The precipitate was filtered, washed with water, and dried at 60° to give the benzylated product.

2,5-Diamino-4-methylamino-6-methylthiopyrimidine (IV, R, R' = CH_3).—To a suspension of 20 g. of 2-amino-4-methylamino-6-methylthiopyrimidine (III, R, R' = CH_3) in 250 ml. of water and 80 ml. of glacial acetic acid, 15 g. of sodium nitrite in 50 ml. of water was added dropwise, with stirring, at room temperature and allowed to stir for 1 hr. The purple nitroso derivative was filtered, washed with water, and then suspended in 300 ml. of water at 60–70°. Sodium hydrosulfite was added with stirring until complete decolorization was afforded. A small amount of gummy material was filtered from the solution, and the filtrate then was adjusted to pH 8–9 with 28% ammonium hydroxide. Upon cooling, the precipitate was filtered, washed with a small portion of cold water, and dried at 50° to give 15.3 g. of product. All attempts to obtain an analytical sample were to no avail due to the fact that in the drying process a small amount of oxidation produced a slightly pink coloration.

2,5-Diamino-4-ethylamino-6-methylthiopyrimidine (IV, R = C_2H_5, R' = CH_3).—Twenty grams of 2-amino-4-ethylamino-6-methylthiopyrimidine (III, R = C_2H_5, R' = CH_3) was nitrosated and reduced in the same manner as 2-amino-4-methyl-amino-6-methylthiopyrimidine above.

Potential Purine Antagonists. XXXII. The Synthesis and Antitumor Activity of Certain Compounds Related to 4-Aminopyrazolo[3,4-d]pyrimidine[1,2]

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A number of new derivatives of 4-aminopyrazolo[3,4-d]pyrimidine (4-APP) have been prepared and examined for antitumor activity against Adenocarcinoma 755. The structure–activity relationships of this group of compounds is discussed. Derivatives of 4-APP with a tetrahydrofuryl or tetrahydropyranyl ring at position 1 were especially active. These compounds can be considered analogs of 4-APP deoxyriboside.

4-Aminopyrazolo[3,4-d]pyrimidine[3] has been shown to prolong

(1) This work was supported by research grants CY-4008(C1), (C2), and (C3) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) Presented in part before the Division of Medicinal Chemistry, 138th Meeting of American Chemical Society, New York, N. Y., Sept. 1960.

(3) R. K. Robins, *J. Am. Chem. Soc.*, **78**, 784 (1956).

the life span of leukemic mice[4-6] and significantly inhibit the growth of Adenocarcinoma 755.[5] 4-Aminopyrazolo[3,4-d]pyrimidine has also been shown to exhibit a differential inhibitory action upon the growth of malignant cells in tissue culture.[7-9] Inhibition of ascites tumor growth by 4-APP has also been reported.[10,11] In an attempt to improve the antitumor activity of the parent compound, a rather large number of related derivatives have been prepared.[12-16]

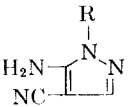
The antitumor activity of the pyrazolo[3,4-d]pyrimidines appears to be confined to derivatives of 4-aminopyrazolo[3,4-d]pyrimidine substituted at position 1 or on the amino group or both.[5] These compounds have been shown to possess a basicity similar to adenine.[17] The present study is a continuation of the effort to select an effective derivative in this series less toxic than 4-APP which has shown signs of hepatotoxicity in man.[18] Such a superior compound would indeed provide a good candidate for clinical trial. Since 1-methyl- and 1-β-hydroxyethyl-4-aminopyrazolo[3,4-d]pyrimidine are significantly active[5] against Adenocarcinoma 755, the preparation of additional derivatives of 4-APP substituted at position 1 was considered. Utilizing the general method of preparation previously devised in this Laboratory,[3,14] the appropriate substituted hydrazine was treated with ethoxymethylenemalononitrile to provide the 1-alkyl-5-amino-4-cyanopyrazoles listed in Table I. Cyclization of the 5-amino-4-cyanopyrazoles with boiling formamide provided the desired 1-alkyl-4-aminopyrazolo[3,4-d]pyrimidines listed in Table II.

Henderson and Junga[19] report that in neoplastic tissue 4-APP was isolated in the form of its riboside and the mono-, di-, and triphosphate derivatives corresponding to the phosphates of adenosine. Way and

(4) H. E. Skipper, R. K. Robins, and J. R. Thomson, *Proc. Soc. Exptl. Biol. Med.*, **89**, 594 (1955).
(5) H. E. Skipper, R. K. Robins, J. R. Thomson, C. C. Cheng, R. W. Brockman, and F. M. Schabel, Jr., *Cancer Research*, **17**, 579 (1957).
(6) J. M. Venditti, E. Frei, and A. Goldin, *Cancer*, **13**, 959 (1960).
(7) T. C. Hsu, R. K. Robins, and C. C. Cheng, *Science*, **123**, 848 (1956).
(8) T. C. Hsu, L. Milofsky, R. K. Robins, and C. C. Cheng, *Antibiotics & Chemotherapy*, **9**, 333 (1959).
(9) T. C. Hsu, L. Milofsky, and R. Fuerst, *Texas Repts. Biol. and Med.*, **16**, 472 (1958).
(10) J. F. Henderson and I. G. Junga, *Cancer Research*, **20**, 1618 (1960).
(11) J. F. Henderson and I. G. Junga, *ibid.*, **21**, 173 (1961).
(12) R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6407 (1957).
(13) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **23**, 191 (1958).
(14) C. C. Cheng and R. K. Robins, *ibid.*, **21**, 1240 (1956).
(15) C. C. Cheng and R. K. Robins, *ibid.*, **23**, 852 (1958).
(16) C. W. Noell and R. K. Robins, *ibid.*, **23**, 1547 (1958).
(17) B. M. Lynch, R. K. Robins, and C. C. Cheng, *J. Chem. Soc.*, 2973 (1958).
(18) R. K. Shaw, R. N. Shulman, J. D. Davidson, D. P. Roll, and E. Frei, *Cancer*, **13**, 482 (1960).
(19) J. F. Henderson and I. G. Junga, *Cancer Research*, **21**, 118 (1961).

