animals and therefore contribute to the careful selection of the most desirable derivative in a series for clinical investigation. (2) It was hoped to provide new purine derivatives which would exhibit antitumor action *via* different biochemical mechanisms. This would provide a powerful tool to combat the resistance often observed after prolonged

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clinical usage of a given purine. (3) Certain purines were designed for synthesis specifically to test current biochemical theories as to the mechanism of action of established purine antitumor drugs. (4) It was hoped to design a sufficient number of compounds so that information might be gained as to the "binding sites" of the biologically active purines on the surface of the purine acceptor enzymes. Since it was known that certain unnatural purines are biologically active, it was deemed worthwhile to determine what structural variations can be made and still retain biological activity. This involves a study of the specificity of the various enzymes which are concerned with the biochemistry of the preformed purines. Such information would be of considerable importance to the biochemist and could also serve as a guide for future synthetic work.

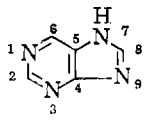
The extent to which these goals have been realized is the major subject of the present report.

Materials and Methods

All testing reported in Tables I–III was performed under the auspices of the Cancer Chemotherapy National Service Center or at the Sloan-Kettering Institute for Cancer Research. Testing procedures, methods, and protocol are adequately described elsewhere.[9a,b–12]

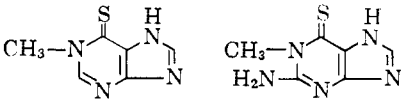
All compounds listed in Tables I–III were prepared in our laboratory, and the procedures employed in their synthesis in most instances have been previously published. Since negative screening results will eventually be published in *Cancer Research* as part of the Cancer Chemotherapy Screening Data, only data on active compounds or compounds exhibiting tumor inhibition at least at one dosage are included in this report. In certain instances a report of antitumor activity and chemical structure in a series of purines and related compounds has already been issued from our laboratory.[4,13–19] Since additional data against a wider spectrum of tumors is now available for some of these active compounds, this information is included in the present work.

Substitution on the Purine Ring and Antitumor Activity against Adenocarcinoma 755.—The results of Skipper, Montgomery, and co-workers[3] in general have been confirmed with similar compounds prepared in our own laboratory. It is now possible to extend this study to include substitution at positions 1, 2, 3, 6, 7, 8, and 9 of the purine ring and to note the corresponding effect on antitumor activity against Adenocarcinoma 755.



Purine

Substitution at Position 1.—Introduction of a methyl group at position 1 of 6-purinethiol gives 1-methyl-6-purinethione (I),[20] a compound which is completely inactive against Adenocarcinoma 755. 2-Amino-1-methyl-6-purinethione (II),[17] on the other hand,



I II

is apparently as active as 6-thioguanine against Adenocarcinoma 755 and possesses a therapeutic index of 64 as compared to 4 for 6-thioguanine against the same tumor.[17] These data strongly suggest that 6-purinethiol and 2-amino-6-purinethiol (6-thioguanine) do not necessarily act as antitumor agents *via* the same biochemical mechanism since substitution at position 1 affects the drugs differently. Such a suggestion is supported by the work of Henderson and Junga[21] who have shown potentiation of antitumor activity by a combination of 6-purinethiol and 2-amino-6-purinethiol. It is of interest that neither 1-methyladenine,[22] 1-methylhypoxanthine,[20] nor 1-methylxanthine[23a] exhibit any significant antitumor activity. 1-Methylguanine[23a] (Table I, **22**), however, does show some inhibition at one dosage level.

Substitution at Position 2.—Introduction of a substituent group, other than hydrogen, amino, or substituted amino, at position 2 usually results in loss of antitumor activity. The only known significant exception is the reported antitumor activity of 2-methyladenine[23b] which is reported to be active against Adenocarcinoma 755. This finding is of interest since 2-methyladenine has been reported as a natural purine present in soluble RNA from various sources.[24] 2-Methyl-6-purinethiol[25] and 6-chloro-2-methylpurine[25] are completely inactive. The presence of a 2-methylthio group[26] similarly renders 6-purinethiol and 6-chloropurine inactive. 2,6-Dichloropurine[27] is essentially inactive. Simple 2-substituted purines such as 2-hydroxypurine,[28] 2-purinethiol,[29] 2-chloropurine,[27] 2-bromopurine,[30] 2-fluoropurine,[31] 2-methylthiopurine,[28]

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Table I
Antitumor Activity of Various Purines and Related Derivatives against Adenocarcinoma 755

Compd. no.	Name	Dose (mg./kg.)	Survivors	Animal wt. diff. (T-C)	Tumor wt. (test/control)	T/C %	Reference to synthesis
1	2-Amino-6-hydroxy-8-methylpurine	125	3/10	−7.0	427/1827	Toxic	70
		62.5	9/10	−4.0	643/2515	25	
		62.5	8/10	−4.0	963/2513	38	
2	6-Methoxypurine	500	8/10	−3.7	0/748	0	46
		250	7/10	−2.7	0/748	0	
		250	8/10	−2.0	90/704	12	
		125	9/10	−2.0	8/748	1	
		62.5	10/10	−1.0	1557/1808	86	
3	6-Amino-8-purinol	250	0/10			Toxic	71
		125	7/10	−10.0	339/1832	18	
		125	8/10	−7.0	552/2230	24	
		62.5	8/10	−6.0	709/2094	33	
		16	10/10	−3.0	1562/2004	71	
4	2-Aminopurine	250	10/10	−6.0	1006/2795	35	28
5	2-Amino-6,8-purinedithiol	250	9/10	−3.3	894/1706	52	72
		125	9/10	−3.1	136/1263	10	
		125	8/10	−2.8	276/1426	19	
		62	9/10	0.1	2083/1706	122	
6	2-Amino-6-chloropurine	125	9/10	−3.7	59/1226	4	34
		62	10/10	−3.3	162/1226	13	
		31	10/10	−1.9	736/1226	60	
		15	6/10	−7.3	0/1504	Toxic	
		10	9/10	−2.5	732/858	85	
		5	8/10	−3.7	6/1504	0	
		2.5	10/10	−2.4	370/1504	24	
7	2,6-Diamino-9-(*p*-tolyl)purine hydrochloride	30	0/10			Toxic	77
		15	7/10	−4.2	471/1074	43	
		7	9/10	−5.5	59/1263	4	
		3	7/10	−1.5	877/1263	69	
		1.5	10/10	−1.6	655/1263	51	
8	2,8-Diamino-6-purinethiol	125	3/10	−6.9	0/900	Toxic	73
		62.5	4/10	−4.0	106/1073	Toxic	
		31	3/10	−0.9	105/556	Toxic	
		15	10/10	−2.3	1452/1604	90	
9	6-Methylthio-9-(tetrahydro-2-pyranyl)purine	250	3/10	−2.5	0/1571	Toxic	87
		125	9/10	−3.1	0/543	0	
		125	9/10	−4.0	70/1348	5	
		62	10/10	−2.4	314/1348	23	
		31	10/10	−1.7	525/1348	38	
		15	10/10	−1.8	830/1348	62	
10	9-(Tetrahydro-2-pyranyl)-6-purinethiol	250	0/10			Toxic	87
		125	9/10	−0.2	41/1099	3	
		62.5	10/12	−7.3	0/1005	0	
		60	9/10	−6.2	7/1550	0	
		31.25	12/12	−5.4	0/1005	0	
		30	10/10	−3.6	12/1550	0	
		15.62	11/12	−2.9	0/1005	0	
		15	10/10	−1.9	71/1077	6	
		7.5	10/10	−1.5	108/1077	10	
		3.8	10/10	−1.0	318/1077	29	
		1.9	10/10	−1.6	214/1099	19	
		0.8	8/10	0.1	1045/1276	81	
11	6-Iodo-9-(tetrahydro-2-pyranyl)purine	250	9/10	−4.4	0/948	0	87
		125	10/10	−2.6	142/948	14	
		62	9/10	−5.4	313/948	33	
		31	10/10	−2.3	297/948	31	
12	6-(*p*-Fluorobenzylthio)-9-(tetrahydro-2-pyranyl)-purine	360	0/10			Toxic	87
		250	10/10	−3.8	196/881	22	
		180	9/10	−4.6	11/1286	0	
		90	9/10	−3.7	0/1106	0	
		45	10/10	−2.6	0/996	0	
		22.5	9/10	−2.7	100/1106	9	
		11.25	10/10	−1.8	157/1106	14	
		6	10/10	−0.8	570/1163	49	
		5.6	9/10	−1.2	433/1106	39	

