Pyrimidines. XVI. 2,4,5-Triaminopyrimidines and Related Compounds[1]

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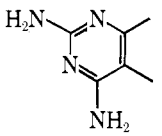
2,4,5-Triaminopyrimidine was found to possess confirmed antileukemic activity against L1210. This activity was not shown by structurally related compounds such as 2,4-diamino-, 2,4,6-triamino-, and 2,4,5,6-tetraaminopyrimidines. Preliminary investigation on the structure–activity requirements for compounds in this series has been initiated. The following three classes of compounds were prepared: 2-(substituted amino)-4,5-diaminopyrimidines, 2,4-diamino-5-(substituted amino)pyrimidines, and 2,5-diamino-4-(substituted amino)-pyrimidines.

It has been suggested that 2,4-diaminopyrimidines and many condensed ring systems containing the 2,4-diaminopyrimidine moiety (I) are competitive antagonists of folic acid in several biological systems.[2] A



I

number of these compounds, among them notably aminopterin and methotrexate, have been found to be beneficial to patients with leukemia. Other compounds containing structure I have demonstrated many interesting biological activities. For example, pyrimethamine [Daraprim, 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine] is active against coccidiosis[3] and the asexual blood forms in malaria parasites[4]; 2,4-diamino-5-(*p*-chlorophenoxy)pyrimidine markedly inhibited the growth of hiochi bacteria[5]; 2,6-diaminopurine possesses activity against a strain of AK 4 mouse leukemia,[6] prevents multiplication of Russian spring and summer encephalitis virus,[7] and has mutagenic effects on T4 bacteriophages[8]; 2,4-diamino-5-(2′-ethyldecyl)pyrimidine exerts a remarkable effect on Japanese encephalitis virus *in vivo*.[9]

Studies on antitumor activities with 2,4-diaminopyrimidines in our laboratories revealed that, although 2,4,6-triaminopyrimidine[10] did not exhibit anticancer activity in preliminary animal tumor systems,[11] the isomeric 2,4,5-triaminopyrimidine[12] has "confirmed" activity against the leukemia L1210 tumor system.[11] The compound is inactive against Ca755. The unique antileukemic activity of 2,4,5-triaminopyrimidine is demonstrated by the fact that the closely related 2,4,5,6-tetraaminopyrimidine,[6,10b,13] 2,4,6-triamino-5-nitrosopyrimidine,[14,15] 2,4,5-triamino-6-hydroxypyrimidine,[16] 2,4-diaminopyrimidine,[17] 2,5-diaminopyrimidine,[18] and 4,5-diaminopyrimidine[19] all failed to demonstrate activity against L1210 in mice.[11] It is also of interest that several compounds containing the 2,4-diaminopyrimidine moiety, *e.g.*, 2,4-diamino-5-nitroso-6-(*p*-bromoanilino)pyrimidine and 2,4,6-triamino-5-(*m*-toluidino)pyrimidine, exhibited "confirmed" antitumor activity against Ca755 but not against L1210.[15]

In order to better understand the structural requirements for anticancer activity of compounds related to 2,4,5-triaminopyrimidine, the amino hydrogen atoms of this pyrimidine were selectively replaced with an alkyl or aryl substituent to yield compounds of the following three categories (II–IV, see Table I).

2-(Substituted amino)-4,5-diaminopyrimidines (II) were prepared as follows. 2-Chloro-4-amino-5-nitropyrimidine, synthesized according to the method of Albert, Brown, and Cheeseman,[12b] was treated with the appropriate amines to form 2-(substituted amino)-4-

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Contract SA-43-ph-3025. Presented in part before the Division of Medicinal Chemistry, 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965.

(2) See, for example, (a) G. H. Hitchings, G. B. Elion, H. VanderWerff, and E. A. Falco, *J. Biol. Chem.*, **174**, 765 (1948); (b) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood, and H. VanderWerff, *ibid.*, **183**, 1 (1950); (c) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, and H. VanderWerff, *Ann. N. Y. Acad. Sci.*, **52**, 1318 (1950); (d) G. H. Hitchings, E. A. Falco, P. B. Russell, and H. VanderWerff, *Federation Proc.*, **10**, 198 (1951); (e) G. H. Hitchings, E. A. Falco, H. VanderWerff P. B. Russell, and G. B. Elion, *J. Biol. Chem.*, **199**, 43 (1952); (f) E. A. Falco, I. G. Goodwin, G. H. Hitchings, I. M. Rollo, and P. B. Russell, *Brit. J. Pharmacol.*, **6**, 185 (1951); (g) G. H. Hitchings, P. B. Russell, and N. Whittiker, *J. Chem. Soc.*, 1019 (1956); (h) S. F. Zakrzewski, *J. Biol. Chem.*, **238**, 1485 (1963).