TABLE I

1-ALKYL-5-AMINO-4-CYANOPYRAZOLES[a]

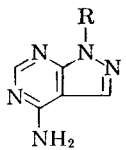


R		M.p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Yield from hydrazine, %	Recrystn. solvent	Ultraviolet absorption, ethanol $\lambda_{max.}$ mμ	ϵ
$CH_2C_6H_5$	$C_{11}H_{10}N_4$	194–195	66.6	66.9	5.1	5.3	57.6	EtOH-H_2O	230	29,640
$(CH_2)_5CH_3$	$C_{10}H_{16}N_4$	144–145	62.5	62.9	8.3	8.2	47.6	EtOH-H_2O	228	8,280
$HC(CH_2)_5$	$C_{10}H_{14}N_4$	108.5–110	63.2	62.9	7.3	7.6	50.4	H_2O	26	8,000
$(CH_2)_3CH_3$	$C_8H_{12}N_4$	164–165	58.6	58.5	7.3	7.3	60.0	H_2O	230	7,400

[a] Compounds listed in this table were prepared by the general method described in detail for 5-amino-4-cyano-1-cyclohexylpyrazole (see Experimental).

TABLE II

1-ALKYL-4-AMINOPYRAZOLO[3,4-d]PYRIMIDINES[a]

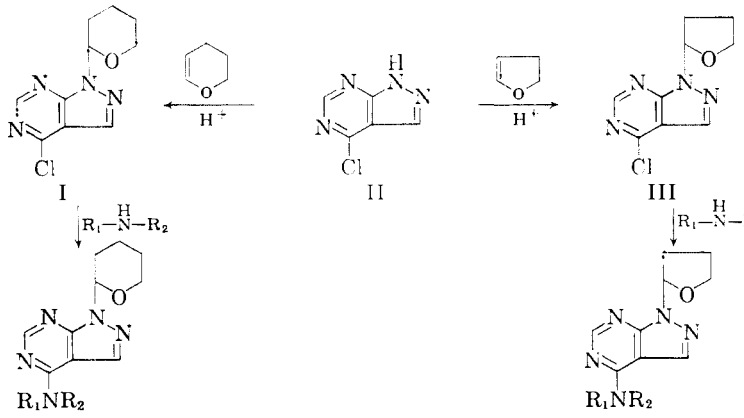


R		M.p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Yield from pyrazole, %	Recrystn. solvent	Ultraviolet absorption pH 1.3 λ max, mμ	pH 1.3 ε	pH 12.2 λ max, mμ	pH 12.2 ε
$(CH_2)_3CH_3$	$C_9H_{13}N_5$	156–157	56.6	56.9	6.8	7.0	47.0	Toluene	220	22,180	260	7,000
									256	8,080	277	7,430
											286	4,670
$(CH_2)_5CH_3$	$C_{11}H_{17}N_5$	170–171	60.2	60.5	7.8	7.6	47.3	H_2O	222	16,200	224	5,260
									260	5,490	262	5,480
											279	5,700
$HC(CH_2)_5$	$C_{11}H_{15}N_5$	196–197	60.9	61.0	6.9	7.1	63.0	H_2O	224	27,600	262.5	8,500
									259	10,000	278	9,120
$CH_2C_6H_5$	$C_{12}H_{11}N_5$	235–236	64.0	63.9	4.9	4.9	59.3	H_2O	258	12,000	262	10,580
											278	11,420

[a] Derivatives listed in this table were prepared by the general method described in detail for 4-amino-1-cyclohexylpyrazolo-[3,4-d]pyrimidine (see Experimental).

Parks[20] and Roy[21] have synthesized the nucleotide of 4-APP with purified enzyme systems. Thus, it seemed quite possible that 4-APP might exert antitumor action as the nucleoside or nucleotide form. Since the synthesis of various 6-substituted-9-(tetrahydropyran-2-yl)purines[22] has recently provided compounds possessing excellent antitumor activity against Adenocarcinoma 755, the synthesis of certain 4-amino-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]-pyrimidines was studied. It was hoped that the tetrahydropyran or tetrahydrofuran ring might provide appropriate nucleoside models of 4-APP.

The reaction of 2,3-dihydropyran[23] and 4-chloropyrazolo[3,4-d]-pyrimidine (II)[3] in 99% ethyl acetate at 35° provided 4-chloro-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (I) in good yield. It was necessary that II be pure since impurities reduced the yield considerably. 4-Chloro-1-(tetrahydro-2-furyl)pyrazolo[3,4-d]-pyrimidine (III) was similarly synthesized from 2,3-dihydrofuran[24] and II. Reaction of I and III with various amines provided the corresponding 1-tetrahydropyranyl (IV) or 1-tetrahydrofuryl (VI) 4-substituted aminopyrazolo[3,4-d]pyrimidines listed in Tables III and IV. Since it is possible that the tetrahydropyran and tetrahydrofuran might have entered position 2 instead of position 1, the following proof of structure was undertaken. Both I and III were con-



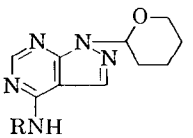
IV, R_1 = H, R_2 = alkyl
VII, R_1, R_2 = CH_3

VI, R_1 = H, R_2 = alkyl
VIII, R_1, R_2 = CH_3

(20) J. L. Way and R. E. Parks, Jr., *J. Biol. Chem.*, **231**, 467 (1958).
(21) J. K. Roy, C. A. Haavik, and R. E. Parks, Jr., *Proc. Am. Assoc. Cancer Research*, **3**, 146 (1960).
(22) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis, and A. Jackson, *J. Am. Chem. Soc.*, **83**, 2574 (1961).
(23) Obtained from Quaker Oats Co., Chemicals Division, Chicago 54, Ill.
(24) R. Paul, *Bull. soc. chim. France*, 668 (1950).