TABLE I (*Continued*)

Compd. no.	Name	Dose (mg./kg.)	Survivors	Animal wt. diff. (T-C)	Tumor wt. (test/control)	T/C %	Reference to synthesis
		3	9/10	−0.4	939/1163	80	
		1.5	10/10	−0.8	710/1163	61	
		0.75	8/10	−0.4	469/1163	40	
13	6-(Benzylthio)-9-methylpurine	504	4/10	−4.2	0/1156	Toxic	162
		252	8/10	−3.5	19/1156	1	
		126	7/10	−2.8	11/1676	0	
		63	8/10	−3.1	6/728	0	
		31	9/10	−2.7	190/1676	11	
		15	6/10	−1.2	435/1676	Toxic	
14	7-Diethylamino-3-methyl-*v*-triazolo[*d*]pyrimidine	250	7/10	−3.2	350/1286	27	128
		250	8/10	−2.5	763/1106	68	
15	5-Amino-7-(2,4-dichlorobenzylthio)-*v*-triazolo[*d*]-pyrimidine	70	0/10			Toxic	128
		35	7/10	−2.9	207/854	24	
		35	7/10	−5.5	78/1119	6	
		17.5	9/10	−3.3	544/1119	48	
16	7-Isobutylamino-*v*-triazolo[*d*]pyrimidine	100	1/10	−2.2	450/963	Toxic	128
		50	8/10	−1.9	656/1378	47	
		50	7/10	−3.9	150/1106	13	
17	9-(6-Ethoxytetrahydro-2-pyranyl)-6-purinethiol	75	0/10			Toxic	87
		37.5	7/10	−3.1	0/1380	0	
		37.5	9/10	−3.3	0/1100	0	
		18.75	10/10	−1.7	10/1100	0	
		9.38	8/10	−1.1	31/1100	2	
		4.69	10/10	−0.5	322/1100	29	
18	2-Amino-6-chloro-9-β-D-ribofuranosylpurine	600	4/10	−2.1	12/475	Toxic	80
		500	8/10	−1.4	329/1042	31	
		300	10/10	−2.8	55/475	11	
		150	9/10	−0.4	1047/1247	83	
19	6-Dimethylamino-9-(tetrahydro-2-furyl)purine	100	2/10	−3.9	250/881	Toxic	88
		50	7/10	−3.6	100/881	11	
		25	10/10	−1.7	760/1290	58	
		12.5	10/10	−0.4	1010/1290	78	
20	6-Bromopurine	300	6/10	−3.1	59/1108	Toxic	30
		200	10/10	−2.1	0/1290	0	
		150	10/10	−3.0	69/1108	6	
		100	10/10	−2.3	0/881	0	
		75	10/10	−2.8	68/1108	6	
		50	10/10	−1.6	5/1290	0	
		25	10/10	−2.3	0/1290	0	
		12.5	10/10	−3.5	139/1837	7	
		6.25	8/10	−2.4	681/1837	37	
		3.12	8/10	−1.3	588/1837	32	
21	6-Chloro-9-(6-methoxytetrahydropyran-2-yl)purine	300	1/10	−2.5		Toxic	87
		150	6/10	−2.1	0/632	Toxic	
		75	10/10	−1.3	30/632	4	
		37.5	9/10	−2.6	133/1403	9	
		18.75	10/10	−3.3	278/1403	19	
		9.37	8/10	−0.5	756/1403	53	
		4.68	10/10	−0.7	735/1403	52	
		2.34	10/10	−1.3	1133/1403	80	
22	1-Methylguanine	75	1/10	−7.0		Toxic	23
		37.5	7/10	−3.2	257/857	29	
		18.7	8/10	−1.0	593/864	68	
23	8-(*p*-[Bis(2-chloroethyl)amino]phenylazo)xanthine hydrochloride	300	0/10			Toxic	163
		200	2/10	−4.1	100/755	Toxic	
		100	7/10	−3.8	58/936	6	
		100	10/10	−6.1	145/755	19	
		50	10/10	−2.5	525/936	56	
		25	10/10	−1.3	695/936	74	
24	9-Acetyl-2-acetamido-6-(benzylthio)purine	65	2/10	−5.7	0/517	Toxic	19
		60	7/10	−2.6	0/661	0	
		30	10/10	−2.0	17/661	2	
		20	7/10	0.8	0/130	0	
		15	10/10	−0.8	28/661	4	
		7.5	10/10	0.1	351/661	53	
25	2-Acetamido-6-(benzylthio)purine	100	2/10	−3.7	0/412	Toxic	19
		50	7/10	−3.1	0/412	0	

TABLE I (*Continued*)

Compd. no.	Name	Dose (mg./kg.)	Survivors	Animal wt. diff. (T-C)	Tumor wt. (test, control)	T/C %	Reference to synthesis
		25	8/10	−0.9	0/412	0	
		12.5	9/10	−0.5	36/412	8	
26	2-(9-β-D-ribofuranosylpurin-6-ylamino)ethanol hydrate	225	1/10	−4.1	/412	Toxic	80
		200	7/10	−2.6	49/676	7	
		100	8/10	−2.9	183/676	27	
		75	10/10	−2.9	74/412	17	
		50	10/10	−2.7	332/676	49	
		25	10/10	−1.6	229/412	55	
27	N-Methylpurine-6-sulfonamide	225	0/10			Toxic	46
		50	9/10	−1.4	0/645	0	
		40	8/10	−3.6	45/1699	2	
		25	9/10	−0.0	0/645	0	
		20	10/10	−3.4	35/1699	2	
		12.5	9/10	0.6	288/645	44	
		10	10/10	−2.4	344/1699	20	
		6.2	10/10	−0.7	234/645	36	
		3.1	10/10	−1.2	499/645	77	
28	(2-Amino-9-β-D-ribofuranosylpurin-6-yl)trimethyl ammonium chloride	400	8/10	−5.0	40/1188	3	80
		240	9/10	−4.0	348/1395	24	
		120	10/10	−2.5	729/1395	52	
		60	10/10	−0.9	1422/1395	101	
29	Trimethyl(9-β-D-ribofuranosylpurin-6-yl)ammonium chloride	400	7/10	−5.8	0/975	0	80
		240	10/10	−4.9	132/1395	9	
		120	10/10	−2.2	404/1395	28	
		60	10/10	−0.2	1533/1395	109	
		30	10/10	−1.4	472/975	48	
30	2-Amino-6-bromopurine	240	2/10	−3.8	0/1286	Toxic	30
		120	7/10	−4.5	0/1286	0	
		60	9/10	−2.8	14/1286	1	
		30	10/10	−3.7	271/1286	21	
31	7-Hydroxy-*s*-triazolo[*d*]pyrimidine	500	9/10	−0.7	0/1132	0	119
		250	9/10	−0.8	57/1132	5	
		125	9/10	−1.6	474/1029	46	
32	7-Amino-*s*-triazolo[*d*]pyrimidine	62.5	0/10			Toxic	119
		62.5	3/12	−5.3	237/1005	23	
		31.25	11/12	−3.4	858/1005	85	
		31	9/10	−1.8	283/556	50	
33	6-Chloro-9-(tetrahydro-2-pyranyl)purine	250	8/10	−5.5	22/1550	1	87
		250	10/10	−4.2	79/1099	7	
		125	10/10	−3.3	20/948	2	
		62	9/10	−3.8	144/948	15	
		60	10/10	−4.0	362/1550	23	
		31	10/10	−2.6	402/948	42	
		30	10/10	−0.0	675/1077	62	
34	4-Amino-1-(2-chloroethyl)pyrazolo[3,4-*d*]pyrimidine hydrochloride	25	0/10			Toxic	162
		12.5	9/10	−4.8	38/411	9	
		12.5	10/10	−3.1	270/1074	25	
		6.2	9/10	−3.3	144/535	26	
		3.1	10/10	−1.5	167/535	31	
		1.5	10/10	−0.4	423/535	79	
35	2-Amino-6-(methylsulfonyl)purine	30	9/10	−2.0	179/1383	12	36
		30	9/10	−2.3	96/1732	5	
		15	9/10	−2.2	562/1732	32	
		7.5	10/10	−0.1	1413/1732	81	
36	6-(Allylthio)-2-aminopurine	64	7/10	−6.4	0/1497	0	34
		32	7/10	−3.7	263/2113	12	
		32	8/10	−4.7	0/1497	0	
		21	9/10	−2.6	159/2370	6	
		16	9/10	−2.5	66/1673	3	
		16	9/10	−5.1	0/1497	0	
		14	8/10	−1.6	110/2370	4	
		8	10/10	−2.6	53/1673	3	
		8	10/10	−1.7	81/1497	5	
37	(2-Amino-6-purinylthio)acetonitrile	125	8/10	−2.8	13/1391	0	34
		125	10/10	−3.0	61/938	6	
		62.5	8/10	−2.1	0/938	0	
		31.25	9/10	−2.0	67/938	7	