(3) R. E. Luex and A. M. Brubaker, U. S. Patent 2,895,874 (July 21, 1959).

(4) See for example, ref. 2f; P. B. Russell and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 3763 (1951); G. Covell, G. R. Coatney, J. W. Field, and J. Singh, "Chemotherapy of Malaria," World Health Organization, Geneva, 1955, pp. 51–53; WHO Technical Meeting of Chemotherapy of Malaria, Geneva, 1961.

(5) S. Teramoto, W. Hashida, and M. Mukai, *Hakko Kogaku Zasshi*, **36**, 443 (1958).

(6) H. E. Skipper, L. L. Bennett, Jr., P. C. Edwards, C. E. Bryan, O. S. Hutchison, J. B. Chapman, and M. Bell, *Cancer Res.*, **10**, 166 (1950).

(7) C. Friend, *Proc. Soc. Exptl. Biol. Med.*, **78**, 150 (1951); A. E. Moore and C. Friend, *ibid.*, **78**, 153 (1951).

(8) E. Freese, *J. Mol. Biol.*, **1**, 87 (1959).

(9) I. Nakata and T. Ueda, *Yakugaku Zasshi*, **80**, 1065 (1960).

(10) (a) S. Gabriel, *Ber.*, **34**, 3363 (1901); (b) W. Traube, *ibid.*, **37**, 4544 (1904).

(11) Testing reports furnished by the Cancer Chemotherapy National Service Center.

(12) (a) O. Isay, *Ber.*, **39**, 255 (1906); (b) A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 474 (1951); (c) P. D. Laudor, and H. N. Rydon *ibid.*, 1113 (1955); (d) D. J. Brown, *J. Appl. Chem.*, **7**, 109 (1957).

(13) O. Gerngross, *Ber.*, **38**, 3406 (1905).

(14) H. Sato, M. Nakajima, and H. Tanaka, *J. Chem. Soc. Japan*, **72**, 866 (1951); E. C. Taylor, O. Vogl, and C. C. Cheng, *J. Am. Chem. Soc.*, **81**, 2442 (1959).

(15) The fact that 2,4,5,6-tetraaminopyrimidine and 2,4,6-triamino-5-nitrosopyrimidine exhibited no activity against Ca755 while closely related 2,4,6-triamino-5-(*m*-toluidino)pyrimidine and 2,4-diamino-5-nitroso-6-(substituted anilino)pyrimidines have demonstrated confirmed activity against the Ca755 system in preliminary screening is, we believe, due to possible riboflavin antagonism of the anilino derivatives. See D. E. O'Brien, F. Baiocchi, R. K. Robins, and C. C. Cheng, *J. Med. Pharm. Chem.*, **5**, 1085 (1962); *ibid.*, **6**, 467 (1963).

(16) W. Traube, *Ber.*, **33**, 1371 (1900).

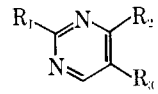
(17) (a) E. Büttner, *ibid.*, **36**, 2233 (1903); (b) T. B. Johnson and C. O. Johns, *J. Am. Chem. Soc.*, **34**, 190 (1912).

(18) G. W. Raiziss and M. Freifelder, *J. Am. Chem. Soc.*, **64**, 2340 (1942); S. Tozaki, *Rept. Sci. Police Res. Inst.* (Tokyo), **27**, 401 (1951); *cf. Chem. Abstr.*, **47**, 2181 (1953).

(19) (a) O. Isay, *Ber.*, **39**, 250 (1906); (b) D. J. Brown, *J. Appl. Chem.*, **2**, 239 (1952).