TABLE III

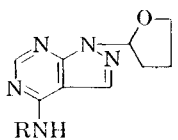
4-ALKYLAMINO-1-(TETRAHYDROPYRAN-2-YL)PYRAZOLO[3,4-d]PYRIMIDINES



R		M.p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Yield, %	Recrystn. solvent	Ultraviolet absorption pH 1 λ max, mμ	pH 1 ε	pH 11 λ max, mμ	pH 11 ε
H	$C_{10}H_{13}N_5O$	182.5–183	54.8	55.0	5.9	5.5	32.0	32.0	54.3	C_6H_5	258	10,620	274	10,800
CH_3	$C_{11}H_{15}N_5O$	158.5–159	56.7	56.5	6.4	6.6	30.1	29.9	51.0	*n*-C_7H_{16}	264	13,150	281.5	13,680
CH_2CH_3	$C_{12}H_{17}N_5O$	155–156	58.3	58.2	6.9	6.7	28.4	28.5	54.6	C_6H_6-*n*-C_7H_{16}	265	14,110	282.5	14,500
$(CH_2)_2CH_3$	$C_{13}H_{19}N_5O$	114.5–116.5	59.9	60.1	7.3	7.5	26.9	27.1	22.8	*n*-C_7H_{16}	265.5	13,350	283.5	15,420
$(CH_2)_3CH_3$	$C_{14}H_{21}N_5O$	106.5–107.5	61.1	61.2	7.6	7.5	25.5	25.2	30.1	Petroleum ether (60–110°)	266	13,250	283.5	14,950
$CH_2C_6H_5$	$C_{17}H_{19}N_5O$	183.5–185.5	66.0	66.2	6.2	6.1	22.7	23.1	34.6	MeOH	267	14,250	282.5	16,850
$CH_2C_6H_4CH_3$-*p*	$C_{18}H_{21}N_5O$	204–204.5	66.8	67.1	6.5	6.5	21.7	21.7	41.7	EtOH-H_2O				
$CH_2C_6H_3Cl_2$-3,4 · H_2O	$C_{17}H_{16}Cl_2N_5 \cdot H_2O$	170–171	51.5	51.8	4.8	4.9	17.7	17.2	48.0	EtOH-H_2O	266.5	16,050	282	17,220

TABLE IV

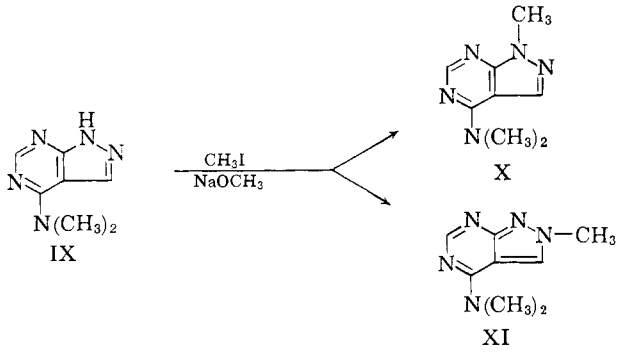
4-ALKYLAMINO-1-(TETRAHYDRO-2-FURYL)PYRAZOLO[3,4-d]PYRIMIDINES



R		M.p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Yield, %	Recrystn. solvent
CH_3	$C_{10}H_{13}N_5O$	180–181	54.8	54.9	5.9	6.0	32.0	31.8	24.4	C_7H_{16}-C_6H_6
C_2H_5	$C_{11}H_{15}N_5O$	172–173.5	56.7	57.1	6.4	6.3	30.1	29.9	38.7	C_6H_6
$(CH_2)_2CH_3$	$C_{12}H_{17}N_5O$	98–99.5	58.3	58.3	6.9	6.9	28.4	28.3	22.8	C_6H_6-C_7H_{16}

verted with dimethylamine to the corresponding dimethylamino derivatives VII and VIII.

4-Dimethylaminopyrazolo[3,4-d]pyrimidine was prepared by the method of Robins[3] and treated with methyl iodide in the presence of base to yield two isomers, X and XI. Similar alkylation of 7-



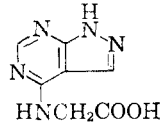
dimethylamino-v-triazolo[d]pyrimidine with ethyl iodide has been reported[25] to yield a mixture of the 1-, 2-, and 3-ethyl derivatives. Thus, the expected isomers were 4-dimethylamino-1-methylpyrazolo-[3,4-d]pyrimidine (X) and 4-dimethylamino-2-methylpyrazolo[3,4-d]-pyrimidine (XI). Since X had been previously prepared by Cheng and Robins[14] by an unambiguous synthesis, the assignment of structure XI to the other isomer was readily made. Additional evidence for structure XI is the fact that at pH 1 the absorption maximum is λ_{max} 294 mμ, ϵ 13,800 as compared to λ_{max} 268 mμ, ϵ 12,900 for compound X. The fixed bond structure, XI, would be expected to absorb at longer wave lengths.[26]

Schmidt and co-workers[27] have shown that 2-alkylpyrazolo[3,4-d]-pyrimidines exhibit a bathochromic shift of 15 to 25 mμ over that of the similarly substituted 1-alkyl derivatives. A comparison of the ultraviolet absorption spectra of 4-dimethylamino-1-(tetrahydropyran-2-yl)- (VII) and 4-dimethylamino-1-(tetrahydro-2-furyl)pyrazolo-[3,4-d]pyrimidine (VIII) was made with the spectra of X and XI (see Table VIII). It is apparent that the compounds VII and VIII are 1-substituted derivatives since the spectral data are almost identical with those of the corresponding 4-dimethylamino-1-methylpyrazolo-[3,4-d]pyrimidine. Thus, the reaction of 2,3-dihydropyran and 2,3-

(25) R. B. Angier and J. W. Marsico, *J. Org. Chem.*, **25**, 759 (1960).
(26) F. Bergmann, G. Levin, A. Kalmus, and H. Kwietny-Govrin, *ibid.*, **26**, 1504 (1961).
(27) P. Schmidt, K. Eichenberger, M. Wilhelm, and J. Druey, *Helv. Chim. Acta*, **42**, 763 (1959).