TABLE I (*Continued*)

Compd. no.	Name	Dose (mg./kg.)	Survivors	Animal wt. diff. (T-C)	Tumor wt. (test/control)	T/C %	Reference to synthesis
		15.62	9/10	−1.2	61/1059	5	
		7.81	9/10	−1.9	44/1059	4	
		3.9	10/10	−1.6	175/1059	16	
		1.95	10/10	−0.7	405/1059	38	
		1	10/10	−0.2	554/873	63	
38	2-(2-Amino-6-purinylthio)heptanoic acid	130	7/10	−5.2	0/1795	0	34
		125	4/10	−3.4	50/1212	Toxic	
		65	10/10	−3.7	5/1141	0	
		32.5	9/10	−4.1	172/1795	9	
		16.25	10/10	−2.0	630/1795	35	
39	2-(9-Methyl-6-purinylamino)ethanol	130	7/10	−2.3	14/587	2	162
		65	8/10	−3.8	38/1378	2	
		65	10/10	−0.7	40/587	6	
		32.5	8/10	−0.4	186/587	31	
		16.25	9/10	−0.8	493/587	83	

TABLE II

ACTIVITY OF VARIOUS PURINES AND RELATED DERIVATIVES AGAINST LEUKEMIA L-1210

Compd. no.	Name	Dose (mg./kg.)	Survivors	Animal wt. diff. (T-C)	Survival (days) (test/control)	T/C %	Reference to synthesis
1	8-Diazohypoxanthine	50	6/7	−0.1	/9.4	113	73
		50	6/6	−0.1	12.0/9.4	159	
2	2-Amino-9-(*n*-propyl)-6-purinethiol	15	6/6	−2.5	10.0/9.5	105	13
		11	5/6	−3.2	11.4/8.5	134	
		7.5	5/6	−2.1	12.6/9.1	138	
		7.5	5/6	−2.1	12.6/8.5	148	
		5	6/6	−2.6	13.0/8.5	152	
		3.3	6/6	−1.0	11.1/9.1	121	
		1.88	6/6	−2.2	11.6/9.5	122	
3	N-Methylpurine-6-sulfonamide	75	6/6	−1.3	10.3/8.5	121	16
		50	6/6	0.3	12.0/9.0	133	
		50	6/6	−1.7	11.2/8.5	131	
4	2-Amino-6-(*n*-propylthio)purine	60	6/6	−1.3	12.5/9.2	135	34
		40	6/6	−1.2	13.2/8.6	153	
		40	6/6	−1.0	13.8/9.2	150	
		26	6/6	−1.7	12.7/9.2	138	
		17	6/6	−1.1	12.8/9.2	139	
		10	6/6	−0.4	12.0/9.0	133	
		6.7	6/6	−0.4	11.3/9.0	125	
		3	6/6	−0.6	11.0/11.4	96	
5	2-Amino-6-(methylsulfonyl)purine	60	6/6	−1.6	10.5/8.8	119	36
		30	6/6	−1.5	12.7/8.8	144	
6	2-Amino-6-(phenethylthio)purine	675	6/6	−4.5	10.5/8.4	125	34
		450	6/6	−2.7	13.2/10.3	128	
		450	6/6	−1.9	12.2/8.8	138	
		450	6/6	−4.0	11.3/8.4	134	
		300	6/6	−1.9	11.0/8.4	130	
		200	5/6	−2.6	10.4/8.4	123	
7	6-Bromopurine	500	6/6	−3.6	11.2/8.9	125	30
		250	6/6	−2.0	12.2/8.9	137	
		200	6/6	−0.9	11.7/9.7	120	
		125	6/6	−1.4	10.8/8.9	121	

and 2-methylpurine[32] are all inactive as antitumor agents. 2-Aminopurine (Table I, **4**) shows borderline activity; however, only one dosage is reported which is accompanied by a significant weight loss. This activity therefore awaits confirmation. It is of interest that 2-aminopurine has been reported to be incorporated into *Escherichia coli* DNA.[33]

It is noteworthy that, in most instances studied, if a simple 6-substituted purine exhibits good antitumor activity, the corresponding 2-amino-6-substituted purine also possesses significant activity, although often of a lower order. This is the case with 6-chloropurine,[3,5,27] 2-amino-6-chloropurine[34] (see Table I, **6**, for activity of 2-amino-6-chloropurine), 6-bromopurine[30] (Table I, **20**), 2-amino-6-bromopurine[30] (Table I, **30**), 6-(methylsulfonyl)purine,[16,35] 2-amino-6-(methylsulfonyl)purine[36] (Table I, **35**), 6-alkylthiopurines,[3,5,16]

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TABLE III

ACTIVITY OF PURINES AGAINST OTHER TUMORS

Compd. no.	Name	Dose (mg./kg.)	Survivors	Animal wt. diff. (T-C)	Tumor wt. or survival (days) (test/control)	T/C %	Ref. to synthesis
	Dunning Ascites Leukemia						
1	2-Amino-6,8-purinedithiol	250	6/6	−24.0	15.0/10.0	150	72
		250	5/6	−20.0	14.0/8.5	164	
		125	6/6	−7.0	15.0/10.0	150	
		125	6/6	−12.0	15.5/8.5	182	
		62.5	6/6	−10.0	15.0/10.0	150	
		62.5	6/6	0	15.0/8.5	176	
2	2-Amino-9-(*n*-propyl)-6-purinethiol	250	4/6	−28.0	11.5/7.5	153	13
		125	6/6	−21.0	10.0/7.5	133	
		62.5	6/6	−8.0	9.0/7.5	120	
3	2-Amino-6-(pentylthio)purine	125	6/6	−16.0	12.5/8.0	156	34
		125	6/6	−8.0	11.0/8.5	129	
		62.5	6/6	−8.0	10.5/8.0	131	
4	2-Amino-6-(isopropylthio)purine	125	1/6	−19.0	5.0/8.0	Toxic	34
		62.5	6/6	−11.0	13.0/8.0	162	
		62.5	6/6	−6.0	14.5/8.5	170	
		31.25	6/6	−5.0	13.5/8.5	158	
5	2-Amino-6-(*o*-chlorobenzylthio)purine	125	5/6	−11.0	9.0/8.0	112	34
		62.5	6/6	−3.0	12.5/8.0	156	
		62.5	6/6	−7.0	14.0/8.5	164	
		31.25	6/6	−5.0	11.5/8.5	135	
6	2-(2-Amino-6-purinylthio)acetamide	250	6/6	−2.0	14.0/9.0	155	34
		250	6/6	−3.0	13.5/10.0	135	
		125	6/6	7.0	12.5/9.0	138	
		62.5	6/6	−2.0	10.0/9.0	111	
7	(2-Amino-6-purinylthio)acetonitrile	125	6/6	−17.0	14.0/9.0	155	34
		125	6/6	7.0	15.5/10.0	155	
		62.5	6/6	−5.0	13.0/9.0	144	
		31.3	6/6	7.0	13.5/10.0	135	
8	1-Methyl-4-(*n*-butylamino)pyrazolo[3,4-*d*]pyrimidine	125	6/6	−16.0	11.0/9.0	122	108
		62.5	6/6	−9.0	11.0/9.0	122	
		31.25	6/6	−1.0	10.0/9.0	111	
9	4-Benzylaminopyrazolo[3,4-*d*]pyrimidine	31.25	6/6	−21.0	12.0/9.0	133	99
		16	6/6	−3.0	8.5/8.0	106	
		15.6	6/6	−8.0	11.5/9.0	127	
	Dunning Leukemia (solid)						
10	6-Ethylthiopurine	100	6/6	−8.0	22.0/14.0	157	137
		100	5/6	−15.0	24.0/15.0	160	
		50	6/6	−7.0	21.0/14.0	150	
		50	6/6	−9.0	20.5/15.0	136	
		25	6/6	−1.0	20.0/14.0	142	
		12.5	6/6	−4.0	17.5/14.0	125	
11	2-Amino-6-(isopropylthio)purine	250	6/6	−2.0	17.5/13.0	134	34
		100	6/6	−10.0	22.5/16.0	140	
		50	6/6	−24.0	22.0/15.0	146	
		50	6/6	−8.0	21.0/16.0	131	
		10	6/6	−24.0	19.5/15.0	130	
	Solid Friend Virus Leukemia						
12	2-Amino-9-(*n*-propyl)-6-purinethiol	9.9	10/10	−2.4	40/327	12	13
		9.9	9/10	−3.3	22/430	5	
		6.6	10/10	−1.9	15/430	3	
		4.4	10/10	−0.7	79/327	24	
		4.4	9/10	−1.6	17/430	3	
		2.9	9/10	−0.8	63/430	14	
		1.5	10/10	−2.3	303/1076	28	
	Murphy-Sturm Lymphosarcoma						
13	2-Amino-1-methylpurine-6-thione	7.5	6/6	4.0	2.0/1.9	105	17
		5	6/6	5.0	3.1/1.9	163	
		2.5	6/6	−2.0	2.6/1.9	136	