TABLE I

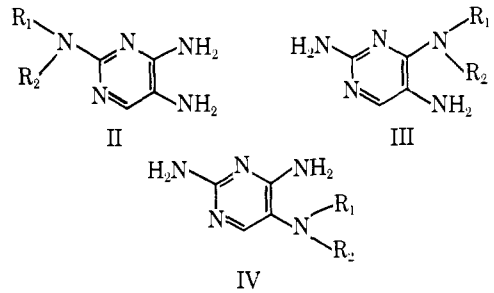
2,4,5-TRISUBSTITUTED PYRIMIDINES



													Ultraviolet absorption					
													pH 1		pH 11		methanol	
				Recrystn.	Yield,		Calcd., %			Found, %			λ_{max},		λ_{max},		λ_{max},	
R_1	R_2	R_5	Formula	solvents	%	M.p., °C.	C	H	N	C	H	N	mμ	ε	mμ	ε	mμ	ε
CH_3NH	NH_2	NO_2	$C_5H_7N_5O_2$	Ethanol	88	225–226	35.5	4.14		35.4	4.36						265	5,100
																	350	25,700
$(CH_3)_2N$	NH_2	NO_2	$C_6H_9N_5O_2$	Ethanol	72	213–215	39.3	4.92		39.0	5.58						272	4,500
																	359	21,300
$CH_3(CH_2)_3NH$	NH_2	NO_2	$C_8H_{13}N_5O_2$	Ethanol	83	118–120	45.5	6.16	33.1	45.6	6.55	32.9					262	6,100
																	252	14,800
C_6H_5NH	NH_2	NO_2	$C_{10}H_9N_5O_2$	Heptane–benzene	65	131–132	50.8	6.35	29.6	50.6	6.53	29.9					266	4,250
																	351	18,800
p-BrC_6H_4NH	NH_2	NO_2	$C_{10}H_8BrN_5O_2 \cdot 0.5H_2O$	Ethanol	82	220–222	37.6	2.82	21.9	37.2	2.80	21.6					250	17,200
																	364	25,200
CH_3NH	NH_2	NH	$C_5H_9N_5 \cdot H_2SO_4$	Methanol	76	202–204	25.3	4.64	24.6	25.2	4.96	29.3					234	19,800
																	297	1,200
																	(sh)	
$(CH_3)_2N$	NH_2	NH_2	$C_6H_{11}N_5 \cdot H_2SO_4$	Methanol	77	215–216	28.7	5.18	27.9	28.4	5.23	27.7					240	14,000
																	315	3,750
																	(sh)	
C_6H_5NH	NH_2	NH_2	$C_{10}H_{11}N_5 \cdot H_2SO_4 \cdot CH_3OH$	Methanol	70	185–186	39.2	6.82	20.7	39.2	6.85	20.5					237	17,200
																	308	1,700
																	(sh)	
p-BrC_6H_4NH	NH_2	NH_2	$C_{10}H_{10}BrN_5 \cdot H_2SO_4$	Dil. H_2SO_4	44	241–243	31.7	3.17	18.5	31.3	3.48	18.8					277	24,600
NH_2	CH_3NH	NO_2[a]	$C_5H_7N_5O_2$	Water	86	245–247	35.5	4.14	41.4	35.2	4.26	41.2					259	7,300
																	293	4,900
																	356	12,600
NH_2	$(CH_3)_2N$	NO_2	$C_6H_9N_5O_2$	Water	61	144–146	39.3	4.92	38.3	39.4	5.26	38.4					270	11,900
																	363	6,800
NH_2	$CH_3(CH_2)_3NH$	NO_2	$C_8H_{13}N_5O_2$	Heptane	56	135–136	45.5	6.16	33.1	45.3	6.58	33.1					260	9,500
																	295	6,500
																	358	14,200
NH_2	C_6H_5NH	NO	$C_{10}H_9N_5O_2$	Heptane–benzene–methanol	12	199–200	50.7	6.34	29.6	50.5	6.54	29.2					258	11,800
																	292	7,800
																	358	20,200
NH_2	CH_3NH	NH_2	$C_5H_9N_5 \cdot H_2SO_4 \cdot 0.5H_2O$	Ethanol	55	213–215	24.4	4.88	28.5	24.8	5.22	28.3					235	17,500
																	302	5,200
NH_2	$(CH_3)_2N$	NH_2	$C_7H_{13}N_5 \cdot H_2SO_4 \cdot 1.5H_2O$	Methanol	34	198–199	26.0	5.75	25.2	26.2	5.87	25.1					247	13,400
																	316	5,600
NH_2	$CH_3(CH_2)_3NH$	NH_2	$C_8H_{15}N_5 \cdot H_2SO_4$	Benzene–methanol	57	201–206	34.5	6.10	25.1	34.6	5.13	24.6					237	17,600
																	304	5,900
NH_2	C_6H_5NH	NH_2	$C_{10}H_{11}N_5 \cdot H_2SO_4$	Ethanol	79	177–178	39.4	6.23	23.0	39.5	6.46	23.1					235	25,500
																	300	8,200
CH_3NH	CH_3NH	NH_2[b]	$C_6H_{11}N_5 \cdot H_2SO_4$	Methanol	40	218–220	28.6	5.19	27.8	28.6	5.44	27.2	...	...	238	12,700	238	22,850
															332	6,800	304	6,800
OH	OH	CH_3NH[c]	$C_6H_7N_5O_2$	H_2O–DMF	71	312–314 dec.	42.5	5.00	29.8	42.2	5.23	30.0	258	7,600	227	8,800		
															289	4,500		
OH	OH	$(CH_3)_2N$[d]	$C_7H_9N_5O_2$	H_2O–DMF	78	306–308 dec.	46.5	5.85	27.1	46.6	5.92	26.6	259	6,200	230	6,100		
															291	4,000		
OH	OH	C_6H_5NH[e]	$C_{11}H_{11}N_5O_2$	H_2O–DMF	93	311–342 dec.	57.4	7.23		57.4	7.35		261	9,100	234	8,600		
															295	5,000		