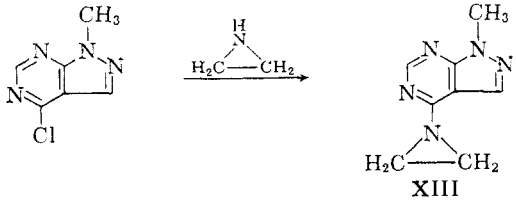
dihydrofuran with 4-chloropyrazolo[3,4-d]pyrimidine must have occurred at position 1. Assignment of the structure of the reaction product of dihydropyran and 6-chloropurine has been made on the basis of ultraviolet absorption spectra in a similar manner.[22] Similar arguments show that 6-chloropurine and 2,3-dihydrofuran give 6-chloro - 9 - (tetrahydro - 2 - furyl)purine.[23] 4 - Amino - 1 - (tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (IV, R_1, R_2 = H) was prepared from I and alcoholic ammonia heated in a bomb at 130°.

A number of derivatives of 4-benzylaminopyrazolo[3,4-d]pyrimidine have been prepared by standard procedures previously described.[3,16] These derivatives are listed in Table V. An interesting compound, N-[pyrazolo[3,4-d]pyrimidin-4-yl]glycine (XII) has been



XII

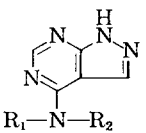
prepared from 4-chloropyrazolo[3,4-d]pyrimidine (II) and glycine in hot aqueous ammonia. Treatment of 4-aminopyrazolo[3,4-d]-pyrimidine with glacial acetic acid and hydrogen peroxide (30%) gave a mono-N-oxide of 4-APP which exhibited an ultraviolet absorption spectrum similar to that of adenine-N-1-oxide.[29] By analogy with adenine the structure of the product has tentatively been assigned as 4-aminopyrazolo[3,4-d]pyrimidine-5-N-oxide, since the highest center of electron density of 4-APP has been assigned[17] to position 5 which is analogous to position 1 in adenine. Although no rigorous proof of structure was undertaken, a strong band in the infrared at 1302 cm.$^{-1}$ (absent in 4-APP) gave definite evidence for the presence of the N-oxide function.[30] Since a number of 1-aziridinyl derivatives such as triethylenemelamine[31] have shown significant anticancer activity, 4-(1-aziridinyl)-1-methylpyrazolo[3,4-d]pyrimidine (XIII)



XIII

(28) L. R. Lewis, F. H. Schneider, and R. K. Robins, *J. Org. Chem.*, **26**, 3837 (1961).
(29) M. A. Stevens and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2759 (1958).
(30) R. H. Wiley and S. C. Slaymaker, *ibid.*, **79**, 2233 (1957).
(31) S. M. Buckley, C. C. Stock, M. L. Crossley, and C. P. Rhoads, *Cancer Research*, **10**, 207 (1950).

TABLE V



				Analyses, %								Ultraviolet absorption			
				Carbon		Hydrogen		Nitrogen				pH 1		pH 11	
R_1	R_2		M. p., °C.	Calcd.	Found	Calcd.	Found	Calcd.	Found	Yield, %	Recrystn. solvent	λ max, mμ	ε	λ max, mμ	ε
H	$CH_2C_6H_4CH_3$-*o*	$C_{13}H_{13}N_5$	261–261.5	65.3	65.1	5.4	5.6	29.3	29.1	85.2	H_2O-EtOH 70:30	268	12,310	277.5	13,860
H	$CH_2C_6H_4CH_3$-*m*	$C_{13}H_{13}N_5$	211.5–212.5	65.3	65.4	5.4	5.5	29.3	29.0	57.2	EtOH-H_2O	268	12,750	277	13,630
H	$CH_2C_6H_4CH_3$-*p*	$C_{13}H_{13}N_5$	222–222.5	65.3	65.6	5.4	5.4	29.3	29.7	55.2	EtOH-H_2O	268.5	13,150	276.5	14,210
H	$CH_2C_6H_3(CH_3)_2$-3,4	$C_{14}H_{15}N_5$	214.5–216.5	66.4	66.2	5.9	6.1	27.7	27.4	32.7	EtOH-H_2O	269	12,900	279.5	15,050
CH_3	$CH_2C_6H_5$	$C_{13}H_{13}N_5$	193.5–196.5	65.3	65.3	5.4	5.3	29.4	29.2	54.5	EtOH	299	12,480	284	13,970

was prepared from 4-chloro-1-methylpyrazolo[3,4-d]pyrimidine[14] and ethylenimine in the presence of triethylamine and benzene. 4 - (1 - Aziridinyl) - 1 - (tetrahydropyran - 2 - yl)pyrazolo[3,4 - d]-pyrimidine was similarly prepared from I and ethylenimine. A number of new 4-alkylamino-1-methylpyrazolo[3,4-d]pyrimidines have been prepared essentially by procedures previously described.[14,16] These compounds (hydroxyalkylamino derivatives) are described in Table VI.

Biological Testing Data.—The antitumor testing data against Adenocarcinoma 755 are listed in Table VII for the more active derivatives prepared. The testing procedures employed have been adequately described previously.[32,33] All testing was conducted under the auspices of the Cancer Chemotherapy National Service Center. Although the compounds in Table II possessed a pK_a in the range suggested for activity for 1-alkyl-4-aminopyrazolo[3,4-d]pyrimidines[17] (4.3 to 4.1), none of these derivatives were active. Presumably the large alkyl group at position 1 renders the molecule (4-APP) inactive due to steric considerations. An exception to this is the tetrahydropyran and tetrahydrofuran derivatives of Table III and IV, which in general, were significantly active. 4-Amino-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine is as active as 4-APP on a similar weight basis. This suggests that the tetrahydrofuran and tetrahydropyran groups might well be acting as deoxynucleoside analogs, and that the deoxyriboside of 4-APP should be synthesized and tested. It is of interest that a similar derivative, 4-amino-1-cyclohexylpyrazolo[3,4-d]pyrimidine (Table II), is without antitumor effect.