TABLE III (*Continued*)

Compd. no.	Name	Daily dose (mg./kg.)	Wk.	Result	Growth index	Wt. change, g.	Deaths	No. inj.	Survival (days)	Ref. to synthesis
				Spontaneous Mammary Tumors						
14	2-Amino-9-isobutyl-6-purinethiol	6	3	±	1.37/1.63	−2.5/−3.0	+1/8	11	33.3/67	13
		8	3	±	1.32/1.63	−3.0/−3.0	+0/8	11	59/67	
		16	3	+	1.18/1.61	−2.0/−4.5	+3/9	11	25.1/39.3	
		16	2	+	1.09/1.30	−10.0/−1.5	+4/9	8	18.9/44.2	
15	6-Bromopurine	125	3	+	1.00/1.21	0/+1.0	+1/11	11	58.5/62.1	30
		125	3	+	1.07/1.36	−4.0/−4.0	+1/10	11	41.7/50.3	
		250	3	−	1.38/1.36	−5.0/−4.0	+1/10	11	37.9/50.3	
16	2-Amino-9-(*n*-butyl)-6-(2-pyridylmethylthio)purine	15	3	+	.92/1.29	−7.0/+1.0	+1/8	11	54.3/36.8	13
		15	3	±	1.33/1.57	−6.0/+2.0	+2/9	11	37.7/44.2	
		30	3	−	1.39/1.57	−5.0/+2.0	+2/9	11	34.4/44.2	
				3,4-9,10-Dibenzpyrene-Induced Fibrosarcomas						
17	2-Amino-9-isobutyl-6-purinethiol	8	2	+	.88/1.3	−3.5/+4.5	2/10	10		13
		6	2	+	1.0/1.3	−1.0/+3.5	3/10	10		
		6	2	+	.94/1.4	−0.5/+3.0	2/10	10		
		3	2	±	1.2/1.4	+3.0/+3.0	1/10	10		
18	6-Bromopurine	125	2	+	.98/1.3	−2.0/+3.5	3/10	10		30
		125	2	+	1.0/1.3	0/+3.5	1/10	10		
		60	2	+	1.1/1.3	−0.5/+3.5	1/10	10		
		30	2	−	1.2/1.3	+1.5/+2.5	1/10	10		
19	2-Amino-9-(*n*-propyl)-6-purinethiol	4	2	+	1.0/1.	−1.5/+2.5	3/10	10		13
		4	2	+	.98/1.2	+0.5/+1.0	3/10	10		
		2	2	±	1.1/1.2	+2.0/+1.0	1/10	10		
		2	2	−	1.1/1.1	−0.5/0	2/10	10		
20	2-Amino-9-(*n*-butyl)-6-(2-pyridylmethylthio)purine	15	2	+	.99/1.3	−1.5/+0.5	2/10	10		13
		15	2	±	1.2/1.6	−2.5/+3.0	5/10	10		
		15	2	+	.98/1.3	−2.0/+2.5	2/10	10		

Compd. no.	Name	Daily dose, mg./kg.	Result	Wt. change, g.	Survival (days)	T/C %	Ref. to syn.
			Leukemia P815				
21	2-Amino-9-(*n*-propyl)-6-purinethiol	25	±	−1.1/+0.2	10.6/7.0	151	13
		10	+	−0.5/+0.2	13.0/7.0	186	
			Leukemia L1210 G				
22	2-Amino-9-(*n*-propyl)-6-purinethiol	20	±	−0.9/+0.4	11.0/7.0	157	13
		10	+	−0.7/+0.4	11.7/7.0	167	
		7.5	+	−0.9/+0.4	11.4/7.0	163	

and 2-amino-6-alkylthiopurines.[15,34] 2-Amino-6-methylpurine is inactive against Adenocarcinoma 755; however, this might have been predicted since 6-methylpurine has shown[3] only questionable activity against this test system. 2,6-Diaminopurine is also inactive[3] against Adenocarcinoma 755. Acetylation of the 2-amino group apparently does not greatly diminish antitumor activity. Thus, 2-amino-6-(benzylthio)purine[3] and 2-acetamido-6-(benzylthio)purine[19] (Table I, **25**) are both active compounds. 2-Acetamido-9-acetyl-6-(benzylthio)purine[19] (Table I, **24**) is also a highly active compound. Perhaps deacetylation occurs *in vivo*.

Substitution at Position 3.—Without exception substitution at position 3 with a methyl group provides only inactive compounds. Thus, 3-methyl-6-purinethione[37] and 3-methyl-6-methylthiopurine[37,38] are completely inactive. 3-Methylguanine,[39] 3-methyladenine,[38] 3-methylhypoxanthine,[37] and 2-amino-3-methyl-6-purinethione[39] are all inactive. The antitumor activity of 2-amino-6-(*o*-chlorobenzylthio)purine[15] was completely eliminated by introduction of a 3-methyl substituent to give 2-amino-6-(*o*-chlorobenzylthio)-3-methylpurine.[39]

Substitution at Position 6.—A number of 6-substituted purines have exhibited significant activity in the Adenocarcinoma 755 test system. Noteworthy are 6-purinethiol,[3,5,6] 2-amino-6-purinethiol,[3,5,40,41] various 6-alkylthiopurines,[3,5–7,16,41,42] and 6-chloropurine.[3,5,6] 6-Hydrazinopurine,[3,43] 6-methylpurine,[3] and purine[44] itself show[3,40] reproducible inhibition against Adenocarcinoma 755 with a therapeutic index of approximately 2.[5] Purine-6-sulfonamide,[16,45] N-alkylpurine-6-sulfonamides[16] (Table I, **27**), and 6-alkylsulfonylpurines[16,35] all show good inhibition at various dosage levels. In general, however, the 6-alkylsulfonylpurines are more toxic than the corresponding 6-alkylthiopurines. Of considerable interest is the ac-

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tivity of 6-bromopurine[30] (Table I, **20**) which has a therapeutic index of 65 as compared to 15 for 6-chloropurine in the same test system.[3] 6-Methoxypurine[46] (Table I, **2**) has shown activity at 250 and 125 mg./kg. This activity is essentially lost with 6-ethoxypurine. Purine-6-isothiocyanate has been reported[47] to be active against Adenocarcinoma 755.