R_1	R_2	R_3	Formula	Recrystn. solvents	Yield, %	M.p., °C.	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N	Ultraviolet absorption pH 1 λ_{max}, mμ	pH 1 ε	pH 11 λ_{max}, mμ	pH 11 ε	methanol λ_{max}, mμ	methanol ε
OH	OH	*m*-$CH_3C_6H_4NH$	$C_{11}H_{11}N_5O_2$	H_2O–DMF	65	317–319	60.8	5.10	19.3	60.5	5.12	19.2	245	13,000	242	12,000		
													312	3,000	277	8,000		
OH	OH	*m*-BrC_6H_4NH	$C_{10}H_8BrN_5O_2$	H_2O–DMF	22	310–312	42.6	2.86	14.9	43.1	2.90	14.7	248	14,300	247	13,000		
															290	9,000		
Cl	Cl	CH_3NH	$C_5H_5Cl_2N_3$	Heptane	35	148–149	33.7	2.83	23.6	33.9	3.15	23.3					258	15,300
Cl	Cl	$(CH_3)_2N$	$C_6H_7Cl_2N_3$	Heptane	69	90–91	37.5	3.67	21.8	37.7	3.75	21.3					260	14,100
NH_2	NH_2	CH_3NH	$C_5H_9N_5 \cdot HCl$	Ethanol	42	225–226	34.2	5.75	39.9	34.2	5.91	40.0	224	17,000	240	9,100		
													292 (sh)	3,200	302	5,400		
NH_2	NH_2	$(CH_3)_2N$	$C_6H_{11}N_5 \cdot HCl$	Ethanol	61	241–242	38.0	6.38	36.9	38.2	6.78	36.6	224	16,000	240	8,600		
													280 (sh)	3,300	298	5,900		
NH_2	NH_2	$C_6H_{11}NH$	$C_{10}H_{17}N_5 \cdot 2HCl^f$	Ethanol	11[g]	250–251 dec.	42.8	6.83	25.0	43.1	6.94	24.9	...	...	240	8,400		
															296	5,200		
NH_2	NH_2	*m*-$CH_3C_6H_4NH$	$C_{11}H_{13}N_5$	Butanol	18[g]	183–184	61.4	6.10	32.5	61.2	6.46	32.3	232	20,200	240	14,400		
													260 (sh)	11,000	278	8,800		

[a] E. C. Taylor and M. J. Thompson [*J. Org. Chem.*, **26**, 5224 (1961)] prepared this compound in 91% crude yield from 2-chloro-4 methylamino-5-nitropyrimidine. [b] The starting material, 2,4-bis(methylamino)-5-nitropyrimidine, was reported by D. J. Brown, *J. Appl. Chem.*, **4**, 72 (1954). [c] T. B. Johnson and I. Matsuo [*J. Am. Chem. Soc.*, **41**, 782 (1919)] reported m.p. 297° dec. [d] H. L. Wheeler and H. F. Merriam [*Am. Chem. J.*, **31**, 603 (1904)] reported m.p. 297° dec. [e] Lit.[28] m.p. >305°. [f] *Anal.* Calcd.: Cl⁻, 25.3. Found: Cl⁻, 25.4. [g] Over-all yield calculated from 5-(substituted amino)uracil.