4-(1-Aziridinyl)-1-methylpyrazolo[3,4-d]pyrimidine is significantly active but not greatly superior to the similar 4-dimethylamino-1-methylpyrazolo[3,4-d]-pyrimidine.[5] In general, the structure-activity relationships previously noted[5] have been confirmed and extended by the present work. The search for a derivative in this series with a therapeutic index considerably superior to 4-APP in experimental mice has not been rewarded. It is of considerable interest, however, that 4-APP is not[5] cross-resistant with a 6-mercaptopurine resistant line of AD 755 and, therefore, in all probability acts *via* a different mechanism from 6-MP. Similarly, an 8-azaguanine resistant strain of L-1210 is also not cross-resistant to 4-APP.[34] Bennett, Brockman, and Smithers[34] have reported that 4-APP produced moderate incorporation of formate ^{14}C into soluble purine and a marked inhibition of incorporation into polynucleotide purines, particularly those of DNA.

Henderson[35] has shown recently that co-administration of 4-APP and thioguanine or 6-mercaptopurine increased the therapeutic effectiveness of thioguanine or 6-mercaptopurine in experimental animals with Ehrlich ascites carcinoma.

Since 4-APP appears to act by a different mechanism from the *usual* purine

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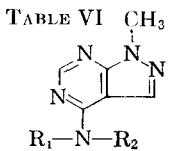
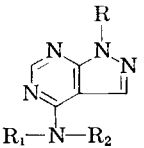
TABLE VI



R_1	R_2		M.p. °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Yield, %	Recrystn. solvent	Ultraviolet absorption pH 1 λ max, mμ	pH 1 ε	pH 11 λ max, mμ	pH 11 ε
H	$(CH_2)_2OC_2H_5$	$C_{10}H_{16}N_6O$	108–110	54.3	54.5	6.8	6.9	31.7	31.9	81.5	EtOH	266.5	11,720	284.5	12,320
H	$C_2H_5CHCH_2OH$	$C_{10}H_{16}N_6O$	143.5–145	54.3	54.1	6.8	7.0	31.7	31.2	21.8	EtOH	268	12,690	284.5	13,890
H	$(CH_3)_2CHCH_2OH$	$C_{10}H_{16}N_6O$	181–182	54.3	54.5	6.8	6.8	31.7	31.9	12.2	Toluene	268	12,620	284	14,280
CH_3	$(CH_2)_2OH$	$C_9H_{13}N_6O$	130–132	52.2	52.4	6.3	6.2	33.8	33.8	66.3	EtOH	272.5	13,080	290.5	15,310
C_2H_5	$(CH_2)_2OH$	$C_{10}H_{16}N_6O$	106.5–107.5	54.3	54.6	6.8	7.2	31.7	32.1	12.0 est.	EtOH	272.5	16,600	290.5	20,800
$(CH_2)_2OH$	$(CH_2)_2OH$	$C_{10}H_{16}N_6O_2$	137–139	50.5	50.2	6.6	6.4	29.6	29.6	68.7	EtOH	275.5	13,820	291	16,280

TABLE VII

ANTITUMOR ACTIVITY OF 4-AMINOPYRAZOLO[3,4-d]PYRIMIDINES AGAINST ADENOCARCINOMA 755



R	R_1	R_2	Dose, mg./kg.	Survivors	Wt. change (test/control)	Tumor wt. (test/control)	T/C
H	H	CH_2COOH	200	0/10	toxic		
			100	10/10	−6.0/−1.9	45/622	0.07
			100	7/10	−4.1/−1.1	21/980	0.02
			50	10/10	−4.1/−1.9	225/622	0.36
			25	10/10	−2.1/−1.9	515/622	0.82
			12.5	9/10	−1.8/−1.9	406/622	0.65
H	H	$(CH_2)_3CH_3$[a]	25	9/10		0/851	0.00
			20	10/10		31/920	0.03
			18.75	10/10		142/851	0.17
			12.5	10/10		147/851	0.17
			10	8/10		313/920	0.34
H	H	$(CH_2)_2CH(CH_3)_2 \cdot HCl$[b]	50	3/10	toxic		
			25	10/10	−2.0/1.0	247/1454	0.17
			12.5	10/10	2.0/2.0	1033/944	1.10
			6.25	10/10	3.0/2.0	815/944	0.86
H	H	$CH_2C_6H_3Cl_2$-3,4[b]	62.5	10/10	−2.0/5.0	214/1985	0.11
H	CH_3	$CH_2C_6H_5$	100	8/10	−4.1/0.0	25/1100	0.02
			50	10/10	−4.5/0.0	120/1100	0.10
			25	10/10	−1.0/0.0	685/1100	0.62
			12.5	10/10	−0.4/0.0	467/1100	0.42

R	R_1	R_2	Dose, mg./kg.	Survivors	Wt. change (test/control)	Tumor wt. (test/control)	T/C
CH_3	Aziridinyl		50	0/10	toxic		
			25	11/12	−1.7/0.6	105/562	0.18
			25	7/10	−3.0/1.0	171/1119	0.15
			12.5	7/10	−0.1/1.0	650/1119	0.58
			6.25	9/10	0.5/1.0	1111/1119	0.99
CH_3	H	$CH_2CHOHCH_3$	100	6/10	−5.4/−1.9	0/622	0.00
			50	10/10	−6.1/−1.9	65/622	0.10
			25	10/10	−2.0/−1.9	190/622	0.30
			12.5	10/10	−1.3/−1.9	590/622	0.94
CH_3	H	$(CH_2)_2OC_2H_5$	200	0/10	toxic		
			100	10/10	−5.7/−1.9	110/622	0.17
			50	9/10	−3.7/−1.9	239/622	0.38
			25	10/10	−2.3/−1.9	410/622	0.65
			12.5	10/10	−0.7/−1.9	700/622	1.12
		CH_3	18.7	3/10	toxic		
			9.3	8/10	−2.6/−0.2	136/954	0.14
			4.6	10/10	−1.6/−0.2	320/954	0.33
			2.3	10/10	−0.9/−0.2	685/954	0.71
		C_2H_5	25	0/10	toxic		
			12.5	9/10	−4.3/0.0	39/1100	0.03
			12.5	6/10	−3.6/−0.2	125/954	0.13
			6.2	9/10	−2.3/−0.2	378/954	0.39
			3.1	9/10	−0.6/−0.2	589/954	0.61
		$(CH_2)_2CH_3$	25	4/10	toxic		
			12.5	8/10	−3.8/0.0	44/1100	0.04
			6.2	8/10	−1.6/−0.2	263/954	0.27
			3.1	9/10	−0.2/−0.2	639/954	0.66