The size of substituent permitted at the 6-position is truly remarkable. A number of 2-amino-6-pyrimidinylthiopurines[48] have shown excellent activity[48] against Adenocarcinoma 755. Elion, Bieber, and Hitchings[49] have studied the activity of 2-amino-6-(1-methyl-4-nitro-5-imidazolylthio)purine which was shown[49] to possess a therapeutic index against Adenocarcinoma 755 superior to that of 2-amino-6-purinethiol when administered intraperitoneally or orally. Purine-6-thioglucoside[50,51] has also shown excellent activity against Adenocarcinoma 755. It appears quite probable[49-53] that with these large substituents attached to the 6-thio group these compounds are metabolized *in vivo* to the active form of the drug, 6-purinethiol or 2-amino-6-purinethiol. The use of 2-amino-6-(1-methyl-4-nitro-5-imidazolylthio)-purine would seem to have no particular advantage over 2-amino-6-purinethiol in clinical trial.[54-56]

The activity of various 1-aziridinylpurines against Adenocarcinoma 755 has recently been reported.[57] N-(6-Purinyl)-L-glutamic acid[58] is another example of an unusual 6-substituted purine active against Adenocarcinoma 755. The selective toxicity of "6-purinylhistamine" against carcinoma cells *in vitro* has been reported.[59-61] S-Ethyl 6-aminopurine-9(or 7)-carbothioate has shown activity against Adenocarcinoma 755 at least at one dosage level.[62]

Substitution at Position 7.—No purine substituted with an alkyl group at position 7 has been found to exhibit significant antitumor activity in experimental animals. Thus, 7-methyl-6-purinethiol,[63] 6-chloro-7-methylpurine,[63] 7-methyladenine,[63] and 2-amino-7-methyl-6-purinethiol[63] are all negative against Adenocarcinoma 755. 7-Butyl-6-purinethiol has been reported[64] to be inhibitory in tissue culture but has not yet been found active against a solid tumor *in vivo*.

A new method of synthesis of 7-substituted purines has recently been reported.[65] Since a number of 7-α-D-ribofuranosylpurines have been isolated from natural sources[66-69a] as degradation products of pseudovitamin B_{12} derivatives, it is quite possible that derivatives of this type may exhibit antitumor properties. In this regard the synthesis of 7-α-D-ribofuranosyladenine has recently been reported.[69b]

Substitution at Position 8.—The introduction of a substituent group at position 8 gives varied results. None of the monosubstituted derivatives such as 8-purinethiol,[28] 8-aminopurine,[28] 8-hydroxypurine,[28] 8-methylthiopurine,[28] 8-methoxypurine,[69c] 8-chloropurine,[27] 8-bromopurine,[30] or 8-methylpurine[32] are active against Adenocarcinoma 755. 2-Amino-8-methyl-6-purinethiol,[34] 8-methyl-6-purinethiol,[70] 6-chloro-8-methylpurine,[70] and 8-methyladenine[70] are essentially inactive. 2-Amino-6-hydroxy-8-methylpurine[70] (Table I, **1**), however, does show inhibition at 62.5 mg./kg.

The introduction of a hydroxyl group at position 8 renders 6-purinethiol inactive. 6-Amino-8-purinol[71] (Table I, **3**) shows activity, but the accompanying weight loss makes these results of questionable value. The introduction of an 8-mercapto group into 2-amino-6-purinethiol (6-thioguanine) gives 2-amino-6,8-purinedithiol[72] (Table I, **5**) which does show activity at rather high dosages. Similarly, 2,8-diamino-6-purinethiol[73] (Table I, **8**), although rather toxic, is quite inhibitory. Introduction of a halogen into position 8 of various active purines results in loss of activity. 6,8-Dichloropurine,[71] 8-chloro-6-purinethiol,[71] 8-amino-6-purinethiol,[70] and 6,8-purinedithiol[71] are all inactive. 8-Chloroadenine[70] is also inert. None of the 2,8-disubstituted purines such as 2-amino-8-purinethiol,[71] 2-amino-8-hydroxypurine,[75] 2,8-purinedithiol,[30] 2-chloro-8-hydroxypurine,[74] 2-hydroxy-8-purinethiol,[71] 2,8-dichloropurine,[27] and 2,8-diaminopurine[74] exhibited any activity against Adenocarcinoma 755.

Although the usual effect of an 8-substituent is to decrease the activity of an otherwise active compound, it does appear that the judicious choice of an 8-substituent could lead to various purines and purine nucleosides of significant biological importance.

Substitution at Position 9.—One may vary the substituent at position 9 within wide limits and still retain antitumor activity. The substitution of phenyl[76] at position 9, however, completely eliminated the antitumor activity of 6-purinethiol and 6-chloropurine. 2-Amino-9-phenyl-6-purinethiol[77] is inactive against Adenocarcinoma 755, while 2-amino-9-benzyl-6-purinethiol[77] is active.[73] 6-(1-Aziridinyl)-9-benzylpurine[57] in-

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hibits Adenocarcinoma 755. The only known 9-phenylpurine derivative exhibiting antitumor activity is 2,6-diamino-9-(*p*-tolyl)purine[77] (Table I, **7**). It is quite possible that this compound showed inhibition due to its structural similarity to aminopterin and is therefore acting as an inhibitor of folic acid. Attempts to improve the activity of this latter type of compound were unsuccessful.

Skipper, Montgomery, *et al.*,[3] have shown that such compounds as 9-ethyl-6-purinethiol and 9-butyl-6-purinethiol are highly active compounds against Adenocarcinoma 755. A recent study[78] employing sulfur-labeled 9-butyl- and 9-ethyl-6-purinethiol has shown that no dealkylation occurred *in vivo* in the rat. A similar experiment[79] has shown that 9-ethyl-6-purinethiol and 9-(*n*-butyl)-6-purinethiol are apparently not dealkylated in humans. The original postulation[3] that the 9-alkyl-6-substituted purines owe their activity to *in vivo* dealkylation is open to question. A case worthy of note is that of 6-(2-hydroxyethyl)aminopurine which is totally inactive against Adenocarcinoma 755, while the corresponding 9-substituted derivatives, 2-(9-β-D-ribofuranosylpurin-6-ylamino)ethanol[80] (Table I, **26**) and 2-(9-methyl-6-purinylamino)ethanol (Table I, **39**), are definitely active.

6-Fluoro-9-methylpurine[81] is totally inactive against Adenocarcinoma 755, possibly because the fluoro group is readily hydrolyzed at room temperature in aqueous solution[81] and thus may be rapidly changed *in vivo*. 9-Ethyl-6-purinethiol has shown activity against chronic adult human leukemia.[82-84] 9-(*n*-Hexyl)-6-purinethiol appears to be the most effective inhibitor[82] of a number of 9-alkyl-6-purinethiols tested against 6-purinethiol-resistant Adenocarcinoma 755 cells[82] in tissue culture. Various 9-cycloalkyl-6-purinethiols[64,85] are active against 6-purinethiol-resistant lines of H. Ep. No. 2 in tissue culture studies. 9-Alkyl derivatives of 2-amino-6-purinethiol have been shown to be especially active against Adenocarcinoma 755.[13] 9-Methyl-[86] through 9-(*n*-pentyl)-2-amino-6-purinethiol[13] all show excellent activity. Longer alkyl chains such as 9-octyl[86] render 2-amino-6-purinethiol inactive.[13] It is of interest that 2-amino-9-cyclohexyl-5-purinethiol[13] is inactive while 2-amino-9-cyclopentyl-6-purinethiol is very active.[13] Such activity of the 9-cyclopentyl derivative suggested that these compounds might be acting as nucleosides with the cyclopentyl ring occupying approximately the same steric configuration as the β-D-ribofuranose ring. This deduction led to the synthesis of a number of purines possessing a 9-tetrahydropyran[87] and a 9-tetrahydrofuran[19,88] ring. These compounds have shown[19] remarkable activity against Adenocarcinoma 755. 9-(Tetrahydro-2-furyl)-6-purinethiol[19] exhibits a therapeutic index of approximately 200 as compared to 30 for 6-purinethiol against the same tumor.[19] It is clear that the tetrahydro-2-furyl moiety is making a substantial contribution to the activity of these derivatives since 9-(tetrahydro-2-furyl)adenine is confirmed as active against Adenocarcinoma 755.[19] Although many 9-(tetrahydro-2-pyranyl)purines[87] are definitely active (Table I, **9–12**, **17**, **21**, **33**), in every case the corresponding 9-(tetrahydro-2-furyl)purines are substantially superior. The requirement for activity must involve more than just a five-membered ring since the corresponding 9-(tetrahydro-2-thienyl)purines[88] were entirely devoid of any antitumor activity. The 9-(tetrahydro-2-furyl) derivative of 6-thioguanine[19] is a potent inhibitor[19] of Adenocarcinoma 755.