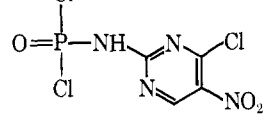
II III

IV

amino-5-nitropyrimidines. These intermediates were then hydrogenated in the presence of Raney nickel to give the desired 2-substituted aminopyrimidines (II), isolated as sulfates.

Brown[12d] has reported the preparation of 2-chloro-4-methylamino-5-nitropyrimidine by the reaction of 2,4-dichloro-5-nitropyrimidine[20] with aqueous methylamine in the presence of acetic acid in dioxane. The reported yield of the monoaminated product[12d] was rather low with the formation of a considerable amount of bisaminated product. This method was repeated in our laboratory under various conditions and was found to be impractical for the synthesis of 2,5-diamino-4-(substituted amino)pyrimidines. Another route *via* 2-amino-4-chloropyrimidine[21] → 2-amino-4-(substituted amino)-pyrimidine → 2-amino-4-(substituted amino)-5-nitropyrimidine also met with little success.

Chlorination of 2-amino-4-hydroxy-5-nitropyrimidine[22] with phosphorus oxychloride yielded an interesting and stable intermediate, 4-chloro-5-nitro-2-pyrimidinylphosphoramidic chlorides[23] (V). Treatment of V with the appropriate amines followed by hydrolysis in



V

dilute hydrochloric acid cleaved the N–P linkage to give 2-amino-4-(substituted amino)-5-nitropyrimidine which, upon reduction, yielded the 2,5-diamino-4-(substituted amino)pyrimidines (III).

Reaction between 5-bromouracil and aliphatic amines to yield 5-(substituted amino)uracils was reported as early as the turn of this century.[24,25] Although Phillips[26] claimed that 5-anilinouracils could not be prepared by this method, it was found in our laboratory that substituted anilines reacted readily with 5-bromouracil in ethylene glycol to yield 5-(substituted anilino)uracils.[27] Subsequent chlorination of these 5-(alkyl- or -arylamino)uracils with phosphorus oxychloride and

(20) N. Whittaker, *J. Chem. Soc.*, 1565 (1951).

(21) (a) S. Gabriel and J. Coleman, *Ber.*, **36**, 3379 (1903); (b) S. Gabriel, *ibid.*, **38**, 1691 (1905); (c) M. E. Hultquist and E. Kuh, British Patent 559,455 (Feb. 21, 1944); (d) E. Kuh and T. W. Clapper, U. S. Patent 2,425,248 (Aug. 5, 1947).

(22) T. B. Johnson and C. O. Johns, *Am. Chem. J.*, **34**, 559 (1905).

(23) Chlorination of an aminopyrimidine with phosphorus oxychloride to give a phosphoramidic dichloride was first reported by T. B. Johnson, *ibid.*, **34**, 191 (1905).

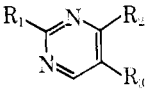
(24) H. L. Wheeler and H. F. Merriam, *ibid.*, **32**, 355 (1904).

(25) (a) T. B. Johnson and I. Matsuo, *J. Am. Chem. Soc.*, **41**, 782 (1919); (b) S. Y. Wang, *J. Org. Chem.*, **24**, 11 (1959).

(26) A. P. Phillips, *J. Am. Chem. Soc.*, **73**, 1061 (1951).

(27) While our work was in progress, British Patent 971,307 (Sept. 30, 1964) describing the preparation of 5-anilinopyrimidines from 2,4-di(bifunctionally substituted)-5-halogenopyrimidine was published. The approach and reaction conditions used are in complete agreement with ours.