R	R_1	R_2	Dose, mg./kg.	Survivors	Wt. change (test/control)	Tumor wt. (test/control)	T/C
		H	1.5	9/10	0.1/−0.2	722/954	0.75
			14	8/10	−5.1/0.4	19/1075	0.02
			7	10/10	−3.1/1.2	278/1132	0.25
			3.5	10/10	−1.7/0.4	545/1075	0.50
		C_2H_5	25	7/10	−3.4/−0.3	71/731	0.09
			12.5	7/10	−3.3/−1.9	36/311	0.11
			6.25	9/10	−2.4/−1.9	39/311	0.12
			3.12	10/10	−1.7/−1.9	105/311	0.33
			1.56	9/10	−1.0/−1.9	111/311	0.35
		CH_3	100	0/10	toxic		
			50	8/10	−6.2/−0.9	0/1042	0.00
			25	10/10	−0.1/−0.9	940/1042	0.90

[a] Ref. 4. [b] Ref. 17.

TABLE VIII

Compound	R	Ultraviolet absorption: pH 1 $\lambda_{max.}$ mμ	pH 1 ε	pH 11 $\lambda_{max.}$ mμ	pH 11 ε	Ethanol $\lambda_{max.}$ mμ	Ethanol ε
VII	1-Tetrahydropyran-2-yl	267.5	13,550	288 267	15,280	286.5 266	14,900
VIII	1-Tetrahydro-2-furyl	269.5	11,780	289 267	15,010	288 266	15,100
X	1-Methyl	267.5	12,900	286 267	17,200	288 265	12,500
XI	2-Methyl	294	13,800	298	18,000	299	15,050

antagonists, the use of combined therapy utilizing 4-APP and 6-mercaptopurine might well be considered for clinical trial.

Acknowledgment.—The authors wish to thank Dr. Brian M. Lynch, Memorial University of Newfoundland, St. Johns, Newfoundland, Canada, for helpful suggestions relative to this work.

Experimental[36]

Cyclohexylhydrazine hydrochloride, m.p. 116–117°, was prepared for the first time by the general method of Fugger, Tien, and Hunsberger.[37] The yield of product based on ethyl chloroacetate was 46.8%.

Anal. Calcd. for $C_6H_{15}ClN_2$: C, 47.8; H, 10.0. Found: C, 47.4; H, 9.9.

5-Amino-4-cyano-1-cyclohexylpyrazole.—Cold 3 *N* ethanolic potassium hydroxide was added slowly to a solution of cyclohexylhydrazine hydrochloride (40 g.) in 160 ml. of absolute ethanol. When the pH of the solution was adjusted to 8, the precipitated potassium chloride was filtered and washed twice with hot ethanol. Ethoxymethylenemalononitrile (32 g.) was added slowly to the filtrate. The solution was heated on the steam bath for 2 hr. to insure completion of the reaction, and then the mixture was evaporated to dryness; 25.4 g. of crude product was obtained. Recrystallization from water gave 19.5 g. of pure 5-amino-4-cyano-1-cylohexylpyrazole, m.p. 108.5–110°. An additional 5.0 g. was recovered from the concentrated mother liquor.

Anal. Calcd. for $C_{10}H_{14}N_4$: C, 63.2; H, 7.3. Found: C, 62.9; H, 7.6.

Other compounds in Table I were prepared in a similar manner.

4-Amino-1-cyclohexylpyrazolo[3,4-d]pyrimidine.—5-Amino-4-cyano-1-cyclohexylpyrazole (10 g.) was added to 80 ml. of formamide, and the mixture was boiled gently for 1.5 hr. Water (50 ml.) was added to the warm mixture, and the mixture was cooled overnight. The yield of crude product was 7.2 g. (63%), m.p 194–197°. The product was dissolved in 140 ml. of 2 *N* hydrochloric acid, and the solution was decolorized with charcoal. Concentrated aqueous ammonia was added to the hot filtrate until pH 8 was reached. When cool, the resulting precipitate was filtered and washed with water to yield 6.5 g. of pale-yellow 4-amino-1-cyclohexylpyrazolo[3,4-d]pyrimidine. Recrystallization from water gave 4.6 g. of pure product, m.p. 196–197°. An additional 0.6 g. was obtained from the concentrated mother liquor.

Anal. Calcd. for $C_{11}H_{15}N_5$: C, 60.9; H, 6.9. Found: C, 61.0; H, 7.1.

N-[Pyrazolo[3,4-d]pyrimidin-4-yl]glycine (XII).—4-Chloropyrazolo[3,4-d]-pyrimidine (II, 5.0 g.) and 2.5 g. of glycine were heated to reflux for 3 hr. with 50 ml. of concd. ammonium hydroxide. The solution then was adjusted to pH 4 with concentrated glacial acetic acid and filtered. The residue was crystallized by precipitation from base (dil. ammonium hydroxide, pH 8) with glacial acetic acid to yield 2.1 g. (33.8%) of product which gradually decomposed above 215°. At pH 1 the compound exhibited: λ_{max} 268.5 mμ, ϵ 13,750; at pH 11: λ_{max} 278 mμ, ϵ 11,300. Neut. equiv. calcd.: 195. Found: 193.