It is becoming increasingly clear that the 9-alkyl-2-amino-6-purinethiols do *not* exert their activity by mere dealkylation to the parent purine. 2-Amino-9-methyl-6-purinethiol has been found[89] to be inhibitory to a 6-thioguanine-resistant cell line of ascites cells. Kimball and LePage[90] have shown that 2-amino-9-(*n*-butyl)-6-purinethiol is neither dealkylated nor incorporated into nucleic acid in the mouse. More recent studies by Kimball and LePage[91] suggest that 2-amino-9-(*n*-butyl)-6-purinethiol may exert its antitumor activity by interference of utilization of preformed adenine for DNA synthesis. This is in sharp contrast to the parent compound, 2-amino-6-purinethiol (6-thioguanine), whose antitumor activity can be correlated with its incorporation into nucleic acids.[92-94]

The purine nucleosides may be considered as purines possessing a special substituent at position 9. 9-β-D-Ribofuranosyl-6-purinethiol (6-mercaptopurine ribonucleoside) has been shown[3,6] to possess the highest therapeutic index of any purine derivative studied for antitumor activity against Adenocarcinoma 755. It should be pointed out however that against a wide spectrum of tumors 6-MP riboside has been found to be much less effective[95] than 6-MP. The synthesis of a number of 6-alkylthio-9-β-D-ribofuranosylpurines has been reported[96]; however, none of these ribosides were significantly more effective against Adenocarcinoma 755 than the corresponding simple 6-alkylthiopurine.[96] In a study of the antitumor effect of various 6-alkylthio-2-amino-9-β-D-ribofuranosylpurines[15] against Adenocarcinoma 755 it was noted that the antitumor activity of the parent purine was generally retained, and in several instances the ribosides were slightly more active than the corresponding 6-alkylthio-2-aminopurine.[15] LePage and Junga[97] have recently shown that in ascites tumor cells 2-amino-9-β-D-ribofuranosyl-6-purinethiol (6-thioguanine riboside) is cleaved rather rapidly to 2-amino-6-purinethiol (6-thioguanine). This

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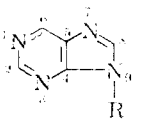
type of enzymatic cleavage could well account for the general results observed in Adenocarcinoma 755 with the ribonucleosides of the derivatives of 6-purinethiol and 2-amino-6-purinethiol. It is of considerable interest in this regard that 2-amino-9-methyl-6-purinethiol[96] acts in ascites cell tumors to inhibit the enzymatic cleavage of 6-thioguanosine.[97] The feedback inhibition by a purine analog which does not form a nucleotide has been noted.[98] Henderson[98] in a study of compounds active against Adenocarcinoma 755 studied the ability of various active purines to form nucleotides. No correlation between feedback inhibitory activity and nucleotide formation was noted. Most compounds which did exert feedback inhibition, however, were carcinostatic, but many of the carcinostatic purines did not exert feedback inhibition. Thus, the use of more than one purine derivative simultaneously could well be rewarding in the clinic. Such a proposal has previously been made[13,15,19] on the basis of preliminary animal testing.

A study of the potentiation of the antitumor activity of 6-purinethiol, 6-chloropurine, and various 6-alkylthiopurines against Adenocarcinoma 755 by the hypoxanthine analog, 4-hydroxypyrazolo[3,4-*d*]pyrimidine,[99] has recently been made.[100] Hitchings and co-workers[100] found that 4-hydroxypyrazolo[3,4-*d*]-pyrimidine inhibited the degradation of 6-purinethiol by xanthine oxidase and thus resulted in increased effectiveness of the drug in both the mouse and man. Henderson[101] has shown that the therapeutic effectiveness of 2-amino-6-purinethiol or 6-purinethiol could be increased by simultaneous administration of 4-aminopyrazolo[3,4-*d*]pyrimidine to mice with Ehrlich Ascites Carcinoma. Recently the potentiation of the bacteriostatic action of the purine nucleoside antibiotic, psicofuranine, by other purine nucleosides has been reported.[102]

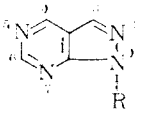
Although the riboside of 2-amino-6-chloropurine (Table I, **18**) is active against Adenocarcinoma 755 as is the parent purine, 2-amino-6-chloropurine (Table I, **6**), the riboside of the highly active compound 6-bromopurine (Table I, **20**), 6-bromo-9-β-D-ribofuranosylpurine,[80] is totally inactive. The riboside of trimethylpurin-6-ylammonium chloride[80] (Table I, **29**) is active against Adenocarcinoma 755 as is the parent compound.[103] The use of trimethylpurin-6-ylammonium chloride has been reported[104] to result in solid tumor regression in certain cases in clinical trials. This is one of the few instances where purine antagonists have been successfully employed against solid neoplasms in the clinic.

In summary of the structure–activity relationships of various purines against Adenocarcinoma 755, it can be stated that in order to possess significant antitumor activity the molecule should not be substituted with large substituents at positions 2, 7, or 8. Substitution at position 2 is essentially limited to amino or alkylamino. Substitution at position 1 with a small alkyl group is permissible if a 2-amino group is present. Substitution at position 3 results only in inactive compounds. The greatest allowable freedom as far as size and kind of functional group change is concerned is that which can be made at position 6. The substitution of various alkyl, cycloalkyl, tetrahydrofuryl, tetrahydropyranyl, and carbohydrate substituents can be made at position 9 often with an increase in antitumor activity.

Structure and Antitumor Activity of Compounds of Related Ring Systems. Antitumor Activity of the



Purine



Pyrazolo[3,4-*d*]pyrimidine

Pyrazolo[3,4-*d*]pyrimidines.—A detailed study of structure and antitumor activity of various derivatives of 4-aminopyrazolo[3,4-*d*]pyrimidines against Adenocarcinoma 755 has been reported.[105,106] The similarities of the allowable positions for substitution in the pyrazolo[3,4-*d*]pyrimidine ring and those in purine are most striking. The active parent compound in this series is 4-aminopyrazolo[3,4-*d*]pyrimidine.[99] Substitution of certain alkyl groups at position 1 (corresponding to position 9 in purine) provided active compounds.[105,106] The presence of a tetrahydropyranyl or tetrahydrofuryl group at position 1 also retained antitumor activity.[106] Substitution of alkyl groups on the amino function at position 4 (corresponding to position 6 in purine) provided compounds less toxic and possessing a therapeutic index superior to that of the parent compound.[105,107] Substitution at both the 1- and 4-position simultaneously[106,108,109] in many instances diminished antitumor activity[105,106] only slightly. The introduction of a phenyl group[108] at position 1 resulted in loss of activity. The preparation of 6-amino-4-hydroxypyrazolo[3,4-*d*]pyrimidine[110] provided an active compound.[105] The presence of an amino substituent at position 6 (corresponding to position 2 in the purines) is therefore permissible. Thus, the general geometric requirements found in the simple purines appear to carry over to the pyrazolo-[3,4-*d*]pyrimidine ring system very well. It is noteworthy however that the compounds pyrazolo[3,4-*d*]-pyrimidine-4-thiol[99] and 6-aminopyrazolo[3,4-*d*]pyrimidine-4-thiol,[110] the corresponding analogs of 6-purinethiol and 2-amino-6-purinethiol, are without activity against Adenocarcinoma 755. Thus, steric requirements alone are not sufficient for activity. The fact that 4-aminopyrazolo[3,4-*d*]pyrimidine is not cross-resistant[3,5] to a 6-MP-resistant strain of Adenocarcinoma 755 and must therefore possess a different mechanism of action has stimulated further studies with this compound. 4-Aminopyrazolo[3,4-*d*]pyrimidine and 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine

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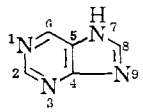
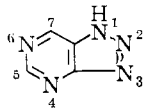
have shown activity against spontaneous mammary adenocarcinoma.[111] The 4-alkylaminopyrazolo[3,4-*d*]-pyrimidines exhibit significant inhibition[3] of solid Leukemia 5178 and definitely prolong the life span of mice given intraperitoneal inoculations of L5178 or L1210 leukemic cells.[3] An L1210/8-azaguanine-resistant strain has been found to be cross resistant with 6-purinethiol[3] but not cross resistant to 4-aminopyrazolo[3,4-*d*]pyrimidine. 4-Aminopyrazolo[3,4-*d*]-pyrimidine has been shown to be active against a wide spectrum of tumors.[12,112] 1-Methyl-4-(*n*-butylamino)-pyrazolo[3,4-*d*]pyrimidine[108] and 4-benzylaminopyrazolo[3,4-*d*]pyrimidine[99] have been found active against Dunning Ascites Leukemia (Table III, 8 and **9**).

Henderson and co-workers[101,113,114] have shown that simultaneous administration of 4-aminopyrazolo[3,4-*d*]-pyrimidine and 6-purinethiol or 2-amino-6-purinethiol showed definite potentiation in experimental animals with Ehrlich Ascites Carcinoma, Sarcoma 180, and 6C3HED Lymphosarcoma. Although 4-aminopyrazolo[3,4-*d*]pyrimidine is incorporated readily into nucleic acids[115] of normal and neoplastic tissue, it probably exerts its antitumor effect at the acid-soluble nucleotide level.[115] There is good evidence that the *de novo* pathway to nucleotide purines is inhibited[116,117] probably by a feedback mechanism. Recent clinical successs[118] of 4-(*n*-propylamino)pyrazolo[3,4-*d*]-pyrimidine[99] against myeloblastic leukemia has stimulated renewed interest in this group of compounds.

Antitumor Activity of *v*-Triazolo[*d*]pyrimidines.—5-





Amino-7-hydroxy-*v*-triazolo[*d*]pyrimidine (8-azaguanine) was one of the first purine analogs to be prepared[119] and studied for antitumor properties.[120,121] This compound has been shown[3] to exhibit excellent activity against Adenocarcinoma 755 and a 6-MP-resistant strain of Adenocarcinoma 755. The incorporation of 8-azaguanine into nucleic acids has been noted,[122–124] and a review of the action of this compound has appeared.[125] Recently 8-azaguanosine triphosphate has been shown to inhibit adenylosuccinic synthetase.[126] 7-Hydroxy-*v*-triazolo[*d*]pyrimidine[119] (Table I, **31**) is definitely active against Adenocarcinoma 755,[3] but 7-amino-*v*-triazolo[*d*]pyrimidine[119] (Table I, **32**) is only weakly active.[3] Although 5-amino-3-ethyl-7-hydroxy-*v*-triazolo[*d*]pyrimidine (9-ethyl-8-azaguanine) is devoid of activity,[127] 7-diethylamino-3-methyl-*v*-triazolo[*d*]pyrimidine[128] (Table I, **14**) has shown a low order of activity against Adenocarcinoma at least at two dosage levels. 7-Isobutylamino-*v*-triazolo[*d*]pyrimidine[129] (Table I, **16**) is also active against Adenocarcinoma 755. This activity is typical of the type observed with the pyrazolo[3,4-*d*]-pyrimidines. Another compound of interest in this series is 5-amino-7-(2,4-dichlorobenzylthio)-*v*-triazolo-[*d*]pyrimidine[128] (Table I, **15**) which is also active. This compound is of interest since it resembles in structure the compound 2-amino-6-(*o*-chlorobenzylthio)-purine[34] which is very active[15] in the purine series. Although the ribonucleoside and ribonucleotide of 6-purinethiol show the same order of effectiveness[130] as 6-purinethiol in inhibiting Adenocarcinoma 755, the ribonucleoside and ribonucleotide of 5-amino-7-hydroxy-*v*-triazolo[*d*]pyrimidine were only slightly active.[130] Thus, the *v*-triazolo[*d*]pyrimidines also show many of the same type of structure–activity relationships characteristic of both the active purines and pyrazolo[3,4-*d*]pyrimidines, although the activity is of somewhat lower order.

Activity of Purine Derivatives against Animal Tumors Other than Adenocarcinoma 755.—A study of various purines related to 6-purinethiol and their activity against Sarcoma 180 has previously been reported.[8,131] In general, it can be said that usually the 6-thiopurine derivatives most active against Adenocarcinoma 755 also exhibit significant activity[13,15,16,131] against Sarcoma 180. Many purines exhibiting antitumor activity against Adenocarcinoma 755 were found to be inactive against Sarcoma 180. This is to be expected since Sarcoma 180 is known to be much more resistant to inhibition by purine antagonists. It is interesting to note that all purines which exert good activity against Sarcoma 180 are also active against Adenocarcinoma 755. The same type of structural modifications which produced active purines against Adenocarcinoma 755 often result in derivatives active against Sarcoma 180; however, a higher dosage schedule is usually required for activity[132] in Sarcoma 180. The highly active compound 2-amino-9-(*n*-propyl)-6-purinethiol[13] is also active[13] against Sarcoma 180. The 9-(tetrahydro-2-furyl) derivative of 2-amino-6-purinethiol[19] is significantly active against Sarcoma 180. Bis(2-amino-6-purinyl) disulfide[133] is a more potent inhibitor of Sarcoma 180 than 2-amino-6-purinethiol itself. It is quite possible that the disulfide is grad-

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(131) C. C. Stock, D. A. Clarke, F. S. Philips, and R. K. Barclay, *ibid.* (supplement), **18**, part 2, 49 (1958).
(132) Compare for example the activity of 2-amino-6-isopentylthiopurine against Adenocarcinoma 755 and Sarcoma 180 (ref. 14).
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ually converted to the active antimetabolite *in vivo*. Purine itself is active[131] against Sarcoma 180.

Many of the purines which exhibit activity against Adenocarcinoma 755 are also active against Leukemia L-1210. 5-Amino-7-hydroxy-*v*-triazolo[*d*]pyrimidine (8-azaguanine) exhibits good activity[134] against Leukemia L-1210. 6-Purinethiol,[3] 2-amino-6-purinethiol,[135] and the riboside[3] of 6-MP are all active against Leukemia L-1210. Purine-6-sulfonamide,[16] 2-amino-6-isopropylthiopurine,[15] 2-amino-1-methyl-6-purinethione,[17] 2-amino-9-(tetrahydro-2′-furyl)-6-purinethiol,[19] and 6-bromopurine[30] (Table II, **7**) are all examples of purines active against Leukemia L-1210 as well as Adenocarcinoma 755. 2-Amino-6-(*n*-propylthio)purine (Table II, **4**) and N-Methylpurine-6-sulfonamide[16] (Table II, **3**) are also active. 8-Diazohypoxanthine,[73] a compound of borderline activity in Adenocarcinoma 755, is active (unconfirmed) as shown in one test (Table II, **1**). 6-(2,2-Dimethylhydrazino)purine has been reported[136] to be 6% as active as amethopterin against Leukemia L-1210. 6-Purinethiol (6-MP) is 34% as active as amethopterin in the same system,[136] while 2-(2-aminopurin-6-ylthio)valeric acid[34] shows[136] 53% of the activity of amethopterin against Leukemia L-1210.

It is quite likely that many of the other purines active against Adenocarcinoma 755 are also active against Leukemia L-1210, but in the majority of instances these tests have not been run.

A number of selected purines active against Adenocarcinoma 755 have been submitted for random screening against other neoplasms. These results for some of the active compounds are tabulated in Table III. It is abundantly clear from inspection of this table that these purine derivatives possess a wide spectrum of activity against a large variety of experimental neoplasms. The 6-alkylthio-2-aminopurines[34] and 6-alkylthiopurines[137] show good activity against Dunning Solid and Dunning Ascites Leukemia (Table III).