TABLE II: PRELIMINARY SCREENING OF SOME 2,4,5-TRISUBSTITUTED PYRIMIDINES



R_1	R_2	R_5	Tumor system[a]	Dose, mg./kg.	Survivors	Wt. dif.	T/C, %	Slope	ED_{50}, μg./ml.
CH_3NH	NH_2	NO_2	SA	500	0/6	...	...		
				125	6/6	−2.5	145		
			CA	100	10/10	−1.2	53		
			LE	100	6/6	0.2	104		
			KB					−1.2	36
$(CH_3)_2N$	NH_2	NO_2	SA	500	5/6	−3.6	78		
			CA	400	10/10	−2.3	72		
			LE	400	4/6	−2.3	89		
			KB					−1.4	32
$CH_3(CH_2)_3NH$	NH_2	NO_2	SA	500	5/6	2.2	45		
				500	5/6	−0.3	156		
			CA	400	10/10	0.6	75		
			LE	400	6/6	−0.7	93		
			KB					−1.1	24
$C_6H_{11}NH$	NH_2	NO_2	SA	500	3/6	1.5	...		
				250	1/6	0.6	...		
				62	4/6	−1.0	198		
				62	7/7	−0.8	111		
			CA	50	10/10	−1.5	101		
			LE	50	6/6	−0.3	106		
			KB					−1.1	25
p-BrC_6H_4NH	NH_2	NO_2	SA	500	4/6	−5.0	33		
				500	6/6	0.8	74		
			CA	400	9/10	−1.7	82		
			LE	400	6/6	−1.3	97		
			KB					−1.1	25
NH_2	CH_3NH	NO_2	SA	500	0/6	...	...		
				125	6/6	0.3	58		
			KB					...	>100
NH_2	$(CH_3)_2N$	NO_2	SA	500	5/6	−1.5	68		
			CA	400	10/10	1.0	89		
			LE	400	6/6	−1.9	95		
			KB					−0.86	38
NH_2	$CH_3(CH_2)_3NH$	NO_2	SA	500	0/6	...	...		
				125	2/6	−0.06	...		
				32	4/6	−2.1	49		
			LE	26	6/6	−1.2	100		
			KB					−0.54	53
NH_2	$C_6H_{11}NH$	NO_2	SA	250	6/6	−1.5	113		
			LE	200	6/6	−1.3	102		
CH_3NH	CH_3NH	NO_2	SA	500	5/6	−3.5	78		
			CA	400	9/10	−2.2	71		
			LE	400	6/6	−2.0	98		
			KB					−0.83	74
Cl_2PONH	Cl	NO_2	SA	500	0/6	...	...		
				125	1/7	4.7	..		
				31	7/7	−0.1	115		
			CA	25	9/10	0.5	76		
			LE	25	6/6	−0.8	97		
			KB					−1.2	29
Cl	Cl	NH_2	SA	500	0/6	...	...		
				125	1/6	−1.1	...		
				31	6/6	−0.3	103		
			LE	25	6/6	1.5	94		
NH_2	NH_2	NH_2	SA	500	6/6	−1.3	48		
			CA	240	10/10	−1.3	96		
			LE	810	4/6	−5.0	86		
				540	5/6	−2.3	134		
				500	5/6	−2.4	160		
				360	6/6	−2.9	159		
				240	6/6	−1.5	132		
				160	6/6	−0.3	135		
				70	6/6	−1.1	125		
				31	6/6	−0.8	106		
				21	6/6	−0.5	101		
			LZ	300	10/10	−0.5	100		
			KB					−0.82	30

TABLE II (*Continued*)