Anal. Calcd. for $C_7H_7N_5O_2$: C, 43.5; H, 3.6; N, 36.3. Found: C, 43.2; H, 4.0; N, 36.1.

4-Chloro-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (I).—4-Chloropyrazolo[3,4-d]pyrimidine (II, 22.0 g.) was added to 250 ml. of 99% ethyl acetate, and the solution was heated with stirring to 35°. *p*-Toluenesulfonic acid (200 mg.) was added, followed by 12.0 g. of 2,3-dihydropyran which was added dropwise over a 10-min. period. The temperature rose to 43°, and II dissolved completely. Heating and stirring were continued until the temperature rose to 45°. The

(36) All melting points are uncorrected and were taken on a Fisher-Johns melting point block unless otherwise stated.

(37) The hexyl-, benzyl-, and butylhydrazine used in this work were prepared according to the method used previously by J. Fugger, J. M. Tien, and I. M. Hunsberger, *J. Am. Chem. Soc.*, **77**, 1843 (1955).

yellow solution was then cooled rapidly to room temperature and washed free of acid with four 20-ml. portions of saturated sodium carbonate solution, followed by four 20-ml. portions of water (until neutral). The ethyl acetate extract was finally dried over anhydrous magnesium sulfate for 5 hr. and removed *in vacuo* employing a water bath (60°) as a source of heat. The viscous, yellow residue was recrystallized from petroleum ether (60–110°). Recrystallization yielded pure, white elongated needles, m.p. 101–102°. The yield was 10.2 g.

Anal. Calcd. for $C_{10}H_{11}ClN_4O$: C, 50.4; H, 4.6; N, 23.5. Found: C, 50.4; H, 4.7; N, 23.3.

4-Chloro-1-(tetrahydro-2-furyl)pyrazolo[3,4-d]pyrimidine (III).—4-Chloropyrazolo[3,4-d]pyrimidine (II, 18.5 g.) and 9.0 g. of 2,3-dihydrofuran[24] were added to 200 ml. of 99% ethyl acetate containing 200 mg. of *p*-toluenesulfonic acid. The mixture was heated slowly with stirring. At 35° the temperature started to rise quite rapidly until 42° when solution was complete. Heating was continued until the temperature reached 45°. The product was isolated in a manner similar to that employed in the preparation of I. The oily, viscous, yellow residue could not be recrystallized and was utilized directly for succeeding experiments. The yield was 27 g.

4-(1-Aziridinyl)-1-methylpyrazolo[3,4-d]pyrimidine (XIII).—4-Chloro-1-methylpyrazolo[3,4-d]pyrimidine[14] (12.0 g.) was added to 100 ml. of benzene containing 12 ml. of triethylamine. Ethylenimine (4 ml.) was added, and the temperature of the reaction was kept at 35° for 1 hr. The mixture was then cooled and filtered and the solid extracted with boiling benzene. The benzene was reduced to dryness *in vacuo*, and the residual solid was recrystallized from *n*-heptane to yield 7.0 g. of white needles, m.p. 141–142°.

Anal. Calcd. for $C_8H_9N_5$: C, 54.9; H, 5.1; N, 40.0. Found: C, 55.0; H, 4.9; N, 40.3.

4-Aziridinyl-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine.—4-Chloro-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (I, 5.0 g.) was dissolved in 100 ml. of benzene containing 10 ml. of triethylamine. Ethylenimine (5 ml.) was added, and the mixture was stirred at 35° for 1 hr. The temperature was raised to 40° and the mixture stirred an additional hr. Gradually the temperature was raised to 50°, and the mixture was filtered. The benzene was removed *in vacuo* from the filtrate using a water bath at a temperature not higher than 40°. The residue was dissolved in 2 portions of 75 ml. of boiling petroleum ether (60–110°), and the solution was allowed to stand overnight. The yield of white crystals was 2.5 g. (48.6%), m.p. 100–102°.

Anal. Calcd. for $C_{12}H_{15}N_5O$: C, 58.8; H, 6.1; N, 28.6. Found: C, 58.9; H, 6.2; N, 28.6.

4-Dimethylamino-1-(tetrahydro-2-furyl)pyrazolo[3,4-d]pyrimidine (VIII).—4-Chloro-1-(tetrahydro-2-furyl)pyrazolo[3,4-d]pyrimidine (III, 10.0 g., crude) and 300 ml. of 25% aqueous dimethylamine were heated to dryness on the steam bath (12 hr.). To the residue was added 6 portions of 30 ml. of benzene, and the solution was again heated to dryness. The remaining solid was dissolved in a minimum of hot benzene and treated with charcoal. Heptane was added until the solution became cloudy. The yield was 1.4 g. (13.5%) of crystals, m.p. 68.5–70.5°.

Anal. Calcd. for $C_{11}H_{15}N_5O$: C, 56.7; H, 6.4; N, 30.0. Found: C, 56.9; H, 6.6; N, 29.7.

Other compounds in Table IV were prepared in a similar manner.

4-Methylamino-1-(tetrahydro-2-furyl)pyrazolo[3,4-d]pyrimidine (VI, R_1 = H, R_2 = CH_3).—4-Chloro-1-(tetrahydro-2-furyl)pyrazolo[3,4-d]pyrimidine (III, 9.5 g.) was added with stirring to 200 ml. of 40% aqueous methylamine. The solution was slowly heated to dryness on the steam bath (7.5 hr.). To the residue was added 2 portions of 30 ml. of benzene, and the solution was again heated to dryness. The remaining solid was recrystallized from a benzene–heptane mixture to yield 2.25 g. (24.4%) of product, m.p. 180–181°.

Anal. Calcd. for $C_{10}H_{13}N_5O$: C, 54.8; H, 5.9; N, 32.0. Found: C, 54.9; H, 6.0; N, 31.8.

4-Dimethylamino-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (VII).—4-Chloro-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (I, 7.0 g.) and 150 ml. of 25% aqueous dimethylamine were heated to dryness on the steam bath (4.5 hr.). To the residue was added 3 portions of 30 ml. of benzene, and the solution was again heated to dryness. The remaining solid was recrystallized from heptane to give 4.0 g. (55.3%) of product, m.p. 114.5–115.5°.

Anal. Calcd. for $C_{12}H_{17}N_5O$: C, 58.3; H, 6.9; N, 28.4. Found: C, 58.1; H, 7.0; N, 28.6.

Other compounds in Table III were prepared in a similar manner.

4-Amino-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (IV, R_1, R_2 = H).—4-Chloro-1-(tetrahydropyran-2-yl)pyrazolo[3,4-d]pyrimidine (I, 5.7 g.) and 250 ml. of saturated, ammoniacal, absolute ethanol (0°) were placed in a bomb, and the solution was heated at 130° for 2.5 hr. Solid potassium hydroxide (3.0 g.) was added to the cooled reaction mixture. The mixture was filtered and the ethanol removed *in vacuo* using a water bath at 60° as a source of heat. The white residue was recrystallized from benzene to yield 2.5 g. (54.3%) of colorless solid, m.p. 182.5–183°.

Anal. Calcd. for $C_{10}H_{13}N_5O$: C, 54.8; H, 5.9; N, 32.0. Found: C, 55.0; H, 5.5; N, 32.0.

4-Aminopyrazolo[3,4-d]pyrimidine-5-N-oxide.—4-Aminopyrazolo[3,4-d]pyrimidine[3] (1.0 g.), 30 ml. of glacial acetic acid, and 4 ml. of 30% hydrogen peroxide were stirred for 3 days at room temperature. After approximately 10 min. the mixture went into solution and gradually darkened to light brown over the 3-day period. Finally, 200 mg. of 5% palladium-on-charcoal was added, and the mixture was stirred a day until it showed a negative starch-iodine test. It was filtered and the solvent removed *in vacuo* at 60°. The brown solid was crystallized from water to yield 0.5 g. (43.8%) of light-brown solid, m.p. >300°. The compound exhibited at pH 1: λ_{max} 257.5 mμ, ε 7,540; at pH 11: λ_{max} 232, 310 mμ, ε 30,000, 3,160.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.7; H, 3.3; N, 46.3. Found: C, 39.5; H, 3.5; N, 46.1.

4-Dimethylamino-1-methylpyrazolo[3,4-d]pyrimidine (X) and 4-Dimethylamino-2-methylpyrazolo[3,4-d]pyrimidine (XI).—4-Dimethylaminopyrazolo[3,4-d]pyrimidine[3] (IX, 10.0 g.), 77 ml. of methanol, 34 ml. of 2 *N* sodium hydroxide and 9.5 g. of methyl iodide were refluxed for 2.25 hr. The solution was then evaporated *in vacuo* on a steam bath. The white, gummy residue was dissolved in 77 ml. of 10% potassium hydroxide. The solution was filtered from a small amount of impurities and extracted with 3 × 200 ml. and 3 × 100 ml. of chloroform. The chloroform extracts were dried overnight over anhydrous sodium sulfate. The excess chloroform was removed *in vacuo* on a steam bath to yield 6.3 g. of light tan solid. The solid was recrystallized from heptane to give 3.4 g.

of X, m.p. 129–129.5°, and 2.2 g. of an insoluble, somewhat tan residue. Further recrystallization from heptane gave 2.6 g. (24.1%) of white solid, m.p. 131–131.5°. This compound exhibited at pH 1: λ_{max} 267.5 mμ, ϵ 12,900; at pH 11: λ_{max} 286 mμ, ϵ 17,200 and 267 mμ (inflection). The R_f values of this compound in the solvents A, B, C, and D[38] were 0.687, 0.295, 0.827, and 0.126, respectively. R_f values of 0.687, 0.297, 0.844, and 0.126 for solvents A, B, C, and D, respectively, were found for the same compound previously prepared by Cheng and Robins.[14]

Anal. Calcd. for $C_8H_{11}N_5$: C, 54.2; H, 6.2; N, 39.6. Found: C, 54.5; H, 6.3; N, 39.6.

The insoluble residue was crystallized from toluene and treated with charcoal, followed by repeated recrystallizations from benzene, to give 0.2 g. (1.9%) of white solid (XI), m.p. 194–195.5°. This compound exhibited at pH 1: λ_{max} 294 mμ, ϵ 13,800 and at pH 11: λ_{max} 298 mμ, ϵ 18,000. The R_f values for this compound were 0.472, 0.273, 0.787, and 0.063 in solvents A, B, C, and D, respectively.

Anal. Calcd. for $C_8H_{11}N_5$: C, 54.2; H, 6.2; N, 39.6. Found: C, 54.2; H, 6.3; N, 39.6.

(38) Solvent A: 1-butanol–water–1% aqueous ammonia; Solvent B: 1-butanol–glacial acetic acid–water (5:2:3); Solvent C: disodium hydrogen phosphate (59% in water) saturated with isoamyl alcohol; Solvent D: 1-butanol–formic acid–water (77:10:13).

The Preparation and Antitumor Activity of Certain Derivatives of 6-Mercaptopurine[1]

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A number of new 6-alkylthiopurines have been prepared and tested against Adenocarcinoma 755 and S-180 in experimental mice. Several derivatives possess a therapeutic index greatly superior to that of 6-mercaptopurine in both S-180 and Ad 755. The synthesis of several N-alkylpurine-6-sulfonamides is described. These derivatives exhibit significant inhibition of Ad 755 and L-1210. Certain 6-alkylsulfonylpurines are also active against Ad 755. The significance of these results is discussed briefly.

The antitumor activity of 6-mercaptopurine and various 6-alkylthiopurines has been studied in Sarcoma 180[2,3] and in Adenocar-

(1) This study was supported by Contract SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Cancer Institute of the National Institutes of Health.

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