The activity of 6-bromopurine[30] against spontaneous mammary tumors (Table III) is noteworthy. 6-Bromopurine and 6-iodopurine have been shown to enhance the carcinostatic activity of azaserine against ascites cell forms of Sarcoma 180 and Ehrlich Carcinoma *in vivo*.[18] 2-Amino-9-isobutyl-6-purinethiol[13] and 2-amino-9-(*n*-butyl)-6-(2-pyridylmethylthio)purine[13] are active against 3,4-9,10-dibenzpyrene-induced fibrosarcomas (Table III).

Mautner and co-workers have prepared 6-selenopurine[138] and 2-amino-6-selenopurine[139] and studied the antitumor properties of these compounds. As an inhibitor of Leukemia L-1210, 6-selenopurine was essentially as good as 6-purinethiol (6-MP).[140] The inhibition of an Ehrlich ascites tumor cell system by 6-selenopurine has been studied.[141] 2-Amino-6-selenopurine exhibits a better therapeutic index[139,142] than 2-amino-6-purinethiol on ascites cells of Sarcoma 180 and against Lymphomas L-1210 and L-51784. The activity of 9-(2-chloroethyl)adenine against C_{1300} experimental tumor has been reported.[143] It is interesting to note that 6-purinethiol has shown activity against 75 out of 120 different experimental animal tumors.[112] 2-Amino-6-purinethiol was active in 19 out of 32 cases, and 5-amino-7-hydroxy-*v*-triazolo[*d*]pyrimidine (8-azaguanine) showed activity against 34 out of 75 different tumors tested.[112]

The interesting purine mustard 9-(3-[bis(2-chloroethyl)amino]propyl)hypoxanthine[144] has been shown to be active against Ehrlich Ascites 6C3HED and several other neoplasms.[145] This compound, however, has shown activity only against alkylating-agent sensitive tumors.[145] Burckhalter and Bariana[146] have recently found that the compound N^6-(α-[bis(2-chloroethyl)-amino]-4-ethoxy-*m*-tolyl)adenine is active against Leukemia L-1210 and Dunning Solid Leukemia. It is interesting to note that these two examples of active purine nitrogen mustards contain the mustard function at either position 6 or 9, the positions of greatest allowable versatility with regard to size and type of substituent.

To date only a relatively few purines have been tested against Dunning Ascites Leukemia. Table III lists a number of purines, prepared in our laboratory, which are active against this neoplasm. 6-Purinethiol exhibits a T/C of approximately 1.4 against Dunning Ascites Leukemia. 2-Amino-6,8-purinedithiol exhibits a T/C of about 1.7 and 2-amino-6-(isopropylthio)-purine[34] a T/C of 1.6 (see Table III, **1**, **4**). Other 6-alkylthio-2-aminopurines are also active (Table III), but too few purines have been screened to draw any general structure–activity correlations. 6-Ethylthiopurine[137] and 2-amino-6-(isopropylthio)purine[34] are examples of purines active against Dunning Solid Leukemia (Table III, **10**, **11**). The excellent therapeutic index of 2-amino-9-(*n*-propyl)-6-purinethiol against Adenocarcinoma 755[13] and the wide spectrum of antitumor activity make this compound a promising candidate for clinical trial. 2-Amino-9-(*n*-propyl)-6-purinethiol,[13] besides exhibiting activity[13] against Adenocarcinoma 755 and Sarcoma 180, is also active against Leukemia L-1210 (Table II, **2**), Solid Friend Virus Leukemia (Table III, **12**), Dunning Ascites Leukemia (Table III, **2**), 3,4-9,10-dibenzpyrene-induced fibrosarcomas (Table III, **19**), Leukemia P815 (Table III, **21**), and Leukemia L1210 G (Table III, **22**). A related compound, 2-amino-9-isobutyl-6-purinethiol,[13] is active against spontaneous mammary tumors (Table III, **14**). 2-Amino-9-(*n*-propyl)-6-purinethiol has not been tested against this latter tumor. The activity of 6-bromopurine[30] against spontaneous mammary tumors (Table III, **15**) and 3,4-9,10-dibenzpyrene-induced fibrosarcomas (Table III, **18**) is noteworthy. Thus, with the limited testing data presently available on purines tested against tumors other than Adenocarcinoma 755, it would appear that the purines which are highly active against this tumor do possess significant activity against a variety of other animal tu-

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mors, in many cases surpassing the activity of 6-purinethiol.

General Summary

Many different metabolic effects have been observed with the active purines and purine analogs, and inhibition has been shown to occur at various sites in the biochemical sequence of nucleic acid biosynthesis.[18,89-94,98,115-117,147-154] Undoubtedly, numerous different enzyme systems are involved. It is quite possible, however, that the enzymes which accept preformed purines and purine nucleosides require certain structural features. Although the binding sites for the natural purines may not be identical, it seems likely that these sites would be restricted. In order for a purine analog to exert significant biological activity, it must become attached to some purine-accepting enzyme system. In view of the number and type of compounds studied, it is tempting to speculate as to the positions of the purine molecule which might be involved in enzyme attachment.

In the purine series positions 3 and/or 7 appear to be the most likely to bond to receptor sites. It is possible that with adenine and adenine analogs, such as the 4-aminopyrazolo[3,4-*d*]pyrimidines, position 5 (purine position 1) is also important. Studies[155] of the pK_a of the active 4-substituted pyrazolo[3,4-*d*]pyrimidines would tend to support this view. It is of interest that even with the pyrazolo[3,4-*d*]pyrimidines a close steric fit is required at position 3 (purine position 7), since 4-amino-3-methylpyrazolo[3,4-*d*]pyrimidine[108] was found entirely devoid of antitumor properties.

LePage and co-workers[94,97] have suggested the synthesis and study of purine 2′-deoxynucleoside derivatives as more proximal precursors for incorporation into DNA. Such compounds might exert a more selective action on tumor since it has been shown that the carcinostatic properties of 2-amino-6-purinethiol can best be correlated with its incorporation into DNA.[92-94,97] Munch-Peterson[156] has shown that 2′-deoxyadenosine can be converted to the nucleotide in Ehrlich ascites cells, and Maley and Maley[157] have noted the enzymatic synthesis of 2′-deoxyribonucleotides from 2′-deoxyribonucleosides *via* a phosphotransferase reaction. This extension of the present work does appear to be worthy of investigation, especially since 2′-deoxyadenosine has been observed to be an inhibitor of DNA synthesis,[158] and 2′-deoxyadenosine and 2′-deoxyguanosine have been shown to inhibit the reproduction of mammalian cells in culture,[159-161] presumably by a type of feedback mechanism which prevents conversion of certain ribonucleotides to 2′-deoxyribonucleotides.

Although there have been relatively few purine derivatives evaluated in the clinic to date, this is probably due in part to the toxicity of certain purine derivatives and the unwarranted assumption that *all* purines and related derivatives would probably behave similarly in human trial. Recent renewed interest in the future clinical possibilities of purines has been stimulated by the work of Adams and Bowman[161] who have shown that 2-amino-6-purinethiol (6-thioguanine) produced striking regression of *established* Sarcoma 180 and Adenocarcinoma 755 tumors.

In the search for compounds of greater specificity against neoplasms and lesser toxicity in man, it would now appear that the animal testing data presently available provide many compounds worthy of further investigation. Much has been learned concerning the desirable structural variations on the purine ring which are most likely to result in selective biological activity. Further refinements in the structure and improvements in the activity of these important compounds and the study of their biochemical relationship to the precursors of nucleic acid and vital coenzymes is a most exciting challenge. It is already abundantly clear from present biochemical studies that the purines and purine nucleosides are capable of highly specific action and represent an area which has only begun to be investigated in cancer chemotherapy.[162,163]

Acknowledgment.—The author wishes to thank Dr. C. C. Stock of the Sloan-Kettering Institute for Cancer Research, Donald S. Walker Laboratory, Rye, N. Y., for permitting us to include certain testing results in Table III.

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