R_1	R_2	R_3	Tumor system[a]	Dose, mg./kg.	Survivors	Wt. dif.	T/C, %	Slope	ED_{50}, μg./ml.
CH_3NH	NH_2	NH_2	SA	500	2/6	−1.0	...		
				250	6/6	−0.3	87		
			CA	200	10/10	−1.1	64		
			LE	200	6/6	−2.9	100		
			KB					−0.49	72
$(CH_3)_2N$	NH_2	NH_2	SA	500	1/6	−2.3	...		
				125	6/6	0.2	73		
			CA	100	10/10	−0.9	155		
			LE	100	6/6	−1.3	115		
			KB					...	>100
$C_6H_{11}NH$	NH_2	NH_2	SA	500	0/6	...	...		
				125	7/7	−0.9	99		
			CA	100	10/10	0.9	110		
			LE	100	6/6	−1.6	101		
			KB					...	>100
p-BrC_6H_4NH	NH_2	NH_2	SA	500	5/6	−3.5	120		
			CA	400	7/10	0.3	85		
			LE	400	6/6	−3.0	101		
			KB					−1.1	25
NH_2	$(CH_3)_2N$	NH_2	SA	500	0/6	...	...		
				100	6/6	−2.7	83		
			KB					...	>100
CH_3NH	CH_3NH	NH_2	SA	500	0/6	...	...		
				125	6/6	−3.5	84		
			CA	100	10/10	−0.1	161		
			LE	100	6/6	−0.4	108		
			KB					...	>100
OH	OH	CH_3NH	SA	500	5/6	−3.3	89		
			CA	400	8/10	−0.8	135		
			LE	400	5/6	−2.5	114		
			KB					...	>100
OH	OH	$(CH_3)_2N$	SA	500	0/6	...	...		
				125	5/6	1.0	123		
			CA	100	9/10	−5.2	77		
			LE	100	6/6	−0.7	102		
			KB					...	>100
OH	OH	$C_6H_{11}NH$	SA	500	6/6	0.3	134		
			CA	400	10/10	0.0	102		
			LE	400	6/6	−0.8	103		
			KB					...	>100
OH	OH	*m*-$CH_3C_6H_4NH$	SA	500	6/6	0.0	85		
			CA	400	8/10	−5.4	63		
			LE	400	6/6	−0.9	87		
			KB					...	>100
OH	OH	*m*-BrC_6H_4NH	KB					−0.33	>100
Cl	Cl	CH_3NH	SA	500	6/6	−0.1	58		
			CA	400	3/10	−6.7	...		
				200	10/10	−2.4	80		
			LE	400	3/6	−3.5	...		
				200	3/6	−3.7	...		
				100	6/6	1.0	96		
			KB					−1.3	3.0
								−1.0	2.4
								−3.2	1.7
Cl	Cl	$(CH_3)_2N$	SA	500	5/6	−2.5	54		
			CA	200	0/10	...	...		
				100	10/10	−1.0	95		
			LE	400	4/6	−1.0	92		
			KB					−1.0	22
NH_2	NH_2	CH_3NH	SA	125	6/6	−0.8	101		
			KB					−0.49	36
NH_2	NH_2	$(CH_3)_2N$	SA	250	0/6	...	...		
				63	6/6	0.1	89		
			LE	50	6/6	−0.2	103		
NH_2	NH_2	$C_6H_{11}NH$	KB					−0.68	22
NH_2	NH_2	*m*-$CH_3C_6H_4NH$	SA	63	6/6	−2.7	87		
			LE	48	6/6	−1.9	101		
			KB					−0.68	40

[a] SA = Sarcoma 180, CA = Adenocarcinoma 755, LE = lymphoid leukemia L1210, KB = tissue culture (cell line), LZ = L1210 (delayed).

N,N-dimethylaniline afforded the corresponding 2,4-dichloro-5-(substituted amino)pyrimidines. These were then treated with alcoholic ammonia at elevated temperature to yield the desired 2,4-diamino-5-(substituted amino)pyrimidines (IV).

Preliminary screening results[11] (see Table II) indicated that substitution of the amino group in either position 2 or 4 of 2,4,5-triaminopyrimidine results in the loss of antileukemic activity. One of the intermediates, 2,4-dichloro-5-methylaminopyrimidine, exhibited confirmed activity in cell culture cytotoxicity tests. The mode of action and specificity of the antileukemic activity of 2,4,5-triaminopyrimidine is still being investigated.

Experimental Section[28]

General Preparation of 2-(Substituted amino)-4-amino-5-nitropyrimidines.—To a mixture of 10 g. (0.057 mole) of 2-chloro-4-amino-5-nitropyrimidine[12b] in 200 ml. of absolute ethanol was added 0.114 mole of the appropriate amine (with lower boiling amines, a fivefold excess of aqueous solution of an amine was used). The mixture was refluxed with stirring for 2 hr., diluted with 500 ml. of water, and allowed to stand at room temperature overnight. The resulting precipitate was filtered, washed with water, and dried *in vacuo* to yield the desired product (see Table I).

General Preparation of 2-(Substituted amino)-4,5-diaminopyrimidines (II).—A mixture of 10 g. of the preceding nitro compound, 200 ml. of absolute ethanol, and 3 g. of Raney nickel was hydrogenated at 4 atm. for 90 min. The mixture was filtered and to the filtrate was added 100 ml. of absolute ethanol followed by careful addition of an equivalent amount of H_2SO_4. The mixture was then stirred for 30 min. and refrigerated overnight. The resulting precipitate was filtered and washed well with petroleum ether (b.p. 35–60°). The product was dried *in vacuo* over P_2O_5 and silica gel.

4-Chloro-5-nitro-2-pyrimidinylphosphoramidic Dichloride (V).—A mixture of 50 g. of 2-amino-4-hydroxy-5-nitropyrimidine and 1 l. of phosphorus oxychloride was refluxed for 5 hr. Excess reagent was removed under reduced pressure. To the syrupy residue was added 400 ml. of dry benzene, and the mixture was heated gently on a steam bath (with drying tube attached) for 2 hr. The mixture was rapidly filtered and the filtrate was quickly cooled in an ice bath with constant stirring. The solid product, which gradually separated from the filtrate by scratching the side of the flask with a glass rod, was isolated and recrystallized twice from 400 ml. of dry benzene to yield, after drying at 70° (15 mm.) for 45 min., 32 g. of white solid, m.p. 132°, $\lambda_{max}^{methanol}$ 224 mμ (ε 11,000) and 324 mμ (ε 14,300).

Anal. Calcd. for $C_4H_2Cl_3N_4O_3P$: C, 16.5; H, 0.69; Cl, 36.6; N, 19.2. Found: C, 17.0; H, 0.75; Cl, 36.5; N, 19.1.

General Preparation of 2-Amino-4-(substituted amino)-5-nitropyrimidines.—Ten grams of V was added at 0° with stirring to 200 ml. of an aqueous solution of at least 2 equiv. of the appropriate amine. The mixture was heated on a steam bath for 30 min. while an excess of the amine was maintained at all times. The hot reaction mixture was acidified to pH 1 with dilute HCl and then heated on the steam bath for 30 min. The solution was decolorized with charcoal and filtered while still hot. The filtrate was adjusted to pH 8–9 by the addition of aqueous ammonia, then chilled. The resulting precipitate was collected by filtration, washed with water, and dried at 80°.

2,5-Diamino-4-(substituted amino)pyrimidines (III) were prepared from the aforementioned nitropyrimidines by essentially the same method used for the preparation of II.

General Preparation of 5-(Alkylamino)uracils and 5-(Dialkylamino)uracils.—A mixture of 19.1 g. (0.1 mole) of 5-bromouracil[24,25] and 200 ml. of a 30% aqueous solution of the appropriate amine was heated on the steam bath for 3.5 hr. During this time a complete solution resulted, followed by gradual reprecipitation of a solid. The reaction mixture was added to 250 ml. of water, and the pH was adjusted to 6–7 with dilute HCl. The resulting solid was separated by filtration, washed with water, and dried at 80°. The product was purified either by dissolving in hot dilute NaOH, decolorizing with charcoal, filtering, and acidifying the filtrate with acetic acid; or by recrystallizing from a mixture of water and dimethylformamide.

General Preparation of 5-(Substituted anilino)uracils.—A mixture of 38.2 g. (0.2 mole) of 5-bromouracil, 0.4 mole of the appropriate aniline, and 100 ml. of ethylene glycol was heated at 195° for 1 hr. During this time a dark solution was formed which was followed by gradual reprecipitation of a solid. The mixture was added to 1 l. of water, and the resulting solid was filtered and washed successively with water, ethanol, and ether. Purification was done in a similar fashion as for the aliphatic analogs.

General Preparation for 2,4-Dichloro-5-(substituted amino)pyrimidines.—A mixture of 20 g. of 5-(substituted amino)uracil, 40 ml. of N,N-dimethylaniline, and 500 ml. of phosphorus oxychloride was refluxed (stirring) for 4 hr. Excess solvent was removed *in vacuo* on a steam bath. The syrupy residue was added, with vigorous stirring, to flaked ice. The resulting icy mixture was stirred for 30 min. and extracted with three 300-ml. portions of ether. The ethereal extract was washed well with cold water and dried over anhydrous Na_2SO_4. The ether was then evaporated to yield the yellow, crystalline chloro derivative.

General Preparation of 2,4-Diamino-5-(substituted amino)pyrimidine.—A solution of 10 g. of 2,4-dichloro-5-(substituted amino)pyrimidine in 250 ml. of ethanol saturated with ammonia at 0° was heated in an autoclave at 180° for 12 hr. After cooling, the mixture was evaporated to dryness. The residue was extracted with 700 ml. of boiling butanol and the butanol extract was evaporated *in vacuo* to yield the crude triamino derivative. The 5-alkylamino derivatives were isolated as monohydrochlorides from the reaction mixture. Since 2,4-diamino-5-cyclohexylaminopyrimidine monohydrochloride was difficult to purify, it was converted to a dihydrochloride salt with ethanolic HCl (see Table I).

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(28) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer.